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Division: Pharmacology Division Head: Harold C. Hodge

Pharmacology Report #562

PROGRESS REPORT OF PRELIMINARY STUDIES OF BERYLLIUM TOXICITY

Dr. Harold C. Hodge, et al

MASTER

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Foreword

Harold C. Hodge

In connection with the symposium on beryllium poisoning at the Saranac Laboratories, preliminary experimental studies dealing with various phases of the beryllium problem have been briefly summarized. These summaries in full make up the body of this report.

A brief statement of orientation might be in order:

Clinical Industrial Beryllium Poisoning. In the report of the Beryllium Symposium held at the Medical School, University of Rochester, in December 1946, a number of first-hand reports were made, in which the several manifestations of beryllium poisoning were described in detail. A summary of the symposium has been distributed as an earlier report from this laboratory. *

Briefly, four conditions have been ascribed to industrial beryllium poisoning:

1. Metal fume fever
2. Dermatitis and ulcers of the skin
3. Acute pneumonitis
4. Delayed, prolonged lung disease

One of the most confusing aspects of beryllium poisoning is the extreme variability of the symptoms. The most common seem to be as follows:

1. Anorexia and loss of weight in mild poisoning
2. Dyspnea and x-ray changes in the lungs in severe poisoning.

Another confusing aspect of beryllium poisoning is the lack of consistent correlation between exposure and poisoning. Individuals working near a beryllium furnace, where exposures might be expected to be severe, may not develop the disease; while office workers in a distant part of the plant may be affected. In fact, the establishment of beryllium as the unique cause may still be said to be open to question.

Seven chapters are included in this special report; the titles are given in the table of contents. As a guide to the subject matter, a series of paragraphs are appended herewith:

Dust Hazards. One of the sources of perplexity in the past has been the nearly total absence of data as to the nature and concentration of beryllium dusts in the beryllium plants. The first report by Laskin, Turner, and Stokinger describes a careful survey which has been made of a typical beryllium plant. Concentrations of beryllium in the factory air were found to vary from high values, e.g. 4.7 mg/m^3, near the metal furnace to low values,

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e.g., 0.05 mg/m^3, in rooms at some distance from the metallurgical operations. The median particle size of the beryllium dusts ranged from 0.9 microns near the beryllium furnace to 2.5 microns near the beryllium fluoride furnace; and even to as large as 10 microns in the ore treatment room. In passing it might be noted that concentrations of fluoride as great as a thousand times those of beryllium were encountered. These dust data possess an importance out of proportion to their numbers, since no other such data are available.

Analytical Methods. In the second report, W. F. Neuman has mentioned the analytical methods available for pure samples of beryllium. Colorimetric or fluorimetric methods can be used for amounts of the order of 10 to 0.5/^{u}g. The spectroscopic method will detect as little as 0.005/^{u}g. Radioactive beryllium is one possibility as a tool in distribution studies. At present, no reliable method for isolating beryllium from tissue samples is available.

Pathology. The cardinal pathological changes in experimental animals have been described by Scott. Three systems of the body reveal the effects of acute, high-grade poisoning: a) the liver shows a mid-zonal necrosis; b) the kidney shows a characteristic necrosis in the distal third of the proximal convoluted tubule, and c) a variety of changes arise in the hematopoietic system, which are reflected in the peripheral blood in a secondary anemia, a leucocytosis, and an increase in the number of platelets.

Acute Toxicity. In adult albino rats, the amounts of beryllium to kill the average rat are as follows: intravenously, about 0.4 mg/kg; intraperitoneally, 5-10 mg/kg; intratracheally, about 1 mg/kg. In each case the sulphate was administered in aqueous solution, but the doses were calculated as beryllium.

There is some evidence of a marked species variation in susceptibility when beryllium sulphate is administered intraperitoneally. The dose to kill the average animal is roughly as follows: mice 10-15 mg/kg; rats 5-10 mg/kg; rabbits 0.1-0.4 mg/kg; guinea pigs 0.1 mg/kg.

The soluble beryllium compounds are quate toxic; the toxicity is a function of the anionic part of the molecule as well. The insoluble beryllium compounds are slightly toxic or almost innocuous.

