epinephrine to adrenalectomized rats, shown to augment muscle AIB accumulation after insulin, significantly reduces the elevated plasma $[K^+]$ of adrenalectomized rats to 4.50 ± 0.28 ($n = 4$), but 6 hr pretreatment with either DOC or cortisol, which had no effect on muscle AIB response to insulin, did not reduce plasma K concentration. Values for other treatments were not obtained, but these preliminary observations indicate that the relationship of plasma $[K^+]$ to the insulin-induced increase in muscle AIB accumulation merits further study.

Independent of the foregoing, the results of this study suggest that epinephrine and corticoids may play a "permissive" or "supportive" role, in the sense proposed by Ingle (8), in the response of muscle to insulin with respect to AIB transport.

Summary. The distribution of AIB *in vivo* in diaphragm and gastrocnemius muscle of intact, hypophysectomized, and adrenalectomized rats has been examined with respect to the effect of insulin and epinephrine and corticoids. Adrenalectomy, but not hypophysectomy, impairs the ability of insulin to increase the accumulation of AIB in muscle to the level observed after insulin in intact rats. Epinephrine alone has slight effect on muscle AIB in adrenalectomized rats, but concurrent administration of epinephrine and insulin to adrenalectomized rats restores AIB distribution nearly to the level observed in adrenal-intact rats receiving insulin. Maintenance treatment of adrenalectomized rats with either DOC or cortisol resulted in normal AIB distributions in diaphragm and both corticoids, especially DOC, also enhance the AIB accumulation response to insulin. The suggestion emerges that these hormonal effects on AIB accumulation may be related to alterations in electrolyte metabolism.

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Effect of External Cooling of the Leg on Muscle Intracellular Water and Potassium.* (26474)

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Patients with the hyperkalemic form of familial periodic paralysis become paralyzed upon exposure to a cold environment(1,2). Localized paralysis of the arm has been produced in experiments with these same patients by swathing the forearm in ice packs for ½ hour(3,4). We have postulated that in this disease there is an exaggeration of the normal flux of potassium from the intracellular into the extracellular fluid and an inhibition of the normal return of potassium into the intracellular fluid. Muscle biopsies from one of these patients demonstrated an elevated intracellular water concentration and a depression of intracellular potassium concentration, but no direct evidence was obtained that potassium moved out of the muscle when paralysis was produced by external application of cold. The present studies were carried out to test the hypothesis that intra-

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TABLE I. Water and Na, K, Cl Content of Leg Muscles after External Cooling of Leg.

	$H_2O_{(t)}$, ml	Na	K	Cl	Na	K
		meq			meq/l $(H_2O)_I$	
	100 g of dry fat-free solids					
Control (38 rats)	276.0 ± 2.72*	9.5 ±.29	48.0 ±.31	5.0 ±.11	9.4 ±.86	173.4 ± 1.85
Exp. (30 rats)	284.6 ± 2.38	10.1 ±.26	46.7 ±.36	5.4 ±.16	9.4 ±.66	164.3 ± 2.19
Significance of difference	$p < .01$	$p > .1$	$p < .01$	$p < .05$	$p > .1$	$p < .01$

* ± stand. error of mean.

cellular potassium concentration diminishes in the muscle of a locally cooled leg of the intact animal.

