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DEPLETION AND REPLACEMENT OF THE ADRENALINE AND NORADRENALINE CONTENTS OF THE RAT ADRENAL GLAND, FOLLOWING TREATMENT WITH RESERPINE

BY

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The effect of reserpine on the adrenaline and noradrenaline contents of rat adrenal glands has been studied. Given subcutaneously to male albino Wistar rats in 3 daily doses of 1 mg/kg, it caused, 24 hr after the final dose, a similar percentage loss of adrenaline and noradrenaline of approximately 50%. Subsequently the adrenaline content slowly increased, until at 14 days from the begining of the experiment there was no significant difference between the reserpine-treated and the control glands. In contrast, the recovery of the noradrenaline content was rapid, exceeding the control value at 7 days by approximately 250%. This increased content of noradrenaline declined to the normal level by 21 days. The total amine content returned to normal by 7 days and remained at this level subsequently. The effect of re-depleting the glands of their amine content was investigated. It was found that re-depletion at 7 days caused a preferential release of noradrenaline, followed at 14 days by a peak of noradrenaline at least as high as that obtained following the initial depletion only. Re-depletion at 21 days caused an effect similar to that obtained initially. Denervation of the left adrenal gland did not alter the degree of depletion caused by reserpine, nor did it alter the subsequent replacement of the amines as compared with that in the innervated right gland.

In 1956 Holzbauer & Vogt showed that reserpine caused a loss of adrenaline and noradrenaline from the adrenal gland of the cat. Carlsson & Hillarp (1956) demonstrated a similar effect in the rabbit adrenal gland, and Parratt & West (1957) noted that the adrenal gland of the rat was markedly depleted by doses of 10 mg/kg of reserpine. Taketomo, Shore, Tomich, Kuntzman & Brodie (1957) found that, following depletion of the rabbit adrenal gland, 5 to 7 days were necessary for the amine stores to be replenished and that the depletion could be prevented by section of the spinal cord at the level of the first thoracic segment. Kroneberg & Schümann (1957a) also demonstrated that recovery was slow and that cutting the splanchnic nerves one week previously only partially protected the glands from depletion. Muscholl & Vogt (1958) found that 14 days were necessary for replacement of the catechol amine content of the rabbit adrenal medulla following depletion by a single intravenous dose of 1.6 mg/kg of reserpine. They also showed that the relative proportions of adrenaline and noradrenaline were normal even in glands still partially depleted at 8 to 9 days.

Kroneberg & Schümann (1957b) found that depletion of rat adrenal glands by doses of 10 mg/kg of reserpine could not be prevented by section of the spinal cord at the level of the sixth cervical segment. Mirkin (1958) could not demonstrate any difference in the response of innervated and denervated rat adrenal glands to 5 daily intraperitoneal injections of 1 mg/kg of reserpine. However, in 1958 Eränkö & Hopsu studied the effects of reserpine on the adrenal glands of rats and mice by histochemical and fluorimetric methods. They obtained a preferential loss of noradrenaline from the rat adrenal gland 24 hr after single doses of either 0.25 mg or 0.5 mg of reserpine. Denervation by cutting the splanchnic nerve and removal of the adipose tissue around the adrenal gland prevented this selective loss of noradrenaline. However, Coupland (1958a) could find no evidence for selective liberation of either adrenaline or noradrenaline following a single intravenous injection of 15 mg/kg of reserpine in Wistar rats or of 5 mg/kg in Sprague-Dawley rats. Subsequently it was found (Coupland, 1959a) that, following a single intravenous injection of 15 mg/kg of reserpine in Wistar rats, the noradrenaline content returned to normal by 6 days, whereas 14 days were necessary for the complete replacement of the adrenaline content. It was of interest, therefore, to study the actions of reserpine on the adrenal glands of Wistar rats in an attempt to resolve some of the different effects that have been reported.

METHODS

Male albino rats of the Wistar strain, weighing 150 to 190 g, were used. In all except the denervation experiments, groups of 5 rats were employed, and each test group had its own control group, also of 5 animals. In the denervation experiments the groups were of 10 rats. In all experiments the animals were distributed in cages, 24 hr before commencing the injections, in such a way that the mean weights were similar in all groups.

Reserpine, dissolved in the solvent used by Pletscher, Shore & Brodie (1955), was injected subcutaneously into the neck of the test rats. Control animals received a corresponding volume of the solvent. The rats treated with reserpine were not specially fed, although the food was placed on the floor of the cages and the water in shallow dishes. The temperature of the animals' surroundings was maintained at approximately 20° C.

