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Protection Afforded Mice Against Tourniquet Trauma Induced Mortality By Ganglion Blocking Drug Chlorisondamine Dimethochloride. (25095)

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Wiggers and co-workers(1) reported that dogs were afforded significant protection against lethal effects of severe hemorrhage by preadministration of the adrenolytic drug dibenamine. They postulated that this protection was due to improved circulation in those tissues whose circulation is usually most severely curtailed as a result of sympatho-adrenal activation, which is a generalized reaction to severe hemorrhage or trauma. Many reports have since described the significant protection afforded experimental animals against lethal effects of traumatizing and/or hemorrhagic procedures, by *pre*-administration of one or more adrenolytic, sympatholytic and/or ganglion blocking drugs. References in 3 of these papers(2,3,4) include most of the literature. Some reports include data on mortalities when the test drug was administered after severe trauma or hemorrhage. The common finding has been failure of the drug to afford test animals significant protection when so administered. In tourniquet trauma, precipitation of the shock syndrome does not occur until after removal of tourniquets. Hence in this type of trauma it should be possible to assure arrival of drug at postulated vital centers before precipitation of the shock syndrome, by lengthening the period of occlusion of circulation to the hind legs, to permit injection of the drug prior to removal of hind leg ligatures. Alternatively, the innate severity of the shock syndrome might be minimized somewhat, at least in theory, by removing the ligatures earlier, and injecting the drug after such removal, when it would presumably be less capable of combating shock. Experiments were therefore initiated with the ganglion blocking drug, chlorisondamine dimethochloride (CD), with the above relationships in mind. While the original problem has not been resolved, our experiments revealed a protective capability of the drug (CD) against tourniquet trauma induced mortality, injected at time of ligature removal, sufficiently great to warrant this report.

Methods. Mice used were Carworth Farms males, about 3 months of age and 20-30 g body weight. Tourniquet trauma was inflicted by occluding circulation to both hind legs for about 2 hours using rubber bands(5). Control mice were injected with water, test mice with an aqueous solution of chlorisondamine dimethochloride. Other procedures are indicated in Table I.

Results. Table I gives data for 2 experiments, performed in the same manner, with similar results. The drug dose (5 mg/kg) was one which earlier experiments indicated would significantly increase the likelihood that mice would survive tourniquet traumatization, when the drug was injected at time of ligature removal. Experiments of Table I were designed to test whether degree of protection was measurably altered when the drug was administered 15 minutes, rather than zero minutes, after removal of hind leg ligatures. Since it was considered essential that the time between application of ligatures and injection of drug or vehicle (water) be kept constant (at 2 hours), this necessarily resulted in a ligation period of 120 minutes for mice of Groups A_1

TABLE I. Protective Action of Chlorisondamine Dimethochloride (Ciba Ecolid) against Tourniquet Trauma Induced Deaths of Mice.

Group	Min. before (−) or after (+) removal of hind leg ligatures: Ligature application	I.P. inj. of water or drug	Drug dose (mg/kg)	No. of mice*: No. traumatized	No. dead to designated hr after ligature removal: 6	24	48	120
A_1	−120	0	Zero	45	10	26	32	32
A_2	−105	+15	"	45	9	27	28	30
$A_1 + A_2$				90	19 (21%)	53 (59%)	60 (67%)	62 (69%)
B	−120	0	5	64	1 (2%)	20 (31%)	27 (42%)	29 (45%)
C	−105	+15	5	65	1 (2%)	26 (40%)	32 (49%)	36 (55%)
Probabilities that observed differences were due to chance, as estimated by Chi Square procedures (NS, p over .05, not significant)								
A *vs* B:					<.01	<.01	<.01	<.01
A *vs* C:					"	.04	.04	NS
B *vs* C:					NS	NS	NS	"

* Carworth Farms males, 20-30 g, about 3 mo of age.

and B, and of 105 minutes for mice of Groups A_2 and B. This alteration in ligation period did not result in an appreciable difference in mortality rate as between the 2 control groups, A_1 and A_2. Hence data for these 2 Groups have been summed for use in estimation of probabilities by the Chi Square method. Since Degrees of Freedom = 1, Yates' 0.5 correction was used in calculation of Chi Square values(6). If a probability of 0.01 or less was used as criterion of significance, the present data indicate that for mice the likelihood of indefinite survival following tourniquet traumatization was significantly increased by administration of chlorisondamine dimethochloride at time of ligature removal, whereas this was not the case for the drug administered 15 minutes after ligature removal. While the data are not in disagreement with the hypothesis that delay in injection of drug is deleterious to survival, they are inadequate to demonstrate this relation. The change in mortality rate associated with the delay is apparently small; hence very large numbers of mice would be needed to demonstrate unequivocally whether the delay was deleterious.

