Biochemical Pharmacology, 1965, Vol. 14, pp. 1621–1633. Pergamon Press Ltd., Printed in Great Britain.

PLASMA CATECHOLAMINE LEVELS AND URINARY EXCRETION OF CATECHOLAMINES AND METABOLITES IN TWO HUMAN SUBJECTS DURING A CYCLE OF MORPHINE ADDICTION AND WITHDRAWAL*

H. WEIL-MALHERBE, E. R. B. SMITH, A. J. EISENMAN and H. F. FRASER

National Institute of Mental Health, Clinical Neuropharmacology Research Center, Saint Elizabeths Hospital, Washington, D.C.; and Addiction Research Center, Lexington, Ky., U.S.A.

(*Received* 18 *March* 1965; *accepted* 17 *May* 1965)

Abstract—Plasma catecholamine levels and the urinary excretion of catecholamines and catecholamine metabolites were studied in two male volunteers during a cycle of morphine addiction and withdrawal.

Plasma catecholamine values were within normal limits except for an elevated epinephrine level in Subject 1 on the first day of withdrawal.

At the beginning of the addiction phase the excretion of epinephrine, norepinephrine, dopamine, 3-methoxy-4-hydroxymandelic acid, and the catecholic acid fraction, consisting predominantly of 3,4-dihydroxyphenylacetic acid, rose significantly in both subjects. The excretion of metanephrine and normetanephrine was also increased in Subject 1, but not in Subject 2. The excretion rates, with the exception of the catecholic acid fraction in Subject 2, returned to normal or below normal during the withdrawal phase. These results indicate a stimulation of catecholamine discharge during the addiction phase but provide no evidence for its stimulation during the period of withdrawal.

STIMULATION of the sympatho-adrenomedullary system by morphine is well known and is attested to by elevated catecholamine levels in the suprarenal vein blood of dogs[2–5] and by the depletion of catecholamines in the adrenal medulla of dogs and cats[6, 7], rabbits,[8, 9] and rats[10]. Morphine is also known to cause changes in the catecholamine concentration of brain, heart, and spleen, their extent and direction depending on species, dosage, and mode of administration.[11–18] Finally, as Gunne[18] has shown, chronic morphine administration increases the excretion of epinephrine and norepinephrine in dogs and rats. This increase disappears as partial tolerance develops and reappears after each step-up in the medication schedule. Sudden withdrawal of the drug is followed by a marked rise of catecholamine excretion, lasting for several days.

It seemed to be of interest to study the effect of chronic morphine administration on catecholamine metabolism in human subjects for several reasons: it was desirable to compare the results obtained in animal experiments with the response in man; methods for the estimation of some of the major metabolites of the catecholamines have become available so that it is now possible to obtain a better integrated picture of catecholamine discharge and metabolism; and, finally, such a study might lead to better understanding and improved management of the withdrawal syndrome.

* A preliminary report of these studies has been made.[1]

EXPERIMENTAL

The experiment was performed on two male volunteers, both former addicts, in the Research Ward of the Addiction Research Center in Lexington, Kentucky. The subjects were maintained on the regular hospital diet except that desserts containing vanilla were withheld.

Urine was collected on a 24-hr basis in bottles containing 10 ml of 10 N sulfuric acid. When the volume of a specimen exceeded 1,000 ml, additional acid was added to make the final concentration 0·1 N. Aliquots of 48-hr pooled specimens were frozen, packed in dry ice, and shipped by air to the laboratory at Saint Elizabeths Hospital for analysis. During the first seven days of the withdrawal period samples were taken from 24-hr specimens. Blood samples (20 ml) were collected 2 hr after administering a placebo or morphine, except during withdrawal when samples were collected 26, 50, and 74 hr after the last full dose of morphine. The specimen was mixed with 5 ml of anticoagulant solution[19] and centrifuged immediately. The plasma was frozen and sent to the laboratory in dry ice.

Body temperature, pulse rate. respiratory rate, and blood pressure were recorded daily. Some additional measurements were aimed at the assessment of physical activity; these procedures have been described by Fraser *et al.*[20]

The course of morphine addiction was preceded by 30 days of preliminary observation. Blood samples were collected on days 15 and 18, 2 hr after the injection of a placebo, and on days 22 and 29, 2 hr after the s.c. injection of 0·3 mg morphine sulfate /kg. The addiction period was started on day 30 with four doses of 10 mg morphine sulfate, s.c. The daily dose was gradually increased over a period of 17 days to a maximum of 3 mg/kg and was continued at this level for another 50 days in Subject 1. Subject 2 became anxious, nervous and apprehensive about withdrawal as the dosage of morphine sulfate was advanced. He requested that the medication be discontinued; this was done 20 days after the maximal daily dose had been attained, i.e. day 37 of addiction. Despite frequent complaints his physical examination was essentially negative throughout intoxication. Subject 1 received no morphine on the first day of withdrawal; doses varying from 5 to 20 mg/day were administered during the subsequent 13 days except for the fourth day when he required 120 mg. Subject 2 received 60 mg on the first day of withdrawal, 8 mg on the second, and 4 mg on the third; 150 mg was administered on the fourth day. The 60 mg given on the first day of withdrawal was a single dose injected at 6 a.m. It may therefore still be considered as part of the addiction regime.

