was observed following HYPO-X and triple operations. Although triple operations caused a lower DNA value than that of HYPO-X alone, there was no significant difference between the two groups. This suggests that the ovaries and adrenals are of secondary importance on maintenance of mammary glands after hypophysectomy.

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## Electrophoresis of Human Hexokinases in Acrylamide Gel.* (32483)

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Recent reports have established that hexokinase exists in multiple forms with distinct structural and kinetic properties in tissues of the rat(1). It has also been shown that the activity of some isoenzymes of hexokinase alter under conditions such as fasting and insulin deficiency(2,3). A systematic survey of the pattern of isoenzymes of hexokinase in human tissues would therefore be of value toward an understanding of the changes occurring in pathological conditions. This paper presents a study of the isoenzyme pattern of hexokinases in normal human tissues using acrylamide gel electrophoresis, and includes a variation of pattern observed in a patient with hemolytic anemia.

*Materials and methods.* Human tissues were obtained during surgical operation from 13 patients. After removal the tissue was transferred to the laboratory in gauze soaked in saline, and, after washing, the tissue was homogenized in an equal volume of buffer containing: KCl 150 mM; 2-mercaptoethanol 10 mM; EDTA 5 mM; and $MgCl_2$ 5 mM and adjusted to pH 7.4 with $KHCO_3$. The homogenate was centrifuged at 15,000 $\times$ *g* for 20 minutes in a refrigerated International Centrifuge (angle head: 856) at 4°C. After centrifugation equal parts of the supernatant and spacer gel were mixed and 100 to 150 $\mu$l were applied to the columns of acrylamide gel. Electrophoresis was performed at 0-4°C using a 4% acrylamide running gel according to the method of Davis(4) except that in place of the spacer and sample gel a sephadex-sucrose-trisCl solution was used(5). Electrophoresis required approximately 50 minutes maintaining 2.5 to 3 m amps per column.

On termination of electrophoresis the acrylamide columns were stained for hexokinase in a solution similar to that described by Katzen and Schimke(1). Each experiment was performed at least in triplicate. Control tubes without ATP were run for each sample to distinguish nonenzymatic bands. Bands additional to those observed in controls were assumed to be due to the activity of hexokinase.

*Results.* Fig. 1 presents the pattern of isoenzymes of hexokinase in a variety of normal human tissues. The controls for each are included to show that non-specific bands develop in addition to those caused by hexokinase. In conformity with other observers employing starch gel electrophoresis, the bands of hexokinase are numbered in order

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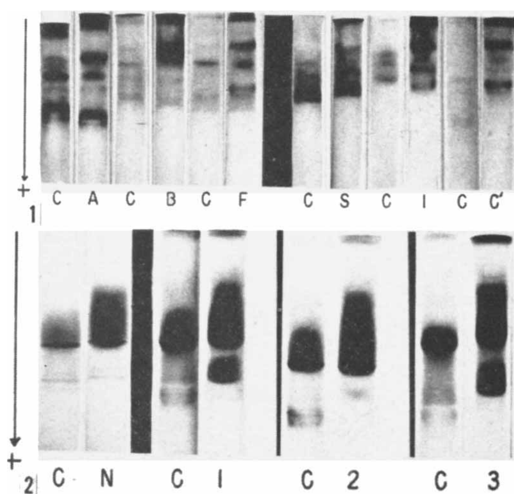


FIG. 1. Photograph of bands of hexokinase activity on acrylamide gel electrophoresis of homogenates of various human tissues. The controls (C) incubated without ATP precede their respective tissues. The tissues are: A, adrenal; B, brain; F, adipose tissue S, Stomach; I, ileum; and C′ colon.

FIG. 2. Photograph of bands of hexokinase activity seen on acrylamide gel electrophoresis of hemolysates of erythrocytes from a normal adult (N) and a family in whom the infant presented with a hemolytic anemia, the father had a reticulocytosis and the mother was hematogically normal. 1, father; 2, mother; 3, infant. Controls (C) incubated without ATP precede their respective tissues.

of increasing anodal mobility. This does not necessarily mean correspondence between the properties of the types of isoenzymes previously described on starch gel electrophoresis, because of the different medium for electrophoretic migration. It is of interest that brain and red blood cells which are thought to be insensitive to the action of insulin contained only one type of hexokinase, when 0.1 M glucose was used in the staining procedure.

