Response of Chicks to Pituitary Gonadotropins and Pregnant Mare Serum[1]

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The variation in the response of various vertebrates to anterior pituitary hormones is of considerable biological importance and in this laboratory we have been concentrating on avian studies of this type. There are some notable differences between bird and mammalian responses to gonadotropins. First, the luteinizing hormone, L.H. stimulates the interstitial cells of the rat testes and this is also accompanied by androgen secretion; in the chick, however androgen secretion is most marked when the seminiferous tubules are stimulated by the follicle-stimulating hormone, F.S.H. (Greep et al., 1936; Fevold, 1937; Engle, 1929; and Breneman, 1935). Second, it is noteworthy that there is no augmentation reaction demonstrated by chick testes when mammalian F.S.H. and L.H. are administered (Breneman, 1936), or by squab testes, (Evans and Simpson, 1934). Chlorophyll, however, did appear to augment pituitary gonadotropin when injected in the chick (Breneman, 1939b) but this was possibly a result of slower absorption of the hormone from pituitary-chlorophyll mixtures. These apparent differences emphasize a need for better understanding of the physiology of the avian gonads.

Earlier reports on the striking response of bird gonads to pregnant mare serum were accompanied by the suggestion that this substance was purely a follicle-stimulating principle. However it was demonstrated (Breneman, 1936) that the testes of chicks which had been given P.M.S. showed both F.S.H. and L.H. effects as measured by tubule and intertubular tissue development. Rowlands and Williams (1941) and Bates and Schooley (1942) likewise have indicated that in the rat the evidence favors the idea that two principles are present in pregnant mare serum.

The availability of more highly purified pituitary preparations in recent years and the improvement in testing techniques, especially the use of inanition experiments, suggested that a reinvestigation of the avian responses to anterior pituitary and anterior-pituitary-like hormones would be profitable. Previous work by Byerly and Burrows (1938) and by Breneman (1939b) indicated that small amounts of anterior-pituitary principle would elicit responses in chicks which were kept without any food or water for a test period of 72 to 96 hours. These animals also were much more uniform in their responses than were animals on a normal diet. This greater uniformity and sensitivity affords a better opportunity for determining the response to gonadotropins.

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Materials and Methods

All chicks used in these experiments were single comb White Leghorn cockerels. Injections were made subcutaneously into the dorsal neck region once daily in the longer series, or more frequently in the short series, as will be noted in the tables. The series referred to as "limited diet" were modified inanition experiments in which food was available only on alternate days. No food was given during the last twenty-four hours of any experiment. The 96-hour experiments were patterned after the Byerly and Burrows (1938) technique in which the chicks were kept in shipping boxes of four compartments each holding 25 chicks. Injections began when the birds were 12 hours old and the animals received no food or water during the period of treatment. The mortality seldom was more than 1% in spite of the severity of the inanition. The stored yolk apparently was able to sustain the animals. The following hormone preparations were used: pregnant mare serum, P.M.S.[2], follicle-stimulating hormone, F.S.H.[2], and luteinizing hormone, L.H.[2]. A total of 650 chicks was used in these experiments.

Pregnant Mare Serum: Two types of inanition experiments were performed with P.M.S. The series were terminated at either the fifteenth or twentieth day after hatching in the first instance or a ninety-six hour test was performed. All dosages used were in terms of international units of the hormone (I.U.). The results of the longer experiments are presented in table I.

It is evident that in the 15-day series dosages of pregnant mare serum ranging from 2.5 I.U. to 40 I.U. had no significant effects on comb or gonad weights of the chicks. Injection of 40 I.U. of P.M.S. however, did produce increases in both comb and gonad weights but these were short of significant. The results suggest that 40 I.U. is near the threshold for the preparation in these experiments. Since this dosage is relatively high, it was hoped that a better response would result if the time of the experiment were extended to twenty days. The results of this experiment appear in the second part of table I.

