oleate solutions, it is apparent that oleate possesses the potential of inducing a decelerating hemolytic chain reaction under proper dynamic circumstances—*i.e.*, when erythrocytes binding oleate lyse after moving into areas populated by normal erythrocytes.

Summary. 1. The initial step in human erythrocyte lysis by oleate at pH 7.2 ± 0.1 and concentrations below 8 x 10^{-4} M involves erythrocyte-oleate binding; oleate binding by a standard number of erythrocytes in a fixed volume is a function of oleate concentration, temperature, and contact time. 2. Once lytic quantities of oleate are bound, hemolysis proceeds at pH 7.2 ± 0.1 as a function of quantity bound and of temperature. Lysis cannot be checked by repeated iced saline washings. A mean minimum of approximately 7 x 10^{-9} μg of sodium oleate is required/erythrocyte at 37°C, pH 7.1 for lysis at rates detectably faster than controls; the sodium oleate binding mechanism appears saturated with a mean of approximately 111 x 10^{-9} μg/erythrocyte. 3. Lowered surface tension of oleate solutions does not influence hemolysis rates under conditions tested. 4. Cyanide retards oleate hemolysis; other nonspecific enzyme inhibitors fail to do so. 5. Following oleate hemolysis, hemolytic substances are released; these probably represent oleate or products of oleate-erythrocyte interaction. It is suggested that oleate possesses the potential of inducing a decelerating hemolytic chain reaction.

1. Bodansky, M., PROC. SOC. EXP. BIOL. AND MED., 1930, v28, 632.
2. Pethica, B. A., Schulman, J. H., *Biochem. J.*, 1953, v53, 177.
3. Ponder, E., Gordan, A. S., *ibid.*, 1934, v28, 748.
4. ———, *J. Gen. Physiol.*, 1944, v27, 483.
5. Tsai, C., Lee, J. S., *Chinese J. Physiol.*, 1941, v16, 165.
6. Ponder, E., Hyman, C., White, L., *Am. J. Physiol.*, 1941, v132, 18.
7. Gordon, R. S., Jr., Cherkes, A., *J. Clin. Invest.*, 1956, v35, 206.
8. Loewy, A., Freeman, W., Marchelle, A., Johnson, V., *Am. J. Physiol.*, 1942, v138, 230.
9. Shafiroff, B. G. P., Mulholland, J. H., Baron, H C., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 224.
10. Creditor, C. M., *ibid.*, 1953, v82, 83.
11. Weld, C. B., Spitzer, J. J., *Am. J. Physiol.*, 1952, v169, 726.
12. Lee, J. S., Tsai, C., *Quart. J. Exp. Physiol.*, 1942, v31, 281.
13. Getman, F. H., Daniels, F., *Outlines of Theoretical Chemistry,* Publ. John Wiley & Sons, Inc., N. Y., 1937.
14. Dervichian, D. G., *Ann. Inst. Pasteur,* 1949, v77, 193.
15. Ponder, E., Ponder, R. V., *J. Gen. Physiol.*, 1958, v41, 609.
16. Goodman, D. S., *J. Clin. Invest.*, 1958, v37, 1729.

Received February 25, 1959. P.S.E.B.M., 1959, v101.

Comparative Effects of Glycogen and Antigen-Antibody Reactions on Serotonin and Histamine in the Rabbit. (24852)

T. PHILLIP WAALKES* AND HARRIETTE COBURN (Introduced by M. B. Shimkin)
Section of General Medicine, Nat. Heart Inst., U. S. Dept. of H. E. W., Bethesda, Md.

