CI. DETERMINATION OF THE KETOGENIC ACTIVITY OF EXTRACTS OF ENDOCRINE ORGANS

By CHARLES HORACE GRAY¹

*From the Graham Research Laboratories, University
College Hospital Medical School, London*

(Received 1 March 1938)

NUMEROUS workers have reported the ketogenic effects of crude extracts of the anterior hypophysis. Burn & Ling [1933] found that an alkaline extract of fresh glands after neutralization with acetic acid increased total ketonuria in seven rats out of seventeen fed on a high fat diet. All seven were females, and no rise was recorded in male rats. This work was confirmed by Black *et al.* [1934], who showed that ketogenic responses to extracts of the anterior pituitary could also be obtained in fasting rats.

Meanwhile, Funk [1933] had isolated a material by benzoic acid adsorption from pregnancy or normal urine which caused an increase in ketonuria of rats; it is not clear from his paper whether these were fasting or on a fat diet. Butts *et al.* [1934] have shown that extracts prepared according to Burn and Ling greatly exaggerate the ketonuria of fasting rats and that of rats fed with acetoacetic acid. As marked responses were obtained with male as with female rats, and with castrated animals as with normal ones. Best & Campbell [1936] using a neutralized alkaline pituitary extract precipitated with alcohol, again confirmed the ketogenic effect on fasting female rats, and noted in addition a very great increase of liver fat.

Parallel with this work, there have been numerous attempts to determine the activity of similar extracts by their power to increase ketonaemia in rats and rabbits. Anselmino & Hoffmann [1931] showed that simple extracts of dried anterior hypophysis produced a rise in the blood ketones of non-fasting rats. The active constituent was water-soluble, but insoluble in ether, chloroform and in high concentrations of alcohol; it was ultrafiltrable and thermolabile. Magistris [1932] confirmed this work using rabbits, and Best & Campbell [1936] obtained similar results using a neutralized acid extract of fresh glands. The degree of ketonaemia showed large variations which bore no constant relation to the dose administered. The extract used also increased liver fat in female rats, but less markedly than did alkaline extracts of the same powder. Unfortunately these workers do not report the ketogenic activity of their acid extract in rats. They state also that "the rise in blood ketones (of the rabbit) was also noted after thyroidectomy up to the eighteenth day". After 18 days the response was negative, but returned after the administration of thyroid by mouth.

Black [1935] and Bennett [1937] have shown that crude extracts lose their ketogenic activity when administered daily over a period of several months to rats fed on a normal diet. The animals were fasted for 2 or 3 days at intervals in order to determine the ketogenic efficacy of the extracts. Anselmino & Hoffmann [1936, 1] have confirmed their previous work and have shown further

¹ Graham Scholar, University of London.

that extracts of sheep anterior pituitaries are more strongly ketogenic than those from cattle and the latter more so than those from the pig.

Thus, there appears little reason to doubt that there is present in the anterior lobe of the hypophysis a factor which stimulates ketosis. The well-known association between ketosis and fat deposition in the liver suggests that the ketogenic factor might be identical with that considered to increase the liver fat of fasting female rats.

More recently extracts of other organs have been found to possess ketogenic activity, e.g. ovarian extracts [Anselmino & Hoffmann, 1936, 2] and massive doses of adrenal cortical extracts [MacKay & Barnes, 1936]. Thus the point is raised as to whether ketogenic activity is shared by a number of endocrine organs, or whether there is one particular organ directly controlling ketogenesis, this organ being in its turn under the control of some other organ. The work of Long & Lukens [1936] stresses the importance of the role played by the adrenal cortex in ketogenesis.

In view of the inconsistent responses obtained by previous workers it becomes important to determine a method of estimating ketogenic activity giving the most consistent results in a simple manner.

