teine, *26*; antioxidants, *24*; sodium pyrophosphate in low concentrations, *23*). The synthesis can occur aerobically and anaerobically (in the presence of an excess of energy-rich phosphate bonds, e.g. adenosinetriphosphate, *14, 30*). The amount of acetylcholine synthesized depends on temperature (*14, 16*) and pH (optimum at alkaline pH, *29*). The enzyme is located intracellularly (*8*).

On the basis of the above data the following postulate is presented. Normally occurring constituents of cells and extracellular fluid (serum, spinal fluid) modify the amount of acetylcholine synthesized in the living organism. Further, there is a dynamic equilibrium between potentiator substances (organic phosphates, metabolites of carbohydrates and fats, amino acids, inorganic ions, hormones, vitamins) and inhibitor substances (unsaturated and higher fatty acids, aromatic and heterocyclic compounds, steroid substances, inorganic ions, some decomposition products of nucleoproteins and organic phosphates). During physiological activity the original dynamic equilibrium is disturbed, and new dynamic equilibria are established. Certain metabolites of muscle released during prolonged work decrease the synthesis of acetylcholine (*23, 25, 26*). The accumulation of such metabolites is important in the production of fatigue resulting from indirect stimulation and secondary to decreased acetylcholine synthesis.

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Granulocytopenia and Anemia in Rats Fed Diets of Low Casein Content[1]

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Granulocytopenia, correctable by crystalline *L. casei* factor (L.C.F., "folic acid"), has been found to occur occasionally in rats fed highly purified diets (*4*) and regularly when sulfonamides are included in such diets (*3, 4, 7*). Anemia (or impairment in erythropoiesis following hemorrhage) correctable by L.C.F. also has been found in rats fed sulfonamide-containing diets (*1, 3, 6*). Recently we have noted granulocytopenia in rats fed highly purified diets deficient in riboflavin and also among pair-fed, riboflavin-supplemented controls (*5*). Further investigation of this influence of inanition on the production of granulocytopenia has revealed limitation of casein intake to be a highly significant factor.

Weanling albino rats (Osborne and Mendel) were fed one of several purified diets differing only with respect to casein content. Diet No. 1055 contained no casein or protein and consisted principally of Crisco, salt mixture, and dextrose.[2] In the other diets casein (Labco) in varying amounts replaced equivalent weights of dextrose. Total white blood cell counts, polymorphonuclear granulocyte counts, and hematocrit determinations were made as previously described (*6*). For the purposes of this report, granulocytopenia was considered to be present when the polymorphonuclear granulocytes numbered 500 or less per cu. mm. Anemia was considered to be present when the hematocrit was less than 30 vol. per cent.

Of 89 rats fed the casein-free diet (No. 1055), 10

[1] Presented in part by one of us (A. K.) before the AAAS Vitamin Conference at Gibson Island, Maryland, July 1945.

[2] The casein-free diet No. 1055 consisted of anhydrous dextrose, 86.76 grams; Crisco, 8.0 grams; salt mixture No. 550[3], 4.0 grams; ferric citrate, 1.16 grams; and copper sulfate · $5H_2O$, 0.08 grams. Into this diet were incorporated 1 mg. of thiamine hydrochloride, 2 mg. of riboflavin, 1 mg. of pyridoxine hydrochloride, 4 mg. of calcium pantothenate, 2 mg. of niacin, 200 mg. of choline chloride, 0.001 mg. of biotin, and 0.4 mg. of 2-methyl-1,4-naphthoquinone. Twice weekly each rat received a supplement of 0.25 cc. of corn oil containing 2,000 units of vitamin A and 200 units of vitamin D (Natola) and once weekly 3 mg. of α-tocopherol in 0.03 cc. of ethyl laurate.

died within 19 days after starting the experimental diet. One or more blood counts were made between the nineteenth and twenty-eighth days on the surviving 79 rats. Granulocytopenia was noted in 75 rats and anemia in 68. The 4 rats without granulocytopenia and the 11 rats without anemia failed to survive 28 days on the experimental diet. The average body weight was 42.5 grams at the start of the experiment and 30.2 grams after 20 days on the diet. Of 6 and 7 rats fed diets containing 2 and 4 per cent casein, respectively, all developed granulocytopenia within 30 days. Anemia was noted only among the rats fed the 2-per cent casein-containing diet. Seven of 8 rats fed an 8-per cent casein-containing diet developed granulocytopenia within 45 days; none had anemia.