Observations were continued for 22 days in Subject 1 and for 65 days in Subject 2, but in the latter case only one sample per week was collected during the last 50 days of the experiment.

The experiment may thus be divided into periods of preaddiction, addiction, withdrawal, and recovery.*

ANALYTICAL

Plasma analysis. Epinephrine and norepinephrine were determined in venous plasma by the modified ethylenediamine method, after purification on columns of alumina and Amberlite CG 50, as described by Weil-Malherbe[19].

* The term recovery should not be interpreted to mean that recovery was complete in all respects and for the entire span of this phase. In fact, there is evidence that many symptoms of withdrawal persist for prolonged periods.

Urine analysis. The following compounds were estimated: the three catecholamines (epinephrine, norepinephrine and dopamine); metanephrine and normetanephrine; 3-methoxy-4-hydroxymandelic acid (vanillylmandelic acid, "VMA") and total catecholic acids (mainly 3,4-dihydroxyphenylacetic acid, "dopac"). The methods used have recently been summarized.[21] Briefly, urine was hydrolyzed at pH 1·5 for 20 min, brought to pH 8·3, and passed through a column of alumina. The column was eluted first with 0·2 N acetic acid (eluate 1), then with 1 N sulfuric acid (eluate 2). Eluate 1 was passed through a column of Amberlite CG 50 at pH 6.0 and the column eluted with 1 M acetic acid. Epinephrine and norepinephrine were determined in the eluate by an adaptation of the method of von Euler and Lishajko.[22] In a separate aliquot of the eluate the sum of the three catecholamines was estimated by condensation with ethylenediamine, and dopamine was determined by difference.[23] Eluate 2 was combined with the filtrate from the Amberlite column. This fraction contained the catecholic acids. It was extracted with ethyl acetate, the extract evaporated, and the residue, at pH 5·0, passed through a column of DEAE-Sephadex, A-25. In the eluate (1 M acetic acid) the catecholic acids were determined fluorimetrically after condensation with ethylenediamine and the results expressed in terms of dopac.[21] Metanephrine and normetanephrine were estimated in the filtrate from the alumina column according to Smith and Weil-Malherbe.[24] VMA was estimated in a separate portion of urine by a modification [21] of the method of Sunderman *et al.*[25]

Attempts were made to estimate 3,4-dihydroxymandelic acid (DHMA) and 3-methoxy-4-hydroxyphenylglycol (MHPG). For the estimation of DHMA the same fraction was used as for the estimation of total catecholic acids, but passage through DEAE-Sephadex was omitted. DHMA was oxidized to protocatechuic aldehyde at 70° (pH 4·5) in the presence of 10^{-4} M cupric ion, a modification of the method of Miyake *et al.*[26] However, subsequent research showed that dopac caused some interference which, owing to the large preponderance of dopac over DHMA in the fraction, was sufficient to vitiate the results. The method used for the estimation of MHPG was similar to that used for the estimation of VMA except that the urine was hydrolyzed and that ethyl acetate extraction was carried out at pH 7·0. Since the validity of this method has not yet been sufficiently established, the results, like those of the DHMA estimations, are omitted, although they demonstrated changes very similar to those observed with other catecholamine metabolites.

RESULTS

Plasma catecholamines

The results of the plasma catecholamine estimations were, on the whole, within the normal range of the method, which extends from 0 to 1·0 μg/liter plasma for both epinephrine and norepinephrine.[19] A concentration of epinephrine in excess of 1 μg/liter was observed only once, i.e. in Subject 1 on the first day of withdrawal when 1·98 μg epinephrine and 0·11 μg norepinephrine/liter of plasma were found. Unfortunately, the corresponding sample of Subject 2 was lost. On the second, third, and fourth days of withdrawal the plasma epinephrine content of Subject 1 was 0·46, 0·05, and 0 μg/liter and his plasma norepinephrine content was 0, 0·04, and 0. The plasma epinephrine concentrations of Subject 2 on the second and third day of withdrawal were 0·46 and 0·25 μg/liter and the corresponding norepinephrine

concentrations were 1·31 and 0·37. Blood samples were also collected from two other subjects on the last day of addiction and 26 hr after the last dose of morphine, but no significant reaction was observed.

A series of plasma samples from both Subjects 1 and 2 was collected after a placebo injection, after injections of isolated doses of morphine, or at various stages during the addiction period, but failed to demonstrate any significant reaction: epinephrine concentrations ranged from 0 to 0·78 and norepinephrine concentrations from 0 to 0·86 μg/liter.

