binding components present in commercial intrinsic factor concentrates of hog origin. The amount of one particular binder has been found to correlate with the clinical activity of the concentrates as measured by the urinary excretion method of Schilling. This provides the basis for a simple *in vitro* assay of potency of hog intrinsic factor concentrates ranging in level of activity from 2.0 mg to 75.0 mg. It is predicted that pure hog intrinsic factor will be active clinically at a level of 0.1 mg; calculations suggest that it will bind 23 μg B_{12}/mg and have a molecular weight of 60,000.

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Histochemical Localization and Metabolic Significance of Glucose-6-Phosphate Dehydrogenase System in Adrenal Cortex.* (24959)

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It has been suggested that the adrenal cortex is one of the few mammalian tissues which utilizes the oxidative mechanism of pentose phosphate pathway for metabolism of carbohydrate in preference to the more usual glycolytic pathways of Embden and Myerhof and the citric acid cycle(1,2). Haynes(3,4) provided evidence that adrenocorticotrophin (ACTH) may act to promote steroid synthesis through a stimulating effect on adrenal phosphorylase with increased production of both glucose-1-phosphate and glucose-6-phosphate. The latter compound enters the pentose phosphate pathway *via* a triphosphopyridine nucleotide (TPN) mediated dehydrogenation to 6-phosphogluconic acid, with reduction of TPN to TPNH. The 6-phosphogluconic acid is in turn oxidatively decarboxylated, again using TPN as an electron acceptor with production of ribulose-5-phosphate and reduced TPN (TPNH). The system at this stage is acting as a TPNH generator. This is particularly significant since TPNH is required in the adrenal cortex as reductant for hydroxylations of the steroid molecule. These data in metabolic studies were obtained by quantitative methods which do not preserve structural relationships in the tissue. It was felt that use of histochemical technics for demonstration of some enzyme systems involved might contribute useful data on localization of these systems within the cortex and thus supplement the quantitative observations.

Materials and methods. Adrenal tissue was obtained from 10 250 g, female, white rats fed a standard laboratory diet and acclimated

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to animal room for at least a week. Animals were sacrificed by blow on head. Both adrenals were removed immediately and 15 μ frozen sections were cut in a cryostat. Each section constituted a cross section of the entire gland. Multiple sections from each gland were taken. Some of these sections were incubated 20 minutes at 37°C in a substrate solution (I) designed to demonstrate activity of the glucose-6-phosphate dehydrogenase system. This solution is composed of 30 mg of potassium salt of glucose-6-phosphate; 5 mg of TPN and 5 mg of a paranitrophenyl substituted ditetrazolium salt (NBT)(5) in 10 ml of 0.1 M veronal buffer at pH 7.4. Potassium cyanide to 0.1 M is added as inhibitor of tissue cytochrome system. The reaction depends on oxidation of glucose-6-phosphate with the passage of electrons to TPN *via* the specific dehydrogenase. TPNH is then reoxidized by means of TPNH diaphorase with the electrons being transferred to the NBT. Reduction of NBT results in formation of a relatively insoluble (fat and water), substantive, blue, granular pigment at site of enzyme activity. It is important to note that a positive reaction is dependent on presence in the tissue of both specific dehydrogenase and TPNH diaphorase activity. Further significant observations can be made on this system by incubating parallel tissue sections for 20 minutes at 37°C in a substrate solution (II) containing 3 mg of TPNH and 5 mg NBT in 10 ml of 0.1 M veronal buffer at pH 7.4. Potassium cyanide to .01 M is added. In this system the reduced coenzyme is used as substrate and thus demonstrates TPNH diaphorase activity directly. If for instance after incubation of a tissue section in substrate I no or little reaction is demonstrated, and if in a parallel section incubated in substrate II a positive reaction for TPNH diaphorase is obtained it can be inferred that the factor lacking for completion of the reaction in substrate I is glucose-6-phosphate dehydrogenase activity. If the reactions in both solutions happen to be negative, one can be certain that diaphorase activity is low or lacking but nothing can be said about the activity of the dehydrogenase.

Results. Sections of adrenal cortex incubated in substrate solution I (glucose-6-phosphate; TPN; NBT) showed extensive deposition of dye in cytoplasm of cells of the zona fasciculata and reticularis with the greatest concentration present in middle and outer fascicular zone. The glomerulosa, however, showed considerably less activity (Fig. 1). Parallel sections incubated in substrate solution II (TPNH and NBT) also showed intense dye deposition in the fascicular and reticular zones, but in sharp contrast to sections incubated in the first medium, these sections showed marked enzyme activity in the glomerulosa. This indicated presence of considerable TPNH diaphorase activity in the glomerulosa. One could infer that the comparatively poor dye deposition in glomerulosa of those adrenals incubated in substrate solution I was due to a relative deficiency of specific glucose-6-phosphate dehydrogenase activity. These results were obtained in all animals studied.

