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

whether pyrexin can be in large part inactivated, while leaving behind an active leukopenic factor. The scheme of extraction adopted follows:

SCHEME OF EXTRACTION OF LEUKOPENIC FACTOR

Exudate
|
$(NH_4)_2SO_4$ at 1/3 saturation
|
precipitate
|
treated with distilled water
|
shake
|
insoluble material
|
dialyze until free of SO_4
|
pyrexin (dried by freezing)
|
reflux with 0.1 N HCl for 10–15 minutes
|
cool
|
N NaOH adjusted to pH 10 or 10.3
|
concentrate on steam bath to about 1/10th volume
|
dialyze
|
evaporate to dryness on steam bath or dry freeze
(*leukopenic factor*)

The final material obtained when extracted from pyrexin, as indicated above, still induces a marked leukopenia. This, as in the case of the whole exudate, develops abruptly, and it may last a few hours. The average fall in 14 experiments is 6,146 cells per cubic millimeter, a drop of over 50 per cent. Yet, this leukopenic factor is now essentially dissociated from pyrexin, for it essentially causes no fever when injected into the blood stream of dogs. Preliminary observations in collaboration with Dr. Frederick Bernheim, to be subsequently reported in detail, on the amino nitrogen concentration before and after hydrolysis indicate that the leukopenic factor seems to belong to the group of polypeptides. It is thermostable, for boiling fails to inactivate its effect. Various controls, such, for instance, as the normal variation in leukocyte counts and in temperature level during the period of study (*i.e.* within about 6 hours) or the reagents themselves that were utilized in dissociating the leukopenic factor from pyrexin, all indicate that the effect of the leukopenic factor is indeed real. These studies are being pursued further and will be reported *in extenso* elsewhere.

In brief, evidence has been advanced to indicate that there exists in inflammatory exudates, particularly when obtained from an area of severe inflammation and therefore usually at an acid pH, a leukopenic factor, which *per se* may offer a reasonable explanation for the development of a state of leukopenia with some of the types of acute inflammation. Furthermore, in the exudative material this leukopenic factor seems to be in close association with pyrexin, the pyrogenic factor from which in turn it can to a large extent be separated. The leukopenic factor affects the granulocytes as well as the mononuclear cells, for the latter are likewise depressed. The effect is a general one. The leukopenia is found to exist both in the peripheral circulation and in samples of blood obtained from the heart.

In subsequent studies since this communication was sent for publication, it has been found that, although the leukopenic factor of inflammatory exudate is mostly found in close association with pyrexin, it can be recovered sometimes to some extent in other fractions of exudative material, indicating that it is apparently not exclusively found in association with pyrexin. Furthermore, additional studies seem to indicate that the leukopenic factor of exudates does not primarily deplete the bone marrow, but rather the mechanism involved appears to be a rapid trapping of leukocytes in the alveolar walls of the lungs, in the sinusoids of the liver, and apparently in the spleen. The latter fact may be of significance in our further understanding of the mechanism involved in the acute splenic tumor accompanying numerous inflammatory processes.

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The Comparative Toxicity of Thiourea to Four Mutants of *Drosophila melanogaster* [1]

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During the course of an investigation of the effects of a number of drugs upon the development of several genotypes of *Drosophila melanogaster,* the striking toxicity of thiourea [2] was observed. A survey of the literature disclosed several reports dealing with thiourea as an insecticide. Two-per cent solutions of thiourea and phenylthiourea were effective against the webbing clothes moth (*9*). Third instar blowfly larvae died in the third or fourth instar when exposed to thiourea incorporated in their food (*6*). McGovran, Richardson and Piquett (*8*) observed a 92-per cent

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