Electrolyte, Water and Glycogen in Rat Muscle Following Ligation of Major Arteries and Veins.* (17713)

FREDERICK A. FUHRMAN AND J. M. CRISMON.

From the Department of Physiology, Stanford University School of Medicine, Stanford, Calif.

In order to elucidate the nature of the physiological changes preceding irreversible damage produced by ischemia we have investigated the electrolyte and glycogen content of muscle following frostbite(1), at various periods after the removal of limb tourniquets(2,3) and after vascular occlusion produced by the intra-arterial injection of glass microspheres(4,5). For comparison with these results we have also determined the electrolyte, glycogen and water content of rat muscle following ligation of the major vessels of the hind leg. The latter method does not produce marked alteration in electrolyte or water content of the muscle. Neither gangrene nor permanent functional changes have been observed in the rat. The chemical changes following severe ischemia are dependent upon the duration of the ischemia(2,3). Failure of their appearance after vessel ligation furnishes evidence that in the rat adequate collateral circulation is established in about 3½ hours.

Methods. Adult albino rats of the Slonaker-Wistar strain, weighing 150 to 300 g were anesthetized with pentobarbital sodium. The desired vessels were then ligated using the aseptic precautions usually observed in rat surgery(6). After 3 or 4 days the animals were again anesthetized with pentobarbital, 3 to 5 ml of blood were withdrawn from the abdominal aorta, and the anterior tibial and gastrocnemius muscles of both legs were removed for chemical determinations. The muscle was dried to constant weight at 110°C. The dried muscles from the same leg were sometimes combined before ashing. Sodium and potassium were determined by means of the flame photometer (Perkin-Elmer Co., Model 18) on solutions of muscle ash from which the phosphate had been removed(7). Glycogen was determined by the method of Good, Kramer and Somogyi(8) with the modifications suggested by Deane *et al.*(9). Skin temperature of the feet was measured by means of a thermocouple as previously described(10).

Results. 1. *Ligation of the femoral artery.* In 5 animals the left femoral artery was ligated a few mm proximal to the superficial circumflex iliac artery 4 days before the animals were killed. The results of the chemical analyses on 4 animals are presented in Table I. A latex mass was injected into the abdominal aorta of the fifth animal. The data show that the mean water, sodium and potassium concentrations are approximately the same in both legs. The glycogen content of the gastrocnemius was greatly reduced in 2 animals, but only slightly reduced in the other 2. The foot temperatures were not significantly different in the 2 legs. All 5 animals had normal use of the leg in which the femoral artery had been ligated. In one animal sodium fluorescein was injected into the tail vein. Observation of its distribution

* Supported by grants from the John and Mary R. Markle Foundation and the Fluid Research Fund of the Stanford University School of Medicine.

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TABLE I.

Skin Temperature and Chemical Analyses of Muscle Following Ligation of the Major Arteries and Veins of the Hind Leg of the Rat.

I. Left femoral artery ligated.
II. Left common iliac artery and vein ligated.
III. Left femoral, saphenous, and popliteal arteries and veins ligated.
All constituents are per 100 g dry tissue.

	I		II		III	
	Right	Left	Right	Left	Right	Left
Water (g):						
Gastrocnemius	306.3	308.3	303.8	306.1	305.4	326.1
Anterior tibial	299.2	299.5	294.2	296.8	295.3	346.4
Sodium (mEq):						
Gastrocnemius	3.8*	4.1*	8.6	9.3	8.8	11.7
Anterior tibial	4.5*	4.9*				
Potassium (mEq):						
Gastrocnemius	25.7*	23.7*	37.7	37.2	42.2	43.8
Anterior tibial	25.1*	25.5*				
Glycogen (g):						
Gastrocnemius	1.69	1.25	1.14	1.72		
No. of animals	4		3		2	
Skin Temp. °C						
Foot: dorsal	30.6	30.1	30.1	29.7		
plantar	31.2	30.4	31.0	30.3		
No. of animals	4		6			

* Determined without phosphate precipitation (7) and therefore low.

under ultraviolet illumination showed that it appeared at the same time in both legs and was of about the same brilliance.

