propyl alcohol (3 to 1) at room temperature. The extracts were dried at reduced pressure, and the lipid was fractionated on columns containing 10 g alumina (Brockman No. 3). Columns were eluted with portions of hexane (50 ml each) containing 0, 2, 4, 6, and 20 percent of ether. Fractions (5 ml) were collected and scanned for ultraviolet-absorbing material. The elution pattern for *B. melaninogenicus* (strain BE1), which was typical of the anaerobes examined, is shown in Fig. 2*A*. The ultraviolet absorption spectrum of fraction 14 contained peaks at 243, 248, 260, and 269 mμ, characteristic of compounds of the vitamin K group. This material was purified by thin-layer chromatography on activated silica gel HF plates developed with a mixture of isooctane, ether, and acetic acid (94 : 6 : 0.1). Compounds absorbing ultraviolet light were eluted with acetone, and their ultraviolet absorption spectra were determined in isooctane. All four anaerobes examined yielded compounds which had an absorption spectrum typical of 2,3-alkyl substituted naphthoquinones of the vitamin K group (Fig. 2*B*). Reduction of alcoholic solution of each with potassium borohydride (KBH_4) resulted in a bleaching of absorption at 260 to 270 mμ, accompanied by an 80 to 100 percent increase in absorption at 245 mμ. The compound from each organism gave a positive reaction in the Irreverre and Sullivan test (*10*) and supported growth of strain K110 of *B. melaninogenicus*. Vitamin K, isolated from *Escherichia coli* in a similar manner, supported growth of *B. melaninogenicus*, wheras ubiquinone failed to do so.

Thus, compounds of the vitamin K group are present in certain strictly anaerobic chemoorganotrophic bacteria and evidently play a vital part in anaerobic metabolism. In the case of the *Bacteroides* species at least, this vitamin must function in processes other than phosphorylation resulting from electron transfer to molecular oxygen, nitrate, or sulfate. The observation that strains of *B. fragilis* synthesize and excrete vitamin K suggests that this organism plays an important role in mammalian nutrition. Synthesis of vitamin K compounds by intestinal bacteria provides the mammalian host with a source of this vitamin (*11*). It has been assumed that the facultative coliform group has been responsible for this synthesis. However, organisms of the *B. fragilis* type are among the most numerous bacteria inhabiting the intestinal tract of man and are present in concentrations 100-fold greater than that of *E. coli* (*11, 12*). Thus, the bulk of vitamin K synthesized by the intestinal microbiota is probably due to these organisms.

RONALD J. GIBBONS
LOIS P. ENGLE
Department of Oral Microbiology, Forsyth Dental Center, Harvard School of Dental Medicine, Boston, Massachusetts

References and Notes

1. O. Isler and O. Wiss, *Vitamins and Hormones* **17**, 53 (1959); C. Martius and D. Nitz-Litzow, *Biochim. Biophys. Acta* **13**, 152 (1954); A. F. Brodie and J. Ballantine, *J. Biol. Chem.* **235**, 226 (1960).
2. R. L. Lester and F. L. Crane, *J. Biol. Chem.* **234**, 469 (1959).
3. D. H. L. Bishop, K. P. Pandya, H. K. King, *Biochem J.* **83**, 606 (1962).
4. B. K. Jacobsen and H. Dam, *Biochim. Biophys. Acta* **40**, 211 (1960).
5. T. Green, S. A. Price, L. Gare, *Nature* **184**, 1339 (1959); R. C. Fuller, R. M. Smillie, N. Rigopoulos, V. Yount, *Arch. Biochem. Biophys.* **95**, 197 (1961).
6. R. H. Baum and M. I. Dolin, *J. Biol. Chem.* **238**, PC4109 (1963).
7. M. Lev, *J. Gen. Microbiol.* **20**, 697 (1959).
8. R. J. Gibbons and J. B. Macdonald, *J. Bacteriol.* **80**, 164 (1960).
9. S. J. Sawyer, J. B. Macdonald, R. J. Gibbons, *Arch. Oral Biol.* **7**, 685 (1962).
10. F. Irreverre and M. Sullivan, *Science* **94**, 497 (1941).
11. R. M. Donaldson, *New Engl. J. Med.* **270**, 938, 994, 1050 (1964).
12. T. Rosebury, *Microorganisms Indigenous to Man* (McGraw-Hill, New York, 1962), pp. 310–355.
13. Supported in part by grant DE-1471 from the National Institute for Dental Research, and in part by a grant from the Colgate-Palmolive Co. R.J.G. was supported by a career development award from NIH.

