THE HOST-PARASITE RELATIONSHIP IN TULAREMIA

I. A Study of the Influence of Bacterium tularense on the Amino Acid Metabolism of White Rats[1]

JOHN M. WOODWARD, ANTHONY J. SBARRA, AND D. FRANK HOLTMAN

Department of Bacteriology, University of Tennessee, Knoxville, Tennessee

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Relatively little is known concerning the manner in which *Bacterium tularense* (*Pasteurella tularensis*) exerts a damaging effect on its hosts. The pathology of tularemia in humans and in animals has been described by many investigators, but there is no report in the literature concerning the influence of this organism on the metabolic activities of the host.

It is well known that in many diseases and in the case of protracted fevers the free amino acid level of the blood is elevated and is accompanied by excessive excretion of these compounds by way of the kidneys. Furthermore, it is recognized that in tularemia the organisms tend to localize in the liver and spleen. Also, it is known that the liver is concerned to a considerable degree with the control of the amino acid levels of the blood. Preliminary data reported by Sbarra *et al.* (1952) have indicated that the free amino acid level in the blood of rats is disturbed during infection with *B. tularense*.

In view of these observations, it has been the purpose of this investigation to determine more extensively the effect of this organism on the free amino acid levels in the blood and urine of albino rats and to attempt to correlate the data obtained with the pathology of the infection, particularly with regard to possible damage of the liver and spleen.

Such a study represents a relatively new approach from which it may be possible to derive greater insight into the nature of the injury that accompanies infection as well as more extensive information concerning the management of infectious disease.

MATERIALS AND METHODS

Cultures. The following strains of *B. tularense* were employed: strain NIH 38 which is avirulent and strain Sm which is highly virulent and has a rat LD_{50} of $10^{-6.5}$ (Reed and Muench, 1938). The cultures were maintained on glucose cysteine blood agar (GCBA) (Downs *et al.*, 1947*a*). Standard suspensions of the organisms for animal inoculations were prepared by adjusting saline suspensions of the organism to a turbidity reading of 160 on the Klett-Summerson photometer. Plate counts of these suspensions averaged approximately 3.5×10^9 viable units per ml. Appropriate dilutions were employed for rat inoculations in the various experiments.

Animals. Adult male rats of the Wistar strain weighing 180 to 250 grams were used.

Experimental methods. Free amino acids in the blood and urine of the rats were determined by the two dimensional paper partition chromatographic technique of Williams and Kirby (1948).

All rats were maintained on a diet of Purina Dog Chow. Test animals from which blood and urine were to be collected were fasted for 12 hours prior to collection of the sample but were allowed water *ad libitum*. Approximately four ml of blood were removed from each animal by cardiac puncture under light ether anaesthesia immediately prior to, and 72 hours after, infection. Urine was collected by placing the animals in a metabolism cage with a false bottom allowing drainage through a large Buchner funnel into a test tube containing a few crystals of thymol for the prevention of bacterial growth. Four to six hours were sufficient for the collection of an adequate urine sample.

The blood serum and urine samples were deproteinized by the addition of equal volumes of acetone, and 0.1 ml of the supernatant was employed for chromatographic analysis. The above procedures were conducted routinely except where otherwise noted in the individual experiments.

