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Received June 11, 1956. P.S.E.B.M., 1956, v92.

Chlorpromazine Counteraction of Trauma Induced Death.* (22634)

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Chlorpromazine(1) has been reported to have a pronounced protective action against death induced in dogs by severe hemorrhage (2,3) and in rats by tumbling trauma(3). This paper deals with the counteraction by chlorpromazine of hemoconcentration and death induced in mice by tourniquet trauma, and by heat trauma. An abstract of part of this work has already appeared(4). Findings similar to certain of those reported here have been published recently by Millican and Stohlman(5).

Methods. Mice used were Carworth Farms males, 2-5 months of age and 20-30 g weight. In any one experiment mice were sorted into such groups that the average body weights for all the groups were nearly the same. Traumatization procedures were those of Rosenthal(6,7). In the tourniquet trauma experiments the hind leg tourniquets were left in place 2 or 3 hours; in the heat trauma experiments etherized mice were submerged to the neck in water at 60°C for 12 seconds. All injections were made intraperitoneally. Chlorpromazine was dissolved in distilled water at such a concentration that the desired dose was secured by injection of .01 ml per g, or in .9% NaCl at such a concentration that the desired dose was secured by injection of .05 ml per g. Since initiation of shock presumably begins at the time of removal of hind leg tourniquets, or at the time of immersion in very hot water, this time has been designated *zero hour* (Tables). Prior times have been assigned minus (—) values, later times plus (+) values. For the present purposes shock in mice is defined as the condition after severe traumatization wherein mice are prostrated, relatively indifferent to stimuli, cold to the touch, and likely to die within 48 hours after traumatization if untreated. *Hematocrit studies*: Hematocrit values were obtained using heparinized fine capillary tubes and an International "Hemacrit" centrifuge. A control tail blood sample was secured for each mouse. In all except the earliest experiments those mice exhibiting tail blood hematocrit values below 40 were replaced. The second (test) blood sample was taken from the heart, since insufficient blood could be obtained from the tail after traumatization. Hemodilution was indicated by a heart blood hematocrit value appreciably smaller than the previously obtained tail blood hematocrit value, hemoconcentration by the reverse. For any one group, the standard error of the average difference between tail and heart blood hematocrit values was calculated using this difference for each mouse as the unit. The probability value, p, was secured from a (t,

* This research was supported in part by USAF School of Aviation Medicine Contract AF 18(600)-555.

† The authors are indebted to Adele M. Bianconi, Ronald Johnson, Yvette Meitner, Gary Patterson, and Donald Trimmer for technical assistance.

TABLE I. Counteraction by Chlorpromazine of Tourniquet Trauma Induced Hemoconcentration.

Procedure timing, in hr, before (−) or after (+) removal of hind leg tourniquets			Avg differences between tail and heart blood hematocrit values (DHV's)*			
			Dist. water inj.		Drug inj. (8 μg/g)	
Ligatures on	Drug or DW inj.	Mouse sacrificed	No. of mice†	DHV's*	No. of mice†	DHV's*
−2	−2.5	+ 2	4/ 4	+ 6.9	4/4	−17.5
		+ 6	3/ 4	+ 5.5	4/4	− 4.5
		+10	4/ 4	+ 3.3	4/4	+ 4.8
−2	−1	+ 2	8/ 9	+ 9.8	6/6	+ 2.3
		+ 4	7/ 9	+13.9	7/8	+ 5.0
		+ 6	10/12	+16.9 ± S.E. 1.87‡	8/8	+ 4.8 ± S.E. 1.56‡
		+10	3/ 4	+15.2	3/4	+ 7.0
−3	−1	+ 2	4/ 4	+20.0	4/4	+ 5.1
		+ 4	3/ 4	+21.8	4/4	+ 6.9
		+ 6	3/ 4	+15.0	4/4	+ 7.4
		+10	6/ 9	+14.7	6/9	+12.4

* DHV marked plus (+) if heart blood hematocrit exceeded tail blood hematocrit, and minus (−) if vice versa.

