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THE ADRENALINE AND NORADRENALINE CONTENT OF THE ADRENAL GLAND OF THE CAT FOLLOWING DEPLETION BY ACETYLCHOLINE

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This paper describes the attempts that were made to obtain a replacement of adrenaline and noradrenaline in the adrenal gland of the atropinized cat, subsequent to the depletion of the gland by repeated intravenous doses of acetylcholine. In the anaesthetized animal, there was no replacement of adrenal amines within 15 hours of the depletion. The further loss of amine that occurred during this time was prevented by the denervation of the gland. If the animal was allowed to recover from the anaesthetic there was some replacement of amines within 2 to 3 days, provided that the condition of the animal was satisfactory. By 6 to 7 days the total amine content had returned to its initial level, but there was now an alteration in the relative proportions of the two amines. Although the adrenaline was still well below the resting level, the noradrenaline was several times its initial value. By one month, the noradrenaline had decreased and the adrenaline had increased to their initial amounts and proportions. Thus this work gives evidence for the formation of adrenaline from noradrenaline.

Many workers have shown that various substances cause the release of catechol amines from the adrenal gland, but few have studied the subsequent replacement of these amines. One of the earliest papers in this field was by Crowden (1929), who depleted the adrenal glands of cats by exposing the animals to a low environmental temperature. He found that there was complete recovery of the adrenaline content in three days and that this occurred in the denervated gland to as great an extent as in the innervated gland. Van Arman (1950) showed that, three days after depletion of rat adrenal glands with physostigmine, the denervated gland contained as much amine as the innervated gland.

These workers only assayed the adrenal glands for total amine, calculated as adrenaline. The first mention of noradrenaline in this type of work was by Burn, Hutcheon, and Parker (1950), who studied insulin depletion in the adrenal glands of rats. Their evidence indicated that noradrenaline is a precursor of adrenaline and that, after depletion of the gland for several hours, the supply of noradrenaline is restored more rapidly than it can be methylated to form adrenaline. Hökfelt (1951) found that it took about six days for the catechol amine stores in the rat gland to be restored, following insulin depletion. More recently, Holland and Schümann (1956), studying the effect of excitation of the splanchnic nerve on the adrenal amines of the cat, suggested that splanchnic stimulation increases the rate of synthesis and methylation of noradrenaline.

Thus it may be seen that in these few papers the variety of species, of experimental conditions, and of depleting agents makes it difficult to reach any definite conclusions as to the rate and nature of the synthesis of the catechol amines in the depleted adrenal gland. Butterworth and Mann (1957b) have shown that acetylcholine releases similar percentages of adrenaline and noradrenaline from the adrenal gland of the cat, and they observed no replacement during the period of liberation of the amines. This paper reports the course of replacement of the adrenal amines following depletion of the adrenal glands by acetylcholine.

METHOD

Healthy, adult cats of both sexes were used.

Short-term Experiments

The animal was given 6 mg./kg. of atropine sulphate intraperitoneally and anaesthetized with ether and chloralose as described in the previous paper (Butterworth and Mann, 1957b). Both adrenal glands

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were depleted of their adrenaline and noradrenaline by repeated intravenous doses of acetylcholine chloride. The dose (0.2 to 3.0 mg./kg.) and the number of doses were such as to cause an approximately 50% depletion. This was estimated by a comparison of the acetylcholine-induced rises in blood pressure with pressor responses to doses of adrenaline and noradrenaline. Following the depletion, one gland was removed as a control depleted gland and the animal was left on a warm operating table for a number of hours. An attempt was made to remove the second gland, at varying time intervals, while the animal was in good condition (as judged by its blood pressure and respiration). In the latter experiments the splanchnic nerve fibres to the second adrenal gland were cut when the first gland was removed. Control experiments were performed in which no acetylcholine was given but one gland was denervated and the animal allowed to survive for a number of hours.

