COMPARATIVE EFFECTS OF HYDROCORTISONE, A DERIVED PYRIDOPYRIMIDINE, AND XEROSIN ON PNEUMONIA PRODUCED IN MICE BY VIRAL AND BACTERIAL TOXIN

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Received for publication February 22, 1956

It is well known that disease is not necessarily associated with experimentally induced or naturally occurring viral infection. Indeed, in recent years, considerable attention has been directed to the possible lack of direct relationship between viral multiplication and the formation of lesions. Sugg (1949) found that egg-propagated but mouse-unadapted influenza virus produced pneumonia in mice on primary inoculation, but despite active viral multiplication in the lung, lesions were not produced on subsequent passage in mice. He later showed (Sugg, 1950) that the production of pneumonia correlated with the infective titer of virus present in the lung. Extensive studies with pneumonia virus of mice (Horsfall and Ginsberg, 1951) and influenza A virus (Ginsberg and Horsfall, 1952) showed that gross lesions appeared only after extensive viral multiplication and that the rate of production of pulmonary lesions was slower than the rate of production of new virus particles. Ginsberg (1951) and Davenport (1952), independently, studied the nontransmissible pneumonia induced in mice by Newcastle disease virus (NDV) and concluded that this pulmonary lesion resulted solely from the injurious or toxic action of a large amount of virus.

Selective inhibition by xerosin of pneumonia produced in mice by the pneumotoxicity of Newcastle disease virus (Groupé, et al., 1953) and by mouse unadapted (Ginsberg, 1955) and mouse adapted influenza virus (Groupé, et al., 1952) in the absence of demonstrable anti-viral effects provided additional evidence pertinent to the role of viral toxicity in the development of lesions. The fact that xerosin was also found to suppress pneumonia induced in mice by a chemical irritant such as the capsular polysaccharide of Klebsiella pneumoniae (Ginsberg, 1955) led to the investigation of the anti-inflammatory properties of xerosin. Waksman and Groupé (1956) showed that xerosin was, indeed, a potent anti-inflammatory agent that suppressed the tuberculin reaction, inflammation induced by turpentine or urease, and the diffusion of dye in guinea pig skin but that it did not affect the histamine wheal and flare. These activities were shown to be strikingly similar to those of cortisone and its derivatives. However, cortisone has been found to be ineffective against pneumonia produced in mice by Newcastle disease virus or mouse unadapted influenza virus (Ginsberg, 1955) and generally to decrease resistance of the host to viral infection (Kass and Finland, 1953).

The data presented in this report concern certain comparative anti-inflammatory properties of hydrocortisone, xerosin, and a derived pyridopyrimidine (DPP) which was found by Selitto and Randall (1956) to suppress yeast-induced inflammation in the rat ankle. In the interest of brevity and clarity the toxic factors associated with Newcastle disease virus and influenza virus will be referred to tentatively as NDV pneumotoxin and influenza neurotoxin.

MATERIALS AND METHODS

Newcastle disease virus (NDV) pneumotoxin. The California strain of NDV was propagated in the allantoic cavity of embryonated eggs in the usual manner and infected allantoic fluid was collected from both dead and living (refrigerated overnight) embryos 48 hr after infection. In the early experiments undiluted allantoic fluid was used for intranasal instillation. In later experiments NDV was sedimented at 30,000 rpm for 30 min in a Spinco Model L ultracentrifuge, the

1 These investigations were supported in part by Grants-in-aid from The National Microbiological Institute, National Institute of Health (E-S11), the Roche Anniversary Fund, and by funds provided by the Rutgers Research and Endowment Foundation.
1.3,6-octahydropyrido[4,3d] pyrimidine dihydrochloride

*Figure 1.* Derived pyridopyrimidine (DPP)

The pellet was resuspended in \( \frac{1}{2} \) the original volume in tryptose broth (Difco), and this concentrated NDV was stored at \(-70\) C in glass-sealed ampoules. The frozen concentrate diluted \( \frac{1}{2} \) in saline produced severe pneumonia in mice.

*Escherichia coli* endotoxin. Strain 123 of *E. coli* was obtained from Dr. Ruth Gordon, curator of the culture collection in this Institute. Semi-purified endotoxin was prepared from acetonedried bacteria by the trichloroacetic acid method described by Boivin et al., (1933). The final product was a white, amorphous substance which was easily crumbled to form a powder. The LD₅₀ of this material, when injected into mice by the intraperitoneal route, was approximately 0.40 mg. The smallest dose which produced a response after intranasal instillation of 0.1 ml amounts into mice was 0.031 mg, and 0.25 mg produced extensive pulmonary consolidation.

