The Effects of Stress on the Enzymes of Peripheral Leukocytes

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by

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Previous work in our laboratory showed an early response of rabbit and human leukocyte enzymes to the stress of bacterial infection. Since these represented a mixed population of leukocytes and since polymorphonuclear leukocytes (PMN) increased in these preparations, it was necessary to establish whether the observed increase in lactate dehydrogenase (LDH) and protein was the result of an increase in any one particular cell type or in all cells.

The need for the development of a simple reproducible method for the differential separation of peripheral leukocytes for the furtherance of our own studies was apparent. It was also becoming increasingly apparent that morphologically similar cells, such as small lymphocytes (L) and macrophages, were capable of different biological functions. A method for distinguishing and separating these would, therefore, serve an important function.

A dextran gradient centrifugation method was developed which has provided an easily reproducible technique for separating L from PMN. The reproducibility of the technique has been assessed and the separation method utilized to evaluate the effects of the stress of restraint, malnutrition, infection with Diplococcus pneumoniae, and infection with Herpes simplex virus of three degrees of severity. The protein, LDH, aldolase, malate dehydrogenase, (MDH), creatine phosphokinase (CPK) content of the differentially separated cells was established before and after infection.

During the course of this work, in which over 250 rabbits were examined, the pattern of daily leukocyte protein and enzyme variation became increasingly more apparent. This information could have some impact on future work with leukocyte enzymes, by our group and by other workers. The differences in normal protein and enzyme levels maintained by some individuals, and some inbred strains, were evaluated and reported separately.

In summary, we have shown that one type of leukocyte may react more to a given stress than other leukocytes. In weanling rabbits on a deficient diet there was a general trend toward lower protein, LDH and aldolase in all cells of weanlings fed a lysine deficient diet, despite the fact that weanlings leukocytes have higher protein and enzyme content than adult cells.

Following restraint in the supine position, there was a marked increase in protein and LDH in the lighter lymphocytes, but no change in aldolase, immediately after a 4-6 hour period of restraint. In the PMN, especially the heavier cells from the bottom of the gradient, there was an increase in LDH but not in protein. Protein, LDH and aldolase returned to normal or below normal levels 24 hours after the removal of the applied stress.
The lymphocytes from the top of a 1.060 specific gravity dextran gradient (C#1) showed an increase in protein and LDH immediately following infection with *Diplococcus pneumoniae*. The protein increase was significant and the elevation was maintained throughout the course of the disease.

Increases in protein and LDH content of lymphocytes removed from the top of the 1.069 (C#2) dextran gradient were noted in a moderate disease produced by *Herpes simplex* virus. In contrast, a more acute disease produced by a higher concentration of virus, resulted in decreased cell protein and enzyme concentrations.

The increases in C#2 protein and LDH following the *Herpes simplex* infection are also in contrast to the effects of infection with *Diplococcus pneumoniae* in which the elevation in protein and LDH occurred in C#1. Another point of contrast between bacterial and viral infection is the elevation of C#5 LDH and MDH in the disease produced by *Herpes simplex* virus which was not observed in the pneumococcal infection.

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In conclusion, it would seem possible to establish a finger print or pattern for an individual, by repeated leukocyte enzyme analyses. Sudden and wide fluctuation from an individual's norm could provide meaningful information concerning a possible exposure to an infectious agent before the onset of clinical symptoms.