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Hypothermia: IV. Study of Hypothermia Induction Time with Various Pharmacological Agents. (24617)

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Hypothermia is of interest from its practical application(1) as well as from the observed basic physiologic alterations. At present, most of the interest in hypothermia concerns its application to cardiovascular surgery and neurosurgery(2). McQuiston(3) reported cases of 25 children subjected to various cardiac operations under hypothermia. Bigelow (4) and Cookson(5) performed dog experiments in which they occluded both vena cavae during hypothermia thus giving the surgeon a bloodless field for intra-cardiac surgery. Application of the principle of hypothermia for diminishing vital tissue oxygen requirements in patients undergong cardiac surgery was successful by Swan and his associates(6). Interest in hypothermia was aroused also by the concept of "artificial hibernation" developed by Laborit and Hugenard(7). They achieved their "artificial hibernation" through use of a "lytic cocktail" (a pharmacological mixture containing chlorpromazine, promethazine, and meperidine). Their major interest was to potentiate general anesthesia, and counteract the shock and other undesirable reactions to surgical or traumatic stress, but it was also noted that with supplementary body cooling, hypothermia could be obtained easily. Physiological alterations resulting from the lytic cocktail in patients undergoing surgery have been summarized by Shackman *et al.*(8). The effectiveness of the lytic cocktail for inducing hypothermia has been tested by Dundee *et al.* (9), who also concluded that muscle relaxants were equally effective. Moyer *et al.*(10) compared the effects of agents which block the sympathetic nervous system on hypothermia induction time. The rationale for this study was that the autonomic response to hypothermia supposedly provides a barrier to hypothermia induction. Adrenergic blocking agents (chlorpromazine and Hydergine)* which also possess central effects, Dibenzyline, an adrenergic blocking agent with minimal central effects, and mecamylamine (some central effect) were compared. They concluded that sympathetic nervous system depression *per se* did not affect hypothermia induction time and that chlorpromazine and mecamylamine (less effect than the former) were the only effective agents which reduced induction time due, apparently, to the anesthetic potentiating effect of these drugs. Our study was a further attempt to compare the effect of various pharmacologic agents on hypothermia induction time and to elucidate the locus of action of some of these agents. Chlorpromazine, the lytic cocktail containing chlorpromazine, promethazine, and meperidine, and d-tubocurarine were studied. Chlorpromazine and the lytic cocktail (promethazine, meperidine, and chlorpromazine) were compared to see how effective chlorpromazine plus adjunctive agents were, in contrast to chlorpromazine alone. Peripheral blockade of corticospinal pathways at the neuro-muscular junction by use of d-tubocurarine was studied to localize sites of action of effective agents. We were also interested in the effect of anesthesia alone compared to the more diverse pharmacological action of the various drugs.

* Equal mixtures of dihydroergocornine, dihydroergokyptine and dihydroergocrystine.

TABLE I. T/W* Values in 12 Dogs.

Group	I	II	III	IV
	Hypothermia alone (no drug, but anesthesia)	Hypothermia with		
		Chlorpromazine	Lytic cocktail	Curare
	11.2	7.6	12.0	11.8
	12.4	9.5	6.3	9.7
	9.5	7.3	8.0	9.0
	11.5	7.9	7.3	9.0
	10.9	9.2	8.2	9.5
	11.6	6.9	8.6	10.0
	11.9	10.2	9.4	9.0
	11.9	10.4	8.7	7.0
	11.7	9.2	7.7	12.2
	10.8	7.3	10.5	11.9
	8.5	9.2		
	10.5			
Mean values	11.0	8.6	8.7	9.9
P values less than		.001	.01	.1
P " comparing Group II & IV				.05

$$* \text{T/W} = \frac{\text{Time in min. to reduce dog's temp. to } 80°\text{F}}{\text{Dog's wt in kg}}.$$