The rats were killed by a blow on the head at varying time intervals from the first injection of reserpine, which was taken as time zero. Both adrenal glands were rapidly removed, care being taken to avoid damage to the capsule. After removal of the surrounding fat and connective tissue, the glands from each group were weighed collectively and a 25 mg/ml. extract prepared in 0.1 N hydrochloric acid. The extracts were stored at just above 0° C.

The adrenaline and noradrenaline contents of the extracts were determined by differential biological assay, using the blood pressure of the cats treated with hexamethonium and the isolated uterus of the rat in dioestrus as test preparations. The adrenaline and noradrenaline contents were calculated, as the laevo isomers of the bases, in μg/gland, by the formula of Bülbring (1949).

Denervation of the left adrenal gland was performed by cutting the left splanchnic nerve fibres. The method used was that of Vogt (1945), except that sodium pentobarbitone (40 mg/kg injected intraperitoneally) anaesthesia was employed. Rats weighing 80 to 100 g were used and 3 weeks were allowed post-operatively for the amine content of the glands to recover from the stress of the operation. The left glands of the control animals were also denervated so that the denervated left glands of test and control rats could be compared, as could the innervated right glands of both groups. If the denervated gland in either test or control animal was atrophied at the time of removal, that animal was rejected.

RESULTS

Depletion experiments

Injections of 1 mg/kg of reserpine were given at intervals of 24 hr to 14 groups of rats. Two groups of test animals were killed at each of the following times after the first injection—4, 8, 12, 16, 24, 48 and 72 hr. Thus rats killed at intervals up to and including 24 hr received one injection; those killed at 48 hr received 2 injections; and those killed at 72 hr, 3 injections. Single groups of control animals were killed at 4, 24, 48 and 72 hr after the first injection of solvent. Only 4 control groups were considered necessary because no alteration in the amine content could be shown in normal rat glands over a period of 3 days. Thus it was possible to use the mean results of the 4 groups as the control value for the whole experiment.

TABLE 1

MEAN AMINE CONTENTS OF RAT ADRENAL GLANDS AT INTERVALS DURING RESERPINE TREATMENT

3 doses of 1 mg/kg of reserpine were given subcutaneously at 0, 24 and 48 hr to rats. Two groups of animals were killed and their adrenal amine content estimated at 4, 8, 12, 16, 24, 48 and 72 hr after the first injection. Control estimates were made at 4, 24, 48 and 72 hr

Hr after first injection	No. of observations	Amine contents (μg/gland) Adrenaline	Noradrenaline	Total amine	% noradrenaline
A. *Reserpine-treated*					
4	2	10·29	1·16	11·45	10·13
8	2	9·67	1·03	10·70	9·63
12	2	8·13	0·89	9·02	9·87
16	2	7·41	0·86	8·27	10·40
24	2	7·49	0·85	8·34	10·20
48	2	6·71	0·75	7·46	10·05
72	2	5·63	0·66	6·29	10·49
B. *Solvent-treated controls*					
4	1	10·29	1·13	11·42	9·89
24	1	12·58	1·44	14·02	10·27
48	1	10·74	1·23	11·97	10·28
72	1	10·99	1·17	12·16	9·62
Mean control	—	11·15	1·24	12·39	10·01

Absolute results are shown in Table 1. Percentage differences between the mean amine contents of the 2 test groups at each time interval and the mean of the 4 control groups are shown in Fig. 1. The greatest loss of amines occurred between 0 and 16 hr. The reserpine given at 24 and 48 hr caused a much smaller reduction in the amine content than that caused by the first injection. Throughout the experiment there was always a similar percentage loss of adrenaline and noradrenaline, there being no indication of a preferential release of either amine at any of the time intervals studied. Thus throughout the depletion the percentage of noradrenaline remained similar to that of the controls.

By 72 hr an approximately 50% depletion of both adrenaline and noradrenaline was obtained. Althcugh by this time the reserpine-treated animals were in poor condition, with loss of body weight, no deaths occurred. There was some increase in the adrenal weight due to cortical hypertrophy. This made it necessary to calculate all results in μg/gland and not per unit weight of gland.