*Inoculation of mice.* Albino mice (Webster strain) weighing 18–22 g each were used. One tenth ml amounts of NDV or endotoxin diluted in saline were instilled intranasally into mice under light ether anesthesia. The cages were previously labelled with the scheduled date of sacrifice and examination. The lungs of each mouse were carefully examined and scored as follows: 1 = 5–25 per cent pulmonary consolidation; 2 = 26–50 per cent; 3 = 51–75 per cent; 4 = 76–100 per cent; 5 = dead mouse with consolidated lungs. The average lesion score was calculated as follows: Total lesion score + total maximum lesion score \( \times 100 \). The average weight of the lungs was determined by weighing the dish plus the pooled lungs, removing the lungs, reweighing the dish less the lungs and dividing the difference by the number of lungs. The average lesion score always paralleled the average lung weight and the former has been omitted in the interest of brevity.

**Anti-inflammatory agents.** The derived pyridopyrimidine (DPP), (figure 1), also known as RO 2-5383, was kindly supplied by Dr. William Davis, Department of Clinical Research, Hoffmann-LaRoche, Inc. Hydrocortisone and its diluent were obtained through the courtesy of Dr. Aleck Borman, Department of Endocrinology, Squibb Institute for Medical Research. The bacterial product, xerosin, was prepared as previously described (Groupé et al., 1954). Diluent (Borman et al., 1954) alone was administered to control mice in experiments with hydrocortisone and saline was similarly employed in experiments with DPP and xerosin. One half ml amounts of suitable dilutions of these preparations were injected subcutaneously once daily as indicated in the text. In all experiments with hydrocortisone and cortisone daily injections were begun 2 days before intranasal instillation of NDV or endotoxin.

**RESULTS**

Attempts to induce pulmonary lesions in mice with chemical irritants. Since pulmonary lesions were readily produced in mice by chemical irritants such as the capsular polysaccharide from *Klebsiella pneumoniae* and such lesions responded favorably to both cortisone and xerosin (Ginsberg, 1955), a number of other substances were tested for toxicity for lung tissue in mice. One tenth ml amounts of various preparations were instilled intranasally into groups of 4 to 6 mice each and the mice were sacrificed on either the third day or on the second and sixth day after inoculation. The lungs were examined for gross lesions and their average weight was recorded. The following substances diluted in saline failed to produce pulmonary consolidation sufficiently extensive to be measurable: desoxyribose nucleic acid, 100 mg/ml; ribose nucleic acid, 33 mg/ml; 20 per cent urea; 20 per cent yeast extract; 20 per cent beef extract; 20 per cent peptone; 20 per cent starch; 10 per cent glycine; 1 per cent formalin; pneumococcus polysaccharide, Type III, 1 mg/ml; and undiluted normal horse serum. However, 0.1 per cent Zephiran (a mixture of cationic detergents) and 0.1 mg of *E. coli* endotoxin did produce pulmonary lesions and an increase in average lung weight of 50 to 70 per cent. Parenteral injection of 50 mg/kg per day.
of xerosin failed to suppress pulmonary consolidation induced by 0.1 per cent Zephiran but did suppress pneumonia produced by 0.25 mg of endotoxin. The latter lesion was selected for further study.

Description of pneumonia produced in mice by E. coli endotoxin. Two groups of 15 mice each were instilled intranasally with 0.1 ml amounts of saline containing 250 µg of endotoxin and saline alone, respectively. All the mice were killed on the third day when the maximum of pneumonia occurred (figure 2). Their lungs were then carefully examined and fixed in formalin. Paraffin sections of 5 lungs from each group were prepared and stained with hematoxylin and eosin in the usual manner. Grossly, the lesions were indistinguishable from those produced by NDV. Sharply outlined, somewhat irregular, areas of hepatization were seen often involving one or more entire lobes of the lung. The excised lungs were not buoyant in water and their weight was greatly increased (figure 4). Histologically, the lesions were characterized by desquamation of the bronchiolar epithelium, atelectasis, marked lymphocytic infiltration of the alveoli, and pulmonary edema. Interstitial infiltration of mononuclear cells, characteristic of pneumonia produced by NDV (Ginsberg, 1951), was not observed.

