EFFECTS OF AVIAN AND MAMMALIAN PITUITARY PREPARATIONS ON FOLLICULAR GROWTH IN HENS TREATED WITH METHALLIBURE OR FASTING

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(Received 10th November 1971, accepted 15th February 1972)

Summary. Hens with regressed ovaries produced by treatment with Methallibure (0.02% concentration in the ration) or by fasting received injections of avian or mammalian pituitary preparations to induce follicular growth in their ovaries. The preparations used were homogenates or acetone-dried powder of fowl, *Gallus domesticus*, anterior pituitary (CAP), glycoprotein-extract obtained from CAP, the unadsorbed fraction of glycoprotein-extract on CM-cellulose, the unadsorbed fraction of glycoprotein-extract on DEAE-cellulose, acetone-dried bovine anterior pituitary, NIH-FSH of ovine origin, ovine NIH-LH, and a mixture of FSH and LH.

The administration of preparations originating from CAP resulted in an increase of the serum vitellin titre and the formation of large yellow follicles. By contrast, the serum vitellin titres in almost all the hens injected with mammalian preparations did not show the same increase. Ovarian stimulation, shown by increase in the size of white follicles or the existence of recently formed atretic follicles, was found in 42% of hens treated with mammalian preparations. Yellow follicles were observed in only a few of these treated hens, and there were not as many follicles of graded size as in hens treated with preparations of CAP origin.

It was suggested from these results that fowl pituitary constituent(s) were necessary to cause a substantial follicular growth in the regressed ovary of the hen.

INTRODUCTION

It has been reported that fowl, *Gallus domesticus*, pituitary material is more effective than mammalian gonadotrophins in stimulating ovarian function in immature pullets (Das & Nalbandov, 1955), starving pullets (Morris & Nalbandov, 1961) and hypophysectomized hens (Opel & Nalbandov, 1961). In addition, Nalbandov, Meyer & McShan (1951) found that mammalian gonadotrophin preparations stimulated testicular function of hypophysectomized cockerels only for a short period, whereas sustained growth and continuous androgen secretion were produced in their testes by the injection of

fowl pituitary. In these studies, however, the effects of desiccated fowl anterior pituitaries were compared with those of partially purified mammalian gonadotrophins. The superiority of the avian material for gonad-stimulating activity in the fowl may lie in a qualitative difference between avian and mammalian gonadotrophins or may be attributable to the difference between crude and partially purified preparations. In the present work, the effects of follicular growth of partially purified fowl and ovine gonadotrophin preparations were compared with those of crude fowl and bovine anterior pituitary glands on the regressed ovaries of hens, which were produced by previous treatment with Methallibure, a non-steroid pituitary inhibitor (Paget, Walpole & Richardson, 1961; Sykes, 1964), or by fasting (Hosoda, Kaneko, Mogi & Abe, 1955).

Follicular growth in the hen is a very pronounced process compared to the same event in mammals commonly used for experiments, since it involves continuous production, mobilization and deposition of large quantities of yolk-precursors for about 10 days (Warren & Conrad, 1939). During the rapid growth phase, the small white follicle of less than 0.5 cm diameter develops to a mature yellow follicle of more than 3 cm diameter.

MATERIALS AND METHODS

Experimental hens

Single comb White Leghorn pullets, aged 7 to 11 months, were maintained in individual cages under a 14-hr light/10-hr dark regimen and at a temperature of $24 \pm 4^\circ \text{C}$. Food and water were freely available. After recording the egg-laying for at least 3 weeks, hens with relatively regular sequences (ranging from four to seven eggs) were selected for the experiments. The hens were allotted to two groups which received different treatments to produce regression of the ovaries.

