fibers of the stalk attached to the surface of the third ventricle would be included as part of the median eminence. In a few cases the fibers of the stalk were dissected free of their attachment to the surface of the third ventricle.

It will be observed that the median eminences of most cattle contain little or no milk "let-down" hormone (Table I). In a few cases there were traces to measurable amounts. Such activity is probably due to stalk fibers retained by the median eminence after dissection.

These data indicate that the suggestion of Bargmann and Scharrer regarding the origin of the posterior pituitary hormones in the hypothalamus apparently does not hold for the milk "let-down" hormone. If the hormone is secreted in that division of the neurohypophysis it must be in an inactive form, becoming active in its passage down the stalk.

In this connection it is interesting to note that the stalk in every case contained measurable amounts of hormone. However, the amount of hormone in the stalk per mg of acetone-dried tissue (specific activity) was roughly of an order of one-tenth or less that of the posterior lobe. These observations suggest that the movement of hormone is from the posterior lobe to the stalk rather than from the median eminence to the stalk.

Summary and conclusions. 1. The neurohypophyses of a series of animals were separated into the median eminence, stalk and posterior lobe. The acetone-dried tissue in each division was extracted and assayed for milk "let-down" hormone content, using lactating sows as assay animals. 2. The median eminence usually contained an insufficient amount of hormone to cause milk "let-down" in the sow. In a few cases some activity was observed. 3. It was concluded that the milk "let-down" hormone is either not secreted in the hypothalamus or, if secreted there, exists in an inactive form which becomes active only as it passes down the stalk into the posterior lobe. Since the posterior lobe contained roughly 10 times the amount of hormone present in the stalk per unit of dry matter, it seems more probable that the posterior lobe is the source of the milk "let-down" hormone.

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Effect of Epinephrine and Insulin on Tissue Electrolytes in Normal and Demedullated Rats. (19569)

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A reduction in the blood potassium level has been reported following the administration of insulin and epinephrine in normal animals and man(1-4) Considerable evidence in the literature(5,8), however, indicates that either epinephrine or insulin reflexly releases the other humoral agent apparently through the mediation of altered glycemic levels. In view of the interaction between these two endocrine substances it appeared desirable to investigate further the effect of insulin and epinephrine on the ionic content of plasma and selected tissues.

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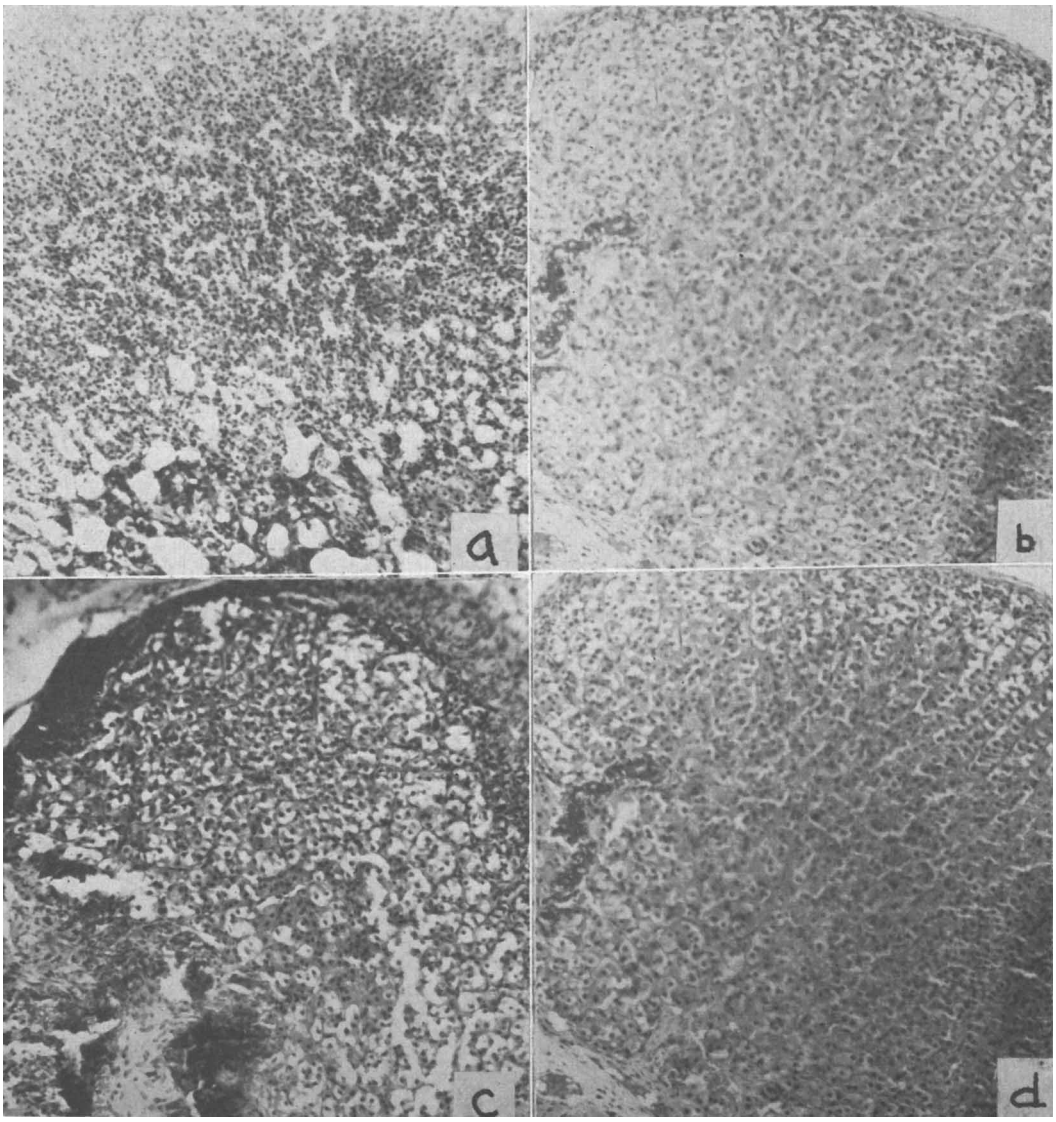

