THE COMPOSITION OF ADIPOSE TISSUE UNDER VARYING CIRCUMSTANCES

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CONTENTS

THE AIM OF THE INVESTIGATION

The stimulus which evoked this thesis was the following description by Davidson, Meiklejohn and Passmore of the morbid anatomy of obesity. "The number of cells in the body capable of accepting stores of fat is said to be strictly limited and not increased in obese people. Each cell is simply distended with fat."(1)

The aim of this investigation is to try and show whether in fact this is true. In obesity do each of a set number of cells become increasingly distended with fat, or is there an increase in the number of fat cells? If the statement quoted above is correct, then the more obese the subject, the greater will be the proportion of fat in adipose tissue. If there is an increase in the number of cells with increased deposition of fat, the proportion of water, fat and protein should remain approximately the same.

It was decided that a suitable way to approach the problem would be to analyse the chemical components of adipose tissue in normal, lean and fat mice, and in normal and obese human subjects.

PREVIOUS STUDIES

The chemical composition of the fat in adipose tissue has become a subject of considerable research; and techniques for the analysis of the triglycerides have developed tremendously during the last few years. This has mainly been due to the realization that adipose tissue participates actively in lipid metabolism and

and may play an important role in the etiology of obesity and atherosclerosis. Very much less attention has been given to the tissue that stores this fat.

Until 1911, average fatty tissue was considered to contain about 90% fat and 10% water. How these figures were obtained is uncertain for no references can be found in the literature. Bozenraad (2) then analysed some human adipose tissue for its water content and found that this ranged from 7 - 46%.

Aware of the unsatisfactory character of information on the chemical composition of the adult human body, Mitchell (3) attempted an investigation into the matter. He had hoped to analyse a number of human cadavers in a satisfactory nutritive condition, but was unable to obtain specimens very readily, and thus published data on a single specimen. This was an adult man 35 years of age who had died from a heart attack. The age-height-weight relationship, when applied to Edwards nomogram, indicated that the subject was about 11% underweight. Individual analyses of the skeleton, musculature, skin and several visceral organs, amongst them adipose tissue, are reported. Mitchell found that 50% of the fatty tissue was water, 42.4% was ether extract, 7.1% crude protein (N x 6.25) and the remainder ash. Phosphorus content was estimated on the ash and this represented .048% of the adipose tissue. The body on which these analyses were done was preserved only by freezing until dissection was started about 6 weeks after death, so it is doubtful that these results can be regarded as very accurate.

In 1953 [4] a similar study was completed on an adult male 46 years of age. His appearance was that of a thin man, in good physical condition, 23% below average weight for his age and height. His death was due to a skull fracture. In this analysis the components of adipose tissue were 23% water, 71.6% fat, 5.8% protein and .2% ash. Phosphorus was .03%. The body was preserved in a frozen state for a month before it was dissected, but precautions were taken to minimize dehydration during storage by wrapping the specimen in a plastic sheet.

Vague and Garrigues [5] were persuaded after a long clinical study that the function of adipose tissue extended well beyond that of storing energy and undertook a chemical study of its components centred on its steroid content. They analysed adipose tissue taken by biopsy or post-mortem from 70 women and 35 men, but they do not mention what state of health the subjects were in or the site the adipose tissue was taken from. The mean age of the men was 46.3 years, and that of the women was 43.7 years. The men weighed a mean of 79.5 kg while the women had a mean weight of 80.3 kg. They found the composition of the adipose tissue was 28.7% and 23.2% water in men (M) and women(W) respectively with 56.4% M and 70.0% W fat and 7.4% M, 8.5% W residue. The iodine number for men was 73.1 and 74.1 for women.

They correlated each of the components of the tissue with age and found that the fat free residue increased with age in men, while the fat increased to a much smaller degree and the water decreased slightly. The residue decreased with age in women with a corresponding decrease in water, while the fat content increased a little.

(6)
Enterman, Goldwater, Ayres and Behnke analysed samples of adipose tissue taken from the abdomen of two men before and after they had lost weight. The change in composition was compared with calculations from data on body weight, body water and body volume in an attempt to obtain additional information on the nature of alterations produced by loss of weight. The subjects were two normal and healthy men aged 53 (B) and 44 (E). Both had attained weights somewhat above their 'desirable' weights by overeating, and had then restricted their caloric intake to 1200 - 1400 Cal. Subject B weighed 101.5 kg at the beginning of the experiment and 86.6 kg at the end. This was a loss of 14.9 kg. in the space of four months. His adipose tissue composition before and after weight loss was 79.2% and 62.3% fat, 18.6% and 32.4% water and 2.8% and 5.3% residue. Subject E weighed 89.1 kg and in just over a month reduced his weight by 8.4 kg to 80.73 kg. There was a similar change in his adipose tissue composition. The water content rose from 12.5% to 17.7% and the residue rose as well from 1.8% to 3.4% while the fat content decreased from 85.7% to 78.9%

Their results lead them to conclude that adipose tissue probably contributes the greatest amount of fat lost during weight reduction, while other soft tissues also contribute significantly to the body weight loss by losing non fat components.

The only analysis of adipose tissue both in lean and obese subjects, to my knowledge, has been done by Pawan and Clode. (7) They obtained biopsy specimens from 6 lean and 7 obese persons and 14 lean and 14 obese post mortem specimens. The specimens were subcutaneous adipose tissue taken from the same area of the abdominal wall. In the lean subjects the biopsy tissue had mean values of 28.6% water, 68.4% fat and 2.1% protein. Post mortem tissue had mean component values of 26.1% water and 72.1% fat. Adipose tissue in the obese had slightly less water, the mean value being 18.8%, and more fat with a mean value of 78.7%. The protein content was no different from that in the lean adipose tissue and had a mean value of 2.0%. The iodine numbers (Wij's) of the fatty acids in the subcutaneous adipose tissue in the lean were 71.1 - 74.8 (mean 73.7) and 67.7 - 71.6 (mean 69.1) in the obese. Pawans' data indicates a wide variability in the gross composition of adipose tissue in human subjects.

There do not seem to be any analytical data on the composition of adipose tissue in mice and the analysis of adipose tissue in animals seems to have been restricted to the rat and the guinea pig. During a study on the relationship between body water and chemically combined nitrogen content with body fat content, Pace and Rathbun analysed the perirenal fat of 20 guinea pigs. They found that the fat and water components in this adipose tissue have mean values of 84.13% and 10.25% respectively.

Babineau and Page (9) analysed perirenal adipose tissue, as well as pelvian and scapular fat, in rats. 69 samples of perirenal fat, with an average weight of 4.8 g., were analysed and it was found that the fat component comprised 87.6% water, 10.3% and fat free dry matter 2.1%. The 39 samples of the panniculus adiposus with an average weight of 18.0 g had a composition of 73.9% fat, 18.6% water and 7.5% fat free dry tissue. Babineau claims that the composition of the pelvian and scapular fat in rats is similar to the composition of fat in man, as described by Brozek and Keys[10]. But it is evident that he has not distinguished between adipose tissue and so called "obesity tissue".

In addition to the study on adipose tissue composition both in mice and men, a further analysis was made of adipose tissue in rabbits, sheep and pigs. A short study on the possibility of mitosis in adipose tissue of mice and rats was also undertaken. Colchicine was used as the mitotic inhibitor.

METHODS

Six components of adipose tissue were analysed. These were, water, fat, protein, potassium, sodium and phosphorus. An estimate of the degree of saturation of the triglycerides was also made using the iodine absorption method. Water, fat, protein and iodine number were all estimated on the same sample, while sodium and potassium were analysed in a separate sample. Most of the components were analysed in triplicate, but when the sample was too small only duplicate analyses were possible. Any tissue sample which was left from the above analysis was stored in the deep freeze and this was later analysed for phosphorus.

Water

Drying in a desicator under vacuum was attempted, but proved unsatisfactory as a skin always formed over the tissue and thus prevented complete extraction of moisture.

Some of the first samples were dried in large test tubes in a water bath at 70°C. The test-tubes were connected to a suction pump and the air was dried by drawing it through a series of calcium chloride and phosphorus pentoxide u. tubes. A steady weight was attained after 8 hours of drying.

Finally when an oven was made available the rest of the moisture determinations were done by drying in the oven at 80°C. Of the many methods described for moisture determinations, oven drying is the oldest and undoubtedly the most widely used technique. The samples were weighed into small 10 ml beakers and it was found, as before, that a steady weight was reached after

8 hours drying. The drying period could be cut down to three hours once a vacuum oven was obtained and no change in the estimations were found when the temperature was raised to 105°C and a vacuum of 130 m.m. Hg. was applied. The difference between the weight of the sample before and after drying was taken as a measure of moisture in the sample.

Fat.

The small soxhlet apparatus was used for fat estimations. The thimble cylinders had a volume of 60 ml and the round bottom flasks a volume of 100 ml. 6 of these sets were set up over a long rectangular water bath and the temperature of the water was kept at 40°C , just above the boiling point of ether.

Because the tissue was already dry prior to extraction it was considered unnecessary to use Bloors alcohol-ether mixture and anaesthetic ether alone was used as the solvent for extraction.

The dried adipose tissue was transferred with a spatula into the soxhlet thimble, which was fixed firmly on to the neck of the cylinder with a clip so as to ensure no loss of either tissue or ether. Once the bulk of the tissue had been transferred to the thimble, the remaining fat in the beaker was dissolved in ether and washed several times into the cylinder.

The samples never exceeded 500 mg and it was found that reflux overnight for 16 hours was sufficiently long for complete extraction. Once the ether had been distilled from the flasks the fat was dried in an oven at 80°C for two hours and then allowed to cool in a desicater under vacuum before being weighed.

These

flasks were carefully cleaned and weighed before each experiment and their increase in weight once the fat had accumulated was taken as a measure of fat in the sample. Weighed amounts of fat were used as standards and recovery was found to be within 1%.

Protein.

The micro-Kjeldahl method was used for protein estimations.

Before digestion the fat free dry tissue was dried completely in the oven and then weighed into small digestion tubes, from the soxhlet thimbles. This was done as rapidly as possible so as to avoid any water absorbtion from the air. A spatula tip of catalyst, (5 parts $CuSO_4$, 15 parts K_2SO_4, 5 parts Hg oxide and 1 part selenium oxide) and 1 ml of concentrated sulphuric acid were added to the tissue and this was allowed to digest on an electric heater for two hours by which time the digests were colourless.

