Immunohistochemical Demonstration of Serine Dehydratase in Rat Liver

Tomoyuki Kitagawa, MD, PhD and Henry C. Pitot, MD, PhD

Serine dehydratase (L-serine hydro-lyase, EC 4.2.1.13) was demonstrated in liver tissue of rats by an indirect immunofluorescent method. In the adult rat liver, serine dehydratase was localized to periportal hepatocytes, diffusely in their cytoplasm. The enzyme-specific fluorescence was absent or extremely low in the centrolobular hepatocytes. It was not demonstrated in nonparenchymal cells. Feeding a 90% protein diet for 5 days caused marked induction of this enzyme in the periportal and midzonal hepatocytes but no induction in the centrolobular hepatocytes. In the newborn rat liver, there was no apparent intralobular heterogeneity seen in the distribution of serine dehydratase, either before or after dietary induction. After 1 week of age, there was a gradual development of the intralobular heterogeneity of the enzyme, which was emphasized by dietary stimulation. A comparative study of the induction pattern between the livers of intact and adrenalectomized rats suggested that there is no heterogeneity among serine-dehydratase-positive cells with respect to hormonal regulation of this enzyme by either glucagon or cortisone. Am J Pathol 78:309–318, 1975)

SERINE DEHYDRATASE is one of the rat liver enzymes whose regulation by environmental factors has been extensively studied both in normal and malignant cells. In the normal liver *in vivo*, it is inducible by several hormones (glucagon, epinephrine and cortisone), as well as by substrate and dietary stimuli,[1–4] while the induction can be prevented or repressed by the administration of glucose or fructose.[2,5] Two forms of serine dehydratase, each of which are regulated by a glucagon and cortisone, respectively,[6] have been reported. The question then arose as to whether the two forms of the enzyme are produced by the same hepatocyte or by different cells under the influence of each hormone.

The enzyme level and the regulation of serine dehydratase is quite variable among a series of transplantable hepatomas in comparison with the same mechanisms seen in normal liver.[7,8] The dietary induction of this enzyme, along with others, in the "preneoplastic" liver obtained by feeding acetylaminofluorene or 3′-methyl-dimethylaminoazobenzene

From the McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisc.

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Address reprint requests to Dr. Henry C. Pitot, McArdle Laboratory for Cancer Research, University of Wisconsin Medical Center, Madison, WI 53706.

was severely impaired.[9,10] While the authors of these reports interpreted these findings as an expression of a characteristic "preneoplastic" change of the liver during carcinogenesis, other investigators have observed retention of the metabolic regulation of other corticoid-mediated enzymes in hyperplastic nodules,[11] which have been considered as the most likely precursors of hepatic carcinoma.[12] On the other hand, histochemical studies have shown that the "preneoplastic" liver is a mixture of degenerated hepatocytes derived from the original parenchyma and various kinds of hyperplastic regions. Furthermore, the phenotype of these hyperplastic lesions changes with time.[13] Apparently more elaborate studies on the regulatory state of the "preneoplastic" liver are required in close correlation with the morphology of these cellular populations. For this purpose we have studied the histochemical variation of serine dehydratase in liver.

This report describes an indirect immunofluorescent method for serine dehydratase, the results being obtained from observations on the liver of normal rats of various ages before and after induction. In addition, the question as to the heterogeneity of hepatocytes in hormonal regulation is partly answered in this report from the observation of the induction pattern of this enzyme in adrenalectomized rats. The histochemical study of the metabolic regulation of this enzyme in "preneoplastic" liver tissue is the subject of a future report.[14]

Materials and Methods

Preparation of Antiserum

Crystalline serine dehydratase containing both forms I and II was prepared, and its specific antibody was raised in rabbits by methods described previously.[2,6] The specificity of the antiserum for serine dehydratase was verified by the Ouchterlony double diffusion test and immunoelectrophoresis, as described previously,[2] and by inhibition of fluorescence by reacting the antiserum with either crude tissue extracts or pure enzyme before staining.

