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THE URINARY EXCRETION OF ADRENALINE AND NORADRENALINE BY RATS UNDER VARIOUS EXPERIMENTAL CONDITIONS

BY

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A study has been made of the excretion of adrenaline and noradrenaline in the urine of normal male rats and in the urine of these animals after the subcutaneous injection of saline, adrenaline, noradrenaline, morphine, insulin, iproniazid and thyroxine. The effects of thyroidectomy, adrenal demedullation and ether anaesthesia on the urinary output of adrenaline and noradrenaline have also been investigated. The findings are discussed against the background of the known effects of these treatments on the activity of the sympathetic nervous system.

The results demonstrate that urinary adrenaline and noradrenaline estimations in the rat can, under favourable conditions, serve as an indicator of an alteration of sympathetic activity following the administration of a drug or some other deviation from normal.

In a contemporary paper (Crawford and Law, 1958) we have described a method for the estimation of adrenaline and noradrenaline in urine. The amines are adsorbed on a cation-exchange resin, Amberlite IRC-50, and eluted therefrom with acid. The sympathin (adrenaline + noradrenaline) in the extract may be estimated fluorimetrically under conditions in which the two amines yield equal intensities of fluorescence so that the sympathin estimate is uninfluenced by the proportions of the two amines in the mixture. The two amines may be estimated separately after paper chromatography of the urine extract.

The method has been used to study the urinary sympathin excretion of normal rats and of rats subjected to various experimental procedures. The study was undertaken primarily with a view to demonstrating that measurements of the urinary sympathin excretion would provide a means of detecting alterations in the level of sympathico-adrenal discharge.

METHODS

Adult male albino rats were used throughout.

Urine Collection.—Each rat was housed in a circular galvanized wire gauze cage with a detachable floor consisting of zinc sheet, 21 cm. in diameter, perforated with holes 0.9 cm. in diameter. A hole in the side of the cage just large enough to allow the animal's head to pass through gave access to a compartment into which fitted a dish 6 cm. in diameter for drinking water. The cage was supported by laterally projecting metal rods on top of a large glass filter funnel resting on a rack of angle iron designed to carry six such funnels. The stem of the funnel was cut and bevelled about 3 cm. from the apex. Situated directly underneath the tip of the funnel stem at 2 to 3 cm. distance was the centre of an inverted 100 ml. Kjeldahl flask with the neck reduced to approximately 3 cm. in length. The bulb of the flask rested on three inverted U-shaped pieces of glass rod placed symmetrically round the lip of a glass jar in which the urine eventually collected.

During urine collection, both top and bottom of the floor of the cage was coated with hard paraffin to prevent access of urine to the metal. Faeces usually passed readily through the perforations in the cage floor, down the funnel stem and were deflected off the rounded surface of the inverted Kjeldahl flask. Urine passed down the outer surface of the flask into the collecting jar. Collected in this way, urine samples were reasonably free from faecal contamination.

Drinking water was in continuous supply. Solid food in the form of 15 g. rat cake (North-East Scotland Agricultural Co-operative Society Ltd., Aberdeen) was offered twice daily for 30 min. at 9.15 a.m. and 8.30 p.m. For the feeding, the rat was transferred to a duplicate cage. Before transference, the animal was induced to urinate by gentle pressure on the abdomen in order to prevent urine loss. Such a feeding regimen was found necessary to avoid gross contamination of the urine sample.

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The glass cylinders for urine collection contained 0.5 ml. 2 N-H_2SO_4 (A.R.) to preserve the excreted sympathin (Euler and Hellner, 1951). At the end of the collection, the glass funnel and inverted flask were washed down into the urine specimen with a small quantity of de-ionized water. The 24 hr. specimen from a normal animal was usually about 15 ml., and had a *p*H of 3.0 to 3.5.

Adrenaline and Noradrenaline Estimation.—Estimations of the urinary sympathin (adrenaline+noradrenaline) or of the two amines separately were carried out by the techniques described by Crawford and Law (1958).

For most experiments, six rats were used and usually the 24 hr. urine specimens from pairs of animals were combined. Two portions of 10 ml. were taken from each of two combined samples. One portion was analysed directly to give an estimate of the "free" amines while the second portion was analysed after hydrolysis at *p*H 2 to give an estimate of the "total" (free+conjugated) amines. The other pooled sample was used for estimating the recovery of the amines in the analytical procedure. Factors based on these recovery experiments were used to correct the analytical values (Crawford and Law, 1958).

On occasions, the urine specimens from single animals were analysed either for "free" or for "total" sympathin.

For assays, a fluorimetric method modified (Crawford and Law, 1958) from that of Lund (1949, 1950) was employed routinely. To corroborate the estimates, parallel bioassays were sometimes also performed using the rat blood pressure preparation (Crawford and Outschoorn, 1951), or the isolated rat uterus (Gaddum and Lembeck, 1949).

Administration of Drugs.—Drugs given subcutaneously were injected usually as a neutral aqueous solution in a volume of 0.5 ml. To prevent the loss of fluid during injection, the animal was wrapped in a towel with the head and a portion of the neck exposed and the injection was made into the subcutaneous tissue of the neck. In this way it was possible to perform an injection single-handed without any apparent distress of the animal. However, during the injection the animal frequently urinated, and therefore, when repeated injections were given, the animal was trained to sit quietly on the floor of the cage while the injection was made so that urine voided was not lost.

