# FAT DEPOSITION DURING POSTEMBRYONIC GROWTH IN THE DOMESTIC DUCK, *ANAS PLATYRHYNCHOS*, WITH SPECIAL REFERENCE TO THE ACTION OF SOME HORMONES.

by

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"laws of nature are delimitations
which, on the basis of our experience,
we set our expectations."

Ernst Mach.

Abstract.

Analysis of the accretionary growth of fat in various depots of the juvenile domestic duck using both Gompertz and allometric equations, has indicated that the growth pattern of this body component is parallel to that of other body components. Although fat is a relatively late growing component, it has been clearly demonstrated, that its growth is not associated with the onset of chemical maturity. These studies have also shown that the accretionary growth pattern of the fat varies between the depots.

The distribution of fat in the domestic duck, whereby the largest deposit is in the subcutaneous region, is consistent with the semi-aquatic habitat of the wild form of this species. Fasting experiments indicate that fat from this deposit can be readily mobilised.

A comparison of the distribution and growth of the fat deposits in the domestic duck with that of two wild breeds has shown that the rate of accretion of fat during growth is greatest in the domestic breed.

By combining the results obtained from total fat extractions and measurements of adipocyte size, of the fat depots, it has been demonstrated that the capacity of the fat depots to store fat is increased during growth both by hyperplasia and hypertrophy. The estimated rate of cell division declined with age. This result was in agreement with electron microscope observations, in which it was found that the primordial adipocytes were common in the adipose

tissue from young birds, but rare in this tissue from older birds. A striking structural feature of avian adipose tissue was the regular array of microfibrils about the lipid droplets.

Insulin was demonstrated to have neither any *in vitro* nor *in vivo* lipogenic action in the duck. The effect of this hormone administered *in vivo* on plasma NEFA levels was investigated.

Incorporation studies using labelled glucose indicated that the liver had much greater *in vitro* lipogenic activity than the adipose tissue. However the liver showed neither any change in activity with age, nor following the *in vitro* addition of 'lipogenic' hormones. The former result has suggested that the adipose tissue represents the most probable site for the regulation of fat deposition during growth. The manner by which such regulation might be achieved has been discussed.

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# 1. Introduction.

## 1. Introduction.

The concept of fat as an inert deposit whose primary roles were in insulation and the provision of mechanical support, persisted into the early 1930s (Wertheimer, 1965). The slow growth of knowledge relating to lipogenesis and adipose tissue, over the previous eighty years has been described by McHenry and Cornett (1944) and Wertheimer and Shapiro (1948) respectively. It was not until Schoenheimer and Rittenberg (1936, 1937) had demonstrated both the rapid rates of turnover and synthesis of fat in the animal body, that the subject managed to attract much scientific interest. Thereafter however a more concerted approach to the study of fat metabolism developed. Research in the late 1930s was restricted almost entirely to the use of liver and consequently the idea became accepted that this tissue represented the major if not only site for lipogenesis in the mammalian body (Barret et al., 1938; McHenry, 1937; Stetten and Schoenheimer, 1940). Then in 1948 Shapiro and Wertheimer demonstrated for the first time the ability of rat adipose tissue to synthesise fatty acids in vitro. This finding was to divert attention almost entirely from hepatic lipogenesis in the following years.

The next major development was the discovery of a highly metabolically active fraction of the plasma lipids, the non-esterified fatty acids (NEFA) (Gordon and Cherkes, 1956; Dole, 1956; Laurell, 1956). These were shown to originate from the fat in the adipose tissue, and to con-

stitute a major source of energy in the body (Gordon and Cherkes, 1958; Dole, 1958; Goodman and Gordon, 1958; Fredrickson and Gordon, 1958; Jeanrenaud, 1961; Gold and Spitzer, 1964; Spitzer and Gold, 1964).

Over the past 20 years the almost explosive increase in the knowledge of the physiology and biochemistry of adipose tissue can be largely attributed to the interest which has been developed in both the active lipogenesis, and the hormonal sensitivity of rat and mouse white adipose tissue. Consequently when two major reviews on the physiology of adipose tissue were published in 1965 (Whipple, and Renold and Cahill) well over 50% of the articles in each dealt with rat or mouse adipose tissue exclusively. The justification for the virtual total use of these tissues has no doubt rested on the belief that they are entirely representative of adipose tissues from other mammals, and that adipose tissue is the major site of lipogenesis in the mammalian body. Recent findings however have shown that there is considerable variability between the lipogenic activity of adipose tissue from different mammalian species (Rudman and DiGirolamo, 1966a). In addition the primary role of the adipose tissue in lipogenesis in the intact rat has been challenged by the findings of Hollenberg and Vost (1968). In view of the species variability already encountered, it has become important to examine the pathways and systems involved in lipogenesis in other homoiotherms. This is particularly relevant to the problem of the increasing incidence of obesity in man in the affluent society,

where diseases associated with this condition are becoming increasingly prevalent. It is also of considerable significance to those involved in animal production since the control of carcase quality requires an understanding of the processes involved in lipogenesis.

Progress in commercial duck production has largely been hindered by problems of obtaining birds of a desirable composition. In the fast growing strains, the advantages of the rapid growth rates are frequently offset by increasing rates of fat deposition. This problem has afforded an opportunity to investigate lipogenesis in the bird, and in particular to investigate the processes involved in the control of fat deposition during postembryonic growth.

At the time this study was begun no account had been published on either the growth of the fat deposits in the duck, or on the possible mechanisms responsible for regulation of avian fat deposition. It has consequently been necessary to adopt the broadest approach possible in investigating this problem.

First, the accretionary growth of fat in the main deposits of the duck was examined. This was intended to provide basic data about the rates of fat deposition and the nature of the growth of the fat deposits.

Second, since adipose tissue represents the actual storage organ for the deposition of fat, a study of the histological changes occurring during growth were undertaken to investigate the role that this tissue plays in fat deposition.

Third, the action of insulin in birds was difficult to reconcile with its action in mammals. This indicated the presence of some striking differences in the regulation of lipid and carbohydrate metabolism in the bird which warranted further investigation.

Fourth, in vitro studies were used to determine the relative lipogenic activity of liver and adipose tissues, the lipogenic action of some hormones, and possible changes in lipogenic and lipolytic activity of the liver and adipose tissues during growth.

## 2. A general review of the literature.

## 2.1. The fat deposits: a phylogenetic approach.

Specialised tissue for the storage of fat appears to have evolved in only two animal groups, the insects and the vertebrates. In the absence of such tissues the role of energy storage has been frequently assumed by other tissues such as liver and muscle.

Vague and Fenasse (1965) have reviewed the distribution of fat storing tissues in the animal kingdom. According to these authors such tissue first appears in some coelenterates, namely the Siphonophores where oil containing cells, oleocytes, aid in flotation. However in many pelagic protozoans such as the Radiolarians (Allee et al., 1949) oil droplets are maintained within the cytoplasm for a similar purpose. Specific deposits of oil which provide sufficient bouyancy to enable animals to maintain a pelagic habitat are also notable in many species of aquatic invertebrates and some fish (Waterman, 1961; Allee et al., 1949).

Less specialised deposits of oil occur in the gastric caeca of Asteroids (Echinodermata) (Vague and Fenasse, 1965). Such deposits also contain protein and both the oil and protein are used in the seasonal growth and development of the gonads. Oligochaete, Annelids store lipids in cholaragogonic cells which occur along the coelomic wall of the intestine. These cells are thought to serve a dual role as storage organs for various nitrogenous waste products, in addition to being energy stores. This two fold function of fat stores has arisen independently in other phyla. In

Chilopods and some insects waste also accumulates in the fat bodies.

The hepatopancreas first appears as a fat storing organ in some arthropods, namely the Crustaceans and Arachnids, and later in molluscs and vertebrates. In Crustaceans lipid depletion of the hepatopancreas occurs during moulting and ovogenesis (Waterman, 1961). The arthropod groups devoid of a liver have usually developed separate perivascular fat bodies. The structure of these tissues in many cases is very similar to that of the adipose tissue of vertebrates. A detailed account of the fat bodies in insects is given by Tietz (1965). These fat bodies also frequently act as stores for protein and glycogen, and again in some cases they are associated with waste storage. The greatest development of the insect fat bodies occurs usually in the immature forms. Their metabolic activity is such that Tietz (1965) considered their normal function to combine that of both energy store and liver. In the group of insects which use fat as the principal source of energy during flight namely locusts, aphids and some Lepidopteran species, lipid is rapidly mobilised from the fat bodies. In the migrating locust large deposits of fat are deposited prior to and consumed during migratory flight. In some species of moths there is sexual dimorphism in the fat content of the adult. The greater fat deposits in the male were related to its need to fly considerable distances to search out and find the females. In Dipteran species in which carbohydrate provides energy for flight, fat deposits

are nevertheless frequently considerable. In the female mosquito Culex pipiens large deposits of fat are stored prior to entering hibernation.

In most molluscs the hepatopancreas forms the major lipid store although in certain Lamellibranchs, such as the oyster, fat is also stored in vesiculous cells in the connective tissue of the mantle and within chromolipoid containing cells of the digestive diverticula. These deposits are depleted after gametogenesis and as a result of variation of various external factors such as temperature and salinity. In the snail Helix pomata lipid is contained both in the hepatopancreas and the calcareous cells. These deposits accumulate prior to the seasonal onset of reproductive activity.

In the vertebrates, adipose tissue has arisen both in the poikilotherms and the homoiotherms, although in the former its distribution is not universal. In the absence of adipose tissue in the poikilothermic vertebrates the role of energy storage has been taken over by the liver. In Elasmobranch fishes, the liver represents the sole lipid store, and consequently its lipid content can reach striking proportions (90% in a selachian, Marcelet, 1944). Vague and Fenasse (1965) have made the interesting observation that both the large size of the liver (up to 25% of body weight in some sharks) and its high fat content might be accounted for by the role it may play in bouyancy, since Elasmobranchs do not possess a swim bladder. In many mid water fishes the incidence of large deposits of wax esters

(slightly less density than triglyceride) has been attributed to the need of such species to achieve neutral bouyancy (Nevenzel et al., 1965, 1966).

Adipose tissue is rare in Teleost fish. Tashima and Cahill (1965) have made the observation that in the bottom dwelling forms practically all the lipid is stored in the liver, whereas in the pelagic species fat is stored in large deposits in and about the skeletal muscle. Muscle lipid is particularly notable in the migrating species such as the salmon. Both spawning and migration result in the exhaustion of such reserves. In both marine teleosts and other fish, both the degree of unsaturation and chain length of fatty acids increases with depth (Lewis, 1967). This is presumed to be an adaptation to the decrease in temperature occurring with depth.

Although adipose tissue first appears in the teleost fishes (Vague and Fenasse, 1965), it accounts for only a very minor part of the total lipid stored in such species. In the Amphibians the adipose tissue becomes more conspicuous, and in reptiles large postcoelomic deposits occur in many temperate-climate species (Miller, 1954; Tinkle, 1962; Bustard, 1967). Large adipose bodies in the abdomen associated with the gonads represent conspicuous deposits in both urodele and anuran amphibia. Gonadal fat bodies are also conspicuous in some lizards. These have been shown to be utilised during ovogenesis (Hahn and Tinkle, 1965; Smith, 1968). In an anuran Rana tigrans considerable amounts of lipid are deposited in the ~~lipid~~ liver prior to hibernation (Singh and Kanungo, 1968). Adipose tissue reaches

full development in the homoiotherms, the birds and mammals, and shows greatest development in the completely aquatic species of these vertebrate classes (Vague and Fenasse, 1965). In such species practically all the adipose tissue can be found in the subcutaneous region where its role in insulation becomes vital, particularly in those homoiotherms which inhabitat the seas in the antarctic and arctic regions. Specialised deposits in the mammals are numerous. In desert ruminants most of the body fat may be contained within a single discrete deposit. Examples of this form of distribution are the camels, zebu, yaks and various breeds of sheep. Such a distribution is presumably an adaptation to temperature regulation in a hot climate. Notable seasonal increases in the fat deposits occur in migrating birds (Odum, 1965) and in hibernating mammals. The latter normally also possess brown adipose tissue which is believed to play an important role in arousal and return to normal temperature regulation at the completion of the hibernation period. However this tissue is distributed more widely amongst the mammals than hibernating species alone. It occurs in the following mammalian orders; Insetivora, Chiroptera, Rodentia, and Carnivora.

In man the distribution of fat within the body is influenced both by sex and race. Geographic variation related to climate is common. The ethnic steatopygia in the Hottentot or Bush woman however may have nothing more than cosmetic value, since there is little evidence that such deposits are mobilised during fasting.

The independent evolution of fat deposits of different types within the animal kingdom suggest that there has been neither a common origin or common set of circumstances responsible for its development. The accumulation of lipid for the regulation of bouyancy has been achieved by many species in various types of cells. In those examples in which lipid deposits are associated with the storage of nitrogenous waste products it is tempting to suggest that lipid may also have been originally accumulated as a waste material and secondarily utilised as an energy store. The extent to which a similar evolutionary process has been responsible for the evolution of adipose tissue in the higher animals cannot be assessed.

Attention has already been drawn to the similarity between the adipose tissues of the insects and the vertebrates. It is further notable that in both groups such tissues have been associated with either the production of or may have originated from blood cells. In the homoiotherms it is now generally accepted that adipose tissue cells most probably arise from a primitive reticulocyte (see Section 4.2.1.).

Adipose tissue in the vertebrates was developed before it was necessary for the thermoregulative requirements of the homoiotherm. This suggests that its function is primarily that of an energy store, and that it has been secondarily adapted to the insulation requirements of the homoiotherms. This is borne out by the observation that even in the completely aquatic mammals in antarctic waters the subcut-

aneous fat can if required be readily mobilised at the expense of the animals thermal efficiency and chances of survival (Bryden, 1968).

## 2.2. Pathways for fat synthesis.

As indicated in the previous section (2.1.) two types of adipose tissue occur in homoiotherms. Brown adipose tissue has not been found to occur in birds, and is consequently not considered in the following discussions. An excellent recent review on all aspects of this tissue has been written by Smith and Horwitz (1969).

This review has been restricted to an examination of lipogenesis in homoiotherms alone and white adipose tissue specifically. Renold and Cahill (1965) provides a source of information about lipogenesis in various poikilotherms.

Although some lipid synthesis occurs in the intestinal mucosa, the two sites considered to be most important for lipid synthesis in the homoiotherms are the liver and adipose tissue. With the discovery of the high lipogenic activity of rat and mouse adipose tissue, the tendency has been to neglect the role of the liver. However results recently obtained with avian species may cause attention to be redirected toward the liver, and recent studies in the rat (Hollenberg and Vost, 1968) indicate that hepatic lipogenesis may well be of primary importance in the production of fat within the intact mammal.

In this section the pathways related to lipid synthesis mainly in rat white adipose tissue have been outlined. In most cases however this biochemistry will be equally applicable to lipogenic tissues in other species.

The two basic pathways for lipid (triglyceride) synthesis start with two different precursor molecules, in

TRIGLYCERIDE DEPOSITION FROM DIETARY TRIGLYCERIDE.

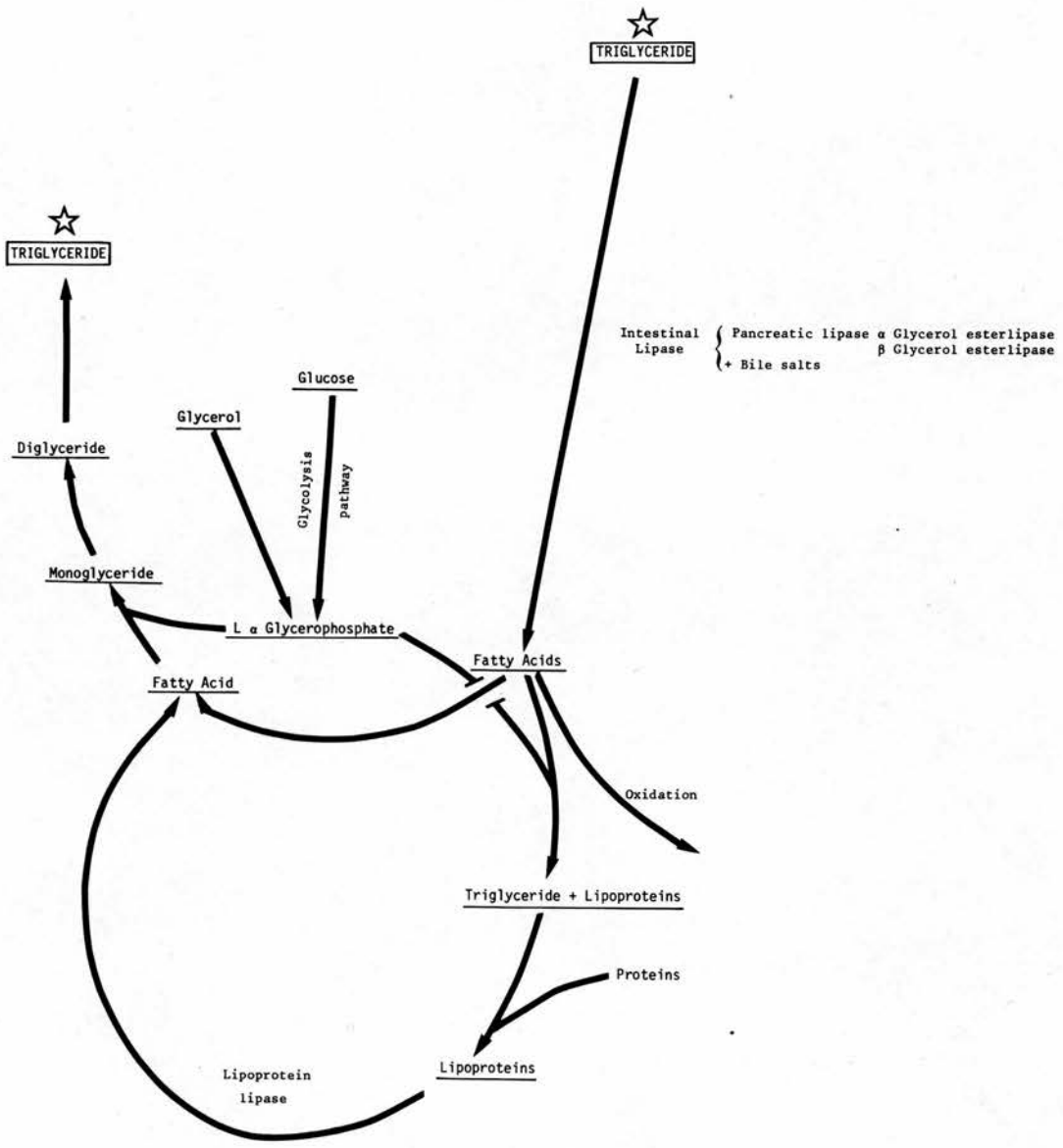



Figure 2.1
(derived from literature cited in text)

the diet, namely triglyceride and glucose. Sugars released from the breakdown of carbohydrate within the alimentary canal are taken up into the bloodstream from which they are absorbed into the lipogenic tissues. Fatty acid synthesis from glucose in these tissues follows essentially similar pathways, which are described in Section 2.2.1. Triglyceride synthesised in situ in adipose tissue is deposited directly into the lipid droplet within the adipocyte. Triglyceride synthesised within the liver however is largely converted into the form of complex particles, the very low density lipoproteins which are released into the bloodstream. The subsequent lipolysis of these lipoproteins, followed by the uptake and re-esterification of the fatty acids by the adipose tissue, constitute pathways common to lipogenesis from dietary fat or carbohydrate. This aspect of lipid metabolism is considered in Section 2.2.1.

2.2.1. Triglyceride synthesis from dietary triglyceride. (Fig. 2.1).

The processes involved in the breakdown and absorption of dietary triglyceride in the small intestine have been recently reviewed by Johnston (1968). The emulsified triglycerides in the intestinal lumen are hydrolysed mainly at the 1,3 positions by pancreatic lipases. The resulting NEFA and monoglycerides form micelles in the presence of bile salts and are then absorbed into the intestinal cells by some energy independent process. Within these cells the fatty acids are re-esterified to the monoglycerides and some endogenously produced α glycerophosphate

to form stable particles, mainly chylomicrons, in combinations with very small amounts of protein, cholesterol, cholesterol esters and phospholipids. On entering the bloodstream these chylomicrons become mixed with lipoproteins synthesised in the liver from either plasma NEFA or glucose. Traditionally the next step as elaborated by Bierman (1965) is that the chylomicrons are removed from the circulation by the liver, where they are degraded and the products mixed with endogenous fat. Particulate fat is resynthesised and reenters the bloodstream as smaller secondary particles containing both dietary and body fat. This explanation has been challenged by Felts (1965) who found that perfused rat liver was incapable of hydrolysing chylomicron triglyceride. More recently this observation has gained further support from the discovery that the lipoprotein lipase in liver is normally in an inactive state (Mayes and Felts, 1968). Lipoprotein lipase is normally responsible for the hydrolysis of the triglyceride in both chylomicrons and lipoproteins in the bloodstream (Robinson, 1963a). It is found in adipose tissue (Rodbell, 1964b), cardiac muscle (Delcher et al., 1965) skeletal muscle (Hollenberg, 1960) and lung tissue (Robinson, 1965). It has now been established that in adipose tissue the lipoprotein lipase is produced within the adipocyte (Rodbell, 1964b; Pokrajac et al., 1967) and not within the capillaries as proposed by others (Ho et al., 1967). Its activity within adipose tissue is increased by glucose loading or feeding fat (Pokrajac and Lossow, 1967) and reduced by fasting and diabetes (Hollenberg, 1959; Pav and

Wenklova, 1960; Kessler, 1963), exercise (Nikkila et al., 1963) and high levels of NEFA (Nikkila and Pykälistö, 1968). In skeletal muscle lipoprotein lipase activity increases during exercise and consequently uptake of NEFA from hydrolysed plasma triglycerides will increase in this tissue at the same time that the uptake into the adipose tissue is decreasing. It will therefore be apparent that the fate of the plasma triglycerides will depend largely upon the relative activities of the lipoprotein lipase in the different body tissues. This situation will be equally applicable to triglycerides derived from either dietary lipid or carbohydrate.

In adipose tissue, hydrolysis of the triglyceride is thought to precede absorption (Bragdon and Gordon, 1958; Bezman et al., 1962; Robinson, 1965) and probably occurs at sites along the capillary lumen (Pokrajac et al., 1967). Little of the resulting glycerol enters the adipocyte (Borgstrom and Jordan, 1959). Adipose tissue however lacks glycerokinase (Lynn et al., 1960) and consequently α glycerophosphate must be generated within the cell, presumably from glucose, as a necessary intermediate in triglyceride synthesis. Consequently the uptake of NEFA into the cell and the preceding step of the hydrolysis of the plasma triglyceride may be dependent upon the level of intracellular glucose (Hollenberg, 1962), since this in turn will determine the concentration of intracellular NEFA. In the rat liver the α glycerophosphate generating enzymes were activated following refeeding and reduced by fasting (Tepperman and Tepperman, 1968). Fasting also reduced the rate of esterification of

palmitate in rat adipose tissue (Daniel and Rubinstein, 1967). This was thought to be due to a reduction in thiokinase activity, the enzyme responsible for the conversion of palmitate to palmitoyl-CoA.

Triglyceride synthesis from dietary triglyceride essentially involves a number of sequences of hydrolysis and re-esterification of the resulting fatty acids. The role of such a pathway is basically to produce intermediary forms of triglyceride which can be transported from the lumen of the small intestine to the lipid storing tissue. The final location of the dietary fatty acids depends largely upon the activity of lipoprotein lipase in different tissues, and this in turn is dependent upon the different states of the body.

Failure of the liver to produce β lipoproteins leads to impairment of lipid absorption in the intestine (Polonovski, 1966). In congenital hyperlipidaemia an absence of lipoprotein lipase in the adipose tissue and most other tissues, results in a considerable reduction of the fat deposits (Genẽes, 1969). Lipoprotein lipase from fowl adipose tissue is different from the mammalian enzyme, in that it is not activated by heparin (Korn and Quigley, 1957; Angervall, 1960). However the significance of this difference is unknown.

2.2.2. Synthesis from glucose (Figs. 2.2,3).

Since most diets are low in fat but high in carbohydrate, synthesis from glucose represents the major pathway for the synthesis of triglycerides. Most of the research directed towards understanding the processes involved in lipogenesis from glucose have been done using rat white adipose

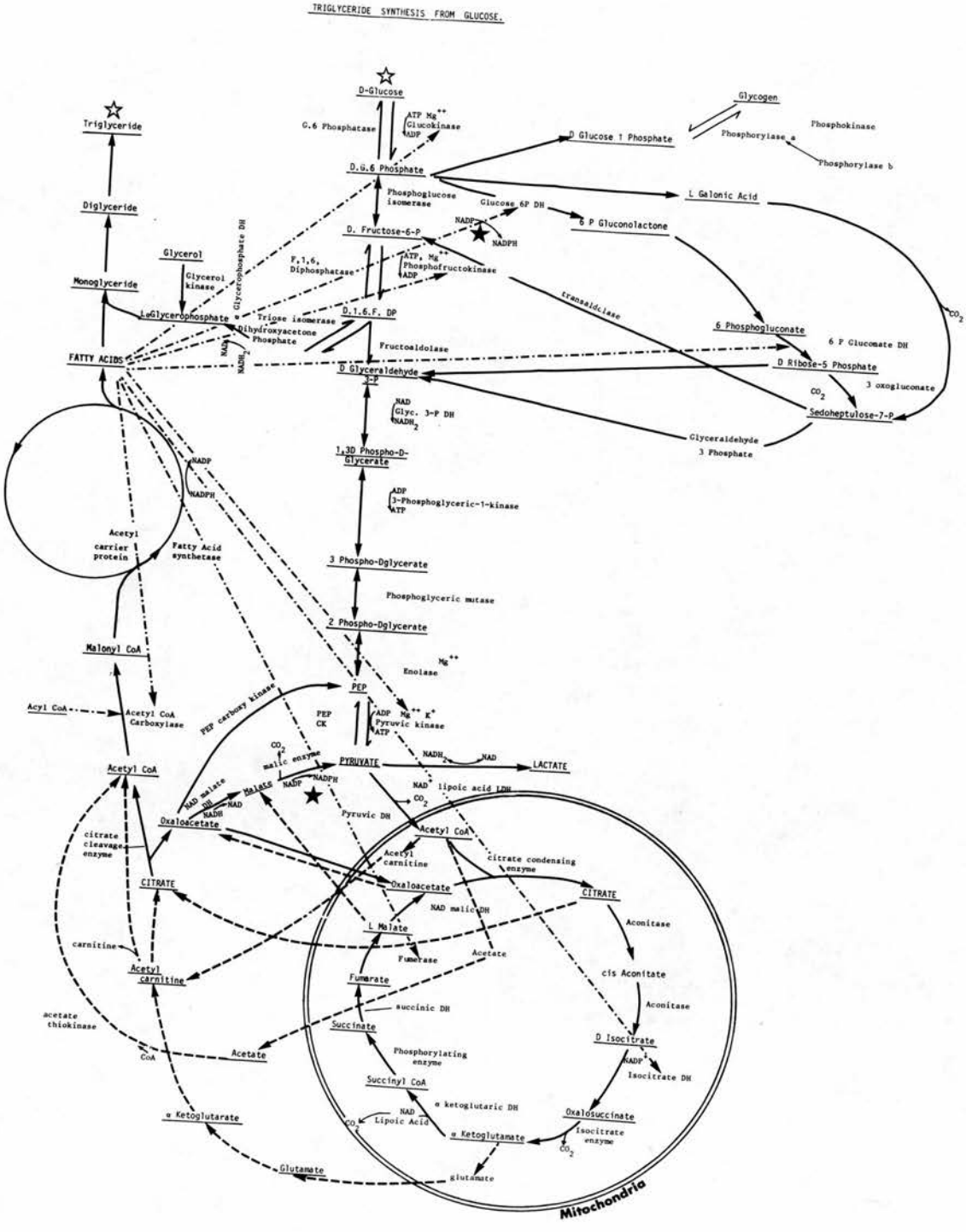



Figure 2.2
(Derived from literature cited in text. Dot dash lines indicate inhibition, other broken lines represent possible pathways for Krebs cycle intermediates to diffuse out of mitochondrion.)

tissue, and for this reason the account that follows has been largely taken from the results obtained with this tissue.

The major pathways involved in lipogenesis from glucose are the Embden Meyerhoff glycolysis (EM) pathway, the Hexose monophosphate (HMP) shunt, the Krebs cycle, and the extramitochondrial fatty acid synthesising cycle. Good general reviews on this subject have been written by Jeanrenaud (1961), Vaughan (1961a) and Ball (1966). The primary step towards lipogenesis from glucose, is the uptake of glucose into the cell. In adipose tissue the rate of uptake appears to be solely determined by the rate of transport across the cell membrane (Crofford and Renold, 1965). A transport system in this tissue stereo-specific for D glucose has been demonstrated (Crofford and Renold, 1965; Crofford et al., 1966). Counter transport has also been demonstrated substantiating the existence of a carrier system (Crofford and Renold, 1965). Intracellular glucose concentrations generally appear to be low which would be explained by the findings of Hernandez and Sols (1963) that the $K_m$ of glucose transport system was considerably higher than the $K_m$ for intracellular hexokinase.

Evaluations of the major pathways of glucose catabolism in adipose tissue have been made by Flatt and Ball (1964), Landau and Katz (1964) and Flatt and Ball (1965). According to these authors in the basal state approximately 65% of the metabolised glucose enters the HMP shunt of which approximately two thirds of the glucose carbons rejoin the

EM pathway as Fructose 6 Phosphate. This pathway provides an important source of reduced NADP required later in the synthesis of fatty acids. The glycolysis pathways produce two intermediates in the synthesis of triglyceride, namely α glycerophosphate which serves as the "backbone" of the triglyceride and pyruvate which is converted to acetyl CoA within the mitochondria. It is now well established however that the synthesis of fatty acids from acetyl CoA takes place outside the mitochondria (Popjak and Tiertz, 1955; Langdon, 1957; Masoro et al., 1962; Abraham et al., 1963; Spencer et al., 1964). Since Srere (1965) has argued that the direct transfer of acetyl CoA across the mitochondrial membrane is unlikely, Krebs cycle intermediates have been favoured as providing an alternative form of transport for the acetyl CoA. Five forms of transfer are shown in Fig. 2.2. The acetyl group may diffuse out of the mitochondria either in the form of acetate produced by the hydrolysis of acetyl CoA (Lowenstein, 1963; Olson, 1966) or by the formation of acetyl carnitine (Lowenstein, 1963; Bressler et al., 1965; Olson, 1966). Although oxaloacetate has been proposed as another possible form of transfer there is little evidence that it can pass through the mitochondrial membrane in any quantity (Spencer and Lowenstein, 1962). Of these it is now generally accepted that citrate represents the main vehicle for the transfer of acetyl CoA across the mitochondrial wall, either by direct diffusion (Spencer and Lowenstein, 1962; Srere and Bhaduri, 1962; Lardy et al., 1964; Srere, 1965; Greville, 1965; Daikuhara et al., 1968)

FATTY ACID SYNTHESIS.

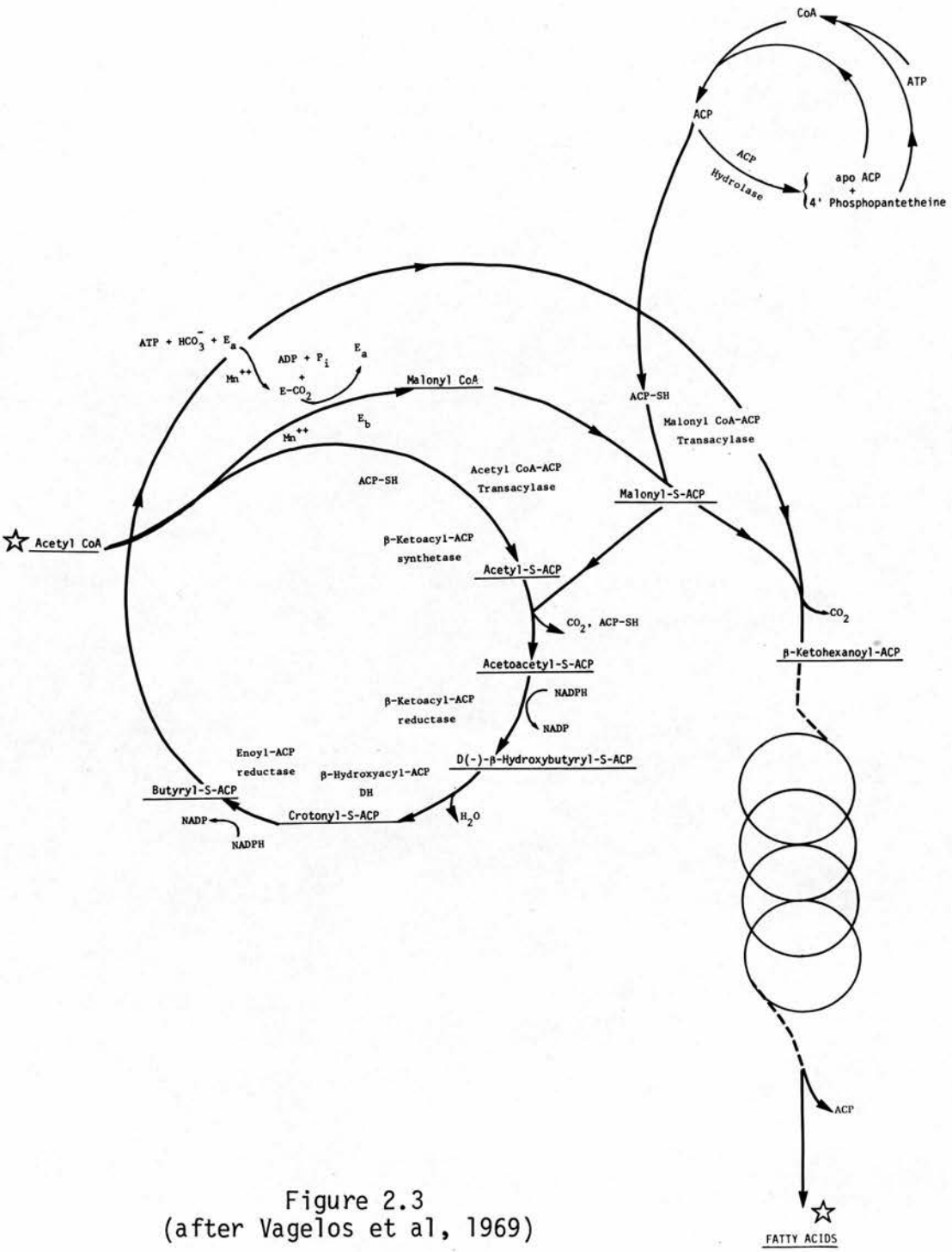



Figure 2.3
(after Vagelos et al, 1969)

or indirectly as glutamate via keto-glutarate (D'Adamo and Haft, 1962, 1965; Hardwick, 1965). Extra mitochondrial citrate is broken down into acetyl CoA and oxaloacetate by citrate cleavage enzyme which was found in the soluble fraction of the cell (Srere, 1959). This enzyme has been assumed to have a regulatory role in the synthesis of fatty acids, since changes in its activity parallel changes in the rate of fatty acid synthesis induced by various nutritional and hormonal states (Kornacker and Ball, 1965; Brown and McLean, 1965; Howanitz and Levy, 1965; Brown et al., 1966). More recent work (Srere and Foster, 1967; Foster and Srere, 1968) however has presented convincing evidence that citrate cleavage enzyme does not play a primary regulatory role in fatty acid synthesis.

Vagelos et al. (1969) have produced an excellent review of the recent findings regarding the pathways involved in the synthesis of fatty acids from acetyl CoA. These findings have been presented in diagrammatic form in Figure 2.3. The initial step in the fatty acid synthesis, the carboxylation of acetyl CoA to produce malonyl CoA is generally regarded to be rate limiting. It is catalysed by the enzyme acetyl CoA carboxylase the components of which aggregate to produce the active form in the presence of citrate or isocitrate. Vagelos et al. (1969) have shown that the acetyl CoA carboxylase consists of two protein particles. One, termed $E_a$, which is a protein-biotin complex, becomes carboxylated, and the second $E_b$ catalyses the transfer of the carboxyl group from the $E_a$ to the acetyl

CoA. $E_b$ was regarded to be the component which gives the acetyl CoA carboxylase its specificity for the substrate.

The enzyme system which catalyses the synthesis of long chain fatty acids from acetyl CoA and malonyl CoA is called fatty acid synthetase. The extra mitochondrial fatty acid synthesising system is common for all mammalian and avian tissues investigated (Martin et al., 1961; Martin and Vagelos, 1965). The initial step in this synthesis is the conversion of the CoA derivatives of acetate and malonate to thio-esters of acyl carrier proteins (ACP) Thereafter only the ACP derivatives are involved in the subsequent reactions, and only these derivatives are catalysed by the enzymes of the fatty acid synthetase system. ACP and CoA are the only major cell constituents containing pantothenic acid. The importance of ACP may therefore be further gauged by the fact that under conditions of pantothenic acid deficiency, ACP is synthesised at the expense of CoA.

A condensation-decarboxylation reaction between acetyl-ACP and malonyl-ACP produces acetoacetyl-ACP which is reduced by NADPH to β hydroxybutyryl-ACP. This in turn is dehydrated to form crotonyl-ACP which in the presence of another molecule of NADPH is reduced to form butyryl-ACP. This in turn undergoes a condensation-decarboxylation reaction with malonyl-ACP producing β ketohexanoyl-ACP which passes through the same sequence of reactions as those involved in the production of butyryl-ACP from acetoacetyl-ACP. This cycle is repeated until the entire fatty acid is synthesised, at which point the end product is freed from the ACP.

The fatty acid cycle represents the stepwise increase in the fatty acid chain length by the addition of two carbon fragments supplied by the condensation and subsequent decarboxylation of the malonyl-ACP. The intermediate reduction reaction between each addition require two molecules of NADPH.

It is not known how the cycle is terminated, and consequently how the chain length is determined. An oxygenase system is responsible for production of unsaturated bonds (Benjamin and Gelhorn, 1964).

The glycerol moiety of the triglyceride is derived by reduction of dihydroxyacetone phosphate synthesised from an EM pathway intermediate, or in the case of tissues other than adipose tissue by phosphorylation of glycerol. In the formation of triglyceride the fatty acids are first activated by CoA and then probably transferred to ACP (Stafford, 1968). Fatty acids are esterified to the free hydroxyl groups of $\alpha$ glycerophosphate, usually with an unsaturated acid in the $\beta$ position. The phosphate group is split off the diglyceride by a phosphatase and a fatty acid esterified to produce the triglyceride. Although this completes the sequence for the synthesis of triglyceride from glucose in adipose tissue, in the liver the triglyceride formed becomes bound to protein phospholipid, cholesterol and cholesterol esters to form very low density particulate lipoprotein which is released into the bloodstream (Albrink, 1962; Bierman, 1965). The fate of this lipoprotein has been discussed in the preceeding section.

In white adipose tissue only about 50% of the NADPH required for fatty acid synthesis is derived from the HMP shunt (Wise and Ball, 1964). Since the rate of diffusion of NADPH across the mitochondrial wall is presumed to be very slow, this indicates that there must be some alternative source of extramitochondrial NAPDH. Since the NADH produced during the oxidation of triose phosphate to pyruvate is in excess of requirements (Flatt and Ball, 1964, 1965) it is believed that the surplus might be converted to NADPH by some transhydrogenation process. The NAD malate dehydrogenase, malic enzyme system, which reduces oxaloacetate via malate to pyruvate has been proposed as a reaction sequence capable of carrying out this transhydrogenation process (Ball, 1965). Oxaloacetate would be provided by cleavage of extra mitochondrial citrate. This idea has been supported by the finding that adipose tissue possesses high malic enzyme activity which is altered in a parallel manner to changes in fatty acid synthesis produced by fasting and refeeding experiments (Wise and Ball, 1964; Leveille, 1966; Leveille and Hanson, 1966; Hahn and Drahota, 1966). More recently Ballard and Hanson (1967b) have proposed the presence of an extramitochondrial malate transhydrogenation cycle in adipose tissue, whereby oxaloacetate utilised by the system described above, could be replenished by the carboxylation of pyruvate. This suggestion was based on the discovery by these authors of a cytoplasmic form of pyruvate carboxylase in adipose tissue.

Accumulation of the end products of lipid synthesis

exerts feedback control on many of the earlier pathways. Thus intracellular NEFA inhibits the activity of many of the enzymes of the EM, HMP and Krebs cycle pathways (Weber et al., 1966a; Lea and Weber, 1968) and acyl CoA inhibits the action of citrate condensing enzyme, pyruvate kinase, and acetyl CoA carboxylase (Weber et al., 1966b; Weber et al., 1967).

At the present, ruminants and birds are the only examples in which lipogenic pathways have been shown to differ from those in rat tissue. In tissues of the ruminant (sheep and cow) it has been found that both citrate cleavage enzyme and malic enzyme activities are very low (Hanson and Ballard, 1967). Using tracers it was established that the main precursor for fatty acid synthesis was acetate. This indicated that citrate is not a normal intermediate in fatty acid synthesis, and consequently that NADPH generated by the malate transhydrogenation reaction would be unlikely. In contrast however the HMP shunt activity was high in ruminant adipose tissue (Hanson and Ballard, 1967) and so presumably this pathway represents the main source of NADPH.

HMP shunt dehydrogenases have been found to be of very low activity in both avian adipose tissue (O'Hea and Leveille, 1968; Goodridge and Ball, 1966, 1967c) and avian liver (O'Hea and Leville, 1968; Goodridge and Ball, 1966, 1967c; Goodridge, 1968c). Further evidence of the lack of activity of the HMP pathways in the avian liver has been obtained using specifically labelled glucose (Duncan and Common, 1967; Leveille et al., 1968). Avian liver,

however, has very high citrate cleavage enzyme and malic enzyme activities (Goodridge and Ball, 1966, 1967c; Goodridge, 1968c), indicating that in this tissue the main source of NADPH is via the malate transhydrogenation pathway. In avian adipose tissue all of the lipogenic enzymes have been found to have very low activity (Goodridge and Ball, 1966; O'Hea and Leveille, 1968), and rates of fatty acid synthesis in this tissue from either glucose or acetate have been shown to be correspondingly low (Goodridge, 1964; Goodridge and Ball, 1966; Goodridge, 1968c; O'Hea and Leveille, 1968) representing only 5-10% of the synthetic activity in rat adipose tissue.

Although no other differences in synthetic pathways have been described for the lipogenic tissues of other homoiotherms, there are striking variations in both basal and hormone stimulated rates of lipogenesis from the tissues of different species. The observations regarding hormones are considered in a later section (2.4.). Species in which the rate of synthesis of fatty acids is similar to the rat are the mouse, the guinea pig (DiGirolamo and Rudman, 1966a) and the domestic pig (O'Hea and Leveille, 1968). Adipose tissue of the rabbit (DiGirolamo and Rudman, 1966a) and the cow and sheep (Hanson and Ballard, 1967) are only approximately half as active as rat tissue, and hamster (DiGirolamo and Rudman, 1966a), dog (Shafrir and Wertheimer, 1965), avian (Goodridge, 1964; Goodridge and Ball, 1966; Goodridge, 1968c; O'Hea and Leveille, 1968), and human (Gellhorn and Marks, 1961; Pozza et al., 1963; Hirsch and Goldrick, 1964; Ostman,

1965) adipose tissues were found to be relatively inactive. In man although lipogenic enzymes of high activity were found in the liver, in adipose tissue the same enzymes were of very low activity (Shrago et al., 1966).

The high lipogenic activity found in the adipose tissue of some mammals, has lead to the belief that this tissue represents the main source for lipid synthesis in the intact animal. In the mouse and rat in situ synthesis in adipose tissue has been said to account for more than 50% of the total fat (Favarger and Gerlach, 1958, 1965a, 1965b; Jeanrenaud and Renold, 1961). More recently Hollenberg and Vost (1968) have questioned these results. Their own findings indicate that the liver is the primary site of lipogenesis in the intact rat. Certainly for those species in which adipose tissue has been shown to be relatively inactive, the liver must undoubtedly represent the main site for lipogenesis. In both the pigeon (Goodridge and Ball, 1967a) and the fowl (Leveille et al., 1968) such a result has been conclusively established.

TRIGLYCERIDE UTILISATION.

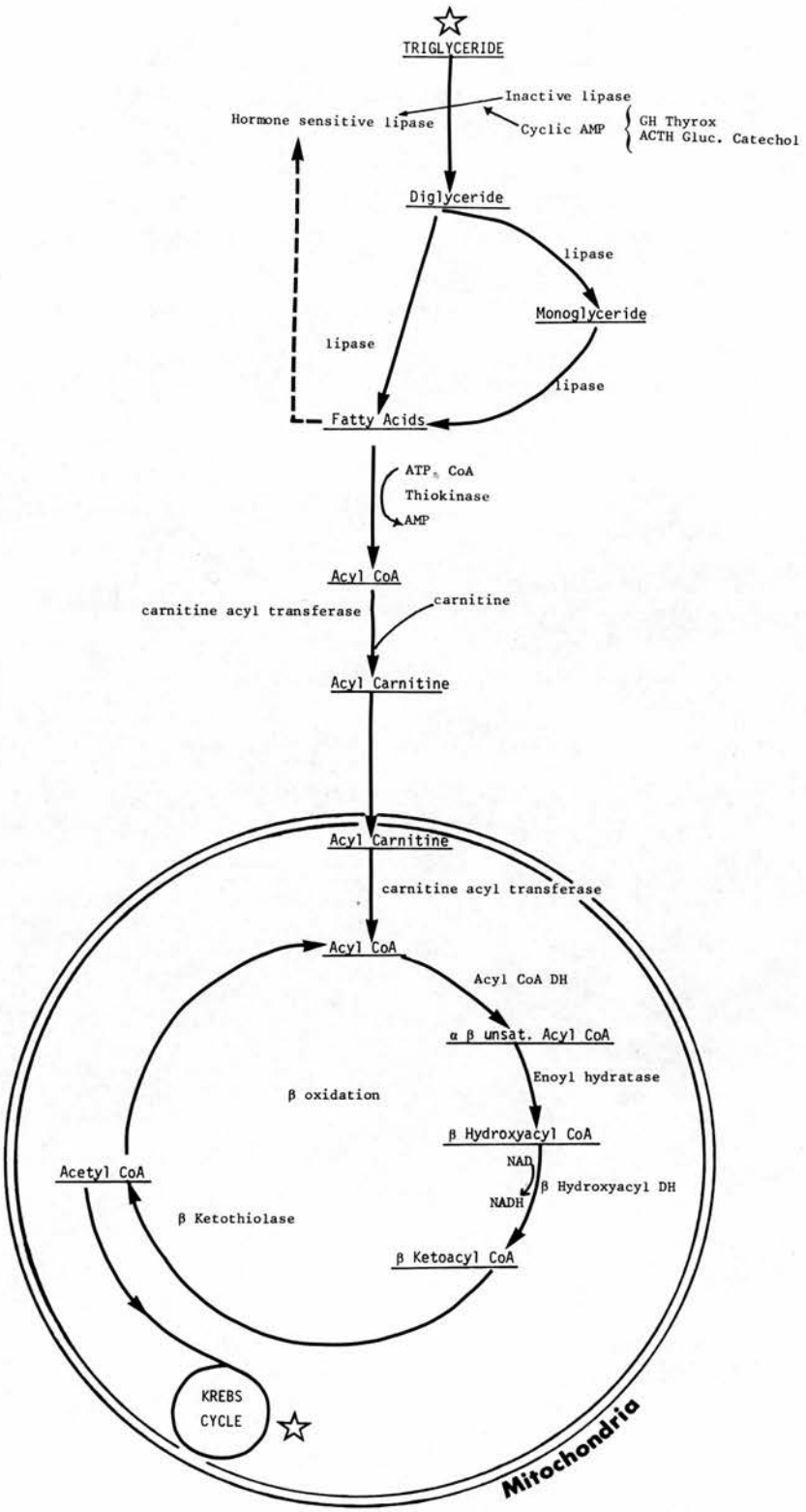



Figure 2.4
(Derived from literature cited in text.
Broken line indicates inhibition.)

## 2.3. Pathways for the utilisation of triglyceride (Figure 2.4.)

With few exceptions, most tissues utilise fatty acids for the production of energy (Fritz, 1961). The main sequence of events in the utilisation of fatty acid, is first their release from the triglyceride stores (mainly in the adipose tissue) by hydrolysis, their transport across the extravascular spaces, bound to albumin, their uptake into the cell and finally their transport across the mitrochondrial membrane where they are finally degraded via the β oxidation cycle, the products of which yield their energy during complete oxidation of the Krebs cycle. The initial step along this pathway involves hydrolysis of the triglyceride by the action of two lipases (Vaughan et al., 1964). Hormone sensitive lipase acts mainly on the triglyceride, and a hormone insensitive lipase hydrolyses the lower glycerides. The initial hydrolysis is considered to be the rate limiting step. The fatty acids produced leave the cell only if an acceptor such as albumin is available (Reshef et al., 1958), otherwise intracellular accumulation of fatty acids results in the inhibition of the hormone sensitive lipase (Rodbell, 1965). Vaughan and Steinberg (1963) have shown that both under basal conditions or hormone stimulation, re-esterification of the released fatty acids occurs continuously. Consequently release of fatty acids from adipose tissue will depend on the rate of lipolysis, the rate of re-esterification of the fatty acids, and availability of carrier protein. Cahil (1964) has suggested that the

lipolysis- re-esterification cycle in adipose tissue may be important in the production of heat for the maintenance of body temperature.

Plasma albumin has 6 to 7 high energy binding sites for fatty acids and a great many more with lesser affinity, per molecule (Goodman, 1958; Spector et al., 1969). Binding of the fatty acid to the albumin serves to facilitate its transport between cells. Entry of the fatty acids into the cells appears to be almost entirely dependent upon their concentration in the plasma (Armstrong et al., 1961; Annison, 1964). On entry the CoA derivative of the fatty acid is formed within the cell. The rate controlling step of the oxidation of the acyl CoA appears to be its rate of passage across the mitochondrial membrane (Fritz, 1963). This transport of the acyl CoA is achieved by conversion to acyl carnitine in the presence of the enzyme carnitine acyl transferase. Consequently factors which influence the activity of this enzyme will determine the rate at which fatty acids are oxidised. The activity of this enzyme has been found to increase in fasted, fat-fed or insulin deficient animals (Fritz, 1967). Thyroxine also enhances its activity (Fritz, 1967). Within the mitochondria the acyl carnitine is reconverted to acyl CoA which is in turn completely oxidised via the β oxidation and Krebs cycles in this order. The effect of glucose utilisation and gluconeogenesis on these processes is now considered.

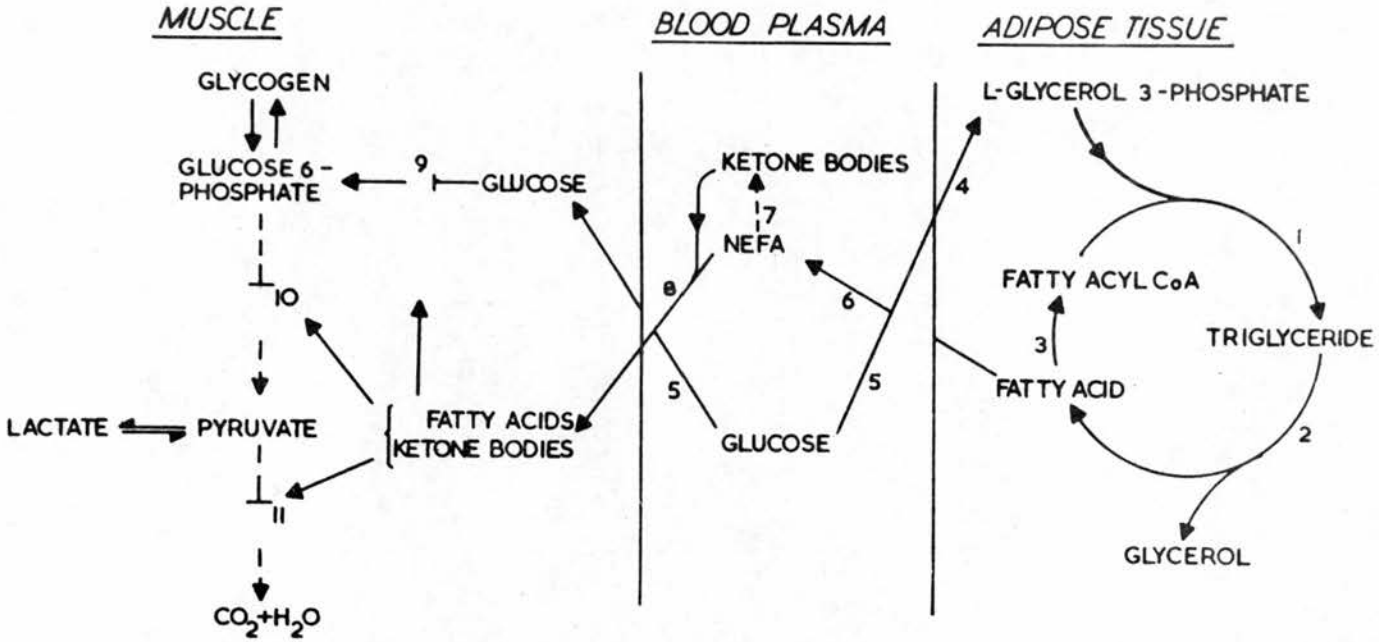



Figure 2.5
(from Randle et al, 1964)

. The glucose fatty acid cycle. 1. Esterification. 2. Lipolysis. 3. Fatty acid activation. 4. Glycerol phosphate synthesis from glucose. 5. Uptake of glucose by muscle and adipose tissue. 6. Release of fatty acids from adipose tissue to plasma albumin (NEFA). 7. Formation of ketone bodies by liver. 8. Uptake of fatty acids by muscle. 9. Phosphorylation of glucose by hexokinase. 10. Glycolysis (controlling reaction phosphofructokinase). 11. Pyruvate oxidation by pyruvate dehydrogenase.

The reactions shown in muscle apply to adipose tissue and *vice versa*, but the emphasis in the two tissues is presumably as shown.

The glucose fatty acid cycle and gluconeogenesis.

Randle et al. (1964) have drawn attention to the inverse relationship which exists between glucose and fatty acid utilisation in the body. This relationship they have termed the "glucose fatty acid cycle" (Figure 2.5.). The switch from glucose utilisation to fatty acid utilisation is triggered by such states as fasting and insulin insufficiency in which gluconeogenesis associated with ketosis may prevail. The interrelationship of these pathways have led to some speculation regarding the overall control of the utilisation of the major energy substrates.

In the glucose fatty acid cycle as elaborated by Randle et al. (1964) increased glucose utilisation reduces fatty acid oxidation primarily by decreasing the rate of lipolysis and increasing the rate of fatty acid re-esterification in the adipose tissue (Shapiro, 1965). Following a fall in plasma glucose, lipolysis will increase resulting in a subsequent increased uptake of fatty acids which will result in reduced glycolysis (see Section 2.2.1.). The resulting decrease in glucose utilisation will tend to restore the plasma levels to normal. Consequently this cycle can have the effect of maintaining the plasma glucose at a constant level.

During fasting gluconeogenesis provides the glucose required as an essential energy substrate by some cells (Fritz, 1961), and the glucose required as a precursor for cell constituents such as ribose-phosphate and mucopolysaccharides. Pathways for the production of glucose from

## Diagram of the pathway of gluconeogenesis from various precursors

The diagram shows the stages where the pathways from amino acids, glycerol, α-glycerophosphate, intermediates of the tricarboxylic acid cycle, and propionate join the pathway from lactate.

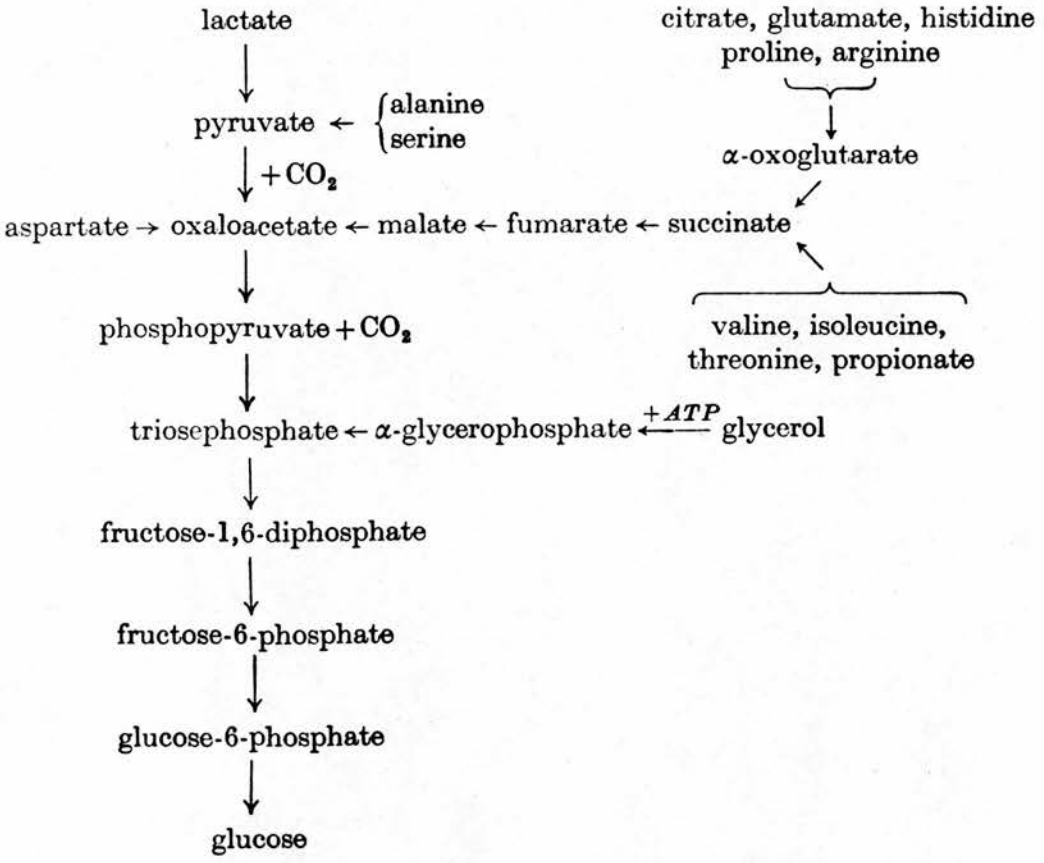



Figure 2.6
(from Krebs, 1964)

various amino acids are shown in Figure 2.6. Krebs (1965) has drawn attention to the close relationship existing between gluconeogenesis and ketogenesis. This relationship he considered to be due to the competition between the gluconeogenetic and fatty acid oxidation pathways for oxaloacetate. Oxaloacetate production is an obligatory step in the pathway of glucose production from amino acids (Krebs, 1964). It is also required to replenish the Krebs cycle intermediates. Consequently when the demand for carbohydrate are great, or the production of acetyl CoA from fatty acid oxidation is high, insufficient oxaloacetate may be available to keep the Krebs cycle primed. Under these conditions complete oxidation of the acetyl CoA cannot be accomplished and consequently ketone body formation results.

There is a further interesting link between fatty acid oxidation and gluconeogenesis which has been elaborated by Fritz (1967). Acetyl CoA, ATP and NADH generated by fatty acid oxidation are essential requirements for the potentially rate limiting steps of gluconeogenesis (Krebs, 1964). In addition increased fatty acid oxidation also inhibits glycolysis (Fritz, 1967). It was therefore concluded that "fatty acids are not only the main energy supply for gluconeogenesis, but that they also play a decisive role in switching on this process by inhibiting pyruvate oxidation, by stimulating pyruvate carboxylation and by promoting malate formation".

## 2.4. The effects of hormones on lipid metabolism.

Hormones are important regulators of lipogenesis and lipolysis in the mammalian body. Much of their current importance in these roles has been established from work performed on rat adipose tissue. The basic findings for the action of hormones on lipid metabolism, presented here, was therefore obtained from research carried out on this tissue. An attempt has been made to compare the results obtained from this tissue with those from the tissues of other species.

