monkeys similarly immunized against Brunhilde and Leon viruses.

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Action of Adrenal Hormones on Lethal Toxicities of Certain Organic Compounds. (19083)

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It has been reported(1) that mice bearing Sarcoma 37 will tolerate larger doses of a number of tumor-damaging trivalent arsenical compounds than will non-tumor-bearing mice. The report by Dalton(2) that tumor growth in mice is accompanied by histologic changes in the adrenals suggested that the observed increased resistance of mice to trivalent arsenical compounds with growth of Sarcoma 37 might be related to a change in adrenal activity. This paper reports results of experiments performed to test for effects of adrenal hormones on the resistance of mice to trivalent arsenical compounds and to certain other organic compounds.

Materials and methods. Injections into mice. Compounds under test for lethal toxicity or tumor-damaging potency were injected in aqueous solution either subcutaneously or intravenously. Hormone preparations under test for ability to counteract toxic or tumor-damaging effects of these compounds were usually injected intraperitoneally. Doses for adrenal extract and for 10% alcohol have been stated as cc per mouse or cc per gram. All other substances employed were dissolved or suspended at concentrations such that the desired doses, in mg per kg, were secured by injecting the test solutions or suspensions into mice in the amount of 0.01 cc of solution or suspension per gram of body weight. The amounts of substances injected, the routes of injection, and the strain, sex and numbers of mice used are indicated in the Tables and Figures.

Optimum time for injecting hormone to secure protection. In any one experiment, each mouse in a control group of at least 25 mice received an LD60-100 amount of the toxic compound, but no hormone preparation. Each experimental group consisted of 10 mice, each of which received the selected dose of a hormone preparation, and the same dose of the toxic compound as that administered to mice in the control group. For each experimental group, a different time interval between administration of hormone preparation and administration of toxic compound was employed (Fig. 1). *Lethal dose determinations.* Experiments and calculations were done in accordance with a simplification of Karber's method described by Cornfield and Mantel(3). Generally a given dose of toxic compound was administered to 10 mice, and the doses were varied in 0.1 $\log_{10}$ steps. The time interval between injection of hormone preparation and of toxic compound was in the range indicated by previous experiments as optimal for securing protection of mice against an LD60-100 amount of the toxic compound. *Estimation of gross and histologic damage induced in mouse sarcoma 37 by administration of compounds to mice:* Tumor mash was

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1. Beck, L. V., Perrault, A., and Gillespie, J., *Cancer Research,* 1949, v9, 626.

2. Dalton, A. J., *J. Nat. Cancer Inst.,* 1944, v5, 99.

3. Cornfield, J., and Mantel, N., *J. Am. Statistical Assn.,* 1950, v45, 181.

TABLE I. LD Values for Trivalent Arsenical Compounds Injected Subcutaneously into Mice, as Affected by Prior Intraperitoneal Injections of Beef Adrenal Extract.

Exp. No.	Group*	Mice used: Strain	Mice used: Sex	Mice used: Total No.	LD values (mg/kg): $LD_{50} \pm$ S.E.	LD values (mg/kg): LD_5	P values for differences in LD_{50} values of C & E groups
				Experiments with arsenite			
1	C	ZBC	♂	95	12.3 ± .70	8.6	.000004
	Ca	ZBC	♂	95	11.1 ± .37	7.8	
	E	ZBC	♂	95	16.6 ± .62	13.4	
2	C	CAF_1	♂	60	11.2 ± .43	8.6	.00002
	E	CAF_1	♂	60	13.5 ± .33	12.1	
3	C	CAF_1	♀	60	12.5 ± .50	9.3	.04
	E	CAF_1	♀	60	13.7 ± .46	10.3	
4	C	CAF_1	♀	40	10.5 ± .64	6.7	.03
	E	CAF_1	♀	40	12 ± .68	8.2	
				Experiments with clorarsen			
5	C	ABC	♂	80	41 ± 1.8	22.5	.005
	E	ABC	♂	80	48.8 ± 1.5	38.2	
				Experiments with oxophenarsine			
6	C	ABC	♂	30	42.7 ± 2.4	27.1	.03
	E	ABC	♂	30	49 ± 2.2	38.2	

* C or Ca = control, E = exp. C mice received arsenical injections only. Ca mice of Exp. 1 were each given two .5 cc intraperitoneal injections of 10% alcohol, one 40 min before, the other 6 hr after injection of arsenite; E mice of Exp. 1 were each given two .5 cc injections of adrenal extract, one 40 min before, and the other 6 hr after injection of arsenite. In all other experiments, E mice were each injected with .5 cc of adrenal extract 60 min before injection of the trivalent arsenical compound. Mice used in Exp. 2 and 3 bore Sarcoma 37 implanted intramuscularly in the right hind leg 6 days before injection of arsenite.

