Tryptophan Pyrrolase Induced in Human Liver by Hydrocortisone: Effect on Excretion of Kynurenine

Abstract. *Administration of hydrocortisone causes two- to fourfold increase in the level of activity of tryptophan pyrrolase in human liver, as measured in needle-biopsy specimens. Correlation of the higher levels of the enzyme with the amounts of urinary kynurenine suggests that the tryptophan pyrrolase level, which is regulated by adrenocortical hormones, may be the important variable in the increased excretion of tryptophan metabolites that accompanies various diseases.*

In rat livers the activity of tryptophan pyrrolase rises significantly within a few hours after administration of glucocorticoids (*1*) or of agents that cause increased adrenocortical secretion secondary to pituitary stimulation (*2, 3*). That increased activity is associated with increase in the amount of the protein moiety of the enzyme system is demonstrable by immunochemical measurements (*4*). Our purpose was to determine whether human tryptophan pyrrolase also responds to this hormone and, if so, whether this change in enzyme concentration alters the metabolism of tryptophan.

Human-liver tryptophan pyrrolase, studied in excision-biopsy specimens from patients undergoing abdominal surgery, has properties similar to those of rat-liver tryptophan pyrrolase: it is localized in the particle-free fraction and is most active when supplemented with a source of heme, such as methemoglobin, and with small amounts of mitochondria and microsomes (*5*). The method of assay, based on that used for rat liver (*5*), was adapted to a microscale. The tryptophan pyrrolase reaction forms formylkynurenine that is rapidly converted by formylase to kynurenine (*6*). Percutaneous-aspiration needle-biopsy samples of human liver (10 to 20 mg, wet weight) were immediately chilled and homogenized within 30 minutes in 0.45 ml of 0.14*M* KCl containing tryptophan (6 m*M*) and sodium phosphate buffer at *p*H 7.2 (0.02*M*). The homogenate was centrifuged for 45 minutes at 100,000*g*, and 0.1 ml of the supernatant was pipetted into a cuvette containing 0.05 mg of methemoglobin, tryptophan (3 m*M*), sodium phosphate buffer at *p*H 7.2 (0.02*M*), and 0.05 ml of mitochondria, to a final volume of 0.5 ml. Because of the small amount of human liver available, mitochondria (without tryptophan pyrrolase) were prepared from rat liver; the 0.05 ml of this suspension used contained approximately 80 μg of protein. The change in optical density of this cuvette at 360 nm (the absorption peak of kynurenine) was followed with a Gilford automatic recording spectrophotometer. After an initial period of lag, the slope of the line was straight for at least 30 minutes. Activity is expressed as micromoles of kynurenine formed per hour at 37°C per 100 mg of soluble liver protein (equivalent to about 1.5 g of liver, wet weight).

The basal tryptophan pyrrolase levels of activity in the three patients (Fig. 1) were similar, being about 10 percent of the value found in rat liver. Repeated determinations on different days gave similar values. Measurements 5 hours after administration of hydrocortisone showed two- to fourfold increases in the tryptophan pyrrolase concentrations (Fig. 1). These inductions are of similar magnitude to those reported in rats (*1, 3*).

Excretion of kynurenine after an oral dose of 2 g of L-tryptophan was measured in the same patients. In normal, control subjects such a dose, in our experience and in that of others (*7*), results in excretion of 5 to 15 mg of kynurenine during the following 24 hours. Before administration of hydrocortisone, excretions by our patients were in the normal range (12, 9, and 8 mg per day, respectively); they increased three- to fivefold (to 37, 26, and 47 mg, respectively) within 24 hours after administration of hydrocortisone. Such values are typical of the great excretions of kynurenine that accompany various diseases. Thus hydrocortisone significantly increased excretion of kynurenine after a standard dose of tryptophan, in addition to increasing the activity of the tryptophan pyrrolase in liver. There was good correlation between the levels of activity in liver of the kynurenine pyrrolase, which forms kynurenine, and the excretions of kynurenine (Fig. 2)—both in the same patient before and after administration of hydrocortisone and in the three different patients. Thus greater activity of tryptophan pyrrolase results in more oxidation of tryptophan, and this effect is reflected in excretion of more kynurenine.

