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THE EFFECT OF IPRONIAZID AND IMIPRAMINE ON THE BLOOD PLATELET 5-HYDROXYTRYPTAMINE LEVEL IN MAN

BY

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Observations are reported on the blood platelet 5-hydroxytryptamine content of six patients receiving imipramine, *N*-(γ-dimethylaminopropyl)-iminodibenzyl hydrochloride. The response was a fall to a level of one-sixth of the original in three weeks, with little change thereafter. This is in sharp contrast to the action of iproniazid which caused a rise of some 200% in the blood platelet 5-hydroxytryptamine level over the same period. Imipramine in a concentration of 1 mg./ml. had no inhibitory action on 5-hydroxytryptophan decarboxylase; 8·0 μg./ml. of imipramine suppressed two-thirds of the *in vitro* uptake of 5-hydroxytryptamine (2·5 μg./ml.) by normal human platelets.

The recently developed pharmacological approach to the treatment of disordered mental states, particularly schizophrenia, with the so-called tranquillizing or ataractic drugs has undoubtedly achieved results (Brill and Patton, 1957), although the individual response is unpredictable and the mode of action is still not clear. Nevertheless, the degree of success has been sufficient to cause the adoption of a similar approach to the treatment of the other major functional psychosis, the manic-depressive syndrome. Of the two aspects of this illness, the depressive is the more frequent in the ratio of 10 to 1 and therefore calls for more urgent attack.

Iproniazid, developed for the treatment of tuberculosis, was among the first drugs to achieve improvement of mood in psychotic depression; it has been known for some time as a powerful inhibitor of amine oxidase (Zeller, Barsky, Fouts, Kircheimer and van Orden, 1952). Imipramine, *N*-(γ-dimethylaminopropyl)-iminodibenzyl hydrochloride (G.22355, Tofranil, Geigy), has more recently been claimed to be of value in the treatment of typical endogenous depressions (Kuhn, 1957, 1958; Kielholz and Battegay, 1958).

The knowledge that iproniazid was an inhibitor of amine oxidase suggested that it might cause an increase in 5-hydroxytryptamine *in vivo*, and when trials of this drug were instituted it was decided to follow the blood platelet 5-hydroxytryptamine levels. Pletscher and Bernstein (1958) have since shown that the expected increase does in fact occur. Imipramine was similarly studied to determine whether it also caused a rise of blood platelet 5-hydroxytryptamine.

METHODS

Blood was withdrawn from an arm vein into a siliconed 10 ml. syringe through a No. 20G needle. The needle was removed and 9 ml. of the blood transferred to a siliconed centrifuge tube containing 1.0 ml. of a 1.0% solution of disodium diaminoethanetetraacetate and 0.7% NaCl and mixed by slow inversion. After removal of blood for a platelet count, the tube was transferred to a chilled 15 ml. centrifuge bucket. This was prepared by placing the 15 ml. bucket without its rubber cushion inside a 50 ml. bucket containing its rubber cushion, filling the intermediate space with water, and storing the combination in a deep freeze cabinet (−15°) until required; the rubber cushion of the 15 ml. bucket was replaced immediately before use. The blood was centrifuged for 20 min. at 150 *g*. The platelet-rich plasma was withdrawn into a siliconed pointed centrifuge tube and centrifuged in a chilled bucket for 15 min. at 2,000 *g*. The button of platelets was freed from supernatant plasma and suspended in 7 ml. of physiological saline, care being taken to obtain a uniform suspension. A sample was taken for a platelet count from this suspension, which was then frozen to −15°.

Platelet counts were carried out by the method of Baar (1948). The recovery of platelets averaged 72%.

