تأثير بعض عوامل الإجهاد على كريات الدم البيضاء، جلوكوز الدم والهرمونات اللحائية الكثيرية في الكثافيات

د. سهير صالح، د. ياسين

الخلاصة

تناول البحث دراسة تأثير بعض عوامل الإجهاد على الهرمونات اللحائية الكثيرية كرديت الدم البيضاء، وخلط الدم في الكثافيات وكانت النتائج كالتالي:

1- تم تقدير الهرمونات اللحائية الكثيرية في دم الكثافيات عمر 0.58 يوماً بطريقة Competitive protein binding assay مختلطة باستخدام تنافس البروتين المصب.

2- ومقارنة الطرقين باستخدام التربيع المرمط لـ H-Corticosterone وامام الهرمونات اللحائية وجد ان الطرقية الأخرى تحتاج إلى مجهود أقل وواضحة.

3- نتائج الحالة الكثيرية تسبب زيادة معنوية في مستوى الهرمونات اللحائية كثرية وكذلك في عدد الخلايا البيضاء الأوزانية، وكاذبة ارتفاع التغييرات في مستوى الفلوبيز والعدد الكلي للكروبات البيضاء كان غير معنوي.

4- لوحظ أنه تحت تأثير درجة حرارة 34.2°م زاد مستوى الهرمونات اللحائية الكثيرية زيادات معنوية كما بينت نقص مستوى الفلوبيز في الدم وكذلك عدد الخلايا البيضاء الأوزانية الحقيقية منها والزائدة هذا وقد تلت الكثافيات بعد هجوم من التعرض لدرجة حرارة 34.2°م.

5- لوحظ ان حركات الكثافيات من الحالة وفَّاقة يسبب زيادة في مستوى الهرمونات اللحائية الكثيرية في الدم بعد 12 ساعة وكانت الفروق الأخرى في محتويات الدم غير معنوية.

6- أكد البحث أن الجهد العضلي المنفي يزيد من مستوى الهرمونات اللحائية الكثيرية في الدم بعد سامة من الجهد كما أزال دائماً الدم من الفلوبيز بعد ساعة، وكانت الزيادة معنوية في الحالة، ولم يغير المدك الكلي للكروبات البيضاء.
لا يمكن قراءة النص العربي من الصورة.
THE EFFECT OF SOME STRESS FACTORS ON BLOOD LEUCOCYTIC COUNT, GLUCOSE AND CORTICOIDS IN CHICKENS
(4 Tables and 3 Figures)

By

Sohair Y. Saleh* and W. Jaksch**

SUMMARY

The effect of some stress factors on blood corticoids, leucocytic count, and glucose in chickens has been investigated.

1.—Blood corticoids in chickens of 52 and 58 days of age have been determined by using two different methods depending on competitive protein binding assay (CPB).

2.—Comparison between these two methods CPB method using $^5$H—B corticosterone and the Cortipac CPB assay using Selenium labelled Cortisol, proved that the last method can be satisfactorily used.

3.—Injection of ACTH significantly increased the level of corticoids, eosinophils and pseudoeosinophils. Variations in total leucocytic and blood glucose level were not significant.

4.—Under heat stress (40°C) significant increase of blood corticoids and significant decrease in the blood glucose, eosinophils, and pseudoeosinophils was evident. Death occurred after two hours.

5.—Deprivation of food and water caused significant increase in the blood corticoids after 12 hours. Other variations in the blood parameters were not significant.

6.—Vigorous exercise significantly increased blood corticoids after one hour and blood sugar after two hours. Leucocytes showed no significant change.

INTRODUCTION

Poultry industry suffers great losses due to stress factors. In stress, blood corticoids are increased and the bird's resistance is decreased (GROOS (1972), and BUCKLAND, BLAGRAVE AND LAGUE (1974).

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During the last few decades a great deal of work has been focused on determination of plasma corticiodis as an index for stress. The use of different methods of investigation yielded variable results. (SILBER, BUSCH and OSLAPAS (1958), NEWCOMER (1957, 1958 & 1958 a, b), NAGRA, BAUM and MEYER (1960), URIST and DEUTSCH (1960), HENRY, CHARLES and MELVIN (1961), SIETGGEL and BEANE (1961), BREITENBACH (1962), SIEGEL and SIEGEL (1966), SEIGEL (1968), DUNN, ALVERSON, CARSON, ROGLER and BOHREN (1970), and FRANKEL (1970).

