In some individuals anoxemia produced a greater change in rate of breathing than in amplitude. In others the reverse occurred. In those dogs in which the rate control was developed at the expense of amplitude control, anoxemia produced the same increase in rate after denervation of the carotid gland, and the same small change in amplitude after double vagotomy with carotid gland innervation intact. In those dogs, in which amplitude control was developed at the expense of rate control, anoxemia produced the same large increase in depth of breathing after double vagotomy, and the same small increase in rate after carotid gland denervation with the vagus nerves intact.

The response to carbon dioxide was relatively little affected by denervation of the carotid gland, by double vagotomy, or section of the pulmonary branches of the vagus nerve. The relative absence of increase in rate and the predominance of increase in amplitude from the administration of carbon dioxide with complete innervation may be due to the depressing effect of carbon dioxide on reflexes. Carbon dioxide saturation, so to speak, produces a partial chemical vagotomy, thereby reducing the rate control of the vagus nerves.

7884 C

Immunologic Studies of Anti-Gonadotropic Sera.

CARL BACHMAN. (Introduced by J. B. Collip.)

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We have reported[1, 2] that the serum of rats and rabbits treated with anterior-pituitary-like hormone (A.P.L.) of pregnancy urine contained substances capable of protecting test animals against the gonadotropic action of this hormone. The A.P.L. used in these experiments was prepared by alcohol-salt precipitation methods and consequently contained nitrogenous substances giving some of the reactions common to antigenic proteins. It was of interest, therefore, to determine whether the A.P.L.-inhibitory sera of treated animals contained antibodies against A.P.L. in the immunological sense.

[1] Selye, H., Bachman, C., Thomson, D. L., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1113.

[2] Bachman, C., Collip, J. B., and Selye, H., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 544.

For this purpose 4 preparations of A.P.L. from different collections of pregnancy urine and one control product prepared by identical methods from male urine were examined. The gonadotropic potency of the pregnancy urine extracts varied between 10 and 100 rat-day-units (R.D.U.) per milligram of dry substance, the differences being accounted for principally by variations in salt content of the preparations. The male urine extract possessed no gonadotropic properties, but like the pregnancy urine extracts, gave the biuret and Millon reactions.

Each of the above preparations was injected intravenously into 5 female rabbits. The A.P.L. treated rabbits received 5 daily increasing doses of from 100 to 1000 R.D.U. in salt solution, and following a rest period of 5 days, a second course of from 500 to 2500 R.D.U. The rabbits treated with male urine extract received similar courses of injections in doses ranging from 10 to 150 mg. of dry substance dissolved in salt solution. The animals were bled from the fourth to the tenth day following the last injection. As previously reported, the serum of rabbits treated with A.P.L. in this manner was highly potent in A.P.L.-inhibitory substance.

When the sera of the treated rabbits were tested for precipitins against homologous injection preparations, preliminary experiments with the "layer" method showed weakly positive results in low dilutions of many of the antigens. This method was therefore abandoned for the more sensitive technic of complement fixation.

In the complement fixation tests 0.2 cc. of heated serum from the treated rabbits was incubated at 37°C. with 1.0 cc. of a 1:20 dilution of fresh pooled guinea pig serum as complement, and 0.2 cc. of urine extract "antigen" in varying dilutions starting from a concentration of 5 mg. (50 to 500 R.D.U.-A.P.L.) per cc. of salt solution. To this mixture, made up to 3.0 cc. with salt solution, was added after one hour 1.0 cc. of a 1:40 dilution of washed sheep corpuscles and 2 units of hemolytic amboceptor. In the proportions of reagents chosen following numerous trials, and in the fact that complement was titrated only after preliminary incubation for one hour, the test set-up provided a sensitive indicator for the presence of complement fixing antibodies. Controls of each dilution of antigen, as well as the usual corpuscle, hemolysin and serum controls were run. In addition to these a control of each serum in the absence of hemolytic amboceptor was also run, in order to reveal the hemolytic activity of serum alone.