TABLE II. LD Values for Arsenite Administered to CAF_1 Mice, as Affected by Prior Intraperitoneal Injection of Cortisone.

Exp. No.*	Group	Dose of cortisone (mg/kg)	Time between hormone & arsenite (min)	Mice used: Sex	Mice used: Total No.	LD values (mg/kg): $LD_{50} \pm$ S.E.	LD values (mg/kg): LD_5	P values for differences in LD_{50}s of C & E groups
1	C	0	—	♂	24	10 ± .75	—	.01
	E	1.25	50	♂	24	12.6 ± .79	—	
2	C	0	—	♂	50	9.8 ± .59	6.4	.03
	E	2.5	60	♂	50	11.2 ± .41	9	
3	C	0	—	♂	37	12 ± .55	8.6	.01
	Cs	0	—	♂	37	11.7 ± .50	8.9	
	E	2.5	60	♂	37	13.6 ± .35	12.2	
4	C	0	—	♂	70	10.7 ± .42	6.9	.001
	E	2.5	60	♂	60	12.7 ± .38	10	
5	C	0	—	♂	40	10.5 ± .37	9	†
	E	5	90	♂	40	11 ± .46	9.1	
6	C	0	—	♂	20	9.8 ± .55	—	.0001
	E	10	50	♂	20	13.5 ± .62	—	
7	C	0	—	♀	24	10 ± .52	—	†
	E	1.25	50	♀	24	10.4 ± .64	—	
8	C	0	—	♀	50	10 ± .60	6.6	−.02‡
	E	5	90	♀	50	8.5 ± .41	6.5	
9	C	0	—	♀	20	10.3 ± .52	—	†
	E	10	50	♀	20	12.3 ± .69	—	

* In Exp. 4 arsenite was injected intravenously; in all other experiments, subcutaneously. In Exp. 3 an extra control group (Cs) was employed, in which mice were injected intraperitoneally with .9% NaCl, .01 cc per g, 60 min before injection of arsenite. In Exp. 1 and 7 mice bore Sarcoma 37 implanted intramuscularly in right leg 5 days before injection of arsenite.

† Not significant.

‡ Minus value for P indicates augmentation, not diminution, of arsenite toxicity for cortisone injected mice.

TABLE III. Effect of ACTH on LD Values of Arsenite for Normal ABC Mice.

Group	Hormone injected	Mice used: Sex	Mice used: Total No.	LD values (mg of As_2O_3/kg): LD_{50} ± S.E.	LD values (mg of As_2O_3/kg): LD_5	P values for differences in LD_{50} values of C & E groups
C	None	♂	40	11 ± .41	9.1	.01
E	ACTH, 100 mg/kg IP, 24 hr before arsenite	♂	40	12.9 ± .60	10.1	
C	None	♀	40	11.8 ± .42	10.5	*
E	ACTH, 100 mg/kg, IP, 25 hr before arsenite	♀	40	12.6 ± .70	8.9	
C	None	♂	40	11 ± .41	9.1	C vs. E_1:
E_1	40 mice received ACTH, 100 mg/kg IP, 24 hr before arsenite. 40 mice received ACTH, 100 mg/kg IP, 24 hr and again 16 hr before arsenite	♂	80	12.6 ± .41	9	.004
E_2	ACTH, 100 mg/kg IP, at 24 and again at 16 hr before arsenite, + beef adrenal extract 45 min before arsenite	♂	40	16.6 ± .64	14.3	C vs. E_2: .00001

* Not significant.

TABLE IV. Data Obtained in Tests for Protection of Mice by Cortisone Against Semi-Lethal Doses of Compounds Other Than Trivalent Arsenical Compounds, Injected Subcutaneously.

