

the comparative anatomy of the reproductive tract; the epididymis of the fowl being an extremely small organ through which the sperm must pass quickly to the long coiled vas in which they spend the greater part of their time subsequent to morphological maturity and before ejaculation.

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## Relationship of Precipitin Titers to Gonadotropic Inhibitory Action of Monkey Sera.

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Selye and Collip and their coworkers,<sup>1-4</sup> Twambly and Ferguson,<sup>5</sup> and Meyer and Gustus<sup>6</sup> have demonstrated that the sera of animals injected with gonadotropic hormone contain a substance or substances capable of inhibiting the action of the hormone in test animals. The conditions under which the gonadotropic-inhibitory substance appears in the blood of animals suggest that the mechanism of formation is similar to that involved in the production of antibodies. We have undertaken experiments to ascertain whether or not there is any correlation between the precipitin titer of the serum of monkeys repeatedly injected with the gonadotropic hormone prepared from the serum of pregnant mares and the presence of gonadotropic antagonistic substance.

For this purpose a highly purified preparation of pregnant mare's serum hormone, made by the method of Evans, Gustus and Simpson,<sup>7</sup> was available. Solutions containing 5 r.u.\* per cc. of this

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<sup>1</sup> Selye, H., Collip, J. B., and Thomson, D. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **31**, 487.

<sup>2</sup> Selye, H., Collip, J. B., and Thomson, D. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **31**, 566.

<sup>3</sup> Selye, H., Bachman, C., Thomson, D. L., and Collip, J. B., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **31**, 1113

<sup>4</sup> Collip, J. B., *J. Mount Sinai Hosp.*, 1934, **32**, 28.

<sup>5</sup> Twambly, G. H., and Ferguson, R. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **32**, 69.

<sup>6</sup> Meyer, R. K., and Gustus, E. L., *Science*, 1935, **81**, 208.

<sup>7</sup> Evans, H. M., Gustus, E. L., and Simpson, M. E., *J. Exp. Med.*, 1933, **58**, 569.

\*A rat unit is that amount of hormone which when injected once daily for three consecutive days produces within 96 hours of the first injection a 5-fold increase in the weight of the ovaries of 21-23-day-old rats.

hormone gave none of the usual chemical tests for protein and reacted only faintly in 1-10 dilution with a potent antihorse serum precipitating serum.

*Macacus rhesus* monkeys, weighing between 2-3 kg., were injected either subcutaneously or intravenously with daily doses of this purified hormone as indicated in Table I. Blood was withdrawn on the days indicated and the amount of serum necessary to inhibit the gonadotropic action of 10 mg. of standard horse serum powder was determined. Precipitin titers were determined in the usual manner, keeping the quantity of monkey serum constant.

TABLE I.  
Comparison of Precipitin Titers and Inhibitive Substance in the Sera of Female Monkeys Injected with Gonadotropic Hormone.

Monkey No.	Days Injected	Rat Units Purified Hormone	Days of Exp.	Precipitin Titers		Serum Injected cc.	Wt. of Ovaries of Immature Rats	
				Hormone	Horse Serum		Exp. mg.	Controls mg.
1	54	245 intrav.	66	—	—	0.5	15	32
			80	16	0	0.5	27	64
			136	4	0	—	—	—
			165	16	0	0.7	16	43
			212	0	0	1.0	17	44
			269	4	2	2.0	14	25
			365	2	0	2.0	13	25
2	54	245 subcut.	66	—	—	0.5	11	52
			136	0	10	0.5	11	40
			212	0	0	0.75	20	44
			365	0	2	1.0	15	59
3	83	629 subcut.	54	—	—	0.5	12	42
			84	0	2	—	—	—
			98	0	0	0.2	10	43
			146	0	0	1.0	24	44
			305	0	0	1.0	24	59
4	83	415 intrav.	54	—	—	0.5	15	56
			82	10	100	0.9	13	56
			98	64	128	0.2	13	43
			146	16	0	1.5	19	50
			208	2	0	0.75	28	50
			316	0	0	2.0	25	34
5	56	390 subcut.	43	0	0	0.5	11	40
6	34*	120 subcut.	43	8	2	0.5	12	40
			119	0	0	1.0	26	48
	19†	95 subcut.	168	0	0	1.0	26	39
			181	0	0	1.0	10	65
			273	0	2	2.0	13	31

\*5 r.u. day for 11 days; 10 days rest; 5 r.u. day for 13 days.

†After 134 days of rest.

Purified hormone solution and normal horse serum were used as antigens. Normal monkey serum, horse serum and hormone controls were negative throughout the experiments and are not included in the table.

In Table I are shown the data obtained by the precipitin test compared with the inhibitive action of monkey serum on a standard amount of hormone in immature female rats. The serum of monkey 1 showed reactions in low dilutions between the 80th and 212th days of the experiment in the absence of a detectable reaction with horse serum. This reaction decreased as the amount of antagonistic substance in the monkey serum decreased. Such results, however, were not obtained in the case of monkeys 2, 3, 5, and 6. The sera of these animals, with the exception of monkey 2 on the 136th day (10 with horse serum†) and monkey 6 on the 43rd day (8 with hormone) gave no significant reactions with either hormone or normal horse serum. Precipitin titers of monkey 4 serum with both hormone and normal horse serum reached a maximum on the 98th day and decreased thereafter. These titers reached their maximum at the time when the amount of antagonistic substance in the serum was greatest but decreased somewhat more rapidly than the latter.

