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ANTERIOR PITUITARY EXTRACTS AND LIVER FAT.[1]

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The original objective of this investigation was the elucidation of the mechanism of action of the ketogenic substance, or substances, present in certain extracts of the anterior pituitary gland. The ketogenic effect of anterior pituitary extracts was first reported by Burn and Ling [1930], who noted that their injection produced an increased excretion of ketone bodies in rats on a fat diet. Anselmino and Hoffmann [1931] found an increase in the ketone content of rats' blood, and Magistris [1932] has reported similar findings in the rabbit. We have repeated the experiments and confirmed the findings of Burn and Ling and of Magistris, and have become interested in the extensive deposition of liver fat produced by the repeated administration of large doses of certain ketogenic preparations to white rats. It is with this phenomenon that we are particularly concerned in this report. The mechanism of action of the ketogenic substance is also briefly discussed.

Fatty livers produced in rats by fasting, by diets rich in fat, cholesterol feeding, phosphorus poisoning, and by diets deficient in choline and in other "lipotropic" factors have been recently studied in these laboratories, but the rate of accumulation of fat in the liver when large doses of the anterior pituitary extract are given is, under certain conditions, greater than that caused by any of these other procedures. The increase in liver fat is associated with a decrease in the fat of the depots. The correlation of our findings with those of Coope and Chamberlain, Steppuhn, Anselmino *et al.*, Raab, Schäfer, and others will be discussed later in this paper.

[1] A preliminary report of this work was made at the Annual Meeting of The Royal Society of Canada, May 1935.

METHODS.

The total ketone bodies of the blood were determined by the method of Engfeld [1925]. The urinary ketone bodies were estimated by van Slyke's method in 24-hour samples of urine from rats kept in Hopkins' metabolism cages. The 24-hour sample of urine from each animal was treated with a copper-lime reagent and made up to a volume of 100 c.c.; 25 c.c. samples were used for the determinations. Liver and body fats were estimated by the saponification procedure which has been used for some years in these laboratories [Leathes and Raper, 1925]. The values for fat represent, therefore, the total fatty acids and the unsaponifiable fraction. The extracts were prepared from anterior pituitary glands which had been carefully separated from the whole gland and defatted by acetone within a few hours after their removal from the animals. The extracts were prepared from the dried anterior lobe powder by two different procedures. The material which was administered to the rats was extracted from the dry powder with 20 volumes of $N/20$ NaOH. After standing for about 2 hours the extract was adjusted to *p*H 5·2 with HCl. A bulky precipitate was removed and re-extracted in the same manner as the original powder, but with half the amount of solvent. The filtrates were combined and 2 volumes of absolute alcohol were added. The resulting precipitate was collected and dried with absolute ethyl alcohol and ether. A weighed amount of this powder was dissolved in faintly alkaline aqueous solution, adjusted to neutrality and the undissolved material removed by centrifuging. 3 c.c. of the clear, light brown, supernatant solution were administered subcutaneously. In all the experiments except that described in Table III, where other figures are given, 1 c.c. of the solution injected contained the active material obtained from 1 g. of the original dried defatted anterior pituitary glands. The extracts of other tissues, to be discussed later, were prepared by exactly the same procedure. The material administered to the rabbits was extracted from the crude anterior lobe powder with 40 volumes of $N/25$ HCl. A bulky protein precipitate was removed from this extract by adjusting to *p*H 5·2 with NaOH. One volume of 95 p.c. alcohol was added to the filtrate and the reaction was adjusted to *p*H 5·2. The precipitate was removed and the filtrate evaporated *in vacuo*, at approximately 35° C., to a volume which corresponded to a 10 p.c. solution of the original powder. This solution was also administered subcutaneously.

Experimental results and comment.

The effect on ketone body excretion and on the size and fat content of the liver.

