EFFECT OF THE ADMINISTRATION OF CORTISONE ON THE RESPONSE OF CHICKS TO THE ENDOTOXIN OF SALMONELLA PULLORUM

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Previous investigations conducted in our laboratory have shown that the arginase system and, consequently, the Krebs ornithine cycle, is reactivated in baby chicks experimentally infected with Salmonella pullorum (Ross et al., 1956). A similar reactivation, as evidenced by an alteration in the nitrogen excretion pattern, was demonstrated in chicks treated with the endotoxin of S. pullorum (Dooley and Holtman, 1957). Under the conditions of both of these experiments, urea replaced uric acid as the major nitrogen excretion compound of the chicks.

Urea synthesis represents the principal pathway of nitrogen excretion during the early stages of embryonic development, but in newly hatched chicks, this synthesis accounts for only a small amount of the total nitrogen excreted. In the developing embryo the production of urea parallels arginase activity (Needham et al., 1935). According to Moog (1952) the peak of arginase activity occurs on the third day of incubation. Then the activity declines rapidly and can not be detected in the 8-day-old embryo.

In studies of the reaction of chick embryos to bacterial endotoxins, Smith and Thomas (1956) observed that the period of maximal susceptibility occurs on the 10th day of incubation. At 6 days, and at 16 days, the embryos appear to be completely insusceptible. The administration of cortisone was reported to protect 10-day-old embryos against the lethal effects of endotoxin.

Since the period of maximal susceptibility to endotoxin occurs subsequent to the period of arginase activity in the developing embryo, it became of interest to us to study the possible relationship between susceptibility and arginase activity in chicks treated with the endotoxin of S. pullorum. Cortisone, previously shown to increase arginase activity (Kochakian, 1951), was included in this investigation because earlier studies (Dooley and Holtman, 1957) suggested that this hormone protects newly hatched chicks against the lethal effects of endotoxin of S. pullorum.

METHODS

One- to three-day old White Leghorn cockerels obtained from a local hatchery were used. The chicks were hatched from eggs from a certified pullorum-free flock. They were maintained on an antibiotic-free starter mash and brooded at 40 C during the experiment.

The virulent strain of S. pullorum (CDC 3522/51), obtained from the Communicable Disease Center, U. S. Public Health Service, Chamblee, Georgia, was used as the source of endotoxin.

The Boivin-type endotoxin was prepared according to a previously described technique (Dooley and Holtman, 1956). The LD₅₀ of the preparation was 6.4 mg for 35-g chicks. The endotoxin was suspended in physiological saline and inoculation was made by the intraperitoneal route.

A saline suspension of cortisone acetate (Merck) containing 25 mg per ml was employed. Chicks received intraperitoneal injections of 5 mg 24 hr prior to administration of endotoxin and again at the time of treatment with the endotoxin.

Blood samples were collected at 24 hr following the experimental treatment. Samples from 4 chicks were pooled for the determinations of nitrogen-containing compounds and heparin was used as an anticoagulant. The concentrations of nonprotein nitrogen, urea, creatinine, uric acid, glucose, and serum inorganic phosphorus were determined by the techniques of Karr (1924),

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Brown (1926), Folin and Wu (1919), Koch and McMeekin (1924), Folin (1929), and Fiske and SubbaRow (1925), respectively. Blood ammonia was determined directly on individual blood samples by the technique of Seligson and Seligson (1951).

Chick body temperatures were taken according to the technique of Lamoreux and Hutt (1939). To insure uniformity all readings were made between 1:00 and 4:00 pm. This represents the period of peak diurnal temperature in chicks.

RESULTS

The administration of cortisone to chicks treated with the endotoxin of *S. pullorum* produced an increase in the nitrogen excretion pattern (tables 1 and 2). Urea represented the principal excretion product of chicks treated with endotoxin. The injection of cortisone did not significantly alter the amount of nitrogen excreted in this form, nor did it affect the excretion of uric acid. The amount of nonprotein nitrogen was increased after administration of cortisone, whereas the synthesis of creatinine was reduced, but still remained above values established for the normal chick.

Blood ammonia levels, shown to be increased following administration of endotoxin, were reduced after treatment with cortisone to near normal levels (table 2). Also, the normal ability of liver to remove ammonia from the portal blood appeared to be unimpaired in the chicks receiving both cortisone and endotoxin (table 3).

The hypoglycemia normally found in chicks at 24 hr following treatment with endotoxin was moderated by treatment with cortisone. Serum inorganic phosphorus, normally increased in the blood of chicks subjected to endotoxin, was not significantly altered by administration of cortisone under the conditions of these experiments.

