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NORADRENALINE AND THE SUPRARENAL MEDULLA

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Noradrenaline is widely distributed in the body and has been found in all nerves containing adrenergic fibres. The amount of adrenaline occurring together with noradrenaline in such nerves is usually only a few per cent of the total catechol amines, showing that noradrenaline is the more important adrenergic mediator.

In extracts of suprarenal glands, however, the relative amounts of adrenaline and noradrenaline vary greatly in different animals. In the ox and pig, 30–40 per cent of the total amines is noradrenaline (Holtz and Schümann, 1949). It has been isolated in pure crystalline form from the adrenals of cattle (Bergström, Euler, and Hamberg, 1949), and has been shown (Holtz and Schümann, 1950) to be present in the adrenal medulla of the dog, cat, and man. Very little has been found in rabbit suprarenals (West, 1950b).

Evidence that noradrenaline is a precursor of adrenaline in the suprarenal medulla has been obtained by several workers. Firstly, Bülbring (1949) showed that minced suprarenal tissue from dogs and cats, when incubated with adenosine triphosphate and choline, converts noradrenaline to adrenaline. Secondly, Bülbring and Burn (1949b) observed a disappearance of noradrenaline and a corresponding increase in the amount of adrenaline in the perfused suprarenal gland of the dog. The proportion of noradrenaline and adrenaline which is released from the gland by nerve stimulation in cats (Bülbring and Burn, 1949a ; West, 1950a) probably depends on the relative amounts present, and there is no convincing evidence that one amine is released alone. Lastly, when the suprarenal medulla of the rat is depleted by the injection of insulin (Burn, Hutcheon, and Parker, 1950b ; Outschoorn, 1951), both adrenaline and noradrenaline are lost from the gland, though it is more depleted of the former amine. When insulin is given to rabbits (West, 1951) similar results are observed. These observations suggest that, when the suprarenal glands are exhausted, mostly adrenaline is poured forth into the blood stream and relatively more noradrenaline is found in the gland.

The proportion of noradrenaline in extracts of tumours of the adrenal medulla of man (Holton, 1949) is higher than that found in normal glands (Shepherd and West, 1951). This difference may be related to the fact that the tumour cells are relatively undifferentiated and thus may be more comparable with embryonic tissue than with adult tissue. Hence the present investigation was undertaken to discover what precursors of adrenaline are present in the suprarenal medulla of young animals, including that of man. If such precursors, or their relative amounts, differed from those found in the adult state, it might be possible by taking animals at different ages to correlate the change-over with a certain stage in development. In addition, another method might be provided of studying adrenaline formation in the body.

METHODS

Preparation of the adrenal extracts.—The human glands were secured from the post-mortem room as soon as possible after death; occasionally the delay was as long as 48 hours, but the majority of specimens were obtained before 24 hours had elapsed. After the capsule and the fatty tissue adhering to it had been removed, the glands were weighed and ground with sand and 0.1–1 ml. of 0.01 N-HCl/g. The acid extracts were centrifuged, and the clear supernatants were assayed for their noradrenaline and adrenaline contents as described below. In many adult specimens, the cortices were separated from the medullae. Each part was then weighed, extracted, and assayed separately.

The adrenal glands of rabbits, cats, guinea-pigs, dogs, and domestic fowl were secured after killing the animals by a blow on the head or by an overdose of urethane. The glands were dissected free of capsules, weighed, and ground in a mortar with sand and 1–5 ml. 0.01 N-HCl/g. After centrifuging, the extracts were assayed. In a few experiments with guinea-pig glands, a similar volume of 10 per cent trichloroacetic acid was used instead of hydrochloric acid for the preparation of the extracts. In these experiments, excess trichloroacetic acid was removed with ether before the solution was assayed.

Assay methods.—All extracts were assayed for their adrenaline and noradrenaline contents by paper chromatography and by the action on the blood pressure and normal nictitating membrane of a spinal cat (Burn, Hutcheon, and Parker, 1950a). In certain experiments with guinea-pig extracts, colorimetric assays (Euler and Hamberg, 1949b) were also carried out. The method is discussed in detail under the results of guinea-pig gland assays.

