LXX. STUDIES ON THE METABOLISM OF ANIMALS ON A CARBOHYDRATE-FREE DIET. IV. THE EFFECT OF PITRESSIN AND PITOCIN ON THE DISTRIBUTION OF FAT AND GLYCOGEN IN THE LIVER AND MUSCLES OF ALBINO RATS.

By ALEXANDER HYND and DOROTHY LILLIE ROTTER.

From the Physiology Department, University of St Andrews.

(*Received March 14th, 1932.*)

In continuing the study of the distribution of glycogen and fat in the liver and muscles of animals under different nutritional conditions, described by us in previous papers [Hynd and Rotter, 1930; 1931, 1, 2], it was planned to investigate not only the effect of exogenous insulin but also the effects produced by the administration of those endocrine secretions, which are at present recognised as antagonistic to the action of the pancreatic hormone.

Though we have accumulated a considerable amount of data regarding the part played by the ductless glands concerned in the metabolism of carbohydrate and fat, the present paper is confined to a description of the results obtained with pitressin and pitocin, as other workers [Bischoff and Long, 1931] are evidently engaged in the same field. A full discussion of the significance of the results will be deferred until a later communication on the effect of pituitrin, and we hope, in subsequent papers, to deal with the effects of other ductless glands, namely the thyroid and the suprarenal.

It is now recognised that extracts of the posterior lobe of the pituitary body influence the metabolism of both fat and carbohydrate. The former was demonstrated experimentally for the first time by Coope and Chamberlain [1925], who found that the injection of pituitrin produced a fatty infiltration of the liver. This result, though denied by Van Dyke [1926], has been corroborated by other workers, and recently Raab [1930] has examined the effect on the blood-phosphatides of the two pituitrin constituents, "pitocin" and "pitressin," separated by Kamm *et al.* [1928]. Several years ago, Burn [1923] demonstrated that pituitrin had the property of checking the activity of insulin, and more recently this same worker [1929] arrived at the conclusion that the insulin-inhibiting effect of posterior lobe extract lay in the vasopressin ("pitressin"), and not in the oxytocin ("pitocin"). Magenta [1930], however, reached just the opposite view and reported that vasopressin and an anterior lobe extract did not modify the effect of insulin, while oxytocin was the active principle in pituitary-insulin antagonism.

In view of the contradictory reports in the literature, and the fact that the changes in glycogen and fat distribution have hitherto never been investigated simultaneously, it seemed desirable to undertake a further examination of the effects produced by the separated hormones of the posterior lobe of the pituitary.

EXPERIMENTAL.

The experiments here reported have been carried out on standard albino rats, 6–7 weeks old, the average body-weight being 80–100 g. The diets employed were (*a*) bread and milk for the carbohydrate-rich diet, and (*b*) cheese for the carbohydrate-free diet. The injections were made subcutaneously, the pituitary extracts used being "pitressin" and "pitocin" supplied by Parke, Davis and Co.

The effect of a single dose of the hormone, given at varying periods before death, was investigated. In this way it was possible to plot "time curves" of the changes in the blood-sugar, liver- and muscle-glycogen, and also liver-fat contents.

(A) *Effect of pitocin.*

The dose of pitocin employed was 20 units per 100 g. rat. Under this dosage, no definite effects on the general behaviour of the animals were noted, and the results of the chemical analyses, carried out on 12 rats, are given in Table I.

Table I. *Analyses of carbohydrate- and non-carbohydrate-fed rats after the subcutaneous injection of pitocin.*

Diet	Time after injection (hours)	Blood-sugar %	Muscle-glycogen %	Liver-glycogen %	Liver-fatty acids %	Iodine value of fatty acids
Carbohydrate-rich	0	0·15	0·55	3·5	3·7	120–140
	5	0·17	1·00	—	3·4	123
	7½	0·16	0·70	2·8	2·7	120
	7½	0·18	0·80	1·16	2·6	124
	15½	0·14	0·86	4·7	4·7	120
	15½	0·17	1·08	5·3	3·3	120
	16½	0·16	1·05	—	2·7	148
	18	0·16	1·00	4·5	2·0	124
Carbohydrate-free	0	0·17	0·63	1·9	5·0–7·0	100–128
	15½	0·16	0·94	1·6	4·9	121
	15½	0·17	1·26	1·6	5·7	—
	16½	0·15	0·73	1·0	9·7	102
	16½	0·15	1·00	1·4	8·5	116
	17	—	0·70	1·9	9·2	—

(B) *Effect of pitressin.*

After the injection of pitressin, many of the rats showed a distinct slowing of respirations. In some cases, slight sprawling of the limbs was noted, and nearly all the animals had a "blanched" appearance and felt cold when handled. These symptoms, however, lasted only for a short time after the injection, and probably correspond to the effect described by Lawrence and Hewlett [1925] as resulting after pituitrin in normal human subjects.

