fluid by way of the kidneys, but the explanation must come from a study of the factors involved in the abnormal distribution of water within the body. That the total water exchange can not account for all the symptoms of adrenal insufficiency is further indicated by the work of Caldwell,[4] who found that the water content of adult cats could be reduced practically to the vital limit without disturbance in the thermoregulatory mechanism, whereas such disturbance is one of the notable features of cortical deficiency.[5, 6]

The values found for insensible weight loss do not differ significantly during adrenal insufficiency from the corresponding values during the normal period. This is interesting in view of the lowered metabolism during cortical deficiency, and the view expressed by some authors[7] that insensible weight loss follows the basal metabolism so closely that the former can be used as a measure of the latter.

7757 C

Anti-Gonadotropic Substances.

C. BACHMAN, J. B. COLLIP AND H. SELYE.

From the Department of Biochemistry, McGill University, Montreal.

Both the gonadotropic hormone prepared from the pituitary gland and the anterior pituitary-like hormone of pregnancy urine (A.P.L.) lose their gonad-stimulating effect after a certain time, if given daily over a long period, but this loss of sensitivity is limited to the gonadotropic preparation with which the animals have been injected previously. Animals which become insensitive to pituitary implants remain sensitive to A.P.L. and *vice versa*.[1, 2] It has also been found that a state of passive A.P.L. resistance may be induced in immature female rats by the administration of the blood of A.P.L.-resistant

[4] Caldwell, G. T., *Physiol. Zool.*, 1931, **4**, 324.

[5] Hartman, F. A., Brownell, K. A., and Crosby, A. A., *Am. J. Physiol.*, 1931, **98**, 674.

[6] Hartman, F. A., Brownell, K. A., and Lockwood, J. E., *Endocrinology*, 1932, **16**, 521.

[7] Benedict, F. G., and Root, H. F., *Arch. Int. Med.*, 1926, **38**, 1.

[1] Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 487.

[2] Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 566.

animals[3] and these findings have recently been confirmed in the rabbit.[4] We here report our more recent experiments concerning the production of sera inhibitory to the gonadotropic hormone of the pituitary and to A.P.L.

Anti-A.P.L. The technique of obtaining and assaying the inhibitory sera followed closely the methods previously described.[3] Adult rats were injected daily with doses of A.P.L. increasing from 10 to 40 R.U. for periods of from 35 to 60 days. Their blood was collected 48 hours after the last injection, defibrinated, and the sera pooled. A typical assay consisted of 6 daily subcutaneous or intraperitoneal injections of 1 cc. of such sera into each of five 21-day-old female rats. During the last 3 days, A.P.L. in doses varying from 2 to 6 R.U. per day were also given. Control animals received only A.P.L. or pooled normal rat serum and A.P.L. The vaginal smears and the ovaries were examined microscopically.

It was found that the sera of 7 adult intact female rats having 50 days of A.P.L. treatment with a total individual dosage of 1400 R.U. completely inhibited vaginal oestrus and corpus luteum formation in immature females treated with 9 R.U. of A.P.L. The weight of the donors' ovaries averaged 48 mg. The sera of a parallel group of 7 similarly treated females was still inhibitory 70 days after treatment was stopped, the donors' ovaries averaging 39 mg. at the time.

In another group of female rats chronically treated with A.P.L. we found that loss of sensitivity to this hormone did not interfere with conception, but pregnancy tended to end in death and resorption of the foetuses during the last half of gestation. The sera of 2 such A.P.L. treated pregnant groups, one with living and one with dead ova, were assayed and found inhibitory.

Inhibitory substances were also demonstrated in the sera of a group of 8 adult castrate females after treatment for 44 days with a total of 750 R.U.-A.P.L., 10 normal adult males after 34 days and 1300 R.U.-A.P.L., and 10 adult castrate males after 32 days and 960 R.U.-A.P.L.

It is also possible to use the rabbit for the production of inhibitory sera.[4, 5] In most of our experiments with rabbits we have employed ascending (100 to 5000 R.U.) daily intravenous injections of A.P.L. in 2 courses of 5 injections each, separated by

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

[4] Twambly, G. H., and Ferguson, R. S., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 69.