FIG. 1. Sections of adrenal glands. (a) Normal sham-operated. (b) Demedullated 21 days; non-treated. (c) Demedullated 21 days; epinephrine-treated. (d) Demedullated 21 days; insulin-treated. Note that the regenerated adrenal cortices appear histologically normal. (All sections × 80.)

Materials and methods. The experiments were designed to compare the effects of epinephrine and insulin on tissue electrolytes and water content in intact groups of rats and rats with apparently functional adrenal cortices but no medullary tissue (adrenal medulla). Greep and Deane(9) showed that approximately 21 days after adrenal-enucleation the regenerated adrenal cortices have attained normal functional capacity. Accordingly, in the experiments reported here groups of sham-operated and demedullated male Wistar rats were identically prepared for use in the experiments 21 days after surgery, as follows: Adrenal-enucleation was done by the method of Ingle and Higgins(10). The sham operation was done similarly except for the final stage of enucleation. After the surgery the rats were maintained on chow biscuit and a 1% NaCl drinking solution *ad libitum* for the

next 10 days. The salt solution was shown(9) to be necessary for the adrenal-enucleated during the first 8-10 days of adrenocortical regeneration. On the 11th day after surgery the 1% NaCl solution was replaced by tap water for the next 11 days. At the end of the 21-day post surgical period the sham-operated and demedullated were fasted overnight and treated as one of the following groups: No treatment (controls); epinephrine injected; and insulin injected. The rats were lightly anesthetized with n-methylcyclo-hexenyl-methyl barbituric acid (EVIPAL) before epinephrine or insulin was administered, and before tissues were taken for analyses. Epinephrine (Adrenalin tablets; Parke, Davis & Co.) was prepared fresh in physiological saline and administered subcutaneously at a dose level of 0.04 mg/100 g body weight (approx. 0.5 ml). Insulin (ILETIN, regular; Lilly) was administered subcutaneously at a dose level of 0.5 *U*/rat (0.6 ml). Cardiac blood was obtained from the control rats immediately after they were anesthetized without further treatment, and at the end of 60 minutes from the rats injected with epinephrine or insulin. Specimens of gastrocnemius muscle and liver were taken immediately after the blood was obtained. The procedures followed for the analyses of electrolytes and water composition of plasma, skeletal muscle, and liver were the same for all animals and have been described in detail in a previous report(11). Tissue chloride content was determined by the method of Van Slyke(12); and plasma chloride by the method of Schales and Schales(13). Plasma glucose was determined by the photometric method of Kingsley and Reinhold(14). Tissue and plasma sodium and potassium were determined with the aid of an internal lithium standard flame photometer.

