EXCRETION AND METABOLISM OF EPINEPHRINE[1]

FRED ELMADJIAN

Worcester Foundation and Worcester State Hospital, Massachusetts

The principal problems of the excretion and metabolism of epinephrine were reviewed by Bacq ten years ago (5). Our objectives were (a) to study the differential excretion of biologically active epinephrine (E) and norepinephrine (NE) under various stress conditions and (b) to ascertain the nature of the metabolism of E in man. This presentation consists principally of a report of the cooperative research conducted by the Worcester Foundation and the Worcester State Hospital, involving the direct participation of Drs. Justin M. Hope, Harry Freeman, Oscar Resnick and Mr. Edwin T. Lamson.

Method. Urine was extracted by the alumina adsorption method of von Euler and Hellner (12). The bioassay was performed by a modification of the method described by Gaddum and Lembeck (15). Infusion experiments were conducted with the use of unlabeled E and NE, and with E labeled with C^{14} at the *beta* position and E labeled at the methyl carbon. Urine from C^{14}-E infusions was processed and paper chromatographic techniques were applied (2, 16).

Excretion of epinephrine

Epinephrine is secreted in most part from the adrenal medulla, since a number of investigators have shown that doubly adrenalectomized human subjects excrete very little, if any E in urine (9, 14). From infusion experiments it is apparent that only 0.5% to 2.0% of the total E infused is recoverable in the urine as biologically active E (8).

There is marked variability in the excretion of E in the course of a 24-hour period (7). In addition to this variability, there is the variability within individuals under the same situation. We have noted the range of excretion in a number of subjects who were in a reclining position (presumably resting) before they were to undergo the mecholyl test and the insulin tolerance test (8). The subjects who were familiar with the nature of the tests and the hospital setting were quiet and waited patiently; they had normal excretion rates of E and NE. On the other hand, students and personnel unfamiliar with the tests and the setting were restless, asked many questions of the attending nurse and physicians and were rather anxious about the experiment; such subjects had very high excretions of NE with normal values for E, or high values for both amines in each instance.

Hypoglycemia induced by insulin administration results in a selective excretion of E (8, 13). Studies were conducted on a male subject who was given an insulin tolerance test for 5 consecutive days, Monday through Friday (7). The

[1] Aided in part by grants from the Army Medical Research and Development Board, Contract No. DA-49-007-MD-438; the Ford Foundation and the Scottish Rite Committee of Research for Dementia Praecox of the National Association of Mental Health.

data indicated a diminution in E excreted on successive days. These results may be interpreted to indicate a depletion in the capacity of the adrenal medulla to produce E under these conditions.

Sixteen samplings were obtained from 3 psychiatric patients receiving insulin treatment (7). One subject was in coma in each of the samplings, another was receiving the standard insulin subcoma treatment, and the third was in the initial phase of the insulin shock treatment, but in none of the samplings was he in coma. We observe an inverse relation of NE to E in these samplings. A sample obtained on the third subject showed an elevated NE excretion which was readily explained by the fact that the patient became extremely agitated during the course of the morning and was emotionally disturbed. A tendency for the reduction in NE excretion with markedly elevated E values was also observed in several subjects during the insulin tolerance tests carried out on the normal subjects (8).

Professional hockey players. Hockey as a sport entails considerable aggressive activity in attack and defense. The game is fast and involves marked activity and man-to-man contact. Pre-game samples of urine were collected at 10 to 30 minutes before the game and the post-game samples were collected some 3 hours later (6). The latter samples included urine formed during the contest. Game time was 8:30 P.M. For samples taken following the game, there was a 6-fold increase in NE excretion. Two players were sampled before the game, but on physical examination by the trainer, they were not permitted to participate. In these players there was no post-game increase in NE, but appreciable increase in E. Both players sat on the bench and watched the game. Both were concerned about their injuries.

One player showed a 9-fold increase in NE and a 20-fold increase in E. He skated his regular turn, but did not play an outstanding game. At the end of the second period, 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 strategy. Data were obtained on the goal tender in 3 games, and on the coach in 6 games. 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. There were, however, 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 (6). Added significance was placed on these finals because the fighters who were ultimately to win 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 pre-fight samples.

The high NE pre-fight values were noted in fighters who engaged in shadow boxing before the contest. The highest pre-fight E excretions were found in the

fighters who showed the greater degree of anticipation preceding the fight. Finally, increase in the post-fight samples over the pre-fight samples of E were observed in those fighters who had to fight for a decision in a close contest.