The laying pullets of one group were fed Methallibure (ICI compound 33828) at a concentration of 0.02% in the normal dry-mash ration based on the results of preliminary experiments using 0.01% and 0.02% concentrations (Sykes, 1964). The 0.02% level of the compound caused cessation of egg-laying in all the treated hens within 5 days and induced a non-detectable level of serum vitellin (a titre of less than 10) within 12 days after the start of feeding in ninety-eight out of a total of 105 birds. Those hens with a non-detectable vitellin level on the 12th day after the beginning of the treatment had regressed ovaries without yellow follicles. After 21 days, the ovaries weighed an average of $2.4 \pm 0.26 \text{ g}$ (eighteen hens) and did not have any yellow or atretic follicles. The hens fed Methallibure produced watery droppings and suffered a slight loss of body weight but were otherwise healthy. On the basis of these results, the serum vitellin titre was measured on the 12th day after the onset of treatment with Methallibure and those hens with a non-detectable level of vitellin were used for the experiments involving administration of pituitary preparations.

The other group of laying pullets fasted for 3 to 5 days. The serum vitellin level of the non-laying hens produced by this treatment was measured on the 6th day after that on which they laid no egg. The hens with a non-

detectable level of vitellin had completely regressed ovaries, weighing an average of 2.9 ± 0.37 g (four hens). These ovaries consisted of small white follicles (less than 0.4 cm in diameter) and sometimes contained small atretic follicle(s); no yellow follicles were present. The ovaries of fasting hens remained in quiescent phase, weighing an average of 2.7 ± 0.31 g (eight hens) until at least 21 days after the cessation of egg laying. The fasting hens with no detectable level of serum vitellin on the 6th day after cessation of lay received the injections of pituitary preparations, which were started on the day following the vitellin determination.

Treatments with hormone preparations

The hens selected following Methallibure treatment were injected intramuscularly with various doses of fowl or mammalian pituitary preparations once daily for 8 days. The control birds received physiological saline under the same conditions. Hens were killed on the day following the final injection, and the ovaries and oviducts were observed and weighed.

The hens with non-detectable vitellin induced by fasting were injected with fowl anterior pituitary homogenate or ovine FSH and/or LH preparations once a day for 6 or 8 days. An autopsy was carried out on the day after the final injection, except for one group in which the hens were killed on the 7th day after the final injection of fowl pituitary homogenate. During the period from the first injection to autopsy, the serum vitellin titre was measured every other day in all of the injected hens.

Serum vitellin levels were measured by the immunological procedure described previously (Imai, Tanaka & Nakajo, 1972). The interfacial precipitin reaction between antivittellin serum and serially diluted test serum was observed in the capillary tube, and the titre was expressed as the maximal dilution rate of the test serum in which the precipitin reaction was positive.

Pituitary preparations

Fowl (chicken) anterior pituitaries (CAP) were collected from broilers of both sexes, quick-frozen and stored at -20° C. The CAP pool was homogenized in cold saline in an ice-box immediately before the injection (CAP homogenate in the Tables). Part of the CAP pool was stored for 2 weeks in cold acetone, replacing with fresh acetone every 3 days, and then dried and stored in a desiccator. The dried CAP pool was homogenized in cold saline before the injection (CAP, acetone-dried).

Batches consisting of 2 g frozen CAP (approximately 380 pituitaries) were used for extraction and partial purification, and the procedures were carried out at 4° C. Glycoprotein hormones from CAP were obtained by extraction with ammonium acetate according to the slightly modified method of Stockell-Hartree & Cunningham (1969). The CAP was extracted twice with 20 ml 6% ammonium acetate in 40% ethanol at pH 5.1. The supernatants obtained from each extraction were combined and 5 vol. cold absolute ethanol were added slowly to the extracts. The precipitates were collected 24 hr later, washed with cold ethanol and freeze dried. The average yield of the product (designated GP-extract) was 34.8 mg/2 g CAP. Adsorption of glycoprotein (GP)-extract on

