rode's solution was diluted 1/16. Staphylococcus was inhibited in dilution of 1/32 (crude material 1/320) and *E. coli* at 1/16.

Other investigators[8-11] have reported the extraction of substances inhibiting cell growth from liver and other sources by methods different from those reported here, and it is not yet clear whether the substance which we have used corresponds in its physical and chemical properties to any of those previously described.

Summary. Acetone extract of sheep cardiac muscle lyophilized and redissolved in Tyrode's solution inhibits the growth of chicken fibroblasts and mouse breast tumor cells *in vitro*. A similar inhibitory activity of varying degree is exhibited against standard strains of tubercle bacilli, hemolytic *Streptococcus aureus* and *E. coli*.

[8] Baker, L. E., and Carrel, A., *J. Exp. Med.*, 1925, **42**, 143.

[9] Heaton, T. B., *J. Path. and Bact.*, 1926, **29**, 293; 1929, **32**, 565.

[10] Shebruja, T., Inaba, M., and Kawaguchi, A., *Tr. Japan. Path. Soc.*, 1935, **25**, 412.

[11] Brues, A. M., Subbarow, Y., Jackson, E. B., and Aub, J. C., *J. Exp. Med.*, 1940, **71**, 423.

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Effect of Adrenal Cortex Extract Upon the Urinary Nitrogen of Rats Following Adrenalectomy.

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The adrenal cortex has been characterized as a regulator of protein metabolism. It is generally true that the adrenally insufficient animal excretes smaller than normal amounts of urinary nitrogen and that the administration of adrenal cortex extract or of the 11-oxy steroids favors an increased loss of nitrogen[1]. The present report concerns an exception to the above rule. Others are known.[2-5]

In studies on the force-fed rat it was noted[4,5] that following adrenalectomy the animals which were given saline to drink without additional treatment excreted more urinary nitrogen than did similar animals which were treated with adrenal cortex extract or than did sham-operated rats. The present study confirms and extends these preliminary observations.

Methods. Male rats of the Sprague-Dawley strain were maintained on stock diets of Purina Dog Chow (Experiment 1) and Archer Dog Pellets (Experiment 2). When the rats reached a weight of approximately 300 g they were adapted to the force-feeding of a medium carbohydrate diet by stomach tube each morning (8:30 to 9:15 A.M.) and afternoon (4:15 to 5:00 P.M.). The technic of force-feeding and the diet (Table I) used

TABLE I.
Medium Carbohydrate Diet.

Constituent	G
Cellu flour (Chicago Dietetic Supply)	120
Osborne & Mendel salt mixture	40
Dried yeast (Pabst)	100
Wheat germ oil	10
Cod liver oil	10
Vitamin K (2-methyl-1,4-naphthoquinone)	100 mg
Mazola oil	200
Casein (Labco)	160
Starch	200
Dextrin	190
Sucrose	200
Water to make total of	2000 cc

[1] Long, C. N. H., Katzin, B., and Fry, E. G., *Endocrinology*, 1940, **26**, 309.

[2] Koelsche, G. A., and Kendall, E. C., *Am. J. Physiol.*, 1935, **113**, 335.

[3] Berman, D., Sylvester, M., Hay, E. C., and Selye, H., *Endocrinology*, 1947, **41**, 258.

[4] Ingle, D. J., and Oberle, E. A., *Am. J. Physiol.*, 1946, **147**, 222.

[5] Ingle, D. J., Ward, E. O., and Kuizenga, M. H., *Am. J. Physiol.*, 1947, **149**, 510.

were modifications of those described by Reinecke, Ball, and Samuels.[6] The rats were brought to a full feeding of 26 cc per rat per day on the 5th day. After the rats had been force-fed for 2 weeks the adrenal glands were removed by the procedure of Ingle and Griffith.[7] In the control animals the adrenal glands were exposed but were not damaged. Asepsis was successfully maintained in these operations. All of the animals were given a solution of 1% sodium chloride to drink during all phases of the experiments. The animals were housed in an air-conditioned room in which the temperature was maintained at 74 to 78°F and the humidity at 30 to 35% of saturation.

Twenty-four-hour samples of urine were collected at the same hour each day and were preserved with thymol with added citric acid (1 g per sample) to insure the acidity of the urines for nitrogen analysis. The determination of urinary nonprotein nitrogen was by the micro-Kjeldahl procedure as follows: proteins were precipitated as the salts of tungstic acid by the Folin-Wu procedure. The organic matter was oxidized by sulfuric acid and hydrogen peroxide. Copper sulphate and sodium sulphate were used as catalysts. The ammonia was distilled off into a standard acid solution and titrated with standard base.

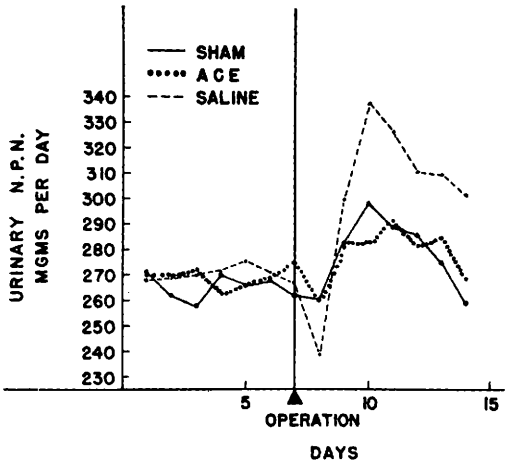

FIG. 1.
The effect of adrenal cortex extract upon the level of urinary nitrogen immediately following adrenalectomy. Averages of 8 rats per group.