The administration of 1 I.U. daily (total 16 I.U.) had no appreciable effect on the comb size or gonad size. Although dosages of 32 I.U. and 48 I.U. increased comb weights, the gonad weights were significantly increased in only the 48 I.U. series. It must be concluded that the longer injection period was probably the better, but the actual percentage increase over the controls produced by 48 I.U. was very little in excess of that which followed the injection of 40 I.U. in the shorter series. The longer series was, therefore, a doubtful technical improvement affording evidence only of an increase in secretory activity of the gonads. It must be concluded that the "limited diet" assay which was an efficient test for

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androgens (Breneman, 1942), is not very effective as a test for pregnant mare serum.

Previous experience has indicated that the 96-hour injection technique is a more sensitive assay in the chick than longer treatments. Three groups of experimental animals were used and these were given P.M.S. in one, three, and five injections. The controls were injected with distilled water in comparable fashion. The literature indicates that P.M.S. probably is not excreted in the rat, possibly due to the large size of the molecule and that multiple injections are no more efficacious than single administrations of the hormone; (McShan and Meyer, 1941; Meyer and McShan, 1941). It was observed in our experiments with chicks that multiple injections were much more effective. This is in harmony with a similar observation made previously concerning testosterone-propionate in which greater responses were produced by multiple injections (Breneman, 1939a).

An analysis of the data in table II reveals several important facts. As was indicated, the multiple injections were more effective at all dosage levels than a single injection of hormone. It is of considerable import that even 1 I.U. of P.M.S. produced significant increases in gonad weight when given in three or five divided doses but a single injection of 1 I.U. did not produce a significant increase. Administration of 2 I.U. in a single injection, however, did increase the testis weight to a significant level. It is also noteworthy that the gonads were larger at every dosage level of the hormone in the five injection series than in the three injection group. Furthermore, the marked plateau in response in the series given one or three injections was not evident at these dosage levels in the experiment which received five administrations of the P.M.S.

It was encouraging to observe the marked response of the gonads to the pregnant mare serum at these dosages, especially in view of the low standard errors observed. This test shows considerable promise for the assay of total gonadotrophic content of a preparation but, of course, does not furnish any evidence of a qualitative nature. It is probably safe to say, however, that the 96-hour test is from thirty to forty times more sensitive than the longer tests shown in table I. The physiological basis for this greater sensitivity is undoubtedly due to several factors, chief among which is the probability that there is no endogenous gonadotrophin present in these chicks. The White Leghorn may secrete minute amounts of pituitary hormone immediately after hatching, but the severity of the inanition reduces this pituitary activity. The response of the birds is, therefore, not complicated by variations in the amounts of endogenous hormones and this is reflected in lower standard errors. Likewise the fact that the chicks are confined in a small area and are less active would probably result in a slower rate of hormone absorption and utilization.

Follicle stimulating and lutenizing hormone — "96-hour test". The amounts of L.H. and F.S.H. injected into chicks are given in terms of rat units, R.U. The hormone was standarized on the basis of micrograms of nitrogen (Greep et al. 1942). A rat unit of the hormone was 2 μ gm. N. of L.H. and 5 μ gm. N. F.S.H. according to these determinations. These amounts, or multiples, were chosen for the chick tests and three injections

were made in a 96-hour period. Two groups of experiments were run and the controls, which were almost identical in range and standard error, were pooled. The data are presented in table III.

The effect of 1 R.U. of L.H. was barely significant but 1 R.U. of F.S.H. produced a very marked increase in gonad weight which was, however, accompanied by a greater variability. Two R.U. of L.H. was only slightly more effective than 1 R.U. and not significantly so. Good responses were also given by 2 and 4 R. U. of F.S.H. and some interesting comparisons can be made with the P.M.S. series. The 1 and 2 R.U. dosages of F.S.H. produced gonads which were almost identical in size to those produced by 4 and 8 I.U. of P.M.S. in the 5 injection series. This does not imply a qualitative similarity, but does indicate a remarkably uniform quantitative response to gonadotrophins in 96-hour chick series.