### 2.4.1. The lipogenic hormones.

#### Insulin.

Insulin has become established in the mammal as the main lipogenic hormone of physiological importance. It greatly increases glucose utilisation both by increasing uptake (Crofford and Renold, 1965) and the activity of the EM and HMP glycolysis pathways (Itshaki and Wertheimer, 1957; Hausberger, 1958a; Winegrad and Renold, 1958a,b; Rodbell, 1964a). In recent years increasing attention has been given to the mechanism involved in the uptake of glucose. A specific transport system in the adipocyte cell membrane which is stereo-specific for D-glucose has been demonstrated (Crofford and Renold, 1965; Crofford et al., 1966). Ouabain was found to increase glucose uptake and stimulate glucose metabolism in a manner similar to that obtained with insulin (Ho and Jeanrenaud, 1967). Since this agent is known to interfere with action of sodium-potassium pump, these authors

suggested that the action of insulin was in some way linked with this mechanism. Subsequent experiments however have demonstrated that there is no simple relationship between the pump activity and insulin action (Letarte and Renold, 1968). These authors have suggested that the glucose carrier system activated by insulin is inactive in its absence. The basal glucose uptake is effected by a sodium and potassium primed carrier system, whereas extracellular sodium alone was necessary for the action of the insulin stimulated carrier system. Other factors which increase glucose uptake have a similar action to insulin in stimulating glucose metabolism. These were cadmium and protein (Flatt and Ball, 1965) and phospholipase C (Rodbell, 1966). It is thus possible to attribute most of lipogenic effects of insulin to its action of increasing glucose uptake.

Insulin has an inhibitory effect on lipolysis, even in the absence of glucose (Jungas and Ball, 1963; Perry and Bowen, 1962; Jungas and Ball, 1964; Mahler et al., 1964; Tarrant et al., 1964; Froesch et al., 1965). This occurs in the presence of various lipolytic agent (Jungas and Ball, 1963; Mahler et al., 1964; Fain et al., 1966). Insulin also rapidly produces substantial reductions in the levels of cyclic AMP (Butcher et al., 1966) and also apparently reduces adenyl cyclase activity (Jungas, 1966). Insulin has also been shown to increase the rate of synthesis of "key" glycolytic enzymes (Weber et al., 1966b), and to inhibit the action of the gluconeogenic enzymes (Weber et al., 1965a,b,c). Some results obtained by admin-

istration of insulin to diabetic animals suggest that insulin also increases lipoprotein lipase activity (Schnatz and Williams, 1963).

An attempt to produce a unified theory for the action of insulin on lipogenesis is based on the results obtained by experiments using phospholipase C, an enzyme which hydrolyses the phospholipid components of the cell membrane. The action of this enzyme has been shown to be almost identical with that of insulin, even to the extent of reducing the rate of lipolysis in the absence of glucose (Rodbell, 1966; Rodbell and Jones, 1966). On this basis it has been proposed that all effects of insulin can be attributed to its action on glucose transport. The results with phospholipase have led to suggestions that this transport is accomplished by alteration of the lipid portion of the cell membrane, so that new sites of the carrier system become exposed.

Insulin given <u>in vivo</u> to rats has a similar lipogenic action on liver and adipose tissue, (Goldman and Cahill, 1964). Addition of insulin <u>in vitro</u> to liver tissue was shown to stimulate lipogenesis by Brady and Geurin, 1950; Felts et al., 1951; Altman et al., 1951. However the following, Masri et al., 1952; Haft and Miller, 1958; Renold et al., 1955, could find no effect. Of the adipose tissue from other species tested, only that from the mouse has proved to be as equally responsive to insulin as rat tissue (Christophe et al., 1961; Stenke et al., 1965).

Although the effects of insulin on human adipose tissue have proved to be rather variable, this appears to be due largely to variation in the experimental procedures. There is now good evidence that insulin both increases glucose uptake and lipogenesis substantially in this tissue (Pozza et al., 1963; Kahlenberg and Kalant, 1964; Fessler et al., 1965, 1967; Gries and Steinke, 1966; Goldrick, 1967a). The action of insulin on lipogenesis in guinea pig adipose tissue is only about one sixth that observed in the rat (Steinke et al. 1965; DiGirolamo and Rudman, 1966a) and only a marginally significant action on lipogenesis has been reported for its action in the adipose tissues from the domestic pig (O'Hea and Leveille, 1968) and the rabbit (DiGirolamo and Rudman, 1966a). Both adipose tissues from the hamster (DiGirolamo and Rudman, 1966a) and birds (Goodridge, 1964; Goodridge and Ball, 1966; O'Hea and Leveille, 1968) have been found to be totally unresponsive to the action of insulin. Species variation to the lipogenic or antilipolytic actions of insulin have been generally attributed to the occurrence of insulin degrading peptidases in the unresponsive tissue, rather than to the species specificity of the insulin tissue interaction (Rudman, 1965; Rudman et al., 1966; Rudman and Shank, 1966).

Prolactin has been shown to have an action very similar to insulin on rat adipose tissue (Winegrad, 1962). Although in vivo administration of prolactin to birds produced increased fat deposition (Meier and Farner, 1964) no in vitro effect on lipogenesis in avian adipose tissue could be demonstrated (Goodridge, 1964).

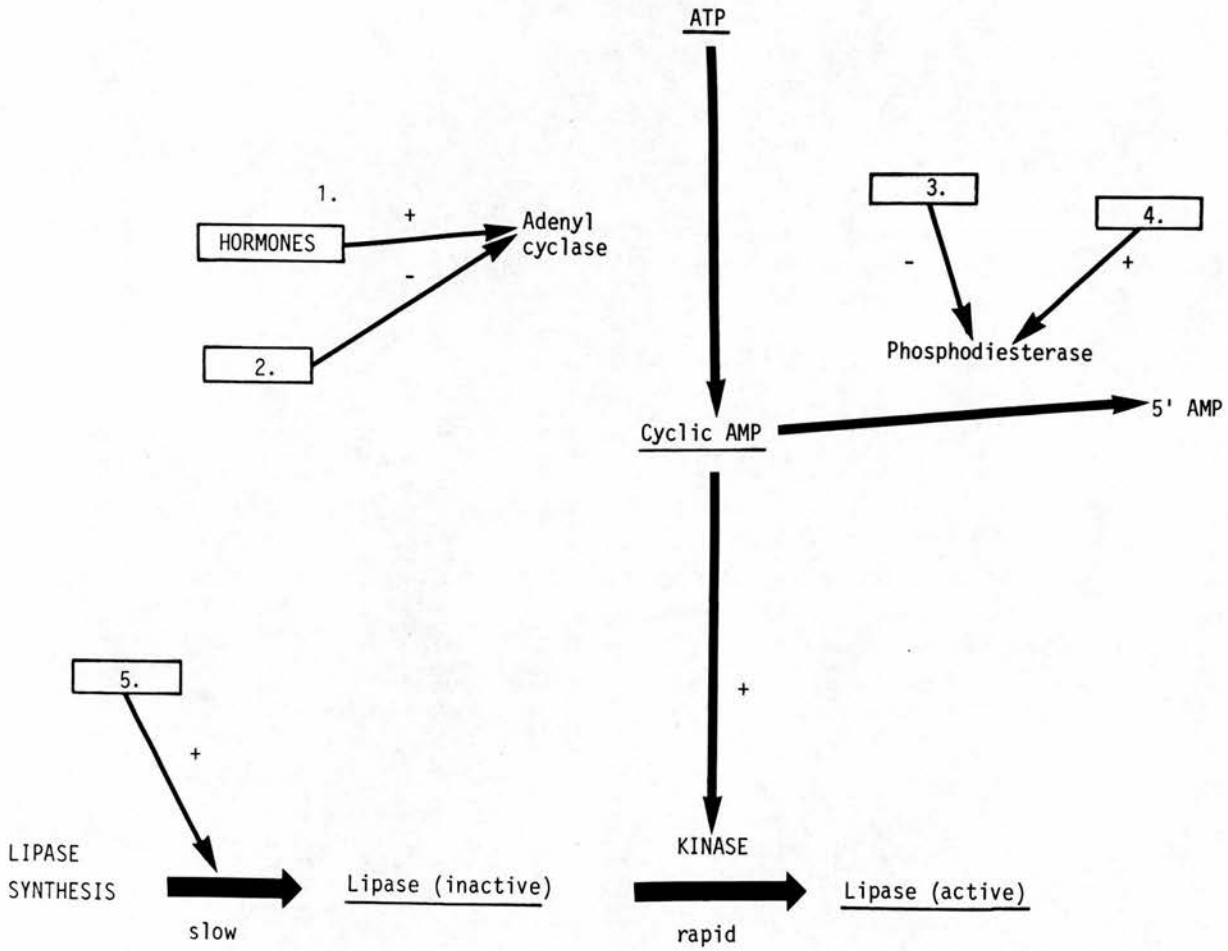
Oxytocin and lysine vasopressin have been found to stimulate lipogenesis in rat adipose tissue (Pittman et al., 1961; Mirsky and Perisutti, 1962; Migliorini and Linder, 1966). Oestrone, oestradiol and oestriol all increase lipogenesis in the adipose tissue of the female rat but have no action on this tissue from the male (Gilmour and McKerns, 1966). In vivo progesterone has also been shown to increase fat deposition in the female rat (Galetti and Klopper, 1964; Hervey and Hervey, 1967). The action of oestrogens in the female is synergystic with insulin. Testerone however had no effect on the lipogenic activity of adipose tissue of the rat from either sex (Jeanrenaud and Renold, 1964). Experiments in which growth hormone has been given to hypophysectomised rats, suggest that this hormone can have a lipogenic action (Goodman, 1965).

2.4.2. The lipolytic hormones.

Hormone stimulated lipolysis is normally brought about by the action of hormones on the hormone sensitive lipase in adipose tissue. This enzyme which attacks triglycerides, is slower acting than the other tissue lipases (Gorin and Shafrir, 1964; Strand et al., 1964; Vaughan et al., 1964; Steinberg, 1966), and consequently its action represents the rate limiting step in the hydrolysis of the lipids.

The discovery by Sutherland and collaborators (Sutherland and Rall, 1958) of the involvement of cyclic AMP in hormone action has lead to their development of a generalised theory for the mechanism of hormone action.

Figure 2.7




Mechanisms controlling the action of hormone-sensitive triglyceride lipase. ( after Stafford 1968.)

This system appears to be involved in the lipolytic effect of many hormones in adipose tissue. A schematic representation of the system is presented in Figure 2.7. The following description of its mode of operation has been taken from Sutherland et al. (1965).

Hormone action is initiated when the hormone is received by a specific receptor site in the cell membrane. Binding of the hormone activates the associated enzyme adenyl cyclase (Step 1, Figure 2.7.) which is also located within the cell membrane, which in turn stimulates the synthesis of cyclic AMP from ATP. Finally cyclic AMP activates the hormone sensitive lipase within the cell. As shown in Figure 2.7. the action of hormones operating through such a system can be regulated in several ways. The rate of degradation of the cyclic AMP is controlled by the action of a phosphodiesterase specific for this nucleotide. Many agents have been discovered which either stimulate or inhibit the action of this enzyme, and thereby modulate the lipolytic activity. Factors which influence the rate of synthesis of adenyl cyclase and hormone sensitive lipase will also influence hormone induced lipolysis.

It has been shown that epinephrine, norepinephrine, adrenocorticotrophic hormone (ACTH), thyroid stimulating hormone (TSH) and glucagon all produce their lipolytic effects via the system described above (Bueding et al., 1966; Butcher, et al., 1965; Fassina, 1966; Handler and Orloff, 1964; Haynes, 1958; Murad et al., 1962; Sutherland et al., 1965; Sutherland and Robison, 1966).

Epinephrine, ACTH, TSH, glucagon and lutenising hormone have all been found to elevate cyclic AMP levels in isolated fat cells (Butcher et al., 1968). Epinephrine and norepinephrine (Gordon and Cherkes, 1958; White and Engel, 1958 ), ACTH (White and Engel, 1958 ), TSH (Freinkel, 1961) and glucagon (Hagen, 1961; Vaughan, 1961b) all produce a prompt increase in lipolysis in rat tissue, either in vitro or in the perfused tissue (Ho and Meng, 1964; Scow, 1965). Associated with the increased lipolysis induced by these hormones is a highly significant increase in the rate of re-esterification of the released fatty acids (Vaughan and Steinberg, 1963). This response is of less magnitude than the lipolytic response so the total effect is that of an increased fatty acid mobilisation associated with an increased turnover of fatty acids within the cell.

The response of adipocytes to the activation of lipase by hormones, additionally depends on the availability of extracellular binding sites for the uptake of released fatty acids, since accumulation of intracellular fatty acids has an inhibitory feedback effect on the hormone sensitive lipase (Rodbell, 1965; Bally et al., 1965).

The strong antilipolytic effect of insulin on these lipolytic hormones is thought to be due primarily to its action in increasing the rate of re-esterification within the cell (Jungas and Ball, 1963; Bally et al., 1965), although, in addition, it has been shown to lower the cyclic AMP level within the cell (Butcher et al., 1966).

The response to these hormones by adipose tissues from other species is very variable. Mouse tissue was as

equally responsive as the rat tissue to the lipolytic action of catecholamines, ACTH and TSH (Payne, 1949; Marshall and Engel, 1960). Likewise adipose tissue from the hamster was highly responsive to the action of both catecholamines and ACTH, but was uninfluenced by TSH (Rudman and Shank, 1966). Catecholamines were also actively lipolytic in dog (Havel and Goldfien, 1959; Rudman et al., 1963) and cat (Aronovsky et al., 1963), although ACTH was inactive in both species (Rudman et al., 1963; Aronovsky et al., 1963). TSH and melanophore stimulating hormone (MSH) was mildly active in the dog (Rudman et al., 1963) but these were inactive in the cat (Aronovsky et al., 1963). In man infusion of catecholamines raised NEFA levels (Gordon and Cherkes, 1956; Dole, 1956; Bogdonoff et al., 1961) although ACTH and TSH had no effect (Raben, 1959; Friesen, 1964; Lerner and McQuire, 1964). In vitro work confirmed the activity of catecholamines, and further, demonstrated the inability of ACTH, TSH and glucagon to stimulate lipolysis in human adipose tissue (Mosinger et al., 1965). The lipolytic response of human adipose tissue was low when compared to the action of these hormones in the other mammalian species already referred to (Hamosh, 1963; Hjorntorp, 1964; Mosinger et al., 1965; Burns and Hales, 1966). There is indirect evidence to indicate that glucagon may have some lipolytic activity when administered in vivo to man (Lefebvre, 1965). Adipose tissues from the rabbit (Rudman et al., 1963; Landolt and Astwood, 1961), the guinea pig (Rudman et al., 1963) and the pig (Rudman et al., 1963) were unresponsive to the lipolytic

action of catecholamines. However rabbit tissue lipolysis was increased slightly by ACTH (Raben et al., 1968), as was that of the guinea pig, in which lipolysis was also stimulated by TSH (Rudman and Shank, 1966 ), although adipose tissue from the pig was unaffected by ACTH, TSH or MSH.

Avian adipose tissue was generally unresponsive to the lipolytic action of catecholamines (Carlson et al., 1964), lipolysis only being observed when high levels of the agent are given (Goodridge and Ball, 1965; Gibson and Nalbandov, 1966a; Langslow and Hales, 1969). In contrast glucagon has been demonstrated to be very strongly lipolytic in this tissue (Goodridge, 1964; Carlson et al., 1964; Hoak et al., 1966; Langslow and Hales, 1969). ACTH like catecholamines was only able to stimulate lipolysis in avian adipose tissue when given in very high concentrations (Rudman et al., 1962; Carlson et al., 1964; Langslow and Hales, 1969). Insulin has no antilipolytic effect on avian adipose tissue (Goodridge, 1964; Goodridge and Ball, 1965; Langslow and Hales, 1969).

Although growth hormone has been shown to increase glucose oxidation (Hamid et al., 1965; Jeanrenaud, 1961; Leboeuf and Cahill, 1961; Winegrad et al., 1959) and lipogenesis (Goodman, 1965) its main function in relation to lipid metabolism, is in the mobilisation of the triglyceride stores. In the intact animal the lipid mobilising action of this hormone has been demonstrated in man, dog, monkey and rats (Engel et al., 1958; Knobil and Greep, 1959; Raben, 1959; Raben and Hollenberg, 1959; Winkler et al., 1964; Rabinowitz et al., 1965). Its effect on lipolysis occurred

after two hours and generally reached a maximum after about 4 hours. Continuous daily treatment of rats with growth hormone has resulted in a marked reduction in the fat deposits, and a serious impairment in the ability of the adipose tissue to synthesise lipids (Goodman, 1963). The *in vitro* action of growth hormone on lipolysis in rat adipose tissue was increased considerably in the presence of dexamethasone (Fain et al., 1965). Both actinomycin D, puromycin and cycloheximide inhibit the lipolytic action of growth hormone (Fain et al., 1965; Fain, 1968; Goodman, 1968) indicating that the action of this hormone is dependent upon protein synthesis. This has been interpreted to mean that growth hormone increases the rate of synthesis of hormone sensitive lipase (Step 5, Figure 2.7.), or adenyl cyclase. Growth hormone also increases lipolysis in the adipose tissue of the rabbit.

Corticosteroids can act directly on adipose tissue to stimulate the release of fatty acids in the rat (Jeanrenaud and Renold, 1960, 1966; Jeanrenaud, 1967). The stimulatory role of cortisol and corticosterone on fatty acid mobilisation *in vitro* was only observed after prolonged incubation (Jeanrenaud and Renold, 1960; Munck, 1962; Herrera et al., 1962; Fain et al., 1963). By using dexamethasone and cortisol, it has been demonstrated that the main lipolytic action of corticoids is produced by their action of inhibiting the rate of fatty acid re-esterification (Lynen, 1961; Vaughan, 1962; Jeanrenaud, 1967). Cortisol in man causes a rapid increase in rate of fatty acid mobilisation, which is thought to be due to an increase in

hormone sensitive lipase activity (Jenkins et al., 1964).

Although thyroid hormones have been found to have no lipolytic action on adipose tissue in vitro (Debons and Schwartz, 1961; Deykin and Vaughan, 1963), hypothyroid animals are less responsive to the lipolytic action of epinephrine, ACTH, TSH and glucagon (Goodman and Bray, 1966) and conversely hyperthyroid animals were more responsive to the action of catecholamines (Debons and Schwartz, 1961; Felt et al., 1962; Deykin and Vaughan, 1963; Goodman and Bray, 1966). A number of explanations have been provided to explain the potentiating action of the thyroid hormone. There are indications that it may act by inactivating cyclic AMP (Rizack, 1966; Waldstein, 1966) or by increasing the rate of synthesis of adenyl cyclase (Brodie et al., 1966; Bray et al., 1966; Krishna et al., 1968) or alternatively by enhancing receptor activity to the lipolytic hormones (Sutherland and Rall, 1960; Waldstein, 1966).

A number of pituitary peptides have been found to possess lipolytic properties. The effect of these on lipolysis in different species has been reviewed by Rudman and DiGirolamo (1967). A more recently isolated pituitary hormone, lipotrophic hormone, has been shown to be strongly lipolytic in the rabbit, weakly active in the rat and inactive in the mouse (Lohman and Li, 1968).

Much attention has been given to the action of drugs which affect the involvement of cyclic AMP in the regulation of lipolysis. Methyl xanthines by inhibiting the action of phosphodiesterase (Step 3 Figure 2.7.) have been shown

to prolong the action of lipolytic hormones (Butcher et al., 1965).

Prostaglandins which are compounds with vasopressor and smooth muscle stimulating properties have been shown to reduce both basal and hormone stimulated lipolysis in in vitro adipose tissue (Steinberg et al., 1964). It has been suggested that this action may be brought about by a possible inhibition of the activity of hormone sensitive lipase (Stock and Westermann, 1966).

The considerable species variation in the response of adipose tissue to the lipogenic and lipolytic peptides and polypeptides has been attributed to the presence of specific inactivating peptidases. Generally the ability of the fat cell to inactivate a hormone has been shown to correspond well with its lack of response to that particular hormone (Rudman, 1965; DiGirolamo et al., 1965; DiGirolamo and Rudman, 1966; Rudman and Shank, 1966; Rudman et al., 1966).

The action of hormones on lipogenesis and lipolysis represents one example of a method whereby the activity of the anabolic and catabolic pathways can be adjusted according to the state of the body's energy reserves.

# 3. Materials and methods

## 3.1. Materials.

### 3.1.1. Animals.

Aylesbury ducks were mainly used throughout this study. These were obtained as day-olds from D. Ralston and Co. Ltd., Moffat, and reared in huts at the P.R.C.-section, Bush Estate. In addition a small breeding flock of Pekin-type ducks, originally obtained from a parent stock supplied by Cherry Valley Farms Ltd., Lincoln., was established at the P.R.C. Kings Buildings. The progeny of this flock was used in some experimental work. All non-breeding ducks were kept in pens on deep litter and illuminated with a constant 14 hour day-length. They were fed on commercial rations; Rank's duck starter for the first three weeks and Rank's duck finisher thereafter. Food and water were always provided ad libitum unless otherwise stated.

Mallard (*Anas platyrhynchos*) and Tufted (*Athya fuligula*) ducks were obtained as whole deep frozen carcases from Cherry Valley Farms Ltd., and The Wildfowl Trust, Slimbridge, respectively. The rearing conditions for these birds were similar to those used for ducks housed at the P.R.C. The Mallard ducks were given the same diet as the domestic ducks but the Tufted were fed on "Ful-O-Pep" (Ful-O-Pep Feeds Ltd., London) chick starter for the first four weeks and thereafter on "Ful-O-Pep" chick grower. Tufted ducks were hatched from eggs collected from a wild population at Loch Leven, Kinross. Day-old eider ducks (*Somateria mollissima mollissima*) were obtained from a wild colony at

Newburgh, north of Aberdeen.

Fowl used were from an inbred line of Brown Leghorns maintained at the Poultry Research Centre. Rats were obtained from a small colony maintained by the British Empire Cancer Campaign Unit at the Poultry Research Unit.

3.1.2. Chemicals.

Phospholipids, DL-α-cephalin synthetic, practical grade, and L-α-lecithin, commercial grade, type II E were from Sigma, London, and DL-α-lecithin, synthetic, A grade, from Calbiochem Ltd., London. The enzymes, pyruvate kinase, glycerokinase, lactic dehydrogenase, and the nucleotides, ATP and reduced NADP, and phosphoenolpyruvate were all obtained from the Boehringer Corp. (London) Ltd., London. Glucose oxidase as "Fermcozyme 653 AM" and peroxidase, R.Z. 1·0 grade, were from Hughes and Hughes (Enzymes) Ltd., Brentwood, Essex. Bovine serum albumin, Fraction V, monopalmitin, grade 99 + % and dipalmitin, synthetic grade II S were from Sigma. Collagenase (bacterial), B grade was from Calbiochem. "Biochemical grade" cholesterol stearate, cholesterol acetate, palmitic acid and stearic acid, and "laboratory reagent" glycerol tripalmitate and glycerol trioleate were obtained from British Drug Houses, Poole. Silica gel G was from E Merck, Darmstadt; Silica gel D-0 was from Camag, Switzerland; prepared silica gel plates were from Antec AG, Birsfelden.

Fixatives for ultramicroscopy, glutaraldehyde, paraformaldehyde and acrolien were obtained from TAAB

laboratories, Reading, and dimethyl sulphoxide (DMSO) grade 1 from Sigma. Radioactive labelled compounds, (D glucose - C14 (U), Iodine-125, Iodine-131, glyceryl tri(palmitate-1-C14), n-hexadecane-1-C14 and cholesterol-4-C14 were obtained from the Radiochemical Centre, Amersham, Bucks.

3.1.3. Drugs and hormones.

Alloxan monohydrate was obtained from Sigma, London; heparin BP was obtained from Boots, Nottingham; veterinary nembutal from Abbot Laboratories Ltd., Kent; Rastinon (sodium salt of tolbutamide) as a 5% solution in ampoules ready for intravenous injection was obtained from Hoechst Pharmaceuticals Ltd., Slough.

Hormones or hormone preparations were obtained from the following. Glucagon-free insulin (Bovine Batch 19 AN 81670, 24·5u/mg) and protamine zinc insulin BP (40 u/ml) from Burroughs Wellcome & Co., Kent; prolactin (ovine, NIH-P-S-8) from Endocrinology Study Section, N.I.A.M.D., Bethesda, Md.; glucagon (less than 0·006 u insulin/mg glucagon) from Eli Lilly, Indianapolis and Novo Industri A/S, Copenhagen; oestradiol monobenzoate in arachis oil (5 mg/ml) from Organon Laboratories Ltd., Morden, Surrey; purified oestrogen from Dr. J. Wells, P.R.C.

Bovine and fowl growth hormone were obtained from Dr. A. Stockell Hartree, Department of Biochemistry, University of Cambridge. Fowl growth hormone was prepared according to the following method. Acetone dried pituitaries were extracted with 6% ammonium acetate in 40% ethanol at

pH 5·1. The insoluble residue was then extracted according to standard procedure described by Raber (1959) for the extraction of growth hormone from the mammalian pituitary.

## 3.2. Methods

### 3.2.1. Measurement of the major chemical components of the body.

#### 3.2.1.1. Dissection and preparation of the body parts for analysis.

Birds were killed by intravenous injection of nembutal, or by neck dislocation. After the cloacal contents had been expelled the bird was weighed and its length and wingspan measured. When the feather weight was required the birds were then plucked. The plucked or unplucked carcases were then placed in polythene bags which were sealed, and the whole kept in a deep freeze until further dissection and analysis could be carried out.

For dissection the carcase was first thawed. The complete skin with the subcutaneous fat attached (but not including the scaley covering of the feet and legs, nor the covering on the bill) was then removed. Following this, the main superficial fat deposits were dissected from the skinned carcase, and the ventral abdominal wall was then opened and the peritoneal fat deposit dissected free. Subsequently the crop and gizzard contents and the liver were

removed. As body parts were dissected from the carcase they were weighed and sealed in polythene bags. The weight of each body component was carefully recorded at each stage in order to determine the evaporative losses during dissection and subsequent procedures. Fluid which accumulated on the dissecting tray from the skinned carcase after the skin had been removed, was always transferred to the polythene bag containing the skinned carcase. The dissected material was stored in a deep freeze until needed.

The accuracy of the chemical analysis of the body components is primarily dependent upon the reliability of the aliquotting procedures. In the case of the fat depots and the liver, this was relatively simple; duplicate samples were obtained by excising with a scalpel a suitably sized portion. The methods used in obtaining aliquots from the skin and skinned carcase are described below.

Frozen skins (with or without feathers) were chopped into small pieces and minced twice in a 1½ h.p. power mincer (Biro, Marblehead, Ohio) using first a coarse and then a fine die. Duplicate samples of the resulting fine mince were taken for chemical analysis.

Frozen skinned carcases were sawn into slices using a power band saw meat cutter with special blade clearing guards to prevent loss of material by the saw action (Biro, Marblehead, Ohio). Loss during this stage was less than 1%. The pieces were then minced twice with a power mincer as described for the skin samples. When the skinned carcase was of greater weight than 1 kg then a 25 quart Hobart (Troy,

Ohio) vertical cutter mixer was used as this could produce a suitable mince in a single operation. Duplicate samples were taken for analysis. Variation between duplicates for total fat, water and protein was generally less than 2%.

3.2.1.2. Chemical analysis of body parts.

Water content. This was determined by heating the sample in a hot air oven at 105°C until a constant weight had been attained. This usually took 14 hr.

Total fat. Dried samples of skins and skinned carcases and wet samples of the fat depots were used for total fat determinations. The method employed was based on the procedure described by Bligh and Dyer (1959). Briefly, this consisted of homogenising approximately 5 g of sample in 150 mls of a chloroform-methanol (1:2) mixture for 30 seconds followed by the addition of 40 mls of water with further blending. A further 50 mls of chloroform and then 50 mls of water were added separately with blending. The resulting mixture was transferred to a separating funnel and allowed to stand overnight for the complete separation of the two phases. The solvent was distilled off from a filtered aliquot of the lower phase, which was then dried by heating in a hot air oven and weighed.

Comparison of results with Soxhlet method.

The Bligh and Dyer method was used in preference to the standard Soxhlet method since it was more convenient to use when handling large numbers of samples. There was

little difference between the results obtained by these two methods (Table 3.1). The Bligh and Dyer method generally gave slightly higher results. These results were in agreement with those obtained by Bligh and Dyer (1959) for a comparison of the two methods.

Recovery results using glycerol tripalmitate were 98·3 ± 0·8% (4) for the Bligh and Dyer method.

Total nitrogen (protein)

Nitrogen was estimated by the standard Kjeldahl method. The factor 6·25 was used to convert N to protein. No effort was made to estimate the non protein nitrogen as the absolute protein value is not of great importance in the present studies. Due to the high fat content in the skin samples the fat was extracted from these samples prior to the digestion in sulphuric acid for the N determination. This was done by leaving the sample in chloroform at room temperature overnight.

Calcium, Sodium and Potassium.

These cations were estimated in dried skinned carcase samples and in some dried and defatted skin samples. The cations were released from the sample by gently refluxing 1 g of the sample with 40 ml of 6N (microanalytical reagent) hydrochloric acid for 1½ hours. After cooling, the insoluble material was removed by filtration and the filtrate was diluted to a suitable concentration for measurement using an atomic absorption spectrophotometer (Techtron assembly AA-100).

Table 3.1. Comparison of the Bligh and Dyer (1959) and Soxhlet fat extraction methods. A 12 hour extraction was used in the Soxhlet method (Mandrigo et al. 1967). The samples used were duck skinned carcases. Values are means ± S.E.M. and shown in brackets.

Soxhlet Solvents

o Petroleum ether 40-60°C fraction

x Toluene

| Sample No. | % Fat in dry sample | | t | p |
|---|---|---|---|---|
| | Bligh-Dyer | Soxhlet | | |
| 6969 | 24·8 ± 0·34 (3) | 23·3 ± 0·34 (4) | 3·061 | > 0·01 < 0·02 |
| 6961 | 20·1 (2) | 19·0 (2) | | |
| 6941 | 21·9 ± 0·37 (4) | 22·3 ± 0·75 (3) | 0·478 | > 0·10 |
| | | 21·2 ± 50[x] (4) | 1·095 | > 0·10 |
| 6968 | 21·7 ± 0·33 (4) | 19·1 ± 0·22 (4)[x] | 6·500 | < 0·001 |
| | | 21·4 ± 0·66 (4)[x] | ·429 | > 0·10 |
| 6948 | 24·6 ± 0·61 (3) | 23·6 ± 0·57 (3)[x] | 1·754 | > 0·10 |

### 3.2.2. Histological methods for the light and electron microscope.

#### 3.2.2.1. Light microscopy.

Adipose tissue. Pieces of fresh adipose tissue were fixed in 10% buffered formalin and embedded in paraffin. 15 and 50 μ sections were stained with haemalum followed by 0·5% aurantia (in 70% ethanol). This was usually followed by Van Gieson's stain. Aurantia staining of the red blood cells made it possible to readily identify all blood vessels.

Pancreas. The main purpose of this work was to investigate the effect of certain treatments on the islet morphology. In order to do this the freshly removed pancreas was divided into the main lobes and the splenic lobe. The former two were divided transversely into five sections. Bouin's solution was used for fixation. Paraffin sections were stained with Gomori's aldehyde fuchsin or haematoxylin and eosin. At least 3 complete transverse sections of the gland were examined from each piece of pancreas (including the splenic lobe).

#### 3.2.2.2. Electron microscopy.

It is difficult to obtain adequate fixation of adipose tissue because of the presence of large quantities of lipid. Fixation methods used with reasonable success on either rat white epididymal adipose tissue or brown adipose tissue (Napolitano and Fawcett, 1958; Napolitano 1963; Shelton et al. 1962; Idelman 1964; Pictet et al. 1968 and Suter 1969) have all proved to be unsatisfactory for avian adipose tissue.

Perfusion of the bird immediately before or after death, with warm 4-5% formalin or glutaraldehyde in Millonig's buffer whether following a prior injection of heparin to prevent clotting, or 1% sodium nitrite to dilate the vascular beds was not found to give better results than those obtained from simply dropping the freshly excised tissue directly into the fixative. Injection of fixative into a subcutaneous vessel supplying the subcutaneous fat was likewise of no benefit.

After many trials with various fixative mixtures the following two methods were found to produce the most satisfactory results. They are based on the use of rapid penetrating agents such as acrolein and dimethyl sulphoxide (DMSO).

Method 1.

Fresh adipose tissue was rapidly cut into small pieces with scissors (less than 1 mm square) and placed immediately into fixative, of the following composition: 2·5% formaldehyde (prepared from paraformaldehyde), 2% acrolein, 4% glutaraldehyde in Millonig's buffer pH 7·3, for 20 minutes at 4°C. The tissue pieces were then trimmed and replaced in the fixative for a further 1½ hours at 4°C. After removal from the fixative and passing through two 15 minute washes of Millonig's buffer the tissue pieces were post-fixed in 2% osmium tetroxide in Millonig's buffer for 1 hour at 4°C. The tissue was then rapidly dehydrated through a graded series of ethyl alcohols, followed by two 5 minute changes in epoxy propane before placing in a mixture of epoxy propane and

araldite overnight. On removal from this mixture the tissue was placed in araldite for two days with a change of araldite after the first day, after which it was placed in fresh araldite at 60°C for another 3 days for embedding.

Method 2.

Fixative. 15% DMSO, 2·5% formaldehyde, 2% acrolein, 4% glutaraldehyde, in pH 7·3 Millonig's buffer.

Initial fixation procedure was identical to that described for method 1. Post-fixing in 2% osmium tetroxide in Millonig's buffer was for 3 hours at 4°C with a change of the fixative after 1½ hours. The embedding procedure was identical with that described above.

Sections were cut using an LKB ultra microtome, stained with lead citrate and uranyl acetate and examined using a Philips EM 300 electron microscope.

### 3.2.3. Measurement of cell diameter of adipocyte.

From each fat deposit examined, usually four pieces of tissue were randomly selected. In order to measure cell diameter it was necessary to obtain fairly uniform thin slices of tissue. Cutting fresh tissue rarely produced satisfactory results and cell damage was common. In contrast if the fresh tissue was cooled to 4°C thin slices could be obtained with a sharp scalpel. This treatment had no effect on cell diameter. The thin tissue slices when placed on a microscope slide rapidly cleared, and photomicrographs were immediately taken using transmitted light. The use of cover slips was avoided as this was found to cause deformation

of the cells. The procedure was so rapid that tissue dehydration was minimal up to the time of photography.

In order to avoid bias in cell diameter measurements a rectangular grid was placed on the photomicrograph, and those cells which were in focus and lying within specified squares on the grid were selected for measurement. A vertical and horizontal diameter was obtained for each cell measured. The mean of these two values were used in the analysis.

3.2.4. Chemical composition of adipose tissue.

Fresh tissue was used for these analyses.

Water. The tissue was first cut into fine strips in order to shorten the drying time. These tissue pieces were then dried to a constant weight in a hot air oven at 105°C.

Potassium and sodium. Small pieces of adipose tissue were placed in platinum crucibles and dried in a hot air oven at 105°C. The tissue was then ashed in a muffle furnace at 600°C for 16 hours. The ashed residue was then dissolved in a small quantity of distilled water to which a drop of 1N HCl (microanalytical reagent) had been added, and transferred to a volumetric flask and made up to volume. After suitable dilution potassium and sodium were measured using atomic absorption spectrophotometry.

Fat. Approximately 0·5 g of the tissue was homogenised in the chloroform-methanol solvent (2:1) using a high speed Polytron homogeniser. Thereafter the method used was that of Folch et al. (1957). Finally the solvent was evaporated

off under a stream of nitrogen, over a water bath at 40°C. The lipid sample was then completely dried in a vacuum desiccator over phosphorus pentoxide in the presence of paraffin chips.

Protein In order to shorten the time required for digestion, most of the fat was extracted from the sample prior to analysis. This was done by disrupting the tissue with a glass rod in the presence of a small quantity of chloroform. The solvent was evaporated off first over a water bath and finally in an oven. The dried residue was then used for nitrogen determination using a standard microkjeldahl procedure employing a Markham still. Protein was obtained by multiplying the result by 6·25.

Collagen was estimated by auto analyses, using the method of Grant (1964).

3.2.5. In vivo studies.

Blood was collected by venepuncture from a wing or leg vein into a syringe wetted with heparin. Plasma was obtained after immediate centrifugation of the heparinised blood.

Plasma glucose. Fresh or deep frozen plasma samples were used. Frozen samples could be held for a week without effecting glucose content. 0·1 ml of plasma was required for D-glucose assay using the glucose oxidase method (Wooton, 1964). Orthodianisidine was used as the chromogen. Protein was precipitated from the plasma samples with 5% trichloracetic acid since this method was more rapid than

the zinc hydroxide method generally employed, and did not effect the glucose estimates in any way.

Plasma NEFA. In order to obtain reproducible results only fresh plasma samples could be used for NEFA determinations. The method used was based on that described by Dole (1956). Fresh plasma (0·5 ml) was extracted with a solvent mixture, consisting of heptane, isopropyl alcohol and 1N sulphuric acid in the proportions of 10:30:1. After gentle shaking for 30 seconds heptane and water were added and the mixture shaken again. On standing the two phases separated and an aliquot of the upper phase was taken. Against this 0·01 N sodium hydroxide was titrated from a Conway microburette, using thymol blue in 90% ethanol as the indicator. The solutions were kept mixed during the titration by bubbling $O_2$ and $CO_2$ free nitrogen through the mixture. Palmitic acid was used for the standard.

Administration of alloxan.

Alloxan monohydrate was prepared as a 10 or 20% solution in physiological saline, and given to the experimental birds by injection into a wing vein.

For the subsequent glucose tolerance test a solution of 20% glucose in physiological saline was injected into one wing vein and blood samples were obtained from the other. The glucose tolerance test was carried out under light diethyl ether anaesthesia.

Administration of insulin, and tolbutamide.

Bovine insulin was dissolved in physiological saline (with the addition of one or two drops of dilute alkali in order to completely dissolve the protein). This solution was injected into a wing vein of the experimental birds and the controls were given physiological saline (with equivalent amount of alkali) by the same route.

Tolbutamide was given as a 5% solution (Rastinon) into a wing vein and the controls were given physiological saline.

Injections and withdrawal of sequential blood samples was performed on unanaesthetised birds. All the experiments were carried out in the morning in order to standardise the conditions as much as possible, since all birds were on ad libitum food.

Administration of protamine zinc insulin.

Burroughs Wellcome protamine zinc insulin BP (40u/ml,) was given daily by subcutaneous or intramuscular injections. Controls were given a similar volume of physiological saline by the same route. Both injections and withdrawal of blood samples were performed on unanaesthetised birds.

Measurement of antibodies to insulin.

Antibodies were detected using a method based on the radioimmunoassay procedure of Yalow and Berson (1960). 0·5 ml of diluted plasma (diluent used 0·5 g of sodium azide, 0·5 g crystalline bovine plasma albumin, 600 mls of barbitone buffer pH 8·6, 400 mls of water) was added to 0·05 ml of

either ($^{125}I$) insulin or ($^{131}I$) insulin of concentration 5 ng/ml. This insulin was iodinated according to the method of Greenwood et al. (1963). The resulting mixture was incubated at 4°C for 12 days.

Bound labelled insulin was separated from unbound labelled insulin using paper chromatography. 0·025 ml of sheep serum dyed with bromophenol blue was added to the incubate and the mixture was applied to a strip of Whatman 3 MC paper which had been wetted with 0·07 M barbitone buffer pH 8·6. The paper was held horizontally with one end dipping into a trough of the buffer. Free insulin remained near the origin whereas antibody bound insulin and the dyed protein migrated away from the origin in the opposite direction of the trough. After separation was complete the paper was dried and cut into two strips representing the bound and unbound insulin. These were then placed in vials and their activity counted in a Nuclear Enterprise (Edinburgh) well-type scintillation counter.

Plasma collected at the completion of the long term insulin treatment experiments were stored in a deep freeze until they could be assayed for antibodies.

Administration of Oral glucose.

A 50% solution of glucose in physiological saline was given to the experimental birds by means of a long polythene tube attached to a large syringe. The tube was passed into the region of the proventriculus, and a measured volume of the glucose solution was expelled from the syringe into the digestive tract. Controls were either given a

similar volume of physiological saline by the same route or no treatment at all.

Sequential blood samples were obtained by venepuncture or unanaesthetised birds.

Administration of growth hormone.

Growth hormone was dissolved by adding sufficient water to produce a thin paste and then a drop of 1 N sodium hydroxide, with mixing, followed by the addition of more water until a clear solution was obtained. It was then diluted to a suitable concentration with physiological saline. It was given by intravenous injection. Both injections and withdrawal of blood was carried out on unanaesthetised birds.

3.2.6. In vitro studies with liver and adipose tissue.

3.2.6.1. Incorporation of ($U^{14}C$) glucose into lipids.

Incubation of tissue. Birds were killed by decapitation, rats by a blow to the head. Liver and fat tissues were removed immediately, cut into small pieces, and placed in ice-cold and warm (rat 37°C; bird 40°C) Krebs-Ringer bicarbonate buffer pH 7·4 (Cohen 1951, with half the suggested concentration of calcium) respectively. The tissue remained in the buffer for a period of approximately ½ to 3/4 hour which was the time taken to transport the tissue to the laboratory and prepare it for incubation. For the in vitro studies the adipose tissue was removed from the buffer washed in fresh warm buffer, cut into small pieces less than 50 mg, washed in warm buffer again, then blotted and weighed rapidly on a torsion balance, before being trans-

ferred finally to the glass flasks for incubation. The flasks contained 3 mls of Krebs-Ringer bicarbonate buffer pH 7·4 which contained 4 mg/ml of glucose and (U $^{14}C$) glucose (0·5 to 1·5 μc). After the tissue was added the flasks were sealed with serum stoppers and gassed for 1 minute with a mixture of 95% oxygen and 5% carbon dioxide. The flasks were kept in a water bath at 40°C with continuous gentle shaking for 3 hours.

Liver was generally prepared and incubated in the same way as adipose tissue. The only exception to this procedure was that thin slices (thickness 0·3 mm.) of this tissue, obtained by using a tissue slicer (Mickel Engineering Laboratory Co. Surrey) were incubated.

Rat tissues were prepared and incubated using the same procedure as used for the avian tissues with the exception, that the incubates were kept at 37°C.

Control tissues were prepared in the same manner, but were added to incubation medium at 4°C containing the same amount of labelled glucose. The flask was gently agitated for approximately 30 seconds, after which the tissue was removed, washed in further cold buffer (label free), blotted onto filter paper and stored in a deep freeze until further analysis. Hormones were added at the beginning of incubation, along with the tissue.

It has been demonstrated that incubation of free adipocytes have much greater incorporation activity than the intact tissues (Rodbell 1964a). This author described a method for the dissociation of rat epididymal adipose

tissue by incubation with collagenase. This method was tried for avian adipose tissue with mixed results. Adipose tissue was cut up into small pieces and incubated in Krebs bicarbonate buffer with collagenase (10 - 20 mg/g of tissue) for from 1 to 5 hours. Tissue from the adult or 10 week old birds produced a good yield of free cells, but only poor yields were obtained from the tissue of younger birds. For this reason the technique was not used in this study, since in order to compare the lipogenic activity between adipose tissue from birds of different ages it was necessary to incubate them under uniform conditions.

Extraction of total lipids.

At the completion of incubation the tissue pieces were removed from the incubation medium, washed in cold label free buffer blotted onto filter paper and placed immediately into a deep freeze where they were stored until further analysis.

Total lipid was extracted from the incubated tissues according to the method of Folch et al. (1957). The tissue was homogenised in 2·5 ml of chloroform-methanol (2:1) mixture using a polytron high speed homogeniser. The mixture was then filtered under vacuum through a sintered glass micro-filter. Subsequently a further 1·5 ml and then 1 ml of chloroform-methanol were separately used to rinse clean the homogeniser and the microfilter. 1 ml of 0·017% magnesium chloride was then added to the filtrate and the whole mixed thoroughly by shaking. The resulting emulsion was separated

by centrifugation and the upper phase removed by aspiration. The lower phase was then washed 3 times with approximately 5 ml quantities of "upper phase solution" (Folch et al. 1957). After each wash the upper phase was removed by aspiration. With the addition of a few drops of methanol the remaining sample was reduced to a single phase. At this stage the sample was divided in half. One half used for the determination of activity in the total lipids was then heated in a water bath at 45°C under a stream of nitrogen (oxygen free) to evaporate the solvent. The lipid was finally dried completely in a vacuum desiccator in the presence of phosphorus pentoxide and paraffin chips.

The total lipids were extracted from the incubation medium using the same procedure. 0·5 ml of incubation medium was used, and only 0·5 ml of the magnesium chloride solution was added after homogenisation and filtration.

Saponification.

Saponification was carried out according to the method described by Denton and Randle (1967). This method employing the use of perchloric acid has the advantage that if the aqueous layer remaining after the extraction of fatty acids is cooled to below 4°C practically all the potassium perchlorate precipitates, and consequently the activity in this phase can be counted without further preparation. The solvent was evaporated from the tissue total lipid extract, and 4 ml of 4% potassium hydroxide in 95% ethanol was added to the residue.

The mixture was gently refluxed over a boiling water bath for 1 hour. Thin layer chromatography was used initially to establish that saponification was complete. Non-saponified lipids were removed by two 5 ml washes with light petroleum (b.p. 40-60). This fraction was pooled and washed twice with 10 mls of water. The saponified material was then acidified by the addition of 4 ml of 10% perchloric acid, and the fatty acids were extracted by three 5 ml washes with light petroleum. These washes were pooled and washed twice with 5 ml of aqueous 20 mN/hydrochloric acid. The remaining aqueous solution after extraction of fatty acids was cooled to 4°C and then centrifuged at 0°C to remove the potassium perchlorate. 1 ml of the supernatant was removed for counting.

Both the saponified and non-saponified fractions were dried by first heating over a water bath at 45°C under a stream of nitrogen and then further dried in a vacuum desiccator in the presence of phosphorus pentoxide and paraffin chips.

Distribution of the radioactivity between the major lipid classes.

In the case of adipose tissue all the activity could be accounted for in the glycerides.

The distribution of the activity in the total lipid extract from incubated liver was examined using thin layer chromatography. The solvent was evaporated from the lipid extract under nitrogen, and the lipids were redissolved in a

small volume of chloroform-methanol (2:1). A small volume of this containing approximately 100-200 µg of lipid was spotted onto silica gel G thin layer plates, from a micro pipette. For the separation of neutral lipids the solvent system described by Freeman and West (1966) was used. Plates were developed initially in a solvent system consisting of diethyl ether, benzene, ethanol and glacial acetic acid in the proportions of 40:50:2:0·2. When the solvent front had reached a point 2/3rd of distance from origin to final position of the new front, the plate was removed from the solvent tank and air dried briefly before placing in the next solvent (diethyl ether and hexane in the proportions 6:94). Development was considered complete when the solvent front had reached a point just short of the end of the plate. After drying the spots were detected by spraying with a reagent containing rhodamine G and 2,7-dichlorofluorescein (Jones et al. 1966) and viewed under ultraviolet light, or after spraying with saturated potassium dichromate in 80% sulphuric acid, and then charring in an oven. Standard solutions of mono-, di- and tri-glycerides, fatty acid, cholesterol, cholesterol esters and phospholipids were run simultaneously, in order to identify the lipids in the tissue extracts.

For separation of phospholipids chromatograms were developed in a solvent consisting of chloroform, methanol, glacial acetic acid and water in the proportions of 102:15:15:4. Standard solutions of phospholipids (cephalin and lecithin) were run with the tissue extracts. Spots on

the plates were detected using a sulphuric acid-molybdenum spray for phospholipids (Dittmer and Lester 1964). Using this solvent system the neutral lipids migrated with the solvent front.

Annther method which was used to determine the amount of activity in the phospholipid fraction of the total lipid extract from the incubate liver slices was that described by Carlson (1963). The lipid extract was dried under nitrogen and redissolved in 10 ml of chloroform. Phospholipid was removed by adding 0·5 g of previously activated silicic acid (100 mesh) followed by gentle shaking. After centrifugation an aliquot of the supernatant was removed for counting. Recovery of trigliceride using glyceryl tri(palmitate-1-$^{14}$C) was 96·5 $\pm$ 0·4% (3). Complete removal of phospholipid from the supernatant was tested by using a standard phospholipid solution as the sample, and concentrating down an aliquot obtained after silicic acid extraction. This was spotted onto a thin layer plate and the colour developing after using sulphuric acid-molybdenum spray was examined. The results obtained always indicated that removal of phospholipid was complete.

Radioactivity assay.

Lipid extracts from the incubated tissue were transferred to counting vials, and the solvent evaporated under nitrogen and in vacuo. 5 ml of scintillator fluid was then added for counting (2-5-Bis-[5'-tert.-butylbenzoxyazolyl-(2') thiophen (BBOT), 4 g/litre of toluene).

For counting of aqueous samples 1 ml of the sample was mixed thoroughly with 5 ml of NE 250 scintillator fluid

(Nuclear, Enterprises, Edinburgh, Dioxan based for aqueous samples). All $^{14}C$ compounds were counted using a Nuclear Enterprise liquid scintillation beta spectrophotometer. Quenching was determined by using n-hexadecane-1-$^{14}C$ as an internal standard. Efficiencies obtained for lipid samples using BBOT scintillator were between 80-90%, and for aqueous samples using the NE 250 were between 20-50%.

Thin layer chromatograms of $^{14}C$ compounds were scanned for radioactivity using a Panax thin layer chromatogram scanner.

Estimation of triglyceride mobilisation in in vitro adipose tissue by measurement of glycerol.

Adipose tissue was prepared and incubated using the same procedure as for the in vitro incorporation studies. However no labelled glucose was added. The incubation medium contained 1·6% bovine serum albumin, fraction V, which had been previously dialysed against 2 changes of Krebs-bicarbonate buffer for 24 hours at 4°C. Glucagon was added to the incubation medium immediately prior to the addition of the tissue. This tissue was incubated for three hours following the addition of the hormone.

Glycerol was extracted from and estimated in the incubation medium by the method of Garland and Randle (1962). The assay was based on the following reactions.

$$\text{glycerol} + \text{ATP} \xrightleftharpoons{\text{glycerol kinase}} \text{L-glycerol 1-phosphate} + \text{ADP}$$

$$\text{ADP} + \text{phosphoenolpyruvate} \xrightleftharpoons{\text{pyruvate kinase}} \text{ATP} + \text{pyruvate}$$

pyruvate + $NADH_2$ $\xrightleftharpoons{\text{lactate dehydrogenase}}$ lactate + NAD

Phosphorylation of the glycerol results in the oxidation of $NADH_2$ which was measured spectrophotometrically at 340 A. The whole reaction is carried out at pH 7·6 which ensures that the forward reaction proceeds rapidly.

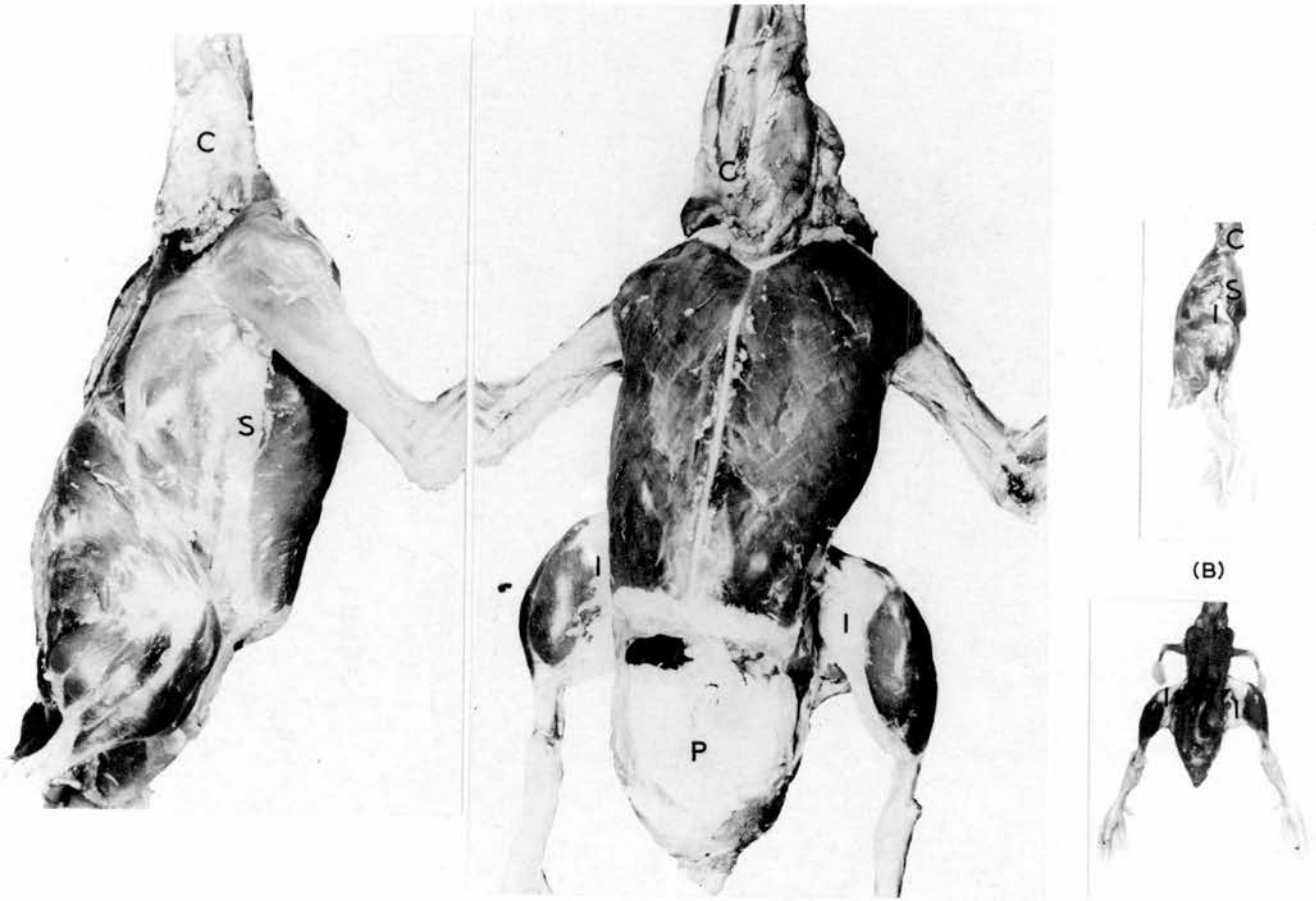
At the completion of incubation an aliquot of incubating medium was withdrawn and deproteinised by the addition of an equal volume of 10% trichloracetic acid. After centrifugation 0·9 ml of the clear supernatant was washed three times with 5 ml of redistilled diethyl ether, in order to remove the trichloracetic acid. Any remaining ether in the sample was removed in vacuo. Glycerol was then assayed in these samples according to the method of Garland and Randle (1962).

## 4. Results.

## 4.1. Fat deposition during post-embryonic growth

Since the purpose of this study was to investigate the process of fat deposition during growth in the domestic duck, a natural starting point was to examine the pattern of development and growth of the fat deposits. For this purpose a group of 60 Aylesbury ducks consisting of 30 females and 30 males divided equally between the following age groups, day-old, 2 week, 4 week, 6 week, 8 week and 10 week old were used. This age range entirely encompassed the period required for the commercial rearing of ducks for meat production. A group of 60 Mallard ducks of similar age and sex composition to the Aylesbury group, and a group of 30 male Tufted ducks (Athya fuligula) of similar age composition were also analysed, in order to compare the pattern of fat deposition in the domestic breed with that of its ancestral form (the Mallard) and a wild diving species (The Tufted). All these groups were reared under similar conditions and thus it is possible to relate observed differences to the genetic variation between the breeds.

To characterise the growth and development of the fat deposits the birds were dissected into the following parts, skin, the main dissectable fat depots and skinned carcase. The fat content of the fat depots and the other dissected body components was determined. Throughout this work the word "depot" is used to describe a morphological unit of adipose tissue inclusive of the fat contained within the tissue. The term depot is synonymous with "fat organ" as used by Liebelt (1963).




The main fat depots in the Aylesbury duck. (A) 10 week-old. (B) 4 day-old. Key: C = claviculo, coracoid, cervical and tracheal depot; S = subalar and lateral thoracic depot; I = Inguino-crural depot; P = peritoneal depot.

Figure 4.1.

An account of the fat depots in the duck follows.

4.1.1. The fat depots.

The distribution of the main fat depots in the chicken embryo Gallus gallus and the white-crowned sparrow Zonotrichia leucophrys gambelli have been described by Liebelt and Eastlick (1954) and McGreal and Farner(1956) respectively. The location and relative shape of the fat depots in the sparrow were sufficiently similar to the chick that the latter authors were able to use Liebelt and Eastlick's (1954) nomenclature. Since these species are members of such diverse orders as Galliformes and Passeriformes it suggested that there may not be much variation in the morphology of the fat depots between different bird species. In the ducks examined few of the individual fat depots described by the above authors could be identified. This seemed to be due mainly to the greater size of the depots in these semi-aquatic species, which could have arisen from the coalescing of smaller depots. Since no attempt was made to trace any of the depots back to their embryonic origins it was not possible to apply the above authors' terminology. Consequently different terminology has generally been used in this study. Where the possibility of synonomy exists this will be discussed.

Below is an account of the main dissectable depots in the duck.

Inguino-crural depot (Fig. 4.1.) This includes all the adipose tissue attached to and loosely associated with the

femoral and tibiotarsal regions of the leg. It is mainly in the form of a loosely attached body of adipose tissue stretching from the region of the sartorius muscle to two thirds along the tibiotarsus, with a smaller amount lying above the sartorius muscle. This is probably synonymous with the lateral abdominal depot of Liebelt and Eastlick (1954).

Subalar and lateral thoracic depot (Fig. 4.1). The subalar depot lies within the axilla of the wing, and the lateral thoracic depot is continuous with this and extends posteriorly along the lateral edge of the sternum.

Claviculo, coracoid, cervical and tracheal depot (Fig. 4.1.). This is a continuous body of adipose tissue forming pads over the claviculo-coracoid regions which coalesces with a layer of varying thickness surrounding the neck to just below the level of the head. It would appear to form from the individual depots of the same name, as described by Liebelt and Eastlick (1954).

Peritoneal depot (Fig. 4.1.). This depot lies within the abdominal cavity, extending ventrally over the intestinal mass, and attached to the central and lateral peritoneal membranes lining the abdominal wall. A prolongation of this depot extends forward and is attached to the ventral surface of the gizzard.

The rest of the body fat which could not be dissected free from the associated tissue was grouped as either subcutaneous or skinned carcase deposits.

The Subcutaneous deposit (Fig. 4.1.). This referred to all the adipose tissue attached to the skin. In the chicken embryo (Liebelt and Eastlick, 1954) a number of depots, the lateral thoracic, the femoral, the synsacral, the spinal and the medio-ventral abdominal all became ultimately connected to the follicles of the feather tracts. Since probably a similar development may occur in the duck, it is unlikely that the subcutaneous adipose tissue can be regarded as a single morphological depot. Certainly the main vascular supply to different regions arises from different major vessels. Intracellular fat other than that occurring in the adipose tissue will represent a negligible proportion of the total fat extracted from the skin component.

Skinned carcase deposit. This represented all the fat remaining in the carcase after all the deposits described above had been removed by dissection. It included inter-muscular and visceral adipose tissue and all the intra-cellular fat of the skinned carcase. A superficial dorsal caudal depot (analagous to the ischio-pubic and part of the synsacral depots of Liebelt and Eastlick (1954)) in which the uropygial glands were embedded was also left attached to the skinned carcase, since it was difficult to separate it cleanly from the underlying muscle.

The criteria for defining the major fat depots were unfortunately determined by practical rather than biological considerations, namely that the deposit was sufficiently

discrete to obtain good reproducibility with dissection. Consequently as already indicated many of the fat depots (with the probable exceptions of the inguino and peritoneal depots) were probably composite organs and therefore it was not possible to make any useful comparisons between the distribution of the depots in the ducks examined and the species for which descriptions are already available. There is however no reason at present to believe that the distribution of the depots in the duck are substantially different from either the chicken or the sparrow.

4.1.2. Some general considerations regarding growth.

The regular changes which occur in organs, tissues and the gross body components during normal growth have been quantitatively described by the allometric equation (Huxley, 1932). This author has demonstrated the almost universal applicability of this equation to growth problems. In this study the allometric equation has been used to compare the accretionary growth of fat, first between depots and secondly with other chemical components of the body. Prior to discussing the application of this equation to the growth data of this study, some more general aspects of biological growth will be considered.

Mathematical descriptions of growth are numerous and diverse. The most commonly used growth equations are the logistic, the Gompertz (Laird et al., 1965) and the assymptotic (Brody, 1945). Other growth equations (Weiss and Kavanau, 1957 and Von Bertalanffy, 1960) are generally

too complicated for use in most growth problems. The general criteria for using any particular growth equation is primarily that it provides the best possible fit for the growth data and secondly that it is based on a mathematical model which accounts for the changes occurring during normal biological growth. On this basis the Gompertz equation is widely used.

