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Received January 3, 1967. P.S.E.B.M., 1967, v124.

ATP Hydrolysis by Isolated Embryonic Heart Nuclei Using a Histochemical Method.* (31980)

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Embryonic myocardial cell nuclei of the chick are able to hydrolyze adenosine triphosphate (ATP) enzymatically; this hydrolysis is characteristic of an ATP phosphohydrolase, EC 3.6.1.3 (ATPase)(1). A number of similar physical and chemical properties of the enzyme can be demonstrated both biochemically using purified isolated nuclei and histochemically using unfixed (or fixed) cryostat sections. A modified method of Wachstein's and Meisel's(2) was used in the latter procedure.

The present investigation was undertaken to demonstrate that the histochemical test for presumptive ATPase activity can be applied to isolated nuclear preparations from myocardial tissue. It is also given as further proof that earlier interpretations using cryostat sections could not be due to diffusion artifacts resulting from phosphate liberation by other cell components.

Methods. Myocardial cell nuclei of the chick were isolated from 12-day embryos and purified as described previously(1). The final concentrated suspension of nuclei was in 0.5 M sucrose. Aliquots were spread on clean glass slides with the edge of a cover slip and allowed to air dry for 5 minutes. Slides with adhered nuclei were placed on a petri dish in a Technicon water bath at 37.5°C and flooded with the desired incubating solution at the same temperature. The enzyme reaction medium contained: 5 mM Mg^{++} (Cl^-, NO_3^- or SO_4^{3-} salt), 5 mM $tris_4$-ATP, 20 mM tris-HCl or tris-maleate-NaOH buffer at the desired pH, 0-130 mM Na^+ (Cl^- or NO_3^- salt) and 5×10^{-4} M Pb $(NO_3)_2$ as the phosphate trapping agent. Calcium ions were substituted for Mg^{++}, and nucleoside and other phosphates for ATP in equal concentration. Media were kept isotonic with sucrose when below 0.31 M in non-electrolyte equivalents. Mersalyl (Salyrgan), ouabain and 2,4-dinitrophenol (2,4-DNP) were used at concentrations between 10^{-7} and 5×10^{-4} M.

Preparations were preincubated for 5 minutes in reaction media without substrate and lead ions. Upon the addition of the latter components, reactions were allowed to proceed for 30 minutes with several medium changes. They were stopped by rinsing with ice cold medium without substrate or lead ions, bathed 3 minutes in cold 0.1% $(NH_4)_2S$, rinsed and mounted in dilute gelatin medium without further staining.

Results. The concentration of Pb^{++} is comparatively low to that often employed. Approximately 3×10^{-4} M gives near maximal staining intensity using hatched chick myocardial sections(1). With isolated nuclei from 12-day embryo heart, about 5×10^{-4} M Pb^{++} is required. Higher concentrations are considered unwarranted as they would only cause increased enzyme inhibition and poorer nuclear preservation. In addition higher concentrations of Pb^{++} can cause non-enzymatic hydrolysis of ATP(3). The latter was tested under present conditions and 5×10^{-4} M Pb^{++} produces no measurable hydrolysis of ATP, or is this affected by Na^+ or Mg^{++}.

Differential analyses of ATP hydrolysis by other cell fractions (myofibrillar, actomyosin, microsomal and mitochondrial) under a vari-

* This research was supported by grant HE-07056 and Research Career Program Award HE-5892 from Nat. Heart Inst., USPHS.

ety of conditions and microscopic observation of isolated nuclear preparations support the belief that the latter are relatively pure((1), and unpublished observations).

In the following Figures, nuclei are unstained except for the lead salt deposit indicative of ATP hydrolysis.

With all components present in the enzyme reaction medium except for the activating Mg^{++}, there is no evidence of nuclear staining, Fig. 1. The nuclei become swollen compared to those in the presence of Mg^{++}, whether or not the preparation has been reacted. Microscopic observation after various periods of time indicates nuclear swelling occurs more readily in the absence of Mg^{++}. In the presence of Mg^{++}, nuclear staining occurs at pH 6.5 (Fig. 2) and more intensely at pH 9.0 (Fig. 3). The pH values 6.0-6.5 and 9.0 correspond to the lesser and greater optima, respectively, for Mg^{++}-activated ATP hydrolysis(1). At pH 9.0 nuclear staining can be observed after 5 to 10 minutes incubation and increases up to about 30 minutes; an hour incubation does not add appreciably to the reaction intensity and is likely to cause unnecessary nuclear deterioration.

Nuclear staining intensity is diminished (Fig. 4) when Na^+ is omitted from the medium; an osmotic equivalent of sucrose is substituted for sodium salt. The reaction can be also inhibited by Mersalyl between 1 and 5×10^{-4} M (Fig. 5). The higher concentration of drug is used in this preparation to increase the difference in visual contrast for comparison with Fig. 3. The addition of Dimercaprol (BAL) at 0.5 times the concentration of Mersalyl will prevent most of the inhibition by the latter. Ouabain and 2,4-DNP have no obvious effect under these conditions.

