of variability of both electrical parameters for a given 100-Å membrane was about 10 to 15 percent and was less for thicker membranes. These parameters were approximately 250 ohm cm^2 and 0.57 μfarad/cm^2 for a 100-Å membrane.

These procedures, including diffusion potential measurements, have been applied to hundreds of membranes of thicknesses ranging from 50 to 1000 Å. In Fig. 2 are shown the typical increases in both resistance and capacitance with decreasing thickness of membrane formed from solution containing 5 mg of parlodion per milliliter of isoamyl acetate.

The resistivities vary inversely with thickness; they are about 2×10^8 ohm cm for 100–Å membranes and about 5×10^6 ohm cm for 400–Å membranes (*4*).

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References and Notes

1. P. H. Carnell and H. G. Cassidy, *J. Polymer Sci.* **55**, 233 (1961).
2. N. L. Gershfeld, *Chem. Eng. News* **38**, No. 9160 (1960); abstracts of papers presented at the 138th meeting of the Am. Chem. Soc. (Div. of Coll. Chem.), p. 3-I (1960).
3. C. D. Hodgman, *Handbook of Chemistry and Physics* (Chemical Rubber Co., Cleveland, ed. 40, 1959), p. 2524.
4. A paper giving details is in preparation. Work supported in part by grant NB-03322-02 from the National Institute of Neurological Diseases and Blindness and by grant G 22235 from the National Science Foundation.

3 May 1963

Erythrocytes: 5′-Adenylic Acid Deaminase Requirement for Ammonia or Monovalent Metal Ion

Abstract. *5′-Adenylic acid deaminase free from sodium and potassium ion is prepared from erythrocytes by a convenient method. Like adenosine triphosphatase and adenylic kinase from erythrocytes, the deaminase is activated by some monovalent cations, but unlike these enzymes it requires the presence of a monovalent cation. In all three instances the pattern of activation by ions is similar and suggests a common mechanism.*

The ability of most cells to accumulate K^+ in preference to Na^+ from a medium with higher concentrations of sodium than potassium is dependent on a process of active transport. The movement of ions against their electrochemical potential gradient requires energy supplied by cell metabolism. In recent years accumulated evidence from several laboratories has indicated a close connection between the Na^+-, K^+-activated adenosine triphosphatase of various membrane preparations and the active transport of monovalent metal ions (*1*, *2*). Evidence for such a relationship is strongest for erythrocytes, in which enzymic activities of the isolated membranes have been correlated with those of active transport in the intact cells (*2–4*). The enhancement of the activity of adenosine triphosphatase of the erythrocyte membrane requires both Na^+ and K^+, and it can be prevented by ouabain (*2*, *4*). When the membranes are broken by ultrasonic vibrations, this activity is enhanced with either Na^+ or K^+, and in this case enhancement is no longer affected by ouabain (*6*).

Preparations of erythrocyte membrane also contain an adenylic kinase which is activated by either Na^+ or K^+ (*6*). In my studies of adenosine triphosphatase and adenylic kinase, a 5′-adenylic acid deaminase was discovered in the membrane preparations. This enzyme, in contrast to the adenosine triphosphatase which is tightly bound to the membrane, can be obtained in soluble form. While adenosine triphosphatase and the adenylic kinase exhibit a basic activity even in the absence of Na^+ and K^+, the deaminase absolutely requires a monovalent ion if activity is to occur. However, the pattern of activation of the three enzymes by Na^+ and K^+ is quite similar. Potassium ion is a better activator for all, and the concentration of each ion for half-maximal activation (*Km*) is the same for the three enzymes. This similarity suggests either a close relationship between the three enzymic activities or a common mechanism of activation of three separate enzymes. In any case, studies on the properties and the mechanism of ion activation of the soluble deaminase should contribute to our understanding of the molecular basis of cation selectivity in erythrocytes.

Partially purified 5′-adenylic acid deaminase was prepared as follows: erythrocytes were obtained by centrifugation from blood-bank blood 3 to 5 weeks old and washed in saline. Packed cells were hemolyzed in ten volumes of ice-cold distilled water. The hemolyzate was allowed to stand at 4°C for 2 hours. The precipitate formed was separated from the supernatant by centrifugation at 2000*g* and washed six times with five volumes of cold distilled water. The fluffy precipitate contained the membranes of erythrocytes and some hemoglobin as was evident from its pink color. It was practically free from Na^+ and K^+, as ascertained by flame photometry tests. The precipitate was suspended in one volume of 0.08*M* tris-HCl buffer, *p*H 7.4, and stirred in cold for 1 hour. The suspension was then centrifuged at 18,000*g* for ½ hour. The supernatant was decanted. Table 1 shows the 5′-adenylic acid deaminase activity of this extract in the presence of various ions.