2. *Ligation of the common iliac artery and vein.* In 6 animals the common iliac artery and its accompanying vein were ligated proximal to the hypogastric artery. Several of these animals showed a temporary partial loss of function of the left leg on the first day after ligation, but on the fourth day after ligation all animals had normal use of the leg. The fluorescein test was performed in 3 animals, at 1, 2 and 4 days after ligation. During the first 2 minutes after injection, the left leg was slightly less fluorescent than the right in all 3 animals. Reduced skin temperatures were found in the left feet of 2 animals on the first day after ligation. In one animal the left foot was 3°C, in the other animal 7°C cooler than the right foot. No differences in skin temperature between the 2 feet were observed on the second or subsequent days after ligation. A similar transient fall in skin temperature has been reported in the dog following ligation of the external iliac artery (11) or the femoral artery proximal to the deep femoral branch (12). Three animals were killed after 4 days for chemical analyses of the tissues. The results are given in Table I. As in Series I there was little difference in water, sodium or potassium concentrations in the 2 legs. In contrast to the animals in which the femoral artery was ligated, in this series the left gastrocnemius contained slightly more glycogen than did the right. The foot temperatures of the right and left feet in the 6 animals in this series and the 4 in Series I were combined for statistical analysis. The method of paired differences showed that the temperatures of the right and left feet were not significantly different 4 days after ligation, ($P<0.4$ for dorsal aspect and <0.2 for the plantar).

3. *Ligation of femoral, popliteal and saphenous arteries and veins.* In 4 animals in which the left femoral, popliteal and saphenous arteries and their accompanying veins were ligated in the popliteal fossa, no functional changes were observed. After 3 days chemical analyses were made on the muscles of 2 animals, and 2 were injected with latex for study of collateral circulation. The in-

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crease in sodium and water content of the gastrocnemius indicated in Table I is the result of change in only one of the animals. In this animal the extra sodium in the ligated leg amounted to 6.0 mEq and the extra water to 34.7 g per 100 g dry tissue. Since the plasma sodium concentration was 148.5 mEq per liter, 5.15 mEq of Na, or 86% of that found, could be accounted for on the basis of the presence of edema fluid containing sodium at the same concentration as that of plasma. Since this is probably within the limits of error of the methods used there is no evidence for the accumulation of sodium in excess of water.

Discussion. Workers disagree as to the extent of gangrene following ligation of large arteries in the hind leg of the rabbit(13,14, 15,16); following ligation of the common iliac artery gangrene has been reported to result in 10%(15) to 71.5%(13) of the animals. In the dog gangrene was not produced by ligation of the external iliac artery, or of the artery and vein(11). To produce it consistently in the dog Murray *et al.*(17) found that it was necessary to ligate not only the external iliac artery but also 12 other arteries supplying the muscles of the hind leg. In the rabbit when gangrene did occur it appeared the second day after ligation(13). In the experiments reported here the rats were only observed for 4 days, but it would seem that gangrene would have been observed within that time if it were to develop. It has been shown previously that a wide variety of severe injuries, produced by frostbite(1), anoxia (18), burning(19) or tourniquets(19,2), result in the accumulation in the injured tissues of sodium in excess of water. This phenomenon appears to be a non-specific event. It is apparent from the table that in the rat extensive sodium accumulation, of the type previously reported to occur after other injuries, did not result from the ischemia produced by ligation of the major arteries of the limb. Since neither gangrene nor permanent functional impairment occurred it is apparent that collateral circulation in this species is sufficient to maintain the integrity of the leg muscles after loss of their major blood supply.

Summary. Ligation of the femoral artery (Series I), the common iliac artery and vein (Series II) or the femoral, popliteal and saphenous arteries and veins (Series III) in albino rats resulted in only slight alteration in the sodium, potassium and water content of the gastrocnemius and anterior tibial muscles 4 days after occlusion. Muscle glycogen was reduced in 2 out of 4 animals in Series I. Edema occurred in 1 animal in Series III, but the extra sodium found in the muscle could be accounted for by the increase in extracellular fluid. No gangrene or permanent functional changes were observed.

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Received January 31, 1950. P.S.E.B.M., 1950, v73.