Compound	Dose of cortisone (mg/kg)	Min between cortisone & compound	Mice employed: Strain	Mice employed: Sex	Mice employed: Total No.	LD_{50} ± S.E.*	P values for differences in LD_{50} values for cortisone & control mice
Alpha-peltatin	0	—	CAF_1	♀	40	60.3 ± 4.3	−.002†
	2.5	120	CAF_1	♀	40	43.7 ± 2.7	
"Acridan"‡	0	—	ABC	♂	40	3.63 ± .21	§
	2.5	120	ABC	♂	40	3.55 ± .21	
	0	—	ABC	♀	40	4.17 ± .24	§
	2.5	120	ABC	♀	40	4.34 ± .21	
P-chloromercuribenzoate	0	—	CAF_1	♂	40	75.9 ± 2.7	§
	5	60	CAF_1	♂	40	79.5 ± 3.1	
Iodoacetamide	0	—	CAF_1	♂	65	42 ± 1	§
	5	60	CAF_1	♂	65	40.8 ± 1.8	

* LD_{50} values are for 7 days after injection of alpha-peltatin; for 48 hr after injection of other compounds.

† Minus probability value indicates *augmentation* of toxicity by cortisone.

‡ 9-phenyl-9-chloro-10-methyl-3-dimethyl amino acridan 2 HCl.

§ Not significant.

implanted in the right hind leg of CAF_1 mice. Six or 7 days later injections of the potentially tumor-damaging compound, and of hormone preparations, were made. The mice were sacrificed 24 hours after injection of the compound under test for tumor-damaging potency and the extent of induced hemorrhagic necrosis was estimated grossly by visual inspections of cross sections of the tumors *in situ* (5 mice per group). Sections were obtained and histological estimations of extent of induced damage were made by criteria described by MacCardle and Downing(4), and by Leiter, Downing, Hartwell and Shear(5). In this paper, attention has been focused on *comparison*, at each dose level of tumor-damaging

4. MacCardle, R., and Downing, V. *Cancer Research*, 1947, v7, 717.

5. Leiter, J., Downing, V., Hartwell, J. L., and Shear, M. J., *J. Nat. Cancer Inst.*, 1950, v10, 1273.

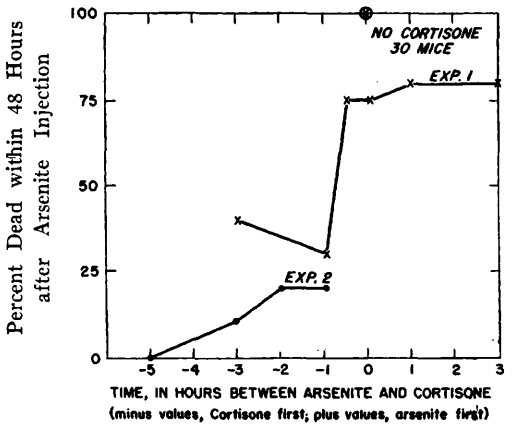

FIG. 1. Relation between time of administration of cortisone and protection afforded mice against arsenite.

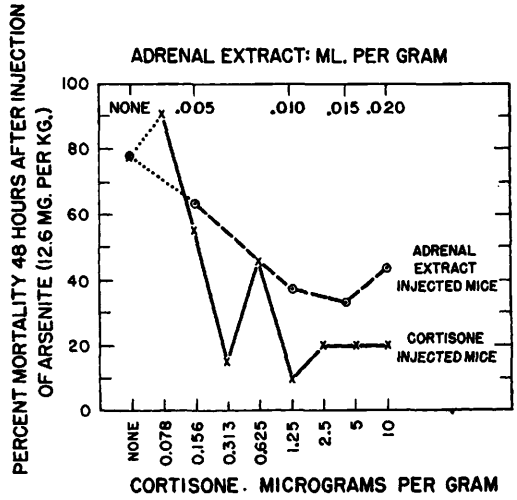

FIG. 2. Protection afforded mice against arsenite by adrenal hormones, in relation to dose of hormone administered.

compound, of the amount of induced damage found in tumors from mice which (a) *did*, and (b) *did not* receive a prior injection of a hormone preparation.

