hearts which had been excised immediately after stunning the animal and had been placed in ice-cold water while still beating vigorously, addition of salt caused a sharp rise in viscosity, comparable in magnitude to the increase costumarily obtained with actin from fresh skeletal muscle. In these instances the steroid glycosides were without effect.

Following the discovery by HORVÁTH *et al.*[1] of the influence of cardiac glycosides on the polymerization of actin a variety of other effects of these drugs on contractile muscle proteins were described in the literature. Whether some of these effects are likewise unspecific in the sense that they can be reproduced by structural analogs devoid of cardiac activity is at present under investigation.

This work was undertaken during tenure of a Life Insurance Medical Research Fellowship. I wish to express my gratitude to Prof. T. REICHSTEIN for a generous gift of emicymarin and alloemicymarin, and to Prof. A. STOLL for a generous gift of scillaren A and hexahydroscillaren A. I am also grateful to Dr. O. SNELLMAN and Dr. B. GELOTTE for their interest and cooperation.

A. WOLLENBERGER[2]

Institute of Biochemistry, University of Uppsala, March 3, 1954.

Zusammenfassung

Die fördernde Wirkung der herzwirksamen Glykoside Emizymarin und Szillaren A auf die Polymerisation von Aktin aus Herzmuskel wird in demselben Masse von den strukturell nahe verwandten, jedoch herzunwirksamen Glykosiden Alloemizymarin und Hexahydroszillaren A ausgeübt.

[1] I. HORVÁTH, C. KIRÁLY, and J. SZERB, Nature *164*, 792 (1949).

[2] Present address: Pharmakologisches Institut der Humboldt-Universität, Berlin NW 7.

Sull'ossidazione dell'acido lattico ed acido piruvico e di alcuni componenti del ciclo citrico in omogenati di epitelio corneale

Le ricerche sulla partecipazione del ciclo citrico di KREBS nell'ossidazione dell'acido piruvico da parte di tessuti oculari sono scarse. In due mie note[1] ho riferito sull'attività deidrogenasica generale della cornea, cristallino e retina mediante il Trifenil-tetrazolio, e sulla localizzazione istochimica della succinodeidrogenasi col Blu Tetrazolio. I miei dati hanno permesso di poter localizzare l'attività deidrogenasica elettivamente nell'epitelio corneale ed in minima parte nell'endotelio. Nella presente nota riferisco i risultati delle mie ricerche sulle deidrogenasi del ciclo citrico di KREBS in omogenati di epitelio corneale bovino mediante il Trifenil Tetrazolio.

Per le mie ricerche ho adoperato la tecnica consigliata da NORDMAN e NORDMAN[2]. L'epitelio di cornee bovine veniva scarificato e si preparava un omogenato al 20% nell'omogenizzatore in vetro di POTTER[3]; per la determinazione dell'attività deidrogenasica la miscela d'incubazione era preparata nelle seguenti proporzioni: In tubi da centrifuga si aggiungevano 1 ml di omogenato al 20% (200 mg), 0,5 ml Puffer Fosfati (0,1 M) pH 7,4, 0,5 ml di substrato (0,2 M) (acido lattico, piruvico) (acido citrico, α-chetoglutarico, succinico, malico) 1 ml di Trifenil Tetrazolio (0,1%) si completava a 4 ml con acqua distillata previa aggiunta di 0,3 ml di ATP (0,01 *M*).

I tubi da saggio si lasciavano incubare a 37°C per 30′ agitando due tre volte durante l'incubazione, si aggiungevano successivamente 10 ml di acetone, si agitava e si centrifugava.

Il liquido soprastante veniva decantato e si leggeva l'estinzione in colorimetro KLETT con filtro 420. In ogni prova si praticavano due controlli uno senza substrato ed il secondo anche senza substrato ma con aggiunta di una determinata quantità di Tetrazolio. Nella Tabella riporto la media dei dati di più esperimenti, l'attività enzimatica è riferita in μg di Tetrazolio ridotto in 30′ da 1 mg di tessuto (peso fresco).

Deidrogenazione dell'Acido Lattico, Piruvico e di alcuni componenti del ciclo citrico in omogenati di epitelio corneale

Substrati	N. Esp.	μg Tetrazolio rid. da 1 mg di tess. 30′	Errore Standard
Acido lattico	12	0,10	±0,04
Acido piruvico	9	0,22	± 0,05
Acido citrico	15	0,63	± 0,12
Acido chetoglutarico	10	0,24	± 0,08
Acido succinico	8	0,78	± 0,10
Acido malico	14	0,12	± 0,03

L'esame della tabella mostra che in presenza di tutti gli acidi del ciclo citrico saggiati è stato possibile mettere in evidenza nell'epitelio corneale una notevole attività deidrogenasica, e pertanto si può ammettere che anche nel tessuto corneale come in tutti i tessuti il ciclo tricarbossilico abbia un'importanza fondamentale nei processi ossidativi.