Methods. White, male rats were anesthetized with intraperitoneal pentobarbital, and the right hand leg was held immersed in a Petri dish containing acetone and ice for 30 minutes. Control rats were similarly anesthetized and immobilized, but the leg was immersed in a Petri dish containing only tepid water. The tests were carried out on groups of 6 to 8 control, and 6 treated animals. After 30 minutes of cold immersion, the middle portion of the rectus femoris muscle was removed, dried on filter paper and placed in tared crucibles for weighing. After the wet weights had been obtained, the muscles were dried in an oven at 105°C until constant dry weights were obtained. The dry muscle was pulverized. The fat of the dried muscle was then extracted with petroleum and ethyl ether. The ether was discarded and the muscle residue once more dried in the oven at 105°C. The muscle was reweighed to obtain the weight of the fat removed and the residue then dissolved in 2 ml of 0.75 N nitric acid. From this, aliquots were taken for sodium, potassium and chloride analysis. Sodium and potassium determinations were performed using a flame photometer with an internal lithium standard. Chloride determinations were made with the Cotlove Chloridometer. Muscles from control animals were treated in the same fashion. Muscles were also removed for analysis from the left hind limbs of 19 of the animals whose right hind limb was chilled. No exact measurements of muscle temperature were made. At the end of experiment the temperature of the cold bath with the immersed right hind limb was 0-3°C. When exposed, the cold muscles were of normal color and appearance and of normal contractility when cut.

Chloride in the tissue was used as an indicator of extracellular water in our computations. The hazards of this calculation are recognized. However, any increase in intracellular chloride in the cooled muscles would accentuate the differences.

Results. The data recorded in Table I show that the muscles from the 38 control animals contained an average of 276.0 ± 2.72 ml (SEM) of intracellular water per 100 g of dry fat free solid. Mean intracellular water content of muscles from the chilled legs was 284.6 ± 2.38 ml (SEM) per 100 g of dry fat free solid. This difference was significant at the 99% level of confidence. Mean potassium concentration in control muscles was 173.4 ± 1.85 meq/l of intracellular water, whereas in the 30 muscles from limbs exposed to cold, mean potassium concentration per liter of intracellular water was 164.3 ± 2.19 meq. This difference and that between potassium concentrations per 100 g of dry fat free solids were also significant at the 99% level of confidence. The sodium per 100 g of dry fat free solids was insignificantly lower in control muscles than in muscles exposed to cold. Concentration of sodium in intracellular water in the 2 groups of muscles did not differ. Intracellular water concentration and concentration of potassium per liter of intracellular water in the muscles obtained from the limb opposite the cooled extremity in the experimental animals were measured only for the first 19 animals since it was impracticable to prevent some chilling of the left hind limb as well as the right hind limb and it was impossible to determine the extent of this chilling. In these 19 muscles, mean intracellular water concentration was

283 ml/100 g of dry fat free solids. There were 170 meq/k per liter of intracellular water.

Discussion. Interpretation of these findings is complicated by possible variations in blood flow in the cooled hind leg and the stressful nature of the procedure. However, the conditions employed for the experiment reproduced as closely as possible those used in production of localized paralysis in patients with hyperkalemic familial periodic paralysis. In the latter experiments, the forearm was swathed with ice bags for 30 minutes and paralysis was produced(3,4). In these experiments with rats, the changes in water and potassium concentration of the muscles demonstrated are analogous to those previouly posulated for the patient with hyperkalemic familial periodic paralysis. Thus, water enters the muscle cell, significantly increasing water content in relation to dry solids of muscle. Presumably, sodium accompanies the water since there is no change in sodium concentration per liter of intracellular water. Concentration of potassium per liter of intracellular water in muscles of the limb exposed to cold becomes significantly decreased. No measurements have been made of muscle water and potassium in patients with hyperkalemic familial periodic paralysis after exposure to cold. Under other circumstances, however, muscle specimens obtained at biopsy from these patients have been shown to contain an increased quantity of intracellular water and a depression of potassium concentration per liter of intracellular water(3,4).