Effect of xerosin on pneumonia produced in mice by E. coli endotoxin. Figure 2 summarizes data showing that xerosin effectively suppressed the extent of the lesion and reduced the weight of the lungs. In this experiment 120 mice each were inoculated intranasally with 250 µg of endotoxin. Two hours later and daily thereafter half of the mice were injected subcutaneously with 1.0 mg each of xerosin. The remaining half were injected with saline. Subgroups of 15 mice from each group were selected at random and placed in cages previously marked with the scheduled date of sacrifice and examination. One subgroup from the control and xerosin-treated group were sacrificed each day from the second through the sixth day after instillation of endotoxin and the lesion scores and average weight of the lungs were recorded.

Figure 3 presents data from 2 experiments showing first that when daily injections of xerosin were delayed until 24 hr after instillation of endotoxin pulmonary consolidation was still suppressed, and second that when daily injections of xerosin were discontinued after 2 successive daily injections beginning 2 hr after instillation of endotoxin, pneumonia, though initially suppressed by xerosin, became more extensive. These experiments were similar in design and detail to the experiment just described. In the first experiment 165 mice each were inoculated intranasally with 250 µg of endotoxin and then subdivided into 11 groups of 15 mice each. Two series of 4 groups each received 5 single, daily, subcutaneous, injections of saline and 1.0 mg of xerosin, respectively, beginning 2 hr after endotoxin, and 1 series of 3 groups each received 2 single, daily, subcutaneous injections of 1.0 mg each of xerosin beginning 24 hr after endotoxin.

One group of 15 mice each from each series was killed and examined on the second, third, fourth, and fifth days, respectively, after endotoxin. In the second experiment, 180 mice were inoculated intranasally with 250 µg of endotoxin and subdivided into 12 groups of 15 mice each. Two series of 4 groups each received daily, subcutaneous, injections of saline, and 1.0 mg of xerosin, respectively, beginning 2 hr after endotoxin, and
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1 series of 4 groups each received 2 single, daily, subcutaneous injections of 1.0 mg each of xerosin. One group of 15 mice each from each series was killed and examined on the second, third, fourth, and fifth days, respectively, after endotoxin. It is clear that daily injections of xerosin were necessary for effective suppression of pulmonary consolidation induced by \textit{E. coli} endotoxin as well as by NDV (Groupé et al., 1953).

Attention should also be drawn to the fact that Jawetz (1954, personal communication) found that parenteral injection of xerosin failed to affect mortality in mice inoculated intraperitoneally with endotoxin and that parenteral injections of xerosin also failed to affect mortality following intravenous inoculation of influenza viral toxin (Groupé and Herrmann, 1955).

Figure 4 summarizes 6 representative experiments in which the anti-inflammatory properties of hydrocortisone, DPP, and xerosin were compared. In each of these experiments groups of 100 or more mice each were inoculated intranasally with 250 \( \mu \text{g} \) of endotoxin or with NDV pneumotoxin. Daily subcutaneous injections of the indicated amounts of these 3 preparations were administered to subgroups of 15 mice each treated with viral or bacterial toxin as indicated. Control groups of 15 mice each received daily injections of diluent alone. Daily injections of xerosin and DPP were begun 2 hr after instillation of toxin while daily injections of hydrocortisone were begun 2 days before instillation of toxin. Dosage levels of 500 mg per kg of hydrocortisone resulted in marked loss in weight and in death of mice which was frequently associated with pulmonary abscesses. Thus, interpretable data

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure4.png}
\caption{Comparative effects of hydrocortisone, DPP, and xerosin on pulmonary lesions produced by NDV pneumotoxin and \textit{Escherichia coli} endotoxin.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{Effect of delayed and discontinued treatment with xerosin on pulmonary consolidation produced by \textit{Escherichia coli} endotoxin. \( \times \) = Xerosin discontinued 48 hours after endotoxin. \( \bullet \) = Xerosin delayed until 48 hr after endotoxin. \( \triangle \) = Daily injections of xerosin. \( \circ \) = Control.}
\end{figure}
were obtained in these systems only with dosage levels of 250 mg per kg or less. All mice were sacrificed on the third or fourth day after instillation of toxin and the lesion score and average weight of the lungs were recorded. The lesion scores paralleled the average weight of the lungs and the former have been omitted in the interest of brevity. The data show that: (a) All 3 preparations effectively suppressed pulmonary consolidation produced by endotoxin; (b) DPP and xerosin suppressed pneumonia produced by NDV but hydrocortisone failed to do so; (c) the minimal effective daily dose of DPP (300 mg per kg was also the maximum tolerated dose) was 6 times greater than that of hydrocortisone or xerosin; and (d) partly purified xerosin was as potent as crystalline hydrocortisone. Additional similar experiments showed that cortisone was as effective as hydrocortisone in suppressing pneumonia produced by endotoxin.