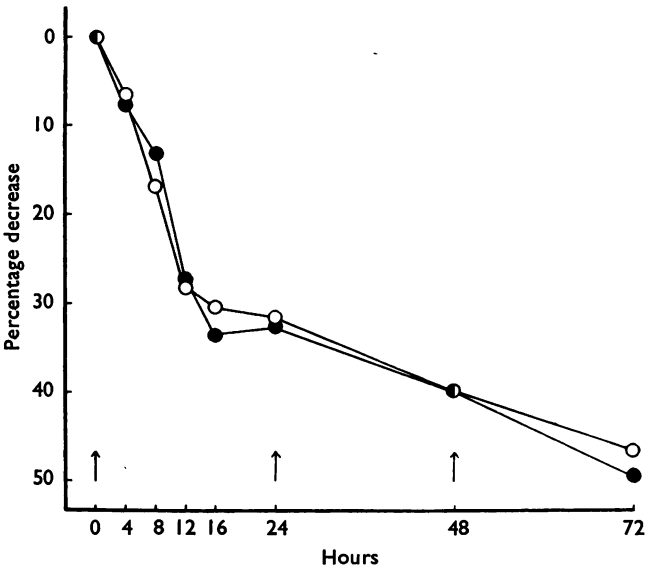

Fig. 1. Mean adrenaline (●—— ●) and noradrenaline (○—— ○) contents of rat adrenal glands at intervals up to 72 hr during depletion with 1 mg/kg reserpine (at arrows) injected subcutaneously, expressed as percentage differences relative to their control values.

Replacement experiments

Having obtained an approximately 50% depletion of adrenaline and noradrenaline at 3 days following the administration of 3 doses of 1 mg/kg of reserpine at intervals of 24 hr, the subsequent rate of replacement of these amines was studied. Test groups were killed at 3, 7, 14, 21 and 28 days and each had its own control group. The results of these experiments are shown in Table 2 and Fig. 2.

TABLE 2

MEAN AMINE CONTENTS OF RAT ADRENAL GLANDS AT INTERVALS UP TO 28 DAYS FOLLOWING RESERPINE TREATMENT

Rats were injected subcutaneously with 1 mg/kg of reserpine on days 0, 1 and 2. Groups of animals were killed and their adrenal amine content estimated 3, 7, 14, 21 and 28 days after the first dose of reserpine. Control estimates were made on animals treated with the solvent only

Days	No. of observations	Amine contents (μg/gland±s.e.) Adrenaline	Noradrenaline	Total amine	% noradrenaline ±s.e.
A. *Reserpine-treated*					
3	14	6·33±0·40	0·72±0·05	7·05±0·44	10·21±0·32
7	9	8·55±0·36	5·81±0·99	14·36±0·95	40·5±4·0
14	5	11·79±0·67	3·03±0·26	14·82±0·90	20·4±0·75
21	5	14·76±0·79	1·43±0·25	16·19±0·69	8·83±1·68
28	2	17·89	1·55	19·44	7·97
B. *Solvent-treated controls*					
3	14	12·12±0·55	1·39±0·11	13·51±0·63	10·29±0·33
7	9	12·87±0·52	1·63±0·18	14·50±0·66	11·24±0·72
14	5	13·33±0·88	1·55±0·17	14·88±0·96	10·42±0·85
21	5	14·48±0·91	1·24±0·22	15·72±0·79	7·89±1·33
28	2	17·69	1·59	19·28	8·25

