Concentrations of Serum Proteins during Aflatoxicosis

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Concentrations of Serum Proteins During Aflatoxicosis. TUNG, H. T., WYATT, R. D., THAXTON, P. AND HAMILTON, P. B. (1975). Toxicol. Appl. Pharmacol. 34, 320-326. The concentrations of different classes of serum proteins of chickens to graded doses of dietary aflatoxin (0, 0.625, 1.25, 2.5, 5.0, and 10.0 μg/g of diet) were measured using disc gel electrophoresis. Total serum proteins were reduced significantly (p < 0.05) by a dose of 1.25 μg/g or greater. The α-globulins and β-globulins were reduced at levels of 2.5 and 1.25 μg/g, respectively. The IgG component was reduced at 2.5 μg/g while the IgM component was not affected significantly at any level. The prealbumin fraction was reduced at 5 μg/g. The most sensitive component was serum albumin that was decreased significantly at the smallest level and was decreased to the greatest extent at the highest level. Its response curve roughly paralleled that of total serum lipids. Serum lipoproteins were decreased at 1.25 and 2.5 μg/g but not at lower or higher levels. These data can be explained by a hypothesis that aflatoxin or an active metabolite binds randomly to template deoxyribonucleic acid and inhibits the larger transcribing units such as those for serum albumin and lipid before the smaller transcribing units are inhibited.

The concentrations of serum proteins of animals during aflatoxicosis has both practical and theoretical implications. It was suggested by Datta and Gajan (1965) that a serum protein index might indicate quantitatively the intake of aflatoxin by ducklings. Brown and Abrams (1965) found that the concentrations of all classes of serum proteins of ducklings were decreased to about the same extent during aflatoxicosis and proposed that this apparently unique lack of specificity be used in the diagnosis of aflatoxicosis. The observation of lack of specificity was confirmed in ducklings (Nemeth and Juhasz, 1968) and in New Hampshire chickens (Brown, 1966). Alterations in the serum electrophoretic patterns of turkeys dosed with aflatoxin was reported by Magwood et al. (1966). However, there are contradictory reports. Carnaghan et al. (1967) reported no changes in the serum proteins of chickens receiving aflatoxin and similar negative findings of no alterations in the concentrations of serum proteins have been reported in swine (Keyl et al., 1968) and calves (Lynch et al., 1972) given aflatoxin.

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AFLATOXIN AND SERUM PROTEINS

The biochemical mechanism whereby aflatoxin exerts its effects in animals is not known with certainty, but it has been generally accepted that its primary effect is to inhibit ribonucleic acid (RNA) polymerase as a result of binding to deoxyribonucleic acid (DNA) (Clifford et al., 1967; Lafarge and Frayssinet, 1970; Pong and Wogan, 1970). More recently, evidence has been obtained that aflatoxin B1 itself is inactive but is metabolized by microsomes to a reactive compound that inhibits RNA polymerase directly (Moule and Frayssinet, 1972) and that reacts with both RNA and DNA to form covalent derivatives (Garner et al., 1972; Garner, 1973). At any rate, the mechanism of gene expression through transcription and translation suggests that alterations in RNA synthesis should be reflected by alterations in protein synthesis. Experimentation has revealed that incorporation of amino acids into the proteins of chick embryo liver cells (Terao and Miyaki, 1968) and rat liver slices (Clifford and Rees, 1967) was inhibited by aflatoxin. Apparently contrary to these findings and assumptions about the mechanism of action, Tung et al. (1972) reported that the lipid transport from the liver of the chicken is more sensitive to aflatoxin than is the RNA content of the liver. Also, it has been reported that aflatoxin does not inhibit liver protein synthesis in vivo under conditions which have dramatic effects on nucleic acid synthesis (Shank and Wogan, 1966; Pong and Wogan, 1969; Clifford and Rees, 1967).

The objective of the present research was to investigate the status of serum proteins in response to graded doses of dietary aflatoxin. Comparison of the serum proteins with the serum lipids should permit assessment of the relative importance of the inhibition of lipid metabolism and the inhibition of protein metabolism in the intact animal during aflatoxicosis.