Table II. *Analyses of carbohydrate-fed rats after the subcutaneous injection of pitressin.*

Number of units	Time after injection (hours)	Blood-sugar %	Muscle-glycogen %	Liver-glycogen %	Liver-fatty acids %	Iodine value of fatty acids
2·5	0	0·15	0·55	3·5	3·7	120–140
	1	0·21	0·65	2·2	5·8	—
	1	—	0·82	2·2	4·6	92·5
	1¾	0·14	1·23	3·8	5·25	92
	2½	0·15	0·6	—	—	—
	2½	0·17	2·3	3·8	4·5	91·4
	5	0·19	0·96	1·15	6·0	88
	7	0·17	1·0	1·35	6·4	—
	12	0·23	0·65	6·75	6·1	99
	12	0·16	0·93	3·49	5·1	99
	17½	0·14	1·04	8·2	3·65	97·5
	24	—	1·4	5·5	4·8	104
	36	0·13	1·4	1·28	3·5	—
20	0	0·15	0·55	3·5	3·7	120–140
	¼	—	1·0	3·6	3·4	121
	½	0·21	0·66	3·5	3·5	148
	1	—	1·83	2·1	4·0	123
	1	0·24	—	2·1	6·15	—
	2½	0·16	0·77	2·2	5·2	99
	5	0·19	0·59	0·68	4·7	92
	7	0·16	1·0	2·0	4·2	82·5
	17	0·16	1·27	7·8	2·9	—
	17½	0·15	1·2	9·3	3·15	76
	17½	0·16	1·1	6·0	—	88
	24	0·17	0·99	4·8	2·55	136
	30	0·16	1·0	3·1	1·64	124
	36	0·17	1·52	3·2	3·0	—
40	0	0·15	0·55	3·5	3·7	120–140
	2½	0·14	0·81	1·0	5·0	86·5
	5	0·15	0·61	0·96	5·5	80
	5	0·15	0·62	1·2	5·1	97
	7	0·15	1·62	2·6	5·5	72
	12	0·16	—	4·4	4·3	112
	17½	0·16	1·24	8·7	3·7	87·7

Table III. *Analyses of non-carbohydrate-fed rats after the subcutaneous injection of pitressin.*

Number of units	Time after injection (hours)	Blood-sugar %	Muscle-glycogen %	Liver-glycogen %	Liver-fatty acids %	Iodine value of fatty acids
2·5	0	0·17	0·63	1·9	5·0–7·0	100–128
	1	0·13	0·93	1·18	5·0	139
	5	0·15	1·58	1·87	4·7	111·5
	12	0·15	1·3	2·6	6·8	84·3
	18	0·14	1·08	3·3	7·2	104·5
	22	0·16	1·05	2·7	13·1?	—
	24	0·15	0·71	4·1	6·9	95·4
	30	0·13	1·08	1·67	6·4	100
	42	0·15	0·95	1·1	—	88
20	17½	—	0·69	2·4	6·8	112
	17½	0·15	0·90	2·8	7·1	85·2
40	17	0·15	1·7	3·2	8·0	—

"Time curves" after the injection of 2·5, 20 and 40 units of pitressin per 100 g. rat were completed for carbohydrate-fed rats, and for non-carbohydrate-fed rats after 2·5 units of pitressin. Forty-six animals have been used in experiments with this hormone, and the results obtained are summarised in Tables II and III.

Results.

Inspection of the foregoing tables reveals the fact that pitocin is much less potent in its effect on either fat or carbohydrate metabolism than is pitressin.

With pitocin no alteration in blood-sugar was detected, as the values obtained were identical with those found in animals under similar dietetic conditions but receiving no injection. This is in agreement with the finding of Gavrila and Mihaileanu [1930] that pitocin was without influence on the blood-sugar level in the human subject, though it should be noted that Nitzescu and Benetato [1930] had previously reported that both pitocin and pitressin given intravenously brought about a marked and comparatively long-lasting hyperglycaemia in dogs and rabbits. Bacq and Dworkin [1930] obtained similar results with intravenously injected cats, namely a prompt rise in blood-sugar lasting for about 2 hours. As our first observation was made 5 hours after a subcutaneous injection of pitocin into a rat, the experimental conditions are not comparable. Consequently, the discrepancy between the results requires no further explanation.

