TABLE I. Blood Pressure Findings in Rats Surviving % Day Period of Choline Deficiency Shortly After Weaning.

Diet	No. of animals	Avg initial wt, g	Avg wt after 5 days on low choline diet, g	Avg wt at 6 mo., g	Avg blood pressure at sacrifice, mm Hg	Animals with blood pressure over 150 mm Hg %
Choline free	28	43.4	50.8	395.4	166 (100-256)	71
0.4 mg % choline	20	43.7	54.9	411.4	118 (95-160)	5
2.0 mg % choline	22	42.9	54.7	392.8	114 (70-145)	0

TABLE II. Blood Pressure Findings in Older Rats Surviving 10 Day Period of Choline Deficiency.

Diet	No. of animals	Avg wt at start of low choline diet, g	Avg wt after 10 days on low choline, g	Avg wt 6 mo. later, g	Avg blood pressure at sacrifice	Avg heart: body wt %	Avg liver: body wt %	Avg kidney: body wt %
Choline free	24	140.2	140.6	353.2	100 (70-128)	.337	3.182	.748
0.4 mg % choline	20	135.1	134.8	346.5	120 (104-144)	.330	3.345	.740
2.0 mg % choline	22	145.6	141.2	324.9	110 (96-136)	.365	3.070	.763

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Leukocytic and Pyrogenic Effects of Typhoid Vaccine and Augmentation by Homologous Plasma. (18298)

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The response of a rabbit to the intravenous injection of a moderate amount of pyrogenic solution is characterized by an initial leukopenia followed by a leukocytosis and hyperthermia. There are varying opinions as to which of these reactions is the best indicator for the presence of pyrogens. Chapman(1) and Bandelin(2) have reported a leukopenia lasting 45-90 minutes following the intravenous injection of pyrogens into rabbits as a reliable test of pyrogenicity. Young and Rice(3) have reported that in dogs, a leukocytosis during the third to sixth hours after injection is a more reliable index of pyrogenicity. Co Tui and Schrift(4) have proposed a pyrogen test using both rabbits and dogs. If there is a positive thermal response

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1. Chapman, C. J., *Quart. J. Pharm. and Pharm.*, 1942, v15, 361.

2. Bandelin, F. J., *J. of the Am. Pharm. Assn.* (Scientific Edition), 1945, v34, 48.

3. Young, E. G. and Rice, F. A. H., *J. Lab. and Clin. Med.*, 1944, v29, 735.

4. Co Tui and Schrift, M. H., PROC. SOC. EXP. BIOL. AND MED., 1942, v49, 320.

in the rabbit, the test is repeated in dogs where a leukopenia (fall of 5000 cells) and a rise in temperature of 0.5°C in 1-3 hours is considered a positive test for pyrogen. A negative thermal response in rabbits is considered of more significance than a positive response. The same authors(5) feel that the phenomenon of leukopenia which accompanies the pyrogenic reaction seems to be more sensitive in dogs and affords an added check. Bose and Ahuja(6), among others, did not find the leukocyte changes in rabbits as reliable as the temperature changes. A comprehensive review of the leukocyte response to pyrogenic agents and nonspecific protein injections prior to 1922 is included in the text by Petersen(7).

The objective of this work was to make a comparison of the leukocytic and thermogenic effects of pyrogens on rabbits with particular emphasis upon the correlation between the amount of pyrogen administered and the magnitude of the response. Early in the course of this study it became apparent that the addition of plasma to the pyrogen altered the effects observed and, therefore, a comparison of the responses obtained with plasma and saline as menstrua was also made.

Materials and methods. Two strains of *Eberthella typhosa* were used as the source of pyrogen in these experiments. The Naval Medical Research Institute strain 901 W *E. typhosa* was employed in most of the work. A lyophilized culture was grown on brain heart-infusion agar slants which were incubated at 37°C for 24 hours. The growth of each slant was washed off with sterile normal saline and the organisms were killed by placing the tubes containing the suspensions in boiling water for 15 minutes. The sterile individual suspensions were pooled and a bacterial count performed using the Petroff-Hausser bacterial counting chamber. A sterile pyrogen-free saline solution (0.85% sodium chloride) was then added to the suspension to give the desired dilution of 125 million organisms per ml. No chemical preservative was added. A few additional studies were done using strain 58 *E. typhosa* obtained from the Army Medical Center, Washington, D. C. It was cultured on tryptose phosphate agar, and was killed by immersing a saline suspension in water at 58°C for 60 minutes.

