The Effect of Haemodialysis on the Excretion of the Mauve Factor in Schizophrenia

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Introduction

In the course of therapeutically applied haemodialysis it was observed that, with a few exceptions, both the blood and the urine of mauve factor (5-hydroxyhaemopyrrole-lactam)-positive schizophrenic patients became negative after 2 to 3 dialyses (Durko et al. 1981,1983).

As demonstrated by the experiments of Gorchein (1980), the mauve factor can not be regarded as a causal factor of schizophrenia, but its occurrence is always connected to some form of the psychosis. Irvine (1973) concluded that schizophrenics with the mauve factor had a poorer prognosis following standard psychiatric treatment than did those without the mauve factor. The molecular mass of this compound is very low, but it nevertheless appeared interesting to examine whether and how its level varies during repeated haemodialysis.

It is currently believed that the mauve factor is exclusively a human metabolic product. It occurs in the highest frequency in the hepatic porphyrias accompanied by neurological, psychical symptoms, such as AIP, PCT and PV, and in acute and chronic schizophrenic patients, but it is also found in about 10 percent of the "normal" control group (Irvine and Wetterberg 1972; Huszak et al. 1972). Hoffer and Osmond (1963) distinguish individuals synthetizing the mauve factor as suffering from malvaria.

As concerns the origin of this compound, numerous theories have been put forward during the past 25 years. The investigations by Irvine (1978) suggested that the mauve factor (3-ethyl-5-hydroxy-4,5-dimethyl- 3-pyrrolin-2-one) is a haeme degradation product formed from a vinyl side-chain bile pigment of unknown structure. Sohler et al. (1974) considered that individuals excreting the mauve factor are generalized pyrrole hyperproducers, in whom the excess pyrrole enters the circulation because of the stress-induced permeability enhancement. Ward (1975) too believed the stress condition to be an important feature in the synthesis of the mauve factor. Pepplinkhuizen and Bruin-vels (1977) observed increased porphyrin and pyrrole syntheses in psychoses and in

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stress situations, and explained this in terms of an increased glycine synthesis.

We came to the conclusion that useful data on the unclarified metabolism of the mauve factor might be provided by the stress condition caused by haemodialysis, as a consequence of the known changes in the ion levels during haemodialysis influencing the synthesis and degradation of haeme.

Table I

Data to the Mauve-factor Positive Schizophrenic Patients

Subject case no	Sex	Age (years)	Diagnosis	Duration of illness (years)	Number of shubs before dialysis	Status under dialysis	Earlier therapies	Drugs during dialysis	Number of dialysis
1	M	29	Sch. paranoides	9	<10	shub	ECT.Ph.	Clozapine	6 + 6
2	F	19	Sch. paranoides	2	3	shub	ECT.Ph.	Metofenazine	6 +6
3	M	20	Hebephrenia	5	?	shub	?	no	5
4	F	37	Schizophrenia	18	?	shub	ECT.Ph.	Thioridazines	
5	M	24	Sch. katatonica	24	<10	shub	ECT.Ph.	no	6
6	F	29	Sch. paranoides	7	1	shub	Ph.	Pimozid Promethazine	6
7	M	30	Hebephrenia	15	15	shub	ECT.I.A. Ph.	Haloperidol Promethazine	6
8	F	38	Sch. paranoides	9	10	shub	ECT.Ph.	Trifluperidol	6
9	M	19	Sch. katatonica	2	3	chronic	ECT.Ph.	noresidualis	6
10	M	37	Schizophrenia	20	<10	chronic	Ph.	noresidualis	6
11	M	32	Sch. paranoides	3	3	chronic	ECT.Ph.	noresidualis	6
12	M	30	Sch. paranoides	12	?	chronic	ECT.Ph.	noresidualis	6
13	M	23	Sch. paranoides	3	?	chronic	?	Thioridaresiduzinealis	3
14	M	37	Schizophrenia	15	<10	chronic	ECT.Ph.	noresidualis	5
15	F	40	Schizophrenia	21	<10	chronic	Ph.	Haloperidol Promethazine	6 + 6

Note: ECT: electroconvulsive therapy Ph : psychopharmacon I : insulin coma A : atropin coma

Patients and Haemodialysis

Some data on the mauve factor-positive, schizophrenic patients who participated in haemodialysis are listed in Table 1. The dialysis was performed either at their own request or at the request of the family. There were 7 females and 14 males (average age 30.2 years). In each case the diagnosis was made independently by two specialists according to International Classification of Diseases (WHO, 1977, 9th revision).

Control experiments involving sham dialysis were not carried out either on other mauve factor-positive patients (e.g. with porphyria) or on "normal" subjects.

The examined cases consumed standard hospital food and did not take part in any diet or receive any calorie-rich foodstuffs.

In spite of the fact that mauve factor excretion does not depend on the drug state, drug level or drug absence, the relevant drug data are given in Table 1.