All chromatograms were carried out by the ascending method in a glass tank (15 by 7 by 22 in.) containing the solvent to a depth of ½ in. The solvent was prepared by shaking *n*-butyl alcohol (4 vol.), glacial acetic acid (1 vol.), and water (5 vol.) together and discarding the lower layer. The butyl alcohol and acetic acid were of ordinary reagent quality and were not purified before use. Sheets of Whatman No. 4 filter-paper, 12 in. wide, were suspended from horizontal glass rods placed 18 in. above the liquid surface, the paper being kept taut by means of a thin glass tube 12 in. long, closed at both ends and threaded through four vertical slits ⅝ in. long and 3 in. apart cut near the bottom of the sheet. The top of the tank was sealed by a vaselined glass plate.

Test solutions were applied from a graduated glass syringe as single or replicate drops each 0.01 ml., 1 in. apart, along a line 2 in. from the foot of the paper and at least 2 in. from the lateral edges of the paper where the flow tended to be erratic. Usually 0.02 ml. was sufficient, although for very weak extracts 0.08 ml. was necessary. When the drops had dried, chromatography was carried out at room temperature for 18 hours, during which time the solvent travelled 12 in. from the starting line. After drying for 15 minutes in a cabinet through which a current of warm air circulated, the paper was sprayed with a 1 per cent (w/v) aqueous solution of potassium iodate. On development in an air oven at 100–110° C. for not more than two minutes, adrenaline and noradrenaline were rendered visible as pink and violet spots respectively. Care was taken to avoid overheating, which causes both spots to become brown and indistinguishable in colour. At room temperature, the spots assume a uniform brown colour within a few hours. Since the colours are viewed against a white background, the method is more sensitive than the original ferricyanide method (James, 1948), in which the spots are viewed against a yellow background. The use of potassium iodate as a developer for adrenaline and noradrenaline on chromatograms has recently been described by Burn, Langemann, and Parker (1951), who employed a 10 per cent solution of the salt in 5 per cent acetic acid. In our experience this solution showed a marked tendency to crystallize in the atomizer during spraying, gave a less striking colour difference between adrenaline and noradrenaline, and would not detect such small quantities as the aqueous solution used by us.

Estimates of adrenaline and noradrenaline contents of adrenal extracts were obtained by comparison of the intensities of the spots with those from standard solutions of the two amines, and values agreed well with those obtained by parallel biological and colorimetric assay. By the iodate method described above, spots containing 1 μg. adrenaline or 2 μg. noradrenaline can be readily detected. In no experiment was dihydroxyphenylalanine or hydroxytyramine detected in adrenal extracts by the chromatographic method. The R_f values and colours of the spots given by these substances as well as by adrenaline and noradrenaline are recorded in Table I.

TABLE I

R_f VALUES OF FOUR AMINES IN BUTANOL-ACETIC ACID-WATER SOLVENT

Amine	Rf value	Colour of spot on development with 1% KIO_3
Dihydroxyphenylalanine	0.19	Greyish-violet
Noradrenaline	0.28	Violet
Adrenaline	0.36	Pink
Hydroxytyramine	0.39	Orange-brown

Solutions of *l*-adrenaline, *l*-noradrenaline bitartrate, hydroxytyramine hydrochloride, and *dl*-dihydroxyphenylalanine in 0.01 N-HCl were used throughout this study.

RESULTS

Human adrenal glands.—The mean values of extracts from 31 adult glands were 209 μg. adrenaline and 31 μg. noradrenaline/g. Hence 13 per cent of the total catechol activity exists as noradrenaline. In general, lower values for total activity were obtained in cases where the cause of death was of an infectious nature or dying prolonged. Higher figures were found in extracts where rapid deaths had occurred. So far we have been unable to obtain for comparison the glands of healthy individuals who died a sudden or violent death. It must not be assumed, therefore, that the mean values quoted above represent the normal amounts found in the healthy gland.

In 10 of the samples, the activities of the medullary and cortical components have been compared. The mean values were 1,260 μg. adrenaline and 214 μg. noradrenaline/g. fresh medulla, and 109 μg. adrenaline and 3 μg. noradrenaline/g. fresh cortex. Hence 15 per cent of the total in the medulla is noradrenaline, whereas in the cortex it is only 3 per cent. This suggests that noradrenaline is formed in the medulla, although no proof has yet been obtained that it is actually produced in the gland.

