ASPECTS OF THE CLINICAL CHEMISTRY OF DESMETHYLIMIPRAMINE IN MAN

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A method is described for the fluorimetric estimation of desmethylimipramine (DMI), an active metabolite of imipramine, in plasma. The plasma levels of DMI were followed in six patients over a period of 4 weeks during the treatment of endogenous depression. The results suggested that there was an inverse relation between the mean plasma DMI concentration and the weight of the patient. Two to 3 weeks treatment with DMI was necessary before the blood platelet 5-hydroxytryptamine was significantly lowered, whereas an improvement in symptoms, if it occurred, usually took place earlier.

Investigation into the biochemical actions of drugs used in the treatment of depression has received an impetus since the discovery that many antidepressant drugs inhibit monoamine oxidase. The rise in brain amines of animals after treatment with these drugs has been postulated to account for their therapeutic activity (Burns and Shore, 1961). Since it is impracticable to follow the levels of 5-hydroxytryptamine (5-HT) in human brain, the levels of 5-HT in the blood platelets have been measured in patients receiving antidepressant drugs. Those drugs which inhibit monoamine oxidase cause an increase in platelet 5-HT (Pletscher and Bernstein, 1958) whereas imipramine (Marshall, Stirling, Tait and Todrick, 1960), which has no inhibitory action on monoamine oxidase, causes a fall in platelet 5-HT.

The fact that only a proportion of depressed patients respond to treatment with antidepressant drugs could be due to individual failure of absorption. An estimate of the amount of drug in the plasma is therefore desirable before attempting to correlate behavioural response with biochemical effects.

Clinical investigation of desmethylimipramine (DMI) has followed the observation of Gillette, Dingell, Sulser, Kuntzman and Brodie (1961) that imipramine owes its activity to this metabolite. Brodie, Dick, Kielholz, Poldinger and Theobald (1961) and Meduna, Abood and Biel (1961) showed that DMI produced improvement in the mood of some depressed patients after only 2 days treatment, in contrast to imipramine which may take 2 to 3 weeks to produce any effect; on the other hand Oltman and Friedman (1962) found that DMI required 7–10 days to exert its therapeutic action.

We report the first stage of an attempt to correlate the behavioural effects of DMI with the level of the drug in the plasma and of 5-HT in blood platelets.

Methods

Blood was collected and a saline suspension of the platelets obtained by differential centrifugation by the method of Marshall, Stirling, Tait and

Todrick (1960). Platelets were counted by the method of Dacie (1956). The plasma, containing anticoagulant solution, was collected separately and both plasma and platelet suspension were stored at −15°.

Estimation of Desmethylimipramine

DMI differs chemically from imipramine only by the absence of one methyl group from the terminal nitrogen atom. Gillette, Dingell and Quinn (1960) have described a method for the estimation of imipramine in human plasma and found concentrations of 0·1–0·6 μg./ml. in patients receiving 150–300 mg. daily; they noted losses due to adsorption on glass in the heptane extraction procedure which they used (Quinn, personal communication). A protein precipitation procedure has therefore been tried in an attempt to avoid this difficulty. It was soon found that tryptophan, present in plasma to the extent of 10 μg./ml. (Duggan and Udenfriend, 1956) and fluorescing strongly with a peak at 280/360 mμ (uncorrected instrument reading), was liable to interfere, and subsequently preliminary investigations were made with DMI solutions containing tryptophan at the expected level. It was observed that the tryptophan fluorescence was high at neutral pH but much less in strong alkali, whereas the DMI fluorescence was lowest in acid solution and increased progressively with rise in pH. The following procedure was therefore adopted.

The plasma is diluted 1 in 5 with normal saline and 1·0 ml. is mixed with saline (1·0 ml.) or standard DMI in saline (1·0 ml.) in a 3 ml. centrifuge tube. 5 per cent zinc sulphate heptahydrate (0·4 ml.) is added followed by 0·3N barium hydroxide (0·3 ml.) (sodium hydroxide is not satisfactory; see Somogyi, 1945) with immediate mixing by inversion (6–8 times). After standing 20 min. in a refrigerator, the tube is centrifuged for 20 min. at 3,000 r.p.m. in an iced bucket (see Marshall and others, 1960).

Exactly 1 ml. of the supernatant is transferred to a quartz cuvette and the fluorescence measured in a spectrophotofluorimeter (Aminco-Bowman), at a setting of 280/360 mμ without altering the pH and at a setting of 280/440 mμ after the addition of 0·25 ml. 5N sodium hydroxide. Standards and blanks are run concurrently using plasma obtained from the same patient on the two days preceding the commencement of therapy.

