(A. H. Thomas Co., size A) through a sac made of hard finish dacron mesh (marquisette, 24 threads/inch in one dimension by 30 threads/inch in the other) into a Thomas grinding vessel, size B containing only 30 ml of CFL at room temperature. While the tissue is pressed gently the individual cells disperse into the bulk of the suspension medium thereby avoiding any additional contact between the free cells and the pestle. When most of the tissue ($\sim$ 85%) has been dispersed in this way the tissue still remaining in the mesh sac (largely connective tissue) is discarded. The suspension is filtered once through a filter of fine mesh rayon (80 threads/inch by 84 threads/inch) to remove any large clumps of cells. The suspended cells are gently sedimented by centrifugation at 50 *g* for 4 min at 4 °C using an International refrigerated centrifuge. The supernatant is removed by suction and the sedimented cells are carefully resuspended in 20 ml of CFL using a pipette having an orifice of 0.90 mm, or larger. The use of a pipette with a finer bore appears to result in rupture of some of the cells during resuspension. The cells are sedimented again for 3.5 min at 50 *g* using the same type of centrifuge. After removal of the supernatant by suction, the cells are resuspended in 15 ml of CFL. The entire procedure can be completed in approximately 35 min (Figure 1).

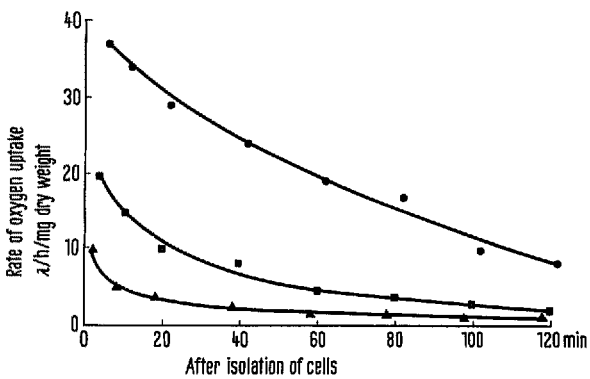

Fig. 2. The effect of increased time intervals between the isolation of mouse liver cells and the measurement of their rate of oxygen uptake. The cells were maintained at 25 °C in the absence of additional substrate during this time. Oxygen consumption was measured in the Gilson Oxygraph at 37 °C, the chamber containing 2.0 ml of calcium-free Locke's solution, 0.3 ml of the cell suspension (3.0 mg dry weight), and, when added, 0.1 ml of the substrate to give a final concentration of $10^{-2}M$. ▲ no addition; ■ pyruvate; ● succinate.

This method has been found to give consistently a yield of from 40–50% of the original tissue mass recovered as intact free cells. Two mouse livers dispersed in the manner described yield 1.8–2.2 ml of packed cells which have a dry weight of 160–200 mg. The suspensions have less than 3% of free nuclei apparent microscopically. There are also very few erythrocytes present. The number of cells in the suspension was determined in the Coulter Counter (Coulter Electronics, Inc.). The yield was found repeatedly to be $35 \cdot 10^6 \pm 10\%$ of cells/g of dispersed liver.

It was found that the free cells maintained at 25 °C in the absence of additional substrates (other than the $5.5 \cdot 10^{-3}M$ glucose present in CFL) exhibit a slowly decreasing capacity to respire. This activity was measured at 37 °C with the Gilson Oxygraph, both with and without additional substrates. The rates of respiration observed in the presence of $10^{-2}M$ glucose are essentially identical to those shown in Figure 2 for cells respiring in the presence of CFL alone. The presence or absence of $5.5 \cdot 10^{-3}$ to $10^{-2}M$ glucose had no effect on the rate of respiration observed when pyruvate or succinate was added to the cell suspension in the Oxygraph chamber. The metabolism of nucleic acids, protein, and lipids of mouse liver cells prepared by this technique is currently under investigation[7].

Zusammenfassung. Einfache und schnelle Methode zur Isolierung freier Mäuse-Leberzellen. Freie Hepatozyten (bis 50% des Gewebes) zeigen so aktiven Stoffwechsel.

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[7] This work was supported by grant No. CA05172 of the National Cancer Institute, U.S.P.H.S.