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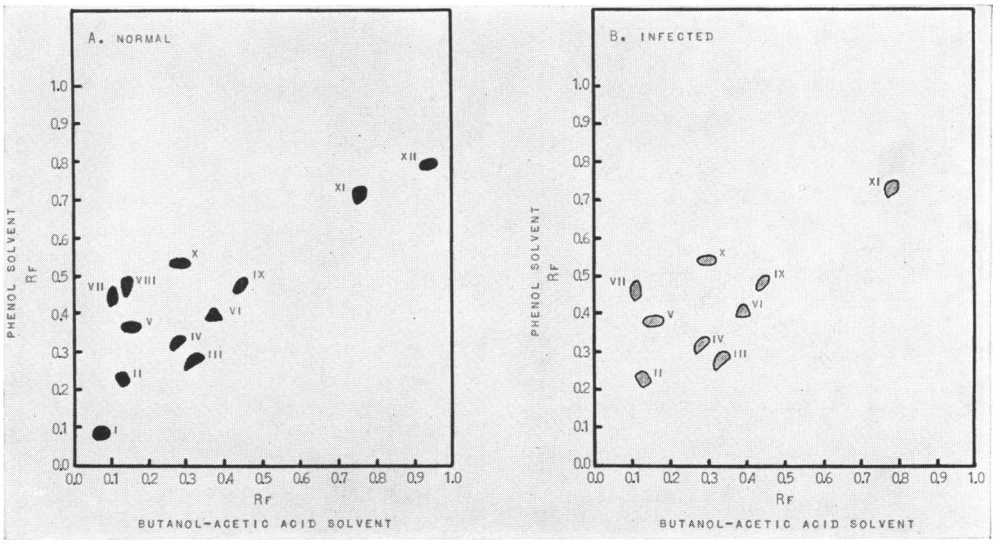

Figure 1. Typical chromatogram of the free amino acids in the blood of normal and infected rats. The spots are identified as follows: spot number I cystine, II histidine, III serine, IV glutamic acid, V glycine, VI threonine, VII alanine, VIII arginine, IX tyrosine, X proline, XI leucine and isoleucine, and XII phenylalanine.

RESULTS

Chromatographic analysis of the blood of normal and infected rats. Blood samples were collected from a total of 46 normal and 45 infected rats, both prior to infection with one LD_{50} (approximately 3.5×10^3 organisms) of the Sm strain and also 72 hours later. Chromatographic analysis for free amino acids in the blood of these animals revealed in every instance that infection caused a pronounced decrease in free amino acids. Typical chromatographic pictures from normal and infected animals are shown in figure 1. These data illustrate the lowered concentration of free amino acids in all identifiable spots and a consistent failure to detect cystine, arginine, and phenylalanine.

Since control observations in normal rats showed that identical patterns were given by chromatographed blood filtrates from the first and second bleedings, it may be concluded that alterations of free amino acid content were due to the organisms injected and not to the procedure employed.

Furthermore, the injection of 3.5×10^9 killed organisms of strain Sm into 10 rats under the conditions of the infection experiment did not modify the free amino acid content of the blood of these animals.

Comparable observations on the blood of 9 rats infected with approximately 3.5×10^9 viable units of strain 38 revealed that this large inoculum of nonvirulent organisms was likewise incapable of altering the free amino acids in the blood. It may be noted that this strain grows on artificial medium more slowly than strain Sm and that it establishes itself less readily in tissues of the mouse (Downs and Woodward, 1949).

The onset of free amino acid deficit following injection of strain Sm. The normal chromatograms of 12 rats were compared with those of blood drawn only 48 hours after infection with one LD_{50} of strain Sm. The free amino acids in the blood of 4 animals were already depressed as described for 72 hours. In the 8 remaining rats the amino acids had been lowered to a lesser extent and in varying and individual degrees. These results suggest a gradual reduction of free amino acids in each animal, perhaps in accordance with individual differences in susceptibility.

Free amino acids in the blood during and after recovery. Chromatograms of 5 rats bled 10 days after infection were identical with those made at the 72 hour interval. However, the pictures for these same animals at the end of 25 days were normal. It appears that the return of the blood amino acids to their usual levels coincides with complete recovery from infection.