Long-term Experiments

The cat was given 6 mg./kg. of pentobarbitone sodium subcutaneously and 6 mg./kg. of atropine sulphate intraperitoneally. Ten minutes later ether was administered in an anaesthetic chamber, and open ether anaesthesia was maintained throughout. Injections were made into the femoral vein and the blood pressure was recorded from the femoral artery of the same limb. Two series of experiments were performed. In Series 1 both glands were depleted with acetylcholine, as in the short-term experiments, and then one gland was removed as a depleted control. In Series 2 one gland was removed as a non-depleted control and then the other depleted in the usual way. After depletion and unilateral adrenalectomy a solution of penicillin sodium was placed in the abdominal cavity, the abdominal walls were sutured together, the femoral artery and vein ligatured and the cut edges of the skin held together with Michel's clips. Aseptic precautions were taken throughout and penicillin was given subcutaneously at the end of the operation. The animal was carefully looked after during the post-operative recovery period. When the second gland was due to be removed (see later) the animal was re-anaesthetized and the adrenal gland removed as in the first part of the experiment.

The results of Series 1 were intended to show the extent to which the amines had been replaced in comparison with the depleted gland, and those of Series 2 whether the amine content had returned to its initial, non-depleted level. In both series the second gland was removed at 2 to 3 days, 6 to 7 days or one month after the first adrenalectomy. Separate control experiments were performed at these various time intervals.

In all experiments, the removal and extraction of the adrenal glands and the subsequent biological and chromatographic analysis of the extracts were made using the methods described by Butterworth and Mann (1957b). The adrenaline and noradrenaline contents of the adrenal glands are expressed as μg./gland (Butterworth and Mann, 1957a) in terms of the laevo isomers of the base.

Results

It has been shown in a previous paper (Butterworth and Mann, 1957b) that acetylcholine causes a similar % depletion of adrenaline and of noradrenaline. Hence in the following experiments any difference in the degree of depletion of the amines must be due to some other factor.

Short-term Experiments

In the experiments in which the innervation of the adrenal gland was intact (Table I) there was, in all but two animals, a further loss of both amines. The longer the time interval between the removal of the two glands, the greater was the loss. If the animal died suddenly after maintaining its initial blood pressure for a number of

TABLE I

SHORT-TERM EXPERIMENTS

Both adrenal glands were depleted of their amines by acetylcholine. One gland was then removed and the other remained in the cat for the number of hours given in the column headed "Interval." The amine content of the second adrenal gland is compared with the first.

Cat No.	Interval (hr.)	Condition of Animal	Total Amine Difference in μg.	% Adrenaline Difference	% Noradrenaline Difference
Group A—Similar % loss of adrenaline and noradrenaline.					
1	0·5	Died suddenly	−11·8	−11·1	−1·6
2	3·5		−40·9	−12·1	−11·4
3	3·5		−138·1	−38·1	−28·2
4	7·0		−179·9	−66·5	−65·5
5	7·0		−120·0	−66·8	−77·2
6	17·0		−265·2	−74·7	−88·3
		Mean		−44·9	−45·4
Group B—Greater % loss of noradrenaline.					
7	3·0	Gradual fall in blood pressure	−53·4	−9·5	−53·6
8	4·0		−102·5	−15·4	−50·3
9	4·0		−190·9	−18·4	−75·2
10	4·0		−59·6	−7·3	−29·8
11	4·5		−30·7	−5·1	−16·3
12	5·0		−72·5	−1·5	−48·9
13	5·0		−146·5	−20·1	−77·2
14	5·25		−198·9	−32·7	−90·3
15	6·0		−175·1	−32·2	−58·6
16	9·0		−104·3	0	−70·4
		Mean		−14·2	−57·1
Group C—Greater % loss of adrenaline.					
17	3·0	Good	+3·6	−30·7	+19·6
18	6·5		−32·1	−37·2	+57·2
19	15·25		−48·1	−47·0	−21·3
		Mean		−38·3	+18·5
In the following groups the gland which remained was denervated immediately after both glands had been depleted.					
Group D—Little alteration in adrenaline or noradrenaline.					
20	1·0	Poor	+3·6	−2·9	+7·6
21	4·75		−6·5	+10·8	−10·4
22	6·5		−28·1	−11·0	−10·7
23	7·75		+3·7	+2·1	+1·8
24	9·0		+3·3	−12·5	+21·1
25	13·0		−15·7	−19·2	+7·3
		Mean		−5·5	+2·8
Group E—Some replacement of amines.					
26	5·0	Good	+19·3	+25·9	+1·4
27	7·75		+30·7	+28·5	+10·9