Addendum. Mentre la presente nota era in corso di pubblicazione, ho potuto prendere visione di alcuni lavori di JAEGER[1] sullo stesso argomento. L'Autore adoperando lo stesso metodo, ha ottenuto risultati che concordano con i miei dati.

E. DE BERARDINIS

Istituto di Clinica Oculistica Università Napoli, il 10 novembre 1953.

Summary

Dehydrogenase Activity of some components of citric cycle in corneal Epithelium homogenates is determined by means of 2-3-5 Triphenyltetrazoliumchloride.

[1] W. JAEGER, Grafes Archiv. Ophthalmol. *154*, 142, 401, 431 (1953).

Cortical Representation of the Cortico-Motoneuronal System in Monkeys

Bunctional evidence has recently been presented by BERNHARD, BOHM, and PETERSÉN[1] and by BERNHARD and BOHM[2] for a direct activation of the spinal motoneurones by descending volleys in corticospinal fibres in the monkey. That fraction of the corticospinal system, the descending volley in which activates the spinal motoneurones directly, i.e. monosynaptically, we refer to as the cortico-motoneuronal system or the *CM system*. We have now attacked the problem concerning the topography of the cortical representation of the CM system for different muscles.

[1] E. DE BERARDINIS, Boll. Soc. ital. Biol. sper. *27*, 7 (1951); *29*, 63 (1953).

[2] I. NORDMAN, R. NORDMAN, O. GAUCHERY, Bull. Soc. Chim. biol. *33*, 1826 (1951).

[3] V. R. POTTER, I. Biol. Chem. *114*, 495 (1936).

[1] C. G. BERNHARD, E. BOHM, and I. PETERSÉN, Exper. *9*, 111 (1953); Acta physiol. Scand. *29*, suppl. 106, 79 (1953).

[2] C. G. BERNHARD and E. BOHM, Acta physiol. Scand. In press (1954).

Single and repetitive square wave shocks of short duration (0·5–1 ms) were used for stimulation of the cortex in narcotized, curarized monkeys (*Macaca mulatta*) and the action potentials were led off from different peripheral nerves in the fore limb. The effect of cortical stimulation on the monosynaptic ventral root reflex in the lumber region was also studied.

Figure 1 shows the shape of the electrical response in the triceps nerve to contralateral cortical stimulation within the fore limb subdivision at a frequency of 25 per s. The pictures (*A–F*) taken at various intervals after the beginning of the repetitive stimulation, show a series of superimposed records of the action potentials following upon each cortical stimulus. During the first two seconds of repetitive stimulation (Fig. 1 *A–B*) an early response appeared, the amplitude of which increased to a maximum (*B*). Later on the early response decreased and disappeared (Fig. 1 *C*) in order to appear and fall again (Fig. 1 *D–F*). These fluctuations of the amplitude are typical for the early response when it is led off from nerves or ventral roots innervating fore and hind limb muscles.

In different types of experiments it was shown that the early response, which in this experiment had a latency of 3·4 ms represents activity in motoneurones which are monosynaptically activated by descending volleys in the corticospinal neurones[1]. According to our earlier investigations, this cortico-motoneuronal system, the CM system, comprises the corticospinal fibres with highest conduction rate, i.e. about 70 M per s corresponding to the diameter values around 12–14 μ given by HÄGGQVIST[2] and LASSEK[3].

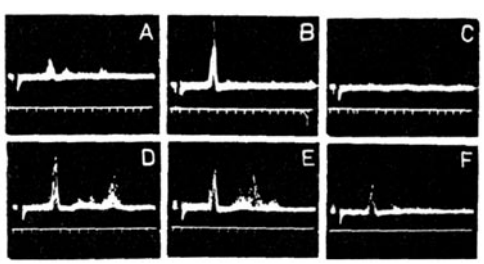

Fig. 1.—Superimposed action potentials in the left triceps nerve following each stimulus in a train of shocks (stimulation frequency 25 per s) delivered to the fore limb subdivision of the right precentral area. *A–F*, records obtained 1(*A*), 2(*B*), 3(*C*), 8(*D*), 9(*E*), and 10(*F*) s after the beginning of the repetitive stimulation. Time in ms.