Results. Photomicrographs of representative histological sections of adrenal glands from normal sham-operated and demedullated rats are presented in Fig. 1. It is apparent that except for the absence of medullary tissue in the glands of the latter group that the adrenal cortices appear histologically normal with good zonal regeneration.

The results of analyses of plasma, skeletal

TABLE I. Comparison of Tissue Water and Ionic Content 60 Min after Epinephrine and Insulin in Normal and Adrenal-Demedullated Rats.

Tissue constituent		Normal sham-operated				Adrenal-demedullated			
		Controls (6)	Epinephrine*(5)	Insulin† (6)	Controls (8)	Epinephrine (7)	Insulin (8)
Plasma‡	% H_2O	93.2 ± .14§	93 ± .11	92.1 ± .28**	93 ± .09	92.5 ± .07	92.7 ± .1
	Cl mEq.	112 ± 1.1	104.9 ± 1 **	109.1 ± 1.2	110 ± .8	106.9 ± 1.1 ¶	110.3 ± .5
	K "	4.97 ± .21	4.29 ± .09**	3.94 ± .24**	5.58 ± .27	4.38 ± .19**	4.86 ± .2
	Na "	154.3 ± 1.1	151.1 ± 2	161.5 ± 1 **	153.3 ± .6	155.3 ± .9	162.4 ± 1.1**
	Glu. mg %	134 ± 7	294 ± 11	34 ± 4	116 ± 6	283 ± 12	26 ± 3
Muscle‖	H_2O g	757 ± 1	761 ± 1.5¶	757 ± .7	757 ± 1.1	763 ± .8**	756 ± .8
	Cl mEq.	10.3 ± .3	12.6 ± .3**	9.5 ± .2	10.6 ± .2	12.7 ± .4**	8.9 ± .2**
	K "	97.8 ± 2.3	88.6 ± 1.2**	98.4 ± 4.3	93.7 ± .9	90.5 ± .3**	96.8 ± 2.2
	Na "	31.5 ± 2.7	40.3 ± 1 ¶	28.6 ± 3	33.7 ± .8	38.2 ± 1 **	31.3 ± .9
Liver	H_2O g	698 ± 1.4	694 ± 3.1	692 ± 2.1¶	696 ± 3.2	694 ± 1.8	692 ± 1.1
	K mEq.	80.6 ± 3.3	78.4 ± 3.5	82.9 ± 3.4	78.7 ± 1.5	85.9 ± 2.3¶	86.5 ± 2.1**
	Na "	35.5 ± 2.4	40.9 ± 2.9	34.9 ± 1.7	36.5 ± 1.7	32.5 ± 1.8	33.8 ± 1

* Epinephrine sub-q at dose level of .04 mg/100 g body wt. † Insulin sub-q at dose level of .5 *U*/rat. ‡ Electrolyte conc. expressed as mEq./kg plasma water. § Mean ± S.E. ‖ Muscle and liver content expressed per kg wet tissue. Differences between means which were significant are indicated: ¶ *P* values = .05–.01; ** *P* values = <.01. No. of rats in each group in parentheses.

muscle and liver of the sham-operated and demedullated groups of rats are presented in Table I. The respective mean values of the tissue composition determined in the epinephrine and insulin treated groups were compared with those of the controls to determine whether the difference between the means were statistically significant. Inspection of these data show the following significant changes: *After epinephrine treatment*—Plasma chloride and potassium concentration were significantly decreased. Muscle water, chloride and sodium content were significantly increased; but muscle potassium was decreased. These changes were found in both the *sham-operated and the demedullated* groups. An increase in liver potassium was determined in the demedullated group alone. *After insulin treatment*—Significant changes in the *sham-operated* group were: decreased plasma potassium and increased plasma sodium concentration; and decreased plasma and liver water content. Significant changes in the *demedullated* group were: increased plasma sodium concentration; decreased muscle chloride and increased liver potassium content. In summary, the results showed that in both the sham-operated and demedullated groups there was a consistent pattern of altered electrolyte composition in certain tissues 60 minutes after epinephrine injection. The effect of insulin on tissue ionic composition was not consistent in the sham and the demedullated groups except for the significantly increased plasma sodium concentration. Particularly relevant to the present study was the evidence that epinephrine induced a significant reduction in the plasma potassium concentration in the sham and the demedullated groups whereas following insulin administration the plasma potassium concentration was decreased in the sham but not in the demedullated group.