14 September 1964

Allosteric Properties of Glutamate Dehydrogenases from Different Sources

Abstract. *The structure and allosteric properties of glutamate dehydrogenases from different sources have been studied by immunologic means. Despite structural differences detected by electrophoretic mobility and antigenicity, all the vertebrate enzymes responded similarly to allosteric modification. Bacterial glutamate dehydrogenase was immunologically unrelated to the vertebrate enzymes and did not respond to allosteric regulation.*

Certain low molecular weight "allosteric" reagents alter the structure and enzymic activity of glutamate dehydrogenase (GDH) crystallized from bovine liver (see *1*). This enzyme activity is widely distributed among microorganisms and higher forms, and, therefore, it was of interest to find out whether the allosteric regulation observed with the bovine liver enzyme also occurred with glutamate dehydrogenases from other sources. Because immunologic techniques show structural modifications in the enzyme induced by allosteric reagents, even in crude tissue extracts (*2*), we used these methods in the present investigation.

Earlier studies revealed three different antigenic forms of crystalline GDH (*2, 3*). The relative concentrations of these forms were altered by allosteric reagents such as adenosine diphosphate (ADP), guanosine triphosphate (GTP), or diethylstilbestrol. In general, ADP, which favored an antigenic form of the molecule with high GDH activity, also decreased the electrophoretic mobility in agar gel and resulted in heavier enzyme-antibody precipitin lines. Guanosine triphosphate and diethylstilbestrol increased the electrophoretic mobility and had opposite effects from those of ADP on enzyme activity. They favored antigenic forms having little or no GDH activity but having greater alanine dehydrogenase activity. These findings correlated well with the effects of these molecules on the physical and catalytic properties of GDH (*1*).

It was further shown (*2*) that sodium dodecyl sulfate dissociated the enzyme into a single immunologic component that lacked enzyme activity; this component probably represents the fundamental subunit of GDH.

In our present study, crude extracts prepared from commercial "acetone powders" of different beef organs and the livers of other vertebrate species were reacted with rabbit antiserum to GDH in double diffusion (*4*) and immunoelectrophoretic (*5*) experiments. The rabbit antiserum was prepared against crystalline GDH obtained from bovine liver as already described (see *2*).