Dialysis was performed on one occasion weekly, on the same day and at the same time, and lasted for 4 to 5 hours. Each series consisted of 6 dialyses. For therapeutic purposes, the series were repeated several times, a dialysis-free period of one week being inserted after each series.

Initially, a Scribner Shunt was applied in 3 patients; later, venous puncture was introduced; a Cordis DAK 1.3m^2 Kapillardial-ysator or an Asahi 1.6m^2 Kapillardialysator was used. The blood flow rate was 150 to 200 ml/min, and that of the dialysing solution was 500 ml/min, without a transmembrane vacuum.

Experimental

The "short" and "long" screening tests (McCabe, 1983) were not used at all to study the mauve factor-positivity of the patients. For qualitative examinations, extracts were prepared both from the blood and from the urine collected during 24 hours and were subjected to two-dimensional thin-layer chromatography with two-directional standards. Identification of the mauve factor was carried out via development with the Ehrlich reagent. As a standard in the present experiments too, use was made of 5-hydroxyhaemopyrrole-lactam synthetized according to Wooldridge et al. (1977).

The extraction method of Irvine (1976) was applied for both qualitative and quantitative studies. Chromatography was performed on a Kieselgel-G plate, in ether in the first dimension, and in the chloroform -acetone (7:3, v/v) system in the second dimension. After reaction with the Ehrlich reagent, quantitative measurement of the mauve factor was made densitometrically within 30 minutes with a Joyce-Loebl 2000 Chromoscan. As the limit of detectability of pyrrole compounds with the Ehrlich reagent is 0.04 /yg, the mauve factor could be measured well.

The 24-hour values measured after dialysis were compared with the radiolysis', basal levels. Urine was in all cases collected in a dark bottle offering protection from light. The examined sample was taken from the combined urine collected during 24 hours.

Simultaneously with the measurements on the urine samples, the serum Fe^{2t}, Cu^{2}* and Zn^{2t} contents were determined by atomic absorption photometry (Perkin-Elmer 306 instrument) both before and after haemodialysis, according to Fernandez and Kahn (1971).

The method of Berko and Durko (1977) was used to determine the effects of haemodialysis on the activity of delta-amino-laevulinic acid dehydratase (ALAD; EC 4.2.1.24) in the same blood samples.

In some cases the above examinations were supplemented with quantitative determination of the Zn-protoporphyrin in the erythrocytes by means of spectrophoto-fluorescence measurements (HITACHI 204A) according to Lamola and Yamane (1974). (Excitation 423 nm, Emission 594 nm).

Results

Our qualitative and quantitative investigations so far permit the following findings:

a) Not every subject who excretes the mauve factor becomes negative as a result of repeated haemodialysis.

b) In every case the haemodialysis lowers the mauve factor levels of both the blood and the urine as compared to the predialysis, basal levels.

c) In our cases the5-hydroxyhaemopyrrole-lactam levels before dialysis were 4-10 /ig/100 ml whole blood, and 0.05-1.0 mg in the 24-hour urine; the best agreement was given by the data of Graham (1977) (0.2-1.0/mg/24 hours).