Discussion. Chlorisondamine dimethochloride is a drug with relatively prolonged ganglion blocking effects which begin shortly after its administration(7). Data here presented suggest that procedures might be developed whereby some drugs of this nature would be usefully employed clinically in certain traumatic situations. However, these situations should be very clearly understood and carefully defined and circumscribed. In experiments performed for other purposes we noticed repeatedly that mice preinjected with chlorisondamine dimethochloride are much more susceptible to lethal effects of severe cold or high insulin dosage than control mice. We reported(8) that while chlorpromazine had protective action against both heat and tourniquet trauma induced mortality, if administered well before traumatization, this drug actually decreased the likelihood that mice would survive heat trauma if administered immediately after traumatization. The general impression from the literature is that for most traumatic situations and most drugs, post-administration of adrenolytic or ganglion blocking drug does not increase but may actually decrease likelihood of survival.

Summary. Survival-mortality data were obtained for tourniquet traumatized, adult Carworth Farms male mice for 120 hours after zero hour (time of removal of both hind leg ligatures). Higher percent survivals were noted throughout for test mice injected intraperitoneally with chlorisondamine dimethochloride, 5 mg/kg, than for control traumatized mice injected with water. The p (proba-

bility) values for test *vs.* control mice were: (A) for mice injected at zero hour, 0.01 or less at 6, 24, 48 and 120 hours thereafter, and (B) for mice injected 15 minutes after zero hour, 0.01 or less ONLY at 6 hours thereafter.

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Normal and Experimental Growth of Rat Mammary Gland.* (25096)

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It is generally accepted that estrogenic hormones stimulate growth of mammary gland duct system while estrogen and progesterone stimulate lobule-alveolar growth in intact and ovariectomized rats. Many investigators have attempted to determine levels of these hormones which produce optimal mammary gland growth(1). Differences in extent of lobule-alveolar growth have been evaluated by visual examination of whole mounts of glands. Kirkham and Turner(2) first suggested the use of desoxyribonucleic acid (DNA) content as an objective index of mammary gland cellular growth. Griffith and Turner(3) have shown that mean DNA content/nucleus of rat mammary gland remained constant during pregnancy. Determination of total mammary gland DNA permits quantitative estimation of gland growth during normal pregnancy and following growth under experimental conditions. In the present study, frequency distribution of total mammary gland DNA of rats pregnant 18-20 days has been compared with that of adult ovariectomized rats treated with 1 μg estradiol benzoate in synergism with graded levels of progesterone for 19 days.

Materials and methods. Groups of pregnant and ovariectomized rats of Sprague Dawley-Rolfsmeyer strain with initial weight of 220-270 g were used. Animals killed 18-20 days of pregnancy and ovariectomized rats killed 34 days postcastration served as controls. Remaining ovariectomized rats received daily subc. injections of 1 μg estradiol benzoate (EB) alone and with 1-10 mg progesterone (P) for 19 days commencing 14 days after ovariectomy. They were killed one day after last injection, skinned rapidly and abdominal-inguinal glands removed and frozen for 4 days. Tissues then were thawed and fat extracted in boiling 95% ethanol for 6 hours followed by ether extraction for another 6 hours. DNA was extracted from 25 mg aliquots of finely ground, dry, fat-free tissue (DFFT) by the method of Schneider(4) with exclusion of fat extraction and cold trichloroacetic acid extraction steps. DNA was determined according to method of Webb and Levy(5). The product of the quantity DNA/mg DFFT and DFFT, expressed as unit body weight, was estimated as total DNA/posterior 6 glands. Visual observations were made for presence of lobule-alveolar development but

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