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Effect of Krebs-Ringer Medium on Muscle Contraction *In situ* in Adrenalectomized Albino Rats. (25566)

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Although it is well established that adrenal cortical insufficiency is accompanied by reduced ability for sustained muscle performance *in vivo*, underlying mechanisms are not clearly understood. Tensile(1,2), electrical (2), or biochemical(3) properties of resting muscle are apparently not sufficiently altered in adrenal insufficiency to be implicated in any major way as direct targets of corticoid action. Nevertheless, corticoids normally may act directly on contractile processes to a limited extent(4). Although weight of evidence (5,6,7,8,9) suggests that impaired muscle performance *in vivo* in adrenal insufficiency is related to well known accompanying circulatory and cardiovascular disturbances(10,11), it is not certain whether these contribute directly by acting on contractile processes or indirectly by interfering with circulatory adjustments normally occurring under physiological demands for sustained muscular activity. Whatever the underlying physiological mechanism, immediate cause of impaired muscle function may be consequential to inadequate transport of metabolic substances vital to sustained muscle activity or to inadequate removal of toxic metabolites. The immediate importance of circulatory factors is suggested by the work of Ramey, Goldstein, and Levine (5) who have shown that differences in work performance *in vivo* of intact and adrenalectomized rats fatigued by swimming are not reflected in diaphragm and oblique abdominal muscles isolated to nutrient medium. However, in these studies differences in muscle work performance prior to isolation were assumed and not demonstrated. In the present paper we have obtained quantitative data from *in situ* experiments designed to demonstrate more definitively the effect of nutrient medium on muscle contractile capacity in adrenalectomized rats.

Methods. Male Rockland-Sherman rats weighing approximately 200 g were bilaterally adrenalectomized or sham operated under

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ether anesthesia and maintained postoperatively on tap water and Purina Chow *ad lib.* Under sodium pentobarbital anaesthesia, the triceps brachii muscle was freed distally at insertion with minimal damage to nervous and vascular elements and attached *in situ,* via the tendon, to a muscle lever so that contractions could be recorded with muscle immersed in oxygenated Krebs-Ringer phosphate medium (pH 7.4, 200 mg % added glucose, temp. 24 $\pm$ 2°C) contained in a chamber between muscle and recording lever. Muscle contractions were recorded by the Everse-DeFremery method(12) with some modifications. The isolated muscle was after-loaded with 10 g weight and under constant tension stimulated tetanically by maximal faradic stimuli through copper electrodes implanted in its proximal end. Each record was obtained by procedures of intermittent stimulation previously described(12). Three separate records were obtained at 25 minute intervals for each test muscle: the first representing initial contraction; the second corresponding to period of immersion in nutrient medium; and the third corresponding to period after removal from medium. Time intervals employed between records were those wherein maximal effects of immersion or removal of muscle from the medium were first observed. For descriptive and quantitative purposes, it has been assumed that, following initial fatigue, reduction in peak amplitudes of the last 3 contraction curves observed typically in each record (Fig. 1-4) is an index of recovery of muscle contractile capacity. By relating average peak height of these 3 curves to that of initial peak contraction, a relative value of recovery (expressed as percent) has been obtained for each record. Values thus obtained are inherently more uniform, since variations in contraction heights resulting from differences in muscle mass, initial tension, and other variables inherent in the recording method are invariably reflected in amplitude of initial curve and are thus internally compensated. Finally blood hematocrit values and body weights were obtained for all animals immediately after obtaining the third record.

