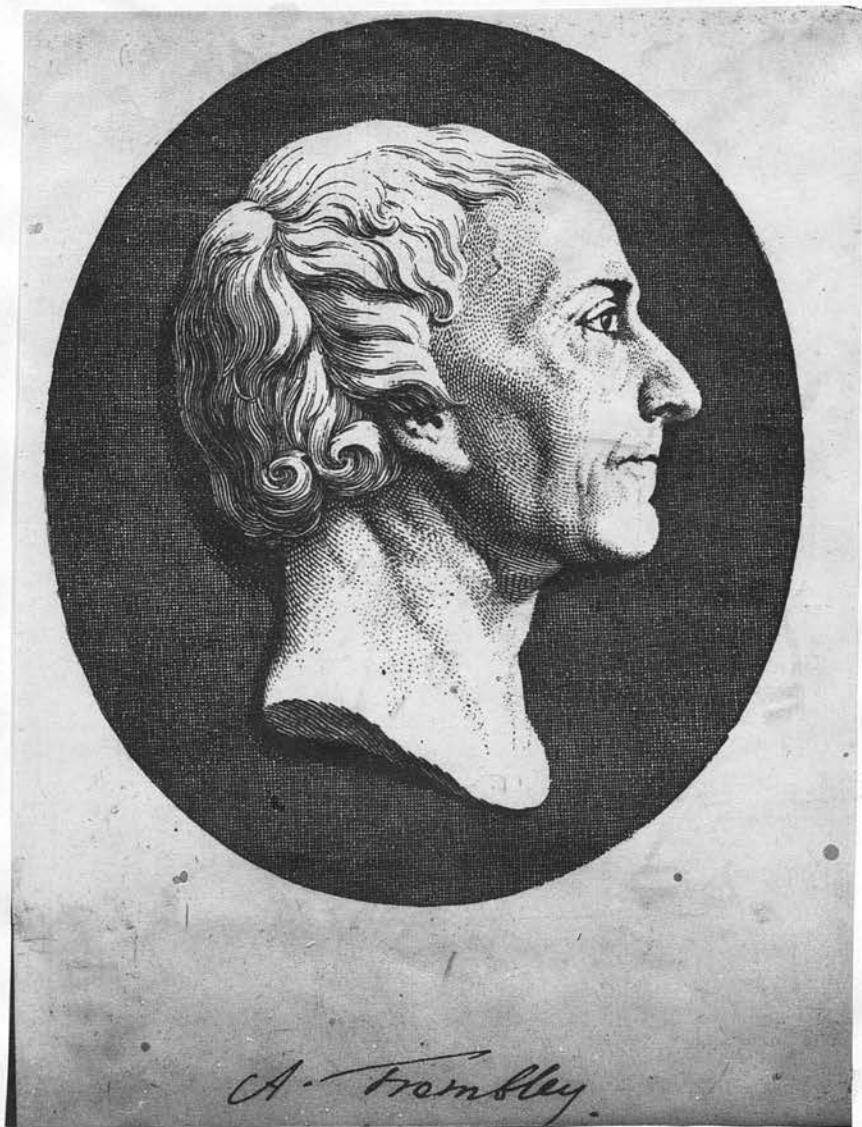


ADRENO-CORTICAL HOMO-TRANSPLANTATION  
as a method of endocrine research.

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Abraham Trembley.

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The Then proudly smiled that old man

To see the eager lad

Rush madly for his pen and ink

And for his blotting-pad -

But, when he thought of publishing

His face grew stern and sad.

Homo-transplantation as a saw (Lewis Carroll).

Gerontological research

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## INTRODUCTION.

The idea of transplantation must have arisen very early in the history of human thought. It presents itself to the mind as a prima facie solution to problems such as the repair of defects, the replacement of diseased parts and the acquisition of additional powers. As such it must have occasionally filled the imaginings of primitive man.

Mankind was not slow to proceed from dreams to deeds in this matter. The technique of horticultural grafting seems to have been perfected very early in civilised development and Aristotle mentions the procedure on a number of occasions as something well established. He also draws attention to the different compatibilities between the various plants used for grafting.

The transplantation of animal tissues meets greater technical and biological obstacles and the only example of its routine use amongst the Ancients was probably rhinoplasty by autoplasmic pediclegraft as practised by the ancient Hindus. This was a modest but very useful beginning at a period of human history when mutilations (due to savage customs and primitive conditions of life) were almost as common as they are now becoming (due to savage customs and civilised conditions of life). The success of this type of operation was a demonstration of the important fact that a secondarily acquired blood supply can maintain the life of a transplanted part. The operation was modified in the Renaissance by

Tagliacozzi of Bologna (1546-1599) who cut his skin flap from the arm instead from the forehead. It is in connection with his activities that we find, what must be one of the earliest reports on the late failure of incompatible homo-grafts. Van Helmont relates the story as follows "A certain inhabitant of Brussels, in a combat had his nose mowed off, addressed himself to Tagliacozzus, a famous Chirurgain, living at Bononia that he might procure a new one; and when he feared the incision of his own arm, he hired a porter to admit it, out of whose arm, having first given the reward agreed upon, at length he dig'd a new nose. About thirteen moneths after his return to his own Countrey on a sudden the ingrafted nose grew cold, putrified, and within a few days dropt off..." The sequence of vascular occlusion followed by necrosis is quite typical, though thirteen months is a longer survival time than one would expect.

Paracelsus (1493-1541) whose exuberances have annoyed every generation of physicians starting with his own, set no limits to his imagination and was therefore able to anticipate most of the developments of modern medecine. He proposed the use of healthy organs to replace diseased ones, but neither he nor his generation were in any way capable of acting upon this proposal. The scientific history of free transplants of animal tissues starts only with the publication in 1744 of Abraham Trembley's book on the hydra. This

work which revolutionized concepts in the field of reproduction and regeneration contained also accounts of the successful grafting of one hydra upon another. Although, strictly speaking, experiments on parabiosis and therefore involving slightly different problems from those of a tissue graft, they proved clearly, and for the first time, the possibility of survival of tissue from one animal in the body of another.

This was just the type of experiment which the curious and playful 18th century would take up with enthusiasm and transplantation has ever since remained one of the techniques of the biological laboratory. John Hunter (1728-1795) probably reported the first successful endocrine homografts, transplanting testes and spurs, and believed in a general "disposition" in all living substances to unite when brought into contact with one another, although they are of different structure, and even although the circulation is only carried out in one of them". Boronio in 1804 published accounts of the successful homotransplantation of skin but it is doubtful whether any of his grafts really survived. The first systematic use of transplantation as an endocrine research tool was the demonstration by Berthold in 1849 that testicular autotransplants had a masculinising influence even when deprived of all nervous connections and placed in an atypical location. This experiment is justly regarded as the start of scientific endocrinology.

In the second half of the 19th century it became clear through numerous investigations that autogenous grafts are often, homogenous grafts seldom, and heterogenous grafts never, successful. The clinical methods of grafting in the repair of skin defects which are still in common use were also elaborated in this period. Halsted in 1909 as the result of experiments on parathyroid auto-transplantation came to the conclusion that the necessary condition for the success of any transplant of an endocrine tissue is the prior existence in the recipient of a deficiency of the corresponding tissue. The phenomenon found later its explanation in the discovery of the trophic hormones. The use of homo-plastic transplantation for purposes of endocrine research had one of its earliest and certainly its most important application in the demonstration by Steinach in 1912 of the feminising effect of implanted ovaries on castrated male animals. Steinach made a number of other important contributions to Biology. He is mainly (though not better) remembered for his gallant if over-sanguine attempt to stem the advances of ageing by his operation of vasoligation.

To-day transplantation has ceased to be a biological tour de force, and is finding daily wider applications. Where survival of the graft for any length of time is not of importance as in the case of blood, bone, skin, and arteries, it has already established itself amongst routine therapeutic procedures. With our daily

increasing knowledge of the factors on which transplant survival depends, we may confidently expect an extension of the therapeutic use of transplantation to other tissues, Transplantation experiments are fructifying every field of biological research and have become of extreme importance in cancer research, embryology and endocrinology.

Viewed against this background the subject of this thesis may appear unduly narrow. The minutiae of procedure are however the real life blood of science. They are the stuff from which general principles spring, and against which they are tested.

They sought it with passion, they sought it with love

They pursued it with tears and blood

They threatened its life with a thousand deaths

They charmed it with smiles and songs

## ADRENAL TRANSPLANTATION.

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Although gonadal grafting has been practiced since the middle of the eighteenth and thyroid grafting since the beginning of the nineteenth century, the first attempt at transplantation of adrenal tissue was not recorded until 1887. In that year, Pierre Danalis, in the course of an investigation of the histology of adrenal regeneration found that small autogenous pieces of adrenal gland, grafted on to the surface of the kidney, would occasionally show signs of regeneration in animals killed on the fifteenth post-operative day.

They sought it with thimbles, they sought it with care  
They pursued it with forks and hope;  
They threatened its life with a railway-share;  
They charmed it with smiles and soap.

(Lewis Carroll).

2) That the kidney is a suitable site for adrenal transplantation.

3) That such grafts can grow in the presence of the animal's own glands.

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## ADRENAL TRANSPLANTATION.

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- 1) That small adrenal auto-grafts can be made successfully.
- 2) That the kidney is a suitable site for adrenal transplantation.
- 3) That such grafts can grow in the presence of the animal's own glands.

The remaining years of the nineteenth century produced a number of unsuccessful attempts at adrenal transplantation some of which will be referred to in detail/

Unfortunately/

detail, but already in 1892 Abelous produced, in the frog, evidence of the functional survival of transplanted adrenal tissue (Abelous, 1892). He introduced pieces of frog's kidney with adrenal tissue attached into the lymph sac of frogs and destroyed their own adrenal tissue some time afterwards. Eight of his animals survived this procedure and six of these died shortly after the removal of the transplants. In addition to functional survival, Abelous therefore demonstrated the feasibility of adrenal homotransplantation. His results were confirmed by Gourfein, using similar methods (Gourfein, 1896).

From 1898 onwards, Poll published a number of studies of the histological fate of adrenal grafts, describing in detail the initial destruction of most of the transplanted tissue; the subcapsular origin of the regenerated cortex in successful cases; and the complete disappearance of the medulla (Poll, 1899, 1899, 1905, 1906). It may be noted that many of his investigations were carried out by homo-graft on animals still possessing their own glands.

The basis of knowledge created by these successful investigations might, at the beginning of this century, have led to rapid progress in this field.

Unfortunately/

Pybus, 1924) alternated with those of complete failure (Parodi, 1903, Coenen, 1906, Mayeda, 1921, Bledinger). Unfortunately, these findings were obscured and rendered impotent by the great number of unsuccessful and contradictory investigations published at the same time, so that the actual state of opinion on adrenal grafting at the turn of the century was one of great confusion. It will be interesting to analyse the reasons for some of the failures.

One group of failures is related to attempts at hetero-transplantation. In 1896 Gourfein (loc.cit) tried to implant guinea-pig's adrenal into the lymph sac of frogs and in the next year Jaboulay grafted dog's adrenals under the skin of an Addisonian patient with disastrous results (Jaboulay, 1897). Although the invariable failure of such attempts was soon generally recognised, attempts to treat Addison's disease by hetero-grafting continued to be made from time to time (Busch et al. 1910, Currie, 1924, Dimitrijevic, 1925, Kanevski, 1929).

While unsuccessful hetero-transplantation was never a cause of serious confusion, much misunderstanding arose from the conflicting results obtained with homoplastic grafts. Reports of occasional successes (Abelous, loc.cit. Busch et al., 1908, Neuhauser, 1909, Pybus/

Pybus, 1924) alternated with those of complete failure (Parodi, 1903, Coenen, 1906, Mayeda, 1921, Blodinger et al., 1926). The main reason for this, as we now understand it, is that random grafts within a species are likely to prove as incompatible as grafts between members of different species. Homo-grafts are successful only if carried out between closely related members of an inbred strain, apart from certain special cases, and ignorance of this fact accounted for many of the contradictions between early workers.