TABLE 1

GRANULOCYTOPENIA AND ANEMIA IN RATS FED PROTEIN-FREE DIETS AND PREVENTION WITH CASEIN

Group*	Diet	No. of rats	No. of rats with granulocytopenia‡	No. of rats with anemia‡
A	0 per cent casein	8	8	6§
B	0 per cent casein — 20 γ L.C.F. daily†	8	8	7§
C	18 per cent casein — pair-fed with Group A ..	8	2	0
D	18 per cent casein	8	0	0

* The 4 groups were equal with respect to sex, litter, and weight distribution. Food intake was ad libitum in groups A, B, and D.
† This crystalline fermentation product (2) was administered by pipette to each rat from the outset of the experiment.
‡ Noted within 28 days.
§ The rats which failed to develop anemia died after 19 to 25 days.

Data on the influence of restriction of food intake and the effect of L.C.F. (2)[3] administered preventively are in Table 1. These data indicate no significant preventive action by L.C.F. Severe restriction of an 18-per cent casein-containing diet (Group C) failed to produce blood dyscrasias in 6 of 8 rats. The 2 cases of granulocytopenia noted in this group may have resulted from inadequate casein intake.

Several materials were tested for their effectiveness in correction of granulocytopenia developed in rats fed the casein-free diet No. 1055. Treatment was administered daily for 4 days. A recount was made on the day following the last treatment. For the purposes of this report, a response was considered "positive" when the granulocytes numbered 1,000 or more cells per cu. mm. Further details and results of treatment are in Table 2. Rats which failed to survive the treatment period are not considered. When no treatment was administered, there was a progressive decline in granulocyte count and hematocrit value terminating in death. Of 13 rats treated with crystalline L.C.F. or a liver concentrate of L.C.F., only 2 reached granulocyte levels of 1,000 cells per cu. mm. Granulocyte values declined in all of 9 rats fed diets containing casein at levels of 18 or 30 per cent[4] in place of the casein-free diet No. 1055. However, the administration of L.C.F. combined with a change to an 18-per cent casein-containing diet resulted in significant increases in granulocyte count in each of 8 rats. Similarly treatments with L.C.F. combined with the dietary administration of a mixture of purified amino acids[5] produced significant increases in granulocyte count in each of 6 rats. Data on the treatment of anemia are incomplete and therefore are not presented at this time.

TABLE 2

TREATMENT OF GRANULOCYTOPENIA

Treatment	No. of rats	No. of rats with positive response	Poly. granulocytes per cu. mm. (average) Before treatment	Poly. granulocytes per cu. mm. (average) After treatment
L.C.F.*—100 γ	8	2	350	750
L.C.F.†—200 γ subcutaneously	2	0	300	750
L.C.F.†—100 γ + L.C.F. conc.‡ —50 γ	3	0	33	417
Casein diets—18 per cent or 30 per cent	9	0	217	39
L.C.F.†—100 γ + L.C.F. conc.‡ —50 γ + casein diet—18 per cent	3	3	83	4,833
L.C.F.*—100 γ + casein diet—18 per cent	5	5	350	3,090
L.C.F.§—100 γ + amino acid mixture	6	6	317	2,433

* Crystalline fermentation L.C.F. (2) or synthetic L.C.F. (Stokstad). No differences were observed between the activity of the two substances in these experiments.
† Crystalline fermentation L.C.F. (2).
‡ Contained in 0.058 grams of liver concentrate.
§ Synthetic L.C.F. (Stokstad).

Further study is required to determine the identity of the amino acids responsible for the granulocytopoietic activity found in casein or in a mixture of purified amino acids. Such data may help in the elucidation of the mechanism of action of *L. casei* factor and the amino acids in the formation of granulocytes.

[3] The crystalline fermentation L.C.F. (2), synthetic L.C.F., and L.C.F. concentrate from liver used in these studies were furnished through the courtesy of Drs. E. L. R. Stokstad and B. L. Hutchings, of Lederle Laboratories, Inc.

[4] Five and 4 rats were fed diets containing 18 per cent and 30 per cent casein, respectively. Average daily intake of these diets per rat was 3.6 grams.