The phasic amplitude variations of the monosynaptic triceps nerve response to cortical stimulation with six different stimulation frequencies, obtained in a similar experiment, are illustrated graphically in Figure 2. The amplitude values of the monosynaptic triceps response are plotted against time from the beginning of the repetitive stimulation. The diagrams show that the latency of the building up of the first phase shortens from about 8 s (at 13 per s) to 1 s (at 25 per s) in order to increase again at a higher stimulation frequency (35 per s). In parallel to this regular shift in the delay of the first phase there is a regular variation of the amplitude of the first phase. As seen the shortest latency of the first phase, as well as the highest amplitude of the monosynaptic response during this phase, was obtained at frequencies around 25 per s.

Since thus, stimulation frequencies of 20–25 per s were found to be most effective for the building up of the monosynaptic response, these frequencies were used in the following mapping experiments.

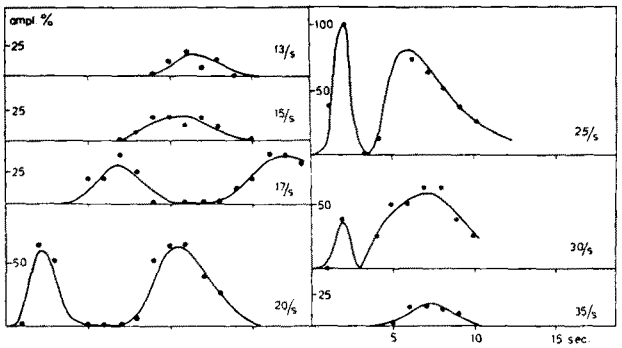

Fig. 2.—Amplitude values of the monosynaptic response in the triceps nerve to contralateral cortical stimulation with different frequencies (13, 15, 17, 20, 25, 30, and 35 per s) plotted against time from the beginning of the repetitive cortical stimulation. The amplitude values are given in per cent of the highest value obtained (100 per cent in curve 25/s).

Experiments were performed in which the action potentials were led off from the *triceps nerve* when different points within the precentral cortical area were stimulated. Figure 3 shows the surface of the precentral cortical area from a typical experiment and the different points numbered, indicate the different sites on the cortical surface on which the stimulating electrode was placed. Curves similar to that in Figure 2 (25/s) were drawn on the basis of the results obtained when different points (1–19 in Figure 3) were stimulated. The latencies of the first phase of the different curves were then measured. It was found that the monosynaptic triceps response to stimulation of point 5 appeared within 1 s after the beginning of the repetitive stimulation. This point is included within the circle drawn in full on the map in Figure 3 (the 1 s "latency field"). The field surrounded by a dashed line includes the points the stimulation of which elicited a monosynaptic triceps nerve response within 3 s after the beginning of the repetitive stimulation (the 3 s "latency field"). When the surrounding cortical areas were stimulated the monosynaptic response appeared still later during the course of stimulation. Thus, when taking into account the rapidity with which the response is built up by repetitive cortical stimulation, the most effective field for elicitation of the monosynaptic response in the triceps nerve is marked by the inner circle drawn in full. It was also found that the largest monosynaptic responses were elicited from the area the stimulation of which was also followed by a rapid building up of the monosynaptic response.

As seen in Figure 1 (*A*, *D*, and *E*) there are also one or several late responses. The late responses may be built up in parallel to, earlier, or later than the monosynaptic response during the course of the repetitive stimulation. In Figure 1 the amplitude of the late responses also fluctuates during the course of the repetitive stimulation, and these fluctuations may also show one or two phases. It was found that the late responses could be elicited from a wider area than the monosynaptic response. A comparison of the characteristics of the monosynaptic response and those of the late responses thus shows that the cortical representation of the direct cortico-motoneuronal system for one muscle is more

[1] C. G. BERNHARD, E. BOHM, and I. PETERSÉN, Exper. *9*, 111 (1953); Acta physiol. Scand. *29*, suppl. 106, 79 (1953). – C. G. BERNHARD and E. BOHM, Acta physiol. Scand., in press (1954).

[2] G. HÄGGQVIST, Acta psychiat. neurol. *12*, 457 (1937).

[3] A. M. LASSEK, J. comp. neurol. *79*, 407 (1943).

restricted than that of the system which is responsible for the late responses.

