zyten in unmittelbarer Weise vom ATP abhängt[6]. Das anorganische Phosphat steigt beim Verschwinden der organischen Phosphorverbindungen entsprechend an.

$$Q = \frac{\text{meq Na/l}}{\text{meq K/l}}$$

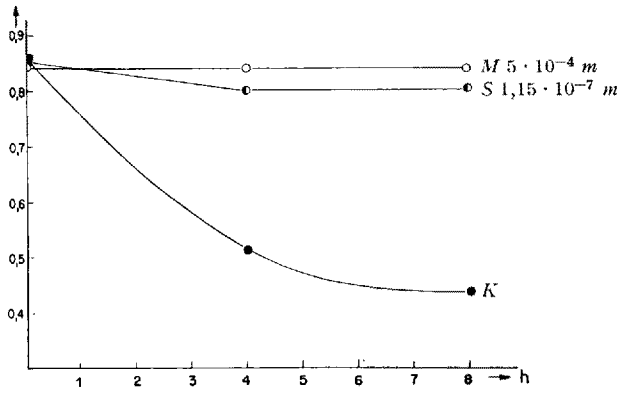

Abb. 3. Hemmung des aktiven Kationentransportes bei Inkubation von «Kälteblut» (gleiches Blut wie Versuch der Abbildung 1, 6 Tage bei 4°C aufbewahrt). S = k-Strophanthosid $1{,}15 \cdot 10^{-7}$ m (10^{-7} g/ml). Übrige Angaben siehe Abbildung 1.

Bei Inkubation von «Kälteblut» (Abb. 3) wird die normale Kationenverteilung zwischen Zellen und Serum durch aktiven Transport wieder hergestellt, das heisst, der anfänglich hohe Quotient Q der relativ natriumreichen und kaliumarmen «Kälteerythrozyten» fällt im Laufe der Inkubation ab und nähert sich wiederum dem «Normalwert». In Gegenwart von MJE oder k-Strophanthosid bleibt dieser Abfall von Q aus. Auch beim «Kälteblut» lässt sich keine Wirkung des Herzglykosids auf den Phosphatstoffwechsel nachweisen (Tab. II): ATP verschwindet wiederum nur in den MJE-Ansätzen. Die «Kälteerythrozyten» enthalten weniger ATP und Adenosindiphosphat (ADP), hingegen mehr 2,3-DPGS und anorganisches Phosphat. Ebenso nimmt der Gehalt von 2,3-DPGS bei Zusatz von MJE schneller ab.

MJE und Herzglykoside greifen also an zwei ganz verschiedenen Stellen in den Ablauf der Reaktionen ein, welche dem aktiven Transport zugrunde liegen. Die vorliegenden Befunde weisen darauf hin, dass die Hemmung des aktiven Kalium-Natrium-Austausches durch Herzglykoside nicht auf einer Blockierung energieliefernder Vorgänge, sondern auf einer direkten Wirkung auf den Membrantransportmechanismus, das «Carrier-System», beruht.

Beim Abschluss der Arbeit erhielten wir Kenntnis vom Autorreferat von R. WHITTAM für das Programm der Englischen Physiologentagung vom 29. bis 30. März 1957, auf welcher er über ähnliche Untersuchungsergebnisse berichtet hat.

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Summary

Inhibition of active cation transport through the red cell membrane by cardiac glycosides is not accompanied by alteration of phosphate metabolism (ATP, ADP, 2,3-diphosphoglyceric acid) in contrast to the action of iodoacetate. This fact suggests that the cardiac glycosides act directly on the membrane transport mechanism.

[6] G. GARDOS, Acta physiol. Acad. Sci. Hungar. *6*, 191 (1954).

Studies of Lipid Extracts from Red Blood Cells

A lipid extract obtained from Rh positive human red blood cells was reported by the writer in 1947[1] and other findings have been published since[2]. Recently a study[3] of the specificity of such an extract was made by means of quantitative nitrogen assays of Rh antibody adsorbed on to the lipid particle. It has been suggested[4] that the amount of specific substance produced by the methods employed is so slight as to be negligible. Since other findings tend to refute this[5], it was resolved to investigate use of identical methods with other than human red cells in order to see if comparable specific material could be derived by the same techniques.

