PULMONARY VASCULAR LESIONS IN CHICKENS FOLLOWING INTRAVENOUS INJECTIONS OF DISINTEGRATED CELLS OF *ESCHERICHIA COLI*

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Intravenous injections of disintegrated cells of *Escherichia coli* in chickens were almost constantly followed by extensive lesions in pulmonary arteries; the alterations consisted of mural fibrinoid necrosis, sometimes with slight intramural occurrence of mononuclear inflammatory cells and eosinophils. Massive perivascular accumulations of the same cell types were also very common findings. Affected arteries were frequently occluded by precipitates, predominantly consisting of the injected material, which were rapidly replaced by proliferating endothelial cells, resulting in obliteratorive lesions, where giant cells sometimes occurred. The conclusion was drawn that the arterial lesions could most adequately be categorized as hypersensitivity angiitis.

Key words: Vascular lesions, pulmonary; *Escherichia coli* cells; intravenous injections.

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In a preceding report, arterial renal lesions in various mammalian species, induced by systemic injections of disintegrated cells of *Escherichia coli*, were described (13). The vascular lesions were found to be of the same type as those occurring in various organs in association with hypersensitivity to drugs and serum (4, 8, 11, 15, 16, 21); hence, they were interpreted as a form of hypersensitivity angiitis, according to the description of Zeek et al. (25–27). It was concluded that the pathogenetic mechanism was related to the Shwartzman phenomenon. Some animal species in the previous experiment did not develop renal arterial lesions. Among these latter animals, however, some individuals in all species tested exhibited pulmonary mural arterial modifications, sometimes also arterial and/or venous thrombosis; the pulmonary lesions were most pronounced in dogs (14). As mammals thus show some variation in the morphological response to systemic administration of crushed cells of *Escherichia coli*, it was decided to perform a corresponding study in an avian species.
 MATERIAL AND METHODS

The strain of haemolytic Escherichia coli type 0 141 a b (NVH 2653) was originally isolated from pig intestine. The bacterial suspension was prepared by the same procedure as described previously (22) and frozen until used; the suspension contained 0.0154 g dry matter per ml. A total of 48 female chickens (Gallus domesticus) of the White leghorn breed, about 4 weeks old and weighing 250-350 grams, were used as experimental animals. The animals received one or two injections, spaced 24 hours apart, given intravenously through the wing vein. Surviving animals were killed at different times after challenge (few min. - 8 days) by cervical fracture and bled by cutting the neck. Doses and times of killing, or death, of animals used as illustrative material are shown in Table 1. Two untreated chickens and 4 animals injected with isotonic saline, were used as controls.

All animals were autopsied and pieces of organs were fixed in a 10 per cent formaldehyde solution for at least 3 days, and/or in Zenker's and Carnoy's fixatives, embedded in paraffin and sectioned at about 5 μ. Sections were stained with haematoxylin and eosin (H&E), methyl green-pyronin, Wilder's silver stain, elastin van Gieson (e1.v.G.), Di-PAS, phosphotungstic acid haematoxylin (PTAH), the acid picro-Mallory method of Lendrum and with the Martius scarlet blue (MSB) method (10). Formalin fixed frozen sections from the lungs were stained with Oil red O and Sudan III.

After centrifugation of the bacterial suspension the sediment was fixed in buffered 3 per cent glutaraldehyde and postfixed in 2 per cent osmic acid. Initial fixation for the electron microscopic study of the lungs of two animals was performed by injecting glutaraldehyde into the trachea and abdominal cavity. Lung pieces were thereafter fixed by the same procedure as the sediment and ultrathin sections from the latter material and the lungs were stained with lead citrate and examined in a Siemens Elmiscop I A.

RESULTS

Most chickens exhibited rapid respiration immediately after challenge. Some animals went into shock and died within a few minutes. Signs of the acute shock included severe dyspnoea with gasping, ruffling of feathers, stretching of the neck, muscular weakness and convulsions.

Macroscopic Lesions

In most animals, the lungs appeared congested and oedematous, whereas all other organs were unchanged.

Light- and Electron Microscopic Investigations

1. Control animals and injected material. Lung sections from the controls revealed evident vacuolization of endothelial cells and distinct external and internal elastic membranes (Fig. 1). Electron microscopic examination of the bacterial material showed that the bulk consisted of easily recognizable bacterial remnants mixed with a homogenous or slightly granular substance.