METHODS. I. CHEMICAL

Preparation of anterior pituitary extract

Aqueous extract. 1 g. of acetone-dried ox anterior lobes was triturated with a little water in a mortar and the volume gradually made up to 20–25 ml. The suspension was then stirred mechanically for 12–24 hr., centrifuged and the supernatant injected, 10 ml. into a rabbit or 3 ml. into a rat.

Saline extract. A simple saline extract of fresh ox anterior lobes prepared by Dr F. G. Young of The National Institute for Medical Research, Hampstead.

Alkaline extract. Prepared according to Burn & Ling [1933].

Acid extract. Prepared according to Best & Campbell [1936].

Thyrotropic extract. Prepared by Dr F. G. Young and found by Marks & Young [1938] to possess fatty liver-producing activity in rabbits.

Adrenaline-free extract of ox adrenal. Prepared by Dr F. G. Young.

Estimation of ketone bodies in the blood

Several methods are available for the estimation of ketone bodies in blood, but choice is limited by the small quantities of blood available in the type of experiment to be described. Thus the Denigès-Van Slyke method is useless owing to its insensitivity. The Behre and Benedict colorimetric method even in the micro-modification of Cantoni [1935] is still not sufficiently sensitive. We are thus left with the more sensitive but much less specific iodimetric method. The determination of the small difference in acetone to be measured is on safer grounds since a titrimetric procedure is used. The Engfeld-Pincussen [1928] method is entirely satisfactory. In the experiments using rabbits, both the Cantoni and Engfeld-Pincussen methods were employed.

Estimation of ketone bodies in the urine

The choice of method here is simpler. The amount of ketone bodies excreted daily by rats is so variable that increases of over 200–300 % alone can be regarded as significant. The actual amount excreted daily by three rats on a fat

diet gives 1–6 mg. precipitate with the Denigès-Van Slyke reagent, so that this method is unquestionably the most suitable.

Estimation of liver fat

The method of Leathes and Raper [1925] was followed in all essential details.

METHODS. II. BIOLOGICAL

Rats. These were all of Wistar strain and were killed for estimation of liver fat by a blow on the head. The urinary output of a single rat is very small and this is particularly the case when anterior pituitary extracts have been administered. Rats were therefore put in groups of three in each metabolism cage and analyses carried out on the mixed urine of the group, the urine being preserved under toluene. In this way, individual variations of the rats were partly eliminated, but even so there still persisted a great variation in the ketone body excretions of different groups of rats treated under identical conditions (see Fig. 1).

Fig. 1.

Fig. 2.

Fig. 1. Ketonuria of 4 groups of rats fed on a 75% butter-fat diet. July, 1937.

Fig. 2. Ketonuria of 3 groups of 3 rats all fed on a butter-fat diet. Group 1, July 1937, 97% fat. Group 2, Sept. 1937, 97% fat. Group 3, July 1937, 75% fat, included one pregnant rat.

Because of the marked sex variation in the ketonuria after injection of pituitary extracts each group of rats should be exclusively of the same sex. Pregnant animals must be excluded since pregnancy greatly increases the ketosis of rats on a fat diet [Burn & Ling, 1933]. This is well shown in Fig. 2,

which also confirms the findings of these workers in showing a very marked seasonal variation in the ketonuria of rats fed on a high fat diet.

The early part of this work was carried out using rats fed on a mixture of 97 % fat and 3 % salts, but it was soon realized that rats varied a great deal in the amount of this diet which they ate so that it was uncertain whether they were on a high fat diet or were partly or even wholly starving. It was therefore considered preferable to starve them over a short period rather than maintain them doubtfully on a fat diet.

All injections were administered subcutaneously.

Rabbits. A single rabbit was used in each experiment, bleeding from the marginal vein being effected without the use of irritants.

RESULTS

Rabbits. Rabbits were injected with various types of anterior pituitary extract, and the blood ketones estimated by the Cantoni method hourly for 5 hr. after the injection. Of 68 rabbits 22 showed slight increases of blood ketones detectable by this method of determination.

