# CXCV. FURTHER OBSERVATIONS ON THE DISTRIBUTION OF FAT AND GLYCOGEN AFTER THE SUBCUTANEOUS INJECTION OF EXTRACTS OF THE POSTERIOR PITUITARY GLAND.

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In a recent paper the effects on the blood-sugar, muscle- and liver-glycogen, and liver-fat, which ensued as the result of a single subcutaneous injection of either pitressin or pitocin, were described [Hynd and Rotter, 1932]. Pitressin, in its effects on the metabolism of both fat and carbohydrate, appeared to be much more potent than pitocin. Indeed, so indefinite were the results obtained with pitocin that it was doubtful whether any significance could be attached to the slight changes observed. Nevertheless, the results of preliminary experiments with pituitrin suggested that pitocin was not entirely without effect. Moreover, Raab [1930] concluded that both pitressin and pitocin exercise a certain influence on the phosphatides of the blood similar to that of pituitrin.

Accordingly, as it seemed likely that some light on the problem might accrue from a careful study of the effects produced by the injection of (*a*) pituitrin, that is the unseparated posterior pituitary extract, and of (*b*) artificial mixtures of pitressin (the pressor constituent) and pitocin (the oxytocic constituent), such an investigation was commenced and the results obtained are dealt with in the present communication.

## Experimental.

As the effect of pituitary extracts on the distribution of glycogen and fat is less definite in the case of animals fed on a carbohydrate-free diet than in those receiving abundant carbohydrate [Hynd and Rotter, 1932], the present series of experiments was confined to albino rats fed on bread and milk. Each rat when used for experiment weighed approximately 100 g. A single dose of the hormone was administered subcutaneously at varying intervals before killing the animal by stunning. The effects on the blood-sugar, liver- and muscle-glycogen, and liver-fat were investigated, the same technique as previously described being followed. The sex of the rat was always noted, but contrary to expectations, no difference in the results obtained with male and female animals was detected. The pituitary extracts used were all provided by Parke, Davis and Co., and as a rule contained chloretone as a preservative. It may be added at this point that through the kindness of Parke, Davis and Co., we were enabled to carry out a set of experiments with pitressin, specially prepared for research purposes and free from preservative. As the results obtained with this material were identical with those already reported [Hynd and Rotter, 1932] for pitressin containing chloretone, it seems reasonable to draw the conclusion that the presence of chloretone as a preservative does not interfere with the effects produced by the posterior pituitary hormones on the metabolism of fat or carbohydrate.

### (a) *Effect of pituitrin.*

Blanching and other symptoms, similar to those described for rats injected with pitressin, were noted in animals receiving pituitrin. Time-curves after the subcutaneous injection of 2·5, 10 and 20 units of pituitrin were completed. In experiments with this extract 24 rats were used, and identical results were obtained with two distinct samples of the hormone. To economise space only the average values of the results obtained are summarised in Table I.

Table I. *Analyses of carbohydrate-fed rats after the subcutaneous injection of pituitrin.*

| No. 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 | 0·165 | 0·78 | 3·34 | 3·44 | 127·5 |
| | 2½ | 0·18 | 0·66 | 2·31 | 4·3 | — |
| | 5 | 0·18 | 1·05 | 2·3 | 3·68 | 104 |
| | 7 | 0·148 | 1·1 | 2·43 | 3·5 | 110 |
| | 12 | 0·17 | 1·34 | 2·65 | 3·5 | 90·5 |
| | 14 | 0·15 | 1·58 | 2·65 | — | 109 |
| | 15 | 0·16 | 1·87 | 3·95 | 3·5 | 105 |
| | 16 | 0·11 | 1·05 | 4·16 | 3·6 | 106·5 |
| | 17½ | 0·16 | 1·59 | 8·8 | 3·1 | 101·5 |
| | 18 | 0·12 | 1·02 | 10·6 | 3·66 | — |
| | 24 | 0·14 | 1·03 | 7·95 | 2·0 | — |
| | 36 | 0·16 | 0·95 | 3·57 | 3·09 | 121 |
| 10 | 2½ | 0·187 | 0·94 | 2·88 | — | 74 |
| | 7 | 0·176 | 1·18 | 2·8 | 3·45 | 110 |
| | 17½ | 0·16 | 1·1 | 4·6 | 3·5 | 96 |
| 20 | 5 | 0·16 | 1·27 | 2·3 | 3·45 | 114 |
| | 7 | 0·12 | 1·11 | 1·95 | 4·05 | 119 |
| | 9 | 0·21 | 0·98 | 2·54 | 4·18 | 111 |
| | 15½ | 0·158 | 1·02 | 5·2 | 4·6 | 88 |