Animals and Treatment for Induction

Sprague-Dawley rats were used in the experiments throughout. They were kept in an air-conditioned room with alternating 12-hour periods of light and dark. Male rats and pregnant rats of various ages were purchased from the Sprague-Dawley Co. of Madison, Wisc. They were fed either standard laboratory diet containing 24% protein, 90% casein diet for 5 to 28 days, or a 0% protein diet for 5 days. The composition of the 90% and 0% protein diets were described previously.[15] By feeding 90% or 0% protein diet for 5 days, greater than tenfold differences in the serine dehydratase level in the livers of rats on the two diets should be expected.[6] Two-, 8- and 14-day-old sucklings were starved for 6 hours starting at 9:00 AM and then force-fed with 17.0% casein hydrolysate in saline six to eight times during 2 days, 0.2 to 0.5 ml at a time, using the smallest bore Standard Tubing available (Tech-

nicon Instrument Co., Tarryton, NY) with the point sharpened. During the period of force-feeding, the sucklings were kept in a warm room at 37 C, separate from the mother. The sucklings were killed at 24 or 36 hours after the initiation of force-feeding.

Enzyme induction following glucagon or cortison injection was found to be much less effective than that of dietary stimulation and was not useful for the present histochemical observation of serine dehydratase induction. Adrenalectomized male rats weighing 150 g were purchased from the Endocrine Labs, Madison, Wisc, and either fed a standard laboratory chow diet or the 90% protein diet for 5 days with 0.9% NaCl in their drinking water.

The anti-serine-dehydratase rabbit serum and nonimmune labeled goat serum had been adsorbed with rat kidney or liver acetone powder, respectively, before staining; 100 mg of the powder was added to each 1.0 ml of serum. After a 1-hour incubation at room temperature, the powder was spun down by centrifugation at 2000 rev/min, and another 100 mg of the powder was added to the supernatant. After incubating for 1 hour at room temperature and then overnight at 0 to 4 C, the powder was spun down in the same way, and the supernatant was used.

Preparation of Sections

The rats, under light ether anesthesia, were killed by bleeding from the jugular vein. Pieces of liver tissue were frozen on dry ice. Frozen sections were cut 6 or 8 μ in thickness in a cryostat at −18 C, placed on nonfluorescent slides, dried, fixed for 30 seconds in cold methanol, and dried again quickly. Fixation for longer periods in methanol caused diffusion of the enzyme into the sinusoids. Other pieces of tissue (2 to 3 sq mm) were fixed in three changes of cold methanol for 3 days, passed through three changes of xylol in 60 minutes and embedded in paraffin at 56 C (principally after Hamashima's method [16]). Sections 3 to 4 μ in thickness were cut, placed on nonfluorescent slides, deparaffinized with xylol and dried.

Indirect Fluorescent Antibody Staining

Sections were dipped in phosphate-buffered saline (PBS), pH 7.2, for 30 seconds, and the water on the slides surrounding the tissue was wiped off. Two drops of serine dehydratase antiserum or control rabbit serum were placed on the slide and allowed to react with the tissue for 30 minutes at room temperature in a moist chamber. The excess serum was washed off with PBS, and then the slides were washed in three changes of PBS for 15 minutes. They were wiped carefully and 2 drops of fluorescein-isothiocyanate-labeled antirabbit goat IgG globulin (Travenol Laboratories, Inc, Calif) were layered on each section. The sections were allowed to react for 30 minutes at room temperature and washed as before. Some of the slides were counterstained with Eriochrome black (Difco Labs, Detroit, Mich), diluted 1:20 in PBS for 10 seconds, washed in PBS and mounted under cover slips with glycerol. Slides without counterstaining were also useful, especially for making black and white pictures.

The slides were examined with a Leiz Ortholux microscope equipped with an Osram HBO 200 high pressure mercury vapor lamp, BG38 and BG12 excitation filters, 510-nm barrier filters and dark field condenser. Photographs were taken on daylight-type high-speed Kodak Ektachrome film (ASA 160).

Staining for glucose-6-phosphatase (Wachstein-Meisel's method [17]) and for histologic observations (hematoxylin and eosin) was performed for a comparative study using serial sections.