A Markham-still was used for the distillation and the digested sample was washed straight into it once it had been allowed to cool. Six small washings were sufficient to transfer the sample. No dilution was necessary as the sample was small enough. The mean weight of the fat free residue was 10 mg.

15 ml of 40% sodium hydroxide were added to each sample to release the ammonia and this was trapped in 10 ml of a .025N solution of boric acid during a period of 10 minutes.

The indicator used was a mixture of methyl red and methylene blue, which was only made up just before use as it tends to deteriorate on standing. The mixture I found satisfactory was a methyl red to blue ratio of 6.1. Six drops of this gave a good pink colour in boric acid and turbed a sharp lime colour as soon as ammonia was released into it. The ammonia was titrated with .025N hydrochloric acid to a faint permanent pink colour.

Sodium hydroxide was standardised with potassium phthalate with phenol phthalein as indicator and then used to standardize the hydrochloric acid.

Sodium and Potassium

Samples specially put aside for these estimations were kept to approximately the same weight at an average of 300 mg. These were first dried to assess the moisture content and then reduced to a white ash by heating in a muffle furnace at 450°C for 16 hours.

The ash was dissolved in 5 ml distilled water and then diluted to give readings on the photometer at a maximum sensitivity. Each solution was then read against a standard solution of approximately the same molar strength and checked 3 times.

The diluted standards used had concentrations of .38 ug Na: 1.30 ug K; .76 ug Na: 2.60 ug K; 1.53 ug Na: 5.21 ug K; 2.30 ug Na: 10.43 ug K per ml and 1.53 ug Na and 2.60 ug K gave full deflection at maximum sensitivity.

Every precaution was taken to avoid contamination of the tissue with Na by using Pyrex Grade glassware. The blank used was the same distilled water used for the dilutions.

The EEL flame photometer was used for these estimations.

Phosphorus.

There are many varieties of Osmonds method of phosphorus estimation, in which phosphorus forms phosphomolybdic acid and then becomes reduced to a blue compound suitable for colourimetric measurements. I have used Allens adaptation[12]. Allen originally estimated phosphorus by a simple adaptation of Kings perchloric acid method,[13] but later substituted amidol in place of amino naphthol-sulphonic acid as a reducing agent.

After the adipose tissue had been dried and extracted for fat, the residue was weighed into small micro-Kjeldahl flasks. The average weight of the tissue was 10 mg. 2.5 ml of perchloric acid was added to the flask and the tissue was digested at a low heat over an electric heater until it was colourless. After cooling, the contents of the flask were thoroughly rinsed into a 25 ml volumetric flask. 1 ml ammonium molybdate was added and lastly 2 ml amidol and water to make up to the 25 ml mark. The amidol and water were added at regulated intervals so that the extinction coefficients could all be read between 5 and 10 minutes after they had been mixed.

A standard solution containing .008 mg phosphorus was digested with the samples and its extinction coefficient when checked against the unheated solution was identical.

SOLUTIONS

Ammonium molybdate solution 8.3 g of A.R. reagent were dissolved and diluted to a 100 ml with distilled water.

Perchloric acid. 60% solution (S.G.1.54) A.R.

Amidol. 2 g amidol and 40 g pure sodium meta-bisulphate were dissolved in glass distilled water and diluted to 200 ml. The reagent was kept in a dark place in the refrigerator and discarded after 10 days.

Standard phosphate solutions.

A stock solution containing 1 mg phosphorus per ml was prepared by dissolving 1.0967 g A.R. potassium hydrogen phosphate in distilled water and diluted to 250 ml. This solution was stored over chloroform at 0^{o}C. Suitable standard solutions for the calibration curve were obtained by dilution of this stock solution. The dilutions used were .004, .008, .014, .016 and .020 mg phosphorus per ml.

The Unicam spectro photometer was used and all readings were taken at 660mμ with a red filter.

Iodine Number.

Hawks (14) adaptation of Wij's method was used. 10 ml of carbon tetrachloride were pipetted into the round bottom flasks containing the weighed ether extract from the soxhlet distillation. After the fat had been dissolved 25 ml of Wij's iodine solution (supplied by B.D.H.) were added. This was well mixed and then stored in a dark place for one to two hours. The solution was then transferred to a 500 ml conical flask. Any solution left in the round bottom flask was washed out, first with 10 ml of 10% potassium iodide and finally with water to make up a volume of about 250 ml.

This was titrated immediately with a 0.1 N standard solution of sodium thiosulphate. Starch powder was added when the solution had become yellow and disappearance of the blue colour was taken as the end point. The untitrated iodine in the carbon tetrachloride revealed by its pink colour, was brought into solution with continued shaking.

To determine the iodine equivalent of the Wij's solution, 10 ml of 10% potassium iodide and about 200 ml of water were added to 20 ml of Wij's solution already in a 500 ml conical flask. This was then titrated with the standard sodium thiosulphate.

The sodium thiosulphate solution was standardized with potassium iodate. 24.8 g pure dry sodium thiosulphate was dissolved in distilled water and washed into a litre volumetric flask. 10 ml of amyl alcohol and 1 ml of 20% sodium carbonate were added to prevent decomposition and the solution was made to volume. The solution was kept in a bottle carrying a soda-lime tube to exclude carbon dioxide.

For the standardization of the thiosulphate 25 ml of 0.1 N potassium iodate were pipetted into a conical flask and 2 ml of 100% KI (2g. KI) were added. A solution of equal volumes of concentrated hydrochloric acid and water, was made up and 10 ml of this was added to the potassium iodate to liberate the iodine. This was titrated immediately. Starch powder was added when the solution became pale yellow, and the end point was reached at loss of blue colour. The thiosulphate was standardized and the iodine equivalent was estimated with each group of titrations. Olive oil was used as a standard fat solution and this gave a constant value of 83.

ANALYSIS OF MOUSE TISSUE

MATERIAL

The mice used for this investigation were the outcome of a selection experiment, carried out by Dr. Falconer at the Institute of Animal Genetics,(15) in which selection was based on growth during the 3rd to 6th weeks of post natal development. A two-way selection was applied to one pair of lines for growth-rate when the individuals were reared on a high plane of nutrition, and to another pair of lines for growth-rate when the individuals were reared on a low plane of nutrition, one line of each pair being selected for increased growth and the other for decreased growth. This programme of selection was extended over 13 generations.

The high plane of nutrition was the normal cubed diet consisting of 56.8% carbohydrate, 18.5% protein, 4.5% fat, 12.9% water and 7.3% ash. The low plane of nutrition was provided by a specially prepared cubed diet made from ingredients of the normal cubes and indigestable fibre, in equal volumes. The fibre was in the form of ground oat husks. This low diet was fed ad. lib over the 3 week period from 3 to 6 weeks of age and then the normal high diet was fed.

Selection for large size on a low plane of nutrition was found to result in mice with less fat than those produced by selection on a high plane of nutrition.

These selected large mice were used for this investigation because of their difference in fat composition and the original stock from which they had been selected was included for comparison. All the mice used were males.

The chemical components in adipose tissue were analysed on the epididymal fat pad of these mice and the weight of the pad was also used as an index of the fatness of the animal.

Hull (16) has shown the weight of this fat body to be highly correlated with the total body fat.

The mice were killed by cervical dislocation at ages between 10 and 14 weeks. Their epididymal fat was removed immediately, without its main vascular trunk, and a section from each pad was snipped off into platinum cups for potassium and sodium estimations. The remainder of the material was weighed into small 10 ml beakers for water, fat and protein estimations. The shortest possible time was taken between the removal of the fat and its weighing. Contamination of the tissue was guarded against by careful washing of stainless steel instruments with glass distilled water.

The mean values of all the data for the 3 mouse groups including number, age, Body weight, Epididymal fat pad weight and its composition

TABLE I

	Age days	No.	Body Weight g	Weight of Fat g	Water g %	Fat g %	Protein g %	Potassium MEQ/100 g	Sodium MEQ/100 g
Control	90	10	31.4	0.63	7.5	90.8	0.79	0.44	0.80
Large fat	86	7	54.6	1.42	6.5	92.8	0.75	0.48	0.69
Large lean	78	7	42.7	0.48	8.2	90.3	1.31	0.65	1.34

TABLE 2

The significant differences between the 3 mouse groups

	15 degrees freedom 1% Probability 2.95 5% Probability 2.13		15 degrees freedom 1% Probability 2.95 5% Probability 2.13		12 degrees freedom 1% Probability 3.06 5% Probability 2.18	
	Normal - Large fat		Normal - Large lean		Large fat - Large Lean	
	Mean diff.	t	Mean diff.	t	Mean diff.	t
Body Weight	- 23.2	8.0	-11.3	8.3	+11.9	4.05
Fat body	- 0.79	3.4	+ 0.15	1.1	+ 0.94	4.69
Water	+ 1.0	1.8	- 0.7	1.1	-0.17	2.90
Fat	- 2.0	1.9	+0.5	.12	+2.5	2.93
Protein	+ 0.04	.27	- 0.52	3.6	- 0.56	4.4
K	- 0.04	1.9	- 0.21	4.6	- 0.17	3.85
Na	+ 0.11	1.5	- 0.54	8.1	- 0.65	10.5

RESULTS

The descriptive and analytical data on each of the three groups of mice, the control, the large lean and large fat, are presented in summary in table 1 with full details in the appendix. They include age, body weight, fat pad weight and the chemical analyses of water, fat, protein, potassium and sodium in the fatty tissue.

An attempt to analyse DNA in the adipose tissue of the control mice, using Ceriotti's (17) method proved unsuccessful, and analysis of potassium was substituted as a means of estimating the cellularity of the tissue.

Potassium and sodium were therefore estimated on the adipose tissue of a second batch of control mice, and it is for this reason that they are listed separately.

The data on the three mouse groups were compared against each other using 'Students' 't' test. These 't' values are listed in table 2.

Body Weight

The most marked difference between the three groups is their body weight. The control mice weighed the least with a mean weight of 31.4g. The large lean mice had a higher mean of 42.7 g and the fat mice had the largest mean weight of 54.6g. It is evident from the 't' values 8.0, 8.3 and 4.05 that the body weights of these three groups are very significantly different.

Weight of Epididymal fat

The mean weights of the fat pads in the control, large lean and large fat mice are respectively, 0.63g, 0.48g, and 1.42g.

When the 't' test is applied no difference between the weights of the fat pads of the control and the large lean mice is evident. The 't' value of 3.4 for the normal and large fat mice shows a significant difference between these two groups and the higher 't' value of 4.69 for the large fat and large lean mice shows the difference between these two groups to be even more significant.

