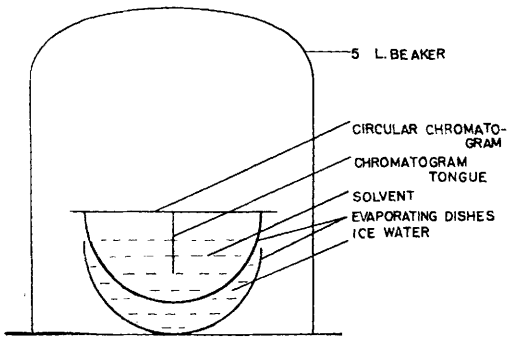

FIG. 1.
Apparatus for chromatographic separation of steroids.

using the same solvent and conditions. The importance of water-vapor and/or temperature was demonstrated, since no separation of cholesterol, ergosterol and vit. D_3 could be obtained on a circular chromatogram at 2°C in a cold room, nor at room temperature (approx. 30°C with the icebath eliminated).

Recently, Kostir and Slavik(9) devised a method for paper chromatography in which the partition is between 2 organic phases—one stable (acetylated filter paper), and the other mobile—in distinction to the ordinary partition between the water of the hydrated cellulose of the filter paper and the organic mobile solvent. They state that this method should enable one to separate compounds having low water solubility such as the fatty acids, steroids, etc., which are not resolved in the water-organic partition. We could not effect separation of sterols using this method. We think it advisable, however, to investigate this aspect of chromatographic separation of sterols further.

Summary. Methods for identification and separation of several non-keto steroids in 10 μg quantities on paper chromatograms are described.

9. Kostir, J. V., and Slavik, J., *Collection of Czechoslovak Chemical Communications*, 1950, v15, 17.

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Effect of Cortisone Acetate upon Plasma Amino Acids in the Eviscerate Rat. (18348)

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The level of blood amino acids in the eviscerate-adrenalectomized rat is lower than that of non-adrenalectomized, eviscerate rats (1,2). The administration of adrenal cortex extract to eviscerate and to adrenalectomized-eviscerate rats causes a rise in plasma amino acids(2). In the present study, it was shown that the administration of cortisone acetate to eviscerate and to adrenalectomized-eviscerate rats caused a rise in plasma amino acids.

Methods. Male rats of the Sprague-Dawley strain were eviscerated at 250 g weight. Adrenalectomy was performed at the same operation. Immediately following operation, all of the animals were given continuous intravenous infusions of 0.9% sodium chloride with glucose and insulin. The volume was 20 cc per 24 hours per rat. The glucose load was 40 mg per 100 g of rat per hour, and the insulin dose (Regular Insulin, Lilly) was 4 units per 24 hours per rat. The details of the methods have been described(3,4). Cortisone acetate (Merck) in saline suspension was administered by subcutaneous injection at the beginning of the experiment and 6 hours later and 24 and 30 hours later in those animals which were studied for 48 hours. Eviscerate rats with and without cortisone acetate were studied simultaneously. Samples of blood

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2. Bondy, P. K., *Endocrinology*, 1949, v45, 605.
3. Ingle, D. J., Prestrud, M. C., and Nezamis, J. E., *Am. J. Physiol.*, 1947, v150, 682.
4. Ingle, D. J., *Exp. Med. and Surg.*, 1949, v7, 34.

TABLE I. Effect of Cortisone Acetate Upon Plasma Amino Acids Following Evisceration.

Group	Operation	No. rats	Hr	Cortisone mg/24 hr	Amino N mg % avg and range
1	Eviscerate	22	24	0	8.03 ± .24
2		11	24	5	8.31 ± .29
3		22	24	10	9.57 ± .30
4		12	48	0	13.15 ± .59
5		13	48	10	16.63 ± .39
6	Eviscerate-adrenalectomized	21	24	0	5.80 ± .20
7		11	24	5	7.71 ± .26
8		12	24	10	9.47 ± .45

were taken from the abdominal aorta at the end of each experiment. Plasma amino nitrogen was determined by the method of Hamilton and Van Slyke(5).

Experiments and results. The data are summarized in Table I.

Group 1 consisted of 22 eviscerate rats without cortisone which were observed for 24 hours. In Group 2, 11 eviscerate rats treated with 5 mg of cortisone acetate during 24 hours showed a statistically insignificant rise in plasma amino nitrogen. In Group 3, the treatment of 22 eviscerate rats with 10 mg of cortisone acetate during 24 hours caused a significant rise in plasma amino nitrogen above the average values of Group 1.