The cortical representation of the CM system for the *biceps nerve* was also mapped using the same experimental procedure as in the triceps experiments. The lower circles in Figure 3 show the representation of the CM system for the biceps nerve. As seen there is a restricted field where stimulation was followed by a monosynaptic biceps response which was built up within 1 s after the beginning of the repetitive stimulation (the 1 s "latency field"; circle drawn in full). The two surrounding circles mark the 2 and 3 s "latency fields".

In a classical investigation SHERRINGTON and HERING[1] described excitation and inhibition of limb antagonists to cortical stimulation. The effect may however be due to–or partly due to–afferent back responses from the muscles during the contraction.

In special series of experiments we found that there is a reciprocal behaviour of the monosynaptic responses in the nerves to the two antagonistic muscles. Stimulation of the lower part of the "triceps field" was found to counteract the monosynaptic biceps response elicited from the upper part of the "biceps field" and *vice versa*. Since the preparations were curarized there were no

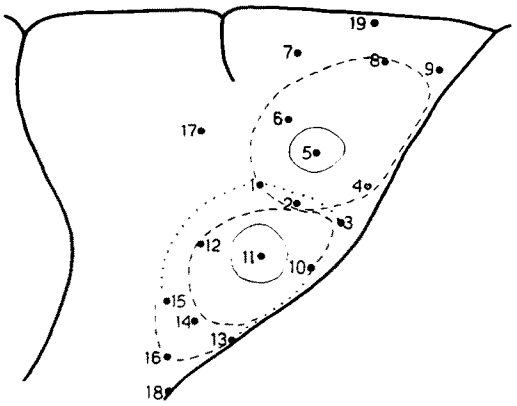

Fig. 3.—Map of the precentral area and orientation of the different points (1–19) stimulated in the experiments described in the text in which monosynaptic responses were recorded from the contralateral triceps and biceps nerves (stimulation frequency 25 per s). Upper set of circles indicate the triceps fields (circle drawn in full, 1 s "latency field"; dashed circle, 3 s "latency field"). Lower set of circles indicate the biceps fields (circle drawn in full, 1 s "latency field"; dashed circle, 2 s "latency field"; dotted circle, 3 s "latency field"). For further explanation see text.

muscle contractions. Thus the reciprocal behaviour of the responses described was not due to afferent back responses from the muscles.

Experiments similar to those described above were performed in which the responses to contralateral cortical stimulation were led off from the *thenar* and *hypothenar* nerves. There is a striking difference between the responses obtained from the thenar and hypothenar nerves and those recorded from the triceps and biceps nerves. The record of the thenar nerve responses were always characterized by a poverty in late discharges whereas the biceps and triceps responses to each single stimulus consisted of a whole set of action potentials following upon the monosynaptic response.

Since the value, representing the synaptic delay between corticospinal neurones and the thenar or hypothenar motoneurones, was also found to be of the same magnitude as that found for monosynaptic transmission elsewhere, we concluded[1] that the thenar nerve motoneurones are also monosynaptically activated by descending volleys in the corticospinal fibres.

