THE EFFECT OF DIMERCAPROL ON THE ADRENAL ASCORBIC ACID IN THE RAT

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Dimercaprol produces a wide variety of metabolic disturbances. It inhibits many enzymes, particularly those that are metal-containing,[1,2] antagonizes the activation of certain enzymes[2] by metals and reduces the respiration of tissue slices.[3] Methaemaglobin it instantaneously reduces to haemaglobin.[1]

Small doses of it produce hypoglycaemia, the cause of which is uncertain.[4] Liver glycogen is reduced,[5] and repeated doses have induced hepatic injury in dogs.[6] The action of insulin is attenuated both *in vitro* and *in vivo*.[3,4] Large doses produce hyperglycaemia as a result of the release of adrenaline from the adrenal medulla.[4] The action of adrenaline is potentiated.[7]

Because dimercaprol produces liver damage and releases adrenaline, its action on adrenal and hepatic ascorbic-acid levels in the rat has now been investigated.

METHODS AND RESULTS

Adult male and non-pregnant female rats of the Wistar strain, weighing 180-300 G., were used. Dimercaprol injection B.P. (dimercaprol 5 G., benzyl benzoate 9·6 ml., and arachis oil to 100 ml.) was given in doses of 10 mg., 50 mg. and 100 mg. per kg. body weight. Controls received injections either of 10% benzyl benzoate in oil or of saline. All injections were intramuscular. At varying intervals after an injection (Table I) pairs of rats were killed. Their adrenal glands and 1 G. of freshly excised liver were then assayed for their ascorbic-acid content using the method of Kennaway and his co-workers.[8,9]

Another group of rats received injections of dimercaprol (75 mg. per kg.) daily for 5 days. A control group received injections of saline daily for 5 days. Two hours after their last injection, both groups were killed and their adrenal glands assayed as above.

Hepatic Ascorbic Acid

The method used did not permit estimation of hepatic ascorbic acid owing to the opalescence of the extract obtained.

Intact Rats given Single Injections only

Rats given injections either of saline or of 10% benzyl benzoate in oil had control values of adrenal ascorbic acid that ranged from 300 to 390 mg. per 100 G. of fresh tissue.

Injections of dimercaprol, in doses of 50 mg. and of 100 mg. per kg., resulted in a depletion of adrenal ascorbic acid in all rats (Table I). After 24 hours, the adrenal ascorbic acid was elevated above control values.

Intact Rats given Daily Injections for 5 Days

The administration of dimercaprol daily for 5 days resulted in a marked depletion of adrenal ascorbic acid (Table II).

Adrenaline has been shown to be released from the adrenal medulla by large doses of dimercaprol.[4] Adrenaline causes a depletion of adrenal ascorbic acid.[10] An investigation was therefore made into the possibility that the depletion of adrenal ascorbic acid following dimercaprol might be due to the release of adrenaline.

Rats, then, were given 'reserpine' by intramuscular injection for 5 days. The dosages used were:

0·5 mg. per kg. body weight for the first 3 days
2·0 mg. per kg. body weight on the 4th day
0·5 mg. per kg. body weight on the 5th day.

TABLE I. EFFECT OF SINGLE INJECTIONS OF DIMERCAPROL ON ADRENAL ASCORBIC-ACID LEVEL

Drug	*Dose mg. per kg.*	*Rats per group*	*Adrenal ascorbic-acid level mg. per 100 G. fresh tissue*					
			¼ hr.	*½ hr.*	*1 hr.*	*1½ hr.*	*2 hr.*	*24 hr.*
Dimercaprol	10	2	—	312	281	300	370	—
Dimercaprol	50	2	274	260	276	266	288	406
Dimercaprol	100	2	295	276	239	220	230	420
	ml. per kg.							
10% benzyl benzoate	4	2	—	330	356	328	340	—
Saline	2	2	348	390	370	358	300	368

Four hours after the last injection of reserpine, one-half of the rats were given dimercaprol by intramuscular injection in a dosage of 100 mg. per kg. body weight. The others received intramuscular saline. Ninety minutes later the rats were killed and their adrenal glands assayed for ascorbic acid.

TABLE II. EFFECT OF 5 DAILY INJECTIONS OF DIMERCAPROL ON ADRENAL ASCORBIC-ACID LEVEL

Dose mg. per kg.	*Rats per group*	*Adrenal ascorbic-acid level mg. per 100 G. fresh tissue*	
		Control group	*Treated group*
75	4	437	239

Reserpine-treated Rats given Single Injections only

Rats given dimercaprol after treatment with reserpine, showed a marked reduction of adrenal ascorbic acid when compared with the control group (Table III).

TABLE III. EFFECT OF DIMERCAPROL ON ADRENAL ASCORBIC-ACID LEVEL OF RESERPINE-TREATED RATS

Dose mg. per kg.	*Rats per group*	*Adrenal ascorbic-acid level mg. per 100 G. fresh tissue*	
		Control group	*Treated group*
100	6	437	249

The adrenal ascorbic-acid levels of rats that received reserpine only for 5 days and rats that received saline only for 5 days were identical (Tables II and III).

Weight of Adrenal Glands

All rats that received daily injections for 5 days were weighed before they were killed and their adrenal glands weighed after death. The relationship of weight of adrenal glands to total body weight when living was expressed in mg. per 1,000 G. total body weight.