Preliminary Feeding Tests. Maynard carried out a few tests in which rats were maintained on diets containing 5% of beryllium sulphate. These animals did not grow; but when placed again on a stock diet resumed growth. Comparable rats placed on a diet containing 5% of beryllium carbonate lost weight and died. The rats receiving either compound developed well-defined rickets.

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Rats fed on diets containing 5% of beryllium oxide or 10% of finely-powdered beryllium metal exhibited no ill effects.

Preliminary Inhalation Tests. Groups of animals were exposed to atmospheres containing beryllium sulphate dust in amounts sufficient to be equivalent to 4-5 mg Be/m^3.

In a two-week study, mice were found to be quite susceptible, rats and hamsters, somewhat susceptible; but dogs, rabbits, and guinea pigs were quite resistant to the exposure. Some skin lesions were observed, and those mice which died early in the experiment showed liver injury; a tendency toward increase in leucocyte count was observed.

In a few experiments, rats were placed in an atmosphere containing beryllium metal fume having a total concentration of the order of 800 mg/m^3. Thirty-minutes' exposure to such an atmosphere will kill the majority of a group of rats. Half this exposure killed none. The effect seems to be that of a primary irritant.

Harold C. Hodge

HCH:ms

September 1, 1947

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ANALYSIS OF DUST AND FUME HAZARDS

IN A BERYLLIUM PLANT

Sidney Laskin, Robert A.N. Turner, Herbert E. Stokinger

A great percentage of this material is included in the following Monthly Reports: #M-1929, M-1936, M-1947. Additional data included in this paper will be included in future Pharmacology Reports

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ANALYSIS OF DUST AND FUME HAZARDS IN A BERYLLIUM PLANT

Sidney Laskin, Robert A. N. Turner, Herbert E. Stokinger

ABSTRACT

A survey has been made of industrial health conditions of a beryllium plant in which many instances of beryllium poisoning have existed. The survey included an analysis of the dust and fume data taken under operating conditions at the plant. The results of this analysis have been correlated with the medical history of the plant.

The plant in question produces a technically pure beryllium metal and beryllium-copper alloy. The operations involve grinding beryl ore, sintering, conversion to the sulfate with sulfuric acid, furnacing to the oxide, treatment of the oxide by acid fluoride followed by a reduction of the beryllium fluoride to yield metallic beryllium. The beryllium-copper alloy is made by heating copper with beryllium oxide in the presence of carbon. The atmospheric contamination associated with these processes is in the form of dusts, fumes and mists. Selected areas were sampled, the selection being based on past medical history of hazard or on obvious contamination at the time of the survey. Sites selected were the ore-treatment area and that about the beryllium fluoride and beryllium metal furnaces. Sampling equipment consisted of a Modified Cascade Impactor, Filter Paper Dust Sampler and a M.S.A. Midget Impinger. Samples were analyzed in most instances, both for beryllium and fluoride.

Of the three areas surveyed, those of the beryllium fluoride and beryllium metal furnace showed the most hazardous concentrations of beryllium and fluoride. Most arresting was the disproportionately high fluoride concentration near the fluoride furnace. Concentrations 1000 fold that of beryllium were found.

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Such high concentration of fluoride may represent a hazard in itself. The concentration of beryllium 3 feet from the furnace opening during the pouring operation was approximately 0.065 mg/m^3. During the pouring operation of the beryllium metal furnace, beryllium concentrations varied from 1.4 to 4.7 mg/m^3 at approximately 3 feet from the furnace. Samples for fluoride in this site were of the order of 1.7 mg/m^3. Near the rotary kiln drier for beryllium ore treatment, beryllium dust concentration varied from 0.050 to 0.53 mg/m^3; fluoride concentrations in the air were of the same order of magnitude. A particle-size analysis of the air contamination samples by the means of the Cascade Impactor showed a mass-median particle size of 0.86 μ in the vicinity of the beryllium metal furnace, 2.36 μ next to the beryllium fluoride furnace, but sizes up to 10 μ in the area about the ore treatment room. The insoluble beryllium dusts were most efficiently sampled by either the Cascade Impactor or the Filter Paper Dust Sampler, but the Midget Impinger gave more satisfactory recoveries for soluble beryllium fumes.