Figure 1 illustrates two simultaneous immunoelectrophoretic experi-

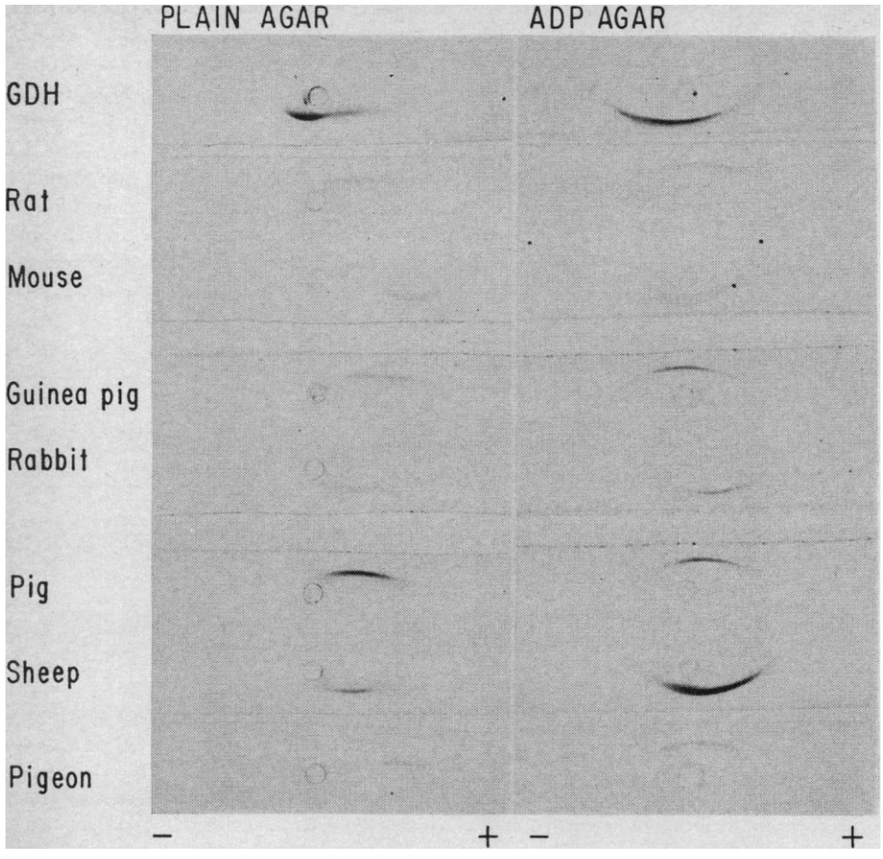

Fig. 1. Immunoelectrophoresis of GDH from different sources. The experiments were carried out in plain and ADP-containing agar with 0.05*M* veronal buffer, *p*H 8.2. All specimens were subjected to electrophoresis simultaneously (200 v, 135 min) on two large agar plates. Precipitin lines were stained for GDH activity.

ments performed in "plain agar" (not containing ADP) and in agar to which ADP was added. The GDH migrated toward the anode as a single precipitin line, and the rate of migration varied with different species. Similar differences in mobility were also seen in starch gel electrophoresis. When ADP was present in the agar, the electrophoretic migration was decreased, the precipitin lines became stronger, and the staining for enzyme activity became more intense. These changes, which presumably represent conformational changes induced by ADP in enzyme molecules, were noted with all extracts and were the same as those observed with the crystalline bovine enzyme (Fig. 1).

Kinetic studies of the GDH activities present in the extracts shown in Fig. 1 demonstrated that ADP stimulated, and GTP and diethylstilbestrol inhibited, the enzymic reaction. These effects paralleled those observed with the crystalline bovine enzyme, as would be expected from the results of the immunoelectrophoretic studies. The GDH activity from *Escherichia coli, Bacillus subtilis*, and *Saccharomyces cerevisiae* did not respond to these allosteric modifiers.

Despite the catalytic and allosteric similarities of the enzymes from different species, their different electrophoretic mobilities (Fig. 1) indicated structural differences. The structural relationships between different GDH's were studied by double diffusion (Fig. 2).

The antiserum to bovine liver GDH was reacted with crystalline GDH, the GDH from different beef organs, and the GDH from several vertebrate livers. A precipitin line did not form with beef heart extract (Fig. 2, *A*6) or pituitary (not shown) because the activity of GDH in these preparations was tenfold less than in the other extracts. Precipitin lines appeared when the heart and pituitary extracts were concentrated. No reaction occurred with a crude extract of *B. subtilis* containing high GDH activity.

The crystalline enzyme, GDH prepared from fresh beef liver mitochondria, and the GDH present in other beef organs showed complete immunologic identity. Reactions of partial identity occurred between the crystalline enzyme and GDH present in extracts from some species, as shown (Fig. 2*B*) by the short spurs formed between crystalline GDH and the GDH present in the extracts of pigeon (*B*5) and rat livers (*B*6).

