THE EFFECT OF A GROWTH-PROMOTING EXTRACT OF THE ANTERIOR PITUITARY ON THE EARLY GROWTH OF THE ALBINO RAT*

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(Received for publication, February 13, 1934)

Data are here presented on the weight and water content of the various organs and parts of castrated male albino rats as influenced by the injection of an anterior pituitary growth-promoting extract during the greater part of the first 2 months of life. The data were accumulated in the course of another investigation (1) in which it was shown that a growth-promoting extract of bovine anterior pituitary does not prevent the increase in gonad-stimulating content of the pituitary of the rat which occurs after castration (2, 3). It was felt that the data on the water content would throw additional light on the question of the composition of the tissues of rats and mice injected with anterior pituitary extract as reported recently by several investigators (4–8), since these investigators in their analyses used the animals *in toto* without attempting, as indeed the magnitude of the task would forbid, to follow the changes in the separate parts and organs. In addition, it was thought that the data on the weight of the different organs and parts of the animals might yield some interesting information as to the early effects of an excess of anterior pituitary growth-promoting principle on the development of the young organism that is still rapidly growing.

Inspection of the literature as to the effects of castration on the growth of the albino rat indicates that the castration of the animals of the early age here employed has not materially altered the results from what they would have been had non-castrates been used. Stotsenburg (9), using three different groups of male albino rats castrated

* This work was supported in part by Biological Grant No. 12 of the Rockefeller Foundation.

on the 14th or 15th day of life and carried up to the 143rd, 181st, and 185th day of life, respectively, found that the growth curve for the castrates was practically the same as that for the normal animals. Hatai (10) castrated his animals at weaning or shortly after and reported that the castrates did tend to become smaller in weight and length than the non-castrate controls, but this difference was but slight after approximately 5 months. Van Wagenen (11) found like Hatai that male albino rats castrated at weaning did not maintain the weight and body length of their unoperated controls, but this failure was not always evident during the first 100 to 150 days of life; after this time there was an increasing divergence. Van Wagenen's graphs of the average weight curves for castrates and non-castrates indicate that there was no divergence at all in the weight of her animals during approximately the first 50 days of life.

The rats here employed were castrated at weaning and killed when the difference in weight between pituitary-injected and control groups had just become manifest. This was when the animals were approximately 56 days old (youngest litter 53 days, oldest 59). It may be assumed, then, with some degree of confidence that so far as total body weight is concerned there was at this age but little difference, if any, in the growth of the castrates from what it would have been had they been normal animals.

Material and Methods

The growth-promoting extract was prepared in accordance with the method described by Bugbee, Simond, and Grimes (12). This method is essentially as follows: Whole beef pituitaries are used which have been placed on ice at the packing house as soon as possible after the death of the animals. The anterior lobes are dissected free from the surrounding tissues and shelled out. The resulting anterior lobe tissue is ground as fine as possible and to this hash is added for every 100 gm. of tissue 400 cc. of 0.05 N sodium hydroxide containing 2.5 per cent butanol. The mixture is put away to stand in the refrigerator overnight. The pH of the mixture is then brought down to 7.2 to 7.6 by the addition of 0.2 N acetic acid. Centrifugation for ½ hour throws down the insoluble residue. The cloudy supernatant liquid is warmed to 37°C. on the water bath and to every 100 cc. is added 20 gm. of sodium sulfate. Again centrifugation for ½ hour separates precipitate and supernatant liquid. The precipitate is dissolved in an amount of 0.02 N sodium hydroxide containing 2.5 per cent butanol so that 2 cc. of the resulting solution contains the material extracted from 1 gm. anterior lobe tissue

(200 cc. of the sodium hydroxide solution for the precipitate from 100 gm. tissue). The gland residue remaining from the first extraction may be re-extracted with one-half the volume of sodium hydroxide used in the first extraction, and the sodium sulfate precipitation from this second extract added to the solution of the first extract. The resulting fluid is filtered through asbestos three times and finally through sterile porcelain filters into suitable sterile containers, such as small glass ampoules or bottles. The material is then kept in the refrigerator until used.