The rats were supplied by the Connaught Laboratories. They were of the Wistar strain and were all in excellent condition at the start of the experiment. Normal females weighing about 160 g. were fasted in individual cages for 3 days. One-half of the animals were injected daily with anterior lobe extract, while the others received the same volume of saline (3 c.c.). All of the animals were given 1·5 c.c. of a 10 p.c. solution of sodium chloride by stomach tube to increase the urinary volume. All the figures in the tables represent averages. The figures in brackets indicate the minimum and maximum values. Certain particularly significant figures are printed in heavy type. The results obtained in the control animals are shown in Table I, series I. It will

Table I. Normal female rats.

Series I: Injected once daily with 3 c.c. saline.
Series I*a*: Injected once daily with 3 c.c. extract from 3 g. anterior pituitary powder.
Fasted during the period of the experiment (3 days).
1·5 c.c. 10 p.c. NaCl administered daily by stomach tube.

Series	No. of rats	Weight g.	Loss of weight g.	Ketone body excretion in 24 hours mg. 1st day	2nd day	3rd day	Total	Liver Weight g.	Total fat mg.	Fat p.c.
I	22	159 (119–200)	17 (3–29)	1·1	1·0	1·8	3·9	3·99 (2·2–4·9)	219 (95–322)	5·46 (2·7–8·0)
I*a*	17	159 (138–196)	10 (1–27)	2·2	36·2	51·5	**89·9**	**6·42** (4·9–7·8)	**673** (300–1210)	**10·38** (5·0–17·2)

Two rats in series I excreted considerably more ketone bodies than the others, a total of 11 and 23 mg. respectively, on account of some uncontrolled factor. In series I*a* all but four excreted more than 23, all but one more than 11 mg., this one only 3·5 mg.

be observed that the daily ketone body excretion is, on the average, less than 4 mg. for the 3 days. The animals receiving the extract (Table I, series I*a*) show a very definite increase in ketone body excretion (average approximately 90 mg. for the 3-day period), although the individual values are variable. The increase is particularly significant on the second and third days. The increase in the liver weight of animals injected with anterior pituitary preparations was apparently first observed by Riddle and Flemion [1928]. In our animals it will be noted that there is a definite increase in the liver weight and also in liver fat. The values for the total fat of the liver in the test and control groups present a rather striking contrast.

In another experiment larger groups of animals were used, but the excretion of ketones was not followed. The results for the control animals are shown in Table II, series II, and for those which were injected, in

TABLE II. Normal female rats.

Series II: Injected once daily with 3 c.c. saline.
Series II*a*: Injected once daily with 3 c.c. from 3 g. anterior pituitary powder.
Fasted during the period of the experiment (3 days).

Series	No. of rats	Weight g.	Loss of weight g.	Liver: Weight g.	Liver: Total fat mg.	Liver: Fat p.c.	Carcase fat*: g.	Carcase fat*: p.c.
II	20	170 (147–208)	31 (7–75)	4·52 (3·57–5·70)	270 (166–369)	6·0 (4·3–8·6)	9·4	7·1
II*a*	19	176 (147–191)	21 (11–32)	**7·44** (6·28–8·45)	**1100** (664–1566)	**14·7** (9·9–19·4)	**7·9**	**5·5**

* "Carcase" = body without liver.

Table II, series II*a*. The contrast in the total liver fats of these two groups is particularly interesting, and the values for the individual animals are as consistent as one can expect in this type of experiment.

When smaller doses of the anterior pituitary preparations are used the response is correspondingly less. The increase in total liver fat and ketone body excretion is apparently elicited by the smallest dose which we have used (Table III). This dose contained the active material secured from 188 mg. of the dry defatted anterior lobe.

TABLE III. Normal female rats.

Series III: Injected once daily with 3 c.c. saline.
Series III*a*: Injected once daily with extract from 188 mg. anterior pituitary powder.
Series III*b*: Injected once daily with extract from 540 mg. anterior pituitary powder.
Fasted during the period of the experiment (3 days).
1·5 c.c. 10 p.c. NaCl administered daily by stomach tube.

