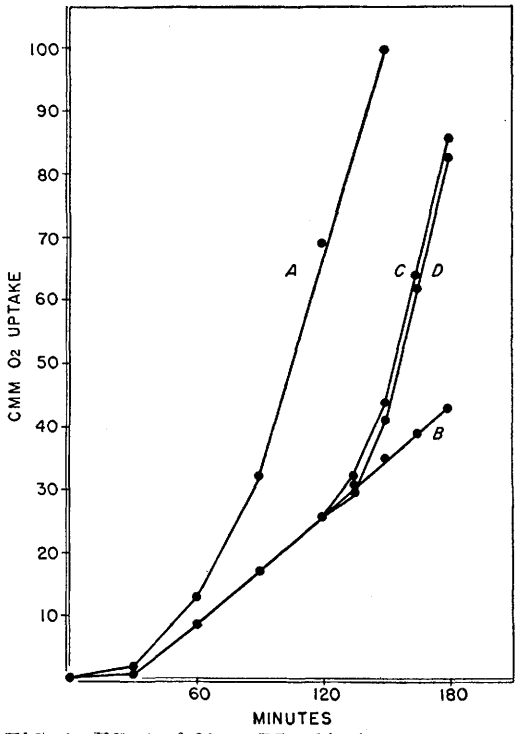

FIG. 1. Effect of 20 μg DL-ethionine and 2 μg of either L- or D-methionine on oxidation of 0.5 mg Na benzoate. Curve A: oxidation of benzoate. At 120 min. either L- or D-methionine or ethionine was added. There was no effect on oxidation rate. Curve B: oxidation of benzoate in presence of ethionine added at zero time. At 120 min. L-methionine (curve C) or D-methionine (curve D) was added.

be mentioned. After an initial period ethionine caused a constant inhibition. If it had merely delayed the entry of benzoate into the cells, *i.e.*, if oxidation rate was limited by amount of substrate available the inhibition should have decreased with time, because benzoate would be entering the cell throughout the entire experimental period. Since the inhibition remained constant, it seems probable that ethionine was not inhibiting a permease. Secondly, if upon addition of methionine the cell synthesized enzyme *de novo* and thus bypassed the ethionine inhibition, any amino acid should have been effective. The specificity of the reaction indicates that ethionine was displaced. Thirdly, if ethionine was incorporated into the enzyme protein during the 120 minute period, then methionine must have rapidly displaced it to convert an inactive into an active enzyme. Fourthly, the slightly slower action of the D-isomer may mean either that it was assimilated more slowly or that it was changed to the L-isomer by an isomerase.

Summary. 1) Ethionine inhibition of induction of enzymes involved in benzoate oxidation by washed cell suspensions of *Pseudomonas aeruginosa* is rapidly reversed by L-methionine and almost as rapidly by D-methionine. Neither amino acids nor ethionine have any effect once the enzymes have been induced. Similar results were obtained with p-fluorophenylalanine.

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Stimulation of Mitosis in Rat Marrow Cultures by Serum From Infected Rats.* (24935)

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Workers have reported substances in blood, urine, or inflammatory exudates which are capable of increasing the number of circulating leukocytes(1,2). However, the specific mechanism by which this effect is produced has not been determined. It has not been demonstrated, for instance, whether the observed leukocytosis results from increased mitotic activity in hematopoietic centers or from increased release of mature cells from these centers or other extravascular storage sites.

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In the present experiments, an attempt has been made to demonstrate at least one of the mechanisms by which granulocytosis, which normally accompanies acute infection, is produced. The effect of serum from infected rats on mitotic index of rat marrow cells in short-term tissue culture has been measured; and the results suggest that, in acute infections, some serum-borne substance increases mitotic activity of bone marrow cells and thus, presumably, increases production of mature granulocytes.

