THE EFFECT OF *SALMONELLA PULLORUM* INFECTION ON AMINO ACIDS OF THE CHICK[1]

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The pathology of pullorum disease has been frequently described, but little information exists concerning the mechanism by which *Salmonella pullorum* exerts a damaging effect on its host. While the organism becomes quite generally distributed throughout the chick during the course of disease, the fact that it tends to be prevalent in the liver suggests the possibility of alterations in both protein and carbohydrate metabolism of this animal. It was shown by Holtman and Page in 1950 (*unpublished data*) that an experimental increase in the blood level of threonine in guinea pigs infected with *Salmonella typhimurium* resulted in a marked reduction of survival time. This amino acid, *in vivo*, was later demonstrated by Page, Goodlow, and Braun (1951) to induce significant bacterial population changes resulting in the establishment of variants of increased virulence. Whether this type of amino acid effect occurs during the progress of natural infection has not been determined although it is evident that the free amino acid levels of the blood may be reduced during the course of disease. For example, Woodward, Sbarra, and Holtman (1954) reported a decrease in all free amino acids and the complete disappearance of cysteine, arginine, and phenylalanine in the blood of rats infected with *Bacterium tularense*.

The study reported here was undertaken to determine if changes occur in the free and bound amino acid levels of the blood and liver of chicks infected with *S. pullorum*. This particular combination was selected because it is a natural host-parasite relationship that can be conveniently studied in the laboratory and, thus, could be useful in shedding light on the alterations in host metabolism during the course of disease.

MATERIALS AND METHODS

The virulent strain 3522/51 of *S. pullorum*, supplied by the Communicable Disease Center, U. S. Public Health Service, Chamblee, Georgia, was employed. This strain has a chick LD_{50} of $10^{2.5}$. It was maintained during the experiment by regular passage through chicks. One avirulent strain was used for control studies. This strain, NE no. 17, produced no deaths in chicks given dosages as great as 10^6 organisms. Furthermore, it attained no virulence through animal passage. Cultures of strain NE no. 17 were maintained on nutrient agar. Standard suspensions of the virulent and avirulent strains were prepared for inoculation by adjusting a saline suspension of a 24 hour broth culture to a reading of 160 on a Klett-Summerson colorimeter. One ml of a 1:10,000 dilution of this suspension was used as an inoculating dose. All inoculations were made intraperitoneally.

One to three day old white leghorn cockerels were used as experimental animals. They were maintained on an antibiotic-free chick starter mash prepared by Security Mills, Inc., Knoxville, Tennessee. All chicks were free of pullorum disease prior to inoculation.

Blood samples were collected by cardiac puncture 48 hours following infection. The chicks were then sacrificed and the livers excised. Sera were deproteinized with aliquot portions of acetone. Each serum-acetone mixture was filtered and 0.02 ml of the filtrate used for chromatographic analysis. Hydrolysis of blood serum for the determination of bound amino acid was accomplished by the addition of concentrated HCl in an amount equal to that of the serum. The mixture was autoclaved for two hours, then evaporated under vacuum to dryness. The original volume was restored by the addition of glass distilled water. The solution was neutralized with silver oxide and filtered. Two hundredths ml of this filtrate was subjected to chromatographic analysis.

One gram of liver was treated according to the method of Awapara as described by Block (1952). Deproteinization was accomplished by homogenization of one part tissue with three parts absolute ethanol in a Waring blender. The mixture was

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filtered, and the filtrate used for the recovery of free amino acids. This was washed in 80 per cent ethanol, then combined in a 1:3 ratio with chloroform.

The hydrolysis of liver for the analysis of bound amino acids was accomplished by treating the original precipitate in the manner described for blood sera. The final solution was diluted 1:10, and 0.02 ml of this dilution was used in chromatographic studies.

The two dimensional paper partition chromatography procedure of Williams and Kirby (1948), employing the ascending technique of Horne and Pollard (1948), was used in all experimentation. Ninhydrin served as the developer.

Blood samples were collected from 30 normal chicks, 30 chicks infected with the virulent strain, and 30 chicks inoculated with the avirulent strain. The chicks were sacrificed and liver specimens removed immediately following withdrawal of the blood. In addition, blood samples were collected from 30 chicks of each group prior to, and 48 hours after, infection.

