9. Wilson, R. E., Hager, E. B., Hampers, C. L., Corson, J. M., Merrill, J. P., and Murray, J. E., New Engl. J. Med. 278, 479 (1968).

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Plasma Corticosterone and Contractility of Glycerinated White Muscle Fibers in the Rat* (33794)

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Insufficient or excessive amounts of circulating adrenal steroid hormones can cause skeletal muscle weakness (1). Excessive glucocorticoids increase gluconeogenesis and the resulting depletion of muscle protein has been considered to be the cause of muscle weakness in hyperfunction of the adrenal cortex (2, 3). The precise mechanism of skeletal muscle weakness in hypofunction of the adrenal cortex is not known, but it is associated with sodium depletion (4) and may represent changes in microsomal activity of the muscle cells (5). This laboratory has previously demonstrated that the contractility of glycerinated muscle fibers from adrenalectomized rats is significantly decreased (4). No information is available, however, relating increased adrenal cortical activity to glycerinated muscle contractility. This report attempts to provide such information. Procedures were adopted which subjected rats to stress and thus achieved adrenal hyperfunction as measured by plasma corticosterone levels. The data relating glycerinated muscle contractility to adrenal hypofunction have been previously published (4). They are included in the present report so that a comparison may be made of the contractility of glycerinated skeletal muscle during adrenal hyperfunction and hypofunction.

Materials and Methods. Sixty-eight male Holtzman rats weighing 150–250 g were divided into four groups, housed in pairs, and maintained on Purina lab chow and water *ad libitum*. The first group was anesthetized with ether, subjected to bilateral adrenalectomy, maintained without replacement therapy for 6 days, and sacrificed. The control group received a sham-operation with bilateral penetration into the peritoneum and was maintained for 6 days before being sacrificed. The third group was subjected to ether anesthesia stress to elevate the naturally occurring plasma corticosterone levels. Light ether anesthesia was administered for 0.5 hr daily for 6 consecutive days before sacrifice. The final group was subjected to a severe stress to drive corticosterone levels as high as possible. A horizontal wheel 4 ft in diameter was constructed with four narrow tubular wire cages placed equidistant around the periphery. An electric motor provided power to rotate the wheel. By varying the angular velocity of the wheel and the distance of the cages from the axis of rotation, a controlled angular *g* force could be administered. These animals were subjected to 10*g* for 0.5 hr daily for 6 consecutive days before sacrifice.

Samples of tail blood were drawn for determination of plasma corticosterone 0.5 hr after stress administration on the third and the sixth or terminal day. Sampling was carried out between 1:30 and 2:30 p.m. to minimize diurnal variations of corticosterone levels. Corticosterone was determined fluorometrically by the method of Glick *et al.* (6) using a Turner fluorometer model 110 with a 47-B primary and a 2A-12 secondary filter. Immediately after drawing the final blood sample the rats were killed with ether and the su-

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perficial portion of the medial head of the gastrocnemius muscle was removed to a 50% glycerol solution for extraction in the cold. Extraction was carried out for 3 days at 4° with daily change of glycerol and then for an average of 76 days at —18°. Embry and Briggs (7) have shown about 84 days of extraction are necessary for dog cardiac fibers to develop maximum tension. The methods and apparatus used to determine muscle fiber tensions have been published (4, 8). Briefly, they consist of teasing the extracted muscle into bundles of 2–6 fibers, cementing the ends to aluminum discs, attaching them to a rigid muscle holder and a Grass FT 0.03 C transducer, and submerging the mounted bundles in a phosphate buffer consisting of 30 m*M* KH_2PO_4, 50 m*M* KCl, and 5 m*M* $MgCl_2$, with a total ionic strength of 0.11 and a pH of 7.45 at 27°. Isometric tension is developed by adding to the buffer adenosine-5′-triphosphate (ATP) made up in the buffer to a final concentration of 0.4 m*M* ATP at pH 7.20. Output of the transducer is amplified and recorded on a Grass model-5 polygraph.

Results and Discussion. Results of the study are presented in Table I. The corticosterone values are means of the levels of the sixth day blood samples. They were essentially the same as the third day samples. Success of the methods employed to alter plasma corticosterone can be seen from the fact that adrenalectomy decreased plasma corticosterone levels to about one-fifth the control value while ether stress increased levels by about one-third and centrifuge stress increased levels about threefold. These changes from control values were all highly significant ($p = <.001$). Isometric tension decreased significantly in muscles from adrenalectomized rats and from those subjected to a high degree of stress. It is interesting that an increase of more than 30% in plasma corticosterone seemed to have no effect upon skeletal muscle contractility. The slight increase in measured isometric tension was not significant. Such refractoriness could be important to the animal in view of the large release of ACTH, and hence corticosterone, following a variety of systemic stresses (9). How long a time period might be required for a chronically maintained 30% increase in plasma corticosterone to cause a change in contractility is not apparent from this study.

