

that actual deposition of increased amounts of magnesium had occurred.

Less striking changes were observed when either insulin or dextrose alone was given following injection of magnesium. It is of interest that magnesium content of both heart and skeletal muscles was increased by administration of dextrose and magnesium, while the sole significant increase in magnesium content following injection of insulin and magnesium occurred in the heart. The combination of insulin and magnesium resulted in the highest mean relative Mg^{28} activities observed in the 4 groups, but the range of values was so wide that no statistically significant differences were elicited.

The biochemical mechanisms whereby dextrose and insulin accelerate Mg^{28} turnover in the tissue are obscure. Animals in this study were subjected to a slight magnesium load. Previous studies indicated that magnesium increases glycogenesis in liver slices(8) and rat-heart slices(9), activates phosphatases from kidneys, intestinal mucosa, liver, bone and plasma, and increases activity of ATP(10). Although it is not known at the present time whether these enzymatic processes are involved in turnover of magnesium, it is of interest that these are the very tissues which in this study showed increased Mg^{28} activity in the presence of insulin and glucose.

Taken as a whole, the observations made in this study and previous studies suggest that metabolism of magnesium is intimately related to that of carbohydrate and that the turnover of magnesium in tissues is regulated by insulin and dextrose. The relationship be-

tween metabolism of magnesium and that of other intracellular electrolytes, such as potassium, remains to be studied.

Summary. In rabbits which had received by intravenous injection 1 to 2 meq of magnesium tagged with Mg^{28} , parenteral administration of insulin and dextrose significantly accelerated Mg^{28} uptake in bone, kidney, heart, liver, appendix, skin and skeletal muscle. Tissue magnesium content was increased in heart and skeletal muscle. Less striking changes were noted when insulin or dextrose alone was given following injection of magnesium. Magnesium metabolism appears intimately related to that of carbohydrate and insulin.

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Effect of ACTH on Regeneration of Adrenal Cortex Following Auto-grafting in Hypophysectomized Rats.*† (25521)

LINDA PLZAK (Introduced by D. J. Ingle)

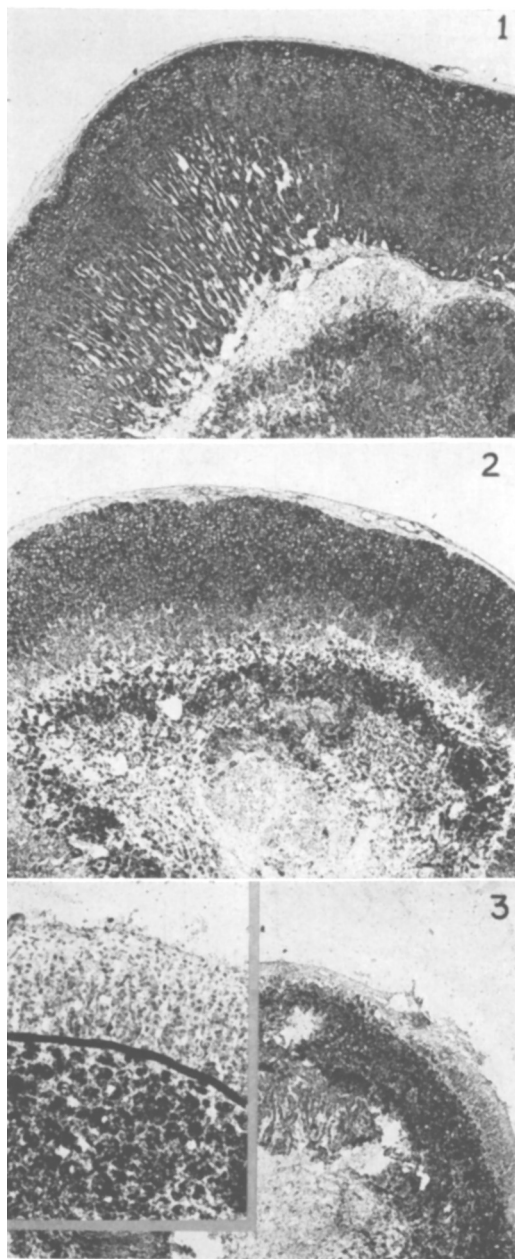
Ben May Laboratory for Cancer Research, University of Chicago, Chicago, Ill.

Although adrenal cortical tissue may survive autogenous transplantation in the hypophysectomized rat, regeneration is less than in rats having intact pituitary glands. ACTH

treatment of hypophysectomized rats did stimulate regeneration of cortical tissue in

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Frozen sections of 3 adrenal glands one mo following autotransplantation (oil-red-O-stain) $\times 25$.

FIG. 1. Regeneration of adrenal cortex in a non-hypophysectomized rat.

FIG. 2. Regeneration of adrenal cortex in a hypophysectomized rat inj. with ACTH.

FIG. 3. Regeneration of the adrenal cortex in untreated hypophysectomized rat. *Insert* — High power photograph illustrating an outer atrophic cortex and an inner zone of lipid-laden debris.

adrenal autografts, but extent of regeneration was less than occurred in non-hypophysectomized rats.