Series	No. of rats	Anterior pituitary powder daily dose mg.	Weight g.	Loss of weight g.	Ketone body excretion in 24 hours, mg. per 100 g. rat: 1st day	2nd day	3rd day	Total	Liver: Weight g. per 100 g. rat	Liver: Fat mg. per 100 g. rat	Liver: Fat p.c. of liver
III	5	0	159	23	0·7	0·5	1·5	2·7	2·71	143	5·29
III*a*	5	188	171	22	0·8	2·9	16·8	20·5	2·94	171	5·82
III*b*	5	540	166	20	1·0	22·1	38·4	61·5	3·32	202	6·04

Similar experiments were carried out on fed rats. The animals were injected once daily for 3 days as in the previous experiment. The results are shown in Table IV. While the increase in ketone body excretion is, in view of the variation in individual values, hardly significant, there is

a definite rise in liver weight and total liver fat in the injected animals. Further observations upon the liver fat of fed animals are reported in the next section.

TABLE IV. Normal female rats.

Series IV: Injected once daily with 3 c.c. saline.
Series IV*a*: Injected once daily with 3 c.c. extract from 3 g. anterior pituitary powder.
Fed a sufficient diet during the experiment (3 days).

Series	No. of rats	Weight g.	Gain in weight g.	Ketone body excretion in 24 hours mg. 1st day	2nd day	3rd day	Total	Liver Weight g.	Fat mg.	Fat p.c.
IV	7	145 (134–158)	4 (0–9)	1·4	2·0	1·3	4·7	5·95 (5·1–7·0)	227 (207–261)	3·8 (3·5–4·1)
IV*a*	8	147 (135–160)	6 (− 13 to + 6)	1·3	5·1	3·0	9·4	7·91 (6·0–8·7)	661 (384–882)	8·4 (5·0–11·6)

Two rats in series IV*a* excreted much more ketones than the other six (309 mg. and 175 mg.); the other six less than 6 mg.

The effect of the anterior lobe extracts on body fat.

The fat content of the bodies of the animals referred to in Table II, series II and II*a*, were estimated, and the values given in that table indicate that a definite decrease has been produced by injection of the anterior pituitary material. Body fat was also investigated in a series of fed animals. As injected rats do not eat quite as much as controls, the diet of the latter was slightly restricted in an effort to maintain the caloric intake of the two groups approximately the same (Table V). It will be noted that the injected animals (series V*a*) gained weight while the controls (series V) lost. On the first day of the experiment the gain in weight of the injected animals exceeded the food intake. This suggests that there was a positive water balance. If so the values for total fat are more significant than those expressed as percentages. The total fat in the bodies of the control animals was higher than in those of the animals receiving the anterior pituitary extract, while the liver weight and total liver fat of this latter series were significantly greater than that of the controls. While it does not appear probable that the slightly smaller caloric intake would exert an appreciable effect on body fat over this short period, we base our conclusion that the depot fat is decreased by the ketogenic preparation on the results obtained in fasting animals. The results in Tables II and VI justify the conclusion that injections of the anterior lobe extracts in fasting animals cause within 3 days a decrease in body fat which is from 15 to 20 p.c. greater than that observed in the control rats.

TABLE V. Normal female rats.

Series V: Injected once daily with 3 c.c. saline.

Series V*a*: Injected once daily with 3 c.c. extract from 3 g. anterior pituitary powder.

Food 8·7 g. per day = 6·1 p.c. of body weight. In series V, two rats ate only 8·1 g. In series V*a* only six rats ate 8·7 g. The average amount eaten was 7·9 g. = 5·5 p.c. of the body weight. The smallest amount eaten was 6·4 g.

Series	No. of rats	Weight g.	Loss or gain of weight p.c.				Carcase*			Liver			Kidney			Intestines fat g.
			1st day	2nd day	3rd day	Total	Weight g.	Fat g.	Fat p.c.	Weight g.	Fat mg.	Fat p.c.	Weight g.	Fat mg.	Fat p.c.	
V	20	143	+2·1	−0·9	−6·2	−4·5	113	9·57	8·4 (3·9–12·6)	4·49	201	4·49 (1·9–5·6)	1·22	45·8	3·77	8·37
V*a*	17	144	+6·9	+1·8	−5·6	+3·1	122	**6·98**	**5·7** (3·9–8·4)	6·12	**405**	**6·64** (3·9–11·6)	1·36	52·8	3·87	**6·67**

* Carcase = body after removal of the liver, kidneys and intestines.

