activity of phosphorylase-a. He observed, however, that in the case of phosphorylase-b, produced by the hydrolysis of the enzyme, this effect did not occur[7].

From these facts it may be concluded that either ACTH itself must be of a high molecular weight to be able to form a protamine complex, or protamine does not directly effect ACTH but exerts the complex-forming and *in vivo*-inhibiting effect by means of the carrier protein of ACTH which is hydrolyzed in the case of our peptide type materials. The investigations will be reported in detail elsewhere.

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Zusammenfassung

Der Mechanismus der Verhinderung des ACTH-Effektes durch Protaminsulfat wurde untersucht. Papierchromatographisch konnte festgestellt werden, dass beide Substanzen einen Komplex bilden und infolgedessen ACTH seine Aktivität verliert. Während die proteinartigen ACTH-Präparate starke komplexbildende Eigenschaft besitzen, vermögen die peptidartigen Präparate nur in geringem Masse Komplexe zu bilden. Dies konnte auch durch *in-vivo*-Verhinderungsversuche (Sayers test) mit beiden Präparaten bestätigt werden.

[7] E. G. KREBS, Biochim. biophys. Acta *15*, 508 (1954).

Etude de l'activité adénosinetriphosphatasique et hexokinasique de cristallins de lapins soumis à une irradiation locale par les rayons X*

Dans le cadre de nos recherches sur les effets biochimiques des radiations, nous avons étudié l'action des rayons X sur l'activité hexokinasique et adénosinetriphosphatasique (ATPasique) du cristallin de lapin.

L'œil droit était soumis à une irradiation locale. (Dose: 1400 r. Tension: 220 kv. Intensité: 12 mA. Débit: 70 r/min. Filtre: 3 Al. Distance foyer-peau: 30 cm. Surface de localisation: 20 mm.) L'œil gauche était protégé et son cristallin servait de témoin. Après le sacrifice des animaux par saignée, les cristallins immédiatement prélevés ont été refroidis et les activités enzymatiques étudiées par des méthodes manométriques décrites ailleurs[1]. Les résultats de nos essais sont consignés dans les Tableaux I et II.

Il résulte de l'examen du Tableau I que l'activité hexokinasique est considérablement abaissée à la suite de l'irradiation et cette réduction persiste pendant longtemps. Ces résultats sont en accord avec ceux obtenus au niveau de la peau par MANDEL et RODESCH[2]. Ainsi contrairement à ce qui a été observé avec de l'hexokinase irradiée *in vitro*[3], cet enzyme s'avère très sensible aux rayons X.

Tableau I

Activité hexokinasique de cristallins normaux ou soumis à une irradiation locale par les rayons X chez le lapin

Expérience n°	Traitement	Durée jours	μM CO_2 dégagées en 20 min/100 g de poids frais	Différence en %
1	non	–	52	– 77
	1400 r	2	12	
2	non	–	112	– 67
	1400 r	2	37	
3	non	–	77	– 100
	1400 r	2	0	
4	non	–	102	– 88
	1400 r	4	12	
5	non	–	87	– 37
	1400 r	4	55	
6	non	–	50	– 70
	1400 r	4	15	
7	non	–	75	– 87
	1400 r	4	10	
8	non	–	145	– 31
	1400 r	103	100	
9	non	–	115	– 52
	1400 r	108	55	
10	non	–	55	– 100
	1400 r	113	0	
11	non	–	55	– 81
	1400 r	113	10	

Tableau II

Activité adénosinetriphosphatasique de cristallins normaux ou soumis à une irradiation locale par les rayons X chez le lapin

Expérience n°	Traitement	Durée jours	μM CO_2 dégagées en 30 min/100 g de poids frais	Différence en %
1	non	–	85	+ 254
	1400 r	2	300	
2	non	–	235	+ 64
	1400 r	2	380	
3	non	–	145	+ 45
	1400 r	2	210	
4	non	–	270	0
	1400 r	3	270	
5	non	–	230	+ 73
	1400 r	3	395	
6	non	–	150	+ 90
	1400 r	4	285	
7	non	–	280	+ 18
	1400 r	4	330	
8	non	–	175	– 14
	1400 r	7	150	
9	non	–	225	+ 8,5
	1400 r	8	250	
10	non	–	170	+ 77
	1400 r	65	300	
11	non	–	260	+ 2
	1400 r	68	265	
12	non	–	235	+ 68
	1400 r	69	395	
13	non	–	265	+ 9,5
	1400 r	70	240	

* Travail effectué avec le concours matériel de l'Institut National d'Hygiène.

[1] P. MANDEL, C. A. QUARANTA et Mme. M. L. SCHMITT, Bull. Soc. Chim. biol. *38*, 139 (1956) et *37*, 847 (1955).

[2] P. MANDEL et J. RODESCH, J. Physiol. *47*, 234 (1955).

[3] E. S. G. BARON, S. DICKMAN, J. A. MUNTZ et T. P. SINGER, J. gen. Physiol. *32*, 537 (1949). – E. S. G. BARRON, Ann. N. Y. Acad. Sci. *59*, 574 (1955).

L'activité ATPasique est accrue entre le 2e et le 4e jour après l'irradiation, à une exception près (Tableau II). Au-delà du 4e jour, les différences ne sont pas homogènes. L'augmentation de l'activité ATPasique constatée les premiers jours après l'irradiation est en accord avec les faits observés par ASHWELL et HICKMANN[4] et VAN BEKKUM[5] au niveau de la rate chez la souris blanche et par DUBOIS et PETERSEN[6] au niveau de la rate et du thymus chez le rat et la souris mâle. Cette majoration de l'activité ATPasique pourrait être rapprochée d'un accroissement similaire mis en évidence dans le cristallin d'animaux diabétiques[7]. Nous l'avons interprété dans ce cas comme une adaptation enzymatique tendant à contrebalancer certains blocages de la dégradation des glucides. Il pourrait en être de même dans les suites après irradiation. Notons encore que le comportement des deux enzymes étudiés *in vivo* s'avère nettement différent de celui *in vitro*. Dans ce dernier cas, on a signalé en effet que l'hexokinase résiste mieux aux rayons X que l'ATPase.

