LXXXVII. THE PRODUCTION OF ANTISERA TO PREPARATIONS OF PROLACTIN CONTAINING THE GLYCOTROPIC (ANTI-INSULIN) FACTOR OF THE ANTERIOR PITUITARY GLAND

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THE discovery of "antihormones" by Collip and his colleagues [see Collip, 1934] stimulated great interest in the production of antisera to anterior pituitary hormones, and sera capable of inhibiting the action of pituitary gonadotropic, ketogenic and thyrotropic factors, as well as urinary gonadotropic substances, have been extensively investigated during the last few years.

The discovery that the "anti-insulin" or "glycotropic" factor of the anterior pituitary gland is present in preparations of prolactin as usually obtained [Young, 1936] suggested that an antiserum prepared against prolactin might be of some interest, and the possibility of its production was investigated.

METHODS

Preparation and assay of prolactin

Prolactin was obtained from commercial (B.D.H. Ltd.) dried ox anterior lobe tissue by a modification of the Bates-Riddle [1935] method described elsewhere [Young, 1938]. The yields, and activities of the different preparations on the pigeon crop gland are shown in Table I.

When 20 mg. of any of these prolactin preparations were injected daily for 5 days into each of a group of immature guinea-pigs, no hypertrophy of the thyroid gland occurred [method of Rowlands & Parkes, 1934]. These preparations therefore contained no significant amount of thyrotropic hormone,¹ but they all possessed glycotropic activity [method of Young, 1936, as subsequently modified, 1938].

Table I

Preparation	Yield % dried gland	Pigeon test*	
		Amount injected daily, mg.	Crop-gland increase/300 g. pigeon, g.
37C	8.62	3	5.0
50C	16.90	3	3.5
52C	12.77	3	4.5

* Method of Rowlands [1937].

Production of antisera

Rabbits and monkeys were the chief donor animals used, although sera from animals of other species, which had received pituitary injections, were also tested.

¹ By thyrotropic hormone is meant the anterior pituitary substance which induces hypertrophy of the thyroid gland. The identity of this substance with that causing increased secretion of the thyroid gland has not been established.

Adult rabbits, bucks or does, received daily subcutaneous injections of prolactin equivalent to 1 g. of dried gland, i.e. 80–170 mg. of prolactin daily. Young female *Rhesus* monkeys (3–4 kg.) generally received twice this dose. Daily injections were maintained for periods up to 9 months. For injection, the prolactin was dissolved in saline at pH 8.0 and Seitz-filtered after the addition of 0.4 % tricresol. 20 ml. of blood were removed every week from the ear vein of each rabbit and from the saphenous vein of each monkey, and the samples from a number of animals pooled before separation of the serum.

Testing of antisera

(a) *Anti-prolactin activity.* Prolactin preparation 50 C constituted a standard preparation against which the activity of the antisera was tested. When 1–3 mg. of this preparation were given daily to adult pigeons for 6 days the increase in crop-gland weight was approximately proportional to the amount of prolactin administered (Fig. 1). Doses of prolactin in this range were therefore used in the assessment of activity of the antisera.

Fig. 1. Dose-response curve for prolactin preparation 50 C. Assays carried out by the method of Rowlands [1937]. Each point represents the average result for a group of at least 10 pigeons.

1 ml. or less of the serum under investigation was injected subcutaneously each day into a pigeon, which simultaneously received a subcutaneous injection of a suitable dose of the standard prolactin into another area of the body. After six such double daily injections the bird was killed and the crop gland weighed. A control test was carried out at the same time using the same dose of prolactin but with normal instead of experimental serum. The average weight of crop gland in a group of 10 birds receiving prolactin together with normal or experimental serum was thus determined. The activity of the experimental serum was indicated by the difference in the average crop gland weight in the two groups. As the average response of a group of our pigeons to a given dose of prolactin is by no means constant from week to week, a control experiment with normal serum was carried out with every test that was made on the experimental sera. For quantitative assay of the inhibitory action of the antiserum it was necessary to adjust the dose of serum to such a level that the response of the crop gland to prolactin was not entirely prevented. If the dose was such that no crop-gland response occurred, the dose of serum used might have been many times that necessary just to prevent the response.

The results are expressed in terms of the weight of crop-gland growth per 300 g. pigeon, which the daily injection of 1 ml. of serum for 6 days could just prevent; e.g. if 0.5 ml. of serum were injected daily the observed difference in crop-gland response of the experimental and control groups was doubled.

(b) *Tests with lactating mice.* When antisera were injected daily into lactating mice nursing their young, any depression of milk secretion of the mothers would be recorded as a subnormal growth of the litters. Accordingly, experiments were carried out in which 0.5 or 1 ml. of antiserum was injected subcutaneously into each of a group of lactating mice, the litters being weighed daily. Control experiments were simultaneously carried out using normal serum. The difference in the weight of the young in the two groups was a measure of the ability of the antiserum to inhibit milk secretion in the mothers.