The net fluorescence due to DMI or its metabolites is obtained by deducting the reagent blank and the residual fluorescence due to tryptophan. The ratio of the fluorescence due to tryptophan in alkali at 280/440 mμ to that in neutral solution at 280/360 mμ, determined in the presence of the reagents, was found to be 0·029.

Estimation of 5-Hydroxytryptamine

The protein precipitation procedure of Weissbach, Waalkes and Udenfriend (1958) developed for estimations in whole blood is applied to the platelet suspension; 10 per cent zinc sulphate monohydrate (0·3 ml.) and 0·42N sodium hydroxide (0·3 ml.) are added to a 2 ml. aliquot of the saline suspension of platelets, the tube being shaken after each addition. After 20 min. in a refrigerator the tube is centrifuged for 20 min. at 3,000 r.p.m. in an iced bucket. Exactly 1 ml. of the supernatant is

transferred to a quartz cuvette and the fluorescence measured in a spectrophotofluorimeter (Aminco-Bowman) set at 300/550 mμ both before and after acidification with concentrated hydrochloric acid (sp. gr. 1·18; 0·3 ml.).

Acidification has been found to quench the blank fluorescence partially and the net fluorescence due to 5-HT has been taken as "fluorescence in acid solution —0·7 × fluorescence in neutral solution" for both unknown and standard solutions. Two standards are run with each batch (0·4 μg./ml. 5-HT creatinine sulphate ≡ 0·174 μg./ml. free base) and the duplicates from each platelet suspension are run in different batches.

This method (Crosti and Lucchelli, 1962; Todrick, 1962) gives results agreeing with those obtained by the extraction procedure of Brodie, Tomich, Kuntzman and Shore (1957). However, owing to the omission of the extraction procedure, interference with the 5-HT estimation by the drug being studied is more liable to occur.

DMI being less fluorescent in acid than in neutral solution would, if present in effective concentration, produce a high blank reading but a negligible alteration in the fluorescence due to 5-HT after acidification; it would therefore cause a fictitiously low 5-HT estimate. The mean blank reading for pre-treatment bloods was 0·037 in arbitrary units (23 estimations); for bloods taken during treatment the mean blank was 0·034 (72 estimates); this indicates that interference has not occurred.

The effect of DMI on the estimation of 5-HT in aqueous mixtures subjected to the precipitation procedure has been checked. For DMI concentrations of 10, 1, 0·1 μg./ml., the estimation of 5-HT expressed as a percentage of the 5-HT found in the absence of DMI, gave 44, 93, 97 per cent respectively. Thus the 1 μg./ml. concentration of DMI will affect the estimation of 5-HT. However, the platelet button containing adhering plasma occupies a volume of approximately 0·1 ml. before being diluted to 5 ml. with saline. This means that the plasma DMI is diluted 50-fold before the 5-HT is estimated. The only way in which DMI could be present in amounts sufficient to interfere would be if it were concentrated in the platelets (as is 5-HT). Long and Lessin (1962) investigated this possibility with a series of drugs including imipramine and found no evidence of uptake against a concentration gradient.

RESULTS

Six female patients recently admitted to one ward of the hospital formed the group studied. The mean time interval between admission and commencement of therapy was 9 days. All six patients suffered from symptoms of depression and not all were first admissions. They received DMI (Pertofran: Geigy) orally in tablet form. The dosage schedule was 75 mg. for the first 3 days (in three doses at 9 a.m., 2 p.m. and 6 p.m.), 100 mg. on the fourth day, 125 mg. on the fifth day and 150 mg. on the sixth and subsequent days. Blood samples were taken on 4 or 5 days in the first week and thereafter at 7-day intervals. Blood was also collected from three controls (staff) not receiving the drug.

Effect of Acetylsalicylic Acid Preparations on DMI *Estimation*

After the observation of an unexpectedly high level of the drug in patient No. 5 at the end of the third week, it was discovered that this patient had received codeine compound tablets, containing 24 grains of acetylsalicylic acid in the 24 hr. before sampling. This compound fluoresces strongly in alkaline solution (peak 320/410 mμ uncorrected instrument reading). Two previous high figures, one in a patient and one in a control, which had been discounted as anomalies arising from a new technique, were scrutinised. Patient 2 had started on acetylsalicylic acid for rheumatoid arthritis a few days previous to the high level although the duration of therapy was not known. Control A admitted that he had probably taken the drug 1 or 2 hr. before collection of the sample showing an apparent high level of DMI. The apparent DMI concentrations (μg./ml. plasma) from two controls who received 10 grains of acetylsalicylic acid were: 25·3, 37·5, 10·7, 0·09 for subject A and 19·8, 39·5, 16·2 and 0·02 for subject D at ½, 2, 7, 24 hr. after dosage.

