Tout se passe donc comme si, dans ces sérums, il y avait captation de l'hémoglobine par une α_2-globuline qui se sature rapidement, après quoi l'hémoglobine migre librement et se place sur le front des β-globulines.

Nous avons d'ailleurs pu montrer que cette captation de l'hémoglobine par une α_2-globuline conduit à un complexe de mobilité intermédiaire entre celle d'une α_2- et d'une β-globuline (Fig. 2). En effet, après l'addition d'hémoglobine, on trouve sur le phérogramme coloré à l'amido schwarz, une fraction apparemment nouvelle, entre les α_2- et les β-globulines, et qui correspond à la tache *h* repérée au crayon. En même temps, l'emplacement des α_2-globulines s'est vidé et il n'y reste qu'une fraction mineure qui ne semble pas avoir réagi. Ceci conduit au diagramme à trois sommets intriqués, que

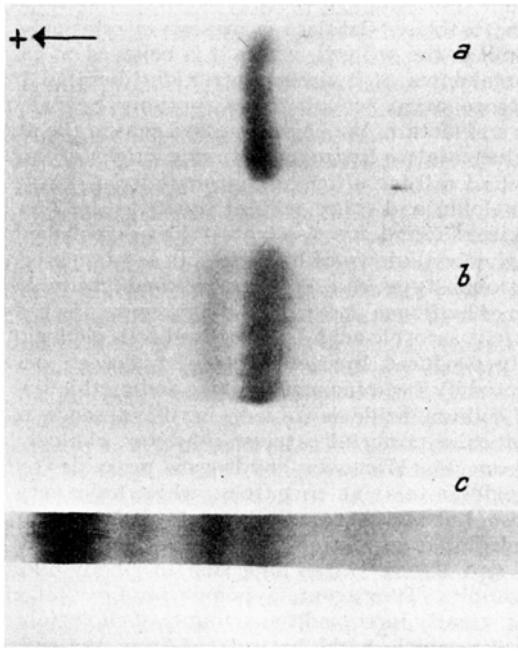


Fig. 1. *a* hémoglobine; *b* hémoglobine additionnée de 10 μl de sérum; *c* hémoglobine additionnée de 1 μl de sérum. La moitié supérieure du phérogramme est colorée à l'amido schwarz. Dans la moité inférieure on perçoit la tache *h* très légèrement indiquée.

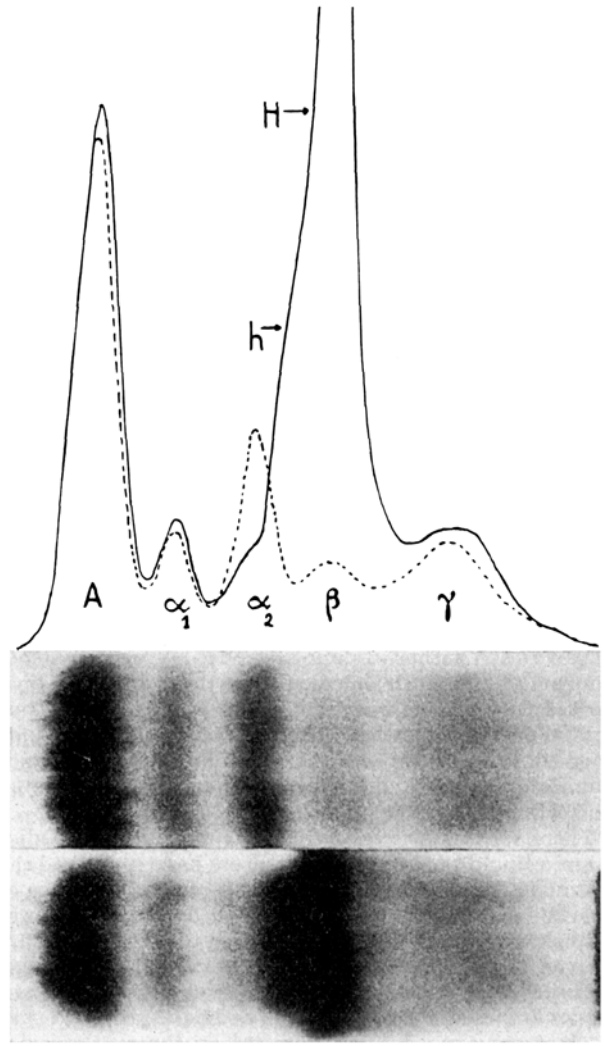


Fig. 2. Diagramme pointillé: sérum à élévation des α_2 globulines; diagramme en ligne continue: même sérum après addition d'hémoglobine. – Phérogrammes: supérieur: même sérum; inférieur: même sérum additionné d'hémoglobine.

nous avions retrouvé sur des sérums légèrement hémolytiques.