The aim of the present investigation is the study of some blood parameters during stress. Comparison of two methods for corticoid estimation is also attempted.

MATERIAL AND METHODS

One hundred and eighty female chickens, 52 days of age were divided into 6 groups. The birds were reared in wire cages. The ambient temperature ranged between 18-20°C and the relative humidity was about 75%, food and water were offered ad lib. Two experiments were carried out on two successive weeks.

In each experiment two groups of birds were treated and the third was kept as control. In the first week the experimental groups were treated as follows. One group was injected I.M. in the breast muscles with ACTH "SANABO, WIEN" (20 I.U. per Kg body weight dissolved in physiological saline solution), the second group was subjected to acute heat stress (40°C). In the second experiment, one group was also kept as control, the second group was deprived of food and water, and the third group was subjected to vigorous exercise.

Blood samples were collected from the wing vein after 1/2, 1, 2, 3, 6, & 12, hours from the start of the experiment. Heparin was used as anticoagulant. At the mentioned intervals, samples were collected from 5 chickens of each group simultaneously.

For counting the total number of leucocytes, fresh blood samples were collected and the method of NATTA & HERRIJK (1946) was adopted. Aceto-phen and pseudo-eosinophils were counted together by using the WISEMAN (1931) method. The heparinized samples were centrifuged in a cooled centrifuge (-4°C) at 4000 R.P.M. Part of the plasma was used for blood glucose estimations according to WERNER and WEILINGER (1970) and the rest was kept at -20°C for corticoids determination by two methods. These are the competitive protein binding (CPB), assay of corticoids in peripheral plasma using corticosterone labelled with 3H-B MURPHY (1967) as
modified by BUCKLAND et al. (1974), and by the Cortipac CPB assay** in which Selenium labeled cortisol was used. The last method is depending on the significant cross reaction between cortisol and corticosterone.

All data were subjected to unpaired "t" test or to correlation comparisons, SNEDECOR (1956).

RESULTS AND DISCUSSION

Tables I, II and Fig. I indicate the data of mean plasma corticosterone, since corticoids in chickens are mainly corticosterone, FRANKEL (1966). In the control group of 52 days old chikens the range of corticosterone was between 1.7 ± 0.5 and 2.3 ± 0.4 ug/100 ml and the corticoids between 2.3 ± 0.6 and 3.7 ± 0.9 ug/100 ml. In the control group of 58 days old chickens the range of corticosterone was from 1.0 ± 0.5 to 1.9 ± 0.7 and corticoids ranged 1.6 ± 0.7 to 2.8 ± 0.9. These results are in complete agreement with the findings of BROWN (1968), DUNN et al. (1970), and SIEGEL et al. (1972). Higher values of corticoids were reported by NEWCOMER (1959 a & b), URIST et al. (1960) and BREITENBACH (1962). Lower values of corticoids however, were reported by BUCKLAND et al. (1974). It was also evident from table I, II and Fig. I, that all stressors significantly increased the corticoids level. In the group injected with ACTH corticoids were significantly increased for more than five times after 1/2 an hour and gradually decreased after one hour to attain their normal level after two hours. The above results are in accord with the findings of NEWCOMER's (1959), NAGRA et al. (1960), URIST et al. (1960) EREITENBACH (1962), SIEGET et al. (1960), FRANKEL (1970), BUCKLAND et al. (1974). During acute heat stress corticoids were significantly increased after half an hour and remained steady for 2 hours after which the birds were dead. Similar finding were reported by CHARLES et al. (1961), DUNN et al. (1970), BUCKLAND et al. (1974), EDEND and SIEGEL (1974), CALHOUN and HUSTON (1974), in the group subjected to starvation the level of blood corticoid was significantly increased after 12 hours. Similar results were reported by CHARLES et al. (1961), and BUCKLAND et al. (1974). In the group subjected to vigorous exercise there was a significant increase in the level of blood corticoids after one hour then returned gradually to their normal level within 3 hours.