Methods and materials. The method for determining hypothermic induction time was the same as that used by Moyer *et al.*(10). Unselected mongrel dogs, varying in weight from 8 to 16 kg were used. A surface cooling refrigerated blanket was the hypothermia apparatus used. The blanket was precooled to 20°F before being applied. The dogs were anesthetized with 30 mg/kg of sodium pentobarbital given intravenously. The hair was shaved from thorax and abdomen to prevent variability due to insulating effects of different thicknesses of hair. The dogs were given the drug, then placed on the blanket immediately. Induction time was the time required to cool the dogs to 80°F as measured by rectal thermometer. During induction, shivering response, depth of anesthesia, interval temperatures, the electrocardiogram and respiration were observed. When respiration became significantly depressed, the dogs were maintained on an artificial respirator. All drugs were given intravenously, in the following dosages: Group I, 12 dogs, Control; Group II, 11 dogs, Chlorpromazine 5 mg/kg; Group III, 10 dogs, lytic cocktail, meperidine 6.6 mg/kg, promethazine 3.3 mg/kg, chlorpromazine 5 mg/kg; Group IV, 10 dogs, d-tubocurarine, .15 mg every 40 min. Dosages of the lytic cocktail, with exception of that of chlorpromazine which for purposes of comparison could not be greater than that of Group II dogs, were based on the method of Laborit. Without pentobarbital, 3 dogs were given varying dosages of chlorpromazine (from 30 to 70 mg/kg) alone and their response noted.

Results. The results are presented in Table I. Group I consists of 12 dogs in which hypothermia induction times were observed with only pentobarbital anesthesia(10). In Groups II, III and IV, induction times were determined witsh pentobarbital plus chlorpromazine, the "lytic cocktail," and d-tubocurarine respectively. About 50% of dogs in Group I showed a significant amount of shivering. None of the dogs in Groups II, III or IV showed any shivering. To compare individual dogs or groups of dogs, an arbitrary ratio of T/W (induction time/weight of dog in kg) was set up. T/W ratio for dogs with hypothermia alone was 11 as compared to Group II (chlorpromazine) T/W of 8.6 ($p<0.001$) and Group III (lytic cocktail) T/W of 8.7 ($p<0.01$). In comparing Group I and Group IV, the p value was >0.1, the T/W of the latter being 9.9. A significant difference existed between Group II (T/W 8.6) and Group IV (T/W 9.9); p value was <0.05.

Of 3 dogs which received chlorpromazine without concomitant pentobarbital, none was sufficiently anesthetized to be kept on the cooling blanket for any length of time. All dogs had a maximal shivering response. At doses of 30 mg/kg and 40 mg/kg 2 dogs showed no temperature reduction and anesthesia was too light for the dogs to be kept on the blanket. The third dog received 70 mg/kg, remained on blanket 60 minutes, and underwent temperature reduction from 100° to 93°F. On Group II dogs (chlorpromazine 5 mg/kg and pentobarbital) the average temperature level after 60 minutes was 87.8°.

Discussion. To be a useful procedure, hypothermia must be induced safely and efficiently. The time factor is important because the longer a patient is submitted to hypothermia, the greater the chance of developing complications (ventricular fibrillation, hemorrhagic tendency)(2). When induction time

is shorter it suggests that the animal is not fully utilizing his defense mechanisms against temperature reduction, shivering, vasoconstriction, and pilo-erection(12). Our experiment does not show whether or not these defense mechanisms (autonomic nervous system and/or hormonal response) are harmful to the patient, but we did show that both lytic cocktail and chlorpromazine reduce induction time. There was no significant difference betwen lytic cocktail and chlorpromazine indicating that giving other agents in the lytic cocktail had no additional effect, as previously suggested(9). The difference between induction times of hypothermia alone and d-tubocurarine was not statistically significant; however, the curare preparation did prevent shivering, suggesting that neuromuscular blockade did not alter induction of hypothermia significantly. Average T/W ratio was 1.1 units less than that of the controls (Group I).

Chlorpromazine, combined with pentobarbital anesthesia was the most effective hypothermia inductant studied. The mechanism of action is of interest. Physiologic thermoregulation is performed by a forebrain center(12) which controls several peripheral mechanisms, by which an animal combats cold. One of these mechanisms of heat gain, shivering, has been shown to involve higher pathways and does not occur as a simple spinal reflex. The hypothalamus seems to contain the thermoregulatory center(12) which is divided into a rostral area which prevents overheating, and a caudal area preventing heat loss. This center is probably stimulated in 2 ways(13)—reflexly from the skin and by the temperature of the blood flowing through the center. Somewhere along these pathways effective agents make induction of hypothermia easier.

