injections. Blood sugar samples were taken from the tail vein, the first few drops being discarded. Samples were taken fasting and at 10, 20, 30, 60, and 120 minutes after injection. Table I gives the average values for the various groups.

TABLE I.

Group	No. Animals	Blood Sugar in mg. per 100 cc. after injection of 125 mg. glucose/100 gm. wt. Fasting	10 min.	20 min.	30 min.	60 min.	120 min.
a. Normals	23	81±1.7	272±4.7	208±2.7	161±2.7	119±1.8	97±1.4
b. Normals, injected	6	88±2.9	269±2.2	206±4.3	157±4.8	120±3.5	97±2.1
c. Adrenalectomized	10	75±3.3	252±9.1	237±4.1	212±4.1	157±2.9	98±2.
d. Adrenalec., injected	9	78±2.7	268±9.8	213±7.8	157±5.7	110±3.3	94±2.
e. Hypophysectomized	18	68±2.7	301±8.6	271±6.5	242±9.9	173±4.3	105±2.
f. Hypoph., injected	6	58±3.0	307±9.5	258±6.8	230±12.7	205±7.0	91±5.3

The defect in ability to remove excess sugar from the blood in the hypophysectomized male rat is not relieved by cortical extract, while that observed in the adrenalectomized animal disappears. It seems then that the deficiency brought on by removal of the pituitary gland is not solely, if at all, a result of atrophy of the adrenal cortex. Since the normal rats injected with cortical extract showed no greater rate of glucose removal than the uninjected controls, the action of the cortical extract in adrenalectomized rats on the tolerance must be due to its specific action in relieving cortical insufficiency.

9083

Inability of Sheep to Develop Antihormone to the Gonadotropic Hormone from Sheep-Pituitary Glands.*

K. W. THOMPSON. (Introduced by Harvey Cushing.)

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Information concerning antihormones does not as yet indicate whether these substances are immune bodies or chalones (hormone antagonists, Sharpey-Schafer). Most observers are agreed that antihormones may be secured in serum after suitable injections of pituitary extracts into animals, as was first described by Collip and his co-workers.[1] Meanwhile, attention has been called to the pres-

* The experiments were aided by a grant from the Josiah Macy, Jr. Foundation.

[1] Anderson, E. M., and Collip, J. B., *Lancet*, 1934, **1**, 76.

ence in the injected extracts of proteins which may be antigenic in the recipient.[2, 3] That certain active protein principles, such as the enzymes, pepsin, and steapsin, are antigenic in heterologous species is well known, and it is conceivable that hormones, which are substances closely related to enzymes, may also be antigenic under certain circumstances.

The purpose of the present communication is to record observations which appear to support the concept that antihormones are the product of the reaction of an animal to an antigen. In these experiments an extract of sheep-pituitary glands, which had produced gonadotropic antihormone in several species of animals, did not produce this antihormone in sheep.

It will be recalled that Twombly,[3] working along somewhat similar lines, found that prolan injected into rabbits produced an antihormone, but he found no evidence that it was present in the sera of patients, even after prolonged injections. Experiments with parabiosis in rats[4, 5] and with implants of rat pituitaries into rats have also failed to show evidence of formation of antihormones in these animals by these methods. The first report of failure to produce antihormones by injections of a thyrotropic extract into a foreign species was made by Werner,[6] who found that injections of a bovine thyrotropic flavianate did not produce antihormones in guinea pigs. With these exceptions the antihormone studies so far reported have concerned injections of the commonly available bovine and sheep-pituitary products, pregnant mare serum, or human pregnancy-urine preparations into the usual laboratory animals, in which heterologous pituitary proteins must be considered.

The present experiments were devised to determine whether antihormones could be produced in the sera of sheep by injections of highly active sheep-pituitary extracts, which were known to produce antihormones readily in other species of animals. Brief descriptions of the extracts employed follow.

Extract No. 1 was prepared by alkaline extraction of fresh sheep-pituitary glands with subsequent alcohol precipitation at a pH of 5.6, using a method described by VanDyke and Wallen-Lawrence.[7]

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2 Bachman, C., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 851.

3 Twombly, G. H., *Endocrinology*, 1936, **20**, 311.

4 DuShane, G. P., Levine, W. T., Pfeiffer, C. A., and Witschi, E., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 339.

