linear increase in the mutation rate with the dosage of the Mutator gene.

4. The Mutator probably is linked to the second chromosome.

5. A total of approximately two thousand mutations was observed; some of the mutations were located.

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*ON THE PHYSICAL BASIS FOR GENETIC RESISTANCE TO MOUSE TYPHOID, SALMONELLA TYPHIMURIUM**

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With most species wide variations in susceptibility to most diseases exist. Some members of the species die, others show different degrees of morbidity. By suitable genetic techniques it is possible to segregate these different levels of disease resistance into consistently breeding groups, some with nearly complete mortality, some of medium resistance, others with little or no morbidity when exposed to the same dose of the causative organism. Using the mouse, *Mus musculus*, as the host and the typhoid organism, *Salmonella typhimurium*, as the disease causing organism investigations at Iowa State College[1, 2] have segregated a mouse population into six different breeding groups characterized in part by their disease resistance. The first two groups designated as *Ba* and *L* have a mortality of 92 and 87 per cent, respectively, when inoculated with 200,000 bacteria; the *E* and *Z* lines have 47 and 42 per cent mortality; and the *R.I.* and *S* lines have 25 and 14 per cent. Each line is now essentially pure breeding for its resistance to *Salmonella typhimurium*. The purpose of this investigation was to investigate one of the several possible physiological causes for this resistance and thus establish a character basis for the observed genetic resistance. The character chosen for study of this correlation is the cellular constitution of the mouse blood.

For the purposes of this analysis the blood of 45 to 50 mice of each strain was examined for the number of erythrocytes, leucocytes and their proportions.

In our mice the cellular constituents of the blood are shown to vary widely, both in total number and the proportion of the different cell types. This variation seems to be characteristic of blood in general, as we have found similar variations in the published results of other investigators. Two major variables and one minor are known contributors to the variation. The major variables are sex of the mouse and the strain from which it is derived. The minor variable is the short age range over which the mice were tested. Analysis of the effect of these variables proves that sex is relatively unimportant in its influence on either the numbers of erythrocytes or leucocytes or the proportion of the cells composing the leucocytes. In its narrow range, age also is not particularly important as the variation attributable to age is irregular, not showing a consistent trend. Genetic differences which have been segregated into the different strains composing our material account for most of the known variation in the numbers of erythrocytes and leucocytes. The same genetic differences affect the proportions of the cell types composing the leucocytes to only a limited degree.

If we contrast the six different strains we find that the levels of the erythrocytes and leucocytes in the blood are characteristic of the particular strains. In the breeding process the variations of these cells in different mice have been reduced and made characteristically higher or lower in the particular strain. It is found that there is no correlation between the degree of fixation of the erythrocytes and the leucocytes indicating that for the six strains they have been fixed independently of each other. The proportions of the different cells composing the leucocytes do not show the same degree of fixation in this hereditary process as do the cell numbers.

The variation in numbers of leucocytes is highly correlated with the degree of resistance which exists for the particular mouse strain (Fig. 1). Those strains of low resistance have low leucocyte numbers whereas those of high resistance have relatively high leucocyte numbers within the range of the customary variation. In the formation of these strains of mice, those of high and those of low resistance have been established through inbreeding and selection for either high survival value or low survival value to the typhoid disease organism. In the process the leucocyte numbers have also been fixed, the correlation between the leucocyte number for the given strain and its resistance being on the order of 0.9. This fact would be as expected if, basically, there was a direct relation between the number of leucocytes which the organism carried and its potentialities for resisting mouse typhoid.