carboxymethyl (CM)- or diethylaminoethyl(DEAE)-cellulose was used for a further purification. The GP-extract obtained from each batch of CAP was dissolved in 5 ml 4 mM-ammonium acetate at pH 5.5. The resulting solution was mixed with 20 ml 4% (w/v) CM-cellulose in 4 mM-ammonium acetate at pH 5.5, and stirred thoroughly for 20 min. The supernatant (unadsorbed fraction on CM-cellulose) was collected and the precipitates were then obtained by addition of 5 vol. cold absolute ethanol to the supernatant. After washing with cold ethanol and freeze drying, the final product (named CM-fraction) was obtained. The average yield of CM-fraction was 9.2 mg/2 g CAP. The GP-extract prepared from 2-g batches of CAP was also adsorbed on to DEAE-cellulose in 0.05 M-disodium hydrogen phosphate at pH 7.5, according to the method described for CM-cellulose. Following the adsorption procedure, the unadsorbed fraction on DEAE-cellulose was collected and the precipitates resulting from the addition of ethanol were freeze dried. The average yield of the final product (designated DEAE-fraction) was 21.3 mg/2 g CAP. A desired amount of the preparations was dissolved in 1 ml cold physiological saline immediately before the injection.

Three kinds of preparations of mammalian pituitary origin were used. Anterior pituitary glands collected from five cows were placed in acetone for several weeks. The glands were then dried, crushed and stored in a desiccator. Immediately before the injection, the pool of dried bovine anterior pituitaries (BAP) was homogenized in saline in an ice-box. Ovine pituitary FSH (NIH-FSH-s7) and LH (NIH-LH-s15) were also used for injection, alone or in combination.

RESULTS

Follicular growth in hens treated with Methallibure

Table 1 shows the effects of the preparations of fowl pituitary origin on follicular growth in hens with ovaries made quiescent by treatment with Methallibure. Although the vitellin titre increased within 3 days of the first injection, it increased more in the groups which received a high dose of the preparations. The increases of ovarian and oviducal weights and the degree of follicular growth found at autopsy tended also to be related to the doses used in each preparation. Of the ovaries of thirty-four hens receiving the fowl preparations, twenty-nine (85.3%) had many growing yellow follicles. No yellow follicle was observed in the ovaries of the remaining five hens in the low-dose groups, but white follicles of 0.5 to 0.9 cm diameter and recently formed yellowish atretic follicles were present; they were never observed in the control ovaries. Apparently normal or slightly overstimulated follicular growth with a graded series of follicles, comparable to those found in the ovary of the laying hen, was induced in the ovaries of hens in five groups injected with the high doses of CAP, GP-extract, CM-fraction and DEAE-fraction for 8 days.

By contrast, mammalian pituitary preparations had little effect on the ovaries of Methallibure-treated hens (Table 2). No increase of serum vitellin was found in the hens receiving the two dose levels of ovine FSH. Moreover, no sign of ovarian and, hence, of oviducal stimulation was seen in these hens at the time of autopsy. In the groups injected with BAP or ovine LH, the vitellin titre did

TABLE 1
EFFECTS OF THE ADMINISTRATION OF FOWL PITUITARY PREPARATIONS FOR 8 DAYS TO HENS TREATED WITH METHALLIBURE

Daily dose of preparation	No. of hens	Serum vitellin titre at autopsy	No. of hens showing follicular condition of:					Average no. of yellow follicles/hen	Ovarian weight (g) (Mean $\pm$ S.E.)	Oviducal weight (g) (Mean $\pm$ S.E.)
			-1	+1	+2	+3	+4			
50 mg, CAP, homogenate	3	40 to 160	—	1	2	—	—	6	5.9 $\pm$ 1.5 ^{ab*}	19.5 $\pm$ 0.8 ^{ab*}
100 mg, CAP, homogenate	5	160	—	—	1	4	—	12	43.7 $\pm$ 9.8 ^c	31.5 $\pm$ 2.2 ^{bc}
10 mg, CAP, acetone-dried	3	80 to 160	—	—	2	1	—	9	17.3 $\pm$ 4.3 ^{abc}	28.8 $\pm$ 2.0 ^{bc}
20 mg, CAP, acetone-dried	3	160 to 320	—	—	—	3	—	16	43.2 $\pm$ 7.1 ^{de}	32.9 $\pm$ 2.7 ^{bc}
100 mg, CAPeq, GP-extract	5	80 to 160	—	—	—	4	—	15	36.1 $\pm$ 5.3 ^{de}	29.9 $\pm$ 5.0 ^{bc}
50 mg, CAPeq, CM-fraction	3	40 to 160	—	1	1	1	—	11	12.6 $\pm$ 7.8 ^{ab}	26.9 $\pm$ 2.6 ^{bc}
100 mg, CAPeq, CM-fraction	3	40 to 160	—	2	—	—	—	10	15.6 $\pm$ 13.0 ^{abc}	20.1 $\pm$ 9.7 ^{ab}
200 mg, CAPeq, CM-fraction	3	80 to 320	—	—	1	2	—	15	39.9 $\pm$ 9.3 ^{de}	38.0 $\pm$ 7.2 ^c
100 mg, CAPeq, DEAF-fraction	3	80 to 160	—	1	—	2	—	16	24.5 $\pm$ 11.7 ^{bcd}	22.1 $\pm$ 9.1 ^b
200 mg, CAPeq, DEAF-fraction	3	160	—	—	1	2	—	17	32.2 $\pm$ 4.0 ^{de}	38.7 $\pm$ 5.4 ^c
Total	34		0	5	—	—	29	—	2.5 $\pm$ 0.6 ^a	5.7 $\pm$ 0.6 ^a
Saline	5	< 10	5	—	—	—	—	—	—	—