In a further series of 12 rabbits similarly injected with various types of extract the blood ketones were determined by the Engfeld-Pincussen method at the time of the injection and 3 and 5 hr. after. In each case a definite rise in β -hydroxybutyric acid content of the blood occurred and in 4 the concentration 5 hr. after the injection exceeded 22 mg. per 100 ml. of blood, and was therefore not accurately measurable without modification of the technique. The normal value varied from 3.6 to 9.0 mg. per 100 ml. of blood. In the other 8 rabbits there were increases varying from 0.9 to 12.0 mg./100 ml.

It therefore appears that of the first group of 68, in which ketones were estimated by the Cantoni method, 22 presumably showed a considerable rise in blood ketones while the remaining 46 may have shown rises not detectable by this method. The fact that all rabbits in the second series exhibited rises in ketone bodies as measured iodimetrically suggests that this was the case. The blood ketones of 6 of these 12 rabbits were estimated by both methods and rises in blood ketones were detectable by the Cantoni method when they were very great as determined iodimetrically.

The determination of ketogenic activity using rabbits and determining the blood ketones by the Engfeld-Pincussen method was tedious and lengthy as it was considered necessary to use at least 12 rabbits for investigating an extract of unknown ketogenic activity.

Rats. The early part of this work was carried out on rats fed on a diet of 97 % butter-fat and 3 % salt mixture. Fig. 3 shows that over 15 days a control group of three rats excreted between 1.2 and 7.5 mg. daily of "total ketone bodies" expressed as acetone. Four injections of a saline extract of anterior pituitary into each rat of the experimental groups produced rises in ketonuria above the level previously existent and two of these rises were above the maximum level attained during the whole period of experiment by the control group. Injection of neutralized alkaline extract also produced an increase in ketonuria with a dose of 2 ml.

Definite conclusions from this experiment must be accepted with reserve, since it is open to two criticisms: firstly, no attempt had been made to segregate the sexes and secondly, repeated injections were made into the same animals. However in general this experiment confirmed the ketogenic activity of anterior pituitary extracts.

Fig. 3.

Fig. 4.

Fig. 3. Ketonuria of 4 groups of 3 rats fed 97% fat, Sept. 1937. 3 groups received anterior pituitary extract.

Fig. 4. Effect of anterior pituitary extracts on rats fed on a 97% fat diet, Oct. 1937.

Fig. 5.

Fig. 6.

Fig. 5. Ketogenic activity of extracts of the anterior pituitary rats fed on a 97% fat diet, Nov. 1937, cross-control.

Fig. 6. Effect of cortin (organon) on ketonuria of rats fed on a 97% fat diet, Nov. 1937.

An attempt to obtain a reliable test of ketogenic activity was made by maintaining four groups of three rats each on a 97 % fat diet over 10 days. Two groups were male and two female. On the 5th day one male and one female group were injected and on the 7th day the others were injected. In this way the uninjected rats acted as controls at the time when the others were injected and the former were later injected with the others as controls. Fig. 4 illustrates the results of this experiment and confirms the finding that female rats are more sensitive than male rats under these conditions to the ketogenic factor in these extracts.

It appeared possible that the time of experiment could be cut down, and accordingly four similar groups were given a fat diet for 7 days, with injections on the 2nd and 4th days with the same system of cross-control as before. Fig. 5 shows that the increases in ketonuria following an injection of thyrotropic extract were greater than in the previous experiments. Again the average response by females was greater than that by males, although one group of males showed a greater response than did one group of females. A sample of Cortin (Organon) was investigated in this way for ketogenic activity with the negative results shown in Fig. 6. This experiment formed an excellent control in demonstrating that ketogenesis is not stimulated by a non-specific tissue extract.

In all of these experiments, rats of Wistar strain had been used and as the supply was limited an attempt was made to employ ordinary stock rats but these produced a more variable ketonuria than those of Wistar strain. In two groups the ketonuria reached 33 and 11 mg. daily per group before any injection had been given. In all succeeding experiments Wistar rats were employed.