Even autogenous grafts, however, often failed to take in these early investigations (Boinet, 1895, de Dominicis, 1897, Langlois, 1897 Hultgren & Andersson, 1899, Coenen loc.cit., Zwemer, 1925, Blodinger et al., loc.cit). The dilemma facing these early investigators lies in the fact that adrenalectomy prior to grafting is followed in most species by death before the graft has time to grow, whilst grafting in the presence of the animal's own adrenals usually results in degeneration of the graft from lack of adreno-cortico-trophic stimulation. Even if such a graft is not destroyed, it will not grow to a functional size and the eventual removal of the animal's own adrenals finds it no better off than if grafting had been carried out at the time of adrenalectomy. It must however be emphasised that the view/

view that a graft will never grow in the presence of the animal's own glands (Wyman & tum Suden, 1932) is erroneous (Canalis, loc.cit., Abelous, loc.cit., Schmieden, 1903, Stilling, 1905 etc). Its chances of functional survival are, however, much impaired.

Implantation of a whole adrenal gland is invariably followed by degeneration of all but the subcapsular zones. This, in successful cases is followed by a regeneration of the whole cortex from the surviving subcapsular cells. The presence of a central necrotic mass introduces the hazard of infection or fibrosis. This was a further impediment to the success of some of the early attempts at adrenal transplantation. The danger is circumvented by utilising only the adrenal capsule for grafting. This is of special importance in the guinea-pig which is very sensitive to the local action of adrenaline liberated from the disintegrating medulla (Elliot & Tuckett, 1906) • Schmieden (loc.cit.) went very carefully into the question of operative technique and proposed a satisfactory routine but his lead was not followed.

The uncertainty created by the variable results obtained, was brought to an end by a series of papers by Jaffe, published from 1926 onwards (Jaffe & Plavska, 1926/

Meanwhile, as the result of the more widespread use of inbred strains of laboratory animals, large scale

1926, Jaffe, 1926, Jaffe, 1927 (1) and (2)) in which a method was demonstrated which could ensure successful auto-grafting in rats and guinea-pigs in the majority of instances. Only now was it possible to direct interest away from the problems of grafting, and use adrenal transplantation as a tool to investigate problems of adrenal function. Jaffe himself used it to show that diminished resistance after adrenalectomy can be abolished by an autogenous graft (Jaffe, 1926). From 1928 onwards, Wyman and his co-workers employed autogenous intra-muscular grafts in adrenalectomised rats to differentiate medullary from cortical function (Wyman, 1928, 1929, Wyman & tum Suden, 1929, 1930, Wyman & Walker, 1929). This method is based on the almost invariable (and in any case easily verifiable) disappearance of the medulla from adrenal transplants. Normal reproductive function was maintained in female adrenalectomised rats by means of autogenous transplants (Pencharz & Olmsted, 1931) and Martin in 1932 restored oestrus cycles in the same animal by the same means (Martin, 1932).

the choice of the rat as the experimental animal. Rats and mice share certain characteristics/

Meanwhile, as the result of the more widespread use of inbred strains of laboratory animals, large scale homo-transplantation had become practicable and it was also realised that a successful homo-graft was functionally equivalent to an autogenous transplant (Wyman & Sudekum, 1937 (2)). The method was put on a firm footing in 1938 by Higgins and Ingle in a series of papers in which the technique of successful homo-transplantation in the rat was described and the dependence on close relationship between host and donor for success was demonstrated (Higgins & Ingle 1938, Ingle & Higgins 1938, Ingle & al., 1938, Lux & al., 1938). These workers also re-described the histological changes after transplantation, confirming and expanding the findings of Poll forty years earlier (Poll, loc. cit., Ingle & Higgins, 1937). It may be said that since 1938 adrenal homografting has been available to endocrine research but, so far, little use has been made of the special applications of this method. This question will be discussed in the next chapter.

The establishment of satisfactory standard techniques for adrenal auto and homo-transplantation was largely linked with the choice of the rat as the experimental animal. Rats and mice share certain characteristics/

characteristics which make them ideal animals for this type of experiment but the rat is a more satisfactory size. First of all, they are the only animals of whom pure strains have been made widely available. Quite apart from the question of compatibility of homo-grafts which has already been mentioned, different strains of the same species show important variations with regard to the length of survival after adrenalectomy, the presence of accessory adrenal tissue etc; hence, comparison of the results of different workers becomes only profitable if they are working with the same strain, and this is one important reason why progress has been quicker since certain strains of rats became universally used. The second advantage is an anatomical one. In the dog, cat, rabbit, guinea-pig and in many other species the adrenal glands have intimate anatomical connections with the big vessels, especially with the vena cava. Complete removal is therefore difficult. In amphibia the adrenal tissue grows diffusely on the ventral surface of the kidney which have to be damaged if complete removal is to be effected. In the rat and mouse the glands are discrete and grow at a safe distance from any important structure. They can be cleanly and safely removed without damage to other parts, /

parts, without important haemorrhage and with a minimum of operative shock. The third and probably the most important advantage is a physiological one and lies in the relative resistance of the rat to bilateral adrenalectomy. We have seen that grafting in the presence of the animal's own adrenals usually fails because of insufficient adreno-cortico-trophic stimulation of the transplant. Grafting after bilateral adrenalectomy, on the other hand, is only successful if the animal can survive the operation for the period (one to four weeks) needed for the transplant to become functional. Until recently, the only available method to ensure survival after adrenalectomy of cats, dogs, rabbits and guinea-pigs was the administration of cortical hormones. This however interferes with ACTH secretion and thus with the growth of the transplant. The rat has a high resistance to shock and, in the absence of undue stress, suffers relatively little from a temporary lack of glucocorticoids. Provided the lack of electrolyte regulating steroids is compensated by the replacement of drinking water by saline, rats will survive the period of transplant fixation and regeneration without difficulty. This salt administration is not likely to interfere with ACTH secretion as the output of mineralo-corticoids is independent/

independent of the pituitary gland.

These factors explain why so many of the experiments on rats have been crowned by success, whilst those on dogs (de Dominicis, 1897, Imbert, 1900, Strehl & Weiss, 1901, Coenen loc.cit., Blodinger et al., 1926, Oldberg, 1929, Johnson & Johnson, 1931, Grieco, 1932) cats (Hultgren & Andersson, loc.cit., Zwemer, 1925) rabbits (Canalis, loc.cit., de Dominicis, loc.cit., Schmieden, 1902 & 1903, Parodi, 1903 & 1904, Stilling, 1905, Busch & Van Bergen, 1906, Busch & al., 1908, Neuhauser, loc.cit., Hsiang Ch'uan Hou, 1928, Lux & al., loc. cit., Turner & al., 1939, Williams, 1945) guinea-pigs (Strehl & Weiss, loc.cit., Elliott & Tuckett, loc.cit., Jaffe & Plavska, loc.cit., Jaffe 1927 (1), Lux et al., loc.cit., Morone, 1948) and other animals (Abelous loc.cit., Gourfein loc.cit., Langlois, 1897, Strehl & Weiss, loc.cit.) have been generally unsuccessful. In fact, it still remains to be proved that functionally successful grafts of adrenal gland can be made in animals other than the rat and guinea-pig. This will soon be achieved, as means have been developed which can overcome the dilemma described above. A method for keeping dogs alive after adrenalectomy which does not involve the use of cortical hormones has lately been described (Grollman, 1952)/

infinitesimally small. The fact that in most cases of

1952) and anti-histamine drugs have been shown to exert a useful action in counteracting the post-operative shock of adrenalectomy. Moreover, grafting in the presence of the animals own glands need no longer be avoided as a sufficiency of trophic stimulation can be ensured by the administration of ACTH. Auto-grafting in laboratory animals other than the rat would therefore seem to be a feasible procedure.

Man occupies a special place amongst the animals that have been used for adrenal transplantation. The aim in these cases was always the relief of Addison's disease and the grafts were therefore all homo or hetero-plastic. All the hetero-grafts failed (see above). Most of the homo-grafts were also unqualified failures (Hurst et al., 1922, Pybus, 1929, Curschmann 1928, d'Abreu, 1933) or extremely doubtful clinical successes (Pybus, 1924, Leschke, 1929, Beer & Oppenheimer, 1934, Bailey & Keele, 1935, Broster & Gardiner-Hill, 1946) but in a few there can be little doubt that the clinical improvement was associated with graft survival (Reinhart, 1928, Goldzieher & Barishaw, 1937).

In this type of experiment the dice are heavily loaded against success. Except in the case of uniovular twins the chances of finding a compatible donor are infinitesimally/

infinitesimally small. The fact that in most cases of Addison's disease some functioning remnant of the patient's own glands still exists, also militates against successful grafting. Moreover, in the cases in which Addison's disease is due to simple strophy of the adrenal gland it is ~~very~~ doubtful whether adequate stimulation to graft tissue is available. If it is further remembered that remissions are common and correct diagnosis often difficult, claims to have treated this disease successfully by grafting will be treated with extreme caution.

Nevertheless, there are factors which might account for the probable success of the above quoted cases. The most important of these factors is the phenomenon of delayed absorption of incompatible transplants.

The fate of an incompatible graft is usual sealed within one week of grafting. There is lymphocytic infiltration, necrosis, giant cell formation and finally the remnants of the graft are engulfed by connective tissue. Sometimes however, a homograft will regenerate into a healthy and functional cortical nodule and remain alive for weeks or months before it undergoes the same fate. This phenomenon is not easily explained on the basis of current immunological knowledge but can be

action of adreno-cortical and pituitary tissues.

be circumscribed by stating that in some cases of incompatible graft the activation of the graft destroying mechanism is delayed, the graft meanwhile behaving like a compatible one. Reinhart's case (v.s) is rather suggestive, for prior to successful grafting his patient had been subjected to irradiation. We know now that irradiation can prolong the life of a transplant by interfering with the reticulo-endothelial system. The important point in Goldzieher's case (v.s) is that he obtained histological proof of transplant survival.

With the coming of efficient cortical preparations the interest in therapeutic adrenal transplantation died down; it may revive when we have learned how to perform adrenal homografting with assurance of success. In the long run, grafting is a more acceptable therapy than indefinitely prolonged administration of hormones.

A few words may be added about the various sites that have been tried for adrenal grafting. The brain offers a favourable soil (Pomerat et al., 1944, Barnicot, 1948, Hwai-Ya Chang, 1951). The intrinsic interest of this observation apart, there are many obvious disadvantages in using this site. It might however become useful for the study of the local interaction of adreno-cortical and pituitary tissues.

The ear as a locus of grafting (Hsiang Ch'ouan Hou, 1928, Kroc, 1942, Williams, 1947) offers the advantage of easy complete graft removal with a minimum of associated trauma. It also permits direct observation of the graft. In my experience, however, function is not as fully maintained as with other sites, and even auto-grafts tend to degenerate after a period. This drawback it shares with other types of subcutaneous transplant. These have often been used (Jaboulay, 1897, Poll, 1898 and 1899, Elliott & Tuckett, 1906, Hurst et al. 1922, Pybus, 1924, Dimitrijevic, 1925, d'Abreu, 1933, Higgins & Ingle, 1938) but the advantage offered by the ease of operation is offset by the frequent failure of the grafts to acquire a satisfactory blood supply.

Intraocular transplants offer even greater ease of observation than transplants to the ear and have the additional advantage of permitting survival of otherwise incompatible homografts (Turner, 1938 and 1939, Turner et al., 1939, Williams, 1945). In this fact lies, at the same time, the main drawback of this method, as such grafts cannot be said to grow in a normal physiological milieu and cannot therefore be readily used for investigating normal adreno-cortical physiology./

Intra-muscular adrenal grafts grow well and have been used widely (Hultgren & Andersson, 1899, Poll, 1899, Strehl & Weiss, 1901, Zwemer, 1925, Jaffe & Plavska, 1926, Jaffe, 1927 (1), Reinhart, 1928, Wyman, 1928, and 1929, Wyman and tum Suden, 1929, 1930, 1932 and 1942, Wyman and Walker, 1929, Nice and Shiffer, 1931, Martin, 1932, Bailey and Keele, 1935, Goldzieher and Barishaw, 1937, Komrad and Wyman, 1950 and 1951).

