at 2.76 Mev is due to pair production and what part is due to photoelectric effect. A rough estimate of the efficiency of the crystal for the annihilation radiation leads to a value of about half photoelectrons and half pair production. The measured energy spacing of the photo and pair peaks gives 0.99 Mev for the thorium curve and 1.00 Mev for the sodium curve. These and other similar values for the self-energy of an electron and a positron are consistently from 2% to 3% below expectation, probably due to the unsymmetrical shape of the lower pair peak. A small peak in the Na^{24} curve at about 0.51 Mev can be seen. This peak is produced by annihilation radiation escaping from the shield wall; it is produced there by the positrons of pairs due to gamma rays from the source that do not enter the crystal. This effect limits the sensitivity with which one can search for annihilation radiation in the presence of high energy gamma rays. The difficulty can be much reduced by using a shield with a liner of low Z material which is thick for annihilation radiation.

With gamma rays of about 7 Mev the lower pair production peak is the most prominent feature of the spectrum. If considerable care is used to make sure of the identity of the peaks, there seems to be no reason why this method of gamma ray measurement could not be extended to much higher energy.

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Phagocytosis during Bacteremia in Mice: A Preliminary Report

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Although the phagocytic property of the neutrophile leukocyte was known to Hayem (*13*), Panum (*22*), and Roser (*24*), it remained for Metchnikoff (*20*) and his school to emphasize the importance of this cell in normal and pathological physiology. Later investigators, including Denys and Leclef (*8*), Leishman (*18*), and Wright and Douglas (*29*), employed the neutrophile leukocyte almost exclusively in their studies on phagocytosis. That certain cells of the reticuloendothelial system also possess the same phagocytic property was clearly shown by Werigo (*28*), Levatidi (*19*), Tchistovtch (*26*), Andrews (*1*), Bull (*3–5*), Kyes (*16*), Bartlett and Ozaki (*2*), Wells (*27*), Orskov (*21*), Cappell (*6*), and Wright (*30*). Gay and Morrison (*11*) in their studies on resistance to streptococcal infections even stated that "tissue macrophages" are, in large part, if not entirely, responsible for the natural resistance of rabbits to experimental streptococcus infection; this in spite of the obvious presence of polymorphonuclear cells, which have so long been held entirely responsible for the cellular protective mechanism in acute infections (Metchnikoff). Recently, Taliaferro and Mulligan (*25*) in their important work on defense against malaria also advanced the opinion that resistance is: "essentially a local immunity in strategically placed organs. Phagocytic activity, lymphoid hyperplasia, and the concomitant cytogenesis of macrophages are initiated in the spleen and are always most pronounced in this organ."

In their studies on infections with *Plasmodium cynomolgi* in *Macaca mulatta* they observed: ". . . an increase in the number of heterophiles (polymorphonuclears) but these cells practically never contain malarial pigment." The same held true in *P. knowlesi* infections.

In the following, an attempt was made to compare the phagocytic functions of the phagocytes in the peripheral blood and of those of the fixed tissues. The work was done in normal and immunized mice subjected to severe bacteremia. Phagocytosis was also determined by the traditional test tube procedure. In addition, the effects of magnesium chloride and gelatin on phagocytosis are herein reported.

Experimental work. The mice were separated into various groups, each comprising animals of similar age and sex and, as nearly as possible, also of similar weight. Prior to the induction of the bacteremia, blood was obtained for total and differential counts. Bacteremia was produced by the intravenous injection of a standardized suspension of a nonpathogenic coccus, *Micrococcus candidus*. Following the injection and at various intervals of time—30 min and 1, 2, 3, 4, 5, and 6 hr—the animals were again bled for total leukocyte and differential counts, for the determination of the percent of active neutrophile leukocytes, and for the number of cocci found per neutrophile leukocyte.

The data thus obtained were treated statistically by the method of Fisher (*9*), Fisher and Yates (*10*), or Pearl (*23*). When the number of animals was greater than 30, the method of the standard error was employed, while for series of less than 30, the Student's T test was applied.

All phagocytic and differential counts were done with complete objectivity. The slides were all recoded before they were examined microscopically.

General observations. Upon injection of the *candidus* into normal mice a drop in the total number of white blood cells occurred in the course of from one to four hours after the injection. This was observed in 49 out of a series of 62 female animals or 79.1 percent. In 22 immunized females, all showed leukopenia in the course of from one to five hours.