Electrophoresis of Mitochondrial α-L-Glycerophosphate Dehydrogenase on Acrylamide Gels

2 enzymes catalysing the interconversion of α-L-glycerophosphate and dihydroxyacetone phosphate exist in the mammalian cell. 1 form, localized predominantly in the cytoplasm, requires NAD for its function[1,2], the other, bound tightly to mitochondrial structure, does not[3]. The 2 enzymes differ further in that the activity of the mitochondrial but not the cytoplasmic form is stimulated several fold in certain tissues of the rat by thyroxine and some of its analogues[4–6]. Although, at least in rat kidney, the soluble, or cytoplasmic form has been found to be heterogeneous by starch gel electrophoresis[7], the bound or mitochondrial form has thus far resisted attempts at its resolution by electrophoresis in solid or semi solid media. This is apparently due to its high degree of adsorption to the medium and consequent failure to migrate[8]. The present paper reports the successful resolution of the mitochondrial enzyme into 2 active units by treatment with urea and electrophoresis in acrylamide gels of special (2.5% acrylamide-bisacrylamide) composition.

Methods. Acetone powders[9] of mitochondria from various tissues were extracted once with de-ionized water and twice with $0.03M$ potassium phosphate buffer, pH 7.5. α-L-glycerophosphate dehydrogenase (α-GPDH) activity was solubilized from the residue by incubation with *crotalus terrificus* venom under the conditions of RINGLER and SINGER[8]. The resulting suspension was centrifuged at 25,000 rpm in the No. 30 rotor of the Spinco preparative ultracentrifuge for 60 min. Portions

of the clear amber colored supernatant were made approximately 15% w./v. in either sucrose or urea (0.5 *M* and 2 *M*, respectively) and 0.1 ml aliquots were electrophoresed on acrylamide gels of 5% or 2.5% total solids. 5% gels were prepared by the method of CLARKE[10]. The 2.5% gels were made by simply halving the concentrations of acrylamide and bisacrylamide used in the 5% gels. Electrophoresis was carried out at 4 °C at a constant current of 2 mA per tube for 30–40 min. To facilitate removal of the gels, the tubes were treated with a preparation of 'Column Coat'[11] prior to use. The gels were stained by incubation in the dark at 37 °C in a mixture of: 7.0 ml 0.05 *M* potassium phosphate, pH 7.5; 3.0 ml 0.25 *M* DL-α-glycerophosphate, pH 7.5; 1.5 mg phenazine methosulfate and 7 mg nitro blue tetrazolium. In case of the cytoplasmic α-GPDH, 3 mg NAD was included.

Spectrophotometric assays of enzyme activity were made by the method of LEE and LARDY[5].

Results and discussion. Figure 1 shows the difference in electrophoretic behavior of mitochondrial α-GPDH, 0.5 *M* in sucrose, on gels of 5% and 2.5% total solids. The enzyme is apparently of too great a size to enter the pores of the 5% gel and, under the force of the electric field, is forced to penetrate between the gel and wall of the glass tube resulting in a drawn out, smeared pattern (Figure 1, A). It is of interest to note that the cytoplasmic form of α-GPDH, in contrast to the solubilized mitochondrial enzyme, readily penetrates the 5% gel and is resolved into 4 well defined zones of activity (Figure 1, B). When the porosity of the gel is increased by lowering the total concentration of monomers to 2.5%, mitochondrial α-GPDH easily penetrates into the gel and the activity is manifested in a single rather broad band (Figure 1, C).

Urea is rather commonly used to effect the dissociation of proteins[12]. This reagent, according to TANFORD[13], favors dissociation if new hydrophobic groups, peptide groups or amide groups are exposed thereby. The addition of urea to mitochondrial α-GPDH and subsequent electrophoresis on 2.5% gels, causes the single band of activity to split into 2 zones (Figure 2). The electrophoretic patterns obtained from the mitochondrial

