THE USE OF HISTAMINE AS A STANDARD TEST FOR DIMINISHED RESISTANCE IN SUPRARENALECTOMIZED RATS.

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The resistance of suprarenalectomized animals to a number of nonspecific poisons is markedly reduced, as has been shown by several independent workers (1–6). These observers employing such poisons as cobra venom, curare, morphine, diphtheria toxin and typhoid vaccine were able to demonstrate a striking difference in resistance between normal and suprarenalectomized rats. In comparing the results obtained in different laboratories with the same poison, considerable differences in the lethal dose appeared to exist. With some of the poisons, as for example, typhoid vaccine, it seemed likely that the difference could be explained by a lack of uniformity of the preparation. Inasmuch as this test may have considerable value in future experimental work, it seemed advisable to secure additional data concerning certain of these poisons in order to find a stable substance having a relatively constant ratio between the lethal dose for suprarenalectomized rats and that for normal rats. This ratio should be at least 1:20. If such a substance could be found, the test might be standardized. In our attempts to standardize this test, we have used, up to the present time, typhoid vaccine, chloral hydrate, sodium cyanide and histamine, and the results obtained may be summarized as follows:

Typhoid Vaccine.—Marine and his coworkers (4) have shown that 75 per cent of rats are killed by 0.25 cc. of a standard typhoid vaccine within 8 days after suprarenalectomy. In a recent paper we showed (6) that suprarenalectomized rats surviving in good condition are killed by typhoid vaccine in doses of 0.4–1 cc. at the height of susceptibility (5–7 days). On repeating these experiments, it was noted

TABLE I.

Effect of Intraperitoneal Injections of Different Batches of Typhoid Vaccine on Suprarenalectomized Rats.

No. of rats	Preparation and Lot No.	Sex	Weight	Interval between operation and injection	Amount of vaccine	Duration life after injection	No. rats killed
			gm.	*days*	*cc.*	*hrs.*	
	N. Y. Board of Health						
3	114–413	F.	165	5	1.0	12	2
		"	172	5	1.0	18	
		"	170	5	1.0	Survived.	
9		"	152	6	1.0	4½	6
		"	150	6	1.0	6	
		"	130	6	1.0	6	
		"	164	6	1.0	5	
		"	144	6	1.0	18	
		M.	192	6	1.0	Survived.	
		"	150	6	1.0	"	
		"	167	6	1.0	"	
		"	190	6	1.0	18	
4		"	180	6	1.5	10	4
		"	184	6	1.5	10½	
		"	178	6	1.5	18	
		F.	160	6	1.5	6	
2		"	132	8	1.0	8	2
		"	152	8	1.0	10	
2		M.	190	8	1.5	7	2
		"	175	8	1.5	18	
9	109–229	F.	135	8	1.0	Survived.	1
		M.	170	8	1.0	8	
		F.	154	8	1.0	Survived.	
		"	120	8	1.0	"	
		"	114	8	1.0	"	
		"	110	8	1.0	"	
		"	124	8	1.0	"	
		M.	179	8	1.0	"	
		"	190	8	1.0	"	
3	115–414	F.	153	7	1.0	"	None.
		"	165	7	1.0	"	
		M.	180	7	1.0	"	

TABLE I—*Concluded.*

No. of rats	Preparation and Lot No.	Sex	Weight	Interval between operation and injection	Amount of vaccine	Duration life after injection	No. rats killed
			gm.	*days*	*cc.*	*hrs.*	
6	115–414	F.	137	7	1.5	5	6
		"	172	7	1.5	18	
		"	167	7	1.5	4	
		M.	189	7	1.5	6	
		"	180	7	1.5	7½	
		F.	145	7	1.5	2	
3*	114–415	F.	157	7	0.2	8½	3
		"	185	7	0.2	18	
		"	192	7	0.2	12–18	
2	U. S. Army triple	F.	195	6	1.5	Survived.	None.
		"	180	6	1.5	"	

* See foot-note 1.

TABLE II.

The Effect of Chloral Hydrate on Normal Rats.