A characteristic of biological growth (i.e. multiplicative growth) is that the specific rate of growth declines with age. Laird et al. (1965) and Laird (1965) have shown that for many organisms the rate of decay of the specific growth rate is exponential. This relationship forms the basis of the model from which the Gompertz equation has been derived.

i.e. The specific growth rate at time t,

$$\frac{1}{W}\ \frac{dw}{dt} = A.e^{\alpha t}$$

where A is a constant equal to the initial specific growth rate and $\alpha$ is a constant equal to the rate of decay of the specific growth rate.

Since $\frac{1}{w}\ \frac{dw}{dt} = \frac{d \ln w}{dt}$

Then $\frac{d \ln w}{dt} = A.e^{-\alpha t}$

Then by integration

$$\ln W = B - \frac{A}{\alpha}.e^{-\alpha t} \quad \ldots\ldots\ldots\ldots \quad (1)$$

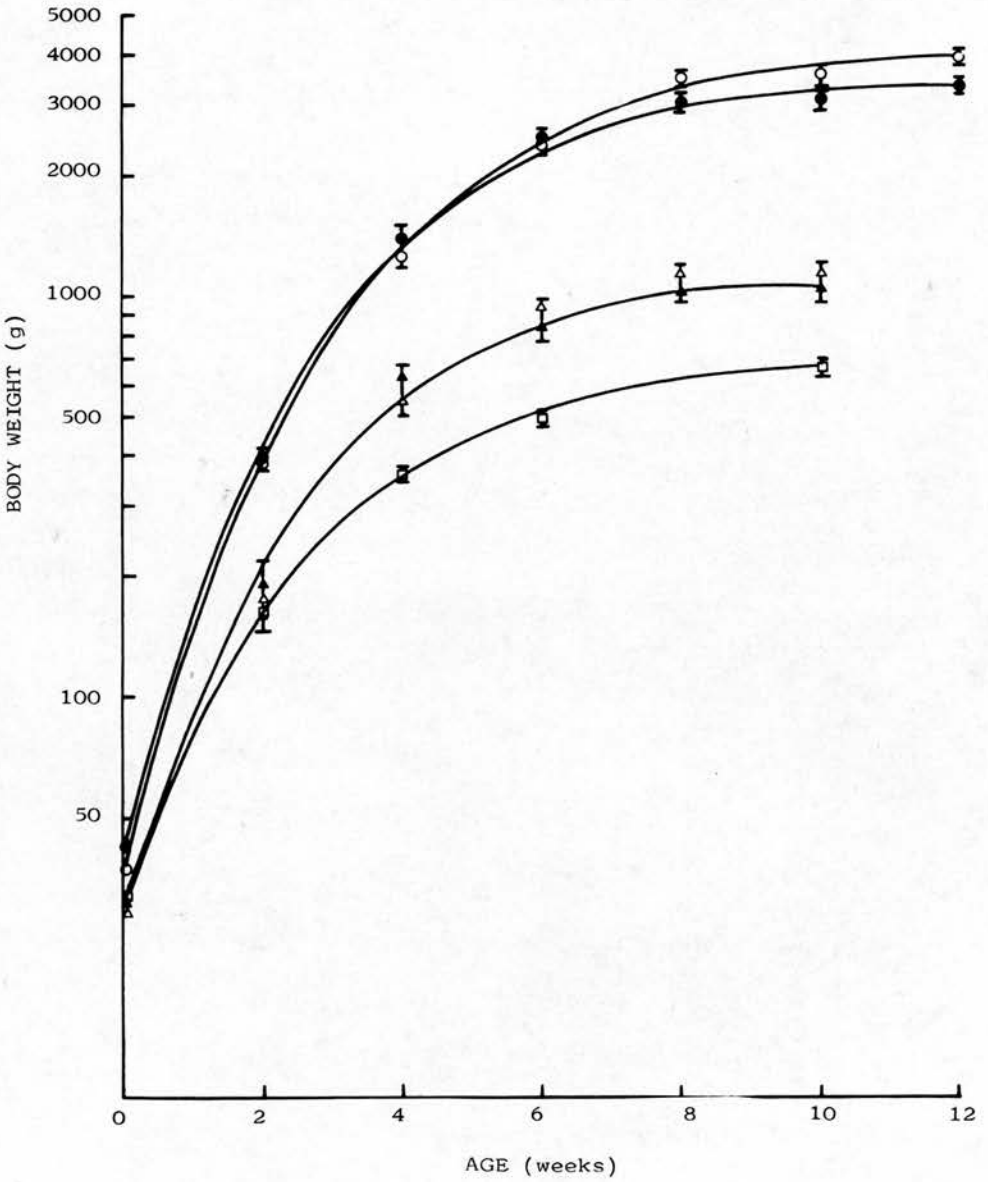
When $t = 0$, $\ln Wo = B - \frac{A}{\alpha}$, where Wo = the initial weight.

Then by substrating $B = \ln Wo + \frac{A}{\alpha}$ in equation 1

$$\ln W = \ln Wo + \frac{A}{\alpha}(1 - e^{-\alpha t})$$

Gompertz parameters

| | Wo | A | $\alpha$ | Point of Inflexion | n |
|---|---|---|---|---|---|
| Aylesbury ♂♂ | 37.4 | 0.229 | 0.048±0.001 | 32.6 | 35 |
| Aylesbury ♀♀ | 42.06 | 0.237 | 0.053±0.002 | 28.4 | 35 |
| Mallard ♀♀ | 30.7 | 0.201 | 0.055±0.004 | 23.5 | 30 |
| Tufted ♂♂ | 32.1 | 0.163 | 0.052±0.003 | 22.0 | 25 |




Gompertz growth curves for three breeds of ducks. Points are observed means ± S.E.M.

- ● Female Aylesbury
- ○ Male Aylesbury
- ▲ Female Mallard
- △ Male Mallard
- □ Male Tufted

Figure 4.2

By taking antilogs $W = Wo \exp[\frac{A}{\alpha}(1 - \exp(-\alpha t))]$

Where W = weight at time t.

Gompertz equation parameters were calculated for some of the growth data obtained from the groups of birds analysed in this study. Curve fitting was accomplished with the aid of a computer, by using an iterative least squares procedure based on the Simplex B programme (Routine 59, 1968, Edinburgh Regional Computing Centre, Program Library). For the growth of body weight the calculated curves fitted the data closely in all cases (Fig. 4.2.).

The domestic breed had both a faster rate of growth and a considerably greater final size than either the Mallard or the Tufted. There was comparatively little difference between the $W_o$ and $\alpha$ values for the different breeds (Fig. 4.2). Differences in the initial specific growth rate (A) constituted the main source of variation between the growth equations for the three breeds. The difference in growth of the body weight between the sexes in the Aylesbury were reflected in the different values for all the parameters (Fig. 4.2.). Sex did not significantly influence the growth rate in the Mallard although in the older age groups the males were consistently heavier than the females. For the Mallard the Gompertz equation was fitted to the female data only.

The point of inflexion of the growth curve is the point at which the absolute growth rate is at a maximum. The growth curves for the Mallard and the Tufted pass through this point earlier than the Aylesbury (Fig. 4.2.) and in

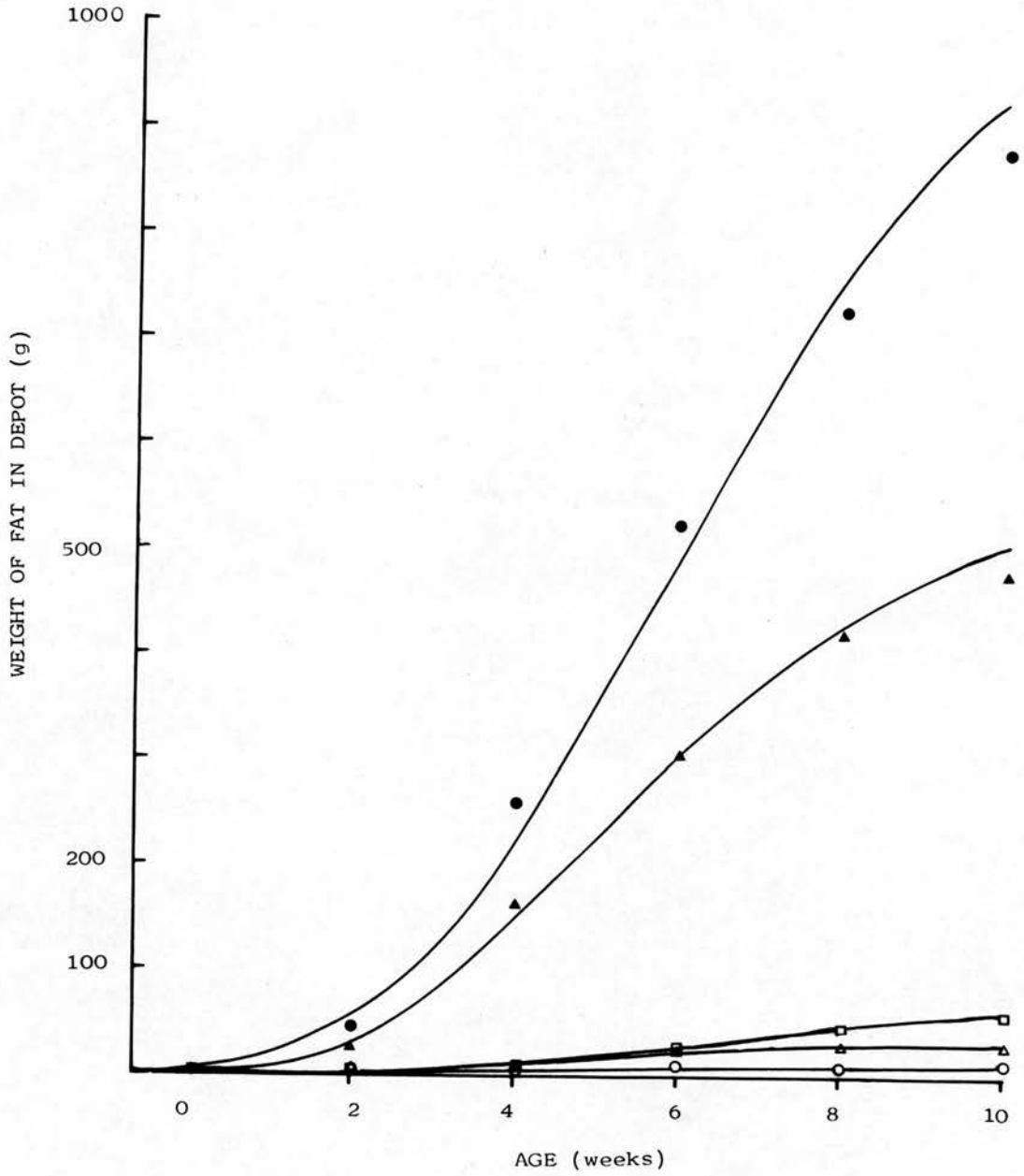
addition both also appear to approach the asymptote faster. From a comparison of the data obtained for the Mallard and Aylesbury it would appear that domestication has produced a growth rate which is greater but which may pass through corresponding developmental stages later. The Gompertz constants given for various birds (Table 4.1.) indicate that intraspecific variation is considerable although less than inter specific variation. The breed from which the Gompertz constants were calculated for the duck in this table was not given. In the turkey domestication has brought about a change in both A and $\alpha$. It would appear, that in the processes of selection involved in domestication, a greater growth rate can be achieved by the alteration of any of the growth constants independently. The experiments of Laird and Howard (1967) using mice indicated that where growth changes were produced, the Gompertz constants frequently varied independently. Fowler (1958) found that selection for high or low body size in mice did not significantly alter the position of the point of inflexion in the growth curve. Presumably both the genetic composition of the parent stock and the mode of selection used will both influence the form by which changes in the growth rate can be achieved.

Allometry and the Gompertz equation.

Fat deposition is an accretionary form of growth. Unlike cellular or multiplicative growth in which the products of the growth reproduce themselves the products of accretionary growth make no further contribution to the growth process. Although accretionary and multiplicative growth

Table 4.1. Gompertz growth parameters for various species of birds (from Laird, 1966b).

| Species | Wo(g) = Computed Birth Weight | α= Rate of decay of Specific Growth Rate | A = Specific Growth Rate at Birth |
|---|---|---|---|
| Quail | 4·16 ± 1·6 | | |
| " (Post-natal) | | ·070 ± ·007 | ·209 ± ·020 |
| Chicken | 31·2 ± 2·9 | | |
| " (Post-natal male) | | | |
| Cornish | | ·0259 ± ·0003 | ·104 ± ·004 |
| N. Hampshire | | ·0276 ± ·0002 | ·107 ± ·006 |
| White Leghorn | | ·0256 ± ·0007 | ·0978 ± ·013 |
| (Postnatal ♀) | | | |
| Cornish | | ·0273 ± ·0006 | ·104 ± ·003 |
| N. Hampshire | | ·0333 ± ·0003 | ·120 ± ·003 |
| White Leghorn | | ·0341 ± ·0005 | ·102 ± ·004 |
| Goose | 45·2 ± 3·6 | | |
| Postnatal ♂ | | ·0645 ± ·002 | ·283 ± ·006 |
| Postnatal ♀ | | ·0525 ± ·003 | ·206 ± ·009 |
| Turkey | 54·4 ± 18·0 | | |
| (Postnatal ♂) | | | |
| Br.Br.Bronze | | ·0223 ± ·0001 | ·108 ± ·003 |
| Beltsv. Small White | | ·0245 ± ·0002 | ·096 ± ·009 |
| Eastern Wild | | ·0155 ± ·0014 | ·083 ± ·005 |
| (Postnatal ♀) | | | |
| Br.Br.Bronze | | ·0232 ± ·0001 | ·109 ± ·002 |
| Beltsv. Small White | | ·0243 ± ·0001 | ·103 ± ·002 |
| Eastern Wild | | ·0156 ± ·0020 | ·078 ± ·007 |
| Duck | 56·3 ± 11·0 | | |
| Postnatal Both sexes combined | | ·0416 ± ·016 | ·188 ± ·027 |




Gompertz growth curves for fat depots in female Aylesbury. Points are observed means; lines are calculated best fit (least squares) curves. Values for Gompertz parameters are given in Table 4.3. .

- ● Total fat
- ▲ Subcutaneous
- □ Peritoneal
- △ Inguino-crural
- O Subalar etc.

Figure 4.3

are different processes there are no a priori reasons why the form of the respective growth curves should differ. Indeed the Gompertz equation gave a satisfactory fit to the growth data for the fat accretion in the fat depots (Fig. 4.3. Tables 4.2-5.) implying that such growth conformed to the model devised for multiplicative growth.

Although the Gompertz parameters can be used to compare the growth of the body components, a more direct and simple method is provided by the allometric equation. This equation is of the form $y = bx^k$, where x and y are the respective sizes of the two parts of the organism, and k is a constant. The allometric equation is derived from the assumption that the ratio of the specific growth rates of the two parts x and y remain constant during growth.

$$\text{i.e. } \frac{\frac{1}{y}\frac{dy}{dt}}{\frac{1}{x}\frac{dx}{dt}} = k \quad \ldots\ldots\ldots\ldots\ldots (1)$$

Since $\frac{1}{y}\frac{dy}{dt} = \frac{d}{dt} \ln y$ and $\frac{1}{x}\frac{dx}{dt} = \frac{d}{dt} \ln x$

Then (1) becomes $\frac{d \ln y}{d \ln x} = k$

i.e. $\ln y = k \ln x + \ln b$

Which taking anti logs becomes $\underline{y = bx^k}$.

Consequently the requirement for establishing allometry between two body parts is that their specific growth rates should decay at the same rate. Therefore body components whose growth can be expressed by the Gompertz equation will be allometrically related if the values of the

Table 4.2. Gompertz parameters for the fat deposits in the male Aylesbury.

| | Wo = Initial body weight | A = Initial specific growth rate | α = rate of decay of specific growth rate (± S.E.M.) | Degrees of Freedom |
|---|---|---|---|---|
| Total body wt. | 37·52 | 0·228 | 0·047±0·001 | 26 |
| Total fat | 3·03 | 0·258 | 0·042 ± 0·002 | 26 |
| Clavic. Fat | 0·072 | 0·260 | 0·041 ± 0·007 | 26 |
| Subal. Fat | 0·021 | 0·302 | 0·045 ± 0·006 | 26 |
| Peritoneal Fat | 0·010 | 0·454 | 0·051 ± 0·003 | 26 |
| Inguino. Fat | 0·299 | 0·271 | 0·054 ± 0·004 | 26 |
| Skinned carcase Fat | 1·464 | 0·175 | 0·028 ± 0·002 | 26 |
| Subcutaneous Fat | 1·216 | 0·310 | 0·049 ± 0·002 | 26 |

Table 4.3. Gompertz parameters for the fat depots in the female Aylesbury.

| | Wo = Initial body weight | A = Initial specific growth rate | α = rate of decay of specific growth rate (± S.E.M.) | Degrees of freedom |
|---|---|---|---|---|
| Total body wt. | 41·613 | 0·236 | 0·052 ± 0·002 | 27 |
| Total fat | 3·310 | 0·268 | 0·046 ± 0·003 | 27 |
| Clavic fat | 0·233 | 0·191 | 0·037 ± 0·005 | 27 |
| Subal. fat | 0·030 | 0·328 | 0·054 ± 0·006 | 27 |
| Peritoneal fat | 0·009 | 0·439 | 0·047 ± 0·005 | 27 |
| Inguino. fat | 0·329 | 0·276 | 0·059 ± 0·005 | 27 |
| Skinned carcase fat | 1·545 | 0·184 | 0·031 ± 0·003 | 27 |
| Subcutaneous fat | 1·111 | 0·341 | 0·055 ± 0·003 | 27 |

Table 4.4. Gompertz parameters for the fat depots in the male Tufted.

| | Wo = Initial body weight | A = Initial specific growth rate | α = rate of decay of specific growth rate (± S.E.M.) | Degrees of freedom |
|---|---|---|---|---|
| Total body wt. | 32·056 | 0·163 | 0·052 ± 0·003 | 21 |
| Total fat | 4·122 | 0·152 | 0·051 ± 0·005 | 21 |
| Clavic. fat | 0·121 | 0·138 | 0·048 ± 0·015 | 21 |
| Subal. fat | 0·133 | 0·241 | 0·080 ± 0·018 | 21 |
| Peritoneal fat | 0·140 | 0·165 | 0·064 ± 0·018 | 21 |
| Inguino. fat | 0·296 | 0·208 | 0·100 ± 0·024 | 21 |
| Skinned carcase fat | 1·750 | 0·098 | 0·030 ± 0·004 | 21 |
| Subcutaneous fat | 1·425 | 0·208 | 0·064 ± 0·007 | 21 |

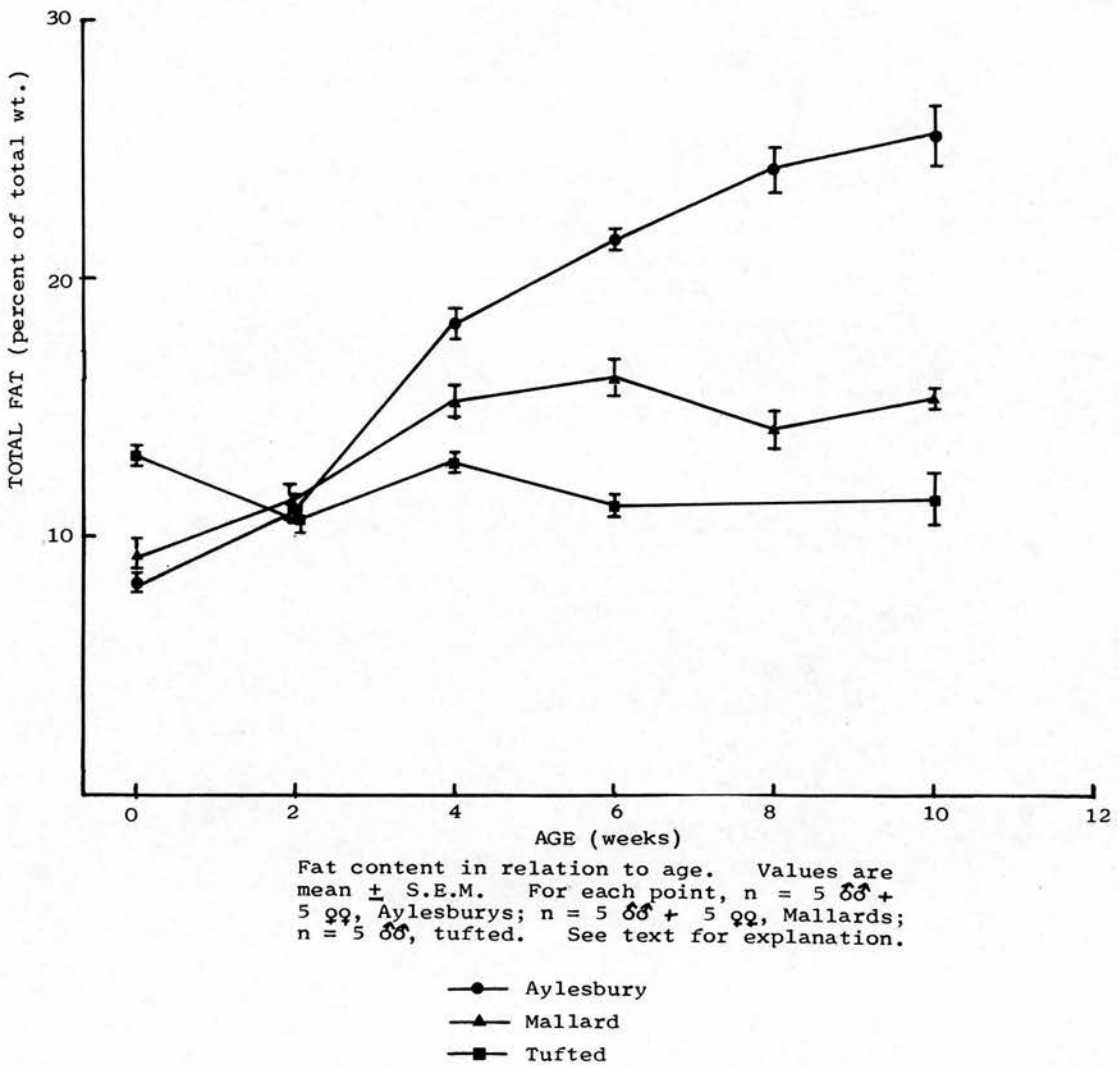
Table 4.5. Gompertz parameters for the fat depots in the female Mallard.

| | Wo = Initial body weight | A = Initial specific growth rate | α = rate of decay of specific growth rate (± S.E.M.) | Degrees of freedom |
|---|---|---|---|---|
| Total wt. | 30·719 | 0·201 | 0·055 ± 0·004 | 26 |
| Total fat | 3·000 | 0·239 | 0·059 ± 0·006 | 26 |
| Clav. fat | 0·137 | 0·108 | 0·029 ± 0·015 | 26 |
| Subal. fat | 0·182 | 0·129 | 0·042 ± 0·009 | 26 |
| Peritoneal fat | 0·051 | 0·359 | 0·067 ± 0·013 | 26 |
| Inguino. fat | 0·422 | 0·233 | 0·082 ± 0·012 | 26 |
| Skinned carcase fat | 1·439 | 0·175 | 0·045 ± 0·004 | 26 |
| Subcutaneous fat | 0·733 | 0·337 | 0·071 ± 0·007 | 26 |

constant α (the rate of decay of the specific growth rate) are identical. On this basis an examination of the Gompertz constants (Table 4.2-5.) indicated that most of the fat deposits were both allometrically related to each other and to total body weight. There were however some exceptions and these are discussed in a following section.

In the most general use of the allometric equation (Huxley, 1932) y represents the size of a body component and x the size of the whole body. For this substitution the power factor, k is termed the growth coefficient of the component. In the case where the growth coefficient (k) is equal to unity then the rate of increase of the component is directly proportional to the whole body. However if k is less than unity this indicates that the growth of the component decreases relative to that of the whole body as the whole body increases in size. Restated in other terms this implies that most of the growth of the component is accomplished earlier than that for the whole body. Similarly when k is greater than unity the component will grow later than the whole body. This interpretation of k has led to its use in distinguishing between early and late maturing tissues during growth (Elsley et al. 1964). In this way the allometric equation has been used to characterise the form of the accretionary growth of fat in the various depots.

Some confusion can arise from the use of the term, maturity. Unless otherwise stated this is used here only as a comparative term. Consequently a late-maturing component in this study refers to one in which growth occurs relatively




Fat content in relation to age. Values are mean ± S.E.M. For each point, n = 5 ♂♂ + 5 ♀♀, Aylesburys; n = 5 ♂♂ + 5 ♀♀, Mallards; n = 5 ♂♂, tufted. See text for explanation.

Figure 4.4

later than body growth. It does not imply that growth occurs in the component after body growth is complete.

A valid theoretical objection to the use of the allometric equation is that if the parts of an organism bear an allometric relationship to each other, then there cannot be an allometric relationship between a part and the whole (Huxley, 1932) since summation of expressions of the form $b.x^k$ with different values for k does not result in a single expression of the same form. However in practise if the values of k are similar this criticism can be largely ignored (Laird, 1965). In the rare cases that the k values are distinctly different, they frequently involve parts whose size is sufficiently small, so as to be without influence on the growth of the organism as a whole. These considerations are discussed in more detail by Laird (1965). Such observations emphasise that the allometric equation represents a close approximation to, rather than an exact expression of relative growth.

4.1.3. Distribution of fat during growth.

Variation of fat content during growth. In the Aylesbury duck there is an approximately three-fold increase in fat content during the first 10 weeks of postembryonic life (Fig. 4.4.). This was considerably greater than that observed for the other breeds (Fig. 4.4.). Data from both sexes for the Aylesbury and the Mallard were pooled since there was rarely any significant difference between the fat content of the different sexes (Table 4.6.). Although the

Table 4.6. Comparison of Percentage fat in whole birds between males and female Aylesbury and Mallard ducks. Mean ± S.E.; ♂ or ♀ shown in brackets.

| Age | ♂♂ | ♀♀ | t | P |
|---|---|---|---|---|
| | | **Aylesburys** | | |
| 0 | 8·30 ± 0·58 (5) | 8·26 ± 0·43 (5) | 0·052 | >0·10 |
| 2 | 11·38 ± 0·69 (5) | 10·85 ± 0·90 (5) | 0·466 | >0·10 |
| 4 | 17·03 ± 0·86 (4) | 19·05 ± 0·77 (6) | 1·718 | >0·10 |
| 6 | 21·35 ± 0·34 (5) | 21·58 ± 0·79 (5) | 0·265 | >0·10 |
| 8 | 24·36 ± 1·08 (5) | 24·08 ± 1·68 (5) | 0·141 | >0·10 |
| 10 | 24·40 ± 0·82 (6) | 27·25 ± 2·01 (4) | 1·480 | >0·10 |
| | | **Mallards** | | |
| 0 | 8·34 ± 0·39 (5) | 9·80 ± 0·58 (5) | 1·953 | >0·05 |
| 2 | 10·73 ± 0·95 (6) | 12·22 ± 0·45 (4) | 1·172 | > 0·10 |
| 4 | 13·91 ± 0·69 (5) | 16·56 ± 0·63 (5) | 2·838 | <0·50>0·20 |
| 6 | 15·38 ± 0·98 (5) | 16·75 ± 1·33 (5) | 0·832 | >0·10 |
| 8 | 13·67 ± 0·84 (4) | 14·38 ± 1·12 (5) | 0·487 | >0·10 |
| 10 | 15·78 ± 0·73 (4) | 15·10 ± 0·52 (6) | 0·777 | >0·10 |

Tufted day-old ducks were significantly fatter than the other day-olds (yolk sac fat was not included in this data) by 10 weeks their fat content was well below that of the other breeds. In fact no appreciable change in fat content occurred during the growth period in the Tufted.

Since fat deposition in the domestic breed was so much greater than in the wild forms, this suggested that the selection for increased body weight in the domestic breed may have inadvertently selected for "obesity" or increased fat deposition. To examine this possibility the coefficients for the allometric equations relating total fat to fat-free body weight were calculated (Table 4.7.). Data for the day-olds frequently showed deviations from linearity for log-log plots for these two variables, and consequently this age group was excluded from the analysis. There are good biological reasons for ignoring such data. These are discussed in a later section.

Data from both sexes were pooled for the regression analysis. The allometric expressions thus calculated relating fat to fat-free body weight differed between the three breeds (Table 4.7.). To test whether the differences between the domestic and wild breed were significant, the data from each pair (Aylesbury and Mallard, and Aylesbury and Tufted) were combined, and the regression equation and residual sums of squares were calculated for this combined data (Table 4.7.). By analysis of variance it was established in each case that the data fitted the individual regression equations significantly better than the combined

Table 4.7. Regression of log total fat (y) on log fat free body weight (x). The regression coefficient and intercept are equal to values, b and k respectively of the allometric equation $y = bx^k$.

| Breed | Age range (weeks) | No. of pairs | Regression equation | r & log x log y | Resid. S.D. | Resid. sums of squares | Allometric equation |
|---|---|---|---|---|---|---|---|
| Aylesbury (males and females) | 2-12 | 62 | log y = 1·499 log x - 2·174 | 0·9913 | 0·062 | 0·2427 | $y = 0·007 \times 1·499 \pm 0·026$ |
| Mallard (males and females) | 2-10 | 49 | log y = 1·207 log x - 1·347 | 0·9800 | 0·072 | 0·2565 | $y = 0·045 \times 1·207 \pm 0·036$ |
| Tufted (males) | 2-10 | 19 | log y = 1·038 log x - 0·981 | 0·9686 | 0·062 | 0·0729 | $y = 0·104 \times 1·038 \pm 0·065$ |
| Aylesbury & Mallards | | 111 | log y = 1·398 log x - 1·860 | | | 0·7226 | |
| Aylesbury & Tufted | | 81 | log y = 1·438 log x - 1·981 | | | 0·5021 | |

$$F_{(2,107)} = \left[\frac{\frac{1}{2}[SS_{A\&M} - SS_A - SS_M]}{\frac{1}{107}[SS_A + SS_M]}\right] = 24·022; \quad p \ll 0·01$$

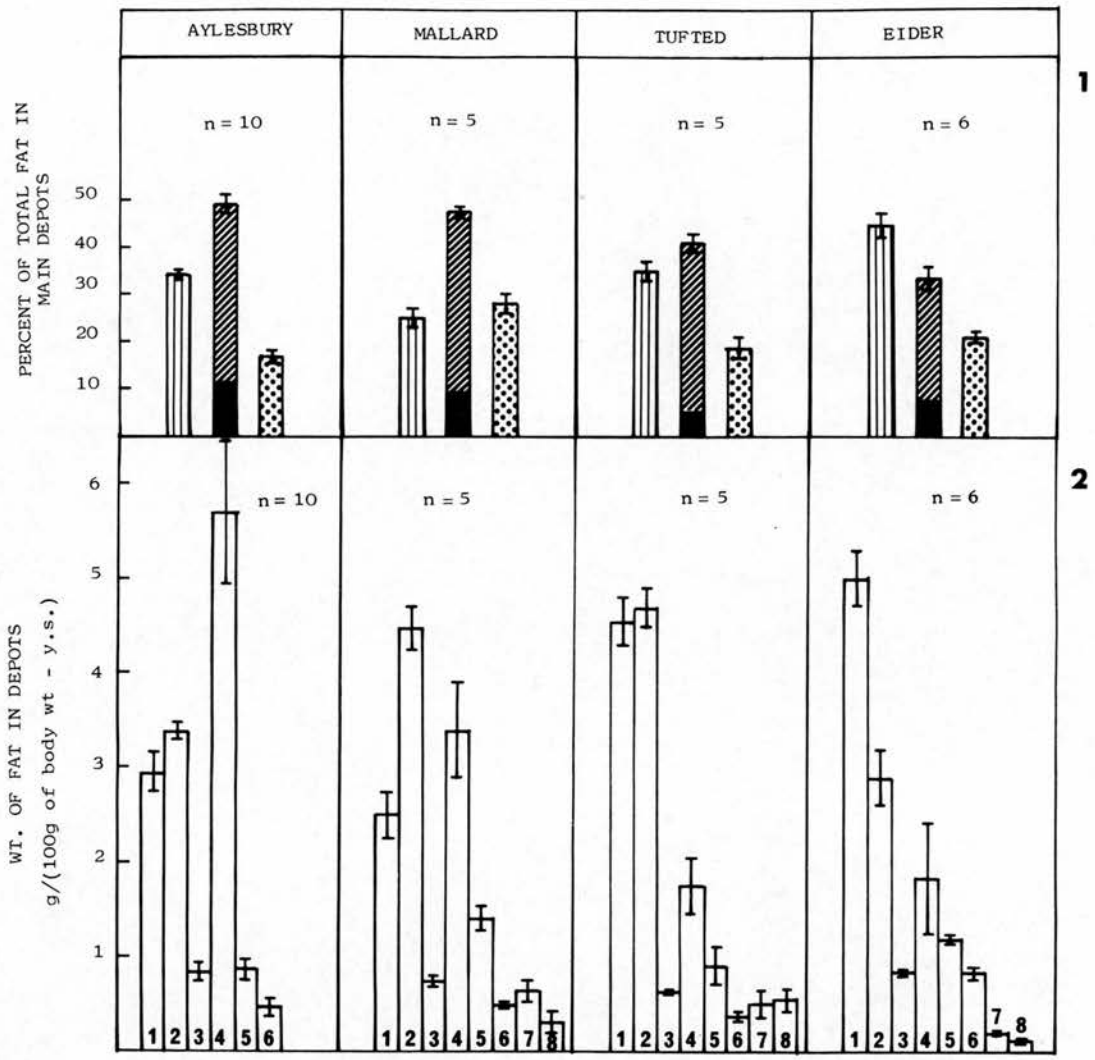
$$F_{(2,79)} = \left[\frac{\frac{1}{2}[SS_{A\&T} - SS_A - SS_T]}{\frac{1}{79}[SS_A + SS_T]}\right] = 22·888; \quad p \ll 0·01$$

regression equations, indicating that the allometric relationship for the Aylesbury was significantly different from that of either the Mallard or Tufted. The higher growth coefficient (k) for fat in the Aylesbury when compared to the Mallard, indicated that the processes of selection responsible for the production of the domestic breed had produced a bird which actively deposited more fat during growth.

Distribution of fat in the day-old (Fig. 4.5.). The distribution of fat in the day-old duckling is considered separately, since the amount and distribution of fat in this stage, is determined by processes occurring during embryonic growth. In the duck, and most other animals the growth rate of the embryo is invariably found to be different to that occurring during the postnatal period (Laird, 1966b). In addition, some of the fat deposits, such as the liver and yolk sac, at hatching, represent only temporary stores which enable the bird to survive the first few days of postembryonic life without food and consequently represent a discontinuity in normal growth. Values for the fat content of the liver in day-olds ranged from 24-30%, 16-28%, 15-16% and 15-20% for the Aylesbury, Mallard, Tufted and Eider (Somateria mollissima) respectively. In the normal postembryonic young or adult Aylesbury the fat content of the liver generally occurred within the range, 3-4%.

Since newly hatched ducklings are brooded less than

# Fat Distribution in Day-old Ducklings




1. Distribution of the total fat (excluding yolk-sac fat) between the major deposits. Values are means ± S.E.M.

Subcutaneous

Skinned carcase

Liver

"Pooled" depots (see Figure 4.6.).

2. The relative amount of fat in each depot. Values are means ± S.E.M.

1, Subcutaneous
2, Skinned carcase
3, Liver
4, Yolk
5, Inguino-crural
6, Clavic. etc.
7, Subalar etc.
8, Peritoneal

Figure 4.5

in most avian species, it has been suggested that the composition of the day-old is determined by the climatic conditions at hatching. Koskimies and Lahti (1964) have shown that both the newly hatched Eider and Tufted ducklings were able to maintain their body temperature for relatively longer periods at low ambient temperatures than the Mallard. The thermoregulative abilities of these species was related to their geographic distribution, and consequently to the climate within their normal breeding range. Populations of Mallards breeding in high lattitudes have been shown to produce young which at hatching have a higher fat content than birds of the same age found in lower lattitudes (Marcström, 1966). Both the Eider and the Tufted analysed in this study were obtained from wild population breeding in Scotland, whereas the Mallard was obtained from a lower lattitude in England. It was particularly notable therefore that both the Tufted and Eider ducklings had greater amounts of fat in the subcutaneous depots than the other breeds.

In the Mallard the amount of fat in the yolk sac, has been shown to be dependent upon the time taken for the hatching process. It also decreases rapidly during the first few days after hatching (Kear, 1965). Consequently, since the exact ages of the day-olds in this study was not known it was not possible to attribute any significance to the variability of yolk fat between the breeds.

Although the liver of the day-old has a very high fat content, the total amount of fat in this organ makes only a relatively minor contribution to the total amount of fat with-

in the bird (Fig. 4.5.). Studies performed with fasting day-old Mallards indicated that the liver acts as a temporary store for the yolk fat, and that it is only after all the fat has been mobilised from the yolk sac that the fat content of the liver begins to decline (Marcström, 1966).

Fat content of the body at hatching may be influenced by the incubation time (Table 4.8. and Fig. 4.5.). Weight at hatching appears related as might be expected to eggsize. When weight was expressed as a proportion of egg size it appeared to be only partially influenced by incubation time (Table 4.8. and Fig. 4.5.).

Compared to the Mallard and Aylesbury the rate of fat deposition in the Tufted was greater during embryonic life but less during postnatal life. Particularly with reference to the above discussion, this seems to indicate that the processes which govern embryonic and postnatal fat deposition are independent and consequently can be selected for separately. Certainly the increased postnatal fat deposition in the Aylesbury has been achieved simultaneously with a decline in embryonic lipogenesis (cf Mallard results Fig. 4.4,5.). The Aylesbury day-old not only had less fat than day-olds of other breeds, but the virtual absence of the peritoneal and subalar depots indicated that the adipose tissue was at an earlier stage of development (Fig. 4.5.).

Distribution of fat in the other age-groups (Fig. 4.6.). The distribution of fat between the major fat depots was found to be uninfluenced by sex in the Aylesbury (Table 4.9.). Since the discrete fat depots (those in which the adipose tissue

Table 4.8. Some data from day-old ducks.

| Breed | Total wt. (g)- yolk sac. | % Fat excluding yolk sac. | Total wt. -yolk sac as percent egg wt. | Egg wt. (g) | Incubation (days) |
|---|---|---|---|---|---|
| Aylesbury | 40·4±1·6 (10) | 8·3±0·3 (10) | 44 | 90±1·2 (30) | 28 |
| Mallard | 30·8±1·1 (10) | 9·1±0·4 (10) | 57 | 54 | 28 |
| Tufted | 32·2±1·0 (5) | 13·1±0·2 (5) | 57 | 56 | 24 |
| Eider | 62·9±1·3 (6) | 12·1±0·2 (6) | 57 | 111 | 25 |

Table 4.9. Comparison between the distributions of the total fat in male and female Aylesbury. Values are Mean ± S.E.M., 'n' shown in brackets.

| Age | ♂♂ | ♀♀ | t | P |
|---|---|---|---|---|
| | | **Percent of Total Fat in the Subcutaneous deposit** | | |
| 0 | 35·92 ± 2·90(5) | 32·24 ± 1·40(5) | 1·143 | >0·10 |
| 2 | 61·99 ± 1·45(5) | 64·20 ± 1·81(5) | 0·948 | >0·10 |
| 4 | 65·22 ± 1·24(4) | 62·28 ± 2·07 (6) | 1·061 | >0·10 |
| 6 | 59·37 ± 1·34(5) | 58·68 ± 0·76(5) | 0·447 | >0·10 |
| 8 | 60·42 ± 1·44(5) | 58·62 ± 2·02(5) | 0·717 | >0·10 |
| 10 | 54·26 ± 0·73(6) | 54·83 ± 0·77(4) | 0·518 | >0·10 |
| | | **Percent of Total Fat in the skinned carcase** | | |
| 0 | 49·28 ± 4·52 | 48·84 ± 1·56 | 0·092 | >0·10 |
| 2 | 22·95 ± 1·69 | 20·98 ± 2·12 | 0·741 | >0·10 |
| 4 | 20·66 ± 0·99 | 21·90 ± 1·65 | 0·561 | >0·10 |
| 6 | 23·28 ± 0·46 | 25·09 ± 1·01 | 1·630 | >0·10 |
| 8 | 25·98 ± 1·23 | 28·20 ± 2·06 | 0·925 | >0·10 |
| 10 | 32·09 ± 0·61 | 31·17 ± 0·82 | 0·812 | >0·10 |
| | | **Percent of fat in the "pooled" depots** | | |
| 0 | 14·80 ± 2·38 | 18·74 ± 1·37 | 1·438 | >0·10 |
| 2 | 15·03 ± 0·67 | 14·82 ± 1·44 | 0·133 | >0·10 |
| 4 | 14·10 ± 0·44 | 15·82 ± 0·69 | 1·840 | >0·10 |
| 6 | 17·38 ± 0·91 | 16·22 ± 0·36 | 1·192 | >0·10 |
| 8 | 13·58 ± 0·61 | 14·14 ± 0·83 | 0·538 | >0·10 |
| 10 | 13·62 ± 0·65 | 13·95 ± 0·56 | 0·317 | >0·10 |

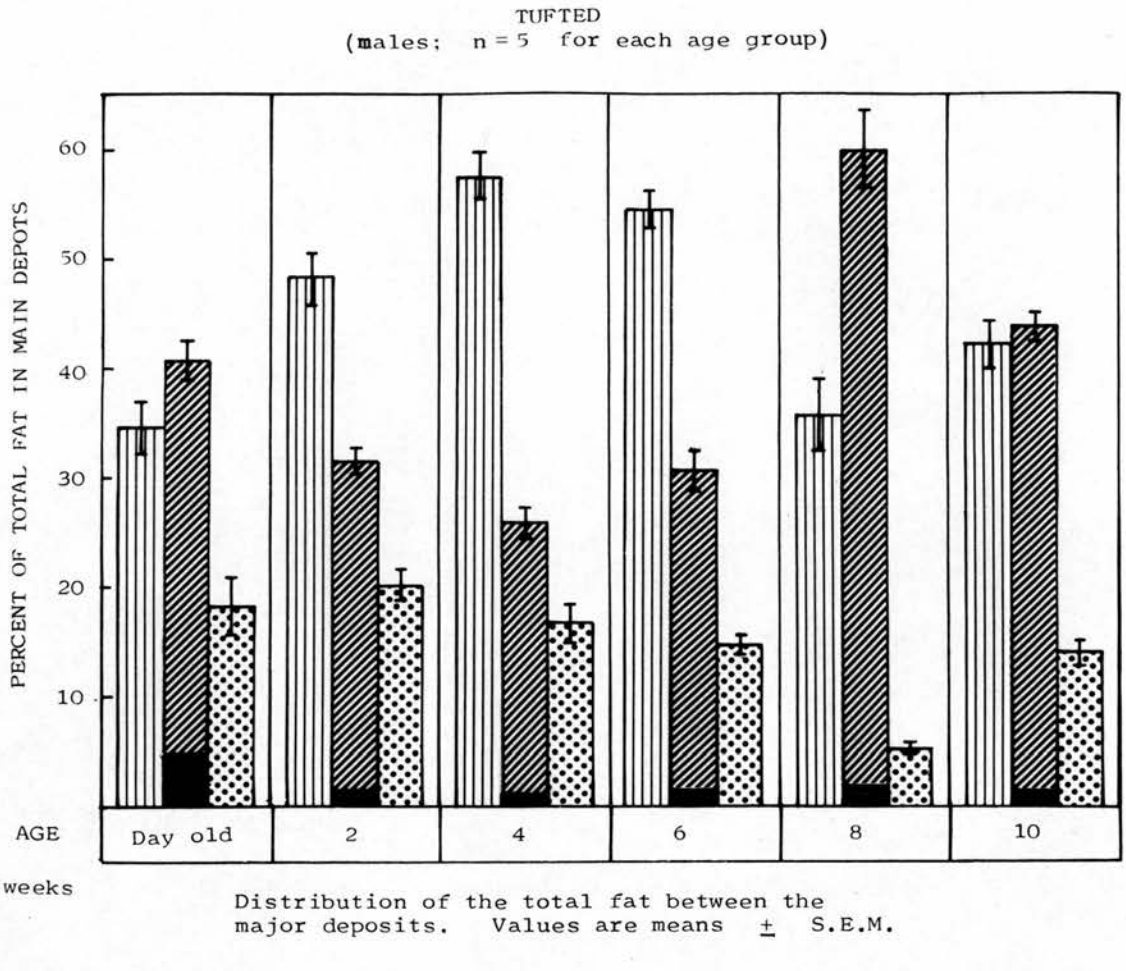
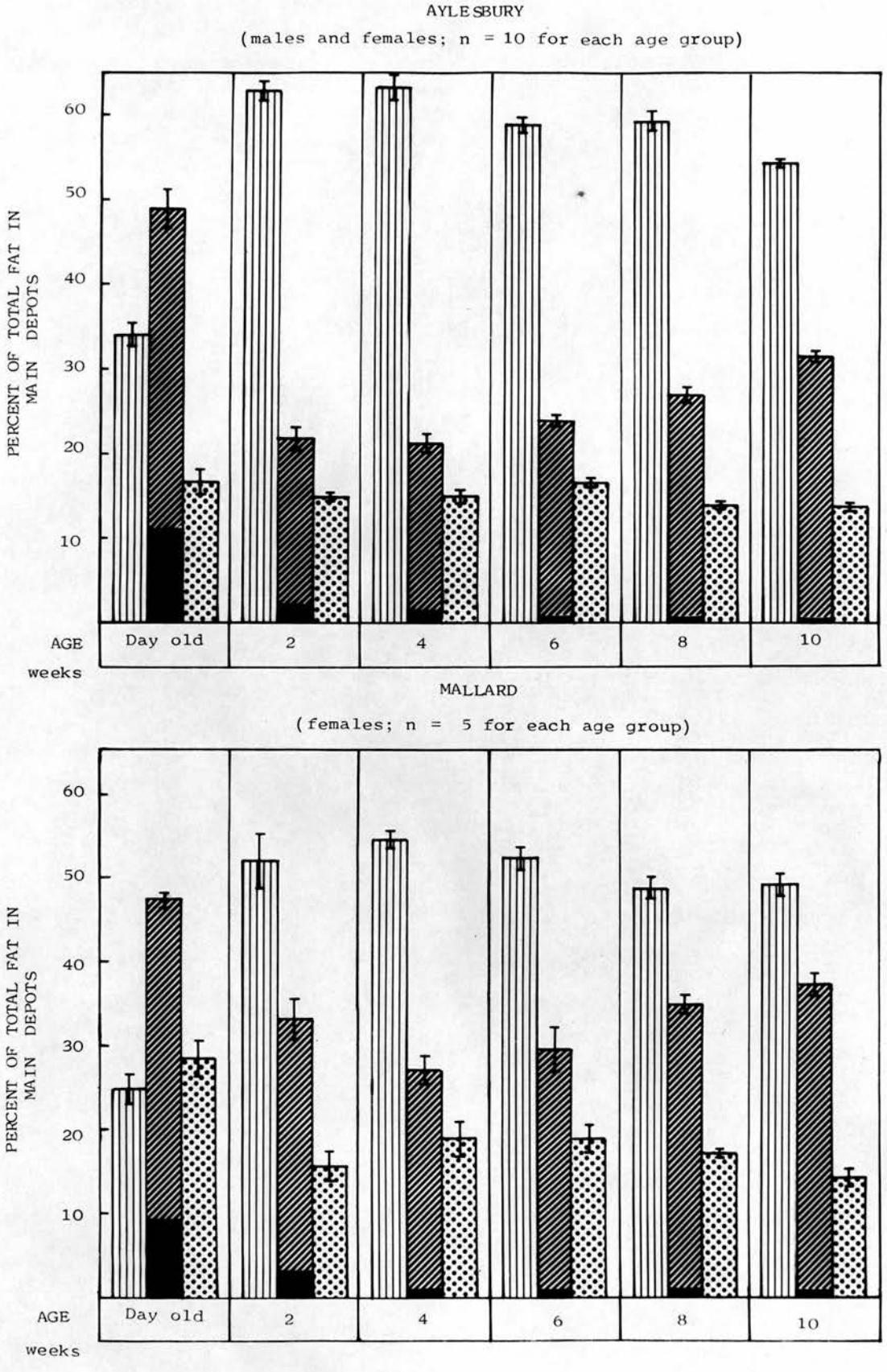

TUFTED
(males; n = 5 for each age group)
PERCENT OF TOTAL FAT IN MAIN DEPOTS
60
50
40
30
20
10
AGE
weeks
Day old
2
4
6
8
10
Distribution of the total fat between the major deposits. Values are means ± S.E.M.
Subcutaneous
Skinned carcase
Liver
"Pooled" fat (includes, inguino-crural claviculo-etc., subalar etc., and peritoneal)

Figure 4.6




(See next page for legend)

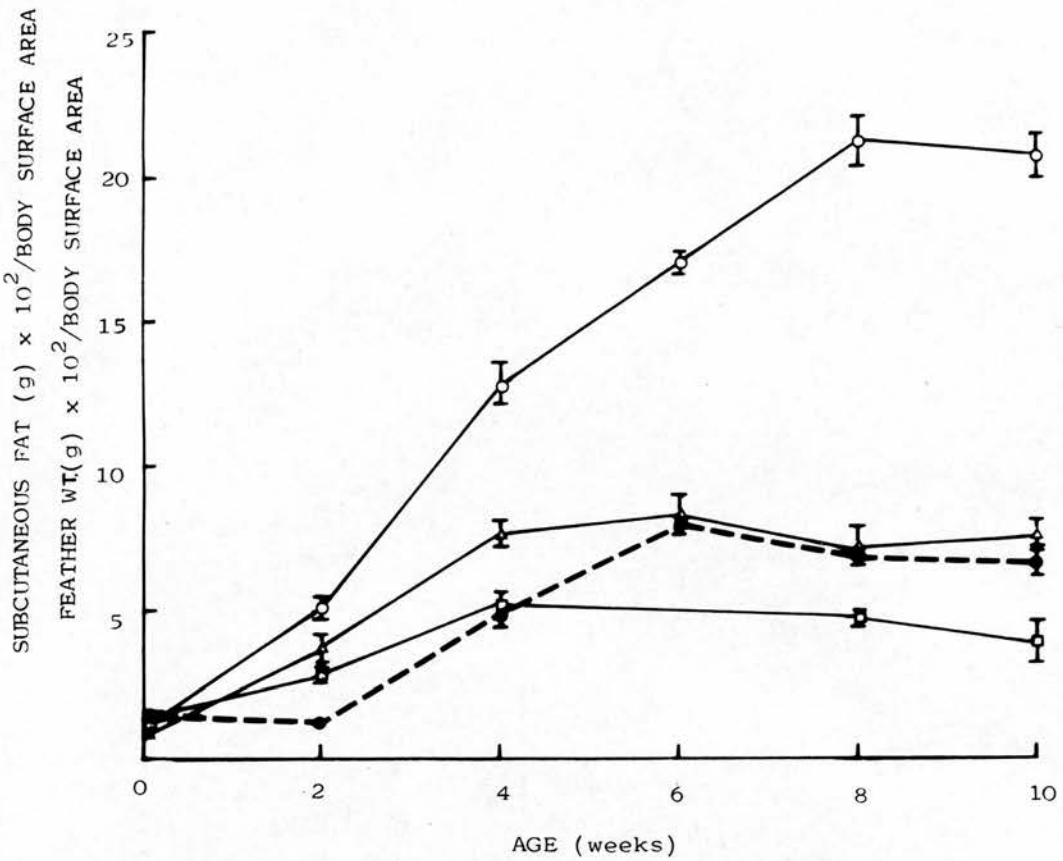
Figure 4.6

could be dissected cleanly from the surrounding tissue) were found to constitute only a relatively minor fraction of the total fat, they have been considered as a single deposit for the purposes of this analysis. This is referred to as a "pooled" depot.

In the Aylesbury the subcutaneous deposit was always the largest fat deposit, ranging from 63·4% of the total fat in the 4 week old bird to 54·5% in the 10 week old bird (excluding results from the day-old). The observed decrease in the proportion of fat occurring in the subcutaneous depots with age was accounted for by the increasing proportion occurring in the skinned carcase. Although the distribution of fat within the skinned carcase was not determined, it would appear from general observation that the rise in the fat content of this body component with age was due to increasing amounts of intermuscular fat. The proportion of fat occurring in the pooled depots varied little with age.

The distribution of fat in the Mallard at different ages closely resembled that for the Aylesbury. Again the pooled depots showed little variation with age. The rather smaller proportion of fat occurring in the subcutaneous depots was the result of a proportionately greater amount occurring in the skinned carcase in the Mallard (Fig. 4.6.). However although the proportion of fat in the skinned carcase was greater in the Mallard than the Aylesbury the amount of fat which it represented as a percentage of the skinned carcase weight (7·9%) was less than the same measurement for the domestic breed (11·6%).

Changes in the distribution of fat during growth in




The effect of age on thickness of the subcutaneous fat. An index of mean thickness of subcutaneous fat has been calculated by dividing the weight of subcutaneous fat by the surface area of the body. Surface area was calculated from body weight according to the formula.

$A = 10.(W)^{0.6667}$ Feather weight/area was calculated as the wet weight of feathers divided by surface area calculated in the same way.

—○— Index of subcutaneous fat thickness Aylesbury
—△— " " " " " Mallard
—□— " " " " " Tufted
- -●- - Feather wt./area, Aylesbury

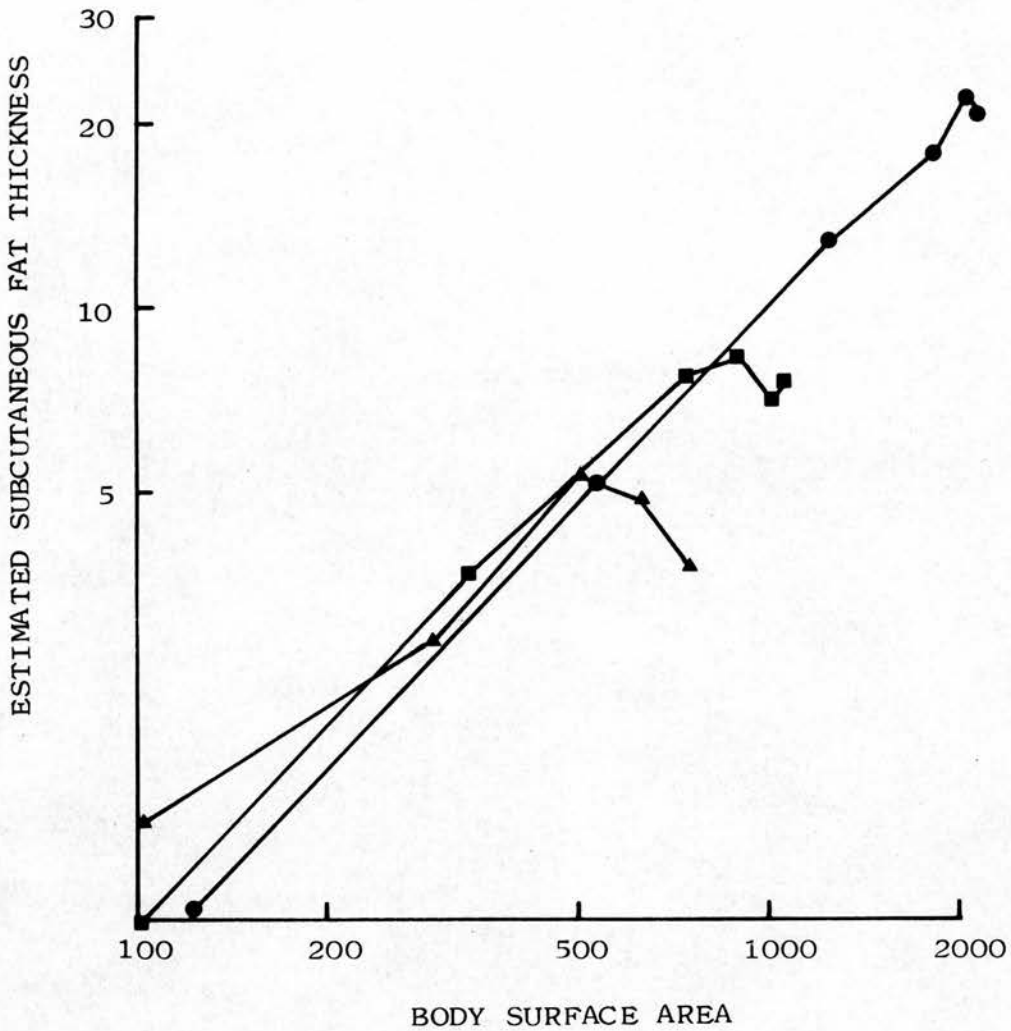
Figure 4.7.1

the Tufted were broadly similar to those occurring in the other breeds (Fig. 4.6.). The 8 week-old birds however were grossly underweight when received, and it is apparent that the distribution of fat in these birds reflects a state of emaciation (cf. results in Sec. 4.1.5.). For this reason this data was not considered to represent a distribution occurring during normal growth. As in the case of the Mallard, compared to the domestic breed, there was a greater proportion of fat in the skinned carcase of the Tufted and a correspondingly smaller proportion in the subcutaneous depots.

These results indicate that the breeds which possess a greater overall fat content, also have a greater proportion of the fat contained in the subcutaneous depot.

The duck is normally a semi-aquatic bird and consequently it might be expected that the largest deposit of fat, in the subcutaneous depots, had been evolved to meet the need for effective thermal insulation. This would be in addition to the insulation provided by the plumage, which in the duck and aquatic birds is notable for the dense layer of down lying between the surface of the skin and the outer covering of contour feathers.

An index of thickness of the subcutaneous fat was calculated by dividing the weight of the subcutaneous fat by the surface area of the body. Surface area was calculated using the formula, Area = $10\ W^{0.667}$, where W represented body weight (Leighton et al., 1966). Similarly an index of feather depth has been calculated on the same basis. These results indicated that during growth there was an approximate 20-fold




The relationship between subcutaneous fat thickness (see Fig.4.7.1.) and surface area

● Aylesbury
■ Mallard
▲ Tufted

Figure 4.7.2

increase in fat thickness in the subcutaneous fat in the Aylesbury (Fig. 4.7.1.). Of course if the distribution of the subcutaneous fat alters during growth then the changes in thickness in some areas will be greater or less than others. Feather "depth" or weight per unit area also increased during this period but to a lesser extent than that for subcutaneous fat. However it is unlikely that the insulating properties of feathers will be related to their weight (wet weight), since down feathers have better insulation properties (on a weight basis) than flight or contour feathers which develop later. Although smaller changes occurred in the calculated subcutaneous fat thickness in the Mallard and the Tufted (Fig. 4.7.1.), when this parameter was plotted against surface area (Fig. 4.7.2.) it became apparent that the fat thickness in this deposit was similarly related to surface area in all the breeds. However departures from linearity were observed for the older age groups of the wild breeds (Fig. 4.7.2.). For marine mammals a somewhat similar relationship between subcutaneous fat thickness and surface area has been established (Bryden, 1968). Part of the explanation for such a relationship may arise from the observation that increasing the thickness of the insulating layer in small animals (i.e. with a surface of high curvature) does not effectively reduce conductance (Newburgh, 1949). The converse of course is that an increase in the thickness of the insulating layer becomes increasingly effective in larger animals.

The distribution of fat in the Aylesbury suggests

that the subcutaneous deposit might usefully provide an estimate of the total fat in the growing bird. A correlation matrix has been calculated for total fat and the fat deposits (Table 4.10.). On this basis a multiple regression equation for estimating total fat from the main fat deposits was calculated. The prime term in this equation, the subcutaneous deposit accounted for a large part of the residual variation (Table 4.10). Therefore, a quite good estimate of total fat could be obtained from measurement of the subcutaneous deposit. Either skinfold thickness measurement techniques which have been used with success in man or punch biopsies of the skin may provide the means of estimating subcutaneous fat in the live bird.

4.1.4. The relative growth of the fat depots.

The growth coefficient (k) of the allometric equation (frequently represented as the regression coefficient of the logarithmic transformation of the allometric equation) has been used as the basis for characterising and comparing the growth form of each fat deposit, and further, to compare the growth of these deposits with some of the major chemical components of the body. In the latter instance it would have been more desirable to first dissect the body entirely into its component organs, muscle groups, bones etc. and to obtain the chemical composition of these parts and therefore to present a more meaningful and naturalistic description of the non-fat components, but the great amount of work involved in such an undertaking precluded this from being considered as a practical possibility.

Table 4.10. Multiple regression analysis for log total fat against log fat deposits.
s/c = skinned carcase.