La captation de l'hémoglobine par une α_2-globuline rappelle le comportement de l'haptoglobine de JAYLE et POLONOVSKI[1]. VAN ROYEN[2] a démontré d'autre part, que l'haptoglobine est congruente à une fraction importante et hautement variable des α_2-globulines. Nous croyons donc pouvoir identifier la tache *h* au complexe haptoglobine/hémoglobine.

Cette observation nous semble d'un intérêt certain: toute trace d'hémolyse diminue apparemment la quantité des α_2-globulines avant d'influencer l'estimation des β-globulines; cet effet sera d'autant plus net que l'α_2-globuline sera en concentration supranormale. Ce phénomène fera donc écarter de l'analyse électrophorétique courante tous les sérums à trace d'hémolyse. Il pourrait, d'autre part, permettre un dosage de l'haptoglobine libre.

R. J. WIEME

Clinique médicale, Université de Gand, le 10 juin 1953.

[1] M. F. JAYLE et ABDELLATIF, Bull. Soc. Chim. Biol. *28*, 80 (1946).

[2] A. H. H. VAN ROYEN dans L. W. JANSEN: *Electrophoretic studies on serum proteins* (1951), p. 94.

Summary

Using paper electrophoresis, the author found that when human hemoglobin is added to human sera, it is captured by a fraction of the α_2-globulin, which results in a compound with an electrophoretic mobility between that of α_2-globulin and β-globulin.

He identifies this fraction of the α_2-globulin with the haptoglobin discovered by JAYLE. This phenomenon may influence the estimation of α_2-globulin even in slightly hemolytic sera.

Catalase Inhibition: A Possible Mechanism for the Production of Heinz Bodies in Erythrocytes

It has long been recognized that inclusion bodies in mammalian red blood cells similar to those originally described by ROBERT HEINZ[1] can be produced *in vivo* and *in vitro* by many toxic substances. So diverse are

[1] R. HEINZ, Virch. Arch. path. Anat. *122*, 112 (1890).

the compounds known to produce HEINZ bodies that no common factor is apparent among them, and WEBSTER[1] in a recent review was forced to conclude that "our knowledge of the relationship between chemical constitution and HEINZ body formation is quite fragmentary".

The appearance of HEINZ bodies in red cells is always associated with haemoglobin degradation, and it is widely accepted that the refractile inclusion bodies consist for the most part of products of haemoglobin breakdown. WARBURG *et al.*[2] and HORECKER[3] showed that HEINZ bodies resemble denatured protein in structure and properties. Denaturation of the protein moiety of haemoglobin, then, would appear to be an early stage in HEINZ body formation. The precise fate of the haem prosthetic group is more problematical. ROSZA and SPICER[4] have effectively separated the denatured globin from HEINZ bodies, which then contain only a small residue of protein, possibly as a contaminant. These extracted bodies are very opaque in electron micrographs, as are the HEINZ bodies developing *in situ* in the red cell[5]. The opacity has been attributed to the iron content of these bodies[6]. The haem group may thus also be degraded in HEINZ body formation, perhaps by opening of the ring at the α-methene linkage to give compounds of the type designated as choleglobin by LEMBERG[7]. A rather ill-defined group of green pigments can be obtained in haemoglobin degradation which appear to resemble one another spectroscopically, but not in other properties; for convenience they will be referred to by the general term "choleglobin". Intact haem, choleglobin and bile pigments can all be extracted from HEINZ bodies[8].

There is also general agreement that HEINZ bodies contain lipoid[9]. Numerous observers have remarked the fact that HEINZ bodies are formed at the periphery of the cell, and this has been confirmed by electron micrograph studies[10]. It has been proposed that the bodies are derived in part from the cell membrane[11], but BRAUNSTEINER *et al.*[12] have shown no connection between HEINZ bodies and the membrane and have proposed that the inclusions are derived from the erythrocyte stroma. In any case, the membrane is affected by the reactions as indicated by the markedly reduced osmotic fragility of red cells with HEINZ bodies[13]. Thus it would appear that HEINZ body formation is associated with considerable alteration of the red cell components and that the breakdown of haemoglobin is only part of the process.