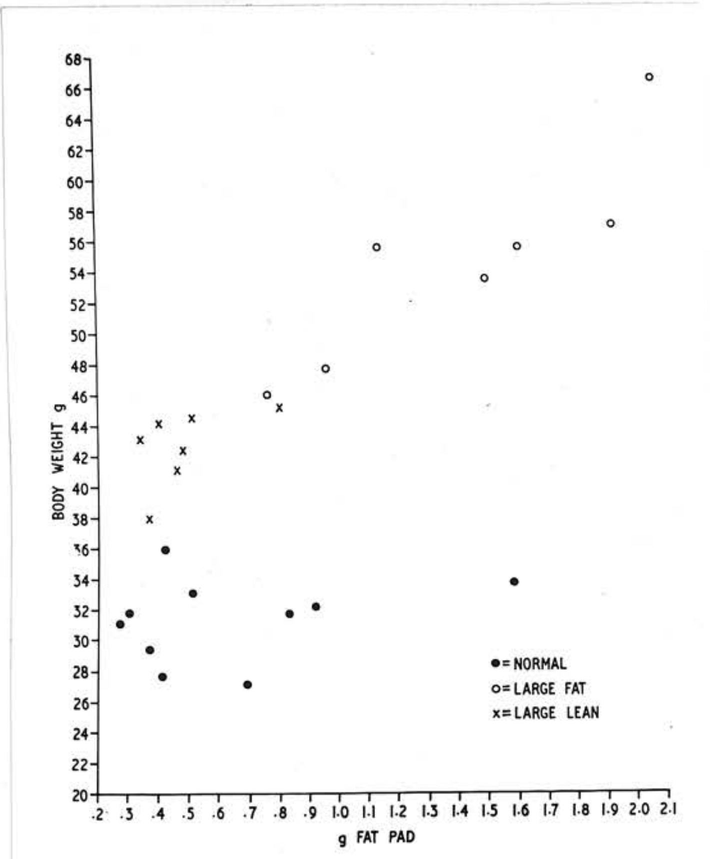
The fat pad weights are correlated with the body weights of these mice Fig. 1. The coefficient, when only the large fat and large lean mice are considered is .94, but when the control mice are included it has a lower value of .72. This is explained by the fact that the control mice have fat pad weights which are insignificantly different from those of the large lean mice, while they have much lower body weights.

The chemical composition of the adipose tissue in the control and large fat mice are not significantly different. The 't' values for each component, water, fat, protein, potassium and sodium for these two groups are all below the 5% level of probability.

When the large fat mice are compared against the large lean mice, however, each component of the adipose tissue of these two groups is shown to be significantly different. But the differences between the fat and water percentages are only significant at the 5% level while those for protein, potassium and sodium are significant at the 1% level. The fat percentage at mean 90.3% in the large lean group is lower than the mean of 92.8% in the large fat group. All the other components have a higher percentage in the large lean group.

The Correlation between Body Weight
and the Weight of the Epididymal Fat Pad

Fig. 1 (r = .72)



The large lean group when compared against the control group also has higher values for protein, potassium and sodium and these differences are significant at the 1% level. There is no significant difference between the fat or the water percentages in the adipose tissue of these two groups.

The relationship of fat to protein in the epididymal fat pad of these mice has been calculated, using the analytical data of this adipose tissue. The milligrams of fat and protein in the fat pads of each group with an additional calculation for the amount of fat supported by 1 mg of protein in each fat pad are listed below in table 3.

Table 3

The composition of the Epididymal fat pads in the 3 mice groups using the mean values.

	fat pad mg.	fat mg.	protein mg.	mg. fat supported by 1 mg. protein
Control	630	572	4.9	117
Large fat	1420	1318	11.1	118
Large lean	480	433	6.2	69

It can be seen that the same amount of fat is supported by 1 mg protein in the control and large fat pads, but only half of this amount of fat is supported by 1 mg of protein in the lean fat pad. This is a clear indication that there has been an increase in the amount of protein in the fat pad of the large fat mice.

DISCUSSION

The results of this investigation can be said to present fairly strong evidence in support of the view that there is an increase in the number of fat cells as the fat they are required to store is increased.

This conclusion is based on the fact that, while the chemical composition of adipose tissue is approximately the same in the large fat and the control mice, the weight of the epididymal fat pad in the large mice is more than twice as great as that in the control mice.

It has also been shown that 1 mg protein in the fat pad of the large fat mice, supports the same amount of fat as is supported by 1 mg protein in the fat pad of the control mice. This clearly indicates that protein increases as the fat pad becomes larger with stored fat. It cannot then be said that the burden of fat is born by a set amount of protein, which becomes increasingly diminished as the fat stores increase, but that as more fat is stored so the protein, that is to say, the cells, increase to accommodate this fat increase.

The fact that there is no significant difference in the fat concentration of adipose tissue in the control and large lean mice whilst the difference in fat concentration between the large lean and large fat is only significant at the 5% level, does suggest that there is not a very wide variability in the fat concentration in lean or fat mice and seems to indicate that the fat cells in mice can only hold a certain amount of fat and no more.

There is a real difference between the adipose tissue composition in the large lean mice, and that of both the control and large fat mice. This is in the concentration of protein, potassium and sodium. The protein concentration in the large lean group is almost twice as high as that in the other two groups, and the ionic concentration also seems to be on a higher plane in the lean group. This seems to be due to the effect of selection for growth on a low plane of nutrition.

It can also be seen that increased weight in the large lean mice above that of the controls is predominantly due to an increase in the amount of protein in the body. For the large lean mice have a much higher body weight than the controls and yet their epididymal fat weights, which have been shown to be correlated with total body fat, are the same as those in the control mice. The age difference between the mice would only account for an increase of approximately 4 g in the lean mice and it is assumed, with a certain amount of justification, that this would have no significant effect on the results of this investigation.

TABLE 4

Mice injected with colchicine

Body Weight g	Colchicine mg
29	.15
29	.15
30	.15
32	.07
28	.07
32	.07
32	.16
29	.15
30	.15
31	.15
32	.16
30	.15

TABLE 5

Rats injected with colchicine

Body Weight g	Colchicine mg
438	2.19
374	1.87
369	1.85
410	2.05
368	1.84

MITOSIS IN MOUSE ADIPOSE TISSUE

INTRODUCTION

Adipose tissue is continually described in the literature, as a tissue which is unable to increase itself by mitosis. In general no experimental evidence is given to justify this statement, and it was decided that an interesting way to test this assumption would be to examine the adipose tissue in mice and rats after they had been injected with colchicine. Cameron's similar work (18) was only discovered after this experiment was completed.

It has long been known that appropriate doses of colchicine will arrest mitosis at metaphase and it was hoped that any mitosis that might be taking place in adipose tissue would be revealed in this manner. This inhibitory effect of colchicine is very evident in the continually regenerating small intestine and sections of this tissue were examined with the adipose tissue to verify the effectiveness of the colchicine used.

Experimental Method

12 mice of the C.B.A. strain all weighing within 4 g of each other, (table 4) and 5 white rats having similar body weights, (table 5) were examined.

The effective dose of colchicine for mice and rats is 5 mg/kg body weight. (19, 20) The appropriate dose for each animal was dissolved in distilled water and injected subcutaneously, in volumes of 0.2 ml in the mice, and 1 ml in the rats. The animals were killed 6 hours later.

Both the rats and mice were asphyxiated with gas and the epididymal fat was dissected from each animal and put straight into 10% formalin. (Lillie, 1948) Additional specimens of fat from different parts of the body were taken from the rats and sections of the small intestine were taken from six of the mice.

After leaving the tissues in formalin overnight, the adipose tissue was transferred straight into a 10% solution of gelatin and allowed to soak during the day. It was then transferred to a 25% solution of gelatin and allowed to soak overnight. The tissue and the gelatin were poured into a mould and allowed to set for an hour. Once the gelatin had set, it was reduced to a small block round each piece of tissue and 10 μ sections were cut. on the freezing microtome.

These sections were then attached to slides treated with 2% gelatin and allowed to stand in formalin vapour for 5 minutes to ensure that they were firmly fixed on to the slide.

The intestine was removed from the formalin, rinsed in water, dehydrated through 50%, 70%, 90% and 100% alcohol, cleared in xylol and then transferred to paraffin. The paraffin was changed three times before the tissue was finally embedded. Microtome sections of each tissue were cut and these were then attached to slides.

Both the intestine and the adipose tissue were stained with Ehrlich's haemotoxylin stain.

The paraffin in the intestine sections was washed out with xylene, xylene and absolute alcohol, 90% and 70% alcohol and finally water. These sections were then transferred straight into the haemotoxylin, allowed to stain for five minutes, and then rinsed in water for five minutes. The section was then dehydrated with 70% and 90% and 100% alcohol, treated with xylene and mounted in balsam.

The adipose tissue once attached to the slide was stained, dehydrated and mounted immediately in exactly the same way.

Results and Discussion

Although numerous nuclei were found in metaphase in the intestine, none could be found in adipose tissue after an extensive search.

This is in agreement with the findings of Cameron [18]. He examined adipose tissue for mitosis after the epididymal fat pad in rats had been subjected to quite severe damage by freezing. He also used colchicine as a mitotic inhibitor and was unable to find any indication of mitotic division within the fat cells.

ANALYSIS OF HUMAN ADIPOSE TISSUE

MATERIAL

Biopsy Material

The original plan was to get equal numbers of adipose tissue specimens from well nourished and obese subjects. It was found however, that it was impossible to get a large series of specimens from obese subjects in the time available.

Under these circumstances it was decided to examine all adipose tissue specimens made available from surgical operations, with the hope that some of them would be from obese people.

Specimens of omental and abdominal subcutaneous adipose tissue were obtained at operation from 76 patients. But only 61 of these have been considered in this investigation because the weights of 10 of the subjects could not be obtained and the remaining 5 were incompletely analysed.

All the specimens save two were taken from subjects undergoing operations for various abdominal complaints. These are listed in tables in the appendix. 44 of the abdominal complaints were gastrointestinal of which 12 were appendicectomies, while 2 were bilateral sympathectomies. There were also 3 prostatectomies and 2 ovariectomies. 8 of the subjects had hernias and of the 2 specimens which were not abdominal, one was subcutaneous adipose tissue from the breast, and the other subcutaneous adipose tissue from the thigh.