Correlation matrix for log total fat and log fat deposits.

| | Log Total Fat | Log Clav. | Log Subalar. | Log Perit. | Log Inguino. | Log S/C. | Log Subcut. |
|---|---|---|---|---|---|---|---|
| Log Total fat | 1·0000 | | | | | | |
| " Clavic | 0·9603 | 1·0000 | | | | | |
| " Subalar | 0·9406 | 0·9701 | 1·000 | | | | |
| " Perit. | 0·9648 | 0·9527 | 0·9244 | 1·000 | | | |
| " Inguino. | 0·9711 | 0·9436 | 0·9212 | 0·9496 | 1·0000 | | |
| " S/C | 0·9913 | 0·9435 | 0·9259 | 0·9490 | 0·9483 | 1·0000 | |
| " Subcut. | 0·9982 | 0·9569 | 0·9374 | 0·9617 | 0·9732 | 0·9828 | 1·0000 |

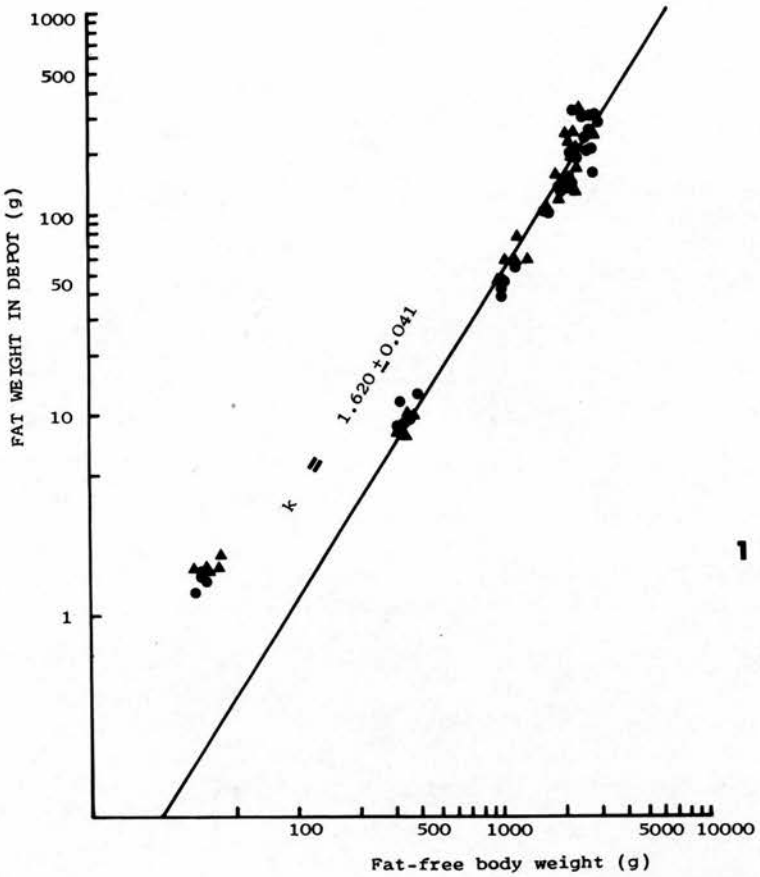
Multiple regression equations

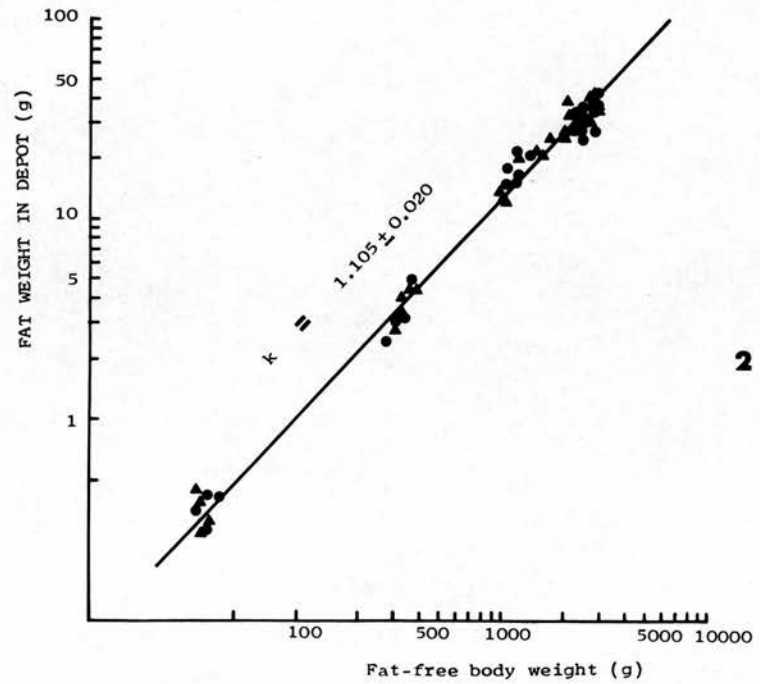
Log Total Fat = 5·78 + 0·032 ± 0·006 (log Clavi. - 2·19) + 0·020 ± 0·005 (log Perit. - 2·61)
+ 0·032 ± 0·011 (log Inguino. - 2·87) + 0·274 ± 0·009 (log S/C. - 4·38)
+ 0·637 ± 0·015 (log Subcut. - 5·26)

Residual Variance

| Variables | Residual d.f. | Regression d.f. | Residual s.s. | Residual s.d. | F(Regression) | T(last variate) | P |
|---|---|---|---|---|---|---|---|
| log Subcut fat. | 48 | 1 | 0·2242 | 0·069 | 13373·14 | 115·64 | < 0·01 |
| log s/Carc. fat | 47 | 2 | 0·0281 | 0·025 | 52412·40 | 18·11 | < 0·01 |
| log Clavic fat. | 46 | 3 | 0·0150 | 0·017 | 63920·48 | 6·32 | < 0·01 |
| log perit. fat | 45 | 4 | 0·0107 | 0·015 | 65778·66 | 4·26 | < 0·01 |
| log Inguino fat | 44 | 5 | 0·0089 | 0·014 | 61815·93 | 2·98 | < 0·05 |
| log Subalar fat | 43 | 6 | 0·0089 | 0·014 | 50342·52 | 0·00 | |

or Log Total Fat = 5·78 + 1·033 ± 0·009 (Log Subcut. - 5·26)

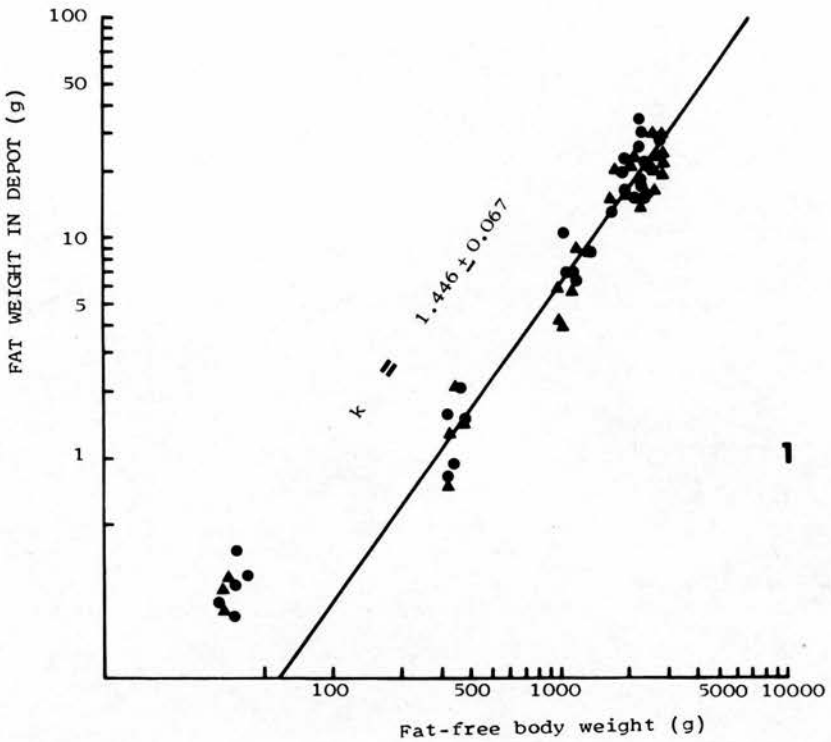


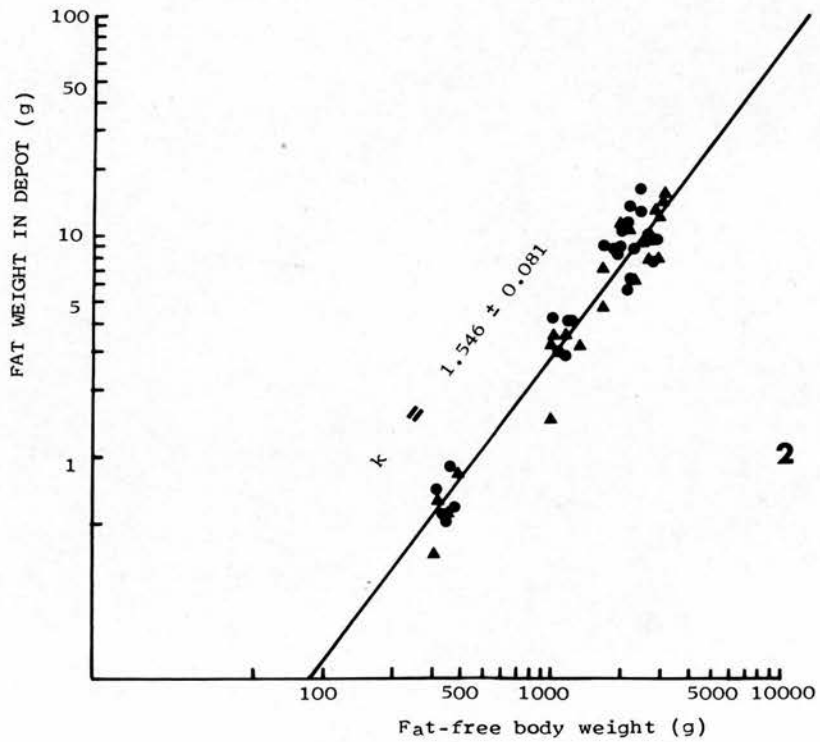




Logarithmic regressions of chemical components on fat-free body weight for the Aylesbury

- ● male
- ▲ female
- **1** Skinned carcase fat
- **2** Inguino-crural fat

Figure 4.9




Logarithmic regressions of chemical components on fat-free body weight for the Aylesbury.

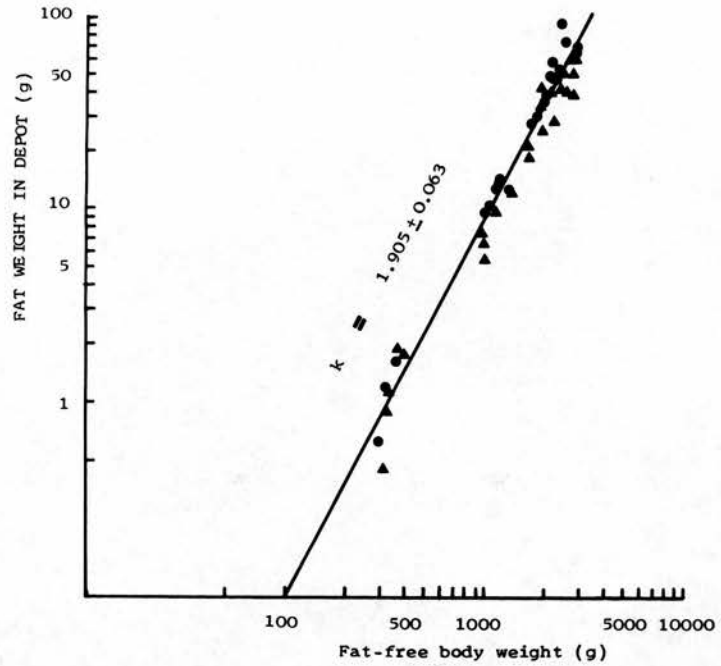
● male

▲ female

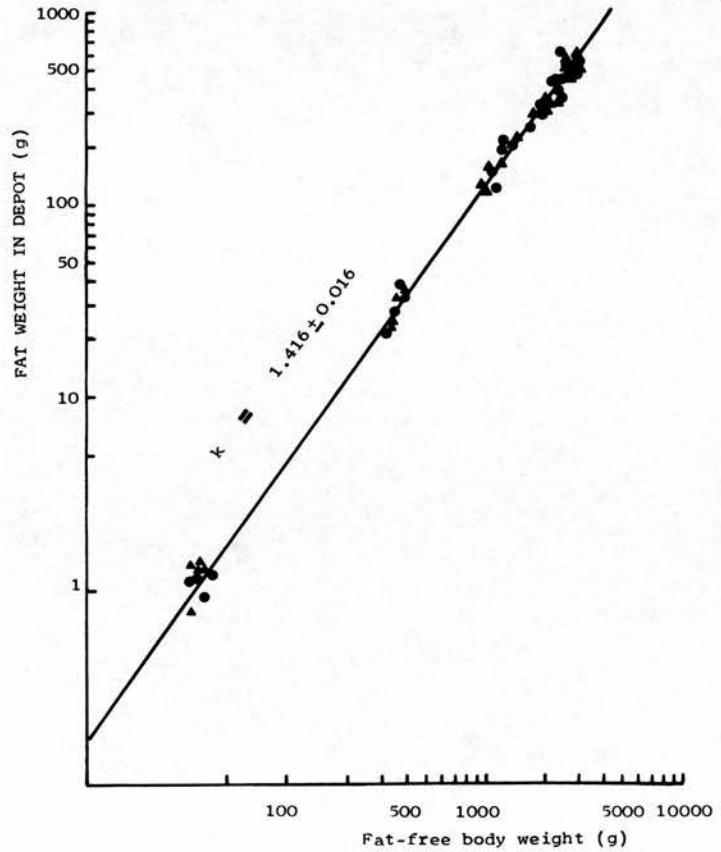
**1** Claviculo etc. fat

**2** Subalar etc. fat

Figure 4.10




1




2

Logarithmic regressions of chemical components on fat-free body weight for the Aylesbury.

- ● male
- ▲ female
- **1** Peritoneal fat
- **2** Subcutaneous fat

Figure 4.8

Using the form of the allometric equation $y = b.x^k$, x represents the fat-free body weight for all these analyses. Where data was available for both sexes it was pooled for the purposes of analysis. Where data from one-day olds showed a departure from linearity in the log-log plots, they were not included in the analysis. The biological reasons for taking this step have already been discussed.

It is apparent from the plotted data that the values for each sex were generally evenly scattered on either side of the calculated regression equation (Fig. 4.8-12.). In the two cases (i.e. clav. and skinned carcase fat) where the Gompertz constants for the decay of the specific growth rate ($\alpha$) from the Aylesbury data, differed from the other calculated $\alpha$s for the other deposits (Tables 4.2,3.) data from the day-olds fell wide of the regression line calculated from the other age groups.

Goodness of fit was indicated by the values for the residual standard deviations (Table 4.11-13). In the Aylesbury, although k values were generally greater than unity, there was considerable variation in the value of this parameter between the deposits (Figs. 4.8-10, Table 4.11.). To analyse this variation fat deposits were ranked in order of ascending values for k, and adjacent pairs were tested for significance (Tables 4.11-13.). On this basis, the sequence of development of the fat deposits occurred in the following order: the inguino-deposit, followed by a group consisting of subcutaneous, claviculo., subalar.,and skinned carcase deposits for which there was little significant variation in

Table 4.11. Growth coefficients (k) of the fat deposits in the Aylesbury, calculated from the equation $\log y = k \log x + \log b$, where y = wt. of fat in deposits; x = wt. of fat-free body. Data for sexes were pooled.

Plotted data is shown in Figs. 4.8-10.

| Deposit | Age (weeks) | No. of Pairs | Growth co-efficient (k) ± S.E. | log b | $r^2$ log x. log y | Residual S.D. |
|---|---|---|---|---|---|---|
| Subcut. | 0-10 | 60 | 1·4164±0·0162 | -2·1443 | 0·9962 | 0·080 |
| Inguino. | 0-10 | 60 | 1·1050±0·0199 | -2·2104 | 0·9907 | 0·101 |
| Subalar. | 2-10 | 48 | 1·5457±0·0812 | -4·2016 | 0·9420 | 0·184 |
| Clavic. | 2-10 | 49 | 1·4461±0·0672 | -3·5579 | 0·9529 | 0·148 |
| Perit. | 2-10 | 48 | 1·9045±0·0626 | -4·7839 | 0·9761 | 0·134 |
| S/Carc. | 2-10 | 50 | 1·6201±0·0411 | -3·1654 | 0·9849 | 0·094 |

Test for significance between growth co-efficient

| Deposit | | d | d.f. | P |
|---|---|---|---|---|
| Inguino. | Subcut. | 12·105 | 116 | <0·001 |
| Subcut. | clavic. | 0·4304 | 105 | >0·10 |
| Clavic. | Subalar | 0·9486 | 93 | >0·10 |
| Subalar. | S/carc. | 0·8176 | 94 | >0·10 |
| S/carc. | perit. | 3·802 | 94 | <0·001 |
| Subalar. | Subcut. | 1·5709 | 104 | >0·10 |
| Subcut. | S/carc. | 4·6363 | 106 | <0·001 |

Table 4.12. Growth coefficients (k) of the fat deposits in the female Mallard, calculated from the equation $\log y = k \log x + \log b$, where y = wt. of fat in deposits; x = wt. of fat-free body.

| Deposit | Age (weeks) | No. of pairs | Growth coefficient (k) ± S.E. | log b | r & log x log y | Residual S.D. |
|---|---|---|---|---|---|---|
| Subcut. | 0-10 | 30 | 1·3535±0·0381 | -2·0389 | 0·9891 | 0·036 |
| Inguino. | 0-10 | 28 | 0·8407±0·0394 | -1·5460 | 0·9726 | 0·116 |
| Subalar | 2-10 | 25 | 1·2111±0·1326 | -3·0235 | 0·8854 | 0·173 |
| Clavic. | 2-10 | 24 | 1·4543±0·3041 | -3·8035 | 0·7140 | 0·371 |
| Perit. | 2-10 | 24 | 1·4221±0·1513 | -3·1425 | 0·8948 | 0·184 |
| S/Carc. | 0-10 | 29 | 1·0490±0·0220 | -1·3370 | 0·9941 | 0·064 |

Test for significance between growth coefficient

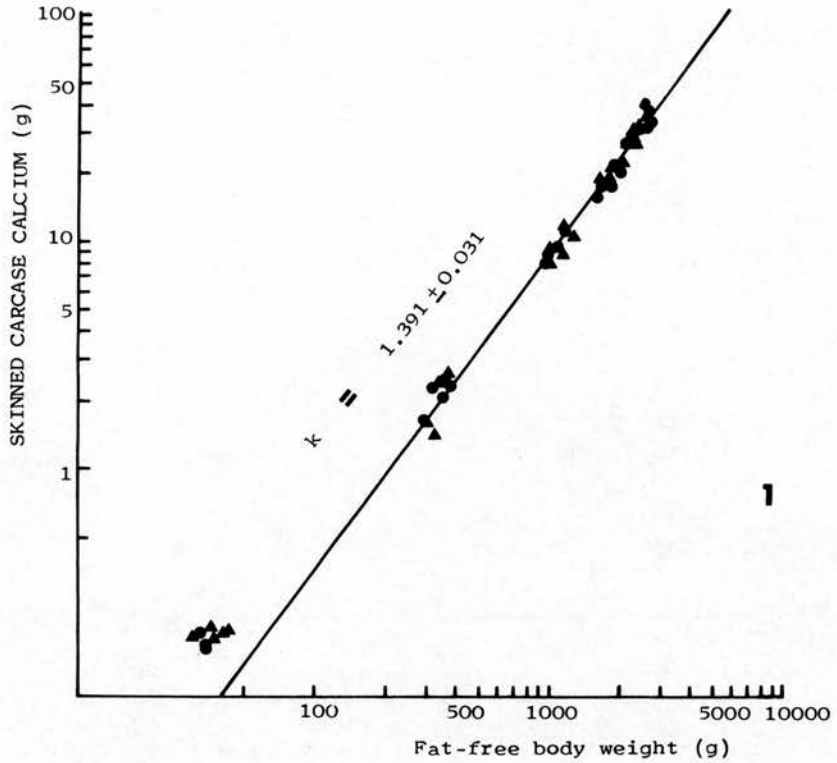
| Deposit | | d. | d.f. | P |
|---|---|---|---|---|
| Inguino. | s/carc. | 4·6289 | 53 | <0·001 |
| S/carc. | Subalar | 1·2007 | 50 | >0·10 |
| Subalar | Subcut | 1·0319 | 51 | >0·10 |
| Subcut. | Perit. | 0·4426 | 50 | >0·10 |
| Perit. | Clavic. | 0·0950 | 44 | >0·10 |
| Clavic. | Subcut. | 0·3294 | 50 | >0·10 |
| Perit. | Subalar. | 1·0498 | 45 | >0·10 |
| Subcut. | S/carc. | 6·9839 | 55 | <0·001 |
| Clavic. | Subalar | 0·7325 | 45 | >0·10 |

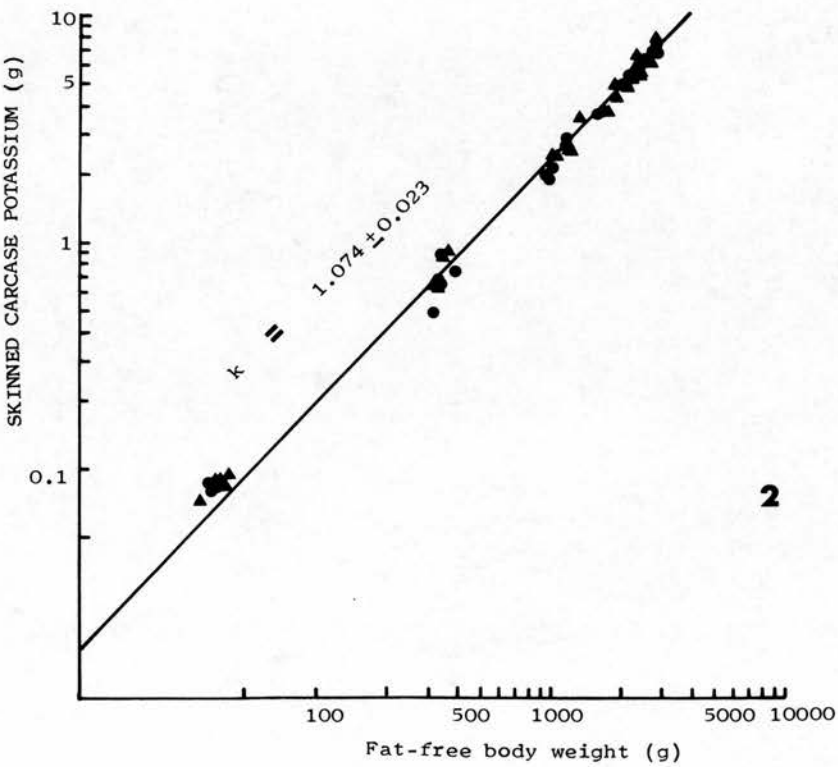
Table 4.13. Growth coefficients (k) of the fat deposits in the male Tufted, calculated from the equation $\log y = k \log x + \log b$, where y = wt. of fat in deposits; x = wt. of fat-free body.

| Deposit | Age (weeks) | No. of pairs | Growth coefficient (k) ± S.E. | log b. | $r_{\log x}^{\log y}$ | Residual S.D. |
|---|---|---|---|---|---|---|
| Subcut. | 0-10(-8) | 24 | 1·0752±0·0429 | -1·3743 | 0·9830 | 0·097 |
| Inguino. | 0-10(-8) | 22 | 0·7801±0·0568 | -1·6204 | 0·9509 | 0·129 |
| Subalar | 2-10(-8) | 18 | 0·8670±0·1429 | -1·8770 | 0·8348 | 0·141 |
| Clavic. | 2-10(-8) | 16 | 0·9046±0·1790 | -2·1680 | 0·8037 | 0·165 |
| Perit. | 2-10(-8) | 18 | 0·9510±0·1968 | -2·2921 | 0·7703 | 0·192 |
| S/Carc. | 0-10 | 24 | 0·8950±0·0425 | -1·1073 | 0·9761 | 0·097 |

Test for significance between growth coefficients

| Deposit | | d | d.f. | P |
|---|---|---|---|---|
| Inguino. | Subalar | 0·5643 | 36 | >0·10 |
| Subalar | S/Carc. | 0·1879 | 38 | >0·10 |
| S/Carc. | Clavic. | 0·0475 | 36 | >0·10 |
| Clavic. | Perit. | 0·1744 | 30 | >0·10 |
| Perit. | Subcut. | 0·6148 | 38 | >0·10 |
| Subcut. | Inguino | 4·1740 | 42 | <0·001 |
| Perit. | Inguino. | 0·8336 | 36 | >0·10 |



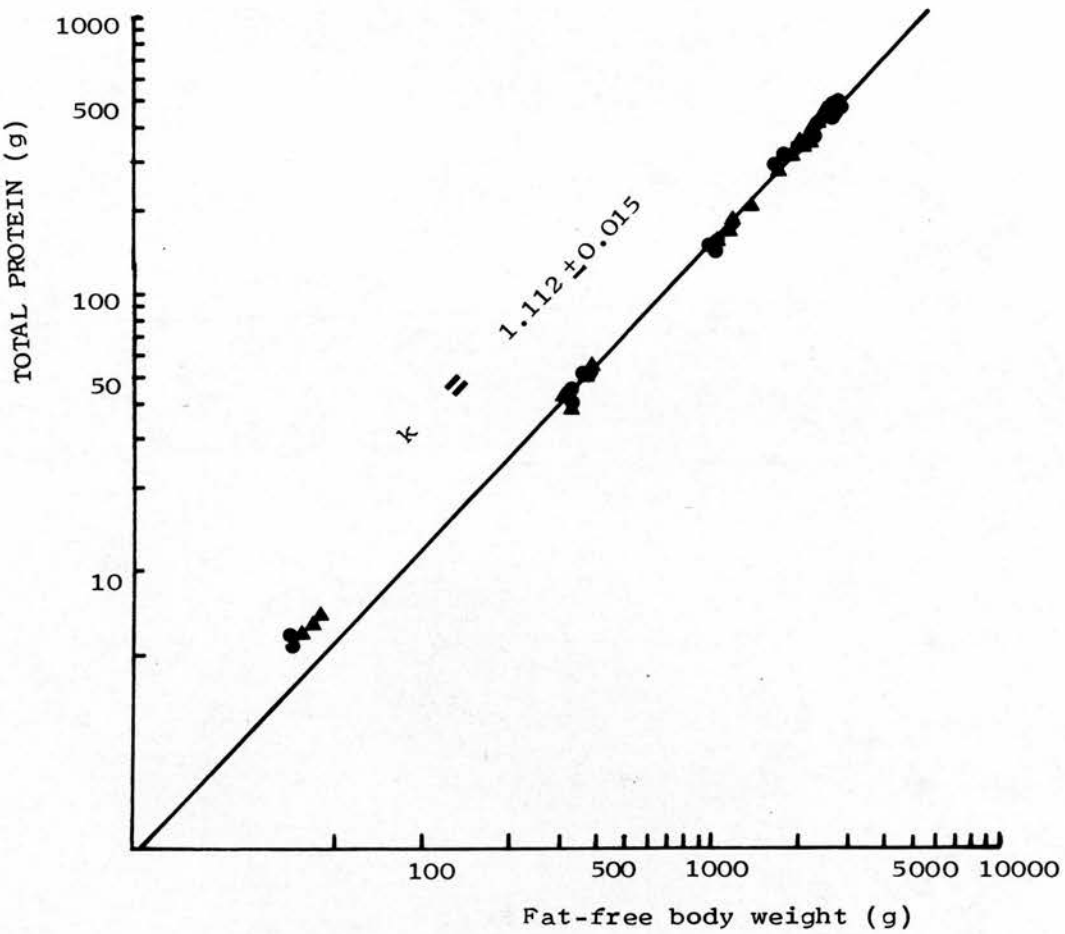




Logarithmic regressions of chemical components on fat-free body weight for the Aylesbury.

● male

▲ female

Figure 4.11




Logarithmic regressions of chemical components on fat-free body weight for the Aylesbury

● male

▲ female

Figure 4.12

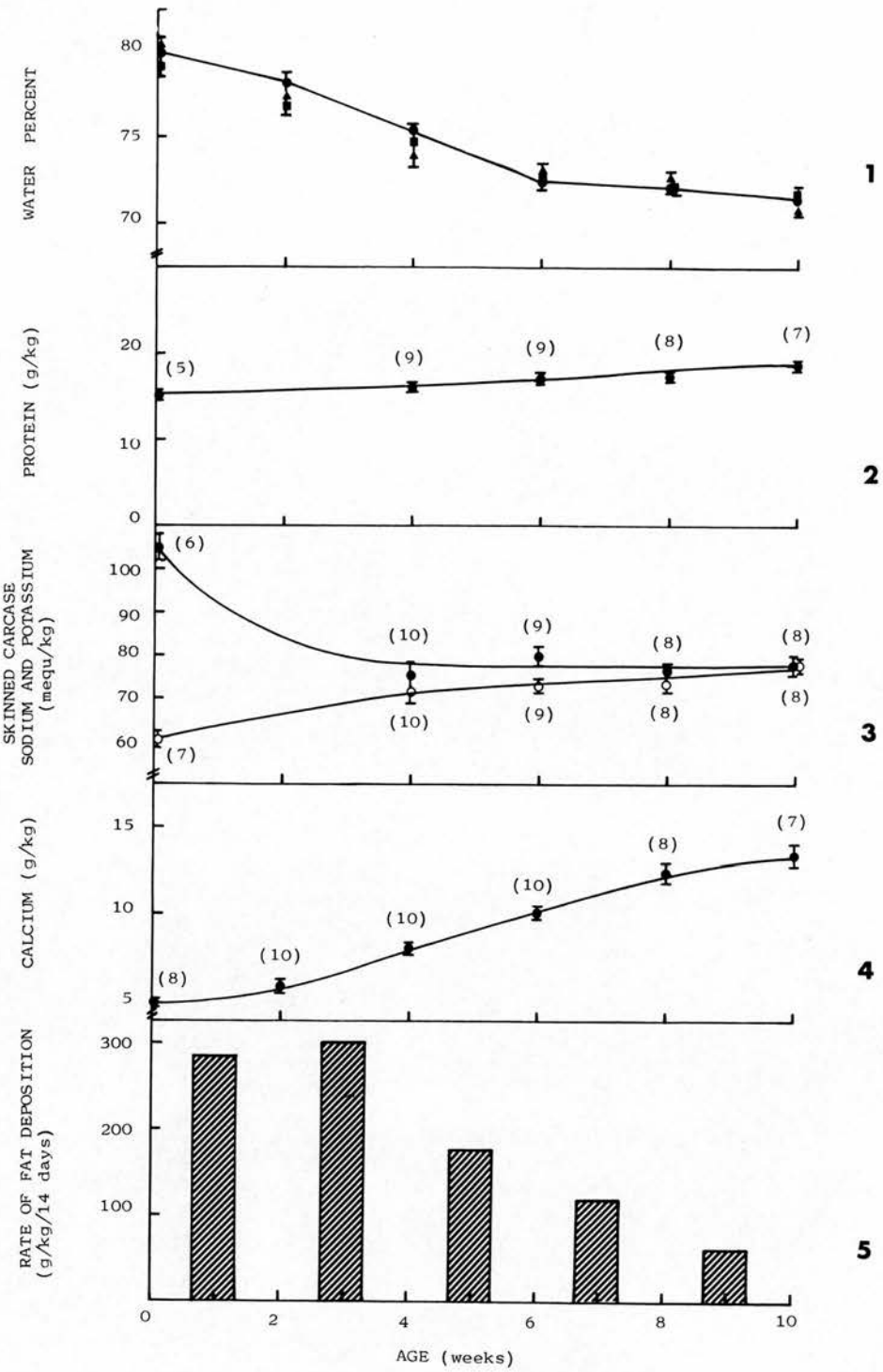
k, and finally the peritoneal deposit. The inguino.,deposit was conspicuously an early growing organ. In the day-old it appeared as the largest single fat depot. It was only slightly later in growth than the fat free body (i.e. k was only slightly greater than unity). In contrast the peritoneal deposit was a distinctly late-maturing deposit. Both the subcutaneous and skinned carcase deposits provided a superior fit to the allometric equation as judged by their residual standard deviations (Table 4.11.).

In other breeds there was less variation in the k value between the deposits, although in all cases the lowest value was obtained for the inguino,deposits (Tables 4.12,13.). It would therefore appear that this deposit retains its early maturing form of growth in all these breeds. An important feature of the results obtained for the Tufted was that the k values were uniformly low being nearly equal to or less than unity, indicating that fat deposition is an early sequence in the overall development of this breed during growth. Generally lower k values were obtained for the Mallard than the Aylesbury. This suggested that in those breeds in which greater amounts of fat are deposited, most of this increased fat deposition occurs later in the developmental sequence of growth.

Log-log regressions were also calculated for other body parts and chemical components of the Aylesbury (Figs. 4.11,12. Tables 4.14, ). Generally the fit of this data to the calculated regressions (as indicated by the residual standard deviations) was much better than that obtained for

Table 4.15. Growth coefficients (k) of various body components in wild ducks, calculated from the equation log y = k log x + log b, where y = wt. of component and x = wt. of fat-free body

| Component | Age (weeks) | No. of Pairs | Growth coefficient (k) ± S.E. | log b | r log x.log y | Residual S.D. |
|---|---|---|---|---|---|---|
| | | | Mallard | | | |
| Total fat | 2-10♂♂♀♀ | 49 | 1·207 ± 0·036 | -1·347 | 0·9800 | 0·072 |
| Total water | o/-10♂♂♀♀ | 54 | 0·9565 ± ·0046 | -0·0188 | 0·9994 | 0·020 |
| FF Skin Wt. | 2-10 ♀♀ | 25 | 1·1180 ± 0·0506 | -1·0410 | 0·9772 | 0·066 |
| Skin Wt. | 2-10 ♀♀ | 25 | 1·1249 ± 0·0518 | -0·8928 | 0·9765 | 0·069 |
| FF S/C wt. | 2-10 ♀♀ | 25 | 0·9760 ± 0·0136 | -0·0331 | 0·9978 | 0·017 |
| S/C Wt. | 2-10 ♀♀ | 25 | 0·9983 ± 0·0139 | -0·0372 | 0·9977 | 0·021 |
| | | | Tufted | | | |
| Total fat | 2-10 ♂♂ | 19 | 1·038 ± 0·065 | -0·981 | 0·9686 | 0·062 |
| Total water | 2-10 ♂♂ | 19 | 0·9551 ± ·0094 | -0·0207 | 0·9992 | 0·024 |
| FF Skin Wt. | 2-10 ♂♂ | 19 | 0·8627 ± ·0698 | -0·3972 | 0·9486 | 0·071 |
| Skin Wt. | 2-10 ♂♂ | 19 | 0·9063 ± 0·0736 | -0·3708 | 0·9482 | 0·077 |
| FF S/C Wt. | 2-10 ♂♂ | 19 | 1·0386 ± 0·0155 | -0·1912 | 0·9981 | 0·016 |
| S/C Wt. | 2-1- ♂♂ | 19 | 1·0455 ± ·0175 | -0·1864 | 0·9976 | 0·035 |




The effect of age on body composition and the specific rate of fat deposition in the aylesbury. All the data have been calculated on a fat-free body weight basis. Values are means ± S.E.M. n shown in brackets. Data pooled from both sex.

1. AYLESBURY (n = 10 for each pair)
   MALLARD (n = 10 for each pair)
   TUFTED (n = 5 for each pair)

3. SODIUM
   POTASSIUM

Figure 4.13

the fat deposits. k values for subcutaneous and skinned carcase fat in the Aylesbury (Table 4.11.) did not appear to be related to the corresponding values for the fat-free skin and skinned carcase components (Table 4.14.). This analysis indicated that although many of the non-fat components have k values close to unity (i.e. water, potassium, and sodium), others were slightly greater than unity (protein) and a few were considerably greater (calcium and feathers). Hence some non-fat components shared with the fat deposits the characteristic of later-maturing growth.

Although it has been demonstrated that fat deposition is generally a late growing or late-maturing phenomena, in the Aylesbury the rate of fat deposition was not related to the state of "maturity" of the body. A commonly held concept of maturity, is that of chemical maturity (Moulton, 1923). This author observed that the chemical composition of the fat free body mass changed in a characteristic way with age. Beyond a certain age there was no further change in composition. This was the point at which the animal had attained chemical maturity.

An examination of certain parameters which are known to change during growth toward a stable level in the fully grown animal, has been made. These are shown in Fig. 4.13, 1-4., along with the calculated relative rate of fat production by the fat free mass (Fig. 4.13,5). By the time the Aylesbury is 10 weeks old the rate of change in chemical composition of the fat-free body is greatly reduced and it is apparent that a state of chemical maturity has almost been reached.

Such changes with age in the parameters considered have all been well established. Similar results to those reported here (Figs. 4.13, 1-4.) have been obtained for the rat, pig, cat and rabbit (Spray and Widdowson, 1950), and for protein and water in the fowl (Osbaldiston, 1967).

It was apparent from the results in Fig. 4.13. that the rate of fat deposition declines as the bird approaches a state of chemical maturity. Therefore although the accretionary deposition of fat is generally a late maturing process, it is not related to the onset of chemical maturity in the duck. Rather it would appear that the factors responsible for the attainment of chemical maturity are also responsible for a partial suppression of lipogenic activity.

4.1.5. The effect of fasting on the fat deposits.

Subcutaneous fat represents the largest single deposit in the duck, (Sec. 4.1.3.). The specific distribution of this fat, mainly on the ventral and caudal surfaces (i.e. below the "plimsole line" when the bird is floating on water) directs attention to its probable function as an insulating layer. It might therefore be expected that when the bird is in negative calorie balance, fat would be mobilised more readily from depots other than the subcutaneous deposit. Such a system of differential mobilisation, which conserved the subcutaneous deposit, would be of considerable adaptive importance to the bird.

Maintenance of body temperature without increasing energy expenditure would thus be ensured during fasting. This hypothesis is examined by observing the effect of fasting on the fat deposits in the duck.

Ten 8 week old female Aylesbury ducks were fasted or kept on a sub-maintenance ration until weight losses of up to 25% had been achieved. Such losses might be expected to occur naturally without causing undue demands on the body's energy reserves. In the Mallard death from fasting does not result until a weight loss of 50% occurs.

Initially ducks were given a ration of 60 g/bird/day. However after 8 days of this regime, there was no change in the plasma NEFA, and surprisingly, a slight increase in weight had occurred (Table 4.16.). This weight gain may have been due to water retention. The normal level of plasma NEFA suggested that little fat mobilisation had occurred. Thereafter food was removed from the birds for three days. Ad libitum water, however, was available. At the end of this period there was a 16·5% loss in body weight and a considerable rise in plasma NEFA (Table 4.16.). Some birds were killed for analysis at this time while the remainder were given a submaintenance ration of 20 g/bird/day. A continuing weight reduction resulted from this regime. Subsequently these birds were killed and analysed when a required weight loss had been achieved.

Eight week old fed females were used as the controls. The fat loss due to fasting was calculated from the difference in weight between fat in depots of the control and fasted birds.

Table 4.16. The effect of the fasting regimes on plasma NEFA and body weight in the female Aylesbury. Values means ± S.E.M.

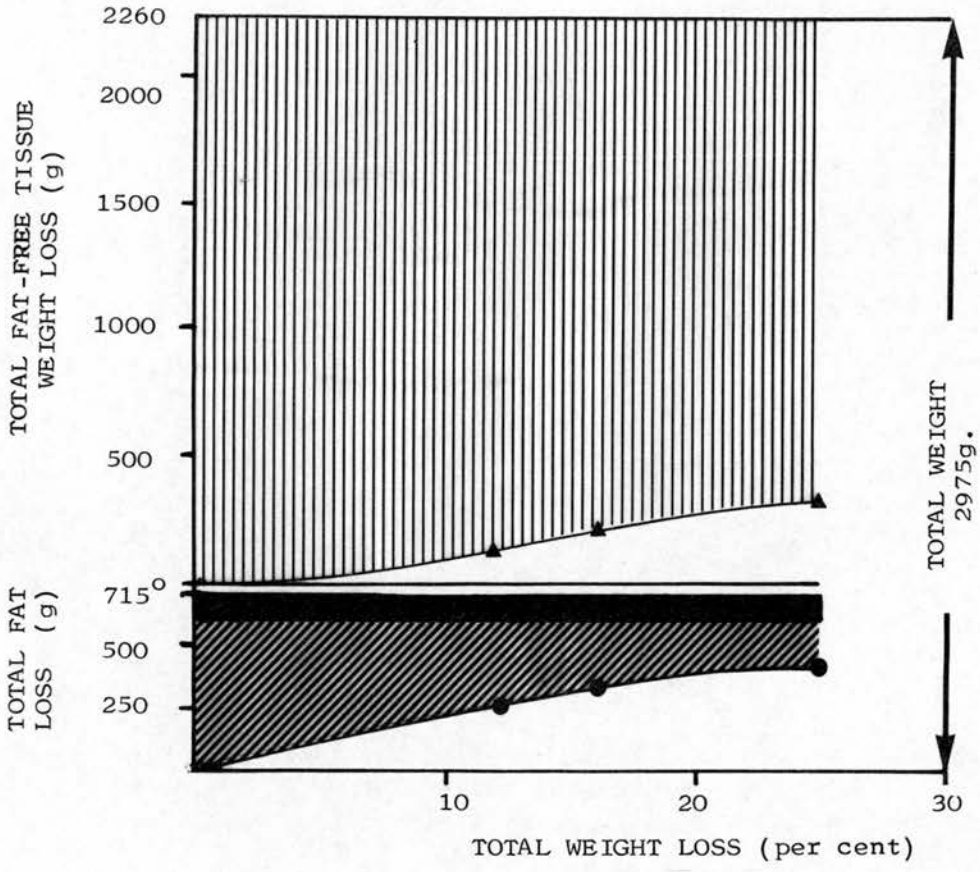
| Treatment | Plasma NEFA μ equ/l | Body Wt. (g) | % Change in body wt. |
|---|---|---|---|
| Initial 5 hour fast | 710 ± 23 (6) | 2945 ± 57 (10) | |
| + | | | |
| 8 days 60g/bird/day | 757 ± 24 (6) | 3019 ± 92 (10) | + 5·9 |
| + | | | |
| 3 days fasting | 1123 ± 46 (6) | 2456 ± 63 (10) | - 16·5 |

Table 4.17. The effect of fasting on the fat deposits in the 8 week old female Aylesbury. "Fat loss %" represents the difference between the weight of fat in the corresponding depots of the fed and fasted birds, as a percentage of the fat weight in the depot of the fed bird. The fat weight in the depot of the fasted bird was first multiplied by a factor which accounted for the difference in mean initial weight between the fasted and fed groups. "Fat Wt." in the tables represents unadjusted weights. Values are means ± S.E.M., n shown in brackets.

| | Fed 8 week old (5) | Fasted Body wt. loss = 12·1 + 0·1% (3) | | Fasted Body wt. loss = 16·2 + 0·5% (4) | | Fasted Body wt. loss = 24·8 + 1·5% (3) | |
|---|---|---|---|---|---|---|---|
| | Fat wt. (g) | Fat wt. (g) | Fat loss % | Fat wt. (g) | Fat loss % | Fat wt. (g) | Fat loss % |
| Initial body wt. (fed 8 week olds) | 2971·4±24·7 | 3053·3 ± 42·9 | | 2862·7 ± 64·5 | | 2946·7 ± 172·4 | |
| Total fat % | 24·0 | 15·1 | | 12·8 | | 10·2 | |
| Total fat | 71·2±25·5 | 462·9±9·4 | 37·1 | 365·0±20·1 | 46·2 | 301·0±56·8 | 57·9 |
| Subcut fat | 416·8±19·3 | 229·7±4·0 | 46·3 | 159·0±12·7 | 60·4 | 115·0±31·0 | 72·2 |
| Inguino fat | 29·6±2·5 | 16·7±0·7 | 45·2 | 11·6±0·1 | 59·5 | 10·7±2·6 | 63·9 |
| Subalar fat | 8·2±2·0 | 4·3±0·9 | 48·8 | 3·5±0·5 | 56·1 | 3·3±0·5 | 58·6 |
| Clavic fat | 19·1±3·8 | 6·8±1·6 | 65·4 | 5·4±1·2 | 70·7 | 4·4±1·9 | 75·9 |
| Peritoneal fat | 41·9±4·1 | 26·7±3·8 | 37·9 | 15·3±1·4 | 62·0 | 15·5±4·8 | 63·0 |
| Skinned carc. fat | 196·4±21·3 | 178·7±8·5 | 11·4 | 167·8±9·0 | 12·2 | 152·3±18·8 | 22·5 |

Table 4.18. The effect of fasting on the distribution of fat in the female Aylesbury. Values are means (± S.E.M.) of percentage of the total fat in each deposit.

| - | Fed | Fasted Body wt. loss 12·1% | Fasted Body wt. loss 16·2% | Fasted Body wt. loss 24·8% |
|---|---|---|---|---|
| Subcutaneous fat | 58·5 ± 2·0 | 49·6 ± 1·7 | 43·2 ± 1·8 | 36·7 ± 4·0 |
| "Pooled" depots fat | 14·0 ± 0·8 | 11·8 ± 1·1 | 10·2 ± 0·9 | 10·9 ± 2·0 |
| Skinned Carcase fat | 27·5 ± 2·1 | 38·6 ± 1·5 | 46·6 ± 1·1 | 52·4 ± 5·2 |




The gross composition of changes in the fat and fat free body components of the female Aylesbury duck during weight loss.

▲ Total fat-free tissue loss. Shaded area represents residual fat-free tissue.

● Total fat loss. Shaded plus black area represents residual fat. Black area represents "bound" fat not available for energy production.

Figure 4.14

For the purposes of analysis,birds which had lost similar amounts of weight were grouped together. The results in Table 4.17. indicate that fat is mobilised readily from all the major fat depots. There was certainly no evidence to indicate that the subcutaneous deposit was in any way spared even when the body weight loss was as small as 12%.

Relatively little fat was mobilised from the skinned carcase component (Table 4.17.). Approximately 4% of the skinned carcase fat (this figure was obtained from the fat content of various organs and tissues in birds obtained from Spector, 1956), is contained within cells other than adipose tissue. Little of this is available as an energy reserve so it has been termed "bound" fat (Fig. 4.14.). If this is subtracted from the values given for the skinned carcase fat (Table 4.17.), then the loss of fat in this body component becomes 22·8%, 24·4% and 45·0% respectively for the three fasted groups (in order of increasing weight loss). It is still therefore apparent that this fat reserve, thought to be mainly intermuscular fat, is mobilised at a slower rate than the other deposits. There is no explanation for this difference.

Fasting also influenced the distribution of fat (Table 4.18.). This result seems to have been due to two factors. Firstly, a proportion of the skinned carcase fat is in the form of bound fat, which will not be reduced to any extent by the level of fasting imposed on the birds in this experiment. Secondly as the above discussion has

already indicated the reserve fat in the skinned carcase appears to be mobilised less readily than the other deposits.

With increasing weight losses, the fat free body component begins to become increasingly depleted (Fig. 4.14.). However a 25% reduction in weight only results in a loss of 15·5% of the fat-free body compared to the loss of 57·9% for fat.

It is well established that during fasting the adipose tissue mass is reduced more rapidly than the other tissues (Kleiber, 1961; Grande, 1964). The extent of the fat loss caused by acute fasting is of such magnitude that mobilisation from all the main fat depots can be inferred. Reed et al. (1930) have shown that fat is mobilised equally from all the main depots (subcutaneous, genital, perirenal, mesenteric, intermuscular and omental) in the rat under conditions of inactive fasting or voluntary exercise. Fat is also readily mobilised from the subcutaneous deposits both in the pigeon during fasting (Chossat, 1843*) and in man during dietary insufficiency (Jansen, 1963).

Some observations in mammals have implied that certain deposits may be relatively resistant to fat mobilisation. In man neither the cheek fat pad of most races or the buttock fat of steatopygic females of certain African races are reduced during weight loss (Vague and Fenasse, 1965). During forced exercise in the rat intermuscular fat appears to be spared at the expense of the other fat depots (Reed et al., 1930). Thus although it is probably generally true that most fat deposits will mobilise their energy on demand, others may be less responsive.

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* Quoted by Grande (1964).

General Discussion.

The most characteristic feature of the fat distribution in the ducks examined in this study, was the relatively high proportion of fat which occurred in the subcutaneous depots. In the Anatidae generally subcutaneous fat is well developed presumably as an adaptation to the semi-aquatic habitat of most of these species. However it is in the wholly aquatic birds and mammals such as the Spheniscids, Pinnepedes and Cetaceans that the unusual distribution of the adipose tissue indicates a complete adaptation to an aquatic environment. Frequently in such species the dissectable fat is located entirely in the subcutaneous depots (Bryden, 1968; and own personal observations). It has been shown in the duck that fat depots other than the subcutaneous depots, are also well developed.

Unfortunately there are few published accounts of the distribution of fat in terrestial homoiotherms. From the limited results available it appears that the proportion of fat which is deposited in the subcutaneous depots in the mammal is quite variable ranging from 12% in the guinea pig (Pitts, 1956) and sheep (Palsson, 1940) to over 50% in the young adult rat (Reed et al., 1930). A result given for a young male fowl was 44% (Wilson, 1954). The paucity of published data on this subject limits useful discussion.

In view of the role of the subcutaneous depots in thermal insulation in the duck it was surprising to find that during conditions of fasting, fat was mobilised as readily from this deposit as from the others. A more extreme

case of subcutaneous fat mobilisation is seen in some subantarctic seals, where for quite long periods it is necessary for them to remain on land (and consequently without food) while rearing young and engaged in reproductive activities. Under such conditions the subcutaneous fat depots frequently become so severely reduced, that they return to the very low temperatures in the sea at some peril to their survival (Bryden, 1968). Such observations indicate that the insulatory role of adipose tissue is subservient to its primary role as an energy store.

It has been established in the young mouse (Liebelt, 1963; Liebelt et al., 1965; Hull, 1960), the rat (Zucker and Zucker, 1963), and the fowl (Delpech and Ricard, 1965) that during growth the size of individual depots are allometrically related to the total amount of body fat or the body weight. This relationship was frequently different for different depots (Zucker and Zucker, 1963; Liebelt, 1963; and Liebelt et al., 1965), indicating, as has been observed in the Aylesbury, that fat depots can have different growth coefficients. In this respect, it was interesting to discover that the abdominal depot (equivalent to the peritoneal depot in the duck) was also found to be a late-growing organ in the fowl (Delpech and Ricard, 1965). In mice, both the sex and breed of the individual markedly influence the growth coefficients of some fat deposits (Liebelt, 1963).

Liebelt et al. (1965) have previously drawn attention to the individuality of the growth form of specific fat depots. Such observations imply that there are local factors

within the adipose tissue of the depot which are involved in the regulation of fat deposition, or the growth of the adipose tissue. Some evidence for this has been obtained by transplant experiments involving adipose tissue. Isografts of adipose tissue have been found generally to retain their normal form and size (reported in Barrnett, 1962). However in homografts where subcutaneous adipose tissue has been transplanted onto an identical site in the recipient the form and size of the tissue was determined by the condition of the recipient (Hausberger, 1959), although in another homograft experiment adipose tissue from genetically obese and lean mice, retained its original activity after transplantation to the ear of the recipient (Liebelt, 1963). These experiments indicate both the presence of general and tissue specific factors which influence fat deposition. Vague and coworkers (Vague, 1968) have established that the distribution of fat in man is largely determined by the sex hormones. Therefore in some cases distribution of fat between the depots can result from the response of certain "target" depots to the effect of hormones. In the duck there was no evidence for sexual differences in fat deposition or distribution. However as the size of each age group was relatively small, not much weight can be given to this conclusion without further work.

In the mouse (Fenton and Dowling, 1953; Larsson, 1967; Liebelt, 1963), the rat (Zucker and Zucker, 1963) and the pig (Elsley et al., 1964) total fat is allometrically related to total body weight or fat-free body weight.

However departures from this relationship are frequently observed in the very young (Zucker and Zucker, 1963) and older animals (Liebelt, 1963). In young mammals the fat content of the body increases considerably during suckling (Spray and Widdowson, 1950) so that dietary induced fat deposition undoubtedly overshadows endogenous lipogenic activity at this time. Similarly a high rate of fat deposition is common in the young of nidicolous birds, particularly amongst seabirds where the parent provides large but infrequent meals. In such cases it is common for the young to be considerably heavier than the parents as a result of their high fat content (unpublished data). Anatidae however are solely nidifugous. With the exception of the data for the day-olds, (which has already been considered) the fat in ducks was allometrically related to the fat free body weight.

In mice dietary induced obesity resulted in a departure from the allometric relationship between total fat and total body weight obtained for the normals, although the extent of the departure was dependent upon the strain of mouse used (Fenton and Dowling, 1953). Deviations from the allometric equation have also been observed in the fat deposition in rats and mice with either hereditary or insulin induced obesity (Liebelt, 1963; Zucker and Zucker, 1963).

Selection experiments for increased growth rate or final size using mice or rats has invariably led to an increased fat content (Biondini et al., 1968; Zucker and Zucker, 1963; Fowler, 1958). In some cases the increase

in growth rates was accompanied by an increased growth coefficient for fat (Fowler, 1958) whereas in other cases this has remained unchanged (Zucker and Zucker, 1963). Although the results obtained for the domestic duck indicated that the weight increase produced by selection (involved in the process of domestication) was accompanied by an increase in the growth coefficient for fat, the results obtained from the above experiments infer that selection for increased final size does not invariably result in the selection for increased lipogenesis.

The sequence for the development of tissues and organs has been adequately described for some domestic animals (Pálsson, 1955). From such studies it has become apparent that fat is characteristically a late maturing component of the body. The growth coefficient for fat is generally greater than that for the other body components and it is estimated with less precision since in these animals fat was found to be a very variable component in the body (Elsley et al., 1964). In both the duck (reported here) and the seal (Bryden, 1968) fat deposition was more closely related to the fat free body weight. It is perhaps reasonable to speculate that in the case of aquatic or semi-aquatic animals where the size of the fat depots is more directly related to survival, selection for this character will no doubt have reduced genetic variability within a population. Both the size and the distribution of the depots will be determined by selection and perhaps develop-

ment of the depots may become more "cannalised". In this regard, and particularly with reference to the foregoing discussion, in such species, the prime factors most likely to be responsible for controlling fat deposition during growth, would be of the same type as those responsible for controlling organ size generally. If such were the case, then dietary influences would be less important in determining the pattern of fat deposition during growth, whereas metabolic and tissue differentiation processes associated with growth would be wholly important.

## 4.2. The structure and composition of adipose tissue.

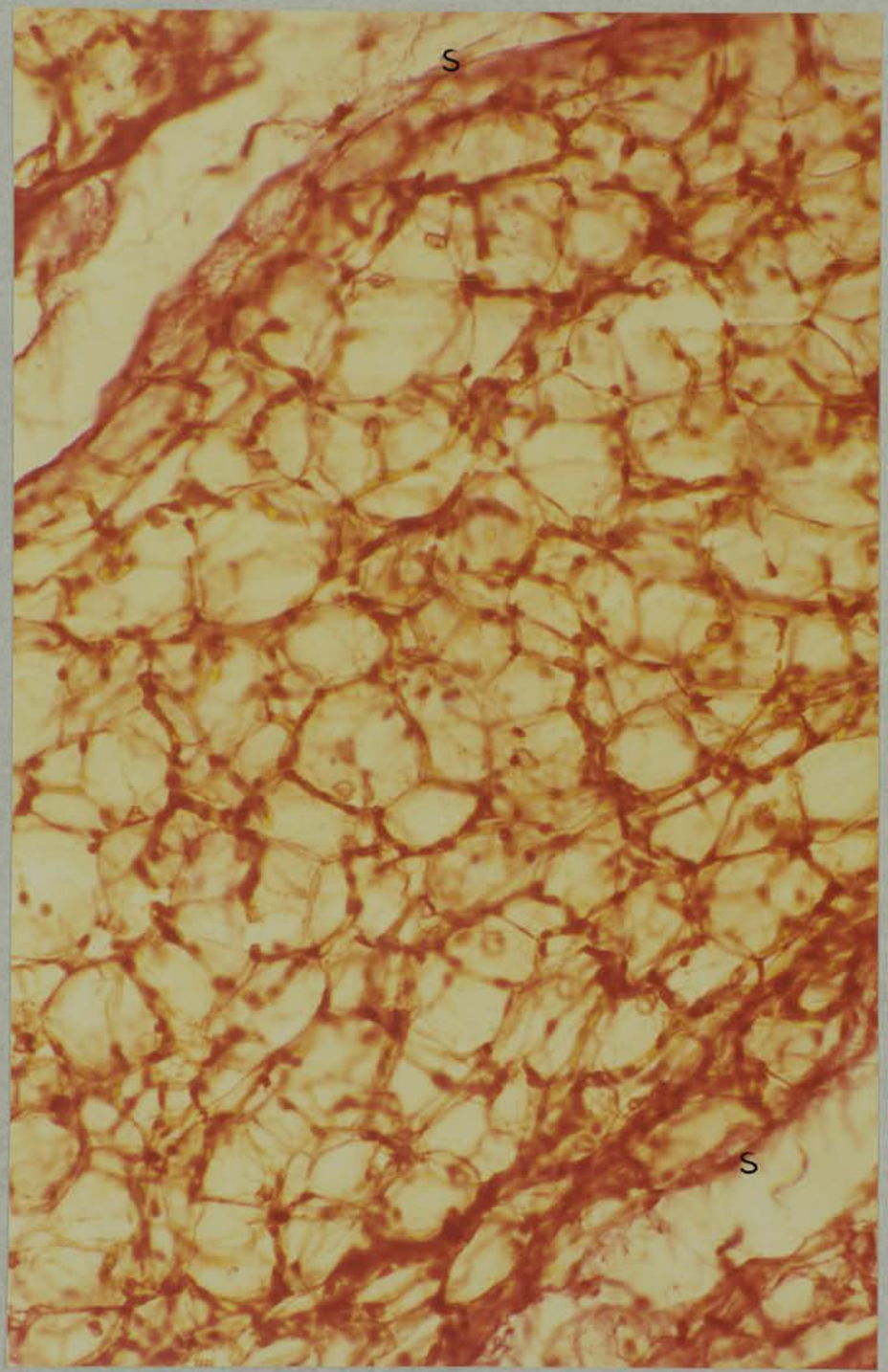
In Section 4.1. it was shown that the fat depots undergo a considerable increase in size during the period of postembryonic growth. A study of the anatomy of adipose tissue was therefore undertaken in order to determine how these changes came about.

### 4.2.1. The structure of adipose tissue.

Wasserman (1965) and Simon (1965) have comprehensively described the differentiation and development of the adipose tissue cell (adipocyte) in the mammal. The brief account of their work given below should however be equally applicable to birds since it is unlikely that the developmental pathways for white adipose tissue differs between these vertebrate classes.

Adipose tissue is thought to arise from cells of reticulo-endothelial origin. In the embryo adipogenesis is initiated by the penetration of a capillary bud into a mesenchymal lobule. Accompanying the axial and branching growth of the capillaries is a multiplication of adventitious perivascular mesenchymal cells. These cells which are very similar in appearance to the endothelial cells of the capillaries were judged to be primitive reticular cells, both on account of their reticular appearance and their ability to store vital dyes. It is from these perivascular cells that the adipocytes finally develop.

The early development of the mesenchymal lobules and




Adipose tissue from the 4 day old Aylesbury (inguino-crural depot).
Stain, Van Gieson's and aurantia.
Magnification x 800.
S = connective tissue septa.

Figure 4.15.