Results and Discussion

In adult rat liver, the specific fluorescence of serine dehydratase was demonstrated in the hepatocytes of the periportal area occupying about one-third of the hepatic lobule. There was a considerable variation in the level of this enzyme among individual rat livers, but generally the fluorescence was very weak when there was no induction (Figures 3 and 7A). After feeding 90% protein for 5 days, the specific fluorescence increased markedly, and the fluorescent-positive zone expanded toward the central portion but did not exceed two-thirds of the lobule, or zones 1 and 2 of Rappaport[18] (Figures 1 and 7A). In the centrolobular hepatocytes, the serine dehydratase was absent or extremely low and not inducible, even when rats were fed a 90% protein diet for up to 28 days. In the hepatocytes with enzyme, the level of the fluorescence was fairly uniform among positive cells except for the abrupt decrease in the cells at the boundary of the negative central zone. Cytologically, the fluorescence was distributed diffusely throughout the cytoplasm of the positive hepatocytes (Figures 2 and 5). The fluorescence was absent in the nucleus, although in paraffin sections it was not possible to avoid some artifactual fluorescence in the nucleus. On cell fractionation, serine dehydratase is found in the cytosol and is considered to be a cytoplasmic enzyme. The cytochemical distribution pattern of this enzyme in the present study is therefore consistent with the biochemical evidence.[19]

Serine dehydratase was not demonstrated in nonparenchymal cells. The specific fluorescence of serine dehydratase disappeared when the antiserum was preabsorbed with purified rat serine dehydratase or crude liver extracts. It was not demonstrable in the liver of rats fed a 0% protein diet for 5 days. In the livers of newborn rats, between 1 and 5 days after birth, there was no apparent zonal heterogeneity in distribution of the specific fluorescence, before and after dietary induction (Figures 4 and 5). After 1 week of age there was a gradual achievement of the intralobular metabolic heterogeneity of this enzyme which was markedly emphasized by dietary stimulation (Figure 7). During the third and fourth weeks of age there was a transient elevation of the endogenous level of serine dehydratase in the periportal area of the liver (Figure 6) which is consistent with the biochemical data.[20]

Generally there was a good parallelism between serine dehydratase and glucose-6-phosphatase in their distribution patterns and intensities in hepatic lobules (Figures 4A, B and 7A, B), although the activity of the latter enzyme was not absent in the centolobular portion. This parallelism seems to be reasonable since both enzymes are related to gluconeogenesis.

The liver from adrenalectomized rats after dietary induction showed an intralobular distribution pattern of serine dehydratase similar to that of intact rats, although generally the intensity of fluorescence was less than that of intact rats (Figure 8). It has been reported that a high protein diet or the forced feeding of amino acids stimulate the secretion of glucagon, which consequently elevates the serine dehydratase level in the liver.[1] Administration of glucagon and cortisone to the intact or adrenalectomized rat, which (like dietary induction) induces both isozymic forms of serine dehydratase,[6] resulted in the same pattern of immunofluorescence as seen in the induced adrenalectomized animal, which has no significant endogenous source of cortiosteroids.[21] The similarity of the distribution patterns of the serine-dehydratase-positive hepatocytes in intact and adrenalectomized rats suggests that there is no heterogeneity among serine-dehydratase-positive hepatocytes in relation to the hormonal regulation of this enzyme by glucagon and hydrocortisone and its two isozymic forms.