Figure 1

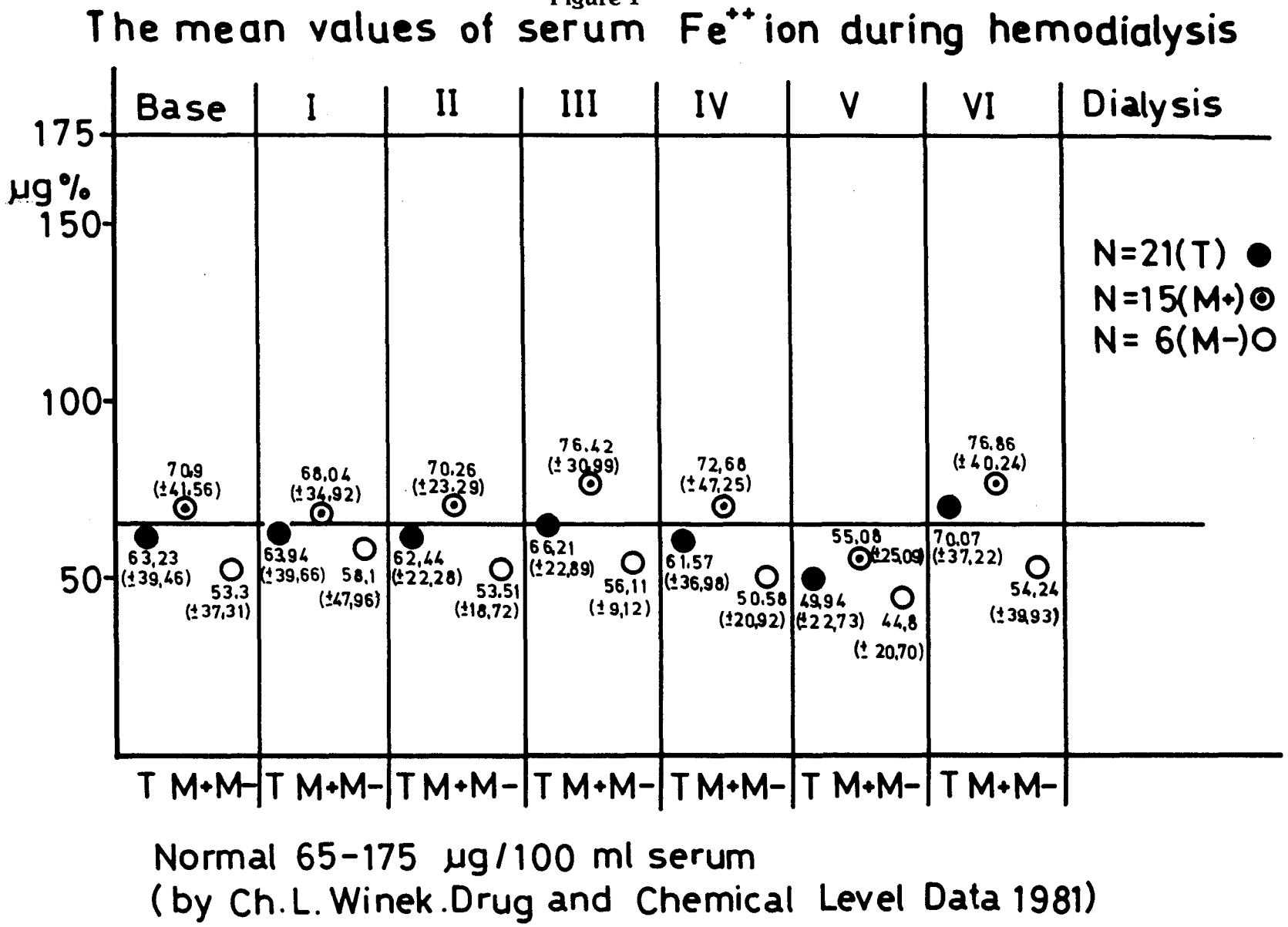

The mean values of serum Fe++ ion during hemodialysis
Base
I
II
III
IV
V
VI
Dialysis
175
µg%
150
100
50
N=21(T)
N=15(M+)
N= 6(M-)
70,9 (±41,56)
63,23 (±39,46)
53,3 (±37,31)
68,04 (±34,92)
63,94 (±39,66)
58,1 (±47,96)
70,26 (±23,29)
62,44 (±22,28)
53,51 (±18,72)
76,42 (±30,99)
66,21 (±22,89)
56,11 (±9,12)
72,68 (±47,25)
61,57 (±36,98)
50,58 (±20,92)
55,08 (±25,09)
49,94 (±22,73)
44,8 (±20,70)
76,86 (±40,24)
70,07 (±37,22)
54,24 (±39,93)
T M+M-
Normal 65-175 µg/100 ml serum
(by Ch.L. Winek. Drug and Chemical Level Data 1981)

