than that of normal lymphocytes, although the DNA values are similar in both cell categories, indicating the lack of a DNA movement in the pathological lymphocytes.

Our findings do not allow us to decide whether the decreased Feulgen/Fast green ratio in the leukemic lymphocytes is due to an absolute increase in histone, or to a modified Fast green stainability.

The biochemical observations of CRUFT *et al.*[9], indicating the abnormal properties of histones from malignant cells, seem to support the latter hypothesis.

In any case, it seems that in leukemic lymphocytes the properties of the desoxyribonucleoprotein complex are different from those of normal lymphocytes.

A detailed account of this work, with statistical analysis of the data, will be published elsewhere.

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Institute of Medical Pathology, University of Modena (Italy), May 23, 1957.

Riassunto

Gli autori hanno determinato con metodo citofotometrico nel visibile il contenuto di acido desossiribonucleico (DNA) e di proteina istonica nei linfociti di 4 soggetti normali e di 4 pazienti affetti da leucosi linfatica.

I valori del DNA sono apparsi uguali nelle cellule normali e in quelle leucemiche, i valori dell'istone sono risultati in queste ultime superiori alla norma, onde il rapporto DNA/istone risulta essere nei linfociti leucemici inferiore a quello dei linfociti normali.

[9] H. J. CRUFT, C. M. MAURITZEN, and E. STEDMAN, Nature *174*, 580 (1954).

Muscle Adenylic Deaminase

After the discovery of adenylic deaminase by SCHMIDT[1], partially purified preparations of this enzyme have been described by a number of investigators[2]. Recently, the extensive purification and crystallization of this enzyme has been reported[3]. Most methods used for the preparation of the enzyme start with procedures which are ineffective in extracting the bulk of the enzyme[2]. The bulk of the adenylic deaminase is extractable only at high salt concentrations. The effect of increased concentration of salt upon adenylic deaminase extraction from acetone powders of tissues of several species is shown in the Table. As indicated in the Table, only after reaching molarities of 0·5 *M* and higher is the bulk of the enzyme extractable. Advantage has been taken of this, and of the denaturation and/or insolubilization of other enzymes by acetone treatment, to obtain with little effort an active preparation free of easily soluble enzymes and of myokinase. The procedure described here is intended to provide a simple reproducible preparation useful for analytical purposes. The data emphasizes the high level in skeletal muscle of this enzymatic activity.

Acetone powders of tissues are prepared by a standard procedure[4]. When kept at 0° in a dessicator they are stable for over 1 year. Some purification and concentration of the enzyme is accomplished as follows: Extract rabbit muscle acetone powder with 10 vol of water w/v for 10 min. All centrifugations were carried out at 5000 × *g* and at 0°C. The ammonium sulfate solutions were saturated and adjusted to pH 7·4 at 0°C. All the steps of the fraction procedure were carried out at 0°C.

The effect of salt concentration upon the extractibility of adenylic acid deaminase

Species	Extracting medium				
	Water	0·2 *M* KCl	0·25 *M* KCl	0·5 *M* KCl	1 *M* KCl
	Units/ml	Units/ml	Units/ml	Units/ml	Units/ml
Rabbit .	185	420	2200	4300	5500
Pigeon .	340	–	777	1200	1400
Chicken .	180	–	1000	1250	1400