### (b) *Effect of a mixture of pitocin and pitressin.*

As pituitrin contains 10 units of pitressin and 10 units of pitocin per cc., the mixture used for injection was prepared so as to contain an equal number of units of each constituent, the amount injected being then adjusted so as to contain 2·5 units of pitocin and 2·5 units of pitressin. Later it was found more convenient to inject the animal in one flank with the necessary amount of pitocin and in the other with the pitressin. No difference in the results obtained for the two methods of administering the extracts was detected.

Experiments have been carried out with the mixture of pitocin and pitressin in the case of 24 rats. The general symptoms produced were similar to those observed in rats injected with pitressin alone. The average values for the results of the chemical analyses are given in Table II.

Table II. *Analyses of carbohydrate-fed rats after the subcutaneous injection of a mixture of pitocin (2·5 units) and pitressin (2·5 units).*

| Time after injection (hours) | Blood-sugar % | Muscle-glycogen % | Liver-glycogen % | Liver-fatty acids % | Iodine value of fatty acids |
|---|---|---|---|---|---|
| 0 | 0·15 | 0·55 | 3·5 | 3·7 | 120–140 |
| 1 | 0·18 | 0·81 | 3·08 | 4·1 | 129·5 |
| 2½ | 0·18 | 1·46 | 2·45 | 3·69 | 116·5 |
| 3½ | 0·13 | 0·63 | 2·05 | 3·83 | 113 |
| 5 | 0·18 | 1·56 | 2·55 | 3·40 | 115 |
| 6 | 0·15 | 1·26 | 2·2 | 3·60 | 127 |
| 7 | 0·187 | 1·23 | 2·65 | 3·1 | 104 |
| 15 | 0·16 | 1·89 | 5·4 | 3·51 | 127 |
| 18 | 0·17 | 0·64 | 3·65 | 4·33 | 130 |
| 19 | 0·15 | 1·45 | 3·67 | 3·48 | 140 |
| 21 | 0·16 | 1·85 | 4·1 | 4·17 | 124 |
| 24 | 0·13 | 0·76 | 1·23 | 2·55 | 108 |

## Discussion.

As the several functions affected by extracts of the posterior pituitary are so diverse, it is probable that each function is influenced by a different hormone. But so far no single hormone of the posterior pituitary gland has been isolated in a pure condition [Schäfer, 1931]. Nevertheless Kamm *et al.* [1928] separated pituitrin into two fractions, pitocin and pitressin, the former containing the oxytocic principle, while the latter is responsible for the vaso-pressor effect. The same workers [1931] claim that there is as yet no evidence that more than two hormones are present in pituitrin, and that on recombining the two separated hormones in the proportion of one oxytocic to one pressor unit, an extract with all the activities of the original pituitrin is reproduced. Hence the mixture of pitressin and pitocin used in the experiments recorded above should produce changes identical with those resulting from an equivalent dose of pituitrin. Though this is doubtless the case with regard to the effect on blood pressure and the contraction of uterine muscle (the two methods usually adopted for assay), comparison of the results summarised in Table I with those in Table II shows that this identity does not apply to the changes in glycogen and fat distribution.

The effect of a mixture containing pitocin and pitressin in the proportions in which they are alleged by Kamm to occur in pituitrin is very similar to that already reported to result from an injection of pitocin alone. As in the case of the latter hormone, the only definite change is an increase in muscle-glycogen. This increase occurs almost immediately, values varying from 1 to 2 % being observed 2½ hours after the injection, and the high level is maintained for at least 24 hours. There is also a possible slight increase in blood-sugar, not noticed after pitocin alone, but no infiltration of fat into the liver and no change in the iodine value of the liver-fat occur. There is a tendency for the liver-glycogen to fall during the initial 5 hours, and then to increase somewhat over the normal. These changes however are not marked, and graphic representation of the results would emphasise the difference between the effects produced by what might be termed "artificial" pituitrin and those resulting from "natural" pituitrin.