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Acknowledgments

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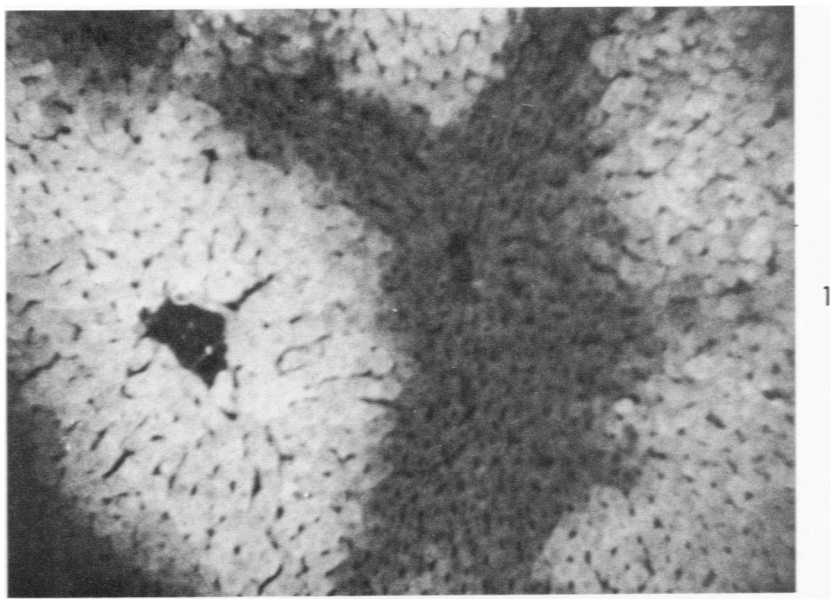
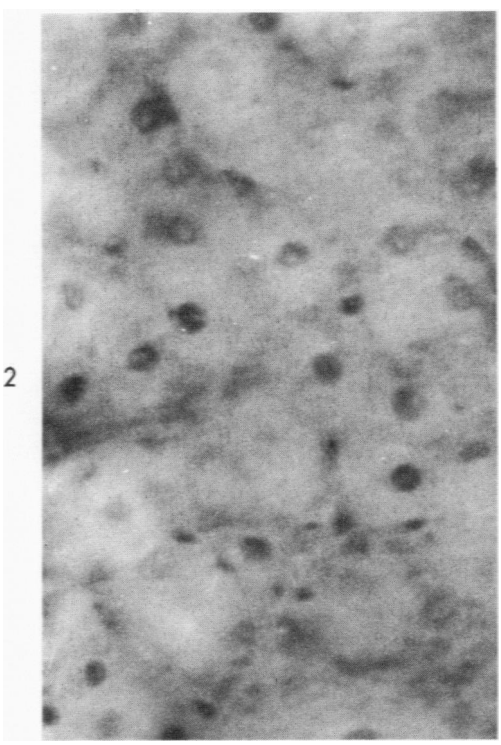




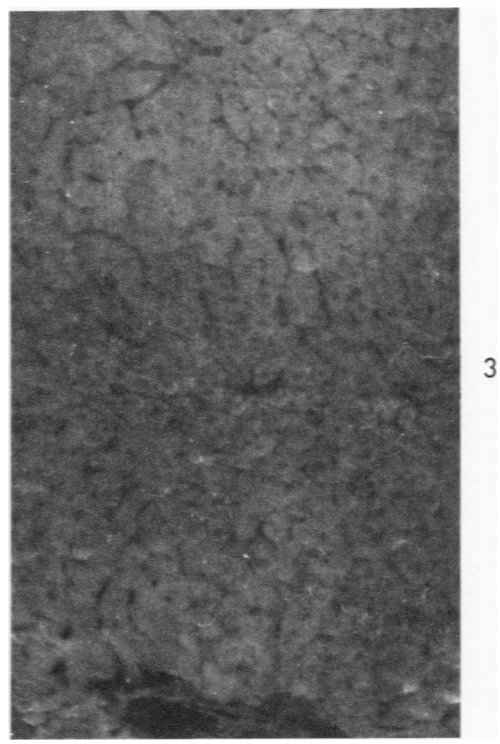


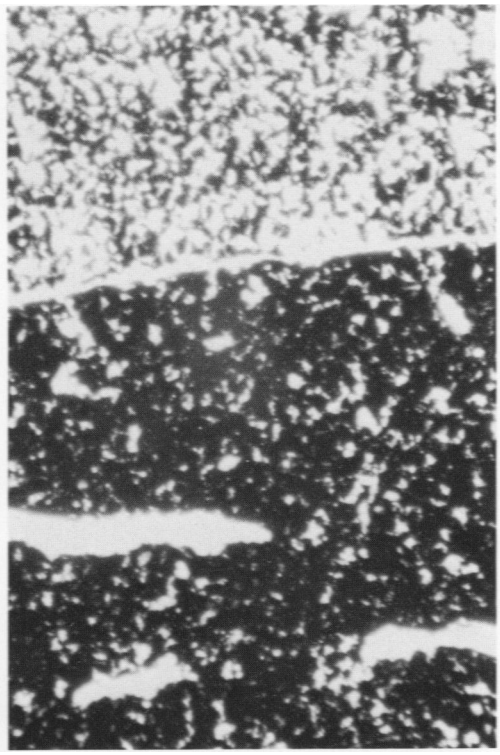
Fig 1—Distribution of serine dehydratase in the liver tissue of an adult rat after dietary induction (90% protein diet fed for 5 days). The remarkable enzyme specific fluoresence is seen only in the hepatocytes of periportal and midzonal areas (Paraffin section, × 90).
Fig 2—A higher magnification shows diffuse cytoplasmic localization of serine dehydratase in hepatocytes (Frozen section, × 450). **Fig 3**—Demonstration of serine dehydratase in the liver tissue of an adult rat before induction. A low level of specific fluorescence is seen in the periportal hepatocytes (Paraffin section, × 90).