Accordingly, it was decided to choose for this purpose beef red cells and to attempt to extract from them the lipid-carbohydrate complex which has been characterized[6] as the antigen reacting with antibody produced in the human as a result of infectious mononucleosis. The use of beef rather than sheep cell extractives rules out the Forssman antigen, which complicates the picture in red cell agglutination tests with antibody from infectious mononucleosis patients. The surface blood cell antigens have been described[7] as chemically similar to the heterophile antigens. Therefore, methods of extraction and assay for the one might prove valid for the other. Although the main objective was to test the value of the extraction method, it was hoped also that an assay using complement fixation technique might be derived which would detect the presence of heterophile antibodies earlier in the course of disease than is now possible.

BARNARD[8] has confirmed the specificity of the hapten extracted from human red cells. This hapten was assayed by him, as well as in this laboratory, through complement fixation techniques. The sample studied by BARNARD was prepared by the writer using techniques reported elsewhere[3]. Beef cells were treated in an identical manner, i.e., one volume of fresh cells is precipitated by two volumes of 95% ethyl alcohol. This is allowed to stand over night at room temperature, then the precipitate is separated by filtration and added to two volumes of dichloromethane. This is mixed and kept at room temperature for 6 h or longer. The liquid is evaporated, leaving a lipid fraction.

The residue is dissolved in a proportion of 100 mg lipid per 1 ml alcohol. This is stable for several months at room temperature. For a complement fixation assay,

[1] B. B. CARTER, Amer. J. clin. Path. *17*, 646 (1947).
[2] B. B. CARTER, J. Immunol. *61*, 79 (1949).
[3] B. B. CARTER, Exper. *12*, 150 (1956).
[4] C. HOWE and R. RUSTIGIAN, J. Immunol. *64*, 505 (1950).
[5] C. J. EHRENBERG, J. Lancet *75*, 275 (1955). – E. MAYR, Geburtsh. Frauenheilk. *11*, 239 (1951). – J. W. GOLDSMITH, Amer. J. Obst. Gyn. *59*, 172 (1950).
[6] J. TOMCSIK and H. SCHWARZWEISS, Proc. Soc. exp. Biol. *69*, 562 (1948).
[7] P. L. CARPENTER, *Immunology and Serology* (W. B. Saunders, Philadelphia 1956), p. 171.
[8] R. L. BARNARD, 5th Int. Congr. Blood Transfusion (1955), p. 85.

the extract is further diluted with alcohol to give a 1:100 dilution. Complement fixation techniques were set up, using both 100% and 50% end-points. The former technique was that described by KOLMER[9] and the 50% end-point method was after KABAT and MAYER[10]. For the tests the beef cell antigen was diluted with 0·9% buffered saline to 1:1000, 1:2000, 1:4000, and 1:8000. These dilutions were tested for hemolytic and anti-complementary properties.

Heterophile antibody was provided by serums from patients suffering or convalescing from infectious mononucleosis. To test specificity, serums from individuals suffering from other diseases, as well as those from apparently normal individuals, were included. Titrations of amboceptor and complement were made according to standard techniques. For the 100% end-point, 0·2 ml serum stood for 10 min with 0·5 ml appropriate antigen dilution, 1 ml complement according to titer was added and, with suitable controls, the tests were kept at 4°C for 18 h. Then they were kept at 37°C for 10 min and to each test was added 1 ml sensitized 2% sheep red cells. A previous sensitization with an excess of amboceptor obviates possible competition between heterophile antibody and amboceptor for the sheep cells.

For the tests with a 50% end-point, 3, 6 and 12 units of complement were incubated for 4 h at 4°C with immune serum plus antigen, sensitized cells were added and results read after 15 min incubation at 37°C, on the basis of VON KROGH's[11] equation: if a given serum permits 20% haemolysis under the conditions of the test, how much complement would be required to produce 50% haemolysis with the same serum?

35 serums from patients diagnosed as suffering or convalescing from infectious mononucleosis were tested in complement fixation reactions with the lipid fraction from beef red cells as antigen. These gave what appeared to be a specific fixation of complement. 15 supposedly normal serums were tested in the same manner and gave negative reactions. 15 serums from persons diagnosed as suffering from a variety of ills were tested also and gave negative results, with the exception of one serum from a person supposedly ill with viral hepatitis. This gave a weakly positive reaction with both the 100% and 50% end-point methods. It may be that here was a cross reaction or possibly the patient had infectious mononucleosis.

