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EXCRETION AND METABOLISM OF EPINEPHRINE AND NOREPINEPHRINE IN MAN*

FRED ELMADJIAN†

H. HOAGLAND, Chairman. *The next paper will be presented by Dr. Elmadjian.*

This is a progress report of a co-operative study between the Worcester Foundation for Experimental Biology and the Worcester State Hospital, which involved the active participation of Drs. Justin M. Hope, Harry Freeman, Oscar Resnick and Mr. Edwin T. Lamson. Our objective was to study stress physiology, especially with respect to the sympathico-adrenal system, and to add to our knowledge of the excretion and the metabolism of the neurohormones epinephrine and norepinephrine.

Responses of the adrenal gland to stress have been the subject of much research.[1] Unlike the adrenal cortex, where emphasis is on the response to the nonspecific stress, the adrenal medulla, as part of the sympathetic nervous system, is intimately connected with specific emotional expression.[2] Another characteristic of the sympathetic nervous system is the various levels of integration represented by structural and functional components involving the cerebral cortices, the midbrain and the hypothalamus, as well as the peripheral effector components. Studies were undertaken to determine the excretion of epinephrine (E) and norepinephrine (NE), the neurohormones of the sympathico-adrenal system, involving stimulation at these various levels of integration. Two general types of stress experiments were conducted: (1) the administration of agents known to stimulate the sympathetic nervous system and (2) stress studies on normal and psychiatric patients in experimental and life situations. Examples of the former included the administration of insulin and the psychotomimetic agent, lysergic acid diethylamide (LSD). The latter included the data obtained from (1) normal subjects in anticipatory states, (2) professional hockey players and their coach, before and after the game, (3) amateur boxers, before and after the contest, (4) psychiatric patients on whom mental status and Malamud-Sands rating scale were obtained during the collection period, (5) psychiatric patients appearing at staff conferences and (6) on both therapist and patient during psychotherapeutic interviews.

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† Worcester Foundation for Experimental Biology, Shrewsbury, Mass., and Dementia Praecox Research Project, Worcester State Hospital, Worcester, Mass.

The excretion rates of E and NE were studied after graded doses of E and NE were infused. Data will be presented on the metabolism of E containing C^{14} in β- and methyl position in man, as well as control data indicating diurnal variation of E and NE excretion. These latter data will be used as control values to estimate the secretion of amines in the various stress conditions studied.

METHOD

Urine was extracted by the alumina-adsorption method of von Euler and Hellner.[3] The maximum efficiency of this method is 70 to 80 per cent, based on recovery data. The bio-assay was performed by a modification of the method described by Gaddum and Lembeck.[4] This method consisted of testing the sample on the rat colon for NE and on the rat uterus for E. The bio-assay is based on the quantitative inhibition by the catechol amines of the contractions induced in vitro in a 2-cc. bath with acetylcholine.[5, 6] The inhibition of NE and E is approximately equal when tested on the colon; but when tested on the uterus, E is 75 to 300 times more potent than NE. The colon assay was used when rapid estimates of total NE + E were desired.

DIURNAL VARIATION

In Table 10, Study A, are presented data on the NE and E excretion during sleeping and waking states on 10 normal subjects. Night samples of urine were collected during a period from approximately 10:00 P.M. to 6:30 A.M., and day samples were collected from 6:30 A.M. to noon. All subjects had breakfast and conducted their usual activities, which consisted

TABLE 10. DIURNAL VARIATION OF NE AND E EXCRETION IN NORMAL SUBJECTS[12]

SAMPLE PERIOD	NE (μg./hr.)	E (μg./hr.)
	Study A (10 subjects)	
Sleep:	1.2	0.02
	$\pm$ 0.12	$\pm$ 0.002
Morning:	2.9	0.40
	$\pm$ 0.48	$\pm$ 0.10
	Study B (6 subjects)	
Sleep:	1.2	0.02
	$\pm$ 0.14	$\pm$ 0.01
Morning:	2.3	0.10
	$\pm$ 0.49	$\pm$ 0.02
Day:	2.4	0.21
	$\pm$ 0.60	$\pm$ 0.07

of laboratory routines. Study B contains additional data on a group of 6 normal subjects consisting of physicians and laboratory personnel who were conducting their usual daily activities.[7] Each collected a sample representing the period of sleeping, a second sample from the time of waking to about 10:00 A.M. and a third sample from 10:00 A.M. to the time of retiring at night. There was an increase in the excretion rate of E and NE during morning and day samples over that observed during sleep. The NE increases were not as large percentagewise as for E values.

INFUSION EXPERIMENTS

Figure 23 depicts the infusion data of 10 E infusions and 9 NE infusions at doses of 0.05, 0.10 and 0.20 μg./Kg./min.[7] Preinfusion urine was collected from 9:00 A.M. to 10:00 A.M., at which time the infusion was started and continued for 30 minutes. The infusion collection represented urine collection ranging from 10:00 A.M. to approximately 11:00 A.M. The data in Figure 23 presents the hourly excretion of each dosage rate above its control collection. In the case of E, 0.5 to 2 per cent of the total dose infused appears in the urine, while in the case of NE, from 3.0 to 6.0 per cent of the total dose appears in the urine in excess of the control sample. In the NE infusion, it appears that the relationship of excretion above control and dosage is

