TABLE I. Effect of Chlorpromazine and Cyanide on Mast Cells and Leucocytes of Rats.

Treatment*	Mast cells in mesentery/3 mm²	Leucocytes (× 1000)
Saline	310 ± 70†	18.2
CPZ	55 ± 14	11.3
CPZ + KCN	235 ± 80	16.6
CPZ + KCL	50 ± 20	

* CPZ = chlorpromazine (10 mg/kg); KCN = 1.66 mg/kg, and KCL = .75 mg/kg.
† Stand. dev.

In a previous study we postulated that some hypothermic effects of chlorpromazine could be explained by the fact that this substance disrupts mast cells(2). Consequently since cyanide prevents this disruption and also prevents some hypothermia of chlorpromazine. the present study supplies additional evidence to our previous speculation on the possible relationship between mast cell disruption and hypothermia caused by chlorpromazine.

Summary. We have shown that chlorpromazine, like compound 48-80 which is a powerful histamine liberator, destroys mast cells *in vivo* and that cyanide prevents this disruption. Furthermore cyanide also prevents some of the hypothermic effect of chlorpromazine. Consequently, our study may be regarded as further evidence to a possible relationship between mast cell disruption and hypothermia caused by chlorpromazine.

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Histochemical Demonstration of 17-β-Hydroxysteroid Dehydrogenase by Use of Tetrazolium Salt.* (24726)

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Talalay has been able to induce specific steroid dehydrogenases in bacteria. and to separate these enzymes into α and β enzymes (1). These include the 3α and 3β and 17β-hydroxysteroid dehydrogenases, which require DPN† as a coenzyme. Farber *et al.*(2) have demonstrated histochemically the distribution of diaphorases within the kidney by means of neotetrazolium chloride. Since hydroxysteroid dehydrogenases require DPN as a coenzyme, the hydrogen transfer goes through a diaphorase intermediate reaction and can therefore be demonstrated histochemically by means of a tetrazolium salt. A fine histochemical localization with permanent slides can be achieved by use of 2,2′-diphenyl-5,5′-di-(*m*-nitrophenyl)-3,3′ - (4, 4′-biphenylene) ditetrazolium chloride introduced by us as substrate for demonstration of dehydrogenases(3). Our object is to demonstrate histochemically 17β-hydroxysteroid dehydrogenase by means of this tetrazole.

Methods. Small blocks of tissue were taken from rats immediately after sacrifice and frozen at −70°C and sectioned at −20°C at 5 μ. The sections were transferred to incubation solution consisting of Tris (hydroxymethyl) aminomethane buffer pH 8.8 60 mM, magnesium chloride 6 mM, DPN 1 mM, 2,2′-diphen-

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† The following abbreviations are used in text. DPN = diphosphopyridine nucleotide; EDTA = Ethylenediaminetetraacetate; DPNH = reduced diphosphopyridine nucleotide; nitro BT = 2,2′-di-p-nitrophenyl-5,5′-diphenyl-3,3′-dimethoxy-4, 4′ - biphenylene)-ditetrazolium chloride.

TABLE I. Formazan Intensities Related to pH in 17β-Hydroxysteroid Dehydrogenase.

pH	Buffer	Estradiol (1.25 mM)	Testosterone (1.25 mM)
6.57	Phosphate	0	0
7.05		0	1
7.50		1	1
7.60		2	2
7.66	Tris	2	2
8.05		2	2
8.38		3	3
8.80		3	3

yl-5,5'-di-(*m*-nitrophenyl)-3,3' - (4,4'-biphenylene) ditetrazolium chloride dissolved in N, N-dimethylformamide 0.56%, steroid dissolved in N,N-dimethylformamide 1.25 mM. 17β-hydroxysteroids used were estradiol and testosterone. Sections were incubated at 25°C, 60 minutes, washed in water, rinsed in 80% alcohol for 5 minutes and mounted in glycerogel. The following controls were all negative; estrone plus DPN, DPN without estradiol, estradiol without DPN, and no DPN or estradiol.

Results. Concentration of steroids as substrates which gave maximum formazan intensities was 1.25 mM in final concentration. Lesser concentrations namely, 0.25 and 0.05 mM final concentrations, gave weaker reactions. Concentrations higher than 1.25 mM were not carried out due to insolubility of the steroid. Final concentrations of DPN of 1.9 mM, 1 mM, and 0.5 mM were tried but 1 mM gave best results.

Differences in pH were noted as to intensity of formazan produced. (Table I). Two buffers were used, phosphate and Tris. Histology of the section was better in Tris buffer at higher pH and gave maximum formazan deposition at 8.8.

The effect of several added substances on the 17β enzymes was studied, *viz.;* $HgCl_2$ (5×10^{-5}M), KCN (1×10^{-3}M), various concentrations of niacinamide from .00 to .03 M final, Zn in the form of sulphate, chloride and acetate (10^{-7}M) and EDTA (3×10^{-3}M). Mercuric chloride inhibited completely the reaction of 17β enzyme. Potassium cyanide showed slight inhibition of reaction. Niacinamide gave marked depression of the reaction as did Zn alone and in combination with EDTA and EDTA alone.

Twenty-one different organs and tissues of the rat were tested histochemically for presence of 17β enzyme activity. A large number of male and female rats were used. The following were devoid of demonstrable 17β enzyme activity; kidney, adrenal, spleen, large intestine, lung, skeletal muscle, ovary, testes, uterus, prostate, pancreas, thyroid, thymus, oviduct, cerebrum, cerebellum, lymph node, stomach and heart. The positive organs were liver and small intestine. The entire length of small intestine including duodenum was positive.