Perhaps the most interesting aspects of the data in table III are to be found in the series which were given F.S.H. and L.H. simultaneously. F.S.H. was administered at two dosage levels of 1 and 2 R.U. each with 1 R.U. of L.H. The response in both instances was greater than that produced by the F.S.H. when given separately. Most interesting, however, was the fact that the responses were within the range of expected additive weights for the L.H. and F.S.H. treatments when the hormones were given separately. Obviously this demonstrates that no augmentation occurred.

F.S.H. and L.H. "older chicks": Relatively heavy dosages of F.S.H. and L.H. (Difco) produced significant increases in comb and gonad weights of 15-day old chicks. These chicks, as well as those in the 20-day series, were not up to the normal weight for chicks of this age but should probably not be considered as limited-diet birds. A total of 20 R.U. of L.H. produced an 80% increase in the gonad weights of the chicks accompanied by more than a 100% increase in comb weight. The administration of 10 and 20 R.U. of F.S.H. likewise increased both comb and gonad weights but in neither instance was the increase as great as that which occurred in the L.H. series. A combination of the two hormones at the 2 R.U. dosage level increased both comb and gonad weight but the effect was less than the additive effect of each when given separately. This was especially noteworthy for the gonads, because the response was only slightly greater in these than that produced by the L.H. alone. This confirms the observation made in table III that combinations of F.S.H. and L.H. did not produce augmentation in the chick.

The series given F.S.H. (Thylakentrin) and L.H. (Metakentrin) shown in the second part of table IV proved to be disappointing. The fine response to small amounts of these substances which was shown in table III suggested that the dosage tried would be ample, but no significant increases were noted except in those chicks which were given the 0.2 R.U. F.S.H. plus 0.5 R.U. L.H. daily. Even in this series only the comb was increased in size, the gonad weights actually being slightly below the control figure. Since these results are comparable to the

observations on the 15- and 20-day P.M.S. series, the experiment adds additional weight to the observation that the 96-hour technique is greatly superior to the longer injection series.

Summary and Conclusions

Pregnant mare serum and pituitary gonadotrophins were injected into chicks at varying time intervals. Chicks which were kept without food or water during the test period from 12 to 96 hours after hatching proved to be very responsive. Injections of 1 I.U. of P.M.S. produced a positive increase in the gonad weight of chicks if given in three or five divided doses, but not if given in a single injection. Divided dosages of the pregnant mare serum were more efficacious in every instance than were the single injections; and the administration of the hormone in five injections was more effective than in three. Only the combs of chicks composing fifteen and twenty day series were increased in weight following the administration of pregnant mare serum and higher dosages were required than in the experiments of shorter duration. The 96-hour test was approximately thirty to forty times more sensitive than the longer assay.

Follicle stimulating hormones were also effective on chicks but showed no evidence of augmentation when combined with L.H. Small dosages of 0.2 R.U. of follicle stimulating hormone and 0.5 R.U. of luteinizing hormone daily were ineffective in 15-day chick experiments and the hormones gave good responses only at the relatively high dosages of 10 to 20 R.U. Comb growth was stimulated following the administration of each hormone. When combination injections were given there was again no evidence of any augmentation. The increases noted were essentially additive effects of the hormone.

It is concluded that chicks used for the 96-hour test are highly sensitive to pituitary gonadotrophin and to pregnant mare serum. There is no evidence of augmentation when relatively low dosages of the hormone are administered. Both F.S.H. and L.H. increased comb size in older birds.

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Table I

Effect of P.M.S. on comb and gonad weights in "limited-diet" chicks

Treatment P.M.S. daily I.U.	No. of chicks	Body gm.	Comb mg.	Gonad mg.
15-day series: injections 4th to 13th day				
(Controls)	13	55.5	14.2 ± 1.17*	11.0 ± 0.56*
0.25	14	56.6	14.2 ± 0.94	11.0 ± 0.71
0.5	16	57.8	16.8 ± 0.95	10.5 ± 0.47
1.0	14	56.9	13.4 ± 1.12	11.0 ± 0.58
2.0	16	58.2	15.5 ± 1.40	11.0 ± 0.89
4.0	13	54.7	17.9 ± 2.54	13.9 ± 1.30
20-day series: injections 4th to 19th day				
(Controls)	20	75.0	27.3 ± 1.87	24.0 ± 1.19
1.0	13	78.5	28.0 ± 3.71	23.7 ± 2.54
2.0	9	76.5	40.1 ± 8.30	26.1 ± 3.65
3.0	10	89.8	51.7 ± 9.26	31.3 ± 3.65

Chicks received food only on alternate days. Injections were given in 0.20 cc. of water.