RESULTS

Chromatographic analyses of both free and bound amino acids of blood and liver from chicks infected with *S. pullorum* revealed a decrease in the concentration of certain amino acids. A total of thirteen free amino acids appeared consistently in the blood of normal chicks (figure 1A). These amino acids also appeared in like concentration, as determined by color intensity of spots on the chromatograms, in the blood of chicks inoculated with the avirulent strain of *S. pullorum*. Chromatograms of like amounts of blood of infected chicks showed a marked reduction in the concentrations of arginine, methionine, glycine, and tryptophan (figure 1B). By further dilution of the filtrates employed as spotting solutions, it was possible to eliminate these four amino acids entirely from the chromatograms, while the others remained in

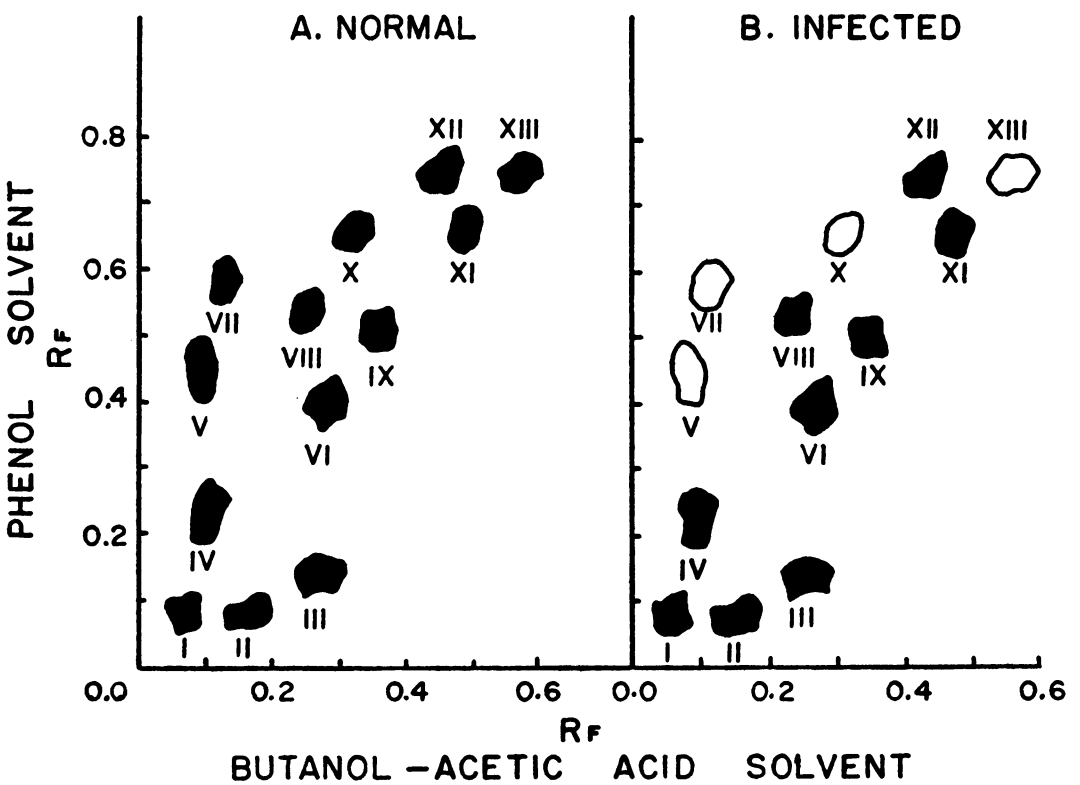

Figure 1. Typical chromatograms of the free amino acids in the blood of normal (A) and infected (B) chicks. The spots are identified as follows: I. cystine, II. aspartic acid, III. glutamic acid, IV. cysteine, V. glycine, VI. threonine, VII. arginine, VIII. alanine, IX. tyrosine, X. methionine, XI. valine, XII. phenylalanine, XIII. tryptophan.