Of particular interest is the fact that determination of corticoid in chicken blood by Cortipac CPB assay is very satisfactory. Comparison of results obtained by the former method and those obtained by the use of competitive protein binding assay in which 3H-B corticosterone was used revealed that the correlation coefficient is more than 0.90. The first method is simple and time saving while the second is elaborate and time consuming.

In... of the variation of blood glucose level during fasting and after injection of ACTH, such variations are statistically not significant under the conditions of the experiment. These results are rather contradictory to the

Cortipac kit for cortisol CPB assay "Radiochemical center Amersham".
findings of BELL (1961)’ SIEGLE of BEANE (1961), FREEMAN, CHUBB & PEARSON (1966) LANGSLOW, BULTER, HALE & PEARSON (1970). However, such results are in complete agreement with these of URIST et al. (1960), who reported that there is no effect on the blood sugar. A significant increase in blood sugar level during vigorous exercise and decrease during heat stress were evident (tables III & IV, Fig II & III).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameter</th>
<th>Sample</th>
<th>Collection</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30 m.</td>
<td>one h.</td>
<td>2 h.</td>
</tr>
<tr>
<td>Control group</td>
<td>Corticosterone ug/100 ml</td>
<td>1.9 ± 0.9</td>
<td>2.1 ± 0.7</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>(30 chickens)</td>
<td>Corticoids in ug/100 ml</td>
<td>2.8 ± 1.1</td>
<td>2.7 ± 0.6</td>
<td>3.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>“Cortipac kit”</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACTH group</td>
<td>Corticosterone ug/100 ml</td>
<td>19.6 ± 4.0</td>
<td>6.5 ± 0.6</td>
<td>2.8 ± 0.4</td>
</tr>
<tr>
<td>(30 chickens)</td>
<td>Corticoids ug/100 ml</td>
<td>21.5 ± 5.4</td>
<td>8.0 ± 1.0</td>
<td>4.1 ± 0.7</td>
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<tr>
<td></td>
<td>“Cortipac kit”</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Heat stress</td>
<td>Corticosterone ug/100 ml</td>
<td>14.6 ± 2.0</td>
<td>13.7 ± 4.5</td>
<td>16.4 ± 2.7</td>
</tr>
<tr>
<td>group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(30 chickens)</td>
<td>Corticoids ug/100 ml</td>
<td>15.9 ± 2.1</td>
<td>15.0 ± 2.9</td>
<td>18.2 ± 2.1</td>
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<tr>
<td></td>
<td>“Cortipac kit”</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Values are means ± standard error
** significantly different from the control group at p < 0.01.

There is a significant rise in the eosinophil and pseudoeosinophils count in the group injected with ACTH after 3 hours (table III). These results are in complete agreement with those obtained by NEWCOMER (1959), JOHN (1962). SIEGEL (1968), SCHUKRO (1974). A decrease in eosinophil and pseudoeosinophil count was observed under the heat stress (table III).

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<table>
<thead>
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<th>Groups</th>
<th>Parameter</th>
<th>Sample</th>
<th>Collection</th>
<th>after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30 m</td>
<td>one h.</td>
<td>2 h.</td>
</tr>
<tr>
<td>Control group (30 chickens)</td>
<td>Corticosterone ug/100ml</td>
<td>1.5</td>
<td>1.4</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.4</td>
<td>±0.5</td>
<td>±0.7</td>
</tr>
<tr>
<td></td>
<td>Corticoids in ug/100 ml Cortipac kit**</td>
<td>2.4</td>
<td>2.5</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.5</td>
<td>±0.9</td>
<td>±0.9</td>
</tr>
<tr>
<td>Fasting group (30 Chikens)</td>
<td>Corticosterone ug/100ml</td>
<td>2.0</td>
<td>2.0</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.7</td>
<td>±0.7</td>
<td>±0.8</td>
</tr>
<tr>
<td></td>
<td>Corticoids in ug/100 ml</td>
<td>—</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.9</td>
<td>±1.5</td>
<td>±1.9</td>
</tr>
<tr>
<td>V. exercise group (30 chickens)</td>
<td>Corticosterone ug/100 ml</td>
<td>1.8</td>
<td>10.0**</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.6</td>
<td>±2.4</td>
<td>±2.9</td>
</tr>
<tr>
<td></td>
<td>Corticoids in ug/100 ml</td>
<td>2.5</td>
<td>12.2**</td>
<td>8.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±1.5</td>
<td>±2.2</td>
<td>±3.4</td>
</tr>
</tbody>
</table>

Values are means ± standard error

** significantly different from the control at $P < 0.01$

Considering the total leucocytic count there is no significant variation under all stressors used. This may be explained on basis of individual variations (tables III and IV).