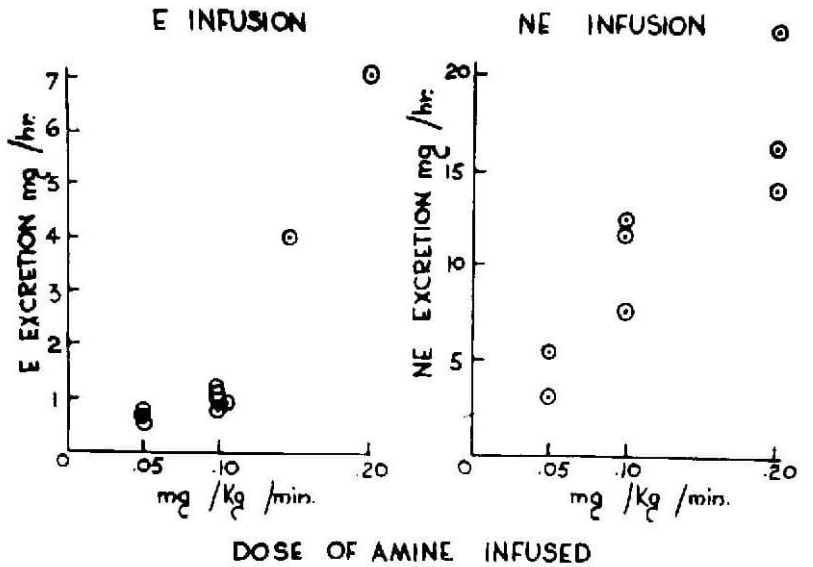
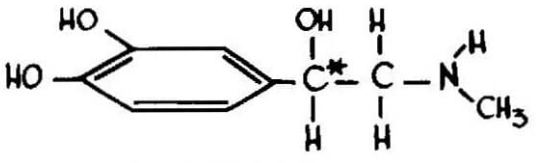
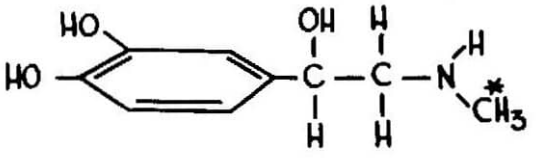


FIG. 23. Excretion of E and NE above control levels after 30-minute infusion of E and NE in doses of 0.05, 0.10 and 0.20 μg./Kg./min. (Elmadjian, F., *et al.*: J. Clin. Endocrinol. **17**:608)

essentially linear. However, in the case of E infusion, there is a sharp change in the slope at doses above 0.10 μg./Kg./min. While there is a twofold increase in the excretion rate with the doubling of the dose from 0.05 to 0.10 μg./Kg./min., there is a sevenfold increase in the excretion rate on subsequent doubling from 0.10 to 0.20 μg./Kg./min. These data will serve us in estimating the approximate secretion rate of E and NE in the studies reported.



BETA-LABELED
EPINEPHRINE



METHYL-LABELED
EPINEPHRINE

FIG. 24. Structures of isotopic epinephrine used. (Resnick, O., and Elmadjian, F.: J. Clin. Endocrinol.**18**:28)

As indicated from these experiments, only 0.5 to 2 per cent of the E infused could be accounted for in the 1-hour postinfusion urine, and 3 to 6 per cent of the NE in a similar experiment. This posed the question regarding the fate of the unaccounted-for material. Dr. Oscar Resnick of our laboratories has undertaken the study of the metabolism of E labeled at the beta position with C^{14}, as well as with E labeled at the methyl position with C^{14} (Fig. 24).[8] As indicated in Figure 25, the excretion of radioactivity is rapidly increased in the urine for the first few hours after the onset of the infusion. In the 7 subjects infused with the β-C^{14}-dl-epinephrine-d-bitartrate, 91 ± 3 per cent of the total radioactivity could be accounted for within 30 hours after the infusion. In the 3 subjects infused with methyl-C^{14}-dl-epinephrine, 34 ± 3 per cent of the total radioactivity could be accounted

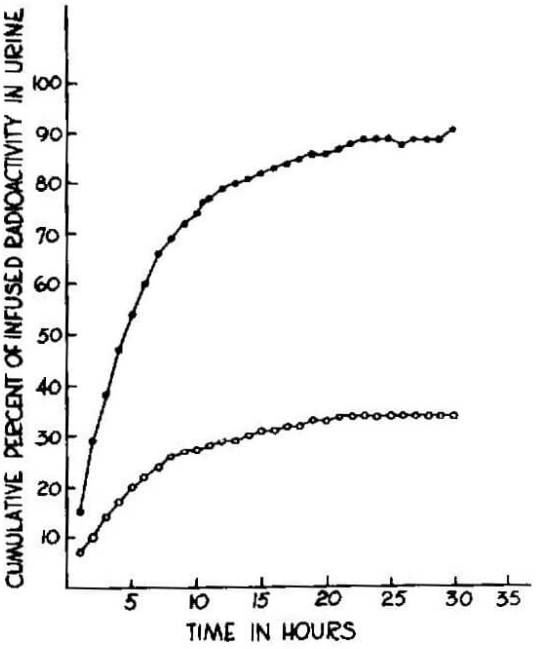


FIG. 25. Average cumulative hourly urinary excretion of total radioactivity. The top line (solid dots) represents the average values for 7 infusions with β-labeled epinephrine. The bottom line (open circles) represents the average values for 3 infusions with methyl-labeled epinephrine. (Resnick, O., and Elmadjian, F.: J. Clin. Endocrinol. **18**:28)

for in the urine. The infusion of both types of isotopically labeled E resulted in the increase in the urinary excretion of nonmetabolized biologically active E. This increase was demonstrable only during the first 2 hours after the onset of the infusion, after which the urinary excretion of biologically active E returned to the control levels. These observations are in agreement with the previous data just shown following the infusion of nonlabeled E in human subjects. The increase in biologically active E in these experiments ranged from approximately 1 to 2 per cent of the infused E. It should be mentioned that the bio-assays of the urine extracts measured biologically active E in the form of its laevo isomers. On the other hand, the radioactivity recovered in the urine was due to the metabolites derived from both dextro and laevo isomers of the isotopically labeled E and the nonmetabolized d-l epinephrine.

These results indicated that the metabolites of E are excreted over a

30-hour period. While the E, measurable by the bio-assay, appears in the first few hours (0.5 to 2% of the dose infused), the radioactivity excreted is 30 to 40 per cent (β-C^{11}-epinephrine) of the count given during the same period. In addition, 65 per cent of the metabolized E loses the methyl group during this process. This latter finding seems to support the hypothesis that oxidative deamination does take place in man.

