and with the parent Challis H strain.

The auxotrophs transformed well to either marker. The frequencies of transformation to streptomycin-resistance were higher than those to prototrophy. We did not score for the presence of transformants to both markers; since we used an excess of DNA, it is likely that some double transformants were obtained. The control sample with the DNA and deoxyribonuclease mixture gave rise to less than 100 colonies per milliliter of sample when plated on MS9 medium or brain heart infusion agar plates containing dihydrostreptomycin (300 μg/ml).

In addition to the stable auxotrophs transformed as reported here, several others are under investigation, and many other auxotrophs were isolated but reverted to prototrophy after several serial transfers. Niederman *et al.* have also reported (*9*) the isolation of an asparagine-requiring auxotroph from *Streptococcus bovis*. These workers used this auxotroph for microbiological assay of asparagine, but they have not tested it for its ability to be transformed. However, the production of some stable auxotrophs and their ability to be transformed permits a start in mapping the streptococcal chromosome.

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10 March 1966

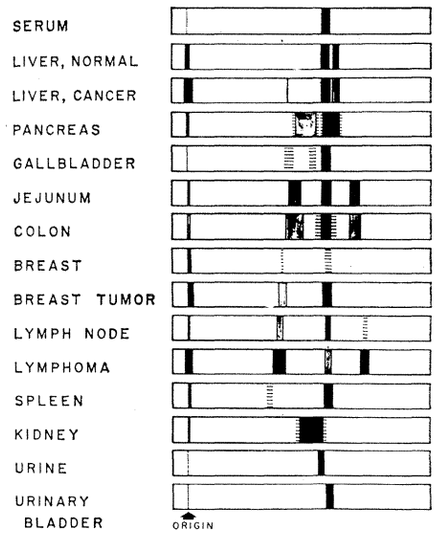

Fig. 1. The electrophoretic distribution on starch gel of naphthylamidases from various human tissues. The liver cancer tissue was obtained from a Carcinoma of the pancreas metastatic to the liver.

Starch-Gel Electrophoresis of Human Tissue Enzymes Which Hydrolyze L-Leucyl-β-Naphthylamide

Abstract. *Electrophoretically distinct forms of the enzyme or enzymes which catalyze the hydrolysis of* L-*leucyl-*β*-naphthylamide were demonstrated in various human tissues. There were characteristic patterns for different tissues. Unlike the enzymes found in normal serum, some tissue-specific enzymes were unaffected or activated by* L-*methionine.*

With L-leucyl-β-naphthylamide as substrate, amino acid naphthylamidases (*1, 2*) have been identified in several electrophoretically distinct forms (presumably isoenzymes) in the serums of patients with various diseases (*3*). We now report the existence of electrophoretically and, in certain cases, biochemically distinct forms of the enzyme in different human tissues.

Specimens of liver, kidney, pancreas, colon, breast, breast adenocarcinoma, heart and skeletal muscle, gallbladder, urinary bladder, jejunum, spleen, lymph node, malignant lymphoma, and adenocarcinoma metastatic to the liver were obtained at operation or immediately after death. Extracts were prepared by homogenizing the tissue in approximately five times its weight of distilled water (20°C) first in a VirTis-45 tissue grinder and subsequently in a Potter-Elvhejem ground glass homogenizer. The homogenates were centrifuged at 900*g* for 30 minutes, and the supernatants were centrifuged again at 20,000*g* for 20 minutes. This supernatant was the extract studied. Each extract was assayed for naphthylamidase activity (*4*) and examined electrophoretically as described below.

Fifty microliters of the extract were subjected to electrophoresis on a vertical starch gel by the method of Smithies (*5*), with a borate-gel buffer, *p*H 8.7, and a borate-bridge buffer, *p*H 8.2. Electrophoresis was carried out at room temperature (23°C) and 4.5 volt/cm for 16 to 17 hours. After electrophoresis a thin layer was sliced from the gel, and the exposed surface of the remaining portion was assayed for the localization of naphthylamidase activity by incubation for 5 hours in a solution of L-leucyl-β-naphthylamide hydrochloride ($6.8 \times 10^{-4}M$) in 0.1*M* phosphate buffer, *p*H 7.0. After incubation, the substrate solution was decanted, and the gel was exposed for 10 minutes to a solution (150 ml) of a stabilized diazonium salt (Garnet GBC; 1 mg/ml). This procedure resulted in conversion of enzymatically released β-naphthylamine to an insoluble red azo dye, which delineated the areas of enzymatic activity. The gel was washed with water and cleared in glycerol overnight.

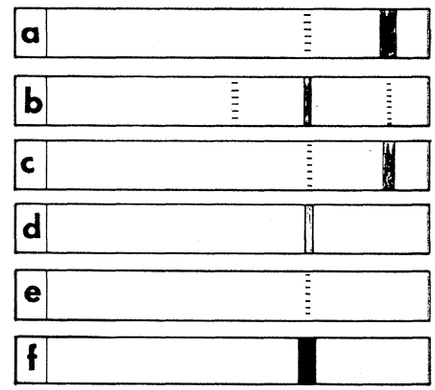

Fig. 2. The effect of 0.01*M* L-methionine on the naphthylamidases of serum and heart and skeletal muscle. (*a*) Skeletal muscle plus methionine; (*b*) skeletal muscle; (*c*) heart muscle plus methionine; (*d*) heart; (*e*) serum plus methionine; (*f*) serum.