Control Values for Sympathin Excretion.—The control values for the sympathin excretion for comparison with the excretion after a drug administration were obtained, whenever possible, from previous analyses of the urine from the same rats after the injection of the vehicle in which the drug was to be administered. The injections matched, both in number and in time relations, the proposed injections of the drug solution. Such control experiments were carried out, as far as possible, just prior to those involving the drug.

Results

All estimates recorded are corrected values (see Methods). The values for sympathin, adrenaline and noradrenaline designated "total" refer to estimates obtained from hydrolysed urine; those designated "free" refer to estimates obtained from urine without preliminary hydrolysis; and those designated "conjugated" refer to the difference between the "total" and "free" estimates.

The Effect of Caging of Rats on the Urinary Sympathin Output.—Portions of the pooled 24 hr. urine specimens from three male rats (210 to 230 g.) were analysed. The "total" urinary sympathin output was estimated as 5.0 μg./rat/day during the first day in the metabolism cage, 4.1 μg./rat/day during the fourth day, 1.9 μg./rat/day during the sixth day and 1.9 μg./rat/day during the tenth day. These results indicated an elevated sympathin excretion during the first few days and, consequently, all experiments were carried out only on animals which had been conditioned to the routine of life in the metabolism cages for at least one week.

Daily Excretion of Sympathin by Normal Rats.—Table I gives the estimated daily output of urinary sympathin by normal rats (250 to 260 g.).

TABLE I

URINARY SYMPATHIN EXCRETION OF NORMAL MALE RATS

The rats weighed 250 to 260 g. Fluorimetric and biological assays. The numerals in column (b) refer to the number of animals whose urines were pooled for sampling. The ranges in column (d) indicate the limits between which the estimate was bracketed.

Expt. No. (a)	No. of Rats (b)	Sympathin (μg./rat/24 hr.) Fluorimetric (c)	Sympathin (μg./rat/24 hr.) Rat Blood Pressure (d)
"Total" sympathin			
1	3	2·0	1·1 (1·0–1·2)
2	3	2·4	2·0 (1·5–2·5)
3	1	1·3	1·35 (0·9–1·8)
4	1	2·0	2·1 (1·5–2·7)
	Mean	1·93	1·64
"Free" sympathin			
5	3	1·0	0·9 (0·6–1··)
6	3	0·9	0·8 (0·6–1·0)
7	3	1·0	0·9 (0·8–1·0)
	Mean	0·97	0·87

Pooled samples were analysed in most cases. Assays of the urine extracts were carried out both fluorimetrically and biologically using (−)-noradrenaline as standard. The estimates by the two methods agreed satisfactorily.

Table II records the estimated daily excretion of adrenaline and of noradrenaline in the urine of normal male rats.

From the combined results cited in Tables I and II, it was estimated that normal rats (250 to 260 g.) excreted 1.15 ± 0.23 (S.D.) (6) μg./day of "free" sympathin, 2.0 ± 0.44 (S.D.) (8) μg./day of "total" sympathin, and 0.67 ± 0.4 (S.D.) (3) μg./day of "conjugated" sympathin. Noradrenaline accounted for 60 to 80% of the "total" sympathin, 60 to 90% of the "free" sympathin and 10 to 100% of the "conjugated" sympathin.

TABLE II

URINARY EXCRETION OF ADRENALINE AND NORADRENALINE BY NORMAL MALE RATS

Samples from individual rats (weighing 250 to 260 g.) were analysed. Fluorimetric assay. The numerals in brackets denote % of noradrenaline or adrenaline in the sum of the two amines

Rat No.	Noradrenaline (μg./rat/24 hr.)		Adrenaline (μg./rat/24 hr.)		Sympathin (μg./rat/24 hr.)		
	"Total"	"Free"	"Total"	"Free"	"Total"	"Free"	"Conjugated"
1	1·19 (62)	1·13 (83)	0·72 (38)	0·23 (17)	1·91	1·36	0·55
2	1·95 (75)	1·3 (88)	0·64 (25)	0·18 (12)	2·59	1·48	1·11
3	1·12 (74)	0·74 (63)	0·4 (26)	0·44 (37)	1·52	1·18	0·34
4	1·86 (79)	Not done	0·48 (21)	Not done	2·34	—	—
Mean	1·53 (73)	1·06 (78)	0·56 (27)	0·28 (22)	2·09	1·34	0·67

Subcutaneous Injection of 0.9% NaCl.—The mean excretion of "free" sympathin after the injection of saline (Table III) showed a significant increase ($P<0.01$) compared with that of untreated rats. The mean amount of "conjugated" sympathin did not show a significant change ($P \sim 0.1$).

Because of these findings the control values for the sympathin excretion for comparison with the excretion after drug administration were obtained, whenever possible, from previous analyses of the urine from the same rats after injection of the vehicle in which the drug was to be administered.

TABLE III

URINARY SYMPATHIN EXCRETION OF MALE RATS IN THE 24 HR. FOLLOWING SUBCUTANEOUS INJECTION OF 0·5 ML. SALINE/RAT

Samples from pooled urine of pairs of rats (each weighing 250 to 260 g.) were analysed. Fluorimetric assay.

Analysis No.	Sympathin (μg./rat/24 hr.)		
	"Total"	"Free"	"Conjugated"
1	4·3	4·9	−0·6
2	4·5	4·2	0·3
3	2·9	2·4	0·5
4	3·2	3·0	0·2
5	2·4	2·5	−0·1
6	2·2	2·0	0·2
7	2·8	2·2	0·6
Mean	3·2	3·03	0·16

Subcutaneous Injection of (−)-Adrenaline or of (−)-Noradrenaline.—Table IV records the excretion of adrenaline and noradrenaline following injection of 1 μg. adrenaline/g. This dose produced visible signs of adrenaline action, such as erection of hair. Table V records the "total" and "free" sympathin excretion following injection of adrenaline (0.1 μg./g.) or of noradrenaline (0.1 μg./g.).