It was soon apparent that while many sera from the injected rabbits were strongly hemolytic in themselves, one or 2 from each

group gave satisfactorily graded inhibition of hemolysis in the presence of urine extract. Complement was never bound, however, in higher than 1:100 dilutions of antigen. Inasmuch as normal rabbit serum controls frequently gave reactions up to 1 in 10 dilution of antigen, it will be noted that the complement fixing titer of potent A.P.L.-inhibitory sera was low.

In one series of rabbits an attempt was made to raise this titer by repeating the A.P.L. injections after a 4 months' rest period. This was not possible. It was found that just before beginning the new injections the original complement fixing titer had been retained both in the preserved serum and *in vivo,* while the A.P.L.-inhibiting potency had been maintained in the preserved serum but was absent in blood drawn at this time. After the new injections the A.P.L.-inhibiting property was restored but complement binding titer was not raised appreciably above the previous level.

When the cross-reactions of the sera were examined, it was found that the sera from the A.P.L.-injected rabbits fixed complement about equally with all A.P.L. antigens, irrespective of the preparation used for treatment. Uniform cross-reactions for comparable dilutions of antigen were, moreover, observed between A.P.L. on the one hand and the sera of the rabbits treated with male urine extract on the other and *vice versa.* Three of the 4 A.P.L.-treated rabbit groups gave cross reactions against human serum in dilutions up to 1 in 5000 to 1 in 25,000. The group treated with the fourth A.P.L. preparation, and the animals injected with male urine extract gave no reactions with human serum. Where this reaction was observed, it was equally strong for normal male and female serum, as well as for pregnancy serum. The reverse of this cross reaction was examined by immunizing 2 series of 5 rabbits each with male serum in the manner above outlined, and testing these sera against urine extract antigens. This procedure, however, gave no consistent cross reactions, although the sera gave titers above 1 in 5,000 against the human serum antigens used for immunization. (Table I.)

A point of difference between the A.P.L.-inhibiting properties and the complement fixing factors of sera has been noted above in the disproportionately rapid loss of inhibitory titer *in vivo* after the injections were discontinued. That these two phenomena do not parallel each other was further suggested by finding that A.P.L.-inhibitory sera from treated male and female rats gave no complement fixation whatever with A.P.L. antigens.

The problem was examined finally as follows: The pooled serum

TABLE I.
Showing maximum dilutions of antigen at which complement fixation was observed.

Antigen	Serum of Rabbits Treated with: A.P.L. No. 1	A.P.L. No. 2	A.P.L. No. 3	Male urine Extract	Human Serum	Normal Rabbit Serum
A.P.L. No. 1	100+	10+	10+	—	—	1+
A.P.L. No. 2	10+	100—	10+	10+	1+	1+
A.P.L No. 3	0	10+	100—	10—	0	1+
Male urine extract	10—	100+	100+	100+	0	1+
Human serum	0	5000	25000	0	5000	1

of rabbits treated with male urine extract, and that of both groups immunized against human serum were assayed for A.P.L.-inhibiting substances by both the rat and rabbit technics described in our previous communications. In no instance was inhibitory activity observed.

We conclude that A.P.L.-treated rabbits yield sera which give weak immunity reactions *in vitro* with solutions of A.P.L and extracts of male urine, and stronger but less constant reactions with human serum proteins. At the present time, however, there appears to be no clear connection between these phenomena and the specific A.P.L.-inhibitory property demonstrable in such sera with biological methods.

7885 C

Cytological Responses of Rat Thyroid to Treatment with Anterior Pituitary and Potassium Iodide.

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Intensified mitotic proliferation and an increase of colloid in the thyroid gland of the normal guinea pig, following potassium iodide administration, have been reported by Loeb.[1] As colloid increased in the follicles, he noted that the follicular epithelium became distended and flattened.

Loeb and Bassett[2] reported that dried and powdered anterior pit-

[1] Loeb, Leo, *Am. J. Path.*, 1926, **2**, 19.

[2] Loeb, Leo, and Bassett, R. B., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 490.