See text for explanation of abbreviations.

* The differences between groups with the same letter were not significant statistically ($P < 0.05$).

¹ The ovary had no follicle larger than 0.5 cm diameter and no recently formed atretic follicle.

² The ovary had white follicles of 0.5 to 0.9 cm diameter and/or recently formed atretic follicle(s), but no yellow follicle.

³ The ovary had yellow follicle(s) of less than 1.4 cm diameter.

⁴ The ovary had yellow follicle(s) of more than 1.5 cm diameter.

TABLE 2
EFFECTS OF THE ADMINISTRATION OF MAMMALIAN PITUITARY PREPARATIONS FOR 8 DAYS TO HENS TREATED WITH METHALLIBURE

Daily dose of preparation	No. of hens	Serum vitellin titre at autopsy	No. of hens showing follicular condition of:					Average no. of yellow follicles/hen	Ovarian weight (g) (Mean $\pm$ S.E.)	Oviducal weight (g) (Mean $\pm$ S.E.)
			—	1	2	+	3	+	4	
20 mg, BAP	3	20 to 40	2	1	—	—	—	—	2.7 $\pm$ 0.4 ^{ab*}	9.6 $\pm$ 1.9 ^{ab*}
40 mg, BAP	3	40	1	2	—	—	—	—	3.9 $\pm$ 0.5 ^{abc}	14.5 $\pm$ 1.6 ^{bc}
100 μ g, FSH	3	<10	3	—	—	—	—	—	3.4 $\pm$ 0.5 ^{abc}	6.8 $\pm$ 0.5 ^a
200 μ g, FSH	3	<10	3	—	—	—	—	—	2.6 $\pm$ 0.4 ^{ab}	4.6 $\pm$ 0.3 ^a
100 μ g, LH	3	40 to 80	1	2	—	—	—	—	4.3 $\pm$ 1.0 ^{bcd}	14.4 $\pm$ 3.6 ^{bc}
100 μ g, FSH + 50 μ g, LH	3	80	—	1	2	—	—	—	5.2 $\pm$ 1.9 ^{cd}	14.7 $\pm$ 2.6 ^{bc}
150 μ g, FSH + 75 μ g, LH	3	80	—	3	—	—	—	—	5.9 $\pm$ 1.1 ^d	16.1 $\pm$ 1.2 ^{bc}
100 μ g, FSH + 100 μ g, LH	3	80 to 160	2	—	1	—	—	—	2.8 $\pm$ 0.3 ^{ab}	11.8 $\pm$ 2.5 ^{abc}
200 μ g, FSH + 200 μ g, LH	3	80 to 160	—	2	1	—	—	—	5.3 $\pm$ 0.7 ^{cd}	19.4 $\pm$ 5.8 ^c
100 μ g, FSH + 200 μ g, LH	3	80	2	1	—	—	—	—	3.1 $\pm$ 0.4 ^{ab}	16.3 $\pm$ 2.4 ^{bc}
Total	30		14	12	4	—	—	—		
Saline	6	<10	6	—	—	—	—	—	2.3 $\pm$ 0.3 ^a	5.7 $\pm$ 0.8 ^a