TABLE IV

URINARY EXCRETION OF ADRENALINE AND NORADRENALINE BY MALE RATS IN THE 24 HR. FOLLOWING THE SUBCUTANEOUS INJECTION OF (−)-ADRENALINE (1 μG./G.)

Fluorimetric assays.

Rat No.	(−)-Adrenaline Injected μg.	"Total" Sympathin Excreted as		
		Adrenaline μg.	Noradrenaline μg.	Sum μg.
1	255	20·0	0·55	20·55
2	230	19·7	0·6	20·3
3	240	21·1	1·0	22·1
4	240	23·0	1·1	24·1
Mean	241	20·95	0·81	21·76

Mean sympathin excretion of saline-injected rats (Table III)	3·2 μg.
Mean additional sympathin excretion of adrenaline-injected rats	18·56 μg.
Additional sympathin as % of injected adrenaline	7·7%

TABLE V

URINARY SYMPATHIN EXCRETION BY MALE RATS IN THE 24 HR. FOLLOWING THE SUBCUTANEOUS INJECTION OF (−)-ADRENALINE (0·1 μG./G.) OR OF (−)-NORADRENALINE (0·1 μG./G.)

Samples of pooled urine from pairs of rats (each weighing 265 to 270 g.) were analysed. Fluorimetric assays.

Amine Injected:	Sympathin Excretion (μg./rat)			
	Adrenaline		Noradrenaline	
	"Total"	"Free"	"Total"	"Free"
	6·65	4·05	7·1	7·4
	6·25	4·55	7·6	7·5
	6·3	6·4	7·0	3·15
	7·4	7·55	6·4	4·5
	—	—	6·25	3·9
	—	—	5·2	6·1
	—	—	6·3	6·15
Mean	6·65	5·64	6·55	5·54
Mean saline control excretion	3·2	3·03	3·2	3·03
Additional sympathin excretion after amine injection	3·45	2·61	3·35	2·51
Additional sympathin excretion as % of amine injected	12·9	9·8	12·5	9·4

Subcutaneous Injection of Morphine.—Four injections of morphine hydrochloride (20 μg./g.) were made into each rat at intervals of 1 hr. Urine was collected during the 24 hr. following the first injection. The urine specimens from pairs of rats were pooled for analyses. Control 24 hr.

specimens of urine were obtained the previous day from the same pairs of rats after four subcutaneous injections of 0.5 ml. saline. The results of this experiment (Table VI) showed a definite rise in the urinary sympathin levels after morphine.

An experiment conducted similarly in which the animals received only one injection of morphine hydrochloride (20 μg./g.) gave no real evidence of a change in the sympathin excretion (Table VII).

TABLE VI

URINARY SYMPATHIN EXCRETION OF MALE RATS IN THE 24 HR. FOLLOWING THE FIRST OF FOUR SUBCUTANEOUS INJECTIONS OF MORPHINE HYDROCHLORIDE (20 μG./G.) GIVEN AT HOURLY INTERVALS COMPARED WITH THE EXCRETION OF THE SAME RATS IN THE PREVIOUS 24 HR. FOLLOWING THE FIRST OF FOUR SUBCUTANEOUS INJECTIONS OF 0·5 ML. SALINE/RAT GIVEN AT HOURLY INTERVALS

Samples of pooled urine from pairs of rats (each weighing 265 to 275 g.) were analysed. Fluorimetric assays.

Urine Sample No.	Sympathin Excretion (μg./rat/24 hr.)					
	After Morphine		After Saline		Increase After Morphine	
	"Total"	"Free"	"Total"	"Free"	"Total"	"Free"
1	6·95	5·7	3·4	3·1	3·55	2·6
2	8·6	8·35	4·8	4·7	3·8	3·65

TABLE VII

URINARY SYMPATHIN EXCRETION OF MALE RATS IN THE 24 HR. FOLLOWING SUBCUTANEOUS INJECTION OF MORPHINE HYDROCHLORIDE (20 μG./G.) COMPARED WITH THE EXCRETION BY THE SAME RATS AFTER INJECTION OF 0·5 ML. SALINE/RAT

Samples of pooled urine from pairs of rats (each weighing 260 to 270 g.) were analysed. Fluorimetric assays.

Urine Sample No.	Sympathin Excretion (μg./rat/24 hr.)					
	After Morphine		After Saline		Increase After Morphine	
	"Total"	"Free"	"Total"	"Free"	"Total"	"Free"
1	1·8	2·2	2·2	2·9	−0·4	−0·7
2	4·85	3·4	2·95	3·45	+1·9	−0·05

Subcutaneous Injection of Iproniazid, 1-isonicotinyl-2-isopropyl Hydrazide.—A study was made of the effect of iproniazid, a monoamine oxidase inhibitor, on the excretion of endogenous and exogenous adrenaline and noradrenaline.

Male rats (265 to 275 g.) were injected subcutaneously with iproniazid [Marsilid phosphate (Roche) 150 μg./g.] and the urine collected during the subsequent 24 hr. Analyses of pooled urine from pairs of rats showed no significant difference in the sympathin excretion/rat from that after a control injection of saline. The excretion of "total" sympathin, estimated in four urine samples, was 2.97 ± 0.64 (S.D.) μg./rat after iproniazid compared with 2.11 ± 0.24 (S.D.) μg./rat after saline in the same rats. The "free" sympathin excretion, estimated using portions of the same urine samples, was 2.08 ± 0.62 (S.D.) μg./rat after iproniazid compared with 2.27 ± 0.3 (S.D.) μg./rat after saline.