In 10 of the 35 infectious mononucleosis cases, it was possible to obtain serum before the convalescent period. Tentative diagnosis had been made on the appearance of the blood smear; the serum was still negative. An attempt was made to demonstrate heterophile antibodies in each serum before they were demonstrable by the PAUL-BUNNELL reaction[12]. In this preliminary assay, the effort failed. Heterophile antibodies were not demonstrable by complement fixation prior to positive reactions with the agglutinating tests. There seemed to be a parallelism in the results with the two techniques. This was a disappointment because absolute diagnosis of infectious mononucleosis at present is a matter of hindsight. The complement fixation reaction as investigated here did not seem to alleviate this situation.

Nevertheless, it would seem that the production of heterophile antigen from beef red cells and its employment in a complement fixation test is both practicable and profitable using the methods outlined above. Since the techniques are identical with those used to obtain fractions from Rh positive human red cells, which appear to react specifically with anti-Rh serums, it would seem to be an easily applied method for obtaining lipid haptens from various biological materials. This inference could have extensive therapeutic as well as experimental implications.

BETTINA B. CARTER

Western Michigan University, Kalamazoo, Michigan, April 23, 1957.

Zusammenfassung

35 Sera von Patienten mit infektiöser Mononukleose (10 Kranke, 25 Rekonvaleszenten), 15 Sera von Patienten mit anderen Krankheiten und 15 normale Sera wurden im Komplementbindungstest geprüft, wobei als Antigen ein Lipoidextrakt von Rinder-Erythrozyten verwendet wurde. Sera von Rekonvaleszenten nach infektiöser Mononukleose zeigten eine spezifische Komplementbindung, während bei Patienten vor der Rekonvaleszenz die heterophilen Antikörper im Komplementbindungstest nur dann nachgewiesen werden konnten, wenn die Paul-Bunnellsche Reaktion auch positiv ausfiel.

Activation de la phosphatase acide par l'acide β-indolylacétique et ses répercussions sur la phosphorolyse de l'amidon

Nous avons récemment montré que l'acide β-indolylacétique (hétéroauxine) aux concentrations phytopathologiques de 10^{-3}–10^{-4} *M* stimule l'activité phosphatasique acide d'extraits de pomme de terre et de racine de maïs[1]. Nous avons retrouvé une telle interaction dans les tissus hypertrophiés des Euphorbes castrées par *Uromyces*[2].

Il nous restait donc à confirmer ces premières données avec de la phosphatase acide purifiée. Les essais rapportés ci-dessous ont été réalisés avec de la phosphatase acide de germe de blé (produit Light).

La méthode et les procédés d'analyse ont été décrits ailleurs[1]. Nous avons simplement remplacé l'extrait phosphatasique brut par 0,5 ml d'une solution à $^1/_{4000}$ de phosphatase pure dans l'eau distillée. L'activité glycérophosphatasique a été mesurée à 25 et 37°, en tampon acétate à pH 5,1 (optimum phosphatases type II[3]).

La Figure résume nos résultats obtenus en présence de différentes concentrations d'acide β-indolylacétique (ABIA).

Ces essais ont donc permis de confirmer la nette activation de la fonction hydrolysante de la phosphatase acide végétale par l'hétéroauxine. Nous pouvons en effet

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[10] E. A. KABAT and M. M. MAYER, *Experimental Immunochemistry* (Charles C. Thomas, Springfield, Ill., 1948), p. 176.

[11] A. B. WADSWORTH, *Standard Methods* (Williams and Wilkins, Baltimore 1939), p. 238.

[12] J. R. PAUL and W. W. BUNNELL, Amer. J. med. Sci. *183*, 90 (1932).

[1] G. TURIAN, Biochim. biophys. Acta *21*, 388 (1956).

[2] G. TURIAN, Phytopath. Z. *28*, 275 (1957).

[3] J. ROCHE et J. COURTOIS, Exposés ann. Biochim. méd. *4*, 219 (1944).