At the same time that these breeding experiments for disease resistance were going on, inbreeding experiments having no particular relation to disease resistance were also molding other different mouse strains. This inbreeding technique should also lead to fixation of particular types of re-

sistance or leucocyte numbers. Such is found to be the case. One of these strains having a low leucocyte number has high susceptibility to mouse

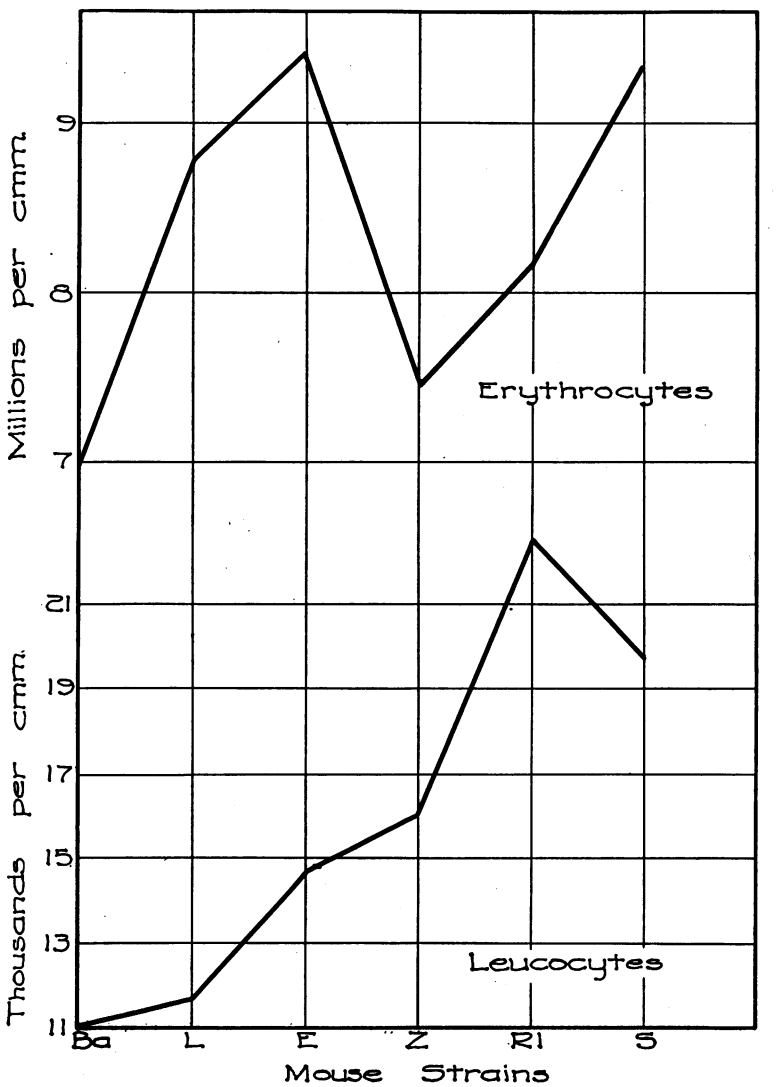

FIGURE 1

Relation of the disease resistance of the different mouse strains to erythrocyte or leucocyte number found in their blood.

typhoid, whereas two of the other strains have intermediate leucocyte number and intermediate susceptibility.

The proportion of the particular types of cells which make up the leucocytes are not fixed in the breeding process. This suggests that the particular cell types are called out by the animal body through different environmental conditions and that a primary cell is capable of developing into any particular type according to these environmental influences rather than through the inheritance control. Such a view would indicate that the important inherited capacity is the production of a few or large number of primary cells that subsequently may develop into leucocytes of the various types according to the environmental need.

The erythrocytes show a similar fixation in their numbers to that of the leucocytes. They do not, however, show any particular correlation to the disease resistance of the given strain. This we might possibly expect in view of the fact that the erythrocytes, so far as mouse blood is concerned, are not known to effect directly the typhoid organism.

In this search for the physical basis of the genetic resistance to mouse typhoid we have studied only two possible types of cells out of many which the body could furnish and be important to the resistance. Both cell types studied have a large variability indicating that there are both hereditary and environmental causes of variation. Analysis shows that our genetic technique would account for about one-fourth of the variations normally present in these mouse blood cells. The other three-fourths of the variation must be due to causes other than those measured in these experiments. The proportions of the different types of cells making up the leucocytes are but little fixed by the genetic techniques used in establishing the different strains.

