An easy method to determine the serotonin content of human platelets

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The functional significance of serotonin (5HT) is still obscure and its tissue concentration is not constant. Carcinoid is the typical condition in which the serotonin content is very high and variable, but probably in many other pathological or physiological conditions the 5HT concentration is modified. Under the influence of certain drugs the serotonin content in tissues undergoes certain variations, which are claimed to be the basis of their mode of action.

The amount of serotonin can be measured directly in the different tissues of laboratory animals but in man this is clinically possible only with blood. This determination, however, is of interest, since the 5HT content of blood usually varies in the same direction as in other tissues, perhaps functionally more important.

Serotonin in human blood is present in a very low concentration (about 0.1 µg./ml.) and is difficult to determine even with special methods (Waalkes, 1959), but as blood serotonin is almost entirely contained in platelets, their isolation provides a means of concentrating the amine. A simple method suitable for clinical research is presented here.

TECHNIQUE

Platelet-rich plasma is prepared from 3 to 5 ml. of blood by the Dillard technique (Dillard, Brecher, and Cronkite, 1951), modified by the use of larger quantities of EDTA solution (about 30%). The thrombocytes are then counted, using white cell pipettes and a Bürker counting cell. After determining the platelet concentration (very constant in our experimental conditions) and the volume of the suspension, the absolute number of thrombocytes is easily obtainable. Platelets are then spun down at 1,600 g for 20 min. at 0 to 4°C.; the supernatant is discarded and the platelets can be stored in a deep-freezer.

Serotonin is determined by a modification of the Weissbach method (Weissbach, Waalkes, and Udenfriend, 1958). Distilled water up to 1 ml. is added to the button of platelets, which is thoroughly resuspended. Then 0.2 ml. of a 10% solution of Zn SO₄·7 H₂O is added, followed, after briefly shaking, by 0.1 ml. of M NaOH. After vigorous shaking for one minute, the test-tubes are centrifuged at 1,600 g for 15 min., when 0.5 ml. of the water-clear supernatant is put in a test-tube, 0.15 ml. of concentrated HCl is added, and serotonin fluorescence is checked at a spectrophotofluorometer (activation 295 mµ,

TABLE I

SEROTONIN LEVEL IN 18 NORMALS

Subject No.	Determination No.	5HT (µg/10 ⁹ platelets)	Average Value
1	1	0.33	0.32
	2	0.31	
	3	0.32	
	4	0.31	
2	5	1.03	1.05
	6	1.07	
	7	1.05	
	8	1.07	
3	9	0.53	0.56
	10	0.55	
	11	0.60	
4	12	0.99	0.95
	13	0.93	
	14	0.93	
	15	0.94	
5	16	0.76	0.78
	17	0.78	
	18	0.79	
6	19	0.68	0.69
	20	0.70	
	21	0.62	
7	22	0.67	0.64
	23	0.62	
	24	0.67	
8	25	0.49	0.52
	26	0.55	
	27	0.66	
9	28	0.72	0.69
	29	0.46	
	30	0.36	
10	31	0.48	0.47
	32	0.45	
	33	0.98	
11	34	1.11	1.04
	35	0.46	
	36	0.70	
12	37	1.08	1.08
	38	0.55	
	39	0.58	

TABLE II

AVERAGE VALUES IN PRESENT SERIES COMPARED WITH SERIES OF OTHER AUTHORS

Authors	Method	No. of Cases	Platelet Serotonin	
			µg/10 ⁹	Variability
Hardisty and Stacey (1955)	Biological	35	0.57	±0.18 (S.D.)
Zucker and Borrelli (1955)	Biological		0.76	
Schmid <i>et al.</i> (1960)	Biological	100	0.73	±0.04 (S.E.)
Weiner and Udenfriend (1957)	Spectrophotofluorometry (Udenfriend <i>et al.</i> , 1955)	94	0.87	
Crosti and Bianchi (1959)	Spectrophotofluorometry (Udenfriend <i>et al.</i> , 1955)	8	0.57	±0.09 (S.E.)
Crosti, Bianchi and Lucchelli (1960)	Spectrophotofluorometry (Udenfriend <i>et al.</i> , 1955)	19	0.57	±0.05 (S.E.)
Bianchi, Crosti, and Lucchelli (1960)	Spectrophotofluorometry (Udenfriend <i>et al.</i> , 1955)	11	0.59	±0.06 (S.E.)
Present paper	Spectrophotofluorometry	18	0.65	±0.06 (S.E.)