Figure 2

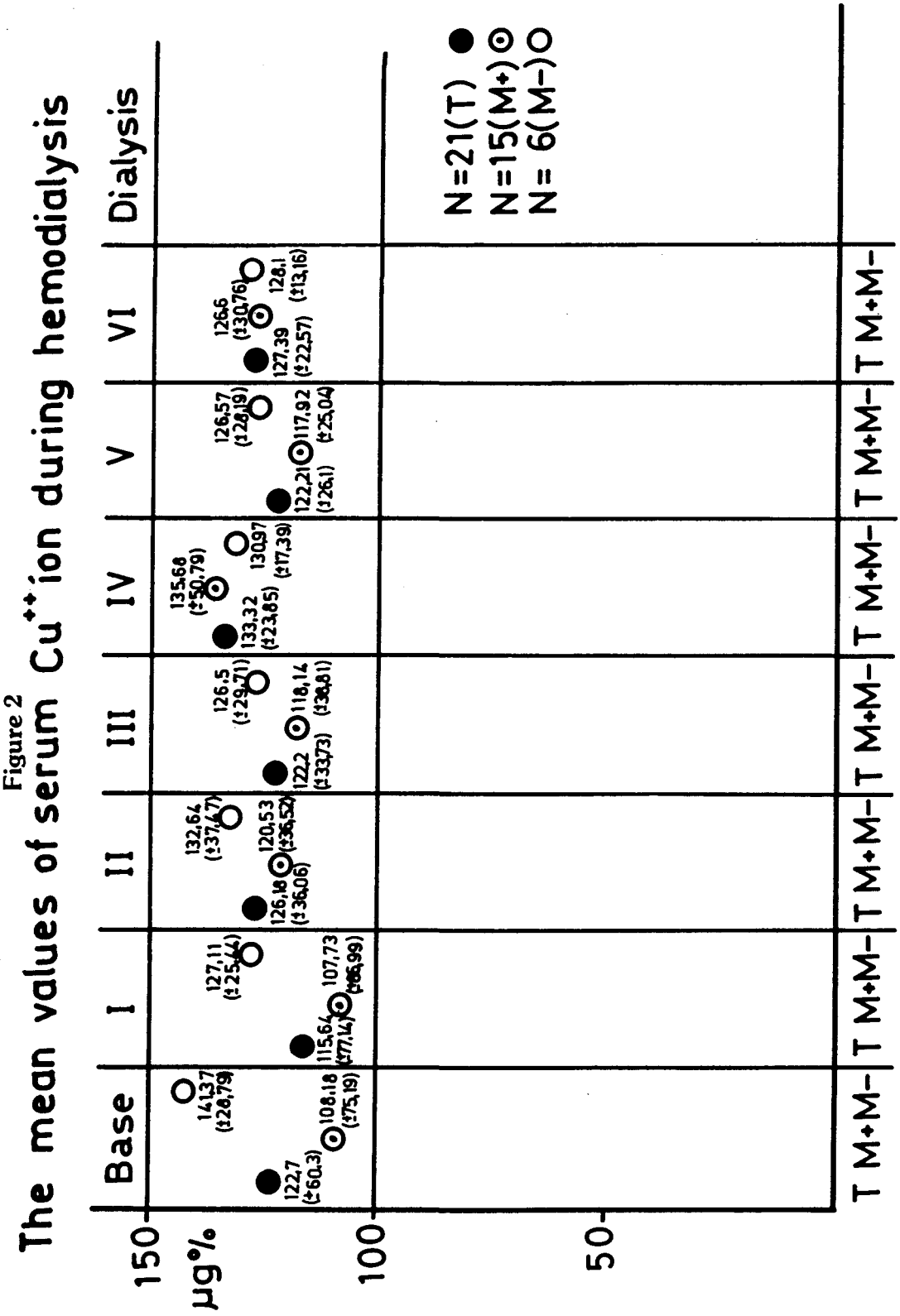
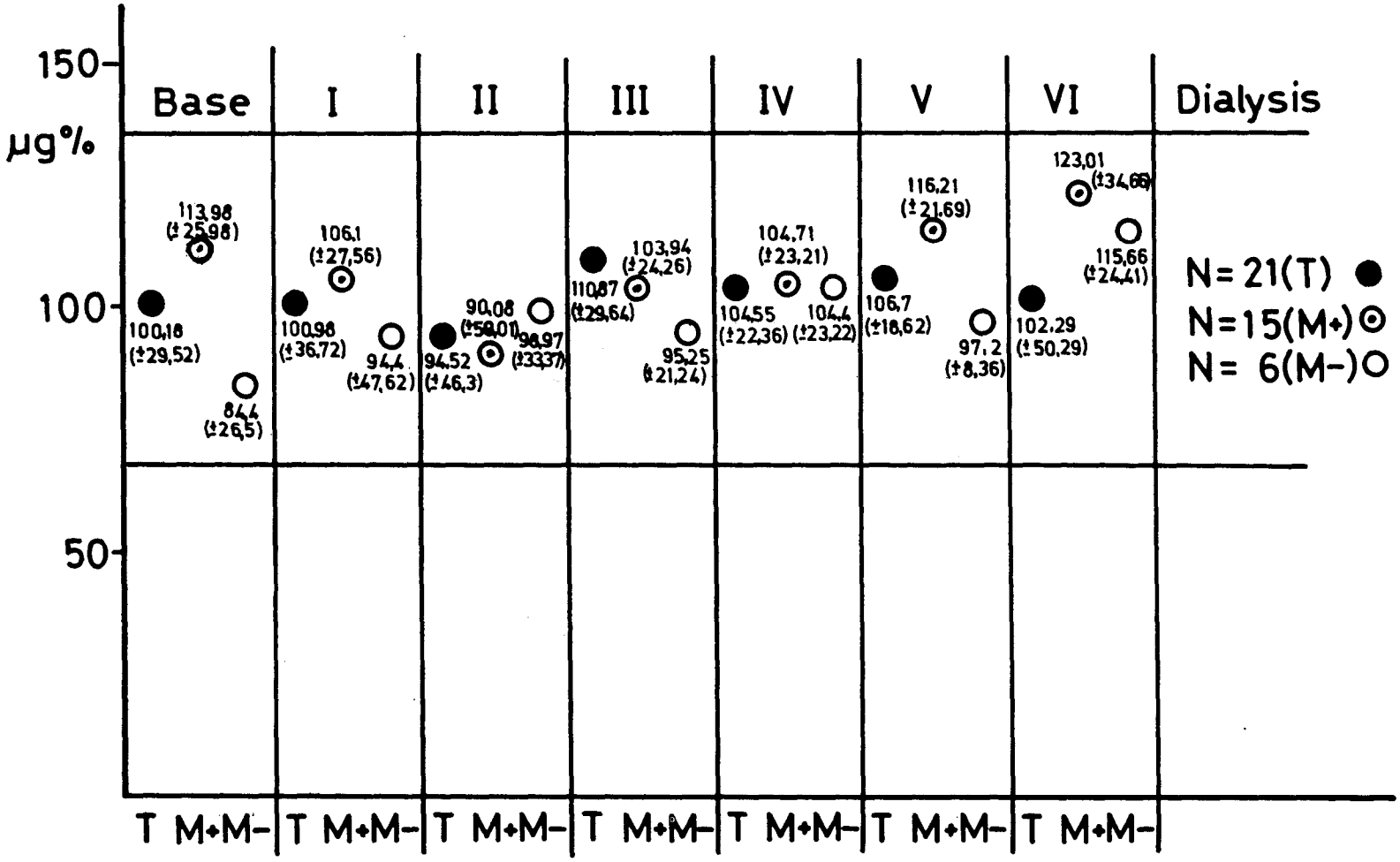