TABLE I

PLASMA DMI (μG./ML.) IN PATIENTS ON THERAPY AND IN CONTROLS

		Patients						Controls		
Day	Dose (mg.)*	1	2¶	3	4	5	6	A	B	C
1	75	0·08	—	—	0·08	0·15	0·20	0·10	0·02	−0·02
2	75	0·35	0·22	—	0·02	0·19	0·32	−0·02	0·04	−0·10
3	75	0·23	—	—	0·28	0·19	—	(0·44)‡	−0·19	—
3 or 4†	100	—	0·14	—	0·50	—	0·36 0·30	(79·0)‡	—	−0·03
5	125	0·40	0·89	—	—	0·75	—	—	0·23	−0·10
6–8	150	—	—	—	0·74	0·54	0·56	—	0·03	−0·09
13–15	150	0·83	1·24	—	0·79	(43·6)‡	0·70	—	0·03	0·06
20–22	150	0·82	(13·0)‡	0·86	1·34	0·53	0·79	0·05	(0·65)§	0·03
27–29	150	0·70	1·51	0·66	0·82	0·69	—	0·06	0·06	—
Mean at 150 mg.‖		0·78	1·38	0·76	0·92	0·59	0·68	0·05	0·03	−0·04
Clinical response at 14–21 days		Moderate	Slight	Moderate	Slight	Moderate	Moderate			
Bodyweight (kg.)**		79·4	46·7	56·0	52·2	63·6	55·3			

Footnotes:

* The dose refers to the total amount of DMI taken in the 24 hr. *immediately* before sampling.

† Due to blood being collected from some patients regularly at 9 a.m. and from others at 2 p.m. the dose on the 24 hr. preceding the third day could be either 75 or 100 mg.

‡ Subject known to have been taking acetylsalicylic acid about this time (see text).

§ Subject has no recollection of having taken acetylsalicylic acid at this time.

‖ Omitting all figures in brackets.

¶ Samples after 150 mg. from this patient were withdrawn 3 days earlier than stated under "day."

** Weights were obtained retrospectively from the ward records; they include clothing.

Relation of DMI *Plasma Levels to other Factors*

Plasma levels of DMI in patients and controls are given in Table I. Mean values for the group at different dose levels are plotted in Fig. 1. After reaching the maximum (150 mg.) daily dose, the levels for a given patient remain fairly constant for three weeks. The range of 0·59 μg./ml. to 1·38 μg./ml. for the group is not excessively wide. The number of cases is rather small to expect any significant correlation with clinical

results. In fact, on simple clinical assessment patients 2 and 4 responded less well than the others; their mean plasma DMI levels at 150 mg. were the highest in the series. Ordinarily, some degree of improvement—largely represented by an increase in psychomotor activity—was evident at one week and a noticeable therapeutic effect by 10–14 days.

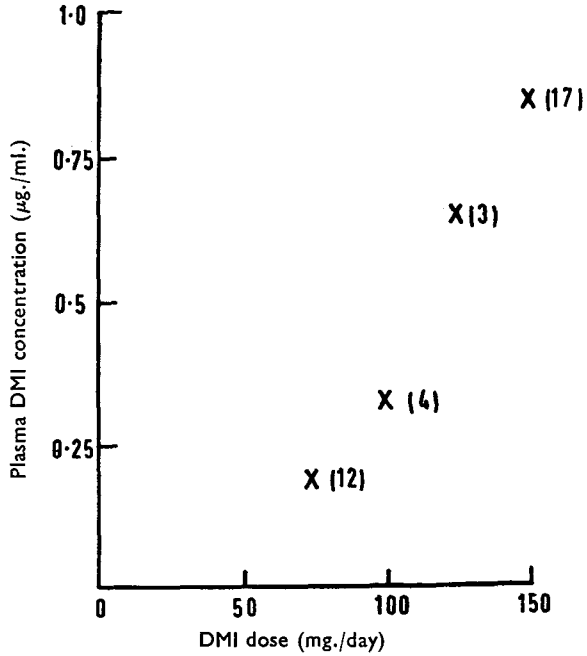

FIG. 1. Effect of dosage level on DMI plasma concentration. Figures in brackets are number of estimates on which value is based.