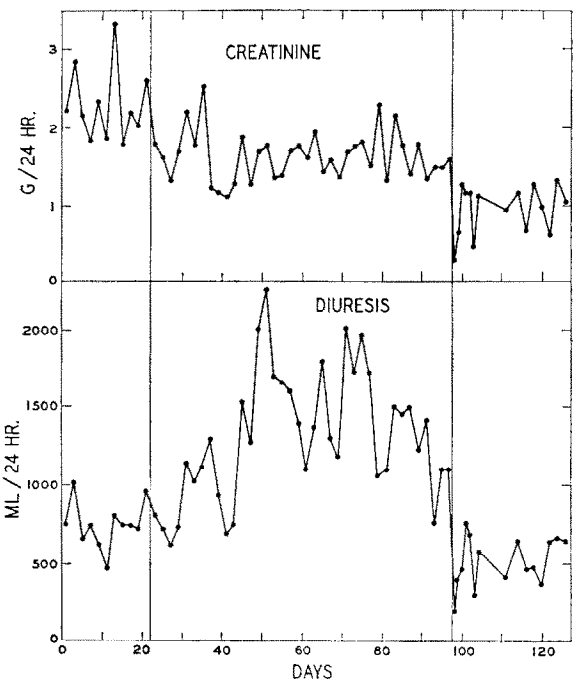

FIG. 1. Creatinine and urine output of Subject 1. The vertical lines separate the period of addiction (center) from the periods of preaddiction, withdrawal, and recovery.

Urine analyses

The point-by-point creatinine and volume output of Subjects 1 and 2 is shown in Figs. 1 and 2. The urine output of Subject 1 increased remarkably during the addiction phase, whereas his creatinine excretion decreased, particularly during the withdrawal and recovery periods. This was possibly due to incomplete collection of urine, since the subject had diarrhea, as is common in withdrawal.[27] Both creatinine and volume output show much less fluctuation in the case of Subject 2. In view of the possibility of urine losses during parts of the experiment, all results were expressed in terms of μg/g creatinine. Since the daily creatinine output of both subjects, at least during the preaddiction period was close to 2 g, the figures given (μg/g) represent about one-half of the μg/24-hr value.

The excretion of catecholamines and catecholamine metabolites in the two subjects during the different phases of the experiment is shown in Table 1. To reveal possible changes during the course of addiction, the addiction phase was subdivided into three

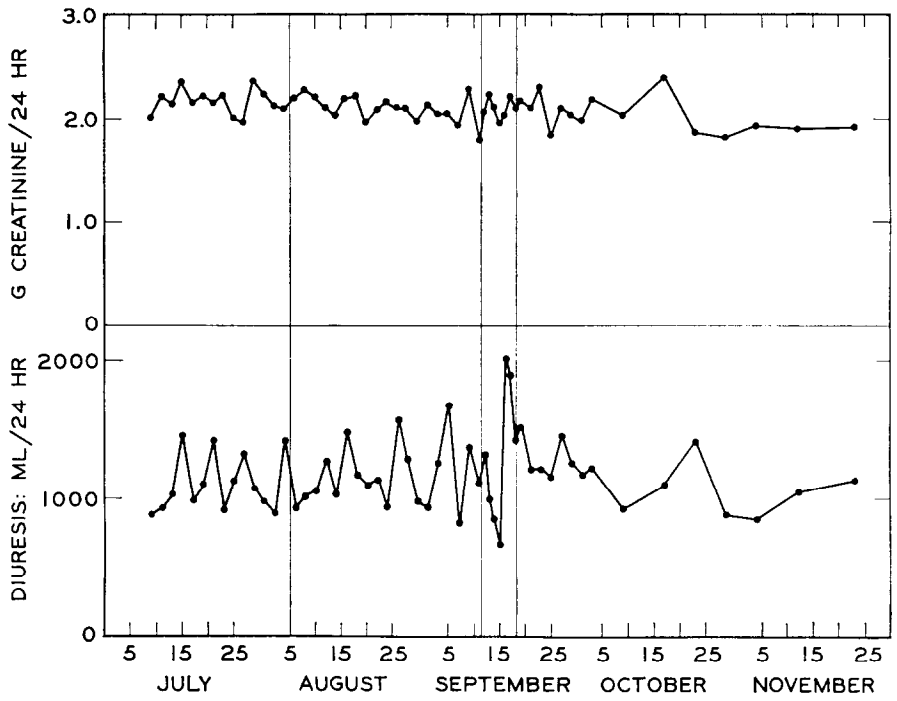

FIG. 2. Creatinine and urine output of Subject 2. The vertical lines separate preaddiction, addiction, withdrawal, and recovery periods.

periods of 22 or 23 days in Subject 1 and into two periods of 20 and 17 days in Subject 2. The statistical significance of the changes observed was checked by the *t* test, and the results thus obtained were assembled in Fig. 3 (relating to Subject 1) and Fig. 4 (relating to Subject 2). The figures consist of a series of squares arranged in horizontal rows and vertical columns which represent the different periods of the experiment. Each square contains a comparison between the period shown on the left-hand side of the rows and an earlier period shown on top of the columns, the earlier period serving as point of reference. Increases are denoted by the symbols shown beneath an arrow pointing upward and decreases by those placed beneath an arrow pointing downward. Both figures are divided into four quadrants by two thick lines. The boxes in the left upper quadrant show the changes on passing from the control period to the addiction periods, those in the right upper quadrant show changes occurring during addiction, those in the right lower quadrant show the changes when passing from the addiction to the withdrawal and recovery phases, and those in the left lower quadrant contain a comparison of the control period with withdrawal and recovery periods.