(c) *Antiglycotropic activity.* The normal procedure for a test of the glycotropic activity of prolactin is as follows. At 5 p.m. on the day previous to a test, a suitable dose (80–150 mg.) of prolactin is injected subcutaneously into a rabbit and at the same time food is withdrawn. The following morning at 9.30 a.m. the same dose of prolactin is again injected, and at 2 p.m. 2 units of crystalline insulin are administered intravenously, and the blood sugar estimated (Hagedorn-Jensen method) at frequent intervals for 4 hr. afterwards. In a test for antiglycotropic activity of sera, 10 ml. of the antiserum to be tested were administered at the same time as the prolactin in some instances, while in other experiments a number of injections of 5 ml. of antiserum were given during the three days immediately preceding the test. Control experiments with prolactin and normal serum were carried out simultaneously with all these tests on antisera, the dose of prolactin being so adjusted that the hypoglycaemic response to insulin in the control experiments was almost, but not quite, abolished. Antiglycotropic activity of the antiserum was indicated by the extent of the fall of blood sugar in the rabbit which received the experimental serum.

RESULTS

Action of the antisera on the response to prolactin of the pigeon crop gland

The results of experiments with rabbit serum are given in Fig. 2, while those with monkey serum are given in Fig. 3. In view of the great variability of the response of the pigeon crop gland to a given dose of prolactin, the points on these

Fig. 2. Rate of appearance of inhibitory activity (pigeon crop-gland test) in the serum of rabbits receiving daily injections of prolactin.

figures fall in a surprisingly regular manner. It will be seen from the curves that the appearance of inhibitory activity is slightly more rapid in rabbits than in monkeys, but that serum with very high activity may be obtained from monkeys which have received daily injections of prolactin for a very long period. The rate of production of inhibitory activity in the monkey appears not to have decreased after 38½ weeks' daily injection of prolactin.

From Fig. 3 it will be seen that sera, obtained after 4, 5 or $6\frac{1}{2}$ weeks of daily prolactin injection, increased rather than decreased the crop-gland response to prolactin in pigeons. The appearance of augmentative action in the serum after 3–7 weeks' daily injection of prolactin has been observed in a number of experiments [Young, 1937], although the augmentation has never been very great. The maximum effect was obtained with the serum of a dog which had received daily injections of prolactin for 5 weeks. In this instance the daily injection of 1 ml. of serum into a pigeon increased the crop-gland response to prolactin by 3.7 g./300 g. bird.

Fig. 3. Rate of appearance of inhibitory activity (pigeon crop-gland test) in the serum of monkeys receiving daily injections of prolactin.

Fig. 4. Curve showing the relationship between the amount of serum injected and the amount of crop-gland growth prevented.

In Fig. 4 is shown the curve obtained from the results of a series of experiments in which different doses of the same monkey antiserum were tested against the same dose of prolactin. The serum used in these experiments was obtained after $38\frac{1}{2}$ weeks of daily prolactin injection.

Action of antiserum on lactating mice

The average weight of the young in the litters of groups of 6 lactating mice receiving daily injections of 0.5 ml. of either rabbit or monkey antiserum was not greatly different from that of a similar control group receiving injections of

normal serum (Fig. 5). For the correct interpretation of this finding, however, it is necessary to take into consideration the fact that many deaths occurred among the young of the mothers which received antiserum, although few young died in the litters of the mothers which received daily injections of normal serum.

Fig. 5. The average rate of growth of the young of groups of 6 nursing mice which each received daily injections of 0.5 ml. of serum. Curve A. Control group receiving normal rabbit serum. Curve B. Group receiving rabbit antiserum. Group C. Group receiving monkey antiserum.

Thus the average number of young in the litters of the mice receiving rabbit antiserum was 7.7 at the beginning of the experiment, 5.8 after 10 days' injection of antiserum and 3.6 after 21 days. The corresponding figures for the litters whose mothers received monkey antiserum were 7.1, 4.8 and 4.2, and for the control group, receiving normal rabbit serum, 7.5, 6.7 and 6.7.

In a repetition of this type of experiment two daily injections of 0.5 ml. of antiserum (monkey or rabbit) were given to groups of 6 pregnant mice beginning 2–8 days before parturition, and continuing for the first 10 days of the lactation period. Two control groups, receiving either normal rabbit or normal monkey serum, were treated similarly. The results were essentially similar to those for the first part of the previous experiments and therefore need not be given in detail.

The antisera used in these experiments were obtained from rabbits or monkeys which had received daily injections of prolactin for 11–15 weeks.