The majority of the subjects were over 40 years of age. There were 32 between 40 and 60 years of age, and 22 subjects between

60 years and 93 years of age. Only 7 of the subjects were between the age of 14 and 40 years.

31 of the subjects were males and 30 were females.

I obtained the heights and weights and some idea of the dietary history of these subjects by sending them a questionnaire. The height and weight measurements given were accepted for the purposes of this investigation, but little information was gained about the true nutritional state of the subjects. An estimate of the degree of fatness of these subjects was therefore made by calculating their weights as percentages of the standard weight given for their sex, age and height. The standard weight table used was that drawn up by an insurance company in the U.S.A. between 1885 and 1900. Their figures are recommended(1) as being preferable to the few studies that have been completed on standard body weight, more recently. The subjects had body weights ranging from 70% to 160% of their standard body weights, those above 120% were considered to be obese. There were 4 subjects in the 70%- 79% range, 12 at 80 - 89%, 15 at 90 - 99%, 9 at 100 - 109%, 12 at 110 - 119% and 8 obese subjects above 120%.

The biopsy tissue was put straight into sterilised, sodium free, air tight bottles by the surgeons and analysed within two hours of excision if possible. When it was impossible to analyse the samples straight away, they were stored in a refrigerator and analysis was started within 24 hours.

Portions of the specimens weighing approximately 300 mg, with as little blood contaminating it as possible, were divided into the platinum cups and the beakers for analysis.

The Composition of Subcutaneous Adipose

Tissue from 10 Human Cadavers

Disease	Age Yrs.	Fat g %	Water g %	Protein g %	K MEQ/100g	Na MEQ/100g	Iodine Number
Myocardial Infarct	62	90.6	7.6	1.3	.26	1.35	62
Cerebral Haemorrhage	58	92.1	5.1	1.4	.26	1.03	66
Pulmonary Embolism	48	88.9	10.5	1.3	.25	1.38	66
Carcinoma	65	89.6	8.4	1.9	.33	1.13	74
Heart Failure	63	87.0	10.8	1.4	.31	1.42	73
Myocardial Infarct	50	93.8	4.9	1.1	.25	.59	67
Carcinoma	35	89.2	9.4	1.4	.30	1.58	60
Head Injury	49	94.7	4.1	1.5	.26	.87	70
Cardiac failure	54	87.6	11.0	1.9	.51	2.10	69
Mean		90.4	8.0	1.5	.30	1.28	67

Autopsy Material

10 samples of subcutaneous adipose tissue were obtained within 24 hours post-mortem. Nearly all of the subjects had died from cardio vascular disease and only 1 of them was moderately obese. The age range of these subjects, of which 6 were female and four male, was from 35 years to 65 years. I was unable to obtain the body weights of these subjects and have merely presented the analytical results of their adipose tissue specimens, without including them in the comparative analysis.

TABLE 6

The composition of Adipose Tissue in subjects weighing between 70% and 160% of their standard weights.

a. Means

% Body Weight	No.	Water g %	Fat g %	Protein g %	K MEQ/100 g.	Na MEQ/100 g
70 - 79	4	14.4	83.5	3.2	.62	1.55
80 - 89	12	16.8	80.0	3.0	.52	2.19
90 - 99	15	13.2	82.5	2.7	.45	1.64
100 - 109	9	10.2	87.2	2.1	.42	1.29
110 - 119	12	10.3	87.2	2.7	.40	1.32
120 - 160	8	9.6	88.2	2.0	.40	1.28

b. Range for each component of the percentage groups

% Body Weight	Water	Fat	Protein	K	Na
70 - 79	6.6 - 25.6	71.7 - 92.4	2.3 - 4.1	.21 - 1.17	1.26 - 3.54
80 - 89	7.1 - 36.1	61.0 - 91.3	1.1 - 6.5	.14 - .89	.49 - 5.14
90 - 99	8.3 - 25.8	69.1 - 91.9	1.2 - 4.6	.32 - 1.02	.90 - 3.04
100 - 109	7.2 - 14.3	80.0 - 91.0	1.4 - 2.8	.26 - .54	.84 - 2.05
110 - 119	4.4 - 15.6	78.2 - 94.1	1.6 - 4.4	.26 - .79	.60 - 1.98
120 - 160	5.9 - 14.1	80.6 - 94.0	1.0 - 3.4	.23 - .57	.71 - 1.76

RESULTS

The number, age, sex, body weight and body height and the operation undergone by each subject are presented in tables in the appendix along with the corresponding chemical analysis of the adipose tissue specimens from the subjects.

The possible effects of body weight, sex, age and gastro-intestinal disorders, on the composition of adipose tissue of these subjects have been studied.

Body Weight

The samples were grouped according to the weights of the subjects, calculated as percentages of their standard weights. The lowest percentage weight being 70% and the highest 160%. The range and mean of each component analysed in the adipose tissue are presented in these weight groups in table 6. The mean water percentage decreased from 14.4% in the 70% group to 9.6% in the obese group, and there was a concomitant increase in the proportion of fat as the weight of the subjects increased. The fat percentage in the 70% group was 83.5% while that in the obese group was 88.2%. The range of both the fat and water proportions decreased with increasing body weight. Protein, as well as potassium and sodium, decreased with increased body weight with hardly any change in their breadth of variation.

The significance of this relationship between the different adipose tissue components and the increasing body weight was tested by correlating the components of each of the 61 samples with the corresponding percentage weight of the subject. The correlation coefficients for water, fat protein, potassium and sodium are respectively:- $\mp$ .35; + .35; $\mp$ .36; $\mp$ .27 and $\mp$ .29. It can be said from these results that there is an increase in the amount of fat in adipose tissue as body weight increases, but this is not a very marked increase.

Fig. 2 to 5 illustrate the relation of water, fat, protein and potassium in adipose tissue with the percentage body weights. The means of the components for each weight group are linked to give the trend of this relationship.

Adipose tissue in the 8 obese subjects was then compared against that in 8 subjects within 6 pounds of their desirable body weight to investigate the possibility of protein increase in adipose tissue in obesity. The protein and fat proportions in each sample were assumed to be representative of adipose tissue throughout the body of the respective subject. This fat - protein ratio was then related to the increase in body fat above that found at desirable body weight.

The percentage value I have used for the fat at desirable body weight in men is 14% and 26% in women[19], and the increase in body fat of each obese subject was estimated as 63%[20] of the 'obesity tissue' assumed to be the difference between their real body weight and their desirable body weight. This additional fat was added to the normal fat and the protein component of the total adipose tissue was calculated from this, using the fat and protein percentages already estimated in the adipose tissue specimens.

In the few controls who weighed a little less than their desirable weight, 63%[21] of the weight difference was taken to be fat and subtracted from the normal fat.

The Relationship between the different Adipose Tissue components and Body Weight.

Fig. 2 (r = - .35)

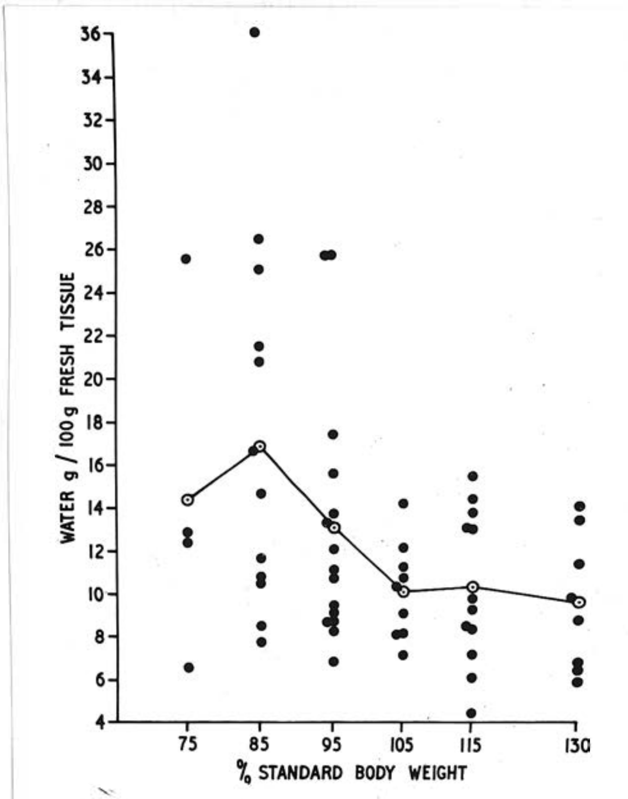


Fig. 3 (r = + .35)

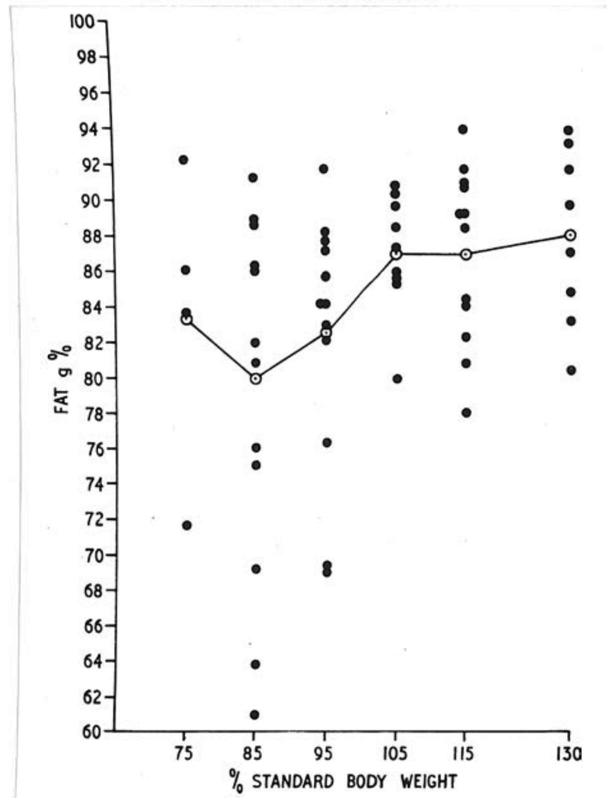


Fig. 4 (r = - .26)

