XI. TETHELIN—THE ALLEGED GROWTH-CONTROLLING SUBSTANCE OF THE ANTERIOR LOBE OF THE PITUITARY GLAND.

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CERTAIN conditions of disordered development, such as acromegaly and gigantism, have long been associated with abnormal conditions of the pituitary body and in consequence the function of this gland in the growth of the animal has been the subject of much investigation. The suggestion that the pituitary body supplies a hormone which regulates the growth of the body, particularly of the skeleton, arose directly from the pathology of the diseases in question and has directed research along two definite lines. On the one hand studies of the effect of extirpation of the gland have been made, on the other, light has been sought in experiments based on the administration of the gland or its extracts to a normal animal.

The extirpation of one or other of the lobes of the pituitary body without injury to the remainder or to surrounding tissues is attended with extreme difficulty owing to the intimate association of the parts and, in consequence, results of such experiments have been conflicting. It is generally agreed that removal of the posterior lobe gives rise to no marked symptoms, whereas the balance of opinion points to the conclusion that with extirpation of the anterior lobe in young animals growth is checked, fatty deposits increase, metabolism is reduced and persistent infantilism results.

The results of experiments on the administration of the gland to normal animals are, again, far from unanimous. Cerletti [1907] injected pituitary emulsion into young animals and reported retardation of bone growth and Cushing [1909] by repeated injection of anterior lobe extracts obtained a fall in weight of young animals. The feeding of pituitary by Sandri [1909] to young guinea-pigs arrested growth whilst Schafer [1912] found that oral administration of anterior lobe was without appreciable effect on the growth and metabolism of young rats. Aldrich [1921] from experiments on puppies came to the same conclusion.

The general opinion at this period was, therefore, that oral administration of anterior lobe did not appreciably accentuate any regulating influence which the gland might normally have upon growth. The value of much of this earlier work is however depreciated by the fact that various forms of administration and different preparations of the gland were adopted by the different workers.

The whole question was reopened by a series of papers by Brailsford Robertson in 1916. He claimed to have demonstrated that the feeding of the fresh anterior lobe of the ox to white mice leads to a "marked retardation of growth in the beginning of the third growth cycle (6-10 weeks) followed by a pronounced acceleration from the 20th to 60th weeks" [1916, 1]. Further, he isolated from the anterior lobe of the ox a substance to which he gave the name *tethelin*, and which he regards as the active growth-controlling principle [1916, 2].

We were, at first, impressed by the care with which Robertson's feeding experiments had been carried out and it did appear that, by the employment of large groups of animals and by a statistical control of his data, he had guarded against all sources of error common to such experiments. A closer inspection of his work, however, revealed many inconsistencies which in our opinion seriously detract from the validity of his conclusions. In particular, it appeared that nothing but a substance of very impure character could possibly be obtained by the method he described for the isolation of tethelin [1916, 3].

The communications referred to were followed by others in which the chemical and physiological properties of tethelin were described and its effects in increasing the rate of tumour growth [1916, 4], in accelerating the healing of wounds and recovery after inanition [1916, 5] were reported. Meanwhile other workers were obtaining varying results. Goetsch [1916] and Marinus [1919] independently observed accelerated growth in rats and Wulzen [1916] accelerated division of planarian worms whilst Pearl [1916] obtained retarded growth of chickens fed upon anterior lobe.

The conflicting nature of all these results is not dispelled by Robertson's later papers in which, from a consideration of his accumulated data, he modifies his former views and comes to the conclusion that the influence of tethelin upon growth consists, as far as the whole animal is concerned, in retardation. His latest explanation of his former conclusions and the development of these views into a theory of growth catalysers is ingenious but quite unconvincing [1919, 1921]. We were led by the conflicting nature of all the evidence to undertake the re-investigation the results of which are reported in this paper. For the opportunity to carry out the work we are indebted to Mr T. O. Kent, who most kindly supplied us throughout with quantities of fresh anterior lobe of the pituitary of the ox.

Isolation of tethelin.

The method for the isolation of tethelin described by Robertson [1916, 3] is briefly as follows. The mixed anterior lobes dried by intimate mixture with an anhydrous inorganic salt are extracted for 48 hours with boiling absolute alcohol. The extract is concentrated *in vacuo* until separation of a precipitate begins. After cooling, one and one half volumes of dry ether are added and a voluminous white precipitate separates, which, after three hours, is filtered, washed with alcohol-ether mixture and dried in an incubator at 30–35°. Robertson appears to claim chiefly from constancy of nitrogen and phosphorus content that the substance is a chemical individual. To anyone familiar with such methods for the extraction of tissues it must be obvious that only a crude product, consisting largely of lipid substances would be yielded by his technique and, further, that no such product would preserve its characteristics unless carefully protected from oxidation throughout the preparation and drying. We have made several attempts to prepare this substance and to obtain a white precipitate such as Robertson describes by following the detail of his method. Our yield varied from 0.4 to 0.8 % of the moist gland, but the products obtained did not show constancy of nitrogen and phosphorus content as reported by Robertson. The analyses of two samples which agree in their content of nitrogen are given in Table I. In all cases our preparation was carried through in an atmosphere of carbon dioxide.