Another problem investigated was that of beryl ores suspected of containing radioactive impurities. One of three suspected ores showed significant beta activity equivalent to 12.6% uranium, which on spectrochemical analysis later revealed 4% U., 0.4% Th., and 0.4% Pb. This degree of activity represents a definite thoron or radon hazard and suggests the possibility that uranium or other radioactive materials may act synergistically or as predisposing agents in beryllium poisoning.

The medical history of the plant over a 4-year period from 1943 to 1946 showed 136 cases of beryllium poisoning. Cases for the most part wereconfined to the preparation of sulfate, the fluoride, the metal, and the beryllium-copper alloy. The symptoms of exposure were confined chiefly to the skin and

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respiratory tract. The former was characterized by dermatitis and skin ulcer, and accounted for 50% of all cases. The dermatitis, affecting exposed areas of the skin, was often severe, being of the edematous, papulovesivular type. When the face was involved, there was invariably associated conjunctivitis. An element of sensitivity was believed demonstrated. It was not uncommon to find individuals showing both dermatitis and bronchitis, especially if permitted to continue work in the areas of the sulfating process and fluoride furnace. Chemical pneumonitis presented the severest type of beryllium poisoning and occurred among workers without respect to type of process. Several deaths from this cause were reported in 1943, typical symptoms were cough, substernal pain, shortness of breath, cyanosis, anorexia, weight loss and increasing fatigue. Vital capacity was reduced. There was a low grade fever. Recovery did not occur if work was continued. Examination of lung tissue sections showed a large number of plasma cells, diffuse pulmonary edema and hemorrhage. X-rays of the lungs were diagnostic.

Previous to 1943 the plant had experienced relatively little trouble, but increase in intensity of operations with the outbreak of the war, together with loss of trained and selected personnel by the operation of the draft, may be listed as possible factors responsible for an epidemic effect.

The general plant history showed a shift in the number of cases through the years 1943 - 1946. During 1943 and 1944, the sulfate and beryllium-copper processes were particularly involved. Following improved conditions at this site, the production emphasis shifted to beryllium fluoride and beryllium metal, where at present, the greatest proportion of cases now appear (1946) The number of cases ranged from 63.5 to 238.1 per million man-hours of exposure, whereas the acceptable number in well-established chemical industries is 4.

Signed E. Lane Larkin

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ANALYSIS OF DUST AND FUME HAZARDS IN A BERYLLIUM PLANT 1)

Sidney Laskin, Robert A. N. Turner[2], Herbert E. Stokinger

In the fall of 1946, an invitation was extended to the industrial hygiene group at Rochester to visit a beryllium plant among whose personnel definite histories of beryllium poisoning were known. A survey of this plant (designated as Plant B) was therefore made with the primary purpose of providing information which would serve as a basis for toxicologic investigations of beryllium at Rochester. This survey included an examination of the plant operations, the medical history, types of beryllium poisoning characteristic of the plant personnel and a survey of the plant environment. This report is a summary of the results obtained and is divided into two parts. Part I is a review of the plant operations and its medical history. These are presented for the purpose of characterizing the types of beryllium poisoning and in order to indicate the major beryllium compounds involved in the reported case histories. The results reported are based upon records of the company health department and reports of the company physician and the district resident safety engineer, Mr. R. E. Turner. Part II is an analysis of the plant environment in terms of atmospheric dust concentrations, dust particle sizes, and types of substances involved in various operations, and are the results of the survey made by the Rochester group at Plant B.

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1) Acknowledgement is gratefully made to Dr. Harold C. Hodge, Head of the Division of Pharmacology, Atomic Energy Project and Professor of Pharmacology, School of Medicine and Dentistry, University of Rochester, for his helpful assistance and guidance in these studies.

2) Resident Safety Engineer, Madison Square Area, Atomic Energy Commission. Present Address: Mallinckrodt Chemical Company, Cleveland, Ohio.

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Part I. Plant Operations and Medical History of Beryllium Plant B.

A. Plant Operations. Plant B is engaged in the manufacture of beryllium metal from raw beryl ore and of alloys of copper and beryllium from copper and beryllium oxide. Table I lists the steps in these processes and shows the types of operations in each process, the beryllium compounds produced, and the major potential hazards at each of the operations. The general history of beryllium poisoning at Plant B is also indicated for each of the processes.

