TABLE II. Effect of Triparanol Feeding on Adrenal Function in Rats in Exp. II.

	Left adrenal wt (mg)	Adrenal cholesterol content (mg)	Adrenal vein plasma flow (ml/hr)	Plasma corticosterone (γ/ml)	Corticosterone secretion rate (γ/hr)
Triparanol treated rats (9)	25.2 ± 1.2 *	3.7 ± .27	4.92 ± .50	9.85 ± .91	45.8 ± 3.3
Control rats (9)	23.4 ± .95	13.8 ±1.6	5 07 ± .74	16.54 ±2.6	76.3 ±10.1

* Mean values ± stand. error of mean.

of adrenal steroids, it seems probable that any agent which inhibits synthesis of this sterol will result in diminished production of adrenal steroids. This hypothesis is borne out by results of the study reported here in which triparanol feeding caused a significant lowering of both adrenal cholesterol and the amount of corticosterone secreted by rats subjected to the stress of surgery and bleeding. Similarly, Melby, St. Cyr and Dale have demonstrated a 45% reduction in urinary excretion of 17-hydroxycorticosteroids in response to ACTH stimulation following a short period of triparanol feeding in humans(10). These investigators also found that urinary aldosterone and plasma cortisol levels were decreased in patients treated with this agent.

The finding that triparanol feeding results in a decrease in adrenal steroid production may have clinical significance in patients taking this compound. It seems conceivable that such an individual might develop acute adrenal insufficiency when exposed to the stress of major surgery or severe infection if not given prophylactic steroid therapy.

Summary. Triparanol feeding produces a significant reduction in the amount of corticosterone secreted by the adrenals of rats subjected to surgery and bleeding. It is postulated that this reduction is secondary to the marked decrease in adrenal cholesterol which results from triparanol therapy.

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Potassium Content of Rat Diaphragms Following Hypophysectomy and Incubation with Growth Hormone.* (26622)

V. S. CONSTANTINE AND J. L. KOSTYO

Departments of Physiology and Medicine and Division of Endocrinology, Duke University, Durham, N. C.

The transport of α-aminoisobutyric acid (AIB) by isolated rat diaphragms is decreased following hypophysectomy and stimulated by addition of various species of growth hormone to the incubation medium(1,

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2). Certain findings suggested that these alterations in amino acid transport might be related to changes in muscle K^+ content. Batts *et al.*(3) reported that the K^+ content of rat skeletal muscle was decreased after hypophysectomy and restored to normal by growth hormone administration. Moreover, the transport of amino acids into Ehrlich mouse ascites tumor cells was found to be related to the level of intracellular K^+(4-6). The present experiments were performed to determine if hypophysectomy and growth hormone influence amino acid transport by muscle through alterations in muscle K^+ content.

Methods. Normal and hypophysectomized female rats weighing 50-80 g were purchased from the Charles River Breeding Laboratories. The rats were hypophysectomized at 24-25 days of age and used for experimentation 2-4 weeks following operation. The animals were killed by cervical fracture. In some instances, the diaphragmatic muscle alone was removed from the rat, washed in Krebs bicarbonate buffer, blotted, weighed and digested in nitric acid in preparation for K^+ measurements. For *in vitro* studies, rib cage preparations of diaphragms were prepared according to the method of Kipnis and Cori(7). They were placed in 125 ml Erlenmeyer flasks containing 50 ml of Krebs bicarbonate buffer, pH 7.4, and 0.01 M glucose, gassed with 95% O_2-5% CO_2, and preincubated with shaking for 10 min at 37°C. Following preincubation they were transferred to flasks containing 30 ml of the same medium and incubated under similar conditions for various lengths of time. In some cases bovine growth hormone (NIH-BGH-1)† was added to the medium to a concentration of 25 μg/ml. At the end of the incubation period, the diaphragmatic muscle was dissected away from the rib cage, blotted, weighed and digested in nitric acid. K^+ determinations were made with a Baird flame photometer with lithium internal standard.