Materials and methods. The tissue culture method used was a slight modification of Osgood's "gradient method"(3). Rat bone marrow was expressed from femurs of young, adult male Webster rats with compressed air and immediately suspended in cold tissue culture medium (TC-199, Difco) with vigorous agitation. The cells were counted and then promptly planted in culture medium consisting of TC-199 and 10% rat serum, with penicillin and streptomycin added. Serum and marrow cells were generally obtained from the same rat. Cultures were planted in small screw-top vials (60 x 25 mm) with initial culture volume of 15 ml and cell concentration of approximately 3×10^6 cells/ml. Ordinarily, enough marrow cells were obtained from one rat for 3 such cultures. A glass slide was placed in each culture bottle at an angle of 60°, and marrow cells settled out and grew on the slide and on bottom of bottle. Cultures were incubated over night at 37°C without agitation. The slide was then removed, stained, and examined for evidence of mitotic activity. In almost all instances, occasional mitoses were observed among marrow cells on the slide. Very rarely, cultures failed to show any mitotic activity and so were discarded at this stage. Otherwise, healthy marrow cells were harvested from culture bottles by discarding approximately ⅔ of the supernatant medium, and then by vigorously agitating the remainder with sterile pipette to loosen cells adhering to bottom. The resultant suspension of cultured marrow cells was used to set up the actual test cultures. Test cultures were similar to primary cultures just described except that, for convenience, volumes of 8-10 ml and cell concentrations of 1.5 to 2.0 x 10^6 cells/ml were used. The composition of test cultures was approximately one-third cell suspension, one-third fresh TC-199, and one-third serum from acutely infected rats or from uninfected control rats. Acute infections were produced by intrathoracic injection of one ml of 24-hour broth culture of *Staphylococcus aureus*. Rats thus injected usually developed, by the following day, an inflammatory reaction in the pleural cavity, characterized by large amounts of cloudy exudate and numerous fibrinopurulent pleural adhesions, as well as marked granulocytosis in peripheral blood. Some variability in response was observed: occasional rats died in less than 24 hours, while others developed little or no inflammatory response. Only rats showing a good exudative reaction and obvious peripheral granulocytosis were included in the study. Serum was obtained from infected rats, as well as from uninfected control animals, by cardiac puncture. Control animals were of same age and weight as experimental rats, and only animals with initial total white blood cell count under 15,000 (less than 30% granulocytes) were used. Preliminary studies had shown that rats with initial counts above these arbitrary levels usually had evidence of infection at autopsy and their sera gave variable results. A few test cultures were set up with serum component replaced by inflammatory exudate, either undiluted or diluted with control serum. Test cultures were incubated at 37°C; and 2 to 3 hours later colchicine, in final concentration of 1×10^{-7} M, was added to arrest, in metaphase, all subsequent mitoses. After additional 17 to 19 hours incubation, the cultures were terminated and the harvested cells washed, treated with hypotonic salt solution, then fixed and stained with acetic-orcein(4). The resultant suspension of stained cells was spread on slides and lightly squashed under a cover slip. Processing the cells in this fashion caused enough spreading of chromosomes to make identification of cells in mitosis extremely easy. Mitotic indices were determined by counting at least 2000 cells from each culture. Differential counts were also made on cell suspensions, distinguishing only between mature granulocytes and all other cells. By this stage, very

TABLE I. Effect of Serum from Infected Rats on Mitosis in Rat Marrow Cultures.

Exp. No.	Mitotic index (No. mitoses/1000 cells) Cultures with serum from infected rats*	Cultures with control serum
1	35	10
2	31	13
3	40	20
	33	
4	11	4
	4	
5	27	13
	24	
Mean†	26	12

* Each figure represents a culture containing serum from different infected rat.
† For difference of means, $P < .05$.

few cells of the erythroid series remained in the cultures.

Results. Table I gives results obtained in 5 experiments in which mitotic activity of 8 marrow cultures containing serum from 8 different infected rats was compared with that of control cultures. All cultures in any one experiment were set up with marrow cells from the same rat. In 7 of the 8 cases, serum from infected rats had a pronounced stimulatory effect on marrow cells, generally resulting in mitotic indices 2 to 3 times that of control cultures. With exception of Exp. 4, mitotic indices in control cultures were quite uniform, ranging from 10 to 20. That both experimental and control values were unusually low in Exp. 4 suggests that viability of marrow cells in this experiment was poor; and examination of slides from the primary cultures of this marrow revealed an exceptionally low number of mitoses.