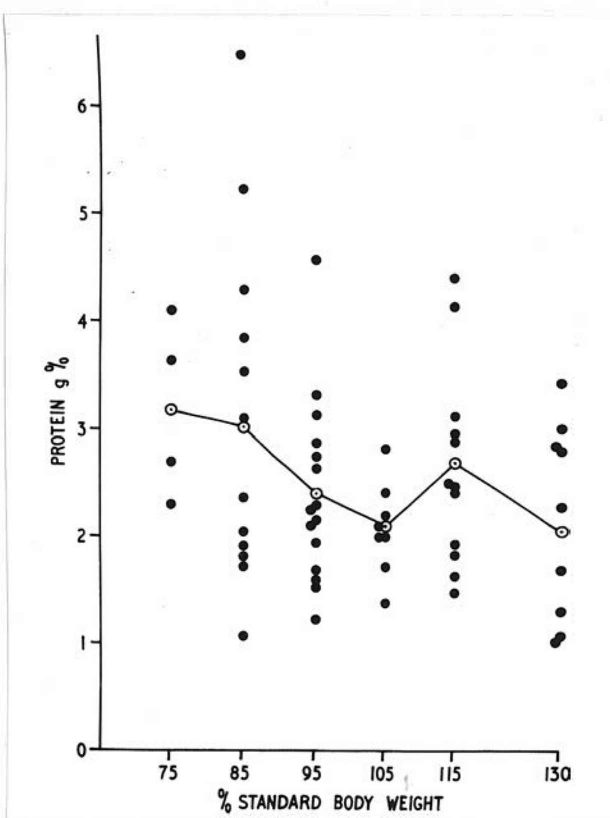
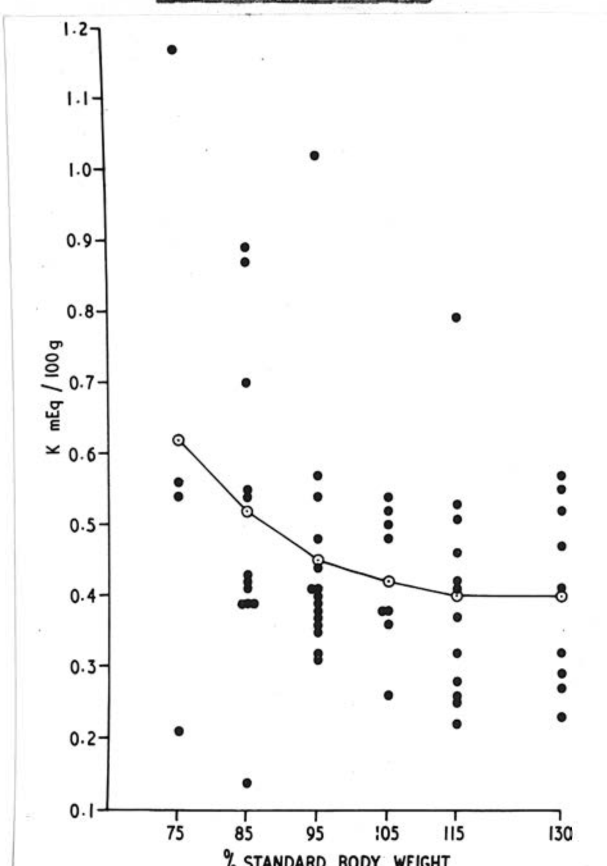


Fig. 5 (r = - .27)



T A B L E 7

An estimation of the Protein Present in Total Adipose Tissue in 8 Normal and 8 Obese Subjects

No.	Age Yrs.	Standard Weight lbs.	Standard Body Fat x lbs.	Actual Body Weight lbs.	Change in body weight lbs.	Fat Content ø of tissue Lost or Gained lbs.	Total Body Fat	Fat g/g A.T.	Protein g/g A.T.	Protein Present in Total A.T. Keys	Protein Present in Total McCance & Widdowson o
					A. OBESE WOMEN						
59	74	143	37.2	180	+ 37	23.3	60.5	.806	.028	955	799
61	20	143	37.2	206	+ 63	39.7	76.9	.940	.010	372	330
60	45	150	39.0	189	+ 39	24.6	63.6	.933	.011	340	285
58	44	151	39.3	245	+ 94	59.2	98.5	.920	.013	633	593
					B. NORMAL WOMEN						
56	46	131	34.1	126	- 5	-3.1	30.9	.843	.031	518	321
54	48	133	34.6	133	0	-	34.6	.886	.022	390	255
57	20	141	36.7	135	- 6	-3.7	32.9	.858	.027	471	290
55	51	144	37.4	142	- 2	-1.2	36.2	.823	.021	420	270

C. OBESE MEN

No.	Age	Standard Weight lbs.	Standard Body Fat x lbs.	Actual Body Weight lbs.	Change in body Weight lbs.	Fat Content of tissue Lost or Gained lbs.	Total Body ø Fat	Fat g/g A.T.	Protein g/g A.T.	Protein Present in Total A.T. Keys	Protein Present in Total A.T. McCance and Widdowson o
29	13	111	15.5	141	+30	18.9	34.4	.872	.023	412	444
31	52	149	20.9	188	+39	24.6	45.4	.834	.034	842	904
30	61	163	22.8	196	+33	20.8	43.6	.899	.017	375	406
28	58	158	22.1	198	+40	25.2	47.3	.850	.028	708	762

D. NORMAL MEN

No.	Age	Standard Weight lbs.	Standard Body Fat x lbs.	Actual Body Weight lbs.	Change in body Weight lbs.	Fat Content of tissue Lost or Gained lbs.	Total Body ø Fat	Fat g/g A.T.	Protein g/g A.T.	Protein Present in Total A.T. Keys	Protein Present in Total A.T. McCance and Widdowson o
19	75	150	21.0	154	+4	2.5	23.5	.875	.014	171	193
3	41	150	21.0	156	+6	3.8	24.8	.860	.020	262	294
17	62	163	22.8	168	+5	3.1	26.0	.910	.021	272	307
22	72	173	24.2	168	-5	-3.1	21.1	.691	.019	270	306

x Assuming that standard male body contains 14% and female 26% fat.

ø Assuming 'Obesity Tissue' and Tissue lost contains 63% fat. Keys.

o According to McCance and Widdowson, standard male body contains 16% fat and female 17% and 'Obesity Tissue' in men contains 64% fat and 71% in women.

TABLE 7 E

Protein present in Total Adipose Tissue in 8 Normal and 8 Obese Subjects

	Keys		McCance and Widdowson	
No.	Individual A.T. Fat & Protein	Mean A.T. Fat & Protein	Individual A.T. Fat & Protein	Mean A.T. Fat & Protein
		OBESE WOMEN		
59	955	627	799	525
61	372	1060	330	707
60	340	877	285	551
58	633	1359	593	958
		NORMAL WOMEN		
56	518	426	321	199
54	390	477	255	234
57	471	454	290	210
55	420	499	270	240
		OBESE MEN		
29	412	474	444	437
31	842	626	704	576
30	375	601	406	557
28	708	652	762	601
		NORMAL MEN		
19	171	324	193	314
3	262	342	294	328
17	272	359	307	346
22	270	291	306	289

These data are presented in table 7A B C&D. It can be seen that the four obese men appear to have considerably more protein in their total adipose tissue than that of the four normal men, while only two of the four obese women have a marked increase in their adipose tissue protein above that in the four normal women. When desirable body fat is taken to be 16% for men and 17% for women and obesity tissue fat is assumed to be 64% for men and 71% for women[22] the same result is obtained.

In view of the fact that the composition of adipose tissue amongst the obese is as variable as that amongst the normal subjects the mean values of the fat and protein components of the two groups were also used to calculate the protein content in the total adipose tissue. The calculations as listed in table 7E now show that there is a marked increase in the protein content in the total adipose tissue of both obese men and women above that in normal men and women. It can be said with a fair amount of certainty, therefore, that there is a cellular increase in the adipose tissue of most patients with obesity.

<u>Sex</u>

When the data were grouped according to sex the only difference in the composition of adipose tissue of the two sexes was a slightly higher protein content in the adipose tissue of the men. All the other components were insignificantly different. The mean values for each component and their standard errors are presented in table 8.

TABLE 9

The composition of adipose Tissue in subjects of different ages

Age Years	No.	Water	Fat	Protein	K MEQ/100g	Na MEQ/100g
10 - 20	2	17.8	78.4	3.4	.77	2.31
21 - 30	4	10.6	87.6	2.3	.37	1.02
21 - 40	2	20.0	78.0	3.3	.84	2.02
41 - 50	14	12.2	84.5	2.9	.42	1.41
51 - 60	14	11.3	86.2	2.5	.42	1.33
61 - 70	8	12.7	85.4	2.2	.47	1.74
71 - 80	10	12.6	83.6	2.5	.44	1.60
81 - 90	3	17.6	78.8	2.9	.56	2.45

TABLE 8

The Composition of Adipose Tissue in Men and Women

		Water g %	Fat g %	Protein g %	K MEQ/100 g	Na MEQ/100 g
Men	Mean	12.9	84.0	2.7	.46	1.52
	s.e.	1.1	1.5	.02	.0003	.01
Women	Mean	12.6	84.3	2.6	.46	1.53
	s.e.	1.6	1.3	.02	.0004	.01

Age

The possibility of a change in the adipose tissue composition with age was examined by grouping the data according to age at intervals of 10 years. The numbers in each group are rather small to be worth investigating in statistical detail, but it can be seen in table 9 that the four groups from 40 years to 70 years with numbers from 8 to 14 in each group, are inconsistantly different with no trent developing with increasing age.

Gastrointestinal Disorders

It was assumed that the subjects who had had hernias and those that had undergone appendicectomies, prostatectomies, ovariectomies, bilateral adrenalectomies, or mastectomy and section of arterial nerves, had sound digestive systems. These were compared against the rest of the subjects with gastrointestinal disorders to see if their adipose tissue differed at all in composition. The means and their standard errors are found in table 10 below.

TABLE 10

The composition of Adipose Tissue in subjects with Normal and Diseased Digestive Systems

No.			Water g%	Fat g%	Protein g%	K MEQ/100 g	Na MEQ/100 g
29	Normal	Mean	13.2	83.7	2.7	.47	1.67
		s.e.	.98	1.3	.02	.003	.01
32	Diseased	Mean	12.68	82.5	2.7	.47	1.37
		s.e.	1.26	1.18	.02	.004	.01

It can be seen from the table that the only significant difference between the composition of the adipose tissue in the two groups is the sodium content.

Chemical Components

The relationship the components of adipose tissue have between themselves are illustrated in fig. 6, 7, 8 and 9 with the correlation coefficient and the regression equation for each pair of components. The water and fat components have a very high negative correlation (r =-.98) and protein and water also have a strong correlation (r =+.72). Protein and potassium (r =+.67) and the sum of potassium and sodium and water (r =+.59) are less strongly correlated.