hours (Table I, Group A), there was a similar % loss of adrenaline and of noradrenaline. Probably this was due to the basal liberation from the gland since, when this loss was calculated in μg./kg./min., it was comparable to the value obtained in the control series, where the amine contents of the innervated and denervated glands were compared. If the blood pressure fell gradually and the second gland was not removed until the pressure was about 40 to 50 mm. Hg (Group B) there was a greater % loss of noradrenaline than of adrenaline. This may have been caused by the gradual fall in the blood pressure of these animals. Under such circumstances it might be expected that there would be a preferential liberation of the more potent vasopressor amine, namely noradrenaline. If the condition of the animal was good (Group C), although there was a loss of adrenaline, there was some evidence of a little replacement of noradrenaline. This is similar to some of the results in the long-term experiments.

To prevent this reflex liberation of amines during the recovery period, the splanchnic nerve fibres to the second gland were cut when the first gland was removed. (Control experiments showed that while the innervated gland lost amine at about 0.07 μg./kg./min., the liberation from the denervated gland was prevented.) In those animals which were in poor condition (Table I, Group D), there was little change in either the adrenaline or the noradrenaline content of the gland for a period of up to 13 hr. The glands of the two cats that were in good condition (Group E) showed a small increase in amine content. Thus it was obvious that, although denervation prevented the loss of amine from the gland, the period of time for which the animal could survive in good condition under anaesthesia was insufficiently long to allow any appreciable increase in amine content to occur. Therefore it was necessary to perform long-term experiments.

Long-term Experiments

Series 1.—In this series the control glands were depleted of their amine content. Thus in these results a negative % difference of amine in the second gland indicated that the gland had lost amine during the recovery period, while a positive sign indicated a replacement of amine. Cat Nos. 28, 29, and 30 (Table II) were in poor condition when the second gland was removed two days after depletion, and there was a further loss of both adrenaline and noradrenaline from these glands. Cat Nos. 31 to 35 were in fair or very good condition. Of these, the glands of cat Nos. 31 and 33 showed a further overall loss of amine,

TABLE II

LONG-TERM EXPERIMENTS

In Series 1 both adrenal glands were depleted of their amines by acetylcholine before removal of the first gland. In Series 2 one adrenal gland was removed before depletion of the second gland. In each series the number of days between the removal of the glands is given in the column headed "Interval." The amine content of the second adrenal gland is compared with the first.

Cat No.	Interval (Days)	Post-operative Condition of Animal	Total Amine Difference in μg.	% Adrenaline Difference	% Noradrenaline Difference
Series 1					
28	2	Poor	−262·5	−95·2	−73·8
29	2		−115·3	−44·0	−57·9
30	2		−98·3	−45·0	−51·3
31	2	Fair	−44·2	−97·3	+35·5
32	2		−0·1	−66·4	+34·2
33	2	Very good	−52·5	−33·4	+18·6
34	3		+41·1	+4·5	+58·2
35	3		+14·2	+9·9	+162·8
36	7	Poor	−147·4	−77·8	−44·4
37	7		−31·6	−46·4	−42·5
38	7	Fair	+13·3	−39·0	+34·8
39	7	Very good	+79·0	+62·9	+102·9
40	6		+104·5	+20·5	+295·3
41	6		+150·3	+21·6	+319·6
42	7		+99·7	+11·7	+481·8
43	27	Poor	+7·2	+10·3	0
44	28	Good	+106·9	+60·0	+47·1
45	27		+162·6	+89·5	+84·5
46	28		+187·5	+101·7	+92·9
47	28	Very good	+254·1	+122·6	+104·9
48	29		+165·1	+199·9	+192·6
Series 2					
49	2	Poor	−113·8	−76·6	−52·9
50	2	Good	−171·6	−46·5	−33·8
51	7	Fair	−91·4	−56·4	−11·1
52	7	Good	−116·9	−49·0	−30·3
53	7		+41·2	−38·9	+48·6
54	6		−18·1	−56·3	+378·8
55	30	Good	+18·9	+8·3	−0·6
56	29		+13·0	+3·8	+9·2
57	30	Very good	+50·4	+18·3	+16·7
58	29		+80·3	+26·8	+23·7

