EFFECTS OF BERYLLIUM SULFATE ON SERUM ALKALINE PHOSPHATASE IN PRIMATES

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FOREWORD

The investigations described in this report were carried out during the period from January 1964 to December 1964. The research was conducted in the Toxic Hazards Division, Toxicology Branch, Biomedical Laboratory, of the Aerospace Medical Research Laboratories, under Project 6302, "Toxic Hazards of Propellants and Materials," and Task 630202, "Pharmacology-Biochemistry."

The assistance rendered by Major Farrel R. Robinson, USAF, VC, of the Pathology Branch, Toxic Hazards Division, is gratefully acknowledged.

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ABSTRACT

The effects of intratracheally administered beryllium sulfate on serum alkaline phosphatase were studied in male Macaca mulatta monkeys. Results showed 80% and 50% inhibition of the serum enzyme at 2 and 4 hours postinjection, respectively. Additional experiments were conducted using aluminum sulfate under identical conditions, and no inhibition of serum alkaline phosphatase was noted.

SECTION I

INTRODUCTION

Since berylliosis was first recognized as a disease in humans, medical investigators have attempted to establish the causal relation of dose-response and in situ identification of beryllium. Much of the research has been done on autopsy material and by statistical analyses of carefully reviewed case histories. Rats, rabbits, and dogs have been the primary laboratory animals used for the study of experimentally-induced chronic effects and for pathological evaluation in acute toxicity studies of beryllium compounds. Several reports have described the in vitro inhibition of rat serum alkaline phosphatase by beryllium. This interesting phenomenon has been used as a test criterion for evaluating the therapeutic effectiveness of certain new chelating agents against the mortality resulting from acute beryllium toxicity (ref 1). No information was available, however, on the in vivo inhibition of this serum enzyme following exposure to beryllium; therefore, the study described herein was undertaken to investigate this biochemical component in primates subjected to an acute dose of beryllium administered by intratracheal route.

SECTION II

MATERIALS

Beryllium Sulfate Solution

$BeSO_4 \cdot 4H_2O$ with a molecular weight of 177.14 and containing 5.09% beryllium was made up in distilled water to a concentration of 10 mg/ml as Be^{++}. The solution was prepared by dissolving 4.912 g of the hydrated salt in a total volume of 25 ml in a volumetric flask.

Aluminum Sulfate Solution

$Al_2(SO_4)_3 \cdot 18H_2O$ (MW = 666.41) solution was prepared by dissolving 6.26 g in 25 ml distilled water. This solution contained the same concentration of SO_4 as the beryllium sulfate solution and was injected in the same manner as the beryllium solution.

Animals

Forty male Macaca mulatta monkeys weighing from 2.0 to 5.2 kg were used in this investigation. Twenty-two monkeys received beryllium, 10 received aluminum sulfate, and 8 received saline. They were allowed food and water ad libitum and were maintained in individual cages throughout the experiments.

SECTION III

METHODS

Time intervals selected for studying the effects of intratracheal (i.t.) injections of the compounds on serum alkaline phosphatase were 2, 4, 8, and 24 hours. Each timed study involved at least one monkey serving as a saline control. All animals were bled before injection for baseline determination of serum alkaline phosphatase so that each animal served as his own control. All beryllium-treated animals received 2 mg/kg Be^{++}.

The experiments were divided into four phases. Phase I involved injection of beryllium sulfate to groups of five animals each and saline to one monkey for each group. The 2, 4, 8, and 24-hour experiments were performed at 1-week intervals.

Phase II was undertaken to compare the effects of an equivalent dose of sulfate, as aluminum sulfate, on serum alkaline phosphatase. This study involved four monkeys that received i.t. injections of aluminum sulfate and two monkeys that served as saline controls for a 2-hour experimental period.

Phase III used six monkeys; two received beryllium sulfate, two received aluminum sulfate, and two served as saline controls. This experiment was designed as a comparative 2-hour study for pathological evaluation without prior knowledge of the compound administered.

Phase IV served to provide additional material for further pathological evaluation of the effects of aluminum sulfate after 24 hours. For this purpose four monkeys received the same dose of aluminum sulfate as previously administered, and necropsies were performed after 24 hours. No serum alkaline phosphatase determinations were made on these animals.

Intratracheal injections were made through the crico-thyroid membrane of the larynx using a 1-cc disposable glass tuberculin syringe and 25-gauge 5/8-inch, or 26-gauge 1/2-inch disposable needle. The unanesthetized animals were restrained in a supine position during injection. Immediately following the injection they were held in an upright position while being returned to their cage. Little difficulty was encountered in making the injections. Peritracheal administration was suspected in two animals, one that received beryllium sulfate and one that received aluminum sulfate, because of resistance encountered at injection. This was not confirmed at necropsy. Gross examination did reveal, however, that in one of the animals in Phase II, the aluminum sulfate solution had been deposited peritrachaeally. Several animals exhibited some degree of coughing following the injection. No expulsion of injected material was observed however.

