ALTERATIONS IN THE NITROGEN EXCRETION PATTERN OF CHICKS INFECTED WITH SALMONELLA PULLORUM

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Investigations in our laboratory have established that infection with Salmonella pullorum strain CDC 3522/51 results in a decrease in arginine, methionine, glycine and tryptophan in the blood and liver of 1- to 3-day old White Leghorn cockerels (Ross et al., 1955a). Subsequent experiments revealed that the administration of arginine within 24 hr following infection produced a marked increase in survival time of the chicks (Ross et al., 1955b). The investigations reported here suggest that the protection afforded by arginine during infection is accomplished through the increased synthesis of urea via the ornithine-citrulline cycle.

Urea synthesis represents the principal pathway of nitrogen excretion during the early stages of embryonic development, but in the hatched chick apparently accounts for only a small amount of all excreted nitrogen. Needham et al. (1935) established that urea production in the chick embryo parallels arginine content and, thus, is the end product of the ornithine-citrulline cycle proposed by Krebs and Henseleit (1932). Accordingly, urea reaches its maximum level on the ninth day of incubation but, with the rapid decline in arginine, falls progressively, and by the end of the incubation period attains the percentage level demonstrated in adult birds. Clementi (1914, 1946) reported arginase and, consequently, the ornithine-citrulline cycle to be absent in hatched chicks. Investigations into the synthesis of urea and metabolism of arginine and other urea cycle intermediates in mammalian tissue have been capably reviewed by Rattner (1954). Arginine synthesis is regarded as minute or absent altogether in avian tissue. Hence this amino acid is presumed to be attained through the diet of the chick.

METHODS

In tracing the fate of free amino acids disappearing from chicks infected with S. pullorum strain CDC 3522/51, blood levels of urea, uric acid and creatinine were determined by the methods of Karr (1924), Folin and Wu (1919) and Brown (1926), respectively. Values were obtained from normal chicks as well as from infected chicks, with and without amino acid treatment.

The hypothesis that arginine, disappearing from the blood of the infected chick, served as the precursor of the corresponding increase in urea was tested by chromatographic and manometric techniques. These were employed as measures of arginase activity in liver of normal and infected chicks.

For chromatographic study, the following protocol was used. Approximately 1 g of fresh liver (wet weight) was frozen and pulverized. Liver arginase was activated by a method proposed by Weil (1935). Arginine hydrochloride (containing 100 mg), 10 ml and borate buffer (pH 9.5), 5 ml were added to liver homogenate, 2.5 ml and incubated at 30 C for 1 hr. The mixture was applied to strips of Whatman No. 1 chromatographic filter paper. Phenol served as the solvent. One strip was developed with ninhydrin, another with sodium hypochlorite.

The manometric technique of Weil and Russell (1934) was employed to measure arginase activity in liver tissue from normal and infected chicks. Arginase activity was measured indirectly by the CO₂ liberated from the urea produced.

The progress of the experimentation to this point made it desirable to determine if ornithine, citrulline or urea increased the survival time of infected chicks as had been observed previously when infected chicks were treated with arginine. The inoculating dose of each was vehicled in 1 ml
of sterile water and administered intraperitoneally immediately following infection. Amounts of each injected were: urea, 1.650 mm; ornithine, 0.822 mm and citrulline, 0.570 mm.

Experiments were performed in vitro to determine if *S. pullorum* produced arginine. Also, observations were made as to whether the ornithine cycle participants had an inhibitory effect upon the organism. Cells were grown in synthetic medium (Gilfillan et al., 1955) or in chick sera. Arginine, citrulline, ornithine and urea were added to either medium in the amounts described for in vivo tests. Growth was observed by means of turbidity measurements using the Klett-Summerson photocolorimeter.

**RESULTS**

Significant differences appeared in blood levels of products of nitrogen metabolism in normal and infected chicks. Within 24 hr following infection with 10° cells of *S. pullorum*, the blood levels of urea, uric acid and creatinine were usually increased. Infected chicks administered a single dose of 0.690 mm arginine intraperitoneally showed a urea blood level more than 10 times that of normal birds, while creatinine and uric acid levels were essentially those of normal controls. Infected, non-arginine treated chicks also showed a significant increase in urea, and in creatinine, but very little in uric acid (table 1).

Curves obtained when urea values were plotted over a time interval of 108 hr after infection or until chicks died from the disease are shown (figure 1). The blood-urea level of chicks receiving the test organism only increased until approximately 60 hr following infection, but then fell sharply until death occurred. Infected chicks treated with arginine showed a progressive increase in blood urea until approximately 84 hr at which time there was a similar decline.