2. Pulmonary arteries. In animals dying

<table>
<thead>
<tr>
<th>Chicken No.</th>
<th>Doses, ml</th>
<th>Killed (K) or died (D), time after the second inject.</th>
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<tbody>
<tr>
<td>3</td>
<td>1.0</td>
<td>K 18 hrs.</td>
</tr>
<tr>
<td>11</td>
<td>0.5</td>
<td>D 12 hrs.</td>
</tr>
<tr>
<td>16*</td>
<td>1.0</td>
<td>K 3 days</td>
</tr>
<tr>
<td>19§</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>0.8</td>
<td>K 24 hrs.</td>
</tr>
<tr>
<td>23</td>
<td>0.8</td>
<td>K 24 hrs.</td>
</tr>
<tr>
<td>29</td>
<td>1.0</td>
<td>K 2 days</td>
</tr>
<tr>
<td>33</td>
<td>0.8</td>
<td>K 8 days</td>
</tr>
<tr>
<td>41§</td>
<td>1.5</td>
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</tbody>
</table>

* This animal received only one injection.
§ These animals died in shock in few minutes after one injection.
shortly after the injections, several distended intrapulmonary arteries occluded by masses, which were frequently laminated, were found; these masses consisted of a eosinophilic, homogenous material, mixed with minor irregular foci of erythrocytic aggregations (Fig. 2). The precipitated homogenous material was strongly PAS positive, but was not positively stained by the fibrin staining methods. These lesions were found independently of whether the animals had been given one or two injections. The endothelium was flattened and greatly stretched, but obvious light microscopic endothelial injury was not ascertained at this stage of involvement. Electron microscopic examination showed that the precipitated occlusive material in the

Fig. 3. Ultrastructure of an arterial occlusion in immediate contact with the endothelium which is in an early stage of disintegration. Chicken No. 41; e = endothelial nucleus. × 39000.
Fig. 4. Incipient proliferation of vacuolated endothelial cells in a pulmonary artery; the lumen is occluded by aggregated erythrocytes. Chicken No. 21. H & E, × 425.

Fig. 5. Mural necrosis and occlusive thrombosis in a pulmonary artery; some erythrocytes can be identified within the fibrin-stained material. Chicken No. 3. PTAH, × 250.

Fig. 6. Mural necrosis in a pulmonary artery. The lumen is in large parts occluded by a hyaline material or an indefinite debris. The external elastic membrane is not obviously damaged, whereas the medial layer and elastica interna are destroyed in the lower part of the figure. Chicken No. 11; el.v. G, × 425.

Fig. 7. Mural fibrinoid necrosis in a pulmonary artery. The lumen is partly occluded by a hyaline material which is stained as fibrin and has coalesced with the necrotic wall. Chicken No. 3. Acid picromallory method, × 680.
arteries had an appearance which was very similar to that of the embedded bacterial sediment. There seemed, however, at times to be some quantitative dissimilarities as the bacterial remnants, although always abundantly present, sometimes did not constitute as great a part of the deposits as in the injected material, while the homogenous or granular components were correspondingly increased. The precipitates were in intimate contact with the endothelium which was variably damaged (Fig. 3).

The occlusive masses were gradually invaded by proliferating cells, probably of endothelial origin, containing PAS-positive granules. In somewhat later developmental stages, remnants of precipitated masses, or fibrin-stained thrombi, which frequently coalesced with the necrotic vascular walls, were found. Early mural lesions were, as a rule, found in the endothelial layer and consisted of oedema, loss of endothelial nuclei and endothelial proliferative changes, with relatively large and highly vacuolated cells. Accumulations of lipid substances could not be demonstrated within these cells. Evident endothelial changes were also observed in arteries which were not occluded by precipitated bacterial material (Fig. 4).

The primary medial injury was localized in the subintimal areas and included degeneration and necrosis of smooth muscle cells; when the lesions were extensive, the mural damage consisted of fibrinoid necrosis (Figs. 5–7). There was often, however, a marked difference between the stains used, as the PTAH method turned out to be negative in many areas where the hyaline material was positively stained with the acid picro-Mallory and the MSB methods. In advanced cases the internal elastic membrane was fragmented, discontinuous or totally destroyed. The lamina elastica externa was also affected in severe cases. Incipient intramural infiltration of mononuclear inflammatory cells and eosinophils was a common finding.
The necrotic areas of the medial walls were rapidly replaced by proliferating cells (Figs. 8-9); in some cases giant cells, of various morphological appearance were involved in the obliterator lesions (Figs. 10-11).