Our observations have interesting corollaries in the work of Reich and Dunning[3] on the effect of leucocyte level and longevity in rats. Six closely inbred lines of rats were tested for the numbers of white blood cells, the per cent of polymorphs and the duration of life. It was found that the higher the leucocytes the longer the rat lived. It was also shown that the neutrophile polymorph represented a higher proportion of the leucocytes in the longer lived rats than in those with a shorter duration. The correlation is of the order of 0.7. Contrary to our findings a sex effect was noted in the leucocyte count and the duration of life. These sex effects favor larger numbers of leucocytes in the females, the sex with the longer duration of life.

Roberts, Severens and Card[4] present a study of the nature of the hereditary factors for resistance and susceptibility to pullorum in the domestic fowl. In this study they analyze the numbers of erythrocytes, leucocytes, lymphocytes and neutrophiles from the fifteenth day of incubation to a week after the chick hatches. It is in this period that the chicks of susceptible strains are killed by *Salmonella pullorum*. After this period most strains of chickens, both susceptible and resistant, are immune to this dis-

ease. The total number of leucocytes is found to increase in both susceptible and resistant strains from the fifteenth day of incubation through the seventh day after hatching. This increase is accounted for in part by a shift in the types of cells composing the leucocytes. The lymphocytes increase from 5 or 10 per cent up to 55 to 65 per cent of the total leucocytes. The chicks which are genetically resistant to pullorum display this rise in lymphocytes earlier than the chicks which are susceptible. The resistant and susceptible chicks have essentially the same lymphocyte numbers seven days after hatching, the period after which both groups are resistant to this disease.

The importance of the lymphocytes is further brought out by x-ray experiments in which the lymphocytes were reduced in number through irradiating the 6-day-old chicks with x-rays. These x-ray chicks, inoculated with pullorum, had a death rate four times that of the untreated birds. These results would point to the leucocytes as important in the defense mechanism to this disease, especially as no difference in bactericidal power of the serum of the susceptible and resistant groups was observed.

In their study of the correlation between the resistance to rat typhoid and bactericidal power of whole blood, Irwin and Hughes[5] showed that rats which were resistant to the disease had less bacteria in their sodium citrated blood, after inoculation with *Salmonella enteritidis* and incubation at 38° for four hours than rats which were incapable of surviving the disease. As the paper stands it is not possible to decide whether this *in vitro* action is due to the serum or to the presence of leucocytes. We are informed, however, that the serum is probably the responsible agent here.

Rous and Jones[6] have presented a study of the *in vitro* reactions of leucocyte, bacteria or other antigens and immune serum. In this paper they properly emphasize the fact that after one hour's incubation bacteria or red blood cells ingested by leucocytes are protected against the action of immune serum in causing bacteriolysis or hemolysis. They point out the significance of this fact to possible bacterial dissemination within the host should the pathogen be eventually freed from the leucocytes. The protective action against immune sera by the leucocytes is found to be a property of the living organism not of the dead leucocytes. The hypothetical significance of these researches to the possible *in vitro* reactions of the leucocytes to disease organisms is of marked significance to our results. The experimental arrangement is excellent. The experiments present data on pathogen death, or erythrocyte hemolysis, when leucocytes are present in the mixture contrasted with their absence from the mixture. In this light the data show that the leucocytes destroy or immobilize many of the bacteria. This reaction may, of course, be looked upon as an exaggerated form of agglutination since each leucocyte collects a fairly large number of bacteria within it. However, the microscopic examination of ingested

bacteria and erythrocytes would indicate rather that the leucocytes' cytoplasm destroys the inclusions rather than simply agglutinating them.

The general evidence of the foregoing papers indicates that the numbers of leucocytes in general, or numbers of particular kinds of leucocytes, play a pronounced part in the immune phenomena controlled by the genetic constitution of the host.

The original data with its complete analysis will appear shortly.

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