In Subject 1 the onset of the addiction phase brought increases in all the urinary constituents measured. The three major metabolites of epinephrine and norepinephrine (metanephrine, normetanephrine, and VMA) were elevated throughout the addiction phase except for metanephrine which returned to a near-normal level in the third period of addiction. Withdrawal of the drug was accompanied by a rapid decrease in the excretion levels of the metabolites. The point-by-point curves (Fig. 5) give a more detailed picture of these changes.

TABLE 1. URINARY EXCRETION OF CATECHOLAMINES AND CATECHOLAMINE METABOLITES (μg/g CREATININE, MEANS ± S.E.M.)

Days of experiment	Phase	Number of samples	Epinephrine	Norepinephrine	Dopamine	Metanephrine	Normetanephrine	VMA	Catecholic acids*
Subject 1									
1–29	Preaddiction	15	1·12 ± 0·24	12·6 ± 1·41	166 ± 22·9	25·4 ± 2·36	79·5 ± 6·93	1,161 ± 83·1	578 ± 81·1
30–52	Addiction I	11	3·46 ± 0·45	21·4 ± 3·96	304 ± 64·9	49·7 ± 5·07	138 ± 13·7	2,418 ± 331	1,753 ± 581
53–75	Addiction II	11	1·76 ± 0·94	18·6 ± 2·99	412 ± 104	41·8 ± 4·00	158 ± 18·7	2,458 ± 219	2,397 ± 423
76–97	Addiction III	12	2·17 ± 1·39	13·4 ± 1·93	308 ± 55·9	30·0 ± 2·62	143 ± 12·4	2,138 ± 194	1,562 ± 370
98–105	Withdrawal	7	2·85 ± 0·95	18·0 ± 3·43	106 ± 14·9	19·6 ± 1·58	94·6 ± 6·84	1,536 ± 227	361 ± 116
110–128	Recovery	8	4·72 ± 0·90	22·0 ± 3·27	335 ± 116·5	20·25 ± 2·51	85·1 ± 9·67	1,124 ± 207	335 ± 145
Subject 2									
1–29	Preaddiction	14	2·36 ± 0·33	13·6 ± 2·00	208 ± 30·5	36·4 ± 3·73	27·2 ± 4·69	814 ± 128	457 ± 107
30–49	Addiction I	10	4·40 ± 0·55	19·0 ± 1·98	349 ± 38·4	31·8 ± 3·61	16·9 ± 3·16	1,543 ± 71·4	1,218 ± 209
50–66	Addiction II	9	1·93 ± 0·68	16·5 ± 2·52	233 ± 43·5	35·5 ± 4·00	23·7 ± 4·94	1,567 ± 231	1,696 ± 290
67–74	Withdrawal	7	2·34 ± 0·64	13·5 ± 1·94	284 ± 54·1	22·5 ± 1·74	39·8 ± 4·51	1,353 ± 203	1,687 ± 292
75–145	Recovery	15	1·56 ± 0·24	12·3 ± 0·66	171 ± 15·2	36·6 ± 3·03	31·8 ± 4·86	1,189 ± 85·6	1,112 ± 104

* Expressed as dopac.