All reserpine-treated rats became markedly dehydrated before death as a result of diarrhoea and disinclination to eat or to drink; consequently, their weights at the start of reserpine treatment were used.

Rats that received dimercaprol daily had heavier adrenal glands than those that received saline only (Table IV).

There was no difference between the weights of the adrenal glands of rats that received reserpine and dimercaprol and those that received reserpine and saline (Table IV).

Reserpine-treated rats had heavier adrenal glands than rats that received dimercaprol (Table IV).

To assess whether dimercaprol produced a depletion of adrenal ascorbic acid as a result of *stimulation of the hypothalamus*, dimercaprol was injected into hydrocortisone-treated rats. Hydrocortisone succinate was first injected into the peritoneal cavity in a dosage of 6 mg. per 100 G. body weight. The first injection was given at 2 p.m. on the day before the experiment, and a second similar injection was given at 9 a.m. the following day. The experimental drugs were given 3 hours later. Half received an intramuscular injection of dimercaprol (100 mg. per kg. body weight) and the rest were injected with saline. Two hours later they were killed and their adrenals assayed.

TABLE IV. EFFECT OF 5 DAILY INJECTIONS ON WEIGHT OF ADRENAL GLANDS

Drug	*Dose mg. per kg. per day*	*Rats per group*	*Weight of adrenal glands mg. per 1,000 G. live weight*
Saline	—	4	130
Dimercaprol ..	75	4	173
Reserpine ..	0·5 (4 days)	6	216
+	2·0 (1 day)		
Saline ..	(1 day)		
Reserpine ..	0·5 (4 days)	6	212
+	2·0 (1 day)		
Dimercaprol	100 (1 day)		

Hydrocortisone-treated Rats given Single Injections only

The depleting effect of dimercaprol on adrenal ascorbic acid was not abolished by pretreatment with hydrocortisone succinate (Table V).

TABLE V. EFFECT OF DIMERCAPROL ON ADRENAL ASCORBIC-ACID LEVEL OF HYDROCORTISONE-TREATED RATS

Dose mg. per kg. per day	*Rats per group*	*Adrenal ascorbic-acid level mg. per 100 G. fresh tissue*	
		Control group	*Treated group*
100	6	470	245

DISCUSSION

The fact that dimercaprol produced a depletion of adrenal ascorbic acid after intensive treatment with reserpine suggests that the depletion noted is not mediated by a release of catechol amines. (Adrenaline has been shown to be released from the adrenal medulla of normal animals after large single doses of dimercaprol.[4])

Reserpine has been shown to cause a depletion of catechol amines and of 5-hydroxytryptamine in many tissues.[11] In rats, reserpine in a dose of 1 mg. per kg. body weight daily for 3 days produces a 50% fall in adrenal catecholamine content.[12] Certain drugs, e.g. tyramine and ephedrine, act peripherally by causing the release of catechol amines from stores.[11] After reserpine depletion, such drugs are no longer effective.[11]

Corroborative evidence for the above view is provided by the fact that adrenaline, when given in huge doses,[13] does not cause as great a fall in adrenal ascorbic acid as that which dimercaprol produced in reserpine-treated animals.

Reserpine, and certain other drugs that cause a depletion of ascorbic acid after a single injection, have been shown to cause no depletion in level after 5 daily injections; and, in the case of reserpine, to result in a significant rise in level.[14] Dimercaprol, unlike these drugs, still produced a depletion after 5 days. The levels of ascorbic acid in rats receiving saline and in rats receiving reserpine for 5 days were very similar. This has not been the experience of other investigators.[14]

There was an obvious hypertrophy of the adrenal glands after injections of reserpine and of dimercaprol, more so with the former. The fact that dimercaprol still produced a depletion of adrenal ascorbic acid after 5 days, and caused a smaller amount of adrenal hypertrophy than reserpine, may be due to the differing durations of their action. Reserpine is a long-acting drug, while dimercaprol is rapidly metabolized and excreted.[15] Hence the stress induced by daily injections of dimercaprol may be of shorter duration than that produced by reserpine.

Pretreatment with hydrocortisone has been shown to abolish the depleting effect of several drugs, e.g. chlorpromazine and reserpine.[14] Hydrocortisone inhibits the release of corticotrophin as the result of a feed-back mechanism. This may be due to action on the hypothalamus, since plasma from the hypothalamico-hypophyseal portal vessels of the dog still causes a depletion of adrenal ascorbic acid when injected into the hydrocortisone-treated rat.[16] Since pretreatment with hydrocortisone did not block the depleting effect of dimercaprol, it is probable that it exerts its action on the adrenal either directly or indirectly *via* corticotrophin release from the anterior pituitary.

In man, dimercaprol, even when given in large doses, has not been shown to cause a deficiency of ascorbic acid; this however applies also to many drugs that deplete adrenal ascorbic acid in the rat, e.g. salicylates and adrenaline.[13]

SUMMARY

Dimercaprol injected intramuscularly produced a depletion of adrenal ascorbic acid in intact rats after single and repeated injections and after single injections in reserpine-treated and in hydrocortisone-treated animals.

Both dimercaprol and reserpine produced an increase in adrenal size.

The adrenal ascorbic levels of rats treated for 5 days with reserpine and with saline did not differ.

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