Chromatography studies of β-labeled E indicated a major radioactive metabolite, which was found to be a phenolic acid having the same Rf values as authentic 3-methoxy-4-hydroxy mandelic acid, generously supplied by M. D. Armstrong.[9] Very little or no phenolic amines, such as methoxy-epinephrine, could be extracted from the urine of these patients; 34 ± 3 per cent of the radioactivity contained in the methyl-labeled E infused could be accounted for in the urine. While under iproniazid therapy, 3 patients were infused with methyl-labeled E;[10] 65 ± 5 per cent of the infused radioactivity was recovered in the urine. Two of the patients were again infused with methyl-labeled E, 2 to 3 weeks following the termination of iproniazid treatment; 50 per cent and 43 per cent of the infused radioactivity were recovered in the urine of these 2 patients, respectively. The urine of iproniazid-treated patients contained a major radioactive metabolite, which was found to be a phenolic amine having the same Rf values as those reported for methoxy-epinephrine by Axelrod.[11] These data clearly demonstrate O-methylation of E in man. Iproniazid treatment in man was observed to inhibit oxidative deamination of E, without influencing the process of O-methylation.

ADMINISTRATION OF AGENTS KNOWN TO STIMULATE THE SYMPATHICO-ADRENAL SYSTEM

Hypoglycemia is known to cause stimulation of the sympathico-adrenal system. The level of integration involved is principally at the midbrain and hypothalamic level. Using insulin as the hypoglycemic agent, a study was undertaken to evaluate the sympathico-adrenal responses of male subjects by measuring the excretion of E and NE. Lysergic acid diethylamide was administered to psychiatric patients to evaluate the accompanying sympathico-adrenal responses which also involve the higher centers such as the cerebral cortex.

A normal male subject was given an insulin-tolerance test for 5 consecutive days, Monday through Friday. A control urine was collected before insulin administration. Two samples of 90 minutes each were collected after insulin administration, representing a 3-hour postinsulin urine.

A second type of study consisted of a total of 16 samplings obtained from 3 psychiatric patients receiving insulin treatment (Fig. 26). All urine represented collections from 7:00 A.M. to about 11:00 A.M.; the former was the

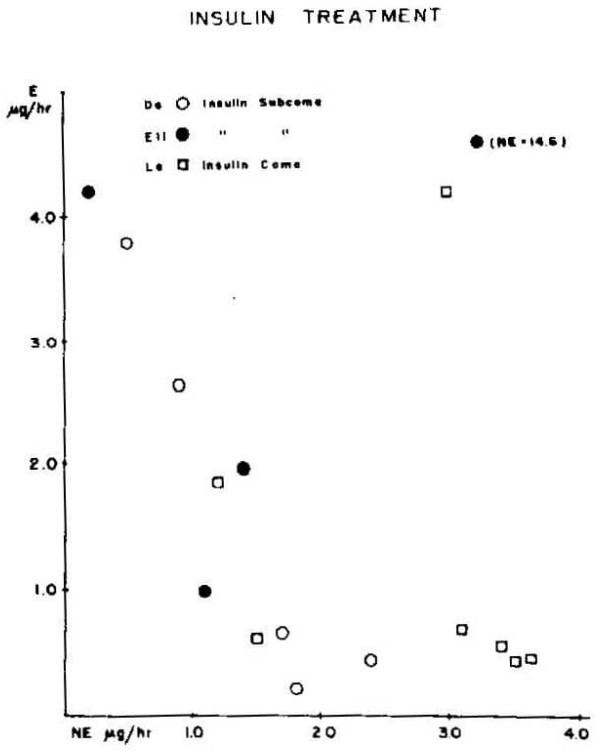


FIG. 26. E and NE in 16 samples from 3 patients treated with insulin. (Elmadjian, F., *et al.*: Rec. Progr. in Hormone Res. **14**:513)

time of insulin administration, the latter the termination time of the treatment. Subject La was in a coma in each of the samplings; Da was receiving the standard insulin treatment, and patient Ell was in the initial phase of the insulin-shock treatment, but in none of the samplings was the patient in coma.

The results obtained on the subject receiving the insulin-tolerance test for 5 consecutive days may be seen in Table 11.[12] We observe that by Thursday and Friday there is a decrease in the E excretion, even though the blood sugar levels at the 30-minute period from the injection of the insulin show no significant differences. The more interesting finding is the successive decrease in the E excretion in the second postinsulin collection, which decreases on the fifth day about 10 per cent of that found on the first day. The subjective report and the clinical observations were consistent in the conclusion that the insulin reaction was markedly reduced at the end of the week when compared with the first day.

TABLE 11. NORMAL SUBJECT RECEIVING INSULIN-TOLERANCE TEST FOR 5 CONSECUTIVE DAYS[12]*

DAY	SAMPLE†	NE (μg./hr.)	E (μg./hr.)	BLOOD GLUCOSE mg.% 0	30 min.
Monday	(C)	2.3	0.10	94	58
	(1)	2.6	0.50		
	(2)	2.3	0.54		
Tuesday	(C)	5.2	0.03	79	45
	(1)	2.6	0.68		
	(2)	2.4	0.36		
Wednesday	(C)	2.3	0.06	94	34
	(1)	2.1	0.64		
	(2)	2.4	0.24		
Thursday	(C)	2.9	0.03	91	42
	(1)	2.3	0.34		
	(2)	2.2	0.14		
Friday	(C)	2.6	0.03	102	44
	(1)	2.4	0.30		
	(2)	3.9	0.05		

* Male subject: weight, 70 Km.; insulin dose, 0.1 unit per Kg., intravenously.

† (C) = preinsulin-test collection; (1) = 90-minute insulin-test collection; (2) = 90-minute postinsulin-test collection.