those of cat Nos. 32 and 35 just maintained their total amine content, and that of cat No. 34 showed an increase in total amine. However, in all five there was an increase in the noradrenaline content.

Of the animals in which the second gland was removed after six to seven days (Table II), cat Nos. 36 and 37 were in poor condition and their glands lost further amine. Cat No. 38 was in fair condition and its adrenal gland just maintained its total amine content. Cat Nos. 39 to 42 were in very good condition and their glands showed a marked increase in total amine content. In all four there was a considerably greater % increase in noradrenaline than in adrenaline. For example, in cat No. 42 there was a 481.8% increase in the noradrenaline content of the gland but only an 11.7% increase in the adrenaline content.

Considering those animals in which the second gland was removed one month after depletion (Table II), only cat No. 43 was in a poor post-

operative condition and it just maintained its adrenal amine content. All the others (cat Nos. 44 to 48) were in good or very good condition, and the glands showed a considerable increase in total amine. However, in contrast to the results of the six to seven day experiments, the % increases of adrenaline and noradrenaline were similar.

Series 2.—Here the control gland was not depleted. In these experiments the exact degree of depletion of the experimental gland was not known, but a 50% loss may be presumed. Thus it was inferred that a negative % difference of less than 50 indicated a replacement of amine, the smaller this negative % the greater the increase in amine content. A positive % difference showed an increase in amine such that the second gland contained more than it did originally. It may be seen (Table II) that in the glands of cat Nos. 49 and 50 two days after depletion there was a slightly smaller % depletion of noradrenaline than of adrenaline. The glands of cat Nos. 51 and 52 examined after seven days showed some increase in noradrenaline but not in adrenaline, while those of cat Nos. 53 and 54 showed a marked increase in noradrenaline but again little change in adrenaline. By one month, the adrenaline and noradrenaline contents of the adrenal glands of cat Nos. 55 to 58 had returned to the original non-depleted values. Actually with cat Nos. 57 and 58 the amine content of the gland that was originally depleted was a little higher than that of the control gland. This may be ascribed to the fact that these two animals were in a much better condition at the end of the experiment than they were at the beginning.

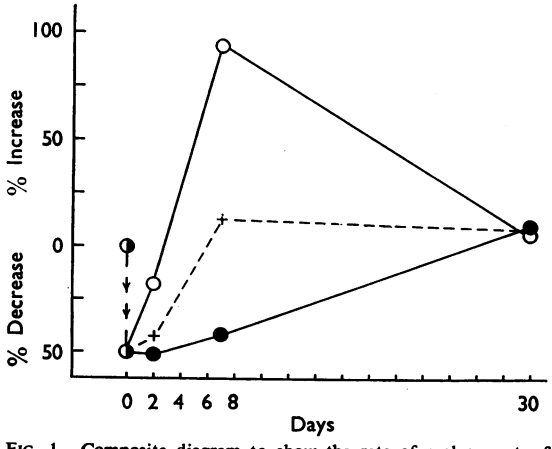


FIG. 1.—Composite diagram to show the rate of replacement of adrenaline (●), of noradrenaline (○) and of total amines (+) in the adrenal gland of the cat at the various time intervals following depletion. ◑ denotes adrenaline and noradrenaline. All results are expressed as % of the initial, non-depleted level.