Some of the substances known to produce HEINZ bodies also lead to the formation of methaemoglobin, and many workers have accepted oxidation of haemoglobin as part of the sequence of events initiated by the toxic agent[1]. However, inclusions of the same type occur in the cells of premature neonates in the absence of methaemoglobin formation[2], and some compounds which form HEINZ bodies, such as methylene blue[3] and Nile blue sulphate[4], actually reduce methaemoglobin in the red cells. Thus methaemoglobin formation does not seem to be an essential stage in the process.

One common property of the toxic substances producing HEINZ bodies which has hitherto been overlooked is their direct or indirect inhibitory action on the enzyme catalase. Catalase is present in relatively large amounts in the red cell, where it is believed to catalyse the breakdown of hydrogen peroxide liberated by the dehydrogenation of substrates, notably hexose phosphate and lactate. We have supposed that in the absence of active catalase hydrogen peroxide might accumulate in the red cell in sufficient concentration to attack the haemoglobin and other cellular constituents. The work of LEMBERG and his associates[5] has established that oxidative breakdown of haemoglobin to pigments of the choleglobin type can be accomplished easily by the action of hydrogen peroxide in the presence of a reducing agent, e.g. ascorbic acid. In intact red cells choleglobin is rapidly produced by the action of hydrogen peroxide generated by D-amino acid oxidase and methionine only when sodium azide is added; in the absence of this substance, a powerful catalase inhibitor, choleglobin is not formed[6]. Moreover, hydrogen peroxide oxidises haemoglobin in avian corpuscles, which have very little catalase, but not in mammalian red cells[7].

Choleglobin formation has been observed by LEMBERG and LEGGE[8] after injection of phenylhydrazine into rabbits. This agent, a potent producer of HEINZ bodies, greatly increased the amounts of choleglobin and biliverdin which could be isolated from the red cells. There is, in fact, a reasonably good correlation between the formation of green pigments of the choleglobin type and HEINZ bodies. Thus, KIESE and SEIPELT[9] have demonstrated the presence of verdoglobin (probably choleglobin[10]) in dogs after the injection of hydroxylamine and aromatic amino and nitro compounds, all of which produce HEINZ bodies. Choleglobin formation together with HEINZ bodies were shown to occur after the administration of alloxan and ascorbic acid[11], and a similar association has been demonstrated by GAJDOS

[1] S. H. WEBSTER, Blood *4*, 479 (1949).
[2] O. WARBURG, F. KUBOWITZ, and W. CHRISTIAN, Biochem. Z. *242*, 170 (1931).
[3] B. L. HORECKER, Pub., Health Rep., No. 285, *46* (1944).
[4] G. ROSZA and S. S. SPICER, Nature *171*, 84 (1953).
[5] F. JUNG, Naturwissenschaften *30*, 472 (1942). – H. BRAUNSTEINER, F. PAKESCH, and E. E. REIMER, Wien. klin. Wschr. *62*, 244 (1950).
[6] G. ROSZA and S. S. SPICER, Nature *171*, 84 (1953). – W. HARTWICH, Folia Haematol., Archiv *13*, 257 (1912).
[7] R. LEMBERG and J. W. LEGGE, *Hematin Compounds and Bile Pigments* (Interscience, New York, 1949).
[8] J. C. WHITE, J. V. DACIE, E. R. HOLLIDAY, G. R. BEAVEN, and E. A. JOHNSON, Brit. Med. J. *2*, 357 (1951).
[9] H. KUNKEL, Folia Haematol. Archiv *14*, 430 (1912–13). – L. HESS and H. MÜLLER, Wien. klin. Wschr. *26*, 1831 (1913). – M. GUTSTEIN and G. WALLBACH, Virch. Arch. path. Anat. *267*, 144 (1928).
[10] F. JUNG, Naturwissenschaften *30*, 472 (1942).
[11] F. JUNG, Naturwissenschaften *30*, 472 (1942). – J. C. WHITE, J. V. DACIE, E. R. HOLLIDAY, G. R. BEAVEN, and E. A. JOHNSON, Brit. Med. J. *2*, 357 (1951). – M. KIESE and L. SEIPELT, Arch. exp. Path. Pharmakol. *200*, 648 (1943). – F. JUNG, Klin. Wschr. *22*, 42 (1943).
[12] H. BRAUNSTEINER, F. PAKESCH, and E. E. REIMER, Wien. klin. Wschr. *62*, 244 (1950).
[13] P. MORAWITZ and J. PRATT, Münch. med. Wschr. *55*, 1817 (1908).

