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

---

[9] J. A. KOLMER and F. BOERNER, *Approved Laboratory Technic* (D. Appleton-Century Co.,.New York 1945), p. 674.

[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).

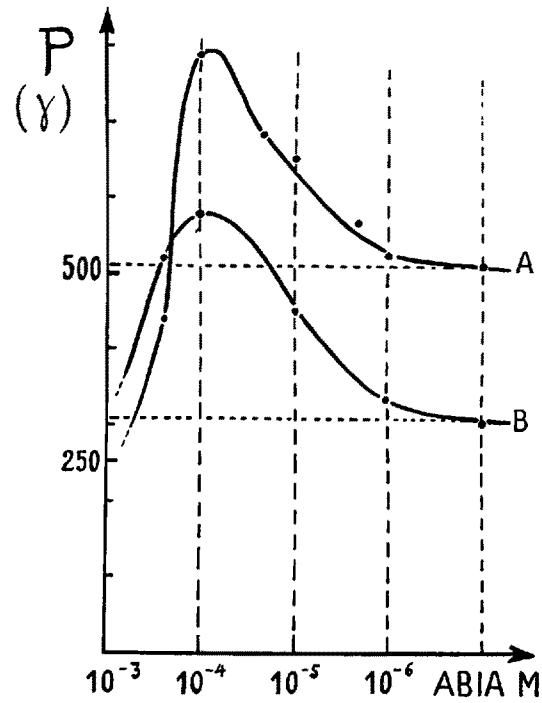
[3] J. ROCHE et J. COURTOIS, Exposés ann. Biochim. méd. *4*, 219 (1944).

calculer qu'en 30 min, à 25°, 36% du glycérophosphate de Na initial (10 mg) est hydrolysé par 1 mg de phosphatase en présence de 10^{-4} *M* d'ABIA et 19% seulement dans les témoins placés dans les mêmes conditions.

La concentration activante optima d'ABIA est plus faible pour la phosphatase purifiée (10^{-4} *M*) que pour les extraits phosphatasiques bruts (10^{-3} *M*). Cette différence relève peut-être d'une réduction du taux effectif d'ABIA par l'activité auxine-oxydasique de ces extraits bruts.

WILDMAN et BONNER[4] ont signalé la simultanéité de la présence de l'ABIA et de l'activité phosphatasique lors de leur purification de protéines foliaires d'épinard. De même, nous pouvions supposer que l'activité relativement élevée des préparations phosphatasiques témoins (Figure) dépendait de traces d'ABIA présentes dans l'échantillon de phosphatase «pure». Nos premiers essais de chromatographie sur papier, avec de la phosphatase (5 mg) soumise à une hydrolyse alcaline (NaOH *N*/100) et extraite au chloroforme, ne nous ont toutefois pas permis de déceler l'ABIA (solvent: butanol-éthanol-eau; révélateur: acide perchlorique-$FeCl_3$). Seule la méthode de détection biologique, plus sensible, permettra d'éclaircir ce point.

Notre confirmation d'une interaction ABIA-phosphatase acide végétale nous a conduit à réexaminer la question d'une intervention des auxines dans le métabolisme de synthèse et de dégradation de l'amidon[5]. L'amylase exocellulaire ne semble en effet pas être influencée par la présence de l'ABIA[6]. Par contre, une médiation phosphorée lors des transformations endocellulaires de l'amidon a été avancée par divers auteurs[7]. Nous avons alors



Activité glycérophosphatasique (à pH 5,1) de la phosphatase acide de germe de blé, en présence de diverses concentrations d'acide β-indolylacétique (ABIA): γ de P libérés par mg de phosphatase, en 30 min à 37° (*A*) et à 25° (*B*). Lignes horizontales en pointillé: activité des témoins.

	1	2	3	4	5	6	7	8	9
Amidon, 0,5 ml sol. 0,5% en tampon phosphate pH 5,4	+	+	+	+	+	+	+	+	+
«Phosphorylase», 0,2 ml	0	+	+	0	0	+	+	+	+
Phosphatase acide, 0,5 ml sol. $^1/_{2000}$	0	0	0	+	+	+	+	+	+
H_2O distillée (ml)	1,0	0,8	0,8	0,5	0,5	0,3	0,3	0,3	0,3
H_2O alcoolisée à 20% (ml)	1,0	1,0	0	1,0	0	1,0	0,75	0,50	0
ABIA 10^{-3} *M*, en sol. H_2O alcoolisée à 20% (ml)	0	0	1,0	0	1,0	0	0,25	0,50	1,0
Résultats: (estimation culot bleu noir à bleu violacé)	++++	+++	++	++(+)	++	+(+)	+	0	0