The plasma was obtained by pooling blood from 5 previously untreated rabbits. Sodium citrate (0.4%) was used as the anticoagulant. Insofar as possible, the plasma was prepared under conditions that simulate the technic used for human plasma production(8). Prior to use, each lot of pooled plasma was cultured and found sterile. The saline injected was isotonic, sterile and non-pyrogenic. A standardized method of preparing the plasma-typhoid and the saline-typhoid suspensions was employed throughout the study. One ml of various dilutions of the stock vaccine was added to a tube containing 9 ml of plasma or saline. For the plasma controls, 1 ml of sterile saline was added to 9 ml of plasma. The tubes were agitated to assure thorough mixing and then stored at 5°C for 12-18 hours. In addition to the precaution of culturing the plasma and vaccine prior to mixing, some experiments were done in which the remaining portion of each solution injected was cultured and found to be sterile. Prior to injection, all solutions were warmed to 37°C and the volume given was always 5 ml.

All glassware and needles used in these experiments were sterile and pyrogen free. This was accomplished by placing the glassware in a chromic acid bath and the needles in a protein digesting solution for at least 24 hours. After a triple rinse in distilled water, each piece of equipment was wrapped in muslin and exposed to 170°C dry heat for 2 hours.

Fifty-eight New Zealand white rabbits were used in this study. The rabbits were males, approximately 6 months of age, and weighed between 3.0 and 3.5 kg. Each rab-

5. Co Tui and Schrift, M. H., PROC. SOC. EXP. BIOL. AND MED., 1939, v42, 549.

6. Bose, B. C., and Ahuja, M. L., *Ind. J. Med. Res.*, 1944, v32, 9.

7. Petersen, W. F., *Protein therapy and non-specific resistance*, 1922, New York, Macmillan Co.

8. Newhouser, L. R. and Kendrick, D. B., *Manual for the Preparation of Human Plasma*, Naval Medical School, National Naval Medical Center, Bethesda, Md., 1941.

bit was used on only one occasion. The animals were studied under conditions controlled with regard to the amount of handling, the environmental temperature and humidity. A standardized technic for taking the leukocyte counts and temperatures was employed. A previous paper gives the details of this procedure and, in addition, describes the leukocyte and temperature variations observed in untreated animals while being studied under these conditions(9). Four basic groups of animals were studied. One group received saline alone, one received saline plus typhoid vaccine, one received plasma alone, and one received plasma plus typhoid vaccine. The volume injected was constant, but the amount of typhoid vaccine was varied as described later. The animals were studied for 6 consecutive hours. Total and differential leukocyte counts were performed every 30 minutes for the first 4 hours and every 60 minutes for the last two hours. In the differential counts, both the monocytes and the lymphocytes were classified as mononuclear leukocytes. Rectal temperatures were taken each hour. The injections were given at the end of the first hour and the leukocyte counts and temperatures taken prior to that time were used as control values. Mean hourly values for the leukocyte counts and temperatures were calculated by pooling the data obtained from each rabbit of a group receiving the same treatment.

Results. Results from representative groups are presented graphically in Fig. 1-5.

Fig. 1 demonstrates the variations found in the leukocyte population and in the temperature of two groups of control rabbits: one group injected intravenously with 5 ml homologous plasma and the other group with 5 ml isotonic saline. There was no essential difference between the response of the two groups. The changes seen in both the plasma and saline injected animals are within the range of variation known to occur in untreated rabbits under otherwise similar conditions(9).

9. Farr, R. S., Schork, P. K., and Gayhart, C. H., Naval Medical Research Institute, *Project NM* 007 039, Report No. 12, 1948.