A total of 120 albino male rats was accumulated consisting of 30 litters, 4 mates to a litter. The animals had at all times access to food and water. Each litter was weaned on the 21st day of life and castrated on the same day. Injections were begun the same day or the day following, and were given subcutaneously, 0.5 cc. twice daily. 1 animal in each group, A, received the growth-promoting extract. A 2nd animal in each group, B, received beef muscle extract prepared in the same way as the preceding. The other 2 animals remained as untreated controls. The injections were continued until the animals were, on the average, 56 days old. At this time 3 animals from each litter were killed. These consisted of the one receiving the growth-promoting extract, the control receiving the beef muscle extract, and that one of the untreated controls which weighed the most. The latter animal thereby served as an untreated age control, AC. The smallest untreated control was allowed to grow until it had reached approximately the same gross weight as had its litter mate receiving the growth-promoting extract, at which time it was killed. This animal thereby served as a weight control, WC.

The animals were disposed of as follows: Each animal was etherized, and through a midline incision the gut was drawn out exposing the inferior vena cava. With a large-bore needle and syringe, blood was withdrawn from the vena cava until the heart had stopped beating. The vena cava was then transected and the remainder of the blood which came out and could be forced out by appropriate pressure was immediately taken up with weighed cotton swabs. The swabs were quickly placed into a weighing bottle kept closed, and when all swabs had been collected in the bottle they were reweighed. Likewise the needle and syringe were weighed before and after collecting the blood. The gut was freed of its mesenteric attachments from esophagus to anus and discarded. The bladder was emptied of any urine present, using a small needle and syringe. The edges of the wound were brought together and the animal was weighed. To this weight was added the weight of the blood previously removed, to give what was called the final weight of the animal. The animal was now stretched on its back on a sheet of white paper, the position of nose and anus marked off, and the distance between the marks measured. The average of several measurements for each animal was taken. The organs and parts were removed in the following order: spleen, adrenals, kidneys, liver, thymus, heart, lungs, thyroid, skin, tail, pituitary. The non-furry portion of the skin was retained on the tail. The small amount of blood which came out following severance of the vessels to the organs was as before taken up with cotton swabs and weighed, and this weight added to that of the blood previously removed. Each organ when removed was placed into a

weighing bottle containing filter paper previously weighed. The paper served to prevent the moisture of the organ from wetting the bottle; also, it served as a vehicle, by grasping one corner with a forceps, for transferring the organ back and forth. The adrenals, thyroid, and pituitary when removed were washed in physiological saline, quickly dried on filter paper, and weighed directly on a torsion microbalance. The posterior lobe of the pituitary was plucked away immediately after the whole gland had been weighed, and the anterior lobe reweighed alone to give the weight of the posterior lobe by difference. The organs, still on the filter paper, were placed in clean beakers to dry in the oven at 100°C., and when dry were reweighed in the weighing bottles. The skin and carcass were placed to dry in the oven in previously weighed beakers. Corresponding portions of litter mates were put in and removed from the oven at the same time. The so called constant weight was taken to be the point where the organs first began losing only a few (1 to 3) mg. in 24 hours, since with organs of a size such as is found in the rat such a loss may keep up for days. The average length of time in the oven was for the smaller organs 5 days, for the larger 7 days.

