lar to those isolated from blood and other tissues. Such investigations by Hardegger, Ruzicka and Tagmann(15) showed that the substances from non-saponifiable extractives of aorta, serum, liver and other tissues are either oxidation products of cholesterol or derivatives of those. This is understandable, considering experiments of Borgstroem and Wintersteiner(16) which show how readily cholesterol can be oxidized by air under conditions similar to the situation of cholesterol in blood, where it is exposed to dissolved oxygen in the presence of solubilizing substances and buffers at a slightly basic pH. More recent contributions to the problem of companions of cholesterol have been made by Fieser(11) and Schwenk *et al.*(17). The impurities may include unknown substances other than steroids. The natural companions of cholesterol, contained in the material used in this work, are evidently different from substances formed when cholesterol is heated(2,3). Such substances enhance the atherosclerotic effect of cholesterol, but the same author(12,18) has found that *in vitro* oxidation of cholesterol decreases its atherogenicity.

Summary. Feeding of highly purified cholesterol to rabbits for 12 weeks resulted in high serum cholesterol and atherosclerotic lesions, which appear more severe than those obtained with commercial cholesterol. This latter contained about 2% impurities isolated from mother liquors after treatment of commercial cholesterol with anhydrous oxalic acid in organic solvents. Such impurities did not produce atherosclerotic lesions when fed to rabbits, but when given together with cholesterol caused less extensive lesions than those obtained with pure or commercial cholesterol.

The help of Erszebet Joachim, Eva Moring and Deirdre McLeod is gratefully acknowledged.

1. Duff, G. L., *Am. Arch. Path.,* 1934, v20, 81, 259.
2. Altschul, R., *Selected Studies on Arteriosclerosis.* Ch. C Thomas, Springfield, Ill., 1950, 135.
3. ———, *Am. Heart J.,* 1950, v40, 401.
4. Cook, R. P., Editor, *Cholesterol.,* Acad. Press Publ., N. Y., 1958, 21, 76.
5. Anitschkow, N., Chalatow, S., *Zentralbl. Allg. Path. pathol. Anatomie,* 1913, v24, 1.
6. Wacker, I., Hueck, W., *Muench. Med. Woch.,* 1913, v60, 2079.
7. Kritschevsky, D., *Cholesterol,* John Wiley Sons, N. Y., 1958, 143.
8. Schwenk, E., Werthessen, N. T., *Arch. Biochem. and Biophys.,* 1952, v40, 334.
9. Smith, L. L., *J. Am. Chem. Soc.,* 1954, v76, 3232.
10. Miescher, K., Kaegi, H., *Helv. Chim. Acta.,* 1941, v24, 986.
11. Fieser, L. F., *J. Am. Chem. Soc.,* 1953, v75, 5421.
12. Alexander, G. J., Schwenk, E., *Arch. Biochem. and Biophys.,* 1957, v66, 381.
13. Kingsley, G., Schaffert, R., *Biochem. J.,* 1949, v180, 315.
14. Abell, L. L., Leny, B. B., Brodie, B. B., Kendall, F. E., *J. Biol. Chem.,* 1952, v195, 357.
15. Hardegger, E., Ruzicka, L., Tagmann, E., *Helv. Chim. Acta,* 1953, v26, 2205.
16. Borgstroem, S., Wintersteiner, O., *J. Biol. Chem.,* 1941, v141, 597; 1942, v145, 309.
17. Schwenk, E., Alexander, G. J., Fish, C. A., Stoudt, T. H., *Fed. Proc.,* 1955, v14, 752.
18. Altschul, R., *J. Am. Med. Assn.,* 1958, v168, 822.

Received May 13, 1959. P.S.E.B.M., 1959, v102.

In vitro Absorption of Serotonin by Thrombocytes of Rheumatoid Arthritic and Non-Arthritic Individuals.* (25137)

GRACE P. KERBY AND S. M. TAYLOR
Dept. of Medicine, Duke University Medical Center, Durham, N. C.