The raw beryllium ore is first processed by grinding and heat treatment and then converted to the sulfate with sulfuric acid. Following a purification of the sulfate, the oxide is then produced from this salt in a calcining furnace. Treatment of the oxide with acid fluoride results in the formation of the beryllium ammonium fluoride, which is then reduced to beryllium fluoride by furnace treatment. Further furnace treatment of the beryllium fluoride results in the final product, beryllium metal. The beryllium copper alloys are produced by direct mixing of beryllium oxide with copper, in the presence of carbon and required temperatures.

The general types of operations involved in the plant process include grinding, treatment in tanks, crystallizing in centrifuges, furnace heating, and furnace product casting. Each of these processes is a potential producer of atmospheric contaminants in the form of dusts, fumes, or mists. The major potential hazards include beryllium dusts and fumes present as the ore, metal, oxide, copper alloy, and the various fluoride compounds, and acid mists and fumes of beryllium sulfate. Also present in the metal production process are dusts and fumes of other fluoride compounds and gaseous hydrogen fluoride.

Table I. Processes and Potential Hazards in the Production of Beryllium Metal and Its Compounds

Process	Beryllium Compounds	Other Compounds Added	Major Potential Hazards	Health History
Ore melting	Raw beryl ore $3BeO \cdot Al_2O_3 \cdot 6SiO_2$		Ore dust SiO_2 fumes	1 hospital case of long duration (?)
Ore treatment	Processed ore $BeSO_4 \cdot 4H_2O$	H_2SO_4	Processed ore dust Acid mists and fumes ($BeSO_4$)	1 fatal case
Crystallizing	$BeSO_4 \cdot 4H_2O$	$(NH_4)_2SO_4$	Acid mists and fumes ($BeSO_4$)	Numerous respiratory and dermatitis cases
Beryllium Oxide Production	BeO	$(NH_4)_2S$	Fumes PbS, CuS, SO_3 Dusts BeO	Very minor dermatitis (inspectors)
Beryllium Copper Production	BeO BeCu	Cu, carbon	BeO, Cu, and BeCu Dusts and fumes	6 respiratory cases 3 fatal (?)
Beryllium Metal Production	BeO $(NH_4)_2BeF_4$ BeF_2 Be metal	Acid fluoride $(NH_4)_2S$	Acid fluoride $(NH_4)_2BeF_4$ mists Fumes and dusts of $(NH_4)_2BeF_4$; BeF_2 HF; NH_4F; Be metal; BeO	Serious dermatitis Many respiratory cases

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B. Medical History. According to the records of the company health department and reports of the company physician, 136 cases of acute beryllium poisoning were reported and observed from 1943 through 1946.

With the exceptions of some very minor dermatitis among inspectors working in oxide production and one respiratory case attributed primarily to silicon dioxide fume, no other reported history was found for these areas. The entire set of case history records was confined to the following areas: processes involving the treatment of beryllium sulfate, beryllium copper production, beryllium fluoride and beryllium metal production. Although all cases reported list specific compounds as causative agents, most of the records are not sufficiently detailed to permit this interpretation. In general little is reported beyond an area or specific operation in which the employee was engaged. The listing of the sulfate, copper alloy, and the metal, therefore, refers to the fact that these compounds were the predominant materials within the area of operations. Beryllium fluoride is used as a collective term to include other fluorides of beryllium such as ammonium beryllium fluoride complexes and beryllium oxyfluoride; it may refer as well to the areas of operations in which these compounds are produced.

Also indicated in Table I are chief types of occupational disease caused by beryllium compounds in Plant B. These are dermatologic and respiratory tract manifestations.

The dermatologic responses comprised contact dermatitis and skin ulcer which occurred in 68 or 50% of the reported cases. The contact dermatitis was often severe, occurring most frequently on the exposed regions of the body namely, the hands, arms, face, and neck. The eruption was an edematous papulovesicular dermatitis, which in cases involving the face, was invariably

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associated with a conjunctivitis. Although the skin lesion frequently was the only manifestation associated upper respiratory tract involvement was found in a large number of cases. All cases were exposed to either beryllium sulfate or beryllium fluoride atmospheres suggesting a somewhat specific etiology.