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One experiment was designed to indicate the degree of amino acid transport by diaphragms which were K^+ depleted *in vitro*. Eight rib cage preparations were placed in a 6 liter Erlenmeyer flask containing 5 liters of bicarbonate buffer, which had the composition of Krebs buffer except that all of the K^+ was replaced by Na^+. Glucose was added to a concentration of 0.01 M. The muscles were incubated in this medium at 37°C with gentle stirring and continuous gassing with 95% O_2-5% CO_2 for 2 hours. This procedure resulted in a 35-40% loss in K^+ by the diaphragm. Following K^+ depletion, the diaphragms were blotted lightly on filter paper and transferred to 50 ml Erlenmeyer flasks containing 10 ml of the K^+-free bicarbonate buffer, 0.01 M glucose and 0.05 mM AIB-$1C^{14}$ (S.A. = 1 mc/mM). The flasks were gassed with 95% O_2-5% CO_2, sealed and incubated with shaking at 37°C for 1 hour. After incubation, the AIB in the diaphragmatic muscle was extracted and counted by previously described methods(2). The results are expressed as the AIB distribution ratio which is the ratio of the cpm/ml of intracellular water to the cpm/ml of incubation medium. This distribution ratio gives an indication of the degree to which the cells concentrated the amino acid during the incubation period. The method used to calculate the radioactivity in the intracellular water has been described previously (*ibid.*).

Results and discussion. Hypophysectomy did not alter the *in vivo* K^+ content of rat diaphragms (Table I). Consequently, the impairment in amino acid transport by diaphragm muscle caused by hypophysectomy cannot be related to a low *in vivo* muscle K^+ level. However, it had been reported previously that rat diaphragm loses large amounts of K^+ when incubated *in vitro* under simulated physiological conditions(8,9,10). This suggested that diaphragms of hypophysectomized rats might lose greater amounts of K^+ than those of normal rats during incubation *in vitro*, resulting in a low K^+ level which would then be responsible for impaired amino acid transport. The results in Table I would indicate that this is not the case. Even after 2 hours of incubation *in vitro* there was no

TABLE I. Effects of Hypophysectomy and Incubation with Growth Hormone on K^+ Content of Rat Diaphragm.

Conditions	Muscle K^+ content (μeq/g wet wt) Normal	Hypox.	Hypox. + G.H.
In vivo	101 ± 1.55*(11)	102 ± .81 (6)	
In vitro, 60 min. incubation†	103 ± 1.15 (5)	106 ± 6.27 (4)	106 ± 1.38 (4)
120 " "	102 ± 2.30 (4)	107 ± 1.38 (4)	110 ± 2.75 (4)

* Mean ± S.E. No. of animals in parentheses. † All diaphragms preincubated for 10 min.

difference in K^+ content between normal and hypophysectomized rat diaphragms. Moreover, when hypophysectomized rat diaphragms were incubated with bovine growth hormone, muscle K^+ content was not altered (Table I). Thus neither hypophysectomy nor incubation with growth hormone exerted a detectable influence on the K^+ content of rat diaphragms *in vitro*.

It was conceivable that hypophysectomy and incubation with growth hormone might produce changes in diaphragm K^+ content which were too small to be detected by the method used and yet large enough to alter significantly the rate of amino acid transport by the cells. Should growth hormone stimulate amino acid transport by shifting small amounts of K^+ into muscle cells, then it should be possible to inhibit the effect of growth hormone on amino acid transport by placing the muscle in a system which does not permit movement of K^+ into the cells. An experiment was performed to test this possibility. Hypophysectomized rat diaphragms were depleted of K^+ and then transferred to flasks containing K^+-free bicarbonate buffer, 0.01 M glucose, AIB-1-C^{14} and in some cases, bovine growth hormone (25 μg/ml) and incubated for 1 hour. K^+ measurements made on muscles similarly treated indicated that K^+ content of the diaphragmatic muscle remained constant during the AIB uptake period. Diaphragms which were incubated with growth hormone had a mean AIB distribution ratio of 2.97 ± .18 ($n = 4$), while the controls had a mean ratio of 1.03 ± .03 ($n = 3$), ($P < 0.01$). Thus diaphragms which were K^+ depleted and could not take up K^+ during the AIB uptake period still responded to bovine growth hormone with an acceleration of amino acid transport.

