of tetracycline on the assembly of hepatic lipoproteins and their release into the space of Disse.

Summary. The increments in plasma triglyceride levels observed after administration of Triton were consistently and significantly lower in tetracycline-injected rats, when hepatic tetracycline concentration was high, than in Triton–injected controls. These data are interpreted as indicating impaired release of hepatic triglyceride in tetracycline-injected animals. Hepatic triglyceride increased in tetracycline-injected rats and most of this could be accounted for by impaired release of liver triglyceride. This mechanism, possibly due to impaired synthesis of hepatic lipid acceptor protein, appears to be the major cause of tetracycline-induced fatty liver.

Appendix. The following calculation provides a rough measure of the decrease in triglyceride (TG) delivery into plasma during the first 7 hours after tetracycline (TC) injection and compares this with hepatic TG increase. The TC-induced decrease in hepatic TG secretion was somewhat variable in different experiments (Table I) and hepatic TG and rates of TG secretion were not measured in the same animals, hence the calculations in Tables II and III are an approximation.

We are indebted to Mrs. Mary Naylor for technical assistance.

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Received Dec. 21, 1967. P.S.E.B.M., 1968, Vol. 128.

In Vitro Release of Histamine from Human Peripheral Leukocytes with Compound 48/80* (32965)

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Many substances have been studied for their effect on the release of histamine from tissues. Some of the more effective histamine releasers include D-tubocurarine (1), opium alkaloids (2), primary amines (3), polyvinylpyrollidone (4), and dextran (5). One of the most extensively studied releasers of histamine is compound 48/80, a polymeric condensation product of *p*-methoxyphenethylmethylamine and formaldehyde.

While the study of compound 48/80 has included its histamine releasing effect on many different tissues from a variety of animal species, very little work has been done on the effect of compound 48/80 in man. Paton (6) demonstrated whealing and flaring on intradermal injection of compound 48/80 in man. Others (7) have confirmed and extended this *in vivo* observation.

* Aided by USPHS Research Support Grant No. 5-SO1-FR 05388.

Recent reports (8, 9) indicating release of histamine from suspensions of human peripheral leukocytes incubated with antigen prompted us to study the effects of compound 48/80 on human peripheral leukocyte suspensions.

Materials and Methods. Subjects. Both atopic and nonatopic subjects served in this study.

Separation of leukocytes. The technique of Lichtenstein and Osler (9) was used with slight modifications. All glassware coming in contact with cells is siliconized with a 5% solution of General Electric SC-87 silicone in n-heptane. Twenty ml of blood is drawn with a plastic syringe and mixed in a 40-ml plastic centrifuge tube (IEC 2802) with 5 ml of 6% dextran in isotonic saline, 2 ml of 1.5% NaEDTA in isotonic saline, and 150 mg of glucose. The mixture is centrifuged in an International PR-2 centrifuge at 400 rpm for 20 min and the platelet and leukocyte rich plasma is aspirated with a siliconized pipette. The plasma is centrifuged at 900 rpm at 4°C for 10 min and the cellular button is resuspended with a siliconized Pasteur pipette and washed twice in Tris-A buffer adjusted to pH 7.6 at 25°C (0.025 *M* Tris[1], 0.120 *M* NaCl, 0.005 *M* KCl, human serum albumin[2], 0.03%). After the final centrifugation, the cellular button is brought to a volume of 13 ml in Tris-ACM buffer (Tris-A buffer, 6×10^{-4} *M* Ca, 1×10^{-3} *M* Mg).

Compound 48/80. The compound 48/80 used in this study was supplied by Dr. S. W. Singleton of Burroughs-Wellcome and Company, Tuckahoe, New York, as a dry powder. The compound was prepared volumetrically in Tris-A buffer in 10-ml volumes and stored at —20°C at concentrations of 1:50, 1:250, 1:1000, and 1:5000. The activity of the frozen preparations remained constant for several months. Compound 48/80 gives off fluorescence at the same wavelength as histamine. Therefore, it was necessary to run a curve for fluorescence with each new preparation of compound 48/80 and subtract the reading of the various dilutions of compound 48/80 alone from the reading of the dilutions of compound 48/80 incubated with white cells.