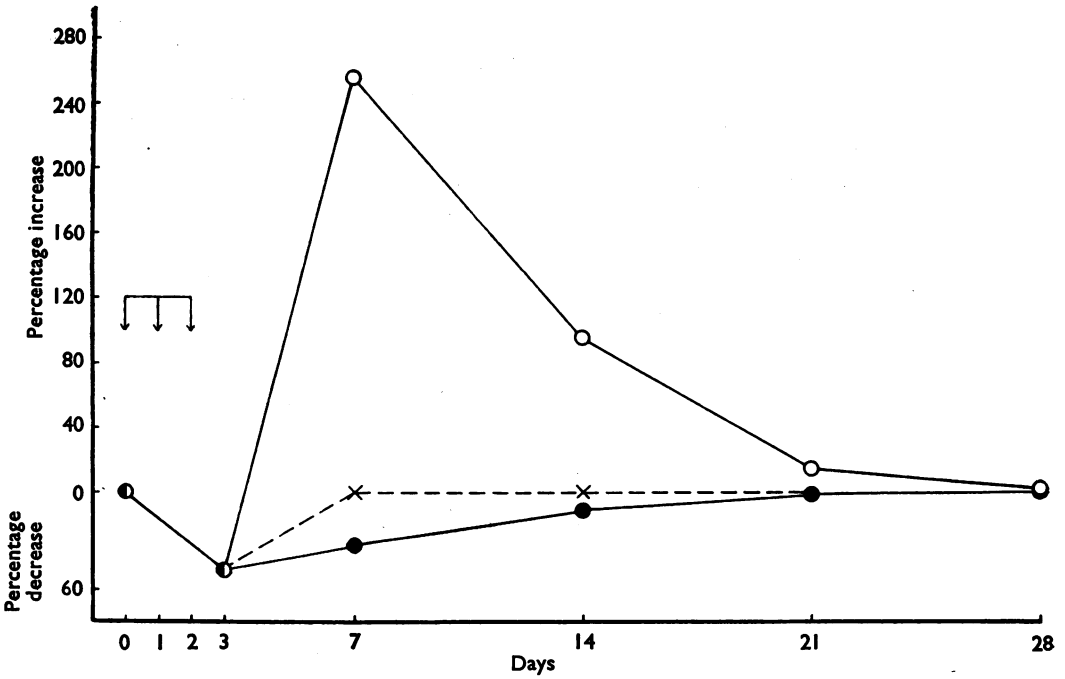

Fig. 2. Mean amine contents (adrenaline ●——●, noradrenaline ○——○ and total amine X- - -X) of rat adrenal glands at intervals up to 28 days following depletion with 3 doses of 1 mg/kg reserpine (at arrows) injected subcutaneously, expressed as percentage differences relative to their control values.

At 3 days the adrenaline content of the test glands was depleted by 47.8%. This difference from the control glands was significant ($P=0.001$). At 7 days the depletion was 33.6%, and this also was significant ($P=0.001$). At 14 days the depletion was 11.6%. This was not significantly different ($P=0.05$) from the control value. Thus the adrenaline content of the reserpine-treated glands had returned to normal by 14 days. At 21 days there was no significant difference ($P=0.05$) between test and control glands. At 28 days the adrenaline content of the reserpine-treated glands was increased by 1.1%.

At 3 days the noradrenaline content of the test glands was depleted by 48.2%, which was significantly different ($P=0.001$) from the control glands. At 7 days the glands of the reserpine-treated rats contained considerably more noradrenaline than their controls, the increase being 256% (significant at $P=0.001$). At 14 days the increase had fallen to 95.1%, but this was still significantly greater ($P=0.01$) than the controls. At 21 days the difference was only 15.3% greater than the controls, which was not significant ($P=0.05$). Thus the noradrenaline content of the test glands had returned to the control level by 21 days. The results of the 2 experiments performed at 28 days showed a negligible loss of 2.5%.

Since the losses of adrenaline and noradrenaline at 3 days were so similar, the percentage of noradrenaline present in the test glands (10.21) was closely similar to that in the control glands (10.29). At 7 days the total amine in the reserpine-

treated glands (14.36 μg/gland) was not significantly different ($P=0.05$) from the control glands (14.50 μg/gland). Thus the total amine content had returned to normal by this time. However this was normal in quantity only, since the percentage of noradrenaline was 40.5, as compared with a control value of 11.24. At 14 days, although the total amine content did not differ significantly ($P=0.05$) from the control value, there was 20.4% of noradrenaline present, which was significantly different ($P=0.001$) from the control value. At 21 days neither the total amine content nor the percentage of noradrenaline in the reserpine-treated glands differed significantly ($P=0.05$) from the control values. Similarly at 28 days there was very little difference between the test and control glands.

Re-depletion experiments

In the previous section it has been shown that, following depletion, there occurs a peak of noradrenaline at 7 days, followed by a return of adrenaline and noradrenaline to normal levels by about 21 days. It was therefore decided to re-deplete the glands at either 7 or 21 days. Re-depletion at 7 days would detect any difference in the sensitivity to reserpine of the replaced noradrenaline, and re-depletion at 21 days would reveal if the glands had, at that time, returned to normal with respect to further reserpine treatment.

Re-depletion at 7 days. Four depletion and recovery experiments up to 21 days were performed, but sufficient animals were used so that, at 7 days after the beginning of each experiment, some of the groups were given a further 3 doses of 1 mg/kg of reserpine at intervals of 24 hr. They were killed, together with their control groups, at 10, 14, 21 and 28 days following re-depletion at 7 days; that is, up to 21 days after commencing re-depletion.