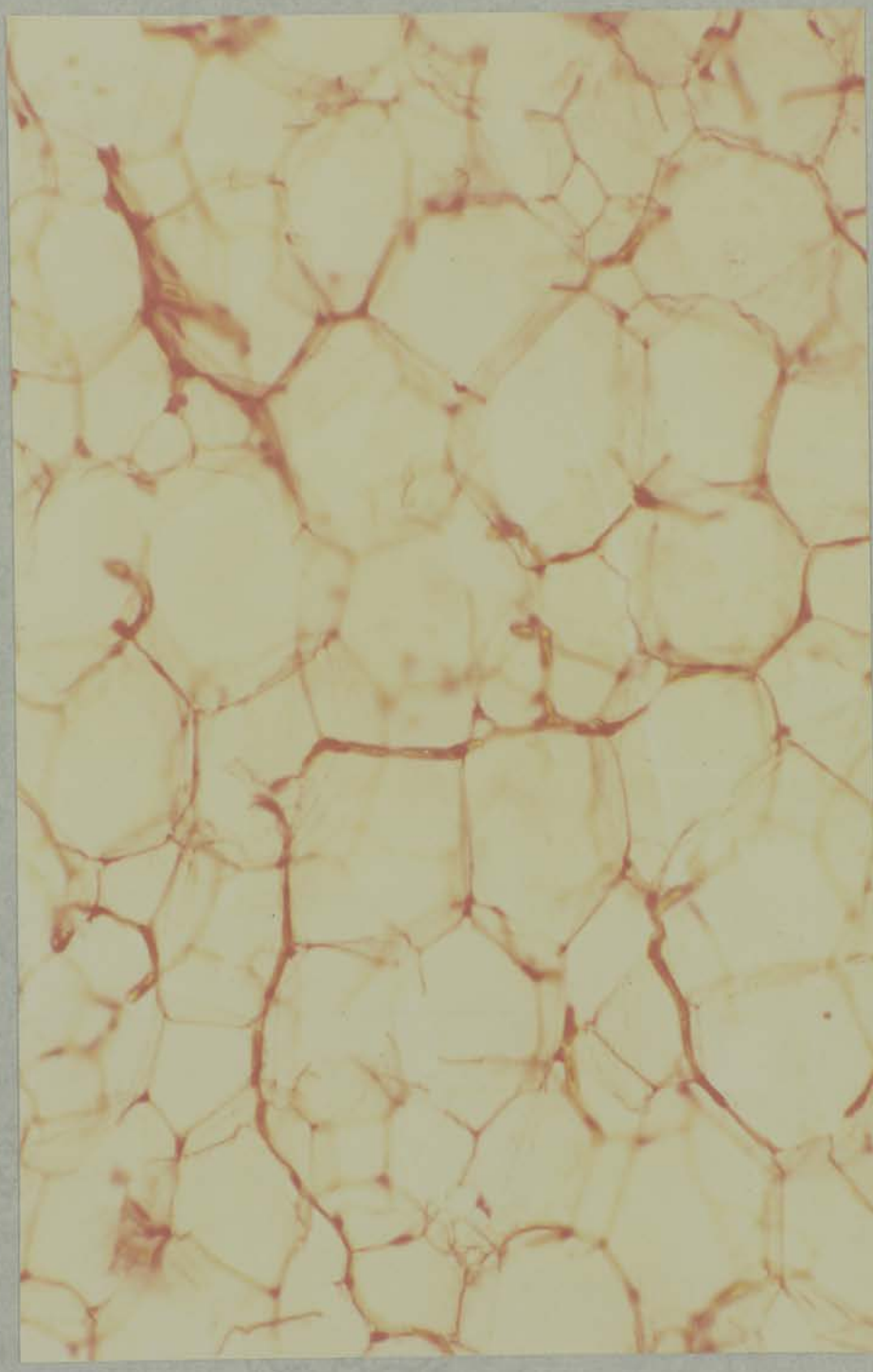
associated tissue gives rise to the formation of small ovoid bodies termed "primitive organs" of the adipose tissue, which have been observed in man, the rodent, the chicken and the reptile.

The differentiation of the perivascular cells ("adipogenic reticular cell") proceeds through the following stages; adipoblast to preadipocyte to adipocyte. The main feature of this developmental sequence is that the cells become progressively laden with more and more fat. Thus in the adipoblast, lipid occurs as relatively small inclusions, whereas in the preadipocyte fat droplets fuse to form larger droplets about a central nucleus giving the cell a "moruloid" appearance. Finally the increasing amount of fat within the cell results in the droplets coalescing to form generally a single large droplet in the centre of the cell causing the nucleus and cytoplasm to be displaced to the periphery. This produces the typical "Signet ring" appearance of the adipocyte.

Good fixation of adipose tissue for the electron microscope has so far proved to be a difficult problem. The results obtained so far are only sufficient to indicate the general aspects of the histology and ultra-structure of the tissue. Birds of ages ranging from 4 days to 10 weeks were used.

The Structure of Avian adipose tissue.

Adipose tissue in the duck is similar in general appearance to this tissue from other species. Adipocytes



Adipose tissue from the 10 week old Aylesbury (inguino-crural depot). Stain, Van Giesons and aurantia, magnification x 800.

Figure 4.16.

in the postembryonic bird range in size from approximately 40-120 μ (see section 4.2.2.), and are generally spherical in shape, although the effect of mututal deformation may cause them to appear polygonal (Fig. 4.15,16). Typically adipocytes in the fat depots occur within lobules which are bounded by connective tissue septa (Fig. 4.15). Light and electron microscopy indicated that within the lobules only three cell types occur; the adipocytes, the endothelial cells of the capillaries and perivascular cells. Mast cells and nervous tissue which occur infrequently in normal white adipose tissue in the rat (Hellman et al. 1963; Boecke, 1933) were not observed in the avian adipose tissue examined. Mast cells increase in number during obesity in the rat (Hellman et al. 1963) and man (Stuchlikova and Selinkova, 1969). Their heparin content has suggested that they may play an important role in influencing adipocyte lipoprotein lipase activity.

Innervation is believed to influence fat mobilisation, mainly through an effect on blood flow (Cantu and Goodman, 1967).

A well developed capillary network is a conspicuous feature of stained light microscope preparations of the adipose tissue from young birds (Fig. 4.15). However as a consequence of the growth of the adipocytes, the capillary bed becomes more diffuse in the older birds (Fig. 4.16). Since no attempt was made to quantify the degree of vascularity in the adipose tissue, it is not known to what extent this represents a diminution of the vascular supply. Examination of light and electron microscope sections indicate that most adipocytes in the tissue from older birds

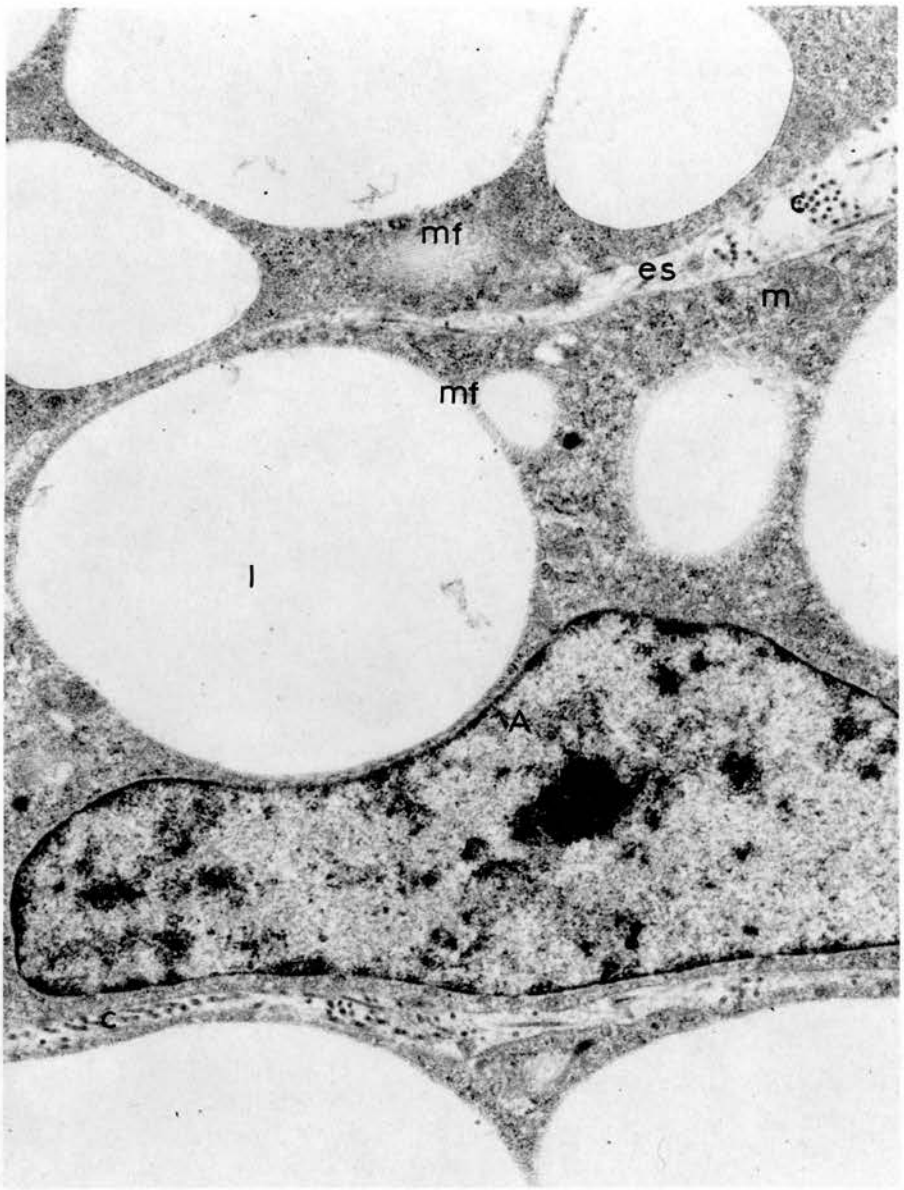



Figure 4.17.2

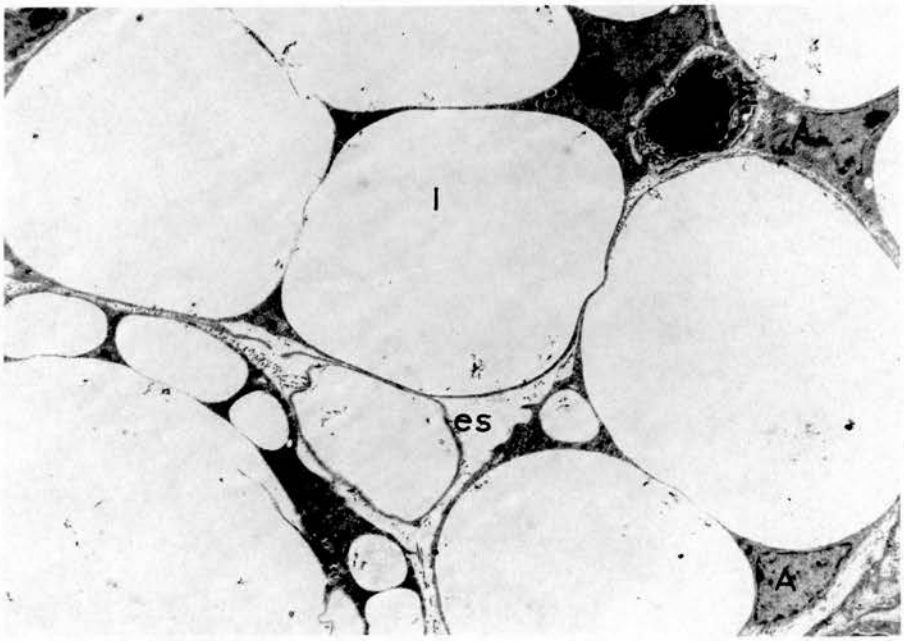



Figure 4.17.1

Figure 4.17.1

Duck white adipose tissue, 5 week old (inguino-crural depot).
Fixation method 1.
Magnification 3, 193.

Figure 4.17.2

Duck white adipose tissue, 4 week old (inguino-crural depot).
Fixation method 1.
Magnification 19, 380.

Key:
A - adipocyte; E = endothelial cell; RBC = red blood cell; l = lipid droplet; m = mitochondrion; mf = microfibrils; es = extracellular space; c = collagen.

remain in contact with at least one capillary.

Electron microscope studies show that in the post-embryonic duck, the fat within the adipocyte is contained within a single or several large droplets, causing both the nucleus and the cytoplasm to become displaced to the periphery (Fig. 4.17.1.). Growth of the lipid droplets may even result in the deformation of the nucleus (Fig. 4.17.2.). The shape of the adipocyte nucleus is typically quite regular which distinguishes it from the more indented outline of the nuclei from endothelial cells (Fig. 4.17, 23,25). Distribution of chromatin within the nucleus appears normal.

The cytoplasmic rim bounding the adipocyte typically varies in thickness between 0·5 to 0·1 μ although it is not infrequently less than 0·1 μ, and about the nucleus it may be slightly greater than 0·5 μ. In the young post natal rat the thickness of the cytoplasmic rim varies between 2 - 0·2 μ. The general absence of organelles in the cytoplasm of the adipocyte from duck adipose tissues (Figs. 4.17, 4.20, 4.23.) may result from poor fixation. Similar techniques applied to rat white adipose tissue however provided a wealth of detail, and indicated the presence of many organelles including Golgi bodies, ~~rough and~~ smooth, and rough endoplasmic reticulum, free ribosomes and various vesicles (Figs. 4.18, 4.21.). Although these results suggest that the rat adipocytes appear to be much richer in cell organelles than the corresponding cells in the duck, until better fixation methods are established for the latter, little reliance can be given to such conclusions.

Mitochondria were relatively more common in tissue from

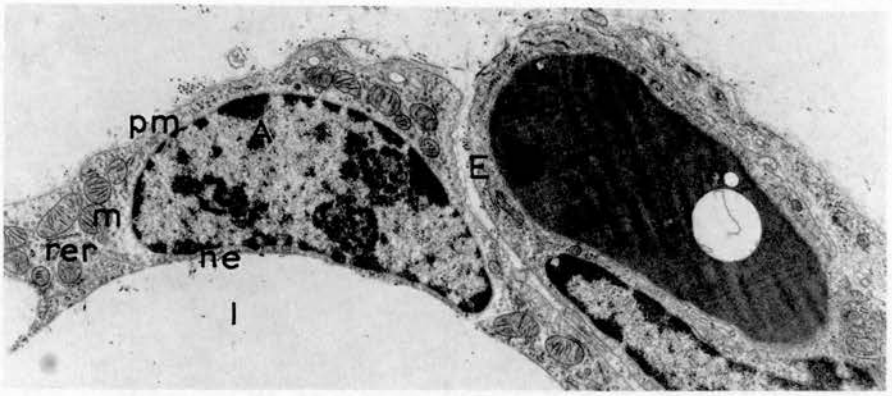



Figure 4.18.1

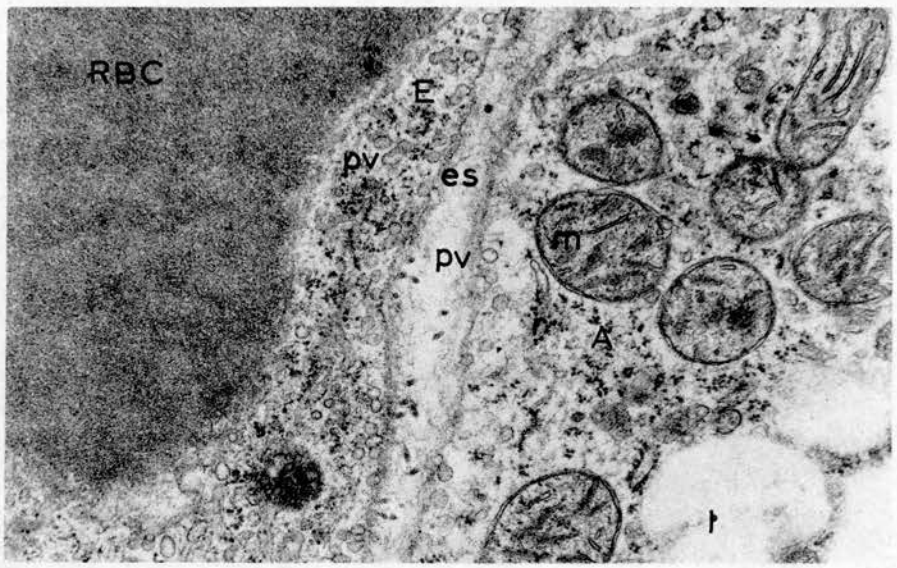



Figure 4.18.2

Figure 4.18.1

Rat white adipose tissue, 4 day old (epididymal depot).
Fixation method 1.
Magnification x 7,000.

Figure 4.18.2

Rat white adipose tissue, 4 day old (epididymal depot).
Fixation method 1.
Magnification x 28,400.

Key: A = adipocyte; RBC = red blood cell; E = endothelial cell; ne = nuclear envelope; l = lipid droplet; pv = pinocytotic vesicles; m = mitochondrion; rer = rough endoplasmic reticulum; ser = smooth endoplasmic reticulum; r = ribosomes; pm = plasma membrane; es = extracellular space.

the young bird, although they were always comparatively rare when compared to their incidence in rat adipocytes. They were normally round or oval in shape becoming cylindrical in older birds, with the dimensions varying between 0·4 and 0·8 μ (Fig. 4.19.1). Cristae were incomplete, generally transverse and plate-like. As in white adipose tissue from the rat (Figs. 4.18, 4.21.1) the mitochondria were fairly evenly distributed through the cytoplasm, and not associated in any way with the lipid droplets (Fig. 4.23.2) as observed in some tissues (Fawcett, 1966). Filamentous mitochondria frequently observed in older rat adipocytes (Napolitans, 1965) were not observed in the avian cells.

A striking feature of the duck adipocytes is the regular array of microfilbrils normally associated with the lipid vacuoles (Figs. 4.17.2, 4.19.2). These fine fibrils of diameter 30 - 60 A, are typically arranged radially around the lipid vacuole so that one end appears to extend into the vacuole and the other back into the cytoplasm, often forming part of an extensive network (Figs. 4.20.1, 4.23.1, 4.24.1). This suggests that there might be lipophilic and lipophobic ends of the microfibrils. In some instances the microfilaments may appear in the cytoplasm as dense bundles (Figs. 4.20.2). There was never any indication that the lipid droplets were membrane bounded. These results were rather in contrast with observation made on the nature of the boundary of the lipid droplets in rat adipose tissue. Neither in this study (Fig. 4.21) or in previously reported

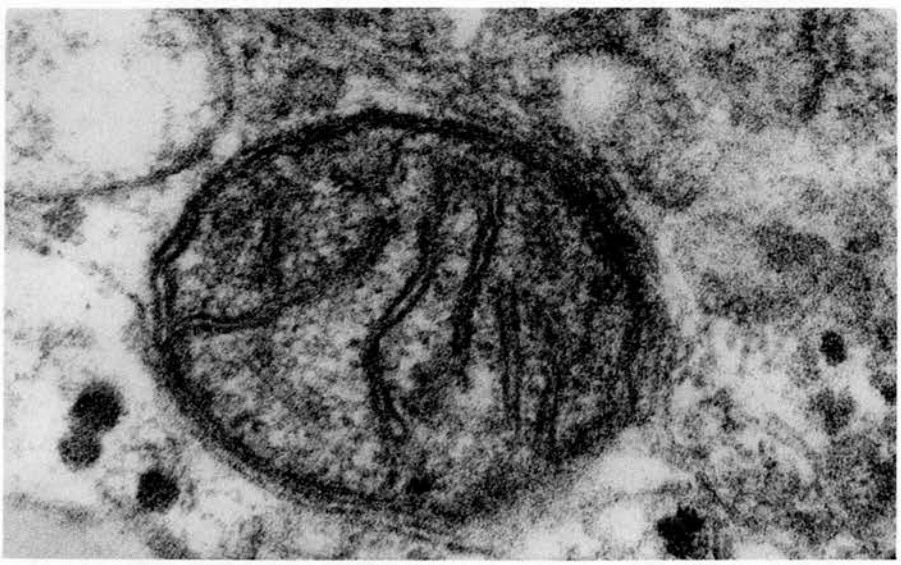


Figure 4.19.1

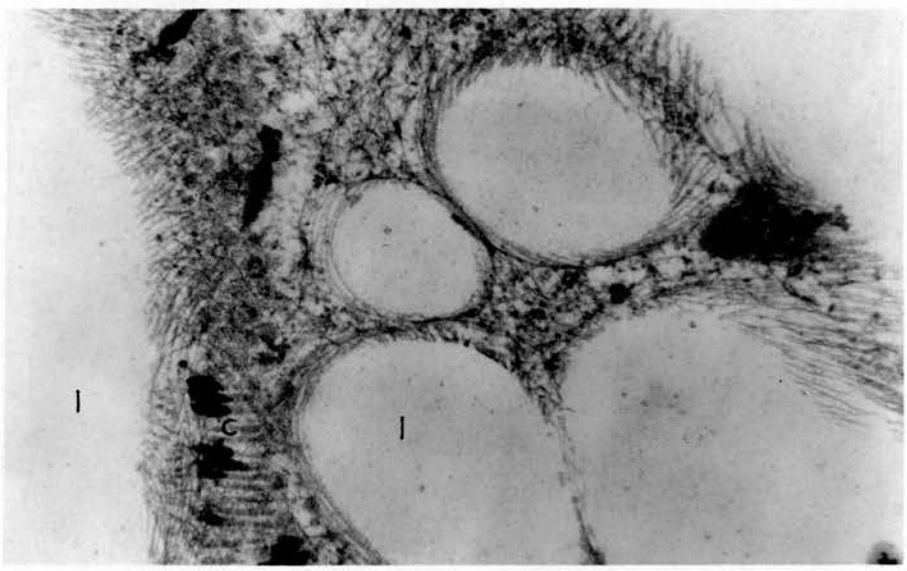



Figure 4.19.2

Figure 4.19.1

Duck white adipose tissue,
5 day old (inguino-crural depot)
showing a typical mitochondrion.
Fixation method 1.
Magnification 106,600.

Figure 4.19.2

Duck white adipose tissue,
10 week old (peritoneal depot),
showing microfilaments.
Fixation method 1.
Magnification 35,230.

Key: l = lipid droplet,
c = collagen.

work (Barnett, 1962; Napolitano, 1965; Sheldon, 1965) for this species, have the presence of microfilaments been observed in association with the lipid droplets, either in brown or white adipose tissue. However a similar arrangement of microfilament about lipid droplets as was described for the duck was observed in the adipocytes of white adipose tissue from the fowl and man. In the white adipose tissue of the rat, particularly associated with the smaller lipid droplets, rough endoplasmic reticulum changing into smooth endoplasmic reticulum appears concentrically arranged about the droplets (Fig. 4.21.1, 4.22.1). A strikingly similar organisation was observed in association with lipid droplets in the main fat storage organ (the liver) in a marine fish (Fig. 4.22.2).

Endothelial cells of the capillaries, represent the other major tissue element in the duck adipose tissue (Fig. 4.23). The capillary lumen is typically formed from two endothelial cells joined end to end. Desmosomes are common at these boundaries of the cells (Fig. 4.24.1, 4.25). Mitochondria, free ribosomes and granular endoplasmic reticulum are notable features of the cytoplasm (Fig. 4.24.1. 4.25). Pronounced marginal folding of the luminal surfaces is common (Fig. 4.23, 4.25). Pinocytotic vesicles are common on both the luminal and basal surfaces of the endothelial cells both in the duck (Figs. 4.24.1, 4.25) and in the rat (4.18.2). Large vacuoles within the cytoplasm of the endothelial cells are particularly common in the tissue from younger birds

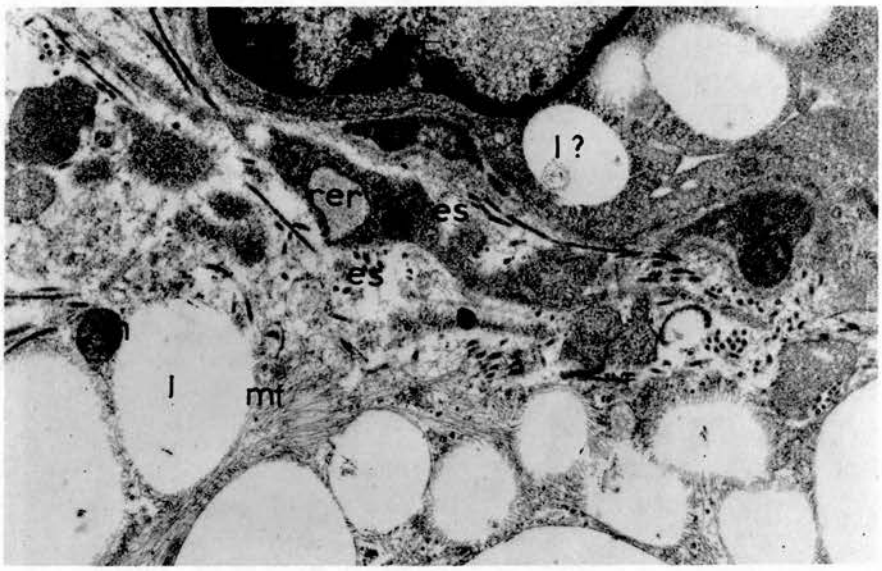



Figure 4.20.1

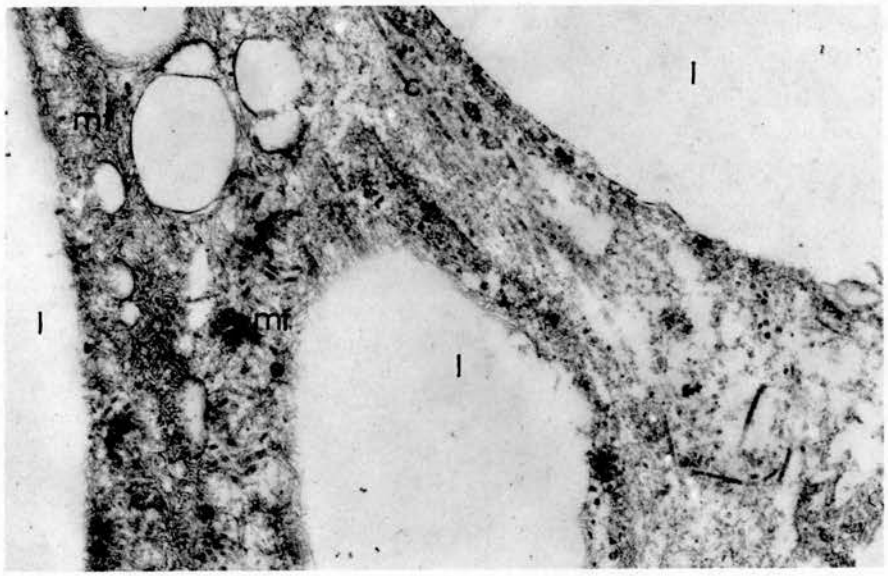



Figure 4.20.2

Figure 4.20.1

Duck white adipose tissue,
5 day old (inguino-crural depot)
Fixation method 1.
Magnification 16,820.

Figure 4.20.2

Duck white adipose tissue
4 week old (inguino-crural depot)
Fixation method 1
Magnification 19,570.

Key:
A = adipocyte; E = endothelial cell;
PV = perivascular cell; l = lipid
droplet; mf = micro filaments;
rer = rough endoplasmic reticulum;
es = extracellular space;
m = mitochondrion.

(Figs. 4.20.1, 4.24.1, 4.25). These vacuoles are similar in appearance to lipid droplets. They are bounded neither by membranes or microfibrils. Microfibrils have never been observed in endothelial cells.

The extracellular space in duck adipose tissue is relatively small and the incidence of collagen fibrils in this space is relatively common (4.17, 4.20, 4.23). Collagen fibrils are undoubtedly an important structural element within the tissue.

In the adipose tissue from the young ducks, perivascular cells similar in appearance to the endothelial cells were commonly found adjoining the capillaries. These cells possess a well developed system of rough endoplasmic reticulum, and large vacuoles, presumed to be lipid droplets are common within the cytoplasm (Figs. 4.24, 4.25). Cytoplasmic processes are also frequently observed (Fig. 4.25). In a single example (Fig. 4.25) the lipid droplets within such a cell were bounded with microfibrils indicating perhaps a transitional stage in the presumed differentiation of an adipocyte from a perivascular cell.

## Discussion

The contrasting quality of the results obtained for white adipose tissue from the rat and the duck, using the same preparative method requires some explanation. White adipose tissue from other species, fowl, man, sheep and rabbit, also produced results comparable with those for the duck. In the case of the duck for which a much greater amount

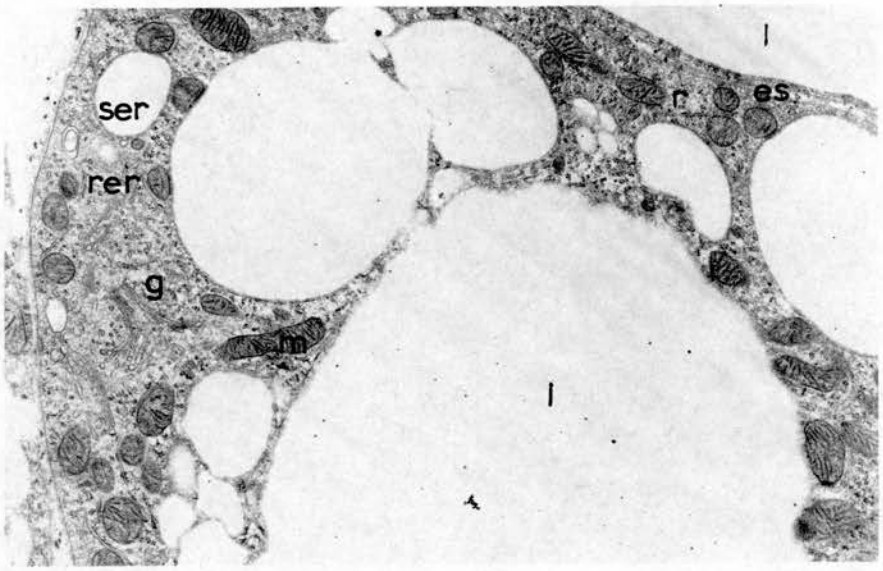



Figure 4.21.1

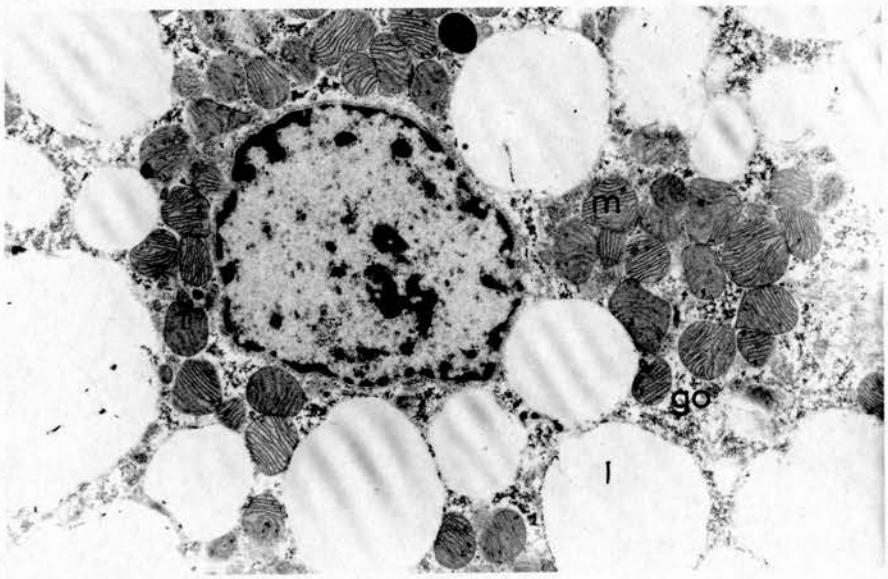



Figure 4.21.2

Figure 4.21.1

Rat white adipose tissue,
4 day old (epididymal depot).
Fixation method 1.
Magnification 11,680.

Figure 4.21.2

Rat brown adipose tissue,
4 day old (suprascapular)
Fixation method 1.
Magnification 7,620.

Key:
l = lipid droplet; ser = smooth endoplasmic reticulum; g = golgi apparatus; m = mitochondrion, pv = pinocytatic vesicles; es = extracellular space; r = ribosomes; go = glycogen.

of material has been examined, the relatively inferior results are attributed to the poorer penetration of the fixative, and in turn this is most probably due to the reduced size of the intercellular space, the relative thinness of the cytoplasm and the relatively greater proportion of collagen present. The latter no doubt would ensure the maintenance of the structural integrity of the tissue during the preparative processes, whereas in some instances rat adipocytes have the appearance of becoming dissociated under such conditions. A higher collagen content in the duck adipose tissue would provide an explanation for the difficulty encountered in dissociating the tissue with a collagenase preparation which was found to be entirely effective for rat tissue (see Section 3). Such structural differences between the tissues may of course have important functional implications, particularly with regard to the lipogenic and lipolytic activity of the depots. These general observations are however largely qualitative at this stage, and consequently require verification by quantitative techniques.

Although microfibrils are considered to be a normal cytoplasmic element of most cells, only in a relatively few cases do they appear in any number (Fawcett, 1966). Their appearance in the adipocytes of the duck, the fowl and man, suggest that they may constitute a normal element of adipose tissue, even though they do not occur in either white or brown rat adipose tissue. The regular distribution of the microfibrils about the lipid droplets indicates that their main function is in some way related to either the production,

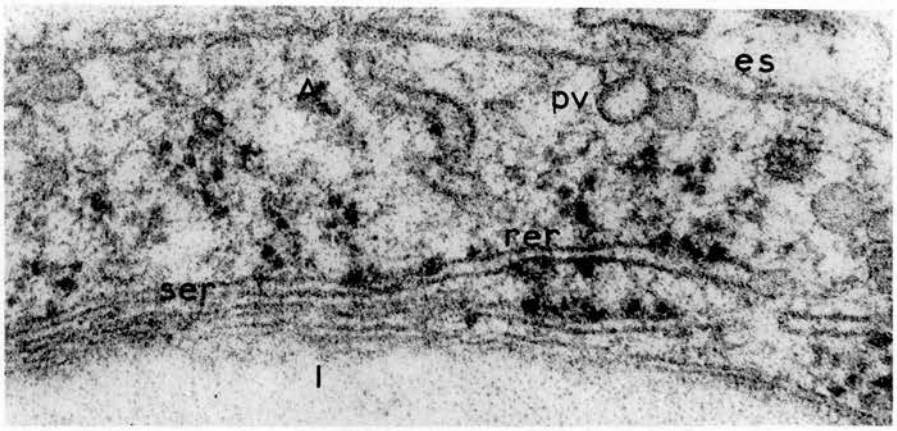



Figure 4.22.1

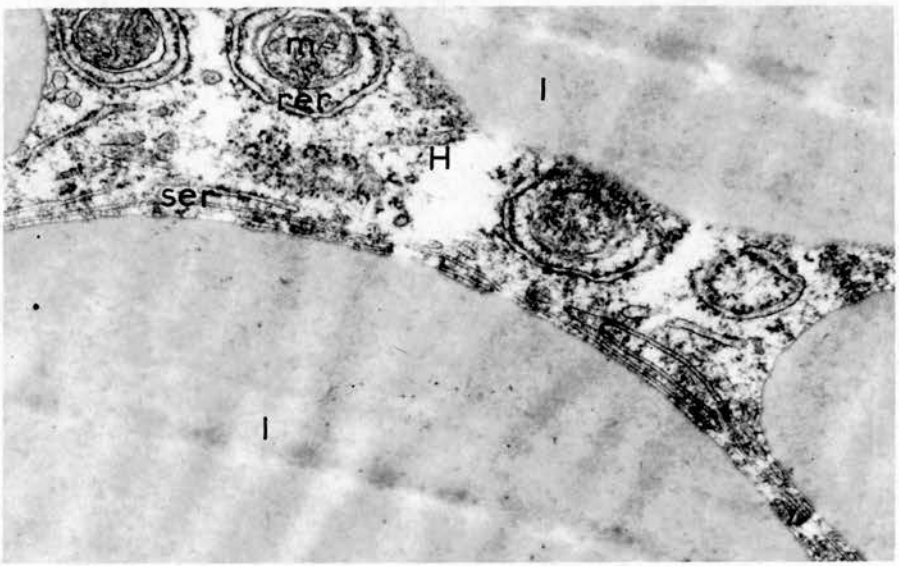



Figure 4.22.2

Figure 4.22.1

Rat white adipose tissue,
4 day old (epididymal depot)
Fixation method 1.
Magnification x 91,040.

Figure 4.22.2

Haddock liver.
Fixation method 1.
Magnification x 25,880.

Key:
A = adipocyte; H = hepatic cell;
l = lipid droplet; es = extracellular space; pv = pinocytatic vesicles;
r = ribosomes; res = rough endoplasmic reticulum; ses = smooth endoplasmic reticulum.

maintenance or dissolution of the droplet. Perhaps their most obvious function may be a supporting one, to stabilise and maintain the form of the large lipid droplets. The structural role of microfibrils in squamous epithelial cells (they are usually termed tonofilaments in such cells) is undoubtedly their primary function (Fawcett, 1966). Their occurrence in this tissue has led to the suggestion that such fibrils are composed of keratin, but this has yet to be adequately demonstrated. However the common presence of microfibrils in other cells such as endothelial cells, neuroglia, neurones, the podocytes of the glomerulus, the interstitial cells of the testis and cells of the adrenal cortex, none of which are known to contain keratin, indicate that it is more likely that they are composed of some fibrous protein other than keratin (Fawcett, 1966). It is interesting to note that since the interstitial cells of the testis frequently contain large droplets of fat (Bloom and Fawcett, 1968) microfibrils may serve a similar function in these to that in the adipocytes.

Adipocytes of rat white adipose tissue contain lipid droplets as large as those occurring in adipocytes of the species which possess the microfibril array (see Section 4.2.2.). This would consequently imply that the microfibrils are unnecessary for the structural maintenance of the lipid droplets alone. The basic difference between rat adipocytes and those from the other species being considered is that the rat cell is capable of active lipogenesis. However since most of the enzymes involved in the synthesis of fatty

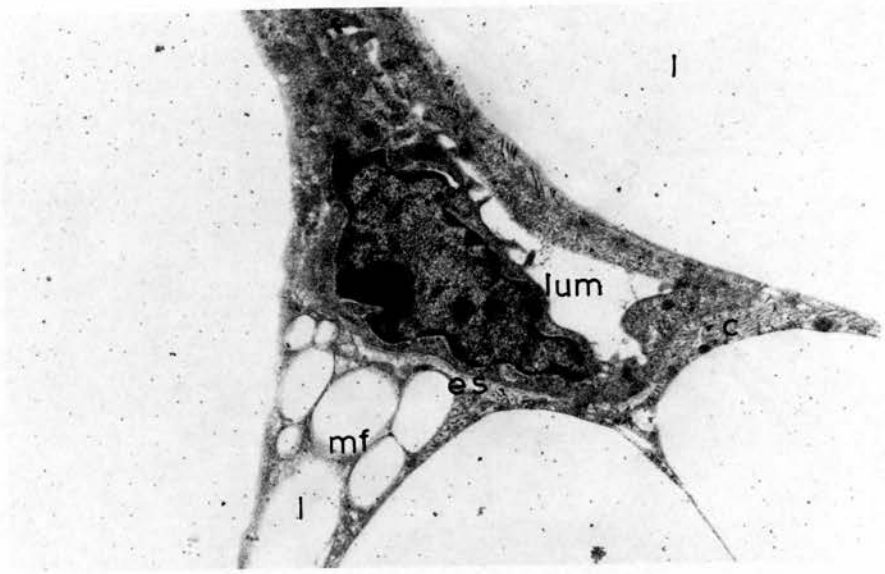



Figure 4.23.1

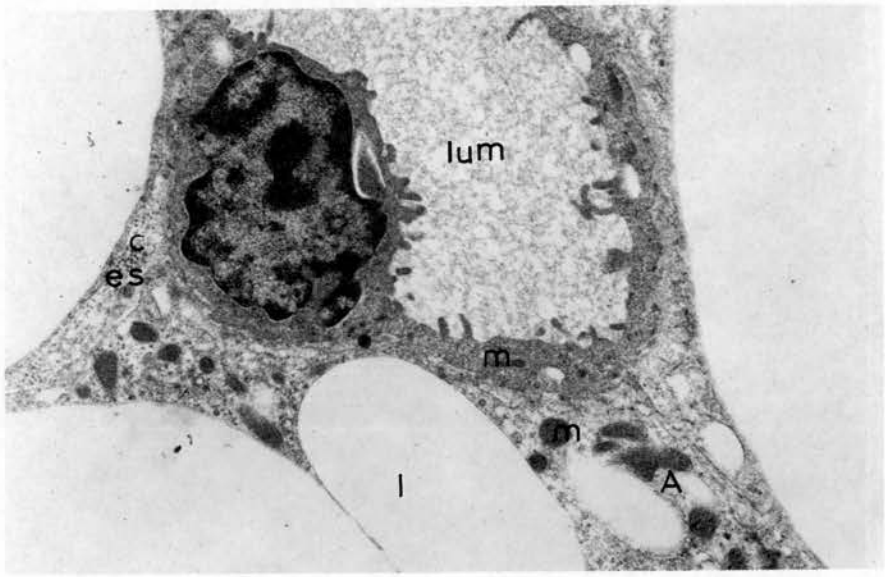



Figure 4.23.2

Figure 4.23.1

Duck white adipose tissue,
10 week old (peritoneal depot).
Fixation, glutaraldehyde.
Magnification x 10,910.

Figure 4.23.2

Duck white adipose tissue
2 week old (inguino-crural depot),
Fixation method 1.
Magnification x 10,790.

Key:
A = adipocyte; E = endothelial cell;
l = lipid droplet; lum = lumen of
capillary; mf = microfibrils; es =
extracellular space; c = collagen;
m = mitochondrion.

acids occur in the clear, organelle-free fraction of the cytoplasm (Vagelos et al, 1969; Angel and Sheldon, 1965) such metabolic differences will not necessarily be reflected in observable ultrastructural differences. The appearance of vesicles, which frequently cannot be differentiated from smooth endoplasmic reticulum, is regarded to indicate lipogenic activity in rat adipocytes (Barrnett, 1962). However the primary cause of such structural changes have yet to be satisfactorily explained since other states bring about similar changes (Sheldon, 1965, Barrnett, 1962). Therefore such ultrastructural criteria of lipogenic activity are currently unsatisfactory. A feature of the rat adipocyte from white adipose tissue was the presence of endoplasmic reticulum arranged in concentric rings about many of the fat droplets. This detail has been observed previously in this tissue (Sheldon, 1965). By using electron microscope radioautography both the smooth endoplasmic reticulum and the Golgi bodies were shown to be the main structures within which esterification of fatty acids (to form triglycerides) occurs in both liver (Jones et al., 1967, and Stein and Stein, 1967) and in cells of the intestinal mucosa (Jersild, 1966). Further the enzymes necessary for esterification are mostly located within the microsomal fraction (Angel and Sheldon, 1965; Jones et al., 1967). Since esterification is a pathway common to adipocytes of all species it might be expected that better fixation techniques would reveal the presence of a similar arrangement of endoplasmic reticulum in all adipocytes. Likewise since the main hormone sensitive

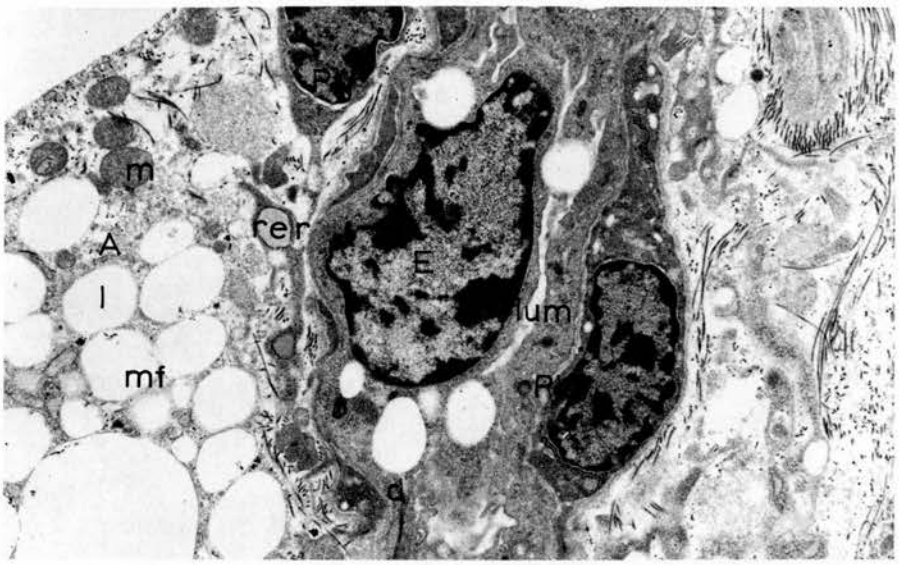



Figure 4.24.1

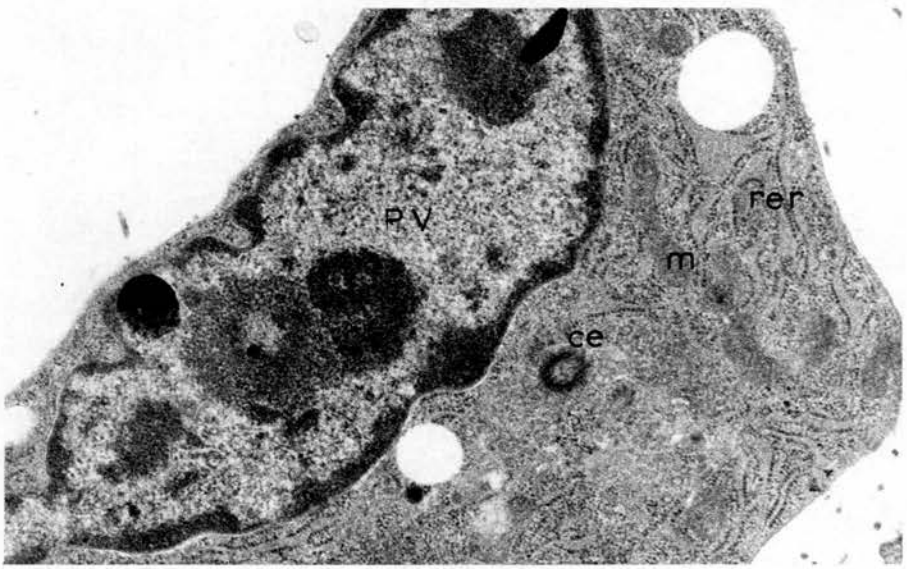



Figure 4.24.2

lipase system which is common to such species probably also resides in the microsomal fraction a similar argument applies regarding the endoplasmic reticulum.

It is apparent from this discussion that no entirely satisfactory explanation of the function of the microfilaments in adipocytes can be provided yet. Their significance would well repay further investigation particularly if the rat should prove to be somewhat different from man and bird.

If as Simon (1965) has suggested, that lipid filled adipocytes are incapable of further division, then the perivascular cells must give rise to all future adipocytes. Since in the duck such perivascular cells were relatively common in the newly hatched bird (5 day old) but extremely rare in the 10 week old bird, this would imply that the tissue from the younger animals would be capable of a more rapid rate of increase of cell number. The rare incidence of microfibrils in the perivascular cells, suggests that such structures probably do not arise until a rather late stage in the differentiation of the cell.

The size of capillary network in adipose tissue is undoubtedly of critical importance to the functional efficiency of the adipocytes. Contrary to popular belief it has been well established by using quantitative techniques that both the size of (Gersh and Still, 1945; Hausberger and Widelitz, 1963) and the rate of blood flow through (Herd et al., 1968), the vascular bed is similar to that in other tissues. Surprisingly if the size of the vascular bed is estimated on the basis of the amount of protein in the tissue,

Figure 4.24.1

Duck white adipose tissue,
5 day old (gastric depot).
Fixation method 1.
Magnification x 7,610.

Figure 4.24.2

Duck white adipose tissue
5 day old (gastric depot).
Fixation method 1.
Magnification x 21,750.

Key:
A = adipocyte; E = endothelial cell;
PV = perivascular cell; lum = lumen of capillary; l = lipid droplets;
d = desmosome; m = mitochondrion;
mf = microfilaments; rer = rough endoplasmic reticulum; ce = centriole.

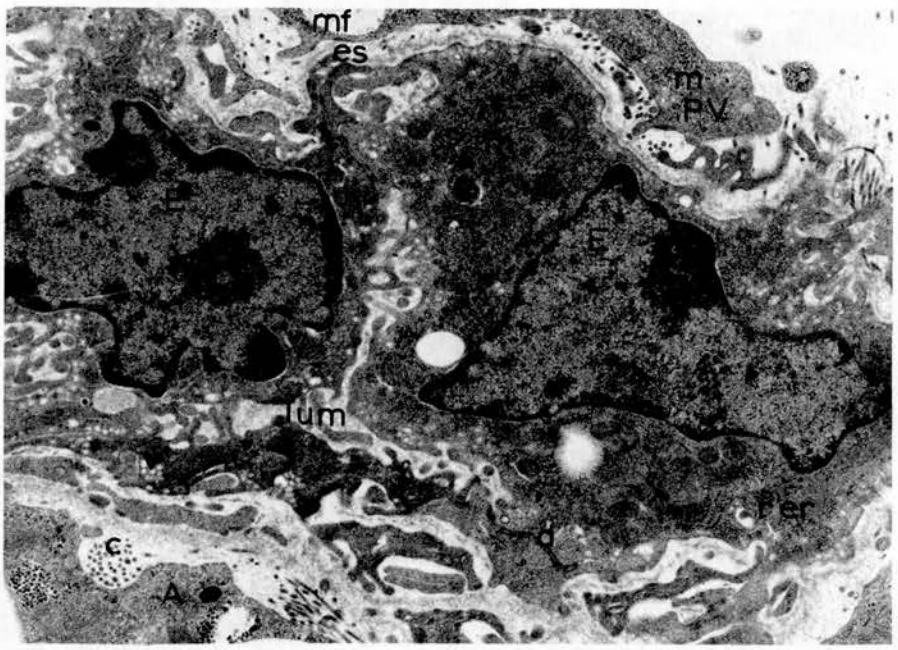



Figure 4.25

Figure 4.25.

Duck white adipose tissue,
5 day old (inguino-crural depot).
Fixation method 1.
Magnification x 14,020.

Key:
A = adipocyte; E = endothelial cell; PV = perivascular cell; lum = lumen of capillary; l = lipid droplet; m = mitochondrion; rer = rough endoplasmic reticulum; r = ribosomes; d = desmosomes; v = vesicles; c = collagen; es = extracellular space, mf = microfilaments.

then adipose tissue has been shown to have a vascular supply 16 times greater than that for skeletal muscle (Hausberger and Widelitz, 1963). These authors also showed that although the size of the vascular bed per unit weight of adipose tissue declined with age, and increasing obesity in the rat, the absolute size of the vascular bed of a fat deposit actually increased during the same period.

4.2.2. Changes in the size of the adipocyte during growth.

Fat depots primarily function as storage organs, which accommodate the increased deposition of fat occurring during growth or periods of positive calorie balance. The increase in storage capacity can be achieved in two ways. By multiplication of the adipocytes, hyperplasia, or by an increase in their size as the fat accumulates within the cell, hypertrophy. Since these two processes are separate (and probably independent) it is important to distinguish between the contribution of each during the accretionary growth of fat in any deposit.

An attempt has been made to do this by measuring the changes in diameter of adipocytes in various fat depots during the first 8 weeks of postembryonic growth in the Aylesbury duck. These results were used in conjunction with measurements of the total fat content of the depots during the same period to obtain an estimate of the changes in cell number and mitotic activity in the adipose tissue. The data presented here was obtained from female Aylesbury ducks. Usually cell measurements were obtained from three fat depots

(subcutaneous, inguino-crural and peritoneal) in each individual bird, and usually five birds were used for each age group.

The data so obtained is shown in Table 4.19. Although it is apparent that there was considerable interindividual variation in cell size this was rarely greater than the variation occurring within a single deposit between similarly sized samples (Table 4.20). Variations, of similar origin, in adipocyte size, have been reported in mammals (Reh, 1953; Bjurulf, 1960). However, the only systematic form of variation discovered was that associated with the position of the adipocyte within a connective tissue lobule. Cells adjoining the periphery of the lobule were slightly smaller than the other cells within the interior of the lobule (Bjurulf, 1960). This source of error has been avoided in this study by the use of a suitable measuring technique (Section 3). Since counting very large samples does not substantially remove interindividual variation (Goldrick, 1967b), it has been considered justifiable, particularly in the interest of describing the general changes occurring during growth, to use the mean values obtained from relatively small samples from individual birds (Table 4.19), as the raw data for analysis when estimating the variation in any parameter or statistic for any age group. It was on this basis that both the means and standard errors have been calculated from the data in Table 4.19 (Fig. 4.26). Figure 4.26 in summarising the data from Table 4.19 shows that in all three depots there is a substantial increase in adipocyte

Table 4.19

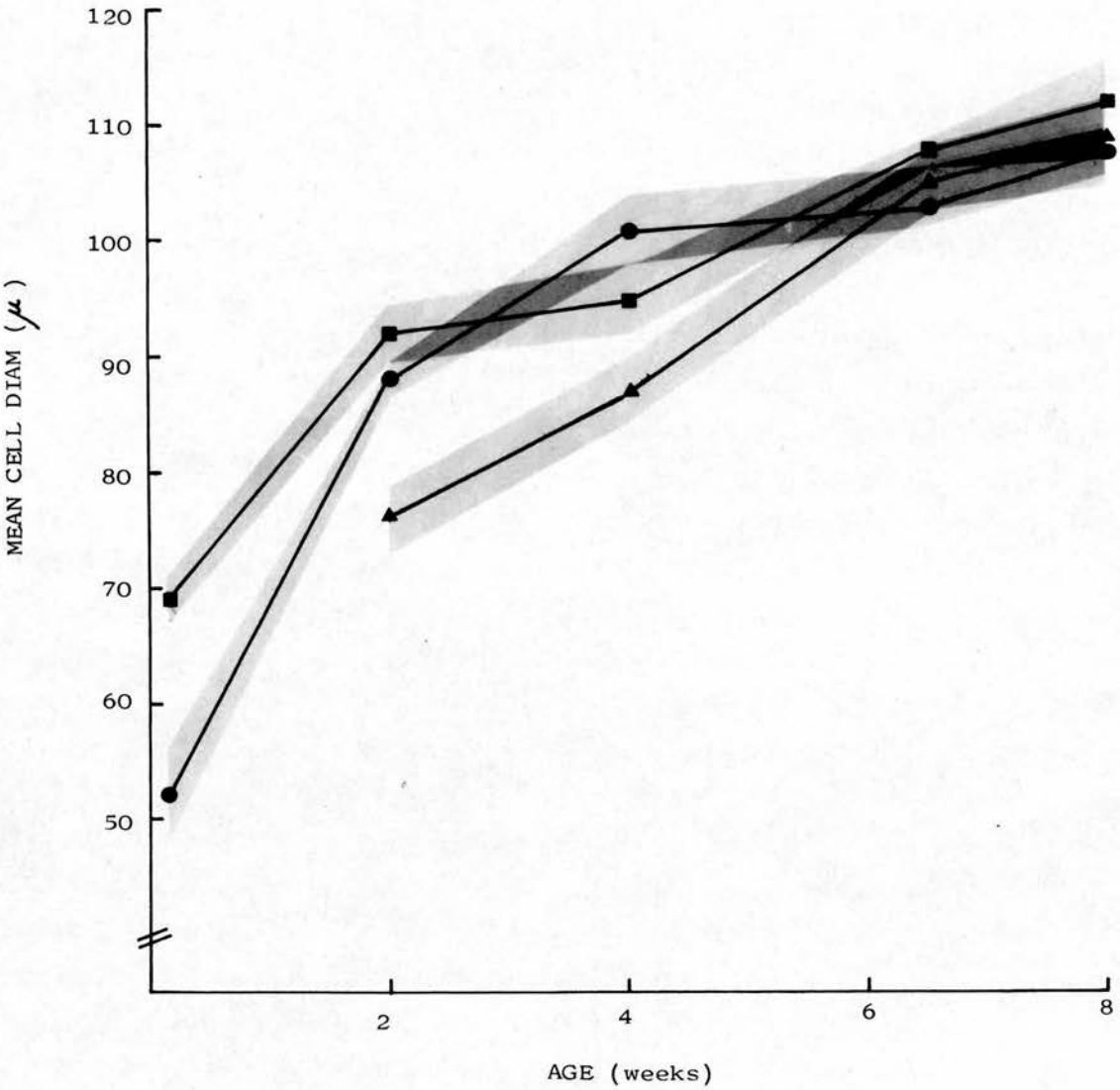
The effect of age and site on adipocyte diameter (μ). 'n' shown in brackets refers to the number of cells measured in each depot in each individual bird. Values are means ± S.E.M.

| Age | Bird No. | Deposit | | |
|---|---|---|---|---|
| | | Subcutaneous | Inguino-crural | Peritoneal |
| 1 day | 1 | 52±1·7 (60) | 66±1·7 (60) | |
| | 2 | 45±1·6 (60) | 71±1·3 (60) | |
| | 3 | 66±1·8 (60) | 71 ±1·3(60) | |
| | 4 | 51±1·3 (60) | 63±1·5 (60) | |
| | 5 | 46±1·8 (60) | 74±1·8 (60) | |
| 2 weeks | 6 | 91±2·8 (60) | 88±1·6 (206) | 84±2·8 (60) |
| | 7 | 88±2·9 (60) | 95±3·2 (60) | 80±3·0 (60) |
| | 8 | 90±2·8 (60) | 86±3·5 (60) | 72±2·6 (60) |
| | 9 | 85±2·5 (60) | 100±2·4 (60) | 70±2·2 (60) |
| | 10 | 88±2·6 (60) | 91±2·8 (60) | 74±3·0 (60) |
| | 11 | | 90±2·3 (60) | |
| 4 weeks | 12 | 105±2·7 (60) | 97±3·8 (40) | 94±2·6 (60) |
| | 13 | 100±3·1 (60) | 105±2·7 (60) | 87±2·4 (60) |
| | 14 | 89±2·7 (60) | 96±3·1 (60) | 95±2·8 (60) |
| | 15 | 103±2·8 (60) | 87±2·1 (60) | 79±2·6 (60) |
| | 16 | 106±2·8 (59) | 91±2·7 (60) | 82±2·1(60) |
| 6·5 weeks | 17 | 98±2·0 (200) | 110±1·7 (212) | 116±1·7 (199) |
| | 18 | 108±3·0 (80) | 106±1·3 (199) | 100±2·0 (80) |
| | 19 | 104±2·6 (80) | 105±1·7 (200) | 104±2·3 (80) |
| | 20 | 103±2·0 (80) | 114±2·7 (60) | 103±2·8 (80) |
| 8 weeks | 21 | 105±3·0 (60) | 106±1·7 (210) | 104±2·7 (60) |
| | 22 | 109±2·6 (60) | 106±2·3 (60) | 116± 2·4 (60) |
| | 23 | 110±3·1 (60) | 106±3·4 (60) | 104±3·4 (60) |
| | 24 | | 120±3·1 (60) | 111±3·4(60) |
| | 25 | | 123±3·6 (60) | |

Table 4.20.

Intradepot variation of cell diameter (μ). No. in brackets refers to the numbers of cells measured. Values are means ± S.E.M.

| Deposit | Inguino-etc. 2 wk. | Subcutaneous $6\frac{1}{2}$ wk. | Peritoneal $6\frac{1}{2}$ wk. |
|---|---|---|---|
| | Bird No.26 | Bird No. 17 | Bird No. 17 |
| | 91 ± 2·6 (60) | 105 ± 3·5 (60) | 116 ± 2·6 (60) |
| | 91 ± 3·0 (60) | 90 ± 4·6 (60) | 119 ± 2·1 (60) |
| | 89 ± 3·2 (60) | 97 ± 3·0 (60) | 113 ± 4·1 (60) |
| Totals | 90 ± 1·7 (180) | 98 ± 2·2 (180) | 116 ± 1·9 (180) |




The effect of age on mean diameter of adipocyte. Width of band ± 1 S.E.M.

■ Inguino-crural
● Subcutaneous
▲ Peritoneal

Figure 4.26

size during this growth period. Initially the size of the adipocyte seems to reflect the growth form of the depot. Thus in the earliest maturing depot (the inguino-crural, see Section 4.1) adipocytes were significantly larger than those in the later maturing subcutaneous depot in day-old birds. Likewise adipocytes in the late maturing peritoneal depot were smaller than those in the other two depots in the 4 week old birds. However these initial differences in size between the adipocytes disappeared as the birds became older.

Generally in all cases the initial rapid rate of increase in cell size declined with age. This led to the speculation that in the latter stages of fat deposition, cell size may be limiting, simply because the amount of lipid within the cell was approaching the maximum which the cell could contain. To examine this possibility the statistics $g_1$ and $g_2$ (based on the 3rd and 4th moments respectively, Quenouille, 1966) were calculated for each sample of cell diameter measurements. The data in Table 4.21 summarise the results obtained. The $g_1$ and $g_2$ values given are the averages of the calculated $g_1$ and $g_2$ values from the same depots from different individual birds of the same age. Since the standard error for $g_1$ and $g_2$ is estimated from the sample size alone, the mean sample size of the averaged group was used for this calculation.