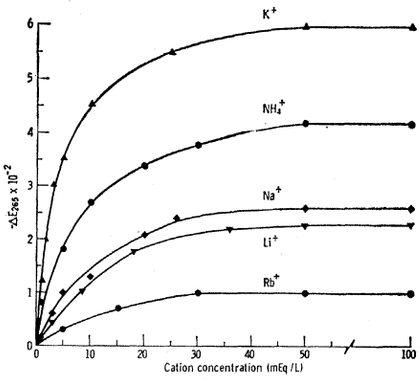

Fig. 1. Effect of varying concentrations of ions on the 5′-adenylic acid deaminase activity of human erythrocytes. The ordinate represents the decrease in the extinction of 1:30 dilutions of the deproteinized reaction mixtures.

The total deaminase activity of eryth-

Table 1. Effect of cations on 5′-adenylic acid deaminase of erythrocytes. The reaction mixture contained 5 μmoles of 5′-adenosine monophosphate, 0.1 mmole of tris-HCl, *p*H 7.1, 0.125 meq of the cations, and 0.5 ml of enzyme solution. Total volume 2.5 ml. Chloride salts were used in all cases. Histidine and imidazole were added as buffer solutions at *p*H 7.1. The solutions were incubated at 37°C for 90 minutes and deproteinized by the addition of 1.5 ml of 8 percent $HClO_4$. Extinctions of appropriate dilutions were measured at 265 mμ and compared with those of the control. A decrease of 60 percent in *E* indicates complete deamination (*8*). Ammonia was determined colorimetrically after the solution had been treated with Nessler's reagent.

Cation	Decrease of *E* at 265mμ (%)	NH_3(μmole)
$Tris^+$	0	0
K^+	58	5
Na^+	56	5
NH_4^+	56	—*
Li^+	57	4.9
Rb^+	49	4.1
Cs^+	2	0
$Imidazole^+$	3	0
$Histidine^+$	0	0
$N(CH_3)_4^+$	1	0
Mg^{++}	0	0
Ca^{++}	1	0

* The added NH_3 was much larger than that produced in the reaction so that no measurement could be made.

rocytes was compared with that of the enzyme extract. Both determinations were conducted in the presence of 50 m*M* K^+. The extract contained ⅕ of the total deaminase activity of the cells and 1/40 of the total hemoglobin content. The deaminase has a broad *p*H optimum at 7.1. The enzyme does not deaminate adenine, adenosine, adenosine diphosphate or adenosine triphosphate. That the ion activation of the enzyme is not due to a specific effect of tris (since this was present in the enzyme preparation) was evident from: (i) the fact that the enzyme could be extracted from the precipitate by salt solutions other than tris and (ii) the variation of tris concentrations over a wide range did not influence the effect of activating ions. When the enzyme solution, or various water dilutions of it, was centrifuged at 115,000*g* for ½ hour, no precipitate was observed, and samples taken from the top and the bottom portions of the centrifuged solution had equal activity. Although some of the enzyme was extracted readily from the precipitate containing the erythrocyte membranes, even repeated extraction did not result in a membrane fraction free from deaminase. In fact membranes prepared by several other methods (*2, 4, 5*) also contained the deaminase activity with properties similar to those of the soluble enzyme.

Figure 1 shows the effect of varying concentrations of the activating ions on the rate of deamination. The reaction conditions were the same as described for Table 1. The reaction time was 20 minutes. During this period the rate of reaction was a linear function of time. The *Km* values for K^+ and Na^+ were 3 and 8. These values are the same as those for adenosine triphosphatase (*5*) and adenylic kinase (*6*).

An unusual feature of the erythrocyte 5′-adenylic acid deaminase is its activation by ammonia, the product of the reaction it catalyzes. Conway and Cook (*7*), studying the deamination of 5′-adenylic acid by erythrocyte hemolyzates at high substrate concentrations (2 to 60 m*M*), found an unusual increase in the rate of reaction with increase of substrate concentration over 6 m*M*. Their results could well be explained by the above-mentioned autocatalytic nature of the 5′-adenylic acid deaminase (*9*).