There was a marked variability in the values obtained from the insulin-treated patients. In general, there was a marked elevation of E excretion. When 16 samples obtained from the 3 patients were plotted on graph, where the ordinate indicated the rate of E excretion and the abscissa the NE, Figure 26 was obtained.[12] We observe an inverse relation of NE to E in those samplings. The sample obtained on Ell which induced an elevated NE excretion was readily explained by the fact that the patient became extremely agitated during the course of the morning and was emotionally disturbed. For the sample obtained on La, which lies outside this distribution, there was no explanation. This tendency for the reduction in NE excretion with markedly elevated E values was also observed in previous studies in several subjects during the insulin-tolerance tests.[13]

LYSERGIC ACID DIETHYLAMIDE (LSD) ADMINISTRATION

Fourteen psychiatric subjects were given from 150 to 300 μg. of LSD orally.[7] Seven were chronic schizophrenics, 4 were manic-depressive patients, and 3 were involutional-depression cases (Table 12). The control collection was made from 10:00 A.M. to 2:00 P.M., at which time the LSD was administered. The LSD collections were made from 2:00 P.M. to 10:00 P.M. In this series the chronic schizophrenics showed no reaction to the

TABLE 12. LSD ADMINISTRATION TO PSYCHOTIC SUBJECTS[7]

PSYCHOTIC STATE	NO. OF SUBJECTS	NE (μg./hr.)	E (μg./hr.)
	A. NONREACTORS		
Chronic Schizophrenics:			
Control	7	2.3 ± 0.4	0.14 ± 0.02
LSD	7	2.0 ± 0.8	0.20 ± 0.03
	B. REACTORS		
Manic-depressives:			
Control	4	2.8 ± 0.5	0.21 ± 0.09
LSD	4	10.4 ± 1.5	1.38 ± 0.65
Involutional-depressives:			
Control	3	5.6 ± 0.2	0.21 ± 0.05
LSD	3	1.7 ± 0.4	1.11 ± 0.84

LSD in mood, affect or perception. They also showed no changes in NE and E excretion. On the other hand, in the manic-depressive patients and in those with involutional depression there was a marked increase in E excretion. In the manic-depressive group there was also an increase in NE excretion; in the involutional-depression group there was no change in NE values. In contrast with the schizophrenics, the other two groups manifested marked psychiatric changes following administration of LSD.

STRESS STUDIES ON NORMAL AND PSYCHIATRIC PATIENTS IN EXPERIMENTAL AND LIFE SITUATIONS

NORMAL SUBJECTS IN ANTICIPATORY STATES

Urine collections were made on 11 normal subjects before they underwent the usual tolerance test and the Mecholyl test described by Funkenstein.[13] The subjects consisted of laboratory personnel and students. There was a varying degree of knowledge and understanding as to the nature of these tests among the group. Note in Table 13 the variability of the excretion rates obtained. Those subjects who were familiar with the nature of the tests and the hospital setting were quiet and waited patiently. These subjects showed normal excretion rates of E and NE. On the other hand, the students and the personnel who were unfamiliar with the tests and the setting were restless, asked many questions of the attending nurse and the physician and were quite anxious about the experiments. Subjects Gi and

TABLE 13. PRETEST NE AND E EXCRETION OF NORMAL SUBJECTS[13]

NORMAL SUBJECTS	BEFORE INSULIN TEST		BEFORE MECHOLYL TEST	
	NE μg./hr.	E μg./hr.	NE μg./hr.	E μg./hr.
Ba	3.4	0.35	1.7	0.13
El*	3.2	0.16	4.9	0.17
El*	4.1	0.17	—	—
Fo	0.9	0.02	—	—
Ma	1.2	0.10	2.8	0.18
Sp	1.5	0.05	1.0	0.20
Zi	1.9	0.15	3.2	0.51
Gi	3.7	0.22	7.7	0.17
Dm	—	—	8.9	0.07
Ms	4.2	0.52	3.2	0.80
Sm	9.0	1.82	3.5	0.96
Pa	—	—	3.2	0.07

* Same subject tested on different days.

Dm showed very high NE excretions with normal E values, while Ms and Sm show high values for both amines in each instance.

PROFESSIONAL HOCKEY PLAYERS

This sport entails considerable aggressive activity in attack and defense. The game is fast and involves marked activity and man-to-man contact. Pregame samples of urine were collected at 10 to 30 minutes before game time, and postgame samples were collected some 3 hours later. The latter samples included urine formed during the contest. Game time was 8:30 P.M. In Table 14 are listed the values for pregame and postgame collections of defensemen and forwards who do the skating.[7] The data are presented in terms of creatinine because it was not always possible to obtain timed samples. For samples taken following the game, there was a sixfold increase in NE excretion. Two players were sampled before the game, but, on physical examination by the trainer, were not permitted to participate. In the same table are presented the data on these players, indicating no postgame increase in NE but appreciable increase in E. Both players sat on the bench and watched the game. Both were quite concerned about their injury.

Table 14 also gives the data on Player No. 16 who showed a ninefold increase in NE and a twentyfold increase in E. He skated his regular turn but did not play an outstanding game. At the end of the second period, however, he got involved in a lively fist fight with an opposing player and was ejected from the game.

The goal tender skates very little but is in constant vigilance in front of the net, ready to defend the goal. The coach remains on the bench directing

TABLE 14. EXCRETION OF NE AND E IN MEMBERS OF A PROFESSIONAL HOCKEY TEAM—DEFENSEMEN AND FORWARDS VS. NONPARTICIPATING PLAYERS*

URINE COLLECTION	NUMBER OF HOCKEY PLAYERS SAMPLED	NE (μg./100 mg. CREATININE)	E (μg./100 mg. CREATININE)
ACTIVE HOCKEY PLAYERS (DEFENSEMEN AND FORWARDS)†			
Pregame	20	2.7 ± 0.43	0.36 ± 0.07
Postgame	20	15.3 ± 2.20	0.95 ± 0.21
		t = 5.66	t = 2.68
		P = <0.001	P = <0.05 >0.01
TWO PLAYERS WHO DID NOT PARTICIPATE IN THE GAME†			
Pregame	1 (No. 18)	2.2	0.23
Postgame		3.3	0.75
Pregame	1 (No. 10)	5.6	0.78
Postgame		5.3	1.42
PLAYER INVOLVED IN FIST FIGHT			
Pregame	1 (No. 16)	3.5	0.18
Postgame		29.3	3.30

* The approximate hourly excretion is 10 per cent less than the figure given in terms of 100 Gm. creatinine when corrected by creatinine coefficient.

† Due to their physical condition.

strategy. Data obtained on the goal tender in 3 games and on the coach in 6 games are presented in Table 15. There was a marked increase in both E and NE excretion in the goal tender, but, on the average, there were no significant changes in the excretion of either amine in the coach. However, there were individual games where the coach showed marked increases in E.

