Comparison of Leukocyte Changes Produced by Pyrogens and by Anaphylaxis in the Guinea Pig.* (18923)

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It is well known that in human beings and animals, the injection of bacterial pyrogens or endotoxins produces a precipitous fall in circulating leukocytes at the expense of the polymorphonuclear cells; this initial reduction is followed by a polymorphonuclear leukocytosis within a few hours(1). Many investigators have also reported that anaphylactic shock in animals is accompanied by an initial reduction in peripheral polymorphonuclear cells followed shortly by leukocytosis if animals survive the acute episode(2). Although the symptoms and physiologic changes produced by pyrogens and those accompanying anaphylaxis are easily distinguished and generally recognized as separate entities, the hematologic changes are not. Current hematology texts lump together as causes of sudden leukopenia "anaphylactoid shock and early stages of reaction to foreign protein"(3), "anaphylactic shock due to introduction of a foreign protein" or "acute protein shock such as is caused by the intravenous injection of typhoid vaccine"(4).

It is the purpose of the present report to show that the variations in circulating leukocytes which accompany anaphylaxis in the guinea pig are not due to pyrogen contamination and to point out that a difference in behavior of the circulating eosinophils makes it possible to distinguish between the two types of reaction in this animal.

Methods. Male guinea pigs weighing 250 to 350 g were used. Diet consisted of laboratory pellets with added green vegetables twice weekly. The following antigens were used: normal horse serum, diphtheria antitoxin (Squibb), and crystalline ovalbumin. Pyrogen preparations were *Escherichia coli* and typhoid vaccines containing 1.0 billion organisms per ml and a filtrate of *Pseudomonas aeruginosa* prepared by the method of Welch (5). Intravenous injections were made into the dorsal penile vein. Total leukocyte counts were performed by the usual methods, employing 3% acetic acid with 0.1% added gentian violet as a diluting fluid. Except for a few experiments in which slide smears were made, cover-slip smears stained with Wright's stain were examined for differential counts. Eosinophils were enumerated by a direct chamber method within 5 minutes after collecting blood. The acetone-water-eosin fluid recommended by Thorn *et al.*(6) was found satisfactory. Pipettes for eosinophil counts were shaken 150 times after loading. Blood was collected from fresh cuts made over ear veins. At least 2 control counts were obtained before injection of test materials and post-inoculation counts were taken at varying intervals. All syringes and glassware were sterilized at 170°C for 2 hours to destroy any contaminating pyrogen. 0.9% sodium chloride solution and solutions of antigens were tested at frequent intervals in rabbits and were always pyrogen free.

Effect of anaphylaxis produced by non-pyrogenic antigens upon leukocyte count. Eight animals were given 0.1 ml of diphtheria antitoxin (Squibb) intravenously, total and

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1. Bennett, I. L., and Beeson, P. B., *Medicine*, 1950, v29, 365.

2. Biedl, A., and Kraus, R., *Wien klin. Wochenschr.*, 1909, v22, 363; Weiss, H., and Tsuru, J., *Z. f. Immunitat.*, 1910, v5, 516; Watanabe, Y., *Okayama-Igakkai-Zasshi*, 1940, v52, 885; Kinsell, L. W., Kopeloff, L. M., Zwemer, R. L., and Kopeloff, N., *J. Immunol.*, 1941, v42, 35.

3. Wintrobe, M. M., Clinical Hematology, Philadelphia, Lea and Febiger, 1946.

4. Whitby, L. E. H., and Britton, C. J. C., Disorders of the Blood, Philadelphia, Blakiston, 1950.

5. Welch, H., Calvery, H. O., McClosky, W. T., and Price, C. W., *J. Am. Pharm. Assn.* (sci. edit.), 1943, v32, 65.

6. Thorn, G. W., Forsham, P. H., Prunty, F. T. G., and Hills, A. G., *J. A. M. A.*, 1948, v137, 1005.

TABLE I. Absolute Polymorphonuclear Leukocyte Counts of Guinea Pigs Given 1 ml Horse Serum Intraperitoneally on 1st and on 21st Experimental Day. Counts 5 min before and 20 min after injection. All figures refer to mean ± standard error.