In a few instances, test cultures were set up containing serum from rats in which the injected staphylococci had failed to "take" and hence a good exudative response had not developed. In these cases, no stimulatory effect of serum was demonstrated; mitotic indices of these test cultures were no greater than control values. Similarly, in studies in which test cultures contained the *inflammatory exudate* instead of serum from infected animals, no stimulatory effect was demonstrated. Undiluted exudate, which undoubtedly contained many viable bacteria, was definitely toxic to marrow cultures; and, even when the exudate was diluted 10-fold with normal serum, mitotic indices in treated cultures were either the same or less than in control cultures.

Differential counts done on harvested marrow cells revealed, in all experiments, essentially the same percentage of mature granulocytes in cultures treated with serum from infected rats as in control cultures. The percentage of mature granulocytes varied only slightly (60% to 75%) from experiment to experiment. It had been hoped that differential counts would indicate whether serum from infected rats increased maturation rate of myeloid cells, since with a relatively constant number of cells in cultures during test period, a higher percentage of mature granulocytes, as compared to controls, would have indicated a more rapid rate of maturation. Unfortunately, in *control* cultures, two-thirds to three-fourths of the harvested cells were mature granulocytes, so that conditions were obviously not suitable for quantitatively demonstrating presence or absence of a maturation-stimulating factor in the experimental cultures. Granulocytes in *all* cultures were maturing at such a rapid rate that any additional maturing effect of serum in experimental cultures would not have been apparent.

Discussion. The results indicate that serum of rats with an acute infection is capable of producing a 2- to 3-fold increase in mitotic rate of rat marrow cells *in vitro*. This finding suggests that peripheral granulocytosis normally accompanying acute infection is due, at least in part, to direct mitogenic effect of some serum-borne substance on myeloid cells in the marrow. It is obvious, of course, that other mechanisms must also be involved. The peripheral granulocytosis during the first 48 hours of acute infection could hardly be due to increased mitotic activity of myeloid precursors since granulocytes ordinarily mature for 2-3 days in the marrow after their last division before being released into peripheral blood(5).

Time and magnitude of observed mitogenic effect is comparable to what Swann, in his recent review of mechanisms controlling cell di-

vision(6), calls a "short latent period" response, the prompt 2- to 3-fold increase in mitotic rate typically seen in tissues with normally high mitotic index upon exposure to various stimuli which speed up energy metabolism.

No attempt has been made, as yet, to identify the agent in serum responsible for its demonstrated marrow-stimulating activity. Perhaps an adrenal hormone, released as part of the body's response to "stress" of acute infection, constitutes the circulating marrow stimulant. It has been demonstrated, for instance, that cortisone may have a myelopoietic effect on bone marrow(7).

However, most earlier studies have indicated that substances responsible for leukocytosis accompanying acute inflammation are produced at the inflammatory site itself(1,8). In our study, this substance might be a product of either the infecting bacteria, disintegrated leukocytes, or the inflamed tissues. Failure to demonstrate a direct marrow-stimulating activity of the exudate itself does not rule out any of these possibilities. The contaminated purulent exudates in our experiments might contain a high enough concentration of substances toxic to marrow cultures to obscure the effect of any marrow-stimulating factor; or it is possible that, *in vivo,* such a locally-produced substance might act on marrow only *indirectly,* through the endocrine system. Such an indirect action of the exudate would, of course, not be demonstrable in our tissue culture system.

Summary. Serum from acutely infected rats produced a 2- to 3-fold increase in mitotic index of rat marrow cells in short-term tissue cultures. These results suggest that a serum-borne marrow-stimulating substance may play a part in producing the characteristic peripheral granulocytosis which normally accompanies acute infection.

The technical assistance of Elizabeth Yoast is gratefully acknowledged.

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Histidine Metabolic Loading Test to Distinguish Folic Acid Deficiency From Vit. B_{12} in Megaloblastic Anemias.*† (24936)

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In the past, differentiation of clinical deficiency of folic acid from that of Vit. B_{12} in megaloblastic anemias has depended upon extended therapeutic trials with Vit. B_{12} and folic acid. Failure of hematologic response to B_{12}, but a typical reticulocyte and red blood cell rise following minute doses of folic acid indicated folic acid deficiency(1). Formiminoglutamic acid (FIGLU) has been shown to accumulate in urine of patients with folic deficiency but not in uncomplicated Addisonian pernicious anemia or normal subjects

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