AMATEUR BOXERS

Six amateur boxers competing in the finals of the Amateur Athletic Union boxing championship were studied.[3] Added significance was placed on these

TABLE 15. EXCRETION OF NE AND E IN GOAL TENDER AND COACH*

SUBJECT	NO. OF GAMES	URINE COLLECTION	NE (μg./100 mg. CREATININE)	E (μg./100 mg. CREATININE)
Goal tender	3	Pregame	3.3 ± 0.07	0.45 ± 0.20
		Postgame	9.2 ± 1.8	1.30 ± 0.81
Coach	6	Pregame	1.8 ± 0.5	0.38 ± 0.09
		Postgame	3.7 ± 0.9	0.43 ± 0.21

finals because the fighters who were to win ultimately would also qualify for the final Olympic tryouts. Enthusiasm and a high state of expectancy characterized the attitudes of the fighters. This was reflected in the elevated E excretion rates observed in most of the prefight samples. Table 16 shows the data obtained on all fighters and the outcome of the fights.

TABLE 16. EXCRETION OF NE AND E IN AMATEUR BOXERS[7]

BOXER	URINE COLLECTION	NE* (μg.)	E* (μg.)	OUTCOME
Ch	Prefight	17.9	1.64	Winner by decision
	Postfight	—	—	in the third round
Sm	Prefight	38.1	1.78	Winner by TKO in
	Postfight	32.4	0.67	the third round
Pe	Prefight	6.7	0.22	Winner by decision
	Postfight	2.8	0.41	in the third round
Br	Prefight	4.2	0.40	Winner by decision
	Postfight	7.0	0.87	in the third round
Wh	Prefight	1.7	1.67	Winner by TKO in
	Postfight	—	—	the second round
Bra	Prefight	—	—	Loser by decision
	Postfight	15.9	1.70	in the third round

* Micrograms per 100 mg. creatinine.

On 3 fighters only 1 sample was obtained; on Ch and Wh the sample was a precontest collection. Both boxers showed elevated E excretion. Each showed ample evidence of tenseness and apprehension in the prefight interview. After the interview, Ch went to the corner of the dressing room and shadowboxed for about 5 minutes, while Wh sat quietly on a bench with his trainer and refrained from shadowboxing before entering the ring. It may be observed that Ch showed an elevated NE excretion, while Wh's NE value was low.

The high NE prefight values were noted in fighters who engaged in shadowboxing before the contest. The highest prefight E excretions were found in the fighters who showed the greater degree of anticipation preceding the fight. Finally, increase in the postfight samples over the prefight samples of E were observed in those fighters who had to fight the distance for decision in close contests.

PSYCHOTIC SUBJECTS ON WHOM MALAMUD-SANDS RATING-SCALE RECORDS WERE OBTAINED

In 10 psychiatric patients a study was made of the relationship between results of Malamud-Sands rating-scale tests and the excretion of E and NE.[7] This scale rates the patient in 22 categories, and the data is recorded on a

check sheet. On the rating scale each item scored appears on the left-hand side. A designated base line appears through the center of the rating scale. Going from the base line to either the left or the right, the deviations are observed. In the preliminary analysis of the data, motor activity and hostility reactions were abstracted, and a composite score including these two items was computed. In Figure 27 is shown the relationship of this score to NE excretion; those with passive self-effacing emotional display had normal levels of NE. In Figure 27 when a line appears through the circles, it indicates that these subjects also had a high excretion of E. The subject indicated by an asterisk had normal NE excretion, but the excretion of E was 2.75μg. per hour, which is extremely high. This subject showed periodic bursts of excitement with expressions of fear and guilt.

Neuropsychiatric Patients Appearing at Staff Interviews

The conditions of the interview were in marked contrast with those of the athletes or the patients on the psychiatric wards. The patient sits across from the psychiatrist who is doing the interviewing, in the presence of some

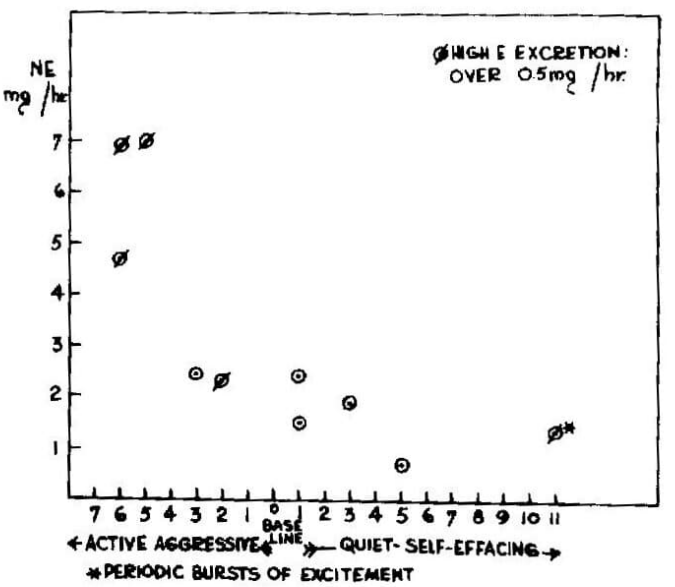


Fig. 27. The relation of NE to emotional states of neuropsychiatric patients. The abscissa depicts the composite score for the functions of motor activity and hostility reactions from the Malamud-Sands rating scale. The ordinate represents the excretion of NE in micrograms per hour during the observation period. (Elmadjian, F., *et al.*: J. Clin. Endocrinol. 17:608.)

20 members of the hospital medical staff. All participants are seated, and the exchange in this case is strictly verbal. The setting is serious, and to the patient it is very important because, on the basis of his performance, decisions are made by the staff which affect his immediate future. Eleven subjects were studied, on 8 of whom control samples were obtained. The interview took place on Thursday morning, and the control sample was obtained at the same time on the next day—Friday. The results are listed in Table 17.[7] There were no changes in NE excretion when the interview

TABLE 17. STAFF CONFERENCE INTERVIEW OF NEUROPSYCHIATRIC PATIENTS[7]

	No. of Subjects	NE (μg./hr.)	E (μg./hr.)
Interview	11	2.6 ± 0.4	0.50 ± 0.14
Control	8	2.3 ± 0.2	0.27 ± 0.12
		NS	P = <0.001

day was compared with the control. However, in every subject on whom a control was obtained there was an elevated excretion of E during the interview. As might be expected, there were marked individual variations in this increase. The subjects, in general, were self-effacing and on their best behavior with no aggressive or active emotional display.