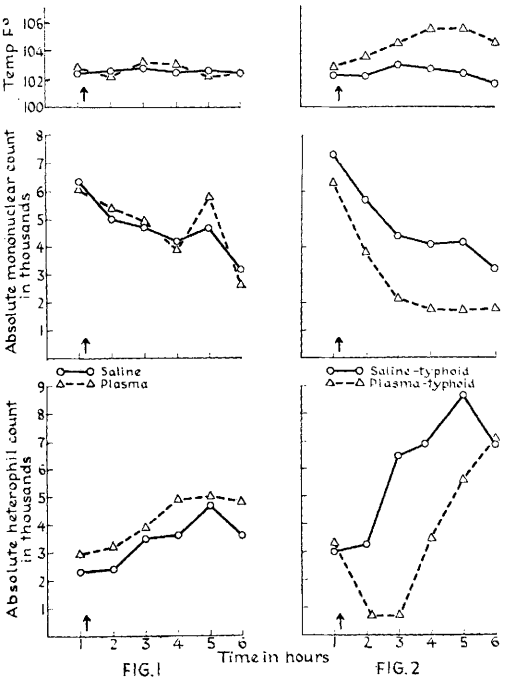

FIG. 1. Mean temperature and leukocyte response of two groups of 4 rabbits injected intravenously with 5 ml 0.85% saline and with 5 ml homologous plasma.

FIG. 2. Mean temperature and leukocyte response of two groups of 4 rabbits injected intravenously with 5 ml 0.85% saline containing 6.25 million *E. typhosa* organisms (strain 901W) and with 5 ml homologous plasma containing 6.25 million *E. typhosa* organisms (strain 901W).

Fig. 2 represents the effect of 6.25 million *E. typhosa* (901 W) organisms added to homologous plasma and isotonic saline. When plasma was used as the menstruum, a different leukocyte and temperature response resulted as compared to the saline suspensions. The animals given the plasma-typhoid solutions developed high fever, an initial heterophile leukopenia, and a secondary heterophile leukocytosis. The rabbits given the saline-typhoid had only an initial heterophile leukocytosis and very little temperature elevation. Both groups developed a mononuclear leukopenia, but the animals given the plasma typhoid injections demonstrated a proportionately greater fall in the mononuclear count than did the animals given the saline-typhoid. The mononuclear leukocyte and temperature changes of these two groups of rabbits suggest that the plasma augments the effects

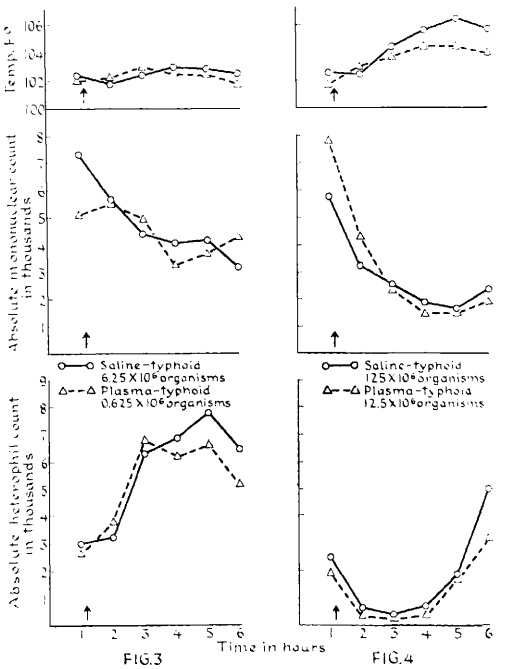

FIG. 3. Mean temperature and leukocyte response of two groups of 4 rabbits injected intravenously with 5 ml 0.85% saline containing 6.25 million *E. typhosa* organisms (strain 901W) and with 5 ml homologous plasma containing 0.625 million *E. typhosa* organisms (strain 901W).

