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.

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** 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 ($\mu = 0{,}10$, pH = 7,1) et de phosphates sodiques + NaCl ($\mu = 0{,}15$, 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 $\mu = 0{,}10$, 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 $\mu = 0{,}15$, 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°, $\lambda = 589$ 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.

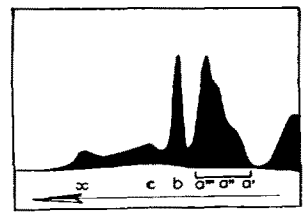

Fig. 1. Protéinogramme (compartiment anodique) d'un liquide de décongélation de muscles de grenouille après 16,300 s d'électrophorèse ($\mu = 0{,}10$, pH = 7,1, champ = 5,2 volt/cm, concentration protéine = 4,95%). Les gradients *x* et *c* peu marqués sont encore repérables; les gradients *a* et *b* 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 $\mu = 0{,}10$ (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 ($\mu = 0{,}10$, 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*).

rapidement; il peut faire défaut. Le gradient x est un peu mieux représenté et reste repérable pendant toute l'expérience.

Quand on soumet un tel extrait à la dialyse contre de l'eau distillée, il se forme un précipité abondant (25%) de globuline X et de myogène dénaturé. Les protéinogrammes indiquent que ce précipité s'est fait aux dépens de b, de a'' et de c, tandis que a', a''' et x ne sont pas modifiés (Fig. 2).

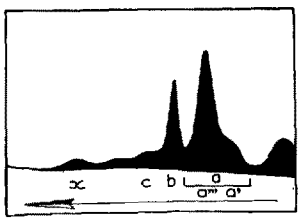

Fig. 2. Protéinogramme (compartiment anodique) du même liquide après dialyse préalable contre de l'eau distillée et élimination du précipité, puis dialyse contre un tampon phosphates sodiques –NaCl μ = 0,10, pH = 7,1 (16,400 s d'électrophorèse; champ: 5,23 volt/cm; concentration protéines: 3,72%). Si x n'a pas changé, c est nettement réduit; b a perdu une partie de ses constituants et a sa fraction moyenne a''.

En conclusion, le liquide exsudant du muscle en contracture de décongélation entraîne une quantité importante de protéines; celles-ci appartiennent au groupe des albumines et de la globuline X, à l'exclusion de tout représentant des myosines. La solution est plus concentrée que celle que l'on peut obtenir en extrayant la pulpe musculaire par une solution phosphatique.

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Summary

Because of cell membrane destruction, fluid escaping a muscle on thawing contains a large amount of proteins (myogen and globulin X). Whilst usual gradients a, b, and c are present in the fluid, the gradients d and e also occurring in extracts of unstimulated frog muscles are replaced by the gradient x already found in extracts of in N_2 exhausted muscles.

Inactivation of Catalase by Phospholipide Extracts

It was reported in a previous paper[1] that phospholipides extracted from yeast or from rat or rabbit liver form a precipitate when added to a solution of cytochrome c. The precipitate contains a large amount of cytochrome c and a phospholipide portion. The cytochrome c can be redissolved from the precipitate with ether, but its absorption spectrum is modified. Phospholipides from yeast or liver produce a precipitate also when added to a solution of iron salts such as ferrous sulphate or ferric ammoniacal citrate. It seemed then possible that phospholipide acts on the iron part of the molecule of cytochrome c. The present paper reports the inhibition *in vitro* by phospholipides of catalase, an enzyme which contains bound iron.

[1] L. MICHELAZZI, Exper. *11*, 389 (1955).

Table I
Inhibition of crystalline beef liver catalase by phospholipide extracts

Phospholipide extract added	Microliters O_2 developed
Rabbit liver lecithin fraction	0
Rabbit liver sphyngomyelin fraction	65
Yeast lecithin fraction	10
Yeast sphyngomyelin fraction	10
Rat sarcoma lecithin fraction	12
Rat sarcoma sphyngomyelin fraction	140
None	467

Each value represents the mean of 3 experiments, with the exception of the value of the controls without phospholipide added, which represents the mean of 9 experiments.

Several investigations on the inhibition of this enzyme by tissue extracts have been reported. HARGREAVES and DEUTSCH[2] have shown that tumor tissue exerts an inhibitory action on liver catalase. GREENFELD and PRICE[3] found a decrease of catalase activity in the liver of tumor-bearing animals. CERIOTTI and SPANDRIO[4] have recently described that an inhibiting action on catalase is present not only in tumor, but also in normal tissues. CERIOTTI, SPANDRIO, and BERTI[5] reported in a further publication that several amino acids are able to inhibit catalase. Phospholipides used in the present investigation were prepared from yeast, from rat or rabbit liver, or from a rat spindle cell sarcoma (Galliera sarcoma), by the same method used in the previous work. Crystalline beef liver catalase was prepared according to the method of SUMNER and DOUNCE[6]. Catalase activity was determined at 37°C with Warburg manometers, by measuring the amount of oxygen liberated in 3 min from 3 ml of a $1^0/_{00}$ perydrol (Merck) solution. The enzyme (0·2 of a $1^0/_{00}$ solution) was added from the side arm of the Warburg flasks after 7 min preincubation. In the experiments in which the influence of phospholipide extracts was to be tested, 0·1 ml of the emulsion containing 100 mg phospholipide in 5 ml water was mixed with the enzyme in the side arm of the Warburg flasks.

The results are reported in Table I. It seems clear that phospholipide extracts from rabbit liver, from rat sarcoma, or also from yeast exert a strong inhibitory action on purified catalase. Yeast sphyngomyeline fraction is more active than that prepared from rabbit liver or from rat sarcoma. In other experiments, the influence of the *in vivo* treatment with phospholipides on catalase activity of the liver and of the kidney of guinea pigs was studied.

Guinea pigs weighing 300 g fed on a semi-synthetic diet were injected in the jugular vein with a dispersion of yeast lecithin fraction containing 100 mg of this substance. The animals were then killed by bleeding, 30 h after the injection. The livers and the kidneys were

[2] A. B. HARGREAVES and H. J. DEUTSCH, Cancer Res. *12*, 720 (1952).

[3] R. E. GREENFELD and V. E. PRICE, J. biol. Chem. *209*, 363 (1954).

[4] G. CERIOTTI and L. SPANDRIO, Biochim. biophys. Acta *18*, 303 (1955); Tumori *41*, 538 (1955).

[5] G. CERIOTTI and BERTI, Biochim. biophys. Acta *23*, 362 (1954).

[6] J. B. SUMNER and A. L. DOUNCE, J. biol. Chem. *121*, 417 (1937); *127*, 439 (1939).