Nevertheless, pitocin sometimes brings about an increase in muscle-glycogen. If this effect is achieved by a transference of glycogen from the liver to the muscles, it may furnish an explanation of the somewhat erratic results obtained for the liver-glycogen content. No definite changes in the liver-fat content were brought about by the subcutaneous injection of pitocin in carbohydrate-fed rats. The values obtained were irregular and may indicate a possible slight decrease in liver-fat content. With cheese-fed rats, the values obtained for liver-fat were somewhat higher than those for cheese-fed controls. However, as the iodine value of the fatty acids in the liver after pitocin remains normal, it is questionable whether the slight changes observed in either carbohydrate- or non-carbohydrate-fed rats, are of any significance.

In marked contrast with the indefinite character of the results obtained with pitocin, were the changes which ensued after the subcutaneous injection of pitressin. Slight hyperglycaemia was evident ½ hour after a dose of 20 units, but on the whole little variation in the blood-sugar level was observed. Nevertheless striking changes occurred in the liver-glycogen content, and also in the amount and character of the liver-fat.

In carbohydrate-fed rats, soon after the injection of pitressin, the liver-glycogen began to fall and the muscle-glycogen showed a tendency to rise. The liver-glycogen reached a minimum between the 5th and 7th hour, at which time the muscle-glycogen was definitely high. Thereafter, a rapid rise in liver-glycogen occurred, a maximum of 6–8 % being attained about the 17th hour after the injection of the hormone. Subsequently there was a slow return to the normal level, which was reached by the 36th hour.

These changes are shown graphically in Fig. 1. The values for liver-glycogen after 2·5 units of pitressin are indicated by triangles, after 20 units by crosses and after 40 units by squares. The continuous curve is obtained by joining the average values found for the medium dose of 20 units. But it is evident that curves of similar type would result if either the average values for the small dose (2·5 units) or those for the large dose (40 units) were joined. Moreover, the maximum and minimum points of these curves would coincide with

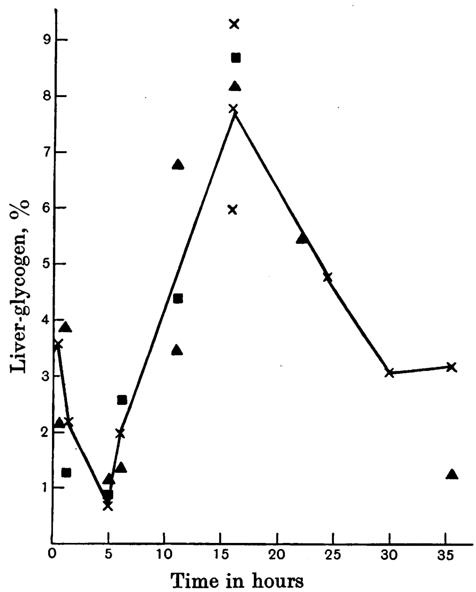

Fig. 1.

those of the curve drawn in Fig. 1, so that, as the same holds for the muscle-glycogen and also for the liver-fat, it appears that the effects produced by the various doses of the hormone are identical. No explanation of this somewhat remarkable result is attempted, but it may be correlated with the well-known fact that after repeated injections of the pressor principle of the posterior lobe of the pituitary, a tolerance to further administration tends to become established.

Recently, Bischoff and Long [1931] have reported that after pitressin "a possible but not clearly established increase in liver-glycogen takes place." This apparently conflicting result is easily explained, as these investigators made their glycogen determinations only at 5 hours after injecting the rabbit with pitressin, and it is clearly demonstrated in Fig. 1 that the liver-glycogen content varies with the interval elapsing after the injection of the hormone.

The results of the present investigation therefore confirm the increase in liver-glycogen noted by Nitzescu and Benetato [1928] to occur after pituitrin, but whereas this rise in liver-glycogen was accompanied by a fall in muscle-glycogen, reference to Table II shows that in our experiments with pitressin the muscle-glycogen tended to rise slowly to a final value approximately double the initial value (over 1 %). A transference of glycogen from the liver to the muscles might explain the fall in liver-glycogen observed during the first few hours and the coincident rise in muscle-glycogen, but such an explanation is quite inadequate to account for the high glycogen content of both liver and muscles at the 17th hour after the injection.