It is evident, from the data obtained, that muscle contractility changes associated with an increase or a decrease in plasma corticosterone must occur at the level of the contractile proteins. The nature of the glycerinated muscle preparation rules out the influence of circulation, neuromuscular transmission, excitation–contraction coupling, or membrane phenomena. As pointed out in the introduction, skeletal muscle weakness is known to occur in both hyperfunction and hypofunction of the adrenal cortex. This study has demonstrated that this relationship applies to glycerol-extracted fibers as well as *in vivo* ones and provides information regarding the magnitude of corticosterone change necessary to bring about tension change in rat muscle.

Summary. Isometric tension developed by glycerol-extracted medial gastrocnemius muscle was measured in rats subjected to procedures designed to alter the level of their

TABLE I. Isometric Tension of Glycerol-Extracted Gastrocnemius Fibers and Plasma Corticosterone Levels of 68 Male Rats.[a]

Condition	*N*	Mean isometric tension (g/mm² ± SE)	*p*	Mean plasma corticosterone (μg/100 ml ± SE)	*p*
Adrenalectomized	15	13.6 ± 1.19	<.001	5.2 ± 0.63	<.001
Control	16	24.0 ± 1.84		27.7 ± 3.26	
Ether stressed	22	26.9 ± 2.16	<.40	39.1 ± 2.05	<.001
Centrifuge stressed	15	13.8 ± 2.07	<.001	93.9 ± 3.39	<.001

[a] All *p* values are compared with control animals.

naturally occurring plasma corticosterone. Glycerol-extracted fibers from rats whose plasma corticosterone has been significantly decreased or significantly increased demonstrated a decreased contractility. This change seemed to be at the level of the contractile proteins.

1. Frawley, T. B., *in* "The Adrenal Cortex" (A. B. Eisenstein, ed.), p. 474. Little, Brown, Boston, Massachusetts (1967).

2. Kochakian, C. D. and Robertson, E., J. Biol. Chem. **190**, 495 (1951).

3. Forsham, P. H., *in* "Endocrinology" (R. H. Williams, ed.), p. 343. Saunders, Philadelphia, Pennsylvania (1968).

4. Sexton, A. W., Am. J. Physiol. **212**, 313 (1967).

5. Katz, A. M., Repke, D. F., and Cohen, B. R., Circulation Res. **19**, 1062 (1966).

6. Glick, D., Von Redlich, D., and Levine, S., Endocrinology **74**, 653 (1964).

7. Embry, R. and Briggs, A. H., Am. J. Physiol. **210**, 826 (1966).

8. Sexton, A. W. and Gersten, J. W., Science **157**, 199 (1967).

9. Scharrer, E. and Scharrer, B., "Neuroendocrinology," p. 170. Columbia Univ. Press, New York (1963).

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Normal Manganese Turnover in Wilson's Disease (33795)

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Similarities in the clinical presentation of manganese poisoning and Wilson's disease and in transport of manganese and copper suggested a study of manganese turnover in patients with Wilson's disease.

Materials and Methods. Two patients with well-established Wilson's disease and three control subjects with impairment due either to spinal cord injury, dystonia, or surgically treated cerebral arteriovenous malformation were each given intravenously 1 μCi of carrier-free ^{54}Mn.[1] Characteristics of the nuclide include a physical half-life of 303 days and a prominent gamma emission at 0.83 MeV. The isotope was injected intravenously as $^{54}MnCl_2$ in saline solution. Oral D-penicillamine treatment of the patients with Wilson's disease was discontinued for 4 weeks beginning on the day prior to injection of the $^{54}MnCl_2$; and on day 28 after Mn injection, oral D-penicillamine was resumed in the patients with Wilson's disease. All subjects were males of similar ages and weights.

Iso/Serve, Inc., Division of Cambridge Nuclear Corporation, 133 Portland Street, Cambridge, Massachusetts 02139.

Total body gamma radiation counts were obtained in a total body counter using a 4 $\times$ 8 in. sodium iodide crystal suspended 18 in. above the subject's trunk in a 4 $\times$ 6 $\times$ 6 ft (120 $\times$ 180 $\times$ 180 cm) counting chamber shielded with 6 in. steel walls. The subject is counted in a partially sitting position. Only those counts in the region of the 0.83 MeV photopeak of ^{54}Mn were utilized.

A control total body count was obtained prior to intravenous injection of the isotope. Subsequent counts were done at 30 min, 24 and 48 hr after the $^{54}MnCl_2$ was administered. Further total body counts were done at appropriate intervals, as indicated in Table I, up to 78 days after the isotope was administered.

Results. The mean effective half-time for the two Wilson's disease cases was approximately 43 days and for the control group approximately 37 days. In Fig. 1, the curve illustrates the average rate of falloff of total body count in each of the two groups of patients. Table I indicates falloff in count of each of the five subjects.

Discussion. Chronic manganese poisoning