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Summary

The irradiation of the lens by a simple dose of 1400 r, gives a very important and lasting reduction of its hexokinase activity. Its ATPase activity shows an increase during the first four days and a return to normal again. The variations observed here (that is *in vivo*) are different from the ones noted after irradiation of these enzymes *in vitro*.

[4] G. ASHWELL et J. HICKMANN, Proc. Soc. exper. Biol. Med. *80*, 407 (1952).
[5] D. W. VAN BEKKUM, Biochim. biophys. Acta *16*, 437 (1955).
[6] K. P. DUBOIS et D. F. PETERSEN, Amer. J. Physiol. *176*, 282 (1954).
[7] P. MANDEL, Mme. M. L. SCHMITT et C. A. QUARANTA, C. r. Acad. Sci., Paris *242*, 193 (1956).

ATPase Activity of Guinea Pig Heart Muscle

The occurrence of several different types of enzymes splitting ATP in animal tissue has been recently described. Biochemical differences among those enzymes concern principally pH optimum, stimulating cations and reaction products. Quite recently, HEPPEL and HILMOE[1] have succeeded in separating three different ATPases from bull seminal plasma. One of these enzymes hydrolyzes ATP to AMP and PP. It is relatively heat-stable, has pH optimum between 8·4 and 8·8 and requires neither Ca^{++} nor Mg^{++} for full activity. The second ATP-splitting enzyme produces *ortho* P and ADP from ATP and has an acid pH optimum. It is completely destroyed by heating at 60°C for 20 min, requires Mg^{++} and is inhibited by Ca^{++}. The third enzyme is a relatively heat-stable alkaline phosphatase which releases *ortho* P from ATP and is more stimulated by Ca^{++} than by Mg^{++}.

[1] L. A. HEPPEL and R. J. HILMOE, J. biol. Chem. *202*, 217 (1953).

The importance of ATP and ATPase in muscular contraction has been particularly emphasized in recent years (SZENT-GYÖRGYI[2], MOMMAERTS[3], NEEDHAM[4]) Some features of muscular ATPase have been then extensively studied. All the above-mentioned authors agree that muscular ATPase shows two pH optima for enzymic activity: the first one is near pH 6·5; the second is much stronger and develops in the presence of Ca^{++} at pH 9·2. Ca^{++} acts as an activator at both pH values; the action of Mg^{++} is, by contrast, still a matter for discussion. BANGA and SZENT-GYÖRGYI[5] have discovered that pure myosin and actomyosin with lower actin contents are always inhibited by Mg^{++}, whereas actomyosins with higher actin contents can be activated.

The purpose of the present investigation was to study the characteristics of ATPase from guinea pig heart muscle. In order to establish it there are differences in enzymic activity in regions of the heart provided with different functions, ATPase activity of both ventricles and atria were studied separately.

Methods.—Male guinea pigs weighing 250–300 g and fed on a standard diet were used. The animals were killed by bleeding and the heart was immediately removed and transferred into the cold room at 2°C. Both ventricles and atria were separated and weighed. 1% homogenates were then prepared by grinding the minced tissues in a Potter-Elvehjem homogenizer with a leucite pestle. 0·067 *M* borate buffer was used as a suspension medium.

ATPase activity was determined according to the method of DUBOIS and POTTER[6] with some modifications described elsewhere[7]. Two pH optima indicated by MOMMAERTS (6·5 and 9·2) were particularly investigated. Incubation temperature was 37°. ATP sodium salt was prepared in solution from dibarium salt supplied by SCHWARZ. Its final concentration was 0·01 *M*. The values reported in the Tables are given as µg P/mg N. P. determinations were made according to the method of FISKE and SUBBAROW[8] and N determinations by the usual microkjeldhal technique. The values obtained were analyzed statistically, standard deviation and the "t" test of STUDENT-FISHER being calculated for each average.

Results.—In a first group of experiments the normal values of ATPase of atria and ventricle homogenates of guinea pig heart were established. ATPase activity was studied without the addition of activating cations at two pH optima 6·8 and 9·2. As shown in Table I, ATPase activity is much stronger at pH 9·2 than at pH 6·8, as the enzyme from skeletal muscle does. No remarkable differences in activity of different parts of the heart were noticed at pH 9·2. At pH 6·8 the activity of the atria is, however, stronger than that of the ventricles.

In a second group of experiments the influence of Ca^{++} and Mg^{++} was studied. The results are represented in Fig. 1 and 2. It is clear that the action of both ions depends upon their concentration and that the activity maximum corresponds always to a low concentration, whereas at

[2] A. SZENT GYÖRGYI, *Chemistry of Muscular Contraction* (Academic Press, New York, 1950).
[3] W. E. H. M. MOMMAERTS, *Muscular Contraction* (Interscience, New York, 1950).
[4] D. M. NEEDHAM, *The Biochemistry of Muscle* (Methuen, London, 1953).
[5] I. BANGA and A. SZENT GYÖRGYI, Stud. Inst. med. Chem. Szeged *1*, 5 (1942).
[6] K. P. POTTER and V. R. DUBOIS, J. biol. Chem. *150*, 185 (1953).
[7] M. A. MOR, Exper. *9*, 9 (1953).
[8] C. H. FISKE and Y. SUBBAROW, J. biol. Chem. *66*, 375 (1925).