In a series of 25 children under 70 days, the mean values were 2 μg. adrenaline and 30 μg. noradrenaline/g. fresh tissue. The position is therefore reversed to that seen in adults (Fig. 1). Over 90 per cent of the total catechol amines in human embryonic tissue is noradrenaline, a result similar to that seen in adult suprarenal

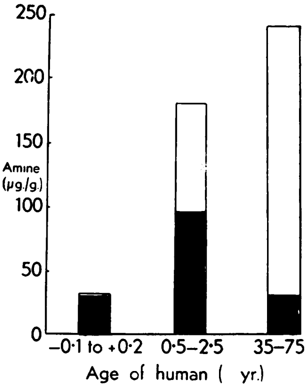

FIG. 1.—The influence of age on the amine content (μg./g. fresh tissue) of suprarenal glands of man. Shaded area is noradrenaline; plain area is adrenaline.

medullary tumours. The glands of three children aged 180, 210, and 912 days were extracted and assayed, and the relative noradrenaline values were 67, 50, and 32 per cent respectively.

Cat adrenal glands.—The mean values for extracts of glands of 12 adult cats were 601 μg. adrenaline and 369 μg. noradrenaline/g. Therefore some 38 per cent of the total activity is noradrenaline. These values agree with those reported earlier by Bülbring (1949) and Euler (1950). When animals between 2 and 3 days old were used, the noradrenaline proportion in the extracts was found to be significantly higher, representing about 82 per cent of the total amines. The mean values for extracts of 20 embryonic glands were 141 μg. adrenaline and 642 μg. noradrenaline/g. The glands of three kittens *in utero* were extracted and found to contain 150 μg. adrenaline and 375 μg. noradrenaline/g. Over 70 per cent of the total amine content of these glands therefore exists as noradrenaline (Fig. 2).

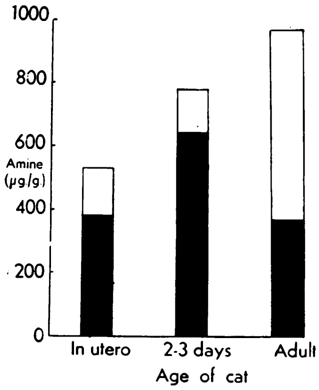

FIG. 2.—The influence of age on the amine content (μg./g. fresh tissue) of suprarenal glands of cat. Shaded area is noradrenaline; plain area is adrenaline.

Rabbit adrenal glands.—The mean values for extracts of glands of 56 adult rabbits were 470 μg. adrenaline and 10 μg. noradrenaline/g. Therefore only 2 per cent of the total activity is noradrenaline. In very young rabbits (secured *in utero*), total activity in the glands was reduced and was composed entirely of noradrenaline.

By the third day of life, total activity had increased four- or five-fold, and large amounts of adrenaline were now present. By using a large series of animals, it was possible to trace the change-over in the gland from almost entirely pure noradrenaline to a high percentage of adrenaline (Table II and Fig. 3).

TABLE II

THE INFLUENCE OF AGE ON THE NORADRENALINE AND ADRENALINE CONTENTS OF THE SUPRARENAL GLANDS OF RABBITS

No. of expts.	Age	Weight (g.)	Activity of gland extract (μg./g.)			% Noradren. in total
			Adren.	Noradren.	Total	
12	*In utero*	45	0	200	200	100
4	1 hour	40	250	250	500	50
23	6 hours	50	181	544	725	75
3	1 day	60	75	670	745	90
3	2 days	50	297	603	900	67
10	2 days	55	574	437	1,011	43
4	8 days	105	667	125	792	16
56	Adult	2,000	470	10	480	2

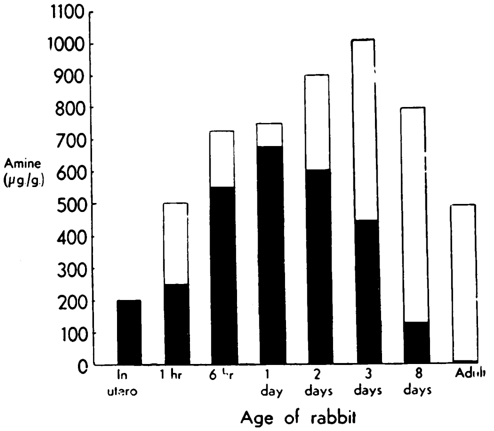

FIG. 3.—The influence of age on the amine content (μg./g. fresh tissue) of suprarenal glands of rabbit. Shaded area is noradrenaline; plain area is adrenaline.