Since Type IV hexokinase is thought to represent glucokinase(6) and has been observed in extracts of human liver(7) the sera of 3 patients with liver disease were examined. In each patient the serum transaminases were greatly elevated. It was not possible to detect any type of hexokinase in the serum of these patients when the stain contained glucose either at a concentration of 0.5 mM or 0.1 M.

The opportunity arose to examine the red cells of a male baby (4 months) who presented at birth with a hemolytic anemia of unexplained origin which necessitated blood transfusions at monthly intervals. The hexokinase pattern of the red cells of this infant and his parents is shown in Fig. 2, and compared with the pattern found repeatedly in normal individuals. Hexokinase isoenzymes were developed at 0.1 M concentration of glucose. A band migrating ahead of the hemoglobin band was observed in the child and father but not in the mother. The father was known to have reticulocytosis, but was otherwise clinically normal.

The fast band did not resemble the other isoenzymes of hexokinase, since it developed increasingly after fixation of the columns in methanol-acetic acid-water (5:1:5 v/v) and became opalescent. However it was not observed in controls which were stained in the absence of ATP, suggesting that its appearance depended on the specificity of the developing solution. When the red cells of the members of this family were ruptured with digitonin the anomalous band failed to develop. The experiment was repeated one month later in triplicate during one electrophoretic run. The cells were ruptured mechanically and the atypical band again appeared in father and infant but was not observed in the controls or in the mother.

*Discussion.* These findings suggest that at least two types of hexokinase are present in human tissues. The presence of only one band in some tissues indicated that the two types were not simply subunits of hexokinase whose dissociation had been produced by the conditions of electrophoresis.

The anomalous band in the zymogram of the patients in Fig. 2 raised the question of specificity of the staining technique. Other workers have assumed that the appearance of additional bands to those seen in controls developed in solutions lacking ATP or glucose represented isoenzymes of hexokinase. However the fast band in Fig. 2 behaved in a manner quite different to the other isoenzymes. Although its appearance depended on the presence of ATP in the developing solution it had an opalescent quality which intensified on standing in the fixative solution. It was abolished when the red cells were ruptured by digitonin. The abnormal

band might have been caused by a similar mechanism suggested by Lewis, Cheever and Seavy(8). They have observed a lipid in pituitary fractions which caused an alteration in the mobility of aldolase on starch gel electrophoresis and caused its dissociation into subunits. It is possible that an abnormal lipid was present in the red cells of the patients in Fig. 2 and complexed with hexokinase thus increasing its anodal mobility. Digitonin would be expected to rupture the lipo-enzyme complex and therefore abolish the anomalous band.

*Summary.* The isoenzymes of hexokinase of several human tissues have been examined by disc electrophoresis. An infant with hemolytic anemia was found to have a band that migrated faster than hemoglobin. His father displayed the same abnormality whereas the mother's zymogram was normal.

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## *Escherichia coli* Resistance to Ethionine.* (32484)

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In a brief report on ethionine resistance by *Escherichia coli,* Martin and Moo-Penn (1) reported a considerable delay in the onset of growth of resistant cells transferred from an ethionine medium to one without the inhibitor. When ethyl-1-$C^{14}$-ethionine was incorporated into the growth medium of resistant cells, labeled $CO_2$, acetic and formic acids were isolated. The specific activity of formic acid approximated that of $C^{14}$-ethionine while specific activities of acetic acid and $CO_2$ were low indicating considerable dilution of these compounds by carbon from other sources. Labeled acetate and $CO_2$ were also isolated from sensitive *E. coli* cultures grown in the presence of subinhibitory concentrations of labeled ethionine but no labeled formate was produced by sensitive cells.

Indirect evidence indicated that this *E. coli* strain was resistant to ethionine by virtue of an ability to decompose this inhibitor to a non-toxic substance, probably hemocysteine. The nature of the growth lag by ethionine resistant cells was not studied. The following report is concerned with further studies on the prolonged lag in growth exhibited by resistant cells and the relationship of this lag to ethionine resistance in *E. coli.*

*Materials and methods. Microorganism.* *Escherichia coli* ATCC 9637 was used throughout this investigation. Ethionine resistant cells were obtained by passage of sensitive cells on agar plates containing increased concentrations of ethionine. The minimum inhibitory concentration of ethionine for 100 sensitive cells inoculated into 10 ml broth was 100 μg/ml after 24 hours incubation at 37C, and 900 μg/ml on agar plates under the same conditions. Resistant cells grew in the presence of 5 mg/ml L-ethionine in broth

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