

Time after stimulation	No. of rats	Dry weight (mg)		No. of rats	TN (mg N)		No. of rats	Non collagen PN (mg N)		Significant to*
		Control	Stimulation		Control	Stimulation		Control	Stimulation	
Immediately	6	72.3 ± 1.7	70.9 ± 2.2	6	10.61 ± 0.25	10.52 ± 0.12	7	9.45 ± 0.28	9.37 ± 0.26	P > 0.5
4 h	9	65.0 ± 2.6	67.7 ± 1.9	9	8.30 ± 0.16	8.65 ± 0.14	8	9.04 ± 0.18	9.51 ± 0.27	P > 0.01
8 h	6	63.8 ± 1.5	64.8 ± 2.3	8	9.36 ± 0.85	9.40 ± 0.85	—	—	—	P > 0.5

* The per cent difference between the muscles of the right and left side of the normal animal are compared with the per cent difference between the muscles of stimulated and control side.

proteins and also a change in the quantitative relations of the known proteins⁴. Moreover contractin, a protein not known in the state of rest, appears⁵.

A change in the turnover of proteins after functional activity is indicated by the observation of increased proteolytic activity after stimulation of the muscle⁶. There is also an increase of amino acids in the muscle of the hind limbs of rats after running in the tread-mill⁷, and finally an increase in the arterio-venous difference of non-protein nitrogen in the muscle of the hind limb of the cat after faradic stimulation of the sciatic nerve⁸.

In this paper we studied the changes in the absolute amounts of muscle proteins after direct stimulation of the m. tibialis anticus of rats. The muscle was stimulated by galvanic pulses at a frequency of 300 impulses/min for 6 min. To exclude the plasma proteins, which are increased after stimulation, due to increased blood flow through the muscle, the extremities of the stimulated and the control side were perfused by Tyrode solution (37°C, oxygenated and containing 100 mg% of glucose). The time of perfusion, i.e. until clear perfusion fluid was obtained, was 15–20 min. Immediately, 4 and 8 h after stimulation the muscle of the stimulated and of the control side were excised and their absolute dry weight and total nitrogen content was determined. The amount of non collagen proteins was determined immediately and 4 h after stimulation with the 'Biuret method' of GORNALL *et al.*⁹.

There are no significant changes immediately after stimulation. However 4 h after stimulation there is an increase of dry weight by 4.1% and of total nitrogen by 4.2%.

The changes in dry weight and total nitrogen can be masked by the changes in non-protein nitrogen which in the experiments reported above increases 4 h after stimulation by 40%.

Using the Biuret method, the absolute amount of non-collagen proteins 4 h after stimulation of the muscle was therefore determined. The homogenized muscle was extracted with 0.1 N NaOH and the proteins were determined in the extract, using the Biuret-method⁹, the accuracy of the method being 0.05 mg N. The values are expressed as milligrams of KJELDAHL's nitrogen, as the method had been calibrated with the standard Kjeldahl method. By this method, specific for proteins, an increase of 5.2% of non collagenous proteins was observed.

The changes are therefore more manifest after elimination of metabolically inert¹⁰ collagen.

This increase in the amount of proteins after stimulation of muscle is only a transient one. 8 h after stimulation of the muscle there is no longer any difference between the muscles of the stimulated and the control side.

We have therefore to deal with an overshoot reaction of similar character described in the metabolism of glycid¹¹ and ions¹². It appears therefore that these overshoot reactions, following functional activity of muscle are significant for the metabolic changes taking place after training of the organism.

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Zusammenfassung

Nach direkter Reizung des Muskels von Ratten mit einer Frequenz von 300 Impulsen/min wurde folgendes beobachtet:

- Direkt nach Muskelreizung kommt es zu keiner statistisch signifikanten Erniedrigung von Trockengewicht, Gesamtstickstoff und Nichtkollagen-Proteinen des Muskels.
- 4 h nach Muskelreizung erfolgt eine Erhöhung des Trockengewichts und des Gesamtstickstoffs um 4,2%, der Nichtkollagen-Proteine um 5,2%.
- 8 h nach Muskelreizung sind die Normalwerte wiederum erreicht.

¹⁰ A. NEUBERGER and J. C. PERRONE, *Biochem. J.* 49, 199 (1951).