METHODS

The chickens (Peterson x Arbor Acres) used in this experiment were obtained as 1-day old broiler males from a commercial hatchery (Raleigh Hatchery, Raleigh, N.C.). They were maintained in electrically heated batteries under continuous illumination, with feed and water available ad libitum. The feed was a commercial starter mash from which all medicants were omitted.

Aflatoxin was produced by growing Aspergillus parasiticus NRRL 2999 on rice according to the method of Shotwell et al. (1966) using the flasks described by Smith and Hamilton (1969). The moldy rice was steamed to kill the mold, dried, and ground to a fine powder that was analyzed spectrophotometrically for its total aflatoxin content by the method of Nabney and Nesbitt (1965) with the modification of Wiseman et al. (1967). The percentages of aflatoxin B1, B2, G1, and G2 (71, 8, 16, and 5%) were determined spectrophotometrically (Nabney and Nesbitt, 1965) after separation on thin-layer chromatograms (Pons et al., 1966).

Aflatoxicosis was induced by incorporating known amounts of aflatoxin into the diet and feeding for 3 weeks when the experiment was terminated. There were six treatments of dietary aflatoxin (0, 0.625, 1.25, 2.50, 5.00, and 10.0 μg/g of diet). There were four replicate groups of 10 birds per treatment.

Serum samples were collected from blood obtained by cardiac puncture. The sera were stored at −15°C until they were analyzed for their components. Total serum proteins were measured by the biuret method (Wooton, 1964). Total serum lipids were
determined by the method of Friedman (1968). Disc gel electrophoresis according to the method of Davis (1964) was used to separate the protein components. Identification of the serum protein components was made according to Morgan and Glick (1972). It should be mentioned that the lipoproteins were visualized by a protein reagent and not by a lipid reagent because it was already known that serum lipids are decreased by aflatoxin (Tung et al., 1972) and because we were interested in the protein component. The amounts of the various components were quantitated in a Digiscreen Scanner R with a Recorder 126 (Gelman Instrument Co., Ann Arbor, Mich. 48106).

The experimental design was completely randomized. The data were submitted to an analysis of variance in which an F ratio was calculated. If the ratio were significant, the least significant difference among the treatment means was calculated (Bruning and Kintz, 1968). Statements of significance were based on $p < 0.05$.

### RESULTS

The effects of graded levels of dietary aflatoxin on the serum components of 3-week old broiler chickens are given in Table 1. Dietary aflatoxin at concentrations of