The mean values of serum Cu++ion during hemodialysis
µg%
150
100
50
Base
I
II
III
IV
V
VI
Dialysis
T M+M- T M+M- T M+M- T M+M- T M+M- T M+M- T M+M-
N=21(T)
N=15(M+)
N= 6(M-)
Normal:100-150 µg/100 ml serum
(by Ch. L.Winek. Drug and Chemical Level Data 1981)

Figure 3

The mean values of serum Zn^{++} ion during hemodialysis



Normal: 68-136 µg/100 ml serum
(by Ch. L.Winek. Drug and Chemical Level Data 1981)

Figure 4

The mean values of ALAD activity in ml RBC during hemodialysis, in schizophrenic patients

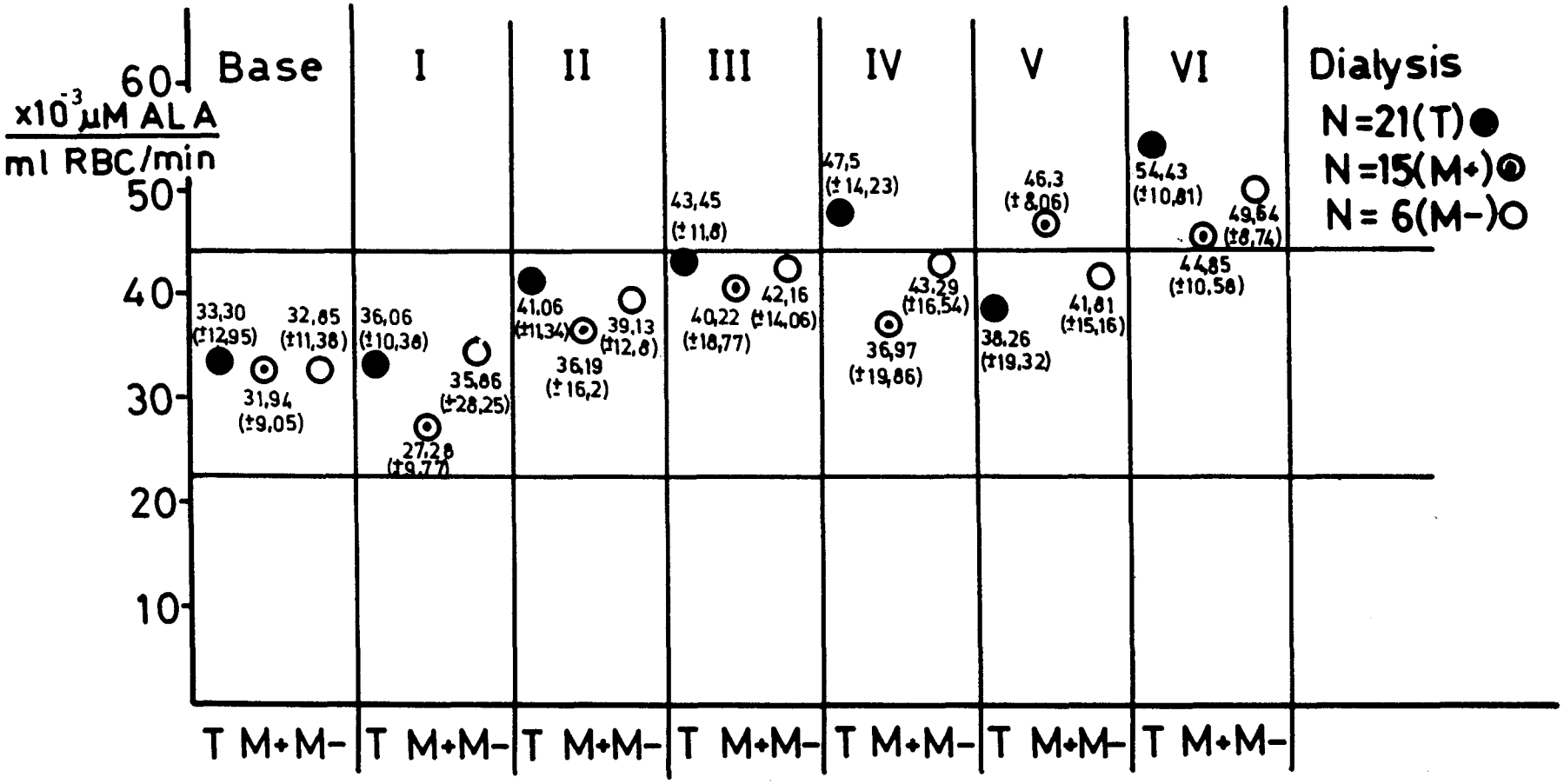


Figure 5

Figure 5

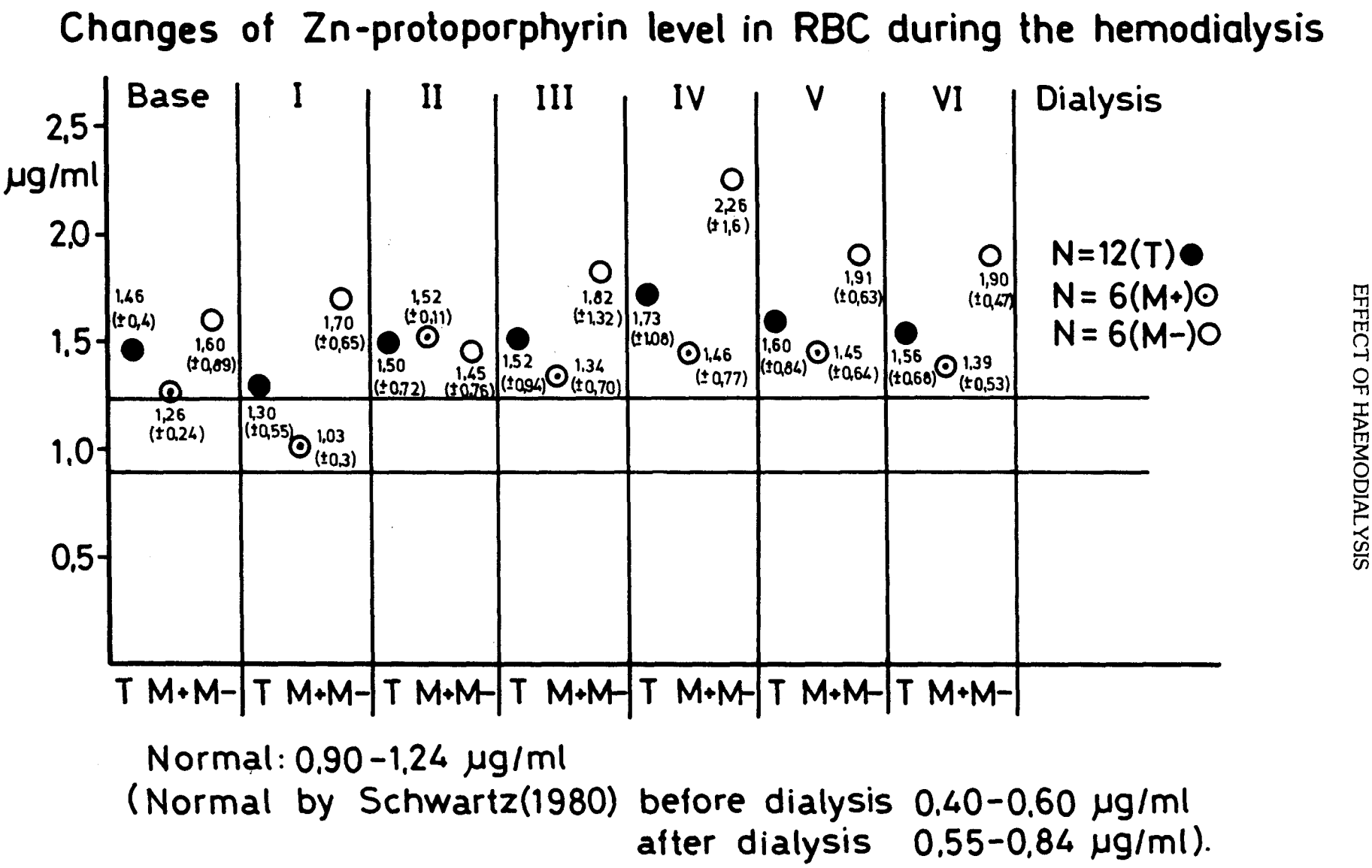
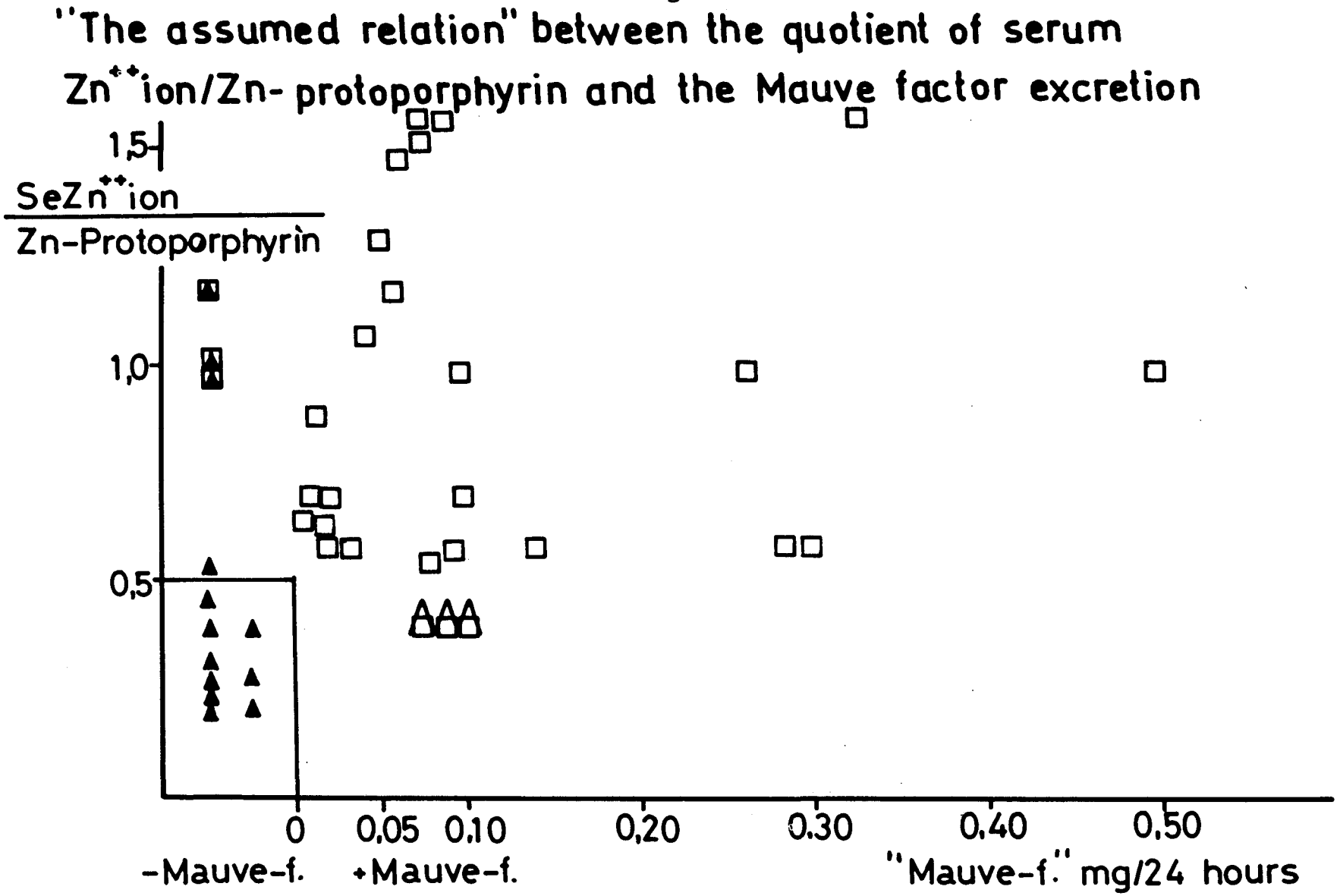