The effect of iproniazid on the urinary output of exogenous adrenaline and noradrenaline was also investigated. As controls, three male rats (255 to 270 g). were injected subcutaneously with 0.5 ml. saline followed, 1 hr. later, by an injection of 0.1 μg. (−)-adrenaline/g. or of 0.1 μg. (−)-noradrenaline/g. Urine specimens collected in the 23 hr. following the amine injection were analysed individually for "total" sympathin. One week later the experiment was repeated using the same animals but injecting iproniazid (Marsilid phosphate, 150 μg./g.) in place of the saline. A comparison of the results of the two experiments is shown in Table VIII.

TABLE VIII

EFFECT OF PRIOR ADMINISTRATION OF IPRONIAZID ON THE URINARY SYMPATHIN EXCRETION OF RATS INJECTED (a) WITH 0·1 μG. (−)-ADRENALINE/G.; OR (b) WITH 0·1 μG. (−)-NORADRENALINE/G.

For details see text. Fluorimetric assay. The rats each weighed 255 to 270 g.

(a) Treatment:	"Total" Sympathin Excretion (μg./rat/23 hr.)	
	Saline followed by Adrenaline	Iproniazid followed by Adrenaline
Rat No. 1 ..	4·7	6·4
Rat No. 2 ..	3·45	5·4
Rat No. 3 ..	4·4	5·15
Mean	4·18	5·65
Mean increase in sympathin excretion after iproniazid		1·47 μg.
Mean dose of adrenaline/rat		26·0 μg.
Mean increase in sympathin excretion after iproniazid as % of dose of injected adrenaline		5·7%

(b) Treatment:	"Total" Sympathin Excretion (μg./rat/23 hr.)	
	Saline followed by Noradrenaline	Iproniazid followed by Noradrenaline
Rat No. 4 ..	5·4	7·55
Rat No. 5 ..	4·35	6·2
Rat No. 6 ..	5·25	7·5
Mean	5·0	7·08
Mean increase in sympathin excretion after iproniazid		2·08 μg.
Mean dose of noradrenaline/rat		26·0 μg.
Mean increase in sympathin excretion after iproniazid as % of dose of injected noradrenaline		8·0%

Iproniazid administration appeared to increase the excretion of exogenous adrenaline and noradrenaline. The mean sympathin excretion of the iproniazid-treated rats was found to be just significantly greater in the case of adrenaline and significantly greater in the case of noradrenaline ($P<0.05$) than the mean excretion in the absence of iproniazid.

It must be mentioned, however, that the accuracy of the estimate from the fluorimetric assays of the urine extracts from the iproniazid-treated animals is open to question since some inhibition of the fluorescence derived from adrenaline and noradrenaline added to the extracts was noted. The figures quoted are corrected values based on the estimate of the degree of inhibition noted for each extract which amounted to 30 to 40%.

Subcutaneous Injection of Soluble Insulin.—Male rats were fed at 6 p.m. on the day previous to the experiment. No food was given thereafter until completion of the experiment, but water was in continuous supply throughout. Control sympathin excretion was measured following injection of 0.5 ml. saline/rat. On the following day, soluble insulin *B.P.* (Burroughs Wellcome) 0.5 unit/100 g. was injected subcutaneously. Convulsions, which developed in all the animals within 2.5 to 3 hr., were relieved by the intraperitoneal injection of 20 ml. 6% (w/v) glucose/rat immediately this indication of hypoglycaemia was noticed. The results (Table IX) showed a significant increase in the mean sympathin excretion after insulin compared with that after the control saline injection. The adrenaline and noradrenaline were estimated separately in the urine from rat No. 1 after insulin. The adrenaline fraction amounted to 4.2 μg./rat and the noradrenaline fraction to 2.4 μg./rat. Comparison with the mean values for normal rats indicated that the elevated sympathin excretion after insulin was to be referred mainly to an increase in the adrenaline fraction.

TABLE IX

EFFECT OF SOLUBLE INSULIN, 0·5 UNIT/100 G., SUBCUTANEOUSLY, ON THE "TOTAL" URINARY SYMPATHIN EXCRETION OF FASTING MALE RATS IN THE 24 HR. AFTER INJECTION COMPARED WITH THAT OF A CONTROL SALINE INJECTION

Fluorimetric assay. The rats each weighed 240 to 260 g.

Rat No.	"Total" Sympathin Excretion (μg./rat/24 hr.)	
	After Saline	After Insulin
1	3·15	6·6
2	4·95	6·45
3	3·35	5·25
4	—	6·3
5	—	7·6
6	—	6·8
Mean	3·8	6·5

A similar experiment was carried out on unstarved rats (240 to 265 g.), which each received a subcutaneous injection of soluble insulin, 1 unit/100 g. Both the "total" and the "free" sympathin excretion showed a slight increase over that following a control saline injection, but the mean difference was not significant ($P>0.1$).

Thyroxine.—The sodium salt of L-thyroxine (British Drug Houses) was dissolved in 0.01 N-NaOH to give a concentration of 1 mg./ml. It was administered subcutaneously to six male rats at 10 a.m. and 8 p.m. for three days and at 10 a.m. on the fourth day. The urine excreted in the 24 hr. following the last injection was collected and analysed. A similarly conducted control experiment had previously been carried out on the same rats when each had received a course of injections of 0.01 N-NaOH. The results obtained in these experiments are compared in Table X. No significant difference ($P>0.1$) in the urinary excretion following the thyroxine treatment was detected.