Compounds under test for lethal toxicity and tumor-damaging potency. Arsenite. Fowler's solution, pH 9.6, was prepared as described in the Pharmacopoeia of the United States, using arsenious acid, C. P., Baker's Analyzed. Any unused portion of this solution was discarded within 4 weeks after preparation. Solutions of arsenite at the desired concentrations for injections into mice were secured by diluting Fowler's solution with water. All doses for arsenite or arsenious acid stated here refer to dry arsenous acid. Arsenite, having certain advantages over various aromatic trivalent arsenical compounds, was used for most of the experiments. It is more stable, both in solid form and in solution than most, if not all, organic trivalent arsenical compounds. The difference in arsenite LD0 and LD100 is relatively small, so that significant changes in arsenite LD50 induced by prior treatment with hormone preparations could be determined without the use of excessively large numbers of mice. The minimum dose of arsenite required to induce marked gross and histological damage to mouse Sarcoma 37 did not vary appreciably from experiment to experiment. *Oxophenarsine.* Preparations of 3-amino-4-hydroxyphenylarsenoxide HCl as a dry powder in sealed ampoules† were used. Just before preparation of a solution of oxophenarsine, it was mixed in the dry form with 4.3 parts by weight of a dry, powdery proprietary citrate-acetate buffer mixture.‡ Solutions of the mixture (pH values about 6.5) were injected into mice as soon as possible after preparation to minimize chemical change in solution. All doses for oxophenarsine refer to the dry form, before mixture with the buffer mixture. *Clorarsen.* Powder preparations in sealed ampoules of 3-amino-4-hydroxyphenyldichlorarsine HCl, mixed with 3.3 parts by weight of a proprietary citrate-acetate buffer, were used.‡ Solutions had pH values about 5.6. Stated doses are for the pure compound. *P-chloromercuribenzoic acid.* In the form of dry powder§ it was dissolved in water plus an equimolecular amount of NaOH. *Iodoacetamide* was secured as powder.¶ *Alpha-peltatin* was secured from the Organic Chemistry Unit, Chemotherapy Section, National Cancer Institute. *"Acridan."* 9-phenyl-9-chloro-10-methyl-3-dimethyl amino acridan 2 HCl was secured as a powder from the Malaria Control Board, National Institutes of Health, Beth-

† Parke-Davis and Co., Mapharsen.
‡ Squibb and Sons, Inc.
§ Donated by Dr. Leslie Hellerman
¶ Eastman Kodak Co.

esda, Md.

Hormone preparations tested for ability to protect mice or mouse sarcoma 37 against toxic and tumor-damaging compounds. Beef adrenal extract‖ obtained in 10 cc sealed ampoules, prepared commercially was generally administered in the amount of 0.5 cc per mouse. In some experiments, control mice were injected with 0.5 cc of 10% alcohol, since the alcohol content of the beef adrenal extract was 10%. *Adrenocorticotropic hormone (ACTH)*** had a stated assay value of 57% of the activity of Armour and Co. standard ACTH preparation. All doses stated here are for the preparation used, uncorrected for difference in assay from this standard. The compound was injected in water solution. *Cortisone.*†† A suspension of 11-dehydro-17-hydroxycorticosterone-21- acetate (25 mg per cc) in 0.9% NaCl was used. Suspensions for injections into mice were prepared by diluting the stock suspensions with 0.9% NaCl, just prior to an experiment. *Desoxycorticosterone acetate.*†† A suspension of the compound (25 mg per cc) in 0.9% NaCl was used. Suspensions for injections into mice were prepared as stated above for cortisone.

Results. I. Experiments with trivalent arsenical compounds. Optimum time interval between hormone and arsenite. Results obtained with ABC mice and cortisone, shown in Fig. 1, indicate that protection against arsenite could be secured by injecting cortisone over a wide time interval prior to injection of arsenite. For the beef adrenal extract, maximum protection against arsenite was secured by injection of extract about an hour before injection of arsenite. In similar experiments in which 2.5 or 5 mg of desoxycorticosterone acetate per kg was used and the time interval between hormone and arsenite was varied from zero to 24 hours, no evidence of protection was secured. Measurable protection was obtained by administration of ACTH 5 to 24 hours prior to injection of arsenite. Measurable protection was not obtained by injecting any of these substances *after* injection of arsenite. *Minimum dose of hormone required to secure protection against arsenite.* Data obtained in tests using ABC male mice are shown in Fig. 2. Hormone doses are plotted on a logarithmic scale. The time interval between injection of cortisone and injection of arsenite was 50 minutes, that between injection of adrenal extract and injection of arsenite, 60 minutes. The data indicate that for mice subsequently injected with 12.6 mg of arsenite per kg, maximum protection obtainable with the extract was obtained by the use of as little as 0.01 cc of extract per gram of body weight, and that considerable protection was secured with as little as 0.31 mg of cortisone per kg. This corresponds to less than one molecule of cortisone for every hundred molecules of arsenite administered. *Lethal dose values obtained for arsenite, oxophenarsine and clorarsen, in male and female mice, as affected by hormones.* Data obtained in experiments in which the beef adrenal extract was injected 40 to 60 minutes prior to the arsenical compound are shown in Table I. This extract afforded male mice significant protection against arsenite, against oxophenarsine and against clorarsen. This protection was not due to the water-alcohol content of the adrenal extract (Exp. 1, Table I). Much less protection was afforded female than male mice against arsenite. From data obtained in experiments using cortisone as the test protecting hormone, and shown in Table II, it is seen that significant protection was afforded male mice against arsenite, injected either subcutaneously or intravenously, by cortisone injected intraperitoneally. In experiments for which data are not included, male mice were found to be protected against arsenite injected subcutaneously, by cortisone injected subcutaneously at another site. Protection by cortisone was not due to the water-NaCl content of the cortisone suspension (Exp. 3, Table II). Cortisone did not afford female mice significant protection against arsenite in any one of the 3 experiments performed. In 8 experiments with male mice in which both LD50 and LD5 values were calculated (Tables I and II), the average percent increase in LD50 value obtained by pretreatment with adrenal extract or cortisone