The mean of the results from animals in good post-operative condition in Series 1 and 2 has been plotted in Fig. 1. It may be seen that, whereas the adrenaline content slowly increased to its non-depleted level by one month, the noradrenaline content exceeded its original value in seven days and then decreased to its normal amount by one month. Although it took one month for the normal proportions of adrenaline and noradrenaline to be regained, the total amine content had returned to normal by seven days and subsequently was maintained at this level.

Control Experiments

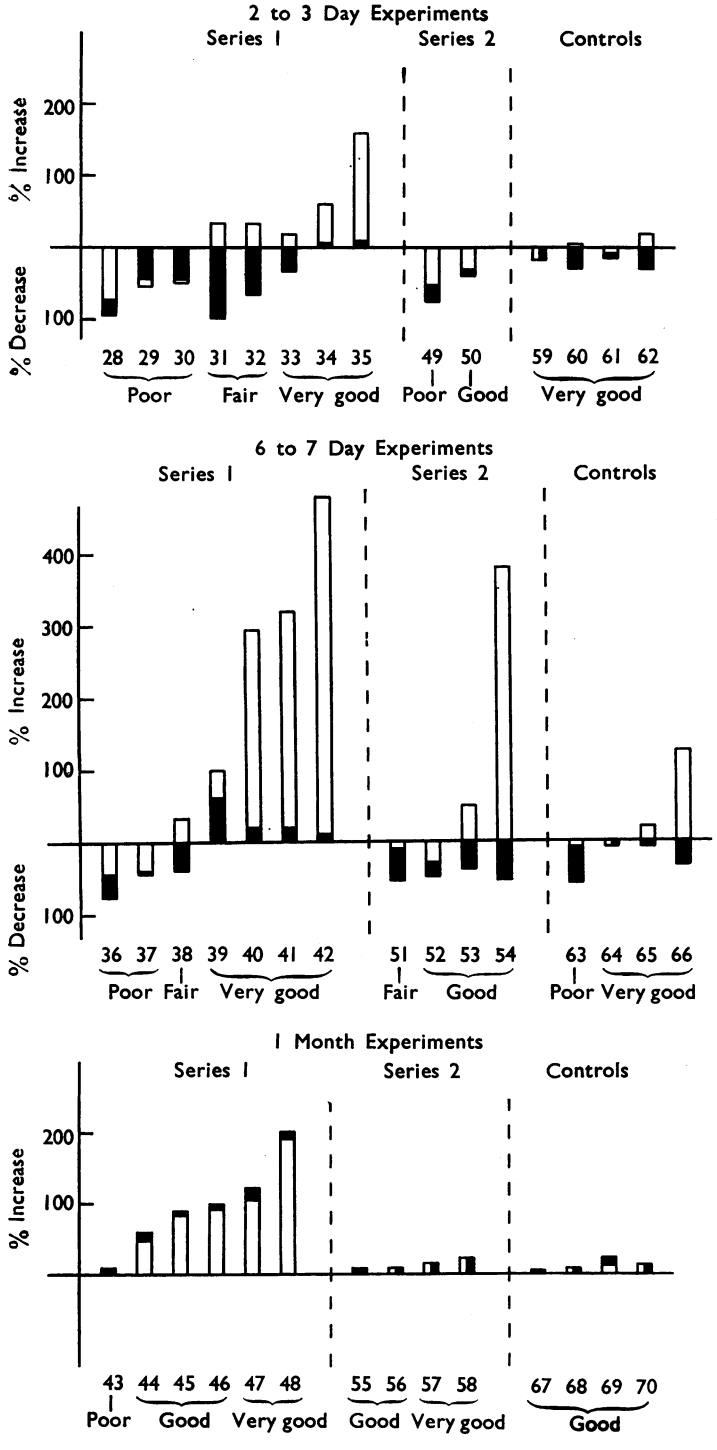
In addition to using one gland of each cat as a control, further control experiments were performed. In each of these experiments the adrenal gland of one side was removed and its amine content measured. Then after an interval of two to three days, six to seven days, or one month, the remaining gland was removed for assay. In all but one experiment out of 12 there was little difference between the total amine contents of the two glands (Table III). Nevertheless the small changes which occurred followed a similar trend to those in Table II. With the two to three day and the six to seven day experiments, the % noradrenaline difference between the two glands was either similar to or a smaller negative or a larger positive quantity than the % adrenaline difference. In the experiments lasting one month the % noradrenaline difference was very similar to that of the adrenaline.