In the treatment of the data, the method given by Fisher (13) is used because of its theoretical advantage over the usual method as far as the experimental set-up here employed is concerned. The usual method for determining whether or not the difference between two means is significant involves the use of the standard deviations of the means. Fluctuations in the value of the standard deviation of a mean may be readily brought about by the introduction of items which deviate widely from the mean. In biological work, wider variations may be expected to exist between non-litter mates than between litter mates. It is theoretically possible, therefore, in an experiment in which the control group is made up of litter mates of the animals in the experimental group, that small but constant differences existing between each experimental animal and its litter mate control would be obscured by the wider variations between the non-litter mates in each group if the mean of the one group as a whole be compared with the mean of the other group as a whole. Fisher's method obviates this possibility by dealing directly with the differences existing between litter mates. For ease of calculation Fisher's formula is recast as follows:

$$t^*_{A,B} = \frac{\bar{\Delta}_{A,B}}{\sqrt{\frac{\Sigma(\Delta_{a,b})^2 - n(\bar{\Delta}_{A,B})^2}{n(n-1)}}} \quad *$$

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* The t of Fisher has the same meaning as the $\frac{x}{\sigma}$ of other statisticians but is more inclusive in that in its table for its distribution he includes those cases where n is small (30 or less) whereas the usual tables for the distribution of $\frac{x}{\sigma}$ apply only where n is large (30 to ∞).

where $\Delta_{a,b}$ = difference between any two litter mates a and b in Groups A and B,

$\bar{\Delta}_{A,B} = \frac{\Sigma\Delta_{a,b}}{n}$ = mean of algebraic sum of all the differences,

and n = number of differences between litter mates.

Since this formula does not involve the use of the standard deviation, this is not given except for the data in Table I. Appended to the original data in each table are the so called P values which indicate whether or not the difference between any two means is to be regarded as significant.[1]

RESULTS

Table I shows that the extract was potent in producing animals heavier and longer than the beef-injected and untreated age controls. (*E.g.*, the P value 0.0001 for the difference between the means A and B indicates that there is but 1 chance in 10,000 of obtaining by random sampling alone a difference as great as the one observed; hence the difference is undoubtedly a significant one.) The weight controls are in turn heavier than the pituitary-injected group, but in nose-anus length these two groups are the same. The P value 0.06 for the difference in nose-anus length between beef-injected and untreated age controls suggests that the former animals are somewhat smaller than the latter.

Table II presents the average group weights of the various parts and organs. The data on the blood are the averages from 22 animals in each group instead of 30, the blood from the first 8 animals in each group having been discarded without weighing. The results are best summarized as follows:

[1] A P value of 0.01 for the difference between any two means indicates that there is but 1 chance in 100 of obtaining again by random sampling alone a difference as great as the one observed. Customarily, the P value 0.01 is taken as a limit on one side of which (0.01 or less) it can be said that the difference observed is undoubtedly significant. Values between 0.01 and 0.05 indicate lesser degrees of significance which must be interpreted with reference to other factors in the experiment. Values greater than 0.05 are not generally considered as indicating that a significant difference is present. This is a safe and conservative procedure to follow. Here, however, because of the variety and interlocking of the data, values as high as 0.08 have been interpreted as indicating a tendency toward a significant divergence where it was felt that there was a sound physiological basis for such an interpretation. The P values given in the tables here were obtained by calculating from Fisher's table of t a table of P corresponding to values of t ranging by tenths from 0.1 to 6.0, for $n = 1, 2, 3 \ldots 20$ and ∞.

1. Adrenals, thymus, and spleen show no significant differences in weight throughout the four groups.

2. The weight of the heart and skin of the beef-injected animals in Group B is significantly decreased in comparison to that of the untreated age controls in Group AC. This adds considerable strength to the suggestion seen in Table I that the animals in Group B tend to be somewhat smaller in nose-anus length than those in Group AC.

TABLE I

Average Final Weight and Length of Animals

	Body weight	Nose-anus length
	gm.	*mm.*
Group A (pituitary-injected)	146.7 ± 23.0	185.8 ± 8.7
Group B (beef-injected)	129.9 ± 15.4	178.6 ± 6.2
Group AC (untreated age controls)	133.9 ± 20.7	180.5 ± 7.6
Group WC (untreated weight controls)	151.4 ± 22.9	185.1 ± 9.2
A, B	0.0001*	0.0001
A, AC	0.002	0.0001
A, WC	0.002	0.23
B, AC	0.16	0.06
B, WC	0.0001	0.0001
AC, WC	0.0001	0.003

* This means that there is but 1 chance in 10,000 of obtaining again as a result merely of the sampling process a difference between the means for Group A and Group B as great as the one here observed.