Experiments with influenza virus neurotoxin in mice. Since it has been shown that parenteral injections of xerosin delayed the onset of induced convulsions in mice previously inoculated intracerebrally with influenza virus neurotoxin (Groupé and Herrmann, 1955), experiments similar in design and detail were carried out with hydrocortisone and DPP. Daily subcutaneous injections of 50 mg per kg of hydrocortisone begun 2 days before intracerebral inoculation of 1, 2, or 4 tonic convulsive doses of influenza virus neurotoxin failed to delay the onset of convulsions induced by twirling the mice. However, daily subcutaneous injections of 300 mg per kg of DPP begun 1 hr after intracerebral injection of 2–4 tonic convulsive doses of viral neurotoxin resulted in a small but definite delay in the onset of induced convulsions (about 10 hr). Thus, it would appear that DPP and xerosin again parallel one another and differ from hydrocortisone in their effects on this type of host-response to viral toxicity.

DISCUSSION

The comparative effects of cortisone, xerosin, and DPP in various diverse biological systems are briefly summarized in table 1. It is clear that all 3 preparations are effective anti-inflammatory agents. The failure of cortisone to suppress pulmonary lesions induced by viruses when the same lesions were beneficially modified by both xerosin and DPP is of particular interest. These data imply either that (a) pulmonary consolidation instigated by these viruses is accomplished by a mechanism which is basically different from that initiated by certain other agents as suggested by Ginsberg (1955), or that (b) the failure of cortisone to suppress lesions produced by viruses is associated with the profound adverse effects of large amounts of cortisone on the physiological state of the host which might also preclude the administration of a

<table>
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<tr>
<th>Host-Response To:</th>
<th>Anti-inflammatory Agent</th>
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<tbody>
<tr>
<td></td>
<td>Cortisone*</td>
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<tr>
<td>Viral infection</td>
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<tr>
<td>Influenza virus</td>
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<tr>
<td>Mouse adapted...</td>
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<tr>
<td>Mouse unadapted</td>
<td>0§ (3)</td>
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<td>Rous sarcoma virus</td>
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<tr>
<td>Yeast irritation...</td>
<td>+ (11)</td>
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* Cortisone or hydrocortisone.
† Aggravation of lesion.
‡ Suppression of lesion.
§ No effect.

sufficient quantity of cortisone to be effective against NDV-induced pneumonia. The fact that DPP, as well as xerosin, were effective in suppressing pneumonia induced by both NDV and bacterial endotoxin favors the latter interpretation. The failure of xerosin to protect mice against the intravenous injection of influenza virus is consistent with the failure of cortisone to protect mice against the intraperitoneal injection of bacterial endotoxin. These data are also consistent with the observation of Ginsberg (1955) who found that xerosin permitted clear definition of at least 2 distinct phases in the pathogenesis of influenza viral pneumonia in mice: (1) Injury by virus of the bronchial and bronchiolar epithelium which was unaffected by xerosin, and (2) secondary reactions leading to edema, hemorrhage, and cellular infiltration manifested as pneumonia which were inhibited by xerosin.

**SUMMARY**

Daily parenteral injections of hydrocortisone, a derived pyridopyrimidine (DPP), or the bacterial product, xerosin, suppressed pneumonia produced in mice by *Escherichia coli* endotoxin. However, only DPP and xerosin suppressed pneumonia induced by the toxicity of Newcastle disease virus (NDV) and delayed the onset of convulsions induced by influenza virus neurotoxin. It is not clear whether the failure of cortisone to affect NDV-induced pneumonia is associated with basic differences between such virus-induced lesions and other inflammatory responses or with the untoward effects of large amounts of hydrocortisone on the host.

**REFERENCES**


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