TABLE VI. Normal female rats.

Series VI*a*, VI*b*, VI*c*, VII*a*: Injected once daily with 3 c.c. extract from 3 g. of the respective gland powders.

Series VII*b*: Injected once daily with "pituitrin" containing 10 international units per c.c.

Series VI and VII: Injected once daily with 3 c.c. saline.

Fasted during the period of the experiment (3 days).

Injection	Series	No. of rats	Weight g.	Loss of weight g.				Liver			Carcase*			Kidney			Intestine		
				1st day	2nd day	3rd day	Total	Weight g.	Fat mg.	Fat p.c.	Weight g.	Fat g.	Fat p.c.	Weight g.	Fat mg.	Fat p.c.	Weight g.	Fat mg.	Fat p.c.
Saline	VI	10	193	14·0	8·8	10·3	**33·1**	4·37	244	5·6	143	10·6	7·45	1·26	64	5·1	9·7	855	8·9
Anterior pituitary	VI*a*	10	184	3·2	7·2	13·2	23·6	6·02	**904**	15·0	138	8·7	**6·39**	1·55	71	4·6	9·8	612	**6·2**
Liver	VI*b*	10	184	9·1	7·0	5·8	21·9	4·16	342	8·2	—	—	—	—	—	—	—	—	—
Pancreas	VI*c*	10	185	6·5	5·7	8·8	21·0	5·21	341	6·6	—	—	—	—	—	—	—	—	—
Saline	VII	10	173	11·5	4·4	2·8	18·7	4·14	276	6·6	144	17·1	11·9	—	—	—	—	—	—
Posterior pituitary	VII*a*	5	162	10·2	5·4	6·4	22·0	6·15	450	7·3	140	12·8	9·12	—	—	—	—	—	—
"Pituitrin"	VII*b*	10	162	10·6	1·7	3·2	15·5	4·16	246	6·0	131	14·0	10·7	—	—	—	—	—	—

* "Carcase" in series VI and VI*a* = body without liver, kidneys and intestines. "Carcase" in series VII, VII*a*, *b* = body without liver.

Extracts from other tissues.

To determine the effect of extracts from tissues other than the anterior pituitary, extracts were prepared from fresh beef liver and pancreas by the procedure described above for the anterior lobe of the pituitary. The results of the injection of these materials and similar doses of anterior pituitary extract are summarized in Table VI, series VI, VI *a*, *b*, *c*. The figures suggest that there may be small amounts of the substance producing the effect on liver fat in liver tissue, but we do not consider the evidence conclusive. With pancreas there is even less evidence. The rate of weight loss of the animals receiving the liver and pancreas extracts, as well as those injected with the anterior pituitary material, was less than that of the controls. This effect, which is probably due to a retention of water, is therefore not specific for the anterior pituitary.

In the latter part of the same table another series of experiments is shown in which an extract of dried posterior pituitary glands and of "pituitrin" (Parke, Davis and Co., 10 units per c.c.) was injected. The extract was prepared by the method outlined above and was given in exactly the same dosage and under the same conditions as that from the anterior pituitary. The effect on liver fat of this preparation and of the commercial "pituitrin" is shown in Table VI, series VII, VII *a* and *b*. On each of the 3 days 1 c.c. of the "pituitrin" was injected subcutaneously. Under the conditions of our experiments, which it will be noted are quite different from those of other workers, the increase in liver fat produced by the commercial preparation containing the oxytocic and pressor principles of the posterior pituitary is no greater than that observed in the control rats which received saline only. There is a slight increase in total liver fat in the animals receiving our posterior lobe preparation. The change is of the same order as that produced by the liver extract. These findings support the conclusion that the substance responsible for the increase in liver fat is associated with the anterior and not the posterior lobe of the pituitary. They must, however, be considered in light of the apparently contradictory results of other workers. In this connection it is necessary to point out that both the posterior lobe preparation which we have made and the commercial "pituitrin" consistently cause, with the doses used, a profound prostration of the animals soon after each injection. While all the animals survived the three doses of "pituitrin", five of those receiving our posterior lobe preparation succumbed. This is not surprising since the