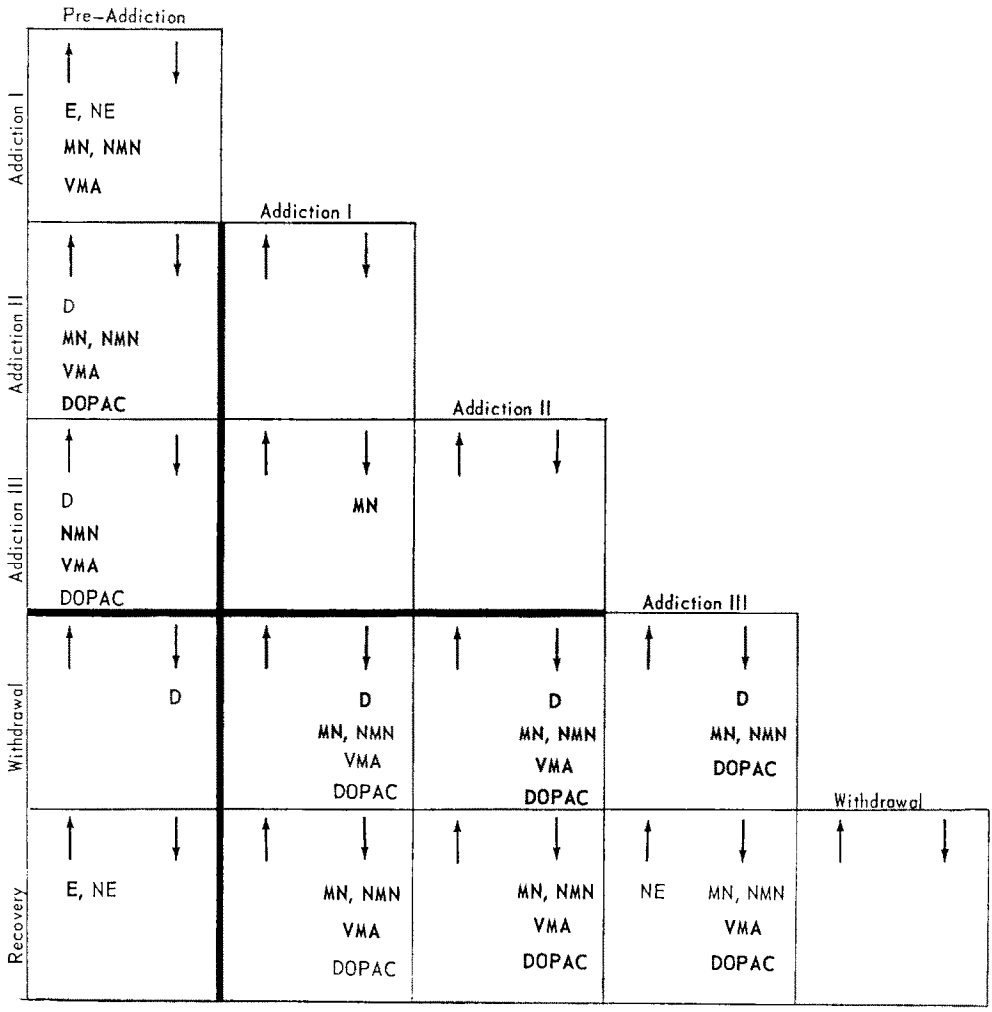

FIG. 3. Significant changes in the excretion of catecholamines and catecholamine metabolites by Subject 1.

E = epinephrine, NE = norepinephrine, MN = metanephrine, NMN = normetanephrine, VMA = vanillylmandelic acid, D. = dopamine, DOPAC = 3,4-dihydroxyphenylacetic acid. Characters in bold type: $P \leqq 0{\cdot}01$. Characters in light type: $P \leqq 0{\cdot}05$. See text for further explanations.

The excretion of epinephrine and norepinephrine rose slightly during the first stage of addiction in Subject 1, but returned to near-control levels during the second and third stage. No rise was observed during the withdrawal phase, but both catecholamines increased during the recovery period. Dopamine excretion was elevated during the second and third addiction stage and decreased markedly to below the control level during withdrawal.

Total catecholic acids, consisting mainly of dopac, a principal metabolite of dopamine,[28, 29] rose and fell more or less together with dopamine.

Subject 2 resembled Subject 1 in showing increased excretion of VMA and catecholic acids in both stages of addiction and of epinephrine in the first stage of addiction.

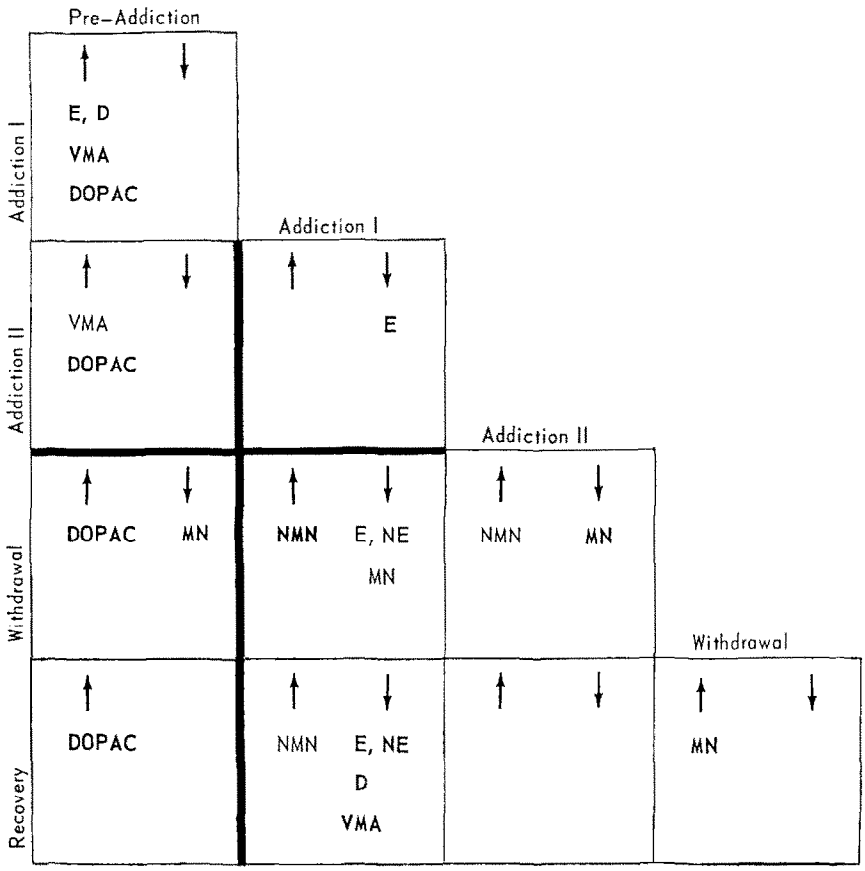

FIG. 4. Significant changes in the excretion of catecholamines and catecholamine metabolites by Subject 2. See legend to Fig. 3.

Dopamine excretion was elevated during the first stage of addiction. The excretion of epinephrine, norepinephrine, dopamine, metanephrine, and VMA was lower during the phases of withdrawal and recovery than during addiction. There were, on the other hand, certain differences, particularly regarding normetanephrine. The level of this metabolite was low to begin with and did not rise during addiction but showed an increase during the withdrawal phase. The metanephrine level did not change in response to addiction either, but dropped during the withdrawal phase as in Subject 1.