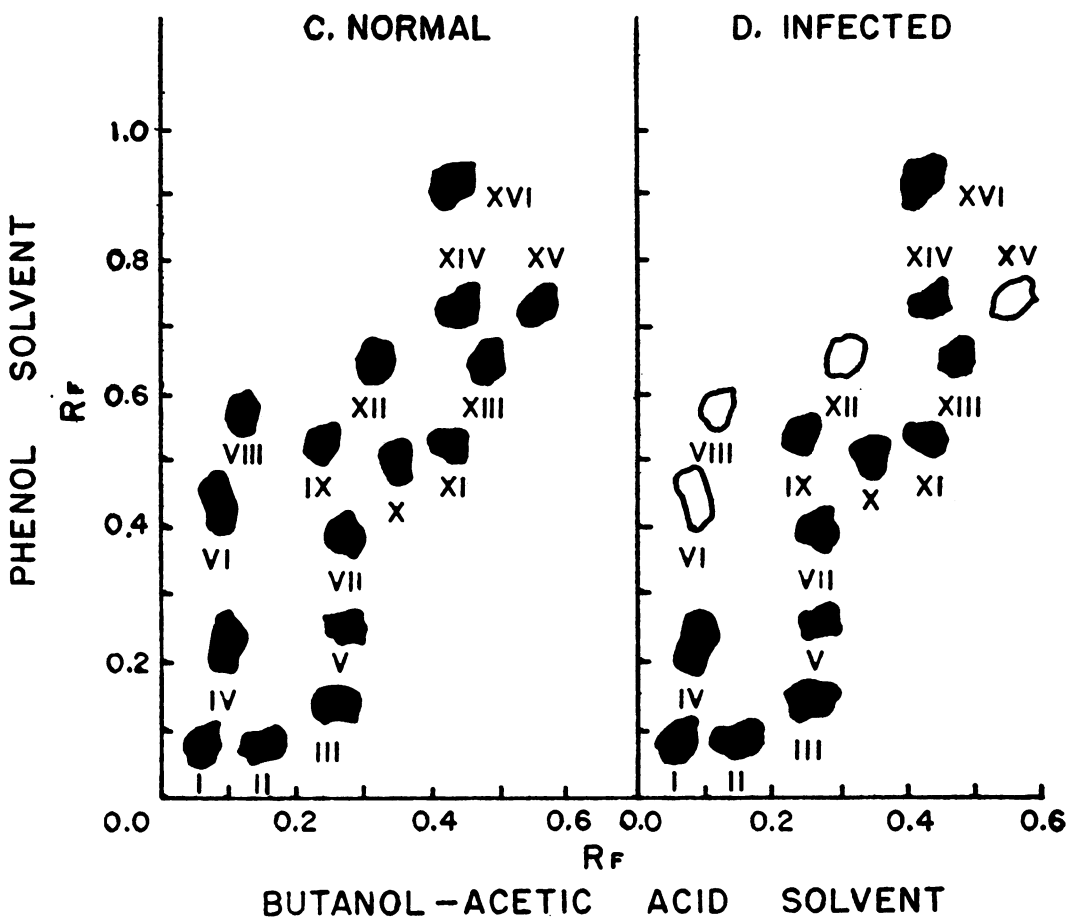

Figure 2. Typical chromatograms of the free amino acids in the livers of normal (C) and infected (D) chicks. The spots are identified as follows: I. cystine, II. aspartic acid, III. glutamic acid, IV. cysteine, V. serine, VI. glycine, VII. threonine, VIII. arginine, IX. alanine, X. tyrosine, XI. hydroxproline, XII. methionine, XIII. valine, XIV. phenylalanine, XV. tryptophan, XVI. proline.

sufficient quantities to produce relatively intense colors with the ninhydrin developer.

Sixteen amino acids appeared in the filtrates of homogenized livers from normal chicks, as well as from those inoculated with the avirulent strain of *S. pullorum* (figure 2C). Again, the four previously mentioned amino acids were significantly reduced in quantity in the livers of infected chicks as shown chromatographically (figure 2D).

Chromatograms of hydrolyzed blood and liver also showed arginine, methionine, glycine, and tryptophan to have been decreased in amount as a result of infection. While hydrolysis of proteins generally yields a large number of amino acids, the hydrolyzed specimens used in these studies occasionally failed to show all the amino acids apparent in filtrates of nonhydrolyzed blood and liver. This was probably due to the technique employed and not to a lack of bound amino acid.

DISCUSSION

While a reduction in the free and bound amino acids of the blood and liver of chicks infected with *S. pullorum* has been demonstrated, the fate of arginine, methionine, glycine, and tryptophan has not been determined. It is conceivable that the organism may utilize these amino acids while parasitizing the host, yet *in vitro* studies have not indicated a need for all four. It is an interesting and important consideration that these four amino acids have been found to be essential in the nutrition of the chick by Almquist (1947). Hence, it seems possible that the host may have need to make use of larger amounts of arginine,

methionine, glycine, and tryptophan than normal in its efforts to defend against the invading parasite and, thus, cause the noticeable decrease in their concentration in blood and liver.

SUMMARY

Chromatographic analyses of blood and liver of chicks infected with *Salmonella pullorum* have shown that, during the course of infection, the levels of arginine, methionine, glycine, and tryptophan are reduced. Chicks inoculated with an avirulent strain of *S. pullorum* presented the same chromatographic pictures as normal controls. Since the organism appears to have no specific need for all four amino acids, it is suggested that the host may be utilizing them in its defense against the pathogen.

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