Fig 4—Distribution pattern of serine dehydratase (**A**) and glucose-6-phosphatase (**B**) in the liver of 4-day-old sucklings after force-feeding with 17.0% casein hydrolystate from the second to fourth days (lower portion of the sections) or without (upper portion) dietary induction. There is no remarkable zonal heterogeneity in the distribution of these enzymes, either before or after dietary induction as in Figure 1 (Frozen section, × 30).

Fig 5—Higher magnification of a portion of Figure 4A (× 90).

Fig 6—The liver from a 21-day-old rat not yet weaned shows a high level of serine dehydratase in the periportal area (Compare with Figure 4A, upper part.) (Frozen section, × 30).

4A



4B

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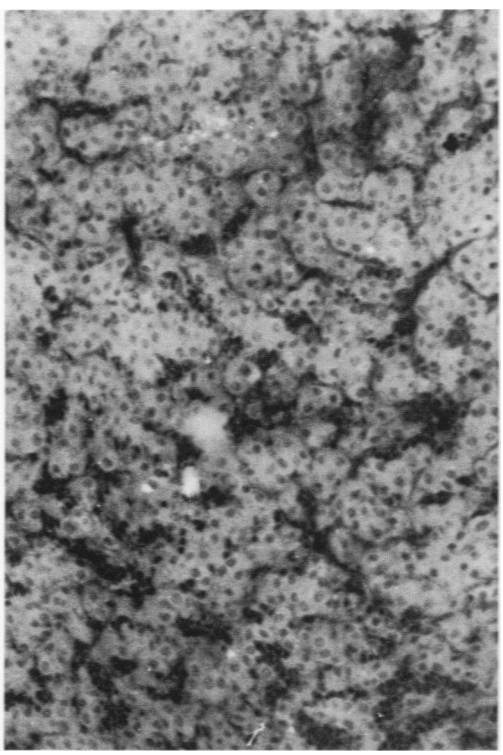