Guinea-pig adrenal glands.—The guinea-pig is an interesting animal, for it possesses relatively the largest adrenal of any animal. Euler, Hamberg, and Purkhold (1949) and Schuler and Heinrich (1949) investigated the guinea-pig suprarenals and estimated by chemical assay that there are 338–400 μg. catechols per g. fresh tissue. Of this total, over 22 per cent has been reported as noradrenaline. Our early results on adult glands indicated that total catechols were about 132 μg./g. and that the proportion of noradrenaline seldom exceeded a few per cent of the total. Noradrenaline was in fact present in only 12 out of 41 experiments. Later determinations on animals secured from another source (Sussex) confirmed this finding, and for 60 animals no more than 3 per cent of the total catechols (mean value 127 μg./g.) was noradrenaline. Since results on total activity and relative noradrenaline content differed widely from those previously reported, further work was carried out to determine the possible sources of error.

We extracted the glands of four adult guinea-pigs with 10 per cent trichloroacetic acid, and, after removal of the excess acid with ether, we subjected the extracts to biological and chemical assay. The chemical assay was that used by Euler, Hamberg, and Purkhold (1949), and depends on the development of colour with iodine. Iodo-adrenochrome is formed in 1½ minutes at *p*H 4, whereas only 10 per cent of iodo-noradrenochrome is formed at this *p*H. On three-minute treatment with iodine at *p*H 6, maximal formation of both quinones is attained. The difference in oxidation rates at these two *p*H values is the basis of this method of estimating the two substances separately (Euler and Hamberg, 1949a). The results obtained on the four extracts are shown in Table III. Two important conclusions are indicated: (*a*) the total amine content estimated by the iodine method was about twice to four times

TABLE III

ESTIMATIONS OF CATECHOLS (μg./g.) IN TRICHLOROACETIC ACID EXTRACTS OF ADRENAL GLANDS OF ADULT GUINEA-PIGS

Extract	Chemical assay				Biological assay			
	Adren.	Noradren.	Total	% Noradren.	Adren.	Noradren.	Total	% Noradren.
1	200	38	238	16	150	0	150	0
2	150	24	174	14	120	2	122	2
3	240	88	328	27	80	0	80	0
4	225	125	350	35	125	0	125	0

that found by biological determination, and (*b*) the proportion of noradrenaline in the mixture was much higher, values being about 10 times those found biologically. It was then found that the presence of precursors of adrenaline may produce erroneous results by this method. Dihydroxyphenylalanine, for example, gives a quinone at *p*H 4, although it is maximal at *p*H 6. Hydroxytyramine gives no colour with iodine at *p*H 4, but is maximal at *p*H 6. Both these substances may be present in adrenal extracts and may affect the iodine reaction, without interfering greatly with the biological assay. A false noradrenaline proportion and raised total activity may result. In addition, Euler (1950) has suggested that extraction of organs with trichloroacetic acid may give a higher yield of active material.

We have carefully examined the extracts of guinea-pig glands by paper chromatography and have never found any hydroxytyramine or dihydroxyphenylalanine. We conclude, therefore, that extraction with trichloroacetic acid and estimation by the iodine reaction yields erroneous results with guinea-pig gland extracts. It is possible that the large cortex seen in the guinea-pig contains a material which enhances the iodine reaction. There may also be a real distinction in the catechol content of the adrenal glands between different breeds. Elliott and Tuckett (1906) have already reported a difference in weights of adrenal glands of English and French guinea-pigs.

When we examined the extracts of glands removed from young guinea-pigs, values for total activity and relative noradrenaline content were much higher than those found in adults. Embryonic tissue of the guinea-pig therefore contains predominantly noradrenaline (Table IV and Fig. 4).