Estimation of 5-Hydroxytryptamine.—Estimations were carried out in duplicate on 3 ml. portions of the saline suspension by a fluorimetric method based on that of Udenfriend, Weissbach and Clark (1955), as modified by Brodie, Tomich, Kuntzman and Shore

(1957). In this method the suspension is brought to *p*H 10 with the addition of borate buffer, and shaken with *n*-butanol; heptane is then added to the butanol solution and the 5-hydroxytryptamine extracted from the solvent mixture into 0.1 N HCl. It is estimated in the Aminco-Bowman Spectrophotofluorimeter using exciting light of wavelength 295 mμ and measuring the fluorescence in the range 330 to 340 mμ.

The Locarte fluorimeter, Pattern LMF/2 (Laurence, 1957), does not incorporate monochromators, and filters had to be employed. Since it seemed likely to be difficult to obtain filters giving good transmission of the fluorescence with complete cut-off of the exciting light, use was made of the fact that the fluorescence shifts into the visible with a peak at 550 mμ if the 5-hydroxytryptamine solution is made strongly acid (Udenfriend, Bogdanski and Weissbach, 1955). The filters used have been, on the primary side, Chance OX7+2 cm. thickness of $NiSO_4$ solution (437.5 g./l.), and, on the secondary side, Chance OY3.

Other modifications have involved the retention of the buffer and solvent volumes given in the original paper of Udenfriend *et al.* (1955b) and the use of 0.5 M formate buffer *p*H 4 for the final extraction. There was a considerable reagent blank, equivalent to five to ten times the fluorescence from the original formate buffer; this appeared to be due to butanol. The procedure adopted to reduce this interference to a minimum was to measure the fluorescence of the formate buffer extracts against a reagent blank prepared by passing water through the extraction procedures. To the solutions, volume about 1.5 ml., 0.1 ml. of concentrated HCl was then added. This addition caused some reduction of the reagent blank fluorescence, which was brought back to the previous scale reading by increasing the sensitivity of the instrument. The standard and unknown 5-hydroxytryptamine fluorescences were then measured, the net values being obtained by deducting the readings obtained before adding HCl. A linear relationship between scale reading and 5-hydroxytryptamine concentration was thus obtained. Each group of estimations carried out included pairs of reagent blanks and 5-hydroxytryptamine standards. In order to reduce systematic errors, the duplicates from one platelet suspension were always estimated in separate groups.

Fluorimetric methods of estimation of 5-hydroxytryptamine have come in for criticism, particularly in relation to brain tissue. A comparison of the results obtained by the technique described above with those of other workers is therefore given in Table I.

The mean value for normal subjects obtained with the simple fluorimetric method is practically identical with the mean value obtained with the spectrophotofluorimeter. They are 25% higher than the mean value obtained by the bioassay technique; the difference, though probably significant, is not large and should not lead to grave errors in serial studies.

The Effect of Imipramine on the Estimation of 5-Hydroxytryptamine.—Another source of error may be the presence of imipramine in the blood. It was early noted that, in mixed aqueous solutions, 3 μg./ml. of imipramine could make the fluorimetric estimation of 5-hydroxytryptamine impossible, since imipramine itself fluoresced at *p*H 4 and the fluorescence disappeared on adding HCl. Herrmann and Pulver (personal communication) found that the blood imipramine concentration in animals receiving higher than therapeutic doses was not measurable by the only method available, that is, it was less than 1 μg./ml.

However, even the simplified procedure involves two steps, (1) the separation of the platelets from the blood and (2) the butanol extraction. In Table II, figures are given for the effect of varying concentrations of imipramine on the estimation of 5-hydroxytryptamine in an aqueous mixture of the two compounds subjected to butanol extraction. The

TABLE I

NORMAL BLOOD PLATELET 5-HYDROXYTRYPTAMINE LEVELS: COMPARISON OF RESULTS OF DIFFERENT WORKERS AND METHODS

Figures in brackets are standard deviations, except those marked with an asterisk, which are limits including 95% of observations.