Day	No. animals	Before injection	After injection
1	8	5536 ± 541	5935 ± 410
21	8	5979 ± 768	990 ± 253

differential counts being done on blood taken 5 minutes before and 5 minutes after injection. No consistent alterations in the number of circulating white cells were noted. The injection of 0.1 ml of antitoxin was repeated 20 days later. Leukocyte counts 5 minutes after injection consistently showed a marked diminution in circulating polymorphonuclear cells. All animals developed typical signs of anaphylaxis after reinjection, 7 of the 8 dying within 10 minutes. The lone survivor was given 0.1 ml of antitoxin intravenously 24 hours later without significant change in leukocyte count. In a similar experiment, 6 animals were given 1.0 mg of crystalline ovalbumin in 1.0 ml of 0.9% sodium chloride solution intravenously; no consistent change in leukocyte count ensued. The intravenous injection of 1.0 mg of ovalbumin was repeated 20 days later. Total leukocyte counts dropped sharply within 5 minutes at the expense of the polymorphonuclear cells. All animals had typical symptoms of anaphylaxis and died within 20 minutes after reinjection. In the third experiment, 8 animals were given 1.0 ml of non-pyrogenic horse serum intraperitoneally, leukocyte counts being performed before injection and 20 minutes after injection. No consistent changes were noted. After 20 days, the intraperitoneal injection of horse serum was repeated. A leukopenia due to disappearance of the neutrophils was noted at 20 minutes, followed by a leukocytosis at 4 hours. Although all animals showed evidence of anaphylaxis with ruffling of fur, dyspnea, etc., none died. Table I shows the polymorphonuclear response in this group of animals. In a fourth experiment, 6 guinea pigs were given 2.0 mg of crystalline ovalbumin intraperitoneally on the first experimental day and this injection was repeated on the 21st day. Leukocytes remained relatively unchanged on the first day, but after sensitization, all animals responded with leukopenia followed by leukocytosis. Table II illustrates the changes in polymorphonuclear cells in this group of animals.

Effect of pyrogenic materials upon leukocyte count. Six animals were given 1.0 ml of *E. coli* vaccine intraperitoneally, total and differential leukocyte counts being done just before injection and at 20 minutes, 1 hour, and 4 hours after injection. The changes in leukocytes followed a pattern entirely similar to those noted during anaphylaxis. In similar experiments, groups of 4 animals received 1.0 ml of typhoid vaccine or 5.0 ml of *Ps. aeruginosa* filtrate intraperitoneally. The leukocyte changes in these animals followed the pattern described above. Table III depicts the changes in neutrophils after pyrogen injection.

Effect of anaphylaxis and pyrogen injection upon circulating eosinophils. Total eosinophil counts were performed upon guinea pigs given 2 injections of 1.0 ml of horse serum intraperitoneally 21 days apart and upon the group given 2.0 mg of ovalbumin intraperitoneally. Two control counts were done before injection with counts at 20 minutes and then hourly intervals up to 4 hours after injection. The first injection of antigen produced no consistent change in circulating eosinophils although there was a tendency for the counts to drop slightly during the 4 hours, perhaps as a result of handling the animals. Reinjection of the antigen produced a striking rise in eosinophils in every animal. This increase was usually noted at about the second hour and was invariably present at 4 hours. The injection of *E. coli* vaccine, typhoid vaccine, or *Ps. aeruginosa* filtrate intraperitoneally produced a sharp drop in circulating eosinophils, at times causing them to disappear entirely by the fourth hour. Tables IV and V contrast the effects of anaphylaxis and pyrogens upon circulating eosinophils.

Discussion. The experiments reported here confirm the observations of others that anaphylactic shock is accompanied by a rapidly developing leukopenia followed by a

TABLE II. Absolute Polymorphonuclear Leukocyte Counts of Guinea Pigs Given 2 mg Ovalbumin Intraperitoneally on 1st and on 21st Experimental Day. All figures refer to mean ± standard error.

Day	No. animals	Before injection	20 min	1 hr	4 hr
1	6	5313 ± 820	5576 ± 564	5942 ± 761	5461 ± 684
21	6	5135 ± 524	1368 ± 314	5351 ± 1179	19470 ± 1757

TABLE III. Absolute Polymorphonuclear Leukocyte Counts of Guinea Pigs After Injection of *E. coli* or Typhoid Vaccine. Figures refer to mean ± standard error.

No. animals	Before injection	20 min	1 hr	4 hr
14	5170 ± 553	1122 ± 650	5096 ± 860	18769 ± 1748

TABLE IV. Circulating Eosinophils After Injection of Pyrogen-Free Antigens on 1st and 21st Experimental Day. Figures refer to mean ± standard error.

Day	No.	Before injection		20 min	1 hr	2 hr	3 hr	4 hr
1	14	52 ± 13	47 ± 12	54 ± 14	52 ± 14	53 ± 18	44 ± 14	50 ± 13
21	14	77 ± 17	72 ± 17	70 ± 12	80 ± 13	122 ± 25	259 ± 13	360 ± 12

TABLE V. Circulating Eosinophils in Guinea Pigs After Injection of *E. coli* or Typhoid Vaccine. Figures refer to mean ± standard error.

No.	Before injection		20 min	1 hr	2 hr	3 hr	4 hr
14	73 ± 11	74 ± 10	74 ± 9	79 ± 6	59 ± 4	23 ± 3	14 ± 3

leukocytosis. This type of hematologic response is also characteristic of the reaction to injection of bacterial pyrogens. However, since pyrogen free antigenic materials produced this response in sensitized animals, these changes are not due to pyrogen contamination.