Basketball players. Basketball as a sport is characterized by skills related to timing and coordination of muscles, especially of the shoulders and arms. Unlike hockey, where aggression is essential, in basketball aggressiveness may very well lose the game. Continuous vigilance with considerable self-control is a basic requirement. Five players of the Boston Celtics were sampled during the first play-off series with Philadelphia in 1958 (11). Samples were obtained before and after the contest; the data were expressed in terms of micrograms of E and NE/100 mg creatinine, in view of the difficulty in obtaining timed samples. Three players showed marked increases in E during the contest. In none of the samples was there marked elevations in NE. One player showed a very high level of E before the game with a very marked elevation in NE as well. During the course of the game his E titer dropped, but his NE excretion rate was further elevated. This player is considered in basketball circles to be one of the most outstanding basketball players of our time, and during this game he was observed to be very tense and preoccupied. His performance during this game was not up to level of that expected of him. Another player showed a drop in both E and NE excretions. Though he did not play his usual game, this drop is unexplained.

The interesting feature of these data is the marked elevations in E excretions, with relatively normal NE excretions.

Baseball team. In general, baseball as a sport is characterized by periods of physical inactivity, interspersed with short periods of maximum effort and vigilance. Boston University baseball team was sampled in the Spring of 1958, during a crucial game with Holy Cross College (11). The game was won by Holy Cross, 3–2, but Boston University had several times in the late innings an opportunity to win the game. Urine was collected during a 2½- to 3-hour period, consisting of a brief period before the game where the team had batting and fielding practice, and during the actual period of the contest. Each sample consisted of the urine formed during this 2½- to 3-hour period. There was great variability in the data. Of particular interest were the elevations of E excretions of the rightfield, firstbase, pitcher and catcher, as well as the coach. The NE excretions of the centerfield, firstbase, leftfield, pitcher and the coach were elevated. The coach in this case was fairly active throughout the game, giving orders and planning strategy, and the data showed marked elevations in E and NE. He was rather disappointed with the outcome of the game, a reaction shared especially by the pitcher, who performed very well, though he was the losing pitcher.

These results were consistent with the general activity of the players during the game, except for the unexplained elevation for E in the rightfield.

Psychotic patients on closed wards. In 10 psychiatric patients a study was made of the relationship between results of Malamud-Sands rating scale and the excretions of E and NE (6). Patients with passive, self-effacing emotional display

had normal levels of NE. One subject had normal NE excretion, but the excretion of E was 2.75 μg/hr, which is extremely high. This subject showed periodic bursts of excitement with expressions of fear and guilt.

Neuropsychiatric patients during staff interviews. The conditions of the interview were in marked contrast to those encountered by the athletes or patients on the psychiatric wards. The patient sat across from the psychiatrist doing the interviewing in the presence of some 20 members of the hospital medical staff. All participants were seated and the exchange in this case was strictly verbal. The setting was serious and to the patient it was very important, because on the basis of his performance decisions were made by the staff which affected his immediate future. Eleven subjects were studied, on 8 of whom control samples were obtained (6). The interview took place one morning and the control sample was obtained at the same time on the next day. There were no changes in NE excretion when the interview 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 displays.

Psychotherapeutic process. In the course of psychotherapeutic treatment, samples were obtained from both therapist and patient. The therapeutic interviews 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 (7). In a typical case, during the first interview both patient and therapist showed normal values during the psychotherapeutic session as compared with their controls. In the second session a slight elevation was 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 indicated that all values were within normal limits. In the fourth psychotherapeutic session, the samples indicated a 2-fold 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 session, elevated 17-hydroxycorticosteroid values were observed for the therapist, accompanied by high values in NE, but E values returned to normal. However, now the patient showed a marked increase in NE excretion with considerable elevation in E excretion, especially for this patient, with no increase in 17-hydroxycorticosteroids. In the sixth interview, again there was a highly elevated 17-hydroxycorticosteroid excretion for the therapist with a moderately high NE value and normal E excretion. The patient's value showed an elevated NE, but E was back to normal.

Information obtained from the therapist indicated that in the fourth session 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 an unpleasant emotional experience. In the fifth session, during the course of the

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 was again shown with the sixth session where the therapist again showed high values of 17-hydroxycorticosteroids and an elevated NE excretion.

