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Potentiation of Chlorpromazine by Insulin.* (25615)

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Soon after its tranquilizing effect had been described(1) chlorpromazine was shown to decrease significantly the oxygen consumption of the whole animal(2). This effect combined with inhibition by chlorpromazine on the heat regulating center of the hypothalamus(1), and with the increased peripheral heat lost(3) constitute the main factors responsible for the marked hypothermia in animals injected with chlorpromazine. Other studies, which have shown that chlorpromazine inhibits activities of both cytochrome oxydase and adenosine triphosphatase(4,5,6) indicate that the decrease in metabolism caused by chlorpromazine may be due to some disturbances in metabolic pathways. Similarly insulin is also known to cause some inhibition in metabolic pathways as evidenced by its effect on hexokinase (7). It is also generally accepted that the depletion of substrate for cerebral metabolism, which is caused by insulin, is the sole explanation for the neural effect of this insulin on the brain and for its use in psychiatry(8). For these reasons it was postulated that chlorpromazine and insulin could potentiate one another on their effect on oxygen consumption or body temperature and on sleeping time of short-acting barbiturates such as hexobarbital.

Materials and methods. Two experiments were performed. In the first male albino rats, weighing 200 to 250 g, were used. The criterium for evaluating response to chlorpromazine was changes in rectal temperature as estimated by inserting a thermistor to a depth of 40 mm. Blood glucose determinations were also made on some groups of the first experiment. In *this experiment* 4 groups of 6 rats were used. Group 1 received chlorpromazine† (CPZ) (1 mg/kg); Group 2, CPZ (5 mg/kg); Group 3, CPZ (10 mg/kg); Group 4, CPZ (1 mg/kg) plus insulin regular (IR) (4 I.U./kg); Group 5 CPZ (5 mg/kg) plus IR (4 I.U./kg); and Group 6, CPZ (10 mg/kg) plus IR (4 I.U./kg). In the *second experiment* 4 groups of mice weighing 20 g were used. All 5 groups received hexobarbital (100 mg/kg). In addition Group 2 received IR (4 I.U./kg), Group 3 CPZ (1 mg/kg) and Group 4 regular insulin (4 I.U./kg) plus CPZ (1 mg/kg). All substances were injected intraperitoneally. Insulin and CPZ were injected, one hour and one half-hour respectively, before injection of hexobarbital. Sleeping time was the length of time between loss and reestablishment of righting reflex(9).

Results. A. *Potentiation of chlorpromazine hypothermia by insulin.* The results obtained in the first experiment are illustrated in Fig. 1 and 2. Fig. 1 shows that if the dose of chlorpromazine is sufficient to induce hypothermia (5 and 10 mg/kg), insulin potentiates this drop in body temperature. Furthermore, a dose of chlorpromazine of 5 mg/kg is potentiated sooner than a higher dose (Fig. 2); however insulin potentiates for a longer time a larger dose of chlorpromazine (10 mg/kg). In other words insulin by itself has no effect on body temperature but it greatly potentiates the hypothermia of chlorpromazine. Blood sugar determinations indicate that

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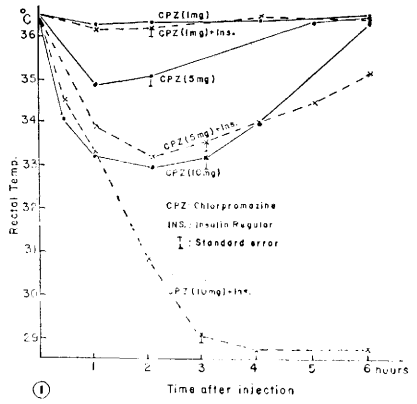
† Chlorpromazine was generously supplied by Poulenc Ltd. under trade name of Largactil.



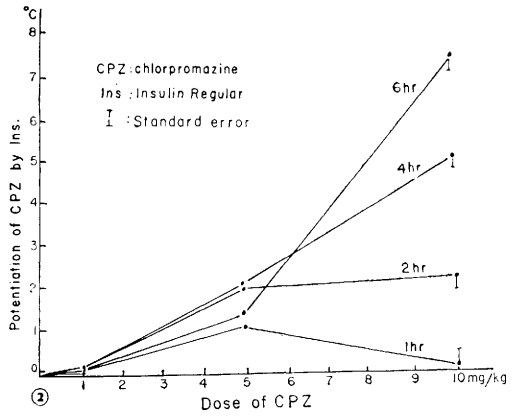


FIG. 1. Potentiation of chlorpromazine hypothermia by insulin (4 I.U./kg).

FIG. 2. Effect of insulin (4 I.U./kg) on hypothermia induced by different doses of chlorpromazine.

chlorpromazine (10 mg/kg) increases blood sugar (132 ± 8 mg %) as compare to the normal value of 98 ± 6 mg %. However simultaneous injection of chlorpromazine (10 mg/kg) plus insulin (4 I.R./kg) produces a marked hypoglycemia (52 ± 4 mg %) which resembles that of insulin when used alone (49 ± 5 mg %).