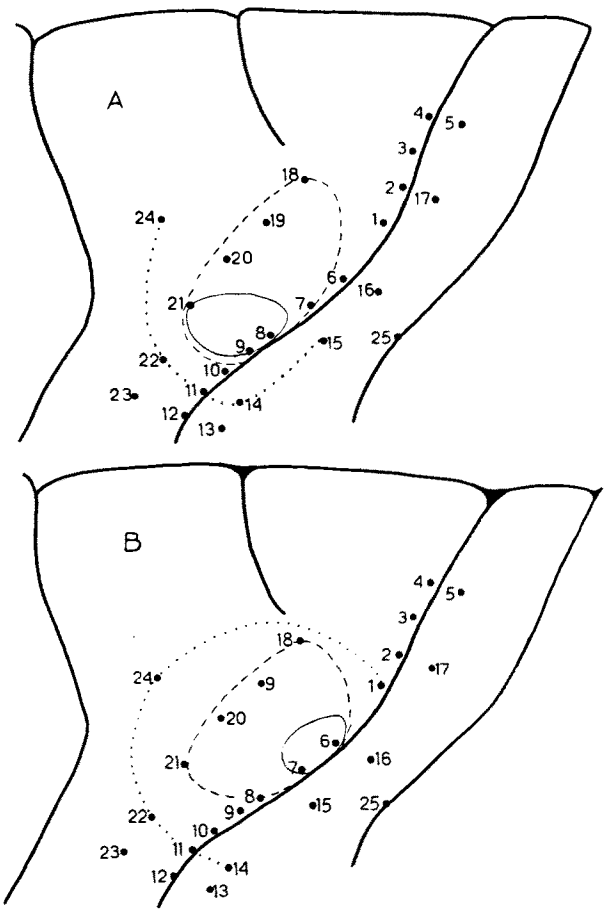

Fig. 4.— *A*. Map of the precentral area and orientation of the different points (1–25) stimulated in the experiment described in the text in which the monosynaptic responses were recorded from the contralateral thenar nerve (stimulation frequency 25 per s). Circle drawn in full, 3 s "latency field". Dashed circle, 5 s "latency field". Dotted line, lower border of the 8 s "latency field". For full explanation see text.—*B*. Same as in Figure 4*A* for the monosynaptic hypothenar nerve response. Circle drawn in full, 2 s "latency field". Dashed circle, 4 s "latency field". Dotted line, anterior border of the 6 s "latency field".

The solid circle (in Fig. 4*A*) shows the field the stimulation of which was followed by a monosynaptic thenar nerve response, which began to rise within 3 s after the beginning of the repetitive stimulation. The dashed circle indicates the 5 s "latency field" and the dotted line the lower border of the 8 s "latency field". Thus the circle drawn in full marks the most potent field for the elicitation of the monosynaptic thenar nerve response. The monosynaptic response of the hypothenar nerve was recorded in the same experiment and Figure 4*B* shows the results of the mapping of the CM system for the hypothenar nerve. The three different circles indicate the 2, 4, and 6 s "latency fields". In this preparation the most potent field for the elicitation of monosynaptic hypothenar nerve responses was situated above that of the thenar nerve. In other similar experiments we found that these two fields may overlap.

[1] C. S. SHERRINGTON and H. E. HERING, Proc. Roy. Soc. *62 B*, 183 (1897).

[1] C. G. BERNHARD and E. BOHM, Arch. Neurol. Psychiat., Chicago 1954 (in press).

Records were also made of the responses in different nerves *ipsilateral* to the cortical region stimulated. The experiments showed that the CM systems for the nerves to the proximal arm muscles also have an ipsilateral cortical representation, whereas they indicate that this is not the case for the CM systems activating the nerves to the distal hand muscles in which only contralateral monosynaptic responses to cortical stimulation could be recorded.

In the investigations made by previous authors on the analysis of the corticospinal system in monkeys (see e.g. review by RUCH[1]), the movements or muscle contractions resulting from cortical stimulation were recorded. In our experiments the responses in different peripheral nerves to single cortical stimuli, as well as to each stimulus in a train of cortical shocks, were recorded. A single cortical shock elicits a descending volley in the fast conducting corticospinal neurones of the pyramidal tract[2]. In our experiments we found that single cortical shocks do not elicit any monosynaptic responses in the different fore limb nerves tested. Repetitive stimulation had to be used and during the course of stimulation the monosynaptic responses were built up in a regular way. It was shown that the repetitive cortical stimulation builds up a "tonic" asynchronous discharge and a long-lasting facilitatory action which successively raises the excitability of motoneurones; and further, that when the facilitatory action reaches a certain level, the monosynaptic responses to the descending volleys in the fast conducting CM fibres break through. In a series of experiments in which the conditioning effect of repetitive cortical stimulation on the monosynaptic reflex was tested, it was found that the cortical field, from which the facilitation of a certain group of spinal motoneurones was elicited, not only covered the restricted cortical field representing the CM system which activates the same group of motoneurones, but also exceeded it. It is of great interest to note that "the facilitatory area" and "the CM area" for the same group of spinal motoneurones have the same posterior border towards the postcentral gyrus, whereas the "facilitatory area" extends in the anterior direction (see BERNHARD and BOHM[3]).

C. G. BERNHARD and E. BOHM

Physiological Department II, Karolinska Institutet, Stockholm, April 21, 1954.

Zusammenfassung

Es wurden Aktionspotentiale verschiedener Nerven registriert, welche auf jede kortikale Reizung in einem Zuge von repetitiven Stimulationen folgten, ferner wurden monosynaptische Reflexe nach konditionierenden kortikalen Stimulationen geprüft.

Die Experimente ergaben, dass unter den verschiedenen kortikospinalen Systemen das direkte kortikomotoneuronale System (das CM-System) die am meisten abgegrenzte kortikale Repräsentation besitzt.

[1] T. C. RUCH, *Handbook of Experimental Psychology*, chapt. 5, p. 154 (1951).