Authors	Method	Description of Subjects	Number	Mean Platelet 5-Hydroxytryptamine of Blood (μg./ml.)
Feldstein, Hoagland, and Freeman (1959)	Spectrophoto-fluorimeter	Normal, male	16	0·19 (±0·08)
Hardisty and Stacey (1955)	Bioassay: rat uterus	Normal, male and female	35	0·16 (±0·06)
This paper	Filter fluorimeter	Normal, male	20	0·20 (±0·05)
Weiner and Udenfriend (1957)	Spectrophoto-fluorimeter	General hospital patients, representative selection, sex unspecified	94	0·23 (0·05–0·49)*
This paper	Filter fluorimeter	Mental hospital patients, male	86	0·24 (0·07–0·50)*

TABLE II

THE EFFECT OF IMIPRAMINE ON THE ESTIMATION OF 5-HYDROXYTRYPTAMINE EXTRACTED FROM AQUEOUS MIXTURES

Aqueous 5-hydroxytryptamine solutions (3·0 ml. samples) were extracted by standard procedure. Percentage recovery was based on parallel extracts of solutions in the absence of imipramine. Figures are means of four estimates, and refer to free base.

5-Hydroxytryptamine Concentration (μg./ml.)	Imipramine Concentration (μg./ml.)	5-Hydroxytryptamine as Percentage of that Extracted from a Solution Containing no Imipramine
0·144	7·5	101
0·144	3·8	100
0·144	1·9	95
0·036	30·0	17
0·036	7·5	85
0·036	1·9	89

higher of the two concentrations of 5-hydroxytryptamine employed is 75% of the normal mean level for platelet suspensions, the lower about 20%. Somewhat surprisingly, since imipramine is a base, the procedure appears to remove it, at any rate up to a concentration eight times the suggested upper limit in blood.

If the imipramine in the blood were equally distributed between the various components, the separation of the platelets, first from the erythrocytes and subsequently from the plasma, would reduce the concentration of imipramine in the saline suspension to one-four-hundredth of that in the original blood. However, since platelets are known to absorb amines, it did not appear wise to make this assumption. Therefore, in experiments on the effect of imipramine on the uptake of 5-hydroxytryptamine by normal platelets, a control was added to give a figure for the effect of imipramine on the estimation of endogenous 5-hydroxytryptamine from platelets. The results are summarized in Table III; they show that imipramine did not interfere with the estimation of 5-hydroxytryptamine in platelets.

Uptake of Exogenous 5-Hydroxytryptamine by Platelets.—The experimental technique was based on that of Born, Ingram, and Stacey (1958). To reduce natural variation, blood for this series of experiments was drawn only from four normal volunteers and no individual's platelets were subjected to the same set of experimental conditions twice. A volume of 22 ml. of blood was taken as previously described and mixed with 2 ml. of anticoagulant; of the platelet-rich plasma, isolated in the standard manner, 1.5 ml. was added to each of four siliconed pointed centrifuge tubes containing 0.5 ml. saline, and either imipramine,

TABLE III

THE EFFECT OF IMIPRAMINE ON THE ESTIMATION OF 5-HYDROXYTRYPTAMINE IN PLATELETS

Three parts of platelet-rich plasma were mixed with one part of saline suspension or drug in saline suspension; mixtures were incubated for 60 min. at 37°. Estimations were in duplicate on each mixture. Imipramine concentrations are as free base; 5-hydroxytryptamine percentages are means.

Imipramine Concentration (μg./ml.)	Number of Experiments	5-Hydroxytryptamine in Presence of Imipramine (Expressed as % 5-Hydroxytryptamine Estimated in Absence of Imipramine)
0·44	3	93
1·77	6	115
7·10	2	105

5-hydroxytryptamine or 5-hydroxytryptamine+imipramine. The final concentrations of imipramine used were 0.5, 2.0, and 8.0 μg./ml.; the 5-hydroxytryptamine concentration was 2.5 μg./ml. except in one set of experiments, where it was 10.0 μg./ml. The final dilutions of the compounds were in saline. After mixing carefully but thoroughly a sample was withdrawn for a platelet count. The tubes were loosely covered and placed vertically in a thermostat at 37° for 60 min., being shaken at sixty double oscillations (6 cm. amplitude) per min. They were then centrifuged under the standard conditions. Since 5-hydroxytryptamine was present in two of the tubes the methods described by Born *et al.* (1958) for the removal of as much plasma as possible were used; the platelets were then resuspended in 5 ml. of saline of which 2 ml. portions were taken for duplicate estimations of 5-hydroxytryptamine. A sample for a platelet count was taken immediately after resuspension.

