Effect of Cyanide on Some Chlorpromazine Responses. (24725)

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We have shown recently that chlorpromazine destroys mast cells *in vivo*(1) and have postulated that some of the hypothermic effects of this substance could be associated with resulting liberation of histamine and serotonine from mast cells(2). After similar studies *in vitro* Hogberg and Uvnas speculated that some of the clinical effects of chlorpromazine may be explained partly by disruption of mast cells(3). On the other hand Junqueira *et al.* have shown that certain substances such as cyanide prevent disruption of mast cells caused by "histamine liberators" (4). In this paper we studied the effect of cyanide on hypothermia and mast cell disruption, normally observed following chlorpromazine injection.

Methods. Male albino rats weighing about 200 g were used. Chlorpromazine* and potassium salts were injected intraperitoneally. In a first experiment, 3 groups of 6 animals were used. After injecting the first group with chlorpromazine (10 mg/kg), the second group with potassium cyanide (0.66 mg/kg), and the third group with chlorpromazine (10 mg/kg) and potassium cyanide (0.66 mg/kg), rectal temperatures were recorded at regular intervals with thermocouple inserted at a depth of 40 mm. In a second experiment, 4 groups of animals received respectively injections of 1) chlorpromazine (10 mg/kg), 2) chlorpromazine (10 mg/kg) and potassium cyanide (0.66 mg/kg), 3) chlorpromazine (10 mg/kg) and potassium chloride (0.75 mg/kg) and 4) saline. Four hours after injection, leucocyte counts were made on blood samples obtained by cutting tip of tail. Finally 24 hours later mast cells of the mesentery were examined as described previously(5).

Results. Fig. 1 shows that although potassium cyanide when used alone has no effect on body temperature, this substance very significantly antagonizes the hypothermic effect normally observed after chlorpromazine administration in rats. Indeed body temperature difference between the group treated with chlorpromazine and the one receiving chlorpromazine and potassium cyanide, is as large as 3°C at third hour after injection.

In addition body temperature returned to normal level in the latter group when it is still almost 2°C lower than normal in the group treated with chlorpromazine alone. Table I also shows the antagonism of potassium cyanide on some of chlorpromazine effect. The destruction of mast cells and leucocytes by chlorpromazine is almost completely prevented by potassium cyanide but not by potassium chloride.

Discussion. Disruption of mast cells by chlorpromazine reported previously(1), is prevented *in vivo* by potassium cyanide. From the work of Motta *et al.*(6), Junqueira *et al.* (4) and the results presented here, it may be said that chlorpromazine and compound 48-80, a powerful histamine liberator, have some common properties. Indeed both substances disrupt mast cells and in both instances this disruption is prevented by cyanide.

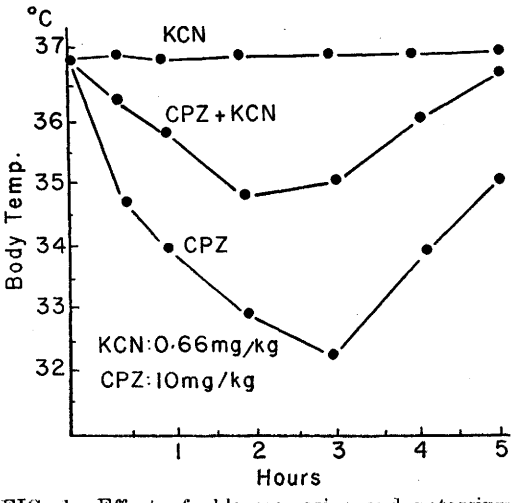

FIG. 1. Effect of chlorpromazine and potassium cyanide on rectal temperatures of rats. (CPZ: Chlorpromazine.)

* Chlorpromazine was kindly supplied by Poulenc, Ltd., Montreal under trade name Largactil.

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.