Centrifuge and discard the supernatant fluid. Re-extract with 10 vol w/v of 0·2 *M* KCl and discard the supernatant fluid. Re-extract with 10 vol of 1 *M* KCl w/v twice and keep the supernatant fluids after centrifugation. The combined supernatant fluids have about 2000 units[5] and 7 mg protein per ml, thus yielding more than 20 times the enzyme activity, per gram muscle, of other procedures[5]. Add to the crude KCl extract ¼ vol of 0·6 *M* disodium phosphate and ⅓ vol saturated ammonium sulfate pH 7·4; discard the precipitate and precipitate the enzyme by adding ²/₃ vol of ammonium sulfate. (All vol refer to the initial volume of the crude KCl extract.) The recovery is from 90–95% and the specific activity is increased approximately 2 fold e.g. 600–700[5]. CONWAY[6] observed that saline was more effective than water for the extraction of the enzyme, however he did not explore further the effect of high concentrations of salts. He also showed that the adenylic deaminase appeared to have a very low affinity for the substrate and he discussed the possible existence of inhibitors. Whether the adenylic deaminase present in the preparation here described possesses tightly bound inhibitors is not known. The addition of crude acetone powder extracts with little adenylic acid deaminase activity, e.g. heart, is not inhibitory; however, when comparing activity at several dilutions, we have observed an apparent higher activity at higher dilutions of the enzyme. We have observed that when using initial velocities and less than 10–20% substrate utilization, there is essentially a doubling of rate by doubling the substrate concentration between 2×10^{-5} *M* and 2×10^{-3} *M*. This is in sharp contrast with the data of NIKIFORUK and COLOWICK[5] but in agreement with the data of CONWAY and COOK[6].

From the activity measurements shown in the Table it has been calculated that at 37°, pH 6·4[5] and 4×10^{-5} *M* 5-adenosine monophosphate, a gram of muscle can deaminate over 15 micromoles 5-adenosine monophosphate/min and this figure would be higher at higher concentrations of 5-adenosine monophosphate. It ap-

[1] G. SCHMIDT, Z. physiol. Chem. *179*, 243 (1928); *208*, 185 (1932); *219*, 191 (1933).

[2] H. M. KALCKAR, J. biol. Chem. *167*, 429, 461 (1947). – G. NIKIFORUK and S. P. COLOWICK, J. biol. Chem. *219*, 119 (1956).

[3] YA-PIN LEE, Fed. Proc. *15*, 298 (1956); *16*, 210 (1957).

[4] S. GRISOLIA, in COLOWICK and KAPLAN, *Methods in Enzymology* (Academic Press Inc., 1955), vol. II, p. 350.

[5] G. NIKIFORUK and S. P. COLOWICK, J. biol. Chem. *219*, 119 (1956).

[6] E. T. CONWAY and R. COOKE, Biochem. J. *33*, 479 (1939).

pears, then, that the adenylic acid deaminase activity of muscle is as high or higher than the ATP-ase activity[7].

Acetone powders of skeletal muscle of rabbit and pigeon and chicken (breast muscle) were extracted for 10 min with 10 vol w/v of extracting medium and centrifuged. The supernatant fluids were then tested at 25° under the conditions described by NIKIFORUK and COLOWICK[5]; succinate buffer pH 5·9, and 4×10^{-5} *M* 5-adenosine monophosphate. Units are expressed as 0·001 O.D. decrease/min at 265 mμ. In addition the range of the assay was extended some 500 fold for substrate concentration by either inserting a spacer in the Beckman cells so as to reduce the light pathway from 10 mm to 1 mm and/or reading the O.D. changes at 288 mμ. At this wavelength 5-inosine monophosphate has about twice the molar extinction coefficient of 5-adenosine monophosphate. As discussed in the text, substrate saturation with crude extracts of adenylic deaminase appears to occur at extremely high concentrations. The studies at high substrate concentration were conducted to the test activities of acetone powder extracts of heart of all species included in the table. Even at high substrate concentrations we detected only very faint activity, less than $^1/_{10000}$ amount present in skeletal muscle. Also extracts of pigeon liver acetone powder contain small amounts of activity, about twice as much as heart.

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Zusammenfassung

Der grössere Teil der Muskeldeaminase und Adenylsäure kann nur bei hohen Salzkonzentrationen extrahiert werden. Die Fähigkeit des Muskels für Adenyldeamination scheint grösser zu sein als seine ATP-ase-Kapazität. Beobachtungen über die Verteilung des Enzymes und seine Kinetik werden beschrieben.

[7] W. F. H. M. MOMMAERTS, in *Phosphorus Metabolism* (Edit. by MCELROY and GLASS, John Hopkins press, Baltimore 1951), Vol. I, p. 551.