FIG. 4. Mean temperature and leukocyte response of two groups of 4 rabbits injected intravenously with 5 ml 0.85% saline containing 12.5 million *E. typhosa* organisms (strain 901W) and with 5 ml homologous plasma containing 12.5 million *E. typhosa* organisms (strain 901W).

seen in saline and that the effect is, therefore, merely quantitative. However, the heterophile response in this experiment could be interpreted as meaning that the two types of leukocyte changes seen in the plasma and saline animals are of a different character and that the reactions vary qualitatively.

A number of groups of animals were used to test the hypothesis that if the difference between the temperature and leukocyte response in the two groups were only quantitative, it should be possible to produce both types of response with either saline or plasma by altering the amount of typhoid vaccine given. Fig. 3 demonstrates that an initial heterophile leukocytosis can be produced with plasma-typhoid suspensions by reducing the number of organisms injected, and Fig. 4 shows that the initial leukopenia can be produced with saline suspensions by increasing the number of organisms. This demonstrated that differences in the leukocyte response are quantitative. Approximately ten times the number of organisms were required in the saline suspension to produce effects comparable to those obtained with a given number of the same organism suspended in plasma. This 1:10 ratio was noted in all groups studied except when high doses were employed. When the amount of vaccine injected became greater, the difference between the plasma and saline groups disappeared since the leukocyte and temperature changes of both groups reached what appeared to be maximum.

Fig. 3 and 4 also show that the fever response is similarly augmented. In addition, the animals given the bacteria suspended in plasma developed a fever approximately one hour earlier than the rabbits given the saline-typhoid suspensions.

A few groups of animals were studied with a new vaccine made from strain 58 *E. typhosa*. Fig. 5 demonstrates that the temperature and leukocyte effect of the plasma-typhoid and saline-typhoid suspensions made with strain 58 were essentially the same as were found

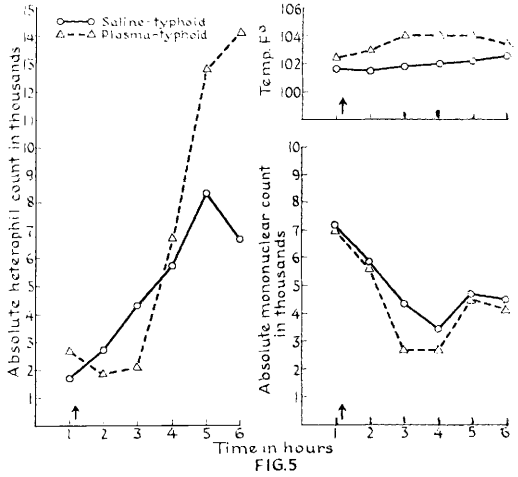

FIG. 5. Mean temperature and leukocyte response of two groups of 4 rabbits injected intravenously with 5 ml 0.85% saline containing 3.1 million *E. typhosa* organisms (strain 58) and with 5 ml homologous plasma containing 3.1 million *E. typhosa* organisms (strain 58).

when using strain 901W, although strain 58 appeared to be more active in producing the leukocyte and particularly the temperature changes. In spite of the relatively potent thermogenic properties of strain 58, the leukocyte changes were still the most sensitive indicator to detect the presence of the vaccine in either saline or plasma. This is demonstrated in the saline-typhoid data in Fig. 5. The leukocyte response was obvious, but the fever response was questionable.

Discussion. The present data and previous literature indicate that there are at least 5 variables which must be considered when using the leukocyte count for a pyrogen test:

1. *Amount of pyrogen given.* Our data with *E. typhosa* agree with the reports(6,7) that the temperature and leukocyte response in the rabbit following the intravenous administration of various numbers of organisms is dependent upon dosage. A relatively small number of typhoid organisms cause very little fever, a heterophile leukocytosis, and a mononuclear leukopenia. When larger doses of vaccine are given, the rabbits respond with a high fever, an initial heterophile leukopenia followed by a secondary leukocytosis, and a mononuclear leukopenia. It therefore follows that a leukopenia alone is an unreliable criterion for pyrogenicity, since small amounts of pyrogen can produce an immediate leukocytosis and a leukopenia is not a part of the reaction.