3. The increased weight of the pituitary-injected animals in Group A over that of the untreated age controls in Group AC is reflected in the case of the heart, lungs, liver, kidneys, blood, tail, and probably also the carcass.

4. Heart, lungs, liver, kidneys, and blood of the pituitary-injected animals exceed in weight the corresponding organs of the weight controls.

5. The increased weight of the weight controls in Group WC over the pituitary-injected animals in Group A is manifested chiefly in the carcass and probably also in the skin; too, the thyroid and pituitary of this group are heavier than in the pituitary-injected.

TABLE II

Average Fresh Weights of Parts and Organs

(a) Endocrines

	Adrenals	Thyroid	Whole pituitary	Anterior lobe pituitary	Posterior lobe pituitary
	mg.	*mg.*	*mg.*	*mg.*	*mg.*
Group A	26.6	20.2	7.0	5.6	1.5
Group B	25.3	19.1	7.9	6.7	1.6
Group AC	27.0	20.3	8.2	6.8	1.7
Group WC	26.3	23.3	9.0	—	—
A, B	0.19	0.16	0.0001	0.002	0.08
A, AC	0.42	0.84	0.0001	0.003	0.04
A, WC	0.76	0.001	0.0001	—	—
B, AC	0.11	0.11	0.16	0.77	0.44
B, WC	0.05	0.0001	0.0001	—	—
AC, WC	0.92	0.0007	0.0003	—	—

(b) Viscera

	Thymus	Spleen	Heart	Lungs	Liver	Kidneys
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Group A	0.574	1.314	0.714	0.977	9.712	1.418
Group B	0.600	1.229	0.611	0.848	7.387	1.206
Group AC	0.560	1.294	0.645	0.912	7.660	1.218
Group WC	0.588	1.298	0.661	0.929	8.088	1.303
A, B	0.84	0.62	0.0001	0.0001	0.001	0.0001
A, AC	0.19	0.84	0.0001	0.02	0.0001	0.0001
A, WC	0.55	0.69	0.0001	0.02	0.001	0.0002
B, AC	0.32	0.42	0.009	0.13	0.19	0.69
B, WC	0.76	0.32	0.004	0.09	0.05	0.04
AC, WC	0.19	0.76	0.48	0.76	0.16	0.01

(c) Parts

	Blood	Tail	Skin	Carcass
	gm.	*gm.*	*gm.*	*gm.*
Group A	10.3	5.699	31.94	84.77
Group B	8.5	4.936	28.47	76.44
Group AC	8.7	5.140	31.18	80.35
Group WC	9.5	5.707	33.67	89.36
A, B	0.0002	0.0001	0.01	0.003
A, AC	0.0002	0.0001	0.29	0.08
A, WC	0.0008	0.62	0.07	0.0004
B, AC	0.49	0.09	0.02	0.11
B, WC	0.008	0.0001	0.002	0.0002
AC, WC	0.01	0.001	0.03	0.0008

6. The pituitary gland in the pituitary-injected animals is smaller in weight than in any of the other groups. Only after this trend of the data from the first half of the animals was noticed were the separate weights of the two lobes determined, so that the averages of the two lobes pertain to 16 animals in each group instead of 30. This number is sufficient to show that the decrease in weight of the anterior lobe is unquestionable. In view of this, it is probable that the posterior lobe is also decreased in weight, but a larger number of animals would be necessary to make this difference in weight as definite as it is for the anterior lobe.