At the end of the 2, 4, 8, or 24-hour postinjection holding periods, blood samples were collected for serum alkaline phosphatase analyses, and the monkeys were necropsied following the intravenous administration of pentobarbital sodium and exsanguination. Gross examinations were performed and tissues removed for pathological evaluation. Particular attention was directed to the lungs, trachea, bronchi, hilar lymph nodes, and spleen. Samples of these tissues were frozen and preserved for beryllium analysis by laser microprobe and emission spectroscopsy. However, the results are not discussed in this report.

SECTION IV

RESULTS

RESULTS OF SERUM ALKALINE PHOSPHATASE DETERMINATIONS

(Klein-Babson-Read Units, Ref 2)

PHASE I

MONKEY NO.	BASELINE	POSTEXPOSURE			
		2 hours	4 hours	8 hours	24 hours
Q-56	14.8	2.3			
Q-68	5.8	0.8			
Q-54	13.0	3.8			
R-00	10.0	2.0			
R-56	17.5	2.8			
R-06 Control	17.3	13.3			
R-88	25.0		10.5		
R-96	22.5		8.3		
S-10	12.3		8.8		
S-18	11.3		12.3		
S-30	22.0		18.5		
Q-90 Control	17.3		26.0		
S-36	21.0			18.0	
R-92	40.0			33.0	
Q-94	39.0			15.0	
R-26	25.0			*	
Q-98	32.0			16.0	
R-74 Control	32.0			50.0	
R-80	35.6				11.6
S-04	32.0				11.6
R-30	47.0				25.0
S-86	24.0				27.6
R-16	29.6				14.0
Q-80 Control	32.0				15.0

*Monkey died 7-1/2 hours after injection.

PHASE II

Monkey No.	Baseline	Compound	2 hours Postexposure
A-50	35.0	aluminum	43.0
A-56	29.0	aluminum	33.0
A-58	41.0	aluminum	28.0
A-64	38.0	aluminum	29.0
A-66	30.0	saline	25.0
A-72	19.0	saline	16.0

PHASE III

A-70	11.0	beryllium	11.3
A-40	20.6	beryllium	9.9
A-60	14.0	aluminum	16.0
A-44	7.3	aluminum	5.5
A-46	7.5	saline	6.3
A-68	13.0	saline	33.4

STATISTICAL ANALYSIS OF ALL SERUM ALKALINE PHOSPHATASE DATA

(Average and Standard Deviation)

Experimental Group	Number of Animals	Baseline	2 hours	4 hours	8 hours	24 hours
All Exposed Animals	32	24.1 s=11.6				
Saline Injected	8	21.0 s=9.89	23.1 s=13.81	26.0	50.0	15.0
Beryllium Treated	22	23.8 s=11.0	4.7* s=3.6	11.7* s=4.1	20.5 s=8.4	18.0 s=7.7
Aluminum Treated	10	27.4 s=10.0	25.8 s=16.5			

*Significantly different from both saline injected controls and individual baselines, p = .01 (Student's t Test).
s = standard deviation

Pathology

Gross lesions seen in the lungs at necropsy were similar in both the beryllium sulfate and aluminum sulfate injected animals and were consistent with a chemical pneumonitis following inhalation of a caustic liquid. Lesions were observed most frequently in the diaphragmatic lobes of the lungs. Two hours after injection, hemorrhagic areas up to 2 cm in diameter, some with apparent central necrosis, were observed. Localized edema was also apparent. In the beryllium treated animals, the hemorrhagic and exudative reaction was more diffuse and the central necrotic areas more prominent at 4 and 8 hours postinjection. No aluminum sulfate treated animals were available for study at these time periods. At the end of 24 hours, massive areas of hemorrhage and consolidation with necrotic centers were observed in both groups of injected animals.

Microscopic examination of tissue from beryllium injected animals revealed irregularly shaped focal areas of congestion, edema, and alveolar hemorrhage with occasional septal necrosis at 2 hours postinjection. Some fibrin thrombi were observed in the associated smaller blood vessels as was a variable degree of exudation into the alveoli and associated bronchioles. At 4 and 8 hours these changes were more pronounced and somewhat more extensive. Erythrophagocytosis, fibrin formation, and increasing neutrophilic infiltration were apparent. A purulent or mucopurulent exudate was present in the adjacent air passages. Large necrotic areas surrounded by extensive hemorrhage, edema, and fibrin formation were present at 24 hours postinjection. In many areas, the inflammatory reaction had advanced to a stage of consolidation with the infiltration of large numbers of neutrophiles and early abscess formation. Purulent and necrotic material was present in associated bronchi.