Potassium and sodium are related in a ratio of 1:3 and since sodium is known to be predominantly extracellular, (23, 24, 25), it is interesting to note that approximately three quarters of the water in adipose tissue must be extra cellular.

The Relationship the components of Adipose Tissue have between themselves.

Fig. 6 (r = - .98) Water = 80.16 + .80 Fat

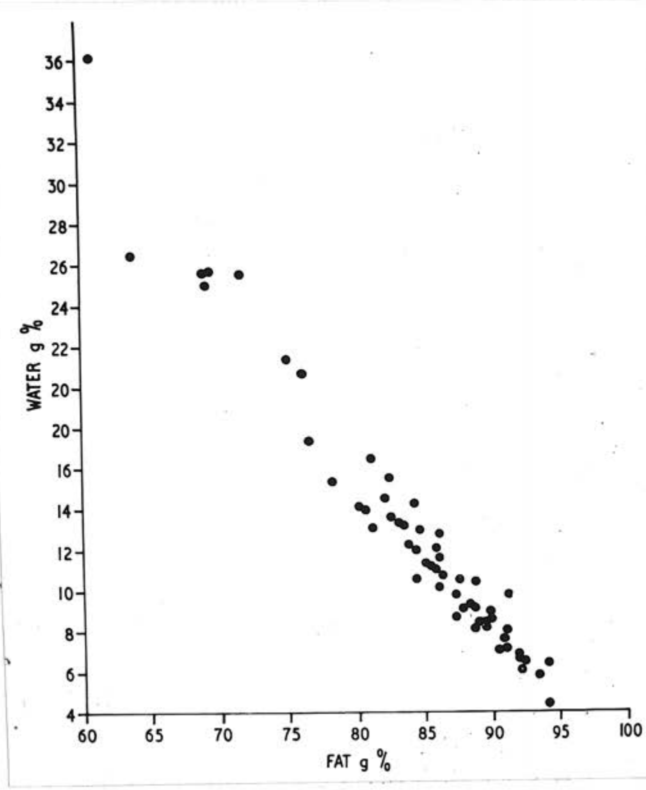


Fig. 7 (r = + .72) Water = 1.38 + 4.31 Protein

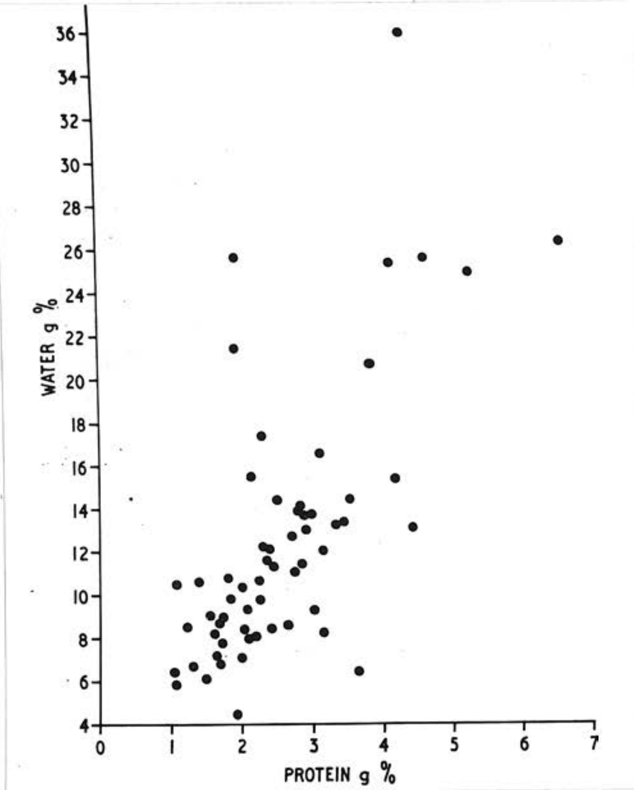


Fig. 8 (r = + .67) Protein = 1.11 + 3.32K

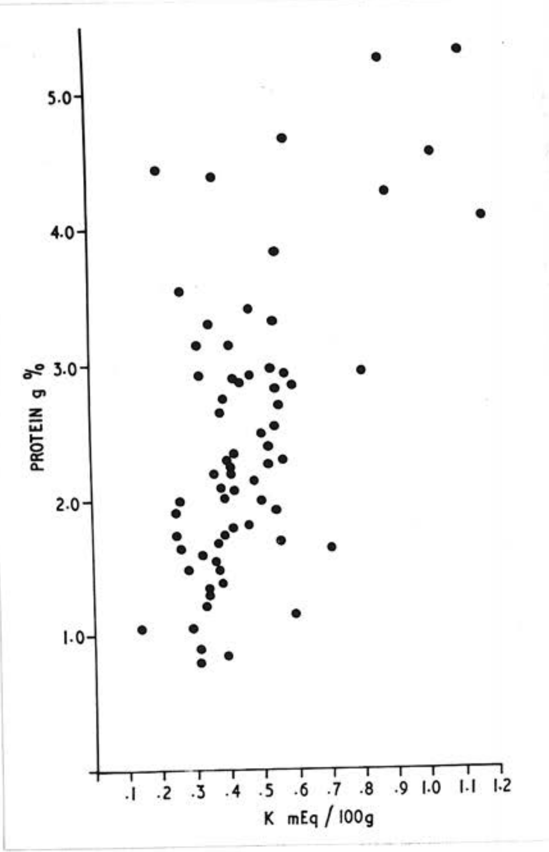


Fig. 9 (r = + .59) Water = 4.21 + 4.32 Na + K

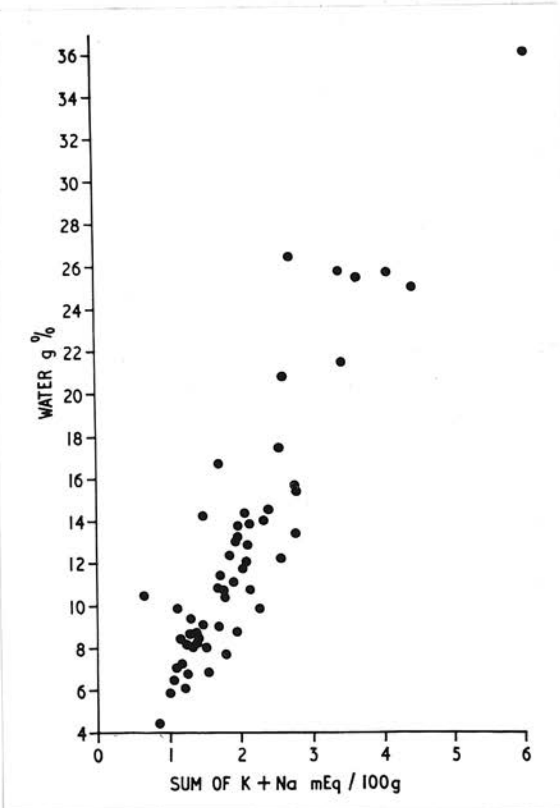


TABLE 11

The Phosphorus Content of Normal and Obese Adipose Tissue

	Normal P mg/g x	Obese P mg/g x
	10.44	8.37
	8.26	11.76
	7.39	6.92
	10.23	7.02
	6.19	5.25
	9.14	7.05
	10.78	8.66
	6.99	7.38
	11.47	
	9.84	
	8.99	
	8.34	
	8.49	
	8.43	
	9.03	
	9.51	
Mean	9.03	7.80

x fat free dry tissue.

The K/N ratio (milli equivalents K/g. N.) was found to be 1.4 which is half that found in muscle and liver. The total phosphorus estimations on fat free dry adipose tissue are presented in table 11. The mean value in the obese subjects is lower than that in the normal subjects.

The iodine numbers of the fatty acids range from 63 to 72 in the adipose tissue of the obese subjects and from as low as 55 to 84 in the normal subjects.

TABLE 12

The Composition of Adipose Tissue analysed by other investigators.

	No.		Water g %	Fat g %	Protein g %
Mitchell[3]	1		50	42	7
Forbes & Mitchell[4]	1		23.0	71.6	5.8
Vague & Garrigues[5]	35	Men	28.7	56.4	7.4
		s.d.	14.3	29.1	9.5
	70	Women	23.2	70.0	8.5
		s.d.	16.2	13.0	7.2
Entenman et al[6]	1 B		18.0	79.2	2.8
			32.4	62.3	5.3
	1 E		12.5	85.7	1.8
			17.7	78.9	3.4
Pawan[7]	6	lean	28.6	68.4	2.1
		range	(18.2 - 50.6)	(61.4 - 52.4)	(1.8 - 2.8)
	7	obese	18.8	78.7	2.0
		range	(12.7 - 30.1)	(67.0 - 85.1)	(1.8 - 2.7)

TABLE 13

The mean values of the Chemical Components of Adipose Tissue in Man, Pigs, Rabbits, Mice and Sheep

No.	Species	Fat g %	Water g %	Protein g %	K MEQ/100 g	Na MEQ/100 g	Iodine Value
61	Man	84.4	12.4	2.6	.45	1.56	68
		7.4	6.3	1.1	.19	.76	
12	Pigs	86.4	9.2	1.9	.33	1.15	42
		3.3	2.9	.6	.03	.27	
5	Rabbits	85.3	11.2	1.12	-	-	-
10	Mice	90.8	7.5	.8			
		3.0	1.3	.2	-	-	-
12	Sheep	96.1	3.4	.6	.23	.67	41
		1.28	.97	.14	.05	.08	

DISCUSSION

The results of this investigation show a wide variability in the composition of adipose tissue in lean normal and obese subjects. This is in accord with previous analytical data as listed in table 12 .

Despite this variability, there appears in obesity, to be both an increase in the concentration of fat in the adipose tissue and an increase in the number of fat cells. For it has been shown, that there is a small, but significant increase in the fat component of adipose tissue with increase in body weight. This small increase in the fat component could, however, hardly account for the increase in weight of from 20 to 60 pounds of body fat, if it represented the concentration of this fat in a set number of fat cells.

This adds weight to the observation, despite the assumption that the adipose tissue sample analysed was taken to be representative of adipose tissue throughout the body of each subject, that there is an increase in the protein of total adipose tissue in obese subjects above that of normal subjects. This has been interpreted as an increase in fat cell number.