[1] S. H. WEBSTER, Blood *4*, 479 (1949).
[2] H. WILLI and F. HARTMEIER, Schweiz. med. Wschr. *80*, 1091 (1950).
[3] S. S. SPICER and E. C. THOMPSON, J. Indust. Hyg. Toxicol. *31*, 206 (1949).
[4] M. GUTSTEIN and G. WALLBACH, Virch. Arch. path. Anat. *267*, 144 (1928).
[5] R. LEMBERG and J. W. LEGGE, *Hematin Compounds and Bile Pigments* (Interscience, New York, 1949).
[6] E. C. FOULKES and R. LEMBERG, Proc. Roy. Soc. [B] *136*, 435 (1949).
[7] V. S. SHAPOT, Biokhimiya *3*, 430 (1938). Quoted by[5].
[8] R. LEMBERG and J. W. LEGGE, Aust. J. Exp. Biol. Med. Sci. *18*, 95 (1940).
[9] M. KIESE and L. SEIPELT, Arch. exp. Path. Pharmakol. *200*, 648 (1943).
[10] R. LEMBERG and J. W. LEGGE, *Hematin Compounds and Bile Pigments* (Interscience, New York, 1949).
[11] D. MERLINI, Folia Endocrinol. *4*, 1 (1951).

and TIPREZ[1] for a number of toxic agents. LEMBERG and LEGGE[2] have concluded that "the changes which are observed in erythrocytes after phenylhydrazine treatment are entirely consonant with choleglobin formation. The spectroscopic evidence, the isolation of free biliverdin, the appearance of denatured globin in the Heinz bodies and the presence of labile iron all represent aspects of a single process".

Perusal of a list of toxic substances producing HEINZ bodies[3] reveals that many are known catalase inhibitors[4], e.g. hydrazine, phenylhydrazine, acetylphenylhydrazine, hydroxylamine, potassium chlorate and potassium cyanide. Since most of these substances will combine with haem iron in general, they might act directly on haemoglobin or indirectly through catalase inhibition. Some of these substances, notably hydrazine, can produce choleglobin in solutions of haemoglobin[1], but it is difficult to fit all the known toxic substances into this pattern. We have therefore examined the possibility that agents producing HEINZ bodies act by inhibition of red cell catalase, and this communication embodies the results of our preliminary studies.

1. *Production of* HEINZ *bodies with sodium azide.*—To our knowledge, the production of HEINZ bodies by sodium azide, a potent catalase inhibitor, has not been previously examined. We have observed that human red blood cells incubated in 10^{-4} *M* solutions of sodium azide in isotonic glucose-citrate-saline contain a large number of HEINZ bodies after 6 h, as shown by supravital staining with 0·5% methyl violet.

The production of HEINZ bodies by sodium azide shows a marked temperature dependence. The optimal temperature is 37°C; at room temperature, few are formed, and at 1°C none at all. This confirms the results obtained by GAJDOS and TIPREZ[1] with other substances, and suggests that activation of one or more enzyme systems is involved.

2. *Production of* HEINZ *bodies with 2:4-dichlorphenol.* It has recently been reported that 2:4-dichlorphenol is a selective inhibitor of catalase[5]. This substance does not appear to combine with haem iron; there are no changes in the spectra of either catalase or haemoglobin in its presence, nor does it inhibit the activity of other iron-containing enzymes such as peroxidase. The inhibitory effect seems to be due to combination with the protein component of catalase. The compound gives 50% inhibition of purified catalase at a concentration of 2×10^{-6} *M*.