TABLE III

Average Water Content of Parts and Organs (Per Cent of Fresh Weight)

	Thymus	Heart	Lungs	Spleen	Liver	Kidneys	Tail	Skin	Carcass
Group A	79.9	78.8	80.4	77.1	72.0	74.4	62.8	60.6	71.9
Group B	80.1	78.6	80.4	78.4	71.7	73.9	62.3	58.1	72.5
Group AC	80.1	78.3	80.1	77.9	72.2	73.9	62.2	55.4	72.2
Group WC	79.4	78.3	79.6	78.0	73.2	73.8	60.8	55.2	71.7
A, B	0.84	0.76	0.37	0.13	0.76	0.004	0.19	0.008	0.07
A, AC	0.76	0.48	0.62	0.84	0.84	0.002	0.16	0.0001	0.5
A, WC	0.009	0.32	0.0001	0.32	0.19	0.003	0.0001	0.001	0.5
B, AC	0.76	0.72	0.69	0.32	0.48	0.76	0.76	0.01	0.33
B, WC	0.002	0.84	0.0001	0.48	0.48	0.48	0.0002	0.02	0.004
AC, WC	0.0001	0.92	0.06	0.48	0.48	0.62	0.0001	0.92	0.09

Table III gives the average water content of the various parts and organs. The pituitary-injected animals show a significant increase in water content of kidneys and skin in comparison to the other groups. However, the skin of the beef-injected animals shows also an increased hydration in comparison to the skin of the age controls and weight controls. The weight controls show less water than all the other groups in thymus, tail, and lungs.

In the attempt to analyze further the nature of the increased hydration of the skin and kidneys of the pituitary-injected animals the data of Table IV were worked up. This shows that the increased water content of the kidneys of the pituitary-injected group is not solely responsible for the increased weight of these organs since there has

been at the same time a definite increase in dry matter. But this is not so in the case of the skin. Here the dry weight in the pituitary-injected group tends to be less, if anything, than in the untreated age controls; it is about the same in amount as in the beef-injected group, which similarly shows a decreased amount of dry matter in comparison to the age controls. In other words, both pituitary-injected and beef-injected animals show the same type of change in the skin with respect to the untreated age controls: an increase in the water content and a decrease in the amount of dry matter.

TABLE IV

Dry Weight of Kidneys and Skin

	Kidneys	Skin
	gm.	*gm.*
Group A	0.327	12.67
Group B	0.282	11.94
Group AC	0.286	14.02
Group WC	0.308	15.13
A, B	0.0001	0.29
A, AC	0.0001	0.02
A, WC	0.005	0.003
B, AC	0.48	0.01
B, WC	0.005	0.001
AC, WC	0.005	0.12

DISCUSSION

Of the investigators referred to at the beginning of this paper, Downs and Geiling, Bierring and Nielsen, and Lee and Schaffer agree that the tissues of pituitary-injected animals show an increased hydration in comparison to controls. Wadehn alone finds that his injected mice show no increase of water in their tissues. According to Wadehn, this difference is due to the fact that he prepares the growth principle by precipitation from extracts which have been carefully dialyzed free from electrolytes. This method, he feels, yields a preparation purer than that used by previous investigators, and he attributes the hydration effect obtained by Downs and Geiling to the presence in their extracts of an "antidiuretic" principle.

The extract used here is one that is essentially similar to the ones used by investigators other than Wadehn, and their finding that such an extract causes an increased hydration is confirmed. This increased hydration, however, is shown here to be confined to the skin and the kidneys, and is not a generalized affair.

Moreover, it is probable that in the case of the kidneys the increased hydration is only apparent. It does not seem likely that we can be dealing here with a true interstitial or intracellular retention of water. The histological structure and functional nature of the kidney makes it difficult to conceive of such an occurrence. More likely, what was measured here was really an increased amount of urine in the kidney tubules subsequent, no doubt, to an increased food and water intake. That the food intake of the pituitary-injected animals was greater than in the weight controls is suggested by the fact that despite the care taken to kill off the weight controls when they had reached exactly the same gross weight as the pituitary-injected animals, the net weight of the weight controls after the gut had been stripped away was greater than that of the pituitary-injected animals, indicating that the gut contents of the latter must have been greater. On the other hand, part of this difference in weight may have been due to an increase in size and weight of the gut itself.