Published reports of intracellular potassium changes during hypothermia have been conflicting. Spurr, Swan, and Taylor found no decrease in cardiac muscle potassium in the hypothermic animal and at times demonstrated actual increases in potassium concentration(5,6,7) but Taylor found decreases in skeletal muscle potassium although Renkin did not(7,8). On the other hand, Gollan reported decreases in concentration of potassium in both cardiac and skeletal muscles in hypothermic animals(9). Finally, Beavers has reported an increase of potassium concentration in cardiac muscles, but a decrease in skeletal muscle in animals undergoing hypothermia(10). He demonstrated an increased intracellular water concentration in both cardiac and skeletal muscles in these animals. All observers have agreed that plasma concentration of potassium is lowered during hypothermia (as it was with local hypothermia in patients with hyperkalemic familial periodic paralysis). Many of the differences have resulted from *in vitro* as opposed to *in vivo* experiments or immersion as opposed to perfusion chilling, etc. Our data on potassium and water content of skeletal muscle from a chilled leg of a rat show the same deviation from control values that Beavers found in animals made totally hypothermic. It cannot be stated whether the smaller changes seen in muscles of the opposite leg were produced by local hypothermia of this limb as well or by cooling elsewhere. It is also impossible to state whether the changes were a direct effect of cooling or were secondary to diminished blood supply and possible anoxia. Nevertheless, the results of this study are consistent with the hypothesis that local exposure of a leg to cold is associated with an increase in that limb in muscle intracellular water and a decrease in muscle potassium concentration without measurable change in sodium concentration. These findings are not inconsistent with the previous postulations concerning patients with hyperkalemic familial periodic paralysis. If these postulations are correct, it would be expected that the changes in such patients on exposure to cold would be of greater magnitude than those demonstrated in normal rats.

Since intracellular content of potassium in muscle decreases at the same time that extracellular potassium concentration falls (without demonstrable increase in extracellular volume) the potassium content of another tissue or compartment must increase with hypothermia. The two obvious areas to be considered first are the viscera, particularly the liver, and the bones. Although this question must still be investigated further, we think that the bones are not the most likely site of potassium gain, because in the previously described human experiments the measurements of A-V differences in the forearm

should have involved bones as well as soft tissues(4). Unfortunately, these data are capable of other interpretations. Attempts to demonstrate increased potassium content in rat liver have not been successful.

Summary. Measurements were made of muscle intracellular water, potassium and sodium concentrations in 2 groups of rats. The right hind limb of the experimental animals was immersed in a bath of acetone and ice, the right hind limb of the control animals in a bath of tepid water. Exposure of the leg to external cooling was associated with an increase in intracellular water in the muscles of that leg and a decrease in potassium concentration per liter of intracellular water. There was no difference in sodium concentration in the muscles from the chilled extremity from that of the control muscles.

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Chemotherapeutic Studies on Cutaneous Leishmaniosis. (26475)

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The majority of experimental studies on antileishmanial drugs were conducted on visceral leishmaniosis. The method most frequently used for testing chemotherapeutic agents is that recommended by van Dyke and Gellhorn(1) using hamsters infected intraperitoneally with *L. donovani.* Stauber *et al.*(2,3) recently described a method which permits rapid completion of the test by counting the number of organisms in liver impression smears 8 days after a heavy intracardial inoculation with *L. donovani.*

Being interested in the therapy of the cutaneous forms we utilized an infection to which the mouse is quite susceptible, obtained with a strain of *L. brasiliensis* from a clinical condition—leishmaniosis tegumentaria diffusa—described by Convit(4). In man, this infection attacks the entire skin surface and resembles lepromatous leprosy. The disease does not affect the viscera and mucous membranes, though it sometimes induces a slight infiltration around the lower nasal septum. The cutaneous lesion is formed "by a specific granuloma composed of large macrophages, which are practically full of parasites in their highly vaculated protoplasm"(5). Mayer, Convit and Pifano(6) have experimentally investigated the infection and induced lesions on the caudal zone of the back in mice, first in ulcerative, later in nodular form, with an impressive abundance of parasites. Pifano(7) in a geographical distribution study concludes that the American tegumentary leishmaniosis is formed by various sub-entities (espundia, uta, *úlcera de los chicleros*), one of them being represented by the leproid form considered here. Interestingly enough, the intradermal Montenegro test with phenolated suspensions of *L. brasiliensis*(8) remained negative only in the latter clinical manifestation (5,6).

The possibility to follow accurately the effect of the treatment by gross and microscopic observation of the mouse infected with