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Effect of Graded Doses of Dietary Aflatoxin on Total Serum Protein, Globulin Fractions, Albumin, Prealbumin, Lipid and Lipoproteins in the Chicken</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serum parameters</th>
<th>Aflatoxin (µg/g)</th>
<th>0</th>
<th>0.625</th>
<th>1.25</th>
<th>2.5</th>
<th>5.0</th>
<th>10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total proteins</td>
<td></td>
<td>2.61*</td>
<td>2.42</td>
<td>2.08*</td>
<td>1.89*</td>
<td>1.58*</td>
<td>1.30*</td>
</tr>
<tr>
<td>(g/100 ml)</td>
<td>±0.08</td>
<td>±0.04</td>
<td>±0.04</td>
<td>±0.05</td>
<td>±0.07</td>
<td>±0.05</td>
<td></td>
</tr>
<tr>
<td>α-Globulins</td>
<td></td>
<td>140</td>
<td>134</td>
<td>142</td>
<td>94*</td>
<td>81*</td>
<td>83*</td>
</tr>
<tr>
<td>(mg/100 ml)</td>
<td>±8</td>
<td>±14</td>
<td>±24</td>
<td>±5</td>
<td>±6</td>
<td>±6</td>
<td></td>
</tr>
<tr>
<td>β-Globulins</td>
<td></td>
<td>522</td>
<td>472</td>
<td>405*</td>
<td>326*</td>
<td>251*</td>
<td>194*</td>
</tr>
<tr>
<td>(mg/100 ml)</td>
<td>±29</td>
<td>±42</td>
<td>±13</td>
<td>±8</td>
<td>±12</td>
<td>±12</td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td></td>
<td>412</td>
<td>442</td>
<td>374</td>
<td>349*</td>
<td>310*</td>
<td>198*</td>
</tr>
<tr>
<td>(mg/100 ml)</td>
<td>±16</td>
<td>±24</td>
<td>±28</td>
<td>±17</td>
<td>±20</td>
<td>±20</td>
<td></td>
</tr>
<tr>
<td>IgM</td>
<td></td>
<td>238</td>
<td>268</td>
<td>203</td>
<td>234</td>
<td>209</td>
<td>195</td>
</tr>
<tr>
<td>(mg/100 ml)</td>
<td>±22</td>
<td>±19</td>
<td>±24</td>
<td>±13</td>
<td>±20</td>
<td>±20</td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td></td>
<td>0.78</td>
<td>0.63*</td>
<td>0.57*</td>
<td>0.51*</td>
<td>0.41*</td>
<td>0.27*</td>
</tr>
<tr>
<td>(g/100 ml)</td>
<td>±0.02</td>
<td>±0.04</td>
<td>±0.04</td>
<td>±0.01</td>
<td>±0.03</td>
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</tr>
<tr>
<td>Prealbumin</td>
<td></td>
<td>83</td>
<td>78</td>
<td>81</td>
<td>71</td>
<td>68*</td>
<td>67*</td>
</tr>
<tr>
<td>(mg/100 ml)</td>
<td>±6</td>
<td>±7</td>
<td>±4</td>
<td>±1</td>
<td>±3</td>
<td>±2</td>
<td></td>
</tr>
<tr>
<td>Lipoproteins</td>
<td></td>
<td>140</td>
<td>120</td>
<td>106*</td>
<td>105*</td>
<td>139</td>
<td>126</td>
</tr>
<tr>
<td>(mg/100 ml)</td>
<td>±6</td>
<td>±6</td>
<td>±8</td>
<td>±7</td>
<td>±10</td>
<td>±3</td>
<td></td>
</tr>
<tr>
<td>Total lipids</td>
<td></td>
<td>923</td>
<td>800*</td>
<td>649*</td>
<td>581*</td>
<td>490*</td>
<td>547*</td>
</tr>
<tr>
<td>(mg/100 ml)</td>
<td>±15</td>
<td>±11</td>
<td>±9</td>
<td>±9</td>
<td>±11</td>
<td>±11</td>
<td></td>
</tr>
</tbody>
</table>

* Each experimental value is the mean ±SE of four groups of 10 birds.

b These values differ significantly ($p < 0.05$) from the corresponding control values.

1.25 µg/g and greater, significantly decreased the total protein concentration of the serum. At the highest level of aflatoxin administration (10 µg/g), the total protein was decreased to about one-half that of normal. In six separate experiments, the threshold
level of aflatoxin required to produce a statistically significant decrease in total serum protein was 1.25 µg/g, although the size of the reduction was somewhat variable.

Aflatoxin at a level of 2.5 µg/g was required to reduce significantly the concentration of α-globulins. Thus, α-globulin concentration was a less sensitive indicator of aflatoxicosis in chickens than was total serum protein which was reduced by a level of 1.25 µg/g. Also, the α-globulins were decreased to a smaller extent than the total serum protein.

The concentrations of β-globulins were reduced at 1.25 µg/g and above, the same levels that affected the total serum proteins. The β-globulins were decreased by about one-half at the higher levels similar to the reduction in total serum proteins by the same doses of aflatoxin.

The IgG component of γ-globulins was reduced significantly at 2.5 µg/g and above. On the other hand, the IgM component was not affected significantly by any level of aflatoxin.

The concentrations of serum albumin and prealbumin were altered and there was a differential effect on the two fractions. The prealbumin was reduced significantly at 5 and 10 µg/g only, while albumin was reduced by even the smallest level. Serum albumin was not only the most sensitive component but it was reduced the greatest extent at the higher levels. At 10 µg/g, it was only about one-third of normal.