dose of this latter material provided several times as much of the pressor principle as did that of the commercial "pituitrin". The same amount of the anterior lobe material has never produced any obvious effect on the general condition of the animals. They have always survived the three injections and appeared to be in excellent health at the end of the experiment.

In some of the experiments the kidneys and the small and large intestine were removed from each animal (Tables V, VI, series VI and VI*a*). The figures for the fat contents suggest that kidney fat is slightly increased and intestine fat slightly decreased by the injection of the anterior pituitary material. This is true in the case of both the fed and fasted animals, but the significance of the finding for the intestinal fat of the fed animals may be doubted since the fat of the intestinal contents was not determined.

Ketone bodies of rabbits' blood.

In confirmation of the results of Magistris [1932] it was found that the maximum rise in the ketone bodies of the blood of rabbits occurred within 2–3 hours after the subcutaneous administration of the extract which, it must be noted, was prepared by a procedure quite different from that used to make the material administered to the rats. The material given to the rabbits does, however, produce an increase in the liver fat of the rat, but the effect is less marked than that elicited by the alkaline extract of the dry powder. In a series of twelve normal male rabbits that received a dose of the extract from 80 up to 170 mg. of anterior lobe powder, ketosis was observed. There were considerable variations in its degree which did not regularly correspond to the size of the dose. But the ketosis rose from an average initial value of 4·35 mg. per 100 c.c. blood (1·3–6·3) to an average of 13·9; the smallest increase was from 4·5 to 6·5 mg. (dose 170 mg.), the largest from 5·6 to 29·6 (dose 155 mg.). In one case with a dose of 85 mg. the rise was from 4·5 initially to 20·7.

The rise was also noted after thyroidectomy up to the eighteenth day. With a dose of 160 mg. of anterior lobe powder in thirteen thyroidectomized rabbits it rose from an average initial value of 4·63 mg. per 100 c.c. blood (2·7–9·6) to the mean figure 10·26. In one case there was no increase but a diminution of 1·2 mg.; in the twelve others the increase varied from 1·6 to 14·5 mg. The length of time that had elapsed since the operation ranged from 4 to 18 days, but within these limits did not seem to affect the results. In two thyroidectomized animals the ex-

periment was repeated after 4 and 6 weeks respectively, and then they did not respond. In these two the effect of the same dose after administration of thyroid by mouth was compared with the effect before the thyroid feeding—the result was a return of the response. Four of the other thyroidectomized rabbits were also tested, after as well as before, thyroid feeding, and they also responded more vigorously as a result of the administration; the one animal for instance which failed to show ketosis within 18 days of the operation responded after receiving thyroid by an increase of 10 mg. per 100 c.c.

Discussion.

It is difficult to compare our results with those of previous workers in this field, since the fractions prepared from extracts of beef anterior pituitary glands, which we have used, may or may not contain the same active substances as those studied by other investigators. Furthermore, we have administered the material daily for a period of 3 days, and have determined the fat content of the liver only at the end of that period. Steppuhn [1934] reported an increase in liver fat within 4 hours after the injection of an anterior lobe extract, when small doses were used. With larger amounts a decrease in liver fat was found. Schultze [1933], on the other hand, reported a consistent decrease in the fat content of the liver. Very recently Anselmino, Effkemann and Hoffmann [1935] reported that there is an increase in the unsaturated fatty acids of the liver within a few hours after the administration of an anterior lobe preparation containing a ketogenic substance, but they find the effect on the total fatty acid content quite indefinite. Raab [1934] believes that a hormone responsible for the decrease in blood fat and the increase in liver fat is found in both the anterior and posterior lobes of the pituitary. He has named the substance responsible for this effect "lipoitrin". The relation of this factor to that which produces the mobilization of fat under the conditions of our experiments is as yet unknown.