In rabbit blood, serotonin (5-hydroxytryptamine) and histamine are present in, or bound to the platelets. When antigen is added to blood from a sensitized rabbit, clumping of platelets and leucocytes occurs, and serotonin as well as histamine is released from platelets (1). During anaphylaxis in the rabbit, similar changes occur. In addition, a decrease occurs in whole blood concentration of these amines(1). This decrease is secondary to disappearance of platelets from circulating blood. The rapid fall in number of platelets and leucocytes in blood is a consistent finding during hypersensitivity reactions in animal species including dogs, monkeys, guinea pigs, and rabbits(2,3,4). In humans, sensitive to

*Present address: Nat. Cancer Inst., Bethesda, Md.

ragweed, intradermal injection of ragweed antigen produces a decrease in number of platelets in peripheral blood(5). Recent evidence shows that during anaphylaxis in the rabbit, platelets are trapped in the lung but not in other organs or tissues(6). When antigen-antibody complex precipitate, suspended in saline, is added to normal rabbit blood *in vitro* or is injected intravenously into normal rabbits, platelet-leucocyte clumps are formed and serotonin and histamine are released. *In vivo,* a marked decrease in whole blood serotonin and histamine occurs, accompanied by trapping of platelets in lung but not in other tissues(6). These findings suggest that direct action of antigen-antibody complex itself is the main causative factor for changes in platelets, serotonin, and histamine which occur during anaphylaxis. Clumping of platelets and leucocytes, associated with disappearance of these blood elements from peripheral circulation has been implicated in Schwartzman (7) and Arthus(8) reactions in rabbits. Platelet-leucocyte clumps have also been found in hearts of patients dying from acute rheumatic fever or rheumatic carditis(9). Glycogen, after intravenous injection into rabbits and dogs, caused rapid clumping and disappearance of platelets and leucocytes from the circulation similar to antigen-antibody reaction(4). Extracts from parasites, *Ascaris lumbricoides* and *Echinococcus granulosus* (hydatid fluid), also have the ability to produce the same blood changes. The active principle in these preparations is polysaccharide in nature(4). Glycogen *in vitro*, however, did not cause release of histamine, and *in vivo* was thought to distribute platelets and leucocytes equally among various tissues. Dextran, on the other hand, when incubated with rabbit blood *in vitro* does release large amounts of histamine from the platelets(10). *In vivo* in rats, dextran produces a typical anaphylactoid response(11). The present study was undertaken to determine the effect of glycogen on serotonin, histamine, and platelets in rabbits both by *in vitro* and *in vivo* technics. The results are compared with those obtained by using an antigen-antibody complex precipitate, in addition to those results found during complete antigen-antibody reaction.

Materials and methods. Male white rabbits (2.5 kg) were used. Ten were sensitized to horse serum as previously described(1). For *in vitro* studies, blood was obtained by cardiac puncture; for *in vivo* studies, blood was obtained by means of plastic tube inserted into carotid artery. Heparin or EDTA (disodium ethylene-diaminetetraacetate, 10% by volume of a 1% solution in n-saline) was used as anticoagulant. Siliconized glassware was used in handling blood. After death of rabbits, their lungs were immediately excised, pressed between porous paper to remove excess blood, and homogenized in 0.1 N HCl. Purified glycogen preparations from 3 different commercial sources were tried and all gave identical results. The antigen-antibody complex was precipitated from blood serum of rabbits sensitized to horse serum and given an additional 3 ml booster injection intraperitoneally of this antigen 3 weeks before blood was drawn(6). The precipitate was washed 3 times with cold saline and suspended in saline. Incubations with rabbit whole blood were carried out as previously reported(1). Serotonin and histamine analyses in whole blood, plasma, and tissues were done by the method of Weissbach *et al.*(12).

Results. Both glycogen and antigen-antibody complex release serotonin and histamine *in vitro* from rabbit platelets, and similar to release of these amines when horse serum is added to blood from rabbits sensitized to this antigen (Table I). For these *in vitro* studies, heparin was used as anticoagulant. When EDTA was used as anticoagulant the release of serotonin and histamine was inhibited in all cases. Previous work by Humphrey and Jaques(13) showed that addition of purified antigen and antibody to normal rabbit platelets suspended in normal plasma released serotonin and histamine. Removal of calcium ion from plasma inhibited this reaction.

