The effect of 3-acetylpyridine on the explanted chick embryo

By B. J. BUCKINGHAM & HEINZ HERRMANN

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A structural analogue of nicotinic acid, 3-acetylpyridine, has been shown to produce morphological and physiological abnormalities in a variety of organisms. The effect of 3-acetylpyridine (AP) on chick embryos has been studied by several investigators using the technique of yolk-sac injection (Ackermann & Taylor, 1948; Zwilling & DeBell, 1950; Landauer, 1957; Herrmann, Clark & Landauer, 1963). Following AP treatment at 96 h of incubation, a reduction in body size, underdevelopment of leg musculature and edema were noted. Teratogenic effects of AP when administered after 24 h of incubation were much more diffuse and included instances of cerebral hernia and muscular hypoplasia (Landauer, 1957). Simultaneous injection of nicotinamide (Ackermann & Taylor, 1948; Landauer, 1957) decreased the incidence and severity of these conditions.

Landauer (1957) noted the similarity of abnormalities found in AP-treated chick embryos to those of the crooked-neck dwarf mutant described by Asmundson (1945). Herrmann et al. (1963) found that different dose levels of AP reduced the wet weight of the leg musculature in such a way as to mimic the two phases of weight loss in the leg musculature of the crooked-neck dwarf mutant embryo.

These observations suggested the need for a more detailed study of the AP-treated chick embryo. In particular, it appeared that studies of protein synthesis in these embryos would be of importance, since the most noticeable defect appeared to be a reduction in muscle growth.

The use of a technique in which analogue administration and the stage of development of the embryos at the time of AP treatment could be closely controlled was desirable. Thus, the technique of explantation of chick embryos as described by Spratt (1947, 1948) was chosen.

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Fertile White Leghorn eggs, obtained from a commercial source, were incubated in a forced-draft incubator at 37.5 °C for approximately 40 h. The embryos were excised from the yolk, using the large trim previously described (Britt & Herrmann, 1959), and those embryos with 11–13 somites selected for explantation. After several washings in chick Ringer solution, to remove adhering yolk, the embryos were explanted as described previously by Hayashi & Herrmann (1959) on to 1 ml of a medium composed of one part of whole egg homogenate and one part of 1.5% agar in chick Ringer solution, with or without added 3-acetylpyridine (Sigma Chemical Co.) and/or nicotinamide. The final concentration of acetylpyridine was 100 μg/ml unless otherwise noted. For the studies of protein synthesis, glycine-l-14C (Nuclear-Chicago, 3.75 mc/mM) was added to the culture medium at a final concentration of 1 μc/ml of medium.

After explantation, the embryos were incubated at 37.5 °C for 24 h, then removed from the medium, washed and transferred to a rubber stopper for dissection. The extra-embryonic membranes and material lateral to the somites were removed as described by Hayashi & Herrmann (1959), and discarded. In most cases the embryos were then separated into two parts, one consisting of brain, optic and otic vesicles and spinal cord (nervous system), and one composed of the remaining trunk primordia. Dissection of embryos into the nervous system and trunk components was carried out under 50% ethanol. The use of ethanol increased the opacity of the tissue and permitted a more precise dissection and greater recovery of tissue for analysis.

Single whole embryos or three pooled nervous system or trunk components were used for chemical analysis. The samples were extracted two times with cold 5% trichloroacetic acid to remove acid-soluble materials. Lipids were removed by chloroform-ethanol 1:3 (v/v) at 70 °C for 15 min. The samples were hydrolysed at 140 °C overnight in 6 N-HCl in sealed tubes. The hydrolysed samples were dried in vacuo, and the residue was dissolved in 0.5 ml of distilled water. Aliquots were plated at infinite thinness on aluminium planchets for counting. The samples were counted in a gas flow counter with a micromil window (Nuclear-Chicago D-47). An aliquot of the hydrolysate was digested overnight with 0.22 ml of 8.2 M-H2SO4 containing 0.2 mg of selenium at 270 °C and this material used for the determination of protein nitrogen by nesslerization.