If cell diameter is normally distributed then $g_1$ and $g_2$ will not be significantly different from zero. If with increasing fat deposition the cell diameter was approaching

Table 4.21. The statistics $g_1$ and $g_2$ calculated for distribution of cell diameter. See text for explanation.

$g_1$ diameter

| Deposit | | Age (weeks) 1 | 2 | 4 | 6·5 | 8 |
|---|---|---|---|---|---|---|
| Inguinn-crural | $\bar{x}$ | -0·784 | -0·111 | -0·278 | -0·868 | -0·785 |
| | se | ±0·309 | ±0·309 | ±0·309 | ±0·269 | ±0·309 |
| | t | 2·537 | 0·359 | 0·900 | 3·227 | 2·540 |
| | P | <0·02 >0·01 | >0·10 | >0·10 | <0·01 >0·002 | <0·02 >0·01 |
| | N | 60(5) | 60(6) | 60(5) | 80(4) | 60(5) |
| Subcut. | $\bar{x}$ | 0·000 | -0·062 | -0·228 | -0·641 | -0·968 |
| | se | ±0·309 | ±0·309 | ±0·309 | ±0·269 | ±0·309 |
| | t | 0·000 | 0·201 | 0·737 | 2·383 | 3·133 |
| | P | >0·10 | >0·10 | >0·10 | <0·02 >0·01 | <0·01 >0·002 |
| | N | 60(5) | 60(5) | 60(5) | 80(4) | 60(3) |
| Periton-eal | $\bar{x}$ | | -0·024 | +0·072 | -0·762 | -0·827 |
| | se | | ±0·309 | ±0·309 | ±0·269 | ±0·309 |
| | t | | 0·077 | 0·233 | 2·833 | 2·676 |
| | P | | >0·10 | >0·10 | <0·01 >0·002 | < ·01 >0·002 |
| | N | | 60(5) | 60(5) | 80(4) | 60(4) |

$g_2$ diameter

| | | | | | | |
|---|---|---|---|---|---|---|
| Inguinc-crural | $\bar{x}$ | 0·917 | -0·328 | -0·333 | 0·914 | 0·524 |
| | se | ±0·608 | ±0·608 | ±0·608 | ±0·532 | ±0·608 |
| | t | 1·508 | 0·539 | 0·547 | 1·718 | 0·862 |
| | P | >0·10 | >0·10 | >0·10 | >0·05 <0·10 | >0·10 |
| | N | 60(5) | 60(6) | 60(5) | 80(4) | 60(5) |
| Subcut. | $\bar{x}$ | -0·380 | -0·163 | -0·405 | 0·074 | 1·957 |
| | se | ±0·608 | ±0·608 | ±0·608 | ±0·532 | ±0·608 |
| | t | 0·625 | 0·268 | 0·666 | 0·139 | 3·22 |
| | P | >0·10 | >0·10 | >0·10 | >0·10 | <0·01 |
| | N | 60(5) | 60(5) | 60(5) | 80(4) | 60(3) |
| Perit-oneal | $\bar{x}$ | - | -0·548 | 1·101 | 0·841 | 0·726 |
| | se | | ±0·608 | ±0·608 | ±0·532 | ±0·608 |
| | t | | 0·901 | 1·811 | 1·581 | 1·194 |
| | P | | >0·10 | >0·10 | >0·10 | >0·10 |
| | N | | 60(5) | 60(5) | 80(4) | 60(4) |

some natural limit then the distribution from this parameter would become skewed to the left and simultaneously the distribution would become increasingly leptokurtotic. This means that with increasing age $g_1$ would become increasingly negative and $g_2$ increasingly positive. In Table 4.21 't' tests have been used to determine whether the estimates of $g_1$ and $g_2$ are significantly different from zero (i.e. in a perfect normal distribution $g_1 = g_2 = 0$). The results shown indicate that cell diameter does become significantly skewed to the left in all the depots examined in the older birds. However except in two examples the skewing was unaccompanied by any leptokurtosis. Consequently there is no strong evidence to support the proposal that the limit in the size of the adipocyte (if such should exist) is being reached during the growth period of the study, particularly since skewing of the cell diameter distribution could arise from other causes.

The volume of the adipocyte was calculated from mean diameter using the following formula

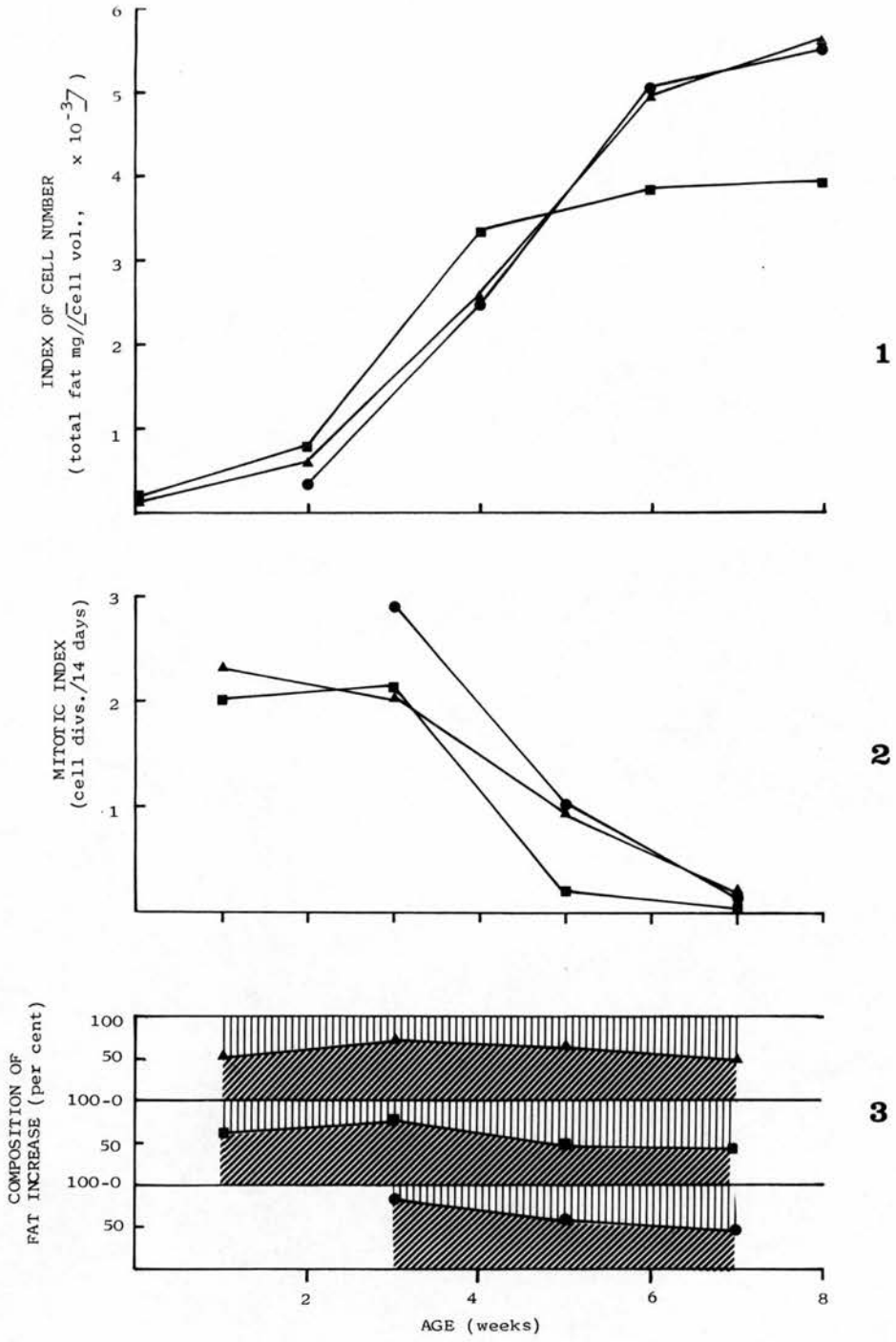
Cell volume = $\frac{\pi}{6}.(3\sigma^2 + \bar{x}^2).\bar{x}$ where $\bar{x}$ and $\sigma^2$ are mean and variance respectively of the cell diameter x, (Goldrick, 1967b). This formula accounts for the fact that if the diameter is normally distributed then volume (or diameter cubed) will be skewed. Consequently volume cannot be calculated from the unadjusted mean of diameter. Table 4.22 shows that the increase in cell volume was respectively 4·5 fold and 8·6 fold for inguino-crural and subcutaneous fat, during the first eight weeks of post embryonic life. Where diameter is not normally distributed as in the case of the older birds,

Table 4.22.

The effect of age and site on the mean volume of the adipocyte. Volume was calculated according to the formula given by Goldrick (1967b), see Text.

| | Age in weeks. | | | | |
|---|---|---|---|---|---|
| | 1 day | 2 | 4 | 6·5 | 8 |
| Inguino-crural | 183,000 | 467,000 | 521,000 | 704,000 | 812,000 |
| Subcutaneous | 86,500 | 419,000 | 616,000 | 631,000 | 748,000 |
| Peritoneal | | 284,000 | 401,000 | 656,000 | 774,000 |

units = $\mu^3$




Estimated changes in the mitotic activity of adipocytes during growth, from three fat deposits.

1. Estimated index of cell number in various fat deposits.
2. Estimated mitotic index in various fat deposits.
3. The composition of the fat increase in various deposits.

hypertrophy  hyperplasia

subcutaneous fat
Inguino-crural fat
Peritoneal fat

Figure 4.27

volume calculated according to the above formula will be slightly underestimated. It is unlikely however to constitute a serious error, since its effect is far less than that produced by the interindividual variation already referred to.

An index of the number of adipocytes occurring in a deposit can be obtained by dividing the total weight of fat extracted from a deposit by the mean volume of the adipocyte. This is based on the assumption that the density of the deposited lipid does not change appreciably during growth, and consequently that there is a constant relationship between weight and volume of the deposited lipid. It is apparent from the result obtained by using such a calculation that there is a considerable increase in cell number during the first eight weeks of postembryonic life (Fig. 4.27.1.).

The multiplication rate required to account for such increases in cell number have been calculated and are shown in Fig. 4.27.2. Again it appears significant that the most early maturing depot shows an earlier decline in the rate of cell multiplication than the others. However in all the fat depots, the rate of cell multiplication was highest initially in the first four weeks, subsequently declining rapidly. This result supports some observation from electron microscope studies. In these it was shown that primordial cells (perivascular cells) were found to be common in the fat depots of young animals, but rare in those of older birds.

It will be noted that a very low rate of mitosis accounts for the increase in cell number during post-

embryonic growth, and therefore it is not surprising that mitosis was never observed during histological examination of the tissue (see section 4.2.1.). The results of this section have been summarised in Fig. 4.27.3, which indicates the relative contributions of the two processes hypertrophy and hyperplasia to increasing the storage capacity of the three fat depots during the first eight weeks of postembryonic growth. It is evident from this that hyperplasia always plays a major role during the early stages of fat deposition, although later, increases in cell size become more or equally as important.

Discussion.

Adipocyte size appears to be largely uninfluenced by the body size of the species (Table 4.23). It is perhaps therefore to be expected that the range of sizesof the adipocytes encountered in the duck appears to be similar to that for other species. From the very limited number of studies made on adipocyte morphology it would appear that several factors influence the adipocyte size. The first is age. Goldrick (1967b), using a technique almost identical to that used in this study, showed that there was a gradual and steady increase in adipocyte diameter from 48 to 102 $\mu$ in the epididymal depot of the rat over the period for which measurements were obtained, namely for the first 140 days following weaning. This represented an approximate 8 fold increase in volume. Similar observations were made in the rat by Hellman et al., 1962. Enesco and Leblond (1962) by

Table 4.23.

Adipocyte size in various species.

| Species | Depot | Condition | Mean Adipocyte Diam. (μ) | Reference |
|---|---|---|---|---|
| Human | Subcutaneous | lean to obese | 17 - 126 | Bjurulf 1960 |
| " | | lean | 80 | Bjorntörp and Martinsson 1966 |
| " | | obese | 125 | " |
| Thrushes | | normal to pre fattened | 50 - 150 | Hicks, 1967 |
| Goose | | obese | 160 | Clara 1929 |
| | | normal | 50-80 | " 1929 |
| Rat | Epididymal | 0 days past weaning | 48 | Goldrick 1967b |
| " | " | 140 " | 102 | " |
| " | " | 64 fasted | 28 (normal 80) | " |

calculating the DNA content of the rat epididymal fat depot, found that cell size increased 18 fold (calculated as weight of material per nucleus) during the first 150 days following birth. This change can be probably considered comparable to the result obtained by Goldrick (1967b) when it is taken into account that the latter author did not measure the changes occurring during suckling when a considerable increase in overall fat deposition is occurring (Spray and Widdowson, 1950).

Adipocyte size is also influenced by conditions which result either in increased fat deposition or an increased rate of fat mobilisation. Consequently fasting decreases adipocyte size (Goldrick, 1967b; Zingg et al., 1962). The period of intense fat deposition which precedes migration in birds is accompanied by a considerable increase in size of the adipocyte (Hicks, 1967). In humans, an overweight condition is generally associated with large adipocyte size (Bjurulf, 1960; Bjorntörp and Martinsson, 1966). These results indicate that abnormal amounts of fat can be wholly or partially accommodated by an increase in adipocyte size. It therefore seems unlikely that adipocyte size is ever limiting. Where the distribution of adipocyte diameter has been examined, a significant deviation from normality was only rarely found (Goldrick, 1967b; Bjurulf, 1960).

During the period of fat deposition associated with development and growth in the rat, there was a 13 fold increase

in cell number in the epididymal fat depot (Enesco and Leblond, 1962). The rate of increase was initially high and declined with age, in a manner somewhat similar to that observed in the duck.

A similar result was obtained for the subcutaneous fat of the rat using tritiated thymidine, and autoradiography of the tissue sections (Hellman and Hellerström, 1961). The rate of cell division appeared to decline after the first 25 days of life and thereafter continued at a reduced rate for the remainder of the study (i.e. until the rats were 150 days old). This study indicated that white adipose tissue was more mitotically active than brown adipose tissue. Zingg et al (1962) found that in the rat both the number of cells and the DNA content of the perirenal fat depot were related to its total fat content, suggesting that chronic starvation may decrease the number of adipocytes and obesity increase the number in a depot. Peckham et al. (1962) obtained similar results in the rat. By measuring DNA content of fat depots they concluded that cell number increased during development and the associated fat deposition and that this increase could be made greater by feeding the rats a high fat diet which resulted in a greater rate of fat deposition. A particularly interesting observation suggested by their work, was obtained for rats which were partially fed a high fat diet and then returned to a normal diet. In these animals which had previously been obese on the high fat diet and consequently acquired an increased cell number in the fat depot, this cell number did not decline when the rats were

returned to a normal diet, although the fat content of their depots returned to normal. This indicated that the increased cell number resulting from obesity once established may be maintained.

Experiments with tritiated thymidine have shown that cell division can occur in adipose tissue of the adult animal. Refeeding following a period of fasting stimulated mitotic activity in the adipose tissue of the rat (Braun et al., 1968; Hazdová et al., 1967). These authors attributed the high lipogenic activity of the tissue, following such dietary manipulation, to the presence of the highly active newly formed adipocytes. Also using tritiated thymidine Hollenberg and Vost (1968b) have demonstrated that an increase in the number of adipocytes in adipose tissue must arise from mitosis of the primordial perivascular cells. Such findings support the conclusions of Wasserman (1965) and Simon (1965) derived from histological studies.

Mitosis in adipose tissue appears to be partially dependent on the presence of growth hormone. This hormone has been shown to increase mitosis in the intact rat (Murakawa and Raben, 1968) and to restore normal activity in rats following pituitary ablation (Hollenberg and Vost, 1968b).

In the work of Enesco and Leblond (1962) their results indicated that in the rat epididymal fat depot hypertrophy played a marginally greater role than hyperplasia in accounting for the increased storage capacity of this tissue during development. They also studied the form of the growth

of other tissues and organs in the rat body during development, and their general conclusions were that the overall pattern of post natal growth can be divided into three periods. The first is one of rapid cell proliferation, with little change in cell size, followed by a middle period in which cell multiplication continues in all locations, but at a slower rate than in the previous period, and cell size increases. Finally the last period is one in which cell proliferation decreases considerably or ceases altogether, while cell enlargement continues.

Adipose tissue was regarded as being similar to skeletal muscle but unlike other tissues and organs which conformed to the above development sequence, in that hypertrophy (regarded here as the amount of material per nucleus) always played a more important role particularly in the middle and final periods, than it did in the other organs examined. In fact in the rat epididymal fat depot, only 6% of the increase occurring after the first seventeen days was due to hyperplasia. The composition of the growth in the duck depots generally conformed to Enesco and Leblond's description for this tissue in the rat.

4.2.3. The chemical composition of adipose tissue.

The histological studies in the foregoing sections (4.2,2) suggest that the increasing amount of fat accumulating in the adipocytes during growth, would have the effect of diluting the other chemical components in the adipose tissue.

It has been the purpose of this study to characterise such changes in composition occurring in the tissue over this period.

It will be obvious from the results presented in section 4.2.1. that adipose tissue consists not only of adipocytes but also of blood cells, endothelial cells and perivascular cells together with fibroblasts and collagen. Consequently the chemical composition of the tissue must reflect the presence of these various components. In rat epididymal adipose tissue only 35% of the total DNA and 50% of the total protein could be attributed to the adipocytes (Rodbell, 1964a). Adipocytes however can be separated from the associated cells in rat white adipose tissue by a relatively simple technique (Rodbell, 1964a), and thus in this tissue these cells can be analysed separately. This procedure however proved to be unsuitable for avian adipose tissue.

The inguino-crural and peritoneal depots were chosen for chemical analysis since they could be obtained uncontaminated by any other tissues. As the peritoneal depots were very small in the 2½ week old bird they were pooled for analysis.

The results obtained indicated that although there was an increasing dilution of the non-lipid elements during the growth of the fat depots (Table 4.24), these elements actually increased slightly in absolute terms (Table 4.25). The difference in composition between the inguino-crural and peritoneal depots in the 2½ week old birds presumably reflects their different states of development (see Section

Table 4.24. The effect of age on the composition of adipose tissue in the inguino-crural and peritoneal depots of the Aylesbury duck. Values are means ± S.E.M., calculated on a wet weight basis.

| Tissue | Age (wks) | No. of birds | mean wt. of deposit (g) | Total fat (g/100g) | Total Water (g/100g) | Total $K^+$ (meqv/100g) | Total $Na^+$ (meqv/100g) | Total Protein (g/100g) | Na/K | Protein/K |
|---|---|---|---|---|---|---|---|---|---|---|
| Inguino | 2½ | 5 | 10 | 78·5±0·8 | 19·9±0·5 | 1·0±0·11 | 1·97±0·06 | 2·90±0·11 | 1·97 | 2·90 |
| Peritoneal | 2½ | 5 (pooled) | 5·5 | 74·3 | 20·7 | 1·15 | 2·24 | 4·84 | 1·95 | 4·20 |
| Inguino | 4 | 5 | 26·5 | 85·7±1·1 | 11·9±0·8 | 0·62±0·03 | 1·63±0·03 | 1·41±0·24 | 2·63 | 2·28 |
| Peritoneal | 4 | 5 | 17·4 | 89·4±0·9 | 9·0±0·9 | 0·81±0·08 | 1·24±0·07 | 1·62±0·07 | 1·53 | 2·00 |
| Inguino | 9 | 5 | 38 | 89·5±0·7 | 8·6±0·6 | 0·43±0·02 | 1·00±0·51 | 0·87±0·08 | 2·52 | 2·02 |
| Peritoneal | 9 | 5 | 68 | 93·8±0·3 | 5·9±0·3 | 0·44±0·03 | 0·51±0·04 | 0·80±0·10 | 1·18 | 1·82 |
| Subcut. | 9 | 2 | | 87·2 | 11·8 | 0·57 | 1·25 | | 2·18 | 2·19 |

Table 4.25.

The effect of age on the growth of chemical components in the adipose tissue in the inguino-crural and peritoneal depots of the Aylesbury Duck.

| Age (weeks) | Depot | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Inguino-crural | | | | | Peritoneal | | | | |
| | Total fat (g) | Total fat-free wt. (g) | Total Protein (g) | Total K (mmoles) | Total Na (mmoles) | Total fat (g) | Total fat-free wt. (g) | Total Protein (g) | Total K (mmoles) | Total Na (mmoles) |
| $2\frac{1}{2}$ | 7·9 | 2·0 | 0·3 | 0·10 | 0·20 | 4·1 | 1·4 | 0·26 | 0·06 | 0·12 |
| 4 | 22·7 | 3·8 | 0·38 | 0·16 | 0·42 | 15·5 | 1·9 | 0·28 | 0·14 | 0·22 |
| 9 | 34·0 | 4·0 | 0·36 | 0·16 | 0·38 | 63·7 | 4·3 | 0·54 | 0·30 | 0·35 |

4.1). Thereafter however the peritoneal tissue contained more fat than the other depot. Whereas the value for the sodium/potassium ratio tended to increase with age in the inguino-crural depot, the opposite occurred in the peritoneal depot.

Collagen was estimated to represent 10% or more of the total protein of the tissue.

Discussion.

The composition of the adipose tissue of the duck was very similar to that of other species (Table 4.26). The results from the 9 week old duck were in particularly good agreement with those from the mouse (Thomas, 1962). Human adipose tissue however contained proportionately greater amounts of protein and sodium which indicated the presence of greater amounts of connective tissue and a greater extracellular space respectively. Certainly estimates of from 15% (Morse and Soeldner, 1963) to 20% (Martinsson, 1967) indicate that the size of the extracellular space in human adipose tissue is relatively large.

Although the most striking difference between the composition of adipose tissue and other body tissues, is its high fat content, conspicuous differences were also apparent in the non-fat components. Thus both in the adipose tissue from the duck and other species, the sodium/potassium ratio was higher than that found either in skeletal muscle of liver (Tables 4.24, 26). Such a result would be consistent with the presence of a greater vascular supply to the adipose

Table 4.26. A comparison of the composition of adipose tissue from various species, and the composition of some tissues from the fowl.
D.F.F.R. = Dried fat-free residue.

| Tissue | Age and Condition | Species | Source Ref. | Total fat (g/ 100g) | Total water (g/ 100g) | $K^+$ (meqv/ 100g) | $Na^+$ (meq v/ 100g) | Total Protein (g/ 100g) | Na/K | Protein /K |
|---|---|---|---|---|---|---|---|---|---|---|
| Epididymal | Adult, normal | mouse | Thomas (1962) | 90·8 | 7·5 | 0·44 | 0·80 | 0·79 | 1·83 | 1·80 |
| " | Adult, obese | mouse | " | 92·8 | 6·5 | 0·48 | 0·69 | 0·75 | 1·43 | 1·53 |
| Omental | lean | human | " | 83·5 | 14·4 | 0·62 | 1·55 | 3·2 | 2·50 | 5·15 |
| Subcut. | obese | human | " | 88·2 | 9·6 | 0·40 | 1·28 | 2·0 | 3·20 | 5·00 |
| " | normal | human | " | 87·9 | 10·2 | 0·42 | 1·29 | 2·1 | 3·28 | 5·00 |
| " | normal | human | Martinsson (1967) | 86·0 | 8·9 | | | 1·63 | | |
| Omental | normal | human | Morse & Soeldner (1963) | | 17·5 | 0·44 | | | | |
| Abd. Wall | " | " | " " | | 13·7 | 0·41 | | | | |
| Breast | " | " | " | | 18·6 | 0·77 | | | | |
| | normal | " | " | | 15·4 | 0·44 | | | | |
| | obese | " | " | | 15·1 | 0·39 | | | | |
| Epididymal | Fat fed | rat | Peckham et al. (1962) | 93-90 | 6·5-10 | | | 1·1-0·8 (D.F.F.R) | | |
| " | normal | " | " | 81-86 | 11-13 | | | 1·4-1·1 (D.F.F.R) | | |
| Abdominal | normal | mouse | Hausberger and | 79 | 18·4 | | | 2·8 (D.F.F.R) | | |
| " | obese | " | Hausberger (1957) | 89 | 9·4 | | | 1·1 | | |
| Abdominal | normal | pigeon | Goodridge & Ball (1966) | | | | | 1·09 | | |
| | 7-day old | fowl | Goddridge (1968a) | | | | | 0·95 | | |
| | 28-day old | " | " | | | | | 1·27 | | |
| Subalar | | Thrush | Hicks (1967) | 90·4-89 | 11·0-7·6 | | | 2·6-1·9 (D.F.F.R) | | |

(Contd.)

Table 4.26 (Contd.)

| Tissue | Age and condition | Species | Source Ref. | Total fat (g/ 100g) | Total water (g/ 100g) | $K^+$ (meqv/ 100g) | $Na^+$ (meqv/ 100g) | Total Protein (g/ 100g) | Na/K | Protein/ K |
|---|---|---|---|---|---|---|---|---|---|---|
| Inguinal | 44 day old | Rat | Benjamin et al. | 58 | | | | 6·9 | | |
| " | 291 day | " | " (1961) | 84 | | | | 3·7 | | |
| Testicular | 44 day | " | " | 74 | | | | 7·0 | | |
| " | 291 day | " | " | 93 | | | | 1·4 | | |
| Subcut. | 28 day | Pig | McMeekan (1940) | 76·8 | 18·5 | | | 4·7 (D.F.F.R.) | | |
| " | 126 day | " | " | 94·3 | 3·8 | | | 1·9 (D.F.F.R.) | | |
| Breast muscle | 70 day old | fowl | Draper (1968) | 1·2 | 72·6 | 11·5 | 3·6 | 23·4 | 0·31 | 2·02 |
| Thigh muscle | " | " | " | 4·8 | 74·6 | 10·3 | 4·5 | 19·1 | 0·44 | 1·86 |
| Liver | " | " | " | 4·3 | 69·7 | 8·9 | 6·7 | 19·8 | 0·75 | 2·22 |

tissue (see Discussion in Section 4.2.1.). Although the sodium/potassium ratio in tissue decreases during normal growth (Draper, 1968, see also Section 4.1), an unexplained increase in this parameter occurred in one depot (the inguinocrural) during growth. In contrast the protein/potassium ratios conformed to the values obtained for other tissues during growth (Table 4.24, Draper, 1968).

Considerable changes in the composition of adipose tissue normally occur during growth (Benjamin et al., 1961; Boshart et al., 1962; Peckham et al., 1962; and McMeekan, 1940). During early growth in the rat the proportion of fat in the adipose tissue increases towards a stable final concentration which is attained by the time the body growth rate has begun to plateau (Peckham et al., 1962; Benjamin et al., 1961). This final fat concentration may vary from depot to depot (Benjamin, et al., 1961). Further fat deposition produced either by hypothalamic lesions, insulin treatment or hereditary processes can be achieved without any significant effect on the composition of the adipose tissue (Hausberger and Hausberger, 1957, 1958). Similarly in the mouse and man, tissue from obese individuals was found to be no different in composition to that in the normal animal (Thomas, 1962), and dietary induced obesity in the rat produced only marginal changes in the composition of the adipose tissue (Peckham et al., 1962). In contrast to these findings premigratory fat deposition in birds is accompanied by considerable changes in the composition of the adipose tissue (Odum, 1965; Hicks, 1967).

Changes in the composition of adipose tissue only indicate changes in the rate of growth of the lipid and non-lipid components relative to each other. It is however important to know the absolute rates of growth of these components in order to understand the processes involved in fat deposition. It has been shown in the duck that although the non-lipid component becomes proportionately smaller during growth, in absolute terms they increase. Consequently deposition in this tissue is accompanied by cellular growth, Liebelt (1959) investigating the growth of the individual fat depots in different strains of mice has shown that the growth of the fat-free component of the depot followed a pattern similar to the growth of other body organs. Growth of this fat-free component was allometrically related to body weight, but appeared to be independent of the rate of fat deposition occurring in the depot. Sexual and genetic factors were found to be important in determining the comparative growth rates of the fat and fat-free components of the depots and consequently the final composition of the adipose tissue in the various depots. A somewhat similar result has been obtained for the fowl (Liebelt and Eastlick, 1954). Early growth of the fat-free component of a depot was found to be allometrically related to body weight, but again such growth appeared to be independent of the rate of fat deposition in the depot. Interestingly the growth coefficient for the fat-free component was considerably less than unity (see Section 4.1).

The increase in the fat-free or cellular component of the adipose tissue, which occurs during normal growth, appears to be achieved by both hypertrophy of the adipocyte (Goldrick, 1967b) and cellular multiplication within the adipose tissue (see Discussion in the preceding section).

In the adult animal since obesity can arise without changing adipose tissue composition it is apparent, that the fat-free component must be capable of growth in the adult. This observation is made with the qualification that changing amounts of collagen in the tissue may also influence the size of the fat free component. Mitosis can certainly be induced in the adipose tissue from the adult (see Discussion in the preceding section).

It has been claimed that premigratory fat deposition in the bird is accomplished without any change in the weight of the fat-free component of the fat depots (Odum, 1965; Hicks, 1967). However subsequent results (King, 1967) have indicated that in one species at least, such fattening is accompanied by an increase in the fat-free component.

Less is known about the changes occurring during fasting. Although typically the fat content of the adipose tissue is reduced (Pandazi et al., 1960) it is not known whether there is a corresponding decrease in the fat-free mass. Investigations in which the effect of fat reduction on cell number within a depot have beendetermined, have produced conflicting results (Zingg et al., 1962; Peckham et al., 1962).

In conclusion it appears that fat deposition during

growth, or in the adult, is generally accompanied by the growth of the fat-free or cellular component of the adipose tissue. However the extent of this growth may vary with the species, the depot, the sex and the conditions inducing fat deposition.

## 4.3. In vivo studies of the action of insulin and growth hormone in the immature fowl and duck.

In homoiotherms, de novo synthesis of lipid from carbohydrate occurs mainly either in the liver or adipose tissue (Wertheimer and Shapiro 1948, Wertheimer and Shafrir 1960). This process of lipogenesis in mammals is largely controlled by the action of insulin. In addition insulin also inhibits both basal and hormone-stimulated lipolysis, and by increasing glucose uptake and consequently the availability of glucose to the cell, fatty acid esterification is enhanced. The important regulatory role of insulin in mammalian lipogenesis is now firmly established, and the literature associated with this is reviewed in detail in Section 2.

Since the pancreas is regarded as the primary source of insulin in homoiotherms it was surprising that pancreatectomy had little effect in birds such as fowl and duck. (Langendorf 1879, Minowski 1892, Weintraub 1894, Kausch 1896, Seitz and Ivy 1929, Mirsky et al. 1941, and Mirsky and Gitelson 1957). This indicated that the role of insulin was relatively unimportant in avian carbohydrate metabolism. However exceptions to these findings are the carnivorous birds, such as the hawk and owl (Minowski 1892, Weintraub 1894 and Nelson et al. 1942), and the goose (Mirsky and Gitelson 1958). Here removal of the pancreas produced a typical diabetes mellitus.

Histological examination of the fowl pancreas (Oakberg

1949, and Mikami and Ono, 1962) indicated that the insulin producing beta cells were in islets separate from the glucagon producing alpha cells. The beta islets were found to be fairly evenly distributed throughout the pancreas. The alpha islets however were mainly restricted to the splenic and third lobes (Mikami and Ono, 1962). In the fowl pancreas alpha islets were slightly less common, but generally much larger than the beta islets (Mikami and Ono, 1962), and there was an overall impression that alpha islet tissue predominated. Analysis of the avian pancreas indicated the presence of an abnormally high amount of glucagon both in relation to the amount of insulin present and in comparison with the normal glucagon content of the mammalian pancreas (Haist, 1944; Vuylteke and De Duve, 1953). The splenic lobe is usually in the form of a small pale nodule shaped organ attached to the main body of the pancreas by a thin strip of tissue, and lying parallel to the ducts to the gall bladder and liver (Mikami and Ono, 1962, and Kaschula et al., 1965). Mialhe (1958) has suggested that many of the results reported from experiments with pancreatectomised birds may well be invalid, since in such cases there was no evidence that the splenic lobe had been removed. Complete pancreatectomy in the duck results in death following the severe hypoglycaemia, although the blood glucose could temporarily be maintained at normal levels with glucagon replacement (Mialhe, 1958). Removal of the splenic and third pancreatic lobes, in the fowl, produces a similar effect and again the bird could be kept alive as long as glucagon replacement was continued (Mikami

and Ono, 1962). These findings indicate that in some birds glucagon may play a more important role than insulin in the regulation of carbohydrate metabolism. In Not only are the glucagon-insulin roles in the regulation of carbohydrate metabolism different, at least in some birds, compared to mammals, but recent work indicates the presence of an extrapancreatic site for insulin production (Mirsky et al., 1964). This makes the past work on pancreatectomy in birds even more difficult to interpret.

The purpose of the experiments described in this section has been to investigate some aspects of the *in vivo* action of insulin, particularly that which is related to lipid metabolism, in immature birds. The latter were chosen to avoid interactions with reproductive activity. *In vitro* effects are examined in the following section. Since the published work discussed above does not indicate any difference in the regulatory action of the pancreatic hormones between ducks and fowl, the latter have been used in these experiments when the former were not available.

### 4.3.1. The effect of alloxan on the glucose tolerance of the immature fowl.

Alloxan (mesoxalylurea, HN-CO-CO-CO-NH-CO) is a cytotoxic agent which causes selective and permanent destruction of the beta cells in the mammalian pancreas (Goodman and Gilman, 1958). Its action is rapid, and disruption of cell function and structure may be complete within 24 hours. No completely satisfactory explanation has been given for the selective action of alloxan. Its cytotoxic

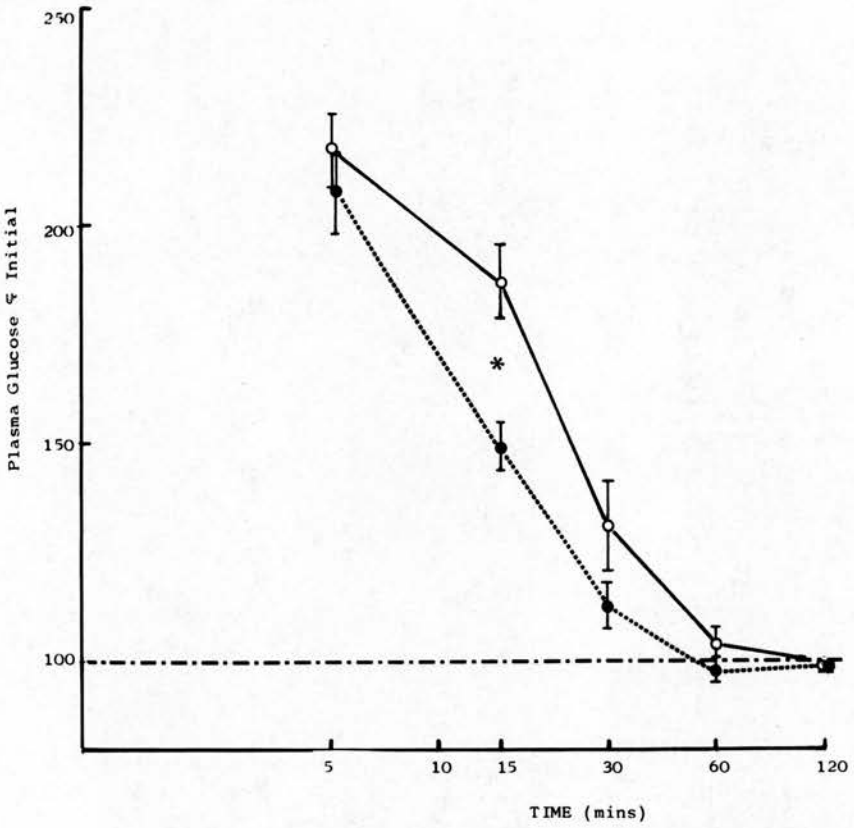
action is associated with its sulph-hydryl oxidising powers, and it has been postulated that in cells other than the beta cells glutathione is present in sufficient amounts to reduce the alloxan, and thus protect the enzyme systems from its destructive action (Ogilvie, 1952).

Associated with the alloxan induced necrosis of the beta tissue is the induction of a hypoglycaemia arising from a reduction of immunoreactive plasma insulin levels. (Morgan and Lazarow, 1965). Alloxan has consequently proved to be a very useful experimental tool in obtaining knowledge of the in vivo action of insulin in mammals. Other related (dehydroascorbic acid) and unrelated (8-hydroxyquinoline and diphenylthiocarbazone) compounds have been reported to induce necrosis of the beta cells of the mammalian pancreas (Ogilvie, 1952).

Since the discovery of the specific cytotoxic action of alloxan by Dunn and coworkers in 1943, numerous attempts have been made to induce diabetes in birds using this compound (Goldner and Gomori, 1945 on pigeons; Mirsky, 1945 on ducks - mallard, canvasback and "white"; Scott et al., 1945, on horned and barn owls, Pekin ducks, pigeons and cockerels; Mirsky and Gitelson, 1957, on fowl and Pekin ducks.) Using blood glucose alone as a criteria for the destruction of the beta cells, alloxan was shown to have no effect in the birds that survived treatment, although instances of degenerative changes in the beta tissue were reported.

Although the results obtained using alloxan were

Figure 4.28




| Initial Plasma Glucose mg/100ml | | | |
|---|---|---|---|
| Normal | Alloxan | t | P |
| 225 ± 8 (6) | 231 ± 7 (10) | 0.541 | 0.10 |

Glucose tolerance of Alloxan treated and control birds. Glucose (20% in physiol. saline) was given intravenously. Dose 1g/kg. Values means ± S.E.M. N = 10 for alloxan treated birds, 6 for control birds.

* $P < 0.001$

—○— Normal

·····●····· Alloxan

negative in birds, the drug has not been tested on immature subjects, and its effect has not been examined by means of a functional test, such as a glucose tolerance test. It was therefore considered worthwhile to investigate the diabetogenic properties of alloxan in the immature fowl as the destruction of beta tissue, if obtained, would afford a valuable opportunity to investigate the *in vivo* role of insulin in lipogenesis.

Alloxan was administered to 8 week old "J" line brown leghorns, which had been fasted for the previous 48 hours, since this procedure had been found to make mammals more susceptible to the action of alloxan (Goodman and Gilman, 1958). Since effective dose levels of alloxan are generally close to the lethal dose, a range of dose levels about the lethal level were given (Table 4.27.1). Death arising from the treatment occurred within 48 hours, and frequently earlier with higher concentration of alloxan. The surviving birds appeared to be outwardly normal and healthy. Four weeks after alloxan injection both body weight and plasma glucose levels were normal (Table 4.27.2, Fig. 4.28). Surprisingly an intravenous glucose tolerance test carried out at this time indicated that the response of the treated birds was slightly superior to that of the controls.

Histological examination of the pancreases of both experimental and control groups, several days after the glucose tolerance test showed that there was no demonstrable difference in the morphology of the alpha and beta islets in the alloxan treated birds.

Table 4.27.1.

Dose levels of alloxan given intravenously to 8 week old "J" line brown leghorns. Controls were uninjected.

| Dose mg/kg | No. of birds injected | No. of birds surviving |
|---|---|---|
| 500 | 2 | 0 + |
| 400 | 4 | 1 o |
| 300 | 5 | 3 o |
| 270 | 3 | 3 |
| 250 | 3 | 3 |
| Controls | 6 | 6 |

\+ deaths within 24 hours.

o deaths within 48 hours.

Table 4.27.2.

Body weight of treated and control birds 4 weeks after injection of alloxan. Means ± S.E.M. ' ' in brackets.

| Controls | Alloxan | t | P |
|---|---|---|---|
| 1080 ± 85 (6) | 1076 ± 56 (10) | 0·040 | > 0·10 |

Discussion.

These results show that alloxan treatment is ineffective in inducing diabetic symptoms in the immature fowl. What could perhaps be interpreted as a superior or improved glucose tolerance in the alloxan treated birds, may have been due to some residual effects of kidney damage. Alloxan produces a considerable increase in urate production in the pigeon (Goldner and Gomori, 1945). These authors found that in birds which died as a result of the alloxan treatment, a whitish layer identified as sodium urate was found associated with all the serous membranes, particularly those of the pericardium, pleura and the surface of the liver. The kidneys were also extensively infiltrated with urate crystals, which had caused dilation of the tubules and distension of the interstitial tissue. These changes were only rarely associated with any signs of degenerative changes in the pancreas. In the experiments described in this section similar whitish granular deposits were found in those birds which died as a result of the alloxan treatment, although no such deposits were observed in the tissues of those birds which survived the treatment. The organs of the surviving birds were entirely normal in appearance. Renal lesions involving the tubules frequently occur when high doses of alloxan are given to mammals (Goodman and Gilman, 1958) although such damage may be subsequently repaired, and the kidney function restored to normal. Kidney malfunction may therefore temporarily accompany the more permanently induced lesions in the pancreas. Renal damage

appears to be independent of the effect of alloxan on the pancreas, and could account for the improved glucose tolerance in the experimental birds. In these birds the rate of loss of glucose from the blood was significantly greater only at higher concentrations, which could be caused by tubular damage which had lowered the renal threshold for glucose (the renal threshold for glucose in the normal fowl is approximately 160% of the plasma glucose value of the fed bird, Martindale, personal communication).

Although no lesions or abnormalities were observed in the beta cells of the alloxanised birds in this experiment, Mirsky (1945) reported that lesions developed in those ducks which survived for more than 12 hours after alloxan treatment, irrespective of the dose given (50-715 mg/kg). However in spite of these lesions no significant change in blood glucose was observed. Scott et al. (1945) found no sign of islet necrosis in ducks, owls, pigeons or cockerels, although similar dose levels were given to those used by Mirsky (1945). Even when the dose was repeated daily for seven or more days no change in blood glucose was observed, although slight signs of hydropic degeneration in the islets of the owl, pigeon and duck were reported. Unfortunately none of these authors have published any direct evidence of islet changes induced by alloxan. The avian pancreas has few beta islets, and the not uncommon presence of large foci of lymphocytes must make the identification of any degenerative changes difficult to establish. Thus it must be concluded that there is little evidence to suggest that alloxan can affect

avian pancreatic islets.

Since some species of bird can be made diabetic by removal of the pancreas (Nelson et al., 1942; Mirsky and Gitelson, 1958) it might be expected that such species could be more susceptible to the cytotoxic action of alloxan. Scott et al. (1945) however found that this was not the case, and that alloxan was equally ineffective in these species. This suggests that the cytotoxic action of alloxan is independent of the status of insulin in the glucoregulatory system of the bird. The basis for the resistance of the avian beta cells to the destructive action of alloxan is still unknown.

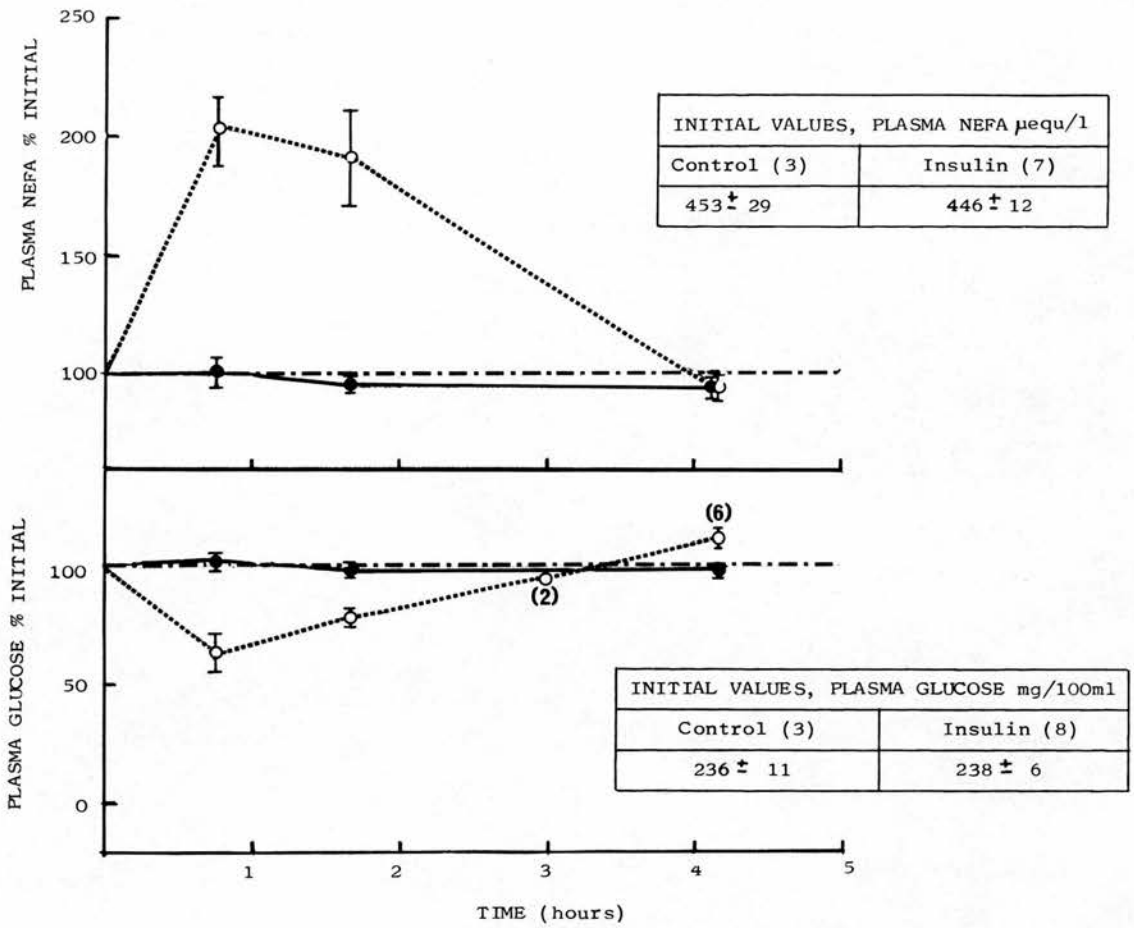
4.3.2. The in vivo effects of insulin and tolbutamide on plasma glucose and NEFA in the immature fowl.

It has been suggested that in the normal mammal "the relationship between the glucose and fatty acid metabolism is reciprocal and not independent" (Randle et al., 1964). This relationship has been termed the glucose fatty acid cycle by these authors, and the details of this cycle have been discussed in section 2. This cycle constitutes a primitive system for the regulation of glucose levels in the mammal. When glucose levels start to decline as a result of exhaustion of available glucose, lipolysis is no longer inhibited, and utilisation of fatty acids is increased. This temporary switch to fatty acid utilisation causes inhibition of both glycolysis pathways and the oxidation of pyruvate. Consequently the inhibition in both uptake and utilisation of glucose, can eventually lead to the restoration or return

of glucose levels to normal. In summary, this cycle is based on a reciprocal relationship in that, if glucose is being used by the cells, lipolysis is inhibited and that, if fatty acids are being mobilised and oxidised then utilisation and uptake of glucose are inhibited.

Heald et al. (1965) reported a contrary relationship between glucose and fatty acid in the fowl. When glucose uptake into the cells was increased by glucose loading or insulin treatment, the plasma NEFA remained unchanged in the former case, and increased in the latter. In both cases, in mammals the increased glucose uptake is always accompanied by a corresponding fall in plasma NEFA (Dole, 1956; Armstrong et al., 1961). Heald's result was particularly surprising since glucose utilisation in the fowl is if anything greater than that in mammals (Riis and Herstad, 1967, Annison et al., 1964). Both glucose loading (Emslie and Henry, 1933) and in vivo insulin (Opdyke, 1942) results in increased glycogen deposition in the fowl. Heald et al. (1965) accounted for the elevated NEFA levels obtained with insulin by postulating that hypoglycaemia stimulated the release of some factor which stimulated lipolysis. They tentatively suggested that this factor was glucagon.

Since their results were published, it has been discovered that a powerful hypoglycaemic agent, tolbutamide, is capable of blocking the lipolytic action of glucagon (Brown and Stone, 1967). It therefore became of some interest to examine the effect of this compound, when administered in vivo on the plasma NEFA of the fowl.




The effect of intravenous insulin (7.5U/kg) on plasma glucose and NEFA in fed 13 week-old brown leghorn pullets. Controls saline injected. Values are means ± S.E.M.

····○····Insulin treated

—●—Controls

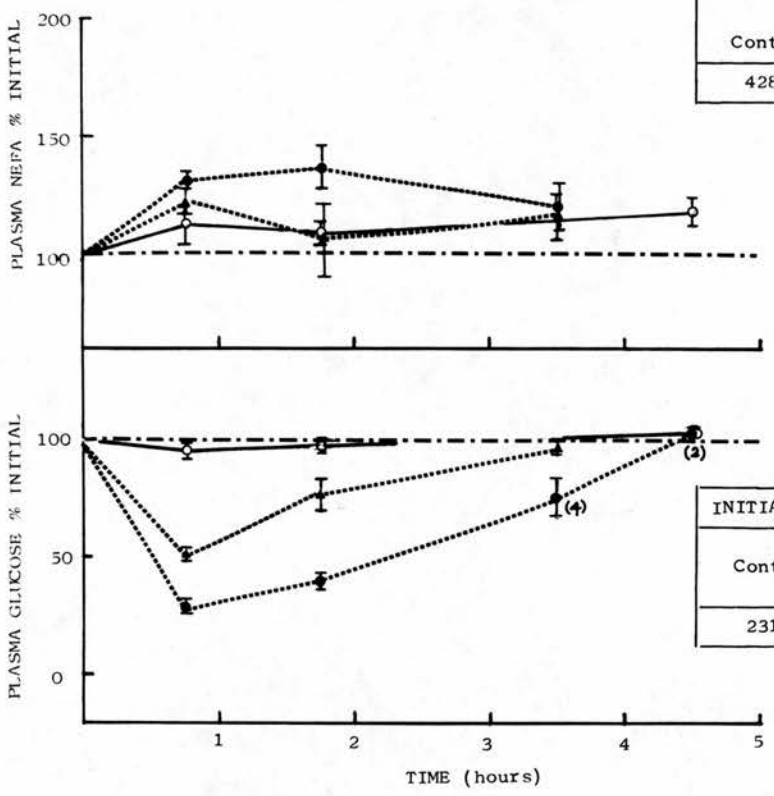
Figure 4.29

Tolbutamide is a sulphonylurea (1-butyl-3-(p-tolyl-sulphonyl)urea, $H_2N$-C$_6$H$_4$-$SO_2NHCONHCH_2(CH_2)_2CH_3$) used commonly in the therapy of maturity onset diabetes. In the mammal, it is effective only in the presence of functional islet tissue in the pancreas (Miller and Craig, 1956; Houssay and Penhos, 1956; Chen et al., 1946, Mirsky et al., 1956) and its hypoglycaemic action is thought to be caused by both prolonging the life of the circulating insulin (Mirsky et al. 1956) and increasing its rate of release from the pancreas (Randle, 1964; Volk and Lazarus, 1958). Its hypoglycaemic action has been frequently demonstrated in birds (Hazelwood and Lorenz, 1957; Mirsky and Gitelson, 1957, 1958).

The findings of Heald et al. (1965) have so far been unconfirmed. Therefore their experiments on the effect of *in vivo* insulin on plasma NEFA have been repeated, on immature "J" line brown leghorn females.

The effect of in vivo insulin on plasma glucose and NEFA

Intravenous injection of bovine insulin (crystalline, glucagon-free, 7·5 u/kg) into fed 13-16 week old pullets produced a reduction in the level of plasma glucose which returned to normal within four hours (Fig. 4.29). This was followed by a subsequent slight but significant rise in plasma glucose. There was also an associated considerable increase in plasma NEFA which reached a maximum within the first two hours. The decline in plasma NEFA subsequent to this was parallel to the return to normal of the plasma glucose. At no time during the experiment did any of the experimental birds show any signs of hypoglycaemic stress.




| INITIAL VALUES | PLASMA NEFA µequ/l | |
|---|---|---|
| | TOLBUTAMIDE | |
| Control (6) | 25mg/kg (3) | 50mg/kg (6) |
| 428 ± 36 | 446 ± 27 | 483 ± 34 |

| INITIAL VALUES | PLASMA GLUCOSE mg/100ml | |
|---|---|---|
| | TOLBUTAMIDE | |
| Control (5) | 25mg/kg (3) | 50mg/kg (6) |
| 231 ± 4 | 221 ± 12 | 229 ± 3 |

The effect of Tolbumatide on plasma NEFA and glucose, in the fed brown leghorn pullet. Number of birds in each group shown in brackets. Values are mean ± S.E.M. Controls, saline injected.

—○— Control
···●··· 25mg/kg Tolbutamide
···▲··· 50mg/kg Tolbutamide

Figure 4.30

The effect of in vivo tolbutamide on plasma glucose and NEFA.

Treatment of fed 14-18 week old pullets with tolbutamide at two dose levels (25 and 50 mg/kg) produced in both cases a considerable hypoglycaemia (Fig. 4.30) which was greater than that induced with insulin in similar birds in the previous experiment. Four out of the six birds given the high dose became temporarily partially paralysed in the legs as a result of the hypoglycaemia, early in the experiment, but completely recovered soon after, and were completely normal by the end of the experiment. In spite of the considerable changes in plasma glucose only slight changes were observed in the plasma NEFA levels. Only at the high dose level was a significant increase in plasma NEFA measured, although this returned to normal by three and a half hours. The increase in NEFA was however very slight and considerably less than that observed accompanying a lesser hypoglycaemia induced with insulin.

Discussion.

The action of insulin in increasing plasma NEFA when given in vivo to the immature fowl confirms the results previously reported by Heald et al. (1965) and work subsequently published (Lepkovsky et al., 1967a). In mammals the fall in NEFA accompanying insulin induced hypoglycaemia is generally attributed to a decreased rate of fatty acid mobilisation arising from the antilipolytic effect of both the insulin and the increased cellular uptake of glucose (Armstrong et al., 1961, see also Section 2). The elevated

NEFA levels found in the bird arising from insulin treatment indicate the release of a lipolytic agent, which overrides any antilipolytic action of insulin or glucose uptake, if any such antilipolytic action occurs. By using agents which either depleted the adrenal medulla of catecholamines or which blocked their action, Heald et al. (1965) indicated that catecholamines were not involved in the plasma NEFA response to insulin. Further they demonstrated that since in vivo ACTH did not significantly alter plasma NEFA, although it elevated plasma glucose, adrenal corticoids were unlikely to have been involved in the insulin action on plasma NEFA. In vivo glucagon however caused a considerable elevation of plasma NEFA. Heald et al. (1965) therefore postulated that the insulin induced elevation of plasma NEFA was due to the secretion of glucagon stimulated by the insulin induced hypoglycaemia.

More recently glucagon has been shown to elevate plasma NEFA in geese, ducks, turkey and fowl (Grande, 1968). In vitro studies with avian adipose tissue have indicated that glucagon is the only hormone which possesses lipolytic activity when given in physiological concentrations (Carlson et al., 1964; Goodridge, 1964; Goodridge and Ball, 1965; and Langslow and Hales, 1969. See also Section 4.4.) Catecholamines, ACTH, growth hormone and adrenal corticoids were all found to be ineffective in mobilising fatty acids from in vitro preparations of avian adipose tissue. Catecholamines infused into young fowl were similarly without lipolytic activity (Carlson et al., 1964). These studies

provide support for the hypothesis of Heald et al. (1965) that glucagon is involved in the NEFA response to insulin treatment in the fowl, since glucagon is the only hormone which has been found to possess lipolytic activity in this or any species of bird.

In humans tolbutamide produces a hypoglycaemia which is accompanied by a depression of plasma NEFA (Bierman et al. 1957; Stone and Brown, 1966). In some subjects tolbutamide produced reduced plasma NEFA levels without lowering plasma glucose (Stone and Brown, 1966). Subsequent investigations of its lipolytic properties on rat epididymal fat pads in vitro, or free cells, demonstrated that tolbutamide reduced basal lipolysis in the absence of insulin and glucose, and that it inhibited the lipolytic action of growth hormone and dexamethasone. It also completely blocked the action of ACTH, theophylline and glucagon (Stone et al., 1966; and Brown and Stone, 1967). This latter group of substances all stimulate lipolysis by a cyclic AMP mediated activation of the adipose tissue lipase. It was suggested therefore that tolbutamide inhibits lipolysis by blocking or inhibiting pathways which lead to activation of the AT lipase. In this way its antilipolytic activity is similar to that of insulin.

The hypoglycaemic response obtained with tolbutamide in this experiment was similar to findings previously published (Hazelwood and Lorenz, 1957; Mirsky and Gitelson, 1957, 1958). The slight changes in plasma NEFA accompanying the tolbutamide induced hypoglycaemia probably result from the blocking action of this compound on the lipolytic activity

of glucagon. This result therefore gives further support to the hypothesis that insulin induced elevation of plasma NEFA is glucagon mediated.

In ducks the presence of the pancreas is not necessary for the production of hypoglycaemia with tolbutamide (Mirsky and Gitelson, 1957). This had lead to the suggestion that endogenous insulin was therefore not involved in tolbutamide action. However since Mirsky et al. (1964) have demonstrated the presence of an extrapancreatic site of insulin production in the duck, this conclusion now appears to be unlikely. An additional action of tolbutamide which could contribute to the reduced NEFA levels associated with hypoglycaemia in the fowl, could arise from a possible antilipolytic action of the endogenous insulin. Although fowl insulin has been isolated (Smith, 1966; and Kimmel et al., 1968) its effect either on lipolysis or lipogenesis has yet to be tested. However since mammalian insulins have no lipogenic or lipolytic activity on avian tissues (Goodridge, 1964; Goodridge and Ball, 1965). This antilipolytic action of tolbutamide mediated by endogenous insulin seems unlikely.

The experiments with insulin suggest that birds mobilise lipid during periods of increased glucose uptake. This state of affairs would appear to be rather peculiar. However Heald et al. (1965) have shown that when the fowl is glucose loaded, plasma NEFA levels remain unchanged. This indicates that the stimulus for glucagon secretion and consequently lipid mobilisation is reduced plasma glucose and not increased glucose uptake. The hypoglycaemia produced by

insulin may therefore represent an unreal situation in the bird. Even during prolonged fasting in the fowl a significant hypoglycaemia does not develop (Hazelwood and Lorenz, 1959). The constancy of NEFA levels during glucose loading suggests that basal lipolysis may not be affected by glucose uptake.

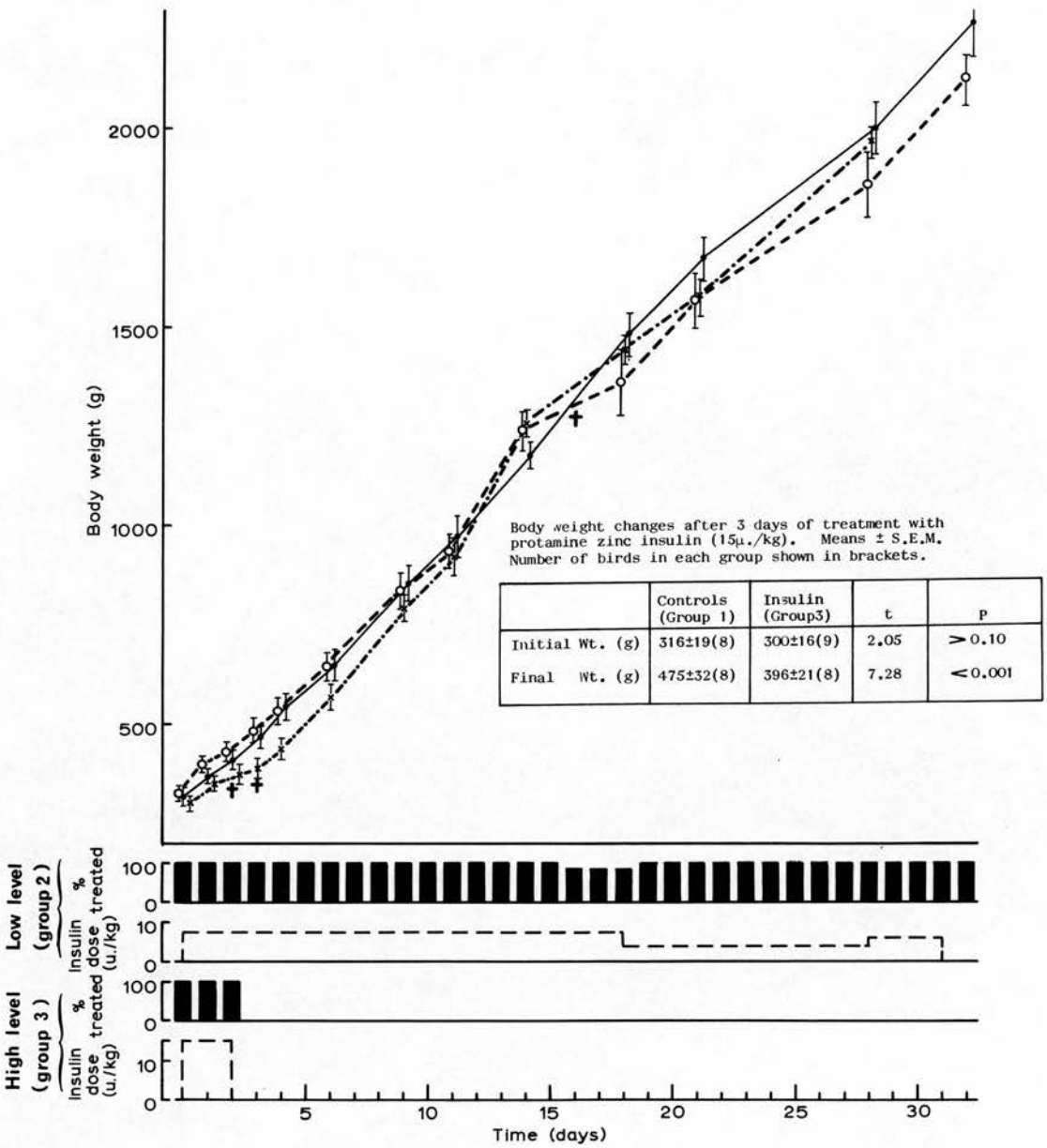
The differences in the response between birds and mammals to insulin, again reflects the different status of the pancreatic hormones in glucoregulatory control. In birds it appears that glucagon overrides insulin activity during hypoglycaemia, whereas the reverse is true for mammals. In man glucagon has been shown to be strongly insulinogenic, even at levels which fail to produce hyperglycaemia (Samols et al. 1966). This action of glucagon is consistent with the experiments of Sokal et al., 1966 in which it was shown that removal of the pancreas abolished glucagon-induced depression of plasma NEFA. The depressant action of glucagon in mammals has consequently been attributed to the overriding action of insulin.