It was noted(1) that the 5-hydroxytryptamine (5-HT, serotonin) content/unit of circulating blood thrombocytes was decreased in patients with rheumatoid arthritis or other inflammatory states, as compared to patients with no evidence of inflammation. Since Spector and Willoughby(2) have shown that 5-HT

* Supported by grants from Nat. Inst. of Arthritis and Metab. Dis., N.I.H., U.S.P.H.S., and Am. Heart Assoc.

appears extravascularly early in evolution of the inflammatory process, it was thought that thrombocyte damage might have occurred and that the decreased 5-HT might simply mean that 5-HT is restored to blood less quickly than are thrombocytes, or it might be conjectured that release of 5-HT from intact thrombocytes had in some way been triggered without equal restoration. However it seemed desirable to look at groups of patients with and without inflammatory states and with rheumatoid arthritis to test the ability of their thrombocytes to absorb 5-HT *in vitro* under standard imposed conditions. There was a possibly significant decrease in amount of 5-HT picked up by thrombocytes from blood of patients with non-rheumatoid inflammatory diseases and rheumatoid arthritis as compared to individuals with no demonstrable inflammation.

Methods. Three groups of patients were studied. These were as previously defined (3) and consisted essentially of Group A with no evidence of inflammatory disease, Group B with significant but non-rheumatoid inflammatory processes,† and Group C with unequivocal active rheumatoid arthritis. Blood was obtained by venipuncture and thrombocyte-rich plasma harvested by the method of Dillard, Brecher and Cronkite(4). Blood volume of total sample was measured by marking the blood level on each tube and finally filling the emptied tubes with water, measuring water volume and correcting for known volume of added anticoagulant; total volume of thrombocyte-rich plasma obtained from the sample was also recorded.‡ Four 2-ml aliquots of thrombocyte-rich plasma were then added to 2 sets of duplicate siliconed 25 ml Erlenmeyer flasks containing, respectively, 0.3 ml of 1% disodium ethylenediaminotetroacetate (EDTA) in 0.7% sodium chloride and either 3 ml (Set A) or 2 ml (Set B) of physiologic saline.§ At zero time, exactly 1 ml of physiologic saline containing 1 μg‖ of serotonin creatinine sulfate (furnished kindly by Abbott Labs) was added rapidly to flask B and immediately immersed in Warburg shaker water bath at 37°C. At exactly 6 minutes,‖ each flask was instantly iced by rapid immersion in ice bath. Contents of each flask were transferred quantitatively with iced saline wash to iced siliconed tubes. The tubes were then centrifuged at 5°C, 500 g, for 30 minutes. Thrombocyte buttons were drained carefully, and walls of tubes were wiped dry. Thrombocytes were then resuspended smoothly and disrupted in 3 ml of 0.14 sodium chloride in 0.02 M sodium hydroxide and subjected as in previous studies(1) to 5-HT extraction and measurement, a standard curve being carried through each extraction procedure as previously. Thrombocyte protein content was es-

† For the first time, due to chance population of hospital wards during the study, Group B series included predominantly acute inflammatory processes particularly lobar pneumonia, whereas prior series included a large proportion of chronic inflammatory processes, e.g. chronic lung disease with secondary chronic bacterial infection.

‡ From this point on, both preparation of platelets for biuret determination and their preparation for 5-HT measurement differed from (1); hence absolute data of present study cannot be compared directly with data of (1).

§ In the study summarized in Table III only, the procedure was varied so that thrombocytes were separated from platelet-rich plasma by centrifuging at 500 g for 30 minutes at 5° C and resuspending in physiologic saline; 2 ml aliquots of saline suspension were then used in each flask, plus 0.3 ml of EDTA and 2 ml of platelet-free autologous or homologous plasma, as indicated in Table III, and in each flask of Set A 1 ml of saline. By this means the effect on recovery systems of inflammatory plasma on noninflammatory thrombocytes and of noninflammatory plasma on inflammatory thrombocytes was observed.