¹¹ L. S. JAMPOLSKAJA, *Fisiol. Zhur. U.S.S.R.* 37, 110 (1951). – E. GUTMANN, Z. VODÍČKA and G. VRBOVÁ, *Physiol. Bohemoslov.* 3, 182 (1954).

¹² Z. DRAHOTA, personal communication.

Serum Phosphoglucomutase Activity in Human Virus Hepatitis

We have investigated the phosphoglucomutase activity in human serum (from normal individuals and from cases of epidemic hepatitis). Phosphoglucomutase activity, as determined according to NAJJAR's method¹ in normal human sera (18 cases) has been found very poor

¹ V. A. NAJJAR, *J. biol. Chem.* 175, 281 (1948).

⁴ M. DUBUISSON, *Exper.* 2, 258 (1946). – M. DUBUISSON, *Biochim. biophys. Acta* 5, 489 (1950).

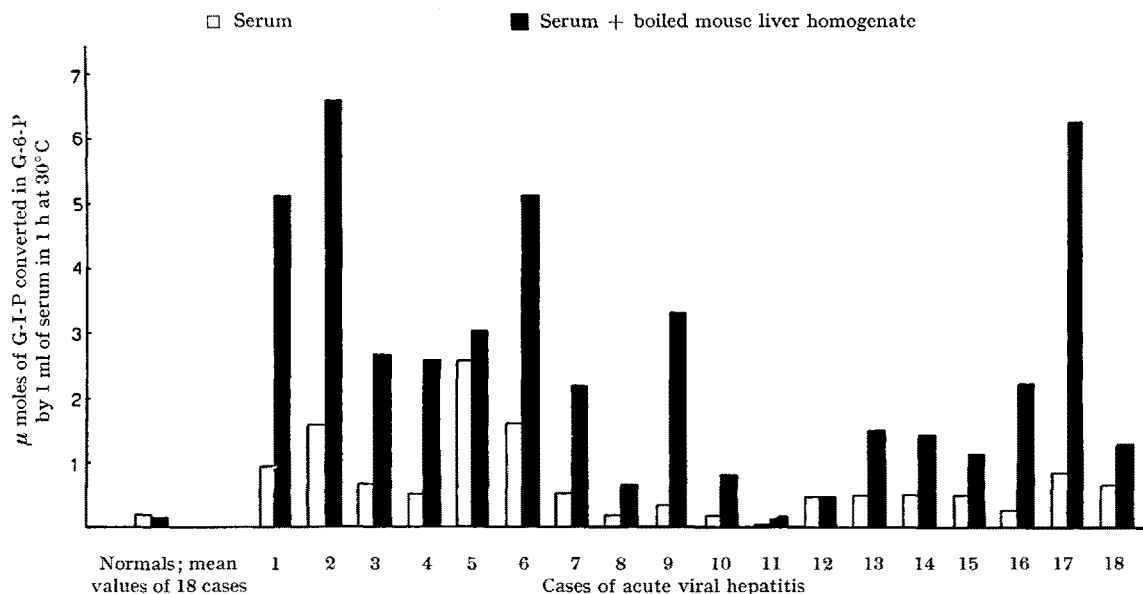
⁵ M. DUBUISSON, *Nature* 166, 1116 (1950).

⁶ N. N. JAKOVLEV, *Fisiol. Zhur. U.S.S.R.* 34, 717 (1948).

⁷ J. W. WILLIAMS, P. E. SCHUR, and C. A. ELVEHJEM, *J. biol. Chem.* 182, 55 (1950).

⁸ N. K. POPOVA, *Fisiol. Zhur. U.S.S.R.* 37, 103 (1951).

⁹ A. G. GORNALL, CH. J. BARDAWILL, and M. M. DAVID, *J. biol. Chem.* 177, 751 (1949).



when not completely absent. In some of 18 sera of cases of acute virus (epidemic) hepatitis, this enzymatic activity has been found to be increased as compared with normal values. Furthermore, in almost all of them it is greatly increased by addition to the sera of boiled mouse liver homogenate. No such increase has been observed in normal human sera.