No. of rats	Sex	Weight	Dose per kg.	Amount injected	Results
		gm.	*mg.*	*mg.*	
3	F.	150	400	60	Recovered.
	"	146	400	60	"
	"	176	400	70	"
2	"	155	600	93	"
	"	150	600	90	"
3	"	155	700	113	Dead 2 hrs. 10 min.
	"	150	700	105	Recovered.
	"	168	700	117	"
3	"	157	800	125	Dead few min.
	"	155	800	125	" 30 "
	"	153	800	125	" 30 "

that different lots, even of so called standard typhoid vaccine, showed wide variations in toxicity.[1]

Chloral Hydrate.—Eleven normal rats were injected subcutaneously with chloral hydrate. Of three rats injected with 800 mg. per kilo, all were killed. Of three rats injected with 700 mg. per kilo, one was killed, while all five rats injected with 400–600 mg. per kilo survived. Three suprarenalectomized rats were tested at the height of susceptibility (6 days after the removal of the glands), but no decrease in resistance could be demonstrated. See Tables II and III.

Sodium Cyanide.—The subcutaneous M.L.D. of sodium cyanide for normal rats was found to be between 9.8 and 11.0 mg. per kilo, approximately the same dose as that found by Voegtlin, Johnson and

TABLE III.

The Effect of Chloral Hydrate on Suprarenalectomized Rats.

No. of rats	Sex	Weight	Dose per kg.	Amount injected	Interval between operation and injection	Results
		gm.	*mg.*	*mg.*	*days*	
3	F.	105	400	42	9	Recovered.
	M.	210	400	84	9	"
	"	210	400	84	9	"

Dyer (7). This amount injected subcutaneously killed five out of six normal rats. Amounts less than 9.8 mg. per kilo did not kill any in a series of six normal rats used. Thirteen suprarenalectomized rats were injected subcutaneously with varying amounts of sodium cyanide. Two rats injected 6 days after removal of the glands

[1] Preparation 114, Lot 415 New York Board of Health vaccine killed with a dose of 0.2 cc. This particular vaccine had been used as a non-specific therapeutic measure in chronic arthritis and cancer, and the clinicians had noted much more marked reactions from a given dose of this preparation than had been obtained with the same dose of other preparations. The variations in toxicity which we had found would explain the differences in results reported by several workers and make typhoid vaccine unsatisfactory as a routine test substance for the resistance of suprarenalectomized rats unless the lethal dose of each lot is separately determined.

TABLE IV.

Effect of Sodium Cyanide on Normal Rats.

No. of rats	Sex	Weight	Dose per kg.	Amount injected	Results
		gm.	*mg.*	*mg.*	
3	F.	147	5.5	.80	Recovered.
	M.	164	5.5	.90	"
	F.	138	5.5	.76	"
3	"	144	4.4	.64	"
	M.	184	4.4	.80	"
	"	176	4.4	.78	"
3	F.	136	9.8	1.33	Dead 5 hrs.
	M.	144	9.8	1.40	" 2 "
	"	179	9.8	1.76	" 2 "
3	F.	144	11.0	1.58	Recovered.
	M.	184	11.0	2.01	Dead 1 hr.
	"	176	11.0	1.93	" 1 "

TABLE V.

The Effect of Sodium Cyanide on Suprarenalectomized Rats.

No. of rats	Sex	Weight	Dose per kg.	Amount injected	Interval between operation and injection	Results
		gm.	*mg.*	*mg.*	*days*	
2	F.	150	5.5	.82	6	Killed 3½ hrs.
	M.	180	5.5	.99	6	" 7½ "
2	"	215	2.75	.59	6	Recovered.
	F.	155	2.75	.43	6	"
6	M.	184	4.8	.9	13	Killed 1 hr.
	F.	129	4.8	.63	13	" 18 hrs.
	"	107	4.8	.52	13	" 18 "
	"	132	5.5	.73	13	Recovered.
	M.	150	5.5	.82	13	Killed 1 hr.
	"	166	5.5	.91	13	" few min.
3	"	164	4.85	.8	30	Recovered.
	"	172	4.65	.84	30	"
	F.	121	3.93	.49	30	"

with 5.5 mg. of NaCN per kilo were killed. Two rats injected at 6 days with 2.75 mg. per kilo were not killed. Five out of six rats injected 13 days after suprarenalectomy were killed with 4.8–

TABLE VI, *a*.

Effect of Intravenous Injections of Histamine Acid Phosphate on Normal Rats.

Rat No.	Weight	Histamine acid phosphate per kg.	Remarks
	gm.	*mg.*	
158–2	195	900	Recovered in 3 hrs. In good condition after 2 wks.
158–1	200	1000	Recovered in 3 hrs. In good condition after 2 wks.
158–4	170	1200	Recovered in good condition in 3 hrs. Lived for 6 wks. Died.
169–3	260	1350	Recovered in 3 hrs. In good condition after 2 wks.
158–3	180	1500	Recovered in good condition after 3 hrs. In good condition after 2 wks.
159–2	165	2000	Dead in 2 hrs. 15 min.