‖ Upjohn Pharmaceutical Co.

** Armour and Co.

†† Merck and Co.

was 18%, whereas the average increase in LD5 value was 42%. Table III gives data obtained in experiments using ACTH and arsenite. In 2 experiments in which male mice were employed a statistically significant protection was noted. In an experiment in which male mice were injected with both ACTH and beef adrenal extract, quite marked protection was obtained against arsenite. In one experiment with female mice, no protection was noted. *Tumor-damaging effects of arsenite, as affected by adrenal hormone preparations.* In an experiment with CAF_1 males and in another with CAF_1 females, the minimum effective dose of arsenite for inducing marked hemorrhagic necrosis in Sarcoma 37 was judged to be 10 mg per kg for control mice, and 12.6 mg per kg for mice which had been injected with the beef adrenal extract, 0.5 cc per mouse, one hour prior to injection of arsenite. In an experiment with CAF_1 males, the minimum effective dose of arsenite for inducing marked hemorrhagic necrosis in Sarcoma 37 was judged to be 8 mg per kg for control mice, and 10 mg per kg for mice which had been injected with cortisone in the amount of 1.25 mg per kg, one hour prior to injection of arsenite. Since (a) the minimum dose of arsenite effective in inducing marked necrosis in Sarcoma 37, and (b) arsenite MTD for mice (LD5 of Tables) were both increased by prior administration of adrenal extract or cortisone, it would appear that in mice prior administration of these hormone preparations was not markedly effective in increasing the margin of safety in the use of arsenite as a tumor damaging agent.

II. *Experiments with non-arsenical organic compounds.* Table IV shows lethal dose values obtained for a number of tumor-damaging compounds. Under the conditions employed, there was no evidence of protection of mice by cortisone against semi-lethal amounts of any of these compounds. The negative findings with p-chloromercuribenzoic acid and iodoacetamide are of particular interest, since they are classified with trivalent arsenical compounds as sulfhydryl poisons(6).

Discussion. The present experiments do not provide a basis for stating the mechanism whereby measurable protection against semi-lethal amounts of arsenite, oxophenarsine and clorarsen was conferred on male mice by certain of the adrenal hormones. Experiments are in progress in this laboratory to test for effects of adrenal hormones on tissue concentrations of sulfhydryl compounds capable of binding and so detoxifying trivalent arsenical compounds. Adrenal hormone preparations have generally failed to increase the resistance of normal (non-adrenalectomized) animals to toxic substances. However, Clark and Barnes (7), using a few animals, have reported protection of rats against colchicine by cortin. Zwemer and Jungeblut(8) have reported some protection of guinea pigs against diphtheria toxin by adrenal extract. Ingle(9) has reported protection of rats against peptone shock by adrenal extracts. Beck and Voloshin (10) have reported protection of mice against *Serratia marcescens* tumor-necrotizing polysaccharide by cortisone. Vollmer (unpublished) has found that mice may be protected against semi-lethal doses of potassium, not only by certain adrenal hormones, but also like arsenite by glutathione.

Summary. Prior administration of beef adrenal extract increased the resistance of mice, particularly male mice, to semi-lethal doses of arsenite, oxophenarsine and clorarsen. Male mice were protected against arsenite by cortisone and ACTH, but not by desoxycorticosterone. Prior administration of cortisone did not increase the resistance of mice to semi-lethal doses of p-chloromercuribenzoate, iodoacetamide, alphapeltatin, or 9-phenyl-9-chlor-10-methyl-3-dimethyl amino acridan. The minimum dose of arsenite required to induce marked hemorrhagic necrosis in mouse Sarcoma 37 was somewhat greater for adrenal extract or cortisone injected mice than for control mice.

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