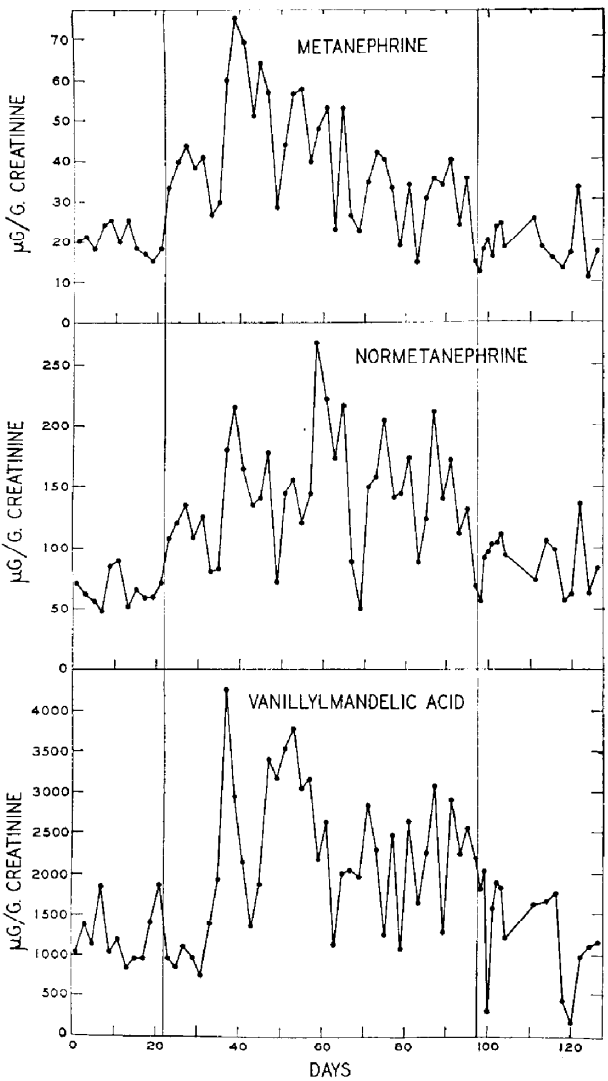

FIG. 5. Excretion of metanephrine, normetanephrine, and vanillylmandelic acid by Subject 1. The vertical lines separate the addiction period (center) from the periods of preaddiction, withdrawal, and recovery.

Clinical observations

After withdrawal of the drug both subjects exhibited a moderate degree of abstinence symptoms which were greatly attenuated by the administration of a large dose of morphine on the fourth day of withdrawal.

The results of routine clinical observations and activity measurements are shown in Table 2. Both subjects showed a drop in pulse and respiratory rate during the phases of addiction and an accelerated pulse rate during the period of recovery, but they were discordant in other respects. Thus, the body temperature of Subject 1 remained normal during addiction but rose during withdrawal by 0·3°, whereas that of Subject 2 was elevated not only during withdrawal but was already elevated during addiction.

TABLE 2. CLINICAL OBSERVATIONS*

Phase	Number of observations	Weights (kg)	Rectal temperature (°C)	Pulse rate (per min)	Respirate (per min)	Systolic blood pressure (mm Hg)	Sleep (hr/day)	Bed rest (hr/day)	Hours off the ward daily	Pedometer miles daily
Subject 1										
Preaddiction	29	72·6 ± 0·118	36·97 ± 0·022	70·0 ± 0·74	14·4 ± 0·32	114·8 ± 0·55	6·7 ± 0·16	9·0 ± 0·24	3·9 ± 0·36	9·9 ± 0·92
Addiction 1	22	72·4 ± 0·092	37·02 ± 0·028	65·8 ± 0·84	12·6 ± 0·32	113·3 ± 0·81	5·1 ± 0·23	7·9 ± 0·80	2·6 ± 0·34	5·9 ± 1·18
Addiction 2	22	71·9 ± 0·073	37·02 ± 0·023	63·9 ± 0·64	12·8 ± 0·28	110·9 ± 0·75	6·2 ± 0·23	9·2 ± 0·27	1·8 ± 0·23	5·8 ± 0·44
Addiction 3	23	71·2 ± 0·092	37·04 ± 0·023	66·4 ± 0·80	13·5 ± 0·29	116·7 ± 0·86	6·9 ± 0·16	9·6 ± 0·23	2·4 ± 0·25	6·6 ± 0·47
Withdrawal	8	68·4 ± 0·251	37·34 ± 0·087	73·6 ± 1·98	14·6 ± 0·71	123·6 ± 1·79	7·7 ± 0·39	12·9 ± 1·30	2·4 ± 0·98	5·3 ± 1·04
Recovery	22	68·8 ± 0·212	37·11 ± 0·044	75·5 ± 0·71	15·1 ± 0·26	121·3 ± 0·72	7·2 ± 0·18	10·4 ± 0·27	4·5 ± 0·59	7·0 ± 0·55
Subject 2										
Preaddiction	29	78·3 ± 0·069	37·0 ± 0·031	78·0 ± 0·16	17·5 ± 0·14	112 ± 0·53	6·3 ± 0·17	9·3 ± 0·32	5·7 ± 0·16	6·4 ± 0·13
Addiction 1	22	78·1 ± 0·094	37·3 ± 0·049	77·0 ± 1·42	14·0 ± 0·60	112 ± 1·28	5·4 ± 0·10	8·1 ± 0·18	7·2 ± 0·43	8·1 ± 0·40
Addiction 2	16	76·7 ± 0·244	37·3 ± 0·003	71·0 ± 1·80	13·0 ± 1·29	110 ± 1·65	6·4 ± 0·30	9·0 ± 0·35	5·2 ± 0·58	7·6 ± 0·20
Withdrawal	9	75·8 ± 0·290	37·3 ± 0·055	86·5 ± 2·30	17·4 ± 1·50	109 ± 6·12	6·1 ± 0·28	11·0 ± 0·42	5·8 ± 0·50	7·5 ± 0·20
Recovery	20	79·0 ± 0·230	37·1 ± 0·028	90·1 ± 0·50	18·0 ± 0·63	118 ± 1·40	6·4 ± 0·14	9·5 ± 0·27	6·4 ± 0·48	6·4 ± 0·62