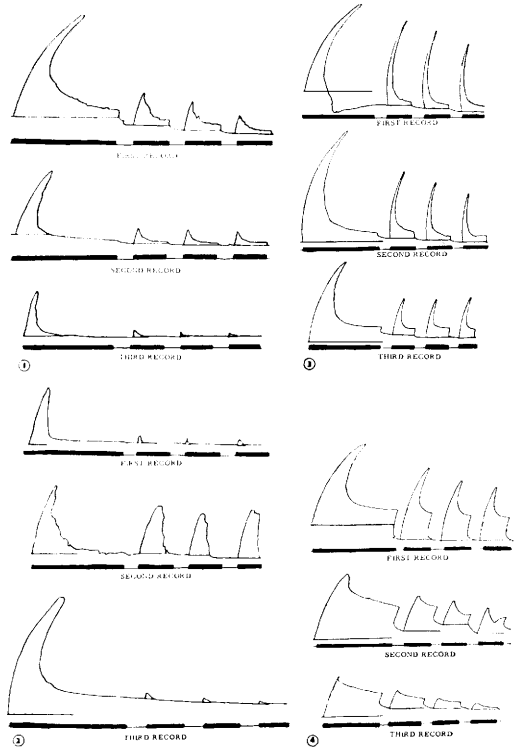

FIG. 1. Representative kymographic recordings of contractile capacity *in situ* of triceps brachii muscle in adrenalectomized rats obtained at 25 min. intervals without immersion of muscle in Krebs-Ringer medium.

FIG. 2. Same as Fig. 1 except that second record was obtained after immersion of test muscle for 25 min. in Krebs-Ringer medium and third record 25 min. after removal of bath.

FIG. 3. Representative kymographic recordings of contractile capacity *in situ* of triceps brachii muscle in sham operated rats obtained at 25 min. intervals without immersion of muscle in Krebs-Ringer medium.

FIG. 4. Same as Fig. 3 except that second record was obtained after immersion of test muscle for 25 min. in Krebs-Ringer medium and third record 25 min. after removal of the bath.

Results. Adrenalectomized Rats. Five rats bilaterally adrenalectomized 5 to 11 days previously and showing overt signs of moderate adrenal insufficiency were tested as described above without subjecting muscles to Krebs-Ringer medium. Fig. 1 shows representative records which demonstrate that recovery of muscle contraction is impaired following adrenalectomy. In 7 rats prepared as above, the muscle was immersed in Krebs-Ringer medium for 25 minutes immediately after completion of first record and prior to obtaining the second record. The medium was then removed, and the third record ob-

TABLE I. Recovery in Contractile Capacity of the *In Situ* Triceps Brachii Muscle Preparation of Intact and Adrenalectomized Male Albino Rats.

Procedure	Muscle treatment	No. of rats	No. of days postoperative	Mean change in body wt	Blood hematocrit	% recovery in muscle contraction		
						1st record	2nd record	3rd record
Sham operation	None	6	8	+35	40 ± 4*	63.8* ± 16.4	46.9 ± 7.7	43.2 ± 12.6
Idem	Krebs-Ringer	6	8	+27	44 ± 4	65.3 ± 18.7	46.0 ± 9.2	33.8 ± 9.4
Adrenalectomy	None	5	9	−29	51 ± 4	17.9 ± 12.9	11.8 ± 10.8	4.5 ± 5.5
Idem	Krebs-Ringer	7	7	−27	52 ± 3	19.2 ± 6.3	49.9 ± 10.1	3.9 ± 3.2
Adrenalectomy (no evident insufficiency)	None	2	9	+29	39	52.7	23.5	

* Mean ± S.D.

tained after 25 minutes. Fig. 2 shows that in adrenalectomized rats recovery of muscle contractile capacity is restored to normal following *in vitro* exposure of test muscle to Krebs-Ringer medium, but falls below pre-immersion levels upon subsequent removal of the medium.

Sham operated rats. Duplicate control experiments were performed with sham operated rats after postoperative recovery periods approximating those of the adrenalectomized group. Muscle contraction was recorded in 2 groups of 6 animals each. In one group muscles were not immersed in Krebs-Ringer medium; while in the second group, muscles were immersed prior to obtaining the second record. Time intervals employed between records in each case were the same as those described above. Fig. 3 shows that in absence of medium recovery of muscle contractile capacity is superior in sham operated rats to that observed in adrenalectomized rats under identical recording conditions (Fig. 1). Furthermore, exposure of the muscle to Krebs-Ringer medium for 25 minutes does not augment recovery of muscle contraction (Fig. 4).