Changes of Zn-protoporphyrin level in RBC during the hemodialysis
Base
I
II
III
IV
V
VI
Dialysis
2,5
µg/ml
2,0
1,5
1,0
0,5
1,46 (±0,4)
1,60 (±0,69)
1,26 (±0,24)
1,30 (±0,55)
1,70 (±0,65)
1,03 (±0,3)
1,52 (±0,11)
1,50 (±0,72)
1,45 (±0,76)
1,52 (±0,94)
1,82 (±1,32)
1,34 (±0,70)
1,73 (±1,08)
2,26 (±1,6)
1,46 (±0,77)
1,60 (±0,84)
1,91 (±0,63)
1,45 (±0,64)
1,56 (±0,68)
1,90 (±0,47)
1,39 (±0,53)
N=12(T)●
N= 6(M+)⊙
N= 6(M−)○
T M+M−
Normal: 0,90-1,24 µg/ml
(Normal by Schwartz(1980) before dialysis 0,40-0,60 µg/ml
after dialysis 0,55-0,84 µg/ml).

Figure 6


"The assumed relation" between the quotient of serum
Zn++ ion/Zn- protoporphyrin and the Mauve factor excretion
1,5
SeZn++ion
Zn-Protoporphyrin
1,0
0,5
0
0,05
0,10
0,20
0,30
0,40
0,50
-Mauve-f.
+Mauve-f.
"Mauve-f." mg/24 hours

d) In general, the blood became negative first, and the urine did so only after further dialysis. However, it did occur that the mauve factor could not be detected in either the blood or the urine after the second or third dialysis.

e) Cases were also observed in which the mauve factor returned after several negative weeks or in the intervals between the dialysis series.

