INVESTIGATION ON CHICK ASSAY METHOD FOR AVIAN GONADOTROPINS*

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One of the major problems in studies on gonadotropin (GTH) is to use the appropriate assay method. While numerous methods of bio-assay for GTH were used by many workers, one of those was based on the weight-increases of gonad and accessory sexual organ of assay animals, and this might be classified into following three groups according to the species of assay animals; rat, mouse and chick method.

Greep *et al.* (1936) and Cartland and Nelson (1938) concluded that gonads and accessory organs of immature rats suited to assay end-point for GTH. The method in which immature mice were used as assay animal was established by Levin and Tyndale (1937). Breneman (1936, 1945a) indicated that small amounts of anterior pituitary principle would elicit the increase of testicular weight of chicks which were kept without food and water for a test period of 96 hrs. When the potency of GTH from the fowl anterior pituitary was assayed, the rat (Meyer *et al.* 1939), the mouse (Riley and Fraps, 1942a,b,) and the chick assay (Breneman, 1945b) were used. However, there are no previous reports on the comparison of sensitivities in three species of assay animal to avian GTH, although Evans *et al.* (1940) used the GTH preparations of mammalian origin as their administered hormone to assay animals for the comparison of sensitivities. Thus, it remains undetermined whether their results on mammalian GTH may be applicable to avian GTH or not.

On the other hand, Byerly and Burrows (1938) used the chicks as assay animal when they assayed gonadotropic activity of chicken pituitary. Breneman (1945b), studying the application of mice and chicks to the assay of fowl pituitary GTH, suggested that the fact that same species of donor and assay animal was used may be a contributing factor in the excellence of the responses in the assay. Recently Nalbandov *et al.* (1951) and Das and Nalbandov (1955) claimed that an avian peculiar GTH (so called an avian third GTH) possessing different properties from mammalian GTH was contained within the avian pituitary and acted only to aves effectively.

The purpose of this study is to compare the results obtained from the assay of fowl pituitary GTH on the immature rat, the immature mouse and the one day-old chick, and afterwards to establish the quantitative assay of the chick method.

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MATERIAL AND METHODS

Cephalic lobes of anterior pituitaries obtained from White Leghorn (W.L.) cocks, ranged in age from 12 to 18 months and in testes weight from 20 to 30 g, were used as source of avian GTH. The anterior pituitaries, removed from the head immediately after the birds were killed, were weighed and separated into cephalic and caudal lobes under the dissecting microscope. The divided fresh lobes were dried with acetone for 60 hrs. and placed in a desiccator for 1 week. The dried cephalic lobes were pulverized and stored in a desiccator until ready for use. The average fresh weights of the anterior pituitary and cephalic lobe were 12.1 mg and 7.6 mg. The average acetone-dried powder weights were 3.0 mg in the former and 1.9 mg in the latter. Before injection, the amount of powder to be used was weighed and suspended in 1.5 ml of physiological saline for rats and mice or in 1.0 ml of distilled water for chicks.

In the comparative assay, rats used as assay animal were 21 day-old immature males and females (35~55 g in body weight) of the Wistar strain, mice were 21 day-old males and females (7~10 g in body weight) of the Kasukabe strain and chicks were one day-old males (35~42 g in body weight) of W.L. Male and female rats were injected subcutaneously twice daily for 3 days, and 24 hrs. after the final injection, ovaries, uteri, seminal vesicles and prostates were removed, cleaned and weighed. Control rats were injected the same volume of physiological saline. Mice of both sexes were injected and autopsied in the same way of rats, and the same organs as described for the rats were removed and weighed.

The chick assay was run by a modified method of Breneman (1945a). Injections for chicks were made subcutaneously into the dorsal neck region at 24, 36, 48, 60 and 72 hrs. after hatching. Chicks were killed 96 hrs. after hatching, and the testes were removed and weighed. Although injected chicks and distilled water injected controls were kept without food and water for the test period, the mortality seldom was more than 4% in spite of inanition.