TABLE X

URINARY SYMPATHIN EXCRETION OF MALE RATS FOLLOWING INJECTION OF THYROXINE SUBCUTANEOUSLY (4 DOSES OF 1·0 μG./G.) COMPARED WITH THAT AFTER CONTROL INJECTIONS OF 0·01 N-NaOH

Samples of pooled urine from pairs of rats (each weighing 265 to 275 g.) were analysed. Fluorimetric assay.

Urine Sample No.	Sympathin Excretion (μg./rat/24 hr.)			
	After 0·01 N-NaOH		After Thyroxine	
	"Total"	"Free"	"Total"	"Free"
1	3·4	4·2	4·05	3·75
2	2·8	2·5	3·0	2·65
3	3·45	3·1	4·4	3·45
Mean	3·2	3·25	3·8	3·3

Urinary Sympathin Excretion by Thyroidectomized Rats.—The urinary sympathin excretion of six male rats (300 to 355 g.), following thyroidectomy under ether anaesthesia seven days previously, was compared with that of six rats in the same weight range which had undergone a sham operation at the same time. Three specimens of 24 hr. urine pooled from pairs of rats from each group were analysed for "total" sympathin. In the thyroidectomized group, the sympathin excretion was 2.97 ± 0.1 (S.D.) μg./rat/24 hr., while in the sham-operated group it was 2.78 ± 0.25 (S.D.) μg./rat/24 hr. The difference was not significant ($P>0.1$).

The two groups of rats, thyroidectomized and sham-operated, were treated with thyroxine as in the experiment with normal animals. The effect on the sympathin excretion is shown in Table XI, the individual estimates from the thyroxine treated animals being compared with those previously obtained from the analyses of the pooled urine from the same pairs of rats before treatment.

In both the thyroidectomized and the sham-operated animals, the urinary sympathin excretion showed a consistent rise after thyroxine treatment. However, the sympathin excretion by the thyroidectomized animals after thyroxine did not show a

significant difference (P 0.1 to 0.05) from that of the controls. In the case of the sham-operated animals, the difference was significant ($P<0.01$).

TABLE XI

EFFECT OF SUBCUTANEOUS THYROXINE (4 DOSES OF 1·0 μG./G.) ON THE URINARY SYMPATHIN EXCRETION OF THYROIDECTOMIZED AND "SHAM-OPERATED" MALE RATS

Samples of pooled urine from pairs of rats were analysed. Fluorimetric assay.

Urine Sample No.	"Total" Sympathin Excretion (μg./rat/24 hr.)		
	Untreated	After Thyroxine	Difference
Thyroidectomized rats			
1	3·0	3·45	+0·45
2	2·85	3·05	+0·2
3	3·05	3·25	+0·2
Mean	2·97	3·25	+0·28
"Sham-operated" rats			
1	3·05	3·6	+0·55
2	2·65	3·25	+0·6
3	2·6	3·35	+0·75
Mean	2·77	3·4	+0·63

While these results indicate some rise in the sympathin excretion following thyroxine administration to both thyroidectomized and sham-operated animals, this might be more apparent than real since the basal levels of excretion were probably not adequately controlled inasmuch as the control animals did not receive, before urine collection, a course of injections with the solvent, matching that of the later course of thyroxine injections.

Exposure to Ether.—Four normal male rats (325 to 335 g.) were exposed to ether fumes in a closed box fitted with a Perspex lid for 10 min. after the loss of the righting reflex. The urine excreted in the subsequent 24 hr. was collected. The urine specimens from two rats were combined and "total" adrenaline and "total" noradrenaline estimated. The two estimates of the noradrenaline output were 1.17 and 1.05 μg./rat and of the adrenaline output 0.88 and 1.06 μg./rat. Comparison of these figures with the estimates of the excretion by untreated animals (Table II) indicated that ether probably had no effect on the noradrenaline output but caused a significant rise in the adrenaline excretion ($P<0.05$).

Urinary Sympathin Excretion of Demedullated Male Rats.—The adrenal medullae of eight male rats (315 to 325 g.) were removed under ether anaesthesia. After operation, the rats were kept in individual metabolism cages in a warm room at 21° to 26°. Experiments involving these animals were begun six weeks after the operation. Analyses of the urine from these rats showed the absence of detectable amounts of adrenaline even when the extracts were tested on the very sensitive isolated rat uterus preparation (Table XII). The mean noradrenaline excretion showed no significant difference (P 0.05) from that of normal rats (Table II).

TABLE XII

URINARY SYMPATHIN EXCRETION OF DEMEDULLATED RATS

Samples of pooled urine from pairs of rats were analysed.

Urine Sample No.	"Total" Sympathin (μg./rat/24 hr.)	"Total" Noradrenaline (μg./rat/24 hr.)	"Total" Adrenaline (μg./rat/24 hr.)	
	Fluorimetric Assay		Fluorimetric Assay	Rat Uterus Assay
1	1·05	1·25	<0·06	<0·008
2	0·8	0·7	<0·14	<0·009
3	0·95	1·0	<0·19	<0·009
Mean	0·93	0·98		

Urinary Sympathin Excretion of Demedullated Male Rats after Morphine.—The six demedullated rats used in the previous experiment received four subcutaneous injections of morphine hydrochloride, 20 μg./g. at intervals of 1 hr. The urine excreted during the 24 hr. following the first injection was collected. The urine from pairs of animals was combined, the individual animals of each pair being the same as in the previous experiment (Table XII). The "total" adrenaline and the "total" noradrenaline contents of the urine samples were determined. The adrenaline was estimated both fluorimetrically and biologically, using the isolated rat uterus.

