CLVI. THE GONADOTROPIC HORMONE AND THE LEVEL OF BLOOD PHOSPHORUS IN THE HEN

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THE presence of phosphoprotein in the blood of laying hens has been demonstrated simultaneously and independently by Roepke & Hughes [1935] and Laskowski [1935, 1]. The phosphoprotein was later isolated in a relatively pure state by Laskowski [1935, 2] and the name serum-vitellin was suggested for it. Roepke & Bushnell [1936] examined its serological properties, and found it to be closely related to ovo-vitellin. Laskowski [1936] investigated the blood of several other species of vertebrates (excluding mammals) and found serum-vitellin during or immediately before the spawning season, in practically all cases.

The object of the present investigation was to examine the influence of gonadotropic hormones on the appearance of serum-vitellin in the blood stream of the hen. That the gonadotropic hormone might be a possible factor in the formation of serum-vitellin was suggested by the work of Bates *et al.* [1935], who found that administration of gonadotropic hormone during 6–16 days increased the true ovarian tissue of resting ovaries by nearly 150 %, and that of active ovaries by 125 %, whilst the weight of resting whole ovaries including yolk was increased by approximately 1000 %; although treatment with gonadotropic hormone does not induce, but on the contrary, inhibits laying. Riddle & Dotti [1936] showed that the administration of gonadotropic hormone results in an increase of serum calcium (which varies in proportion with serum-vitellin during normal laying) in normal, hypophysectomized or thyroidectomized pigeons, but not in castrates.

EXPERIMENTAL

Methods and material. Fully grown Leghorn, Rhode Island Red and Sussex hens were taken for the experiments. The birds were not of uniform age, some of them being in their first, and others in their second laying seasons, and finally, part were in the winter break. The hens were on an all-mash diet, given *ad lib.* Administration of hormone and blood sampling were performed without reference to times of feeding, in view of our previous finding [1934] that the level of phosphorus compounds in plasma is unaffected by feeding.

The injections (usually single) of hormones were made into the pectoral muscle, in the morning. Blood (2–3 ml.) was taken from the wing vein, usually every 48 hr. during 10–12 days following the injection. Total P (P_t) was determined in 0.1–0.2 ml. of citrated plasma, and the individual P fractions, acid-soluble (P_{in}), lipin (P_l) and protein P (P_p) were determined in the same 0.5–1.0 ml. sample by the procedure previously described [Laskowski, 1935, 1]. In those experiments in which only P_t was determined the presence of serum-vitellin was sought qualitatively, by tenfold dilution of the plasma with water.

Phosphorus was determined by Martland & Robison's modification [1926] of Briggs' method [1922]. Most of the hormone preparations used were commercial

samples; in some cases preparations were made according to Bates & Riddle [1935] from fresh pituitaries, and one of these preparations was further purified according to Cartland & Nelson [1937].

Results

1. *Antex Leo (Lovens Kemiske Fabrik, Copenhagen)*. Fig. 1 shows the results of single injections of 30 mg. of Antex (240 mouse units), into two non-laying hens. In both cases P_t rose sharply, to a maximum of 140 mg. per 100 ml. plasma on the 6th day, an increase of about 1000 %. According to our previous findings

Fig. 1. Effect of a single injection of 30 mg. Antex (240 mouse units) on the individual fractions of plasma P in non-laying hens. Curves 1. Brown Leghorn No. 11, 2nd year, winter break in laying began 5 weeks before injection. Curves 2. Rhode Island Red No. 10, 2nd year, exhibited partial broodiness since laying of last egg 3 days before injection.

[Laskowski, 1935, 1], the P_t content of the plasma of non-laying hens varies between 8 and 20 mg., and of laying hens between 18 and 60 mg. per 100 ml.; the highest values now obtained are thus about three times higher than the maximum physiological value. The plasma becomes turbid and strongly opalescent following the injection. All the P fractions participate in the effect, though in different degrees. Thus P_{in} (practically identical with inorganic P in plasma) changes least, its content being only doubled. The bulk of the rise in P_t is due to P_l , which attains a value of 90 mg. per 100 ml. The greatest relative increase is shown by P_p , only traces of which are found in non-laying hens, while after Antex injections it amounts to over 30 mg. per 100 ml., corresponding with a serum-vitellin content of 2.7 %. The curves are symmetrical, return to normal being attained after 10–12 days. Eggs were not laid during this period.

It appears probable that the appearance of serum-vitellin under physiological conditions (during laying) is also due to gonadotropic hormone, since the changes in the individual P fractions are of the same order, although of different magnitudes. The chief difference appears to be that normally the serum-vitellin goes to form the developing ova, which is not possible in the conditions of the present experiments.

The response of different individuals to identical doses is very variable, and this applies both to the maximum increments in P_i and to the time required to attain this maximum. Curves 1-2, Fig. 1, and 3-6, Fig. 2, represent changes in P_i after injection of 30 mg. of Antex into different birds. A comparison of curves 5 and 6 with the remaining ones shows that the curves are less symmetrical in the case of laying than in that of non-laying hens, as a result of the higher initial values in the former case. Curve 3, in which the injection was given on 3 successive days instead of in one dose, differs from curve 4 chiefly in that the peak is attained later; however in other cases of single injections, maximum P_i was also found on the 6th day.