Effect of Imipramine on 5-Hydroxytryptophan Decarboxylase. — The manometric technique of Davison and Sandler (1958) was employed.

Liver Function Tests.—Determinations of serum bilirubin, thymol turbidity, and alkaline phosphatase activity were carried out by standard methods (King and Wootton, 1956).

RESULTS

Except where otherwise stated, this study refers to male patients who were receiving either imipramine or iproniazid for therapeutic purposes.

Effect of Iproniazid on Platelet 5-Hydroxytryptamine.—Four patients received this drug in tablet form; the dose was raised over the course of 4 days to 225 mg./day. Two patients were taken off the drug after 11 and 13 days; the other two continued to receive it for 33 and 38 days.

The response of the blood platelet 5-hydroxytryptamine level to the drug, calculated as the percentage change from the mean for the pre-treatment values, is given in Fig. 1. Where drug treatment continued for an adequate length of time increases of 200% and upwards were observed. On stopping the drug a return to normal levels occurred.

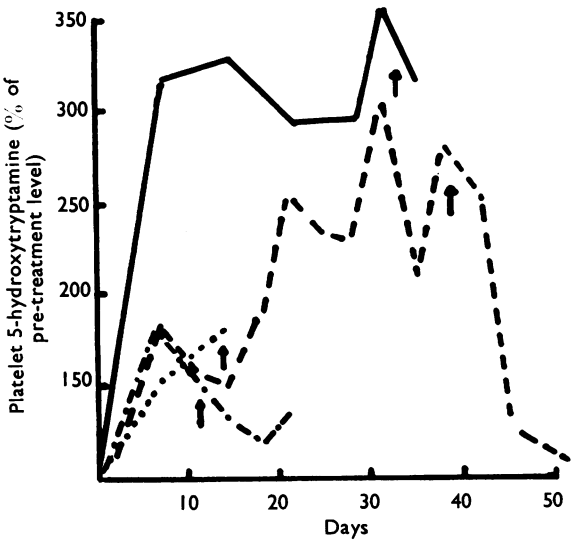

FIG. 1.—The effect of iproniazid on platelet 5-hydroxytryptamine in man. Each line represents one patient. Arrows indicate termination of treatment.

Effect of Imipramine on Platelet 5-Hydroxytryptamine.—Six patients received a standard course of imipramine hydrochloride, commencing with the parenteral therapy recommended for severe cases of depression but passing over to oral therapy at the maximum level of 300 mg./day by the 9th day. The detailed dosage schedule is given in Table IV. Subsequently the dose was adjusted to meet the patients' requirements and ranged from 150 to 300 mg./day.

There was no great variation in response between individuals when this was calculated in terms of the percentage change from the pre-treatment level. The mean values have therefore been given (Fig. 2). Imipramine caused a fall in platelet 5-hydroxytryptamine to 17% of the pre-treatment level after 3 weeks. There was no further significant change up to 6 weeks. A sharp rise in one patient to 50% of the pre-treatment level after 6 weeks was subsequently found to correlate with the patient's refusal to take the drug over a period of 10 days.

TABLE IV

DAILY DOSAGE SCHEDULE FOR IMIPRAMINE

Figures refer to mg. of imipramine hydrochloride. The total daily doses were distributed between three administrations.