Omental, mesenteric, splenic and hepatic grafts have been used to study the effect of portal drainage on cortical function (Strehl & Weiss, 1901, Cristiani & Cristiani, 1902, Parodi, 1903 and 1904, Coenen, 1906, Mayeda, 1921, Oldberg, 1929, Johnson & Johnson, 1931, Grieco, 1932, Martin, 1932, Butcher, 1948, Bomze & Friedman, 1950, Bernstein, 1950 (1) and 1950 (2), Head, 1951, Rather, 1952).

Grafts to the testis (Stilling, 1905, Busch et al., 1908 and 1910, Hurst et al., 1922, Johnson & Johnson, 1931) or ovary (Pencharz et al., 1930 and 1931, Pencharz & Olmsted, 1931, Martin, 1932, Ingle and Harris, 1936, Ingle et al., 1936), apart from surviving well in these situations, can furnish information on the local inter: :action of steroid-producing glands. The secondary sexual organs have been used as transplantation sites to/

to study the question of the local effect of adrenal sex steroids (Katsh et al., 1948, Weinstein et al., 1950).

From the earliest attempts onwards, the kidney has been a favourite organ for adrenal grafting (Canalis, 1887, de Dominicis, 1897, Imbert, 1900, Strehl & Weiss, 1901, Schmieden, 1902 and 1903, Parodi, 1903 and 1904, Busch and Van Bergen, 1906, Busch et al., 1908, Neuhauser, 1909, Mayeda, 1921, Johnson & Johnson, 1931, Rutishauser & Guye, 1936, Turner et al., 1939). Partly this preference rested on mistaken theories about reno-adrenal relations but there are important advantages in using the kidney as the site for transplantation. These will be discussed in the chapter on technique.

The whole question of adrenal transplantation has been reviewed from time to time notably by Biedl (1912) Jaffe (1927 (3)) Grollman (1936) and by Hartman and Brownell (1949). The references to this and the next chapter constitute a bibliography of adrenal transplantation which, although not complete, is far more extensive than any so far published.

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THE HISTORY AND SCOPE OF ADRENAL BOND-GRAFTING

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THE HISTORY AND SCOPE OF ADRENAL HOMO-GRAFTING.

But the Judge said he never had summed up before;  
So the Snark undertook it instead,  
And summed it so well that it came to far more  
Than the witnesses ever had said!

(Lewis Carroll).



Senile rat with adreno-cortical homo-transplant on the left kidney. The animal was 19 months old at operation and 29 months when it died. The dark colour of the graft is due to discharge of lipoids before death.

### THE HISTORY AND SCOPE OF ADRENAL HOMO-GRAFTING.

As we have seen, it took forty years before auto-grafting of the adrenal gland was perfected to a degree which made it useful as a tool of endocrine research. Homo-grafting is still only used occasionally and in an unsystematic fashion. Yet, the only additional requirement for the routine performance of homoplastic grafts is the maintenance of inbred strains of experimental animals. The experimenter must breed his own animals, keep careful stock records and select donor and recipient from animals as closely related as the purpose of the experiment will allow. If this is done, success of the operation can be expected in the majority of cases. The exact procedure to be followed will be described in the next chapter; here we are concerned with reviewing the uses that have been made of adrenal homo-grafting in the past and of outlining the special indications of the method; in other words, to define the problems which can best or solely be studied by it.

Naturally, many of the investigations of the past concerned themselves with the problem of homo-transplantation as such, attempting to discover its feasibility, the conditions of success

and the histological changes undergone by the transplanted tissue. For reasons which have already been discussed many of these investigations were complete failures (Parodi 1903, and 1904, Coenen 1906, Blodinger et al. 1926, Grieco 1932, Kroc 1942,). Others, whilst failing to obtain permanent and functional graft survival were able to study and describe the histological stages through which an incompatible graft passes on its way to destruction (Poll 1898, Schmieden 1903, Jaffe 1927, Hsiang Ch'ouan Hou 1928, Lux et al. 1937,). Some obtained histological survival of the grafts for long periods but no proof of the functional efficiency of the transplant tissue (Poll 1899, Neuhauser 1909, Hwei Ya Chang 1951).

The first experiment in which the capacity of adrenal homografts to maintain the life of adrenalectomised animals was demonstrated was published by Abelous in 1892. His animal was the frog. In 1908 Busch and his co-workers reported successful homografting of rabbit's adrenal into the kidney. From 1931 onwards reports of successful adrenal homografting became more frequent, the rat being the animal used by most

workers. After Wyman and tum Suden had established the functional equivalency of auto and homo grafts (1932 & 1937) the importance for success of close genetic relationship was elucidated by Ingle and his collaborators (Higgins and Ingle 1938, Ingle et al. 1938, Ingle and Higgins 1938, Lux et al. 1938) a matter which had already received some attention by Rutishauser and Guye in 1936.

As a result of all these investigations it was established that adreno-cortical function could be adequately maintained in an adrenalectomised animal by means of homoplastic adrenal grafts and the road was free for an application of this method to the investigation of endocrine problems.

A rather special application of adrenal homografting has already been discussed in the foregoing chapter. These were the attempts to treat Addison's disease by the implantation of human adrenal tissue.

A further group of investigations which have little interest from our point of view are those where homografting was carried out for the study of problems which could have equally well been attacked by autogenous grafts.

The first specific use of adfenal homografting as tum Suden (1937) in which these authors investigated

The cause of adrenal activity after hypophysectomy.  
a research tool was probably made by Nice and Shiffer who, in 1931 thought to induce hyperadrenalism by multiple glandular implants. Similar reasoning prompted the investigations of Morone (1948) and of Murphy and Sturm (1950). An erroneous concept underlies this group of experiments as it is impossible to produce hypercorticism by multiple implants. The total extent of adreno-cortical activity is limited not by the amount of adrenal tissue but by the amount of ACTH secretion. To produce hyperadrenalism it is necessary either to administer large doses of ACTH or to cause an excess of ACTH to be secreted, when the normally present amount of adrenal tissue will be sufficient to produce hyperadrenalism.

Adrenal homo-grafts into the anterior chamber of the eye (Turner 1938 and 1939, Turner et al. 1939) and into the brain (Pomerat et al. 1944) served to elucidate the special immunological properties of these transplantation sites but are of no special endocrinological interest.

The only set of experiments in which it can be said that adreno-cortical homotransplantation was used in a specific manner was that of Wyman and tum Suden (1937) in which these authors investigated

the cause of adrenal atrophy after hypophysectomy. By implanting the atrophied glands of hypophysectomised rats into animals with intact pituitary and demonstrating that they would grow, whilst normal adrenals failed to grow in hypophysectomised rats, they produced physiological proof of the existence of an adrenotropic pituitary principle.

This solitary example in the literature of a specific application of adfenal homo-grafting illustrates the place which the method has as an endocrine research tool. This place may be defined as follows:

adrenal homo-grafting is the only practicable method by which intrinsic adreno-cortical properties can, at present, be differentiated from those imposed upon the cortical tissue by other parts of the organism.

Adrenal function has been shown to vary more than the function of any other organ. It seems to be important in the foetus, undergoes a radical change at birth and again at puberty, differs considerably in the two sexes and exhibits another set of characteristics in post-reproductive life. In many diseases it seems to play a part. Which of these variable characteristics are the result of

genetically determined and autonomically proceeding changes of adreno-cortical reactivity and which are secondarily induced reactions of the cortical cells to changes occurring in the rest of the body? A conclusive answer to this important question can only be obtained by transplanting the gland from one type of body into another and noting the changes in function which such a transplantation brings about.

It must of course, be realised that what one studies in such experiments is not the function of the adrenal gland but the function of an adreno-cortical transplant; comparisons can therefore in the first place, only be made with other transplant bearing animals and not with normal ones. This, provided it is realised and acted upon, does not limit the usefulness of the method.

Homo-grafts in the investigation of age changes in adreno-cortical function:

The assessment in the intact animal of functional age changes of any tissue meets insuperable analytical difficulties because of the presence and interaction of other organs of the same age.

It would, therefore, be of obvious advantage if we could transplant (without loss of function and

without exciting adverse reactions) aged organs into youthful bodies and youthful organs into aged bodies. Ideally, this would enable us clearly to distinguish intrinsic age changes from those induced by ageing or disease of the environing organism.

The execution of such a plan, founders in most instances on technical or biological obstacles. Nervous tissue is biologically intransplantable. Its function depends on connections which have to be severed in the process of transplantation and most of which will not regenerate or could not be relied upon to re-establish normal contacts. The same applies, though to a lesser degree, to skeletal muscle and to any tissue which functions mainly by virtue of its nerve supply.

Organs like the heart, lung, liver, alimentary canal and kidney depend for their continued function after transplantation upon immediate and accurate re-establishment of their correct anatomical connections (vascular and ductal). The technical difficulty of achieving this, precludes the attempt in the small laboratory animals. In bigger animals where such procedures might be undertaken with some hope of success, we are still hampered by lack of sufficiently inbred strains. Homotransplantation of

potentially antigenic tissues can at present only be successfully achieved in biochemically homogenous strains.

The only organs with which this type of experiment can be attempted on a sufficient scale to yield useful results are the endocrine glands. They are independent of ducts, relatively independent of nervous control, and they can discharge their specific functions with the diffuse blood supply, secondarily acquired after implantation. This disposes of most of the technical difficulties, and makes it possible to use the method in the small laboratory animals in which strains, sufficiently pure to allow successful homotransplantation, exist.

The success of endocrine transplants is favoured, at least in the case of <sup>the</sup> adrenal gland, the gonads, the thyroid, and probably also in the case of the islet tissue, by the action of trophic hormones which stimulate the growth of glandular implants in animals deprived of the glands in question. In the case of the adrenal cortex, transplant survival may be further enhanced by the fact that cortical hormones have a local protective effect against the absorption of incompatible transplants (Billingham et al. 1951,

Butterfield et al. 1952) so that adreno-cortical transplants may be able to protect themselves during the critical time beyond which a homo-transplant may become safe from the danger of absorption (Woodruff and Woodruff, 1950).

In one of the following chapters an account will be given of the application of this technique to gerontological problems.

Adrenal homo-grafts in the investigation of intrinsic sex differences of cortical function.

The participation of the adrenal gland in all phases of reproductive function is beyond doubt although the exact mechanisms involved are poorly understood. This participation starts already in foetal life, the adrenal gland being the earliest endocrine organ to differentiate and to show evidence of secretory activity, and probably play a part in counteracting and regulating the effects of maternal hormones on the development of the foetus. The functional importance of the adrenal cortex at this early stage of life is illustrated by the dramatic changes occurring in its structure shortly after birth in many species, by the marked histological differences between the neonate

gonads. For example, the size of the female adult

cortices in the two sexes in other species and by the frequent concurrence of congenital malformations of the sexual organs with abnormalities of the adrenal gland.

The question arises whether genetic differences in the bio-chemical make-up of the glands of the two sexes account for their different behaviours i.e. whether adrenal tissue enters primarily into the mechanism of sex differentiation, or whether adrenal behaviour at this stage represents entirely a reaction to surrounding bio-chemical influences. Whilst it is possible to gather suggestive information on this point by experiments involving the administration of active steroids to normal foeti (Witschi, 1953), a definite answer could only be obtained by investigations in which male and female foetal adrenal glands are successfully interchanged.

Similar problems arise at the time of sexual maturation. There is bio-chemical evidence that adrenal function at puberty begins to show sex differences (Elftman, 1947, Wood and Gray 1949) and it is also easily demonstrated that many of these differences are secondarily induced responses of the adrenal cortex to the secretory activity of the gonads. For example, the size of the female adult

cortex in the rat can be reduced to adult male proportions by ovariectomy and, conversely, castration is followed by growth of the male adrenal cortex to approximately adult female size.