Reaction mixture. Two ml aliquots of cells are mixed in plastic tubes with 2 ml of Tris-ACM and 0.2 ml of Tris-A containing compound 48/80 and incubation is carried out at 37°C for 60 min. The cells are maintained in suspension by gentle agitation at 15-min intervals. After incubation, 0.5 ml of 4.4 *N* $HClO_4$ is added to tube No. 5, the "complete release" tube, and the tubes are centrifuged at 1200 rpm at 4°C for 20 min. Histamine extraction is then carried out on the supernates.

Histamine extraction and assay. The technique of Lichtenstein and Osler (9) was used, the only modification being that readings for fluorescence were taken in a Turner Fluorometer, model 110 using a no. 7-60 primary filter and 47B, 2A and 10% neutral density filters on the photocell side of the fluorometer at a wavelength of 360 mμ. Briefly, the supernatants are extracted into alkalinized butanol and then reextracted from heptane–butanol into 0.1 *N* HCl. After removal of the acid, the histamine is conjugated with *o*-phthaldialdehyde[3] for 40 min at 4°C. The *o*-phthaldialdehyde is recrystallized in petroleum ether and stored in methanol solution at —20°C. The solution is made fresh every 10–14 days.

Experimental design. The experimental design can be seen in Table I with the actual readings from a typical experiment. Tubes 1–4 represent cells incubated with varying dilutions of compound 48/80. Tube 5, the "complete release" tube, represents cells mixed with perchloric acid. In tube 6, the cell blank, cells are incubated without compound 48/80. Tube 7 is the internal control and represents a known amount of histamine carried through the entire extraction procedure. In tube 8, the external control, this same amount of known histamine is conjugated with *o*-phthaldialdehyde and assayed. By comparing tubes 7 and 8, a check is made on the extraction efficiency. Tube 9 represents the internal or reagent blank carried through the extraction procedure. Subtracting the reading of tube 9, the reagent blank, from tube 6, the

[1] Triszma 760 tris (hydroxymethyl) aminomethane; Sigma Chem. Co., St. Louis, Missouri.

[2] Hoechst Laboratories, Cincinnati, Ohio.

[3] Sigma Chemical Company, St. Louis, Missouri.

TABLE I. Design of Experiment Measuring Release of Histamine from Human Leukocytes by Compound 48/80.

Tube	Reaction mixture	30 × Scale readings	48/80 Blank	Nonspecific release[c]	Final reading	Release (%)
1	Cells + 48/80 (1:50) in Tris ACM	102	71	2	29	63.8
2	Cells + 48/80 (1:250) in Tris ACM	54	31	2	21	44.7
3	Cells + 48/80 (1:1000) in Tris ACM	32	21	2	9	19.1
4	Cells + 48/80 (1:5000) in Tris ACM	20	17	2	1	2.1
5	Cells + perchloric acid in Tris ACM	63			47	
6	Cells in Tris ACM	18				
7	Histamine (150 μg/liter) in Tris ACM	89			73	
8	Histamine (150 μg/liter)[a]	177			168	
9	Reagents in Tris ACM	16				
10	Reagents[b]	9				

[a] Not extracted; "external" controls.

[b] Readings over 100 are first read on 10× scale and then multiplied by three.

[c] Tube 6—Tube 9.

cell blank, gives the "nonspecific" release which is an estimation of the care used in handling the cells. In tube 10, the external blank, the reagents are not carried through extraction, but are simply assayed for fluorescence.

Calculations. The amount of histamine released is expressed in terms of percentage release; 100% is tube 5, the "complete release" tube. The 48/80 blank for each dilution of compound 48/80 includes the reagent blank. This 48/80 blank is subtracted from the dial readings for tubes containing cells plus compound 48/80. From this number, the nonspecific release reading (tube 6 — tube 9) is subtracted to give the final histamine reading for tubes 1–4. The percentage release is obtained by dividing the final histamine reading by the final complete release reading (tube 5—tube 9). Sample figures and calculations are given in Table I.