TABLE III

CONTROL LONG-TERM EXPERIMENTS

In these experiments no acetylcholine was given. The number of days between the removal of the two glands is given in the column headed "Interval."

Cat No.	Interval (Days)	Post-operative Condition of Animal	Total Amine Difference in μg.	% Adrenaline Difference	% Noradrenaline Difference
59	2	Very good	−34·0	−17·5	−17·4
60	2		−17·1	−29·6	+2.6
61	3		−18·6	−12·7	−6·9
62	2		−37·2	−31·8	+21·2
63	7	Poor	−94·6	−61·8	−8·0
64	7	Very good	−17·1	−5·1	−9·0
65	6		+0·5	−9·8	+21·7
66	7		−11·4	−36·7	+124·9
67	28	Good	+3·3	+4·5	−1·0
68	28		+16·4	+7·4	+6·9
69	31		+41·8	+26·3	+12·5
70	34		+36·8	+16·7	+13·3



A comparison of the results of Series 1 and Series 2 with those of the control experiments at two to three days, six to seven days, and one month is shown in Fig. 2.

By means of the chromatographic analysis the presence of adrenaline and noradrenaline in all the glands was confirmed. No other catechol amines were detected.

Discussion

The results of the short-term experiments showed the necessity for experiments of longer duration. In the latter experiments a comparison was made between the amine content of the control gland and that of the second gland, removed some days after the first. An increase in the amine content of the second gland, expressed as a % of that of the first, was termed the degree of replacement. This replacement of amines must be somewhat greater than the amount measured because, at the same time as the gland was re-forming amine, it was also liberating a small basal quantity. The assays measured only the store of amines in the gland at a given time. In order to minimize this basal liberation of amines, denervation of the gland would be necessary, but this, in its turn, might affect the process of synthesis.

The results of Series 1 recorded such replacement of adrenaline and noradrenaline as had taken place. The results of Series 2 enabled an estimate to be made of the time required for complete replacement of the amines. Thus the results of both series, taken together, give

FIG. 2.—The % difference of adrenaline (■) and noradrenaline (□) of the second adrenal gland of the cat, as compared with the first (control), at various time intervals after removal of the first gland. In Series 1 the control gland was depleted, and hence any replacement of amines is shown by the columns above the abscissa. In Series 2 the control gland was not depleted, and any value above the 50% depletion level indicates replacement of amine. The numerals refer to the cat Nos. in the tables. The post-operative condition of each cat is recorded below the cat Nos.

some indication as to the rate at which the amines are replaced in the gland. Although the results of each experiment varied, there was a similar trend throughout. Considering only those animals that were in good post-operative condition, there was some replacement of noradrenaline at two to three days, and the quantity of this amine present at six to seven days was considerably more than initially. In contrast to this, there was no increase of adrenaline at two to three days and only a small increase by seven days. By one month noradrenaline had decreased and adrenaline had increased to their initial amounts and proportions. However, the total amine content had returned to normal by seven days, and thereafter it was maintained at this level. This relatively slow rate of formation of the amines is in agreement with the work of Hökfelt (1951) and others, who found a slow increase in the amine content of the gland following insulin depletion, but is in contrast to the work of Holland and Schümann (1956). They obtained some evidence of synthesis while the gland was being depleted by splanchnic nerve stimulation, and thus they suggested that this more rapid rate of synthesis might be due to the electrical stimulation of the nerve. In this connexion, Bülbring (1949) has shown that adrenal glands removed after prolonged splanchnic stimulation possess a higher methylating capacity than normal glands. It is possible for the rates of formation to vary according to the experimental conditions and the type of depleting agent.