A definite element of sensitivity in the dermatitis can be shown. Approximately six of ten new employees in contact with the causative atmospheres develop varying degrees of contact dermatities. In most cases, the dermatologic manifestation appeared within from one to two weeks after employment. The severity of the dermatitis was dependent upon the individual's sensitivity and the length of contact with the atmospheres. Further evidence of the specificity of the suspected compounds can be seen in the fact that the dermatitis tended to disappear after removal of the individual from the suspected atmosphere. There was, however, a high percentage of recurrences on repeated exposure. Because a number of cases were also reported, in which individuals showing the dermatitis, were allowed to continue work and which eventually developed chemical bronchitis and pneumonitis, the occurrence of dermatitis in new workers, therefore, has been used in Plant B as a rough barometer of individual susceptibility to respiratory tract involvement.

The second type of skin manifestation, the beryllium ulcer, occurred in the same operations as those responsible for the dermatitis cases. The prerequisite for this condition appeared to be a small abrasion of the skin into which small crystals of beryllium fluoride or sulfate became embedded. There was a tendency of the surface layer of theskin to heal over the embedded crystal and form a small indurated papule. The papule underwent necrosis and finally formed a small abscess superficially apparent by a surrounding area of erythema.

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Microscopically, the ulcer showed typical tissue reactions to a foreign body with a definite increase of the surrounding epithelial layers and an increase in the numbers of fibroblasts surrounding the ulcer. In most instances a definite crystal fragment could be identified.

The ulcer occurred on exposed parts, particularly the forearm and hands, was discrete, and as a rule, single. It caused little distress unless infected or located near a joint. If neglected, the lesion persisted until traumatic extrusion of the inclusion center occurred. Healing did not occur until the crystal was removed by incision of the nodule and curettage of the fibrous base of the ulcer.

Respiratory tract manifestations were classified in the case histories according to the regions of the respiratory system involved. These include chemical nasopharyngitis, chemical tracheitis, chemical bronchitis and chemical pneumonitis. For purposes of this discussion, the tracheitis and bronchitis cases are collectively grouped as tracheobronchitis.

Chemical nasopharyngitis occurred principally among tenders of furnaces where beryllium sulfate was ignited to produce beryllium oxide and where beryllium fluoride was used in the production of pure beryllium metal.

The irritation of the nasal and pharyngeal mucous membranes varied in degree and was frequently associated with a dermatitis of the face. The chief complaint was soreness of the nose and throat associated with mild epistaxis, manifested by blood clots being blown from the nose. As a rule, cough was not present. There was a diffuse swelling of the mucous membranes with a considerable hyperemia. Epistaxis was secondary to vascular enlargement in the nasal mucosa. In some untreated cases, fissures occurred and persisted for two months. The patients were afebrile and chest and laboratory findings were negative.

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Chemical tracheobronchitis occurred from the same operations as the nasopharyngitis cases. It was characterized by cough, râles in both lungs, but by normal serial roentgenograms. The commonest complaint was the cough, nonproductive except for occasional blood-streaked mucoid sputum. Symptoms of nasopharyngitis were usually concurrent, and in addition some patients showed mild dyspnea, anorexia and weight loss. Râles, characteristically present in the early phase of inspiration over the lower lung fields, were fine early in the disease, later becoming coarse. Vital capacity was reduced. There was an occasional low grade fever; associated chill, however, never occurred. Complete rest was required for recovery. Continued work during this illness proved dangerous in either delaying recovery, or in a subsequent development of true pneumonitis.

Chemical pneumonitis represented the severest form of beryllium poisoning. It developed in workers on many different jobs under varying conditions. No single operation could be generally isolated as the source of exposure. Four deaths were reported in 1943, on which case histories are available for three. The findings showed that the pneumonitis progressed either to complete recovery or to death. Typical symptoms were cough with occasional blood-streaked sputum, substernal burning pain, shortness of breath, cyanosis in most cases, abnormal taste, anorexia with some weight loss, and increasing fatigue. The dominant physical findings were rapid pulse, râles in both lungs, and a reduced vital capacity. Signs of infection were conspicuously absent. The temperature was not greatly elevated except in the terminal states of the fatal cases. Erythrocyte sedimentation rate was essentially normal. Blood counts and chemical findings were also generally normal.

