It is to be further noted that L-methionine alone and aminopterin only in combination with DL-homocysteine are inhibitory to tetracycline biosynthesis.

In Tables III and IV, it can be seen that L- and DL-ethionine are quite inhibitory to tetracycline synthesis, in part due to growth inhibition, but that these compounds as well as D-ethionine cause production of 7-chloro-6-demethyltetracycline. Certain chemical agents can increase or eliminate biosynthesis of this substance and overcome the general inhibition of tetracycline production in the presence of ethionine. These agents include methionine, methionine sulfoxide, methoxinine, glycine, serine, threonine, homocysteine, cyanocobalamin, and Co^{2+} ions.

The inhibition of the 6-methylation of 7-chlorotetracycline by aminopterin supports the hypothesis offered in an earlier report[4] that the insertion of this methyl group is a folic acid-dependent reaction. The effects of ethionine may be ascribed to either a direct competition with methionine or an ethylated folic acid derivative acting as an antagonist of the normal methylated coenzyme.

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Received September 7th, 1962

Biochim. Biophys. Acta, 71 (1963) 199–201

SC 2203

Unavailability of chromaffin granule adenosine triphosphate for metabolic reactions

The adrenal medulla contains considerably more ATP than any other tissue thus far studied[1]. Almost all of this ATP is held within the chromaffin granules[2], which store the adrenaline and noradrenaline[3]. The adrenaline and noradrenaline are known to be held within the chromaffin granules in a biologically inactive form. Thus, when a preparation of isolated adrenal medulla "large granules" suspended in isotonic sucrose is injected intravenously into a cat only a small fraction of the pressor activity of the contained amines is immediately apparent. On the other hand, if the granules are first "lysed" in distilled water and then injected the full pressor activity of the contained amines is immediately observed[4].

In order to find out whether the large amounts of ATP within these granules might also be held in such a way as to be unavailable for reactions in which ATP is known to participate, we have studied the ability of chromaffin granule ATP to drive the hexokinase (EC 2.7.1.1) reaction. In these experiments ATP or ATP-rich "large granules" were incubated in isotonic sucrose with glucose, hexokinase, glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and TPN^+. The progress of the reaction was determined by measuring spectrophotometrically the reduction of TPN^+.

The suspension of the large granules (mitochondria and chromaffin granules) was prepared from a homogenate of ox adrenal medulla in 0.3 M sucrose. Centrifugation for 70 min at 900 × *g* sedimented nuclei and unbroken cells. The supernatant was then centrifuged for 30 min at 11000 × *g* and the "large granule" sediment resuspended in 0.3 M sucrose and kept at 3° until used.

The reaction mixture contained either ATP (at concentrations less than 0.64 mM) or ATP-rich "large granules", a purified yeast hexokinase*; excess glucose-6-phosphate dehydrogenase**; 6.6 mM glucose; 0.04 M Tris buffer (pH 8.0); 7 mM $MgCl_2$; 0.33 M sucrose; and 0.125 mM TPN^+. 2 ml water was first placed in the cuvettes and then the other reactants except glucose were added as concentrated solutions. The reaction was started by adding glucose to one of the cuvettes at zero time. The change in absorbancy at 340 mμ of the cuvette containing the glucose was then continuously recorded with the cuvette lacking glucose set to read zero in a Beckman DB spectrophotometer with a photovolt recorder. When intact granules were being studied they were added to the cuvette after the addition of the sucrose so that they were always in an isotonic medium. When "lysed" granules were studied, a small volume (usually 0.2 ml) of the granule suspension was added to the cuvette first, then 2 ml of water was added, the contents were quickly shaken and then the other reactants added.

It was possible to calculate the amount of ATP participating in the reaction from the amount of TPN^+ reduced. This value could be compared with the ATP content of an acid extract of the "large granules", as determined by separating the adenine nucleotides on a Dowex-1 chloride ion-exchange column. The elution of AMP and ADP was effected with a gradient from water to 0.05 N HCl and the ATP eluted with 0.05 N NH_4Cl in 0.05 N HCl and the amount of each nucleotide calculated from the absorbancy at 260 mμ.

Fig. 1 shows the effect of lysis of the granules on the hexokinase reaction. The top tracing shows the rate of the hexokinase reaction (TPN^+ reduction) when lysed "large granules" were used. The lower tracing shows the rate of the hexokinase reaction when intact "large granules" were used. No reduction of TPN^+ was observed with the intact "large granules". This indicates that the ATP in the intact chromaffin granules is not available for the hexokinase reaction. However, when exogenous ATP was added, the hexokinase reaction proceeded. Thus the intact granules were not inhibiting the enzyme system. That all the ATP in the lysed chromaffin granules was available for the reaction is indicated by the correspondence between the amount of TPN^+ reduced in the course of the reaction and the amount of ATP recovered by ion-exchange chromatography of acid extracts of the large-granule fraction (Table I).

* Sigma Practical Grade.
** Sigma Type V.

The results indicate that ATP is stored in intact chromaffin granules in a form in which it is unable to participate in enzymic reactions so long as the granule remains intact. Lysis of the granules in distilled water can set free all the ATP in a form in which it can participate in enzymic reactions. Even after the "large granules" were stored in 0.3 M sucrose for 50 h at 3° the ATP was still unavailable to drive the hexokinase reaction unless the granules were first lysed with distilled water. This confirms the observation of HILLARP, HÖGBERG AND NILSON[1] that isolated granules do not break down even after several days' storage.

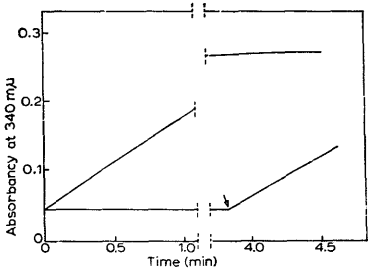

Fig. 1. The figure shows change in absorbancy at 340 mμ when lysed large granules served as the source of ATP for the hexokinase reaction (upper tracing), when intact large granules served as the source of the ATP (lower tracing). ATP was added at point indicated by arrow to give a 0.64 mM ATP concentration.

TABLE I

RELATIONSHIP BETWEEN AMOUNT OF ATP AVAILABLE FOR HEXOKINASE REACTION AND ATP CONTENT

Values are μmoles/g wet wt. of tissue from which the granules were derived.

ATP available for the hexokinase reaction		*ATP content*
Intact granules	*Lysed granules*	
0	4.35	4.12
0	3.4	3.6
0	2.7	3.2
0	3.9	3.1

This work was supported by grants from the U.S. Public Health Service (B2282) and from the Medical Research Council of Canada.

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Received October 8th, 1962