There is however a strong suggestion of an inverse relation between the mean final plasma concentration and body weight. The correlation coefficient $t_{10} = -0{\cdot}55$ (for $P = 0{\cdot}05$, $t_{10} = -0{\cdot}57$).

Platelet 5-HT *Levels in Patients Receiving* DMI

The physiological factors affecting platelet 5-HT level are not well understood and considerable day-to-day variation sometimes occurs in individuals even when they are not receiving drugs known to affect 5-HT metabolism. In one patient (No. 4) the pre-treatment levels were less than half the lower limit of normality and all data from her have been omitted. The remainder had normal pre-treatment levels (Todrick, Tait and Marshall, 1960) with a mean of 0·18 μg./ml. of whole blood.

Data from each individual have been converted to percentages of her mean pre-treatment level and these subsequently grouped. The change in 5-HT level during therapy is shown in Fig. 2. The length of the bar on each side of a point is the product of the standard error of the mean and t for $P = 0{\cdot}05$ for the appropriate number of degrees of freedom.

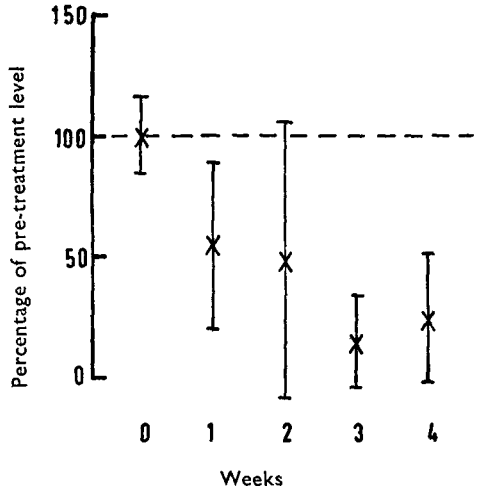

FIG. 2. Effect of DMI on blood platelet 5-HT level.

DISCUSSION

It has been shown that the antidepressant drug imipramine is metabolised in the body both by successive demethylation of the nitrogen atom on the side chain, and by hydroxylation followed by glucuronide formation; a compound involving both pathways has also been isolated from the urine (Herrmann, Schindler and Pulver, 1959; Herrmann and Pulver, 1960).

It has been suggested (Gillette and others, 1961) that the antidepressant action is due to the first product of demethylation, desmethylimipramine, the subject of this paper, and that this substance produces a more rapid therapeutic effect.

The fluorescence spectra and quantum efficiencies of imipramine and desmethylimipramine are so similar that the estimation of these individually in a mixture would involve considerable difficulty. The choice of desmethylimipramine for this study of the correlation between biochemical and behavioural response to antidepressant medication was made partly with a view to avoiding this difficulty; however, it now appears that the metabolite produced by the further demethylation of DMI also possesses the same fluorescence characteristics, though the hydroxylated metabolite does not (Haydu, Dhrymiotis and Quinn, 1962); the plasma levels quoted therefore refer to total DMI and its demethylated metabolite.

From the point of view of the hypothesis that differential clinical response is due to failure in absorption this is not so critical. The plasma content of DMI is a small fraction of the total present in the body (Herrmann and Pulver, 1960) but it is reasonable to assume and the data suggest that after 2–3 weeks on a fixed dosage schedule equilibrium conditions exist. The concentration range 0·59–1·38 μg./ml. observed does not indicate any major failure of absorption in any individuals. Indeed if the apparent

inverse relation between plasma DMI concentration and body weight should be confirmed this would mean that the actual amount absorbed would vary even less between patients than the individual plasma levels suggest. The hypothesis is therefore not supported by the evidence so far presented.

Haydu and others (1962) have recently reported briefly on a similar investigation made with imipramine. They observed a significantly higher mean plasma "iminodibenzyl" level in four patients who failed to respond. Our clinical observations do not contradict this unexpected conclusion.

The reduction in platelet 5-HT level which occurs during DMI therapy appears to follow much the same time course as that caused by imipramine (Marshall and others, 1960). The relationship of this to the clinical response is a matter of uncertainty since Kivalo, Rinne and Karinkanta (1961) and Schanberg and Giarman (1962) have observed that imipramine causes a slight rise in the brain 5-HT levels of rats, though much less than that caused by amine oxidase inhibitors. Himwich, Costa and Himwich (1961) have however found in dogs a fall in free 5-HT coupled with a rise in bound 5-HT. There was little overall change in the amount of 5-HT in the hippocampus, pons and midbrain, but a fall in the amygdala and a rise in the caudate nucleus.

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