Preliminary experiments using rats under fasting conditions gave promising results although there was still some variation in the responses of the four groups. Best & Campbell [1936] have shown that female rats given daily subcutaneous doses of anterior pituitary extract during a 3-day fast show marked increase of liver fat as well as greatly augmented ketonuria. It appeared possible that by administering an active extract more frequently during a shorter period of fasting the onset of these effects might be accelerated.

Actually three groups of rats were fasted overnight; one group was used as control, another given injections at 8 a.m., 4 p.m., 12 midnight and 8 a.m. the following morning and the remaining group given a single injection of extract at 8 a.m. on the first morning after the preliminary fast of 12 hr. Urine was analysed for ketone bodies every 8 hr. Table I, exp. 1 shows that under these conditions the control groups produced no ketonuria, but those receiving a single injection showed a ketonuria commencing between 8 and 16 hr. after the injection and reaching a maximum of 2.7 mg. for the 8-hr. period ending 24 hr. after the injection. The liver fat of these animals averaged 0.36 g./100 g. rat as compared with the controls which had 0.16 g./100 g. rat. Those receiving repeated doses of extract similarly showed a ketonuria commencing between 8 and 16 hr. after the first injection and steadily rising to 4.8 mg. at the end of the experiment. The liver fat of these averaged 0.65 g./100 g. rat. Table I also shows results of further experiments on these lines. Exp. 3 suggests that the ketogenic response of males under these conditions is greater than that of females. In exp. 2 and 3 the urinary volumes were increased by giving each rat 6 ml. of water by stomach tube every 8 hr.

The ketonuria following a single injection in females was very small, but the increases in liver fat very definite, so the experiments were repeated with a single dose of extract and modified in such a way that the relationship between ketonuria and liver fat could be followed. The rats were fasted overnight and, with the exception of the controls, given an injection of an active extract. Groups

Table I. *Ketonuria and liver fat following thyrotropic extract of the anterior pituitary administered after 12 hr. fasting*

Series I. No injection.

Series II. Four injections of thyrotropic extract at 8-hr intervals.

Series III. A single injection of thyrotropic extract at commencement of the experiment.

All injections consisted of 3 ml. of extract from one anterior lobe.

Series	Rat	Wt. g.	Liver fat g./100 g. rat	Ketonuria mg./3 rats/8 hr.			
				0-8 hr.	8-16 hr.	16-24 hr .	24-30 hr
Females							
I	1	250	0.17	Av. = 0.16	0.0	0.0	0.0
	2	230	0.13				
	3	215	0.19				
II	4	245	0.68	Av. = 0.65	0.0	2.5	3.4
	5	200	0.65				
	6	200	0.61				
III	7	260	0.17	Av. = 0.36	0.0	1.7	2.7
	8	200	0.50				
	9	225	0.42				
Females							
I	1	215	—	Av. = 0.19	0.0	0.0	0.0
	2	205	0.21				
	3	220	0.17				
II	4	240	0.60	Av. = 0.55	0.3	5.0	8.8
	5	220	0.47				
	6	195	0.57				
III	7	190	0.42	Av. = 0.32	0.0	0.8	0.4
	8	200	0.28				
	9	200	0.27				
Males							
III	1	200	0.22	Av. = 0.29	31.4	128.6	126.1
	2	200	0.40				
	3	230	0.25				

In this series using male rats only three injections were given during the experimental period.