The protective effect of cortisone against the lethal action of endotoxin was evidenced by a reduction in the number of deaths. It was also shown in the lengthened time of survival of chicks that later succumbed to the intoxication (table 4).

The antipyretic effect of cortisone was demonstrated against the endotoxin of *S. pullorum* (figure 1). This observation is in agreement with the results obtained by Atkins *et al.* (1955) using Pyromen and typhoid bacterin.

**TABLE 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>Non-protein-N</th>
<th>Normal</th>
<th>Urea-N</th>
<th>Normal</th>
<th>Creatinine</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>33.8</td>
<td>100</td>
<td>0.33</td>
<td>100</td>
<td>0.70</td>
<td>100</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>37.5</td>
<td>111</td>
<td>2.28</td>
<td>690</td>
<td>1.27</td>
<td>182</td>
</tr>
<tr>
<td>Endotoxin + cortisone</td>
<td>41.8</td>
<td>124</td>
<td>2.29</td>
<td>693</td>
<td>0.92</td>
<td>130</td>
</tr>
<tr>
<td>Cortisone controls</td>
<td>37.5</td>
<td>111</td>
<td>0.37</td>
<td>114</td>
<td>0.80</td>
<td>114</td>
</tr>
</tbody>
</table>

Average values from 4 determinations on pooled samples from 4 chicks taken at 24 hr.

**TABLE 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>Uric Acid</th>
<th>Normal</th>
<th>Ammonia</th>
<th>Normal</th>
<th>Glucose</th>
<th>Normal</th>
<th>Inorganic-P</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>3.18</td>
<td>100</td>
<td>0.67</td>
<td>100</td>
<td>195.4</td>
<td>100</td>
<td>3.43</td>
<td>100</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>4.25</td>
<td>134</td>
<td>1.01</td>
<td>150</td>
<td>155.6</td>
<td>80</td>
<td>4.10</td>
<td>120</td>
</tr>
<tr>
<td>Endotoxin + cortisone</td>
<td>4.31</td>
<td>136</td>
<td>0.70</td>
<td>104</td>
<td>177.1</td>
<td>91</td>
<td>4.10</td>
<td>118</td>
</tr>
<tr>
<td>Cortisone controls</td>
<td>3.20</td>
<td>102</td>
<td>0.73</td>
<td>108</td>
<td>188.9</td>
<td>98</td>
<td>3.53</td>
<td>103</td>
</tr>
</tbody>
</table>

Average values from 4 determinations on pooled samples from 4 chicks taken at 24 hr.
cortisone or endotoxin must be able to reactivate an enzyme system in newly hatched chicks which normally exists in an active form only in the developing embryo.

The excretion of uric acid and creatinine by newly hatched chicks is also increased by treatment with cortisone or endotoxin. As in the case of urea, however, the increases produced by cortisone are not as great as those produced by endotoxin. When used in combination, the effect of cortisone and endotoxin appears to be additive in the case of nonprotein nitrogen and uric acid. The excretion of nitrogen in the form of urea, however, is not significantly increased by the combined treatment whereas the excretion of creatinine is reduced.

Blood ammonia, previously shown to be increased in the peripheral circulation of endotoxicated chicks (Dooley and Holtman, 1957), is reduced to near normal levels in chicks given the combination of endotoxin and cortisone. Thus, the normal ability of the liver to remove ammonia from the blood is apparently preserved through the action of the hormone. The toxicity of abnormal amounts of ammonia in the blood of uricotelic animals is well known and the capacity of cortisone to prevent the occurrence of ammonia toxicity in the endotoxicated chick may represent one way by which the protective effect of this hormone is exerted.

The source of the increased levels of ammonia occurring in blood of chicks treated with endotoxin is not known. The results of this investigation suggest that it is of intestinal origin. Blood entering the liver via the portal vein has been shown to carry increased amounts of ammonia (table 4) which escape into the periph-

### Table 3

**Effect of cortisone on the ammonia levels in chicks intoxicated with the endotoxin of *Salmonella pullorum***

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood Ammonia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Portal vein</td>
</tr>
<tr>
<td></td>
<td>μmoles/ml</td>
</tr>
<tr>
<td>Normal</td>
<td>1.10</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>1.28</td>
</tr>
<tr>
<td>Endotoxin + cortisone</td>
<td>0.95</td>
</tr>
<tr>
<td>Cortisone controls</td>
<td>1.00</td>
</tr>
</tbody>
</table>