TABLE VI, *b*.

Effect of Intraperitoneal Injections of Histamine Acid Phosphate on Normal Rats.

Rat No.	Weight	Histamine acid phosphate per kg.	Results
	gm.	*mg.*	
94	165	1000	Recovered after few hrs. in good condition.
95	232	1200	" " " " " " "
96	150	1300	" " " " " " "
97	195	1400	" " 10 " " " "
98	185	1500	" " 10 " " " "
99	155	1600	Dead after 18 hrs.
100	170	1700	" " 11 "
101	188	1800	" " 4 "

5.5 mg. per kilo. Three rats injected at 30 days with 4.5 mg. per kilo were not killed. It appears therefore that suprarenalectomized rats at the height of susceptibility are about twice as susceptible

TABLE VII.

Effect of Intravenous Injections of Histamine Acid Phosphate on Suprarenalectomized Rats.

No. of rats	Sex	Weight	Interval between operation and injection	Histamine acid phosphate per kg.	Histamine acid phosphate dose; amount injected	Duration of life after injection	No. of rats killed
		gm.	*days*	*mg.*	*mg.*	*hrs.*	
5	F.	165	20	298.1	49.2	10	5
	M.	200	20	406.0	81.2	½	
	"	220	20	491.7	108.2	¾	
	"	220	20	245.9	54.1	9½	
	"	210	20	351.4	73.8	8	
5	"	220	7	346.3	76.2	5	5
	F.	170	7	520.6	88.5	2¼	
	M.	220	7	171.2	37.9	Few min.	
	"	175	7	140.6	24.6	6¼	
	"	225	7	141.7	31.9	3	
7	F.	165	6	123.0	20.3	6	7
	"	165	6	123.0	20.3	4½	
	M.	200	6	246.0	49.2	2½	
	"	200	6	246.0	49.2	6	
	"	210	6	199.0	41.8	18	
	"	240	6	205.0	49.2	3	
	"	230	6	164.7	37.9	5¼	
3	F.	160	21	187.5	30.0	3	3
	"	165	21	181.8	30.0	12	
	"	175	21	171.4	30.0	18	
4	M.	225	6	80.0	18.0	3	3
	"	243	6	80.0	19.4	3	
	F.	180	6	80.0	14.4	1½	
	"	160	6	80.0	12.8	Survived.	
2	M.	250	6	60.0	15.0	"	0
	"	200	6	60.0	12.0	"	
1	"	240	6	40.0	9.6	"	0
4	"	285	6	20.0	5.7	"	0
	"	185	6	20.0	3.7	"	
	"	230	5	20.0	4.6	"	
	F.	175	5	20.0	3.5	"	
2*	M.	220	7	172.2	37.9	"	0
	F.	195	21	153.8	30.0	"	

* Controls—one adrenal intact.

TABLE VIII.

Influence of the Time Interval after Suprarenalectomy on the Effect of Intraperitoneal Injections of Histamine Acid Phosphate.

No. of rats	Sex	Weight	Interval between operation and injection	Histamine acid phosphate; amount injected*	Duration of life after injection	No. of rats killed
		gm.	*days*	*mg.*	*hrs.*	
6	M.	200	2	20.0	Survived.	1
	F.	165	2	16.5	2	
	"	200	2	20.0	Survived.	
	"	190	2	19.0	"	
	"	195	2	19.5	"	
	"	170	2	17.0	"	
5	"	220	3	22.0	"	2
	"	220	3	22.0	12	
	M.	250	3	25.0	Survived.	
	"	240	3	24.0	48	
	"	260	3	26.0	Survived.	
7	"	230	4	23.0	"	2
	F.	190	4	19.0	"	
	"	180	4	18.0	13	
	"	200	4	20.0	Survived.	
	"	165	4	16.5	"	
	"	190	4	19.0	"	
	M.	220	4	22.0	2	
12	"	190	5	19.0	2	12
	"	220	5	22.0	3	
	"	210	5	21.0	18	
	"	179	5	17.9	1	
	"	194	5	19.4	1	
	"	181	5	18.1	1	
	"	181	5	18.1	$2\frac{1}{2}$	
	"	201	5	20.1	$\frac{1}{2}$	
	"	193	5	19.3	3	
	F.	168	5	16.8	3	
	"	132	5	13.2	4	
	"	162	5	16.2	9	

* 100 mg. per kilo of body weight.