The reactions of complete immunologic identity between glutamate dehydrogenases from different beef tissues suggest that these enzymes are structurally identical or very closely related. However, the partial cross-reactions between glutamate dehydrogenases from various vertebrate livers, as well as their different electrophoretic mobilities, suggest that these enzymes have similar but not identical conformations. Interestingly, in spite of these differences, all of the vertebrate enzymes have common allosteric as well as catalytic properties. Bacterial

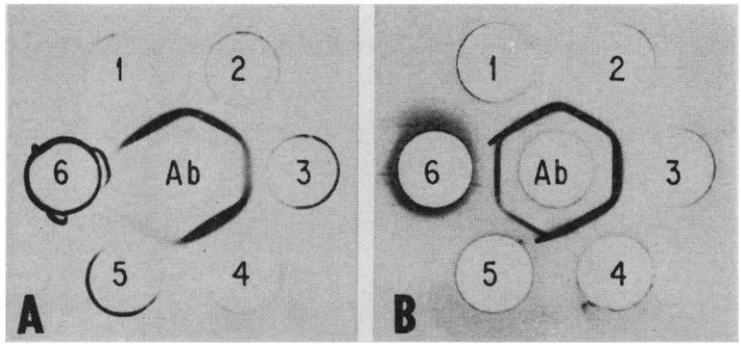

Fig. 2. Agar gel double diffusion experiments in which rabbit antiserum to bovine liver GDH (*Ab*, center wells) was reacted with crystalline GDH (*A*1, *A*4; *B*1, *B*4) and with crude extracts from beef liver (*A*2), beef spleen (*A*3), beef brain (*A*5), beef heart (*A*6), pig liver (*B*2), sheep liver (*B*3), pigeon liver (*B*5), and rat liver (*B*6). The crude tissue extracts were prepared from commercial "acetone powder" in 0.05*M* tris buffer at *p*H 7.6. Precipitin lines were stained for GDH activity by reduction of tetrazolium in the presence of L-glutamate and diphosphopyridine nucleotide as described (*2*).

GDH, by immunologic and kinetic criteria, appears quite different in structure and does not have the same allosteric response.

NORMAN TALAL
GORDON M. TOMKINS
National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland

References

1. G. M. Tomkins, K. L. Yielding, N. Talal, J. F. Curran, *Cold Spring Harbor Symp. Quant. Biol.* **28** (**1963**), 461 (1963).
2. N. Talal, G. M. Tomkins, J. F. Mushinski, K. L. Yielding, *J. Mol. Biol.* **8**, 46 (1964).
3. N. Talal and G. M. Tomkins, *Biochim. Biophys. Acta* **89**, 226 (1964).
4. O. Ouchterlony, *Acta Pathol. Microbiol. Scand.* **32**, 231 (1953).
5. P. Grabar and C. A. Williams, *Biochim. Biophys. Acta* **17**, 67 (1955).

14 September 1964

Actinomycin D: An Effect on Rat Liver Homogenates Unrelated to Its Action on RNA Synthesis

Abstract. *Liver homogenates prepared from rats injected with high doses of actinomycin D show a decrease in polyribosome content and in amino acid incorporation. These effects are not observed in rat liver not subjected to homogenization and are independent of the inhibition of RNA synthesis caused by the antibiotic.*

Actinomycin D inhibits DNA-dependent RNA synthesis in both mammalian (*1*) and bacterial (*2*) systems. However, certain effects of the antibiotic cannot be explained on this basis alone. For example, a rapid loss of pre-existing RNA has been described in mouse fibroblast cells (*3*) and in Ehrlich ascites tumor cells (*4*) when these cells are exposed to the antibiotic. Further, although cell-free preparations from the livers of rats given large doses of the drug show diminished amino acid incorporation into protein and a striking reduction in the number of polyribosomes (*5*), our studies indicate that these effects are not demonstrable in the same livers *not* subjected to cell fractionation. Our observations indicate that actinomycin D diminishes the protein-synthesizing capacity of rat liver homogenates by a mechanism independent of its action on RNA synthesis.

Table 1. Differences in effects of actinomycin (5 mg/kg, injected intraperitoneally) on rat liver slices and on microsomal fractions. The data in each line are from a single experiment. The results shown in Fig. 4 are from a separate experiment not shown in this table.

Time after injection (hr)	Effect (% of control)			
	Liver slices		Microsomes	
	Orotic acid-C^{14}	Leucine-C^{14}	Leucine-C^{14}	Polyribosomes*
11	29	103	37	
11	18	90	34	
11				21
11				37
2.5	88	96	62	
2.5	100			65

* Estimated from sucrose gradient analyses.