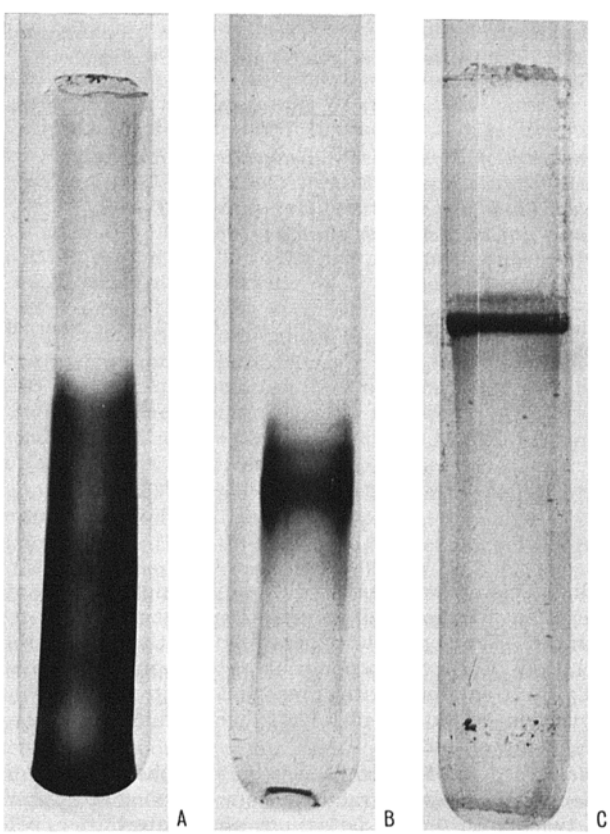

Fig. 1. (A) Mitochondrial α-GPDH on 5% gel. (B) Purified preparation of rabbit muscle α-GPDH (obtained from Sigma Chemical Co.) on 5% gel. (C) Mitochondrial α-GPDH in 0.5 *M* sucrose on 2.5% gel. The cathodic end (origin) of the gels is at the bottom. The thin, faint band above the prominent formazan band in (C) is due to a translucent zone of inactive, denatured protein.

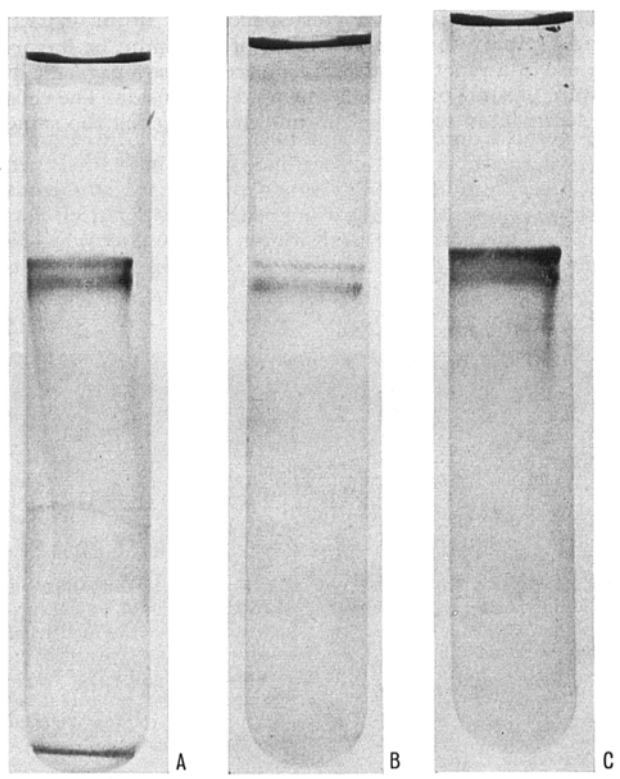

Fig. 2. 2 *M* urea treated mitochondrial α-GPDH from (A) rat brain, (B) rat liver, and (C) liver from triiodothyronine treated rats. The cathodic end (origin) of the gels is at the bottom.

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enzyme of brain, normal liver and liver of rats which had been injected with triiodothyronine (2.5 mg/kg body weight) for 3 consecutive days prior to sacrifice in order to increase the level of this enzyme, are identical. The same two-band pattern was also obtained after 2 *M* urea treatment of α-GPDH obtained from mitochondria of the following tissues: mouse liver, rat kidney, spleen, and testis and also Hepatoma 5123A.