Influence of antiserum on the glycotropic action of prolactin

After the daily injection of prolactin for 18 weeks, the donor rabbits exhibited a normal hypoglycaemic response to the intravenous injection of 2 units of insulin (Fig. 6). The response to this dose of insulin remained undiminished by the administration of two injections, each of 250 mg., of prolactin, although the initial blood sugar level was somewhat raised. Normal rabbits receiving half this amount of prolactin exhibited practically no hypoglycaemic response to the same dose of insulin (Fig. 6).

It might therefore be expected that the serum of the donor animals would possess the ability to inhibit the glycotropic action of prolactin in normal rabbits. Nevertheless, although indications of a slight antiglycotropic activity of the sera were found in some experiments, the results were so inconsistent that no definite conclusion could be drawn.

Fig. 6. Blood sugar response in a rabbit receiving 2 units of crystalline insulin, injected intravenously at the time indicated by the arrow. Curve A. In a normal rabbit after two injections each of 125 mg. of prolactin. Curve B. In a rabbit which had received daily injections of prolactin for 18 weeks. Curve C. In a rabbit which had received daily injections of prolactin for 18 weeks and two extra injections of prolactin, each of 250 mg., within the 21 hr. preceding the insulin injection.

Miscellaneous observations

(a) *Effect of prolactin injections on pregnant rabbits.* Three pregnant rabbits receiving daily prolactin injections for 5, 11 and 14 days respectively, before parturition, began to secrete milk 2–3 days after the beginning of the period of daily injection, but gave birth to normal healthy litters of 7–10 young. The results of de Fremery [1936], in which abortion or death of the foetus *in utero* always ensued within a few days of the onset of lactation artificially induced during pregnancy by prolactin injections, were presumably not due to the injections of prolactin *per se*.

(b) *Effect of prolactin injections on the condition of the mammary glands.* A milk-like fluid was present in the mammary glands of all the female animals—rabbits and monkeys—2–10 days after the beginning of daily prolactin injections. In most cases the amount of fluid which could be squeezed from the mammary glands increased for 2–3 weeks, and in some rabbits a copious secretion of milk was produced. The animals were not milked unless, as in a number of rabbits, it became necessary because of mammary distension. No animal was ever milked on more than two occasions. The mammary glands of all the monkeys ceased to contain fluid after 3–4 weeks' daily injections, but the majority of the rabbit mammae still contained a milk-like fluid, as shown by squeezing and by *post mortem* examination, 18 weeks after the daily injections of prolactin had begun. All the female rabbits used in these experiments were almost certainly parous, but the monkeys were certainly not.

In a number of instances the mammary glands of the male rabbits contained traces of an opalescent fluid after 1-2 weeks' daily prolactin injection. The nipples of both male and female animals appeared to increase substantially in size as the result of the prolactin injections, although, in most instances, the prolactin preparations used possessed no detectable gonadotropic activity.

(c) *Antithyrotropic activity of the antiserum to prolactin.* As the prolactin used to prepare the antisera possessed no detectable thyrotropic activity, the antisera might be expected to possess little or no antithyrotropic activity. The rabbit antiserum did, however, possess the power to prevent hypertrophy of the thyroid gland of the young guinea-pig receiving injections of a pituitary thyrotropic preparation containing no detectable amount of prolactin. Thus, when a group of 10 immature guinea-pigs received daily injections for 6 days of 1 mg. of a thyrotropic preparation together with 1 ml. of normal rabbit serum, the average increase in weight of the thyroid glands [method of Rowlands & Parkes, 1934] was 29 ± 3.3 mg. When, instead of normal rabbit serum, serum from donor rabbits which had received daily prolactin injections for $16\frac{1}{2}$ weeks was given, the average increase in thyroid weight was 8 ± 2.4 mg. Thus, the serum raised against a prolactin preparation possessing no detectable thyrotropic activity possessed substantial antithyrotropic activity.

(d) *Effectiveness of antiserum to ox-prolactin against prolactin from other species.* Although extensive investigations were not made on this point, it may be stated that the antiserum (rabbit) to ox-prolactin inhibited the hypertrophic action on the pigeon crop gland of prolactin from sheep pituitaries.

DISCUSSION

The interpretation of the results of the experiments in which sera were injected daily into lactating mice is a matter of some difficulty, owing to the deaths in the litters of the experimental groups. It seems improbable that these deaths were due to a toxic action of the antisera on the mothers, as very few deaths occurred in the litters whose mothers received daily injections of normal rabbit or normal monkey serum. Another possible explanation is that the mice receiving the antisera provided insufficient milk for the whole litter, and the weakest members of the litters died in the struggle for existence.