Homogenates in 15% concentration in H_2O from mouse liver tissue (washed in H_2O and then dried by filter paper) were prepared at room temperature in Potter-Elvehjem homogenizers; the homogenates were immersed for 30 s in water bath at $100^\circ C$. After centrifugation at 2000 r.p.m. for 15 min, the supernatant was separated and 0.1–0.2 ml of it was added to each test, which contained 0.2 ml of serum and was incubated for 60 min at $30^\circ C$. In each determination controls were performed with only liver homogenate and only serum. The boiled liver homogenate is devoid of any enzymatic activity.

It is assumed that during the liver necrotic process of the virus hepatitis, besides some active enzyme, a greater part of incomplete enzyme reaches the blood flow, which may then be fully activated by some unidentified compound contained in the liver tissue. Experiments are in progress to ascertain the possible identity of the activating compound contained in the homogenate with the known coenzyme of phosphoglucomutase, glucose-1, 6-diphosphate.

The present observations, together with those concerning the increase in the sera of other enzymatic activities found by us and by others (alanine-ketoglutaric transaminase²; aspartic-ketoglutaric transaminase³; aldolase, phosphohexoseisomerase⁴) in cases of epidemic hepatitis and found by us in experimental virus (MHV)

hepatitis (transaminases⁵, phosphoglucomutase⁶, fumarate⁷), emphasize the significance of the enzymatic pattern of the blood plasma in the liver virus 'necrosis syndrome' both human and experimental.

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Riassunto

Nell'epatite virale umana acuta l'attività fosfoglicomutasi del siero è notevolmente aumentata rispetto ai valori normali.

L'aggiunta di omogenato bollito di fegato di topo produce ulteriore notevole incremento dell'attività fosfoglicomutasi del siero di soggetti affetti da epatite epidemica; non influenza tale attività nel siero di soggetti normali.

² F. DE RITIS, M. COLTORTI, G. GIUSTI, *Science* **124**, 32 (1956).

⁶ F. DE RITIS, M. COLTORTI, G. GIUSTI, *Boll. Soc. It. Biol. Sper.* **1956**, in press.

⁷ F. DE RITIS, G. GIUSTI, M. COLTORTI, *Boll. Soc. It. Biol. Sper.* **1956**, in press.

Jahreszeitliche Schwankungen der Blutgerinnung beim Hunde

In letzter Zeit wurde mehrmals festgestellt, dass die Blutgerinnung bei Tieren im Winter- bzw. Sommerschlaf langsamer verläuft (Ziesel¹, Hamster², Igel³, Frosch⁴, Fledermaus⁶). Wir untersuchten während einer

¹ A. SVIHLA, H. BOWMAN und R. RITENOUR, *Science* **114**, 298 (1951).

² A. SVIHLA, H. BOWMAN und R. PEARSON, *Science* **115**, 272 (1952).

³ P. SUOMALAINEN und E. LEHTO, *Arch. Soc. «Vanamo»* **6**, 94 (1952).

⁴ J. J. SPITZER und J. A. SPITZER, *Canad. J. med. Sci.* **30**, 420 (1952).

⁵ D. E. SMITH, Y. S. LEWIS und G. SVIHLA, *Exper.* **10**, 218 (1954).

² F. DE RITIS, M. COLTORTI, and G. GIUSTI, *Boll. Soc. Ital. Sper.* **31**, 394 (1955); *Minerva Med.* **46** *I*, 1207 (1955); **47** *I*, 167 (1956); *Communication 1er Congrès international de Pathologie infectieuse, Lyon 24–26 mai 1956*. *Chimica Clinica Acta*, 1957, in press.

³ F. DE RITIS, M. COLTORTI, and G. GIUSTI, *Boll. Soc. Ital. Biol. Sper.* **31**, 394 (1955); *Minerva Med.* **46** *I*, 1207 (1955); **47** *I*, 167 (1956); *Communication 1er Congrès international de Pathologie infectieuse, Lyon, 24–26 mai 1956*. – F. WROBLEWSKI and J. S. LADUE, *Ann. int. Med.* **43**, 345 (1955).

⁴ F. H. BRUNS, *Klin. Wschr.* **1954**, 1041. – F. H. BRUNS and W. JACOB, *Klin. Wschr.* **1954**, 656. – F. H. BRUNS and J. NEUHAUS, *Arch. Biochem. Biophys.* **55**, 588 (1955).