* Means ± S.E.M.

Only Subject 2 showed a significant rise in pulse rate during withdrawal. Systolic blood pressure fell in Subject 1 during the second stage of addiction, returned to normal during the third stage, and rose above the normal level during withdrawal; in Subject 2 systolic blood pressure was unchanged during addiction and withdrawal, but rose during recovery. Addiction markedly depressed motor activity (pedometer miles) in Subject 1; motor activity remained below normal during withdrawal and recovery. In Subject 2 motor activity was stimulated during addiction and withdrawal, although the hours spent in the recumbent position were increased during withdrawal in both subjects.

The changes mentioned in this section are all statistically significant ($P < 0.01$).

DISCUSSION

The observations made during this investigation are in accordance with animal experiments, inasmuch as they show an increased excretion of catecholamines and catecholamine metabolites during a course of chronic morphine administration, particularly during the phase of increasing dosage. In Subject 1, not only the three catecholamines but also all the metabolites estimated take part in the increase of excretion rate, whereas in Subject 2 only VMA, but not metanephrine and normetanephrine, are excreted at a higher rate. This might suggest that metanephrine and normetanephrine are not reliable indicators of increases in catecholamine discharge. We were unable, on the other hand, to demonstrate a clear-cut sympatho-adrenomedullary activation during the withdrawal phase. On the contrary, our results, particularly those pertaining to Subject 1, seem to indicate a rather abrupt fall in catecholamine discharge. The similarity of the changes in the various parameters measured lends added weight to this conclusion. Two facts seem to point in the opposite direction: the isolated observation of an increased plasma epinephrine level on the first day of abstinence in Subject 1, and the isolated increase in normetanephrine excretion during the withdrawal phase in Subject 2. Their significance is doubtful and can be assessed only by further experiments.

In his experiments with dogs and rats Gunne[18] found large increases in epinephrine and norepinephrine excretion from days 2 to 5 of abstinence. But it must not be forgotten that, while the maximum dose during chronic administration was 360–1,800 mg/kg in rats and 90–180 mg/kg in dogs, it was only 3 mg/kg in the human experiments. Moreover, whereas in the animal experiments withdrawal was absolute, it was more gradual in the human experiments. The degree of stress was therefore presumably considerably more severe in the former than in the latter. The presence of stress under the conditions of the human experiment was demonstrated by Eisenman and colleagues,[30, 31] who found increased levels of 17-hydroxycorticosteroids in urine and plasma during the withdrawal period. The question arises whether the continuous stimulation of the sympatho-adrenomedullary system during the addiction period raised its stress-response threshold with the result that the stress of abstinence was sufficient to excite the adrenocortical system (depressed during the addiction phase) but insufficient to elicit a significant response from the sympatho-adrenomedullary system.

It may be noted that in some respects the excretion pattern during the recovery period differs from that of the preaddiction period. In Subject 1 the excretion of epinephrine and norepinephrine, but not of their metabolites, is increased, suggesting

a metabolic inhibition, possibly at the level of monoamine oxidase. Other changes, although they are not at the level of statistical significance, viz. a slight decrease in the excretion of catecholic acids and an increase of dopamine excretion, point in the same direction. In Subject 2, on the other hand, the excretion of the three catecholamines is about the same as in the preaddiction period, while the excretion of VMA and of catecholic acids is increased.

The fact that the excretion of dopamine and the catecholic acid fraction is subject to changes akin to those affecting the excretion of epinephrine, norepinephrine, and their metabolites confirms numerous observations of a similar nature made by the group at St. Elizabeths Hospital. As far as the composition of the catecholic acid fraction is concerned, unpublished chromatographic studies of this laboratory have shown that dopac accounts for 60–80 % of it. 3,4-Dihydroxymandelic acid, if it can be detected at all, is present only in traces.