It remains, however, to be seen whether the adrenal cortex plays an entirely reactive part in this adrenal-gonadal relationship or whether there is such a thing as an independent adrenal puberty; some evidence suggests that this latter may be the case (Zuckerman and Bourne 1940).

Further, it would be important to know whether male adrenal tissue is capable to respond in the same way and to the same degree to ovarian influences as the female gland or whether there is a basic difference in adrenal reactivity in the two sexes.

The whole question of extra-gonadal sex differences is still very inadequately investigated. The influence of the testis and ovary in determining sexual characteristics is so overwhelming that it is often overlooked that the difference between the two sexes ante-dates and outlasts the activity of the gonads. It can be assumed on genetic grounds and has been demonstrated morphologically (Barr et al. 1950, Graham and Barr 1952, Moore et al. 1953) that even the somatic cells show sex differences

and it may be thought that some tissues will show such sex differences to a more marked degree than others. Of all the extra-gonadal tissues of the organism, the adrenal glands (as the secondary producers of sex steroids) are the most likely to show such sex differences to a measurable degree. The only possible way to decide these questions at present is by exchange of adrenal tissue between gonadectomised animals of the two sexes i.e. by homo-transplantation. Experiments along these lines will be related in the following chapters.

Other uses of the methods of adrenal homo-grafting.

Whilst the investigation of age and sex differences in adrenal cortical function (reactivity) by homo-grafting constitutes in itself a field which could be tilled with profit for many decades there is still another set of problems to which this method is specifically suited. These problems arise with reference to morbid alterations in adrenal function. When such alterations occur the question poses itself whether they are irreversible or whether they could be abolished by removing the gland from the environment in which



at least two components, a renal and an adrenal one.  
they have developed. Two examples may be given to  
make this point clear:

It was shown by Woolley (1949) that tumors of  
the adrenal cortex can be produced with regularity  
in a certain strain of mice by the simple expedient  
of gonadectomy. We are in this case obviously deal-  
ing with a genetic predisposition and it remains un-  
decided whether this special predisposition resides  
in a peculiar sensitivity of the cortical cells to  
the normal effects of ovariectomy or in an abnormal  
set of effects of ovariectomy acting upon normal  
adrenal tissue. If it were found possible to implant  
adrenals from a non-tumor strain into these animals  
and their own adrenals into those of a non-tumor  
bearing strain, this question, with its important  
bearing on the problem of tumor induction, could be  
answered.

Constriction of the renal vessels in the rat in  
the presence of the adrenal glands leads to hyper-  
tension. Removal of the adrenal glands, however,  
abolishes this hypertension even although the renal  
abnormality is allowed to persist. (Goldblatt 1937).  
The same applies to the hypertension which follows  
nephrectomy (Wilson, 1953). We are faced in these  
instances with a hypertensive mechanism comprising

at least two components, a renal and an adrenal one. Is the adrenal component based on normal adrenal function or on a morbid alteration of adrenal function following interference with renal function? If based on a morbid alteration of adrenal function, is this alteration reversible or irreversible i.e. will it persist if the adrenal gland is placed into the body of a normal rat? Homo-grafting could go a considerable way towards answering these questions. It will be apparent that experiments of this nature could become of great importance in testing the validity of the concept of diseases of adaption as put forward by Selye.

What has been said about the scope of adrenal homo-grafting applies, mutatis mutandi, to the other tissues of the body. It may be predicted that as these possibilities are being realised and to the degree to which inbred strains of various laboratory animals are being made available, homo-grafting will make important contributions to biological knowledge.

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THE METHOD

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Then, with an aspect cold and grim,

Regardless of its battered rim,

She took it up and gave it him.

(Lewis Carroll).

THE METHOD.

Then, with an aspect cold and grim,  
Regardless of its battered rim,  
She took it up and gave it him.

(Lewis Carroll).



Left kidney of rat bearing a adreno-cortical  
homo-graft. The transplant nodule is the size  
of two normal adrenal glands and entirely  
made up of cortical tissue. The animal was  
killed one year after transplantation.

### The Method.

Based on the findings of the investigators whose work has been reviewed in the preceding chapters, and on the failures and successes of well over 250 transplantation experiments, the following basic technique is recommended for adreno-cortical homo-transplantation in the albino rat.

The choice of donor and recipient: All experiments in this investigation were performed on rats of the Wistar Glaxo strain. This strain has been propagated by brother-sister mating for forty years, has a high mortality after bilateral adrenalectomy, and is very placid. Thus, it is very suitable for transplantation experiments. It must not be imagined, however, that even such a strain is genetically homogenous.

Inherited variations constantly crop up; indeed, it must be taken as axiomatic that mere inbreeding can never produce a stably uniform race of animals. We cannot, as yet, inhibit the 'spontaneous' variability of protoplasm. If Glaxo rats, being second cousins, are used for purposes of homografting, about two thirds of the grafts will be permanently successful. However, with more distant relations, the success rate rapidly dwindles down to very low levels. This rate/

rate varies from time to time in the same colony and between different colonies. The most important variable (concerning the results of random experiments) is probably the size of the colony, since, in order to obtain sufficient numbers of experimental animals, sub-strains must be used. The rate of success will therefore decrease rapidly with an increase in the size of the colony.

For these reasons it is important, even within a 'pure' strain, to choose host and donor from closely related animals. Litter mates and individuals from successive litters of the same parents yield, in the Glaxo strain, an almost 100% success rate and should therefore be used whenever possible. When it is wished to study age changes the best results are obtained by using early and late litters of the same parents. If it becomes necessary to use animals which are less closely related, a certain number of transplant failures must be allowed for in the planning of the experiment.

The age of the recipients: Rats under thirty days of age and under 40 grammes of weight rarely survive bilateral adrenalectomy for long enough to allow growth of the transplant. To approach 100% post-operative/

operative survival one has to use animals fifty days or more old and weighing 50 grammes or over. After the age of one year, post-operative mortality rises again. This is due mainly to the chronic respiratory infection which most laboratory rats have acquired by then. There is also a higher incidence of wound infection in older animals. In addition, old rats survive much longer without adrenal tissue which complicates the assessment of results. A further difficulty of analysis is introduced by the fact that old rats, used as normal controls, often react unfavorably to a period on saline.

Unless the aim of the experiment forbids it, the recipients should therefore be between two months and one year old and weigh more than 50 grammes. By preference, one operates at the lower end of this range as the transparency of the body wall, the relative lack of peri-renal fat and the accelerated rate of healing render the procedure much easier in the young rat.

The sex of the recipients: This is not critical. Whilst females of all ages withstand adrenalectomy much better than males, by the same token, it may be concluded that they are not ideal subjects for the evaluation/

evaluation of transplant function. The nature of the experiment will usually determine the sex of the recipient and it is perhaps unnecessary to add that, in view of the sex differences in adrenal function, an experimental group must be made up of animals of one age and sex receiving grafts of one age and sex.

Moreover, the effect of gonadal function upon the adrenal cortex is so marked that many problems of transplant function can only be studied satisfactorily in gonadectomised animals.

Choice of controls: Only litter mates are satisfactory as controls in experiments involving quantitative judgments. If at all possible all animals of an experiment should be housed in the same cage. Experiments involving simple qualitative judgments (such as survival) may be controlled with non litter mates of the same age and sex.

Pre-operative measures: none.

Anaesthesia: After adrenalectomy animals are in a state of shock and of greatly increased sensitivity to drugs. They should therefore receive the lightest possible anaesthesia if occasional deaths are to be avoided. When it is desired (as it usually is) to return all animals taking part in the same experiment to the same cage/

cage immediately after the operation, it is important to ensure quick return of consciousness. Unconscious animals with operation wounds are liable to be eaten up by the others. For these reasons a light inhalation anaesthesia is the most suitable and I usually employ ether.

Asepsis and antisepsis: Whilst ordinary cleanliness in performing the operation is not contra-indicated, strict aseptic precautions are unnecessary in dealing with healthy rats. Septic complications are practically unknown. Antisepsis, in the form of preparing the skin or treating the wound with germicidal or antibiotic substances, may be actually harmful to the survival of the transplant. In old animals wound infection becomes fairly frequent and interferes on occasion with the success of the grafting. Aseptic precautions are therefore justified in rats over eighteen months of age.

Adrenalectomy: This is the necessary preliminary to successful transplantation and should immediately precede it. Nothing is gained by two stage operations; in fact they probably interfere with the growth of the transplant by depriving it during the interval between the two stages of the full benefit of adrenocorticotrophic action.

The adrenals are removed by the lumbar route after a dorsal midline skin incision (Hair need not be clipped; chemical epilation is harmful). The animal is turned on its side, the skin is retracted and dissected off a little way and the body wall is incised just below and in line with the last rib. The adrenal pedicle is grasped with forceps and the gland brought out of the wound. Another forceps is placed below the first one to avoid undue pull on surrounding structures and the gland is pulled off. Grasping the gland itself should be avoided as much as possible and it should certainly never be squeezed or ruptured. If this happens, there is considerable danger of spilling cortical cells which can proliferate and spoil the experiment. No haemostatic measures are necessary and the incision should be small enough not to require suturing. Occasionally an incisional hernia is seen but it very rarely interferes with the animals well being.

Preparation of the transplant: Adreno-cortical tissue can be maltreated and stored in a number of ways without losing its regenerative power. Unless the design of the experiment demands it, it is however best to remove the donor gland shortly before it is needed.

In the interval which may elapse between its removal and its implantation, the gland is kept in normal saline. The gland may be implanted whole but since the medulla and the inner layers of the cortex invariably undergo necrosis, it is better only to implant the adrenal capsule. For this purpose the gland is freed from the surrounding fat and connective tissue, and a small cut is practised in its surface. It is then squeezed between two clean slides. The contents are extruded leaving the capsule to be used for implantation. When necessary it may be divided and used for multiple implants.

Precautions to ensure identity of the graft: This is a very critical point and deserves every attention.

Because of the great regenerative powers of cortical tissue it is important that no adrenal cells other than those of the intended donor are introduced into the host. To achieve this with certainty it is necessary (a) not to damage the host's adrenals during removal, (b) to use a different set of instruments for the adrenalectomy and for the transplantation, (c) to keep only glands of the same kind in one saline vessel and (d) to boil forceps before using them on another set of transplantations.

Site/

Site of implantation: In an adrenalectomised rat a compatible adrenal graft will grow and function almost anywhere, at least for a time. Sometimes the purpose of the investigation will dictate the site, e.g., the anterior chamber of the eye for direct observation, the gonads to study local interactions of steroid producing tissues etc. When such special reasons do not obtain, the following principles govern the choice of implantation site. (1) There should be no doubt about the identity of the transplant; therefore, it must not be placed in the adrenal bed or in any location likely to harbour accessory cortical nodules, although transplants grow well in these situations. (2) The transplant must be capable of easy and entire removal without damage to the animal significantly in excess of that due to removal of the transplant. (3) If information on normal cortical function is sought, the site of implantation must be a physiological one with regard to arterial supply and venous drainage. Ignorant as we still are about the possible influences of local factors, it is therefore not safe to remove the site of implantation too far from the adrenal area.

These/

These considerations together with the favorable experiences of many previous workers have led me to adopt the kidney as the routine site of implantation. On the right side adhesions with the liver usually form which are physiologically undesirable and also lead to difficulties in transplant removal. The lower half of the left kidney is therefore the site of election.

Technique of implantation: The adrenalectomy incision is prolonged downwards and medially to allow dislocation of the kidney into the wound. The renal capsule is grasped with fine forceps and a hole is made in it. Through it the transplant is inserted between the kidney and its capsule. It is dangerous to become too adept at this delicate manoeuvre because a minimal amount of trauma is essential for transplant fixation. A little blood oozing from the kidney surface is, therefore, quite desirable. No sutures are used to keep the transplant in place and the kidney is returned to the abdominal cavity. The lumbar incision should be sufficiently valvular and small not to require sutures either. There is however no absolute contra-indication to one or two points of catgut and these will not often cause a deleterious reaction. The skin incision is closed with Michel clips.