Kynurenine in vivo is further metabolized, through at least two alternate pathways of limited capacity. Our results demonstrate that, if the concentration of tryptophan pyrrolase is increased in the patient's liver by hydrocortisone while the enzyme is satu-

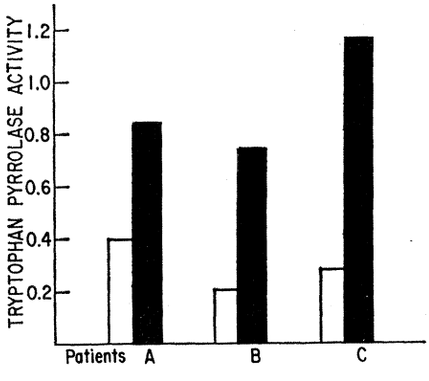

Fig. 1. Effect of administration of hydrocortisone on activity levels of tryptophan pyrrolase in human livers. Samples of liver were obtained by needle biopsy from well-nourished adult males with mild rheumatoid arthritis (*A*), healed homologous-serum hepatitis (*B*), and ankle fracture (*C*), before and after administration of hydrocortisone. The height of the light bars represents the tryptophan pyrrolase activity before, and that of the dark bars, 5 hours after, parenteral administration of 250 mg of hydrocortisone hemisuccinate (Solucortef, Upjohn Co.).

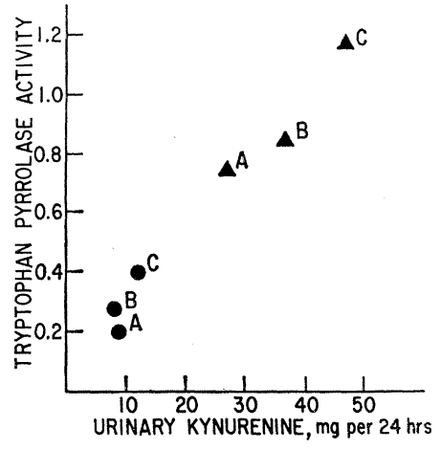

Fig. 2. Correlation of concentration of tryptophan pyrrolase in the liver with urinary excretion of kynurenine. Urinary excretion of kynurenine during 24 hours after an oral dose of 2 g of L-tryptophan (abscissa) was determined (*7*). Administration of tryptophan and collection of urine were begun immediately after the liver specimen was obtained. The concentrations of tryptophan pyrrolase (ordinate) are those of Fig. 1. Circles are values before administration of hydrocortisone; triangles are values after administration. *A*, *B*, and *C* are the three patients.

rated with a loading dose of its substrate, more of this intermediary kynurenine accumulates. This is the result of enzymic imbalance: the larger amounts of kynurenine produced by more tryptophan pyrrolase exceed the unchanged capacity of enzymes that catabolize it. Another enzymic imbalance, but with a different mechanism, is responsible for the increased excretion of kynurenine that accompanies vitamin-B_6 deficiency; kynurenine is produced in normal amounts but accumulates because of the low activity of pyridoxal phosphate–requiring enzymes (such as kynureninase) that metabolize it.

Increased excretion of tryptophan metabolites, in the amounts found by us after administration of hydrocortisone, have been reported in patients having rheumatoid arthritis, schizophrenia, renal tuberculosis, Hodgkin's disease, scleroderma, and various other diseases (*8*). Many of these diseases may be associated with "stress", which results in increased adrenocortical secretion by stimulation of the pituitary-adrenal axis. There may also be greater sensitivity to the adrenocortical hormones in some of these disease states. Our results demonstrate correlation between the activity level of tryptophan pyrrolase in liver and the amount of kynurenine in urine, and also show that the tryptophan pyrrolase is induced in man by hydrocortisone. It is therefore possible that induction of tryptophan pyrrolase by adrenocortical hormones may represent the common factor leading to increased excretion of tryptophan metabolites in diverse diseases in man.

KURT ALTMAN
OLGA GREENGARD
Department of Internal Medicine, New York Medical College, and *Institute for Muscle Disease, New York*

References and Notes

1. W. E. Knox and V. H. Auerbach, *J. Biol. Chem.* **214**, 307 (1955).
2. W. E. Knox, *Brit. J. Exp. Pathol.* **32**, 462 (1951).
3. O. Greengard and P. Feigelson, *Nature* **190**, 446 (1961).
4. P. Feigelson and O. Greengard, *J. Biol. Chem.* **236**, 153 (1961).
5. O. Greengard, N. Mendelsohn, G. Acs, *ibid.* **241**, 304 (1966).
6. W. E. Knox and A. H. Mehler, *ibid.* **187**, 419 (1950).
7. S. L. Tompsett, *Clin. Chim. Acta* **4**, 411 (1959).
8. J. M. Price, *Univ. Mich. Med. Bull.* **24**, 461 (1958); J. Musajo and C. A. Benassi, *Advan. Clin. Chem.* **7**, 63 (1964).
9. We thank G. Acs for advice and discussions and I. A. Jaffe for support. Work supported by the Arthritis Foundation and by PHS grant CA 08676-01.