In vivo, the effects of glycogen and antigen-antibody complex after intravenous injection into non-sensitized animals were similar to those produced by intravenous injection of antigen into sensitized rabbits. Serotonin and histamine were released into the plasma

TABLE I. *In Vitro* Release of Serotonin and Histamine.*

Agent	No. determinations	γ/ml plasma (avg values) Heparin anticoagulant Serotonin	Heparin anticoagulant Histamine	EDTA anticoagulant Serotonin	EDTA anticoagulant Histamine
Antigen-antibody reaction (1:100 horse serum in n-saline)	10	4.0	3.1	.3	.4
Antigen-antibody complex	4	5.0	3.2	.4	.4
Glycogen	6	5.5	3.5	.4	.5
n-Saline	10	.3	.4	.2	.2

* Plasma serotonin and histamine were determined after incubation of rabbit whole blood 30 min. at 37°C with 50% by volume of above agents, using heparin or EDTA as anticoagulants. For antigen-antibody reaction, blood was taken from rabbits sensitized to horse serum. Blood from normal rabbits was used for other agents. Amount of antigen-antibody complex for each experiment was precipitated from 1 ml of rabbit serum. Final dilution of glycogen was 1.3 mg/ml.

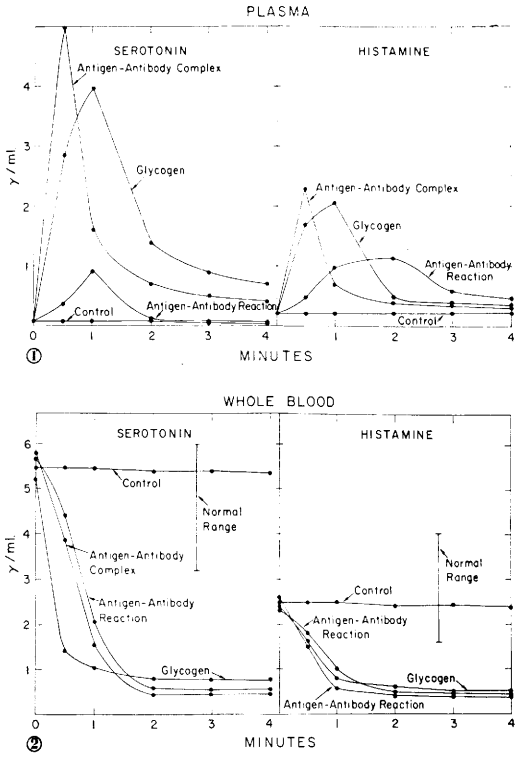

FIG. 1. Blood plasma concentration of serotonin and histamine was determined after intrav. inj. of each agent as follows: antigen-antibody reaction, 2 ml/kg of horse serum into previously sensitized rabbits; antigen-antibody complex, total precipitate from 13 ml of rabbit serum was used for each experiment and inj. into normal rabbits; glycogen, 100 mg/kg; and for normal controls, 2 ml/kg of n-saline. Five animals were used for each agent; only 2 were used with the antigen-antibody complex. Each point is avg value.

FIG. 2. Whole blood serotonin and histamine were determined on samples of blood, prior to centrifugation for plasma, used in Fig. 1.

TABLE II.

Agent	Lung serotonin and histamine,* γ/g (avg values) Serotonin	Histamine
Antigen-antibody reaction	21.5	14.3
Antigen-antibody complex	20.0	13.5
Glycogen	13.0	9.0
n-Saline	2.6 (1.2–4.6)†	4.5 (3.0–6.0)†

* Lung content of serotonin and histamine was determined in rabbits to obtain data for Fig. 1 and 2. Animals were killed exactly 4 min. after inj. of each agent.

† Normal range.

(Fig. 1), whole blood serotonin and histamine content was reduced (Fig. 2), and lung concentration for these amines was increased (Table II). The latter 2 effects were attributed to occlusion of platelet-leucocyte clumps or emboli in the lung. Other tissues, including heart, brain, intestine, and liver from these rabbits, did not show any significant difference in amount of serotonin and histamine compared to same tissues from untreated animals, except for a slight increase in serotonin in the liver.