**ACKNOWLEDGMENT.**

I am greatly indebted to Prof. Dr. J. LEIBETSEDER for helpful criticism and advice and to Mrs. M. SKALICKY for her assistance.

The help of Dr. Desser in Krebsforschungs Institut WIEN, is greatly acknowledged.
Fig. 1.—Changes in plasma corticoids concentration under the effect of different stress factors.

Fig. 2. Changes in plasma glucose level under the effect of some stress factors.
Fig. 3.—Changes in plasma glucose level under the effect of some stress factors.

### TABLE 3. Blood parameters in the first experiment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose mg/100 ml of plasma</th>
<th>Count of Eosinophils per mm³</th>
<th>Count of total leucocytes per mm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>260 ± 13</td>
<td>4750 ± 29</td>
<td>19120 ± 5982</td>
</tr>
<tr>
<td>Group II</td>
<td>264 ± 12</td>
<td>4310 ± 39</td>
<td>15575 ± 2436</td>
</tr>
<tr>
<td>Group III</td>
<td>277 ± 26</td>
<td>397 ± 10</td>
<td>15302 ± 2436</td>
</tr>
</tbody>
</table>

**Note:** Values are means ± standard error. *Significantly different from control at \( P < 0.05 \), **significantly different from control at \( P < 0.01 \). N (number of chickens in each group) = 30.
<table>
<thead>
<tr>
<th>Blood parameters</th>
<th>Groups</th>
<th>30'</th>
<th>one hour</th>
<th>2 hours</th>
<th>3 hours</th>
<th>6 hours</th>
<th>12 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose</td>
<td>Group I</td>
<td>258 ± 10</td>
<td>241 ± 14</td>
<td>250 ± 7</td>
<td>240 ± 25</td>
<td>264 ± 33</td>
<td>237 ± 16</td>
</tr>
<tr>
<td>mg/100 ml of plasma</td>
<td>Group II</td>
<td>236 ± 16</td>
<td>227 ± 24</td>
<td>217 ± 24*</td>
<td>218 ± 13</td>
<td>221 ± 15</td>
<td>199 ± 6</td>
</tr>
<tr>
<td></td>
<td>Group III</td>
<td>233 ± 13</td>
<td>243 ± 26</td>
<td>331 ± 24*</td>
<td>284 ± 17</td>
<td>216 ± 5</td>
<td>238 ± 18</td>
</tr>
</tbody>
</table>
| Count of Fos-    | Group I      | ± 6990     | ± 6625   | ± 6990    | ± 7020     | ± 7820     | ± 7813     | ± 357
| + Pseudosino     | Group II     | ± 4210     | ± 1286   | ± 1987    | ± 1622     | ± 1977     | ± 5470     | ± 2227
| phils per cmm    | Group III    | ± 4210     | ± 1542   | ± 4110    | ± 3700     | ± 4910     | ± 3753     | ± 3753
| Count of total   | Group I      | ± 23680    | ± 22020  | ± 23810   | ± 21250    | ± 31250    | ± 24325    | ± 2145
| Leucocytes per cmm| Group II     | ± 18310    | ± 19800  | ± 19813   | ± 3488     | ± 4393     | ± 2145     | ± 20330
|                  | Group III    | ± 1876     | ± 3242   | ± 4542    | ± 18825    | ± 21325    | ± 8228     | ± 19683
|                  |              | ± 16110    | ± 20770  | ± 25480   | ± 23800    | ± 28920    | ± 5684     | ± 2822

Values are means ± standard error.
* Significantly different from the control at $P < 0.01$
Number of chickens in each group = 30
Group I (control), Group II (fasting and Group III vigorous exercise).
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or Medicinae Veterinariae der Tierarztlchen Hochschule in Wien.