The estimated noradrenaline excretion, μg./rat, was 1.56, 1.67 and 1.63 in the three samples analysed, and the mean excretion was just significantly greater ($P<0.05$, >0.02) than that of the untreated demedullated animals. The estimate of the adrenaline excretion was less than 0.1 μg./rat (fluorimetric) and less than 0.002 μg./rat (rat uterus) for all three samples indicating the absence of any detectable adrenaline from the urines (<0.2% of the noradrenaline content).

Exposure of Demedullated Rats to Ether.—Four demedullated rats (315 to 325 g.) were exposed to ether fumes and the urine collected and analysed in the same manner as in the experiment on normal rats. The estimates of the noradrenaline excretion were 0.96 and 0.83 μg./rat/24 hr. They did not indicate any real difference between the excretion by ether-exposed and by untreated demedullated animals (Table XII). Neither urine sample showed the presence of any detectable amounts of adrenaline, the excretion being estimated at <0.05 μg./rat/24 hr. (fluorimetric) and <0.007 μg./rat/24 hr. (rat uterus).

Discussion

The urinary sympathin (adrenaline and noradrenaline) excretion of male rats under various experimental conditions has been studied using the analytical techniques described by Crawford and Law (1958), who have discussed the accuracy and specificity of these methods

In the following discussion of the results, estimates classified as " total " refer to the sympathin which is detectable after hydrolysis of the urine at *p*H 1.8 to 2.0 and which includes the " free " sympathin, detectable without hydrolysis, and some, but not all, of the " conjugated " sympathin (see Crawford and Law, 1958, for discussion and references).

Normal Rats.—For the first few days in the individual metabolism cages the animals were restless and disinclined to eat. After 5 to 6 days they appeared to have become used to their new environment. The initial restlessness was paralleled by a relatively high " total " sympathin excretion which subsequently fell to a more or less steady level. This observation pointed to the necessity for using conditioned rats for the study of the effect of drugs and other deviations from the normal on the sympathin excretion. The animals were therefore kept in the metabolism cages for at least one week before use.

Male rats thus conditioned and weighing about 250 g. had a " total " sympathin output of about 2.0 μg./day of which some 50 to 70% was " free." Noradrenaline was the predominant amine in the " free " sympathin (60 to 90%) but not always in the " conjugated " sympathin (10 to 100%). Pitkänen (1956) found the " free " sympathin excretion by rats over a 5 hr. collection period to be 0.36 ± 0.16 (S.D.) μg. of which 14 to 80% was noradrenaline.

The main source of adrenaline in normal rat urine would appear to be the adrenal medulla, since none was detectable in the urine of demedullated rats. The urinary noradrenaline levels of such animals did not differ significantly from that of normal rats, indicating that this amine was derived mainly, if not entirely, from extramedullary sources. Similar conclusions for men were drawn from studies of the adrenaline and noradrenaline excretions of adrenalectomized (Euler, Franksson and Hellström, 1954 ; Elmadjian, Lamson, and Neri, 1956) and sympathectomized subjects (Goldenberg and Rapport, 1951). Pitkänen (1956) found that the mean adrenaline excretion of adrenalectomized rats receiving cortisone acetate was about one-third of the mean normal excretion, but the amine was still present in detectable amounts in all urine specimens analysed.

Saline.—The subcutaneous injection of saline increased the excretion of " free " sympathin. This observation indicated that it would be more accurate to compare the sympathin excretion following a drug injection not with that of the normal untreated animal but with that of the same animal after the injection of whatever vehicle was to be used in administering the drug.

Adrenaline and Noradrenaline.—After the subcutaneous injection of (−)-adrenaline or (−)-noradrenaline the 24 hr. " total " sympathin excretion was increased by an amount corresponding to about 10% of the injected dose irrespective of the amine. The experimental results for both amines indicated that the additional sympathin might be wholly or only partly in the " free " form. The injection of adrenaline did not affect the normal level of noradrenaline excretion. These results agree with previous findings that only a small fraction of injected adrenaline or noradrenaline can be accounted for by urinary excretion of the amines in the rat (Schayer, 1951a and b), in the rabbit (Fischer and Lecomte, 1950), in the dog (Bacq, Fischer, Lecomte, and Verly, 1951) and in man (Euler and Luft, 1951 ; Goldenberg, 1951 ; Euler, Luft, and Sundin, 1954 ; Euler and Zetterström, 1955 ; Pekkarinen and Pitkänen, 1955 ; Elmadjian *et al.*, 1956). It is inferred therefrom that the main bulk must suffer enzymatic destruction in the body, and it has been postulated that monoamine oxidase is an important enzyme in this connexion (Blaschko, 1952 ; Burn, 1951, 1952). It might be expected, therefore, that the administration of a monoamine oxidase inhibitor to an animal would result in an increased urinary sympathin excretion. One substance highly active as an amine oxidase inhibitor is iproniazid, 1-*iso*nicotinyl-2-*iso*propyl hydrazide (Zeller, Barsky, Fouts, Kirchheimer and Orden, 1952 ; Zeller and Barsky, 1952).