In both the depletion and replacement experiments, the control glands were obtained from rats given the control solvent only. However, in these re-depletion experiments the 10-day control gland was one that contained the amine content

TABLE 3

MEAN AMINE CONTENTS OF RAT ADRENAL GLANDS FOLLOWING RE-DEPLETION BY RESERPINE

Rats were treated with reserpine 1 mg/kg on days 0, 1 and 2 and again either on days 7, 8 and 9 or on days 21, 22 and 23. Estimates of adrenal amine content were made on days 10, 14, 21 and 28 and days 24, 28, 35 and 42 respectively

		Reserpine-treated			Controls		
		Amine contents (μg/gland)			Amine contents (μg/gland)		
Days	No. of observations	Adrenaline	Noradrenaline	% noradrenaline	Adrenaline	Noradrenaline	% noradrenaline
A. *Re-depletion at 7 days*							
10	4	8·30	0·86	9·40	10·59	3·01	22·13
14	4	8·35	5·90	41·4	12·44	1·42	10·25
21	4	13·43	3·03	18·4	14·50	1·45	9·09
28	4	15·19	2·30	13·2	15·60	1·75	10·09
B. *Re-depletion at 21 days*							
24	2	9·87	1·06	9·70	14·71	1·70	10·36
28	2	9·99	5·91	37·2	12·27	1·30	9·58
35	2	16·71	3·80	18·5	15·75	1·85	10·51
42	2	15·49	1·66	9·68	15·66	1·78	10·21

that the depleted gland would have contained if it had been allowed to recover normally from the initial reserpine treatment. Thus the most suitable control for the 10-day point was obtained from the glands of rats treated with reserpine, killed at 10 days. This represented the amine content that the re-depleted gland would have attained if it had not been further treated with reserpine. This 10-day control group (Table 3) therefore had a raised noradrenaline content of 22.13%. Subsequent to the 10-day point, the amine contents of the re-depleted glands were compared with the solvent injected controls, because it was found that the relative amounts of adrenaline and noradrenaline following re-depletion were similar to the amounts found following depletion. Thus at 10 days the control was a "depletion control" and subsequently the controls were "solvent controls."

The results of re-depletion at 7 days are shown in Table 3 and Fig. 3. At 10 days the loss of adrenaline was 21.6% and of noradrenaline 71.4%. At 14 days there was a further slight loss of adrenaline to 32.9%, but there was now an increase

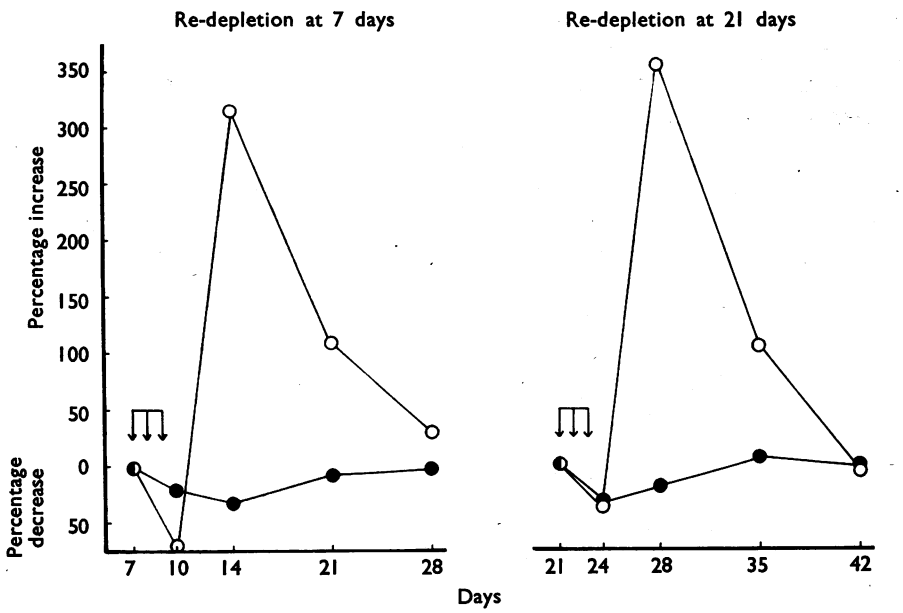

Fig. 3. Mean adrenaline (●—●) and noradrenaline (○—○) contents of rat adrenal glands following re-depletion with 3 doses of 1 mg/kg reserpine (at arrows) subcutaneously, expressed as percentage differences relative to their control values.

of noradrenaline to 315% above the control value. At 21 days there was a decrease (7.4%) in the adrenaline content and an increase (109%) in that of the noradrenaline, relative to the control values. At 28 days the adrenaline content had returned to normal but there was some indication that the noradrenaline level was slightly raised. The preferential loss of noradrenaline at 10 days was such that the percentage present in the glands at this time (9.40%) was similar to that in normal control glands. This value then rose to 41.4% at 14 days and fell to 18.4% at 21 days and 13.2% at 28 days.

