X. TETHELIN:—A GROWTH-CONTROLLING SUBSTANCE OBTAINABLE FROM THE ANTERIOR LOBE OF THE PITUITARY BODY.

BY THORBURN BRAILSFORD ROBERTSON.

*From the Darling Laboratories of Physiology and Biochemistry of the
University of Adelaide, South Australia.*

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IN a recent communication, Drummond and Cannan [1922] have published an account of certain experiments and critical considerations which, in their estimation, invalidate the conclusions drawn by the author concerning the effects of pituitary (anterior lobe) substance upon the growth of animals [1916, 2, 3; Robertson and Ray, 1919] and the presence of a growth-controlling agent in the precipitate produced by ether from an alcoholic extract of the anterior lobe tissue (Tethelin).

The paper of Drummond and Cannan consists of two parts, the one dealing with the question of the composition of tethelin, and the other describing their failure to influence the growth of mice by the administration of pituitary (anterior lobe) tissue. These sections of their paper are presented as if they were logically related and mutually confirmatory, but in fact, save by the common bond of indiscriminate criticism, they are in no way connected. I propose to deal with the two parts separately in the order in which they are presented by Drummond and Cannan.

Isolation of tethelin.

In my original article describing the isolation of tethelin from the alcoholic extract of pituitary (anterior lobe) tissue [1916, 4] I was at pains to point out that the substance is exceedingly susceptible to oxidation. Drummond and Cannan declare that they "have made several attempts to prepare this substance and to obtain a white precipitate such as Robertson described by following the details of his method." This phraseology appears to be meant to create the impression that they failed. The obvious course for Drummond and Cannan to pursue would have been to procure the substance from the manufacturer¹, in which case they would have ascertained that others, besides myself, have been able to prepare this product without pronounced dis-

¹ H. K. Mulford and Co., Philadelphia.

coloration¹. Instead of this, they introduced an "improvement" into my method and employed an atmosphere of carbon dioxide throughout its preparation. Now, as a matter of course, this was the first method which occurred to me to protect tethelin from oxidation, and it was abandoned because I found that carbon dioxide itself caused softening and darkening of the product. A possible reason for this became apparent later, when Schmidt and May [1917] found that heating of tethelin, even in faintly acid solutions, induces decomposition which may be rendered manifest by physiological tests².

From the material which they prepared in this manner Drummond and Cannan now endeavoured to separate several fractions in order to demonstrate that it was a mixture of substances. After hydrolysis by alkalis and by acids I also have separated various fractions from the hydrolysate, and these experiments are described in detail in my paper. After procuring three fractions which presented differing characteristics they abandoned further attempts, owing to their "conviction that the matter was not worthy of further consideration." In agreement with this conviction we may terminate our discussion of this portion of Drummond and Cannan's contribution.

Feeding experiments.

The question of the chemical unity or composition of tethelin is, of course, not in the least involved in the separate question whether or not it contains the whole or a portion of those substances in the pituitary gland which are capable of affecting the growth of tissues. This latter question has been approached in my experiments by the direct administration of tethelin to normal growing animals and to animals inoculated with carcinoma. Drummond and Cannan approach it by the administration of pituitary (anterior lobe) tissue to young mice. In their expressed belief their results invalidate conclusions which have been based by numerous observers besides myself upon experiments extending over many years upon a variety of animal types. They state in the first place that administration of the tissue of the anterior lobe of the pituitary body to animals is devoid of effect upon their growth, in contradiction to the previous findings of Schafer [1912], Cushing [1909], Aldrich [1912], Wulzen [1914], Maxwell [1916], Goetsch [1916], Marinus [1919], Pearl [1916], Uhlenhuth [1921, 1922], and Robertson and Ray [1916, 2, 1919, 1920], all of whom have reported more or less decided effects upon growth as the result of such administration. One would expect to find an opinion, advanced in opposition to such united testimony, supported by a wealth of experimental evidence and an exhaustive analysis of technical detail, but, on

¹ Under laboratory conditions discoloration may be totally avoided, but to attain this the alcohol and ether used must be neutral and anhydrous, the salts employed must be strictly neutral (we manufactured our own calcium sulphate for this purpose) and a heated and evacuated desiccator must be employed [Robertson and Schmidt, 1916].

² Doubtless an atmosphere of dry nitrogen would overcome these difficulties, but we have not found it necessary to resort to this.

the contrary, the evidence presented, in so far as any quantitative evidence is advanced at all, is derived from data furnished by the growth of *ten* animals of each sex during a period of administration which extended over nine weeks¹.

It is urged by Drummond and Cannan that the testimony of former observers is conflicting, in that some report acceleration of total growth as a result of administering pituitary tissue, while others report retardation. The testimony is not conflicting, however, if we view it in the light of the experiments of Schafer, Wulzen, Maxwell, and Robertson and Ray, which have shown that the retardation occurs at one age (and possibly affects one kind of tissue), while the acceleration occurs at another. In seeking to ascertain a reason for Drummond and Cannan's failure to confirm the results of their predecessors one is struck by the fact that they employed *dried* anterior lobe tissue in their feeding experiments, while others have employed *fresh* tissue. Having regard to the susceptibility of tethelin to oxidation, upon which Drummond and Cannan have rightly laid so much emphasis, this procedure was hardly calculated to display its effects.

In the drying of pituitary tissue prior to its extraction with alcohol during the preparation of tethelin, the tissue is mixed with a large bulk of anhydrous calcium and sodium sulphates, whereby the period of drying is abbreviated and the tissue is covered and protected to a considerable extent from the access of oxygen. This procedure is naturally impossible when the tissue is to be administered to animals. When the unprotected tissue is dried with free access to air intense discoloration occurs and it may be conjectured that the greater part of the tethelin is destroyed.