‖ Despite obvious disadvantage of working in the range of a relatively steep slope on curve(5), the information desired concerned ability of thrombocytes to absorb small amounts of 5-HT rapidly. Amount of 5-HT added and test period were constants selected after considerable number of preliminary tests covering periods from 1 to 30 minutes, 6 minutes was selected as time sufficient for few normal thrombocyte-rich plasma samples (prepared as and in amounts specified) to absorb entire added 1 μg of serotonin creatinine sulfate. Disadvantage of the relatively steep slope of curve was minimized by meticulous attention to and care in timing of interval available for absorption of added 5-HT by the thrombocytes. Absorption of 5-HT at low temperatures is much decreased(5) from absorption at 37° C.

TABLE I. Spontaneous Levels of 5-HT in Human Thrombocytes Used in Absorption Experiments of Table II.*

Group (No. of subjects)	Per thrombocytes from 100 ml blood: mg protein	Per thrombocytes from 100 ml blood: μg 5-HT	Per 100 mg of thrombocyte protein, μg 5-HT
A. Noninflammatory (11)	*34.5 ± 13.4*†	*14.4 ± 6.1*	*41.3 ± 12.8*
B. Inflammatory (9)	*47.4 ± 21.5*	*12.7 ± 8.3*	*27.6 ± 13.7*
t, p (*vs* Group A)	1.645, >.05	.515, >.05	2.306, <.04
C. Rheumatoid (11)	*50.2 ± 12.6*	*13.7 ± 6.6*	*27.2 ± 12.0*
t, p (*vs* Group A)	2.841, <.012	.250, >.05	2.665, <.02
" (" " B)	.374, >.05	.294, >.05	.075, >.05

* As noted in footnote (see *Methods*), a difference both in technical methods used and in composition of Group B precludes direct comparison with a previous report(1). Although both (1) and the present work were designed solely for comparison of groups within the individual study, the above data on thrombocyte proteins have absolute validity (because of a washing step introduced) in contrast to(1) where only comparative validity was sought.

† S.D.

timated as before(1) by biuret determination.¶ A 0.7 ml aliquot of thrombocyte-rich plasma was centrifuged at 1000 g for 15 minutes at 5°C, the thrombocytes resuspended and washed in 5 ml saline, recentrifuged and resuspended to original volume in demineralized water for the determination.

Results. Table I summarizes data concerning overall amounts of spontaneous 5-HT content of human thrombocytes, expressed both/unit of blood and/unit of thrombocytes. As in previous studies(1), the amount of 5-HT/unit of blood was uniform in the 3 groups. Amount of thrombocyte protein/unit of blood was increased above noninflammatory Group A levels in the rheumatoid Group C, however, so that the 5-HT content/unit of thrombocytes was decreased ($p<.02$). A corresponding decrease in the inflammatory Group B approached but did not reach significance ($p<.04$). This was in contrast to corresponding findings in (1) where the inflammatory Group B also was decreased ($p<.02$) and may reflect the inclusion of more acute inflammatory processes in Group B of present series, as previously noted.

Table II summarizes data which constituted the chief objective. Expressed as serotonin creatinine sulfate (in contrast to Table I data), column 2 of data suggests that throm-

TABLE II. Absorption by Thrombocytes of Serotonin Creatinine Sulfate Added to Thrombocyte-Rich Plasma.

Group (No. of subjects)	mg thrombocyte protein/2 ml thrombocyte-rich plasma aliquot	μg serotonin creatinine sulfate absorbed, of 1 μg added to 2 ml thrombocyte-rich plasma aliquot
A. Noninflammatory (11)	*1.84 ± .44*†	*.72 ± .22*
B. Inflammatory (9)	*1.95 ± .91*	*.44 ± .27*
t, p (*vs* Group A)	.354, >.05	2.568, <.02
C. Rheumatoid (11)	*1.96 ± .53*	*.52 ± .24*
t, p (*vs* Group A)	.578, >.05	2.131, <.05
" (" " B)	.031, >.05	.688, >.05

† S.D.