Day	Injection mg.	Oral mg.	Total mg.
1	75	0	75
2	100	0	100
3	125	0	125
4	100	50	150
5	75	100	175
6	50	150	200
7	25	200	225
8	0	250	250
9	0	300	300

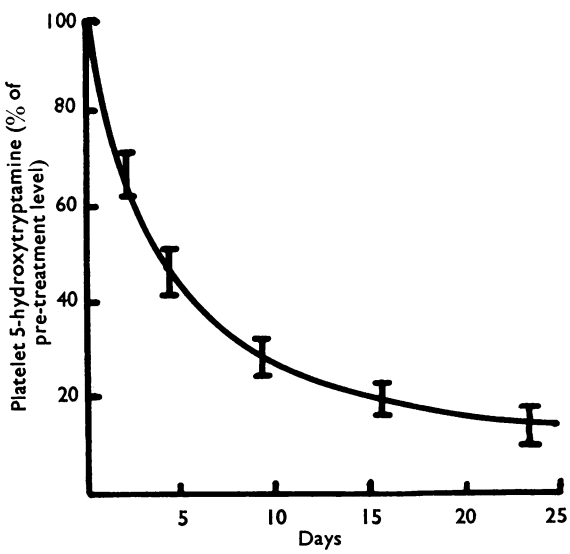

FIG. 2.—The effect of imipramine on platelet 5-hydroxytryptamine in man. Points represent mean percentage of pre-treatment level for six patients, with standard deviations of means.

Observations on the Mode of Action of Imipramine.—Investigation of the action of imipramine on 5-hydroxytryptophan decarboxylase gave negative results: there was no measurable inhibition of enzyme activity at an imipramine concentration of 3×10^{-3} M (1 mg./ml.).

In one patient, the level of excreted 5-hydroxyindoleacetic acid was investigated during a course of therapy. There was no fall in 5-hydroxyindoleacetic acid excretion over a

period of 3 weeks. It has recently been pointed out, however (Milne, 1959), that a fall in the level of excretion is not conclusive proof of decreased synthesis but may also be due to reduced urinary clearance.

In preliminary observations, comparison of the uptake of 5-hydroxytryptamine by normal platelets and by those from patients who had been on continuous imipramine therapy gave equivocal results. It was therefore decided to study the effect of imipramine on normal platelets *in vitro* in the first instance, in order to permit adequate controls with known concentrations of the compounds to be made.

The results of this series of experiments are given in Table V. The uptake of 5-hydroxytryptamine from a diluted plasma containing 2.5 μg./ml. averaged about 300% of the endogenous platelet level. An imipramine concentration of 8.0 μg./ml. reduced the uptake to one-third, and even 0.5 μg./ml. appeared to cause some inhibition. A four-fold increase in 5-hydroxytryptamine concentration markedly reduced the extent of the inhibition, though it raised the uninhibited uptake of 5-hydroxytryptamine by only 40%.

Liver Function Tests on Patients Receiving Iproniazid and Imipramine.—A frequent check is kept on the possibility of liver damage in patients receiving iproniazid (Pare and Sandler, 1959). Liver function tests were done not on the six male patients receiving imipramine but on nine female patients receiving much the same dosage. The results are summarized in Table VI. The percentage of results of these tests above the recognized normal upper limits (Varley, 1954) averaged 22% for iproniazid and 4% for

TABLE V

THE UPTAKE OF 5-HYDROXYTRYPTAMINE BY HUMAN PLATELETS IN THE PRESENCE OF IMIPRAMINE

Platelet-rich plasma (1·5 ml.) was added to 5-hydroxytryptamine and imipramine in saline (0·5 ml.) and incubated for 60 min. at 37° with shaking (60 × 6 cm. double oscillations per min.). Initial 5-hydroxytryptamine contents are the means of values for saline controls and imipramine controls, as in Table III. Drug concentrations are as free base.