RESULTS

When chick embryos had been maintained for 24 h on media containing acetylpyridine at various concentrations, the protein nitrogen (PN) content of the embryos was lower than that of control embryos maintained in the absence of AP, as shown in Table 1. A final AP concentration of 30 μg/ml reduced the PN content slightly, but at a level of 100 μg AP per ml the PN of the treated
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Embryos was only 45% of the control value. To determine if this effect could be prevented by simultaneous administration of nicotinamide, the embryos were explanted on to media containing both nicotinamide and AP. Nicotinamide alone reduced the protein content of the embryos, indicating some toxicity of nicotinamide at the levels used. However, when embryos were exposed to both nicotinamide and AP, the protein nitrogen values were higher than those obtained after treatment with AP alone. This indicated that nicotinamide was partially able to prevent the toxic effect of acetylpyridine in the explanted chick embryo.

Table 1. Effect of acetylpyridine and nicotinamide on protein nitrogen content of whole embryos

<table>
<thead>
<tr>
<th>Acetylpyridine (µg/ml)</th>
<th>Nicotinamide (µg/ml)</th>
<th>Protein nitrogen content (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>—</td>
<td>—</td>
<td>20.51 ± 0.76 (26)</td>
</tr>
<tr>
<td>—</td>
<td>300</td>
<td>16.80 ± 0.87 (5)</td>
</tr>
<tr>
<td>—</td>
<td>500</td>
<td>13.84 ± 0.27 (4)</td>
</tr>
<tr>
<td>30</td>
<td>—</td>
<td>18.78 ± 0.84 (9)</td>
</tr>
<tr>
<td>100</td>
<td>—</td>
<td>11.26 ± 0.53 (11)</td>
</tr>
<tr>
<td>100</td>
<td>300</td>
<td>17.17 ± 1.04 (7)</td>
</tr>
<tr>
<td>100</td>
<td>500</td>
<td>14.94 ± 0.02 (7)</td>
</tr>
</tbody>
</table>

Table 2. Protein nitrogen (PN) content of components of embryos after 24 h in vitro

<table>
<thead>
<tr>
<th>Component*</th>
<th>µg PN/component</th>
<th>Accumulation of PN/component †</th>
<th>Accumulation of PN (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CN</td>
<td>7.6 ± 0.6 (16)†</td>
<td>5.0 ± 0.6</td>
<td>100</td>
</tr>
<tr>
<td>APN</td>
<td>4.0 ± 0.2 (10)</td>
<td>1.3 ± 0.2</td>
<td>26</td>
</tr>
<tr>
<td>CT</td>
<td>7.7 ± 0.7 (15)</td>
<td>4.8 ± 0.7</td>
<td>100</td>
</tr>
<tr>
<td>APT</td>
<td>5.6 ± 0.5 (10)</td>
<td>2.7 ± 0.5</td>
<td>56</td>
</tr>
</tbody>
</table>

* Abbreviations are as follows: CN = control central nervous system (N) component; APN = acetylpyridine-treated N component; CT = control trunk (T) component; APT = acetylpyridine-treated T component.
† Accumulation of PN represents the difference between PN content after 24 h in vitro and that at the time of explantation. PN at time of explantation: N = 2.7 µg; T = 2.9 µg.
‡ Figures in parentheses represent the number of determinations on pooled samples of three components.

The differences in PN accumulation between control and acetylpyridine-treated components and between APN and APT are highly significant by the t test (P < 0.001).

Gross morphological observations of AP-treated chick embryos suggested that the size of the central nervous system was reduced to a greater extent than the size of the whole embryo. No other distinct abnormalities were noted. Therefore, comparative studies of the protein nitrogen content of the central nervous system and of the remaining embryonic tissues were initiated. In this series of experiments the embryos, after 24 h of maintenance in vitro in the
presence or absence of AP, were dissected into two parts, as explained under Materials and Methods.

The protein nitrogen contents of the central nervous system and trunk components are shown in Table 2. It can be seen that protein nitrogen in both acetylpyridine-treated fractions is less than that in the corresponding controls. However, the central nervous system was more severely affected than the remainder of the embryo, since these parts had, respectively, 52 % and 72 % of their normal counterparts. When these samples are compared in terms of the