of rats were killed every 4 hr., the controls being killed simultaneously with the 12-hr. group. All surviving rats except the controls were given 6 ml. of water by stomach tube every 4 hr., in order that the urinary volumes should be adequate. In this way, urinary ketones and liver fats were determined 4, 8 and 12 hr. after injection. Controls were not killed at 4 and 8 hr. since any changes would be still more obvious at 12 hr. Table II illustrates the results obtained in such experiments. In exp. 1 with male rats there was practically no ketonuria, while the liver fat increased from an average control level of 0.093-0.34 g./100 g. rat after 4 hr. and to 0.29 g./100 g. rat after 8 hr. Unfortunately only one figure for the liver fat of the group at 12 hr. was obtained. In exp. 2 of Table II a ketonuria occurred beginning during the first 4 hr. after the injection and rising to 36.2 mg. for the whole period of experiment, while the liver fat showed no significant change. In exp. 2 of Table II rats were not killed at 4 hr. after the injection. In a further experiment in which an accidentally inactivated thyrotropic extract was used there was no ketonuria and no significant change in liver fat.

It was considered possible that by increasing the duration of the previous fast more consistent results might be obtained and therefore the experiment was redesigned. The total period of fasting was 48 hr. and began at 9 p.m.; 24 hr. later the 12 rats were transferred to 4 Hopkins metabolism cages, 3 to each cage.

Table II. *Ketonuria and liver fat following thyrotropic extract of the anterior pituitary administered after 12 hr. fasting*

All the injected animals received 3 ml. of extract from one anterior lobe.

	Rat	Wt. g.	Liver fat g./100 g. rat	Mg. ketones/ 3 rats	
Exp. 1. Males					
Control	1	200	0.095	Av. = 0.093	0.0
	2	195	0.092		
	3	205	0.093		
4 hr.	4	190	0.49	Av. = 0.34	0.1
	5	220	0.25		
	6	190	0.28		
8 hr.	7	215	0.30	Av. = 0.29	0.0
	8	240	0.25		
	9	200	0.32		
12 hr.	10	215	—	0.14	0.0
	11	200	0.14		
	12	200	—		
Exp. 2. Males					
Control	1	125	0.17	Av. = 0.13	0.0
	2	135	0.12		
	3	125	0.11		
4 hr.	4	—	—	—	1.8
	5	—	—		
	6	—	—		
8 hr.	7	160	0.13	Av. = 0.14	9.5
	8	110	0.15		
	9	150	0.14		
12 hr.	10	175	0.16	Av. = 0.20	36.2
	11	125	0.23		
	12	140	0.22		

The urine passed by all groups for the next 12 hr. was collected, pooled and analysed for ketones. At 9 a.m., 12 hr. after the rats had been transferred to the cages, injections of extract subcutaneously and 6 ml. of water by stomach tube were given to all rats of the first three groups; 4 hr. later a group of rats was killed and their liver fats and excreted ketones determined. The remainder of the injected rats were given 6 ml. more of water by stomach tube and 4 hr. later another group of rats was killed. The remaining three injected rats were again given 6 ml. of water and 4 hr. later, at 9 p.m., all the surviving rats were killed. In this way the ketone bodies excreted and the liver fats could be determined 4, 8 and 12 hr. after the injection.

The results of this type of experiment were very satisfactory. With male rats there was marked ketonuria beginning during the first 4 hr. after the injection and steadily rising until the end of the experiment, the controls excreting no ketones. The liver fats were appreciably increased 8 hr. after the injection and showed a still further rise at 12 hr. after the injection. This is clearly shown in Tables III and IV. It is impossible to determine whether there is an increase in the liver fat after 4 hr. without employing a very much larger number of animals.

Such experiments carried out on female rats were less satisfactory in that there was a much less marked ketonuria with greater increases of liver fat. The relationship of this to the findings of other workers will be discussed later.

Table III. *Ketonuria and liver fat following saline extract of the anterior pituitary administered after 36 hr. fasting*

All the injected animals received 2 ml. of extract from one anterior lobe.