Iproniazid.—The subcutaneous injection of iproniazid produced no detectable change in the urinary excretion of endogenous sympathin in the 24 hr. following the injection. The excretions of exogenous adrenaline and noradrenaline showed significant increases over the control levels when the amines were injected 1 hr. after iproniazid. The increases were, however, not marked. These observations would indicate that monoamine oxidase plays a minor rôle in the metabolism of the catecholamines. A similar conclusion may be drawn from the observation of Euler and Zetterström (1955) that, in man, subcu-

taneously injected cobefrine (1-(3',4'-dihydroxyphenyl)-2-aminopropanol), a catecholamine resistant to monoamine oxidase was inactivated in the body to about the same extent as subcutaneously injected adrenaline and noradrenaline. On the other hand, Schayer and his co-workers (Schayer, Smiley, and Kaplan, 1952; Schayer, Smiley, and Kennedy, 1953; Schayer, Wu, Smiley, and Kobayashi, 1954) obtained evidence that, in the rat at least, monoamine oxidase destroyed about half of a dose of injected adrenaline while most of the remainder was metabolized by some other enzyme system. In the presence of a monoamine oxidase inhibitor (iproniazid or choline-*p*-tolyl ether), this other enzyme system took over almost the entire metabolism. Such being so, monoamine oxidase inhibition would not be expected to lead to any marked increase in the urinary excretion of injected adrenaline (or noradrenaline), a conclusion consistent with the results of the present study.

Morphine.—The administration of morphine to animals leads to the release of adrenal-medullary hormones, principally adrenaline (Elliott, 1912; Stewart and Rogoff, 1916, 1922; Outschoorn, 1952; Vogt, 1954). In the present study, a single dose of morphine hydrochloride (20 μg./g.) produced no significant rise in the sympathin excretion in the subsequent 24 hr. urine. A significant rise, mainly related to the "free" sympathin, followed administration of this same dose of morphine four times at intervals of 1 hr. Demedullated rats treated similarly did not show such a marked rise in sympathin excretion, pointing to the adrenal medulla as the main source of the excess sympathin in the urine of normal rats treated with morphine. These findings are consistent with the observations of Outschoorn (1952), who detected no significant change in the catecholamine content of rat adrenal glands after a single injection of morphine hydrochloride (20 μg./g.) but a considerable depletion after four such doses given at intervals of 1 hr.

The urinary excretion of noradrenaline by demedullated rats was slightly increased after morphine, indicating a general sympathetic stimulation by the drug rather than an action limited to the adrenal medulla. This is consistent with past observations (Elliott, 1912; Vogt, 1954).

Ether.—The excretion of noradrenaline following exposure of normal rats to ether did not differ significantly from that of untreated animals, but there was an indication of an elevated adrenaline excretion. No adrenaline was detected in the urine of demedullated rats treated with ether, and the noradrenaline output of such animals showed no significant alteration from that of untreated demedullated animals. The changes in urinary sympathin were thus found to reflect the changes in plasma adrenaline levels found by Vogt (1952b) to occur under essentially similar experimental conditions.

Insulin.—Insulin causes a depletion of adrenal medullary hormones, principally adrenaline (Vogt, 1947; Burn, Hutcheon, and Parker, 1950; Hökfelt, 1951; West, 1951; Outschoorn, 1952; Udenfriend, Cooper, Clark, and Baer, 1953). A rise in the plasma adrenaline concentration following insulin administration has been demonstrated in the fasting man and dog by Holzbauer and Vogt (1954), in man by Millar (1956), and in the cat by Dunér (1954). In the present work, soluble insulin injected subcutaneously into fasting rats produced a two- to three-fold increase in the "total" sympathin excretion. Evidence from one experiment indicated that the increase was mainly of adrenaline. The administration of insulin to unstarved animals produced a small but not significant ($P>0.1$) rise in the sympathin excretion. Pitkänen (1956) found up to a twenty-fold increase over the basal adrenaline excretion of fasting rats in the 5 hr. following insulin, while Euler and Luft (1952) have reported increases of up to ten-fold in the adrenaline excretion of man after insulin.

Thyroxine and Thyroidectomy.—Neither thyroxine administration nor thyroidectomy produced significant alteration in the urinary sympathin excretion of male rats. Thyroxine did, however, raise the output of "total" sympathin in both thyroidectomized and "sham-operated" animals, the increase being almost significant ($P<0.1$, >0.05) in the case of the thyroidectomized animals and significant ($P<0.01$) in the case of the "sham-operated" animals.

Spinks and Burn (1952) detected a small decrease in the monoamine oxidase content of the liver following thyroid feeding in rabbits, while thyroidectomy produced a small effect in the opposite direction in rabbits and rats. Trendelenburg (1953) confirmed the earlier findings of Burn and Marks (1925) that thyroid feeding increased adrenaline hyperglycaemia in rabbits, an effect which he suggested might be due to a decrease in the monoamine oxidase content of the liver.

In view of the absence of a marked effect on the sympathin excretion of rats after iproniazid, it seems unlikely that the slight increase noted after thyroxine was due to alteration in the monoamine oxidase levels in the animals. In this connexion it may be remarked that Schayer (1953)

has found that tyramine metabolism in mice was significantly altered by amine oxidase inhibitors such as iproniazid but not by thyroxine, a finding which pointed to the absence of any marked alteration of the monoamine oxidase levels following administration of the latter drug.