† Numerator, No. of mice surviving to time of sacrifice; denominator, No. of mice alive at beginning of experiment.

‡ For mice of this experiment, sacrificed 6 hr after removal of hind leg tourniquets, the probability that chlorpromazine, administered as indicated, did not counteract tourniquet trauma induced hemoconcentration is estimated as less than .001.

n) table(8). t being calculated in the usual manner. *Survival studies*: Each experiment was set up to permit calculation of Chi Square. The value p was then secured from a one degree of freedom Chi Square table(8).

Results. 1. *Hematocrit studies*: For 25 control mice an average tail blood hematocrit value of 46.22 ± S.E. 0.81% and an average heart blood hematocrit value of 44.12 ± S.E. 0.91% was obtained, p value for difference over .05. No effect of varying time between tail and heart blood samples, from 0 to 48 hours, was noted. *Chlorpromazine on hematocrit values of normal (non-traumatized) mice*: Mice were sacrificed at intervals ranging from 3 to 11 (usually 6) hours after administration of water or drug. No differential effect of time was noted. Average hematocrit values obtained for 35 mice injected with water were: (a) tail blood, 45.9; (b) heart blood minus tail blood, —3.86 ± S.E. .44. Average hematocrit values obtained for 34 mice injected with chlorpromazine, 8 μg per g, were: (a) tail blood, 46.4; heart blood minus tail blood, —8.16 ± S.E. .62. The probability that the moderate hemodilution associated with the use of the drug was due to chance is estimated to be less than .001. *Chlorpromazine on tourniquet trauma induced hemoconcentration*: When administered either prior to application of hind leg tourniquets or after their application but prior to their removal, chlorpromazine at a dose level of 8 μg per g was effective in some degree in counteracting tourniquet trauma induced hemoconcentration (Table I). During these experiments a few limp mice were encountered which failed to show a beating heart when the chest was opened. Data obtained for these moribund mice were not used in connection with Table I. However, it is interesting to note that appreciable hemoconcentration was encountered only for heart blood samples of those moribund mice which had been injected with water rather than the drug, prior to removal of the hind leg tourniquets. Drug counteraction of trauma induced hemoconcentration was not entirely equivalent to prevention of trauma induced death. In still other experiments it was found that if the drug was administered after *removal* of the hind leg tourniquets, no appreciable counteraction of the trauma induced hemoconcentration occurred. *Chlorpromazine on heat*

TABLE II. Effect of Chlorpromazine Alone on Survival of Mice Subjected to Tourniquet Trauma.

Exp.	Dose of drug (μg/g)	Timing of procedures, in hr, before (−) removal of hind leg ligatures: Ligatures on	Drug or DW inj.	Survivals after removal of hind leg ligatures: Up to 24 hr No. of mice†	% of mice	Up to 48 hr No. of mice†	% of mice	p from chi square (48 hr)*
A	None	−2	−2½	14/41	34	13/41	32	
	8	−2	−2½	44/52	85	43/52	83	<.001
B	None	−2½	−2½‡	22/43	51	18/43	42	
	8	−2½	−2½‡	34/44	77	34/44	77	<.001
C	None	−2	−2 ‡	4/13	31	3/13	23	
		"	−1½	1/12	8	1/12	8	
		"	−1	5/12	42	4/12	33	
		Totals for controls:		10/37	27	8/37	22	
	8	−2	−2 ‡	23/25	92	23/25	92	<.001*
		"	−1½	19/25	76	19/25	76	<.001*
		"	−1	17/25	68	16/25	64	.001*
D	None	−2	−1	5/15	33	2/15	13	
	8	−2	−1	10/25	40	2/25	8	.58 Not significant

* In Exp. C, each treated group was compared with the *entire* control group.
† Numerator, No. of survivors; denominator, No. of mice alive at start of experiment.
‡ Injections made immediately after application of hind leg ligatures.