Volume réactif total = 2,5 ml. Concentrations d'ABIA = 10^{-4} *M*, $2 \cdot 10^{-4}$ *M* et $4 \cdot 10^{-4}$ *M*.

pensé que l'accélération de la dégradation de l'amidon en présence d'ABIA pourrait résulter de l'activation hormonale de la fonction hydrolysante de la phosphatase (selon schéma plus bas). L'expérience suivante a permis de confirmer cette représentation.

Une série de petits tubes à essais contenant chacun 0,5 ml d'amidon soluble à 0,5% (sol. tampon pH 5,4), en présence ou en absence d'un extrait de germe de pomme de terre (source de phosphorylase), de phosphatase acide et d'ABIA, est incubée à 37° pendant 36 h. La durée d'incubation précédant la révélation de l'amidon résiduel par le réactif de Lugol (I_2 + IK) a été fixée à la suite de divers essais préliminaires qui nous ont permis de constater qu'elle fournit la meilleure réaction différentielle, c'est-à-dire la disparition complète de l'amidon dans le mélange le plus actif (Tableau).

Il ressort de ce tableau que la dégradation de l'amidon est la plus complète en présence du mélange «phosphorylase»-phosphatase acide-ABIA. On peut donc admettre que l'ABIA contrôle le métabolisme endocellulaire de l'amidon grâce à son rôle de régulateur de l'activité phosphatasique acide dans les tissus végétaux, selon le schéma suivant:

Amidon $\rightleftharpoons$ glucose-phosphate $\rightleftharpoons$ glucose + phosphate

phosphorylase *phosphatase*

↑

ABIA

[4] S. G. WILDMAN et J. BONNER, Arch. Biochem. *14*, 381 (1947).

[5] M. K. BRAKKE et L. G. NICKELL, Arch. Biochem. Biophys. *32*, 28 (1951). – S. C. BAUSOR, Bot. Gaz. *104*, 115 (1942).

[6] P. E. PILET et G. TURIAN, Bull. Soc. vaud. Sci. nat. *65*, 403 (1953).

[7] H. WANNER, Ber. schweiz. bot. Ges. *62*, 205 (1952). – F. M. GEROLA et E. VACCARI, Rend. Ist. Lombardo Sci. Lett. *88*, 575 (1955). – R. FUJII, Bot. Mag. Tokyo *69*, 34 (1956).

Nous remercions M. le Prof. F. CHODAT de ses précieux conseils. Ces recherches sont subventionnées par le Fonds national suisse de la Recherche scientifique.

G. TURIAN

Institut de Botanique générale, Université de Genève, le 21 mai 1957.

Summary

IAA-induced activation of purified acid phosphatase is reported and shown to determine an acceleration of phosphorolysis of starch.

Some Relationships Between the Pancreatic β-Cells and the Metabolism of the Epiphyseal Cartilage

I. *Cartilage ATP Concentration of Young Alloxan Diabetic Rats*

DE MOOR[1] and MARTINETTI, and ANDREANI[2] demonstrated that insulin deficiency provoked by alloxan diabetes produces, besides other biochemical and physiological alterations, a diminution of the body weight and of skeletal growth.

As the epiphyseal cartilage participates to the growth of the bone in length, and as in enchondral ossification the participation of the energetic phosphoric bonds is considerable (*i.e.* ATP, see CARTIER[3]), the author wishes to study whether the diminished growth observed in alloxan diabetic rats is accompanied in epiphyseal cartilage by a reduced concentration of ATP.