At any rate, it appears that actually there is but one organ in the pituitary-injected animals that shows an increased amount of water and that organ is the skin. Moreover, this increased hydration has been produced without an increase in total weight of the skin in comparison with the weight of the skin in the untreated age controls. It seems highly improbable that the same principle that would cause this type of change in the skin would effect the increase in weight without an accompanying increase in hydration that is observed, for example, in the case of the heart. Further, there is in the increased hydration of the skin of the beef-injected animals the suggestion that at least part of the skin hydration of the pituitary-injected animals may be non-specific.

All in all, it appears that the data of this experiment might reasonably be interpreted as favoring Wadehn's view that the increased hydration observed in the tissues of animals injected with anterior pituitary extracts is not due to the growth principle. But the question as to

whether or not the hydration is produced by a specific water balance principle remains unsettled, since there is evidence that at least part of the increased hydration of the skin noted here is due to a non-specific factor or factors.

The fact that the beef-injected animals show in comparison to the untreated age controls a tendency toward a smaller nose-anus length and a significantly smaller heart and skin suggests that the beef-muscle extract exerted a mildly deleterious effect on the growth of the animals in this group.

The decreased weight of the pituitary gland in the pituitary-injected animals may be explained as due to the fact that the parenteral injection of the growth principle rendered unnecessary the production by the gland cells of as much of the principle as they would otherwise have had to produce so that we are dealing here at least to some extent with a process akin to the atrophy of disuse.

By the use of weight controls in this experiment it is demonstrated that the increase in weight of the heart, lungs, liver, and kidneys of the pituitary-injected animals constitutes a true splanchnomegaly, since these organs in this group are significantly heavier than the corresponding organs in the weight controls despite the fact that the total body weight of the latter is greater. It has thus been possible to reproduce experimentally during what might be termed the prepuberal or early puberal life of the rat two characteristics of hyperpituitarism, visceral and skeletal overgrowth, which are in man not seen until the onset of adult life. As far as I am aware, the simultaneous production of these two consequences of an excess of anterior pituitary substance at so early an age has not hitherto been reported.

How is the increase in weight of the blood removed from the pituitary-injected animals to be interpreted? It may simply mean, of course, that the vessels in these animals were more dilated than they were in the controls so that more blood could be removed by the method employed. More attractive, however, is the hypothesis that it represents actually an increase in the amount of the vascular bed, since from this hypothesis one may draw the inference that this increase in vasculature may be a primary effect of the growth-promoting principle, and the visceral and skeletal growth secondary to this. The actual meaning of the phenomenon remains, of course, to be

demonstrated by further experimentation, and work is now under way in which is being studied in greater detail the effects of anterior pituitary extracts on the blood and cardiovascular system.

SUMMARY

Castrate male albino rats were injected with a growth-promoting extract of bovine anterior pituitary from the 21st day of life (day of castration) to the 56th day (average for group) at which time the difference in weight between the pituitary-injected animals and controls was first clearly discernible. In comparison to controls the pituitary-injected animals at this stage showed: (1) a decrease in the weight of the pituitary gland; (2) an increase in nose-anus length; (3) an increase in the weight of heart, lungs, liver, and kidneys which is shown to be of the nature of a splanchnomegaly; (4) an increase in the weight of the blood removed; (5) an increase in the water content of the skin and the kidneys; (6) a tendency toward an increase in weight of the carcass. The significance of these findings is discussed.

I wish to express my appreciation to Dr. Sewall Wright of the Department of Zoology for his advice concerning the statistical treatment of the data. I am very grateful to Miss Helen Goldfield for her aid with the labor of the calculations. I am especially indebted to Professor F. C. Koch of this department for his unfailing and heartening assistance which made it possible to complete this work.

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