5 McCahey, J. F., Solway, D., and Hansen, L. P., *Pennsylvania M. J.*, 1936, **39**, 223.

6 Werner, S. C., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 390.

7 VanDyke, H. B., and Wallen-Lawrence, Z., *J. Pharm. and Exp. Therap.*, 1933, **47**, 163.

For the purpose of this communication it is sufficient to state that the extract was rich in gonadotropic and thyrotropic hormones. The protein of the extract may well have been partially or completely denatured by the exposure to alcohol. This extract has aroused gonadotropic antihormone in rats, guinea pigs, rabbits, dogs, monkeys, and a horse.

Extract No. 2 was prepared by alkaline extraction from an acetone-dried powder of whole sheep-pituitary glands. This extract also was rich in thyrotropic and gonadotropic hormones. In this instance, although acetone is said to be less likely to denature proteins than alcohol, some denatured protein may have been present. Injections of this extract have been followed by the formation of antihormones both in rats and dogs.

Two sister ewes 4 months old were used for the experiment, and injections were started after samples of their sera had been taken for control tests. Ewe No. 1 was injected subcutaneously each day with 25 cc. of extract No. 1, and ewe No. 2 was injected with the same amount of extract No. 2. The animals were bled at intervals, and tests of each specimen of serum were made upon immature rats. After 6 months of injections, when it would appear from previous experience that antihormones had been given adequate time to develop, the animals were sacrificed. Hypertrophy of the external genitalia and signs of sexual stimulation, which appeared shortly after the injections were started, remained during the period of the injections. Within the period of treatment an area of skin on each ewe's back was shaved, and the fact was noted that the wool grew normally. Neither ewe developed hypercholesterolemia.

The power of the sera to modify the gonadotropic effects of the 2 pituitary extracts was tested in 25-day-old immature rats. The standard activity of each extract was determined by a hundred-hour test in which the rats of each group were given 3 subcutaneous injections each day for 2 days, and the animals were sacrificed one hundred hours after the first injection of the extract.

Another group of rats was injected with serum twice daily beginning 2 days before the hundred-hour period and continuing through the hundred-hour period. The object was to have the serum in the process of absorption during the action of the injected gonadotropic hormone. The average weight of the ovaries of each group of rats was used as a measure of the gonadotropic effect.

Study of the results revealed no evidence to indicate that gonadotropic antihormones were produced in either of the sheep by the injections. Two of the specimens of serum were tested in July and

August during days of extreme heat, and these tests (starred in the table) were unsatisfactory because the rats grew poorly and the ovaries of the controls did not respond adequately to the stimulation of the gonadotropic hormone. The other tests were entirely satisfactory. The results are shown in Table I.

TABLE I.
The Average Weights of Ovaries of Rats Tested with Sheep-Pituitary Extracts and with the Sera of 2 Sheep which Received Prolonged Injections of Sheep-Pituitary Extracts.

	The day serum was taken				
	0 day	85th day*	115th day*	145th day	180th day
Controls; no serum; Extract No. 1, 0.5 cc.	59	32	24	54	50
Serum, Ewe No. 1, 0.5 cc. plus Extract No. 1, 0.5 cc.	61	16	23	38	50
Serum, Ewe No. 2, 0.5 cc. plus Extract No. 1, 0.5 cc.	63	16	33	44	38

Summary. Sheep-pituitary extracts, which had produced gonadotropic antihormones in several species of animals, were injected into 2 sheep for 6 months, during which time no gonadotropic antihormone was found in the sheep-sera.

9084

Non-Specificity of Thyrotropic Antihormone.*

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The preparation of an antiserum which inactivated pituitary gonadotropic hormone of several species of animals, including man, has been reported.[1] The present study of the same serum was made in order to extend the observations to include its effect upon pituitary thyrotropic hormone from several species of animals. Along somewhat similar lines, Gregerson, Clark and Kurzrok[2] injected prolan into rabbits and prepared an antiserum which inhibited the gonadotropic activity of an extract of bovine pituitary glands. Prior

* The experiments were aided by a grant from the Josiah Macy, Jr. Foundation.

1 Thompson, K. W., and Cushing, H., *Proc. Roy. Soc., London*, s.B., in press.

2 Gregerson, H. J., Clark, A. R., and Kurzrok, R., PROC. SOC. EXP. BIOL. AND MED., 1936, **85**, 193.