A study of the curves in Fig. 2 renders the simultaneous changes in liver- and muscle-glycogen, just referred to, as well as those in liver-fat content,

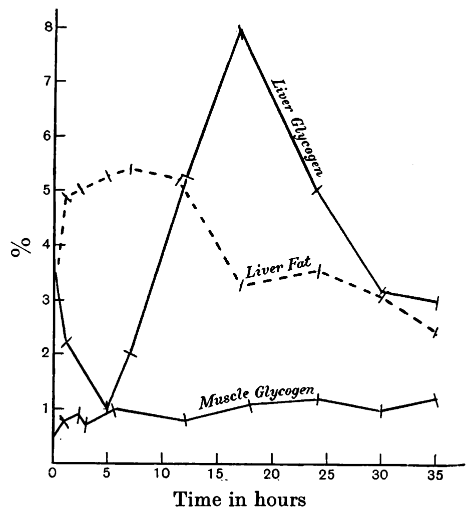

Fig. 2.

more easily appreciated. In carbohydrate-fed rats, pitressin produces an infiltration of fat, which is accompanied by a decrease in the iodine value of the fatty acids present in the liver. The rise in fat commences within an hour after the injection and may extend over a period of 12 hours—a maximum usually occurring about the 5th hour, at which time, it should be noted, the liver-glycogen content is at a minimum. Further, it is noteworthy that while the liver-glycogen passes from a minimum to a maximum between the 5th and 17th hours, during this period the liver-fat falls from a maximum to a more or less normal value.

With non-carbohydrate-fed rats, similar results were obtained. These are summarised in Table III and shown graphically in Fig. 3. It will be noticed that a possible slight increase occurred in muscle-glycogen, as after the first few hours it was maintained at a higher level than that found in cheese-fed controls. Also, the initial fall in liver-glycogen was more rapid and the subsequent rise was less marked but more protracted than in the carbohydrate-fed animals. As in cheese-fed rats the liver-fat content was high (5–7 %) previous to the injection of pitressin, it was less certain whether the hormone caused a further infiltration of fat. But evidence that fatty infiltration occurred to some extent is furnished by the fact that the percentage of fatty acid between the 12th and 18th hours tended to be higher than those obtained earlier and later. Moreover, there was a decided fall in the iodine value which can only be explained by an alteration in the character of the liver-fat. Further, when the liver-glycogen is approaching its maximum value, the liver-fat content shows a tendency to decrease.

The results here recorded not only confirm the work of Coope and Chamberlain, but also point either to the pressor constituent of the posterior lobe of

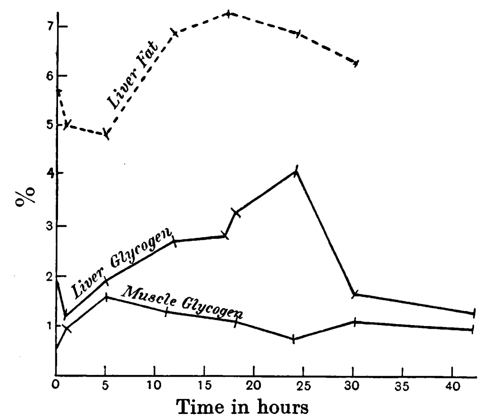

Fig. 3.

the pituitary, or to a hormone which accompanies it, being responsible for the infiltration of fat into the liver. Further, it is clearly demonstrated that that portion of the posterior pituitary extract in which the pressor constituent is concentrated also exercises a remarkable and somewhat unexpected effect on the glycogen reserves. Whether the hormone concerned with the effect on fat metabolism is identical with that affecting the metabolism of carbohydrate cannot be stated at present. Further experiments on this aspect of the problem are in progress and the results, as already indicated, will be communicated at an early date.

Summary.

No significant changes were observed to result from the subcutaneous injection of pitocin into rats on either a carbohydrate-rich or a carbohydrate-free diet.

Pitressin, on the other hand, produced striking changes in carbohydrate-fed rats.

As an early effect, there was observed slight hyperglycaemia accompanied by a fall in liver-glycogen and an increase in muscle-glycogen.

Simultaneously there occurred a marked infiltration of fat into the liver.

As the liver-fat content increased, the iodine value of the fat fell correspondingly.

The liver-fat reached a maximum at the 5th–7th hour, at which time the liver-glycogen was at a minimum, and the muscle-glycogen increasing.

The liver-glycogen reached a maximum about the 17th hour, at which time the liver-fat had returned to normal.

Similar but less striking results were obtained with non-carbohydrate-fed rats.

"Time curves" are given showing the simultaneous changes occurring in liver- and muscle-glycogen and liver-fat during a period of 36 hours after the injection.

Grateful acknowledgment is made to the Carnegie Trust for a Research Grant and Scholarship. The authors are also greatly indebted to Messrs Parke, Davis and Co. for generously presenting the pitressin and pitocin required for this investigation.

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