Re-depletion at 21 days. Two depletion and recovery experiments up to 21 days were performed, but sufficient rats were used so that at 21 days some groups were given a further 3 doses of reserpine. They were killed, together with their solvent control groups, at 24, 28, 35 and 42 days after the beginning of the experiment, that is, 3, 7, 14 and 21 days after the beginning of re-depletion. The results are shown in Table 3 and Fig. 3.

At 24 days there was a similar percentage loss of adrenaline (32.9) and of noradrenaline (37.6). At 28 days there was some recovery of adrenaline, the depletion now being 18.6%, and an increase in the noradrenaline content to 355% above the control value. The adrenaline content had returned to normal by 35 days and the noradrenaline content by 42 days. Thus the noradrenaline content at 24 days was 9.70%, rising to 37.2% at 28 days, falling to 18.5% at 35 days and returning to normal (9.68%) at 42 days.

Denervation experiments

Depletion and replacement experiments similar to those in the depletion section were performed, using rats whose left adrenal glands had been denervated 3 weeks previously.

TABLE 4

MEAN AMINE CONTENT OF INNERVATED AND DENERVATED RAT ADRENAL GLANDS AT INTERVALS UP TO 21 DAYS FOLLOWING DEPLETION WITH RESERPINE

Rats were injected subcutaneously with 1 mg/kg of reserpine on days 0, 1 and 2. Estimations of adrenal amine content were made on days 0·5, 1, 2, 3, 7, 14 and 21. Control estimates on animals treated with solvent only

		Amine contents (μg/gland)					
		Innervated			Denervated		
Days	No. of observations	Adrenaline	Noradrenaline	Total amine	Adrenaline	Noradrenaline	Total amine
A. *Reserpine-treated*							
0·5	1	9·49	0·99	10·48	9·50	1·05	10·55
1	1	10·13	1·06	11·19	9·89	1·05	10·94
2	1	7·31	0·79	8·10	7·16	0·81	7·97
3	4	6·90	0·78	7·68	6·58	0·74	7·32
7	2	10·03	4·55	14·58	8·97	3·79	12·76
14	2	14·20	2·95	17·15	13·51	2·60	16·11
21	2	15·04	1·89	16·93	15·00	1·67	16·67
B. *Solvent-treated controls*							
0·5	1	12·23	1·32	13·55	12·23	1·34	13·57
1	1	12·81	1·41	14·22	12·49	1·40	13·89
2	1	11·20	1·23	12·43	11·03	1·21	12·24
3	4	13·48	1·49	14·97	12·83	1·42	14·25
7	2	13·94	1·55	15·49	12·40	1·35	13·75
14	2	14·93	1·61	16·54	13·57	1·45	15·02
21	2	14·82	1·68	16·50	15·47	1·76	17·23

As can be seen from Table 4 and Figs. 4 and 5, the adrenaline and noradrenaline contents of both the innervated and denervated glands fell similarly, until at 3 days the adrenaline contents were depleted by 48.8% and 48.7% and the noradrenaline contents were depleted by 47.7% and 47.9% respectively. The course of recovery of innervated and denervated glands was also similar. In the innervated glands