TABLE VIII—*Concluded.*

No. of rats	Sex	Weight	Interval between operation and injection	Histamine acid phosphate; amount injected*	Duration of life after injection	No. of rats killed
		gm.	*days*	*mg.*	*hrs.*	
10	F.	180	6	18.0	2	8
	"	200	6	20.0	18	
	M.	240	6	24.0	4	
	"	218	6	21.8	Survived.	
	"	240	6	24.0	1	
	F.	185	6	18.5	2	
	M.	210	6	21.0	Survived.	
	F.	159	6	15.9	1	
	"	150	6	15.0	2	
	M.	216	6	21.6	1½	
6	F.	200	7	20.0	Survived.	3
	"	210	7	21.0	"	
	"	160	7	16.0	2	
	"	225	7	22.5	1	
	"	190	7	19.0	Few min.	
	"	190	7	19.0	Survived.	
3	"	250	8	25.0	2	1
	"	180	8	18.0	Survived.	
	"	240	8	24.0	"	

to NaCN as are normal rats. This difference in the lethal dose of NaCN for suprarenalectomized and normal rats is, however, so small that this drug cannot be advantageously used as a test for decreased resistance. See Tables IV and V.

Histamine.—the increased susceptibility of suprarenalectomized animals to histamine has been shown by numerous investigators (8–12).

The intravenous M.L.D. of histamine acid phosphate for normal rats has been found to be approximately 900 mg. per kilo by Voegtlin (13) and 500–700 mg. per kilo by Crivellari (11).

In our experience (Tables VI,*a* and VI,*b*) the M.L.D. for normal rats is approximately 1600 mg. per kilo. As the preparation of histamine used in our experiments was the same as that employed in

the experiments of Voegtlin (13) and Crivellari (11) it is possible that the difference in the lethal dose is due to a difference in the age or in the susceptibility of the albino rats that we and they have used. Our rats were approximately 5 months of age and were reared in the laboratory from Wistar stock.

The increased susceptibility of suprarenalectomized rats to the intravenous injection of histamine is shown in Table VII. As the intravenous M.L.D. for these animals is approximately 80–100 mg. of histamine acid phosphate per kilo the suprarenalectomized rats are about 20 times more susceptible to histamine than the normal rats of the same age and strain. Banting and Gairns (10) found that suprarenalectomized dogs are about thirty times more susceptible to histamine than are normal dogs.

As the intravenous injection of histamine acid phosphate in doses as high as 2000 mg. per kilo involves the injection of comparatively large amounts of phosphoric acid, it seemed advisable to exclude by control experiments a possible effect of phosphoric acid. We therefore injected two normal rats with the amount of phosphoric acid per kilo contained in 2000 mg. of histamine acid phosphate per kilo. Instead of the phosphoric acid solution, an equivalent amount of $NaH_2PO_3 \cdot H_2O$ was used. The rats were sick for about 3 hours but recovered completely. Smaller amounts of $NaH_2PO_3 \cdot H_2O$ (1/10th of the dose used for the normal rats) had no effect on two suprarenalectomized rats.

The results of intraperitoneal injections of histamine acid phosphate in 49 suprarenalectomized rats are given in Table VIII. Of 18 suprarenalectomized rats injected with 100 mg. of histamine acid phosphate per kilo between the 1st and 4th days after the removal of both glands, 5 were killed, whereas 24 out of 31 suprarenalectomized rats injected at 5, 6, 7 and 8 days after the operation were killed.

A comparison of Tables VII and VIII shows that the difference in the M.L.D. of histamine acid phosphate for suprarenalectomized rats when given intravenously and intraperitoneally is negligible. Since the technique of intravenous injection in the rat is quite difficult, these results show that the intravenous method may be abandoned for this purpose.

We should like to call attention again to the latent period after

suprarenalectomy. As pointed out previously (6), the great drop in resistance does not occur until about the 5th day after the operation. This is illustrated in Table VIII where of 18 rats injected between the 2nd and 4th days, only 5 were killed, whereas of 31 rats injected with the same amounts between the 5th and 8th days, 24 were killed.

SUMMARY.

Of all the drugs thus far used in testing the resistance of suprarenalectomized rats, histamine has been found to be the most satisfactory. It is a readily obtainable and comparatively stable drug, producing characteristic symptoms. Its M.L.D. for a given strain of rats can be established within narrow limits, and the difference between the M.L.D. for normal and suprarenalectomized rats is comparatively great (1:20).

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