Brown to black particulate pigment was frequently observed in phagocytes or free in the tissue or exudate. Since the animals were infested with Pneumonyssus simicola, it was not possible to determine whether this was injected material or pigment usually seen in the presence of these lung mites.

The results of microscopic examination of tissues from aluminum sulfate injected animals were similar to those seen in the beryllium injected animals. Necrosis was less evident at 2 hours, however, and at 24 hours the lesions were possibly somewhat more discrete.

Some erythema and edema was observed in the trachea around and distal to the injection site in both groups of monkeys. Microscopic examination revealed congestion, edema, and focal desquamation of tracheal and bronchial epithelium. Hypertrophy of goblet cells and a mucocellular exudate with an increase in neutrophilic content over the 24-hour period was present. Hilar lymph nodes developed a slight to moderate edema. Pigment similar to that seen in the lungs was occasionally observed in both the hilar lymph nodes and spleen.

SECTION V

DISCUSSION AND CONCLUSIONS

As initially designed, this experiment sought only to investigate the time-phased levels of serum alkaline phosphatase in monkeys receiving 2 mg/kg beryllium sulfate (calculated as beryllium ions) by the intratracheal route of administration. Since this dose is considered to be acute in the monkey, no complete pathological evaluation was deemed either necessary or pertinent. At the time of the study, however, a new capability for detecting beryllium in tissue preparation using the laser microprobe in conjunction with emission spectrography was in the process of being established in our laboratory, and the experimental protocol was modified to include gross necropsies and the preservation of selected tissues for subsequent analysis. As a direct result of the pathologist's gross observations of the monkeys described in Phase I, additional studies were undertaken to more clearly delineate the cause of the pulmonary lesions. Therefore, Phases II, III, and IV were conducted to compare not only serum alkaline phosphatase levels, but also pulmonary lesions in monkeys receiving intratracheal injections of either beryllium sulfate or aluminum sulfate under the same experimental conditions. Saline injected monkeys were considered necessary to serve as controls for the intratracheal method of administration with its possible traumatic effect on the parameters being measured.

Examination of results of serum alkaline phosphatase levels indicates strongly that the 80% and 50% inhibition of this enzyme at 2 and 4 hours, respectively, is indeed attributable to beryllium per se. The mechanism of this interesting biochemical phenomenon is not clear. Pathological changes, essentially the same in both groups, were due apparently to caustic action of solution injected. The inhibition of alkaline phosphatase levels, occurring only in beryllium treated animals however, appeared to have been reversed by 8 hours, and no further inhibition of activity was noted at 24 hours, the time when pulmonary damage was most severe.

That serum alkaline phosphatase levels are severely inhibited in acute beryllium intoxication in monkeys and rats is only of academic interest at this time. Apparently the activity of this enzyme is restored to normal within a comparatively short time after exposure, and its measurement would not provide a good tool for clinical diagnosis. In addition, the monkey and the rat, unlike the dog or human, have fortuitously high normal levels of serum alkaline phosphatase, thus making determination of in vivo inhibition much easier.

Recent reports in the literature have indicated that there may be several isoenzymatic forms of alkaline phosphatase, these presumably emanating from different organ sources into the peripheral blood. The studies described here have made no attempt to identify which alkaline phosphatase isoenzyme is being inhibited. Further work must be done to clarify this point.

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Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY *(Corporate author)* Aerospace Medical Research Laboratories, Aerospace Medical Division, Air Force Systems Command, Wright-Patterson Air Force Base, Ohio 45433	2a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED 2b. GROUP N/A

3. REPORT TITLE

EFFECTS OF BERYLLIUM SULFATE ON SERUM ALKALINE PHOSPHATASE IN PRIMATES

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*

Final Report - January 1964 to December 1964

5. AUTHOR(S) *(Last name, first name, initial)*

Pinkerton, Mildred K.
Ziegler, Ralph F., USAF, VC

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
December 1966	7	2

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. * 6302	AMRL-TR-66-198
c. Task No. 630202	9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*
d.	

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	Aerospace Medical Research Laboratories Aerospace Medical Division, Air Force Systems Command, Wright-Patterson AFB, O.

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DD FORM 1 JAN 64 1473

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14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Beryllium sulfate Serum alkaline phosphatase Berylliosis Macaca mulatta Primates						

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