Group 4 consisted of 12 eviscerate rats without cortisone which were observed for 48 hours. The values for plasma amino nitrogen were significantly higher than at 24 hours. In Group 5, 13 eviscerate rats treated with 10 mg of cortisone acetate daily for 48 hours showed significantly higher values for plasma amino nitrogen than did the control animals of Group 4.

Group 6 consisted of 21 adrenalectomized-eviscerate rats without cortisone which were observed for 24 hours. The average value for plasma amino nitrogen was significantly lower than the average value for the non-adrenalectomized rats of Group 1. In Group 7, the treatment of 11 adrenalectomized-eviscerate rats with 5 mg of cortisone acetate each during 24 hours caused a significant rise in plasma amino nitrogen above the untreated controls (Group 6) and almost to the average values for the untreated non-adrenalectomized rats of Group 1. In Group 8, the treatment of 12 adrenalectomized-eviscerate rats with 10 mg of cortisone acetate each during 24 hours caused an average rise in plasma amino nitrogen which was significantly above the values for the untreated controls (Group 6) and significantly above the average values for the untreated non-adrenalectomized controls of Group 1.

Discussion. In support of earlier observations(1,2), these results show that the level of blood amino acids in the eviscerate rat is suppressed by adrenalectomy. It was further shown that the administration of cortisone acetate caused a rise in the concentration of plasma amino acids. It seems probable that these changes in the concentration of plasma amino acids represent an effect of the adrenal cortical hormones upon the equilibrium between free amino acids and proteins. Since the liver and other intraabdominal organs had been removed, it is clear that the effect of the cortical hormones upon nitrogen metabolism is not limited to these tissues. Long, Katzin and Fry(6) recognized the possibility that the cortical hormones may stimulate the catabolism of proteins in the peripheral tissues. It is also possible that the cortical hormones inhibit the rate of synthesis of proteins from amino acids(7) and thereby cause an increase in the concentration of free amino acids in the body water.

The possibility should be recognized that the observed changes might have been due to shifts in the amount of diluent (water) of the blood amino acids. The intake of water was

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6. Long, C. N. H., Katzin, B., and Fry, E. G., *Endocrinology*, 1940, v26, 309.

7. Albright, F., *Harvey Lectures*. 1942-1943, 122.

constant in all of the animals. No differences in the excretion of water as related to the presence and absence of the adrenal glands and to cortisone were observed. The amounts of amino nitrogen in the urine were negligible. It seems probable that the amino acids of the plasma would move freely with water lost into the extracellular fluid of the tissues, but this is uncertain. The average increase in concentration of plasma amino acids caused by treating adrenalectomized-eviscerate rats with cortisone was over 60 per cent. If this change were due to a relative loss of diluent, there should have been a significant change in the hematocrit and in the concentration of hemoglobin. Such was not observed. The factors responsible for the effects of adrenal cortical hormones upon the concentration of plasma amino acids remain to be fully elucidated, but we suggest that it probably represents an extra-hepatic effect of the hormones upon protein metabolism.

Summary. Male rats were eviscerated and eviscerated-adrenalectomized. All of the animals received continuous intravenous infusions of saline with glucose and insulin for 24 and 48 hours. The administration of cortisone acetate in doses of 5 and 10 mg per 24 hours to one rat of each pair caused significant increases in the concentration of plasma amino acids above the values for untreated animals. It is postulated that the observed change represents an extra-hepatic effect of cortisone upon protein metabolism.

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Marked Acute Hyperglycemic Response of Depancreatized Chicks to Adrenal Cortical Extract (ACE).* (18349)

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Neither pancreatectomy nor alloxanization effects a persistent hyperglycemia in chicks. Birds chronically surviving these procedures exhibit normal blood glucose levels and glucose tolerance curves(1-6). The reasons for this remain obscure. Previously, Golden and Long demonstrated that adrenal cortical extract (ACE) elicits gluconeogenesis in normal chicks; moderate hyperglycemia supervenes (2). These workers did not study the response of pancreatectomized birds to ACE.

The possibility presents itself that ACE-induced hyperglycemia may be enhanced in depancreatized birds due to a relative insulin lack present despite normal control plasma glucose levels(7). Since such a finding would throw light on the metabolic relationships prevailing in pancreatectomized birds, we undertook to study their glycemic responses to ACE.

Methods. A total of 9 depancreatized and 8 unoperated (control) cockerels were used.

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