trauma induced hemoconcentration: Two experiments were performed in which mice were injected with water or drug, 8 μg per g, 1 hour *before,* and heart blood samples secured 2 hours after heat traumatization. Values for the factor "increase in hematocrit occurring in water injected mice, *minus* increase in hematocrit occurring in drug injected mice" were as follows: Exp. 1, + 8.7 ± S.E. 2.74; Exp. 2, + 5.19 ± S.E. 1.62. In both experiments, t (factor/S.E. of factor) was 3.2 and the corresponding p less than .02. It is concluded that *pre*-administration of chlorpromazine resulted in significant counteraction of heat trauma induced hemoconcentration. However, significant counteraction of heat trauma induced hemoconcentration was not secured in two experiments in which the drug was administered at a dose level of 8 μg per g immediately *after* heat traumatization.

Survival studies: A. *Chlorpromazine alone on tourniquet trauma induced mortality*: The data of Table II indicate that maximum protection was secured by administering the drug before application of hind leg ligatures, as in in Exp. A, or immediately thereafter, as in Exp. B and C, and that protection became progressively weaker as the time of drug administration was moved away from time of application of hind leg ligatures toward the time of their removal.

B. *Chlorpromazine supplemented with isotonic saline on tourniquet trauma induced mortality*: The lessening of tourniquet trauma induced hemoconcentration which was secured by use of chlorpromazine administered some time after placement of hind leg tourniquets, but before their removal (Table I) suggested that the drug may delay onset of irreversible shock, even when administered such a short time before removal of hind leg tourniquets that it might be ineffective, by itself, in promoting indefinite survival. If so, administration of large volumes of isotonic saline some time after shock had been precipitated by removal of the hind leg tourniquets should result in a survival difference between control and chlorpromazine injected mice in favor of the latter, even when the drug alone was ineffective in promoting survival. A number of experiments were performed in which each mouse was injected with either water or the drug, 8 μg per g either 15 or 60 minutes before removal of the hind leg tourniquets, and with .9% NaCl, in the amount of

5% of the body weight, 2 hours after removal of the tourniquets. Of 69 mice injected first with water and later with saline, 22 survived to 24 hours, and 13 to 48 hours after removal of tourniquets. Of 68 mice injected first with the drug and later with saline, 42 survived to 24 hours and 32 to 48 hours after removal of tourniquets. The probability, p, that in these experiments the drug plus saline did not enhance likelihood of survival to 48 hours over that obtainable with saline alone is estimated to be less than .001.

Chlorpromazine plus saline failed to enhance survival over that obtainable with saline alone, when both drug and saline were administered *after removal* of hind leg tourniquets. For example, in an experiment in which injections were made 2 hours after removal of tourniquets, survivals to 48 hours were as follows: 16 of 30 mice injected with saline only, 5% of body weight; but only 4 of 30 mice injected with saline plus chlorpromazine, 8 μg per g.

C. *Chlorpromazine on heat trauma induced mortality.* Of 50 mice injected with water one hour before traumatization, 21 survived to 24 hours, and 18 to 48 hours after traumatization. Of 40 mice injected with the drug, 8 μg per g, one hour before traumatization, 40 survived to 24 hours and 35 to 48 hours. The probability that the drug, administered one hour *before* traumatization, did not increase likelihood of survival to 48 hours is estimated to be less than .001.

Of 75 mice injected with distilled water or isotonic saline immediately *after* heat traumatization, 29 survived to 24 hours and 27 to 48 hours after traumatization. Of 100 mice injected with the drug at a dose level of 4, 8, or 16 μg per g, in distilled water or isotonic saline, immediately after traumatization, 15 survived to 24 and also to 48 hours. No matter what procedure was tested, the drug was completely ineffective in promoting survival when administered immediately *after* heat traumatization.