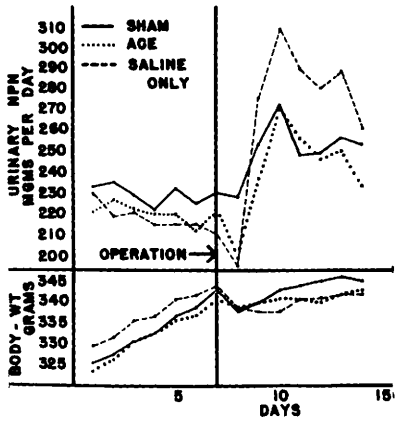

FIG. 2.
The effect of adrenal cortex extract upon the level of urinary nitrogen immediately following adrenalectomy. Averages of 10 rats per group.

Experiments and results. In Experiment 1 (Fig. 1) 24 rats were observed during a control period of 2 weeks. At this time 8 rats were adrenalectomized and were given 4 cc of beef adrenal extract (Upjohn) per day for 7 days; 8 adrenalectomized rats received injections of 4 cc of 0.9% sodium chloride solution for 7 days; and 8 sham-operated rats received injections of 4 cc of 0.9% sodium chloride solution for 7 days. The adrenalectomized rats showed some decrease in urinary nitrogen during the first 24 hours with a subsequent rise. The increase in nitrogen loss was significantly greater in the saline-injected adrenalectomized animals than in either the treated adrenalectomized group or the sham-operated controls.

Experiment 2 (Fig. 2) was identical with Experiment 1 except that 30 rats were used and it was carried out during the summer months instead of early winter. The relative changes following operation were identical with the results of Experiment 1. The level of urinary nitrogen was significantly lower than for Experiment 1 during all phases of the experiments.

Discussion. These data support our earlier

[6] Reinecke, R. M., Ball, H. A., and Samuels, L. T., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 44.

[7] Ingle, D. J., and Griffith, J. Q., Chapter 16, The Rat in Laboratory Investigation, J. B. Lippincott Co., Philadelphia, 1942.

observations[4,5] that the administration of adrenal cortex extract to force-fed rats immediately following adrenalectomy tends to suppress the post-operative increase in urinary nitrogen as compared with that of the untreated animal. The adrenalectomized rat usually shows a temporary decrease in urinary nitrogen which is not shown by sham-operated animals. This may possibly reflect a difference in the breakdown of thymus and other lymphoid tissue or it may reflect an increase in nitrogen retention in the body fluids. Noble and Toby[8] reported a temporary decrease in loss of nitrogen after adrenalectomy followed by a rise above the pre-operative values.

Koelsche and Kendall[2] found that adrenal cortex extract tended to inhibit the catabolic effect of thyroxin in the adrenally insufficient dog. Berman *et al.*[3] noted that regeneration of liver is suppressed in the adrenalectomized rat and that regeneration is supported by treatment with adrenal cortex extract.

The possibility must be considered that adrenal cortex extracts favor anabolism under certain conditions because of the presence of androgenic steroids. However, such extracts have never been shown to possess androgenic properties.

The average level of urinary nitrogen was higher in all phases of Experiment 1 than in Experiment. 2 Such shifts in the average level of constituents of blood, urine and tissues are not uncommon in this laboratory. The reasons have never been elucidated beyond the demonstration that even small variations in temperature are important. Values on the nitrogen content of our fluid diets, on standards and unknowns have remained uniform. Because unknown variables influence the results of experiments which are thought to be under rigid control, it is essential that all control studies be done in parallel with the experimental study.

Summary. Two experiments were carried out as follows. Force-fed rats given saline to drink were subjected to adrenalectomy or to sham operations. Half of the adrenalectomized rats were treated with 4 cc of beef adrenal extract per day. All of the animals showed an increase in urinary nitrogen following operation but it was significantly less in those animals which either had their adrenal glands intact or were treated with adrenal cortex extract.

8 Noble, R. L., and Toby, C. G., *J. Endocrinol.*, 1948, **5**, 303.

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Augmentation of Femoral Venous Flow in the Dog by Electrical Stimulation of Muscles.

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The procedure was performed 6 times on 2 mongrel dogs, weighing approximately 11 kg. They were anesthetized with intravenous nembutal. Eighteen mg of heparin was given intravenously at the beginning of the procedure. The femoral vein was exposed and cannulated. On one dog a straight glass cannula was used. On the other a T-shaped cannula was inserted, so that no backing up of blood was allowed between periods of flow. The diameter of the T-cannula was smaller than that of the straight one; consequently the minute flow was less. The blood was caught in a sterile graduated cylinder, filtered and reinfused at the end of the experiment.

The stimulation was achieved by means of faradic current. The intensity of the current and the placing of the electrodes on the limb were so arranged that a gentle contraction of the muscles of the whole limb was obtained. The stimulation was intermittent, 2 seconds of exercise being alternated with 3 seconds of