Monkeys 1 and 4, whose sera gave precipitin titers, were injected intravenously; monkeys 2, 3, 5, and 6, subcutaneously. This may afford a partial explanation for the lack of reactions in the latter group, since intravenous injection usually gives greater antigenic stimulation. Since inhibiting substance was present in the sera of all monkeys in approximately the same amount regardless of the route of injection, it seems likely that the precipitations obtained were non-specific or due to the slight trace of protein present in the hormone preparations.

In order to check this point, 3 additional monkeys were injected subcutaneously with 95, 140, and 265 r.u., respectively of a purified preparation of hormone which had been freed from protein by a method which will be the subject of a future communication by one of us (E. L. G.). This preparation gave no reaction for horse serum protein serologically. Sera of the monkeys so injected gave no significant precipitin reactions with purified hormone, hormone serologically free from horse serum proteins, or normal horse serum, although inhibitory substance for the gonadotropic hormone was present.

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† Titer is expressed as highest numerical dilution of antigen giving positive reaction; 0 indicates no reaction with undiluted antigen; —, not determined.

The gonadotropic-inhibitory substance found in the serum of monkeys repeatedly injected with gonadotropic hormone of pregnant mare serum is specific in the antagonistic action and is in this respect similar to an antigen-antibody reaction. Thus we have determined that 2 cc. of serum of monkey 6 (obtained on the 273rd day of the experiment) does not significantly prevent the action of gonadotropic hormone contained in the extracts of human pregnancy urine, whole sheep pituitary gland and whole human pituitary gland. However, the action of the gonadotropic hormone prepared from the serum of pregnant mares was completely inhibited (Table II).

TABLE II.  
Serum of Monkey No. 6 Injected in Combination with Various Gonadotropic Hormones.\*

—Gonadotropic Material—		Amt. of Serum cc.	—Wt. of Ovaries, mg.—	
Kind	Amt.		Exp.	Control
Prolan	2 r.u.	2.00	23 (5)	35 (5)
Whole sheep pit.	25 mg.	2.00	34 (5)	34 (5)
” human ”	15 mg.	2.00	108 (4)	124 (2)
Pregnant mare serum	10 mg.	2.00	13 (5)	31 (7)

\*Rats injected with .40 cc. serum each of 5 consecutive days; gonadotropic substances on the 3rd, 4th, and 5th days. Autopsy 48 hours after the last injection. Figure in parenthesis indicates number of rats used in determination.

There is evidence that non-specific stimulation of the antibody-forming mechanism results in an increase in antibodies which previously had been produced—the so-called “anamnestic” reaction.<sup>8</sup> Thus monkey 4, whose serum previously contained inhibitory substances, was given 3 weekly injections of typhoid vaccine, to determine whether or not the gonadotropic-inhibitory content of the serum could be increased. The amount of inhibitory substance present in the serum before the injection of typhoid vaccine was very small as determined by the injection of 2 cc. of serum, together with gonadotropic hormone of pregnant mare serum. Although a high typhoid agglutinin titer (5120) was present following these injections, no increase in inhibitory substance occurred. Therefore, non-specific stimulation of the antibody-forming mechanism in this monkey caused no increase in the inhibitory action of the serum.

The results reported in this paper indicate that although the gonadotropic-inhibiting substance developed in monkeys by repeated injections of gonadotropic hormone prepared from the serum of pregnant mares is specific in its action, no reliable indication of its

<sup>8</sup> Topley, W. W. C., “Outline of Immunity,” Wm. Wood and Company, Baltimore, 1933, 183-185.

formation or presence is provided by the precipitin test. These results are in essential agreement with those reported by Bachman,<sup>9</sup> who determined the complement-fixation titer of the sera of rabbits repeatedly treated with A P L, although no data showing the concentration of inhibitory substances in the sera of the rabbits were presented.

Ehrlich<sup>10</sup> presented data which showed that by the injections of a hormone preparation ("Präpitan"), rabbits produced specific antibodies in the animal sera which reacted positively with gonadotropic hormone prepared from urine of pregnant women; but not with thyreotropic anterior pituitary gland substance. He did not make any attempt to determine whether the sera contained specific or non-specific gonadotropic-inhibitory substances, nor whether the reactions obtained were due to specific or non-specific antibodies.

It is, of course, difficult in all cases to demonstrate by the presence or absence of *in vitro* reactions the existence of an immune state. For example, individuals following typhoid fever or vaccination occasionally show no demonstrable antibodies against *B. typhosus*, but exposure proves them to be immune. The absence of *in vitro* immunological reaction between the hormone and the monkey serum containing the gonadotropic-inhibitory substance does not necessarily eliminate the possibility that the latter is formed and reacts in a manner similar to that of antibodies.

*Conclusions.* The repeated injection of highly purified gonadotropic hormone prepared from the serum of pregnant mares into female monkeys produced definite quantities of a specific gonadotropic-inhibitory substance and in some cases small amounts of precipitins. There was no predictable correlation between the presence or absence of the precipitins and the gonadotropic-inhibitory substance.

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<sup>9</sup> Bachman, C., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 851.

<sup>10</sup> Ehrlich, H., *Wien. Klin. Wschr.*, 1934, II, 1323.