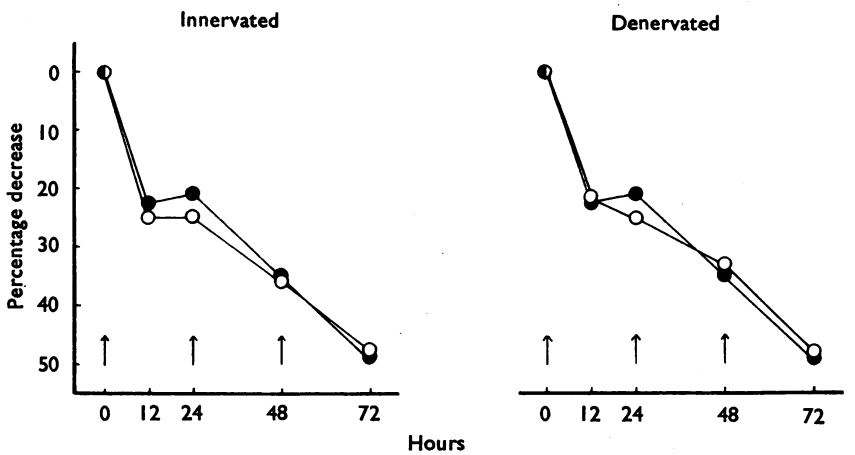

Fig. 4. Mean adrenaline (●——●) and noradrenaline (○——○) contents of innervated and denervated rat adrenal glands at intervals up to 72 hr during depletion with 1 mg/kg reserpine (at arrows) subcutaneously, expressed as percentage differences relative to their control values.

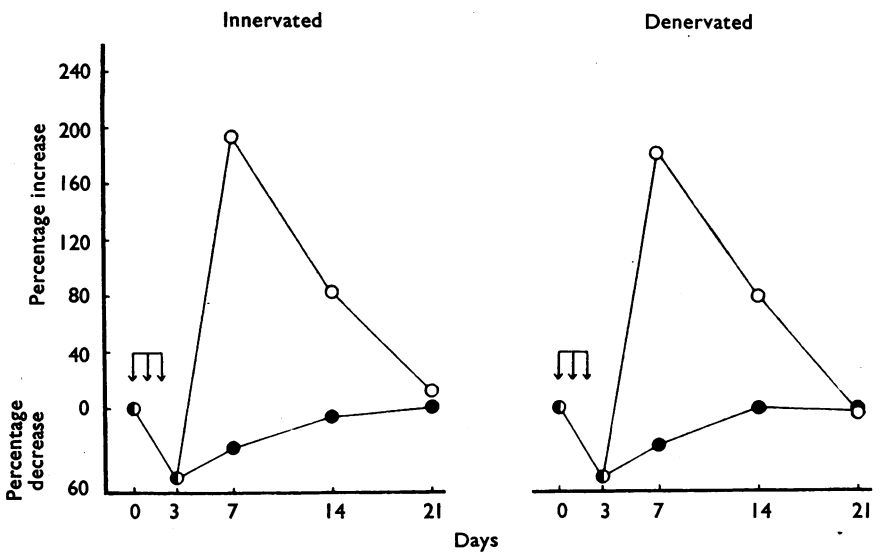

Fig. 5. Mean adrenaline (●——●) and noradrenaline (○——○) contents of innervated and denervated rat adrenal glands at intervals up to 21 days following depletion with 3 doses of 1 mg/kg reserpine (at arrows) injected subcutaneously, expressed as percentage differences relative to their control values.

the noradrenaline increase was 194% and in the denervated glands it was 181% at 7 days. At 14 days, again in both innervated and denervated glands, the adrenaline had almost returned to normal and there was a fall in the noradrenaline content. Both amine levels had returned to normal at 21 days. Insufficient results were obtained to enable a statistical assessment of the results to be made.

DISCUSSION

Three doses of 1 mg/kg of reserpine given subcutaneously at intervals of 24 hr caused a depletion of approximately 50% of both the adrenaline and noradrenaline contents of the rat adrenal glands at 3 days. The maximum depletion following a single dose occurred at 16 hr and two further doses were needed to increase the depletion to 50% at 3 days. This time course is much slower than that found for the depletion of noradrenaline in either the central or peripheral nervous system. In the brain of rabbits (Brodie, Olin, Kuntzman & Shore, 1957) a maximum depletion of 90% occurred 4 hr after 5 mg/kg intravenously of reserpine. This was also the time at which maximum depletion occurred in the cervical and solar ganglia of the rabbit (Muscholl & Vogt, 1958).

No preferential release of either amine could be shown during these experiments. The assay method employed was insufficiently sensitive to detect changes in amine content before 4 hr, when it is possible that selective liberation of either amine could have occurred. Stjärne & Schapiro (1958) showed a preferential rise in the noradrenaline content of the adrenal venous effluent in anaesthetized cats, which rose to a maximum 1.5 to 2 hr after a single intravenous injection of 3 mg/kg of reserpine. However, Coupland (1958a) found no evidence of a preferential release of either amine 24 hr after a dose of 15 mg/kg intravenously of reserpine in rats. Cammanni, Losana & Molinatti (1958) have obtained histological evidence of a preferential release of noradrenaline. Eränkö & Hopsu (1958), whilst showing a preferential loss of noradrenaline 24 hr after single doses of 0.25 or 0.5 mg of reserpine, found a loss of both amines after 9 daily doses of 0.1 mg. It would seem therefore that the amines may possess different sensitivities to reserpine in the adrenal gland, but when the dose is large or the treatment prolonged this difference is not apparent.