Moyer and his associates showed that a potent adrenergic blocker (Dibenzyline) was not an effective inductant; supposedly this would have blocked the vasoconstrictor response. We showed that there was a significant difference between effect of chlorpromazine (Group II) and d-tubocurarine (Group IV) and also that there was not an impressive difference between control (Group I) and curare (Group IV). However, both the d-tubocurarine and the chlorpromazine (with pentobarbital) eliminated shivering indicating that abolition of shivering is not all-important to the chlorpromazine effect. From our experience with the 3 dogs which received chlorpromazine without pentobarbital, it is evident that the latter is essential to the chlorpromazine effect. Also chlorpromazine (with pentobarbital) does have a marked effect when compared with pentobarbital alone. This anesthetic potentiating effect of chlorpromazine when combined with pentobarbital appears to be the primary factor by which induction time is reduced. The results gained by use of peripherally acting agents lead to a more complete understanding of the action of chlorpromazine as follows: 1) Prevention of shivering is not the only action of chlorpromazine, because this also is eliminated by d-tubocurarine. 2) The adrenergic blocking action of chlorpromazine is not significant enough to account for its effect on induction time. 3) The effect of chlorpromazine in accelerating hypothermia induction time is apparently central where it probably provides an effective block of heat gain mechanisms.

Summary and conclusions. Thirty-one dogs were cooled down to 80°F and the ability of various pharmacologic agents to facilitate hypothermia induction was observed. 1) Chlorpromazine and the "lytic cocktail" accelerate hypothermia induction time. 2) Chlorpromazine is apparently the only effective agent in the "lytic cocktail." 3) Chlorpromazine accelerates hypothermia induction by a central action. 4) Pentobarbital anesthesia is essential for production of the chlorpromazine effect.

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Hematology in Vit. B_{12} Deficient Newborn Rat.* (24618)

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With isolation of Vit. B_{12}(1,2), it was shown that this vitamin fulfilled the role of Castle's extrinsic factor as well as that of the hemopoietic factor in liver(3). A number of investigators(4-7) reported the effect of Vit. B_{12} deficiency on blood of animals with rather wide variations in effects produced among different species. This paper includes results of blood and related tissue studies of newborn rats littered to Vit. B_{12}-depleted dams.

Methods. Newborn offspring of Vit. B_{12}-deficient and Vit. B_{12}-supplemented dams were used. Dams were depleted of Vit. B_{12} by maintaining them on soybean meal-glucose type diet from time of weaning. Methods employed for production of the deficiency and for histological studies were described previously(8). Blood was obtained by decapitation and hemoglobin was determined by direct photometric method(9). A Hellige-Diller colorimeter, calibrated with a sample of blood whose hemoglobin content was calculated from its iron analysis, was used for measurement. Red cell, leucocyte and differential counts on blood obtained as described above, were made by standard procedures. Erythrocytes were examined for sickling by mounting a small drop of blood with a drop of fresh, 1% aqueous solution of sodium bisulfite under coverslip and examining microscopically after 5 minutes. Hematocrits were determined in Van Allen hematocrit tubes using 1.3% sodium oxalate as diluent.

Results. Table I shows that a significant difference in blood picture existed between controls and Vit. B_{12} deficient newborn rats. Hemoglobin, hematocrit values and erythrocyte counts were considerably decreased in the deficient rats. Leukocyte counts were reduced to about one-half that of controls and the differential counts showed this difference to be manifest mainly in percentage of mature neutrophils. Neutrophilic granulocytes appeared unable to mature properly leaving more cells in the myelocytic and metamyelocytic stages.

Erythrocyte concentration was reduced significantly in peripheral blood and large numbers of nucleated red cells were present in the deficient animals. Peripheral blood films showed polychromatophilia, anisocytosis, poikilocytosis, smudge cells, and objects resembling degenerate erythrocytes (Fig. 1). Sickling mounts indicated that these were not true sickle cells.

Bone sections showed that the bone was poorly developed. There was a lack of normal calcification and a failure of normal endochondral bone growth and remodeling sequences. Osteoblasts were reduced in number and those present appeared less active than controls. Both cartilage cells and osteoblasts contained very little glycogen compared to controls. Clumps of cartilage cells were observed in the bony collar, and in many cases tongues of nucleated cartilaginous cells extended down into the marrow cavity from the epiphyseal plate (Figs. 2 and 3). Bone marrow from deficient animals showed active hemopoiesis and a 2- to 3-fold higher concentration of pro-erythrocytic elements, a large portion of which were pronormoblasts (Figs. 4

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