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FIG. 1. Activation and fluorescence spectra of extracted serotonin compared with those of a standard solution of 5HT

FIG. 2

FIG. 3

FIG. 2. Correlation between number of platelets and 5HT extracted.

FIG. 3. Serotonin content of platelets after iproniazid, 150 mg./day (—) and syrosingopine, 12.5 mg. intravenously (-----)

fluorescence 540 m μ). A blank (1 ml. of distilled water) and one or more standards (1 ml. of a 0.5 μ g./ml. solution of 5HT base) are run throughout the procedure.

RESULTS

The compound extracted from the platelets shows the same fluorescent characteristics as pure serotonin (Fig. 1) and are known to be highly specific (Weissbach *et al.*, 1958). There is a good correlation between the number of platelets and 5HT extracted, in the range considered (Fig. 2). Table I shows the serotonin level per 10⁹ platelets in 18 normal subjects.

In Table II the average values and variability obtained by our findings are compared with those described by other authors, using different methods.

DISCUSSION

The method described is based on two facts: 1, the highly specific fluorescent characteristics of serotonin, which make its isolation in tissues containing a high level of this substance unnecessary, and 2, blood serotonin is concentrated in platelets. This procedure does not determine free plasma serotonin, which is generally considered to be extremely low, even in carcinoid (Robertson and Andrews, 1961). The obtained results agree well with those already described in the literature.

Platelet serotonin has often been evaluated by the method of Udenfriend, Weissbach, and Clark (1955), but the procedure is tedious and the amount of blood required relatively high (about 20 ml.).

Both these disadvantages seem to be overcome by our method. The facility with which it can be carried out, the short time and the small amount of blood necessary, and the possibility of running many samples at the same time make this procedure particularly suitable for clinical research. The reliability of the method is also shown by the study of platelet serotonin modifications after administration of iproniazid or Rauwolfia alkaloids (Fig. 3). These variations are in agreement with those detected by other methods and reported in the literature.

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Book reviews

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CEREBRAL INFARCTION: THE ROLE OF STENOSIS OF THE EXTRACRANIAL CEREBRAL ARTERIES By Peter O. Yates and Edward C. Hutchinson. (Pp. viii + 95 + index; 20 plates + many diagrams. 14s.) London: Her Majesty's Stationery Office. 1961.

This special report presents in extended detail the material upon which the authors' well-known papers on carotico-vertebral stenosis are based. There can be no doubt that this work has been of great importance in directing the attention of both pathologists and clinicians to the arteries in the neck—so long and so inexplicably neglected as they were.

There are naturally some features open to criticism. The group of cases on which the report is based has a very mixed structure and it is therefore doubtful if some of the analyses to which the group is subjected have any real significance. The assumption that certain arbitrary degrees of arterial stenosis are 'significant' without serious consideration of the haemodynamic problems involved seems unjustified. Definitions of stenosis are not always clear and it is a pity that differing standards were used for the intracranial (p. 11) and the extracranial (p. 16) arteries. At least one reader is left in doubt as to the meaning of such expressions as 'narrowing of the lumen of any degree up to three quarters'. Surely, also, it is an overstatement to say (p. 14) that intramural vessels 'usually' arise from the lumen: most workers have regarded this as the exception rather than the rule.

The most serious criticism of this study, however, is the absence of any satisfactory control group: it is surely essential as a baseline to know the incidence of carotico-vertebral disease in a group of subjects of similar age and in whom there is no evidence of ischaemic cerebral disease, and the absence of such a control group has already led to challenge of the conclusions from one quarter.

The importance of the work, however, remains. There are few who can claim, as these authors can, to have changed post-mortem technique; for it is now obvious that any pathologist who fails to examine the carotico-vertebral system in a case of ischaemic cerebral disease is neglecting an important part of the necropsy.

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FEARON'S INTRODUCTION TO BIOCHEMISTRY, 4th ed. Edited by W. J. E. Jessop. (Pp. viii + 473; illustrated. 30s.) London: William Heinemann.

Because of Fearon's untimely death in 1959, the work has been edited by Professor Jessop, and it is never an easy task to take over another man's structure, especially so here because the design of the work is somewhat unusual. The approach to biochemistry of the earlier editions has been retained: it provides the answers to a number of questions which an intelligent beginner will ask and which remain unanswered in the more orthodox books. Unfortunately, the revision of the text for this edition has not been adequate, and the result shows a lack of balance