THERAPIST AND PATIENT DURING INTERVIEWS

In the course of psychotherapeutic treatment, samples were obtained from both therapist and patient.[12] The therapeutic interview took place between 8:00 A.M. and 9:00 A.M. Samples were obtained before and after each therapeutic session, as well as on control days during the same hours when neither subject was involved in a psychotherapeutic interview. Six psychotherapeutic sessions and 6 control days were studied. Urine samples obtained were analyzed for NE, E and 17-hydroxycorticosteroids.[14] Figure 28 contains the data on both therapist and patient which was obtained during the therapy interview. Along the abscissa are indicated the control values for both therapist and patient. During the interview we observe that both patient and therapist showed normal values during the first psychotherapeutic session as compared with their control. In the second session a slight elevation is observed in the NE and 17-hydroxycorticosteroid excretion for the therapist, with the patient still within normal limits for these determinations. In the third session the results indicate that all values are within normal limits. In the fourth psychotherapeutic interview the samples indi-

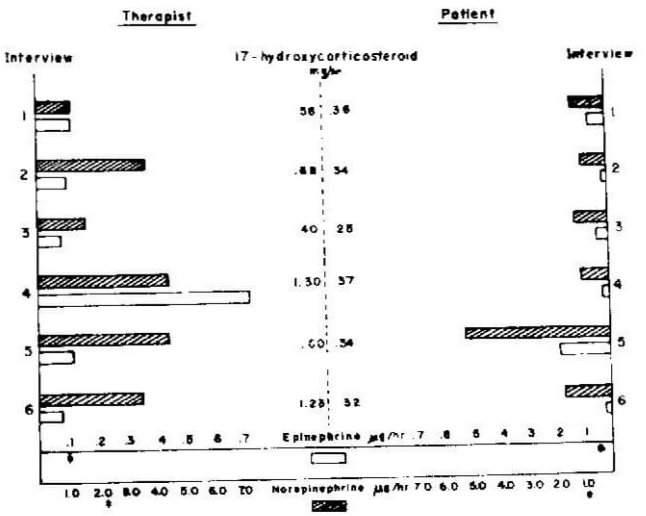


FIG. 28. Data on therapist and patient during 6 psychotherapeutic interviews. Asterisk indicates normal values for E and NE for patient. Double dagger represents normal values for therapist for same time of day when not engaged in psychotherapeutic process. Open bar indicates E excretion (μg. per hour); hatched bar depicts NE excretion (μg. per hour); 17-hydroxycorticosteroids of same samples given in center of figure as mg. per hour. (Elmadjian, F., *et al.*: Rec. Progr. in Hormone Res. **14**:513)

cated a twofold increase in the 17-hydroxycorticosteroid excretion, with marked elevations for both E and NE for the therapist. The patient still showed normal values. In the fifth psychotherapeutic session we observe elevated 17-hydroxycorticosteroid values for the therapist accompanied by high values in NE, but E values returned to normal. However, we now observe the patient showing a marked increase in NE excretion with considerable elevation in E excretion, especially for this patient with no increase in 17-hydroxycorticosteroid excretion. In the sixth interview we note again a highly elevated 17-hydroxycorticosteroid excretion for the therapist, with a moderately high NE value and normal E excretion. The patient's value shows an elevated NE, but E is back to normal.

Information obtained from the therapist indicated that in session 4 the therapist was severely criticized by the patient, and the interview got "out of hand." The therapist was aware of his predicament subjectively and did admit unpleasant emotional experience. In the fifth session, during the course of the psychotherapeutic interview the patient cried, showing considerable emotional expression. The therapist was quite concerned about

the turn of events, which had now taken an unexpected course with regard to the therapy. This aspect is again shown with the sixth session, where the therapist again shows high values in 17-hydroxycorticosteroid and an elevated NE excretion. These data indicate that there is a possibility to study by means of these measurements the emotional exchanges between the therapist and the patient during a psychotherapeutic process. From the physiologic point of view, it may also be noted that the excretion of those substances during such an interview apparently reaches levels which are identified with stressful tasks, such as participation in athletics. These results again show that NE excretion under certain circumstances in the therapist or the patient increases without an elevation in E, which further emphasizes the fact that these hormones may be differently secreted, possibly depending on the nature of the emotional stress and the accompanying sympathetic-adrenal responses.

SUMMARY

The excretion of epinephrine (E) and norepinephrine (NE) was determined (1) after the administration of agents known to stimulate the sympathetic-adrenal nervous system and (2) in stress studies on normal and and psychiatric patients in experimental and life situations. The results support the hypothesis that active aggressive emotional displays are related to increased excretion of NE, with or without excretion of E, whereas, tense, anxious, but passive, emotional displays are related to increased excretion of E in association with normal excretion of NE.

From infusion studies it was found that under the foregoing conditions of stress, secretion rates of NE rose to levels comparable with those during infusion of 0.2 μg. of NE per Kg. per minute, whereas the highest E levels were comparable with those during infusion of 0.1 μg. of E per Kg. per minute.

Metabolism of C^{14}-epinephrine indicates that oxidative deamination may be one of the pathways of E metabolism. The isolation by Armstrong of 3-methoxy-4-hydroxy mandelic acid as a metabolite of NE and the tentative identification of the latter compound as a metabolite of E, as well as 3-methoxy-epinephrine, further supports this view. Data obtained from patients treated with iproniazid is interpreted as indicating inhibition of oxidative deamination without change in O-methylation mechanism.

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Discussion

HOAGLAND. Dr. Elmadjian's paper is now open for discussion.