The goose may represent an exception (apart from carnivorous birds which have not been as adequately investigated) to the system of glucoregulation by pancreatic hormones described for birds. Not only does pancreatectomy result in typical diabetes, but insulin in vivo apparently lowers plasma NEFA (Lepkovsky et al., 1967a). Glucagon given to the intact goose, however, elevates plasma NEFA, indicating that the action of this hormone in this species is similar to that in other birds.

4.3.3. Some effects of protamine zinc insulin on immature ducks.

Protamine zinc insulin (PZI) has the slowest onset and the longest duration of all the insulin preparations. The period of its maximum activity is between 12 and 24 hours after injection, and the overall duration of its activity is from 6-8 to 48-72 hours after injection (Goodman and Gilman, 1958). Given once or twice daily to rabbits and rats PZI substantially increased food intake and body weight (MacKay et al., 1940; Hoebel and Teitelbaum, 1966; Hausberger, 1958b). The excess ingested food was mainly converted into fat (Hausberger, 1958b). PZI induced hyperphagia may be primarily due to hypoglycaemia (Anand, 1967), increased glucose utilisation (Anand, 1967) or the effects produced by the direct stimulatory action of insulin on the lipogenic pathways in adipose tissue (Hausberger, 1958b).

Insulin has no effect on <u>in vitro</u> lipogenesis in avian liver or adipose tissue (Goodridge, 1964; Goodridge and Ball, 1965, see also Section 4.4). However the effect of long term treatment with insulin on lipogenesis in birds has not yet been investigated. To do this immature ducks were used since it was also the purpose of this experiment to investigate the possible role of insulin in fat deposition in these birds during growth. Since antibodies to insulin, which could neutralise its effect, may arise during long term treatment, blood samples were tested for these at the completion of the experiment. Immature Pekin-type ducks were used in all the experiments.




Body weight changes after 3 days of treatment with protamine zinc insulin (15μ./kg). Means ± S.E.M. Number of birds in each group shown in brackets.

| | Controls (Group 1) | Insulin (Group3) | t | P |
|---|---|---|---|---|
| Initial Wt. (g) | 316±19(8) | 300±16(9) | 2.05 | >0.10 |
| Final Wt. (g) | 475±32(8) | 396±21(8) | 7.28 | <0.001 |

The effect of protamine zinc insulin on body weight (mean ± S.E.M., n=8,8,9 initially for group 1, 2 and 3 respectively). The percentage of birds receiving daily insulin injection and the dose per day are indicated in the last four diagrams. Experimental groups, ●——● group 1, O– –O group 2, x- - -x group 3. † mortalities.

Figure 4.31

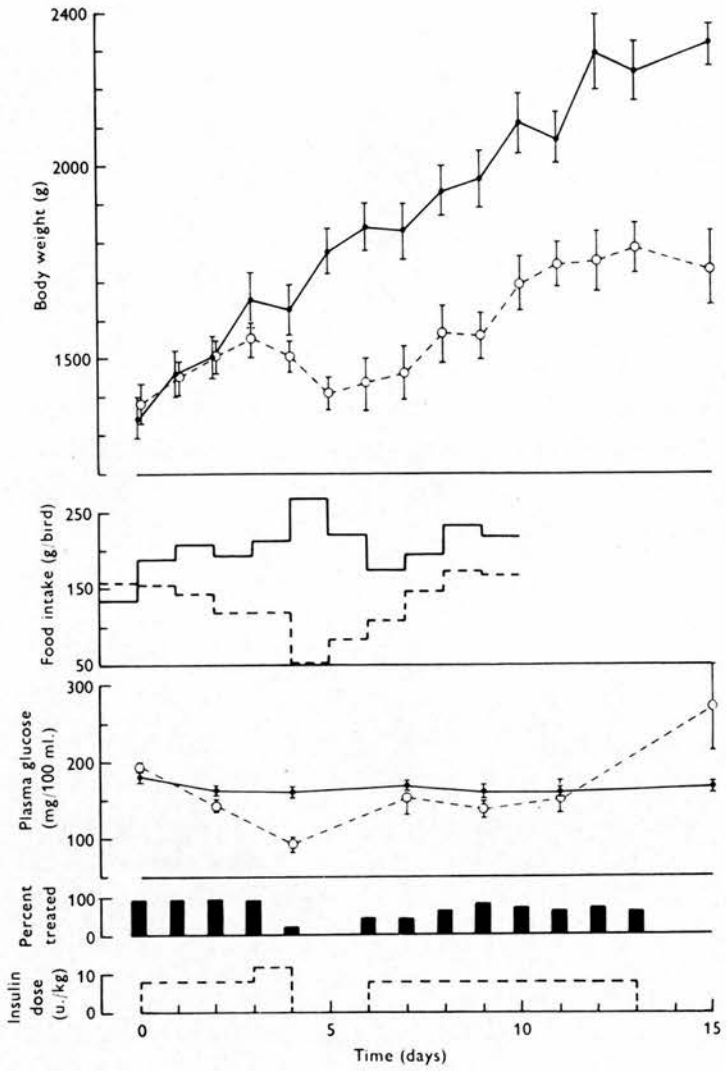
The effect of protamine zinc insulin on growth, food intake and fat deposition.

Initial trials indicated that a dose level of 20u/kg/day was invariably lethal to young ducks. PZI was therefore administered at two levels, a "low" level (Group 2, 7·5 u/kg/day) and a "high" level (Group 3, 15 u/kg/day) to 10 day old ducks.

The control group ~~were~~ was saline injected.

Since the action of this long acting form of insulin was difficult to control, when signs of hypoglycaemic stress became apparent it was necessary to discontinue or decrease the dose level of the PZI injections, until the bird returned to normal health. In spite of this precaution two deaths occurred in the group given the "high" insulin treatment for only three days (Fig. 4.31.) For this reason the high insulin treatment was completely discontinued. However significant depression of the growth rate had already occurred as a result of this "high" insulin treatment (Fig. 4.31). Following the cessation of this treatment the surviving birds, had a compensatory increase in growth rate which was subsequently normal (Fig. 4.31). This indicated that the insulin treatment produced growth impairment without causing any permanent injury to the birds.

The "low" insulin treatment caused a single mortality, 16 days after the commencement of treatment. At the same time 3 other birds showed signs of hypoglycaemic stress, and consequently the dose level for the whole group was reduced to 4u/kg/day, until all birds had recovered. Following this the insulin level was increased to 6u/kg/day for the remaining




The effect of protamine zinc insulin on body weight, food intake and plasma glucose in growing ducks (mean ± S.E.M., N = 8, for glucose n = 6). The percentage of birds receiving daily insulin injections and the dose per day are indicated in last two diagrams. Broken lines, insulin treated; continuous lines, saline injected controls.

Figure 4.32

duration of the experiment. This treatment had no effect on the growth rate (Fig. 4.31) or the final weight and composition of the treated birds (Table 4.28.1), apart from a marginally significant reduction in feather weight. However at the completion of the experiment plasma glucose levels were slightly but significantly lower in the experimental than the control birds (Table 4.28.2). Histological sections of the treated birds showed no abnormalities in islet morphology.

In a second experiment 30 day old ducks were used. The insulin dose was determined by the magnitude of the hypoglycaemic response. It was the intention of the treatment that only sufficient insulin should be given to produce a significant but slight degree of hypoglycaemia. On this basis the treatment was started at a dose level of 8u/kg/day which produced a marginally significant hypoglycaemia (Fig. 4.32). Consequently the dose level was increased to 12u/kg/day. The effect of this dose increase was to produce a very considerable fall in plasma glucose (Fig. 4.32), a further decrease in food intake and a sharp fall in body weight. At this time all the treated birds appeared normal, although 3 of them later showed signs of hypoglycaemic stress, and one of these subsequently died. The other two birds recovered entirely. The treatment was therefore temporarily discontinued and thereafter maintained at a level of 8u/kg/day. If birds became stressed by the treatment, the injections were temporarily discontinued for the particular bird until it made a complete recovery.

Table 4.28.1.

The effect of protamine zinc insulin on various body parameters. Means ± S.E.M., N = 6 for both groups. "Control" = Group 1; "Low Insulin" = Group 2.

| | Control | Low Insulin | t | P |
|---|---|---|---|---|
| Initial wt. (g) | 319·8±24·6 | 332·7±24·9 | 0·37 | >0·10 |
| Final wt. (g) | 2277·2±92·1 | 2126·0±65·1 | 1·35 | >0·10 |
| % Fat | 17·3±1·1 | 18·7±1·1 | 0·89 | >0·10 |
| % Subcut. Fat / Total Fat | 62·0±1·5 | 58·7±1·9 | 1·35 | >0·10 |
| % Skinned Carc. Fat / Total Fat | 25·2±1·3 | 27·3±1·8 | 0·94 | >0·10 |
| % Perit. Fat / Total Fat | 7·7±0·6 | 7·3±0·1 | 0·57 | >0·10 |
| Pancreas wt. (g/kg.b.w) | 2·55±0·15 | 2·81±0·24 | 0·92 | >0·10 |
| Liver wt. (g/kg.b.w) | 36·8±1·4 | 36·9±2·3 | 0·04 | >0·10 |
| Dried Feather wt.(g) | 66·5±3·2 | 54·3±3·5 | 2·54 | <0·05 >0·02 |

Table 4.28.2.

The effect of protamine zine insulin on plasma glucose. Blood collected on final day of experiment. Mean ± S.E.M., N = 6 for each group. "Control" = Group 1, "Low Insulin" = Group 2.

| | Control | Low Insulin | t | P |
|---|---|---|---|---|
| Plasma glucose (mg/100 ml) | 169·3±4·7 | 154·0±5·8 | 2·87 | <0·02 >0·01 |

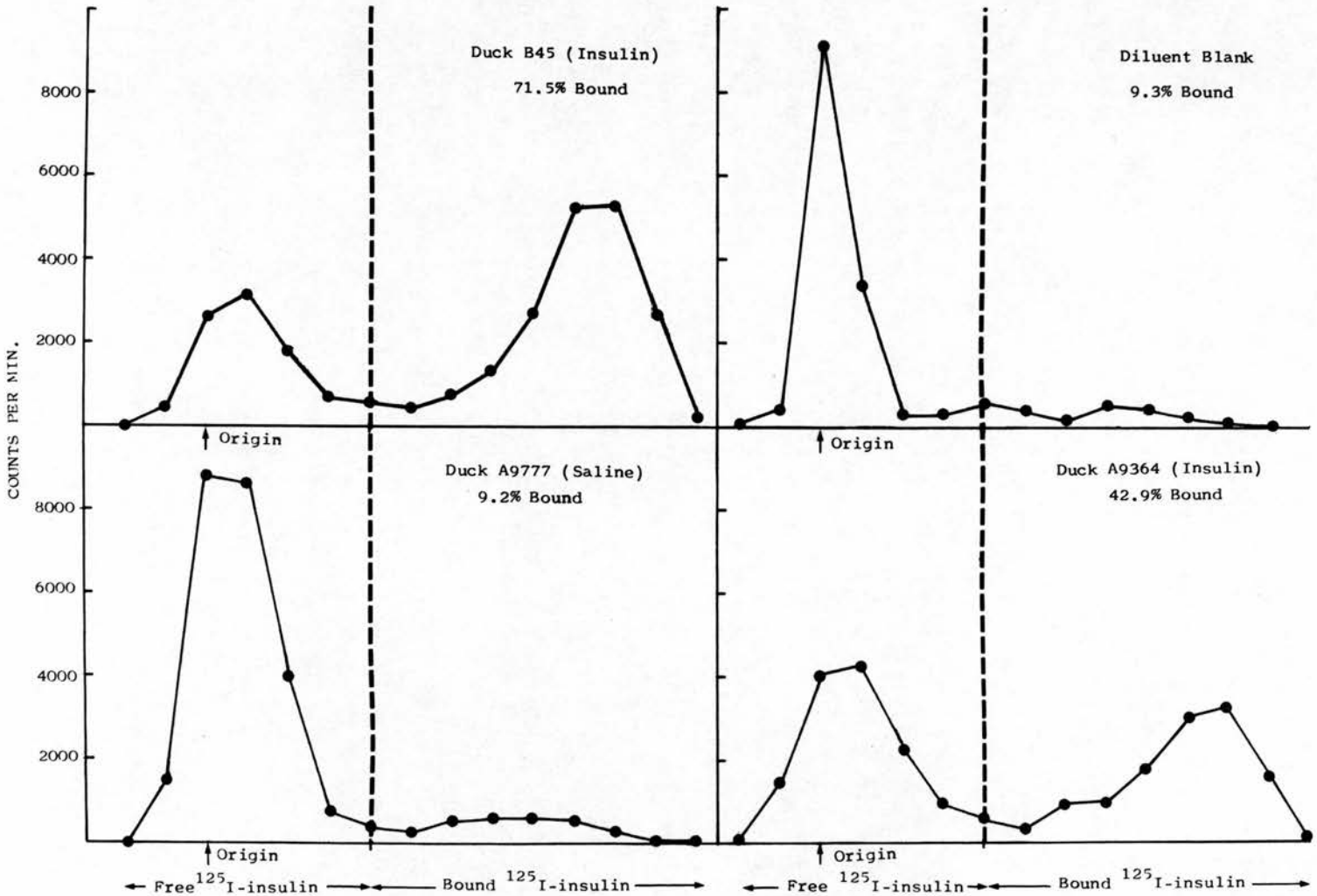



Figure 4.33

The detection of insulin antibodies in duck plasma following protamine zinc insulin treatment. This figure shows the separation of bound and free $^{125}$I-insulin using paper chromatography.

The high variability of the plasma glucose values in the latter half of the experiment were due to the higher values obtained from birds for which treatment had been temporarily discontinued. The symptoms of hypoglycaemic stress frequently persisted beyond the time that glucose values had returned to normal. At the end of the experiment several days after insulin treatment had been finally discontinued a considerable rise in plasma glucose was observed (Fig. 4.32).

Although the insulin treatment in this experiment had decreased food intake and consequently retarded growth (Fig. 4.32 and Table 4.29), it had no significant effect on fat deposition (Table 4.29). Feather growth however was retarded, and the relative weight of the viscera was greater than in the controls (Table 4.29).

The production of antibodies to exogenous insulin.

Blood from both insulin treated (low insulin group only) and control birds was collected at the end of the first experiment, and the plasma was tested for the presence of antibodies to porcine insulin. Plots of the chromatograms used for separating bound from unbound labelled insulin is shown in Fig. 4.33. Only two of the plasma samples from the insulin treated birds showed a significant binding capacity for the labelled porcine insulin (Table 4.30).

In the second experiment blood was collected from the insulin and control groups two days after the final insulin injection. Again only two plasmas bound significant amounts of labelled insulin (Table 4.31) Binding capacities

Table 4.29.

The effect of protamine zine insulin on various body parameters. Mean ± S.E.M., N = 7 for both groups.

| | Control | Insulin | t | P |
|---|---|---|---|---|
| Initial wt. (g) | 1363·7±58·5 | 1345·3±54·7 | 0·21 | >0·10 |
| Final wt. (g) | 2319·9±61·6 | 1806·1±72·3 | 5·50 | <0·001 |
| Length (cm) | 68·8±1·2 | 62·1±1·7 | 3·24 | <0·01 >0·002 |
| Wingspan (cm) | 68·7±1·6 | 61·2±2·1 | 2·82 | <0·02 >0·01 |
| % Fat | 18·4±0·5 | 16·3±1·5 | 1·35 | >0·10 |
| % Subcut. Fat / Total Fat | 63·3±2·3 | 60·9±1·4 | 1·01 | >0·10 |
| % Skinned Carc. Fat / Total Fat | 23·1±1·9 | 26·2± 1·8 | 0·96 | >0·10 |
| % Peritoneal Fat / Total Fat | 7·3±0·8 | 6·0±0·4 | 1·59 | >0·10 |
| % Water (f.f.b.w.) | 74·4±0·9 | 74·5±0·8 | 0·08 | >0·10 |
| Viscera (g/kg b.w.) | 118·4±2·9 | 137·3±5·9 | 2·86 | <0·02 >0·01 |
| Dried Feather wt.(g) | 70·9±3·2 | 49·7±2·5 | 5·21 | <0·001 |

Table 4.30.

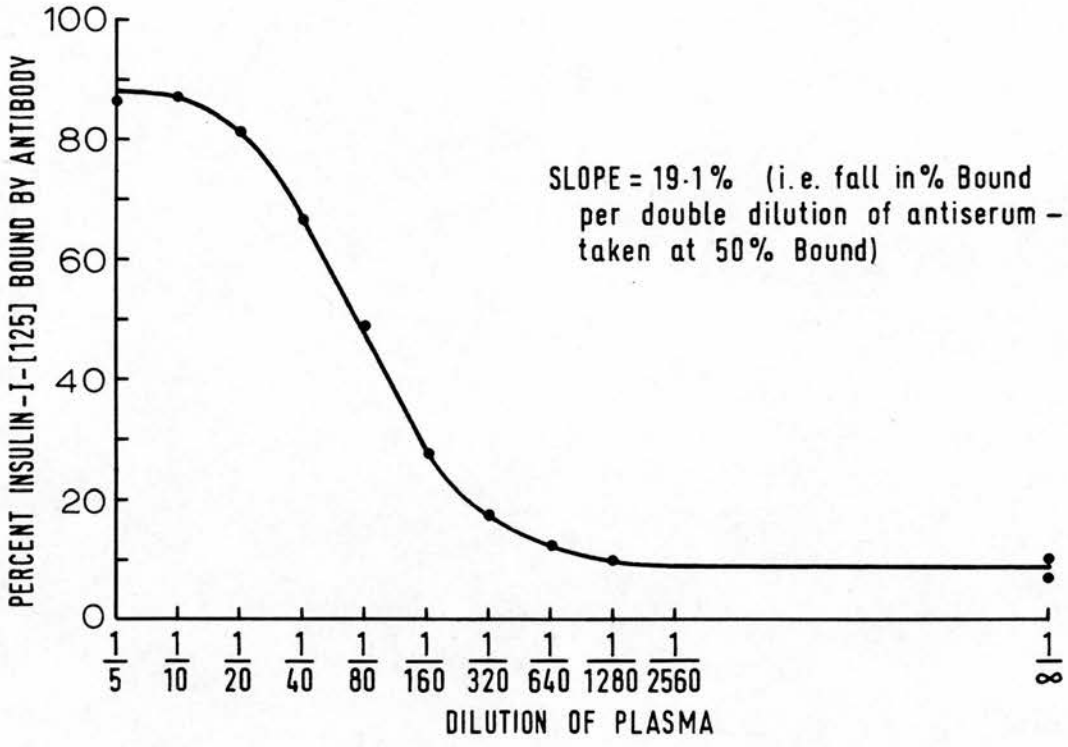
Insulin antibody levels in plasma after protamine zinc insulin treatment. "Control" = Group 1; "Low Insulin" = Group 3.

| Bird No. | Treatment | Percent $^{125}I$-insulin (1 ng/ml) bound to 1:10 diluted plasma | ~Binding capacity of undiluted plasma |
|---|---|---|---|
| A8025 | Control | 10·1 | - |
| A9362 | " | 10·2 | - |
| A9777 | " | 9·5 | - |
| A9596 | " | 9·2 | - |
| A9350 | Low Insulin | 14·8 | - |
| A9963 | " | 10·9 | - |
| A9642 | " | 16·9 | - |
| B45 | " | 71·5 | 150 mu/l |
| B9634 | " | 9·1 | - |
| A8694 | " | 42·9 | 80 mu/l |
| | Blank | 9·5 | - |

Table 4.31.

**Insulin antibody levels in plasma after protamine zinc insulin treatment.**

| Bird No. | Treatment | Plasma Glucose (mg/100 ml) | Percent $^{131}$I-insulin (1 ng/ml bound to 1:8 diluted plasma | ~ Binding capacity of undiluted plasma |
|---|---|---|---|---|
| 5397 | Control | 168 | 12·3 | - |
| 9094 | " | 174 | 12·6 | - |
| 5633 | " | 161 | 11·9 | - |
| 5394 | " | 160 | 11·5 | - |
| 5618 | " | 160 | 12·2 | - |
| 9430 | " | 168 | 12·0 | - |
| 5386 | Insulin | 216 | 11·0 | - |
| 5434 | " | 200 | 33·3 | 80mu/l |
| 5625 | " | 360 | 81·0 | 250mu/l |
| 9198 | " | 166 | 13·1 | - |
| 9594 | " | 250 | 15·8 | - |
| 9634 | " | 580 | 14·6 | - |
| 9298 | " | 228 | 15·7 | - |
| | Blank | | 10·0 | - |




Titration curve of antibodies to bovine insulin in plasma of duck A5625 with $^{125}$I-porcine insulin (final concentration 1ng/ml). Separation of bound from free insulin by paper chromatography.

Figure 4.34

of the plasmas which were determined approximately from these results indicate that the levels of antibody to insulin were of the same order in each experiment. Table 4.31 also shows the plasma glucose values for the same plasma samples which were also those used for the last points in the graph of glucose levels in Fig. 4.32. Titration curves were obtained for the two plasmas in Table 4.31 containing antibodies. Only one of these samples had a reasonable measure of avidity (Fig. 4.34). On examination of histological section of pancreas at the completion of the experiment, the islet morphology of the insulin treated birds was found to be normal.

Discussion.

These experiments indicated that protamine zinc insulin does not produce hyperphagia, weight gain or fat deposition in the immature duck. PZI, however did produce hypoglycaemia which, when of sufficient magnitude, was accompanied by a decreased food intake and growth rate. Although the dose levels of PZI required to produce this effect are close to the lethal level, growth impairment could be induced without causing any apparent permanent damage to the bird.

The absence of a growth promoting effect of protamine zinc insulin could not be attributed to the production of neutralising antibody. Although antibody to exogenous insulin was detected in the plasma of several of the insulin treated birds, it was insufficient to neutralise the effect

of the exogenous insulin (Yalow and Berson, 1961). However in one instance, A 5625, it had sufficient binding capacity and was sufficiently avid, that it could effectively bind all the endogenous insulin, if the levels of insulin in the duck are similar to those in the human (Hunter, personal communication). Consequently the presence of antibody could account for the high glucose value in this sample (Table 4.31). Mirsky et al. (1964) has shown that antibody to mammalian insulin was effective in producing hyperglycaemia in the duck.

PZl produces weight gain and increased fat deposition in mammals by increasing the food intake (Hoebel and Teitelbaum, 1966) and by its direct action of activating lipogenic pathways (Hausberger,1958b). The role of plasma glucose in regulating the food intake in mammals has been reviewed recently by Anand (1967), and Mayer and Thomas (1967). In the rat it appears that insulin produces hyperphagia by lowering the plasma glucose, which in turn activates the feeding centre in the hypothalamus. As yet there is no evidence that similar centres controlling the intake of food occur in the fowl (Lepkovsky et al., 1967b) or any other bird, except perhaps the goose (Monachan, personal communication). The finding that hypoglycaemia does not stimulate appetite in the duck indicates that glucose may not be involved in the regulation of food intake in this bird. Lepkovsky et al. (1965) obtained results similar to those described here, by injecting white leghorn pullets with PZl. Richardson (personal communication) has recently demonstrated that appetite in the fowl is not influenced by administration

of oral or intravenous glucose. Thus there is a considerable amount of evidence which indicates that plasma glucose levels are not important for the control of feeding behaviour in the fowl, and possibly the duck. The full implication of this observation as it relates to maintenance of energy balance in birds is considered in detail in a later section (Section 5.) It is interesting to note that glucose has not been found to be involved in the regulation of appetite in ruminants (Baile, 1968).

The second possible mode of action of PZl in increasing weight gain in mammals is by its influence on fat deposition. It seems clear that insulin has no important lipogenic action on avian liver or adipose tissue (see Section 4.4. and Section 2).

The depression of food intake and general retardation of growth induced by near lethal doses of PZl was similar to that obtained in the fowl by Lepkovsky et al. (1965). It is probable that these effects were nonspecific and due to the induction of a hypoglycaemia of such magnitude, that normal body function was impaired. In the previous section (4.3.2.) it has been shown that elevated plasma NEFA levels accompany insulin induced hypoglycaemia. This effect is thought to be mediated by glucagon. Increased glucagon and NEFA levels could provide an alternative explanation of the depression of food intake, and consequently the retardation of growth, produced by higher levels of PZl. There is some indirect evidence that elevated NEFA levels in the plasma can depress

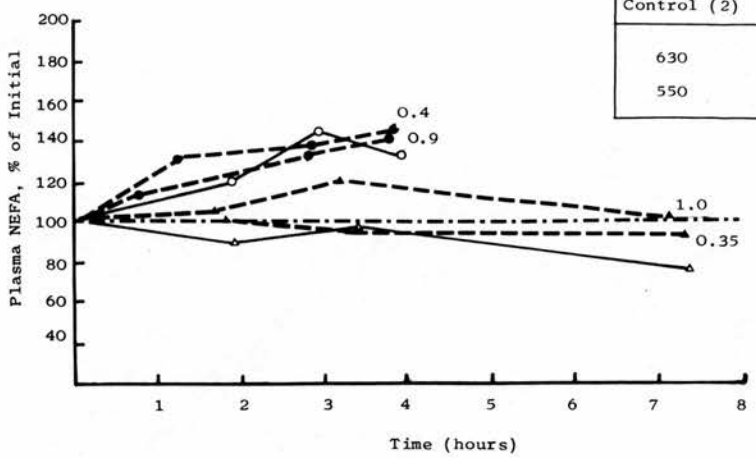
appetite in both mammals (Van Itallie and Hashim, 1960) and in the fowl (Brambila and Hill, 1967). Glucagon appears to depress the food intake and retard growth in the adult rat, (Salter, 1960) although it has no such effect in the young rat (Holloway and Stevenson, 1964). Although both the alpha and beta islets appeared normal in the insulin treated birds at the end of each experiment, the presence of high plasma glucose values, usually in the absence of insulin antibody, at the end of the second experiment, several days after the final insulin injection, suggests that elevated glucagon levels might have been produced in response to the continued insulin induced hypoglycaemia. Degranulation of beta islets which is sometimes produced in mammals by long term insulin treatment (Logothetopoulos, 1966) was not observed. Suppression of endogenous insulin production therefore seemsa less likely explanation of the final elevated plasma glucose values.

4.3.4. The effect of Chicken "growth hormone" pituitary fraction on plasma NEFA and glucose.

Administration of growth hormone to mammals produces elevation of plasma NEFA levels. Growth hormone stimulated lipolysis has been observed in both intact mammals (Raben and Hollenberg, 1959, and Rabinowitz et al., 1965) and in *in vitro* preparation of mammalian adipose tissue (Fain et al. 1965). Gibson and Nalbandov (1966a) suggested that obesity in hypophysectomised cockerels was due to impairment of fat mobilisation, since they were able to demonstrate that plasma

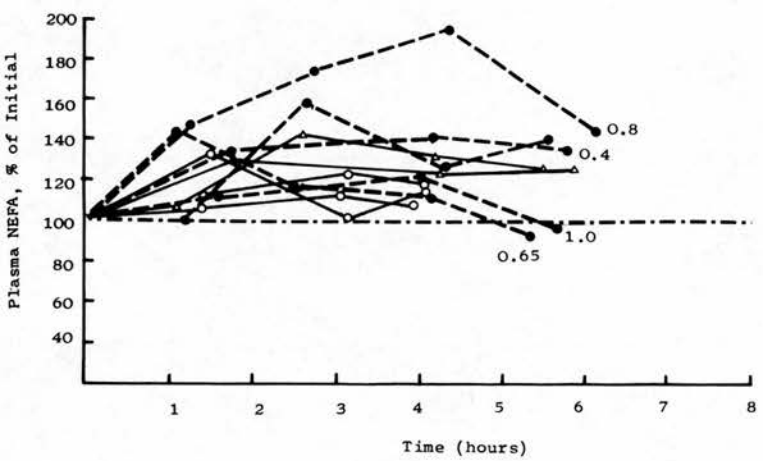
**1**

| Initial Plasma NEFA.(μequ/l ) | |
|---|---|
| Control (2) | Experimental (4) |
| 630 | 580,550,650 |
| 550 | 550 |




**2**

| Initial Plasma NEFA.(μequ/l ) | |
|---|---|
| Control (5) | Experimental (5) |
| 692 ± 69 | 628 ± 45 |




The effect of intravenously administered growth hormone on plasma NEFA.

1. 16 week old Brown Leghorn pullets

   Fed, saline
   Fed, chicken growth hormone
   Fasted, saline
   Fasted, bovine growth hormone

2. Fed adult hypophysectomised Brown Leghorn females

   Uninjected
   Saline
   Chicken growth hormone

Dose level units mg/kg

Figure 4.35

NEFA levels were raised by the injection of whole avian pituitary extract (Gibson and Nalbandov, 1966b). Heald and Rookledge (1964a) however found that although avian pituitary extract stimulated lipolysis in the rabbit, it produced no effect in the fowl.

Since the action of growth hormone is highly species specific (Knobil and Greep, 1959) when some chicken "growth hormone" became available as a gift from Dr. Hartree, Cambridge, this was used to determine the effect on lipolysis in the fowl.

In an initial trial experiment in which chicken and bovine growth hormone were injected into intact fed or fasted pullets, neither plasma NEFA (Fig. 4.35) or plasma glucose (Table 4.32) were substantially different after treatment, from the levels in the control saline-injected birds. Since it has been shown that the effect of growth hormone on lipolysis can be increased by hypophysectomy (Knobil and Greep 1959), all further experiments were carried out using hypophysectomised birds, provided by Dr. M. Mitchell. The effect of the injection of chicken growth hormone at various levels in these birds is shown in Fig. 4.35. and Table 4.33. With the exception of a single case (0·8 mg/kg chicken "growth hormone") the treatment had no observable effect on the NEFA plasma levels. The single positive result could not be explained. Plasma glucose was not appreciably effected by the treatment (Table 4.33). Consequently these limited results suggested that chicken "growth hormone" has no effect on lipid mobilisation.

Table 4.32.

Effect of "growth hormone" on plasma glucose in 16 week old brown Leghorn pullets. Values - plasma glucose as percent of initial value, initial values in mg/100 ml in brackets. Time to nearest 15 mins. Fasted birds had food removed 2 hours before experiment.

| Time (hrs) | Fed | | | Fasted | | |
|---|---|---|---|---|---|---|
| | Control | Fowl "growth hormone" | | Control | Bovine growth hormone | |
| | | 0·35 | 1·0µg/kg | | 0·4 | 0·9 µg/kg |
| 0 | 100 (251) | 100 (256) | 100 (251) | 100 (217) | 100 (238) | 100 (243) |
| 1·45 | 101 | 102 | 101 | 104 | 93 | 91 |
| 3·00 | 100 | 101 | 104 | 101 | 96 | 89 |
| 4·00 | | | | 99 | 95 | 97 |
| 7·00 | 101 | 104 | 104 | | | |

Table 4.33.

Effect of fowl "Growth Hormone" on plasma glucose in fed Hypophysectomised adult female Brown Leghorns

Values - plasma glucose percent of initial value; initial values as mg/100 ml in brackets. Time to nearest 15 mins.

| Time (hrs) | Control | | | Fowl "Growth Hormone" | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 0·4 | 0·45 | 0·65 | 0·8 | 1·0 mg/kg. |
| 0 | 100 (234) | 100 (220) | 100 (212) | 100 (200) | 100 (216) | 100 (218) | 100 (224) | 100 (224) |
| 1·10 | | 100 | 96 | | 95 | 101 | 99 | |
| 1·40 | 100 | | | 98 | | | | 92 |
| 2·45 | | 91 | | | 98 | 102 | 99 | |
| 3·05 | | | 103 | | | | | |
| 4·05 | 87 | 92 | 93 | 100 | 101 | 100 | 101 | 98 |
| 5·30 | 90 | 94 | | 102 | 98 | 99 | 107 | 101 |

Discussion.

While Heald and Rookledge (1964a) have shown that avian whole pituitary preparations have no effect on plasma NEFA in the intact fowl, Gibson and Nalbandov (1966b) have demonstrated that such preparations increase plasma NEFA in the intact and hypophysectomised fowl. More recently Langslow and Hales (1969) have demonstrated that a chicken "growth hormone fraction" stimulated lipolysis in in vitro preparations of fowl adipose tissue. This effect was increased by the addition of hydrocortisone sodium succinate to the incubate. Unfortunately since both Gibson and Nalbandov (1966b) and Langslow and Hales (1969) used only a single concentration of the pituitary preparation, no dose -response relationship was established. In the absence of any such positive results the lipolytic action of avian pituitary preparations must remain in doubt.

It has yet to be convincingly established that the avian pituitary produces a growth hormone. Hazelwood and Hazelwood (1961) using the rat tibia test found that a crude anterior pituitary lobe extract from the fowl was one eighth as active as a similar extract from the rat, and approximately one twentieth as active on the same basis as bovine growth hormone. Also using the rat tibia test Solomon and Greep (1959) found that an extract of whole pituitary from the fowl had no detectable growth promoting activity. The chicken pituitary fraction used in this study was prepared according to the method used for obtaining growth hormone from the human pituitary (Raben, 1959). The growth promoting activity

of this preparation was tested by injection into hypophysectomised rats and measuring the weight gain. On this basis the chicken "growth hormone" was found to be 100 times less active (i.e. of activity 0·02 i.u./mg) than purified bovine or human growth hormone (personal communication Dr. Hartree). The failure of such tests to detect any substantial growth activity in avian pituitary preparations may of course only be indicative of the shortcomings of the tests used. In view of the species specificity of growth hormone (Knobil and Greep, 1959), it would seem essential that some chick assay technique must be devised before further progress can be made, first to establish the existence of an avian growth hormone and secondly to investigate its properties.

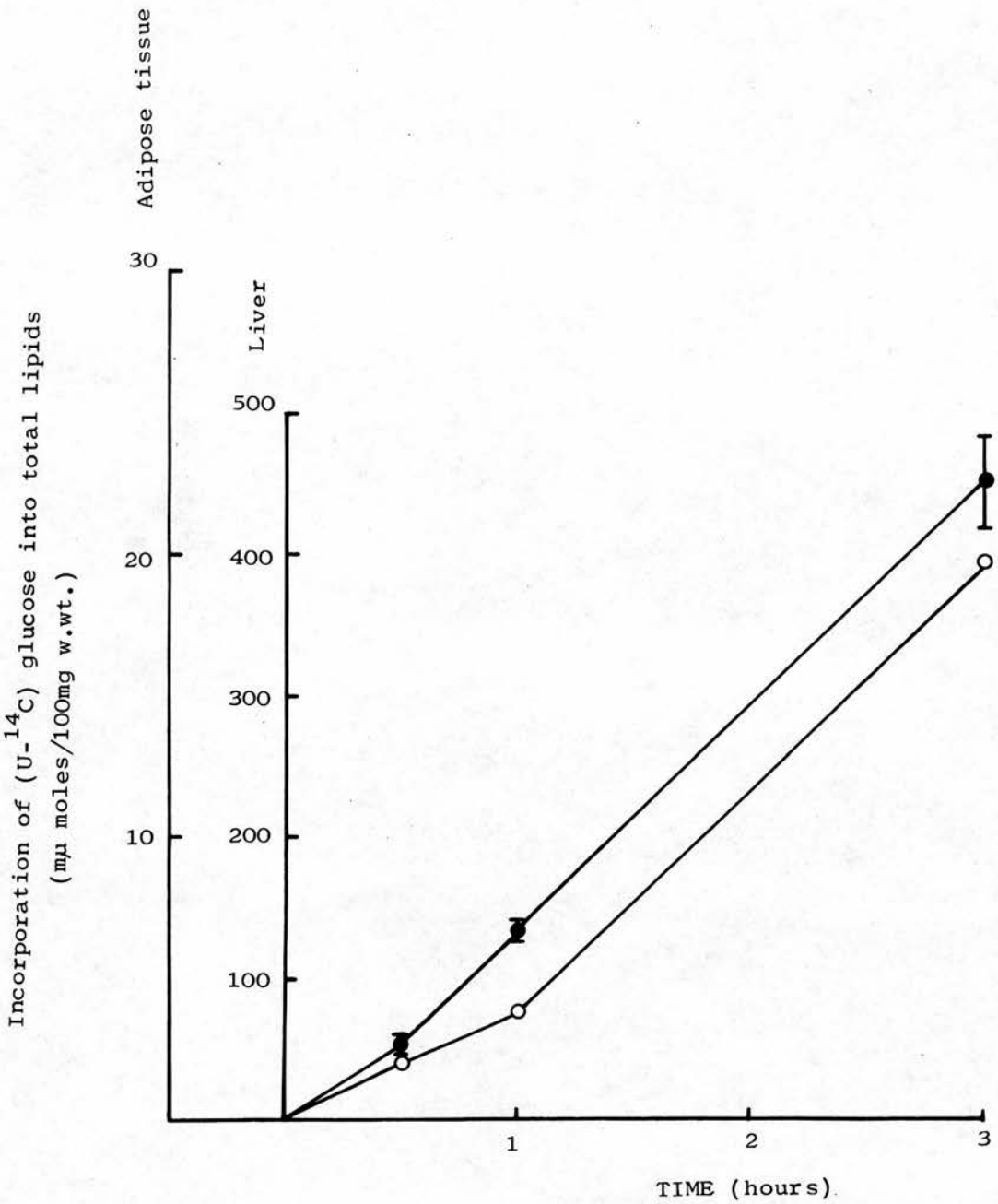
## 4.4. The effect of age and hormones on the in vitro lipogenic and lipolytic activity of liver and adipose tissue

It has been shown in a previous section (4.1.) that the specific rate of fat deposition in the duck, following hatching is initially high, but thereafter declines with age. It was therefore of interest to determine whether there were age dependent changes in the *in vitro* lipogenic activity of the liver and adipose tissues which would reflect developmental changes in these tissues. Since the diet of these birds was not rich in fat, most of the fat deposition must result from *de novo* synthesis from carbohydrate. Consequently glucose was used as the substrate in these studies, where lipogenesis was measured as the rate of incorporation of (U-$^{14}$C) glucose into total lipids or fatty acids. The lipogenic potential of the liver was tested by using three anabolic hormones (known to be lipogenic in the mammal or bird) or by glucose loading the tissue donor. Age dependent effects on lipolysis were examined in the adipose tissue using glucagon as a lipolytic agent.

In all these experiments tissues from female Aylesbury ducks were used.

### 4.4.1. The effect of the duration of incubation and preincubation on the in vitro lipogenic activity of liver and adipose tissue.

Usually it took one hour from the dissection of the tissue, to the commencement of incubation proper. The effect of this preincubation period on the subsequent lipogenic activity of the tissue was first determined. The




The effect of time on the incorporation of ($U^{-14}C$) glucose into total lipid.

Liver n = 3

Adipose tissue (duplication on incubates pooled from 3 birds)

Figure 4.36

activity of the liver tissue was preserved by storing it in ice-cold buffer. However adipose tissue solidifies under these conditions and so it was kept at 40°C (pre-incubation at this temperature) in Krebs-Ringer bicarbonate buffer, without glucose, for up to 2⅓ hours. This did not however appear to affect the subsequent lipogenic activity of this tissue (Table 4.34.). In fact a slight but insignificant increase was obtained by increasing the period of preincubation.

In all the in vitro experiments, a three hour incubation period was used when determining the lipogenic activity of tissue. Figure 4.36. shows that there is no diminution of lipogenic activity either in liver or adipose tissue over this period.

The intracellular accumulation of free fatty acids is known to inhibit lipogenesis (Tubbs and Garland, 1964). Consequently the addition of a fatty acid acceptor such as albumin, to the incubation medium could prevent such an action occurring. This possibility was tested by measuring the activity in the tissue and medium lipids following the addition of labelled glucose to incubates with or without albumin. The results obtained showed that addition of albumin had no effect on the rate of incorporation of glucose into the total lipids, either of the tissues, or those extracted from the incubation medium (Table 4.35.). For this reason albumin was omitted from the incubation medium in subsequent experiments on lipogenesis. Table 4.35 also shows that the amount of activity in the lipids extracted

Table 4.34.

The effect of duration of preincubation on the *in vitro* lipogenic activity of adipose tissue.

1·3 μC of [U-$^{14}$C] glucose were added to the incubation medium. Values are means ± S.E.M. 'n' shown in brackets.

| Duration of preincubation (hours) | [U-$^{14}$C] glucose incorporated into total lipid (counts per minute/100 mg w.wt/hr) | |
|---|---|---|
| 0·5 | 141 ± 33 | (3) |
| 1·0 | 158 ± 20 | (3) |
| 2·5 | 173 ± 34 | (3) |

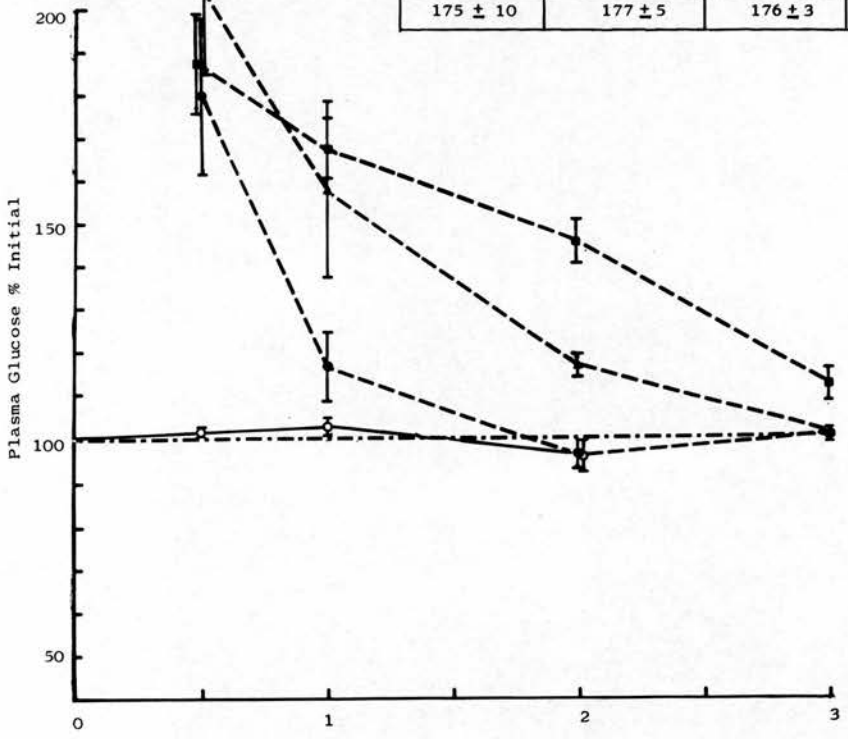
Table 4.35.

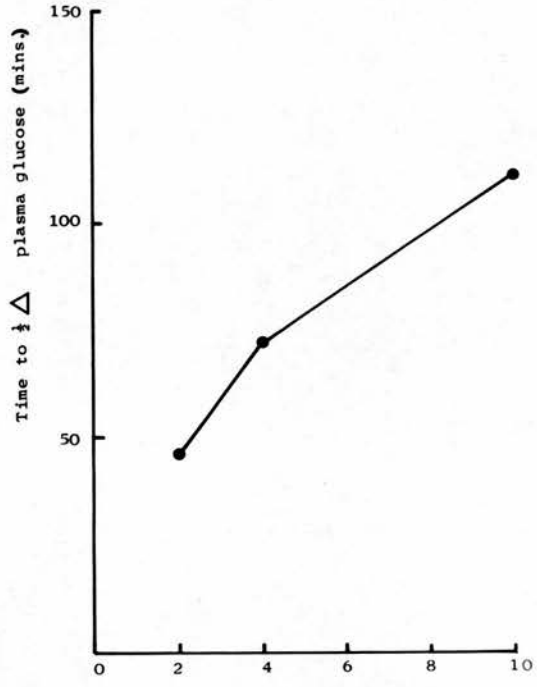
The effect of addition of albumin to the incubation medium, on *in vitro* lipogenesis in liver and adipose tissue of the duck. The albumin (bovine serum albumin, Fraction V) was dialysed overnight in cold Krebs-Ringer bicarbonate buffer pH 7·4, and then dissolved in the incubation medium to give a final concentration of 4%. 1·5 μC of [U-$^{14}$C] glucose was added to the medium. Values are means ± S.E.M. and represent the activity in total lipid extracts from tissues or 0·5 ml of incubation medium expressed in counts per minute/100 mg wet weight of tissue/hour. 'N' shown is in brackets. Controls (see Section 3.2.6.1) represent reaction blanks.

| | 4% Albumin | No Albumin | t | P |
|---|---|---|---|---|
| Liver tissue | 16,080 ± 600 (4) | 17,400 ± 1,800 (4) | 0·957 | >0·10 |
| Liver tissue control | 168 | 84 | | |
| Liver medium | 177 ± 27 (4) | 136 ± 17 (4) | 1·787 | >0·10 |
| Liver medium control | 198 | 120 | | |
| Adipose tissue | 294 ± 32 (3) | 348 ± 44 (4) | 1·071 | >0·10 |
| Adipose tissue control | 52 | 73 | | |
| Adipose tissue medium | 56 ± 9 (4) | 56 ± 8 (4) | 0·000 | >0·10 |
| Adipose tissue medium control | 52 | 90 | | |

Figure 4.37

| Controls (5) | 2wk. (6) | 4wk. (4) | 10wk. (6) |
|---|---|---|---|
| 175 ± 10 | 177 ± 5 | 176 ± 3 | 174 ± 7 |




1. Oral glucose tolerance curves. Glucose load 10g/kg.

—●— 2 week old female duck
—▲— 4 week old female duck
—■— 10 week old female duck
—○— Controls

2. The effect of age on the time for the reduction of the plasma glucose increase at half an hour to 50%. Data obtained from 1.

from the incubation medium was negligible.

4.4.2. The effect of age and glucose loading on the in vitro lipogenic activity of liver and adipose tissue.

Although the lipogenic potential of tissues is frequently measured by using fasting-refeeding dietary regimes, such procedures are not representative of the normal physiological processes occurring during growth and development. It was considered that a better measure of lipogenic potential might be obtained by determining the increase in lipogenic activity following the administration of a carbohydrate meal to the fed bird. In this study the carbohydrate meal was in the form of a glucose solution, introduced into the proventriculus by intubation. The amount given was 10 g/kg (live weight). The birds had free access to water following the glucose loading.

Figure 4.37. shows the effect of glucose loading on plasma glucose levels in three different age groups. There was no significant difference between the glucose levels of these groups in the initial values obtained half an hour after treatment. Thereafter however the rate of decline of plasma glucose varied strikingly between the groups. Glucose uptake was rapid in the youngest birds, but progressively declined with age. This result is shown more clearly in Figure 4.37.2. The control group used in this experiment consisted of three untreated birds, one each of age 2, 4 and 10 weeks respectively and two saline loaded birds, consisting of one 2 week old and one 10 week old bird.

Table 4.36 shows that neither age nor glucose loading had a significant effect on the lipogenic activity of liver,

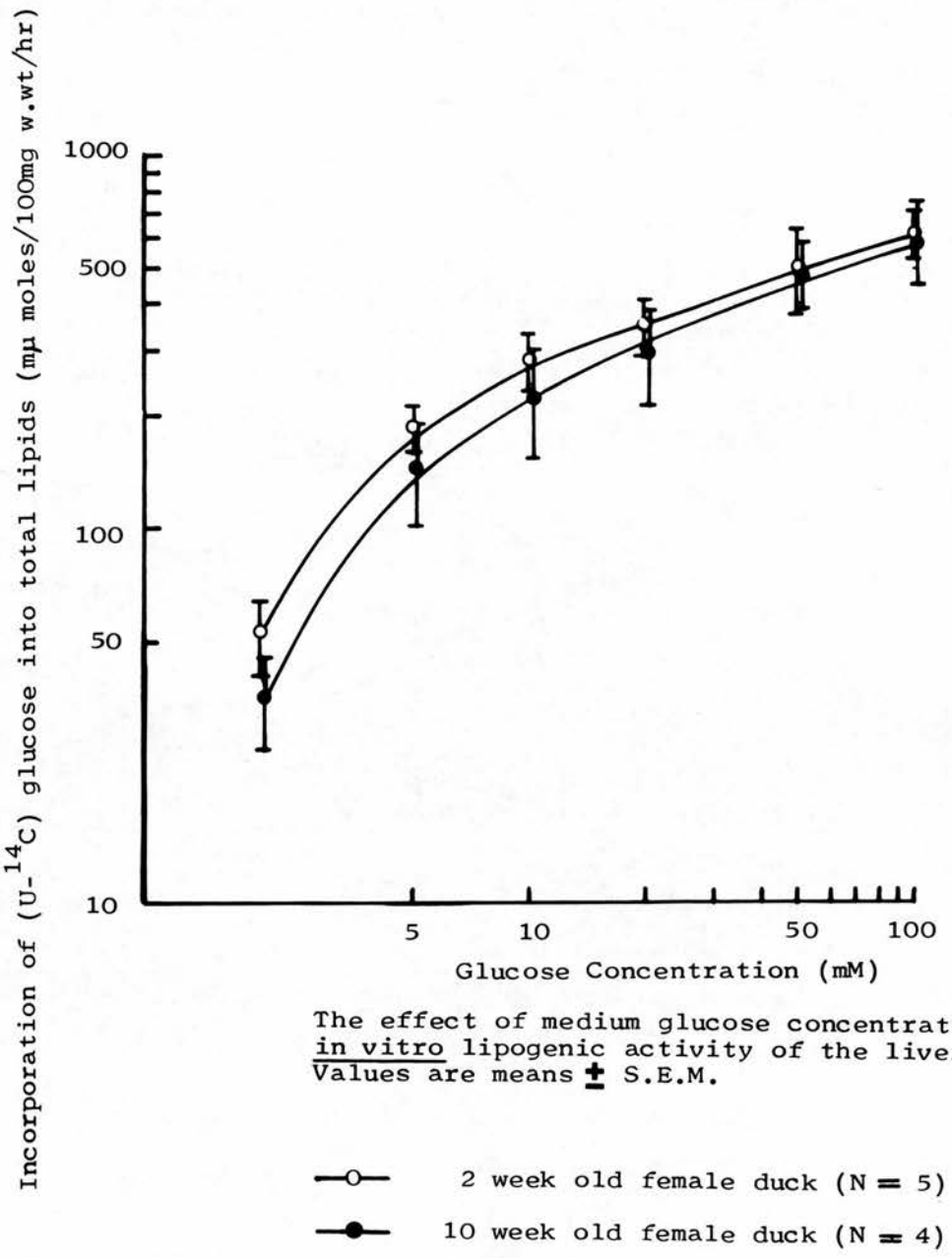
Table 4.36.

The effect of age and glucose loading (oral dose 10g/kg) on the *in vitro* lipogenic activity of the liver. Values are means ± S.E.M., number of birds used is shown in brackets.

| Age (weeks) | Incorporation of [U-$^{14}$C] glucose into total lipids (in mμmoles/100 mg w.wt/hr) | | t | P |
|---|---|---|---|---|
| | Fed | Glucose loaded | | |
| 2 | 238·5 ± 31·1 (17) | 287·8 ± 26·5 (11) | 1·096 | >0·10 |
| 4 | 192·0 ± 37·0 (9) | 249·3 ± 40·5 (10) | 1·023 | >0·10 |
| 10 | 217·9 ± 23·4 (16) | 261·7 ± 30·1 (15) | 1·184 | >0·10 |

't' between Fed 2 and 4 week old tissues = 0·766 (P > 0·10)

't' between Fed 4 and 10 week old tissues = 0·632 (P > 0·10)




The effect of medium glucose concentration on <u>in vitro</u> lipogenic activity of the liver. Values are means ± S.E.M.

—○— 2 week old female duck (N = 5)

—●— 10 week old female duck (N = 4)

Figure 4.38

although glucose loading consistently produced a slight increase in all the age groups.

Similarly glucose loading had no effect upon lipogenic activity in adipose tissues (Table 4.37.). There was however a slight decline in lipogenic activity with age which was observed whether the activity was determined on a tissue wet weight basis, or as the activity of the fat-free tissue mass. There was no significant difference between the lipogenic activity of adipose tissue from different sites for birds of the same age.

The rate of incorporation of glucose into total lipids was notably lower in the adipose tissue than in the liver, whether this was calculated on a wet weight or fat-free tissue weight basis.

4.4.3. The effect of substrate concentration on the in vitro lipogenic activity of liver.

Liver from 2 and 10 week old birds was incubated in various glucose concentrations. As shown in Figure 4.38., increasing the glucose concentration in the medium from 2-100 mM produced an eleven fold increase in lipogenic activity in the tissue from the 2 week old birds and a sixteen fold increase in the tissue from the 10 week old. However this difference cannot be considered to be of any importance, since at no concentration were the results significantly different between the two age groups. Lipogenesis continued to increase with increasing substrate concentration over the whole range tested, for both age groups.

Table 4.37. The effect of age and glucose loading (oral dose 10g/kg) on the *in vitro* lipogenic activity of adipose tissues. Values are means ± S.E.M., number of birds used is shown in brackets.

| Age (weeks) | Tissue | Incorporation of [U-$^{14}$C] glucose into total lipids (in mμmoles/100 mg w.wt/hr) | | t | P |
|---|---|---|---|---|---|
| | | Fed | Glucose loaded | | |
| 2 | Inguino-crural | 20·3 ± 1·8/92·5* (8) | 20·10 ± 1·2 (12) | 0·091 | >0·10 |
| 4 | Inguino-crural | 10·8 ± 1·8/76·0* (6) | 8·7 ± 0·7 (13) | 1·135 | >0·10 |
| " | Peritoneal | 11·4 ± 2·2/98·0* (6) | 10·1 ± 1·3 (8) | 0·542 | >0·10 |
| " | Subcutaneous | 13·5 ± 2·6/100·0* (6) | 10·0 ± 0·7 (8) | 1·500 | >0·10 |
| 10 | Inguino-crural | 5·3 ± 0·6/50·5* (13) | 4·1 ± 0·9 (9) | 1·091 | >0·10 |
| " | Peritoneal | 4·1 ± 0·5/67·0* (8) | | | |
| " | Subcutaneous | 5·7 ± 0·6/45·5* (10) | | | |

(Contd.)

Table 4.37.(Contd.)

* These are mean values of incorporation of [U-$^{14}$C] glucose into total lipids expressed as mμmoles/100 mg of fat-free tissue wt/hr.

t between fed 2 week inguino-crural and 4 week subcutaneous = 2·267 ($P < 0·05$)

t between fed 4 week subcutaneous and 4 week inguino-crural = 0·871 ($P > 0·10$)

t between fed 4 week inguino-crural and 10 week subcutaneous = 3·400 ($P < 0·01$)

t between fed 10 week peritoneal and 10 week subcutaneous = 2·000 ($P > 0·05$).

4.4.4. The effect of age and anabolic hormones on the in vitro lipogenic activity of liver.

Unless otherwise stated, hormones were added directly to the in vitro preparations. Eight incubates were prepared from the liver of each bird. These consisted of duplicate controls (i.e. no hormone added), and duplicates for each hormone level. The results have been calculated from the means of these duplicates. The effect of hormone treatment is expressed as the lipogenic activity (rate of incorporation of labelled glucose into total lipids) of the treated tissues as a percentage of the controls.

Although insulin produced a marked increase in lipogenic activity in the epididymal adipose tissue from the young male rat, no effect could be demonstrated on the avian liver, except a slight inhibition of activity at the highest dose in the 10 week old bird (Table 4.38.). Similarly prolactin had little effect upon lipogenesis, although a slight reduction in activity was produced with higher levels in tissues from 10 week old birds (Table 4.39.). This inhibition was not however dose dependent.

Table 4.40. shows that oestrogen significantly reduced lipogenic activity in the tissues from birds of both age groups. This inhibition was not dose dependent for the ranges of hormone levels used. Although it is shown that the inhibition was not significant for the highest dose level, the response was essentially the same for all doses. However when the birds were given oestrogen by intraperitoneal injection (10 mg/kg oestradiol monobenzoate in arachis oil)

Table 4.38. The effect of *in vitro* insulin on the incorporation of [U-$^{14}$C] glucose into total lipids in duck liver and rat epididymal adipose tissue. Values are means ± S.E.M. Values in brackets give P values for t tests between experimental and control groups, or incorporation rate in mμmoles/100 mg w.wt/hr.

| Age (weeks) | No. of animals | Tissue | Control | Insulin (% control rate) 0·01U/ml | 0·1U/ml | 1·0U/ml |
|---|---|---|---|---|---|---|
| 2 | 6 | Duck liver | 100 (297·3 ± 63·3) | 94 ± 8·2 (>0·10) | 103·4 ± 19·6 (>0·10) | 115·9 ± 25·3 (>0·10) |
| 10 | 6 | Duck liver | 100 (327·5 ± 106·9) | 104·1 ± 19·4 (>0·10) | 111·6 ± 14·6 (>0·10) | 82·3 ± 8·8 (>0·05) |
| | | | | | | 0·35U/ml |
| Young male | 3 | Rat adipose tissue | 100 (178 ± 22) | | 927 (1650 ± 70) | 1074 (1915 ± 101) |

Table 4.39. The effect of *in vitro* prolactin on the incorporation of [U-$^{14}$C] glucose into total lipids in the liver. Values are means ± S.E.M. Values in brackets give P values for t tests between experimental and control groups, or incorporation rate in mμmoles/100 mg w.wt/hr.