Number of implants: Each additional transplant adds to the number of variables and makes analysis of results more difficult. Moreover, the chances of immunity against the graft being set up probably increase with the number of transplants. There is, of course, a limit to the size to which a free transplant can grow since it relies for its nutrition and function on a secondarily acquired diffuse blood supply. For this reason, it may be necessary in bigger animals to implant multiple grafts in order to ensure the development of sufficient cortical tissue to maintain life. In the rat, however, the nodule obtained from one successful implant is sufficient to provide cortical function at near normal levels.

Post-operative care: The animals must be protected from excessive fluctuations of temperature for a few days after the operation, the colony room being kept at about 72° F. Any stock diet which keeps normal rats in good health and at a normal rate of growth is also suitable for adrenalectomised rats, provided that drinking water is replaced by 0.9% saline. Ten days after operation the transplant has grown sufficiently to preserve a normal electrolyte balance and the animals are returned to tap water. To keep them longer on/

on saline jeopardises the chances of transplant survival. If only a fragment of a capsule has been implanted the period on saline may have to be prolonged to a fortnight to allow for sufficient growth.

Weight control will indicate whether the change-over to water has been done too early. From the second post-operative day the animals should be gaining weight and this increase should continue without a break after restoration of water. The important fact to remember is that it is equally deleterious to the success of the experiment to remove the saline too soon as to continue it too long. Cortical hormones have no place in the post-operative management and would interfere with transplant development. The Michel clips are removed one week after the operation.

Verification of transplant function: At the conclusion of the experiment the transplant bearing kidney is removed, the controls being at the same time subjected to unilateral nephrectomy. This is well borne by healthy adult rats. If the transplant was the only substantial functional cortical tissue, its removal will be followed by death in from 4 to 25 days depending on the age and sex of the animal. Deaths occurring within the first 48 hours after operation cannot be accepted as proof of transplant function and/

and are usually due to nonspecific effects of the operation. In such cases and where transplant removal before death is impracticable, anatomical and histological evidence must be relied upon. When a histologically normal transplant nodule of the size of at least one normal adrenal gland (normal for the sex and age of the animal) coexists with the absence, after thorough search, of macroscopic nodules of cortical tissue in other locations, it is probably safe to assume that adreno-cortical function was mainly carried out by the transplant. The presence of microscopic accessory cortical nodules is irrelevant in this connection.

Summary: The following standard technique ensures uniformity of results and a high rate of success in adreno-cortical homografting in the rat.

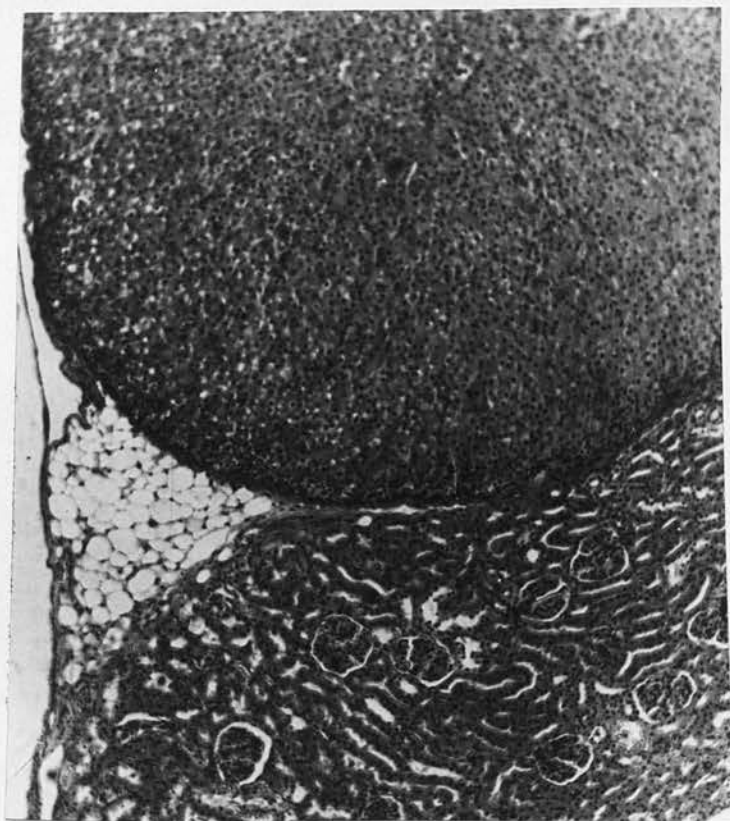
Litter mates from an inbred strain are used as donor and recipient. The animals should be two months to one year old and the experiments should be controlled with littermates of the same weight and sex. Under light ether anaesthesia the donor gland is removed, enucleated and the capsule is put into normal saline. The instruments are laid aside to be used again for the implantation. Under light ether anaesthesia, bilateral/

bilateral adrenalectomy is now performed on the recipient using the lumbar route and avoiding the use of antiseptics. Special care is taken not to squeeze or rupture the glands during removal. The instruments used are laid aside and must not be used for the im: :plantation. The left kidney is now exteriorised through the enlarged adrenalectomy incision, its cap: :sule is cut and the adrenal capsule of the donor is inserted between the kidney and the renal capsule. The renal surface should be slightly traumatised, Kidney and transplant are returned to the abdomen and the skin incision is closed with Michel clips. No internal sutures are employed. The animal, which should be conscious before being returned to the cage, is kept on the ordinary stock diet, the only special measure being replacement of water by normal saline for ten days. At the conclusion of the experiment the transplant bearing kidney is removed. If the transplant has maintained the life of the animal, this removal will be followed by death in from 4 to 25 days.

REPRODUCTIVE FUNCTION OF ADRENALECTOMISED FEMALE RATS  
WITH ADRENO-CORTICAL HOMO-TRANSPLANTS.

Judith has cast her old-skin and got new;  
And walks fresh varnisht to the publick view.

(Herrick).



Adreno-cortical homo-transplant.

Junction of kidney and graft.

( x 65 )

Reproductive function of adrenalectomised female rats with adreno-cortical homo-transplants.

This chapter illustrates the use of homotransplantation for the investigation of primary sex differences in adreno-cortical function. The questions asked were (1) How is the reproductive performance of a female albino rat modified after adrenalectomy followed by successful adreno-cortical homotransplantation? (2) Are these modifications significantly influenced by the sex of the donor? The second question is, of course, a part of the wider question, whether there exist any primary sex differences in adreno-cortical function (reactivity).

#### EXPERIMENT:

Three female littermates, aged 26 days and weighing 41, 42 and 45 grammes were adrenalectomised and the capsule of one adrenal gland of a littermate was inserted under the capsule of one kidney. The donor in one case was a female and in the other cases a male. Drinking water was replaced by 0.9% saline for 14 days. At the age of 85 and again at the age of 163 days all three were mated with the same male. The cyclical activity of the animals was investigated by daily vaginal smears by an independent observer, who was kept in ignorance of the sex of the transplant. At the age of 313 days the animals

were deprived of the transplant bearing kidney. In each case there was a nodule of cortical tissue of the size of at least one normal adult adrenal gland. The operation was well tolerated, and the animals continued quite well for some days. The first death occurred 15 days after operation in the animal with the female transplant (No.3). Next day another death took place (No.1.). The remaining animal continued well, and was killed 50 days after operation, when a small adreno-cortical nodule was found growing subcutaneously at the site of the nephrectomy (No.2.). The results of this experiment are tabulated in Table 1.

#### EXPERIMENT:

Eight female littermates, aged 56 days, were adrenalectomised and the capsule of one adrenal gland of a littermate was inserted under the capsule of one kidney, the donor in three cases being a brother, and in five cases a sister. The animals were kept on 0.9% saline for 12 days after operation, and were mated at the age of 77 days in two groups. One rat (No.6) was found dead 22 days after mating with a full term litter of 12 in utero. (This is seen every now and then in any colony, and is probably due to obstructed labour). Adrenalectomy was complete and the left kidney bore a healthy transplant nodule the size of one normal adult adrenal. At the age of

Number	Age at Operation	Sex of Transplant	Days after mating before littering	No. in litter	Sex ratio	Vag. Smear	Result of Transplant removal	P.M. Findings	Comments
1.	26d.	M.	1st mating 22	11	7:4	Irregular, never came into full oestrus.	Died.	Nil of note.	
2.	26d.	M.	2nd mating 26	10	3:7				
			1st mating 22	10	3:7	Irregular, only one oestrus seen.	Survived	Adrenal nodule in operation wound.	
			2nd mating 25	13	5:8				
3.	26d.	F.	1st mating 30	13	3:10	Normal cycles	Died.	Nil of note.	
			2nd mating 31	14	8:6				
1.	56d.	M.	1st mating 25	6	2:4	Suggestive of cycles.	Died.	Nil of note.	
			2nd mating 26	11	7:4	No full oestrus smear.			
2.	56d.	M.	1st mating 29	10	5:5	Only one oestrus seen followed by metoestrus and dioestrus alternately	Died.	Nil of note.	First litter partly by Caesarian.
			2nd mating 31	7	3:4	Normal cycles.	Died.	Nil of note.	
3.	56d.	F.	1st mating 30	13	8:5		Died.	Nil of note.	
			2nd mating 39	14	6:8				
4.	56d.	F.	1st mating 26	9	4:5	Normal cycles.	Post op. death.	Nil of note.	
			2nd mating 33	13	8:5				
5.	56d.	M.	1st mating -	-	-	Not done.	Killed	Nil of note.	1st litter absorbed.
			2nd mating 24	11	8:3				
6.	56d.	F.	1st mating -	12	3:9	Not done.	-	Transplant nodule in kidney.	Died in first labour.
			2nd mating -	-	-				
7.	56d.	F.	1st mating 26	8	2:6	Normal cycles.	Died.	Nil of note.	
			2nd mating 26	13	7:6				
8.	56d.	F.	1st mating 33	9	8:1	Normal cycles, but longer oestrus than normal.	Died.	Nil of note.	
			2nd mating 33	9	3:6				

Table 1.

137 days the seven survivors were again mated, this time in one group. The evaluation of cyclical activity by daily vaginal smears was again left to an unbiased observer who was unaware of the sex of the transplants. One animal (with a male transplant) was killed at the age of 182 days (No.5). Adrenalectomy was complete and the right kidney bore a healthy adreno-cortical nodule of approx. 1½ times the size of a normal adrenal. The six survivors continued to the age of 287 days, remaining perfectly well. At this date they were subjected to removal of the transplant bearing kidney. In each case a healthy adreno-cortical nodule, of at least the size of one normal adult adrenal gland, was found to be growing in the kidney. One animal died shortly after the operation from haemorrhage, the ligature round the renal pedicle having slipped (No.4). Adrenalectomy was complete, and there were no accessory nodules to be seen. The remaining five animals, having made a good recovery from the operation died from the 7th to the 15th post-operative day. The results of this experiment are tabulated in Table 1. The animals used in both experiments were Wistar albino rats of the Glaxo strain.

#### RESULTS:

From Table 1 it can be seen that mating was followed in all but one case by prompt conception and

normal parturition. The litters were brought up normally, and reached weights within normal limits for the colony. Litter size, interval between mating and conception, and sex ratios showed insignificant differences between the animals bearing male, and those bearing female transplants, nor did the time which had elapsed since adrenalectomy seem to influence these modalities. The results of daily vaginal smears showed normal cyclical activity in all animals with female transplants, and in none of the animals with male transplants. Even on the mathematically most unfavourable assumption, namely of there being a 50% chance of the normal cycle being abolished either spontaneously or as the non-specific result of the operation, the chance of this result coming about is only 1 in 500. With such a small sample any error on the part of the observer will markedly effect the significance of the results, but since the question at issue was simply whether there were or were not normal cycles, such an error is very unlikely. The abnormality characterising the male transplant group, was the absence or extreme infrequency of oestrus smears.