Dog adrenal glands.—Previous workers (Bülbring, 1949; Bülbring and Burn, 1949b) obtained evidence that noradrenaline was present with adrenaline in the

TABLE IV

THE INFLUENCE OF AGE ON THE NORADRENALINE AND ADRENALINE CONTENTS OF THE SUPRARENAL GLANDS OF GUINEA-PIGS

No. of exp's.	Age	Weight (g.)	Activity of gland extract (µg./g.)			% Noradren. in total
			Adren.	Noradren.	Total	
5	*In utero*	10	0	50	50	100
3	At term	33	250	125	375	33
1	1 day	40	75	150	225	66
2	2 days	45	75	150	225	66
1	7 days	60	50	250	300	83
1	18 days	135	250	100	350	40
1	20 days	150	250	50	300	17
1	23 days	167	250	0	250	0
2	26 days	175	185	0	185	0
1	31 days	250	125	0	125	0
60	Adult	500	124	3	127	3

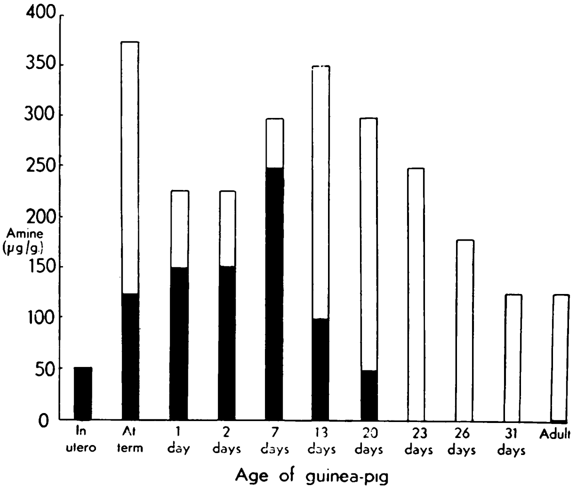

FIG. 4.—The influence of age on the amine content (µg./g. fresh tissue) of suprarenal glands of guinea-pig. Shaded area is noradrenaline; plain area is adrenaline.

suprarenal glands of the dog, but the amount varied considerably (from 0 to 52 per cent of the total amines). We investigated the adrenal glands of puppies to see if their embryonic tissue produces predominantly noradrenaline. Results shown in Fig. 5 indicate that there is not such a marked difference in this species between the relative amine contents of glands of young and old animals, as is seen in guinea-pigs, rabbits, and cats. Perhaps dogs younger than 6 days may provide the necessary data.

Adrenal glands of domestic fowl.—Whereas the adrenal glands of the guinea-pig, rabbit, and cat increase in size as the body grows, those of the fowl do not increase in weight after the third month of life (Elliott and Tuckett, 1906). In addition, the medulla forms a very large fraction of the whole gland, and there is little change with age in the ratio of cortical size to medullary size. The results we have obtained using the adrenal glands of fowls of all ages are shown in Table V. Noradrenaline predominates in the gland even in the adult state, and it is interesting to note that there is little change with age in the relative noradrenaline content.

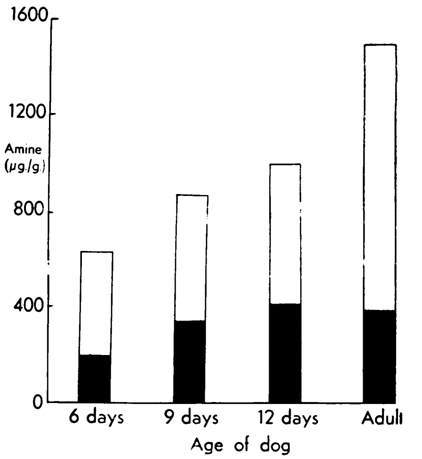

FIG. 5.—The influence of age on the amine content (µg./g. fresh tissue) of suprarenal glands of dog. Shaded area is noradrenaline; plain area is adrenaline.

TABLE V

THE INFLUENCE OF AGE ON THE NORADRENALINE AND ADRENALINE CONTENTS OF THE SUPRARENAL GLANDS OF DOMESTIC FOWL

No. of expts.	Age (days)	Weight (g.)	Activity of gland extract (µg./g.)			% Noradren. in total
			Adren.	Noradren.	Total	
25	1	40	3,300	4,950	8,250	60
10	7	70	3,325	6,175	9,500	65
2	77	650	4,000	6,000	10,000	60
17	110	1,000	2,020	8,080	10,100	80
13	200	2,000	3,375	7,875	11,250	70

DISCUSSION

The results indicate that embryonic tissue in suprarenal glands of man, cat, rabbit, guinea-pig, and dog contains a high proportion of noradrenaline and a very small amount of adrenaline, a finding which is also characteristic of human medullary tumours. While this work was in progress, Holton (1951) obtained a similar result when she compared the adrenal glands of calves with those of bullocks. Although we have been unable to obtain any evidence of the presence of hydroxytyramine and dihydroxyphenylalanine in embryonic gland extracts, large amounts of noradrenaline show that this amine must be a precursor of adrenaline in these mammals. In fact, noradrenaline itself may be the hormone of the gland in the early days of life.