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References and Notes

1. J. C. Skou, *Biochim. Biophys. Acta* **42**, 6 (1960).
2. R. L. Post, C. R. Merritt, C. R. Kinsolving, C. D. Albright, *J. Biol. Chem.* **235**, 1796 (1960).
3. R. Whittam, *Biochem. J.* **84**, 110 (1962); J. F. Hoffman, in *Biophysics of Physiological and Pharmacological Actions*, A. M. Shanes, Ed. (AAAS, Washington, D.C., 1961), p. 3.
4. E. T. Dunham and I. M. Glynn, *J. Physiol.* **156**, 274 (1961).
5. A. Askari and J. C. Fratantoni, *Biochim. Biophys. Acta* **71**, 232 (1963).
6. ———, *Federation Proc.* **22**, 212 (1963).
7. E. J. Conway and R. Cook, *Biochem. J.* **33**, 457 (1939).
8. H. M. Kalckar, *J. Biol. Chem.* **167**, 445 (1947).
9. I thank Prof. Walter F. Riker, Jr. for encouragement and advice. Supported by research training grant 2G-99 from U.S. Public Health Service.

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"Apocatalase" of Catalase-Negative Staphylococci

Abstract. *Crystalline catalase of high purity and activity was prepared from a heme-requiring* Staphylococcus aureus *mutant, JT/52, grown in the presence of heme. A crystalline protein was obtained from the same cells grown without heme. This enzymatically inactive protein and the catalase cross-react strongly when tested by a variety of immunochemical tests.*

Staphylococcus JT/52 requires a heme source for the synthesis of catalase (*1*). Cells of this mutant strain grown in the absence of heme, washed free of nutrients, and resuspended in a buffer solution become catalase positive upon addition of hemin (*2*). Maximum catalase activity (similar to that of the heme-independent parent strain SG 511) is obtained within 30 minutes (37°C) after hemin is added to the resting cells. This rapid formation of catalase, which, depending on the condition of the cells, requires the presence of coenzyme A and glucose (*3*), was taken as an indication for the presence of both preformed apocatalase and a synthesizing system that combines the apoenzyme with the prosthetic group.

Numerous futile attempts have been made to demonstrate this "terminal synthesis" in a cell-free system (*4*). Since no trace of activity can be observed in such experiments, the question was raised whether apocatalase is indeed formed independently in the absence of heme. This would mean, then, that free heme had a profound influence on the protein synthesis of hematin enzymes. On the other hand, the synthetic deficiency of the cell-free system could well be due to the absence of essential structural elements, the liberation of inhibitors, or other factors.

This report contains the main results of an investigation undertaken to decide this question of long standing and some consequence. Our experimental approach was based on the assumption that apocatalase would cross-react immunologically with catalase. Antibody against pure bacterial catalase could then be used as a guide for the detection of the enzyme's protein moiety.

The catalase to be used as antigen was prepared by a modification of the method of Herbert and Pinsent (*5*) from JT/52 cells grown on the surface of nutrient agar containing 3.0 μg of hemin (Pentex) per milliliter. The catalase activity of such cells could not be enhanced by further addition of hemin. The final preparation of the crystalline enzyme was of high purity. It had the following characteristics: The Kat. f. (Katalasefähigkeit or catalase ability), calculated from velocity constants (k) obtained spectrophotometrically at 240 mμ (*6*), was 124,000. Assuming a molecular weight of 232,000 (*5, 7*), the specific activity was calculated as $k'_1 = 5.3 \times 10^7\ M^{-1}\ sec^{-1}$, and the molar extinction coefficient as ε 406 (max) = 420 $mM^{-1}\ cm^{-1}$. The ratio OD_{406}: $OD_{276.5\ (max)}$ was 1.05. The sedimentation pattern in the ultracentrifuge was that of a single component with a sedimentation constant $s_{20,b} = 10.5 \times 10^{-13}$ sec. In double diffusion plates the catalase formed a single, sharp band with its corresponding antibody. Immunoelectropherograms showed a single arc. The enzymatic activity was significantly and specifically inhibited by anticatalase (80 percent inhibition with excess antibody).

The JT/52 cells grown in the absence of heme were submitted to a similar extraction procedure. Guided by double-

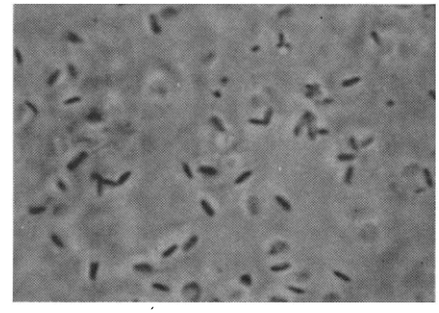

Fig. 1. Crystalline "apocatalase" from the catalase negative mutant (about × 1300).