* Haskell Fellow.

** Established Investigator of the American Heart Association.

Composition du suc de décongélation du muscle strié de la grenouille

La contracture de décongélation, que manifeste pendant son dégel un muscle strié *vivant* congelé, s'accompagne de l'exsudation massive d'un liquide riche en protéines[1]. Nous avons analysé ce liquide notamment par la méthode de l'électrophorèse[2] après une dialyse de 40 h contre les solutions de tampon de phosphates sodiques (μ = 0,10, pH = 7,1) et de phosphates sodiques + NaCl (μ = 0,15, pH = 7,2) utilisées dans ce laboratoire.

Le liquide a un pH légèrement acide, compris entre 6 et 6,5. Il renferme des phosphates, sous forme inorganique (74%), d'I.M.P. (16%) et d'hexoses-phosphates (6%): la répartition de ces composés est identique dans le jus et dans la pulpe[3].

Les vitesses moyennes des protéines de notre liquide de décongélation sont en -10^{-5} cm²/volt/cm, à μ = 0,10, pH 7,1

Vitesses	*x*	*c*	*b*	*a'''*	*a''*	*a'*
Anodique . .	6,34	4,00	3,12	2,16	1,93	1,28
Cathodique .	5,73	–	2,36	1,48		0,99

et à μ = 0,15, pH 7,2

Vitesses	*x*	*c*	*b*	*a'''*	*a''*	*a'*
Anodique . .	5,34	–	2,40	1,93	1,46	1,27
Cathodique .	5	–		1,64		

La concentration en protéines (mesurée au réfractomètre plongeant, à 25°, λ = 589 mμ, I = 0,00182% prot.) oscille entre 3,5 et 4,95%. Le liquide n'est ni visqueux, ni biréfringent et ne contient aucun représentant du groupe des myosines. Il est coloré en rose par l'hémoglobine du sang présent dans les muscles malgré leur rinçage au liquide de Ringer avant congélation. Aux pH d'électrophorèse, le gradient correspondant migre lentement vers la cathode ou reste immobile.

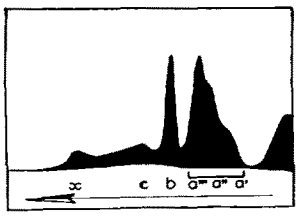

Fig. 1. Protéinogramme (compartiment anodique) d'un liquide de décongélation de muscles de grenouille après 16,300 s d'électrophorèse (μ = 0,10, pH = 7,1, champ = 5,2 volt/cm, concentration protéine = 4,95%). Les gradients *x* et *c* peu marqués sont encore repérables; les gradients *a* et *b* sont distincts, *a* étant lui-même subdivisé en ses trois fractions (*a'*, *a''*, *a'''*). A droite, l'hémoglobine se superpose au gradient salin.

Les autres protéines présentes dans le liquide de décongélation appartiennent au groupe du myogène. On distingue quatre gradients identifiables par leurs vitesses de migration (Tableau) aux gradients *a*, *b*, *c* et *x* de DUBUISSON et JACOB[4].

Ces gradients sont d'importance variable. Leur séparation (compartiment anodique) est meilleure à la force ionique μ = 0,10 (Fig. 1). Le gradient *a* est complexe et se scinde en trois composantes *a'*, *a''*, *a'''*; leur séparation reste cependant incomplète après 8 h d'électrophorèse (μ = 0,10, pH = 7,15, champ = 5 volt/cm). Le gradient *b* est le second gradient important; il dépasse le gradient *a*. Le gradient *c* est peu marqué et s'étale

[1] J. GODEAUX, Arch. int. Physiol. *58*, 299 (1950).

[2] M. DUBUISSON, A. DISTÈCHE et A. DEBOT, Biochim. biophys. Acta *6*, 97 (1950).

[3] Nous remercions vivement Mme GOSSELIN-REY qui a eu l'obligeance de faire cette détermination.

[4] M. DUBUISSON et J. JACOB, Bull. Soc. Roy. Sci. Liège 145 (*1945*).