The responses of the gonad and accessory organ weights of all assay animals were expressed as an increase-rate of the experimentals over the controls. The increase-rate was calculated by following formula;

$$\frac{\text{Exp. wt.} - \text{Control wt.}}{\text{Control wt.}} \times 100 = \text{Increase-rate (in \%)}$$

RESULTS AND DISCUSSION

Comparative Assay of Avian GTH on Rats, Mice and Chicks.

The sensitivities of rats, mice and chicks to acetone-dried cephalic lobe powder of anterior pituitary from W.L. cocks were compared. The results on the comparative assay are summarized in Table 1.

In the rat assay, 1 mg of pituitary powder did not cause increment in weight of sexual organs in both sexes (difference between organ weights of injected and controls was not statistically significant: $P=0.05$). Thus, the pituitary powder of 1 mg had no effect on male and female rat. In the mouse assay, 0.5 mg of pituitary powder acted effectively on female sexual organs but did not cause increase in weight of male sexual organs (difference was not significant: $P=0.05$). When the dose was decreased to 0.25 mg, increases in ovarian and uterine weights did not occur (difference was not significant: $P=0.05$). Therefore, the pituitary powder of 0.25 mg had no effect on mouse. On the other hand, significant increase occurred in testicular weight of chicks even at 0.25 mg dosage level ($P=0.01$). On the contrary, testes of rats and mice did not increase in weight at all dosage levels. This sug-

Table 1. The Comparative Assay of avian GTH on Rats, Mice and Chicks

	1 mg injection			0.5 mg injection			0.25 mg injection		
	Average organ weight (mg) Treat. (N)	Increase-rate (%)	Response	Average organ weight (mg) Treat. (N)	Increase-rate (%)	Response	Average organ weight (mg) Treat. (N)	Increase-rate (%)	Response
Chick									
testes	(5) 13.3	(8) 4.6	190.1	(5) 12.4	(10) 5.4	129.6	(5) 10.9	(8) 5.5	98.3
uterus	(4) 38.6	(5) 13.5	185.9	(5) 21.9	(5) 10.0	119.0	(5) 10.2	(4) 8.4	21.4
ovaries	(7) 12.8	(5) 5.8	120.7	(5) 7.3	(5) 4.8	52.1	(5) 4.3	(4) 4.6	-6.9
Mouse									
s.v.+p.	(7) 24.3	(7) 15.9	52.8	(5) 8.6	(5) 8.2	4.9			
testes	(5) 59.6	(7) 54.3	9.8	(5) 47.5	(5) 43.2	10.0			
Rat									
uterus	(5) 19.0	(5) 17.3	9.7						
ovaries	(5) 9.8	(5) 10.3	-5.1						
s.v.+p.	(5) 16.6	(5) 14.8	12.1						
testes	(5) 187.4	(5) 169.2	10.8						

Abbreviations:

s.v.: seminal vesicles

p.: prostate

N: No. of animals

gests that mammalian testes do not respond in respect of weight-increase to GTH as avian testes.

The results of the comparative assay showed that the chick testes were most sensitive to GTH from fowl anterior pituitary. This fact did not agree with the conclusion of Evans *et al.* (1940) that mouse uterine response was more sensitive than that of the sexual organs of rat and chick. This discrepancy may be due to the fact that they used GTH of mammalian origin as administered hormone, while we used GTH of avian origin. The fact that the same species of donor and assay animal was used may consequently be an important contributing factor in the excellence of the responses.

In the chick assay method advocated by Breneman (1945a), injections were carried out at 12, 24, 36, 48 and 60 hrs. after hatching and chicks were sacrificed 36 hrs. after the final injection (i.e. 96 hrs. after hatching). For the convenience of treatment, chicks in our experiment were injected at 24, 36, 48, 60 and 72 hrs. after hatching and killed 24 hrs. after the final injection. Therefore, comparison on the degree of response between two methods, Breneman's original and our modified, was made. These comparative results are presented in Table 2 in which increase-rates are 133.3% in the former and 129.6% in the latter. Since the results did not signify difference between two assay methods, the degree of response may not be affected by injected times when the first injection was carried out