These results support the hypothesis that active, aggressive, emotional displays are related to increased excretion of NE, whereas tense, anxious, but passive, emotional displays are related to increased excretion of E in association with normal excretions of NE.

Metabolism of epinephrine

Circulatory responses are observed within a minute after the initiation of an infusion of E or NE; on termination of the infusion there is a prompt return of the hemodynamics back to normal levels. It is safe to assume from these experiments that there is a rapid inactivation of the catecholamines. Antoniades *et al.* (1) have reported on the transport of E and NE in human plasma. After fractionation of the blood by methods described by E. Cohn and his collaborators, chemical analysis was made of the catecholamines by methods of Weil-Malherbe and Bone as modified by Aronow (3) and the bioassays were done by the modification of the method described by Gaddum and Lembeck (15). The results suggested that E is almost completely bound to plasma proteins and the protein responsible for the major binding and transport of E is albumen. NE was also found to be bound to the albumen fraction, but far more NE was observed to be unbound than E. Acid hydrolysis yielded increased titer; therefore, it seems that conjugates of these amines are also bound to proteins.

Epinephrine and NE appear in the urine in the free form and as conjugates (10, 12). Acid hydrolysis adds to the measurable titer of E and NE. Examination of the nature of the conjugate indicates that NE is apparently in the form of a glucuronide and not a sulfate (10). Experiments concerned with E have been negative, both with respect to hydrolysis with *beta* glucuronidase and with mylase-P which was the source of phenolsulfatase (10).

As indicated previously, only 0.5% to 2.0% of the E infused could be accounted for in the 1-hour postinfusion urine, and 3.0% to 6.0% of the NE in a similar experiment (9). This posed the question regarding the fate of the unaccounted 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}. 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 $\pm$ 3% 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 $\pm$ 3% of the total radioactivity could be accounted 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 following the infusion of nonlabeled E in human subjects. The increase in biologically active E in these experiments ranged from approximately 1 to 2% of the infused E. It should be mentioned that the bioassays of the urine extracts measured biologically active E in the form of its *levo* isomers. The radioactivity recovered in the urine, on the other hand, was due to the metabolites derived from both *dextro* and *levo* isomers of the isotopically labeled E and the nonmetabolized *dl*-E.

These results indicate that the metabolites of E are excreted over a 30-hour period. While the E measurable by the bioassay appears in the first few hours (0.5 to 2.0% of the dose infused), the radioactivity excreted is 30 to 40% (β-C^{14}-epinephrine) of the count given during the same period. In addition, 65% of the metabolized E loses the methyl group during the process. This latter finding seems to support the hypothesis that oxidative deamination does take place in man (16).

It seemed reasonable to assume that subjects placed on iproniazid therapy should excrete more than 34% of the methyl-labeled E upon infusion. Three such patients who were infused with methyl-labeled E after several weeks of iproniazid treatment, showed 59%, 74% and 63% of the infused radioactivity in the urine. Upon cessation of the treatment, the patients were again infused with methyl-E and the counts excreted were 50% and 43% (17). The urine from these patients was collected following the infusion of either *beta*-labeled or methyl-labeled E. The urine samples were lyophilized and stored at 0 to 5° C. The lyophilized urine was reconstituted with water and extracted for phenolic acids, according to the procedure of Armstrong *et al.* (2). The extracts were concentrated down to a small volume, *in vacuo*, at 45° C. An aliquot of the concentrated extract was chromatographed in the butanol:acetic acid:water system (4:1:5). Another aliquot was chromatographed in the two-phase solvent systems of Armstrong *et al.* (2). The phenolic acids were visualized by spraying with diazotized *p*-nitroaniline reagent. Autoradiograms were made from the chromatograms, in order to visualize these metabolites, which were derived from the infused labeled E. The urine which had been extracted for phenolic acids was hydrolyzed and selectively extracted for methoxy-E in accordance with the procedures outlined by Axelrod (4). The extracts were concentrated down to a small volume, *in vacuo*, at 45° C and subjected to paper chromatographic analysis, as outlined above.