Injection of pituitrin brings about changes in both liver- and muscle-glycogen similar to those which follow the injection of pitressin. During the first 5 hours the liver-glycogen falls to about 1 %. Thereafter there is a steady and well-marked increase, which reaches its maximum of over 9 % about the 17th hour after injection. Return to the normal value of 3·5 % is attained by the 36th hour. During these changes in liver-glycogen, the muscle-glycogen is maintained at an abnormally high level, namely about 1 % or twice the normal control value for rats under the same dietetic conditions. Consequently, though glycogen may be transferred from liver to muscles and *vice versa*, such a transference is altogether inadequate to account for all the changes observed in the distribution of the glycogen reserves. Moreover, attention has previously been drawn to the fact that the magnitude of the changes in glycogen distribution produced by pitressin is independent of the size of the dose employed. Similar results are obtained with pituitrin, which in this regard appears as efficient as pitressin.

On the other hand, pituitrin is much less effective than pitressin in altering the distribution of fat, and the effect produced varies somewhat with the dose employed. Thus after the injection of 2·5 units of pituitrin no infiltration of fat into the liver was detected, though a fall in iodine value (less than 120) made it evident that the character of the fat had changed to some extent. Even after 20 units of pituitrin fatty infiltration did not always occur. When it was obtained, it was never so marked as that after 2·5 units of pitressin. As Raab has reported that pituitrin caused a more marked decrease in the blood-fat level than pitressin, this finding is not what one would expect. Nevertheless it is in agreement with the observation of Coope and Chamberlain [1925]. These workers showed that while a comparatively small dose of pituitrin brought about an infiltration of fat into the liver of a rabbit, a large dose of at least 20 units was not always successful in effecting a like change in the liver of a rat. To reconcile Raab's finding that pituitrin has a more definite effect than pitressin in decreasing the blood-fat, with the results now reported that pitressin is more potent than pituitrin in increasing liver-fat, it is necessary to postulate that pitocin also plays a definite rôle in fat metabolism.

Even though pitocin acting alone was found to have little or no effect on the fat content of the liver, the presence of the oxytocic constituent in pituitrin evidently interferes with the accumulation of fat in the liver. It is remarkable that insulin, as shown previously by Coope [1925], has a like function. Though the same result is achieved by pitocin as by insulin, it is highly probable that these two hormones bring about this effect on the liver-fat by entirely different processes. However, in the present state of knowledge it is impossible to say whether pitocin prevents the accumulation of saturated fat in the liver by inhibiting the actual transference of fat, or by facilitating the oxidation and utilisation of fat brought to the liver, thereby keeping the liver-fat approximately normal both in character and amount. Certain observations of Raab [1928] are opposed to the former view, and the alternative mechanism would generally be regarded as characteristic of the pancreatic hormone. However, there is considerable experimental evidence which shows the interdependence of the pituitary body and the islets of Langerhans, and clearly indicates that the injection of insulin stimulates the pituitary gland to increased secretion [Eaves, 1926; Geiling and Britton, 1927; La Barre, 1930, 1, 2]. Consequently it must not be overlooked that the facilitation of fat metabolism ascribed to insulin may not be due directly to insulin, but to a hormone (pitocin) secreted by the posterior lobe of the pituitary.

Further, such a view would be consistent with the fact recently established by Himwich and Haynes [1931] that pitocin brings about an increase in metabolism of 15 % on the average. These same workers have shown that pitressin, on the other hand, reduces the basal metabolic rate by 22 %. In pituitrin, therefore, there are present two hormones which are antagonistic with regard to their effect on the total metabolism. Elmer and Ptaszek [1930] have also demonstrated that pitressin and pitocin are antagonistic with regard to their action on intestinal peristalsis. The results now communicated show that while pitressin during the first 5–7 hours after injection causes an accumulation of liver-fat and between the 12th and 24th hours storage of liver-glycogen, pitocin entirely inhibits the former and depresses the latter change to a considerable extent. Many factors, such as dosage, method of administration of the hormone, the species of animal and the experimental conditions employed, contribute to the confusion which exists in the literature regarding the effect of posterior pituitary extracts on the metabolism of fat [Raab, 1930] and carbohydrate [Geiling, Campbell *et al.*, 1927]. The opposing actions of the two recognised constituents of pituitrin doubtless explain many of the inconsistencies reported, since the extracts employed by different observers may have contained varying amounts of the pressor and oxytocic principles.