See text for explanation of abbreviations

*1, 2, 3, 4 See the notes of Table 1.

not rise as it did in the groups injected with fowl pituitary preparations. Although a stimulated ovary, consisting of white follicles of increased sizes or recently formed atretic follicles, was found in about half of the hens treated with BAP and LH, no yellow follicle was observed in any hens. Treatments with mixtures of ovine FSH and LH in the ratios of 2:1, 1:1, or 1:2 resulted in an increase of the vitellin titre, reaching 80 to 160, which is within the normal range of the laying hen, but the oviducts remained small compared with those of hens injected with fowl preparations. Small yellow follicles of 0.5 to 0.9 cm diameter were found in the ovaries of only four hens out of a total of thirty birds injected with mammalian preparations. The ovaries of hens treated with mixtures of FSH and LH did not contain as many yellow follicles as those of hens that had been treated with fowl pituitary preparations.

Follicular growth in the fasting hens

The effects of injected avian and mammalian hormone preparations on ovarian stimulation in hens with quiescent ovaries produced by fasting were similar to the results in the Methallibure-treated hens (Table 3). A follicular growth as good as that in the laying hen was induced in all the fasting hens by the administration of CAP homogenate for 8 days. The ovaries of hens killed on the 7th day after the final injection of CAP did not have any yellow follicles, but contained many atretic follicles. In these hens, the serum vitellin increased and remained at a high level during, and for a few days after, the injection period, then decreased again to a non-detectable level for 1 to 3 days before autopsy. These results indicated that follicular growth was induced by the administration of CAP but that developing follicles were not supported by the hormonal condition of the hen itself.

Yellow follicles were only found in three of the hens treated with mammalian FSH and/or LH. In one, which received 40 μ g ovine LH for 6 days, only one yellow follicle of 1.3 cm diameter had developed and the ovarian weight was 5.1 g. In the other two hens, which were injected with a mixture of 20 μ g FSH and 50 μ g LH, there was one yellow follicle in each ovary; the follicle diameters were 1.4 and 2.0 cm, and the ovarian weights were 5.4 and 7.4 g, respectively. The ovaries of the remaining twenty-seven hens receiving mammalian gonadotrophins did not contain any yellow follicle, but evidence of ovarian stimulation, shown by increase in the diameter of white follicles and/or the existence of recently formed small atretic follicles, was found in six of the hens. It was also noted that, in the fasting hens, ovine FSH, in the doses used, did not raise serum vitellin levels or cause follicular growth in the regressed ovary.

Additional experiment

From hens treated with Methallibure and injected with the mixture of 200 μ g FSH and 200 μ g LH once daily for 8 days (the highest dose group in Table 2), blood samples were collected on the day following the final injection and the serum of each hen was tested by Ouchterlony's double diffusion technique in agar gel (Henry & van Dyke, 1958) in order to demonstrate whether the antibodies against injected FSH and LH were formed in the sera of these hens.

TABLE 3

EFFECTS OF THE ADMINISTRATION OF FOWL PITUITARY HOMOGENATE OR OVINE FSH AND/OR LH PREPARATIONS TO HENS THAT HAD BEEN ON A REDUCED FEED INTAKE