2. *Time at which the observations are made.* The plasma-typhoid data of Fig. 5 demonstrate the need for frequent serial sampling of the leukocyte population to detect pyrogenic solutions, especially if total leukocyte counts are used as the criterion. If only one count had been made after the injection, it could appear that the total count had increased, decreased, or remained unchanged, depending upon the time interval at which the count had been taken. Since the leukocyte count can change so rapidly from the initial leukopenia to the secondary leukocytosis, and since the time at which the rapid increase occurs is dependent upon the amount of pyrogen given, "spot" determinations at a given time interval after the injection are not reliable.

3. *The differential composition of the leukocyte population.* Situations arise when even serial sampling of the total leukocyte count is not reliable. Such a situation, which is not uncommon, is demonstrated in the saline-typhoid data of Fig. 2. By adding the absolute heterophile and mononuclear values of the group receiving saline-typhoid, it can be seen that the total count was approximately 10, 9, 10, 11, 13, and 10 thousand per cu mm during the 6 hours of study. The total counts similarly calculated from the saline control group in Fig. 1 are 8, 7, 8, 7, 9, and 7 thousand per cu mm. The increase above the initial total count observed in this particular saline-typhoid group is not sufficiently greater than the increase observed in the control animals to justify an interpretation that the test was definitely positive. However, consideration of the absolute heterophile and mononuclear count clearly indicates that a pyrogenic substance was present.

4. *Variety of bacteria used as a source of pyrogen.* It is well known that bacteria vary in their pyrogenicity. In the present work it was found that the relative amounts of fever-producing and leukocyte-affecting properties in the vaccines made from two strains of *E. typhosa* were moderately different. In both of these strains, the leukocyte variations were more sensitive than the temperature changes. It is conceivable, however, that other strains of *E. typhosa* or other types of bacteria could affect the temperature more than the leukocytes.

5. *The media in which the organisms are suspended.* The magnitude of the temperature and leukocyte response of the rabbit to typhoid vaccine is dependent upon whether the organisms are suspended in plasma or saline. Ten times the number of organisms were required in a saline suspension to produce a thermogenic and leukocytic response comparable to those obtained with a given number of the same organisms suspended in plasma. The difference between the two menstrua is quantitative except that the onset of fever is always earlier when the organisms are given in plasma. Probey and Pittman

(10) found that only 1/10 as many viable organisms are required to produce a given thermogenic response in rabbits when the organisms are grown in rabbit serum for 2-14 days. It is evident that growth in serum is not a necessary factor for augmentation since it also occurred in our experiments when killed *E. typhosa* organisms were suspended 12-18 hours in homologous plasma.

Summary and conclusions. The effects of intravenously administered typhoid vaccine upon the leukocyte population and the temperature of the rabbit were studied.

The response of the temperature and of the leukocyte count was dependent upon the amount of typhoid vaccine given. Small amounts of the vaccine caused little or no fever, a heterophile leukocytosis, and a mononuclear leukopenia. When larger doses of vaccine were given, the rabbits responded with high fever, an initial heterophile leukopenia followed by a secondary leukocytosis, and a sustained mononuclear leukopenia. Ten times the number of organisms were required in saline suspensions to produce a thermogenic and leukocytic response comparable to that produced when the vaccine was given in homologous plasma. The plasma-typhoid and saline-typhoid reaction differed only in degree except for the earlier onset of fever when the organisms were given in plasma. These experiments on rabbits with typhoid vaccine indicate that when leukocyte changes are used as a test for pyrogens, it is necessary to employ a series of total and differential counts on each animal.

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The Absorption and Distribution of Labeled Fatty Acids in the Rat.* (18299)

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The immediate distribution and deposition of ingested fatty acids into the lipid fractions of tissues have not been extensively studied. Various investigators(1-7) observed the passage of unsaturated fatty acids into tissues. Cavanagh and Raper(8) found the concentration of deuterium from "deuterated" linseed oil to be greater in the glyceride fatty acids of rat livers and less in the acetone-insoluble phospholipids. Similar technics were used by Sperry *et al.*(9) who showed signifi-

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