Incubation of human erythrocytes at 37°C for 6 h with solutions of 2:4-dichlorphenol in glucose-citrate-saline shows that large numbers of HEINZ bodies are produced in concentrations as low as 10^{-5} *M*; few of the red cells contain HEINZ bodies with concentrations of 2×10^{-6} *M* or less. There is the same temperature dependence as exhibited by azide.

The concentrations of 2:4-dichlorphenol that produce HEINZ bodies are similar to those known to inhibit catalase. As mentioned above, this substance does not combine with haem iron, and although the possibility does exist that it could combine with globin and degrade the haemoglobin by direct action, this seems unlikely.

These experiments suggest that catalase inhibition may be the first step in the reactions leading to the production of HEINZ bodies. This does not exclude direct action of toxic substances on haemoglobin and it is probable that methaemoglobin is formed as an independent reaction with many of the compounds used. The hypothesis of catalase inhibition is supported by further evidence. Sulphapyridine has been shown by CLYMAN[1] to produce *in vivo* inhibition of catalase in the red cell and this drug is well known as a causative agent in the production of HEINZ bodies[2].

Interference with the action of catalase further provides a basis for explaining the production of HEINZ bodies by methylene blue[3] and by Nile blue sulphate[4]. Dyes with a relatively high oxidation-reduction potential, notably methylene blue, accelerate the oxygen uptake of intact red cells[5]. Autoxidation of the dyes in this system leads to the production of hydrogen peroxide, which can combine with the haemoglobin and oxidise it. Such degradation of the haemoglobin would be prevented by active catalase, but excess hydrogen peroxide in this system inactivates the catalase irreversibly[6]. This type of inhibition by inactivation is typical of ascorbic acid[6] and both choleglobin and HEINZ bodies can be produced in red cells by excess ascorbic acid[7].

The view that catalase may function as a protective agent in the red cell is by no means new. BINGOLD[8], in particular, attempted to prove that the function of catalase in the red cell is the protection of haemoglobin against destruction by hydrogen peroxide. However, his experiments were carried out with relatively enormous concentrations of hydrogen peroxide which bring about an unphysiological breakdown of haemoglobin to colourless products. The experiments cannot be accepted in support of his hypothesis, nor can his assumption that separation of catalase from haemoglobin in the kidney causes haemoglobin breakdown. Indeed, KEILIN and HARTREE[9] have expressed doubts about the biological activity of catalase as a means of preventing accumulation of hydrogen peroxide in the cell; they suggest that it may have a peroxidative function in most cells. This view is not shared by LEMBERG and LEGGE[10], and the results of our investigations support the earlier belief that, in the erythrocyte at least, catalase functions as a protective agent for the whole cell against the products of respiration. Substances inhibiting catalase and thereby leading to an accumulation of hydrogen peroxide would produce degradation of haemoglobin and also other cell components, in particular the lipoids and proteins of the membrane and the stroma. Further experiments are being carried out to investigate the relation of breakdown of these components to the formation of HEINZ bodies.

S. BRENNER[11] and A. C. ALLISON[12]

Physical Chemistry Laboratory, Oxford and Clinical Pathology Laboratory, The Radcliffe Infirmary, Oxford July 24, 1953.

[1] A. GAJDOS and G. TIPREZ, Sang *18*, 35 (1947).

[2] R. LEMBERG and J. W. LEGGE, loc. cit.

[3] S. H. WEBSTER, Blood *4*, 479 (1949). – A. GAJDOS and G. TIPREZ, Sang *18*, 35 (1947).

[4] H. BLASCHKO, Biochem. J. *29*, 2303 (1935).

[5] P. L. GOLDACRE and A. W. GALSTON, Arch. Biochem. Biophys. *42*, 169 (1953).

[6] M. CLYMAN, Ann. Int. Med. *14*, 406 (1940).

[7] S. MOESCHLIN, Schweiz. med. Wschr. *21*, 786 (1940).

[8] S. S. SPICER and E. C. J. THOMPSON, J. Indust. Hyg. Toxicol. *31*, 206 (1949).

[9] M. GUTSTEIN and G. WALLBACH, Virch. Arch. path. Anat. *267*, 144 (1928).

[10] G. A. HARROP and E. S. G. BARRON, J. Exp. Med. *28*, 207 (1928).

[11] R. LEMBERG and J. W. LEGGE, Biochem. J. *37*, 117 (1943).