Injection	No. of hens	Serum vitellin titre at autopsy	No. of hens showing follicular condition of:					Average no. of yellow follicles/hen	Ovarian weight (g) (Mean $\pm$ S.E.)	Oviductal weight (g) (Mean $\pm$ S.E.)
			-1	+2	+3	+4	+5			
70 mg, CAP $\times$ 6	5	80 to 320	—	—	2	3	—	11	48.5 $\pm$ 14.3 ^{ab} *	38.9 $\pm$ 5.7 [†] *
70 mg, CAP $\times$ 8	4	160 to 320	—	—	—	4	—	19	55.0 $\pm$ 13.6 ^{ab}	50.0 $\pm$ 9.5 [†]
70 mg, CAP $\times$ 8	3	<10	—	3	—	—	—	—	5.3 $\pm$ 0.2 ^{ab}	11.0 $\pm$ 0.8 ^{bcd}
(Autopsy: 7 days after the final injection)										
50 μ g, FSH $\times$ 6	3	<10	3	—	—	—	—	—	2.6 $\pm$ 0.4 ^a	7.0 $\pm$ 0.8 ^{ab}
100 μ g, FSH $\times$ 6	3	<10	3	—	—	—	—	—	2.8 $\pm$ 0.5 ^a	9.1 $\pm$ 1.7 ^{abcd}
200 μ g, FSH $\times$ 6	3	<10	3	—	—	—	—	—	3.4 $\pm$ 0.9 ^{ab}	8.6 $\pm$ 2.0 ^{abc}
40 μ g, LH $\times$ 6	3	20 to 40	1	1	1	—	—	1	4.1 $\pm$ 0.6 ^{ab}	12.1 $\pm$ 0.7 ^{cde}
80 μ g, LH $\times$ 6	3	10 to 40	2	1	—	—	—	—	2.7 $\pm$ 0.4 ^a	10.0 $\pm$ 1.4 ^{bcd}
50 μ g, FSH + 20 μ g, LH $\times$ 6	3	10 to 20	3	—	—	—	—	—	2.6 $\pm$ 0.3 ^a	8.4 $\pm$ 1.1 ^{abc}
100 μ g, FSH + 40 μ g, LH $\times$ 6	3	10 to 40	2	1	—	—	—	—	3.4 $\pm$ 0.2 ^{ab}	13.6 $\pm$ 1.0 ^e
200 μ g, FSH + 80 μ g, LH $\times$ 6	3	10 to 40	2	1	—	—	—	—	3.2 $\pm$ 0.9 ^a	10.3 $\pm$ 2.2 ^{bcd}
50 μ g, FSH + 50 μ g, LH $\times$ 6	3	20 to 80	1	2	—	—	—	—	5.0 $\pm$ 1.1 ^{ab}	12.5 $\pm$ 2.8 ^{cde}
20 μ g, FSH + 50 μ g, LH $\times$ 6	3	40 to 80	1	—	1	1	—	1	5.2 $\pm$ 1.3 ^{ab}	13.1 $\pm$ 1.2 ^{de}
Saline	5	<10	5	—	—	—	—	—	2.5 $\pm$ 0.5 ^a	5.2 $\pm$ 0.3 ^a

See text for explanation of abbreviations.

*1, 2, 3, 4 See the notes of Table 1.

The serum of each hen was placed in a central well and FSH or LH solutions at concentrations of 25 to 100 μ g were arranged in surrounding wells in the agar plate. The distance between the well centres was 1.8 cm. The plates were stored at 4° C and observed every 2 days for 8 days. In all cases, no precipitation band formed between the test sera and the antigen solution. These results showed that no antibodies to FSH and LH were formed in the hens receiving the stated doses of FSH and LH for 8 days.

DISCUSSION

It is concluded from the results of the present study that hormone preparations which originated from fowl anterior pituitaries had the ability to cause follicular growth in regressed ovaries produced in hens by treatment with Methallibure or by fasting. Oestrogen secretion was also induced in the follicles developed by these preparations as judged from both the increase in serum vitellin and the oviducal redevelopment which occurred.