Although the change in amine content was very small in the control long-term experiments, the same shift in the balance of amines was observed as in both series of depletion experiments. This indicates that the greater degree of replacement of noradrenaline is not solely a result of the depletion of the gland by the doses of acetylcholine, but may occur physiologically. The loss of a small amount of adrenaline and noradrenaline was probably due partly to the anaesthetization of the animal and partly to the excitement that occurred during the recovery from the anaesthetic.

The most important factor affecting the amine content of the gland, subsequent to the depletion with acetylcholine, appears to be the condition of the animal. When the post-operative recovery of the cat was good the amines were replaced, the degree of replacement depending upon the time interval between the removal of the two glands. When, on the other hand, the animal was in poor post-operative condition, the second gland lost a further quantity of amine.

It has long been established (Elliott, 1912) that general anaesthesia causes a certain release of catechol amines from the adrenal gland. Whatever depletion occurred due to the ether in the first part of the experiment, also occurred when the animal was re-anaesthetized. Thus both glands of the animal were affected. A greater factor in this connexion is the excitement which may occur during the immediate post-operative recovery phase. This probably accounts for the small depletion of the second gland in the control experiments lasting two days.

No study of the adenosine triphosphate (ATP) content of the adrenal glands has been made, but it is of interest that Carlsson and Hillarp (1956) have found that morphine given to cats causes a similar % depletion of catechol amines and of ATP from the glands. It might be useful to investigate whether the increase in ATP content of the glands following depletion is similar to the increase in adrenaline and noradrenaline. Giving ATP during the recovery phase might hasten the formation of the catechol amines. The administration of a methyl donor substance, such as was used by Bülbring and Burn (1949), during the recovery phase might also be of interest.

Hillarp and Hökfelt (1953), Eränkö (1955) and others have studied the histology of the adrenal glands following depletion. Hillarp and Hökfelt have suggested that " All the cells of the adrenal medulla can form noradrenaline while only certain specific cells are able to effect a mechanism for the methylation of noradrenaline to adrenaline and to store this hormone. Those cells which lack this methylation capacity store noradrenaline." It would be of interest to study the histology of these cat adrenal glands at seven days and at one month after depletion. It might be expected that at seven days most of the medullary cells contain noradrenaline, while at one month some of these cells now contain adrenaline. Experiments of the type described above are now in progress.

The aim of the present study was to obtain some evidence of the rate of synthesis of adrenaline and noradrenaline. It has been shown (Butterworth and Mann, 1957b) that acetylcholine causes a similar % loss of both amines. The results of the long-term experiments suggest that there is an initial replacement of noradrenaline and a subsequent conversion of the noradrenaline to adrenaline; the rate of methylation thus being slower than the rate of noradrenaline formation. Other possible explanations of these results are that either there was an

independent synthesis of the two amines, or there was a preferential liberation of one or other amine occurring concurrently with the synthesis, or that more than one mechanism was working at any one time. However, it is improbable that there was an independent synthesis because, if that were so, it is unlikely that noradrenaline would increase to an amount greater than its original value and then subsequently return to its normal value. It is very likely that there is a basal liberation occurring concurrently with the replacement of amines, but it is probable that this would be of similar percentages of the two amines, as was found in some of the experiments. The possibility of more than one mechanism occurring at any one time cannot be ignored, for example the depleted gland could take up adrenaline and noradrenaline from the blood as well as synthesizing these amines. However, van Arman (1951) has shown that the rat adrenal gland does not take up either amine from the blood stream. Thus this work gives strong support for the view that adrenaline is formed from noradrenaline in the adrenal gland of the cat.

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