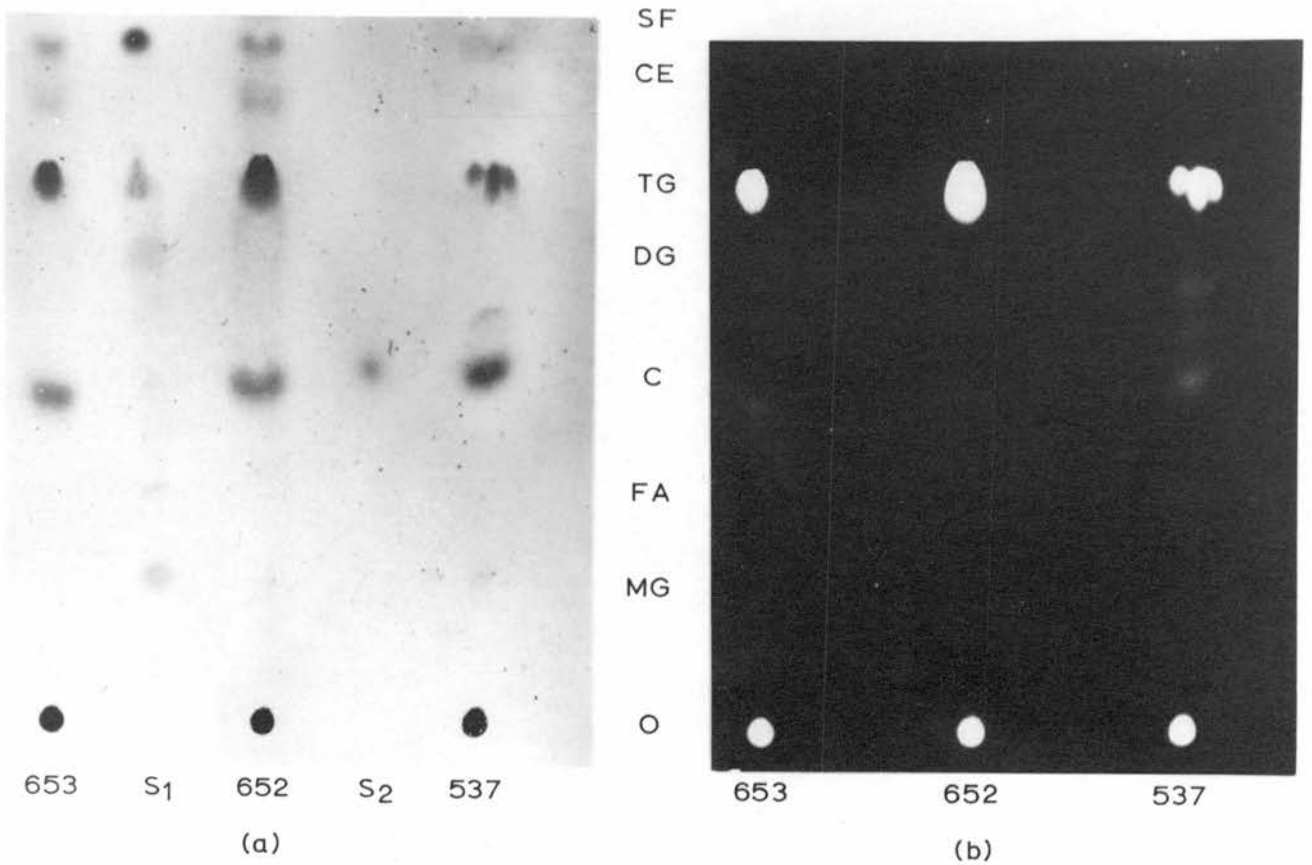
| Age (weeks) | No. of birds | Control | Prolactin (% of control) 0·01U/ml | 1·0U/ml | 10·0U/ml |
|---|---|---|---|---|---|
| 2 | 8 | 100 (305·8 ± 63·2) | 104·6 ± 10·1 (>0·10) | 114·1 ± 6·8 (>0·05) | 115·0 ± 19·9 (>0·10) |
| 10 | 8 | 100 (313·6 ± 32·3) | 90·4 ± 7·4 (>0·10) | 77·9 ± 3·4 (0·001) | 79·9 ± 7·9 (0·05) |

Table 4.40. The effect of *in vitro* oestrogen on the incorporation of [U-$^{14}$C] glucose into total lipids in the liver. Values are means ± S.E.M. Values in brackets give P. values for t tests between experimental and control groups, or incorporation rate in mμmoles/100 mg w.wt/hr.

| Age (weeks) | No. of birds | Control | Oestrogen (% of Control) 10μg/ml | 40μg/ml | 100μg/ml |
|---|---|---|---|---|---|
| 2 | 6 | 100 (292 ± 27·9) | 57·8 ± 12·7 (P < 0·02) | 59·2 ± 15·1 (P < 0·05) | 66·2 ± 26·3 (P > 0·10) |
| 10 | 6 | 100 (233·4 ± 34·4) | 55·0 ± 16·8 (P < 0·05) | 63·7 ± 25·2 (P > 0·10) | 68·5 ± 17·0 (P > 0·10) |

The effect of *in vivo* oestrogen (10 mg/kg) on the incorporation of [U-$^{14}$C] glucose into total lipids in the liver (mμmoles/100 mg w.wt/hr). Values are means ± S.E.M. See text for details of treatment.

| Age (weeks) | No. of Birds | In vivo oestrogen |
|---|---|---|
| 2 | 3 | 468·7 ± 41·3 |
| 10 | 3 | 484·0 ± 72·9 |




Thin layer chromatograms of total lipid extracts from liver slices incubated in a medium containing (U-$^{14}$C) glucose. (a) Charred plate, (b) Radioautograph of same plate. Key: $S_1$ = standard soluyion of cholesterol oleate, glycerol tripalmitate, glycerol dipalmitate, glycerol monopalmitate, palmitic acid; $S_2$ = standard solution containing cholesterol; SF = solvent front; CE = cholesterol esters; TG = triglycerides; DG = diglycerides; C = cholesterol FA = fatty acids; MG = monoglycerides; O = origin.

Figure 4.39.

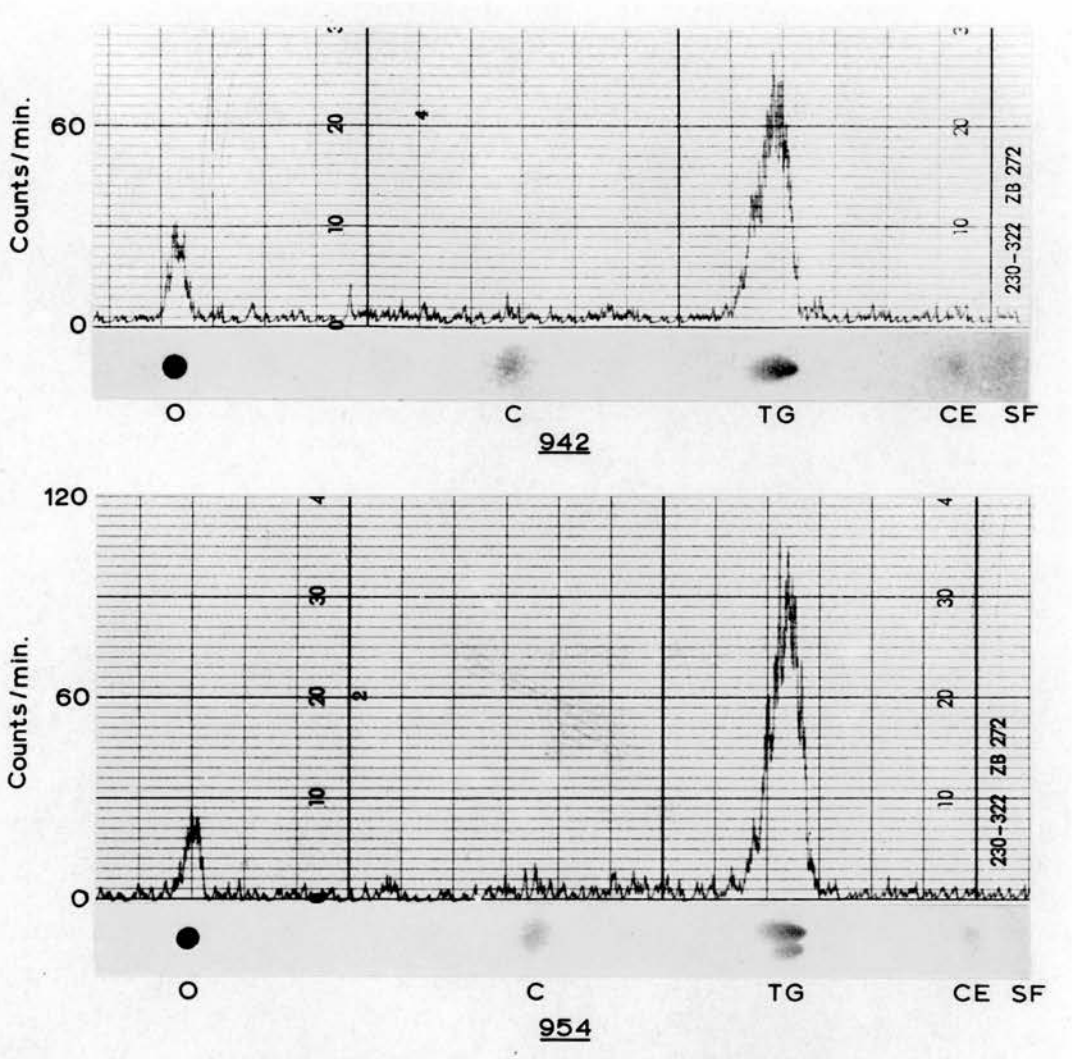
5 hours before the liver tissue was collected, a substantial increase in the *in vitro* lipogenic activity of the liver of a similar magnitude for both age groups was observed.

4.4.5. Incorporation into different lipid classes.

Thin layer chromatography was used to measure the distribution of radioactivity between the main lipid classes in the total lipid extracts of the incubated liver slices. Figure 4.39. shows the separation of the neutral lipids obtained. The accompanying radioautograph in this figure indicates that practically all the label is distributed between the triglycerides and phospholipids. Scanning of the chromatorams produced a similar result, and in addition demonstrated that most of the label occurred in the triglycerides (Figure 4.40.). This latter method provided a means of quantifying the distribution of the radioactivity between the two lipid classes in which virtually all the activity occurred. The results obtained by this procedure compared favourably with those from an alternative method (Carlson, 1963) for obtaining the proportion of the radioactivity in the total lipid extract which resided in the triglyceride fraction (Table 4.41.).

The proportion of activity occurring in the triglyceride fraction was not influenced by age and only slightly influenced by the various treatments (Table 4.42.).

With the solvent system used to obtain chromatographic separation of the neutral lipid classes, the phospholipids remain at the origin. Therefore in order to verify that all the activity occurring at the origin was associated




The incorporation of (U-$^{14}$C) glucose into the major lipid classes in the total lipid extract from incubated liver slices. Separation of lipid classes by thin layer chromatography shown below the radioactivity scans. Key: O = origin; C = cholesterol; TG = triglycerides; CE = cholesterol esters; SF = solvent front.

Figure 4.40

Table 4.41.

Comparison of the results obtained using scanning method and silicic acid extraction method (Carlson, 1963) for the estimation of the percentage of total lipid radioactivity occurring in the triglyceride fraction.

| Sample No. | Percent of activity in triglyceride fraction | | Percent diff. |
|---|---|---|---|
| | T.L.C. Scan. | Carlson (1963) | |
| 497 | 84·0 | 86·3 | 2·7 |
| 1041 | 87·0 | 84·9 | 2·5 |
| 712 | 88·5 | 91·3 | 3·0 |
| 1142 | 88·3 | 91·9 | 3·9 |
| 942 | 90·5 | 87·4 | 3·5 |
| 954 | 85·0 | 88·7 | 4·1 |
| 694 | 84·0 | 85·6 | 1·9 |
| | | mean diff. = | 3·1 ± 0·3% |

Table 4.42.1. Percentage of radioactivity in total liver lipids occurring in the triglyceride fraction. Values are means ± S.E.M., n shown in brackets.

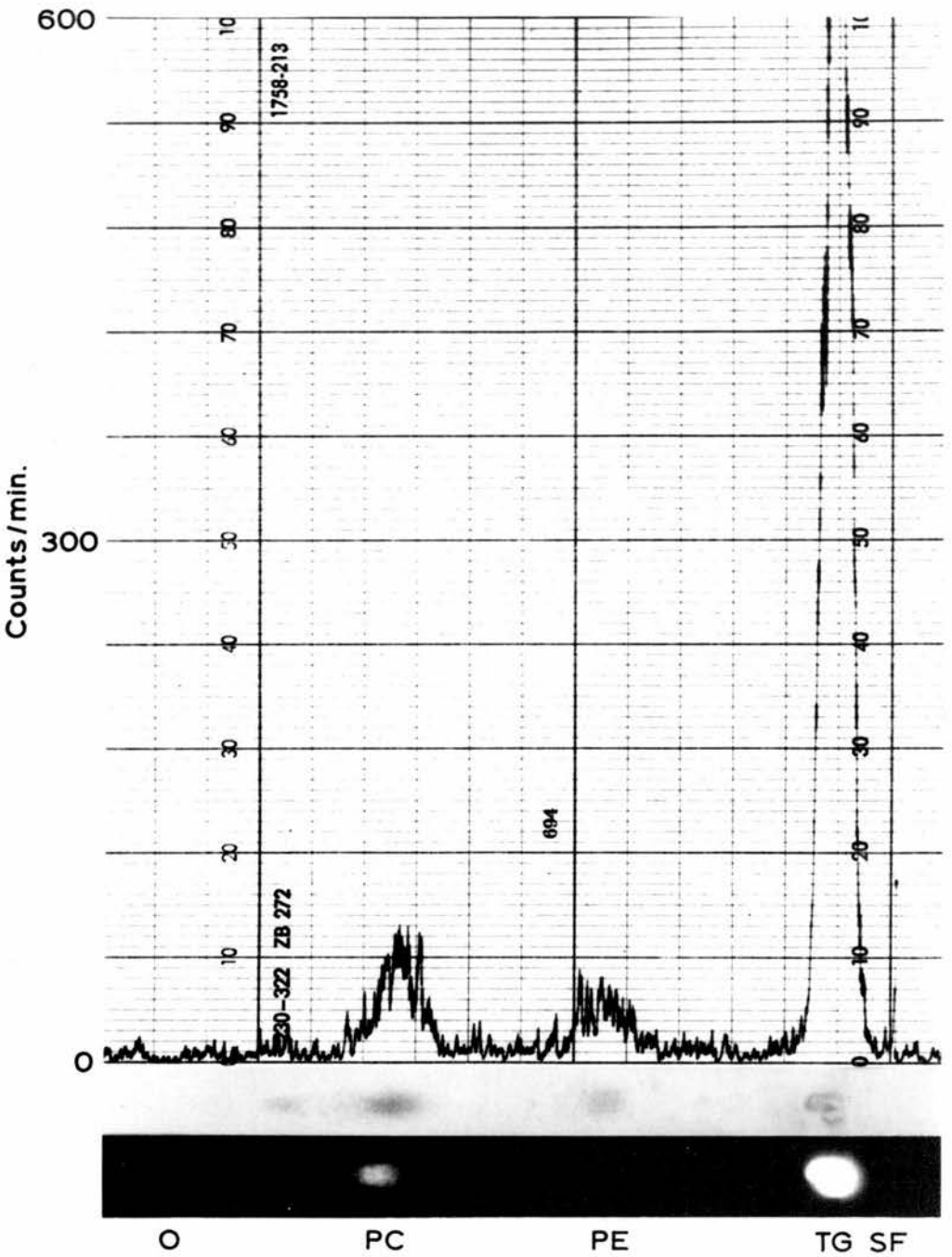
| Treatment | Age (weeks) | | t | P |
|---|---|---|---|---|
| | 2 | 10 | | |
| Fed | 84·0 ± 1·1 (7) | 86·0 ± 1·3 (8) | 1·170 | >0·10 |
| Glucose loaded | 79·6 ± 1·8 (11) | 80·7 ± 2·1 (6) | 0·203 | >0·10 |

t between 2 week fed and glucose loaded = 4·11 (P<0·001)

t between 10 week fed and glucose loaded = 2·28 (P<0·05)

Table 4.42.2. Percentage of radioactivity in total liver lipids occurring in the triglyceride fraction.

| Tissue No. | Age (weeks) | Treatment | % Activity of total lipid in triglycerides |
|---|---|---|---|
| 954 | 10 | Insulin | 87·5 |
| 950 | 10 | Insulin | 80·5 |
| 1041 | 2 | Prolactin | 87·0 |
| 1142 | " | " | 88·3 |
| 1148 | " | " | 84·2 |
| 1025 | " | Oestrogen | 81·0 |
| 1023 | " | " | 80·0 |
| 1026 | " | " | 76·0 |




The incorporation of (U-$^{14}$C) glucose into the phospholipids in the total lipid extract from the incubated liver slices. Thin layer chromatogram and radioautograph of this are shown below the radioactivity trace. Key: O = origin; PC = phosphatidyl choline; PE = phosphatidyl ethanolamine; TG = triglycerides; SF = solvent front. Sample No. 649.

Figure 4.41

with the phospholipids, the liver lipid extracts were also run on plates using a solvent system to provide good separation of the phospholipids. A typical chromatographic separation obtained by this system is shown in Figure 4.41. Also shown in this figure is a radioactive scan and radioautograph of the same chromatogram. With this system all the neutral lipids were swept along with the solvent front. Practically all the activity in the phospholipid class was distributed between phosphatidyl choline and phosphatidyl ethanolamine (Figure 4.41., Table 4.43). The ratio of the amount of activity in these two phospholipids was very similar to the ratio of the absolute amounts occurring in the normal fowl liver (Sgoutas, 1966). In contrast the proportion of activity in the triglycerides compared to the phospholipids was considerably higher than the ratio of the absolute amounts occurring in the fowl liver (Sgoutas, 1966).

The proportion of the activity in the total lipid extracts from both incubated liver and adipose tissues, which resides in the fatty acids is shown in Tables 4.44,45. Some treatments slightly decreased the proportion of incorporation of the label into the fatty acids (Table 4.44.).

### 4.4.6. The effect of age and glucagon on the in vitro lipolysis in adipose tissue.

For each age group the inguino-crural fat depots from four fed birds were pooled. Treated and control tissues were obtained as random samples from the combined tissues. Glucagon was added directly to the incubation medium at the beginning of each incubation.

Table 4.43.

Incorporation into phospholipids.

Percentage of total phospholipid radioactivity in individual phospholipids.

| Sample No. | Age (weeks) | Treatment | Phosphatidyl Ethanolamine | Phosphatidyl Choline | Ratio PC/PE |
|---|---|---|---|---|---|
| 699 | 2 | Glucose | 27 | 73 | 1:0·37 |
| 712 | 10 | Fed | 41 | 59 | 1:0·7 |
| 497 | 10 | Glucose | 39 | 61 | 1:0·64 |
| 694 | 10 | Glucose | 38 | 62 | 1:0·62 |

Table 4.44.

Percentage of radioactivity in total liver lipids occurring in fatty acids.

Values are means ± S.E.M., n shown in brackets.

| Age (weeks) \ Treatment | Control (fed) | Glucose loaded | Insulin 1 U/ml | Prolactin 10 U/ml | Oestrogen 100 µg/ml |
|---|---|---|---|---|---|
| 2 | 75·2 ± 2·1 (5) | 72·4 ± 2·8 (5) | 72·2 ± 3·1 (5) | 68·8 ± 5·3 (5) | 65·2 ± 2·5 (5) |
| 10 | 74·4 ± 2·1 (5) | 74·0 ± 2·9 (5) | 74·4 ± 3·9 (5) | 66·6 ± 2·1 (5) | 63·4 ± 3·9 (5) |

Table 4.45.

Percentage of radioactivity in total adipose tissue lipids occurring in fatty acids. Values are means ± S.E.M. n shown in brackets.

| Age (weeks) | Treatment: Control (fed) | Glucose loaded |
|---|---|---|
| 2 | 15·9 ± 1·4 (10) | 15·4 ± 1·5 (10) |
| 4 | 14·2 ± 1·5 (10) | 15·0 ± 1·5 (10) |
| 10 | 9·0 ± 0·8 (10) | 10·4 ± 0·9 (10) |

Table 4.46 shows the effect of various concentrations of glucagon on the rate of lipolysis in the adipose tissues. The lowest concentration produced a considerable increase in lipolysis in the tissues from birds of both ages. Thereafter the additional response declined with further increases in glucagon concentration. There is no indication from these results that age affects the lipolytic response of the adipose tissues to glucagon. However the absolute rates of lipolysis expressed on a fat-free wet weight basis were greater for tissue from the 10 week old birds.

Discussion.

The results obtained in this section are notable in three main respects. First the demonstration that the lipogenic activity in adipose tissue is considerably less than that occurring in the liver, second that there are no marked developmental changes either in the lipogenic activity or potential of the liver or adipose tissues, and third that the lipogenic pathways in the liver are entirely unresponsive to the *in vitro* action of some important anabolic hormones.

The first observation is examined further by combining the results in Tables 4.36. and 4.44., and Tables 4.37. and 4.45.

From this it is apparent that the rates of incorporation of glucose into fatty acids are markedly different for liver and adipose tissues (Table 4.47.). The unaccounted activity in the total lipids must reside mainly in the

Table 4.46. The effect of *in vitro* glucagon on lipolysis (μmoles glycerol released/g wet wt./hr.) in inguino-crural adipose tissue. Values are means ± S.E.M., n shown in brackets.

| Age (weeks) | Control | Glucagon | | |
|---|---|---|---|---|
| | | 0·1 μg/ml | 1·0 μg/ml | 10·0 μg/ml |
| 2 | 0·44 ± 0·035 (8)<br>2·0* | 1·81 ± 0·168 (7)<br>8·2* | 2·24 ± 0·164 (7)<br>10·2* | 2·75 ± 0·222 (7)<br>12·5* |
| 10 | 0·42 ± 0·038 (8)<br>4·0* | 1·79 ± 0·123 (8)<br>17·1* | 2·56 ± 0·299 (7)<br>24·4* | 2·37 ± 0·174 (8)<br>22·6* |

* Glycerol released expressed as μmoles/g fat free tissue wt/hr.

t tests

| Tissues | N | t | P |
|---|---|---|---|
| 2 week control, 0·1 μg/ml | 15 | 8·57 | <0·001 |
| 2 week 0·1 μg/ml, 1·0 μg/ml | 14 | 1·95 | >0·05 |
| 2 week 1·0 μg/ml, 10·0 μg/ml | 14 | 1·97 | >0·05 |
| 10 week control, 0·1 μg/ml | 16 | 10·52 | <0·001 |
| 10 week 0·1 μg/ml, 1·0 μg/ml | 15 | 2·51 | >0·02, <0·05 |
| 10 week 1·0 μg/ml, 10·0 μg/ml | 15 | 0·57 | >0·10 |

Table 4.47. Comparison of rates of lipogenesis between liver and adipose tissue.

| Units | Age (weeks) | | |
|---|---|---|---|
| | 2 | 4 | 10 |
| **a. Liver** | | | |
| Incorporation of [U-$^{14}$C] glucose into fatty acids (mμmoles/100mg w.wt/hr) | 179 | 144 | 162 |
| Incorporation of [U-$^{14}$C] glucose into fatty acids (mμmoles/liver/hr) | 50,100 (28°) | 118,200 (82°) | 154,000 (95°) |
| **b. Adipose Tissue** | | | |
| Incorporation of [U-$^{14}$C] glucose into fatty acids (mμmoles/100 mg fat free w.wt/hr) | 14·7 | 12·9 | 4·9 |
| Incorporation of [U-$^{14}$C] glucose into fatty acids (mμmoles/total adipose tissue/hr) | 1,265 (8·6*) | 4,225 (32·8*) | 4,320 (88·0*) |
| Percentage of total lipogenic activity in liver | 97·5 | 96·5 | 97·3 |

° Mean liver wt (g)

* Mean estimated total body adipose tissue wt (g).

glycerol moiety in the triglycerides of the adipose tissue, and of the triglycerides and phospholipids of the liver. If the capacity to synthesise fatty acids from glucose is calculated as the incorporation rate per total weight of tissue in the body, the relative contributions of these tissues to total lipogenesis becomes more apparent (Table 4.47.). On this basis it can be seen that the liver could contribute over 96 per cent of the combined lipogenic activity of the liver and adipose tissues in birds during early growth. This result however must remain speculative until it can be verified by suitable in vivo experiments.

The rates of fatty acid synthesis in the duck liver slices were intermediate in value between those obtained in the young fowl (Goodridge, 1968b) and the young pigeon (Goodridge and Ball, 1967c). In all cases the lipogenic activity of the avian liver was consistently greater than that of the rat liver incubated under similar conditions (Ballard and Hanson, 1967a, and Cahill et al., 1958). Such results are supported by observations that the activity of the important and perhaps rate limiting enzymes involved in lipogenesis, namely citrate cleavage enzyme and malic enzyme are much greater in the young fowl (Goodridge, 1968c) and the pigeon (Goodridge and Ball, 1966, 1967c) liver than either that in the rat liver or epididymal adipose tissue (Goodridge and Ball, 1966, and Ballard and Hanson, 1967a, b). In contrast hexose monophosphate (HMP) shunt dehydrogenases are of very low activity both in fowl adipose tissue (O'Hea and Leveille, 1968), fowl liver (O'Hea and Leveille, 1968,

and Goodridge, 1968c) and pigeon liver and adipose tissue (Goodridge and Ball, 1966, 1967c). These results indicate that little glucose oxidation can occur via the HMP pathways. This has been confirmed in the fowl by measuring the relative rates of oxidation of specifically labelled glucose (Duncan and Common, 1967). This result is perhaps rather surprising with respect to the high lipogenic activity found in the avian liver, since in the rat the HMP shunt is a major source of the NADPH required for fatty acid synthesis. This means that NADPH must be largely supplied by the malate transhydrogenation cycle in the bird. The high activity of malic enzyme found in the avian liver would be consistent with such a conclusion.

In adipose tissue of the duck, rates of synthesis of fatty acids from glucose were similar to the values obtained in the sparrow and junco (Goodridge, 1964), young pigeon (Goodridge and Ball, 1966) and the young fowl (Goodridge, 1968e; O'Hea and Leveille, 1968). Such values represent only approximately 5-10% of the synthetic activity found in adipose tissues from the rat (Goodridge, 1968e, O'Hea and Leveille, 1968; Winegrad and Renold, 1958b). Acetyl CoA carboxylase and malic enzyme activities were very low in the adipose tissue of the pigeon and unaffected by dietary manipulation (Goodridge and Ball, 1966).

Goodridge and Ball (1967a) by injecting labelled glucose into young pigeons and following the changes in the amount of label appearing in the lipids of the liver, the blood and the adipose tissue over a two hour period, demon-

strated that the liver is the prime site of lipogenesis in this bird. Hepatic lipogenesis accounted for 96% of the total body lipogenesis. Leveille et al. (1968) indicated that hepatic lipogenesis in the young fowl accounted for 70% of the total. Such results are in marked contrast with those obtained for the rat. In this species and the mouse, hepatic lipogenesis represents only 4% of the total (Favarger, 1965; Jeanrenand, 1968). The minor role of adipose tissue in lipogenesis in the duck has been previously emphasised. These results and those referred to above, indicate that adipose tissue in the bird represents a relatively trivial source of production of fatty acids. Presumably its main function is the re-esterification of extracellular fatty acids produced by the hydrolysis of plasma lipoproteins synthesised in the liver. In this respect therefore it is not surprising to find that the lipoprotein lipase activity in pigeon adipose tissue is twice the activity of that occurring in rat adipose tissue (Goodridge and Ball, 1967a.)

The superior glucose tolerance of the younger ducks (Section 4.4.2.) indicated that glucose uptake into the tissues of these birds was more rapid than in the older birds. However the amount of glucose which is available for lipogenesis in the liver and adipose tissue will depend both upon the relative uptake of the different tissues, and also on the competition between the different pathways for the substrate within the lipogenic tissues. Unfortunately nothing is known about the relative uptake of glucose during early growth in the bird. Consequently the absence of any

marked effect of glucose loading on *in vitro* lipogenesis cannot be adequately explained at present. Goodridge's (1968d) results suggest that hepatic lipogenesis in the young fowl is not maximally stimulated in the fed state, since refeeding-fasting regimes can produce a twofold increase in activity. However the regime used was somewhat harsh for a young bird and consequently may represent a different response from that usually observed under normal conditions.

Although glucose tolerance is reduced in ageing man (Hecht et al., 1961), the ageing rat (Hajdn et al., 1968) and temporarily in the human neonate (Schwartz et al., 1966), changes during early growth have not been reported. Mialhe (1958) found that seasonal changes occurred in the glucose tolerance of the domestic duck, which he attributed to seasonal changes in gonad activity. It would however seem unlikely that the onset of reproductive activity could be an explanation of the decrease in glucose tolerance observed in the ducks in these studies. Glucose tolerance is reduced in obesity, in man (Vague et al., 1969) and in the rat made obese by feeding a high fat diet (Bloom and Fenton, 1956), but was not substantially effected by obesity in rats resulting from hypothalamic lesion (Tucker, et al., 1965). However by using the criterion discussed in Section 4.1. to define obesity (see also Fenton 1956), the ducks used in this study were judged not to be obese. If the glucose loads given were recalculated as the amount given on a lean weight basis, they became 11.2, 12.2 and 13.2 g/kg for 2, 4 and 10 week old birds respectively. This difference in loading is unlikely

to account for the observed differences in their response to the glucose load.

The anabolic hormones used in these experiments to test the lipogenic potential of the liver from birds of different ages, were chosen because of their known ability to stimulate lipogenesis either in mammals or birds.

The role of insulin in mammalian lipogenesis is well established (see Section 2.) However it has been shown to have no effect on the rate of fatty acid synthesis from acetate or pyruvate in fowl adipose tissue (O'Hea and Leveille, 1968) or on lipogenesis in the pigeon (Goodridge and Ball, 1966) or finch (Goodridge, 1964) adipose tissues. In young fowl adipose tissue, insulin produced a very slight increase in the rate of incorporation of glucose into carbon dioxide, glycogen, glyceride glycerol and fatty acids (Goodridge, 1968e), although it had no effect on glucose uptake and oxidation in pigeon adipose tissue (Goodridge and Ball, 1965). Further insulin did not inhibit lipolysis in the pigeon (Goodridge and Ball, 1965) or fowl (Langslow and Hales, 1969) adipose tissue. In vivo protamine zinc insulin given daily to the pigeon either had no effect, or at higher levels, depressed liver in vitro lipogenesis and decreased liver malic enzyme activity (Goodridge and Ball, 1967c.) The inability of insulin to stimulate hepatic lipogenesis in the duck therefore is in agreement with the result obtained from other avian tissues.

Daily in vivo administration of prolactin produces marked fat deposition in sparrows (Meier and Farner, 1964).

Subsequently it has been shown that such treatment in pigeons results in an increased hepatic lipogenesis and an increased turnover of plasma lipids (Goodridge and Ball, 1967b) and an increase in malic enzyme activity in the liver (Goodridge and Ball, 1966). These authors (Goodridge, and Ball, 1967c) have further demonstrated that in the pigeon daily prolactin injections, also increase both the *in vitro* lipogenic activity and the activity of other lipogenic enzymes, such as citrate cleavage enzyme and malate dehydrogenase, in the liver. In addition the increase in liver weight produced by *in vivo* prolactin administration (Riddle, 1963; Goodridge and Ball, 1967b) would further augment the lipogenic capacity of the hormone treated bird.

Ovine prolactin has been shown to increase glucose oxidation particularly via the HMP pathways, and also increases fatty acid synthesis in the rat epididymal adipose tissue (Winegrad, 1962). Its action was found to be very similar to but not identical with that of insulin, since it does not restore lipogenic activity to normal in the alloxanised diabetic rat (Winegrad, 1962). However no lipogenic action could be demonstrated in adipose tissue of finches, although at high concentrations glucose uptake in this tissue was slightly increased (Goodridge, 1964). The absence of any lipogenic action on hepatic lipogenesis in the duck is therefore not inconsistent with previous results. Goodridge and Ball (1967b) have suggested that the primary *in vivo* action of prolactin might arise from an increased food consumption. The effect on lipogenesis of *in vivo*

prolactin treatment in the bird could be adequately explained in this way.

Oestrogens mainly in the form of diethylstilboestrol have been shown to increase fat deposition in turkeys (Lorenz, 1944), and fowl (Lorenz, 1945, Bird, 1946 and Herbert and Brunson, 1957). Many of these results have been described in a review article written by Lorenz (1954). In vivo oestrogen also increases plasma NEFA, total lipids and lipophosphoprotein in the fowl (Heald and Rookledge, 1964b, and Heald and McLachlan, 1963). Liver slices of immature fowl given oestradiol, promoted incorporation of amino acid into lipophosphoprotein, but the addition of the hormone in vitro produced no effect (Heald and McLachlan, 1963). Attention has been directed to the disparity between the in vivo and in vitro action of oestrogen in the mammal (McKerns and Bell, 1960). Oestrogen accelerates glucose oxidation via the HMP shunt in both rat epididymal adipose tissue (McKerns and Clynes, 1961) and rat liver (McKerns and Bell, 1960). This effect has been considered to be responsible for the increased hepatic lipogenesis in the rat resulting from such treatment (McKerns and Bell, 1960). However in vitro oestrogen either depresses lipogenic activity in rat epididymal adipose tissue or has no effect at all (McKerns and Clynes, 1961). Further the HMP shunt activity of the liver is reduced when oestrogen is either administered in vivo to hypophysectomised rats or added in vitro to liver slices (McKern and Bell, 1960). The results of such experiments have led these authors to suggest

that *in vivo* oestrogen exerts its influence on glucose oxidation in the liver, probably by first inhibiting adrenal corticoid secretion, which in turn results in an increased release of pituitary factors which are finally in some way responsible for the increase in glucose oxidation and associated effects.

The *in vitro* effect of oestrogen on hepatic lipogenesis in the duck is therefore similar to that obtained in the rat. It is unlikely that an inhibitory action of oestrogen could be primarily caused by depression of the HMP shunt activity, as this pathway has been demonstrated to be of only minor importance in glycolysis and lipogenesis in the bird. In fact Duncan and Common (1967) have shown that neither *in vivo* nor *in vitro* oestrogen has any effect on the activity of the HMP pathways in the fowl liver. However an inhibitory effect of *in vitro* oestrogen related to dose could not be established in the duck liver.

The action of glucagon as a powerful stimulant of lipolysis in avian adipose tissue is now well authenticated in the pigeon (Goodridge and Ball, 1965), finches (Goodridge, 1964) and the fowl (Goodridge, 1968a; Langslow and Hales, 1969). The lipolytic response induced by glucagon in adipose tissue from the duck was of a similar magnitude to that obtained previously for other avian species, and was not influenced by age. However the absolute rates of lipolysis when calculated on a fat-free tissue basis were greater in the older birds. In the fowl although the response to the lipolytic action of glucagon is very low in

the embryo following hatching it becomes uniformly high (Goodridge, 1968a) thereafter.

From the foregoing discussion it is apparent that the lack of responsiveness of the duck liver to anabolic hormones may obscure any real changes in lipogenic potential of the tissue during development. More suitable test conditions require to be devised before a real absence of any such developmental changes could be established.

The liver of the adult rat contains two enzymes for the phosphylation of glucose (Di Pietro et al., 1962; Walker, 1962). One of these, specific for glucose (glucokinase) has a high $K_m$ (10-40 mM) whereas the other is a non-specific hexokinase with a low $K_m$. It has been shown that glucokinase activity does not develop in the rat until about two weeks after birth (Ballard and Oliver, 1964a; Walker, 1965). Consequently rat foetus glucose phosphorylation is maximal at glucose concentrations well below the physiological range, whereas increasing the concentration over a wide range markedly increased lipogenesis and glycogenesis in the adult liver (Cahill et al., 1958; Ballard and Oliver, 1964b; Ballard and Hanson, 1967a). It is not known whether a similar system of glucose phosphorylating enzymes occurs in avian liver. However increasing glucose concentrations over a wide range (2-100 mM) produced an increase in lipogenesis of similar magnitude in the liver of both 2 and 10 week old ducks. This suggested that there was unlikely to be any difference between the glucose phosphorylating enzymes in these two age groups.

Few studies have been made of the postnatal development of lipogenesis in homoiotherms. Available reports however indicate that diet is the single most important factor influencing lipogenesis during this period. Goodridge (1968b) showed that although hepatic lipogenesis was initially low in the first few days following hatching in the fowl, subsequently lipogenic activity increased rapidly so that by the time the bird was 6 days old it had reached a stable level which was maintained throughout the remainder of the study. Both these changes and parallel changes in enzyme activity could be attributed to dietary changes. The depression of hepatic lipogenesis in both the embryo and the newly hatched bird were believed to result from the high fat diet of yolk, and the following elevation of lipogenic activity was considered due to the transition from this diet to one rich in carbohydrate. High fat diets have been shown to depress lipogenic enzyme activity in the liver of the young fowl (Pearce, 1968), and the laying fowl (Balnave and Pearce, 1969).

In the rat, hepatic lipogenesis is high in the foetus, declines during suckling and thereafter rises rapidly to adult levels (Ballard and Hanson, 1967a). Parallel changes were observed in lipogenic enzymes (Ballard and Hanson, 1967a; Taylor et al., 1967) with the

exception of NADP-malate dehydrogenase which is apparently absent from the foetal liver (Ballard and Hanson, 1967a; Taylor et al., 1967). If after weaning rats were immediately transferred to a high fat diet, the activity of the lipogenic enzymes remained low (Taylor, et al., 1967).

Hepatic lipogenesis in the rat could be influenced by the onset of sexual maturity (Taylor et al., 1967). During ageing in the rat both lipogenic activity (Benjamin et al., 1961; Taylor et al., 1967) and the activity of the HMP pathways (Benjamin et al., 1961) decrease, although old animals retain similar basal rates of mobilisation and oxidation of fatty acids. However their ability to mobilise and oxidise fatty acids in the presence of epinephrine is reduced in comparison to younger animals (Benjamin et al., 1961).

It is obvious that further research into the development of lipogenesis in homoiotherms is required. At the present time however the results obtained for the duck are consistent with those from other species. During early growth, lipogenic activity appears to be primarily only influenced by diet. In the duck no marked changes in diet occurred during the period studied.

Since the specific rate of fat deposition in the duck declines during development factors other than changes in the inherent lipogenic activity of the liver are required

to explain the pattern of fat deposition during growth. Although a decline in lipogenic activity of the adipose tissue was observed during this period, the contribution of this tissue to total fat synthesis at all times is probably so small that it can be essentially disregarded.

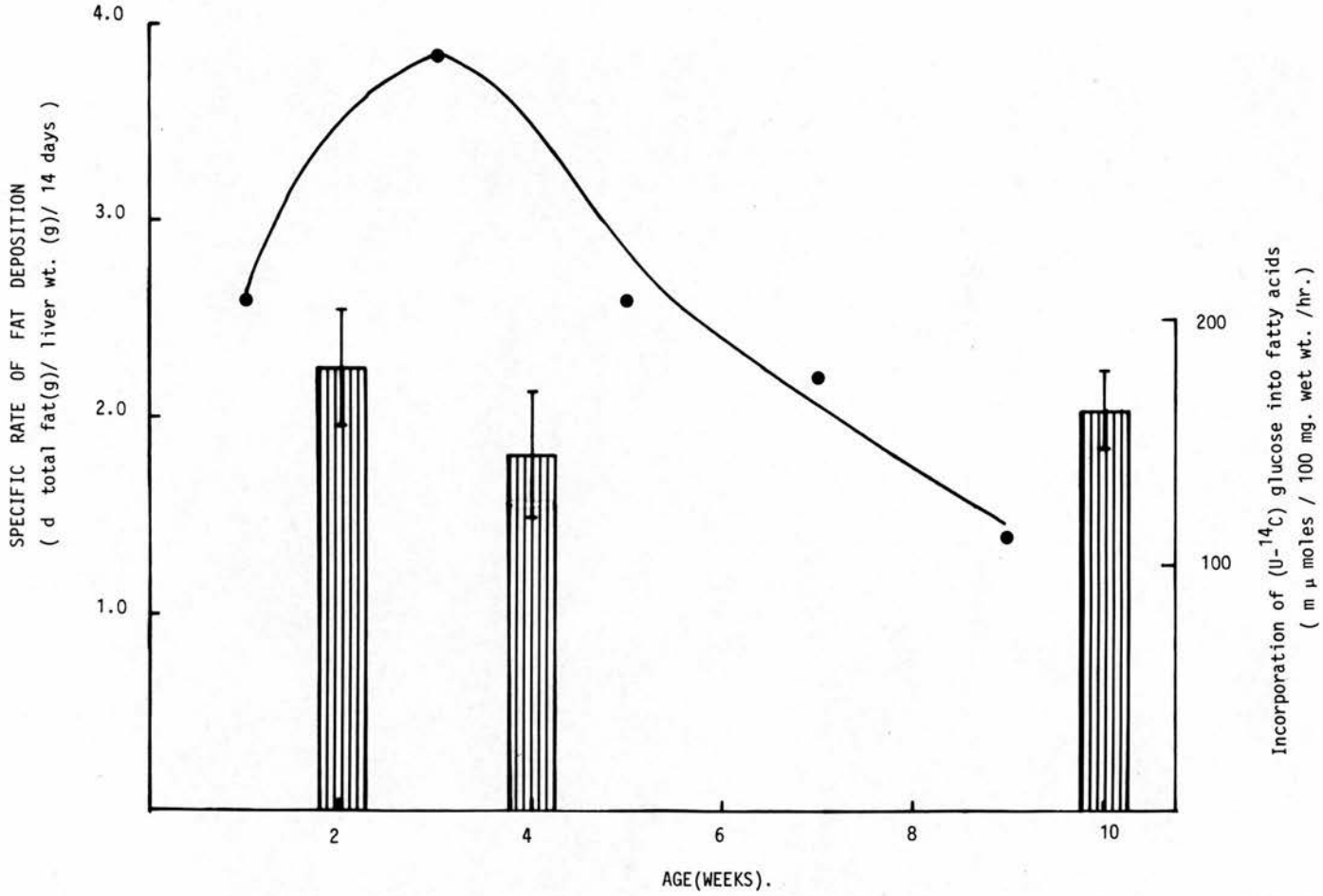
## 5. General Discussion

A survey of the distribution of the fat deposits in various vertebrate species, indicates that the number, size and location of the deposits are adaptive features which reflect the particular ecological needs of each species. It is apparent that there is both an optimal amount, and form of distribution, of the fat in each species, and that the development of the fat deposits proceeds in a genetically predetermined manner. In adult animals homeostatic control operates to maintain the energy store in the mature fat depots at a constant level, under conditions of varying energy input and output.

This study has shown that the fat deposits in the duck develop in a manner which conforms to the processes of normal growth and development of other tissues. In addition the distribution of fat in this bird is consistent with the semi-aquatic habitat of the species. Changes in the structure of the adipose tissue, and the lipogenic activity of the liver and adipose tissue during growth have been examined in order to assess the relative importance of the roles of these two tissues in the development of the fat deposits. It is the purpose of this discussion to consider some of the more general implications of these results.

Studies of the accretionary growth of fat in the various main depots of the duck have produced two main results. First, it has been shown that the growth of the fat depots parallels the growth of the major body components. Second, the individuality of the growth of the separate depots has

Figure 5.1




The in vitro and estimated "in vivo" lipogenic activity of the duck liver during growth. The in vitro activity is represented by the histogram, and the scale for this is on the right hand side.

been established. These results indicate that the processes involved in fat deposition are influenced by general growth factors whose action can be modulated by local factors specific to each depot.

*In vitro* studies with liver and adipose tissue support previous findings which signify that the liver is the primary site of lipogenesis in the birds so far examined. The studies of Goodridge and Ball (1967a,b) have established this result conclusively for the pigeon. Such findings suggest that calculation of the specific rate of growth of the total fat, should be related to the size of the liver rather than to any other body component. The specific rate of deposition of fat has therefore been recalculated on this basis (Figure 5.1.). In passing, it should be noted that although this produces a slightly different result from that shown in Figure 4.13/5., the new result will not effect the conclusions made in Section 4.1.4. The calculated specific growth rates of total fat have been used here to represent an estimate of the *in vivo* lipogenic activity of the liver during this period. The absence of agreement between these values and those obtained from *in vitro* measurements, indicate that changes in liver activity cannot be responsible for producing the observed changes in fat deposition.

In this study it was shown that although oestrogen administered *in vivo* stimulated the lipogenic activity of *in vitro* liver, addition of the hormone directly to the *in vitro* preparation had no effect. Similarly although prolactin has been shown to induce lipogenic activity in the

liver when given *in vivo* (Goodridge and Ball, 1967c), in this study, no effect was observed after the addition of the hormone *in vitro*. Two alternative explanations could account for the absence of an *in vitro* effect. First, the action of the hormones may be mediated by another agent. Second the lipogenic effect of the hormones may be dependent upon protein synthesis (i.e. enzyme production), in which case the reaction might be too slow to produce an observable effect within the incubation period. Alternatively a lack of suitable substrates in the incubation medium could be responsible for inhibiting protein synthesis. The results obtained with both *in vivo* and *in vitro* insulin confirmed previous findings that this hormone has no lipogenic action in birds. From these observations it could be concluded that if hormones were responsible for producing the changing pattern of fat deposition via their action in the liver, then their effects would be detected *in vitro*. It would therefore seem likely that neither developmental or hormone induced changes in the liver activity can account for the observed pattern of fat deposition in the duck.

Adipose tissue, as the repository of the deposited fat is therefore considered as a possible alternative site at which regulation of the growth of the fat depots might occur. From an examination of the cellular changes in various depots it was possible to conclude that an upper limit in size for the adipocyte was not approached in the young duck during growth. Previous studies indicated that adipocyte size has never been found to be limiting

even in extremely obese animals. Such results indicate that the storage capacity of adipose tissue can continue to increase simply by hypertrophy of the adipocyte. However both this and previous studies have shown that adipocyte number increases, particularly during early post-embryonic growth. This increase in adipocyte number might therefore be regarded as the cause of increased fat deposition rather than as its effect. The following discussion examines the means by which an increase in adipocyte number could bring about an increase in fat deposition.

In the bird, the main role of the adipocyte in fat deposition, can be assumed to be first, the hydrolysis of plasma triglyceride, secondly the uptake of the released fatty acids and thirdly, their re-esterification to form triglyceride. The initial step, namely the uptake of fatty acids is controlled by the activity of the enzyme lipoprotein lipase. This enzyme has been shown to be a significant factor in the control of fat deposition in the rat (Garfinkel et al., 1967). In a form of congenital hyperlipaemia, in which lipoprotein lipase was found to be virtually absent from the adipose tissue, the fat deposits were poorly developed (Gennes, 1969).

The main enzymes involved in the re-esterification of the fatty acids are acyl CoA thiokinase, α-glycerophosphate acyl transferase, and acyl CoA deacylase (Daniel and Rubinstein, 1968). High activity in these enzymes occurred during periods of increased fat deposition (Shapiro, 1965; Daniel and Rubinstein, 1967). An increased activity of the

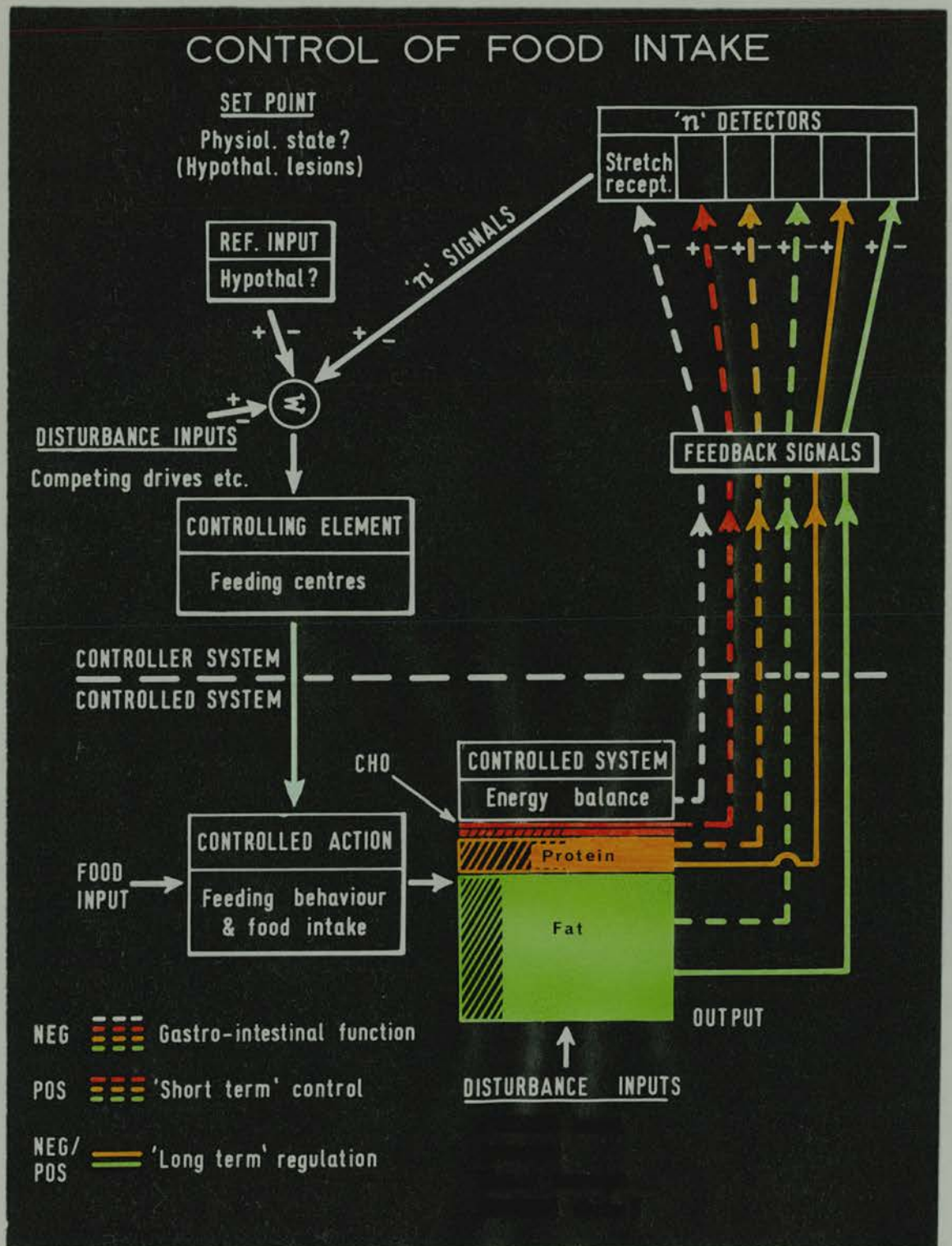



Figure 5.2
('+' stimulates, '-' inhibits feeding activity)

esterifying enzymes would have the effect of maintaining the intracellular concentration of fatty acid at a low level thereby preventing any reduction of lipoprotein lipase activity.

Previous studies have shown that newly formed adipocytes are more metabolically active in several respects than the more mature cells (Braun et al., 1968; Hahn and Greenberg, 1968). It is proposed that a higher activity of either lipoprotein lipase or the enzymes of the re-esterifying system in the newly formed adipocyte could account for an increased rate of fat deposition in the younger animal.

Ultimately, the tissue activity responsible for influencing the rate of fat deposition, must also in some manner influence food intake. In this respect regulation of food intake in the young growing bird may share common elements of control with the system which is responsible for the homeostasis of the fat deposits in the adult. Regulation of the food intake in the adult is directed towards maintaining the energy reserves at a constant level. The manner in which such control could be achieved is shown in Figure 5.2. There is as yet little convincing evidence for the existence of discrete feeding centres in the avian brain (Lepkovsky et al., 1967b). However feeding centres of some form are likely to occur, and have been postulated for the purpose of this model. Other necessary elements of the system are transducers which generate signals indicating the state of the energy reserves, and detectors which convey the information in these signals to the feeding centre to

elicit the appropriate behavioural response.

The sequence of events in energy depletion resulting from fasting, will be first a rapid and virtually complete depletion of the carbohydrate reserves, followed by a reduction in the fat reserves which will be accompanied at least initially by a relatively small loss of protein. Since carbohydrate reserves, namely hepatic glycogen, are very small (Hazelwood, 1965) they can be made replete by the food consumed in a single meal. In contrast both protein and fat losses from prolonged fasting can be considerable. That these can be restored to their former energy level implies that in some way the extent of the deficiency must be gauged so that the response can be of an appropriate duration. In addition adipose tissue has a unique role in the body, in that it is the final recipient of any excess energy taken, as a result of errors in the system regulating food intake. Consequently it would be expected that the feedback signal arising from an energy surfeit would arise from the adipose tissue alone. In Figure 5.2. "short term" feedback loops represent a non quantitative form of control, in that such signals will arise early in energy depletion to signal the need to eat. On the other hand, the "long term" feedback controls arising from the fat and protein stores must be quantitative in that feeding must be continued until the total energy lost by the stores are recovered. Indirect evidence for the involvement of the adipose tissue or the fat deposits in the regulation of food intake in the mammal

is plentiful (Kennedy, 1950; 1953; Hervey, 1959; Hoebel and Teitelbaum, 1966). However Hervey (1969) has been the first to suggest how such lipostatic control might be achieved. His theory proposes the existence of a humoral factor which can influence feeding behaviour, which is continuously produced, and which though soluble in water has a high partition coefficient in favour of fat. He proposes that this hypothetical substance is a steroid, and that it acts as a "tracer" in a dilution measurement technique, whereby its level in the blood is determined by the size of the fat stores.

Liebelt et al. (1965) using mice have shown that surgical removal of a fat depot, results in compensatory hypertrophy in the remaining depots, which is accompanied by hyperphagia in the recovery phase. Although this result could be accounted for by Hervey's theory, the striking similarity between the regenerative capacity of adipose tissue and other tissues such as liver and kidney suggests the involvement of tissue growth regulators such as have been proposed to account for compensatory growth and wound healing. Bullough (1965) has proposed a theory for the control of growth which accounts for both special and general aspects of growth. This theory is based on his discovery of tissue specific growth inhibitors called chalones. These agents inhibit mitosis. According to Bullough's theory, chalones may be responsible for determining the pattern of growth of organs and tissues. The level of the chalone within the cell in which it is being

produced, is largely determined by its concentration within the extracellular fluids, since it is believed to be lost from the cell by simple diffusion. Consequently if the quantity of a particular tissue within the body is reduced, the total production of its chalone within the body will fall, resulting in a fall of intracellular chalone concentration in this tissue. If the drop in chalone concentration is sufficient to fall below a threshold value, then the cell will be released from the inhibitory effects of the chalone and mitosis will proceed. Like Hervey's theory this theory also proposes the utilisation of a dilution technique to relate the size of the organ or tissue to the total body size. In this case the "tracer" is the chalone. Although Bullough has made no such claim, it could be inferred from the action of such a regulatory system that the chalone either directly or indirectly exerts some control on the regulation of food intake.

As shown in the present and previous studies both the cellular mass and the fat mass of the fat deposits increase during growth. In a review of the effect of various experimental conditions on the composition of adipose tissue, Hausberger (1965) reported that during fasting, reduction of the fat deposits was accompanied by atrophy of the fat-free tissue. There was however no evidence for any reduction in cell number. Alternatively during the induction of obesity by dietary or hormonal means, both hypertrophy and hyperplasia of the cellular component of the adipose tissue was produced. Hervey's theory for the regulation of the fat depots does not really account

for the changes in the cellular component of adipose tissue, and Bullough's cannot account for the control of the fat component in this tissue.

A more recent experiment by Liebelt's group (Liebelt et al., 1968) cannot be satisfactorily explained by Hervey's theory. In this experiment syngeneic grafts of adipose tissue were shown to become established only in those animals from which fat depots had been previously removed. These authors drew attention to the similarities between adipose tissue and blood cells. First, it is now accepted that adipocytes and blood cells arise from similar primordial cells. Secondly, there is evidence to suggest that the cellular mass of each tissue is regulated as a single unit in the body. On this basis they have proposed that humoral factors such as erythropoietin or granulopoietin may be involved in the regulation of the growth of the adipose tissue. Their experimental results can be adequately explained by Bullough's theory of growth.

It is apparent that both Hervey's and Bullough's theories may be required to explain the two aspects of growth of the fat depots, particularly since it has been suggested that the growth of the fat component does not appear to bear any definite relationship to the growth of the non-fat or cellular part (Liebelt, 1959).

To summarise, it is proposed that regulation of the growth of the fat depots could be accomplished in the following way. Early growth of the depots could be due to a proliferation of adipocytes and associated tissues. The

role of vascular tissue during the early development of the depots may well be important, but has yet to be assessed. The primary cause of the cellular growth may be the low concentrations of tissue specific growth inhibiting substances (chalones). In addition it has been indicated that the growth of the fat depots is also influenced by a general growth factor. This role could be filled by a hormone such as growth hormone, since this hormone markedly increases the rate of cell multiplication in adipose tissue (Murakawa and Raben, 1968; Hollenberg and Vost, 1968b). Moreover Bullough (1965) has shown that hormones can influence the activity of the tissue specific growth inhibitors. It should be noted that the existence of a growth hormone in the bird has yet to be established.

An implication of such a system is that a restriction of food intake would be expected to impair the growth of all those components influenced by the general growth factor. In this respect the results described in Section 4.3.3, were consistent with such an hypothesis. In this experiment a decreased food intake imposed on growing birds as a result of prolonged protamine zinc insulin treatment, was shown to have no effect on the fat content of the birds.

In mammals growth hormone also has an important physiological role in mobilising fatty acids from the fat reserves in the adipose tissue (Glick et al., 1965; Hunter et al., 1965). This action of this hormone is in direct contrast to its growth promoting effects on this tissue. Korner (1965) has suggested that the different actions of

growth hormone may be determined by the availability of substrates. In a state of repletion growth hormone would influence cell growth whereas, in a negative energy balance it would act to mobilise further reserves. This explanation is supported by the results obtained by Hunter et al., 1968. An alternative explanation of the twofold action of growth hormone on adipose tissue is provided by Daughaday and Kipnis (1966). They propose that the growth promoting action of growth hormone is brought about by its ability to maintain an additional growth factor in the blood which in turn is responsible for the "orderly stimulation of growth".

In addition to a general growth factor, specific factors must be postulated to account for the individual pattern of growth of each depot. These factors are no doubt inherent within the tissue of each fat depot.

The effect of the proliferation of adipocytes in the young animals would be to produce cell populations in the fat depots in which newly formed cells predominate. If as it has been suggested either the fatty acid uptake or re-esterifying systems were more active in the newly formed cells, adipose tissue would represent the main site at which regulation of the fat deposition occurred. There is some slight evidence from this study which could support such a conclusion. It was shown that there was a slight but significant decline in the ability of the adipose tissue to synthesise glycerol <u>in vitro</u> with age. A somewhat similar result was obtained with rat adipose tissue (Hahn and Greenberg, 1968). The availability of α-glycerophosphate is

considered to be of prime importance for the activity of the re-esterifying system (Leveille, 1967).

The two growth regulating systems proposed separately by Bullough and Hervey could represent together or separately opportunities for feedback control from the adipose tissue to the centres regulating the level of food intake. It might however be expected that a shorter feedback loop would operate between the adipose tissue and the liver, since the latter is the primary site of lipogenesis. As yet there is no evidence to indicate how this would be achieved.

The absence of developmental changes in the lipogenic activity of the liver is perhaps not very surprising. Basic pathways for gluconeogenesis and glycogenolysis are well developed in the avian embryo (Goris and Merlevede, 1969). Such general pathways as these and lipogenesis might be expected to be fully developed at an early age. In addition it has been shown in previous studies (see discussion Section 4.4.) that diet is the most important factor influencing the activity of the lipogenic pathways both in pre- and post-natal life.

The absence of the action of insulin on avian lipogenesis may be related to the fact that the liver is the primary site of lipogenesis. In addition however there are also a number of mammals in which insulin has been shown to have no effect on lipogenesis. Some of the anomolous actions of insulin on lipid metabolism in the bird could be attributed to the effect of glucagon since this hormone appears to be the most important glucoregulatory and lipolytic hormone in the bird. In this respect the bird appears to

represent one end of the spectrum for the development of glucagon function in the vertebrate phylum. The bird has relatively large amounts of glucagon in the pancreas, it has a high normal plasma glucose (approximately 200 mg/100 ml), and with the exception of the rat represents the only vertebrate so far tested in which glucagon is lipolytically active at physiological levels (Lefebvre and Luyckx, 1969, and see Discussion, Section 4.4.). At the other end, cyclostomes lack glucagon (Falkmer, 1966), have a low plasma glucose (16 mg/100 ml) and were unresponsive to the glycogenolytic action of glucagon (Falkmer and Matty, 1966). Foa (1968) has suggested that the main role of glucagon may be related to its ability to stimulate the activity of certain pathways in a manner related to the body's metabolic needs. In mammals glucagon increases both the rate of hepatic gluconeogenesis and glycogenolysis. A further function of glucagon in the mammal may be its role in stimulating insulin secretion. The insulinogenic action of this hormone has been demonstrated by Marks and Samols (1969). In birds however this function may be reduced or absent since in contrast to the arrangement of pancreatic islet tissue in the mammal, the α cells are in separate islets to the β cells. It is apparent that the role of glucagon is of major importance both with respect to the lipid and carbohydrate metabolism of the bird, and will require further investigation.

The hypothesis proposed to account for the control of fat deposition during growth obviously represents an over-simplification of a very complex problem. Biological

control systems characteristically have a high level of "redundancy", and feedback loops are frequently of variable complexity. However the main function of this hypothesis is to indicate future areas for research. Should the adipose tissue prove to be the main site for the regulation of fat deposition as proposed here, then a number of interesting possibilities would arise. Since adipose tissue biopsy samples are easy to obtain, the lipogenic activity or potential of the bird might easily be measured or characterised. This could possibly provide a valuable approach as a basis for selection programmes directed towards improving body composition of commercially reared birds. Furthermore avian adipose tissue may provide an ideal situation for the study of the development of activity in the adipocyte. Electron microscope studies have drawn attention to an abundance of microfilaments in this tissue arranged in an orderly array about lipid droplets. Following the appearance of this structure during the development of the adipocyte would provide useful information regarding the development of function in these cells.

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(including additional Bibliography)

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