It will be noted in Table II of this paper that the extent of fat deposition is in some cases almost as great as that which can be consistently produced in the white rat by any of the means at our disposal. In four of the nineteen animals the fat content was greater than 17 p.c. A histological examination of these livers made by our colleague, Dr MacLean, shows that the fat is present in small globules, and that the condition resembles that produced by cholesterol feeding more than

that usually seen in the livers of animals receiving a diet low in "lipotropic" factors. Histologically the picture is that of intense fatty infiltration with some signs of degenerative changes. The results might be quite different, however, if the deposition were produced more gradually by smaller doses of the extract. While it is practically certain that in the fasted animals the excess liver fat will be chemically similar to that present in the depots, we propose to investigate the point in subsequent experiments.

Very little comment on the increased ketone body excretion is necessary, since this has been discussed by previous workers. The responses are extremely variable, although, in a general way, in the fasted animals the extent is proportional to that of the deposition of fat in the liver. It is interesting that while there is a very definite increase of liver fat in the fed animals receiving the extract, the ketone body excretion may be very slight. In our hands the change in the amount of liver fat provides a more satisfactory foundation for a biological test than does the ketone body excretion. It must, however, be pointed out quite definitely here that there is no direct evidence for the assumption that the substance which produces ketosis is identical with that which causes an increase in liver fat. It appears that the whole subject can be advanced most rapidly by adopting a suitable biological test and attempting to obtain the substance responsible for the particular effect in a state of chemical purity. The increase in liver fat is being used for this purpose in these laboratories.

The decrease in body fat which may be produced by injection of anterior pituitary extracts was apparently first observed by Schäfer [1931]. In our fasted animals the decrease in total body fat has been, in each experiment, significantly greater in the groups which receive the anterior lobe extract. The figure suggest that this is also the case in the fed animals. *While the amount of fat lost from the depots is much greater than that which can be accounted for by the increase in liver fat plus the amount necessary to provide the ketone bodies, the finding that over* 50 *p.c. of the fat lost by the depots accumulates in one organ, the liver, cannot be without significance in fat metabolism.* A further discussion of this situation may profitably await the results of metabolism determinations which we hope to make. The adiposity associated with pituitary disturbance or hypothalamic lesions need only be mentioned for its possible significance in relation to the fall in depot fat produced by injections of anterior pituitary preparations to be appreciated.

Examination of the tables reveals the interesting fact that in the

fasting animals the average loss in weight of those receiving the anterior pituitary extracts was less than in the controls that received saline, while in the fed animals the gain in weight, where this occurred, was slightly greater than in the controls. In some cases the increase in weight of the animals receiving the extract was greater than that of the food eaten over the same period. This result indicates a positive water balance, but direct determinations of water content have not as yet been made. This phenomenon is not of particular interest to us at the present time since it is elicited by injections of liver and pancreas extracts as well as by those from the anterior pituitary.

The findings of Coope and Chamberlain [1925], which have been confirmed by Oshima [1929], Steppuhn *et al.* [1929], Hynd and Rotter [1932], and White [1933], raise a very interesting point. These workers observed a deposition of liver fat when very large doses of posterior pituitary extracts were administered to rabbits and rats. The results in rats are in no case as definite as under the conditions of our experiments and in several instances it is permissible to suspect that the injection of "pituitrin" produced little if any effect on liver fat. The question arises, however, whether the posterior lobe preparations used by these investigators were contaminated with the substance, obtainable from the anterior lobe, which produces an increase in liver fat under the conditions of our experiments. Several of these workers have suggested that the effect on liver fat is attributable to the pressor principle of the posterior pituitary. Coope and Chamberlain obtained only a slight effect with a commercial anterior lobe preparation and they suggest that this may be due to a trace of "pituitrin". The anterior lobes of the pituitaries which we have used were, as stated above, carefully separated from the posterior lobes, and the extracts did not contain more than an insignificant amount of the pressor principle. Acid and alkaline extracts of the dried anterior lobe powder were administered intravenously to etherized and also to pithed cats. A very slight fall in blood-pressure was obtained in the etherized cats, and an almost insignificant rise was found in pithed preparations. Our negative findings with one commercial posterior lobe preparation and the slight effect of an extract of dried posterior lobes which contained large amounts of the pressor principle and which was prepared by the method which has provided us with active material from the anterior lobes, strongly favour the view that the anterior and not the posterior lobe of the pituitary is the primary source of the principle which causes the deposition of liver fat we have described.