Mitchell (1966) injected acetone-dried CAP daily for 8 days into hens treated with Methallibure and found that follicular development was variable in response to the particular dose levels used, but daily injection with 25 mg of the powder elicited apparently normal or overstimulated follicular growth in four out of six hens. Since this dose level of the powder was equivalent to about twenty pituitaries, her result coincides very well with the present findings on the induction of follicular growth by 100 mg CAP homogenate (equivalent to eighteen to twenty pituitaries) or 20 mg acetone-dried CAP (equivalent to twenty pituitaries). It was also reported by Mitchell (1967) that fowl pituitary gonadotrophin (CPG), prepared from acetone-dried powder by an extraction procedure essentially similar to the method used for obtaining GP-extract in the present work, was an effective stimulator of follicular growth in Methallibure-treated hens. She found that 1.5 mg CPG was an effective dose to induce the growth of a graded series of follicles and this dose corresponded to an extract from approximately thirty pituitaries. In the present work, the effective dose of GP-extract to cause follicular growth in a regressed ovary was 100 mg CAP equivalent. Thus, GP-extract appears to be slightly more potent than CPG (Mitchell, 1967) with respect to the induction of follicular growth in the regressed ovary. Recently, Mitchell (1970) found that a CM-1 fraction, prepared from CPG by column chromatography on CM-cellulose, had no stimulatory effect on the regressed ovary of hypophysectomized hens. This finding is completely different from the results of the present study. The reason for this discrepancy may lie in differences of procedures for purification and/or of recipients for injection, or may be due to the different dose levels used in the experiments.

The CM- and DEAE-fractions in the present study possessed about equal ability to induce follicular growth in the regressed ovary, similar doses of both fractions resulting in almost the same degree of follicular development. However, it has recently been observed that the activity of the DEAE-fraction in causing ovulation in the hen is considerably higher than that of the CM-fraction (K. Imai, unpublished observations). These findings suggest that the follicular

growth-inducing activity in CAP is different from the activity for induction of ovulation, and the relative concentration of the two activities contained in the CM-fraction differs considerably from that in the DEAE-fraction. Stockell-Hartree & Cunningham (1969) and Furr & Cunningham (1970), examining the gonadotrophic properties of the preparations obtained from fowl pituitary by assays for FSH and LH activities, suggested that FSH (ovarian augmentation response—OAR) and LH (ovarian ascorbic acid depletion—OAAD) existed separately in the pituitary because each activity was concentrated in different preparations. Nelson, Norton & Nalbandov (1965) and Imai & Nalbandov (1971) measured pituitary and blood OAAD and OAR activities during an ovulatory cycle of the laying hen and the latter authors suggested that separate secretion mechanisms existed for these two activities. Further experiments will be required to demonstrate whether follicular growth is initiated and maintained by either of the fowl gonadotrophins or by a complex, and whether a different combination is formed to cause ovulation.

With the dose levels and the combined ratios of ovine FSH and LH used in the present work, it appears that the mammalian pituitary preparations were less effective than CAP in inducing follicular growth in hens with regressed ovaries. It was considered possible that the materials injected during the experimental period provoked the formation of antibodies which disturbed normal ovarian function. However, this possibility may be ruled out by the following considerations. Firstly, the low doses used for the injection and the short duration of the experiment (9 days after the initial injection) were probably inadequate conditions for the production of antibodies. Injections of 1.5 mg ovine FSH or LH with an adjuvant once a week for at least 4 weeks are required to produce anti-FSH or -LH serum in the rabbit (K. Kawakura & K. Imai, unpublished observations). Secondly, it was demonstrated that no precipitin band was formed between the sera of hens injected with the highest dose of FSH and LH used in the present study and the antigen solutions in agar gel. Nalbandov *et al.* (1951) reported previously that no antihormones existed in the sera of cockerels following daily injections of an unfractionated sheep pituitary preparation for 33 days.

Careful observation of the responses of ovarian follicles to the mammalian pituitary preparations suggests that mammalian gonadotrophins are likely to be effective for the initial stimulation of the inactive ovary but inadequate for maintaining further follicular development. In the present results, evidence of ovarian stimulation was found in 42% of the sixty hens receiving the mammalian preparations, but yellow follicles existed in the ovaries of only seven hens (Tables 2 and 3). Even when a yellow follicle was produced by the mammalian gonadotrophins, the number of yellow follicles in the ovaries was few compared with those found in hens treated with the fowl pituitary preparations. Several atretic follicles regarded as having been formed within the period of injection were noted in these ovaries. It may be concluded from the results in the present work that the mammalian gonadotrophins are inadequate to produce continuous development of follicles in the fowl ovary and constituent(s) of the fowl pituitary are essential to induce and maintain a graded series of follicles in the regressed ovary of the hen. Immunochemical investigations are necessary to

clarify any qualitative differences between fowl and mammalian pituitary gonadotrophins.