These findings are in agreement with those of Bjurulf[26]. His is the only other work that has been done on this subject. He found that a person with a thick panniculus adiposus has larger and many fat cells, while a person with a thin panniculus has small and few fat cells, which means that the individual differences in thickness of subcutaneous tissue is ascribable to changes in size as well as in number of fat cells.

Bjurulf's investigation was carried out on autopsy material taken from 110 male cases between the ages of 25 and 88 years. The specimens were subcutaneous adipose tissue taken from the chest, abdomen, thigh and great toe and the subcutaneous fat thickness ranged from .1 mm to 37.1 mm. The cell size was measured by projection, on to paper, of a photographic image 500 times the size of the cell, and the cell number was represented by the number of cells present in the subcutaneous fat along an imaginary straight line perpendicular to the skin and the muscle fascia.

Bjurulf found during his investigation as did Reh[27] that there is a very wide range in the size of fat cells. This could possibly account for the great variability in the composition of adipose tissue in both normal and obese subjects. Reh found cells ranging from a size of 10 u to 170 u and using the same method for measuring cell size, Bjurulf detected cells as small as 7 u while the largest cell was 200 u in diameter.

The higher protein concentration I found in the adipose tissue of men above that in women was not detected by Vague and Garrigues[5]. The variability of the adipose tissue composition of their subjects was so great that the apparent difference between the sexes was found to be insignificant. When they correlated each component of adipose tissue with the age of each subject they found that, in men, the residue increased with age as did the fat, but to a lesser degree, while the water also decreased a little. In women the residue decreased with age as did the water and there was a slight increase in the fat content. I was unable to show any trend of change with age.

Considering the relationship between the various components of adipose tissue, the low $K/_N$ ratio of 1.4 as opposed to 3 in liver[28] and muscle[29, 30] can possibly be explained on the grounds that probably half or more of the protein in adipose tissue is probably extra cellular and not actually part of the fat cell.

This would probably explain the strong correlation between protein and water since approximately three quarters of the water in adipose tissue is extra cellular; as judged by the Na/K ratio.

The very high ratio of sodium and potassium in adipose tissue is in keeping with analyses that have been done on skin and tendon[31] both of which contain much more extra cellular fluid than muscle.

The lower phosphorus content in the fat free dry adipose tissue of the obese tends to suggest that there are fewer nuclei in the tissue or perhaps that the enzyme phosphates are lower in obese adipose tissue.

The iodine numbers of omental fat obtained by Cuthbertson[32] are slightly lower than those he obtained for subcutaneous fatty acids. This probably explains the wider range of iodine number. I obtained as opposed to the small variation found by Pawan and Vague and Garrigues. The majority of the adipose tissue samples I analysed were from the omentum. In an earlier investigation, Cathcart and Cuthbertson[33] found very little difference in the property of fat in the adipose tissue of very fat women and that in normal women. The difference Pawan found in the iodine numbers for obese and normal depot fat has not been shown to be significant. Possibly on the grounds that insufficient samples were analysed.

Comparative Composition of Adipose Tissue

Material

Adipose tissue from pigs, sheep and rabbits as well as that from man and mice was analysed.

The pig and sheep fat was obtained from the abattoir. Almost directly after the sheep had been slaughtered, a strip of the large omentum was cut off and put straight into the universal containers used for the human specimens. The same precautions were taken to avoid any contamination of the sample with either sodium or potassium. The fat taken from the pigs was treated in a like manner. This was obtained about an hour after the pigs had been slaughtered, and was taken from the lower abdominal subcutaneous layer.

Analysis of these specimens, both from the sheep and pigs was started an hour after they had been taken, and it was not necessary to keep them in a refrigerator.

Perirenal fat was taken from experimental rabbits as soon as they had been concussed and dissected. Analysis of the tissue was started directly as the experimental laboratories were close at hand.

Analytical Data

The collective analytical data from these three animals are presented in tables in the appendix.

In table 13 the means & s.d. of each of the components in the adipose tissue of these three animals are listed with those of the human and mouse adipose tissue.

It can be seen that human adipose tissue has the highest water, protein, potassium and sodium content as well as a higher degree of unsaturation in its fatty acids. It also has the lowest percentage of fat. The adipose tissue composition in the pig is remarkably similar to that in man. The only real difference being the degree of saturation of its fatty acids. Although potassium, sodium and iodine numbers were not estimated on rabbits adipose tissue it can be seen that the water fat and protein also have very much the same relationship as that found in man.

Mice have rather a higher fat concentration in their adipose tissue with the water content correspondingly lower and the protein content markedly reduced.

The fat content in sheep adipose tissue is so high as to be almost the sole component. It has a percentage value of 96% while water only comprises 3.4% of the tissue and protein only .6%

GENERAL DISCUSSION

It appears that fat cells in both mice and men increase in number as an increased amount of fat is stored in the body. This has been shown more clearly in mouse adipose tissue as the necessary data for more conclusive evidence have been more accessible.

The increase in the concentration of fat in adipose tissue with an increase in body weight in man was not detectable in mice. This seems largely due to the fact that even the adipose tissue in lean mice has a remarkably high concentration of fat in it.

Since no mitosis has been detected in the adipose tissue of either mice or rats it seems that the increase in the number of fat cells with increasing adiposity must develope from undifferentiated mesenchymal cells or from mature connective tissue cells. It has been claimed by some that the fat cell, which develops from the undifferentiated mesenchymal cell, is a specific cell.[13, ~~33~~, 34, 35] But other investigations[36,37, 38] support the view that the fat cell is merely connective tissue which has taken on the new task of storing fat. The root of the difference between these views lies in the lack of true evidence that fat cells actually develop from undifferentiated mesenchymal cells and not from fibroblasts. Until undifferentiated mesenchymal cells have been clearly distinguished from fibroblasts and fat deposition has actually been seen to take place in them, it can only be assumed that fat cells are connective tissue cells. This means that there is really no limit to the amount of fat that can be accumulated in the body. But on this assumption we are led to wonder why more fat accumulates in subcutaneous connective tissue and predominantly in the abdomen, as opposed to connective tissue in other parts of the body. This is surely a subject which is in need of further investigation.

It appears that the general composition of adipose tissue is very similar in man, pigs and rabbits, while the fatty acid composition of the fat deposited in man and pigs is distinguishably different. Although the fatty acid composition of fat in rabbits was not studied it is known (39) that their composition is also peculiar to the rabbit species.

The fat cells in mice and sheep seem to be able to concentrate more fat than those in man, pigs and rabbits. Little else can be said of the data accumulated.

SUMMARY

The aim of this investigation was to try and show whether, in obesity, a set number of cells becomes increasingly distended with fat, or whether they increase in number as more fat is deposited.

Selected large mice, one line with little fat and the other with excess fat, as well as mice of the original stock from which they had been selected were used for an investigation into the composition of mouse adipose tissue. The chemical components in adipose tissue were analysed on the epididymal fat pad of these mice and the weight of the fat pad was also used as an index of the fatness of the animal. It was found that the chemical composition of adipose tissue was approximately the same in the large fat and control mice, but the weight of the epididymal fat pad in the large mice was more than twice as great as that in the control mice. It was also shown that 1 mg protein in the fat pad of the large fat mice supported the same amount of fat as is supported by 1 mg protein in the fat pad of the control mice. This finding was taken as an indication that the number of fat cells had increased.

The large lean mice had approximately the same epididymal fat pad weight as the control mice, but the concentration of the protein potassium and sodium components of their adipose tissue were higher than that in the adipose tissue of the control mice. This seems to have been due to the effect of selection.

61 biopsy samples of human adipose tissue, taken from subjects undergoing abdominal operations were analysed. Of the

61 subjects, 31 were male and 30 were female, some of which were lean, others well nourished and 8 were obese. The subjects were from 14 to 93 years of age.

The composition of the adipose tissue in the lean normal and obese was found to be very variable. When, however, each component of adipose tissue was correlated with the corresponding percentage weight of each subject, it was shown that there is a small, but significant rise in the amount of fat in adipose tissue as body weight increases. Water, protein, potassium and sodium decreased.

Adipose tissue in the 8 obese subjects was compared against that in 8 subjects within 6 pounds of their desirable body weight to investigate the possibility of protein increase in adipose tissue in obesity.

It appears that the protein content in the total adipose tissue of the obese is higher than that in the normal subjects, and this has been interpretted as an indication that the fat cell number has increased.

A short study on the possibility of mitosis in adipose tissue of mice and rats was undertaken using colchicine as a mitotic inhibitor. No mitosis was found.

The composition of adipose tissue in rabbits, pigs and sheep was also analysed and compared with that in mice and man.

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APPENDIX

COMPOSITION OF THE EPIDIDYMAL FAT PAD IN

10 NORMAL MICE AGED 90 DAYS

	Wt. g.	Wt. of fat g.	Water g. per cent	Fat g. per cent	Protein g. per cent
	33.9	1.58	5.7	89.6	.65
	27.2	0.69	5.5	96.7	.65
	33.2	0.51	7.7	87.8	.81
	29.5	0.37	7.4	86.2	1.08
	27.7	0.41	9.0	91.6	1.17
	36.0	0.42	8.3	93.7	1.05
	32.3	0.92	7.0	91.2	.53
	31.9	0.30	8.2	91.1	.69
	31.8	0.83	6.7	92.9	.73
Mean	31.4	0.63	7.5	90.8	.79
Standard Deviation	2.57	0.38	1.29	3.0	0.22

Composition of the Epididymal Fat Pad in 7 Large Fat Mice

	Age Days	Body Wt. g	Wt. of fat g	Water g %	Fat g %	Protein g %	K MEQ/100g	Na
	99	47.8	.96	6.7	93.7	.71	.40	.61
	100	46.1	.76	7.1	94.8	.71	.57	.79
	79	66.6	2.08	5.8	92.0	.72	.46	.71
	80	55.7	1.61	7.5	91.2	.80	.52	.84
	81	53.6	1.50	6.0	93.0	.76	.46	.66
	81	57.1	1.93	5.9	93.2	.75	.46	.67
	83	55.7	1.14	6.7	91.9	.79	.48	.59
Mean	86	54.6	1.42	6.5	92.8	0.75	0.48	0.69
Standard Duration		6.7	0.47	0.85	1.6	0.03	0.033	0.027

Composition of the Epididymal Fat Pad in 7 Large Lean Mice

	Age Days	Body Wt. g	Wt. of fat g	Water g %	Fat g %	Protein g %	K MEQ/100g	Na
	95	41.2	.46	8.7	93.2	.97	.67	1.26
	99	42.5	.48	10.2	87.8	1.51	.70	1.46
	70	43.2	.34	8.4	-	1.33	.71	1.49
	70	38.0	.37	8.4	90.2	1.55	.71	1.62
	70	44.2	.40	7.7	87.8	1.28	.66	1.48
	70	44.6	.51	7.7	90.0	1.61	.63	1.20
	70	45.3	.80	7.8	93.0	.95	.49	.85
Mean	78	42.7	0.48	8.2	90.3	1.31	0.65	1.34
Standard Deviation		2.5	0.14	1.1	1.9	0.27	0.071	0.22

RANDOM NORMAL K and Na MEQ/100 g.