Fig. 2. Variability of response to the same dose (30 mg.) of Antex in different hens (curves 3 and 4 non-laying, 5 and 6 laying). Curve 3. Brown Leghorn No. 6, 1st year, has not yet laid. 3 daily 10 mg. doses. Curve 4. Rhode Island Red No. 16, 2nd year, winter break in laying began 6 weeks before single injection. Curve 5. White Leghorn No. 15, 2nd year, laid an egg on the day of injection. Curve 6. Rhode Island Red No. 27, 2nd year, laid an egg on the day preceding, and a second egg on the second day after injection.

Fig. 3. Effect of different doses of Antex on the P_i of non-laying hens. Curve 7. Brown Leghorn No. 7, 1st year, has not yet laid. One injection of 50 mg. Curve 8. Rhode Island Red No. 8, 2nd year, winter break in laying began 8 weeks before injection. Three successive daily injections of 15 mg. each. Curve 9. Rhode Island Red No. 6, 2nd year, winter break in laying began 2 weeks before injection of a single 15 mg. dose.

In view of this individual variability of response no close correlation between dose and response could be expected. Curves 7-9, Fig. 3, in conjunction with the curves of Figs. 1 and 2 illustrate this point.

These differences in response may probably be ascribed to the variable physiological condition of the bird. Some light is thrown on this point by the experiments represented in Fig. 4, in which the injections were repeated after the effects of the first dose were no longer apparent. It is evident that the birds were still refractory to the action of Antex at this stage. In two other experiments a

positive response was obtained on the 17th day after the first injection in one case, and a negative response in the other.

In view of the lack of uniformity of response, and of the large amounts of hormone needed, it seems improbable that the effect in question could be applied to the assay of gonadotropic hormone.

Fig. 4. Effect of repeated injection of the same (30 mg.) dose of Antex into the same hen. Curve 11. Sussex No. 16, 2nd year, winter break in laying commenced 8 weeks before the first injection, and the first egg of the new season was laid on the day preceding injection. Curve 12. White Leghorn No. 10, 2nd year, winter break in laying began 8 weeks before injection.

2. *Other gonadotropic hormone preparations.* Reiss *et al.* [1933] showed that the growth of the testes and comb of young cockerels is accelerated by pituitary and serum hormones but not by those of urinary origin, and this finding was confirmed by Hamburger [1934] and Pfaltz [1936]. It was concluded therefrom that birds are insensitive to urinary hormones, and experiments were therefore instituted with the object of testing the validity of this conclusion, with regard to blood P.

Fig. 5. Action of pituitary hormones. Curve 13. White Leghorn No. 3, 2nd year, winter break in laying commenced 1 week before injection. 0.5 g. of anterior lobe pulp and 30 mg. of Antex injected simultaneously. Curve 14. Rhode Island Red No. 12, 2nd year, laying well. 30 mg. of Antex and 100 mg. of Allen and Hanbury's prolactin injected. Five eggs were laid during the 5 days following, after which laying ceased. Curve 15. Rhode Island Red No. 11, 2nd year, winter break in laying set in 6 weeks before injection of 150 mg. of an anterior lobe preparation made according to Bates & Riddle. The injection was repeated after 6 days. Curve 16. Rhode Island Red No. 10, 2nd year, winter break in laying set in 6 weeks before injection of 52 mg. (equivalent to 30 fresh pituitaries) of purified anterior lobe preparation.

In our first experiments injections of 0.5–0.8 g. of fresh ox pituitary anterior lobe pulp produced no effect, whilst higher doses were lethal to the birds. This

negative result might have been due to presence of inhibitory or antagonistic factors in the pulp; this possibility was tested by experiments in which 30 mg. of Antex and 0.5 g. of anterior lobe pulp or 100 mg. (180 crop-sac units) of Allen and Hanbury's prolactin were injected simultaneously (curves 13 and 14, Fig. 5). The results were positive in both cases and afford no support to the hypothesis of interference by antagonistic substances. It is of interest that the hen injected with prolactin did not cease laying immediately, but only on the 6th day; in all other cases Antex caused immediate cessation of laying.

In the next experiment 150 mg. of an anterior lobe preparation, equivalent to 20 fresh ox pituitaries, made by the method of Bates & Riddle [1935], were injected. The effect (curve 15) was definite, though small. A second injection on the 6th day had no effect. A purer preparation (52 mg., equivalent to 30 pituitaries) also gave a slight, but definite effect (curve 16). Positive effects were also obtained with an injection of 300 rat units of a mare-serum gonadotropic hormone (Dr Roussel, Paris).

All urinary preparations tested (300–500 rat units of Schering-Kahlbaum, Organon, Hoffman-La Roche, Richter, Klawe gonadotropic hormones) were inactive when injected into non-laying or laying hens; they did not affect blood P, nor did they interrupt laying.

SUMMARY

1. After a single suitable dose of gonadotropic hormone (pituitary or mare-serum) the appearance of serum-vitellin in the blood stream of resting hens was observed. The other P fractions of the plasma rose simultaneously. In some cases a value for total P of 140 mg. per 100 ml. was found, being ten times higher than the normal value for resting hens and three times higher than for laying hens.

2. Gonadotropic hormones of urinary origin do not influence blood P.

3. The effect cannot be applied to assay of the hormones, but may serve for the differentiation of urinary and pituitary hormones.

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