RESNICK. I would like to make a few comments on the Marsilid studies that were carried out on 4 female schizophrenic subjects. According to the clinical report, 2 of these patients showed marked psychiatric improvement, and 2 showed no signs of psychiatric response to the Marsilid treatment. Nevertheless, all 4 patients showed a remarkably similar alteration in the metabolism of exogenously administered epinephrine, both with respect to the rate of excretion and the type of metabolites found in the urine. When a schizophrenic patient or a normal control, not under Marsilid therapy, was infused with labeled epinephrine, very little radioactive methoxy-epinephrine could be extracted from the postinfusion urine samples. However, these urine samples consistently contained a radioactive metabolite, which was a phenolic acid having the same Rf values as authentic 3-methoxy-4-hydroxy mandelic acid. When schizophrenic patients were placed under Marsilid therapy for several months, 150 mg./day, and then infused with labeled epinephrine, the postinfusion urine samples contained very little radioactive 3-methoxy-4-hydroxy mandelic acid but contained large amounts of a radioactive phenolic amine having the same Rf values as methoxy-epineph-

rine. Approximately half the effect of Marsilid on monamine oxidase activity, as reflected by the metabolism of exogenously administered epinephrine, was still evident 2 to 3 weeks after cessation of Marsilid therapy. Following infusion of labeled epinephrine into these patients, the methoxy-epinephrine in the urine began to diminish and 3-methoxy-4-hydroxy mandelic acid began to reappear in the urine. On the basis of this very limited number of experiments, one may tentatively conclude that no relationship was found to exist between psychiatric response to Marsilid and its effect on the overall metabolism of exogenously administered epinephrine. However, these experiments do not shed any light on possible actions occurring in the central nervous system.[1] Recently, Udenfriend *et al.*[2] reported that iproniazid treatment in animals had no effect on the metabolism of exogenously administered serotonin or tyramine and had little effect on the peripheral depots of serotonin. However, there was a large increase in the stockpiling of serotonin in the brain. Whether such a stockpiling of catechol amines occurs in the brains of human subjects under Marsilid therapy is not at present definitely known. Some workers explain the central actions of Marsilid and other compounds on the basis of their effects on serotonin storage or metabolism, others on their effects of catechol amine storage or metabolism and still others on their effects on other amines found in the brain. This greatly colors the views held concerning the action of Marsilid and other drugs in psychiatric patients.

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KETY. I would like to express my great admiration for these studies which Dr. Elmadjian and Dr. Resnick have done in the metabolism of epinephrine in normal man under emotional states and their studies in schizophrenia. With regard to the studies with the C^{14}-tagged epinephrine, I think that their group is the first to have undertaken a study of the in vivo metabolism of epinephrine in man. Dr. Elmadjian referred to the work of Dr. Axelrod, and, lest the group here get the impression that at the National Institute of Mental Health we do nothing but try to disprove everybody else, I wonder, Mr. Chairman, whether it might be possible for Dr. Axelrod to spend a few minutes elucidating some of the contributions, positive contributions, which he has made to this field.

HOAGLAND. Yes, by all means.

AXELROD. For the past 20 years, the catechol amines were considered to be metabolized primarily by oxidative deamination; however, recent studies from Zeller's group[1] and others have indicated that there are other

pathways in the metabolism of the catechol amines. Armstrong, last February, reported that a major metabolite of norepinephrine[2] is 3-methoxy-4-hydroxy mandelic acid. This suggested to us that this compound may arise in 3 ways: (1) by oxidative deamination followed by methylation, or (2) methylation followed by deamination or (3) both pathways occurring simultaneously. Armstrong favored the first pathway, that is, oxidative deamination followed by methylation. We examined rat urine for the presence of 3-methoxy-4-hydroxy metabolite of epinephrine, which we named "metanephrine" and the corresponding congener "normetanephrine." We found present in the rat urine both metanephrine glucuronide, which was reported in *Science* about 8 months ago,[3] and normetanephrine glucuronide. We then administered epinephrine and measured the excretion of the methoxy compounds. We conclusively identified the structure of these compounds found in the urine (Table 18). After the administration of epinephrine to the rat,

TABLE 18. O-METHYLATION OF EPINEPHRINE IN THE RAT

Compound Administered	(Free Form) Metanephrine Excreted (%)	(Glucuronide Form) Metanephrine Excreted (%)	Total Metanephrine Excreted (%)
Epinephrine	3	22	25
Metanephrine	8	27	35
Epinephrine + Iproniazid	4	51	55
Metanephrine + Iproniazid	10	60	70

we found about 3 per cent metanephrine excreted in the free form and 22 per cent as a glucuronide. Total excretion of metanephrine was 25 per cent. This amount does not necessarily represent the total amount of epinephrine which is methylated. Some of the methylated metanephrine could, in turn, be further metabolized. In order to correct for the further metabolism of metanephrine, we administered authentic metanephrine and found that 35 per cent was excreted, indicating that about 65 per cent of any metanephrine which is formed is further metabolized. Thus, we calculate that about 70 per cent of epinephrine is methylated. Then we administered iproniazid to rats. This compound inhibits the deamination by monamine oxidase. After the administration of iproniazid, we found that the excretion of metanephrine had increased more than twofold, indicating that epinephrine is first methylated and then deaminated to yield Armstrong's compound. After the administration of metanephrine, we observed that the

excretion of metanephrine also increased twofold or threefold. Figure 29

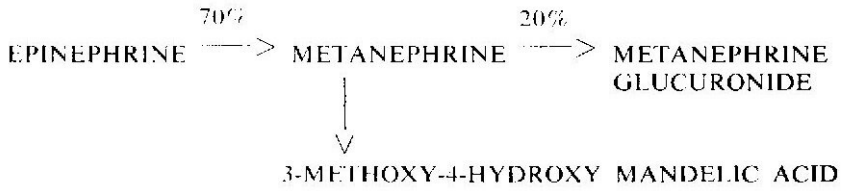

FIG. 29. Pathway for the metabolism of epinephrine in the rat.

indicates the possible pathway in the rat for the metabolism of epinephrine. About 70 per cent of epinephrine is O-methylated to metanephrine; 20 per cent of metanephrine in turn is conjugated as a glucuronide and the remainder metabolized, presumably deaminated to 3-methoxy-4-hydroxy mandelic acid. Normetanephrine follows an analogous pathway. We have also found large amounts of normetanephrine present in the urine of man with pheochromocytoma tumors.