	K. MEQ/100g.	Na. MEQ/100g.
	.56	1.20
	.56	.81
	.32	.59
	.43	1.04
	.50	.99
	.40	.84
	.48	.86
	.38	.56
	.41	.62
	.36	.63
	.40	.81
	.45	.76
	.41	.57
	.48	.88
Mean.	.44	.80
S.D.	.06	.19

DESCRIPTIVE DATA

MALES

No.	Age Yr.	Body Weight lb.	Desirable Weight lb.	Height ft.	in.	Operation
1	58	119	150	5	5	Gallstone removal
2	58	128	150	5	5	Partial Gastrectomy
3	41	156	150	5	6	" "
4	67	130	150	5	5	Gastro-Jejunectomy
5	93	130	150	5	5	Hernia
6	61	200	179	5	11	Appendicectomy
7	88	178	154	5	6	Gall Stone removal
8	26	140	160	5	8	Hernia
9	72	162	154	5	6	Duodenal Ulcer
10	53	140	158	5	7½	Gall Bladder removal
11	79	154	163	5	8	Colostomy
12	73	147	163	5	8½	Prostatectomy
13	28	162	150	5	8	Appendicectomy
14	45	189	177	5	11	Hernia
15	44	140	149	5	5	Stomach Ulcer
16	48	192	161	5	8	Hernia
17	62	168	163	5	8	Duodenal Ulcer
18	56	158	158	5	7½	Gastrectomy
19	75	154	150	5	5½	Prostactectomy
20	61	151	165	5	9½	Partial Gastrectomy
21	41	138	159	5	8½	Duodenal Ulcer
22	72	168	173	5	10	Hernia
23	78	154	140	5	2	Perforated Ulcer
24	66	210	179	5	11½	Duodenal Ulcer
25	58	189	173	5	10	Bile Duct Stone

No.	Age Yr.	Body Weight lb.	Desirable Weight lb.	Height ft.	in.	Operation
26	45	168	161	5	8½	Gastrectomy
27	45	176	156	5	7	Appendicectomy
28	58	196	158	5	7½	Hernia
29	13	141	111	4	11	Appendicectomy
30	61	196	163	5	8	Section of Arterial Nerves in leg
31	52	188	149	5	5	Hernia

F E M A L E S

No.	Age Yr.	Body Weight lb.	Desirable Weight lb.	Height ft.	in.	Operation
32	32	106	137	5	4	Cholecystectomy
33	57	185	158	5	7	Gastrectomy
34	80	112	140	5	2	Cholecystectomy
35	72	174	146	5	4	Ovariectomy
36	48	116	139	5	3	Resection of colon
37	77	126	140	5	2	Appendicectomy
38	14	91	100	4	10	"
39	71	129	140	5	2	"
40	57	100	134	5	0½	Duodenal Ulcer
41	68	127	146	5	4	Gastroenterostomy
42	35	150	129	5	2½	Appendicectomy
43	44	154	136	5	3½	Ovarian Abcess
44	53	164	152	5	6	Appendicectomy
45	55	98	140	5	2	Gastroenterotomy
46	52	112	135	5	1	Partial removal of Stomach
47	64	129	143	5	3	Appendicectomy
48	45	126	133	5	1	Duodenal Ulcer
49	59	128	140	5	2	Gall Bladder Removal

No.	Age Yrs.	Body Weight lb.	Desirable Weight lb.	Height ft.	in.	Operation
50	70	172	146	5	4½	Gall Bladder Removal
51	62	126	134	5	0	Hernia
52	57	112	140	5	2	Cholecystectomy
53	47	130	155	5	8	Bilateral Sympathectomy
54	48	133	133	5	0½	Duodenal Ulcer
55	51	144	142	5	4	Jejunostomy
56	46	126	131	5	0	Appendicectomy
57	20	135	141	5	8	"
58	44	245	151	5	7½	Appendicectomy
59	74	180	143	5	3	Carcinoma of Colon
60	45	189	150	5	5	Mastectomy
61	20	206	140	5	7	Bilateral Adrenalectomy

ANALYTICAL DATA

No.	Water %	Fat %	Protein %	Potassium MEQ/100g	Sodium MEQ/100g	Iodine Number
1	12.9	86.0	2.7	.55	1.56	68
2	22.2	67.1	5.3	1.11	2.77	60
3.	10.4	86.0	2.0	.50	1.29	70
4	36.1	61.0	4.3	.89		70
5	21.6	75.1	1.9	.54	2.89	79
6	7.3	90.9	1.6	.26	.90	66
7	6.1	91.9	1.5	.28	.93	66
8	11.8	86.1	2.4	.43	1.60	65
9	7 .2	90.5	2.0	.26	.84	63
10	8.5	89.0	2.0	.39	1.01	66
11	8.8	87.2	2.6	.38	.90	67
12	13.4	83.1	3.3	.54	2.23	65
13	13.1	84.5	2.9	.42	1.51	60
14	12.3	85.8	2.4	.52	2.05	70
15	9.1	87.9	1.5	.36	1.11	67
16	13.2	81.0	4.4	.37	.58	68
17	8.1	91.0	2.1	.38	1.13	65
18	14.6	82.0	3.5	.39	2.00	-
19	10.8	87.5	1.4	.38	1.75	54
20	9.4	88.3	2.1	.35	. 95	70
21	26.6	63.9	6.5	.70	2.00	65
22	25.8	69.1	1.9	.57	2.82	62
23	8.5	89.4	2.4	.21	.95	-
24	9.9	91.1	1.8	.46	1.82	69

No.	Water g%	Fat g%	Protein g%	Potassium MEQ/100 g	Sodium MEQ/100 g	Iodine Number
25	9.1	89.8	1.7	.48	1.21	53
26	11.3	85.5	2.5	.41	1.47	-
27	9.3	88.6	3.0	.41	1.52	-
28	11.4	85.0	2.8	.32	1.38	69
29	9.9	87.2	2.3	.52	1.58	70
30	8.8	89.9	1.7	.55	1.41	63
31	13.5	83.4	3.4	.47	1.50	66
32	25.6	71.7	4.1	1.17	2.49	61
33	13.9	82.4	3.0	.53	1.49	70
34	25.1	69.3	5.2	. 87	3.54	72
35	15.6	78.2	4.2	.79	1.98	67
36	16.7	80.9	3.1	.41	1.29	62
37	17.6	76.4	2.3	.40	2.15	72
38	25.8	69.5	4.6	1.02	3.04	65
39	17.4	78.0	2.9	.80	2.67	61
40	6.6	92.4	3.6	.21	.85	84
41	10.8	86.2	1.8	.42	1.26	70
42	14.5	84.2	2.5	.51	1.56	61
43	8.3	89.4	3.1	.32	1.05	71
44	14.3	80.0	2.8	.54	.94	66
45	12.4	83.7	2.3	.56	1.30	58
46	10.5	88.7	1.1	.14	.49	51
47	11.0	85.5	2.9	.59	1.39	62
48	8.1	88.8	2.2	.31	1.11	66
49	6.9	91.9	1.7	.37	1.16	55

No.	Water g%	Fat g%	Protein g%	Potassium MEQ/100g	Sodium MEQ/100g	Iodine Number
50	4.4	94.1	1.9	.25	.60	65
51	10.8	84.4	2.2	.41	1.34	-
52	7.7	91.3	1.7	.39	1.42	55
53	20.9	76.0	3.8	.55	2.05	-
54	8.2	88.6	2.2	.36	.97	65
55	15.7	82.3	2.1	.48	2.28	57
56	12.2	84.3	3.1	.41	1.68	66
57	11.2	85.8	2.8	.39	1.51	55
58	6.8	92.0	1.3	.27	.97	-
59	14.1	80.6	2.8	.57	1.76	71
60	5.9	93.3	1.1	.29	.71	72
61	6.5	94.0	1.0	.23	.95	-

The Composition of Omental Adipose Tissue
in 12 Normal Sheep

Fat g%	Water g%	Protein g%	K MEQ/100 g	Na MEQ/100 g	Iodine Number
97.6	3.0	0.6	.24	.60	40
94.3	4.3	0.6	.23	.66	40
96.8	5.0	0.5	.38	1.40	44
97.6	3.5	0.9	.27	.98	30
93.6	4.5	0.7	.25	.59	41
95.4	3.4	0.6	.20	.56	44
95.8	3.8	0.9	.23	.53	44
95.5	3.7	0.8	.23	.53	44
96.9	2.3	0.5	.16	.44	-
97.1	2.0	0.5	.16	.40	-
95.5	3.5	0.6	.23	.79	-
97.6	1.7	0.8	.24	.62	-
96.1	3.4	0.7	.23	.67	41

The Composition of Subcutaneous Adipose Tissue in 12 Normal Pigs.

	Fat g %	Water g %	Protein g %	K MEQ/100g	Na MEQ/100g	Iodine Number
	90.0	8.4	1.2	.36	1.20	47
	79.0	16.4	3.3			45
	81.8	11.1	1.7	.50	1.55	48
	82.1	12.5	1.5	.37	1.10	33
	91.7	6.3	. 9	.22	.90	28
	81.8	9.5	2.7	.38	1.26	32
	94.4	5.1	1.4	.30	1.41	47
	83.7	9.7	2.4	.27	1.23	52
	85.8	8.4	1.9	.37	.96	46
	90.0	7.9	1.9	.26	.93	—
	89.8	6.3	1.5	.27	.98	38
	87.0	8.4	2.0	.34	1.14	46
Mean	86.3	9.2	1.9	.33	1.15	42

The Composition of Perirenal fatty Tissue

in 5 Normal Rabbits

	Fat g%	Water g%	Protein g%
	93.3	5.9	0.8
	91.8	5.4	1.2
	71.0	25.8	3.0
	84.0	10.7	1.4
	86.8	8.3	1.9
Mean	85.3	11.2	1.1

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