The following results were obtained. The urine of non-iproniazid-treated patients infused with *beta*-labeled E consistently showed a major radioactive metabolite, which was a phenolic acid having the same R_f values as authentic 3-methoxy-4-hydroxymandelic acid. Very little methoxy-E could be extracted from the urine of these patients. The urine of iproniazid-treated patients infused with methyl-labeled E consistently showed a major radioactive metabolite, which was a phenolic amine having the same R_f value as authentic methoxy-E (17). The increase in excretion of radioactivity by the iproniazid-treated patients infused with methyl-labeled E could be accounted for by the accumulation of methoxy-E with a decrease in formation of 3-methoxy-4-hydroxymandelic acid.

The autoradiograms of urine obtained from patients infused with *beta*-labeled E showed the presence of another phenolic acid metabolite of E. This metabolite occurred in very much smaller concentration than 3-methoxy-4-hydroxy mandelic acid and had the following R_f values: isopropyl alcohol ammonia, 0.22; benzene propionic acid, 0.12. Authentic dihydroxy mandelic acid has R_f values of 0.25 and 0.19 in the aforementioned solvent systems. This radioactive metabolite, occurring in trace quantities, is tentatively considered to be 3,4-dihydroxy mandelic acid.

The results of these experiments clearly indicate that iproniazid treatment in man inhibits the action of monamine oxidase, but does not influence those enzymes which are responsible for the O-methylation of E.

REFERENCES

1. ANTONIADES, H. N., GOLDFIEN, A., ZILELI, S. AND ELMADJIAN, F.: Transport of epinephrine and norepinephrine in human plasma. Proc. Soc. exp. Biol., N.Y. **97**: 11-12, 1958.
2. ARMSTRONG, M. D., SHAW, K. N. F. AND WALL, P. E.: The phenolic acids in human urine. Paper chromatography of phenolic acids. J. biol. Chem. **218**: 293-303, 1956.
3. ARONOW, L. AND HOWARD. F. A.: Improved fluorometric techniques to measure changes in adrenal epinephrine-norepinephrine output caused by veratrum alkaloids. Fed. Proc. **14**: 311, 1955.
4. AXELROD, J.: O-Methylation of epinephrine and other catechols *in vitro* and *in vivo*. Science **126**: 400-401, 1957.
5. BACQ, Z. M.: The metabolism of adrenaline. Pharmacol. Rev. **1**: 1-26, 1949.
6. ELMADJIAN, F., HOPE, J. M. AND LAMSON, E. T.: Excretion of epinephrine and norepinephrine in various emotional states. J. clin. Endocrin. **17**: 608-620, 1957.
7. ELMADJIAN, F., HOPE, J. M. AND LAMSON, E. T.: Excretion of epinephrine and norepinephrine under stress. Recent Progr. Hormone Res. **14**: 513-553, 1958.
8. ELMADJIAN, F., LAMSON, E. T., FREEMAN, H., NERI, R. AND VARJABEDIAN, L.: Excretion of epinephrine and norepinephrine after administration of insulin and methacholine. J. clin. Endocrin. **16**: 876-886, 1956.
9. ELMADJIAN, F., LAMSON, E. T. AND NERI, R.: Excretion of adrenaline and noradrenaline in human subjects. J. clin. Endocrin. **16**: 222-234, 1956.
10. ELMADJIAN, F., LAMSON, E. T. AND NERI, R.: The nature of noradrenaline and adrenaline in normal human urine. J. clin. Endocrin. **16**: 216-221, 1956.
11. ELMADJIAN, F.: Unpublished.
12. EULER, U. S. VON AND HELLNER, S.: Excretion of noradrenaline, adrenaline, and hydroxytyramine in urine. Acta physiol. scand. **22**: 161-167, 1951.
13. EULER, U. S. VON AND LUFT, R.: Effect of insulin on urinary excretion of adrenalin and noradrenalin. Metabolism **1**: 528-532, 1952.
14. EULER, U. S. VON: Some aspects of the clinical physiology of noradrenaline. Scand. J. clin. Lab. Invest. **4**: 254-262, 1952.
15. GADDUM, J. H. AND LEMBECK, F.: The assay of substances from the adrenal medulla. Brit. J. Pharmacol. **4**: 401-408, 1949.
16. RESNICK, O. AND ELMADJIAN, F.: The metabolism of epinephrine containing isotopic carbon in man. J. clin. Endocrin. **18**: 28-31, 1958.
17. RESNICK, O., WOLFE, J. M., FREEMAN, H. AND ELMADJIAN, F.: Iproniazid treatment and metabolism of labeled epinephrine in schizophrenics. Science **127**: 1116-1117, 1958.