Methods. Female Sprague-Dawley rats, approximately 3 months old and weighing 185-235 g, were maintained on Rockland Rat diet. Operations were done as described by Ingle(1). The gland was excised, freed of fat, and attached by a fine silk suture to the corresponding ovary. Hypophysectomy was by the usual parapharyngeal approach. All rats received 5,000 units of penicillin and 5 mg of streptomycin on day of operation only. Long-acting ACTH in gelatin (Depo-ACTH, Upjohn, 40 units per cc) was given twice daily by subcutaneous injection. Adrenal cortex extract in saline (1 u per cc, Upjohn) was given in amounts of 1 cc per rat/day for 7 days following hypophysectomy. One month after transplantation, adrenal grafts were removed from the ovaries and weighed, and frozen sections prepared with oil-red-O-stain. Extent of regeneration was rated on the basis of width of the fasciculata zone and appearance of cells, on a scale of 0 to 5; 2.5 approximated the normal adrenal cortex of an unoperated rat. The amount of lipid present was also rated on scale of 0 to 5, 2.5 approximating amount of lipid in normal cortex of the rat. After removal of the grafts, the animals were observed for signs of adrenal insufficiency. At necropsy a careful search was made for accessory adrenal tissue and for remnants of pituitary tissue. When such were found, the animal was discarded from the series.

Results. In non-hypophysectomized rats, adrenal autografts showed good regeneration (Fig. 1, Table I) with more lipid deposition than is seen in the normal adrenal cortex. Observations on regeneration were as described by Ingle and Higgins(2). The medulla of the adrenal does not survive.

Regeneration of cortical tissue was observed in 2 groups of hypophysectomized rats, one given 10 and one given 20 units of ACTH daily (Fig. 2, Table I). Since weights and appearance of grafts were not significantly different between one group and the other, the data are averaged together (Table I). Each of the 3 zones of the cortex was fairly well developed although narrower and possessing less

TABLE I. Mean Weights of Adrenal Glands and Microscopic Evaluation of Regeneration and Lipid Deposition in Adrenal Glands One Month after Transplantation.

Group	Procedure	No. of rats	Adrenal wt*		Adrenal cortex†		
			(mg)	(mg/100 g body wt)	Width (mm)	Regeneration	Lipid deposition
A	No hypophysectomy; no ACTH given	14	29.6 ± 1.8	10.3 ± .6	1.15	3.0 ± .4	3.0 ± .3
B	Hypophysectomy; 5 and 10 units ACTH given twice daily	17	14.8 ± 1.9	7.3 ± .7	.77	2.3 ± .8	2.7 ± .3
C	Hypophysectomy; no ACTH given	10	6.8 ± .9	3.5 ± .5	.18	1.8 ± .7	.6 ± .8

* Mean ± S.E.

† Regeneration and lipid deposition are rated on scale of 0 to 5, a value of 2.5 being comparable to appearance in unoperated normal rats.

lipid than autografts in non-hypophysectomized rats. The cells were vacuolated and composed of light cytoplasm, suggesting possible overstimulation. Autografts in hypophysectomized rats without ACTH showed some viable lipid-laden cortical tissue, but most of each graft was necrotic and regeneration remained minimal (Fig. 3, Table I).

Discussion. The results show that regeneration of an apparently well organized adrenal cortex can be stimulated by ACTH alone in the hypophysectomized rat. Extent of regeneration is less than occurs in presence of the anterior pituitary. Amounts of long-acting ACTH used in these experiments were very large. It is possible that the response of

the adrenal graft to exogenous ACTH could be normalized if the ACTH were given continuously. It is also possible that some hypophyseal hormone other than ACTH plays a role in regeneration of adrenal cortical grafts.

Summary. Large doses of ACTH stimulated autografts of adrenal glands in hypophysectomized rats to regenerate cortical tissue. Extent of regeneration was less than occurs in the non-hypophysectomized rat.

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Colorimetric Determination of Amino Acids in Presence of a Peptide. (25522)

G. P. LAMPSON AND H. O. SINGER

Ortho Research Foundation, Raritan, N. J.

In investigating the splitting of peptides by peptidases, there has been need for a simple, quick photometric method to determine quantitatively the amino acids present after hydrolysis. The familiar Moore and Stein ninhydrin method(1) for determination of amino acids is not applicable as color intensity from peptides, on a molar basis, is nearly the same as that from amino acids. Most investigators use a titration method(2) for estimation of liberated carboxyl groups. In the present investigation, it was observed that when pH of

the buffer in the ninhydrin reagent was 4.0 instead of 5.5, and development of ninhydrin color was at 60°C instead of 100°C, as used by Moore and Stein, amino acids gave the usual amount of color while peptides remained almost colorless. The glycyl peptides, which gave about 50% as much color as amino acids, were an exception to this. The hydrolysis of peptides can be readily followed, when these modifications of the Moore and Stein procedure are used to determine ninhydrin-reacting substances, since the color developed is