Parkes [1926] has carefully studied the question of the growth of young mice according to the size of the litter, considering only those litters in which no deaths occurred. He found that, from birth to 21 days, the average increase in weight of the mice in the litters containing 7 young was only about 75 % of that of the members of litters containing 4. At the end of the present experiments the control litters averaged 6.7 young each, while the litters of the mice receiving rabbit antiserum averaged only 3.6. From the consideration of the size of litter, therefore, the expected increase in the average weight of the young in the litters of mice receiving rabbit antiserum is greater than that actually found in the control group (Fig. 5), and the observed growth of the litters whose mothers received rabbit antiserum is probably significantly subnormal. The sharp rise in the growth curve for this group, beginning on the 19th day, is probably associated with weaning, and provides support for the belief that the amount of milk supplied by the mothers was inadequate for the proper growth of the young. Nevertheless, it is clear that the inhibition of milk secretion brought about by the daily injection of a relatively large amount of the antiserum fell far short of that caused by hypophysectomy [cf. Nelson, 1936]. Many investigators have failed to initiate or reinstate lactation in hypophysectomized animals by the administration of prolactin and even crude pituitary

extracts and implants of whole gland have been ineffective in some instances [cf. Selye *et al.* 1934]. These observations suggest that pituitary substances besides prolactin influence the secretion of milk by the mammary gland. The suggestion has been made that the factor or factors from the anterior lobe concerned with carbohydrate metabolism may play a part in the secretion of milk [Young, 1936; Gomez & Turner, 1936]. The antiserum produced in the present investigation which is active in preventing the pigeon crop-gland response to prolactin, may be lacking in ability to inhibit the action of other factors concerned in milk secretion. In this case the ability of the sera to prevent the pigeon crop-gland response would not necessarily be a measure of its power to inhibit the secretion of milk.

Although the donor rabbits were resistant to the glycotropic action of injected prolactin, no satisfactory evidence was found that this resistance could be transmitted to other animals in the serum of the donor animals. No evidence was obtained that an "antihormone" was present in the serum of the resistant animals, and the cause of their resistance should possibly be sought elsewhere. It cannot be concluded, however, as the result of these experiments, that an antiserum to the glycotropic substance cannot be produced. Investigations with other fractions and with glycotropic preparations from the pituitary glands of species other than the ox may throw light on this question.

The presence of substantial antithyrotropic activity, in the antiserum prepared against a prolactin preparation which contained no detectable thyrotropic activity, may be explicable on the basis of the presence of traces of thyrotropic hormone contaminating the prolactin. In the light of other experiments, however, this explanation appears improbable, and the significance of these observations will be discussed elsewhere. At present it is only desired to put them incidentally on record.

SUMMARY

1. Large doses of ox prolactin possessing no detectable thyrotropic activity were administered daily to rabbits and monkeys for long periods. The serum of animals so injected for 12 weeks or more possessed marked ability to prevent the hypertrophic response of the pigeon crop gland to prolactin.

2. The average weight of the young reared by lactating mice which received daily injections of rabbit antiserum was slightly, but probably significantly, less than that of the young of control animals receiving similar injections of normal serum. This was possibly due to partial inhibition of milk secretion in those animals receiving antiserum.

3. Rabbits which had received daily injections of prolactin for 18 weeks exhibited a normal hypoglycaemic response to insulin after the administration of a potent pituitary glycotropic preparation, i.e. they were insensitive to the glycotropic action of the extract. No evidence was found, however, that this resistance to the glycotropic action of pituitary preparations was transmissible to normal animals by the serum of the resistant rabbits.

4. Although the prolactin used to prepare the sera contained no detectable thyrotropic activity, the antiserum obtained from the rabbits possessed substantial antithyrotropic activity. By thyrotropic activity is meant the ability to induce hypertrophy of the thyroid gland of the immature guinea-pig.

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REFERENCES

- Bates & Riddle (1935). *J. Pharmacol.* **55**, 365.
Collip (1934). *J. Mt Sinai Hosp.* **1**, 28.
de Fremery (1936). *J. Physiol.* **87**, 50 P.
Gomez & Turner (1936). *Proc. Soc. exp. Biol., N.Y.*, **34**, 404.
Nelson (1936). *Physiol. Rev.* **16**, 488.
Parkes (1926). *Ann. appl. Biol.* **13**, 374.
Rowlands (1937). *Quart. J. Pharm.* **10**, 216.
—— & Parkes (1934). *Biochem. J.* **28**, 1829.
Selye, Collip & Thomson (1934). *Endocrinology*, **18**, 237.
Young (1936). *J. Physiol.* **87**, 13 P.
—— (1937). *J. Physiol.* **90**, 22 P.
—— (1938). *Biochem. J.* **32**, 513, 524.