Discussion. The alterations in water and ionic content of tissues reported here after epinephrine administration in sham-operated and demedullated groups of rats were the same (except for the increased liver potassium in the demedullated group) as reported(11) in intact and adrenalectomized rats 60 minutes after epinephrine administration. These data indicate that epinephrine's effect on certain tissue ionic constituents was consistently the same pattern in several different kinds of animal preparation. An explanation of the changes in tissue water and ionic content after epinephrine was presented in the discussion of a previous report(11).

It has been shown(5-8) that certain phases of carbohydrate metabolism and indices referable to increased anterior pituitary-adrenocortical activity believed to be an effect of insulin, *per se,* were actually responsible to an epinephrine action. Apparently the insulin-induced hypoglycemia "reflexly" elicited increased secretion of epinephrine. Since the evidence presented here and in related studies (15,16) indicates that epinephrine has a significant role in the physiological regulation of potassium balance in the fluid compartments of the body, then the difference in insulin's effect on the plasma potassium concentration in the sham and demedullated groups can be explained as another illustration of the interaction of these 2 humoral agents in the intact animal.

Summary. 1. The effect of epinephrine and insulin administration on the water and ionic content of plasma, skeletal muscle, and liver was compared in normal sham-operated and adrenal demedullated (21 days after surgery) groups of rats. Histological evidence was presented that the regenerated adrenal cortices appeared normal and medullary tissue was absent. 2. A significant reduction in plasma and muscle potassium concentration was determined 60 minutes after epinephrine administration in the sham and demedullated groups. A decreased plasma potassium level 60 minutes after insulin administration was found in the sham group but not in the demedullated group. However, a significant increase in the plasma sodium concentration after insulin was determined in both sham and demedullated groups. 3. The results were discussed as indicating that the apparent effect of insulin upon the plasma potassium level in the intact animal was the resultant of "reflexly" elicited epinephrine secretion following insulin-induced hypoglycemia and not the effect of insulin *per se.*

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The Effect of Diet on Riboflavin and Xanthine Oxidase in Rat Liver and Intestine.* (19570)

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The effect of low protein and low riboflavin diets on liver riboflavin has been reported previously(1,2). The present study shows a proportional reduction in the riboflavin content of the sedimentable particles and the supernatant fractions of a liver homogenate in both of these types of dietary restrictions. The corresponding changes in intestinal riboflavin, and the independent fluctuation of total riboflavin vs. the flavin enzyme, xanthine oxidase, in both liver and intestine have also been determined.

Methods. Male weanling rats were placed on 4 diets that varied as follows:

Diet	A	B	C	D
GBI Casein (vit. test)	31%	8%	31%	28.5%
Riboflavin (mg/100 g)	.5	.5	0	.5
Soy flour (50% protein)†	0	0	0	5

† A. E. Staley Mfg. Co., Decatur, Ill. Extracted soya flour.

The remainder of each diet consisted (in g/100 g) of: Crisco 4, Wesson oil 2, cod liver oil 1, Phillips and Hart salt mixture 4, and glucose (89—casein and soy flour). The vitamin supplement added (mg/100 g); nicotinic acid 2.5, calcium pantothenate 1.0, thiamine 0.4, pyridoxine 0.4, biotin 0.05, inositol 50.0, p-aminobenzoic acid 10.0, vit. B_{12} 0.05, mixed tocopherols 50.0, ascorbic acid 25.0, 2-methyl-1,4-naphthoquinone 0.25, choline chloride 100.0. In comparison with the control diet A, diet B was low in protein, C was low in riboflavin, and D contained soy flour as a source of the unidentified xanthine oxidase (X.O.) factor(3). After 2 or 4 weeks of *ad libitum* feeding, groups of rats from each diet were sacrificed by decapitation for analysis of the livers and small intestines. Riboflavin was determined microbiologically, using *L. casei* as the test organism and a Difco synthetic medium. The tissues were homogenized with cold 0.04 M phosphate buffer, pH 7.4, and aliquots were prepared and assayed by the procedure of Strong(4). The liver homogenates obtained at 2 weeks were also separated into a combined particulate (nuclei plus mitochondria plus microsomes) and supernatant fractions by centrifuging at 0-5° and approximately 30,000 x g for 90 minutes(5). The precipitate was washed by resuspending it in buffer and centrifuging again for 90 minutes; the wash was added to the super-

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