In the adventitia and the pulmonary tissue surrounding the affected arteries cellular accumulations frequently occurred. The great majority of the cells were immature mononuclear cells, with great variations in the nuclear sizes and chromatin content; some of the cells had considerable morphological similarities with plasma cells and were pyroninophilic. Eosinophils were frequently present, and the cellular infiltrations consisted occasionally predominantly of eosinophilic cells. In several cases apparently unaffected or moderately damaged arteries were also circumscribed by cellular accumulations (Figs. 12-14) or oedema. The remaining pulmonary tissue was congested; any precipitates of material resembling that occurring in the arteries were not found in the blood capillaries.

The arterial lesions and perivascular cellular accumulations appeared to be qualitatively independent of whether the animals had been given one or two injections of the bacterial material, and seemed to increase with the length of time after the inoculations. Animals dying in shock after a second injection frequently showed both acute occlusive lesions and perivascular alterations. Only pulmonary vessels were affected, whereas the bronchial arteries were spared.

3. Extrapulmonary tissue. Evident vascular lesions comparable to the changes in the lungs were not noted in extrapulmonary sites, including the kidneys.

**DISCUSSION**

Avian lungs are anatomically quite different from those of mammals, but their vascular system is similar (1). The present investigation demonstrates that disintegrated cells of *Escherichia coli* induce necrotic mural arterial lesions in the lungs of chickens and arterial obliterator modifications, following occlusions by precipitates of the injected material, when this is administered intravenously. As endothelial proliferation and perivascular accumulation of inflammatory cells were commonly present in the absence of occlusive lesions, one may probably conclude that this precipitation was not a prerequisite for the mural damage. The arterial alterations may, therefore, be interpreted as equivalent to the arterial injury observed in the kidneys (13) and in the lungs (14) in previous experiments in which mammalian experimental animals were injected with the same bacterial material. As some of the animals in the former experiments developed renal cortical necrosis, and as pulmonary vascular involvement is quite common in association with hypersensitivity reactions in man (6, 7, 24), the findings in this experiment tend to support the view that hypersensitivity plays a role in the development of the generalized Shwartzman reaction in mammals.

The injected material appeared to constitute the main component of the arterial precipitates, but it seems likely that plasma proteins were also included to a certain extent. As considerably higher doses of this material, if the body weights are taken into account, have been injected intravenously into mammals (mice) without corresponding arterial occlusions, one may conclude that the
arrest of the material in pulmonary arteries must depend on a special response in avian pulmonary tissue, possibly of the anaphylactic type. The mural lesions obviously started in the endothelium and the major part of the proliferating tissue components seemed to arise from the intimal layer, but the possibility that also medial elements participated in the reactive processes cannot be excluded in this study. Proliferation of vacuolated endothelial cells has also been recognized as an early event in association with atherosclerosis in chickens (18).

Zeek et al. (1948) suggested that pulmonary involvement distinguished hypersensitivity angiitis from the acute form of polyarteritis nodosa. Polyarteritis nodosa may however, also occur in the lungs (6, 7, 12, 17, 20, 24), and other authors have found it doubtful to maintain the distinction of hypersensitivity angiitis from polyarteritis nodosa (16). The findings of this experiment indicate, if seen in connection with previous investigations (13, 14), that species and to some extent also individual differences exist as to the key organs affected after systemic exposure of disintegrated cells of Gram-negative bacteria. A further conclusion may possibly be that the observations reported in this and the previous articles support the belief that hypersensitivity angiitis is closely related to the acute type of polyarteritis nodosa.

Fowls are reported to be relatively resistant to endotoxin shock (2, 9, 19), and although several experimental endotoxin studies have been carried out in poultry, histopathological descriptions are not included in most reports (2, 3, 9, 23). Skjørt and Evensen did not observe any lesions in the lungs of cocks injected with large doses of Serratia marcescens endotoxin (19). It seems thus apparent that the bacterial material used in the present experiment possessed toxic properties in addition to its content of endotoxin, possibly depending on the bacterial particles.

Birds are not prone to develop polyarteritis nodosa (5), and the authors of this study are unaware of any previous paper dealing with arterial lesions in birds, thought to be associated with hypersensitivity reactions.

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