The oxidation of tyrosine to 3,4-dihydroxyphenylalanine (dopa) is now considered to be the rate-limiting step in the reaction sequence leading to the synthesis of norepinephrine.[32] It is assumed that norepinephrine synthesis is under feedback control, the formation of dopa from tyrosine is the most likely point where such a regulation might be exerted. Since dopamine and its metabolites are situated "downstream" of the control point, they would be expected to be excreted at an increased rate when norepinephrine synthesis is accelerated.

It seems paradoxical that in our two cases pulse rate, respiratory rate, and systolic blood pressure were decreased during the addiction phase, when catecholamine discharge appeared to be raised, and that the situation was reversed during withdrawal and recovery. It suggests that chronic morphine administration leads to a depression, and abstinence to an increase, of sympathetic reactivity. On the other hand, the extreme lassitude and lethargy so frequently complained of by patients during withdrawal might well be considered consistent with a lowered catecholamine excretion during withdrawal and, to some extent, during recovery. Objectively, these feelings are observable by the increased hours of bed rest during withdrawal.

REFERENCES

1. H. WEIL-MALHERBE, E. R. B. SMITH, A. J. EISENMAN and H. F. FRASER, *Fed. Proc.* **22**, 567 (1963).
2. H. SATO and F. OHMI, *Tohoku J. exp. Med.* **21**, 411 (1933).
3. M. WADA, H. TANAKA, T. HIRANO and Y. TANEITI, *Tohoku J. exp. Med.* **34**, 52 (1938).
4. Y. SATAKE, *Tohoku J. exp. Med.* **60**, Suppl. 2 (1954).
5. A. F. DESCHAEPDRYVER, *On the Secretion, Distribution and Excretion of Adrenaline and Noradrenaline*. St. Catherine Press, Bruges (1959).
6. T. R. ELLIOTT, *J. Physiol. (Lond.)* **44**, 374 (1912).
7. N. EMMELIN and R. STROMBLAD, *Acta physiol. scand.* **24**, 261 (1951).
8. T. ABE, *Abstr. Jap. J. med. Sci.* **4**, 100 (1929).
9. H. TADA, *Tohoku J. exp. Med.* **19**, 405 (1932).
10. A. S. OUTSCHOORN, *Brit. J. Pharmac.* **7**, 605 (1952).
11. M. VOGT, *J. Physiol. (Lond.)* **123**, 451 (1954).
12. G. QUINN and B. B. BRODIE, *Med. exp. (Basel)* **4**, 349 (1961).
13. E. W. MAYNERT and G. I. KLINGMAN, *J. Pharmac. exp. Ther.* **135**, 285 (1962).
14. G. I. KLINGMAN, and E. W. MAYNERT, *J. Pharmac. exp. Ther.* **135**, 300 (1962).
15. M. SEGAL and G. A. DENEAN, *Fed. Proc.* **21**, 327 (1962).
16. J. W. SLOAN, J. W. BROOKS, A. J. EISENMAN and W. R. MARTIN, *Psychopharmacologia (Berl.)* **3**, 291 (1962).

17. J. W. SLOAN, J. W. BROOKS, A. J. EISENMAN and W. R. MARTIN, *Psychopharmacologia (Berl.)* **4** 261 (1963).
18. L.-M. GUNNE, *Acta physiol. scand* **58**, Suppl. 204 (1963).
19. H. WEIL-MALHERBE, in *Methods in Medical Research*, J. H. QUASTEL, Ed., vol. 9, p. 130. Year Book Medical Publishers, Chicago (1961).
20. H. F. FRASER, E. E. JONES, D. E. ROSENBERG and A. K. THOMPSON, *Clin. Pharmac. Ther.* **4**, 188 (1963).
21. H. WEIL-MALHERBE, *Z. klin. Chem.* **2**, 161 (1964).
22. U. S. VON EULER and F. LISHAJKO, *Acta physiol scand* **52**, 137 (1961).
23. H. WEIL-MALHERBE and A. D. BONE, *J. clin. Path.* **10**, 138 (1957).
24. E. R B. SMITH and H. WEIL-MALHERBE, *J. Lab. clin. Med.* **60**, 212 (1962).
25. F. W. SUNDERMAN, Jr., P. D. CLEVELAND, N. G. LAW and F. W. SUNDERMAN, *Am. J. clin. Path.* **34**, 293 (1960).
26. H. MIYAKE, H. YOSHIDA and R. IMAIZUMI, *Jap. J. Pharmacol.* **12**, 79 (1962).
27. H. ISBELL and W. M. WHITE, *Amer. J. Med.* **14**, 588 (1953).
28. P. HOLTZ and G. KRONEBERG, *Biochem. Z.* **320**, 335 (1950).
29. K. N. F. SHAW, A. MCMILLAN and M. D. ARMSTRONG, *J. biol. Chem.* **226**, 255, (1957).
30. A. J. EISENMAN, H. F. FRASER, J. SLOAN and H. ISBELL, *J. Pharmac. exp. Ther.* **124**, 305 (1958).
31. A. J. EISENMAN, H. F. FRASER and J. W. BROOKS, *J. Pharmac. exp. Ther.* **132**, 226 (1961).
32. T. NAGATSU, M. LEVITT and S. UDENFRIEND, *J. biol. Chem.* **239**, 2910 (1964).