Fukui's observation [1925] that posterior pituitary extracts produce no significant changes in the glycogen reserves seems to find support from recent work by Lawrence and McCance [1931] on the fasting rat, and by Bischoff and Long [1931, 1, 2] on rabbits injected with pitressin or pituitrin. Nevertheless, using somewhat different experimental conditions, it is evident from the results of the present experiments on rats that the posterior pituitary has an important influence on both carbohydrate and fat metabolism, but the mechanisms involved are much more complex than have been supposed.

Hitherto, in experimental studies of the effects of pituitrin, attention has been confined to the first few hours after administration of the hormone. It seems expedient therefore to emphasise that the experiments in the present investigation have been continued until a return to the normal was obtained. Under the experimental conditions adopted this required from 24 to 36 hours. In this way, as already indicated, remarkable changes in liver- and muscle-glycogen have been detected, and, as these changes are at a maximum about the 15th–17th hour after injection, they have been missed by other observers.

During the initial stages, in addition to the infiltration of fat into the liver, a transference of glycogen from the liver to the muscles seems to be the effect produced. However, in the later stages when glycogen is being stored in the liver, a shift of glycogen from the muscles cannot explain the observed accumulation of glycogen in the liver, since the muscle-glycogen remains high. Evidently a synthesis from some outside source must have taken place, but it is not yet clear what this source is. The simultaneous changes in the fat and glycogen of the liver after the injection of pitressin provide an excellent example of Rosenfeld's law, and are suggestive of a conversion of fat into carbohydrate. However, the fact that in pituitrinised animals the glycogen changes are not necessarily accompanied by an infiltration of fat makes it doubtful whether such a conversion is involved.

The further question arises as to whether the effects occurring after a period of 15 hours should be attributed to the hormone actually injected, or whether they arise as secondary effects, produced by the compensatory secretion of some other hormone or hormones. Thus, La Barre [1930, 1, 2] holds that the hyperglycaemia which occurs after the administration of posterior pituitary extracts is followed by a hypoglycaemia, and he attributes the former to an increased excretion of adrenaline and the latter to hyperinsulinaemia. Many other observers, including Lambie [1926], Geiling and his co-workers [1927], and Bischoff and Long [1931, 1], have suggested that the effects of the posterior pituitary on carbohydrate metabolism involve the interaction of the suprarenals. The last-named authors, however, as a result of further experiments on adrenalectomised animals, have now changed their opinion [Bischoff and Long, 1931, 2]. Consequently until the exact mechanism by which the changes in question are produced is elucidated, it seems best to refer them to the posterior pituitary. Nevertheless the interplay of the endocrine secretions is certainly an important factor [Schäfer, 1931] but one regarding which speculation is better avoided.

## Summary.

The subcutaneous injection of pituitrin into carbohydrate-fed rats produces effects on the liver-glycogen similar to those obtained with pitressin, namely a fall during the first 5 hours, followed by a rise, which is at a maximum about the 18th hour after injection.

The effect on the muscle-glycogen is also similar to that with pitressin, an increase during the first 5 hours to twice the normal, at which level it is maintained for at least 24 hours.

On the other hand, pituitrin is much less efficient than pitressin in producing an infiltration of fat into the liver.

An artificial mixture of equal parts by volume of pitressin and pitocin does not produce the same changes in liver-glycogen and fat as does the "natural" pituitrin, fatty infiltration being entirely inhibited and the increase in liver-glycogen greatly depressed.

From the results obtained it is concluded that pitocin is antagonistic to pitressin in its effects on the liver-fat and the liver-glycogen.

As the effects produced by the several extracts vary with the interval elapsing after the injection, it is important to take account of the time during which the extract is allowed to act.

It is suggested that failure to do so, as well as the hitherto unrecognised antagonism between pitocin and pitressin, may account for many of the conflicting reports in the literature regarding the effect of posterior pituitary extracts on carbohydrate and fat metabolism.

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