Discussion. In the present experiments, variation in type of trauma used and time of administration of chlorpromazine in relation to induction of trauma resulted in considerable variation in the activity of this drug in counteracting: (a) trauma induced hemoconcentration, and (b) trauma induced death. However, (a) and (b) activities of chlorpromazine ran roughly parallel. Since hemoconcentration associated with shock is indicative of decreased circulating blood volume, chlorpromazine probably protects mice against trauma induced death, in part at least, by minimizing a trauma induced decrease in circulating blood volume. Chlorpromazine is effective in increasing the likelihood of survival of rats after tumbling trauma(3) or of mice after heat trauma (this paper) *only* if administered prior to traumatization. On the other hand, the drug increased likelihood of survival whether administered before or some time after severe hemorrhage in the dog(2, 3), or before or shortly after application of hind leg ligatures in tourniquet traumatization of the mouse (this paper). The above relations are considered important in relation to possible clinical use of this or related drugs in traumatic or hemorrhagic shock. Wiggers(9) has reviewed the considerable body of evidence that toxic substances are formed in tissue areas subjected to severe trauma and/or anoxia associated with hemorrhage or circulatory occlusion, and that these toxic substances play an important role in the development of irreversibility of the shock syndrome. It is likely that such substances are formed rapidly in association with tumbling or heat trauma, more slowly in association with severe hemorrhage. Toxic substances formed in tissue areas deprived of circulation by use of tourniquets could not reach the general body circulation until after removal of the tourniquets. It is therefore postulated here as a working hypothesis that chlorpromazine, when administered a sufficient period of time before removal of hind leg tourniquets, decreases the reactivity of the cardiovascular system of the mouse to toxic substances formed in the hind legs during occlusion of circulation to such a degree as to prevent development of irreversibility of the tourniquet trauma shock syndrome in some mice.

Summary. Chlorpromazine appreciably counteracted tourniquet trauma induced

hemoconcentration and death when administered either before or after *placement* of hind leg ligatures, but not after *removal* of these ligatures. Maximal counteraction was secured by administering the drug before or immediately after placement of the ligatures. Chlorpromazine was effective in appreciably counteracting heat trauma induced hemoconcentration and death only if administered prior to traumatization.

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Received June 13, 1956. P.S.E.B.M., 1956, v92.

Production of Aldosterone by Rat Adrenal Glands *in vitro*.*† (22635)

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Rat adrenal glands have been shown to produce corticosteroids *in vitro*(1,2,3) and a stimulation of this production by the addition of ACTH to the incubation medium had been demonstrated(1). Small amounts of aldosterone were found by Wettstein and Anner(4) in the incubation medium of beef and hog adrenals, and by Rosenfeld(5) and associates in the perfusion medium of beef adrenals. The present work shows that the incubation medium from rat adrenals contains a sodium-retaining substance with the properties of aldosterone and describes the results of experiments performed to investigate some of the factors which may affect its secretion.

Methods. In each experiment adrenal glands were obtained from 40 to 90 male rats weighing between 170 to 180 g. These animals were subsequently used for bioassay purposes. The glands were freed of fat and quartered. One quarter of each adrenal was placed in each of 4 flasks containing 1.5 ml per 25 mg of fresh tissue of Krebs-Ringer-bicarbonate solution with added glucose (200 mg%)(6). Thus the 4 flasks contained an equal amount of tissue from each rat in the group. The incubation of the tissue was carried out in a water bath at 38°C. After the first hour the medium was poured off and replaced by an equal quantity of the same solution. The incubation was allowed to continue for an additional 2 hours. A gentle stream of 95% O_2 - 5% CO_2 was passed through the medium continuously. At the end of this period the medium was decanted. A 0.5 ml aliquot was extracted with methylene chloride for determination of the total Δ^4 3-ketosteroid content, according to the method of Saffran and Schally(6). The rest of the medium was extracted with 3.5 times its volume of redistilled chloroform in glass stoppered cylinders. The two phases were separated by centrifugation and the chloroform layer was carefully transferred with a syringe and a long needle into a round bottomed flask and evaporated to dryness *in vacuo* at a water bath temperature of 50°C.

* Supported by grants from National Research Council of Canada, Eli Lilly Co., and Federal Provincial Mental Health Grant.

† We are grateful to Mr. K. Antoft, Nordic Biochemical Ltd., for generous gift of ACTH and to Dr. L. M. Mitchell, F. W. Horner Ltd. for Growth Hormone.