Quantitative evaluation. Table I shows average muscle contraction recovery values for each of 3 records on all groups tested. From percent values of the second record, Krebs-Ringer medium had no apparent effect in sham operated rats. In contrast, adrenalectomized rats, compared with sham operated controls, showed an overall reduction of 40% (average of 3 records) in recovery of muscle contraction in absence of Krebs-Ringer medium with subsequent increase ($P<0.01$) in presence of medium to levels observed in second record of sham operated rats under similar conditions. Upon removal of the medium, recoveries declined to levels observed in third record of adrenalectomized untreated controls. Significantly, 2 adrenalectomized rats not showing overt signs of adrenal insufficiency were intermediate between sham operated rats and adrenalectomized rats with evidence of insufficiency. Reduction in muscle performance in complete insufficiency was accompanied characteristically by hemoconcentration and weight loss.

Discussion. Our demonstration that impaired muscle performance in adrenal insufficiency is restored to normal by immersion of isolated muscle in physiological medium strongly suggests, as responsible factors, impaired transport of ionic and/or metabolic factors or inadequate removal of toxic metabolites possibly as a consequence of impaired circulation. Wyman, Fulton, and Shulman (13) described extensive microcirculatory impairments in the cheek pouch of adrenalectomized hamsters. Further, it has been suggested(5,6) that impaired muscle performance in adrenal insufficiency may be, secondarily, a function of failure to make adequate neurocirculatory adjustments in time of physiological need. The effective role of circulation is further suggested by our observation that extensive chance circulatory damage to the triceps of sham operated rats prior to recording contraction invariably resulted in reduced performance which was restored by immersion of muscle in physiological medium. Alternatively, alterations in circulatory levels of ionic and/or metabolic factors necessary for efficient muscle contraction might be implicated. Hemoconcentration resulting from fluid loss implies ionic concentration and associated osmotic imbalance. Coupled with this are primary disturbances in serum electrolyte and carbohydrate levels. The latter do not appear to be important factors contributing to muscle fatigue of adrenal insufficiency, since it has been demonstrated that salt treated animals are incapable of sustained muscle activity despite normal glucose and serum electrolyte levels(14,15). In our experiments, however, hypoglycemia and serum electrolyte imbalance may be additional complicating factors, since adrenalectomized rats were not maintained on salt. Our limited preliminary observations on the role of metabolic factors indicate that omission of glucose and oxygen from Krebs-Ringer medium does not significantly impair its effect on increasing muscle performance in adrenal insufficiency.

Ingle(16) and Ingle, Hales, and Haslerud (17) demonstrated that, despite absence of overt signs of insufficiency, partial adrenalectomy reduces performance of muscle made to work over relatively long periods of time. Our present findings show that, presumably under similar conditions of insufficiency, muscle performance is likewise reduced although under relatively shorter periods of contraction.

Summary. Experiments were performed to evaluate quantitatively the effect of Krebs-Ringer medium on contractile capacity of muscle from adrenalectomized albino rats. The triceps brachii was used as test muscle and contraction recorded *in situ* using the method of Everse and DeFremery. Recovery in contractile capacity subsequent to initial fatiguing of test muscle was expressed as percent of initial contraction height. An overall reduction of 40% in recovery of contractile capacity was observed following adrenalectomy with accompanying hemoconcentration and body weight loss. Following immersion of test muscle in Krebs-Ringer-glucose-phosphate medium for 25 minutes, recovery was restored to levels observed in sham operated controls under similar conditions, but declined to control levels 25 minutes after removal of the nutrient bath. Possible underlying mechanisms are discussed.

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