5-Hydroxytryptamine Concentration (μg./ml.)	Imipramine Concentration (μg./ml.)	5-Hydroxytryptamine Content (ng./10^8 Platelets)			Percentage Inhibition of Uptake Due to Imipramine
		Initial	After Incubation without Imipramine	After Incubation with Imipramine	
1·1	7·1	82	307	169	61
		40	160	76	70
		90	314	172	63
					Mean 65
1.1	1·77	79	283	168	56
		74	266	177	46
		123	291	200	54
					Mean 52
1·1	0·44	62	229	222	4
		48	361	173	60
		72	271	224	24
					Mean 29
4·3	1·77	56	305	273	13
		69	385	308	24
		115	399	428	−11
					Mean 9

TABLE VI

RESULTS OF LIVER FUNCTION TESTS ON PATIENTS RECEIVING IPRONIAZID AND IMIPRAMINE

Test	Percentage of Tests Giving Figures above Recognized Upper Limit of Normal for Bed Patients		Percentage of Tests Giving Figures Higher than: Bilirubin, 0·5; Thymol Turbidity, 2·0; Alkaline Phosphatase, 8·0	
	Iproniazid	Imipramine	Iproniazid	Imipramine
Bilirubin	17	0	35	15
Thymol turbidity	17	0	92	0
Alkaline phosphatase	33	12	54	19
All tests	22	4	60	11

imipramine. These limits refer to bed patients; the upper limits for normal individuals (and possibly for ambulant mental patients) might be somewhat lower. Varley (1954) gives an upper limit of 2.0 units for the thymol turbidity test in normal subjects; King and Wootton (1956) state that the great majority of normal bilirubin values are below 0.5 units. The percentage above these arbitrarily fixed and more stringent upper limits was 60% for iproniazid and 11% for imipramine.

DISCUSSION

The findings on iproniazid confirm those of Pletscher and Bernstein (1958). The observed fall in platelet 5-hydroxytryptamine in patients receiving imipramine is of interest. Aspects of the pharmacology of this compound have been studied (Bradley and Key, 1959; Mörsdorf and Bode, 1959; Domenjoz and Theobald, 1959), but the findings do not appear to relate directly to the present observation. Himwich (1959) quotes a personal communication by Costa that imipramine increases brain 5-hydroxytryptamine in the rabbit.

The results of the experiments *in vitro* suggest a possible mode of action of imipramine *in vivo*; they do not provide independent confirmation of the validity of the fluorimetric technique nor, assuming this to be valid, do they necessarily account for the quantitative aspects of the findings *in vivo*. An attempt to confirm the present findings using a bioassay technique is obviously desirable, but cannot be undertaken in this laboratory. The exact concentrations of neither imipramine nor 5-hydroxytryptamine in human plasma are known; a value of less than 1.0 μg./ml. has been quoted for imipramine (Herrmann and Pulver, personal communication) and figures of 0.002 and of less than 0.0001 μg./ml. have been given for 5-hydroxytryptamine (Humphrey and Jaques, 1954; Armin and Grant, 1957). The concentrations chosen for the experiments *in vitro* were considerably higher than the concentrations occurring *in vivo*. It appears to be the ratio of the concentrations which is important; however, no extrapolation can be attempted with so many factors unknown. Using lower concentrations of both compounds *in vitro* is not necessarily going to provide the answer, since *in vitro* uptake appears to stop within 75 to 90 min. (Born *et al.*, 1958), whereas *in vivo* the changes would hardly be significant in the first 24 to 48 hr.

Our results suggest that imipramine resembles reserpine in inhibiting the uptake of 5-hydroxytryptamine by platelets (Brodie *et al.*, 1957), but beneath this similarity there lie a number of differences in detail. Reserpine in therapeutic dosage reduces the platelet 5-hydroxytryptamine level to zero in 24 hr., whereas imipramine requires the same number of days to achieve its maximum effect; an equilibrium state appears then to be attained, rather than a complete inhibition. The experiments *in vitro* suggest that, unlike reserpine (Shore, Carlsson and Brodie, 1957), imipramine does not set free the endogenous 5-hydroxytryptamine. With imipramine, depletion may involve competition rather than destruction.

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