* Standard error.

Table II

Effect of P.M.S. on gonad weights of chicks in "96-hour test"

Treatment (Total Dosage) P.M.S. I.U.	No. of chicks	Body gm.	Gonad mg.
Single injection 12 hours after hatching			
1	15	26.3	4.9 ± 0.41*
2	15	27.7	6.0 ± 0.32
4	14	26.9	6.9 ± 0.41
8	14	26.5	5.9 ± 0.30
10	15	27.9	7.2 ± 0.50
Three injections: 12, 36, and 60 hours after hatching			
1	11	30.8	6.6 ± 0.38
2	14	30.6	7.0 ± 0.55
4	14	28.8	8.1 ± 0.74
8	14	29.1	7.7 ± 0.55
Five injections: 12, 24, 36, 48, 60 hours after hatching			
1	12	31.3	7.2 ± 0.58
2	15	31.5	7.5 ± 0.60
4	15	31.0	9.4 ± 0.94
8	14	29.8	11.4 ± 0.42
Controls	40	28.0	4.6 ± 0.22

Chicks were kept in shipping boxes, 25 chicks in each of four compartments. All injections were in 0.20 cc. of water.

* Standard error.

Table III

Effect of F.S.H. (Thylakentrin) and L.H. (Metakentrin) on gonad weights of chicks in "96-hour test"

Treatment (Total Dosage)	No. of chicks	Body gm.	Gonad mg.
1 R.U. L.H.	25	39.8	7.7 ± 0.36*
2 R.U. L.H.	10	41.1	8.1 ± 0.66
1 R.U. F.S.H.	15	37.1	9.3 ± 0.47
2 R.U. F.S.H.	15	37.4	11.3 ± 0.42
4 R.U. F.S.H.	10	40.3	15.6 ± 1.70
1 R.U. F.S.H. plus 1 R.U. L.H.	22	35.0	10.4 ± 0.28
2 R.U. F.S.H. plus 1 R.U. L.H.	9	34.8	12.9 ± 1.21
Controls	32	32.6	5.4 ± 0.25

Chicks were kept in shipping boxes, 25 chicks in each of four compartments. All injections were in 0.20 cc. of water.

* Standard error.

Table IV

Effects of pituitary hormones on 15- and 20-day-old chicks

Treatment (daily)	No. of chicks	Body gm.	Comb mg.	Gonad mg.
15-day series—injections from 5th to 14th days				
Controls	18	77.1	30.8 ± 4.6*	21.9 ± 1.7*
2 R.U. L.H.	17	82.6	67.2 ± 10.0	39.6 ± 3.2
1 R.U. F.S.H.	18	87.7	41.9 ± 2.9	28.8 ± 3.6
2 R.U. F.S.H.	17	87.2	48.0 ± 7.1	38.8 ± 2.4
2 R. U. F.S.H. plus 2 R.U. L.H.	19	90.2	88.1 ± 12.2	45.2 ± 4.1
20-day series—injections from 4th to 19th days				
Controls	11	101.6	48.9 ± 12.9	30.5 ± 3.9
0.2 R.U. F.S.H.	9	87.1	37.1 ± 8.1	28.1 ± 3.8
0.5 R.U. F.S.H.	13	86.6	41.7 ± 9.0	25.9 ± 3.9
0.8 R.U. F.S.H.	10	93.1	40.8 ± 7.1	31.6 ± 3.8
1.0 R.U. L.H.	10	85.6	28.4 ± 5.2	23.2 ± 3.4
0.2 R.U. F.S.H. plus 0.5 R.U. L.H.	10	84.4	57.5 ± 21.3	27.8 ± 5.9

All injections were in 0.20 cc. of water.

* Standard error.