The results of the various experiments reported in this paper indicate that the estimation of the sympathin excretion in the urine of rats can, under favourable conditions, serve to demonstrate alterations in the normal level of circulating adrenaline or noradrenaline. Its usefulness appears, however, to be somewhat restricted since it is likely that only relatively large changes will be detected. Only about 10% of injected adrenaline or noradrenaline is excreted in the urine in a form which can be estimated by the method. On the assumption that a similar fraction of the endogenous amines is excreted, a mean output of about 2 μg./day represents the release into the blood stream of the animal of some 20 μg. of sympathin, or about 1 μg./hr. The additional release of, say, 5 μg. of sympathin during the hour following a drug injection would result in an increase of only 0.5 μg. in the sympathin in the urine sample collected during the 24 hr. after the injection although this additional quantity would be excreted within a much shorter period. Owing to the variability of the normal 24 hr. urinary sympathin excretion (S.D. about 0.5 μg.), a mean rise of this order obtained in an experiment involving only two or three animals would probably not be significant. It seems probable that this argument affords an explanation of the failure in some of the experiments to demonstrate definitely an expected stimulation of the sympathetic system as, for example, during ether anaesthesia. This difficulty could be overcome to some extent at least by decreasing the interval of urine collection after the drug administration and thus decreasing the normal background level of the sympathin so that any additional excretion would be more likely to reach a statistically significant level. This possible modification of the technique has not been investigated by us, but Pitkänen (1956) has reported experiments on urinary sympathin estimations in rats in which the excretion was collected over periods as short as 2 hr., the urine output being increased by prior administration of water by stomach tube.

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References

Bacq, Z. M., Fischer, P., Lecomte, J., and Verly, W. (1951). *Arch. int. Physiol.*, **59**, 315.
Blaschko, H. (1952). *Pharmacol. Rev.*, **4**, 415.
Burn, J. H. (1951). *Irish. J. med. Sci.*, 345.
—— (1952). *Brit. med. J.*, **1**, 784.
—— Hutcheon, D. E., and Parker, R. W. O. (1950). *Brit. J. Pharmacol.*, **5**, 417.
—— and Marks, H. P. (1925). *J. Physiol.*, **60**, 131.
Crawford, T. B. B., and Law, W. (1958). *J. Pharm. Pharmacol.*, **10**, 179.
—— and Outschoorn, A. S. (1951). *Brit. J. Pharmacol.*, **6**, 8.
Dunér, H. (1954). *Acta physiol. scand.*, **32**, 63.
Elliott, T. R. (1912). *J. Physiol.*, **44**, 374.
Elmadjian, F., Lamson, E. T., and Neri, R. (1956). *J. clin. Endocr.*, **16**, 222.
Euler, U. S. von., Franksson, C., and Hellström, J. (1954). *Acta physiol. scand.*, **31**, 1.
—— and Hellner, S. (1951). Ibid., **22**, 161.
—— and Luft, R. (1951). *Brit. J. Pharmacol.*, **6**, 286.
—— —— (1952). *Metabolism*, **1**, 528.
—— —— and Sundin, T. (1954). *Acta physiol. scand.*, **30**, 249.
—— and Zetterström, B. (1955). Ibid., **33**, Suppl. 118, 26.
Fischer, P., and Lecomte, J. (1950). *Arch. int. Physiol.*, **58**, 121.
Gaddum, J. H., and Lembeck, F. (1949). *Brit. J. Pharmacol.*, **4**, 401.
Goldenberg, M. (1951). *Amer. J. Med.*, **10**, 627.
—— and Rapport, M. M. (1951). *J. clin. Invest.*, **30**, 641.
Hökfelt, B. (1951). *Acta physiol. scand.*, **25**, Suppl. 92.
Holzbauer, M., and Vogt, M. (1954). *Brit. J. Pharmacol.*, **9**, 249.
Lund, A. (1949). *Acta pharm. tox., Kbh.*, **5**, 231.
—— (1950). Ibid., **6**, 137.
Millar, R. A. (1956). *J. Pharmacol.*, **118**, 435.
Outschoorn, A. S. (1952). *Brit. J. Pharmacol.*, **7**, 605.
Pekkarinen, A., and Pitkänen, M-E. (1955). *Scand. J. clin. and lab. invest.*, **7**, 8.
Pitkänen, M-E. (1956). *Acta physiol. scand.*, **38**, Suppl. 129.
Schayer, R. W. (1951a). *J. biol. Chem.*, **189**, 301.
—— (1951b). Ibid., **192**, 875.
—— (1953). *Proc. Soc. exp. Biol., N.Y.*, **84**, 60.
—— Smiley, R. L., and Kaplan, E. H. (1952). *J. biol., Chem.*, **198**, 545.
—— —— and Kennedy, J. (1953). Ibid., **202**, 425.
—— Wu, K. T. T., Smiley, R. L., and Kobayashi, Y. (1954). Ibid., **210**, 259.
Spinks, A., and Burn, J. H. (1952). *Brit. J. Pharmacol.*, **7**, 93.
Stewart, G. N., and Rogoff, J. M. (1916). *J. exp. Med.*, **24**, 709.
—— —— (1922). *J. Pharmacol.*, **19**, 59.
Trendelenburg, U. (1953). *Brit. J. Pharmacol.*, **8**, 454.
Udenfriend, S., Cooper, J. R., Clark, C. T., and Baer, J. E. (1953). *Science*, **117**, 663.
Vogt, M. (1947). *J. Physiol.*, **106**, 394.
—— (1952a). *Brit. J. Pharmacol.*, **7**, 325.
—— (1952b). *J. Physiol.*, **118**, 588.
—— (1954). Ibid., **123**, 451.
West, G. B. (1951). *Brit. J. Pharmacol.*, **6**, 289.
Zeller, E. A., and Barsky, J. (1952). *Proc. Soc. exp. Biol. N.Y.*, **81**, 459.
—— —— Fouts, J. R., Kirchheimer, W. F., and Orden, L. S. von. (1952). *Experientia*, **8**, 349.