Herrick and, more recently, Campbell(7), using various antigens, have shown that the reinjection of antigen in a sensitized animal results in peripheral eosinophilia. These workers recorded eosinophils in terms of per cent or computed absolute values by the relatively inaccurate method of simultaneous total and differential counts. None of their studies was done with serial counts at brief intervals after injection; for the most part, only single daily counts were recorded. Ishihara(8), however, using a modification of Dunger's original direct counting method, found a marked increase in circulating eosinophils within 1 to 3 hours after administration of a non-fatal shocking dose of antigen to sensitized guinea pigs.

It has been pointed out that the injection of typhoid vaccine in human subjects is followed by eosinopenia, similar to that seen as a result of adrenocortical stimulation(9).

The initial leukopenia followed by polymorphonuclear leukocytosis which occurs after reinjection of an antigen in a sensitized animal is separate and distinct from the hematologic changes after pyrogen injection. These two reactions can be distinguished by the difference in behavior of circulating eosinophils which rise after anaphylaxis and fall after pyrogen injection.

Summary and conclusion. (1) Changes in circulating leukocytes which occur after anaphylaxis have been compared with those after pyrogen injection. Following both of these reactions, there is immediate leukopenia followed by polymorphonuclear leukocytosis. (2) Two types of response, although superficially very similar, are distinguishable by direct eosinophil counts which reveal uniform

7. Herrick, W. W., *Arch. Int. Med.*, 1913, v11, 165; Campbell, D. H., *J. Infect. Dis.*, 1943, v72, 42.

8. Ishihara, A., *J. Chosen Med. Assn.*, 1939, v29, 392.

9. Altschule, M. D., Parkhurst, B. H., and Promisel, A., *Arch. Int. Med.*, 1950, v86, 505.

rises with anaphylaxis and uniform falls with the pyrogenic reaction. (3) The mechanism of these hematologic changes is not touched upon in the present study. (4) It is concluded that the leukocyte changes after anaphylaxis are not due to pyrogen contamination.

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Serologically Reactive Material in Spinal Fluid, Blood, and Urine from a Human Case of Cryptococcosis (Torulosis).* (18924)

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The purpose of this paper is to report the occurrence and the serological detection of reactive substances ("soluble antigens") in the spinal fluid, blood, and urine of a patient with cryptococcal meningitis; and also to describe the serological reaction of the capsules of the *Cryptococcus* cells contained in the sediment of the spinal fluid.

Materials and methods. The materials were from a patient with cryptococcal meningitis at Okmulgee, Okla.; a clinical report of the case will be published elsewhere by Dr. McCauley(1). The samples were obtained about 5 weeks after the patient's admission to the hospital and about a week before the fatal outcome; the spinal fluid was taken 2½ days earlier than the blood and the urine.

The spinal fluid was clear in gross appearance and the urine (a first morning sample of about 200 ml) proved normal in routine chemical analysis. *Cryptococcus* cells were present in considerable number in the spinal fluid, but none were demonstrable in either the blood or the urine by culture or by microscopic examination. The samples were transported to New York City for the serological tests.†

The serological tests on the spinal fluid, blood serum, and urine were made with supernatants cleared by centrifugation. In the case of the urine, tests were also made with solutions of alcohol precipitable material, prepared by 3 precipitations with 2.5 volumes of alcohol and extractions of the precipitate with water; the final solution, made in one-fourth the original volume, was water clear and more suitable for serological tests than the original urine. The precipitation test mixtures consisted of equal volumes (0.2 ml) of antigen and of serum; observations were made after 1½ to 2 hours at room temperature, and again after 3 days storage in the icebox. The complement fixation tests were made with similar mixtures of antigen and of serum plus 2 units of complement (0.2 ml); after overnight storage at 5°C followed by a 10 minute period in a 37°C water bath, 0.2 ml of sensitized sheep r.b.c. were added to the mixtures which were then put at 37°C for a one-hour hemolysis test period.

Experimental. A series of dilutions of the patient's fluids were tested for both precipitat-

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1. McCauley, D. W., *Okla. State Med. J.* in press.

† Merthiolate (0.01%) was added to all of the samples immediately after they were taken from the patient, and an equal volume of 95% alcohol was added to the major portion of the urine. The blood and the urine samples were maintained below 5°C throughout the entire time of transport, but the spinal fluid which was shipped separately by air mail was not packed in ice. Although the merthiolate (0.01%) did not kill all of the cryptococci in the spinal fluid, it was apparently sufficient to have prevented any growth during the transport of the sample, since cultures of the strain isolated failed to grow when large inoculums were tried in broth and in Berkefeld filtered spinal fluid (from other patients) to which either 0.01 or 0.001% merthiolate had been added.