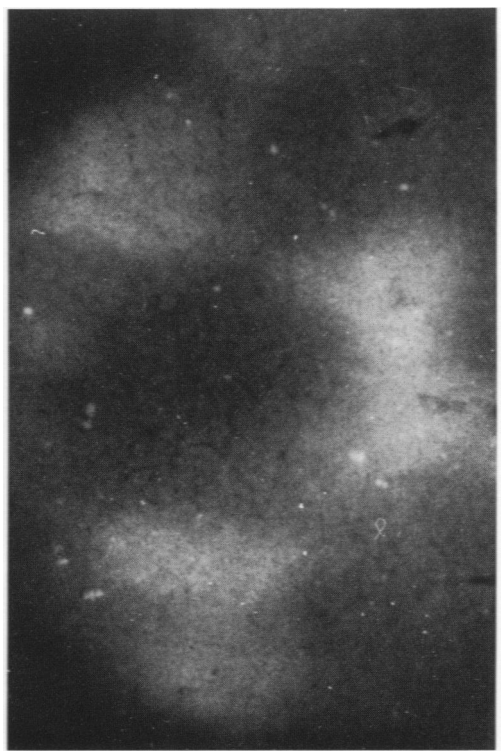

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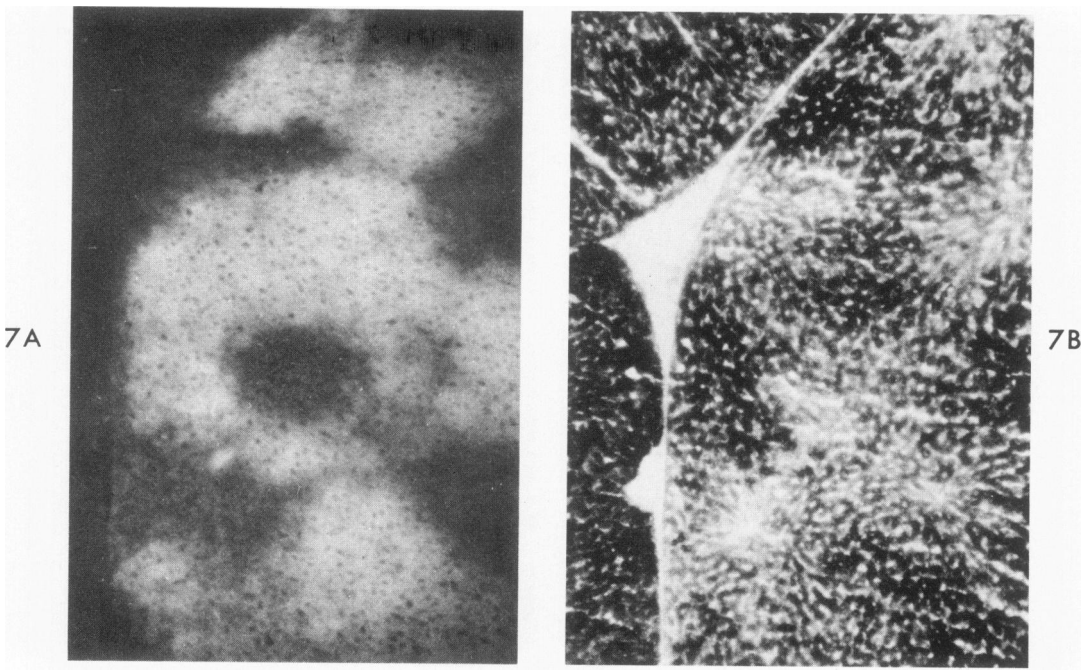

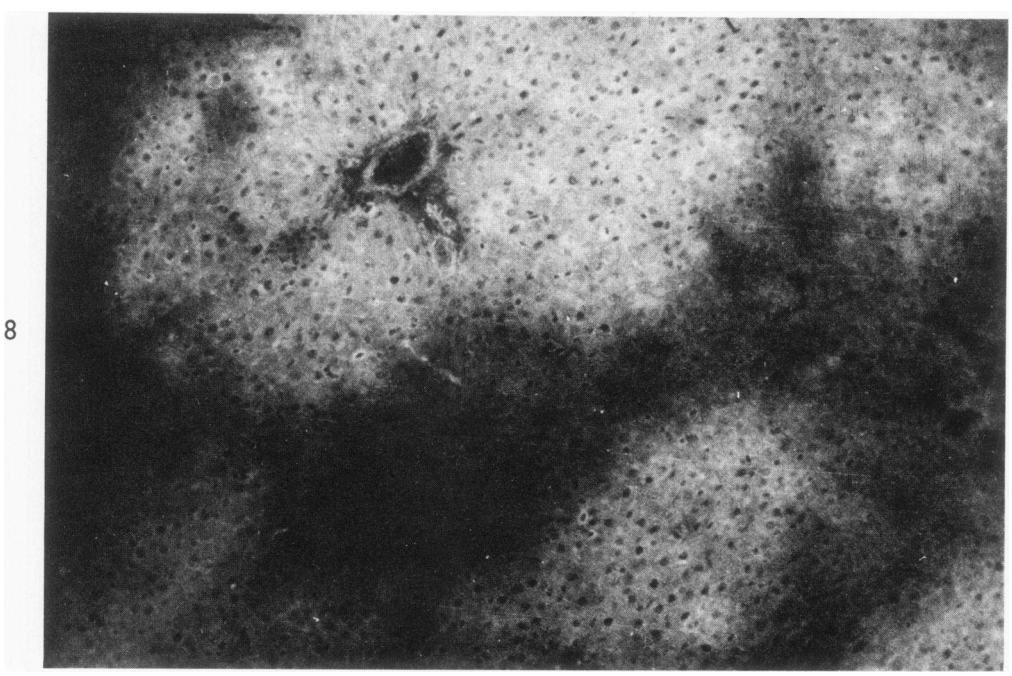

Fig 7—Serine dehydratase (**A**) and glucose-6-phosphatase (**B**) in the liver of weanling rats (5 weeks old) with (right portion of the section) or without (left portion) dietary induction (90% protein diet for 5 days). Before induction, the serine dehydratase level was very low at this age as is demonstrated in this picture (left portion) (× 40). **Fig 8**—The liver from an adrenalectomized rat after dietary induction (90% protein diet for 5 days) showing a similarity in the distribution pattern of serine dehydratase to that of intact rats after dietary induction (see Figures 1 and 7A) (Frozen section, × 50).