Results. The results on 12 subjects can be seen in Table II. In each case, incubation of peripheral white cells with decreasing amounts of compound 48/80 resulted in the release of a smaller percentage of histamine. Percentage release of available histamine at a 1:50 cencentration of compound 48/80 varied from 54 to 90%. Concentrations of 48/80 stronger than 1:50 resulted in readings higher than 100% of available histamine. At a concentration of compound 48/80 of 1:250, percentage of available histamine released ranged from 30.2 to 52.3%, while the range at 1:1000 compound 48/80 was 10.6–31.8%. A significant percentage of release was consistently found at a concentration of compound 48/80 of 1:5000, while in several instances a 1:10,000 concentration of compound 48/80 resulted in release of detectable amounts of histamine. The atopic history in a subject seemed to bear little relationship to percentage of histamine released. The first 6 subjects in Table II are nonatopic, the last 6 are atopic. As can be seen, there is no essential difference in histamine release between the two groups.

Discussion. The potent histamine releasing property of compound 48/80 has been demonstrated in a wide variety of tissues and species, including dog, cat, mouse, rat, guinea pig, chicken, rabbit, and man. Paton (6) injected compound 48/80 intravenously in the dog and cat and produced a marked fall in blood pressure and greatly increased plasma histamine. It was later demonstrated (10) that injection of compound 48/80 into the saphenous artery of the dog depleted up to 58% of the histamine content in the skin of the limb. Mongar and Schild (11) obtained significant amounts of histamine after incubating compound 48/80 with thin pieces of different tissues from guinea pig suspended in Tyrode's solution. Mota *et al.* (12) brought histamine release to a cellular level by showing that compound 48/80 was extremely

TABLE II. Percentage Release of Available Histamine from Human Peripheral Leukocytes by Various Dilutions of Compound 48/80.

Subject	Dilutions of Compound 48/80				
	1:50	1:250	1:1000	1:5000	1:10,000
M.C.	79.9	34.1	18.4	9.4	5.0
G.G.	75.2	42.7	20.1	9.6	4.3
R.S.	66.3	31.1	21.8	4.2	0
G.S.	60.0	33.3	16.7	1.7	—
R.T.	84.1	52.3	24.7	4.5	—
A.B.	91.3	41.3	13.0	4.3	—
R.W.	63.8	44.7	19.1	2.1	—
P.S.	90.7	40.7	18.5	7.5	—
P.G.	74.2	36.4	10.6	4.5	—
T.T.	54.7	30.2	11.3	9.4	—
V.K.	67.5	47.7	31.8	4.0	—
H.H.	86.0	51.3	25.6	14.1	6.3

effective in disrupting rat mast cells *in vivo* and *in vitro*. Marks (13) made the first observation of the degranulation of basophilic leukocytes of the chicken and rabbit in response to compound 48/80.

While most studies were dealing with histamine release from tissues and degranulation of mesenteric mast cells and basophilic leukocytes in situ, some investigators, notably Lagunoff (14) and Ùvnas (15), had turned to the isolated mast cell and demonstrated that, when exposed to compound 48/80, the isolated mast cell reacted promptly, not only with degranulation, but also with release of histamine. Some (16) have looked on compound 48/80 as a specific mast cell histamine releaser and have cited that there is no established case where compound 48/80 releases histamine from any cell other than a mast cell.

Because of the successful demonstration of antigen induced histamine release from suspensions of human peripheral leukocytes, it seemed reasonable to investigate the effect of compound 48/80 on human white cells. The present study indicates that suspension of peripheral leukocytes affords a system for studying *in vitro* histamine release by compound 48/80 in man. The peak of release was seen with a concentration of 1:50 while significant amounts of histamine were consistently found with a concentration of compound 48/80 as small as 1:5000. Preliminary observations would indicate that there is no difference between atopic and nonatopic subjects so far as leukocyte release of histamine in response to compound 48/80 is concerned. Studies relating skin reactivity and *in vitro* histamine release and the effect of antihistamine on this release are in progress.

Summary. The histamine releasing property of compound 48/80 has been studied in a variety of tissues and species. *In vivo* and *in vitro* studies in the experimental animal have shown effects of compound 48/80 on tissue mast cells and basophiles. The only studies in man have involved intradermal skin testing. The present report indicates that suspensions of human peripheral leukocytes affords a system for studying *in vitro* histamine release by compound 48/80 in man. There is no difference in the response of *atopic* and *nonatopic* subjects.

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Received Dec. 22, 1967. P.S.E.B.M., 1968, Vol. 128,