Further Studies of Interrelationship Between Xanthine Oxidase and Influenzal Pneumonia in Mice.* (22292)

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It has been previously reported(1) that an increase in xanthine oxidase occurs in the lungs of mice infected with PR8, Type A influenza virus. In order to define further the interrelationship between the increased levels of enzyme and virus proliferation additional experiments have been carried out. Levels of the *in vitro* activity of xanthine oxidase in mouse lung infected with influenza virus have been compared with those of the same tissue either infected or injured with: (a) Nigg pneumonitis virus, (b) Newcastle disease virus, (c) *Klebsiella pneumoniae,* and (d) toxic chemicals, fats, Friedlander's polysaccharide and leukotaxine. The results indicate that although the increase in xanthine oxidase is not directly related to influenza virus proliferation *per se,* the enzyme may be concerned in some way with the pneumonic process in mouse lung.

Methods. Four to 6 weeks old albino Swiss mice of the Webster strain were employed. Xanthine oxidase assays on homogenized mouse lung tissue were carried out in a conventional Warburg apparatus as described previously(1). The values reported represent the oxygen uptake, stimulated by addition of substrate, of 20 mg tissue (dry weight equivalent) per 20 minutes; in each experiment the oxygen uptake representing the endogenous respiration has been subtracted from the recorded values. Hemagglutination titrations were performed employing a pattern end point (2). In determining the virus infectivity titers, equal volumes of a sample of homogenate from all the animals assayed at a given time were pooled, serial 10-fold dilutions prepared and 4, 10-day chick embryos inoculated with 0.2 ml of each dilution. After 48 hours incubation at 35°C, samples of allantoic fluid were removed and tested for the presence of virus by the spot plate hemagglutination technic(3). The ID_{50} titers were calculated according to the method of Reed and Muench (4). The lungs from each animal were examined and the pulmonary consolidation scored according to the method of Horsfall (5).

Results. The results of an experiment with a mouse adapted strain of PR8, Type A influenza virus are presented in Table I. Mice were infected via the intranasal route with 4,000 LD_{50} doses of stock virus. The day-to-day change in enzyme level together with the change in virus level as determined by hemagglutination titers for chick-embryos were determined on combined aliquots of tissue from each day's experiment; these results are directly comparable to the recorded averages of enzyme activity. Levels of enzyme activity are roughly paralleled by virus titers.

Consolidation is somewhat slower than is the increase in enzyme activity in developing; the presence of gross lesions is not essential for increased xanthine oxidase activity although the maximal levels of enzyme are found in partially consolidated tissue. A lethal dose of virus was employed in this experiment and at the end of 100 hours all the animals were dead. When sub-lethal amounts of virus were employed comparable results were obtained, *i.e.,* the development of enzyme levels and virus titers roughly paralleled each other although the levels reached were lower and slower in developing than in lethally infected mice.

An experiment showing the changes in xanthine oxidase in mouse lung following infection with Nigg pneumonitis virus (Greb

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TABLE I. Correlation of Changes in Virus Concentration and in Xanthine Oxidase Activity in Mouse Lung Infected with PR8 Influenza Virus.

Time (hr)†	Group 1* E.A.‡	H.T.§	C.‖	Group 2 E.A.	H.T.	C.	Group 3 E.A.	H.T.	C.	Avg E.A.	H.T.	EID_{50}
0	2.3			2.5			2.3			2.4		
24	6.9	160	0 0	5.3	160	+ 0	4.9	160	0 0	5.7	160	$10^{-7.75}$
48	8.5	320	0 0	7.2	320	+ +	5.9	320	0 +	7.2	320	$10^{-8.5}$
72	13.2	320	2+ +	9.8	320	4+ 2+	8.0	160	3+ 2+	10.3	266	10^{-9}
96	12.0	80	3+ +	4.8	320	3+ 2+	3.9	80	2+ +	6.9	240	$10^{-7.5}$