¶ However, in(1) a thrombocyte sonicate was prepared, using entire thrombocyte sample drained and tube-wiped but unwashed to minimize prior thrombocyte damage. Aliquots of sonicate were then used for determinations, precluding any opportunity for washing thrombocytes prior to biuret determination. Plasma contamination was thus minimized but present; biuret determinations on dilute plasma samples revealed no significant difference in plasma protein levels between the 3 groups used. The nature of present study required early division of sample into aliquots while thrombocytes were intact and undamaged. This reduced sample volumes below that required for sonication. Since thrombocytes could not be sonicated, an aliquot of intact thrombocytes was available for biuret determination, and these thrombocytes were washed carefully as noted above. Overall amount of biuret positive material in thrombocyte preparations/100 ml of blood was thereby lessened although comparative relationship between Groups A and C of (1) and of the present study was essentially unchanged. The composition of Group B in present study differed from (1) in inclusion of more acute inflammatory states, so the A to B relationship cannot be compared between series.

TABLE III. Effect of Non-Autologous Plasma on Absorption by Thrombocytes of Serotonin Creatinine Sulfate.

Thrombocyte donor	mg thrombocyte protein /100 ml blood	mg thrombocyte protein/ recovery flask	Donor of 2 ml plasma /recovery flask	μg serotonin creatinine sulfate absorbed of 1 μg added
A (Noninflammatory)	25.6	1.71	A	.490
"		"	B	.392
B (Inflammatory)	63.5	2.14	B	.207
"		"	A	.164
C (Noninflammatory)	25.1	2.12	C	.688
"		"	D	.598
D (Inflammatory)	41.3	1.36	D	.146
E (Noninflammatory)	34.1	1.85	E	.682
"		"	F	.589
F (Rheumatoid)	57.7	2.11	F	.282

bocytes from patients with both non-rheumatoid and rheumatoid inflammatory states absorbed serotonin creatinine sulfate less completely than did thrombocytes from patients with no evidence of inflammation, although the added 5-HT/ml plasma was the same in all groups, number of thrombocytes/flask was essentially equal in all groups, and time interval for absorption was rigidly controlled. The decrease was probably significant ($p<.02$) in the inflammatory Group B and possibly significant ($p<.05$) in the rheumatoid Group C.

Table III shows the effect of non-autologous plasma on absorption by thrombocytes of added serotonin creatinine sulfate. Absorption of 5-HT by inflammatory thrombocytes showed the usual lesser value on comparison with noninflammatory thrombocytes when in autologous plasma. When plasmas were reversed so that homologous but not autologous plasma was added to thrombocytes, some depression of absorption of 5-HT was noted. However, the effect of inflammatory plasma on non-inflammatory thrombocytes and of noninflammatory plasma on inflammatory thrombocytes was essentially the same. The noninflammatory plasma did not improve the ability of inflammatory thrombocytes to absorb 5-HT. In presence of inflammatory plasma, noninflammatory thrombocytes were still able to absorb 5-HT in greater amounts than could the corresponding inflammatory thrombocytes in autologous plasma.

Summary. Under controlled *in vitro* conditions (involving equal numbers of human thrombocytes in autologous plasma to which was added an amount of serotonin creatinine sulfate that could be absorbed completely by a few normal specimens in the limited time period allowed), a possibly significant decrease in absorption of serotonin was noted with thrombocytes from patients with non-rheumatoid inflammatory states ($p<.02$) and with rheumatoid arthritis ($p<.05$), as compared to individuals with no demonstrable inflammation.

1. Kerby, G. P., Taylor, S. M., *J. Clin. Invest.,* 1959, v38, 1059.

2. Spector, W. G., Willoughby, D. A., *Nature,* 1957, v179, 318.

3. Kerby, G. P., *J. Clin. Invest.,* 1958, v37, 962.

4. Dillard, G. H. L., Brecher, G., Cronkite, E. P., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 796.

5. Stacey, R. S., Platelets and 5-hydroxytryptamine, pp. 116-118, *5-Hydroxytryptamine,* Edited by Lewis, G. P., Pergamon Press, 1958.

Received May 15, 1959. P.S.E.B.M., 1959, v102.