Notwithstanding the inadequate character of their own technique, Drummond and Cannan have not hesitated to make indefensible assertions concerning the work which their experiments are designed to invalidate. In view of the controversial methods which they adopt it is curious to find that they accuse the author of "biased interpretation," for the following reasons.

In my first experiments upon the growth of mice a batch of 72 animals (36 males and 36 females) were taken from the breeding stock without selection² and set aside to serve as normal controls. Fresh batches of animals were taken from the same stock, as rapidly as the available numbers permitted, to serve as the subjects of experimental additions to the diet. These constituted, of course, so many separate experiments for which the one group of animals served as controls.

Now the variability of mice at four or five weeks of age is over 20 %³, which means that one animal in three may be expected to deviate by over 20 % from the average. This being the case, batches of 36 or 24 animals of

¹ The mothers of these animals were fed with pituitary tissue until the young were weaned (at three weeks). But the investigations of Robertson and Delprat [1917] have shown that administration of tethelin to the mother is without effect upon the growth of suckling young.

² Excepting that involved in the rejection of animals which were obviously sickly.

³ Computed as the ratio of the standard deviation to the average weight.

the same sex, chosen without selection at this age, could not possibly be expected to display equal average weights. To attain such an end would necessitate either selection of individuals chosen to display equal averages, a procedure of very questionable theoretical validity, or else the employment of impossibly huge numbers. Each experimental group, therefore, deviated initially in some degree from the normal group and these deviations were sometimes in a negative sense and sometimes in a positive¹.

This being the case, Drummond and Cannan proceed as follows: "For example it is significant that, in his experiments the effect of feeding egg-lecithin to 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."

The maximum divergence to which Drummond and Cannan refer occurred at 15 weeks of age, when the variability had fallen to 9 %. The extreme variates had approached one another as they always do during growth [Robertson, 1916, 1; Thompson, 1917] and the animals by now represented a much more uniform type. They differed from the normals by an amount equal to no less than seven times the probable error of the difference computed from the variabilities of the normal and tethelin-fed groups. Yet, according to Drummond and Cannan, this means no more than a deviation of three times the probable error, observed in a different group of animals! To anybody acquainted with the elements of statistical analysis, however, a deviation of seven times the probable error is 19,000 times as significant as a deviation of three times the probable error, since in the first case there is but one chance in 420,000 that the observed deviation could have been accidental, while in the second case there is one chance in 22.

However, let us accept Drummond and Cannan's strange conclusion and suppose that the initial differences of highly variable young are continued in the same proportion in slightly variable animals of more advanced age. It then becomes of crucial importance to know what was the initial deviation of the *tethelin-fed* group from the normals. By a strange oversight Drummond and Cannan omit to mention this. The initial deviation of this experimental group (males) from the average of the normals, as stated in my tables, was + 0.60 g. (1.3 times the probable error of the difference), or $4\frac{1}{2}$ % of the mean weight at that age *in the opposite direction to the maximum divergence from the normal which was subsequently displayed*. How are we to interpret this? On

¹ The consequences of the extreme deviation noted in the lecithin-fed class, to which Drummond and Cannan refer, are carefully considered in our communication of 1919 under the headings "Effects of Egg Lecithin" and "Possible errors of sampling" [Robertson and Ray, 1919].

Drummond and Cannan's own showing the initial divergence of the tethelin-fed group from the normal actually emphasises the significance of the subsequent outcome, and yet they forbear from mentioning it.

The objection is urged that the ages of the mice employed were not identical, since some mice were three days in advance of or behind the others. This arises from my procedure in taking, for example, mice of from 25 to 31 days of age inclusive as having been weighed on the 28th day. This bracketing is employed throughout for obvious technical reasons. The maximum divergence of age is three days, an important item without doubt at three or four weeks of age. But will Drummond and Cannan seriously maintain that it is important at 15 weeks of age?

It is stated by Drummond and Cannan that many other examples of "biassed interpretation" occur throughout my 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. 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."

How shall I satisfy these critics? My contention was that the condition of mice fed for a prolonged period upon pituitary tissue resembled that of acromegaly in that size-for-size, their weights were in excess of normal, while weight-for-weight they were smaller than normal. My photograph illustrated this difference of "build." What alternative photograph should have been taken? If I had taken a pituitary-fed mouse of average weight and displayed it beside a normal mouse of the same weight, then this would necessarily have been an "extragantly abnormal member" of the control group. If I had displayed two mice resembling each other neither in weight nor size, the comparison would have been meaningless. If I had been able to exhibit mice from each group resembling one another both in size and in weight, the effect to which I drew attention would not have existed. It is clear that no means of comparison could have been so devised as to meet with the approval of Drummond and Cannan, but that by their criticism they have unintentionally contributed eloquent testimony to the alteration of bodily proportion which was induced in my mice by pituitary feeding.

CONCLUSIONS.

1. The experiments of Drummond and Cannan upon the composition and properties of tethelin are, in part, repetition of experiments previously performed by the author and, in part, vitiated by a faulty method of preparation.
2. The experiments of Drummond and Cannan upon the administration of anterior lobe tissue to mice afford no evidence either for or against the view that tethelin contains a growth-controlling constituent of the pituitary body.

3. The statistical method of comparing the growth of normal animals with that of animals in receipt of dietary additions must of necessity be superior to the haphazard method of comparing individual growth-curves or curves constructed from the growth of a few animals. It is, in fact, the only method properly applicable to this type of problem. The criticisms directed by Drummond and Cannan against the author's employment of this method are based in part upon misstatements of fact and in part upon misapprehension of the method.

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