The disappearance of arginine with a corresponding increase in the blood level of urea in the infected chick would suggest arginine to be the precursor of the urea formed. Through use of chromatography, spots were obtained on paper strips that were identifiable as arginine and ornithine. The latter, incidentally, had not been observed in blood or liver of normal chicks. Urea was readily detected on chromatograms through use of sodium hypochlorite as the developer (figure 2). The appearance of ornithine and urea would strongly suggest arginine activity in liver tissue of chicks infected with *S. pullorum*. A similar test performed with liver of normal chicks

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**TABLE 1**

*Urea, uric acid, and creatinine levels in blood of normal and infected chicks and infected chicks treated with arginine*

<table>
<thead>
<tr>
<th>Groups of Chicks</th>
<th>Mg Per Cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urea</td>
</tr>
<tr>
<td>Normal</td>
<td>0.37</td>
</tr>
<tr>
<td>Infected</td>
<td>2.26</td>
</tr>
<tr>
<td>Arginine treated</td>
<td>4.16</td>
</tr>
</tbody>
</table>

* Values represent the average obtained from 10 chicks per group.

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*Figure 1.* Blood urea levels in infected chicks and in infected chicks treated with arginine.

*Figure 2.* Chromatographic evidence of arginase activity in liver of infected chick.
showed no arginase activity. Likewise, no arginase was demonstrated in cultures of the test organism alone.

When the manometric technique of Weil and Russell (1934) was used to measure arginase activity in liver tissue of normal and infected chicks, it became apparent once again that there was increased activity of that enzyme in the infected birds (table 2).

The protection previously observed in chicks treated with arginine evidently was not due to the amino acid alone but to increased arginase activity producing degradation of arginine during infection. In an effort to determine what role the ornithine-citrulline cycle and synthesis of urea plays in the course of the disease, 3 groups of infected chicks were treated, separately, with ornithine, citrulline and urea, and survival times were noted (table 3). Ornithine induced some protection, but was not nearly as effective as arginine. Either citrulline or urea, on the other hand, afforded a protection equal to that of arginine.

No inhibitory effect upon the growth of *S. pullorum* was noted in liquid cultures fortified with arginine, ornithine, or citrulline. Urea, however, reduced growth at concentrations as low as 250 mM and was completely inhibitory at concentrations of 1 mM.

**DISCUSSION**

The blood levels of urea, uric acid and creatinine observed in chicks inoculated with *S. pullorum* strain CDC 3522/51 indicate a definite alteration in amino acid catabolism during the course of infection. The most marked change is a 7- to 10-fold increase in blood urea. The arginolytic concept of the origin of urea appears to be valid on the basis of results obtained by chromatography, which showed an exceptionally high concentration of blood urea in infected chicks treated with arginine.

From the techniques employed to measure arginase activity, the pathway of urea formation appears to be that of the ornithine-citrulline cycle normally inactive in chicks and other uricotelic animals. Manometric experiments indicate that arginase exhibits an increased activity within 24 hr after infection and remains at an increased level until death. Since *S. pullorum* alone shows no arginase activity, it would seem illogical to attribute any increase in the enzyme to the metabolism of that organism *per se*. Hence, the mechanism responsible for the activation of liver arginase during infection must exist in the host.

The protective role of arginine reported in a previous paper now appears to be explainable on the basis of the activation of the ornithine-citrulline cycle in infected chicks. Whereas the administration of arginine, citrulline, or ornithine increased the survival of the infected birds, none of these amino acids was inhibitory to the growth of *S. pullorum*. Urea, on the other hand, showed a definite protective effect *in vivo* and was inhibitory to the test organism *in vitro*. This suggests that the efficacy of arginine, ornithine, or citrulline is due to a precursory or intermediate effect in the synthesis of urea. Since it is well known that urea in sufficient concentration is toxic to uricotelic animals, the protective role of this substance must be based on some differential level of toxicity to host and parasite. Work is
under way attempting to clarify the mechanism of action. The possibility that the accumulation of products of nitrogen metabolism in the blood results from kidney tissue damage induced by endotoxin of the infectious agent has not been investigated.

SUMMARY

Investigations concerning the host-parasite relationship which exists in chicks infected with *Salmonella pullorum* have revealed that the pattern of nitrogen excretion normally present in this bird is altered during the course of infection. Blood urea is increased 7- to 10-fold and represents the principal form of amino nitrogen in the circulatory system of the infected chick. The urea presumably is of arginolytic origin and is formed via the ornithine-citrulline cycle. Arginase activity, which is present in minute quantities or absent altogether in normal chicks, is increased within 24 hr following infection. The protective action of arginine previously reported has been attributed to its role in synthesis of urea which is inhibitory to the growth of *S. pullorum*.

REFERENCES