[12] E. C. FOULKES and R. LEMBERG, Proc. Roy. Soc. [B] *136*, 435 (1949). – A. GAJDOS and G. TIPREZ, Sang. *18*, 35 (1947).

[13] K. BINGOLD, Klin. Wschr. *14*, 1287 (1938).

[14] D. KEILIN and E. F. HARTREE, Biochem. J. *39*, 289 (1945).

[15] R. LEMBERG and J. W. LEGGE, *Hematin Compounds and Bile Pigments* (Interscience, New York, 1949).

[16] Overseas Science Research Scholar of the Royal Commission for the Exhibition of 1851.

[17] Staines Medical Research Fellow of Exeter College, in receipt of a grant from the Medical Research Council.

Zusammenfassung

Experimentell wird gezeigt, dass Heinzsche Körper durch niedere Konzentrationen von Natriumazid und 2:4-dichlorphenol gebildet werden. Diese Substanzen hemmen Katalase stark, und es wird angenommen, dass die Katalasehemmung die erste Stufe beim Abbau von Hämoglobin und anderer Zellbestandteile ist. Dies führt zur Bildung von Heinzschen Körpern.

Über einen muskelaktiven Wirkstoff aus der Adrenalinreihe (Adrenochrom)[1]

Die Isolierung des ausserordentlich flüchtigen und im Gewebe – vor allem im quergestreiften Muskel – verbreiteten Wirkstoffes Adrenochrom (GREEN und RICHTER[2]) und die Darstellung eines stabilen Präparates Monosemikarbazonderivat des Adrenochroms (BRACONIER, LE BIHAN und BEAUDET[3]), nämlich das Adrenoxyl[4], hat neuerdings unser Interesse erweckt und neue Gesichtspunkte für das Studium des Muskelstoffwechsels eröffnet.

Bekanntlich wird Adrenochrom aus dem Adrenalin über das entsprechende Chinon aufgebaut (Formel).

Das Adrenochrom ist als ein Oxydationsprodukt des Adrenalins und wirkt als hochaktiver Wasserstoffüberträger für bestimmte Oxydoreduktionsprozesse. Dies geht auch aus dem hohen Redoxpotential des Adrenalinchinons hervor, nämlich E' + 380. Es spielt also eine wesentliche Rolle bei katalytisch-fermentativen Prozessen im Organismus des Säugetieres.

Eine Analyse der biologischen Wirkung des Adrenochroms war erst mit Hilfe des stabileren Adrenoxyls, das im Körper zu Adrenochrom abgebaut und schliesslich durch die Nieren zum Teil als Indol (6%) ausgeschieden wird (FISCHER und LECOMTE[5]), möglich.

Diese Studien zeigten einige für die Physiologie des Muskelstoffwechsels sehr interessante Ergebnisse.

Methodik

1. Die indirekte Reizung des freipräparierten Musculus gastrocnemius führt beim Kaninchen (unter Urethannarkose) nach Adrenoxyl-Vorbehandlung - intraarterielle Injektion von 1 × 50 γ Adrenoxyl/2 kg - zu einer deutlichen und signifikanten Kontraktionsverstärkung. Diese tritt schon nach 5 min ein und erreicht nach 15 min eine hohe, nach 40 min die höchste Wirkung. Nach etwa 60 min fällt die Kurve wieder langsam, nach einem Tetanusreiz sehr steil ab.

2. Einen analogen Effekt erzielt man bei Ratten (in Narkose). Nach intravenöser Injektion einer Adrenoxyllösung (1 mg/Tier), führt die indirekte Reizung des Musculus gastrocnemius zu einer rasch ansteigenden, langanhaltenden (über 60 min) Kontraktionsverstärkung, die wir graphisch (in Kurve Nr. 1*b*) festhielten.

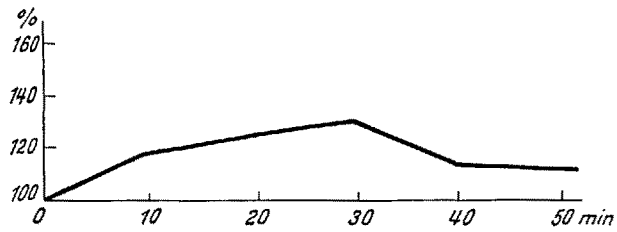


Abb. 1*a*. Mittelwertskurve (mittleres Summenpolygon) des Verhaltens vom Musculus gastrocnemius (Ratten) nach indirekter Reizung, je 3 Reize nach 2 min ohne Adrenoxylvorbehandlung.