The changes in the serum Fe^{2+} (Fig. 1), Cu^{2}* (Fig. 2) and Zn^{2}* (Fig. 3) levels during a dialysis series are given as mean values. The data for the mauve factor-positive and the mauve factor-negative cases are reported separately. Every value is within the normal range before and after dialysis. The serum Fe^{2}* values for the examined schizophrenic patients lie on the lower limit of the normal level.

The variations in ALAD within one dialysis series are illustrated in Fig. 4. The measured activity get increase higher than the normal limits, and even on an individual basis they cannot be brought into correlation with either the variation in the Zn^{2+}concentration or the decrease (or disappearance) of the mauve factor.

An increase in the quantity of Zn-proto-porphyrin (Fig. 5) could be observed in some of our cases, i.e. a change in the free and bound Zn^{2}*. However, from the aspect of the mauve factor, a correlation was found only if (completely arbitrarily) a quotient was formed from the values referred to 1 ml for the serum Zn^{2}* concentration and the erythrocyte Zn-protoporphyrin concentration. Figure 6 demonstrates that if this quotient has a value of around 0.5, the mauve factor can not be detected in the urine. It is highly conceivable that this value holds only for the much lower than normal 5-hydroxyhaemo-pyrrole-lactam levels resulting from haemo-dialysis.

Discussion

Numerous data suggest that the mauve factor is a haeme degradation product. It is believed that the enzyme determining the rate of haeme degradation is haeme oxygenase, the activity of which is not independent of the concentrations of the divalent metal ionsZn^{2}*, Cu^{2}*, Fe^{2}*, Co^{2}*, etc., and the ratios of these. A specific inhibitor of this enzyme in the liver, kidney and other organs is Zn-protoporphyrin (Maines, 1981). Thus, it must be assumed that the concentrations of Zn^{2}* and of Zn-protoporphyrin (i.e. free and bound Zn^{2+}) are determining as concerns haeme degradation. Extensive studies have been made of the effects of haemodialysis on the serum ion levels. Schwartz et al. (1980) reported tht haemodialysis causes an increase in the quantity of Zn-protoporphyrin. It is conceivable, therefore, that the large amount of ZN-protoporphyrin produced in response to haemodialysis in some cases blocks haeme degradation to such an extent that the amount of 5-hydroxyhaemopyrrole-lactam formed is below the limit of detect-ability. Since the substrate of haeme is haeme oxygenase, enzymes influencing haeme synthesis can not be neglected either. As the subject of our investigation we chose to follow the activity of ALAD, since this is an SH-containing, Zn-requiring enzyme; it responds sensitvely to changes in Zn^{2+} and Cu^{2+}, and the increase of its activity on haemodialysis has been observed by Meredith et al. (1979) and by Levi and Purdy (1980).

In a discussion of our results on the effects of haemodialysis on the mauve factor, the findings of Pfeiffer and Iliev (1973) must be taken into consideration. They reported that the appearance of the mauve factor presumes a Zn- and vitamin B_6-deficient state. On treatment of their patients with Zn and vitamin B_6, after a certain level the mauve factor disappeared or could not be detected. In their view, a Zn, vitamin B_6, mauve factor molecular complex is then formed, which is eliminated from the organism.

However, we consider that there is another possibility: Zn administration favours the formation of Zn-protoporphyrin as a result of the shift in the ratio of Fe^{2}* and Zn^{2}* (e.g. in the serum, where they are present in nearly the same proportions under normal conditions), and the disappearance of the mauve factor is due to the shift in the ratio of free and bound Zn^{2}*. Bloomer et al. (1980) concluded that Zn^{2}* competes with Fe^{2}* for the haeme synthetase (ferrochelatase), thereby inhibiting the terminal step of haeme biosynthesis. The changed serum Zn^{2}* levels following haemo-dialysis were within the normal range in our

cases, whereas the Zn-protoporphyrin levels in the erythrocytes were higher in mauve factor negative cases (Fig. 5).

Many experimental data are still necessary to decide whether haemodialysis affects the actual metabolism of 5-hydroxyhaemo-pyrrole-lactam, or only diminishes its de-tectability. It is not easy to assess the effect of stress from the aspect of synthesis of the mauve factor. At any event, it may be stated that haemodialysis as a stress situation did not enhance the quantity of the mauve factor in our cases, and it did not cause the mauve factor-negative cases to become positive. Naturally, it cannot be excluded that haemodialysis gives rise to a decrease in the level of a protein or a peptide that is an important precursor in mauve factor synthesis. Many questions remain open. Nevertheless, we believe that the results obtained in the course of the therapeutic application of haemodialysis will help to clarify currently unanswered points relating to the origin of the mauve factor.

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