animals were shaved with electric clippers from the waist down and immobilized on a board according to a previously described technique[2]. Then they were placed vertically (hind feet down) for 30 min in a tank filled with an ice-water mixture which reached just to the waist.

Immediately after freezing the rats their hind legs were stiff (as if in a rigor mortis) and pale, while during the next few hours they became flaccid and somewhat cyanotic. On the following day, the cyanosis of the skin disappeared but the hind legs remained completely or almost completely paralyzed in all animals. This paralysis persisted, and during the next week an acute involution of the musculature ensued so that the contours of the long bones became easily visible through the skin. Upon autopsy on the 12th day, the involuted muscles exhibited a light 'fish flesh' type of discoloration which, upon histologic examination, proved to be the result of an intense generalized lysis of striated muscle fibers associated with proliferation of the sarcolemma cells and of the surrounding connective tissue (Figure). The vessels, nerves and skin showed no evident change. Similar cold exposure or aorta ligature alone produced no comparable muscle lesions.

Apparently, ligature of the aorta produces only a latent vascular deficiency which becomes manifest, however, upon exposure to cold. The procedure is thus suitable for the consistent production of selective striated muscle lesions in a predetermined territory. It should lend itself especially to biochemical studies on myolysis for which it is important that a large muscle mass of predetermined size be affected simultaneously.

Zusammenfassung. Nach vollkommener Ligatur der Aorta knapp unterhalb der Nierenarterien gelingt es bei der Ratte, durch Abkühlung der Hinterbeine regelmässig eine streng auf die kaudale Körperhälfte beschränkte Dystrophie der Skelettmuskulatur hervorzurufen.

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[2] H. SELYE, *The Pluricausal Cardiopathics* (Charles C. Thomas Publ., Springfield 1961).

The Effect of Limb Ischaemia on the Serum Glycerol Concentration of the Rat

An increase in the concentration of non-esterified fatty acids (NEFA) in the blood usually indicates a greater rate of mobilization from the fat stores. Changes in the NEFA concentration of plasma during and after 4 h bilateral hind limb ischaemia in rats have been investigated by STONER[1]. The general response was the same whether the samples were obtained by decapitation or under nembutal anaesthesia but the actual concentrations depended on the sampling procedure. The plasma NEFA concentration rose whilst the tourniquets were in place and remained high after release. There was little difference between control and experimental values 3 h after release, owing to a rise in the NEFA concentration of control animals during the afternoon. STONER[1] also showed that the increase was dependent on the presence of an intact sympathetic nervous system.

In the case of rats after tourniquet release, an increase in the plasma NEFA concentration might reflect not only a greater rate of release from the depots, but also impaired utilization of NEFA. Changes in the rate of fat mobilization might be followed more easily by measuring the concentration of circulating glycerol[2,3].

Male albino rats of the Porton strain (body weight 223 g ± 22 S.D.) fed on M.R.C. diet 41B were used. Bilateral hind limb ischaemia was produced by rubber tourniquets applied under ether anaesthesia[4]. Each experimental animal was paired with a control which was killed at the same time. Control animals were also anaesthetized to compensate for any effects of ether. Only one such pair was studied at any particular time on any day. Glycerol was estimated enzymatically[5] on filtrates of serum deproteinized with barium hydroxide and zinc sulphate[6].

The serum glycerol concentrations varied widely under all conditions (Table). The process of deproteinizing contributes to this variation, as the unavoidable dilution involved can bring the concentrations of the weaker samples close to the limit of reliability of the method (about 5 μM).

A chi-square test showed that the concentrations in the whole group of injured rats were significantly greater ($P < 0.01$) than in the controls from noon onwards, but comparison of the means ('t'-test) at specific times showed a significant difference between the control and injured rats only at 3 p.m.

The effect of hind-limb ischaemia on the concentration of glycerol in serum. Bilateral hind-limb tourniquets were applied during a 3 min period of ether anaesthesia at 10 a.m. Control animals were similarly anaesthetized with the exception of the 10 a.m. group. Blood samples were obtained by decapitation at the times indicated. Tourniquets were removed at 2.0 p.m. in the experimental groups killed at 3 and 5 p.m. Glycerol concentrations μM/l serum ± S.E.M. Number of rats shown in parenthesis

Time	Control	Experimental
10.00 a.m.	162 ± 17 (8)	–
10.10 a.m.	124 ± 17 (7)	–
12.00 a.m.	154 ± 31 (6)	188 ± 17 (6)
2.00 p.m.	113 ± 8 (6)	138 ± 16 (6)
3.00 p.m.	112 ± 10 (6)	182 ± 11 (6)[a]
5.00 p.m.	137 ± 7 (7)	169 ± 18 (7)

[a] $P < 0.001$.

[1] H. B. STONER, Brit. J. exp. Path. *43*, 556 (1962).
[2] M. VAUGHAN, J. biol. Chem. *237*, 3354 (1962).
[3] L. A. CARLSON and L. ORO, Metab. clin. Exp. *12*, 132 (1963).
[4] H. B. STONER, Brit. J. exp. Path. *39*, 251 (1958).
[5] P. B. GARLAND and P. J. RANDLE, Nature *196*, 987 (1962).
[6] N. NELSON, J. biol. Chem. *153*, 375 (1944).