Table I. *Analyses.*

	Our preparations			
	I	II	Robertson	Protagon
Nitrogen	2.88	2.80	2.58	2.39
Phosphorus	1.76	0.81	1.41	1.07

Our preparations resembled in many ways the mixture of lipid substances which one can prepare from animal tissues by following a method essentially that of Robertson, and the analytical figures as well as the appearance and properties of the products show a resemblance to admittedly impure mixtures of this type such as protagon. Robertson described a number of properties of tethelin which are typical of such lipid mixtures but we were unable to confirm the colour reactions upon which he is rash enough to suggest the presence in the molecule of inositol and of an iminazolyl radicle.

On the assumption that tethelin represents an impure mixture of substances of the lipid class, perhaps contaminated by other cell constituents—no purification of his product appears to have been attempted by the author—we submitted our preparations to a simple fractionation. By extraction with hot alcohol they could be divided into a fraction readily soluble in that solvent and an insoluble fraction.

The soluble fraction deposited, on standing, a white pseudo-crystalline product of which 1.68 g. were obtained (T_1). The mother liquor of T_1 yielded

on evaporation a sticky brown residue, T_3 , whilst the fraction insoluble in alcohol was designated T_4 . On analysis these products gave the figures:

	T_1	T_3	T_4
Nitrogen	1.63	3.43	3.23
Phosphorus	0.88	1.54	3.94

We endeavoured to carry the fractionation further but were led to abandon it owing to the small amount of material at our disposal and to our conviction that the matter was not worthy of further consideration.

We did, however, examine the fraction T_1 which appeared to have the properties of an impure mixture of galactosides.

A determination of the melting point as described by Rosenheim [1914, 1] showed the following:

	Phrenosin (Rosenheim)	T_1
Softens	130–140°	—
Shrinks	170–190°	190°
Darkens	—	209°
Melts	205–215°	214°

In order to determine if the substance were mainly galactosides we hydrolysed 0.5 g. of T_1 by the method described by Rosenheim [1913]. 0.34 g. of impure esters was obtained and this upon hydrolysis yielded 0.23 g. of fatty acids, having a melting point of 58°. Recrystallisation from alcohol gave a crop of white needles melting at 70° which after a second recrystallisation melted at 76°.

A few oily drops were separated from the products of hydrolysis of T_1 and may have been the sulphates of the bases, but there was insufficient for examination.

The hydrolysate, freed from esters, was examined in the polarimeter. The rotation, if any, was slight and laevo in direction. A minute amount of a methylphenylosazone which melted at 182° was obtained (methylphenyl-galactosazone M.P. 190°). A further amount of T_1 was extracted with pyridine following the method of Rosenheim [1914, 2] and yielded fractions of varying nitrogen and phosphorus content. There was insufficient of these for further examination.

There appeared, from all the data, to be little doubt that the product obtained by following the method described by Robertson for the isolation of tethelin is a very impure mixture of substances of the lipid class and that this product is capable of separation by simple means into fractions of variable composition.

Feeding experiments.

Robertson appears to be well aware of the high variability of the growth curves of white mice but it is his claim that, by the employment of large groups of animals, a composite growth curve can be constructed which will to a high degree of probability reproduce the characteristics of growth of the individual. On this statistical basis he feels entitled to draw definite and far reaching conclusions from small differences in such composite curves for

normal and tethelin-fed groups. An examination of his methods and data led us to query the justice of this argument.

For example, it is significant that, in his experiments on the effect of feeding egg-lecithin [1916, 6], two batches of normal mice chosen at random at the age of four weeks showed a difference of 1.5 g. in mean weight, *i.e.* 13 % of body weight—a difference of the same order as the defection of weight of tethelin-fed mice at the maximum divergence of this group from the normal. Yet this, in the former case, is explained away as being due merely to the high variability at that age, whilst in the latter case it is reported as being a marked defection due to the effect of tethelin. Many other examples of such biased interpretation occur throughout this series of papers but one further example will suffice. In support of his contention that mice fed upon pituitary are, size for size, heavier than those normally fed. Robertson reproduces a photograph of two mice of the same age and linear dimensions, one a normal mouse weighing 30 g. the other a pituitary-fed mouse weighing 37 g. [1916, 1]. When it is pointed out that the weight of the normal was about the mean for his batch whereas the other weighed 27 % more than the mean for his batch—*i.e.* was an extravagantly abnormal member—the fallacy of the argument is apparent.