	Rat	Wt. g.	Liver fat g./100 g. rat	Mg. ketones/ 3 rats	
Exp. 1. Males					
Control	1	155	0.15	Av. = 0.17	0.0
	2	150	0.19		
	3	145	0.17		
4 hr.	4	175	0.23	Av. = 0.19	1.8
	5	175	0.18		
	6	160	0.16		
8 hr.	7	195	0.26	Av. = 0.26	24.8
	8	155	0.28		
	9	160	0.24		
12 hr.	10	160	0.30	Av. = 0.30	108.0
	11	150	0.32		
	12	140	0.29		
Exp. 2. Males					
Control	1	160	0.13	Av. = 0.16	0.7
	2	155	0.19		
	3	145	0.17		
4 hr.	4	155	0.10	Av. = 0.13	1.0
	5	185	0.14		
	6	185	0.14		
8 hr.	7	160	0.23	Av. = 0.23	22.3
	8	150	0.27		
	9	185	0.20		
12 hr.	10	180	0.24	Av. = 0.24	182.0
	11	175	0.25		
	12	155	0.23		
Exp. 3. Females					
Control	1	175	0.22	Av. = 0.22	0.0
	2	170	0.21		
	3	170	0.22		
4 hr.	4	185	0.21	Av. = 0.19	0.0
	5	160	0.21		
	6	180	0.16		
8 hr.	7	170	0.24	Av. = 0.27	6.8
	8	220	0.27		
	9	155	0.31		
12 hr.	10	180	0.40	Av. = 0.38	9.1
	11	170	0.36		
	12	200	0.39		
Exp. 4. Females					
Control	1	150	0.19	Av. = 0.23	0.0
	2	165	0.27		
	3	160	—		
4 hr.	4	135	0.26	Av. = 0.28	0.0
	5	170	0.26		
	6	155	0.32		
8 hr.	7	175	—	Av. = 0.23	3.0
	8	165	0.26		
	9	165	0.18		
12 hr.	10	155	0.23	Av. = 0.34	10.2
	11	135	0.40		
	12	135	0.40		

Table III (cont.)

	Rat	Wt. g.	Liver fat g./100 g. rat	Mg. ketones/ 3 rats
Exp. 5. Females				
Control	1	165	0.18	0.0
	2	150	0.17	
	3	160	0.16	
4 hr.	4	150	0.23	0.0
	5	165	0.30	
	6	175	0.22	
8 hr.	7	135	0.37	1.3
	8	160	0.24	
	9	160	—	
12 hr.	10	160	0.34	6.3
	11	150	0.37	
	12	160	0.34	

Table IV. *Ketonuria and liver fat following thyrotropic extract of the anterior pituitary. Male rats after 36 hr. fasting*

All the injected animals received 3 ml. of extract from one anterior lobe.

	Rat	Wt. g.	Liver fat g./100 g. rat	Mg. ketones/ 3 rats
Control	1	125	0.15	0.0
	2	135	0.12	
	3	125	0.16	
4 hr.	4	—	—	3.5
	5	—	—	
	6	—	—	
8 hr.	7	165	—	36.0
	8	140	0.14	
	9	165	0.18	
12 hr.	10	135	0.18	100.0
	11	125	0.21	
	12	120	0.24	

Table V shows an experiment carried out using an adrenaline-free extract of ox adrenal. There was no ketonuria and no increase in liver fat and this experiment together with that shown in Fig. 6, forms an excellent control in showing that ketogenesis is not stimulated by non-specific tissue extracts.

DISCUSSION

From the results it seems preferable to use fasting rats for the determination of ketogenic activity of extracts of endocrine organs, rather than fat-fed animals as the former give more consistent results. Black *et al.* [1934] similarly preferred to study the influence of pituitary extracts on the ketone body excretion of the fasting animal, thereby obtaining consistently low control values. Butts *et al.* [1934] also found regular responses in fasting rats.