Albino male rats, Italicus strain, 50 days old, were divided into two groups. In the first group, after 12 h fasting, alloxan diabetes was provoked (Merck alloxan 200 mg/kg intraperitoneally); the second, which was the control group, was not treated. Ten days after alloxan treatment, and after 12 h fasting, blood sugar was measured according to NELSON[4], and in the diabetic rats (blood sugar 2·8–4·9 g/1000 cm³), analogously to those of the control group, body weight and blood sugar were controlled every 5th day for 20 days. Thirty days after the alloxan injection, having controlled body weight and blood sugar, all diabetic and normal rats, 12 h fasted, were anesthesized with Nembutal (5 mg/100 g body weight) and then killed by decapitation. The anterior and posterior limbs were removed as rapidly as possible, and placed in a beaker containing acetone and dry ice: the epiphyseal cartilages were weighed, homogenized with quartz sand and CCl_3COOH 10% at 0°C. After centrifugation, to the supernatant fluid, neutralized to pH 8·2, barium acetate was added. In the precipitate (barium insoluble fraction) ATP was measured after LE PAGE[5]. P was dosed according to the BEREMBLUM and CHAIN method[6]. It is necessary to emphasize the importance in such experiments of choosing rats of the same age: as a matter of fact, in a preliminary research, I observed that the ATP concentration of the epiphyseal cartilage shows, even for difference in their ages of 15–20 days, statistically significant variations. The decrease with age in ATP concentration in ossifiable cartilage is to be attributed to a progressive decrease during the growth of the intensity of the osteogenic power. Analogous observations, relative to the enchondral ossification, were made by ZAMBOTTI[7] on the behaviour of the cocarboxylase and β-glucuronidase, and by LORENZI on the behaviour of concentration of pyruvic acid[8].

The results obtained can be summarized as follows:

(1) The alloxan diabetic rats showed a remarkable diminution of the body weight with a check of growth (mean body weights at the start = 60 g; 30 days after alloxan treatment = 30 g. In the normal rats, the body weight increased from 60 g to 160 g in 30 days).

(2) The ATP concentration in epiphyseal cartilage of alloxan diabetic rats decreased by about 40% relative to normal rats (epiphyseal cartilage of normal rats = 20 mg P/ATP/100 g of wet tissue; epiphyseal cartilage of diabetic rats = 12 mg P/ATP/100 g of wet weight).

Such results clearly show that in alloxan diabetes the inhibition of skeletal growth is accompanied with a diminution of the ATP concentration in the epiphyseal cartilage.

To control whether the decreased ATP concentration in epiphyseal cartilage of alloxan diabetic rats can be attributed exclusively to the insulin deficiency, a group of albino male rats (of age and strain identical to those used in previous experiments) were treated with insulin (Zn-insulin protaminate 4 U.I./kg daily subcutaneously), ten days after alloxan treatment (blood glucose 2·8–4·8 g/1000 cm³). The results showed that in insulin-treated diabetic rats, hyperglycemia was much less intense than in untreated diabetic rats. In addition, 30 days after alloxan injection, the body weight was seen to be the same as that of the normal rats (*i.e.* 160 g) as was also the ATP concentration in epiphyseal cartilage (mg 20 P/ATP/100 g wet weight).

Therefore, in alloxan diabetic rats, both the decreased ATP concentration and the diminished osteogenic power of the epiphyseal cartilage, are to be referred exclusively to the insulin deficiency.

It may also be suggested that insulin plays an important role in regulating enchondral ossification, especially through its marked control of the multienzyme system which pertains in ATP metabolism and consequently in all biochemical reactions which are dependent on ATP (i.e. biosynthesis of glycogen from glucose; biosynthesis of cocarboxylase and chondroitin sulphate, etc.). However, this hypothesis requires further evidence.

These experimental results, besides explaining one of the mechanisms by which insulin influences enchondral ossification, are in good agreement with CARTIER's *in vitro* findings: the primary importance of ATP for normal behaviour in the osteogenic process[3].

E. BARBIERI

Institute of Clinical Orthopedics G. Gaslini, University of Genoa (Italy), April 23, 1957.

[1] P. DE MOOR, *Exper. Diab.* (Ed. Masson, Paris 1953).

[2] R. MARTINETTI and G. ANDREANI, Boll. Soc. ital. Biol. sper. *23*, 582 (1947).

[3] P. CARTIER, Exposés Ann. Biochim. Méd. *14*, 73 (1952).

[4] N. NELSON, J. biol. Chem. *153*, 375 (1944).

[5] G. A. LE PAGE, *Manometric Techniques and Tissue Metabolism* (Burgess Publishing Co., ed., 1949), p. 185.

[6] I. BEREMBLUM and E. CHAIN, Biochem. J. *32*, 295 (1938).

[7] V. ZAMBOTTI, Il Farmaco, ed. scient. *10*, 1017 (1955). – V. ZAMBOTTI and G. L. LORENZI, Boll. Soc. ital. Biol. sper. *29*, 1953).

[8] G. L. LORENZI, Minerva Ortopedica *7*, April 1956.