#### DISCUSSION:

These results are only of interest if the animals were dependent entirely or almost entirely on the transplants for their adreno-cortical function.

The role of accessory cortical tissue in this connection has been discussed above, (p.70).

It is however advisable to prove the function of the transplant in each case by a survival test after removal. In seven of the eleven animals this gave a clear indication that they had been living on their transplants. Of the remaining four, one (No.6) died during labour. Adrenalectomy was complete, no accessory tissue was seen, and the kidney bore a big healthy cortical transplant nodule. Two others (Nos 4&5) were killed at the time of transplant removal and had the same post-mortem findings. One animal (No. 2 of the first experiment) survived removal of the transplant by virtue of a cortical nodule which grew in the nephrectomy wound; clumsy removal of the transplant must have been responsible for dissemination of cortical cells in the field of operation. Again, adrenalectomy was complete and there were no macroscopic accumulations of accessory tissue. It is reasonable to assume that these four animals also lived by their transplants.

Pencharz and Olmsted (1931) have demonstrated that adrenalectomised rats, living on an adreno-cortical auto-transplant, can go through normal pregnancies. It is not surprising to find that the same applies to homo-transplants as there is no reason to assume that

a successful homo-transplant is functionally inferior to a successful auto-transplant.

Histological examination of the transplants in the present investigation showed complete absence of medullary tissue in each case. This is the rule, even with whole gland grafts, although occasional re-growth of the medulla has been reported (Cristiani & Cristiani 1902, Turner et al., 1939). Apart from other considerations, re-growth of the medulla in the present series was extremely unlikely as only the adrenal capsule was used for transplantation. It may therefore be concluded that the adrenal medulla is not essential for successful reproductive function in the female albino rat. There is however a constant hypertrophy of accessory chromaffin tissue following adrenalectomy in the rat, the possible functions of which, remain to be determined.

Rats living on one adreno-cortical transplant nodule, although normal in many respects, are in a state of mild chronic hypo-adrenalism (Jaffe 1926, Wyman 1928 & 1929, Wyman & Tum Suden 1930, 1937 & 1942, Komrad & Wyman 1950). It can therefore further be stated that full adreno-cortical activity and reserves do not seem necessary for the successful accomplishment of reproductive function in the female albino rat.

Homo-transplantation was used to demonstrate the

primary sex differences of gonadal tissue by Steinach (1912). Similar investigation of adreno-cortical tissue have not so far been published. The adrenal cortex in the normal animal shows many sex differences. Most of these are clearly dependent on and induced by gonadal activity, but it is reasonable to inquire whether some of them represent a primary sex difference in the reactivity of adreno-cortical tissue, *i.e.* whether they can persist after the gland has been placed into the organism of an animal of the opposite sex.

The answer, in so far as ability to conceive, to give birth, and to rear a litter are concerned, seems to be that there is no important sex difference. The last of these is of interest in view of the evidence linking lactation in the rat with adreno-cortical activity (Brownell et al. 1933, Spoor et al 1941, Nelson et al. 1943). There were slight differences between the two groups with regard to litter size, interval between mating and conception and sex ratio of litters. Only an analysis of a larger series can show whether these differences are constant and this question is now being pursued with regard to sex ratios.

On the other hand, this investigation indicates a primary sex difference in the ability of adreno-cortical transplants to maintain normal cyclical

vaginal changes. The influence of adrenal secretions upon the sexual cycle of the female rat is well established. Adrenalectomy usually results in cessation of the cycle and administration of cortical hormones may equally interfere with it (Corey & Britton, 1934). Zuckerman and Bourne (1940/41) have shown that a cyclical change takes place in the adrenal gland, corresponding to the oestrus cycle, which exercises a direct effect on the accessory reproductive organs. They demonstrated that this adrenal cycle is independent of ovary and pituitary. It would be interesting to know whether a male adrenal gland undergoes these cyclical changes when placed in a female organism. The results here presented, furnish two lines of indirect evidence affecting this question. The maintenance of normal reproductive performance in females with male transplants suggests either that such a transplant exhibits this cyclical change (which would then have to be considered as secondarily induced) or that such a cyclical adrenal change is not essential for the maintenance of normal reproductive function. The absence of normal cyclical vaginal changes in animals with male transplants supports the second of these suppositions. The occasional occurrence of oestrus smears suggests that the sex difference is quantitative rather than qualitative.

When considering the question of cyclical adrenal

changes it must be remembered that ovarian activity has marked effects on the adrenal cortex and that therefore, in the intact animal, the primary adrenal cycle must be overlaid and supplemented by an important secondarily induced one.

#### SUMMARY:

Eleven female albino rats were adrenalectomised. Six were given adreno-cortical transplants from female and five from male littermates. They were mated with normal males. There was no abnormal delay in conception, and both groups produced normal litters and reared them normally. The animals with female transplants showed normal cyclical vaginal changes, those with male transplants did not.

These results illustrate the usefulness of adrenal homo-transplantation as a tool of endocrine research and support the concept that there may be a small but demonstrable primary sex difference in the reactivity of the adrenal cortex of the albino rat.

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HOMO-TRANSPLANTATION AS A METHOD OF GENETOLOGICAL

RESEARCH.

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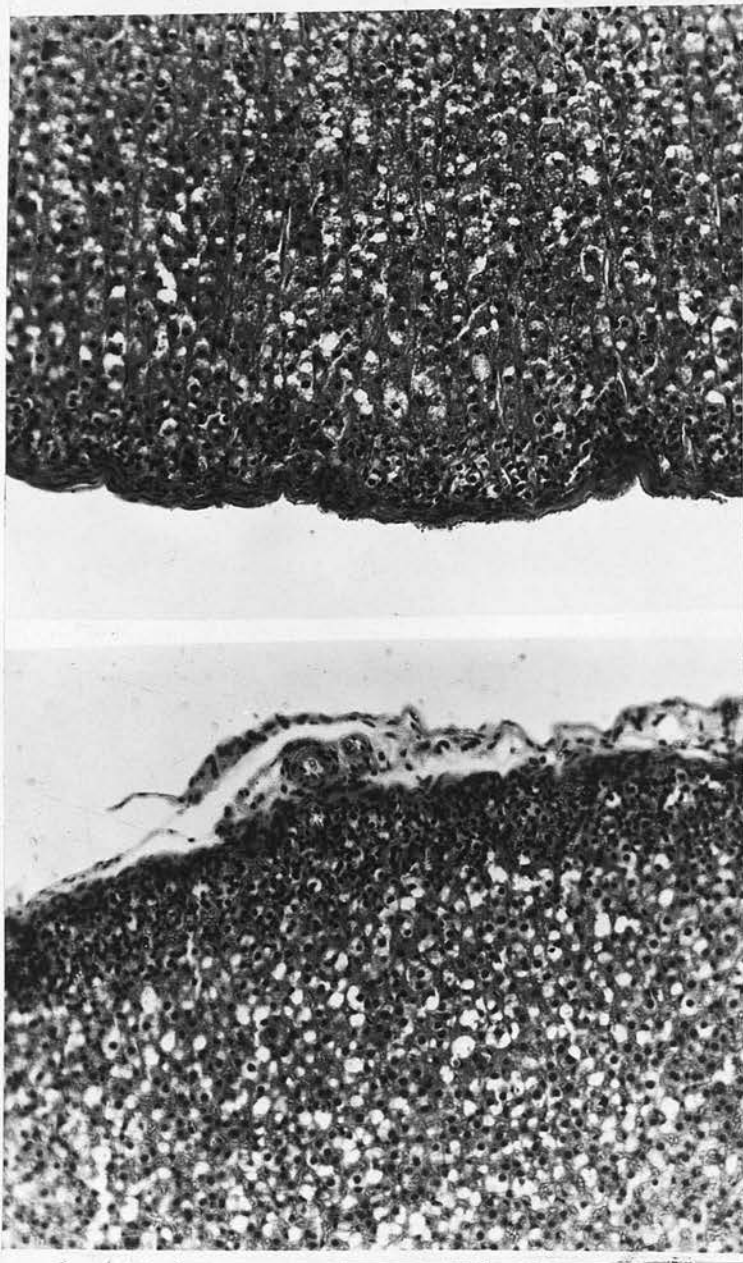
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HOMO-TRANSPLANTATION AS A METHOD OF GERONTOLOGICAL  
RESEARCH.

\* My First is ageing day by day:  
My Second's age is ended:  
My Third enjoys an æge, they say,  
That never seems to fade away,  
Through centuries extended.

(Lewis Carroll).

\* see p. 100.



Juxtapposition of normal rat adrenal cortex(above)and adreno-cortical homo-graft(below)to show identity of histological features.( x 200 ).

Labore p. 83.

Homotransplantation as a method of Gerontological research.

The experimental study of mammalian ageing cannot, in the nature of things, be a matter of short term investigation. Comparison of various age groups and attempts to study the influence of experimental interference upon the ageing process imply the necessity for following at least two generations of animals through their full life span, not allowing for the time spent in becoming familiar with the problems and techniques. This explains why two of the four experiments here reported are still in an interim stage. Indeed, there are indications that to wait for the conclusion of the last of these four experiments, might turn out to be the job of more than a life time. Experimental mammalian Gerontology is not yet in its infancy and the following experiments, crude and inadequate as they are, represent probably the first deliberate attempt to come to grips with the intrinsic mechanisms of ageing, if indeed any scientific meaning attaches to this term.

AGE OF TRANSPLANT AND GROWTH RATE.

Question: Is the difference in growth rate between immature and mature rats contributed to by a primary age/

age difference in the reactivity of adreno-cortical tissue?

Experiment.

15 males and 13 females from four litters, obtained by mating one male with four female siblings, and all born within 5 days of one another, were divided into four groups at the age of 8 months and treated in the following fashion:

Four animals of each sex served as normal controls.

Three animals of each sex were subjected to bilateral adrenalectomy. Four males and two females were subjected to bilateral adrenalectomy, followed by the insertion of the adrenal capsule of a sibling of the same sex under the capsule of one kidney.

Four animals of each sex were subjected to bilateral adrenalectomy followed by the insertion of the adrenal capsule of a newborn rat of the same sex under the capsule of one kidney. The donors were related to the hosts in being the off-spring of mating the grandmother with one brother.

For 20 days after the operation the animals were given 0.9% saline to drink. The weight of the animals was determined at frequent intervals. Six and a half months after the operation the experiment was terminated/

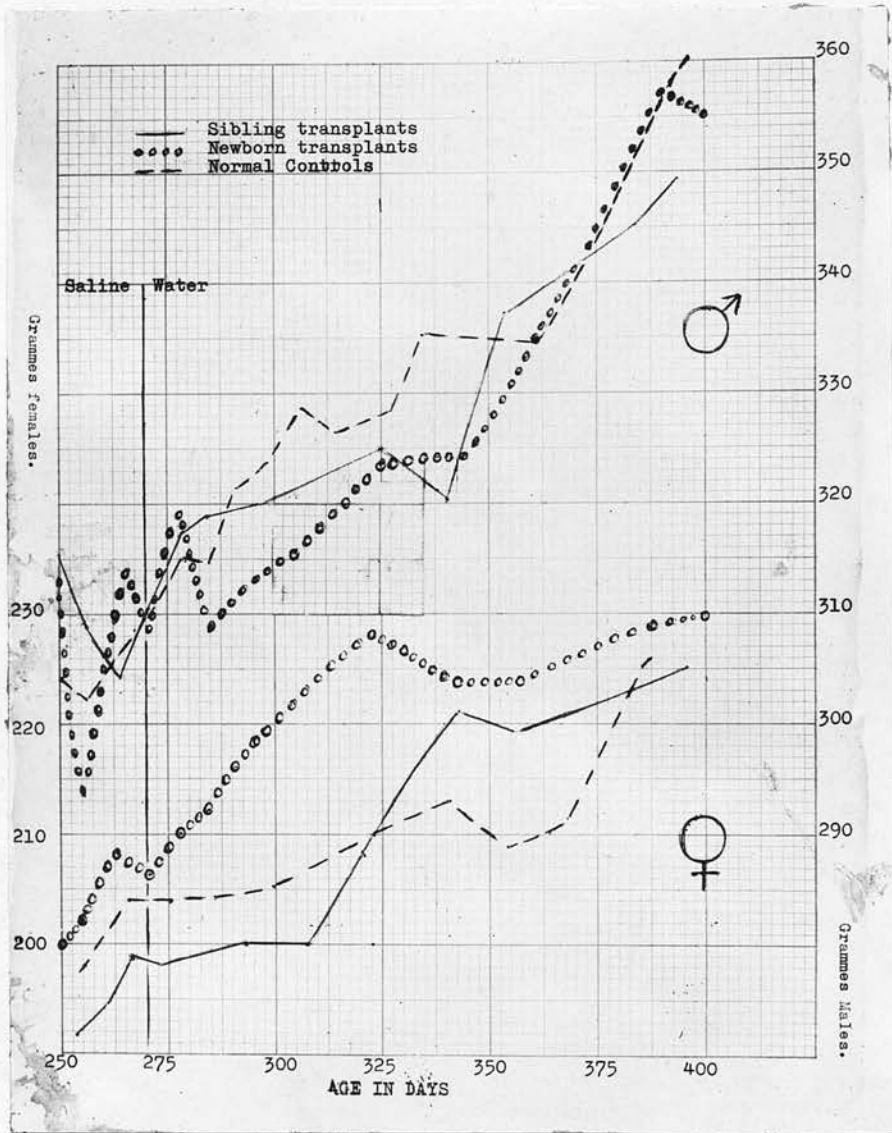


Chart 1.

ated, by killing the survivors.