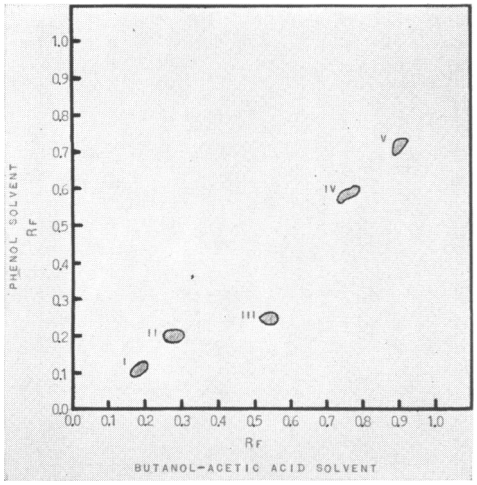

Figure 2. Typical chromatogram of the free amino acids in the urine of normal and infected rats. The spots are identified as follows: spot number I cystine, II histidine, III not identified, IV leucine and isoleucine, and V phenylalanine.

Free amino acids in the blood of recovered and reinfected rats. Normal chromatograms of blood from 10 recovered rats were compared with those made 72 hours after reinfection with 100 LD_{50} of strain Sm. No differences were observed in either case. Recovered rats have a high degree of immunity and are able to restrict growth of the organisms (Downs *et al.*, 1947*b*) which may explain why strain Sm did not alter the blood amino acid levels in these animals.

Free amino acids in the urine of normal and infected rats. Urine chromatograms of 22 normal rats were compared with those made 72 hours after infection with one LD_{50} of strain Sm. No significant differences could be detected in either instance. However, the number and concentration of amino acids in urine were found to be much lower than in blood. A typical chromatogram of urine amino acids is presented in figure 2. These results indicate that the amino acids lost from the blood of infected animals are not excreted as such by way of the kidneys.

DISCUSSION

We have observed that highly virulent and actively metabolizing cells of *B. tularense* stimulate the depletion of the free amino acids in the blood of white rats while high concentrations of either killed virulent or living avirulent organisms do not effect such a change. This may indicate a significant relationship of the parasite to the host in tularemia in view of the fact that it was not possible to account for the loss of the amino acids by way of the kidneys using chromatographic methods.

Several explanations for these observations may be considered. It seems possible that since the organisms tend to localize in the liver and spleen they may utilize the free amino acids directly from the blood as it filters through these organs. This might account for the depletion of these compounds observed during the infection. Also, the fact that cystine, which is an essential metabolite for *B. tularense*, disappears from the blood of infected animals according to our chromatographic technique lends further support to the idea that the organisms themselves utilize the free blood amino acids of the host.

Another explanation involves the possibility that the organisms exert a damaging effect on the liver in a manner which either suppresses enzymatic mechanisms that maintain the free amino acid levels or stimulates amino acid oxidase activity in the degradation of these compounds. In either instance, it follows logically that the free amino acid level of the blood would be disturbed.

Consideration also must be given to the concept that other metabolic activities such as cellular respiratory functions of the liver may be interfered with during infection. The complexities of liver metabolism provide many avenues of approach in the investigation of the influence of the parasite on the host.

On the basis of the data obtained thus far, it is evident that more extensive investigation is necessary particularly with reference to the effect of *B. tularense* on the metabolic activities of the host that are concerned directly with liver function and liver metabolism. Studies are in progress at the present time concerning this problem.

ACKNOWLEDGMENT

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SUMMARY

Chromatographic analysis of the blood of normal rats and rats infected with *Bacterium tularense* has shown that during infection there

is a pronounced decrease of 12 free amino acids in the blood of the infected animals.

Removal of blood during routine procedures was shown, in itself, to have no effect on the concentration of free amino acids in the blood of the rats.

No change in the free amino acid levels in the blood was observed in rats which were inoculated with high concentrations of killed cells of the Sm strain or with living avirulent organisms of strain 38.

Upon recovery from infection with the highly virulent Sm strain, the concentration of free amino acids in the blood of the rats returned to normal within 25 days following infection.

Normal concentrations of the free amino acids remained unchanged in the blood of rats recovered from tularemia and reinfected after an interval of 30 days.

Analysis of the urine of normal and infected animals has demonstrated the presence, in low concentration, of five to nine amino acids. No significant differences could be detected in the number or concentration of these compounds in the normal and infected picture.

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