In animals sacrificed in the course of the leukopenia it was observed that the neutrophile leukocytes accumulated in their lungs. However, phagocytosis by the mononuclear cells in this area *preceded* the leukopenia. Large numbers of injected cocci were trapped in the lung.

A study of the neutrophile leukocyte of the peripheral circulation showed that 30 min after the injection of the coccal pabulum relatively few cells acted as phagocytes and that few cocci were ingested. During the leukopenia the number of active cells was at its maximum, and it was at this time that the neutrophiles contained their greatest number of cocci. With the passage of the leukopenia the percent of active cells and the number of cocci per cell reverted to the preleukopenic levels. During the entire course of the experiment, however, there was noted a relative increase in the percent of neutrophiles. In addition, the total leukocyte count increased following the leukopenia; the ultimate effect of these changes led to a rise in the number of cells phagocyting per cubic millimeter.

The observations described above do not imply that phagocytosis takes place in the peripheral circulation.

The phagocytic cells of the liver, spleen, bone marrow, and axillary lymph nodes were also active in the removal of the injected bacteria but the number of neutrophile leukocytes observed in these organs did not appear to differ markedly from that observed in the control animals.

In animals previously immunized by repeated intraperitoneal injections of the *candidus*, leukopenia occurred more consistently in the course of the first to the third hours. Tissue sections showed that a greater number of neutrophile leukocytes appeared in the lungs of the immunized mice. In the axillary lymph nodes of the immunized animals a greater number of cocci were noted than were seen in the control nodes; these, however, were ingested by the mononuclear cells rather than by the neutrophiles present in the subcapsular areas.

The uptake of the cocci per neutrophile leukocyte was less in the immunized animals than in the nonimmunized controls. In animals showing an agglutinin titer of (1: 160 to 1: 320) the uptake of the cocci by the neutrophile leukocyte was less than was observed in the controls. In the immunized animals whose antibody titer had fallen in the course of 60 days (no further injections given) the difference became insignificant.

It is possible that the fixed tissue phagocytes of the immunized animal possess greater phagocytic activity than those of the nonimmunized animals. It is therefore not improbable that the same may obtain in the case of the fixed tissue phagocytes.

In both normal and immunized animals, only the mature neutrophile leukocytes exhibit marked phagocytic activity. Participation of the immature cells was negligible. Neutrophile leukocytes with nonsegmented smoothly contoured ring-shaped nuclei were considered immature. In experiments *in vitro*, and with the use of whole blood, however, the immature neutrophile leukocytes proved to be phagocytic and even to contain large numbers of the injected cocci.

Employing a constant number of neutrophile leukocytes and varying numbers of organisms in the test tube procedure, it was found that the degree of phagocytosis depended on the ratio of the number of cocci to the number of neutrophile leukocytes present in the mixture. This was also observed by Ledingham (*17*) and by Hanks (*12*). When the ratio is small (9 cocci to one cell) the difference in the degree of phagocytosis is also small. When the ratio is large (185 cocci to one cell) the difference is markedly reflected in an increase of the total number of organisms ingested. This was also borne out by comparison of the results obtained when NaCl, $MgCl_2$, NaCl and gelatin, and $MgCl_2$ and gelatin were used in solutions for suspension of the organisms. When the higher ratios of cocci to cell were used an increase of phagocytosis was also noted. Again, in the test tube procedure, where a high ratio of bacteria to cell was employed, many immature cells participated. This fact gives the impression that both mature and immature neutrophile leukocytes are active in phagocytosis, with the obvious result that the percentage of cells participating would indeed be high. (Ratio of 185 cocci to one cell.)

Gelatin, originally introduced in order to increase the viscosity of the suspension menstruum for the leukocytes, exhibited, rather uniformly, an "opsonin-like" effect. The gelatin employed contained 3.6 μg of magnesium in 0.2 ml of a 1% solution. The blood of the mouse contained about 3.5–4.5 mg %. The determinations were made by the method of Kunkel, Pearson, and Schweigert (*15*).