The results with an extract of pancreas were essentially negative, but the fraction prepared from beef liver produced a slight but apparently definite increase in the concentration of liver fat in the fasted animals. The total fat content of the livers, however, was only slightly greater than that of the control animals. No great significance can, at present, be attached to these results.

Very little comment on the increase in the ketone body content of rabbits' blood produced by the injection of the anterior pituitary extracts is required. Our results confirm those of Magistris, who used the same species, and in so far as the effects of thyroidectomy are concerned, those of Black, Collip and Thomson [1934], who studied the ketone body excretion in rats. The rise in ketone body content of the blood of thyroidectomized rabbits can be demonstrated soon after the operation, but when the signs of hypothyroidism are more definite the effect is not elicited. The responses can be restored by the administration of thyroid material. These results and those previously obtained by Black, Collip and Thomson indicate that the failure of Eitel, Löhr and Loeser [1933] to obtain a response in thyroidectomized animals was due to the fact that their animals were in an advanced stage of hypothroidism when the injections were made. Their inference that the thyrotropic hormone of the anterior pituitary is the ketogenic substance is, therefore, not justified by the evidence at present available. The mechanism by which the ketogenic response is restored by thyroid feeding is not known, but since the liver appears to be the principal, if not the only, site of formation of ketone bodies, the action of thyroxine on the ketogenic ability of this organ will probably repay further study.

Only two other points need be mentioned at this time. Clinicians have already administered to patients large doses of crude anterior pituitary preparations containing the ketogenic substance. In view of the very rapid and extensive increase in size of the liver which we have observed, it will not be surprising if similar observations are made on human subjects. The increase in liver fat produced by large doses of the anterior pituitary preparation is not prevented by the administration of choline, but we have some evidence that the removal of the fat from the liver when the injection of the ketogenic extract is discontinued, is accelerated when choline is added to the diet. This latter point is being investigated in greater detail.

A detailed discussion of the physiological significance of the effects described in this paper may profitably await the results of further experiments.

Summary and conclusions.

1. A rapid increase in size and an intense fatty infiltration of the liver, with some signs of degenerative changes, may be produced in fasting white rats by the daily administration for a period of three days of a fraction prepared from beef anterior pituitary glands. The average figures for the total liver fat and percentage liver fat for forty-seven animals injected with the anterior pituitary preparation were 892 mg. and 13·4 p.c., while those for approximately the same number of controls were 244 mg. and 5·7 p.c. The relation of these findings to those of other workers in this field is discussed.

2. In the fasted rats the increase in liver fat produced by the anterior pituitary preparation is accompanied by a decrease in total body fat and an increased excretion of ketone bodies.

3. Similar but less marked effects on the size of the liver and its fat content were produced by the same means in fed rats. The results suggest but do not prove that there may also be an increased ketone body excretion and a decrease in body fat in the fed animals.

4. An extract of posterior lobes of the pituitary, prepared by an identical method, produced only a slight rise in liver fat. Similar results were secured with material from liver and even less effect when pancreas was the source. A commercial "pituitrin", in the dose used, had no effect.

5. The rise in the ketone body content of rabbits' blood after a single injection of an anterior pituitary preparation reached a maximal value in from 2 to 3 hours, confirming Magistris. This effect could be demonstrated soon after complete thyroidectomy, confirming Black, Collip and Thomson. The rise was not elicited in animals exhibiting signs of advanced hypothyroidism but was observed again in the same animals when this condition had been alleviated by thyroid feeding.

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