### Table 4

**Effect of cortisone on the survival time of chicks intoxicated with the endotoxin of *Salmonella pullorum***

<table>
<thead>
<tr>
<th>Group</th>
<th>Deaths/ Survivors</th>
<th>Avg. Hr of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline controls</td>
<td>0/10</td>
<td>---</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>10/16</td>
<td>30.2</td>
</tr>
<tr>
<td>Endotoxin + cortisone</td>
<td>3/7</td>
<td>43.0</td>
</tr>
<tr>
<td>Cortisone controls</td>
<td>0.10</td>
<td>---</td>
</tr>
</tbody>
</table>

*Figure 1. Effect of cortisone on the body temperature response of chicks to the pyrogenic action of 0.5 mg endotoxin of *Salmonella pullorum*: A, endotoxin; B, endotoxin and cortisone; C, normal controls.*
eral circulation through the inferior vena cava. In the case of nonintoxicated chicks treated with cortisone, the increased ammonia could be a result of protein catabolism which is accelerated under such treatment.

A hepatic system involving a combination of the known pathway for synthesis of creatine with the Krebs ornithine cycle, previously proposed (Dooley et al., 1958) to relate data obtained from a study of the nitrogen metabolism of endointoxicated chicks, could also help clarify the similarity between the actions of cortisone and endotoxin in chicks. This hepatic system provides a pathway for the conversion of ammonia into arginine and, subsequently, into urea. Arginine regenerated in this system, however, could also enter into a pathway for the synthesis of creatine. Since creatinine nitrogen cannot be used in a biosynthetic process, it may well represent an end product of nitrogen metabolism.

The strategic role of arginase in the hepatic cycle, to which reference has just been made, can be quite readily understood. The enzyme is essential to the operation of the ornithine cycle and for the synthesis of urea. It is of interest to note here that both endotoxin and cortisone are capable of stimulating arginase activity in experimental animals, including chicks (Kochakian, 1951; Dooley and Holtman, 1957). Cortisone has also been shown to increase the methylation of guanidoacetic acid to form creatine and to increase the excretion of uric acid (Sprague et al., 1950; 1951).

On the basis of the results of this study it seems that one aspect of the over-all "non-specific antitoxic effect" of cortisone might be due to its ability to simulate arginase activity, not only in cases where such activity normally exists, but also in cases where a potential system exists, i.e., in fowls. Thus, it appears that cortisone, as well as ornithine, citrulline, arginine, glycine, and methionine, might be added to the list of compounds which serve to remove ammonia from the blood by increasing the capacity of the hepatic system. It is interesting to note also that the protective effect of cortisone against the lethal action of endotoxin, both in the developing embryo and in the newly hatched chick, is exerted after the arginase system has been established.

It must be admitted that generalization on the actions of hormones, based on a study of nitrogen metabolism and excretion, is dangerous and may lead to oversimplification. It is known that various tissues and organs react differently to cortisone and often in opposing directions. The complexity of the metabolism of nitrogen in animals makes the understanding of the mode of action of cortisone a difficult one. The changes in nitrogen metabolism consequent to cortisone treatment, as reflected by increased nitrogen excretion and gluconeogenesis, may be secondary to changes in carbohydrate metabolism, particularly at the tricarboxylic acid cycle level (Gilfillan et al., 1956). It is at this level that the glycojenic amino acids are converted to glucose after deamination and transformation to smaller intermediate compounds. In this connection it is interesting to note that endotoxins (Berry et al., 1954; Dooley and Holtman, 1957) have been found capable of blocking the tricarboxylic acid cycle causing an accumulation of citrate in various tissues and organs.

SUMMARY

The treatment of chicks with endotoxin or cortisone was shown to increase the excretion of total nitrogen. These increases were evidenced in nonprotein nitrogen, urea, creatinine, and uric acid. Chicks treated with both cortisone and endotoxin presented alterations in the nitrogen excretion pattern which were additive in the case of nonprotein nitrogen and uric acid. The excretion of urea did not appear to be altered by treatment with cortisone and endotoxin whereas the level of creatinine was reduced. Hypoglycemia in animals treated with endotoxin was moderated by the administration of cortisone. Serum inorganic phosphorus and blood ammonia, normally increased in chicks treated with endotoxin, was not significantly altered or was reduced to near normal levels by cortisone treatment. The antipyretic effect of cortisone toward the action of endotoxin in chicks was demonstrated. Similarities between the effects of cortisone and endotoxin on nitrogen metabolism and excretion in chicks have been discussed.

REFERENCES

acid cycle to bacterial infection. IV. The effect of three metabolic inhibitors and Salmonella pullorum on the citric acid content of mouse tissues. J. Infectious Diseases, 95, 246–259.