It is of considerable interest that the rib cage preparation of rat diaphragm does not lose K^+ during incubation *in vitro*. In one experiment normal rat diaphragms were incubated for 5 hours and still no change in K^+ was noted. Since total tissue water remained stable during incubation, the constant K^+ to wet wgt. ratio observed cannot be due to concomittant loss of K^+ and water. K^+ concentration of the medium increased by about 1 meq/l during the 2 hour incubation in 30 ml of Krebs buffer presumably due to a loss of K^+ from extraneous cut muscle tissue on the preparation. This increase in medium K^+ concentration was not responsible for the stability of the K^+ content of the diaphragm since lowering initial medium K^+ concentration from the usual 5.9 meq/l to 4.8 meq/l failed to cause any change in K^+ content after 2 hours' incubation. Previous studies which indicated that the rat diaphragm loses K^+ during incubation (*ibid.*) were made using the "cut" diaphragm preparation which is prepared by dissecting the diaphragmatic muscle away from the rib cage and tendon before incubation. It was found in the present study that when just a few muscle fibers are cut by bisecting the rib cage preparation anterioposteriorly, the diaphragm lost K^+ during incubation. Hence, it appears that the integrity of the muscle fibers is essential for maintenance of constant K^+ levels during incubation *in vitro*.

Summary. Experiments were performed to determine if hypophysectomy and growth hormone influence amino acid transport by rat diaphragm through alterations in muscle K^+ content. Hypophysectomy did not alter *in vivo* K^+ content of rat diaphragm. Neither hypophysectomy nor incubation with growth

hormone exerted a detectable influence on K^+ level of the diaphragm *in vitro*. Moreover, diaphragms which were K^+ depleted and could not take up K^+ during the incubation period, still responded to bovine growth hormone with an increase in amino acid transport. Thus it appears unlikely that the effects of hypophysectomy and growth hormone on amino acid transport by rat diaphragm are mediated through changes in muscle K^+ content.

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The Effect of Metrazol in Isolated Mammalian Cells.* (26623)

GEORGE J. ALEXANDER AND RITA B. ALEXANDER (Introduced by Warren M. Sperry)
Dept. of Biochemistry, N. Y. Psychiatric Institute and College of Physicians and Surgeons, Columbia University, New York, N. Y.

In vitro studies in rat brain slices show that addition of Metrazol (pentamethylenetetrazole), inhibits 20-25% of the oxygen uptake(1). Studies of the mode of action of Metrazol in intact animals indicate that it stimulates oxygen consumption and causes symptoms akin to those of hypoxia(2). We decided to investigate the effect of Metrazol on a homogeneous cell population, propagated *in vitro*. Strain L mouse fibroblasts (3) were selected for the initial investigation because they are stable, sturdy, and can easily be grown in quantity.

Materials and methods. L strain, NCTC 929, mouse fibroblasts and all media were obtained from Microbiological Associates, Inc., Bethesda, Md. Metrazol was purchased from the Bilhuber-Knoll Corporation, Orange, N. J. Cells were maintained and incubated in Eagle's minimum essential medium (4) supplemented with 10% horse serum and a penicillin-streptomycin mixture. They were grown in monolayer cultures with three changes of medium a week. Harvested cells were introduced into a U-shaped chamber and grown in submerged aerated cultures with continuous inflow of fresh medium and outflow of spent broth. Agitation was sufficient to keep the cells dispersed and to prevent adhesion to the walls. Portions of the suspension were withdrawn at regular time intervals and viable cells were counted in a haemocytometer. Other portions of the suspension were transferred to Warbug vessels equipped with CO_2 traps for measurement of oxygen uptake.

Results. The normal oxygen uptake of strain L cells measured on portions of a suspension culture shaken in Warbug vessels is 0.25 μl per mg of cells per minute. Addition of Metrazol to the Warbug vessels brings about an immediate decrease in the oxygen uptake. Thirty minutes after addition of Metrazol (4×10^{-4} M) the rate of oxygen uptake decreases 94% (Fig. 1) and then remains constant for several hours. Comparison of the rates of oxygen consumption 30 min. after addition of various amounts of

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