3. Beim Menschen liess sich ein «muskeldynamischer Effekt» des Adrenochroms nach Verabreichung des Derivats *Adrenoxyl* ebenfalls feststellen. Die Wirkung trat schon am 3., deutlicher jedoch am 6. Tag nach täglicher Injektion von 0,5 mg *Adrenoxyl* (1 Ampulle) intramuskulär oder 3 × 1 bis 4 × 2 Adrenoxyltabletten zu 2,5 mg täglich ein. Beim Menschen lässt sich die Wirkung nicht am einzelnen Muskel, sondern nur in der Gesamtheit und bestenfalls an einem Muskelbündel studieren. Die Prüfung fand am 3. und am 6. Tag nach Adrenoxylverabreichung durch Messung und Registrierung der Arbeitsleistung des rechten oder linken Mittelfingers am Ergograph nach Mosso statt. Durch Fixierung des Unterarmes am Prüfungstisch wird nur eine Beugung-Hebung des gestreckten Mittelfingers ermöglicht. An seinem Endglied wird ein 2-kg-Gewicht mit einer Lederschlinge befestigt. Dieses Gewicht soll durch Beugung des Mittelfingers in der Zeiteinheit (je Sekunde) maximal gehoben werden. Die Hubhöhe sowie Hubzahl können durch Zeiger auf die rotierende Trommel übertragen und so registriert werden bis zur völligen Ermüdung des betreffenden Muskelbündels (Musculus flexor und extensor digiti III). STANGL[1]; STANGL, JOCHUM, KWERCH[2]; STANGL[3].

Zu analogen Ergebnissen kam GOFFART[4], der die muskelaktive Wirkung des Adrenochroms in Form seines Monosemikarbazonderivates (*Adrenoxyl*) am Rattenzwerchfell nachwies, und CONTAMIN und PARROT[5] am isolierten Froschmuskel. Sie berichten nicht nur über die deutliche Kontraktionsverstärkung des arbeitenden Muskels, sondern letztere wollen auch eine Sympathikuserregung entsprechend der unter dem Namen Orbeli-Phänomen bekannten klassischen Adrenalinwirkung festgestellt haben.

[1] Herrn Professor Dr. HANS VON EULER, Stockholm, zum 80. Geburtstag.

[2] D. E. GREEN und D. RICHTER, Biochem. J. *31*, 596 (1937).

[3] F. BRACONIER, H. LE BIHAN et C. BEAUDET, Arch. internat. Pharm. Ther. *69*, 181 (1943).

[4] Für die freundliche Überlassung der Versuchsmengen des Präparates *Adrenoxyl* sei den Herstellern, Labaz AG. (Basel, Brüssel, den Haag, Paris), bestens gedankt.

[5] P. FISCHER und J. LECOMTE, J. Rev. Med. Liège *6*, [9], 224 (1951).

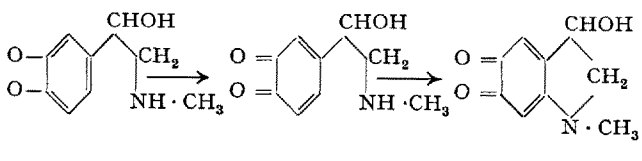
[1] E. STANGL, Praxis (Schweiz) *35*, 713 (1951).

[2] E. STANGL, H. JOCHUM und H. KWERCH, Praxis (Schweiz) *3*, 46 (1952).

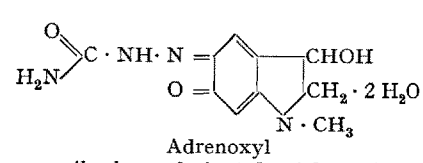
[3] E. STANGL, Ther. Umsch. *4/5*, 64 (1953).

[4] M. GOFFART, Soc. Belge Biol. 1020 (séance 30. avril 1949).

[5] F. CONTAMIN und J. L. PARROT, C. r. Soc. Biol., 28. Mai 1949.



Adrenalin Adrenalin-Chinon Adrenochrom



Adrenoxyl
(Monosemikarbazonderivat des Adrenochroms)