TABLE I. Increase by Chlorpromazine of Hexobarbital Sleeping Time and Potentiation of This Increase by Insulin.

Treatment	Sleeping time (min.)	No. of animals	Value of "p"
Hexobarbital (100 mg/kg)	30 ± 3	9	>.01
Idem + chlorpromazine (1 mg/kg)	51 ± 5	8	.05
Hexobarbital (100 mg/kg) + insulin (4 I.U./kg)	36 ± 4	9	>.01
Idem + chlorpromazine (1 mg/kg)	82 ± 9	7	

B. *Potentiation of chlorpromazine increase in hexobarbital sleeping time by insulin.* The results are summarized in Table I. Insulin has no effect on hexobarbital sleeping time. However when used with chlorpromazine it greatly potentiates the effect of this substance. The increased sleeping time induced by chlorpromazine in animals receiving hexobarbital is larger still if the animals are injected simultaneously with insulin.

Discussion. Insulin, at the levels used in our study, has no effect on body temperature of rats or on sleeping time of hexobarbital in mice. However when used with chlorpromazine it exaggerates the hypothermia and prolonged hexobarbital sleeping time normally observed with this tranquilizer. Consequently a marked potentiation of chlorpromazine by insulin was obtained. However chlorpromazine has no effect on insulin hypoglycemia, since our results show that the marked hypoglycemia of insulin is not changed by chlorpromazine. This result confirms previous reports(10,11) that chlorpromazine does not alter the hypoglycemia of insulin.

The 2 criteria used in this study are hypothermia and hexobarbital sleeping time. Since these two functions are centrally controlled and both exaggerated by chlorpromazine, it is possible that potentiation of chlorpromazine by insulin originates in the central nervous system.

Summary. The action of insulin as potentiator of chlorpromazine was tested. Under conditions of our experiment, insulin had no significant effect on body temperature of rats and on sleeping time of hexobarbital in mice. However insulin greatly potentiates the hypothermia and prolonged hexobarbital sleeping time normally caused by chlorpromazine. On the other hand the hypoglycemic effect of insulin was not potentiated by chlorpromazine.

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Cytopathogenicity and Plaque Formation with Lymphocytic Choriomeningitis Virus.* (25616)

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During experiments(1) aimed at a better understanding of pathogenesis of lymphocytic choriomeningitis (LCM) virus infection in mice and ability of infant mice to develop immunological tolerance to it, an attempt was made to obtain cytopathic effects (CPE) and a plaque assay with this virus. Previous work indicated that LCM virus is not readily cytopathogenic in tissue culture, except in KB cells(2). An earlier attempt to obtain a plaque assay with these cells was not successful. In our hands several other cell lines including HeLa, Detroit 6, human intestine, human liver, human kidney, FL, primary human amnion and L cells have shown no effects in spite of virus multiplication. However chick embryo tissue cultures appeared to have possibilities since infected cultures, kept 3 weeks or longer, showed decreased acid production with less luxuriant growth than controls. Experiments were therefore set up in which LCM mouse brain virus (the WE strain obtained originally from Rockefeller Inst.)† was passed in series through chick embryo tissue cultures.

Methods. Cells were prepared from 9-day chick embryos using Dulbecco's method(3). One ml of tissue suspension at a concentration of 300,000 cells/ml in growth medium was used to seed 16 × 125 mm screw-capped test tubes. The nutrient medium consisted of 20% calf serum, 5% chick embryo extract, 75% Medium 199 with penicillin and streptomycin. After 2 days' incubation, cultures were washed with Hanks tris pH 7.2 (M/50 tri (hydroxymethyl) amino methane in Hanks saline) and infected with LCM virus. A cytopathic effect was produced in the first chick embryo tube passage. In 6 days LCM infected cultures appeared slightly more granular than controls and the sheets were beginning to break. In 9 days the sheets in infected cultures were completely broken up, only single cells remaining, whereas cell sheets in control tube were intact. At this time pH of control culture fluid was 6.8 and pH of LCM culture fluid was 7.5 or higher. Forty serial passages of this virus have been made using at first 3- or 4-day and then 7-day fluid from LCM infected cultures as inoculum; the final passes produced a moderate CPE in 5 days and a moderate color change in 7 days. Control culture fluid has been passaged similarly, and has shown no CPE.

Results. Mice inoculated intracerebrally with LCM culture fluids at various passage levels, diluted 10^{-3} or 10^{-4}, produced typical LCM convulsions and deaths. Undiluted control fluids never caused illness or death of mice. Titrations indicated that maximum virus titer in tubes was reached by 7th day. CPE was obtained in terminal dilution (10^{-4}) tubes after 12 days; no new CPE appeared later. In a neutralization test in mice, virus

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