Experiments have also been carried out to determine the effect of this cation on phagocytosis. Previous work (*7*, *14*) dealt with its effect on the neutrophile leukocyte in the test tube procedure. In this work, magnesium ($MgCl_2$) showed a variable effect in the test tube method. In the living animal, however, and in a series of 100 animals (females), there was no significant difference between the percent of neutrophile leukocytes phagocyting in the control animals and in the animals receiving $MgCl_2$ (intravenously) up to the third postinjection hour. At the fourth postinjection hour the number of neutrophile leukocytes acting as phagocytes was definitely greater in the magnesium-injected group than in the control group. The probability of statistical reproduction was about 275 to 1. In the case of the number of cocci ingested per neutrophile the probability of statistical reproduction was about 1 in 27.

Elimination of the magnesium ion from the diet of the animals led to a leukocytosis which was noted by the 9th or 10th day. Some of the animals succumbed between the 10th and 14th days. Although the number of cocci ingested per neutrophile leukocyte was no greater in the magnesium-depleted animals, the increase in the number of neutrophile leukocytes per cubic centimeter apparently led to the uptake of a greater number of cocci by these cells. As a result of magnesium depletion the animals lost weight and showed an average total leukocyte count of about 32,000, but with a regular distribution of the white cells.

In conclusion, it may be said that phagocytosis, if it is to be considered from the standpoint of the host, should not be interpreted, as has been done generally, from phagocytic indices derived solely from test tube procedures. An exploration of the phagocytic function

of the reticuloendothelial system is indispensable and should be studied concomitantly.

The experimental details of this work will be published elsewhere.

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A Method for Collecting and Sterilizing Large Numbers of *Drosophila* Eggs

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The techniques used for collecting and sterilizing *Drosophila* eggs, which techniques have been developed mainly for work on the nutritional requirements of the fly (*1*), proved unsatisfactory when employed by one of us (J. H. S.) for experiments on mutation rates. The sterilization was incomplete when the large numbers of eggs necessary for this work were handled and the labor of removing the eggs individually from the agar media was too time-consuming. An elaboration of the earlier technique was, therefore, developed, and is reported here.

In this context, the most desirable kind of medium for collecting the eggs is one which (1) allows the flies to lay their maximum and (2) can be easily and completely separated from the eggs. The two media listed in Table 1 were found to provide a reasonable compromise between

TABLE 1

CASEIN MEDIUM	
Casein (light white soluble)	1 g
Fuller's earth	1 g
1% Acetic acid in 2% ethyl alcohol	3.0 ml
YEAST AUTOLYZATE MEDIUM	
D.C.L. yeast autolyzate	0.4 g
Fuller's earth	1.8 g
1% Acetic acid in 2% ethyl alcohol	1.0 ml

these necessary characteristics. The casein medium, which is placed in watch glasses and applied to the mouths of the usual *Drosophila* bottles, is usually the more efficient oviposition medium; whereas the yeast autolyzate, which is smeared over a 2% agar gel in watch glasses, is the more readily separated from the eggs. In both cases, the routine procedure is much the same. Eggs are collected for a short period from three- to four-day-old flies (*2*) and the surface of the medium is scraped off and dissolved either in 3% sodium bicarbonate (if casein is used) or in water (if the autolyzate is used). The eggs freed by this procedure are then separated from large and small particles by sieving through a coarse and then a fine sieve. The latter has a mesh of 100 to the inch, which is sufficient to retain the eggs but allows the fuller's earth to be washed through.

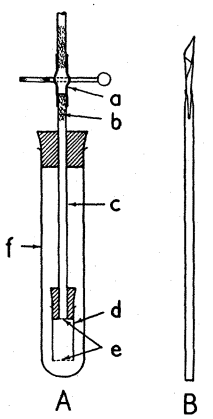

FIG. 1. A. *a*, Rubber connection ; *b*, cotton wool ; *c*, glass tube ; *d*, glass container for eggs ; *e*, platinum grid ; *f*, outer tube. B. Paper spoon in glass tube.

After washing in running water, the eggs are cleaned by immersion for 10 min in a mixture of 5% antiformin in 10% formalin and are then transferred to the sterilization tube (Fig. 1A) which has been autoclaved prior to use. A series of outer tubes are also autoclaved and filled with the sterilizing fluids. It was found empirically that exposure of the eggs for 20 min to the fluid described in Table 2, followed by 35-min exposure to sterile 70%