We have recently presented evidence (*6*) that the livers of rats injected with actinomycin D in an amount of 1.5 mg/kg of body weight incorporate amino acid into protein normally, despite a reduction of over 90 percent in labeling of the RNA of the microsomal fraction, which contains the bulk of cytoplasmic messenger activity (*7*). This work supports the proposition that the bulk of rat liver cytoplasmic messenger RNA is stable. On the other hand, Staehelin *et al.* (*5*) have reported, and we have confirmed, that ribosome preparations from livers of rats given 5 mg of actinomycin per kilogram show a profound impairment in amino acid incorporation. As described by the same authors (*5*), these preparations manifest a marked reduction in polyribosomes and an increase in monomeric ribosomes (Fig. 1). Similar effects can be demonstrated in microsomal fractions from rats treated with 5.0 mg of actinomycin D per kilogram.

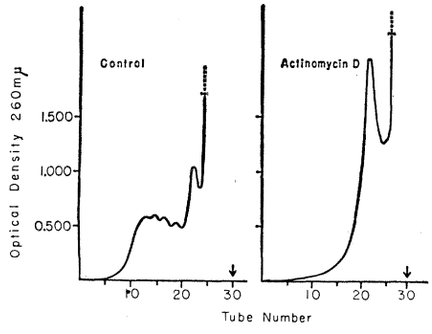

Fig. 1. Sucrose gradient centrifugation of liver particles from control and actinomycin D-treated rats. Livers from control, male, white rats (200 to 220 g) and from rats injected intraperitoneally 11 hours earlier with actinomycin (5 mg/kg) (dissolved in propylene glycol, 5 mg/ml, and diluted with an equal volume of 0.9 percent NaCl immediately before administration) were homogenized in 3 volumes of a medium containing tris *p*H 7.5 (0.01*M*), KCl (0.01*M*), and $MgCl_2$ (0.002*M*), at 0°C. The postmitochondrial supernatant fractions were made 1 percent with respect to sodium deoxycholate, and 1 ml was layered on a 25 ml, 10 to 30 percent sucrose gradient prepared in the same buffer. Centrifugation was at 25,000 rev/min for 2.5 hours at 4°C. After centrifugation the tube contents were removed through a needle inserted into the bottom of the tube and were analyzed for absorbance at 260 mμ by passage through a Gilford continuous flow cell. Polyribosomes were found in tubes 8 to 20; the peak at tube 22 represents "monoribosomes" (80*S* particles).

These preparations incorporate 35 percent as much C^{14}-labeled leucine into protein as material from untreated rats and show an apparent detachment of single ribosomes from endoplasmic reticulum (*8*) (Fig. 2). However, inhibition of isotope incorporation into microsomal RNA is only slightly greater in the livers of rats given 5 mg of the drug than that observed in rats given 1.5 mg/kg (*6*). Indeed, 3.0 mg of actinomycin results in 99 percent inhibition of isotope incorporation into microsomal RNA, but in no reduction in incorporation of C^{14}-labeled leucine by the microsomal fraction in vitro. Therefore, the effects of the higher dose of actinomycin D on amino acid incorporation and on polyribosomes in homogenates require an

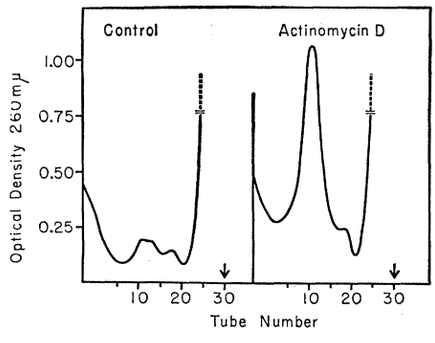

Fig. 2. Sucrose gradient centrifugation of microsomal fractions from control and actinomycin D-treated rats. Conditions were the same as those described for Fig. 1, except for the omission of deoxycholate. In both control and treated samples the bulk of the optically dense material was recovered from the pellet. This material had ratios of protein to RNA that are characteristic of microsomes. There was a large quantity of membrane-free, 80*S* particles in the fraction from treated animals (tubes 10 to 12).