[5] To mixture XII-c of W. C. Rose and S. S. Fierke (*J. biol. Chem.*, 1942, **143**, 115) containing 18 amino acids were added 7.8 grams of dl threonine and 7.8 grams of dl aspartic acid. Of this final mixture, 26.7 grams (representing 18.0 grams of "active" amino acids) replaced an equal weight of dextrose in the casein-free diet No. 1055. Average daily intake of this diet per rat was 2.7 grams.

SUMMARY

Severe granulocytopenia and anemia were developed uniformly in rats fed protein-free diets. Casein (18 per cent) prevented these dyscrasias, but crystalline *L. casei* factor ("folic acid") did not prevent them. In the correction of granulocytopenia in rats fed protein-free diets, *L. casei* factor alone was only slightly effective, diets of higher casein content (18 or 30 per cent) were ineffective under the experimental conditions described. However, *L. casei* factor combined with an 18-per cent casein-containing diet or *L. casei* factor combined with a mixture of purified amino acids were found to be highly effective in correcting the granulocytopenia.

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The Presence and Significance of a Leukopenic Factor in Inflammatory Exudates

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A number of inflammatory conditions are accompanied by a fall in the number of circulating white blood cells, a so-called state of leukopenia. Fitz-Hugh and Krumbhaar (*1*) regard agranulocytosis as the result of an arrested development of leukocytic elements. The disease involves lymphoid elements as well as granulocytes. These authors therefore speak of the condition as a pernicious leukopenia. A profound leukopenia referable to a virus infection has been recently described to occur in cats (*2, 3*). It is interesting to note on close scrutiny the frequent occurrence of some infection accompanying an agranulocytic process.

The writer has demonstrated the presence of an injury factor located in, or at least closely associated with, the euglobulin fraction of inflammatory exudates (*5*). This substance has been termed necrosin. Recent studies indicate its more frequent recovery in exudates from a severe area of inflammation in which there is usually an appreciable degree of acidity (*7*). The whole euglobulin fraction of exudates not only induces marked cutaneous injury, but likewise it causes in dogs a marked degree of fever and a profound leukopenia (*5, 6*). Subsequent investigations have revealed that the pyrogenic property of the whole euglobulin fraction of exudates is really not referable to necrosin, but that this fever-inducing capacity is caused by a completely different, but closely associated substance, termed by the writer pyrexin (*6*). The present preliminary communication indicates that in inflammatory exudates there exists a leukopenic factor which is not one of the biological attributes of necrosin *per se*. It is closely associated with pyrexin. Yet, it can readily be dissociated, at least to a large extent, from this pyrogenic factor. The presence of such a leukopenic factor in inflammatory exudates may in large part explain, perhaps, the state of leukopenia accompanying numerous inflammatory processes. The leukocytosis-promoting factor present in exudates may well mask the ultimate effect of this leukopenic factor (*4*). In brief, the final blood picture accompanying an acute inflammatory process may to a large extent depend on the relative concentration of either the leukocytosis-promoting factor (LPF) or the leukopenic factor now under discussion, both of which factors are produced at the site of an acute inflammation.

An inflammatory exudate at an acid pH will, when injected into the circulation of a dog, tend to induce a rapid and sharp fall in the number of circulating leukocytes. This is a conspicuous feature within the first hour or so. The average fall in 8 experiments has been found to be 3,778 white blood cells per cubic millimeter or 32.3 per cent. Pyrexin, as isolated from such exudates, is the fraction obtained which has been found to induce a marked leukopenia. The average fall in 10 experiments is 9,980 white blood cells per cubic millimeter, a drop of 79 per cent. It is possible that the simultaneous presence of the LPF in the whole exudate counteracts somewhat the full effectiveness of the leukopenic factor. Such a state of affairs would account for the more striking effect obtained with pyrexin where the LPF is absent. Purified necrosin or normal blood serum utterly fails to induce any such drop in the leukocyte count. Within the usual period of study (about 6 hours) the maximum decrease in the number of circulating leukocytes is, under normal circumstances, negligible.

An attempt has been made to dissociate the leukopenic factor from pyrexin. Some recent evidence indicates that the latter is, or is at least associated with, a polypeptide. It is possible that the leukopenic factor also belongs to this group, especially since it is derived from pyrexin. For this reason pyrexin has been partially hydrolyzed with 0.1 N HCl for about 10 to 15 minutes in an effort to determine