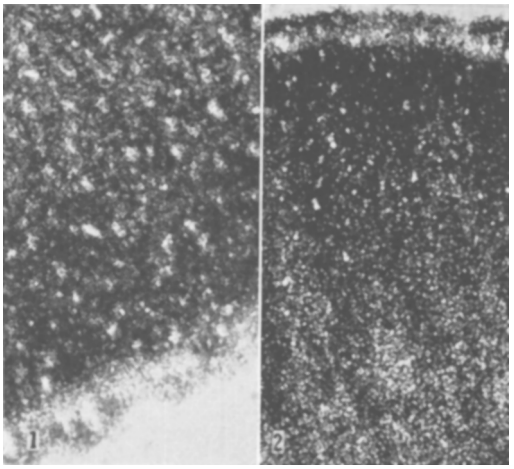

FIG. 1. A section of adrenal cortex incubated in substrate solution I (glucose-6-phosphate; TPN; NBT) showing sharp contrast in activity of glucose-6-phosphate dehydrogenase system between fascicular cortex and glomerular cortex. ×80.

Fig. 2. A section of adrenal cortex taken parallel to Fig. 1 incubated in substrate solution II (TPN; NBT) showing intense activity of TPNH diaphorase system in both fascicular and glomerular cortex. A zone of cells one to 2 cell layers thick lying between fascicular and glomerular zones shows reduced activity. ×53.

Discussion. Activity of glucose-6-phosphatase dehydrogenase system was low in the glomerular zone of the adrenal cortex. In contrast, considerable activity of this enzyme

system was present in both fascicular and reticular zones. The comparatively low concentrations in the glomerulosa were due at least in part to a relative lack of specific dehydrogenase activity. One can not rule out by this method the possibility that TPN may also be low in the glomerulosa, because TPN is added in reaction I. If as has been suggested one of the roles played by this enzyme system in the cortex is to act as a TPNH generator, providing this reductant for important hydroxylations of the steroid molecule, it is reasonable that it be most active in the fascicular and reticular zones. These are the areas which seem to react most strikingly in situations of acute and chronic stress and following ACTH administration. Morphologic and histochemical criteria of response to stress including ascorbic acid depletion, lipid and cholesterol depletion and hypertrophy all appear to involve the reticular and fascicular zones(6). It has not been demonstrated that the glomerulosa participates actively in these adjustments, at least in the early stages. This zone appears to react demonstrably in a specific fashion to alteration in electrolyte concentration. In the light of our present knowledge it would seem that the fascicular and reticular zones are the areas of the cortex where increased synthesis of steroids occurs in response to acute and chronic stress, and it is here that one would suspect a requirement for increased TPNH and potential TPNH generator systems.

Summary. A histochemical technic was used to localize the activity of glucose-6-phosphate dehydrogenase system in zones of the adrenal cortex. It was very active in the fascicular and reticular zones. Activity in the glomerulosa was comparatively low. Since this enzyme system may be indirectly influenced by ACTH and is a potential provider of TPNH, a reductant important in steroid synthesis, demonstration of its predominance in a zone where active steroid synthesis is presumed to occur is reasonable and of considerable interest.

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Pathway of Enterogastric Reflex.* (24960)

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That acid in upper small intestine inhibits emptying of stomach was first described at end of 19th century by Serdjukov in Pavlov's laboratory(1). This phenomenon present in dogs exists also in human subjects (Barsony and Egan(2)). Thomas and co-workers(3,4, 5) pointed out that delay in gastric evacuation was not due to pylorospasm, but to reduction in motility of the whole stomach. They found the mechanism undisturbed by splanchnicectomy but reduced by division of the vagi. The name "enterogastric reflex" was coined for the phenomenon observed. Quigley *et al.*(6,7) reported similar findings in that vagotomy elevated the threshold for acid inhibition of the stomach. In human subjects we found the inhibitory effect of acid in the duodenum or upper jejunum, as measured by balloon-transducer-recorder system, persisted after complete supradiaphragmatic vagotomy, thoracolumbar sympathectomy, partial gastric resection, or transection of

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