[2] C. G. BERNHARD, E. BOHM, and I. PETERSÉN, Acta physiol. Scand. *29*, suppl. 106, 79 (1953).

[3] C. G. BERNHARD and E. BOHM, Acta neurol. Psychiat., Chicago 1954 (in press).

PRO EXPERIMENTIS

Die Isolierung eosinophiler Leukozyten und ihrer Granula

Frühere Versuche zur Gewinnung eosinophiler Zellen und Granula beruhten auf chemischen Methoden. WEISS[1] beschrieb schon 1891 die Resistenz der Granula gegen Fermentlösungen. Die Reindarstellung eosinophiler Granula aus Pferdeblut gelang PETRY[2] 1908 mittels Trypsin. NEUMANN[3] isolierte die Granula später unter Verwendung anderer lytischer Prozesse (NaOH, Autolyse) und stellte mit der gewonnenen Reinsubstanz umfangreiche physikalisch-chemische Untersuchungen an. In neuer Zeit hat VERCAUTEREN[4] eosinophile Granula auf rein physikalischem Weg dargestellt, indem er die Gesamtleukozyten in einem Homogenisatorapparat zertrümmerte und die freien Granula durch wiederholtes fraktioniertes Zentrifugieren gewann.

Eine von BEHRENS und TAUBERT[5] entwickelte Technik erlaubt nun erstmals, die einzelnen Leukozytenarten des Blutes auf Grund des spezifischen Gewichtes voneinander zu trennen. Es gelingt damit, aus Pferdeblut eine Suspension eosinophiler Granulozyten herzustellen. Somit steht uns ein sehr zweckmässiges Ausgangsmaterial zur relativ ergiebigen Gewinnung der eosinophilen Granula zur Verfügung. Wir verwenden folgende Arbeitsmethode:

1. *Isolierung eosinophiler Granulozyten.* Leukozyten werden gewaschen, entwässert und nach dem spezifischen Gewicht getrennt. Als Ausgangsmaterial dient Pferdeblut, das steril aus der Halsvene entnommen wird und durchschnittlich 3–5% Eosinophile enthält. 200 cm³ 3,5%ige Na-Zitrat-Lösung werden mit 800 cm³ Blut bei der Entnahme gut gemischt und in einer Standflasche stehengelassen, bis sich die Erythrozyten auf halbe Höhe der Flüssigkeitssäule gesenkt haben. Die erforderliche Zeit beträgt je nach Senkungsgeschwindigkeit des Blutes ½–2 h. Dann wird das überstehende, deutlich rotgefärbte Plasma abgehebert. Es enthält neben Leukozyten noch massenhaft Erythrozyten. Die gewonnene Plasmamenge von etwa 500 cm³ wird in Portionen zu 100–150 cm³ 4 min lang bei einer Tourenzahl von 1700/min zentrifugiert (Zentrifuge Stock, Marburg). Das Sediment wird vereinigt und in 30 cm³ 1%iger NaCl-Lösung aufgeschüttelt. Durch die etwas hypertonische Salzlösung kommt es zu einer leichten Schrumpfung der Erythrozyten und der neutrophilen Granulozyten, während die eosinophilen Zellen ihr Volumen kaum verändern. Bei den Eosinophilen des Pferdes handelt es sich ohnehin um relativ grosse Zellen, und der Unterschied in der Zellgrösse lässt sich so noch akzentuieren.

Die Zellen werden wiederum während 4 min bei einer Tourenzahl von 1700/min zu Boden zentrifugiert. Das Sediment wird zehnmal gewaschen, jedesmal in 30 cm³ 1%iger NaCl-Lösung kräftig aufgeschüttelt und kurz zentrifugiert (2½ min bei 1700 T.). Dadurch lässt sich nicht nur das Plasmaeiweiss weitgehend eliminieren,

[1] A. WEISS, Zbl. med. Wiss. *29*, 881 (1891).

[2] E. PETRY, Wien. klin. Wschr. *21*, 1360 (1908); Biochem. Z. *18*, 92 (1912).

[3] A. NEUMANN, Biochem. Z. *148*, 524 (1924); *150*, 256 (1924); Fol. haematolog. *36*, 95 (1928); Hdb. allg. Haematol. *1*, 354 (1932, Urban & Schwarzenberg).

[4] R. VERCAUTEREN, Enzymologia *16*, 1 (1953).

[5] M. BEHRENS und M. TAUBERT, Hoppe Seyler Z. physiol. Chem. *289*, 63 (1952); *290*, 228 (1953).