Since L-methionine competitively inhibits hydrolysis of the substrate by

the serum enzymes (*6*), its effect on the tissue enzymes was examined in parallel experiments in which L-methionine was added to the substrate solution to yield a final concentration of 0.01 mole per liter.

The electrophoretic mobilities of the tissue enzymes were compared with the mobility of the normal serum enzyme (Fig. 1). An enzyme identical in electrophoretic behavior to the serum enzyme was present in almost all tissues. The ubiquitous presence of this band may have resulted from contamination by blood since the tissues were not perfused prior to extraction. This may not be the case with liver, however, since a particle-bound enzyme similar to that in serum has been isolated from this organ (*2*).

In addition to the serum-like enzyme, the electrophoregrams of several tissues showed other distinct components of enzymatic activity with mobilities greater or less than that of the serum enzyme.

Extracts of normal and cancer tissue specimens of the same organ (liver, breast, lymph node) exhibited similar electrophoretic patterns. However, the enzymatic activity in the bands of cancer tissue extracts were generally higher than those of the normal tissue.

The electrophoretic patterns of tissue enzymes incubated with substrate in the presence of L-methionine indicated definite inhibition in all cases of the enzyme band corresponding to serum naphthylamidase. However, the tissue components which differed from the serum enzyme were either inhibited, unaffected, or activated by this amino acid. These differences in response to L-methionine were also demonstrated when 1-ml portions of tissue extracts were assayed for their total naphthylamidase activity in the presence and absence of this amino acid. L-Methionine (0.01 mole/liter) yielded 60 percent inhibition of the normal serum enzyme. The effect on tissue enzymes expressed as percentage of inhibition varied as follows: pancreas, 62; kidney, 58; jejunum, 53; colon, 52; liver, 47; breast, 32; urinary bladder, 29; placenta, 24; lymph node, 19; and spleen, 15. In addition, the total naphthylamidase activity of skeletal (diaphragmatic and rectus) and cardiac muscle was increased rather than decreased by L-methionine. The electrophoregram of the muscle extracts showed that it was the fast band of naphthylamidase activity which was activated (Fig. 2).

These results indicate the heterogeneity of tissue enzymes which catalyze the hydrolysis of L-leucyl-β-naphthylamide. These data do not permit the absolute classification of these enzymes either as isoenzymes or totally different molecular species, since their association with other proteins in different tissues may contribute significantly to observed dissimilarities in electrophoretic mobility. In any event, the varied response of the tissue amino acid naphthylamidases to L-methionine serves to establish the existence of biochemical differences among these enzymes.

Chromatographically distinct forms of amino acid naphthylamidase have been demonstrated recently in human liver, small intestine, and pancreas (*7*).

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References and Notes

1. This enzyme was originally considered to be identical with "leucine aminopeptidase" [E.C.-3.4.1.1. in *Enzyme Nomenclature,* (International Union of Biochemistry, Elsevier, New York, 1965)], which catalyzes the hydrolysis of leucylglycine and leucineamide. However, other studies (*2*) have shown that the enzyme is neither identical with aminopeptidase nor specific for leucyl napthylamide. The enzyme has not been given an International Union of Biochemistry code number. However, according to I.U.B. recommendations, its systematic name would be "aminoacylnapthylamide amidohydrolase." In this report we have chosen to use the trivial name "amino acid napthylamidase" (or simply "napthylamidase") in referring to the enzyme.
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Natural Human Antibodies to Gram-Negative Bacteria: Immunoglobulins G, A, and M

Abstract. *Significant amounts of immunoglobulins G and M, and a small amount of natural antibodies reactive with* Neisseria gonorrhoeae *and* Escherichia coli, *have been detected in human adult serums by immunofluorescent techniques. Umbilical cord serums also contained substantial immunoglobulin-G antibody but little or no M or A. These findings challenge the concept that natural antibodies reactive with Gram-negative bacteria are primarily of the immunoglobulin-M class.*

In this report we present evidence that natural human antibodies reactive with Gram-negative bacteria are not limited to the IgM class, but are present in all three major classes of immunoglobulins: IgG, IgA, and IgM. In general, we used fluorescein-conjugated antiserums specific for IgG, IgA, and IgM in an indirect type of fluorescent antibody procedure.

Natural antibodies may be defined as those not related to obvious immunization or specific infection (*1*). Natural antibodies to Gram-negative bacteria are widespread among mammalian species and have long been the subject of investigation and controversy regarding their relation to antibodies associated with immunization or infection (*2*). Studies (*2*) based on a sensitive bactericidal test have suggested that natural and immune antibodies differ in physicochemical properties. Other studies (*3–5*) have resulted in the conclusion that natural human antibodies to Gram-negative bacteria are principally of the γ-macroglobulin (IgM) class (19*S*), and it has been suggested (*3*) that differences between natural and immune antibody result from the properties of two different classes of immunoglobulin (IgG and IgM). However, we have demonstrated in human serums significant natural IgG as well as IgM (and some IgA) antibodies that react with Gram-negative bacteria (*Neisseria gonorrhoeae* and *Escherichia coli*).

We used the following fluorescent-antibody method: A uniform concentration of bacteria (*6*), treated with Formalin (3 percent) or heat (100° or 121°C), was fixed to a circumscribed area of a glass slide. One drop of the