### RESULTS:

The adrenalectomised animals in whom no transplan: tation had been carried out, all died shortly after the substitution of water for saline. There was a marked sex difference in the survival time, the males dying 3, 4 and 8 days, and the females 7, 14 and 15 days after return to water. In both sexes the loss of bodyweight preceding death was cca. 20%, but in the case of the males, this decline started whilst they were still on saline.

The average weights throughout the experiments of the three other groups are given in Chart I. It can be seen that there was no significant difference in either sex in the all-over growth rate between the animals living on transplants and the normal controls. The females bearing newborn transplants, however, showed an accelerated growth for the first ten weeks of the experiment gaining, during this period, as much as the males. Thereafter, their growth rate reverted to that of the other females.

### Comment:

The growth rate of the normal adult rat shows a marked sex difference. Up to the age of ten weeks the two/

AVERAGE GROWTH CURVE IN A COLONY OF  
WISTAR ALBINO RATS (GLAXO STRAIN).  
(BY WEIGHT)

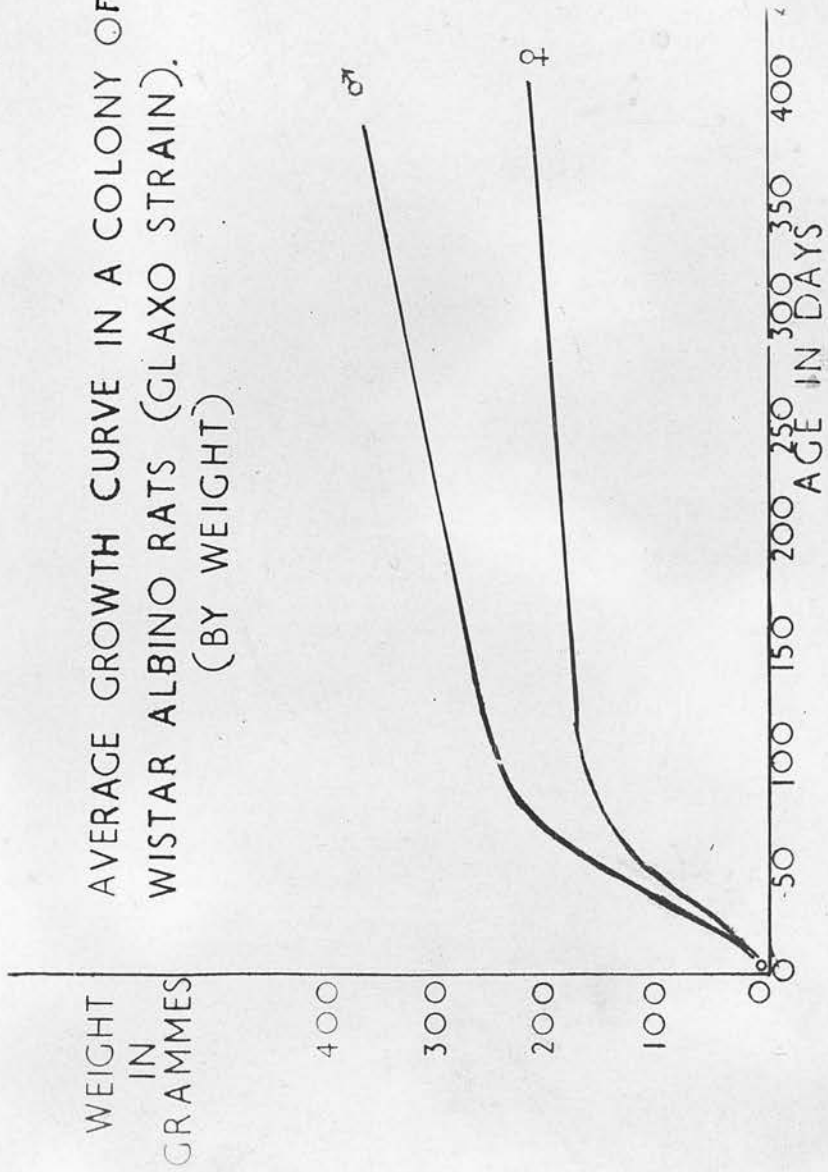


Chart 2.

two sexes grow at approximately the same rate. Growth then slows down rather abruptly in both sexes but more so in the female (Chart II). This sex difference is partly due to gonadal activity since it can be diminished but not abolished by gonadectomy (1, Chart III). The result of the above experiment suggests that the depression of growth in the female may also be contributed to by the activity of the adult adrenal cortex. This adreno-cortical effect could be autonomous or, more likely, the result of adreno-ovarian interaction. In the later case the ten weeks of increased growth represent a period during which the maturing adrenal gland is refractory to ovarian influence or during which it is incapable of supporting normal ovarian function (2,3). The sex difference in the behaviour of the two groups with newborn transplants is either a true primary one or depends on the different biochemical relations of testis and adrenal as compared with ovary and adrenal.

That the adrenal cortex plays a part in the regulation of growth and that the prepubertal gland functions differently from the adult organ is indicated by many observations and it is not proposed to discuss these questions here. Our purpose is merely to/

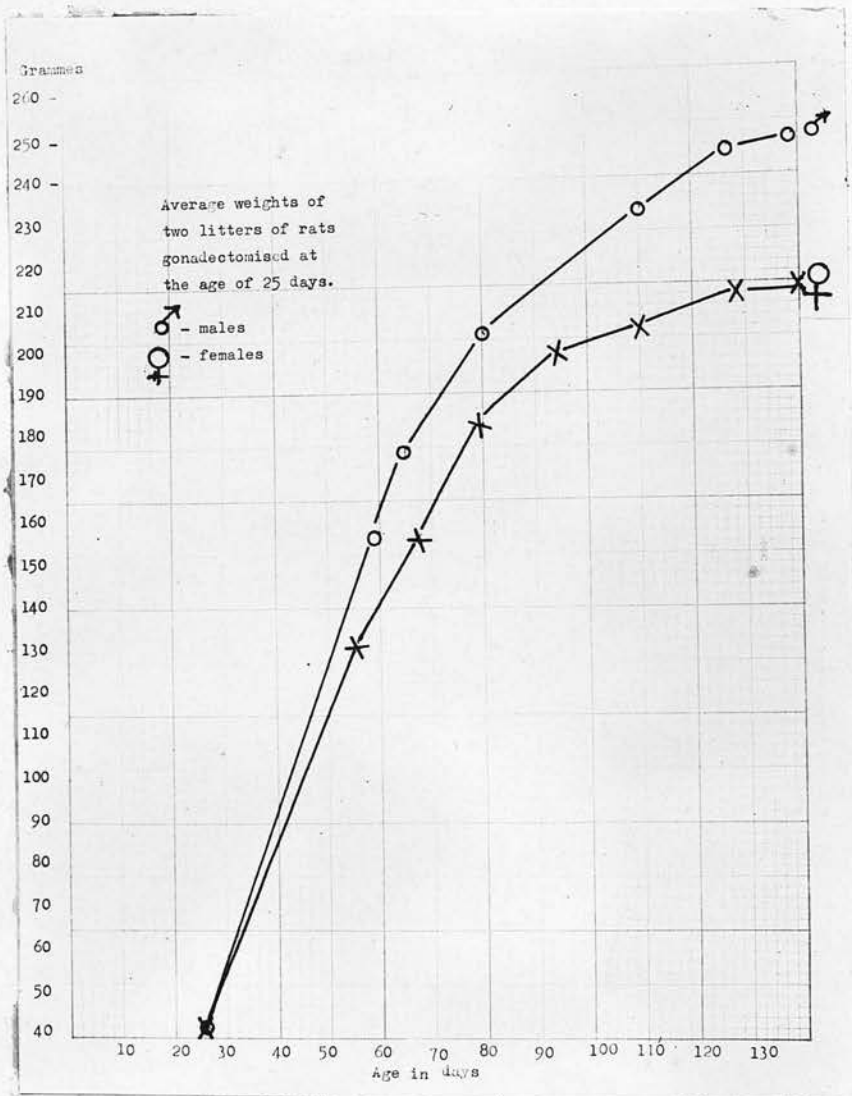


Chart 3.

to demonstrate how suitably arranged experiments involving homo-transplantation can help to elucidate the exact nature of age changes in adrenal function. Until there has been achieved a considerable refinement of assay methods of adrenal hormones coupled with a perfect understanding of the biological significance of each fraction, no other method can be employed to attack this question. A repetition of the above experiment, using gonadectomised animals could decide whether the change of adrenal function at ten weeks is dependent upon gonadal activity or not.

#### SUMMARY:

Adult male and female adrenalectomised albino rats with coeval adreno-cortical transplants and adult male adrenalectomised albino rats with newborn adreno-cortical transplants grew at the same rate as normal controls. Adult female adrenalectomised albino rats with newborn adreno-cortical transplants grew for ten weeks at an accelerated rate. This indicates a primary age difference of adreno-cortical reactivity, at any rate, in the female.

#### ADRENO-CORTICAL TRANSPLANTS AND LIFE SPAN:

Question: Has the age of the transplant any influence on the length of survival of adrenalectomised adult rats?/

rats? How does the length of survival of transplant bearing rats compare with that of normal animals?

#### Experiment.

Eleven females, first cousins, and born within eleven days of one another were divided into three groups at the age of 19 months and treated as follows: Three animals were not interfered with and served as normal controls. Four animals were adrenalectomised and the adrenal capsule of a sibling of the same sex was inserted under the left renal capsule.

The remaining four animals were adrenalectomised and the adrenal capsule of a four week old female from the same strain was inserted under the left renal capsule. All animals were put on saline for seven days. The animals were kept in the same cage and their weights were determined at frequent intervals.

#### RESULTS:

Within seven months of the experiment all the normal controls were dead having reached the age of one year eleven months, two years, and two years two months respectively.

In the group with the sibling transplants no deaths occurred and the animals at the time of writing are two years five months old. They have on the average/

average gained 6 grammes per month since the operation, which is considerably in excess of the normal female growth rate at this age.

In the group with the young adrenal transplants one death occurred four months after the operation, that is at the age of 1 year 11 months. At post-mortem the transplant was found to be in process of absorption. The remaining three animals are still alive at the age of 2 years 5 months and have on an average gained 5 grammes per month since the operation which is considerably in excess of the normal female growth rate at that age.