The sex variations in the responses of the rats are difficult to explain. Burn & Ling [1933] found no increased ketonuria in fat-fed male rats given anterior pituitary extracts. Best & Campbell [1936] employed only female rats and do not refer to results obtained in males; more recently [1938] they say "the production of ketone bodies (due both to fasting and to fasting with the administration of anterior pituitary extract) is greater in female guinea-pigs than in males". However these differences were slight. Deuel *et al.* [1937] find that

Table V. *Ketonuria and liver fat following an extract of the adrenal administered after 36 hr. fasting*

All injected animals received 3 ml. of extract.

	Rat	Wt. g.	Liver fat g./100 g. rat	Mg. ketones/ 3 rats
Exp. 1. Females				
Control	1	125	0.20	Av. = 0.17
	2	180	0.14	
	3	130	0.18	
4 hr.	4	95	0.16	Av. = 0.15
	5	110	0.14	
	6	95	0.14	
8 hr.	7	125	0.18	Av. = 0.16
	8	140	0.19	
	9	155	0.12	
12 hr.	10	115	0.12	Av. = 0.15
	11	115	0.16	
	12	105	0.17	
Exp. 2. Females				
Control	1	75	0.12	Av. = 0.13
	2	75	0.17	
	3	90	0.11	
4 hr.	4	175	0.11	Av. = 0.14
	5	140	0.14	
	6	120	0.17	
8 hr.	7	135	0.22	Av. = 0.25
	8	115	0.23	
	9	95	0.31	
12 hr.	10	80	0.09	Av. = 0.12
	11	90	0.14	
	12	105	0.14	

female rats consistently have a higher level of ketonuria than males during a fast following the administration of a high fat diet. The earlier findings described in this paper are in agreement with this, and indicate that on a high fat diet female rats exhibit a greater increase in ketonuria following the administration of extract of the anterior pituitary than males. The results of experiments using fasting rats in which males exhibit a greater ketonuria than females in response to anterior pituitary extracts are of great interest in view of the general agreement that in fat-fed rats experimental ketonuria is more easily induced in female than in male animals. Butts *et al.* [1934] found that fasting male and female rats gave equal ketogenic responses to anterior pituitary extracts. It would be unwise to speculate on this sex difference in the light of present knowledge.

Similarly, it is unwise to speculate on the cause of the enormous ketosis occurring in the pregnant rat on a high fat diet. That this is not peculiar to the rat has been shown by Bullock [1930] who finds that pregnant women pass acetone in their urine much more readily than non-pregnant women do, a finding which has been confirmed by the present author (unpublished observation).

Geelmuyden [1920] believes that the sequence of events in the production of ketones is:

Fat migration → fatty liver → ketosis.

Dible [1932] observes that in fasting rats "an animal whose urine gave a rapid and intense acetone reaction by the nitroprusside method at the time of

killing could be confidently expected to show a high fat content of the liver with an abundance of depot fat". Gray [1937], referring to infantile vomiting, observed that cases with high ketonaemia showed at autopsy marked hepatic fatty change. Best & Campbell [1938] believe that it is probable that the rise in liver fat precedes the increase in ketonuria.

The experiments of Table III show clearly that in female rats the increase in liver fat precedes the ketonuria when anterior pituitary extracts are administered after 36 hr. fasting; the rise in liver fat commences within 4 hr. of the injection. This is in agreement with Best and Campbell who find that the increase in liver fat occurs within 24 hr. of the injection and with those of Steppuhn [1934] who reports an increase in liver fat within 4 hr.

On the other hand, in male rats there was much greater ketonuria and much less increase in liver fat than in females. In males the ketonuria began within 4 hr. of the administration of the extract, but it is difficult to assess the time of onset of the increases in liver fat as they were so small.