The exact area in the adrenal gland of the adult where methylation of noradrenaline occurs is still an open question. The medullary component of the adult human gland is about one-tenth of the total, so that in the glands where the cortices and medullae were divided and extracted separately it is possible to calculate the amounts of each amine present in each component. Thus, much more noradrenaline is present in the medulla (214/10=21 µg./g. gland) than in the cortex (3 × 0.9=2.7 µg./g. gland), although the adrenaline concentrations are of the same order (126

μg./g. in the medulla and 91 μg./g. in the cortex). We have noted that, as the cortical size relative to that of the medulla increases, so the relative amount of noradrenaline in the adult gland decreases ; even within the same species, this finding is true (Table VI). When there is little change with age in the ratio of cortical size to medullary size (as in the fowl, Table V), there is also little change in the relative amount of noradrenaline in the gland. The ratio of cortical size to medullary size is therefore related to the proportion of noradrenaline found in the adrenal gland.

TABLE VI

THE RELATIONSHIP BETWEEN THE MEDULLA-CORTEX RATIO AND THE RELATIVE NORADRENALINE CONTENT OF ADRENAL EXTRACTS OF ANIMALS

Animal	Medulla: Cortex	% Noradrenaline in total amines
Fowl	1:1	80
Whale	1:2	80*
Pigeon	1:2	55
Dog	1:5	27
Man	1:9	13
Cat	1:18	38
Rat	1:20	9
Rabbit	1:40	2
Guinea-pig	1:63	3
Young guinea-pig (7 days)	1:6	83

* Burn, Langemann, and Parker (1951).

It has been found that the adrenal cortex of the guinea-pig contains a material which may enhance the iodine reaction of Euler and Hamberg (1949) and so produce erroneous results both for total activity and for the proportion of each amine present. When the cortical extract of some human glands was tested colorimetrically (iodine method), biologically, and by paper chromatography, the results by the last two methods were in agreement, but the chemical method gave an answer which was about twice this amount. On the other hand, medullary extracts of the same glands gave comparable results by all three methods. In addition, an erroneous result (three times that found biologically) was obtained when the iodine reaction was applied to some extracts of rabbit glands where a large cortical component is present, but, when applied to extracts of cat and whole human glands, good agreement was reached by all tests. All these findings suggest that there is a cortical material which may upset colorimetric estimations of total amine activity and of proportions of each present.

It is of course accepted that the human glands obtained at death are in an exhausted state, and hence values found must of necessity be low. To obtain a rough estimate of the true value, we first examined the gland extracts of four adult cats which had been dead for more than 20 hours. Despite the fact that the noradrenaline percentage for this animal was within the normal adult range, total activity had dropped to 204 μg./g., i.e., about one-quarter of the normal value. Secondly, in two guinea-pigs which apparently were not feeding well, and which died after being very subdued for two days, we found that the total amine content of the adrenal gland was reduced to 25 μg. adrenaline/g., a value about one-fifth of that normally found. Lastly, in 11 experimental rabbits subjected to ether anaesthesia for long periods, the mean value for total activity was 110 μg. adrenaline/g. This is about one-quarter of that found in glands of freshly killed rabbits. Hence we conclude that the values we have obtained on human adrenal glands are significantly less than those present in the normal unexhausted gland.

CONCLUSION

1. The sympathomimetic amine present in embryonic adrenal glands of cat, rabbit, guinea-pig, dog, and man is noradrenaline: very small amounts of adrenaline may be found.

2. In the adult glands of these mammals, it is suggested that the degree of methylation of noradrenaline is related to the relative cortical size. In animals where the cortex is large relative to the medulla (e.g., the rabbit and the guinea-pig), methylation of noradrenaline is almost complete, and often only adrenaline is found in gland extracts.

3. The total activity in exhausted adrenal glands of man is about 0.24 mg./g. (31 estimations). The results of animal experiments indicate that a total activity of about 1 mg./g. might be found in the healthy individual.

4. In no experiments were dihydroxyphenylalanine or hydroxytyramine detected in adrenal extracts by the chromatographic method.

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