COMMENT:

The evaluation of this experiment turns to some extent on the question of the normal life span of the laboratory rat and on the actual causes of death. Under the conditions obtaining in this colony, the majority of rats die between the ages of 2 and 3 years from a chronic broncho-pneumonic process which usually starts at the age of about one year. The average life span of the Wistar albino rat under laboratory conditions is said to be about 2 years (4,5) but until studies are published of a colony in which chronic lung infection has been prevented, the term life span must/

must be understood in a restricted sense meaning the age at which the laboratory rat usually dies of broncho-pneumonia. Any procedure which prolongs survival under these conditions must be interpreted as retarding the progress of the broncho-pneumonic changes rather than as having a fundamental influence upon natural life span.

None of the animals in the above experiment have so far lived beyond the extreme ages reached in this colony. The survival of the transplant bearing animals as opposed to the death of the control group shows, at any rate, that adrenalectomy followed by homo-transplantation need not shorten the life of the laboratory rat. A comparison of the two transplant groups has not so far revealed any difference in the behaviour of the animals with coeval transplants and those with transplants  $1\frac{1}{2}$  years younger than themselves. The one death in the latter group was due to late absorption of the transplant. It is to be understood that compatibility of transplants was more difficult to ensure in this group than in the controls with sibling transplants. When it is remembered that, regardless of its age, each transplant has to regenerate completely from a few surviving sub-capsular cells, /

cells, the lack of difference between young and old transplants becomes less surprising.

The striking increase in the growth rate in both transplant bearing groups is therefore not a function of the age of the transplant but must be due to a factor common to both groups. It has been shown that animals living on adreno-cortical homo-transplants are in a state of mild chronic hypoadrenalism (6,7,8). It is also known that adreno-cortical secretions interfere with somatic growth (9,10). These facts taken together might supply a sufficient explanation for the acceleration of growth. Such an increase of growth potential might go hand in hand with an enhanced ability for repair and resistance especially in view of what has become known about the interference with reactions to disease by adreno-cortical secretions.

It is therefore possible that normal adreno-cortical function may, under certain circumstances, be inimical to the survival of the senile rat; in other words, that part of the ageing syndrome in the albino rat may be a relative hyper-adrenalism. There are clinical and physiological grounds for thinking that the picture of human senescence (especially in the/

the female) may sometimes be partly conditioned by an absolute or relative hyper-function of the adrenal cortex. A full discussion of this intriguing problem would merit a paper to itself. Our intention is merely to show how the technique of homo-transplantation can be used to acquire more precise notions on these fundamental points.

It may be mentioned that the advantage conferred upon the transplant bearing animals may consist partly in the denervation and demedullation of the adrenal gland which is a concomitant of adrenal grafting. Suppression of the fright and flight reaction might be a disadvantage to a wild living animal but may have an actual survival value to a laboratory animal, especially to one in a poor state of health. Whether this could account for the increase in growth rate is however doubtful. It would be easy to devise experiments to answer these questions.

Summary: In a group of senile rats the normal controls died first. The experimental animals, which were adrenalectomised and had received adreno-cortical homo-grafts, survived and have now exceeded the average life span for the colony. They have also shown accelerated growth since the operation. No difference has/

has so far been noticed between animals with coeval and those with transplants  $1\frac{1}{2}$  years younger than themselves. It is concluded that adreno-cortical homo-transplantation need not shorten the life of an albino rat and that depression of adreno-cortical function in a senile animal may, under certain circumstances, prolong its survival.

This experiment takes us also one step further in the interpretation of the experiment on age of transplant and growth rate. In that experiment acceleration of growth occurred only in animals with immature adrenal transplants, while it occurred in both animals with mature and with immature transplants in the second experiment. Moreover in the second experiment this acceleration was maintained indefinitely, whilst in the first it ceased after 10 weeks.

The animals in the first experiment were mature females, in the second experiment they were postclimacteric senile females.

It appears, therefore, that an increase of adult female growth rate occurs under the following circumstances:

(a)/

(a) After cessation of ovarian activity with either mature or immature adreno-cortical transplants, but not with normal adrenals.

(b) During sexual maturity but only with immature adreno-cortical transplants. In addition, there is rapid female growth before puberty.

The most economical hypothesis to cover these findings is that the depression of the adult female growth rate is a function largely of ovarian and partly of adrenal activity in the mature female and entirely of adrenal activity in the senile female. Gonado: tropic effects on the senile adrenal cortex provide the most likely mechanism for this functional trans: formation. If this is correct it would imply that an immature adreno-cortical transplant is not capable of maintaining normal ovarian function. This hypo: thesis can be tested in all its parts by experiment.



— Senile Alopecia

CORTICAL TRANSPLANTS AND SENILE ALOPECIA.

Question: What is the ability of very old rats to withstand the operations of adrenalectomy and transplantation?

This experiment yielded, as a byproduct, some information giving a little support to the concept of a relative hyperfunction of the adrenal cortex in the senile rat.

Experiment.

Fourteen female rats between twenty-six and thirty months of age were used in an attempt to study the feasibility of homo-grafting in animals of extreme old age. They were all in a weak condition and in various stages of senile decrepitude. Particular care was taken to record the skin changes at the start of the experiment. The skin of these animals was papyraceous, inelastic and affected to a varying degree by a characteristic baldness seen chiefly in the nuchal region, over the back haunches and on the lower (and later the upper) abdomen.

Operation was poorly tolerated and eight animals died in the postoperative fortnight.

Two normal controls survived the stress of saline administration and continued their senile existence until they were killed two months after the start of the/

the experiment. There was no noticeable change in their skin condition.

Two transplant bearing animals settled down to a normal senile existence and showed healthy big transplants when killed two months after the start of the experiment. Both had sibling transplants. One showed no change in his skin whilst in the other the alopecia had progressed slightly.

Two transplant-bearing animals, when killed two months after the operation showed only small transplant nodules in process of absorption. One had a sibling, the other a newborn transplant. In both cases there was observed during life a remarkable regrowth of hair over the bald areas. At the same time the rest of the fur improved in quality. This improvement was maintained until the end.

COMMENT:

Spontaneous cure of senile baldness has never been seen in this colony. Neither the controls nor the animals with healthy transplants showed any regrowth of hair which was seen to a striking extent in the animals with poor transplant growth. It has been shown (11,12) that adrenalectomy encourages hairgrowth, especially in the poorly nourished rat. It seems possible/

possible therefore that adrenal insufficiency was a factor in the regrowth of hair observed in this experiment. Since baldness of the type described is only, and very commonly, seen in the senile rat, this observation supports the idea that in respect to some features of senility the laboratory rat may be relatively hyper-adrenal. That adrenal function may aggravate or bring out co-existing pathology (diabetes, hypertension, renal diseases etc.) is beginning to be understood and it is in this sense that we shall probably have to interpret these findings in the rat.

SUMMARY:

A form of senile alopecia seen in the Wistar Albino rat was unaffected in control animals, and in adrenalectomised animals with healthy adreno-cortical homo-transplants. Adrenalectomised animals with poor transplant growth showed a regrowth of hair over the denuded area.

As it turned out, the results of this experiment could have probably been obtained by any method producing adrenal insufficiency and homo-transplantation was not essential. The next problem however cannot be tackled in any other way.

LIFESPAN/

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LIFESPAN OF ADRENO-CORTICAL TISSUE:

Questions: Can the lifespan of adreno-cortical tissue be prolonged beyond that of the rat by repeated passage: age from old to young animals? What is the extreme of survival obtainable by this method? Does such tissue remain functionally active?

Experiment.

Two male controls from the previous experiment were killed at the ages of 817 and 827 days. The capsules of their adrenal glands were inserted under the renal capsules of four male rats aged 154, 154, 164 and 164 days respectively, the recipients having first been adrenalectomised. The animals were kept on saline for ten days and were then allowed to live the normal life of the colony. At the age of one year and two months and being perfectly well two of the four animals were sacrificed having reached the weights of 392 and 326 grammes respectively. In both cases a healthy transplant nodule was found growing on one kidney, adrenalectomy being complete. Fragments from both transplants were implanted under the renal capsules of four adrenalectomised male rats, weighing 85, 87, 95 and 115 grammes and being 44 days old. They were kept on saline for ten days. These young animals/

animals have now kept well for **two** months after the operation gaining weight at a normal rate. At the time of the second transplanted the adreno-cortical tissue was three years old.

COMMENT:

In evaluating this experiment one has to bear in mind what we have said before about our ignorance of the natural life span of the rat. The experiment is being extended to a great number of young recipients to insure against an accidental dying out of the strain of cortical tissue and it will be interesting to see how long the survival of this tissue can be prolonged. It is already clear that the actual limits set to the life span of the laboratory rat at present are not due to an intrinsic functional deterioration of adrenal cortical tissue. This may be concluded from the fact that three year old adrenal tissue **was** capable of keeping adult male rats in a state of good health and is now supporting the normal life and growth of young animals. Apart from the maximum survival of adreno-cortical tissue which can be achieved by this method, a number of other interesting questions remain to be settled. Can such a transplant support the life of infant rats? Does the active/

active regeneration following transplantation play a part in keeping this tissue youthful? etc.

The experiment reminds one of the demonstration by Carrell of the indefinite survival of chicken fibroblast in tissue culture. The present instance is, however, of greater significance to students of Mammalian Gerontology since it can demonstrate the limits of survival in full functional activity not of a primitive cell in an artificial medium, but of a highly specialised tissue within the animal organism. The possibility of thus segregating by a series of investigations, "immortal" tissues of the body from those subject to intrinsic functional deterioration (ageing) is very attractive.

Here is a possible solution to Lewis Carroll's riddle which prefaces this chapter. The First, which is ageing day by day is the animal bearing the transplant; the Second, whose age is ended is the animal which gave the transplant; the Third, which enjoys an age which may possibly become extended for an indefinite period is the transplant itself.

In solving this riddle we are proposing to ourselves an even greater puzzle. What seems to be true of adrenal cortical tissue probably applies, on the basis of actual biological knowledge, to many

other tissues such as the liver, the connective tissue, the haematopoetic tissue etc. What then is the cause of ageing? The answer lies in three possible directions. Firstly, the notion of ageing, i.e. of automatic and intrinsic functional deterioration, may be a mirage, having no scientific meaning, and dissolving before the advance of biological knowledge. If, however, the concept of intrinsic ageing has scientific validity there are two possibilities; it may turn out that one or more tissues of the body however manipulated by the experimenter, do not possess potential immortality and have a definite life span. In this case ageing of the whole organism would have to be conceived as the effect of these ageing tissues upon the potentially immortal remainder of the body. On the other hand, it may eventually be shown that under suitable conditions the survival of any tissue can be prolonged indefinitely, indicating that ageing is the effect of a genetically determined flaw in the inter-action of potentially immortal tissues. There is an intermediate possibility, namely that tissues subject to ageing may do so at different rates when placed into organisms of different ages.

CONCLUSION.

These experiments are a small beginning and they beg more questions than they answer, but they indicate the methods by which these questions may be solved. Homo-transplantation is the only method at present at our command for the study of intrinsic age changes within the animal organisation. It can easily be seen how an orderly extension of the experiments here reported would unravel the exact part played by the adrenal cortex in the ageing processes of the albino rat, and there is no reason why this type of experiment should be confined to the adrenal gland. To extract all possible information from this procedure, patient and undisturbed team work over many years would be required, at Institutions where generations of animals could be followed under well standardised conditions throughout their lives. Enough has been shown, however, to demonstrate that here we have a hopeful experimental approach to the study of ageing mechanisms.

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Edinburgh University provided the means for the establishment of an inbred colony of rats and for the pursuit of the preliminary investigations whilst a grant from the Medical Research Council allowed the work to continue. Professors F.A.E. Crew and (M.A.) Brennan have kindly and helpfully supervised this investigation. Dr. E.W. Humphries evaluated the vaginal smears in the experiments on reproductive function. To all these and to the technical staff who supported me, I express my gratitude.

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