On considering all the results obtained irrespective of sex those animals producing the greater ketonuria showed smaller increases in liver fat, and vice versa. If one considers that the ketonuria occurs at the expense of the fat brought to the liver, then clearly the relationship of the times of onset of ketosis and liver change cannot be rigid. The amount of liver fat must depend on the balance between fat mobilized from the depots and that catabolized by the liver. If fat catabolism can occur at a greater rate than fat can be brought to the liver, then clearly ketonuria will be more marked than and may precede the increase in liver fat. If, on the other hand, fat mobilization to the liver occurs more rapidly than that organ can catabolize it then the increase in liver fat may be more marked than the ketonuria and the sequence of events will depend on the relative rates of mobilization and catabolism.

These findings throw no light on the identity or otherwise of the ketogenic and "fatty liver-producing" factors of the anterior pituitary. It is therefore essential to determine the two activities simultaneously in any investigation of these actions of extracts of endocrine organs.

SUMMARY

1. The results of previous workers in regard to seasonal, sex and pregnancy variations of ketonuria in rats on a high fat diet are confirmed.
2. The ketogenic action of extracts of the anterior pituitary gland on fasting and fat-fed rats, as well as on fasting rabbits, has been confirmed.
3. Fasting rats give more consistent responses to ketogenic extracts than either fat-fed rats or fasting rabbits.
4. Ketogenic response to such extracts is greater in fasting male rats than in fasting female rats, while the increases in liver fat are greater in females.
5. After 36 hr. fasting, the administration of a ketogenic preparation to female rats resulted in a ketonuria which followed the increase in liver fat. In male rats under similar conditions the resulting increase in liver fat was so small that it was not possible to determine the time of onset in relationship to the commencement of ketonuria.
6. Evidence is presented suggesting that a large ketogenic response to anterior pituitary extracts is accompanied by a small increase in liver fat, and conversely; and that the effect on ketonuria should be studied together with that on liver fat. A test in which this may be done is described.

The expenses of this investigation were defrayed partly by a grant from the Medical Research Council and partly by a grant from the Graham Fund Committee of the University of London to both of which bodies my thanks are due. I have also to express my thanks to Dr F. G. Young of the National Institute for Medical Research for supplying many of the extracts used and also for much helpful criticism.

REFERENCES

- Anselmino & Hoffmann (1931). *Klin. Wschr.* **10**, 2380, 2383.
—— — (1936, 1). *Endokrinologie*, **17**, 1.
—— — (1936, 2). *Arch. Gynaek.* **162**, 176.
Bennett (1937). *Proc. Soc. exp. Biol.* **37**, 29.
Best & Campbell (1936). *J. Physiol.* **86**, 190.
—— — (1938). *J. Physiol.* **92**, 91.
Black, Collip & Thomson (1934). *J. Physiol.* **82**, 385.
Black (1935). *J. Physiol.* **84**, 15.
Bullock (1930). *J. Okla. med. Ass.* Abstracted in *J. Lab. clin. Med.* (1930), **16**, 214.
Burn & Ling (1933). *Quart. J. Pharm.* **6**, 31.
Butts, Cutler & Deuel (1934). *J. biol. Chem.* **105**, 45.
Cantoni (1935). *Biochem. Z.* **277**, 448.
Deuel, Hallmann & Murray (1937). *J. biol. Chem.* **119**, 257.
Dible (1932). *J. Path. Bact.* **35**, 451.
Engfeld (1925) in Pincussen (1928). *Mikromethodik.* (Leipzig: Thieme.)
Funk (1933). *Proc. Soc. biol. Chem. J. biol. Chem.* **100**, xliii.
Geelmuyden (1920). *Acta. med. skand.* **54**, 5.
Gray (1937). *Lancet*, **2**, 1017.
Leathes & Raper (1925). *The Fats.* (London: Longmans.)
Long & Lukens (1936). *Amer. J. Phys.* **116**, 96.
MacKay & Barnes (1936). *Proc. Soc. exp. Biol., N.Y.*, **35**, 177.
Magistris (1932). *Endokrinologie*, **11**, 176.
Marks & Young (1938). *J. Physiol.* (in the Press).
Steppuhn (1934). *Wien. Arch. inn. Med.* **26**, 87.