<table>
<thead>
<tr>
<th>Component</th>
<th>$\mu g \text{PN/ component}$</th>
<th>C.p.m.</th>
<th>C.p.m./$\mu g \text{PN/ component}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CN</td>
<td>$2.9 \pm 0.4 (5) \dagger$</td>
<td>$312 \pm 14$</td>
<td>116</td>
</tr>
<tr>
<td>APN</td>
<td>$3.1 \pm 0.1 (5)$</td>
<td>$260 \pm 23$</td>
<td>83</td>
</tr>
<tr>
<td>CT</td>
<td>$3.1 \pm 0.3 (4)$</td>
<td>$256 \pm 15$</td>
<td>127</td>
</tr>
<tr>
<td>APT</td>
<td>$2.8 \pm 0.4 (5)$</td>
<td>$309 \pm 19$</td>
<td>119</td>
</tr>
</tbody>
</table>

* Abbreviations are given in Table 2.
† Figures in parentheses represent the number of determinations on pooled samples of three components.

Table 3. Protein nitrogen content and incorporation of glycine-1-$^{14}$C into components of chick embryos after 8 h in vitro

<table>
<thead>
<tr>
<th>Component</th>
<th>$\mu g \text{PN/ component}$</th>
<th>C.p.m./component</th>
<th>C.p.m./component</th>
</tr>
</thead>
<tbody>
<tr>
<td>CN</td>
<td>$6.5 \pm 0.5 (10) \dagger$</td>
<td>$1276 \pm 19$</td>
<td>100</td>
</tr>
<tr>
<td>APN</td>
<td>$3.7 \pm 0.3 (10)$</td>
<td>$622 \pm 41$</td>
<td>49</td>
</tr>
<tr>
<td>CT</td>
<td>$5.1 \pm 0.6 (10)$</td>
<td>$1102 \pm 75$</td>
<td>100</td>
</tr>
<tr>
<td>APT</td>
<td>$4.5 \pm 0.5 (9)$</td>
<td>$844 \pm 53$</td>
<td>76</td>
</tr>
</tbody>
</table>

* Abbreviations are given in Table 2.
† Figures in parentheses represent the number of determinations on pooled samples of three components.

The differences in total activity between control and experimental samples are highly significant by the $t$ test ($P < 0.001$).

accumulation of protein nitrogen during the 24 h period of explantation (i.e. protein nitrogen content after 24 h in vitro minus PN content at time of explantation) it is seen that the accumulation of protein nitrogen is reduced by 74 % in the nervous system and by only 44 % in the trunk. These data clearly indicate some specificity of acetylpyridine effects for the central nervous system.

Lower protein values could result from a lower rate of protein synthesis, an increased rate of protein degradation, or both. To investigate the first possibility, studies of the rate of protein synthesis were undertaken, using the incorporation of glycine-1-$^{14}$C as an index.
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Table 3 shows that after 8 h of explantation on to a medium containing AP and glycine-\(^{14}\)C there was essentially no effect of the AP on the protein nitrogen content, nor on the incorporation of labeled glycine into protein. After 24 h of explantation the effect of acetylpyridine becomes apparent in both the PN content and the incorporation of glycine-\(^{14}\)C into protein of the components, as shown in Table 4. The accumulation of isotopically labeled protein was decreased in both of the AP-treated samples, but to a greater extent in the central nervous system than in the trunk fraction, as shown by a difference of 27% in their total activities (counts per minute per component).

If glycine-\(^{14}\)C incorporation into protein is a valid measure of the amount of protein synthesized, decreased protein synthesis would be sufficient to account for about two-thirds and one-half the reduction in protein accumulation (Table 2) in the AP-treated central nervous system and trunk fractions respectively.

DISCUSSION

The observation that acetylpyridine treatment reduces the protein nitrogen content of the whole embryo is in accord with observations of several investigators (Ackermann & Taylor, 1948; Zwilling & DeBell, 1950; Landauer, 1957; Herrmann et al. 1963) that AP-treated chicks have a reduced body size. The prevention of the effect of acetylpyridine by simultaneous administration of nicotinamide also confirms observations of others (Ackermann & Taylor, 1948; Landauer, 1957). In this study, sufficient nicotinamide could not be administered to completely prevent the effects of AP at the dose used, since nicotinamide was also somewhat toxic to the explanted chick embryo. However, the partial preventative action of nicotinamide indicated that the acetylpyridine was acting as a nicotinamide antagonist.