The effects of urea on the enzyme do not appear to be time dependent since the same two-band pattern was obtained after 2 min of exposure of the enzyme to 2 *M* urea as after an exposure of 48 h. The pattern also did not change when the concentration of urea was varied from 1–5 *M* although considerable decrease in activity was noted in both the spectrophotometric assays (Figure 3) and in visual estimation of the formazan formed in the gels. Both bands, however, appeared to be inhibited to the same extent. Removal of urea from the enzyme by dialysis against 0.03 *M* phosphate buffer, pH 7.5, leads to a reversion of two-band pattern to the basic one-band pattern. Addition of urea again causes a two-band pattern, and the cycle can be repeated, indicating the reversible nature of the dissociation.

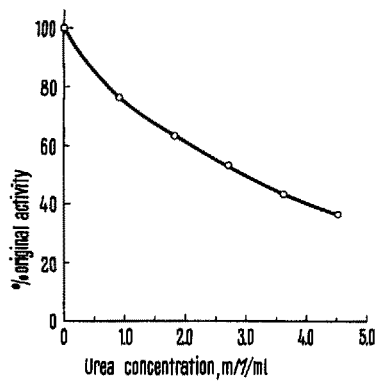

Fig. 3. Inhibition of rat liver mitochondrial α-GPDH by urea. Spectrophotometric assays in a total volume of 1 ml were performed according to LEE and LARDY[5].

The results with α-GPDH presented in this paper may be of more general interest since the same method can in all probability, be applied to electrophoretic investigations of other large proteins or protein complexes. Preliminary results in fact indicate that succinic dehydrogenase activity solubilized from rat liver mitochondria by action of snake venom can be resolved into 3 active bands on 2.5% gels[14].

Zusammenfassung. Es wird ein Verfahren zur elektrophoretischen Trennung am Polyacrylamid der an die Mitochondrien gebundene α-L-Glyzeraldehydphosphat-Dehydrogenase in 2 aktive Bestandteile beschrieben: Partielle Spaltung der Eiweissmolekeln mit Harnstoff. Die Auflösung der Enzyme in 2 aktive Komponenten wurde mit Extrakten aus Mitochondrien verschiedener Rattenorgane (Leber, Niere, Hirn, Hoden und Hepatoma 5123 A) sowie Mäuseleber verfolgt.

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[14] The author is grateful to Mr. B. DIXON for excellent technical assistance.

Über die Stabilitätskonstanten von Pyrimidinderivaten mit Kupfer

Über die biologische Wirkung von Halogenpyrimidinen ist viel gearbeitet worden. Die meisten Untersuchungen befassten sich mit chemotherapeutischen Wirkungen bei Carcinomen[1] und ihren Anwendungen als Radiosensibilisatoren.

5-Fluoruracil erwies sich von den genannten Verbindungen zur Anwendung bei festen menschlichen Tumoren in Verbindung mit Bestrahlung am wirkungsvollsten[2]. Über den Einbau von Halogenpyrimidinen in Nucleinsäuren und deren mutagene Wirkung wurde verschiedentlich berichtet[3]. Auch als Inhibitoren bei enzymatischen Reaktionen können sie zielgerichtet eingesetzt werden[4]. Der vergrösserte van-der-Waals-Radius der Halogene im Vergleich zur Methylgruppe am Pyrimidin verursacht keine sterische Hinderung in der Doppelhelix[5]. Halogendeoxyuridin kann an Stelle von Thymidin in DNS eingebaut werden, z.B. auch in Tumorzellen, wie OEHLERT zeigen konnte[6]. Es zeigt sich sogar, dass sich die Bindungsstärke der DNS-Doppelhelix erhöht, wie sich durch thermische Denaturierungsversuche an DNS mit eingebautem Bromodeoxyuridin herausstellte[7].

5-Fluoruracil kann aber auch durch seinen Einbau in die m-RNS zu veränderten Proteinen und damit auch zu Enzymen mit geringerer oder keiner biologischen Aktivität führen. Die Anwendung neuer empfindlicher Methoden zum Nachweis dieser Substanzen in subzellularen Partikeln und Makromolekülen[8] brachten neue Gesichtspunkte über ihren Wirkungsmechanismus her-

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