* Group of 10 mice, 2 chosen at random and pooled for examination at indicated time.
† No. of hours between initial infection and titration.
‡ Enzyme activity: μl O_2 uptake/20 min./20 mg dry lung. Substrate: 10 μM hypoxanthine/flask.
§ Hemagglutinin titer: Highest dilution of virus showing complete hemagglutination.
‖ Amount of consolidation determined by gross macroscopic examination and scored on a 0-5 point basis.
EID_{50}—50% embryo infectivity end point.

strain) is recorded in Table II. The animals were infected intranasally with 10-100 lethal doses of virus. Although the titer of virus in the tissue examined for enzyme activity was not determined, the presence of virus was established by mouse inoculation. In comparison to the PR8 infection, a somewhat greater level of enzyme activity was reached on each successive day reaching a peak value of 12.4 on the fourth day. Only 3 of the animals survived to the fifth day; the value obtained for the enzyme activity of these lungs was 11.4 which may have indicated a leveling off of activity.

Table III shows the changes in xanthine oxidase in mouse lung following the administration of Newcastle disease virus (NDV). The mice were given 0.05 ml of infected allantoic fluid (ID_{50} titer for eggs of $10^{-7.6}$) by the intranasal route. There was a sharp rise in enzyme activity almost reaching a peak level within 24 hours and remaining relatively constant through the fourth day, while the macroscopic consolidation developed gradually over a three day period.

For comparative purposes, studies were made of the effect of a pulmonary bacterial infection on xanthine oxidase. Virulent *Klebsiella pneumoniae,* when introduced via the intranasal route, produced a rapidly fatal pneumonitis. Within 48 hours the lungs were almost completely consolidated, and the ani-

TABLE II. Change in Levels of Xanthine Oxidase Activity in Mouse Lung after Infection with NIGG Pneumonitis Virus.

Time (hr)†	Group 1* E.A.‡	C.§	Group 2 E.A.	C.	Group 3 E.A.	C.	Avg E.A.
0	2.6		2.0		2.1		2.2
24	6.4	+ —	5.6	+ +	3.8	+ —	5.26
48	11.4	4+ +	8.1	3+ 2+	8.0	3+ +	9.16
72	11.0	4+ 2+	10.7	3+ 3+	10.0	3+ 3+	10.56
96	14.6	3+ 3+	11.8	4+ 3+	10.9	4+ 3+	12.43

* Group of 10 mice, 2 chosen at random and pooled for examination at indicated time.
† No. of hours between initial injection and assay.
‡ Enzyme activity: μl O_2/20 min./20 mg dry lung. Substrate: 10 μM hypoxanthine/flask.
§ Consolidation.

TABLE III. Change in Levels of Xanthine Oxidase Activity in Mouse Lung after Administration of Newcastle Disease Virus.

Time (hr)†	Group 1* E.A.‡	C.§	Group 2 E.A.	C.	Group 3 E.A.	C.	Avg E.A.
0	2.6		2.0		2.1		2.2
24	10.0	0	8.3	0	6.5	+	8.26
		+		+		+	
48	9.0	4+	8.1	+	7.7	+	8.26
		3+		0		+	
72	11.8	5+	8.9	2+	7.3	4+	9.33
		0		+		4+	
96	11.6	+	9.4	5+	7.0	+	9.33
		+		3+		+	

* Group of 10 mice, 2 chosen at random and pooled for examination at indicated times.
† No. of hours between initial inoculation and assay.
‡ Enzyme activity: μl O_2/20 min./20 mg dry lung. Substrate: 10 μM hypoxanthine/flask.
§ Consolidation.

mals seldom survived a lethal dose beyond this time. Enzyme assays showed that the level of xanthine oxidase was increased concomitant with the progressing *K. pneumoniae* infection: Values of 4.0, 5.4, and 6.5 (μl O_2 uptake/20 minutes/20 mg dry lung) at 24 hours and 5.4, 5.6, and 6.7 at 48 hours were obtained in a typical experiment. These enzyme levels, when compared with the levels of lungs infected with influenza virus are comparable at the same time intervals; however, the maximal levels of enzyme in the influenza lungs at the end of 96 hours, when the lesions are more comparable, are about two times these values. That the enzyme activity was not due to the bacteria present in the homogenate was shown by testing the organisms for xanthine oxidase activity. The strain of *K. pneumoniae* employed in these experiments showed almost no activity, and efforts to produce an "adaptive" type of this enzyme were not successful. The results indicate that infection of mouse lung with *K. pneumoniae* produces a severe pneumonitis and gives rise to an increase in xanthine oxidase activity.