The lower accumulation of protein in both the nervous system and trunk components is due, at least in part, to lower rates of protein synthesis, since incorporation of glycine-\(^{14}\)C was inhibited in both components. Low rates of protein synthesis could result from an insufficient supply of energy for protein synthesis in the AP-treated chick embryo. Such a mechanism would appear reasonable since acetylpyridine can be incorporated into the pyridine nucleotides in place of nicotinamide, forming the acetylpyridine analogues of DNP and TNP (Kaplan & Ciotti, 1956). These analogues react more slowly with a variety of dehydrogenases than do DNP and TNP (Kaplan, Ciotti & Stolzenbach, 1956). Thus, formation of the acetylpyridine analogues of the pyridine nucleotides may limit the rate of phosphorylation reactions leading to the synthesis of adenosine triphosphate, the source of energy for protein synthesis.

If acetylpyridine does, in fact, prevent the synthesis of normal pyridine nucleotides, the effect may be particularly severe in the early chick embryo, where both nicotinic acid (Snell & Quarles, 1941) and DNP (Levy & Young, 1948) are present at very low levels.
The greater specificity of the AP effect for the nervous system may be related to similar observations by other investigators. Landauer (1957) found that chick embryos treated with AP by yolk-sac injection after 24 h of incubation showed a scattered incidence of cerebral hernia. Hicks (1955) found that AP-treated rats and mice developed necrotic lesions in the nervous system. Sternberg & Philips (1958) also observed that a variety of mammals treated with AP developed lesions in the nervous system.

The observed specificity of the effect of AP treatment may be the result of any of the factors discussed by Hicks (1955). However, the possibility of differential detoxication of AP in various tissues is of particular interest, since Kaplan and co-workers (Kaplan et al. 1954) found that AP is converted to the AP analogue of DNP, but not to nicotinamide, in mouse brain, whereas mouse liver detoxifies AP by conversion of the analogue to nicotinamide.

The contribution of protein degradation to lowering the protein accumulation was not determined in this study. However, this may be a significant factor since protein degradation influences the magnitude of protein accumulation in chick embryo explants maintained under suboptimal conditions of growth (Herrmann & Marchok, 1963).

**SUMMARY**

1. Exposure of 11–13 somite chick embryos to 3-acetylpyridine for 24 h in vitro inhibited their growth, as measured by the accumulation of protein nitrogen in the embryos. Simultaneous administration of nicotinamide partially prevented this inhibitory effect. Measurements of the protein nitrogen content of fractions of the acetylpyridine-treated embryos indicated that the growth of the central nervous system was inhibited to a greater extent than that of other embryonic trunk primordia.

2. Protein synthesis, as measured by the incorporation of glycine-14C into protein, was inhibited in the acetylpyridine-treated chick embryos. The incorporation of glycine-14C was reduced to a greater extent in the central nervous system than in the remainder of the embryo.

3. It was concluded that much of the inhibition of protein nitrogen accumulation in both the central nervous system and the lateral mesoderm fractions of acetylpyridine-treated chick embryos could be accounted for by lower rates of protein synthesis. Some contribution from protein degradation by proteolytic enzymes or from cell loss could not be eliminated.

**RÉSUMÉ**

*L’influence de la 3-acetylpyridine sur l’embryon de poulet cultivé* in vitro

1. La croissance, mesurée par accumulation d’azote protéique, d’embryons de poulet explantés au stade de 11 à 13 somites, est inhibée par une incubation de 24 h en présence de 3-acétylpyridine. La nicotinamide, administrée simul-
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Hané, contrecarré, dans une certaine mesure, cet effet inhibiteur. La croissance du système nerveux central est plus fortement inhibée que celle des autres régions, ainsi que le démontrent des mesures effectuées sur des fragments appropriés.

2. Une autre mesure de la synthèse protéique consistait en une étude de l'incorporation de la glycine-14C chez des embryons de poulet traités à l'acétylpyridine: un effet inhibiteur a été observé. Cet effet est plus marqué dans le système nerveux central que dans les autres régions de l'embryon.

3. En conclusion, il semble que l'inhibition de l'accumulation de l'azote protéique dans le système nerveux central et dans le mésoderme latéral d'embryons traités par l'acétylpyridine serait due à une diminution de l'intensité de la synthèse protéique. On ne peut exclure, cependant, qu'une partie de l'inhibition observée soit due à l'activité d'enzymes protéolytiques ou à des pertes cellulaires.

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REFERENCES


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