Discussion. The results show a marked similarity between the effects of antigen-antibody complex and glycogen on rabbit platelets and leucocytes, and in release of serotonin and histamine from these platelets. The mechanisms involved appear to be the same. Other substances such as peptone(14) and trypsin(15) have been reported to cause a decrease in circulating platelets and leucocytes when injected into rabbits and dogs. Initial

observations, however, show that total results with these agents are different from those found with glycogen in this study.

Prior work with dextrans indicated that ability to release histamine from rabbit blood was dependent upon molecular size of the dextran(10). The same factor might apply to glycogen and its ability to release serotonin and histamine. The nature of end groups in the glycogen molecule, and alterations in its structure formed as a result of isolation and purification process, may also produce some of the properties in the polysaccharide needed to cause the effects found.

The distinct importance of platelets and leucocytes during anaphylaxis in the rabbit should be recognized. The role of these blood elements during allergic reactions in other animal species and in man could be of equal importance. In addition, the possibility that glycogen, or a polysaccharide-like material, may be involved in hypersensitivity processes needs further investigation.

Summary. When glycogen is incubated with normal rabbit blood, both serotonin and histamine are released from the platelets. EDTA inhibits this release. Intravenous injection of glycogen into rabbits produces a marked fall in whole blood concentration and a rise in lung content of serotonin and histamine. These results are secondary to trapping of platelets in the lung. In addition, both serotonin and histamine are released into the plasma. All of these changes are identical to those obtained when antigen is injected intravenously into a sensitized rabbit or when an antigen-antibody complex precipitate is injected intravenously into a normal rabbit.

1. Waalkes, T. P., Weissbach, H., Bozicevich, J., Udenfriend, S., *J. Clin. Invest.*, 1957, v36, 1115.

2. Dean, H. R., Webb, R. A., *J. Path. and Bact.*, 1924, v27, 65.

3. Kopeloff, N., Kopeloff, L., *J. Immunol.*, 1941, v40, 471.

4. Rocha e Silva, M., *Ann. N. Y. Acad. Sc.*, 1950, v50, 1045.

5. Storck, H., Hoigne, R., Koller, F., *Int. Arch. Allergy*, 1955, v6, 372.

6. Waalkes, T. P., Coburn, H., *J. Allergy*, in press.

7. Stetson, C. A., *J. Exp. Med.*, 1951, v93, 49.

8. ———, *ibid.*, 1951, v94, 347.

9. ———, *ibid.*, 1951, v94, 493.

10. Haining, C. G., *Brit. J. Pharmacol.*, 1955, v10, 87.

11. Halpern, B. N., Histamine (Ciba Foundation Symposium, Little, Brown and Co., 1956) p92.

12. Weissbach, H., Waalkes, T. P., Udenfriend, S., *J. Biol. Chem.*, 1958, v230, 865.

13. Humphrey, J. H., Jaques, R., *J. Physiol.*, 1955, v128, 9.

14. Scroggie, A. E., Jaques, L. B., Rocha e Silva, M., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 326.

15. Dragstedt, C. A., Rocha e Silva, M., *ibid.*, 1941, v47, 420.

Received March 10, 1959. P.S.E.B.M., 1959, v101.

Sources of Excess Taurine Excreted in Rats Following Whole Body Irradiation.* (24853)

D. N. STERN AND E. M. STIM (Introduced by Abraham White)

Dept. of Biochemistry, Albert Einstein College of Medicine, Yeshiva University, N. Y. City

Excessive urinary excretion of taurine has been observed in rat and man in the 24-hour period following exposure to whole-body x-irradiation(1-4). This taurine response appears to be an extremely sensitive and early indicator of radiation exposure, being detectable at doses as low as 35 r(4) and as early as 3 hours post-irradiation(2). Urinary taurine excretion increases semilogarithmically with roentgen dosage in the range between 0 and 250 r. Above this degree of radiation, taurine response does not increase(2). The magnitude of urinary excretion of taurine is a valuable index of survival in rats exposed to LD_{50} dose of x-ray(1). Since taurine is one of the major end products of SH oxidation

* This investigation was supported by Public Health Service Training Grant.