Table 2. The Comparison of Breneman's Method and Method used in our Experiment in Chick Assay for avian Pituitary Powder

Group	No. of chicks	Material & injected dose	Testes weight (mg)	Increase-rate (%)
A	6	0.5 mg ant. pit. powder in 1 ml of distilled water	12.6 ± 2.06^1	133.3
B	5		12.4 ± 2.25^2	129.6
Control	10	1 ml of distilled water	5.4 ± 0.28^3	

A; Breneman's method

B; Our modified method

1)2)3); These values are confidence limits ($P=0.05$).**Table 3. Responses of Chick Testes to graded Doses of avian Pituitary Powder**

Dose of powder (mg)	No. of chicks	Testes weight (mg) (confidence limits)	Increase-rate (%)
0.05	7	8.3 ± 1.91	72.1
Control	10	4.8 ± 0.27	
0.25	5	10.9 ± 2.90	98.3
Control	8	5.5 ± 0.32	
0.50	5	12.4 ± 2.45	129.6
Control	10	5.4 ± 0.28	
0.75	6	14.1 ± 2.05	161.9
Control	6	5.4 ± 0.41	
1.0	5	13.3 ± 2.78	190.1
Control	8	4.6 ± 0.31	
2.0	5	20.0 ± 2.54	285.2
Control	10	5.2 ± 0.28	

during the period from 12 to 24 hrs. after hatching.

Characteristic of the Dose-Response-Line in Chick Assay obtained by the Increase-Rate of Testicular Weight to Avian GTH.

In the above experiment, it was found that chick testes were highly sensitive to GTH from fowl anterior pituitary. However, there are no previous investigations on the response-curve obtained by the increase-rate of chick testicular weight to GTH. If the chick testes could respond in proportion to the graded doses of pituitary powder, the chick assay would be able to avail as quantitative assay method to GTH. Accordingly, the graded doses of pituitary powder were administered to groups of chick in order to detect relationship of increase-rate of chick testicular weights to various dosage levels of pituitary powder. These results are shown in Table 3. The increased response of chick testes could be detected at the 0.05 mg dosage level of pituitary powder. In this dosage, there resulted 72.1% of the increase-rate and difference between average testes weight of treated chicks and that of controls was statistically significant ($P=0.01$). When the dose was increased to 0.25 mg or more, further increases in testicular weight occurred.

The relationship of the responses to graded doses is illustrated in Figure 1. In this figure, the data for average per cent-increase in testicular weights of injected chicks were plotted against the total doses in mg dry powder. A goodness-of-fit test was applied and the regression line to this response-line was calculated by the least squares, yielding the equation;

$$Y = 113.9X + 70.1$$

where Y is the increase-rate and X is the dose of fowl pituitary powder.

In view of the above experiment, weight of chick testes to avian GTH increased straightly in proportion as the graded doses within 2 mg dosage level of fowl anterior pituitary powder. Thus, it was demonstrated that the chick assay method was able to avail as quantitative assay for avian GTH equivalent to dose within 2 mg of acetone-dried cephalic lobe powder of anterior pituitary from W.L. cocks.

Fig. 1. Response of chick testes to avian pituitary powder. Average increase-rate (as per cent) was plotted against the dose of pituitary powder. Spots indicate average increase-rate of testicular weight in each dose. Vertical dotted lines are confidence limits. Solid line represents regression line.

SUMMARY

The sensitivities of rats, mice and chicks to acetone-dried cephalic lobe powder of anterior pituitary from W.L. cocks were compared in order to determine the highest sensitive bio-assay method for avian GTH.

Since the chick showed the highest sensitivity to avain GTH in three species of assay animal, the chick assay method is adequate to use as qualitative assay for avain GTH.

The response-line of chick testes to avain GTH was determined, and it was found that the chick testes response in terms of increase-rate showed a straight line relationship to dosage of avain pituitary powder. Therefore, the chick assay method is able to avail as quantitative assay for avian GTH.

Moreover, effect of the time of injection on responsiveness of the chick testes was examined, and the time of first injection during the period from 12 to 24 hrs. after hatching might have no influence on the degree of response.

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