The following compounds were employed in attempts to produce a sterile pneumonia in mice: dilute ammonia, Friedlander's polysaccharide, olive oil, starch, heparin, leukotaxine, serum and turpentine. Intranasal, intratracheal and intrapleural routes of inoculation were tried. In no instance was a pneumonic process produced equal in magnitude to that produced by virus or *K. pneumoniae*. In 10-25% of the animals one lobe, or perhaps 2 lobes, showed some consolidation; xanthine oxidase was not increased in these lungs. The most successful procedure was that of intrapleural injection of turpentine; at the end of 96 hours some of these lungs showed a moderate amount of consolidation and a concomitant increase in xanthine oxidase of about the same magnitude as the 48-hour influenza lung. Therefore, it is apparent that the increase in xanthine oxidase can be elicited in the absence of an infectious process and may be the result of an inflammatory reaction.

In order to determine if basic similarities existed in the pathology of the lesions produced by these various agents, microscopic sections were examined. Sections of influenza lungs showed diffuse interstitial pneumonitis with a mononuclear infiltrate causing a thickening of septal walls. Necrosis and desquamation of the epithelial lining of the bronchi and bronchioles were noted. The lesions produced by NDV were very similar in character although less severe in degree. Sections of the lesions produced by *K. pneumoniae* and by Nigg pneumonitis virus showed severe bronchopneumonia with diffuse areas of cellular infiltrate, predominantly polymorphonuclear leucocytes. There was associated distortion and destruction of lung architecture. The mucosae and walls of the bronchi and bronchioli appeared to be relatively uninvolved although the lumina were plugged with exudate. A correlation between lesions with characteristic infiltrates and xanthine oxidase activity was not found.

Discussion. In mouse lungs infected with the viruses of influenza and Nigg pneumonitis, a fundamental relationship between purine catabolism and virus synthesis is suggested by an increase in xanthine oxidase activity which occurs during the phase of rapid increase in virus concentration and precedes the development of maximal macroscopic and microscopic lesions. The fact that a similar change occurs after NDV which does not multiply in mouse lung(6,7) might contradict such a hypothetical relationship. On the other hand, it is conceivable that a change in purine metabolism is initiated by invasion of the host cell by viral particles whether or not subsequent multiplication of the virus takes place. Comparative studies of other types of injury to mouse lung indicate that infection with *K. pneumoniae,* and the intrapleural injection of turpentine also result in significant increases in xanthine oxidase activity. These latter findings negate the intriguing possibility that xanthine oxidase is concerned with a phase of nucleic acid metabolism intimately related to viral synthesis. The fact that a variety of microorganisms capable of producing severe and rapidly fatal pneumonias all elicit a rapid increase in xanthine oxidase activity remains of some interest. One may ask whether or not this represents a biochemical disturbance of host-cell metabolism which contributes to the over-all disease process.

Conclusion. Previous observations that xanthine oxidase increases in mouse lung concomitant with influenza virus infection have been confirmed and extended. Comparable increases in xanthine oxidase also occur in murine pneumonitis produced by Nigg pneumonitis virus and by NDV. Comparative studies with other types of infection and injury indicate that infection with *K. pneumoniae* and the intrapleural injection of turpentine also result in significant increases in xanthine oxidase activity in mouse lung thus suggesting that an increase in the level of enzyme is closely associated with injury and death to lung tissue.

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Fluorescent Antibody Studies of Demyelination in Canine Distemper.* (22293)

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The literature on central nervous system lesions in canine distemper has been adequately reviewed by Winqvist(1). Demyelination and glial cell proliferation are the outstanding changes while vascular hyperplasia and perivascular infiltrations occur less frequently. The cerebellum, pons and medulla are the principal sites of involvement. No attempt has been made to correlate the demyelination with localization of the virus. This report describes the demyelination process as it occurs in the cerebellum, and by means of the fluorescent antibody technic(2) to demonstrate the sites of viral concentration.

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