On the basis of such criticisms we submitted the author's papers to Professor Karl Pearson under whose direction, Mr H. Soper, of the Department of Applied Statistics, University College, London, undertook the examination of Robertson's statistical arguments, and who has kindly permitted us to publish extracts from his report. He says "if some mice are apt to be 3 days in advance of or behind the others in stages of growth as seems indicated, then the curve of mean growth will not reproduce the characteristics of individual growths. The addition of curves at different epochs or phases will tend to distort or obliterate the detail." It is significant that just such a possible variation in stage of growth was introduced into the experiments by the empirical grouping together of animals for weighing. Thus animals weighed between the 25th and 31st days inclusive were regarded as having been weighed upon the 28th day. A similar bracketing of ages was employed throughout. Mr Soper sums up in these words: "The conclusion to be drawn is that on the figures presented and statements made there is a significant defect in weight in all the treated groups from the normal group. The normal group was not changed and was not large in numbers and it has been assumed that it was a random sample of the whole community under observation. The figures given are not so complete as they might have been and statements are made and procedures taken which could only be supported by data not detailed."

It is significant that the normal group was not strictly a "random sample of the whole community under observation" as their growth curve antedates that of the pituitary mice by three weeks and that of the tethelin mice by three months. The stages of growth of the contrasted batches were not con-

temporary and there is no evidence that undetected variations in environment incident to the two groups at different ages were not responsible for the differences in growth. Robertson himself [1919] has acknowledged that the normal growth curve varies year by year.

Any experiment, claiming acceptance upon statistical considerations, which in the methods employed introduces a possibility for variability from that statistical basis must be open to criticism. Finally an inherent source of error vitiates all the conclusions. The animals were in possession of their own functioning pituitary glands, the activity of which could be neither measured nor controlled.

EXPERIMENTAL.

It was not possible with the material at our disposal, nor in view of our previous findings was it considered worth while, to carry out feeding experiments on the scale of those of Robertson, but all the other precautions employed by him in respect to weighing, elimination of sickly mice and general care were taken and the errors which it is suggested existed in his methods have as far as possible been avoided.

The mice were placed as soon as weaned in groups of six of the same sex in special wooden boxes and fed on a basal diet of mixed grains and dry bread with meat and vegetables once a week and water *ad libitum*. To the treated animals dried anterior lobe was administered in a dose of 50 mg. per mouse per day (equal to about 3 mg. of tethelin). This was given in a paste of dried milk powder and starch in equal parts and was greedily consumed before the day's ration of ordinary food was given. The same quantity of the paste, without pituitary, was given to the normal animals.

In the first experiment mice were taken at an age between 3-5 weeks and divided into batches of 25 normal and 25 pituitary-fed mice of each sex. The individual growth curves were plotted until maturity. In view of the uncertainty of age no composite growth curves were constructed but it was thought that a careful inspection of such groups of individual curves would reveal the general characteristics of the two rates of growth and the distortion of the normal curve found in Robertson's tethelin curve would be detectable. No general tendency towards a differentiation of the shapes of the two batches of curves was to be observed, a close inspection merely emphasising the high variability of growth.

In a second experiment mice were observed from birth so that composite curves might be drawn. The mothers of the treated mice were fed with pituitary until the young were weaned, when the latter were removed and treated as in the first experiment. The experiment was abandoned at 13 weeks owing to pituitary supply having become exhausted but as Robertson reports marked effects from 7 weeks onwards a comparison was possible.

These growth curves show no characteristic differences. Admittedly the curves are built up from a statistically too limited number of mice but, in conjunction with the other work reported their negative result is significant.

Age	Male mice (ten in each group) Mean weight. g.		Female mice (ten in each group) Mean weight. g.	
	Normals	Pituitary-fed	Normals	Pituitary-fed
Birth	1.60	1.44	1.53	1.50
1 week	3.92	4.20	4.00	4.10
2 weeks	5.55	5.90	5.80	5.75
3 "	7.44	8.00	8.02	8.30
4 "	9.70	10.0	10.1	10.1
5 "	12.0	12.1	12.4	12.0
6 "	14.3	14.0	14.3	13.4
7 "	15.1	15.6	15.4	14.8
8 "	16.0	16.9	16.4	16.0
9 "	17.2	18.0	17.6	17.6
10 "	18.7	19.5	19.0	18.4
11 "	19.8	20.4	19.6	19.0
12 "	21.1	21.4	20.3	19.8

CONCLUSIONS.

1. The product obtained by the method described by Robertson for the isolation from the anterior lobe of the pituitary gland of the substance he has called *tethelin* is a very impure mixture of substances of the lipid class.

2. Criticisms are advanced of the deductions made by Robertson as to the effect of tethelin and of anterior lobe of the pituitary gland upon growth and experiments are reported which fail to point to any influence upon the growth of mice of oral administration of anterior lobe of the pituitary gland.

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