A number of other factors were tried but are not illustrated. Reactions can be greatly reduced or eliminated by lowering the temperature to about 0°C. When Ca^{++} is substituted for Mg^{++}, only low intensity staining results. At pH 6.5 the presence of ATP results in a lead staining reaction (Fig. 2), but substitution of other nucleoside triphosphates (inosine, uridine or cytidine),

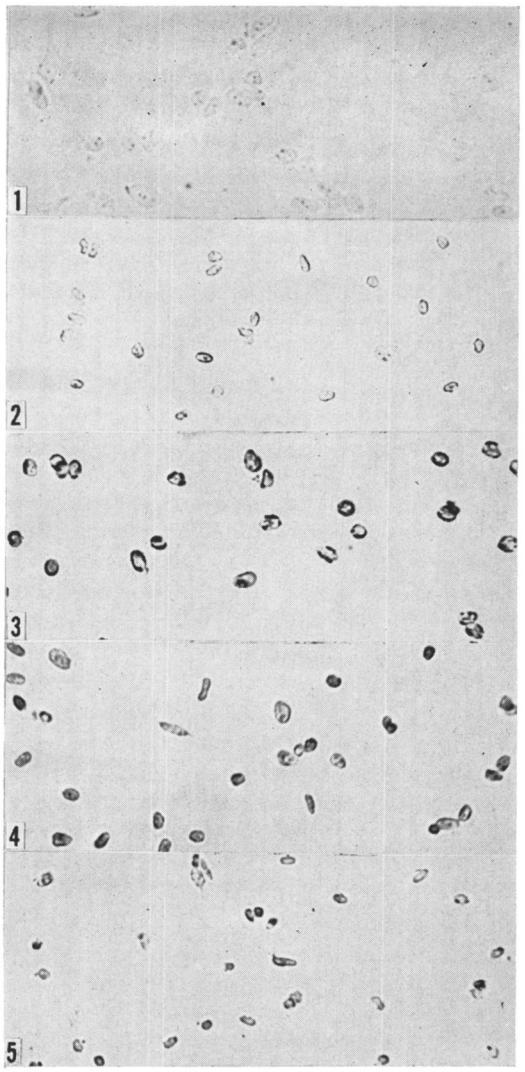

FIG. 1. Mg^{++} omitted, 5 mM ATP, 20 mM tris buffer at pH 6.5, 130 mM Na^+, 37-38°C, reacted 30 min.

FIG. 2. 5 mM Mg^{++}, 5 mM ATP, 20 mM tris buffer at pH 6.5 130 mM Na^+, 37-38°C, reacted 30 min.

FIG. 3. Same as Fig. 2, except at pH 9.0.

FIG. 4. Same as Fig. 3, except Na^+ omitted.

FIG. 5. Same as Fig. 3, except 5×10^{-4} M Mersalyl added.

adenosine diphosphate or monophosphate, β-glycerophosphate, thiamine pyrophosphate or inorganic pyrophosphate give no or greatly reduced reactions. Certain of the latter phosphates do not permit comparison at pH 9.0 due to lead salt precipitation unrelated to ADP hydrolysis.

Discussion. A modified histochemical meth-

od of Wachstein and Meisel can be used to demonstrate presumptive ATP hydrolysis by isolated nuclei from embryonic myocardium. Characteristics of the reaction are in agreement with those previously found using unfixed (or fixed) cryostat sections and with enzymatic studies on the isolated nuclei(1). There can be no appreciable diffusion artifacts resulting from inorganic phosphate liberation by other cell components in the present study. Intense reactions occur at the nuclear surface as if the enzyme is located at least partly in the membrane; less intense reactions are associated with components of the nucleoplasm. On the basis of present experiments, it cannot be ascertained whether Na^+ stimulation or Mersalyl inhibition occurs more at one nuclear locus than another. ATPase activity has also been reported to be associated both with the nuclear membrane and the nucleoplasm of liver nuclei (4). Certain properties of the myocardial enzyme have been compared(1) to those of isolated kidney(5,6) and liver(4) nuclei.

Under carefully controlled conditions, the present method using isolated nuclei may be suitable for quantitative and statistical analysis through dry mass determination in terms of electron opacity(7).

Summary. A histochemical method was modified to demonstrate ATP hydrolysis by nuclei isolated from embryonic myocardium. Under a variety of experimental conditions, the results are in agreement with previous histochemical studies using cryostat sections and with enzymatic studies using isolated nuclei. The data give further support to the localization of ATP phosphohydrolase activity both at the nuclear surface and in the nucleoplasm, under conditions which eliminate possible diffusion artifacts resulting from enzymatic activity by other cell components and non-enzymatic hydrolysis of ATP by Pb^{++}.

The author is indebted to Mrs. A. P. Breland and Mrs. A. T. Klein for technical assistance and to the University Medical Center Illustration Department for photography.

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Received January 5, 1967. P.S.E.B.M., 1967, v124.

Incorporation of Selenium into Spermatogenic Pathway in Mice.* (31981)

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During preliminary studies in male mice on tissue distribution of a single subcutaneous injection of high specific activity Se-75, it was noteworthy that, whereas most tissues acquired and then lost the radioisotope quickly, the testis continued to cumulate selenium. Little information was available on distribution of an actual tracer amount of selenium to male reproductive organs of mammals. But the fact that in fowl Se-75 was retained in testis(1,2) and found in the protein fraction of spermatozoa(2), suggested that the question of possible selenium incorporation into the mammalian spermatogenic pathway merited further investigation.

* This investigation was supported by USPHS Research Grant CA-07004-04 from Nat. Cancer Inst.

Materials. A total of 115 male mice of