ACKNOWLEDGMENTS

I wish to express sincere thanks to the National Institute of Health, U.S.A., for supplying NIH-FSH-s7 and NIH-LH-sl5 and to the Imperial Chemical Industries Ltd, U.K., through Sumitomo Chemical Co. Ltd, Japan, for giving Methallibure. I am indebted to Dr S. Nakajo for helpful discussions, to Mr K. Kawakura for immunological studies, and to Mr T. Niwa, Mr T. Yoshida, Mr Y. Ando, Mr R. Mizuguchi and Mr M. Wakamatsu for providing fowl pituitary preparations. The technical assistance of Mr R. Ogawa is gratefully acknowledged.

REFERENCES

- DAS, B. C. & NALBANDOV, A. V. (1955) Responses of ovaries of immature chickens to avian and mammalian gonadotrophins. *Endocrinology*, **57**, 705.
- FURR, B. J. A. & CUNNINGHAM, F. J. (1970) The biological assay of chicken pituitary gonadotrophins. *Br. Poult. Sci.* **11**, 7.
- HENRY, S. S. & VAN DYKE, H. B. (1958) A study of the antibodies produced in response to purified preparations of sheep interstitial cell stimulating hormone. *J. Endocr.* **16**, 310.
- HOSODA, T., KANEKO, T., MOGI, K. & ABE, T. (1955) Effect of gonadotropic hormone on ovarian follicles and serum vitellin of fasting hens. *Proc. Soc. exp. Biol. Med.* **88**, 502.
- IMAI, K. & NALBANDOV, A. V. (1971) Changes in FSH activity of anterior pituitary glands and of blood plasma during the laying cycle of the hen. *Endocrinology*, **88**, 1465.
- IMAI, K., TANAKA, M. & NAKAJO, S. (1972) Gonadotrophic activities of anterior pituitary and of blood plasma and ovarian response to exogenous gonadotrophin in moulting hens. *J. Reprod. Fert.* **30**, 443.
- MITCHELL, M. E. (1966) *Effect of fowl gonadotrophin following treatment with a pituitary inhibitor*. In: Physiology of the Domestic Fowl, p. 14. Eds. C. Horton-Smith and E. C. Amoroso. Oliver & Boyd, Edinburgh.
- MITCHELL, M. E. (1967) The effects of avian gonadotrophin precipitate on pituitary-deficient hens. *J. Reprod. Fert.* **14**, 257.
- MITCHELL, M. E. (1970) Treatment of hypophysectomized hens with partially purified avian FSH. *J. Reprod. Fert.* **22**, 233.
- MORRIS, T. R. & NALBANDOV, A. V. (1961) The induction of ovulation in starving pullets using mammalian and avian gonadotropins. *Endocrinology*, **68**, 687.
- NALBANDOV, A. V., MEYER, R. K. & McSHAN, W. H. (1951) The role of a third gonadotrophic hormone in the mechanism of androgen secretion in chicken testes. *Anat. Rec.* **110**, 475.
- NELSON, D. M., NORTON, H. W. & NALBANDOV, A. V. (1965) Changes in hypophysial and plasma LH levels during the laying cycle of the hen. *Endocrinology*, **77**, 889.
- OPEL, H. & NALBANDOV, A. V. (1961) Follicular growth and ovulation in hypophysectomized hens. *Endocrinology*, **69**, 1016.
- PAGET, G. E., WALPOLE, A. L. & RICHARDSON, D. N. (1961) Non-steroid inhibitors of pituitary gonadotrophic function. *Nature, Lond.* **192**, 1191.
- STOCKELL-HARTREE, A. & CUNNINGHAM, F. J. (1969) Purification of chicken pituitary follicle-stimulating hormone and luteinizing hormone. *J. Endocr.* **43**, 609.
- SYKES, A. H. (1964) Some actions of an anti-fertility compound in the fowl. *Vet. Rec.* **76**, 393.
- WARREN, D. C. & CONRAD, R. M. (1939) Growth of the hen's ovum. *J. agric. Res.* **58**, 875.