8 October 1965

Microinjection of Mouse Eggs

Abstract. *A technique has been developed for injecting known amounts of liquids into fertilized one-celled mouse eggs by use of a calibrated ocular micrometer superimposed on the terminal regions of the injection pipette. From 128 pronuclear eggs, each injected with 180 or 770 cubic microns of bovine gamma globulin at a concentration of 25 milligrams per milliliter in citrate–Locke's solution and then transferred to the oviducts of pregnant foster mothers, 18 living fetuses developed; from 74 eggs, each injected with 2730 cubic microns, 5 fetuses survived. The living fetuses that developed from the injected eggs were smaller than normal in 6 of 23 surviving experimental fetuses as contrasted to only 1 of 19 control fetuses.*

Microinjection of living cells other than mammalian eggs has been practiced for about half a century (*1*). Micrugical studies have even utilized the living eggs of the rat (*2*), rabbit (*3*), mouse (*4*), and human (*5*) during the last two decades, but the methods have not been able to safeguard the structural or functional integrity of the whole ovum. The mammalian egg is

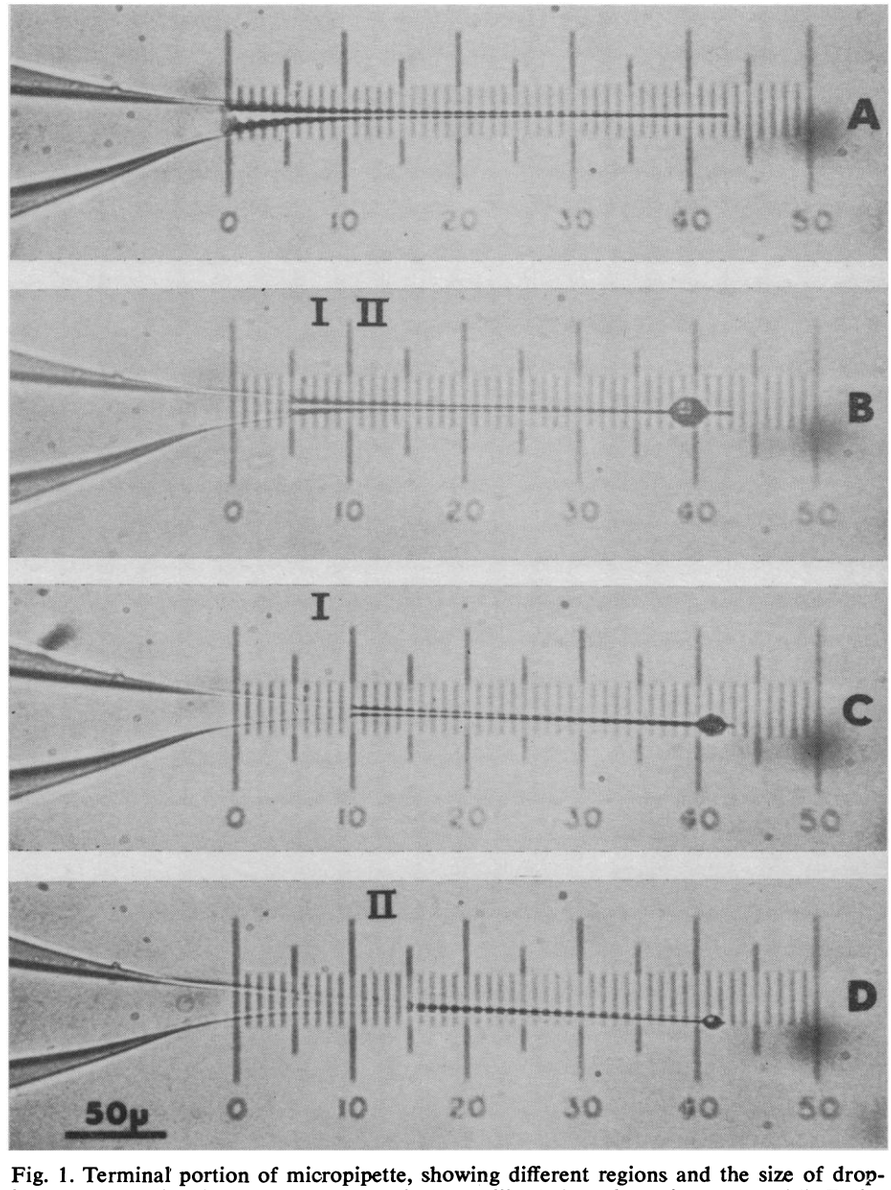

Fig. 1. Terminal portion of micropipette, showing different regions and the size of droplets corresponding in measurement against a calibrated ocular micrometer. (*A*) Entire terminal portion of pipette filled with solution; (*B*) upper region (2730 μ^3); (*C*) middle region I (1340 μ^3); and (*D*) middle region II (180 μ^3). Details in text.