The sensitivity of serum albumin to aflatoxin administration rivaled that of serum lipids. The total serum lipids were significantly reduced by the lowest level (0.625 µg/g) and at the highest level (10 µg/g) were about one-third of normal. Thus, there is an approximate identity between the response curves of serum lipids and serum albumins. There was an unexpected diphasic response of the lipoproteins that were reduced significantly at 1.25 and 2.5 µg/g but not at lower or higher levels.

**DISCUSSION**

The foregoing results demonstrate clearly that serum protein concentrations were decreased from normal values during aflatoxicosis in the chicken. The decrease was dependent on the dose of dietary aflatoxin and on the particular component being measured. Thus, our results do not agree entirely with those of other workers who observed a uniform decrease in all components nor the workers who found different responses with different components including increases as well as decreases. It is possible that the apparent difference among the reports of various workers can be reconciled by the use of different doses, different times, and different types of birds. At any rate it seems reasonable that a decrease in total serum proteins can be of diagnostic value in aflatoxicosis.

The differential effect of dietary aflatoxin on IgG and IgM implies a differential effect of aflatoxin on the progenitor cells of IgG and IgM which traditionally are believed to originate in the bursa of Fabricius (Morgan and Glick, 1972). It should be noted that aflatoxin causes a severe regression of the bursa of Fabricius in both chickens (Smith and Hamilton, 1970) and turkeys (Hamilton et al., 1972). This differential effect recalls the results of Glick et al. (1956) and Lerner et al. (1971) who reported that surgical bursectomy depressed IgG but not IgM values.
The differential effect of aflatoxin on the various serum protein components during the essentially steady-state conditions of these experiments implies a differential effect of aflatoxin on their synthesis. However, a mechanism calling for the general inhibition of RNA polymerase by aflatoxin does not allow for a differential effect on specific proteins. A similar situation exists with serum lipids and marrow lipids that are severely decreased by low doses of aflatoxin while growth is normal and the RNA content of the liver and marrow is unaltered or even increased (Tung et al., 1972; unpublished results). Apparently, a mechanism for a general inhibition of RNA polymerase by aflatoxin does not hold true in the intact, growing chicken.

The mechanism for inhibition of RNA polymerase can be modified to allow for the observed specificities exhibited by aflatoxin in chickens. This modification is based on the observations (Lafarge and Frayssinet, 1970; Roy, 1968; Pong and Wogan, 1969) that not all classes of RNA are affected similarly by the injection of aflatoxin into rats. Thus, long chain RNA (mRNA) is more severely affected by aflatoxin than is the short chain mRNA. The differential inhibition of RNA synthesis is common to many inhibitors that bind at random to the DNA template, and the amount of inhibition of specific RNA synthesis is directly related to its molecular size (Muramatsu et al., 1964) with the result that at lower doses of inhibitor larger transcribing units would be inactivated with a higher probability than smaller transcribing units (Sibatani, 1966).

To apply these considerations to aflatoxicosis, it needs only to be assumed that aflatoxin or its active metabolite (Moule and Frayssinet, 1972; Garner, 1973; Garner et al., 1972) is bound in a random fashion to DNA. Then the genes or operons which are the largest segments of DNA would be affected first. Therefore it need only be assumed that the mRNAs which code for serum albumin and lipid synthesis in chickens are longer than average in order to account for their sensitive and specific inhibition. This reasonable assumption has not been proved in animals, but in yeast the genetic information for the fatty acid synthesizing system occurs as a single gene cluster which is transcribed coordinately as a single gigantic chain of mRNA (Schweizer et al., 1971).

Another attraction of this modified hypothesis is that it permits a reduced rate of synthesis without complete blockage of the various components measured. Also, it accounts for the dose dependency of the multitudinous effects of aflatoxin in the chicken. Higher concentrations of aflatoxin would be expected to inhibit the formation of ever shorter chains of mRNA. This would occur until enough physiological systems are impaired to overwhelm the compensatory abilities of the birds and death would result.

ACKNOWLEDGMENT

We thank Sharon West for technical assistance.

REFERENCES


