Zusammenfassung

Die Eigenschaften des Plättchen-Ac-Globulins wurden mit Rücksicht auf die Thromboplastinbildung untersucht.

Investigations on the ACTH-Protamine complex

One of the authors reported that protamine sulphate could, to a high degree, inhibit the action of ACTH on adrenal ascorbic acid and on the involution of the thymus[1]. In the paper concerned it was mentioned that to clarify the mode of action of protamine in this respect, the authors have carried out electrophoretic and paper chromatographic investigations. The results of these investigations are as follows:

Methods. (1) In the electrophoretic investigations the materials were run on Macherey-Nagel paper No. 214 with a stabilized direct current of 4–10 V/cm voltage and of 2–4 mA intensity for 8–16 h. An acetate buffer of pH 4·7 and of 0·1–0·03 ionic strength and a borate buffer of pH 8·6 and of 0·1 ionic strength was used. The paper strips were dyed with 0·2% acid fuchsine diluted in absolute alcohol containing 10% acetic acid. The differentiation was carried out with methanol containing 10% acetic acid and with 10% acetic acid.

(2) The paper chromatographic investigations were carried out in the case of the polypeptides in a phenol-water system, in the case of the hydrolysates in a phenol-water system or in a butanol-acetic acid-water system. The polypeptides were indicated with acid fuchsine, the amino acids with ninhydrine.

(3) The frog melanophore investigations were carried out on frogs decolorized with light, according to a method modified by the authors[2]. The sensitivity of the method renders it possible to measure the quantities of the materials eluted from the chromatograms. The main point of the method is that the darkening of the frogs (resulting from the action of ACTH or from the melanophore content of ACTH) is measured by comparison with a fixed scale of 1–10. Each determination was carried out at 3 dose levels on 10 frogs per dose. The experiments were evaluated by repeated comparison with a standard (cross-over test).

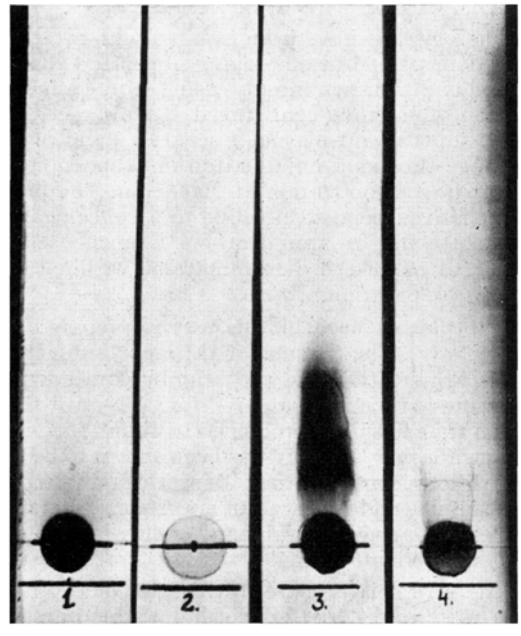
(4) The ACTH of protein type was produced according to SAYERS, WHITE and LONG[3], the peptide type material was produced by acidic hydrolysis of a material prepared according to ASTWOOD, PAYNE and RABEN[4].

(5) Pure protamine sulphate was prepared by purification of commercial protamine sulphate with oxycellulose and by precipitation with hot water. Its purity was checked by chromatographic investigations of the hydrolyzed product.

Results. In the electrophoretic investigations the diverse ACTH products split up—according to their types—into different components. Purified protamine sulphate proved to be homogenous.

In case of simultaneous running of both materials a new component appeared. A complete separation of the latter and its investigation was not possible however, since it could not be delimited from the components of ACTH.

In chromatographic investigations it was established that ACTH progressed together with the solvent, and protamine sulphate appeared as a homogenous fixed spot. In the case of simultaneous running of both materials the appearence of a new component in the form of a well defined spot was observed. The investigation of the complex-forming capacity of different ACTH types showed that in the case of materials of the protein type the greater part of the ACTH activity, in the case of hydrolyzed products only a small part of it was incorporated in the complex (Figure).



Ascending chromatograms. *1* Protamine sulphate. *2* ACTH. *3* Protein ACTH + Protamine sulphate. *4* Peptide ACTH + Protamine sulphate.

This phenomenon was indicated on the one hand by the size of the spot, on the other hand by the melanophore assay of the material eluted from the spots. By elution of the complexes with n/100 sulphuric acid it was established that the amino acids characteristic for ACTH could be found in their hydrolysates, and on the other hand the great quantity of arginine indicated the presence of protamine.

The different abilities of different ACTH preparations to form protamine complexes were also studied in investigations where the depletion of adrenal ascorbic acid (Sayers test[5]) was found to be influenced by protamine; in the case of protein type materials the effect of eightyfold quantities of ACTH could be inhibited by pretreatment with protamine, in the case of peptide type materials by only a two-threefold quantity[6].

The observations resemble those of KREBS who could —by application of salmine—essentially diminish the

[1] GY. FEKETE, Acta med. Acad. Sci. Hung. *8*, 81 (1955); Exper. *11*, 310 (1955).

[2] A. HEGYELI (in press).

[3] G. SAYERS, A. WHITE, and C. N. H. LONG, J. biol. Chem. *149*, 425 (1943).

[4] E. B. ASTWOOD, R. W. PAYNE, and M. S. RABEN, J. biol. Chem. *187*, 719 (1950).

[5] G. SAYERS, M. A. SAYERS, and L. G. WOODBURY, Endocrinology *42*, 379 (1948).

[6] GY. FEKETE, J. NURIDSÁNY, and A. HEGYELI (in press).

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.

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[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).