[5] Collip, J. B., *J. Mount Sinai Hosp.*, 1934, **32**, 28.

a rest period of 5 days. The material to date comprises 22 adult females treated by this short method, and 3 adult females, 1 adult castrate female and one adult male treated with small (1-100 R.U.) daily doses for 6 months.

Rabbits given large doses of A.P.L. by the above method showed very little continued gonadal stimulation after the 5th day. By the 10th day all corpora hemorrhagica and lutea present were apparently ageing. Blood drawn within 48 hours of the last treatment, and injected in 1 cc. doses for 4 days into immature female rats provoked oestrus and corpus luteum formation. Blood drawn from the 4th to the 10th day after treatment has proven uniformly and strongly inhibitory in 9 assays to date on the immature rat, using test dosages of A.P.L. up to 60 R.U. Blood drawn one and 2 months after treatment has been found to be no longer inhibitory. The inhibitory titer of such donor rabbits has again been raised by a course of 5 daily A.P.L. injections.

The normal female rabbit has been found to be a suitable test object for anti-A.P.L. substances. Four adolescent virgin does of over 1800 gm. body weight, and 4 non-pregnant isolated parous females were given A.P.L. in 4 intravenous doses of 25 R.U. at intervals of 12 hours, each such dose preceded 2 hours earlier by the injection of 1 cc. of anti-A.P.L. rabbit serum into the opposite ear vein. Twenty-four hours after the last injection their ovaries showed no change compared with the condition in which we found them at a preliminary laparatomy before the experiment was begun. Four control animals given only the A.P.L. all showed numerous large corpora hemorrhagica and a few apical corpora lutea.

Anti-Maturity. The assays of sera for substances inhibitory to gonadotropic extracts of the anterior pituitary have been modelled on those described above. A standard dosage of 0.25 cc. of an alkaline extract of sheep pituitary, 1 cc. representing 0.25 gm. of anterior lobe given subcutaneously twice daily for 3 days, has been used. Such a dosage has given ovaries averaging 40 mg. in the control animals.

Serum donor rats were treated for 6 to 10 weeks with twice daily subcutaneous doses of 0.25 to 1.0 cc. of the same and similar extracts prepared from hog pituitaries.

Under the conditions of assay it was found that the anti-maturity potency of donor sera from treated adult female rats was independent of the length of treatment, but appeared to parallel the development of the refractory state as evidenced by eventual regression in the weight of the donors' ovaries.

A group of 18 animals treated for 10 weeks was divisible into 3 groups: One group of 8 rats had ovaries averaging 26 mg.; the pooled sera of this group inhibited the action of maturity hormone in the immature female assay animals. In the second group of 7, and the third group of 3 animals, with average ovarian weights of 66 and 267 mg. respectively, the sera failed to show inhibitory potency.

Summary. From these experiments we conclude that the chronic administration of gonadotropic extracts from the pituitary or from pregnancy urine leads to the formation of substances inhibitory to their action, and that a passive resistance to both these hormones may be produced by the administration of serum obtained from animals chronically injected with these gonadotropic substances.

7758 P

Total Creatinine Content of Perfused Rabbit Hearts.

GEORGE HERRMANN, GEORGE DECHERD AND PETER ERHARD.

From the Department of Medicine, University of Texas, Galveston.

In our efforts to determine the part played by creatine and its possible precursors in heart muscle metabolism, we perfused isolated rabbit hearts in Dawson's modification[1] of the Gunn-Locke apparatus. We used oxygenated Ringer-Locke solution to which 0.1% dextrose was added in one apparatus, and in the other the same solution plus 0.1% glycocoll. About 250 cc. of each mixture was placed in the reservoirs, and that which perfused through the coronaries was collected and returned automatically to the reservoirs and oxygenated by an oxygen pump system.

Thirty isolated rabbit hearts, or 15 pairs, were perfused from 1 to 5 hours, beating spontaneously, or stimulated at the rate of 60 per minute when spontaneous contraction was too slow. Thirteen isolated hearts were perfused for a minute each in order to wash the blood from the coronary system, and used as controls. All hearts were weighed, cleaned of fat and connective tissue, and the ventricular muscle minced. Part of the tissue was dried at 105°C. for 22 hours to give the percent of solids; the remainder was used for determination of the total creatinine content by the method of

[1] Dawson, W. T., *J. Lab. and Clin. Med.*, 1925, **10**, 853.