While both products of lipolysis increase in the blood after the injury the time courses of the changes in glycerol and NEFA concentrations do not run parallel. The NEFA concentration is maximal at the time of release of the tourniquets and shortly afterwards, whereas the glycerol concentration reaches its peak about 1 h later. This could be explained by the different volumes of distribution of the two products of lipolysis, but the possibility remains that injury affects the subsequent metabolism of one more than the other.

The fat stores may not be the only source of blood glycerol and NEFA in injured rats. In rabbits, the total lipid content decreases in the muscles below the tourniquets[7], and the release of material from the injured muscles could have complicated the interpretation of the present experiments[8].

Considering these experiments with those on plasma NEFA, it is clear that both the glycerol and NEFA concentrations are high within 1 h of releasing the tourniquets, suggesting that fat mobilization is increased. Further evidence is necessary to confirm this point, and experiments are being undertaken to search for better indices of fat mobilization[9].

Résumé. Le sérum des rats présente une concentration élevée de glycérine après l'application de garrots aux membres postérieurs pendant quatre heures. L'auteur compare ses résultats avec ceux des recherches dans lesquelles les acides gras non-estérifiés ont été soumis à des mesures quantitatives.

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June 23, 1965.

[7] I. B. FRIDLYAND, Uch. Zap. 2 Moskov. Med. Inst. *17*, 79 (1958); Chem. Abstr. *55*, 3767b (1961).
[8] S. R. JOHNSON and L. B. WADSTROM, Scand. J. clin. lab. Invest. *8*, 323 (1956).
[9] I wish to thank Dr. H. B. STONER for his helpful criticism and interest in this work.
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Reticulospinal Inhibition of Transmission through Interneurones of Spinal Reflex Pathways

It has been shown that many reflexes are facilitated from the corticospinal tract and from the rubrospinal tract[1]. This facilitation is achieved by excitatory action on the interneurones of these reflex pathways. Descending inhibition of reflex transmission can be very profound and is tonically active in the decerebrate state and is also found after electrical stimulation of the brain stem[2,3]. The mechanism by which the reflex pathways are inhibited has been difficult to analyse in that it could be exerted either at a primary afferent or at an interneuronal level. Indirect evidence suggests that the tonic decerebrate inhibition is interneuronal[2,4], although it is known that a large primary afferent depolarization (PAD) can be evoked from the brain stem[5]. In order to investigate this problem further, it is necessary to employ electrical stimulation of the brain stem at a strength that does not evoke a PAD. In experiments of this type, it has been shown that transmission to primary afferents can be inhibited at an interneuronal level through pathways descending from the reticular formation in the ventral part of the spinal cord[6].

The tonic descending inhibition in the decerebrate state is mediated via dorsal spinal pathways[7]. The present experiments were made on decerebrate cats with the spinal cord transected except for the dorsal quadrant contralateral to the side tested in the lumbosacral region (see diagram in Figure 1). Electrical stimulation in the ventromedial part of the medullary reticular formation gives an effective depression of the synaptic actions evoked in flexor and extensor motoneurones by volleys in the flexor reflex afferents (FRA). This is shown in Figure 1 for a motoneurone belonging to the ankle-extensor, gastrocne-

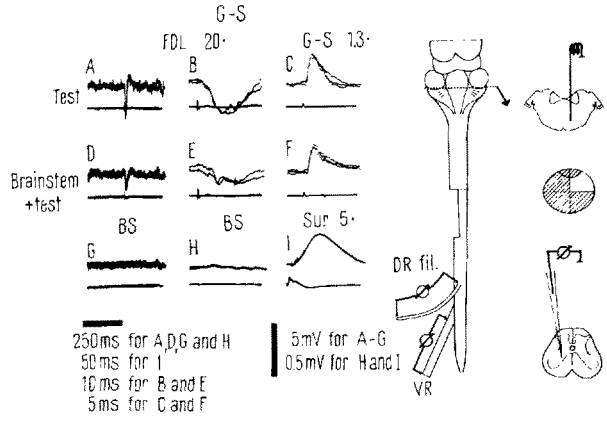

Fig. 1. Inhibition from the reticular formation of transmission in the inhibitory pathway from the flexor reflex afferents (FRA) to an extensor motoneurone. Experiment on a decerebate, decerebellate cat with the spinal cord transected except for the dorsal part of the lateral funicle contralateral to the side of recording (see drawings). The upper traces in A–G are intracellular (citrate electrode) recordings from a gastrocnemius-soleus (G-S) motoneurone. The upper traces in H and I are from the most caudal dorsal rootlet in L6. The lower traces in all records are from the dorsal root entry zone in L7. A and B, taken simultaneously at different sweep speeds, show the IPSP evoked from high threshold muscle afferents in the flexor digitorum longus (FDL) nerve. In the corresponding records D and E the IPSP from FDL is depressed by a conditioning stimulation of the brain stem reticular formation (150/sec for 300 msec at the site shown in the right upper drawing). This stimulation of the brain stem has no effect on the Ia EPSP (test alone in C and conditioned in F). G shows that brain stem stimulation alone does not evoke any postsynaptic potential in the motoneurone. H illustrates that this stimulation of the brain stem does not evoke any dorsal root potential (DRP); for comparison the DRP from sural (Sur) is shown in I.