We also found an enzyme which can carry out the O-methylation of catechol amines. This enzyme requires the presence of S-adenosylmethionine as a methyl donor, as well as a divalent metal (Table 19). We found that

TABLE 19. ENZYMATIC O-METHYLATION OF EPINEPHRINE

	METANEPHRINE FORMED (μMOLS)
Complete system	0.059
AMe omitted	0.000
Mg++ omitted	0.004

Epinephrine was incubated at 37° with rat liver, S-adenosylmethionine (AMe) magnesium chloride

TABLE 20. SPECIES DISTRIBUTION OF O-METHYLATING ENZYME

SPECIES	METANEPHRINE FORMED μMOL/GM./LIVER
Monkey	10.0
Rat	5.0
Cow	3.3
Pig	2.9
Mouse	2.3
Guinea pig	1.0
Man*	0.4
Cat	0.3
Rabbit	0.1

* Liver was kept at −10° for 3 months before enzyme assay.

this enzyme can attack only catechols. For example, catechols such as dopamine, dopa, norepinephrine and 3,4-dihydroxy mandelic acid are also methylated. This enzyme is widely distributed in a number of mammalian species, including man (Table 20). The enzyme is widely distributed in many tissues (Table 21). All tissues which we examined, except skeletal

TABLE 21. TISSUE DISTRIBUTION OF O-METHYLATING ENZYME

ORGAN	METANEPHRINE FORMED μMOL/GM./TISSUE
Liver	7.0
Kidney	2.1
Spleen	0.5
Small intestine	0.3
Lung	0.3
Brain	0.2
Heart	0.1
Skeletal muscle	0.0

muscle, contained the enzyme. I meant to point out that we found metanephrine and normetanephrine present in the adrenal glands of rats and normetanephrine present in the spleen of rats. After the administration of iproniazid we found normetanephrine present in the brain. We found an enzyme in the brain which can methylate normetanephrine and an enzyme which can deaminate normetanephrine. This deamination is blocked by iproniazid.

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RESNICK. Dr. Armstrong very generously sent me a sample of authentic dihydroxy mandelic acid. In our work we extract urine of patients infused with beta-labeled epinephrine for phenolic acids, according to the methods of Armstrong *et al.*[3] 3-methoxy-4-hydroxy mandelic acid is the only radioactive phenolic acid detectable by our counting technics. If, however, we place our paper chromatograms on x-ray film and keep them in the dark for 25 weeks, another phenolic acid appears when the radioautographs are developed. Thus, this phenolic acid is present in the urine in very small amounts and could very possibly be dihydroxy mandelic acid. I might add further that the in vitro and the in vivo studies of Dr. Axelrod

on the metabolism of epinephrine in the rat and mouse correspond beautifully to the results obtained in this laboratory in man.

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GAUDETTE. Armstrong's observations with cases of pheochromocytoma, which he presented at the Federation meetings, prompted us to investigate, like Dr. Axelrod, the metabolism of epinephrine in liver homogenates, fortified with the Cantoni methylating system. We also were able to show the presence of both VMA, i.e., 3-methoxy-4-hydroxy mandelic acid and 3-methoxyepinephrine. In our current investigations we find that the extent of methylation is dependent on the methionine concentration. Paper chromatograms of our incubated mixtures give rise to a fast-running metabolite which we believe to be dimethoxylated, i.e., 3,4-dimethoxy mandelic acid. However, if the amine oxidase system is blocked by Marsilid, the predominant metabolite is believed to be 3,4-dimethoxyepinephrine. The interesting aspect of these studies concerns the metabolic pattern found in stressed rats. By stressing rats in a rotating cage, and incubating liver homogenates therefrom in our fortified system, the predominant metabolite is again believed to be the 3,4-dimethoxyepinephrine. In view of the psychotomimetic action of such compounds as mescaline, these findings permit interesting speculation. A more complete study will be presented at the spring Federation meetings.[1]

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KETY. This morning Drs. Resnick and Elmadjian indicated that they had some data relevant to the possible presence of adrenochrome as an abnormal metabolite in schizophrenia. I wonder if the sensitivity of the isotopic technics which they have used is sufficient to exclude the possibility of significant quantities of adrenochrome being formed from epinephrine in schizophrenics?

ZELLER. I am quite interested to hear about using iproniazid as a tool in the study of the metabolism of catechol amines in human subjects. In this connection, I would like to ask first if anybody has used iproniazid as well as isoniazid. It is an easy way to check whether monamine oxidase is involved, since isoniazid has no action whatsoever on monamine oxidase. Second, I wonder if by using iproniazid we do not disturb the system under investigation. Do we get more methylation by blocking monamine oxidase,

since obviously more catechol amines remain available for methylation? Thus we might get a distorted picture about the relative amount of oxidation and methylation taking place in an inhibited situation.

AXELROD. I do not know whether you recall Schayer's work where he found two major metabolites, Peak I and Peak II. Peak I from the Rf values was metanephrine, whereas Peak II was VMA, and they were both excreted to about an equal extent, and this subject was given physiologic amounts of epinephrine. When he was given Marsilid, Peak I rose at the expense of Peak II, which would explain our results and Dr. Elmadjian's. That is, what is happening is probably several things: first, epinephrine is methylating and deaminating, the deaminating product is methylated, and the methylated product is being deaminated. All of these things are occurring simultaneously.

ELMADJIAN. May I answer Dr. Kety? Whether our methods are sensitive enough, I cannot say. Dr. Resnick has tried, and Dr. Schayer tried before him. Both have come up with negative answers to the question of whether adrenochrome is a metabolite of epinephrine. That is all we can say at the present time. It is not too often that an interesting and startling finding is reported in the literature and found to be confirmed, as Dr. Kety has indicated by the NIH group. I want to state that in this particular matter of the metabolism of epinephrine, NIH was first; we were in the position of confirming results as they applied to humans, since Dr. Axelrod's paper appeared some months ago. I believe Dr. Resnick's results will be published this month.