To compare activity of 17β-hydroxysteroid dehydrogenase and diaphorase, experiments were carried out using DPNH as substrate for the diaphorase reaction. Using incubation time of 10 minutes and pH 7.6 diaphorase activity was very high in all organs tested with the exception of heart, spleen and brain. The adrenals, small intestine and large intestine gave highest activity as shown by formazan deposition. In contrast to this short incubation time of 10 minutes the 17β-hydroxysteroid dehydrogenase required 60 minutes for maximum reaction and the distribution of formazan was different. Histochemically distribution of 17β-hydroxysteroid dehydrogenase as demonstrated by resultant formazan gave a fine deep blue precipitate within the cytoplasm of the cell. In the liver, only the hepatic cells were involved. Bile ducts, vessels and Kupfer cells were negative (Fig. 1). Enzyme activity was distributed evenly in various portions of the liver lobule, in contrast to succinic dehydrogenase which is absent from areas aroung the central veins(3). In the small intestine 17β-hydroxysteroid dehydrogenase is distributed as fine formazan granules in the cytoplasm of the cell with accentuation of its luminal portion. Nuclei and cells of interstitial tissue were negative (Fig. II).

Discussion. While this work was nearing completion Wattenberg(4) demonstrated 3β-hydroxysteroid dehydrogenase histochemically in tissue sections by use of dehydroepiandrosterone (Δ^5-androstene-3β-ol-17-one) and neotetrazolium and nitro BT. DPN served

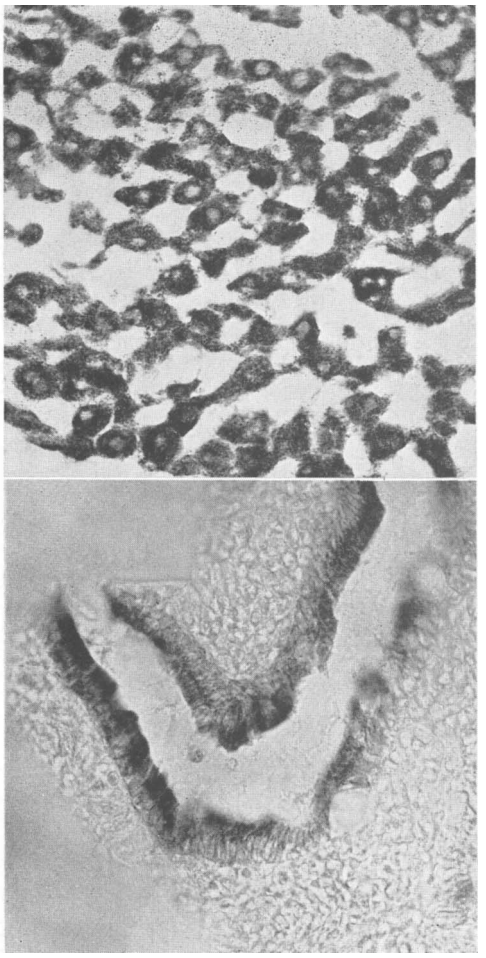

FIG. 1 (top). Liver showing 17 β-hydroxysteroid dehydrogenase activity. Diffuse distribution is present throughout liver cells. 180×.

FIG. 2 (bottom). Small intestine showing 17 β-hydroxysteroid dehydrogenase activity. Enzyme is present in cytoplasm of cells of villi. Stroma is negative. 180×.

as coenzyme and optimum pH was 8.0. All tissues and organs were negative except adrenals, ovary, testes, and liver. This is in contrast to our findings on the 17β-hydroxysteroid dehydrogenase which shows a negative reaction in adrenals, ovaries and testes. Our findings show a positive reaction only in liver and small intestine. Since niacinamide was used in his incubation media we again repeated our results in 17β-hydroxysteroid dehydrogenase with the inclusion of this substance, but found an inhibitory reaction as previously noted.

As estrone is similar to estradiol except that it has a ketone instead of an hydroxyl group in the 17 position, we expected that no dehydrogenation could take place by the action of 17β enzyme in tissue. Estrone was therefore used as control with extensive experiments. No reaction was present in liver and intestine which was positive for the 17β enzyme.

As indicated by Talalay using homogenate material, $HgCl_2$ inhibited almost completely the hydroxysteroid dehydrogenase activity (1). This was also true in our histochemical material. Using homogenates of human placenta, Langer *et al.*(5) demonstrated that Zn ion (10^{-7} to 10^{-3}M) increased the enzymatic activity of estradiol 17β dehydrogenase by as much as 34%. In contrast to this we found a definite inhibition histochemically.

Summary. We demonstrated for the first time the 17β-hydroxysteroid dehydrogenase histochemically by using 2,2′-diphenyl-5,5′-di-(*m*-nitrophenyl)-3,3′-(4,4′-biphenylene) ditetrazolium chloride. Two 17β-hydroxysteroids were used as substrates, *i.e.*, estradiol and testosterone. The optimum pH of the reaction was 8.8 and steroid concentration was determined. There was no apparent difference in activity between the 2 substrates. Controls with estrone in which the 17 group is incapable of dehydrogenation were entirely negative. The liver and small intestine were positive for 17β-hydroxysteroid dehydrogenase. This differs from histochemical results recently reported for steroid 3-β-ol dehydrogenase in that here the organs showing a positive reaction were liver, testes, ovaries and adrenal.

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