During the study of the replacement of the amines the adrenaline content recovered slowly, returning to the normal level by 14 days. This agrees with the observations of Coupland (1959a). However, the recovery of the noradrenaline content differed from that of adrenaline. At 7 days the noradrenaline content exceeded the control value by 250% and then it fell gradually, returning to the normal level by 21 days. It would appear that the noradrenaline is formed fairly rapidly but that methylation to adrenaline is slow. The total amine content had returned to normal by 7 days. This increase in the noradrenaline content has been shown to occur in the cat adrenal gland following depletion with acetylcholine (Butterworth & Mann, 1957). However, Muscholl & Vogt (1958) showed that in the rabbit, following depletion by 8 to 18 daily doses of 0.1 to 0.2 mg/kg intravenously of reserpine, the ratio of adrenaline to noradrenaline was normal at 8 to 9 days although recovery of the total amine content was not complete until 14 days. They also showed an increase in the percentage of noradrenaline in the glands during depletion. Thus they suggested that replacement was occurring during the time of depletion. No evidence of a similar effect in rats was obtained in the work reported here.

Re-depletion of the rat adrenal glands at 21 days indicated that the glands had returned to normal with respect to their response to reserpine, except that the degree of depletion was slightly reduced. It was also noticed that the time lapse between injection and the appearance of signs of reserpine treatment was reduced. Assays

should have been undertaken at this time to determine whether the depletion of the adrenal glands occurred after a shorter time interval or not. Re-depletion at 7 days, during the peak in the noradrenaline content, caused a preferential release of noradrenaline so that the absolute content of the re-depleted gland was similar to that of the depleted gland. Thus it is possible that the reserpine affects the same binding sites whether they contain adrenaline or noradrenaline.

No difference between innervated and denervated adrenal glands could be shown in either their response to depletion of their amine content or in their subsequent recovery. It would seem that the effect of denervation varies with the species, the dose of reserpine and the duration of treatment. In the cat Muscholl & Vogt (1958) confirmed earlier work (Holzbauer & Vogt, 1956) by showing that denervation protected the adrenal glands of cats from depletion by doses of reserpine as high as 2.5 mg/kg. In the rabbit the reports of the effects of denervation are conflicting. Section of the spinal cord at the level of the first thoracic segment (Taketomo *et al.*, 1957) prevented the release, but section of the splanchnic nerves caused either no protection (Carlsson & Hillarp, 1956) or partial protection (Kroneberg & Schümann, 1957a). In the rat the results reported are even more conflicting. Eränkö & Hopsu (1958) reported that denervation of the adrenal gland protected it from the preferential loss of noradrenaline. However, Coupland (1958b), studying the depletion of the amines in transplants of adrenal medullary tissue in the eye of the rat, found that 48 hr instead of 24 hr were needed to reach maximal depletion. Kroneberg & Schümann (1957b) could not demonstrate any protection against 10 mg/kg of reserpine even after section of the spinal cord at the level of the sixth cervical segment. Mirkin (1958) also did not find any difference between innervated and denervated glands following treatment with 5 daily doses of 1 mg/kg of reserpine injected intraperitoneally. Thus reserpine may act centrally in small doses to cause depletion of the adrenal gland, but if the dose is large a powerful peripheral effect is superimposed to cause depletion by a direct action on the storage sites.

The times needed for recovery of the amine contents of both innervated and denervated adrenal glands were similar. This has been shown to be so for rat adrenal glands depleted by deserpidine (Greenberg, Jeffay & Toman, 1960). Coupland (1959b) found that the time of recovery of the amine content of an adrenal transplant in the rat eye was also similar to that of the innervated adrenal gland.

During this study the adrenaline and noradrenaline contents of the glands have been determined solely by biological assay. The results were not confirmed fluorimetrically since a fluorimeter was not available. However, no other substance was detected chromatographically during the recovery phase. The results obtained agree with the suggestion that reserpine in large doses exerts an effect on the adrenal medulla which is not mediated through nervous pathways.

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