Roentgenologic changes in the lung fields did not usually appear until two or three weeks after the onset of symptoms and physical signs. Changes

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were bilateral and diffuse in all cases and varied with the severity of the disease. In order of appearance the changes were: (1) diffuse haziness of both lungs, (2) development of soft irregular areas of infiltration with prominence of peribronchial markings. In the terminal cases, further signs of consolidation appeared. In those cases which recovered, there was an absorption of the soft infiltration and the appearance of discrete conglomerate nodules scattered throughout both lung fields. This was followed by clearing of the lung fields after from one to three months. As a rule clearing of the lungs on roentgenologic examination occurred before complete subsidence of symptoms or disappearance of all physical signs.

Necropsies on several cases showed a typical pneumonitis. The pathological findings were similar. Significant changes were found only in the lungs. The tissue sections showed large numbers of plasma cells, the relative absence of polymorphonuclear infiltration, diffuse pulmonary edema, and hemorrhage. Fibroblasts with evidence of organization were present, although no fibrosis was found on roentgenologic examination of recovered patients.

Table II shows the history of beryllium poisoning at Plant B as correlated with the principal compounds involved in the respiratory and dermatologic disorders. Tables III-V present an analysis of the plant medical records showing the relation of the compounds to the case frequencies. This analysis cannot be considered entirely accurate because the available records were incomplete with respect to individual occupations, the total number and sex of the personnel exposed and time-production data for each of the operations. In addition, several of the case histories represented recurrences. The analysis, therefore, is presented as a guide in describing the extent of the beryllium hazard at the plant.

1946

Table II. History of Beryllium Poisoning at Plant B
(Based on Available Records of Plant B Health Department)

Predominant Compound	Manifestations of Beryllium Poisoning	Year	No. of Cases			Range of Ages	Range of Service	Range of Time Lost
			Total	Sex M	Sex F	Years		Days
Beryllium Sulfate	Dermatologic	1943	8	6	2	16-58	2-12 days	2-20
		1944	6	5	1	21-45	3-14 days	6-25
		1945	8	8	0	24-64	4-14 days	5-19
		1946	4	2	2	27-35	12-42 days	7-19
Beryllium Sulfate	Respiratory	1943	22	14	8	18-66	4 day-6½ yr	2-120 and 1 fatal
		1944	6	3	3	19-45	10day-1½ yr	4-91
		1945	8	8	0	33-54	1 mo-3 yr	8-90
		1946	0	0	0			
Beryllium Fluoride	Dermatologic	1943	5	3	2	17-41	8-42 days	3-27
		1944	2	2	0	17-61	9 days-6½mo	6
		1945	16	15	1	21-55	6-77 days	5-32
		1946	19	19	0	18-56?	6-61 days	0-20
Beryllium Fluoride	Respiratory	1943	6	6	0	24-41	7 days-14 yr	3-103
		1944	1	1	0	47	34 days	150
		1945	9	9	0	21-65	3 wk-3½ yr	8-60
		1946	7	7	0	22-29?	9 days-14 yr	7-19
Beryllium Copper Alloy	Dermatologic	1943 to 1946	0	0	0			
Beryllium Copper Alloy	Respiratory	1943	4	4	0	18-48	1-10 mo	4-20 and 2 fatal?
		1944	2	2	0	41-47	2 mo-7½ yr	15-124
		1945	0	0	0			
		1946	0	0	0			
Beryllium Metal	Dermatologic	1943 to 1946	0	0	0			
Beryllium Metal	Respiratory	1943	0	0	0			
		1944	0	0	0			
		1945	0	0	0			
		1946	3	3	0	25-29	5 wk-5½ yr	6-14

1946

In Table II four beryllium materials compose the list of substances implicated in the entire number of 136 reported cases; beryllium sulfate, beryllium fluoride, beryllium metal, and the beryllium copper alloy. During the 4-year period reported, case histories of respiratory tract manifestations were imputed to all four substances. To only the sulfate and fluoride operations, however, were ascribed cases with both respiratory tract and dermatologic manifestations.

The general plant history shows 45 cases for 1943, a large number for a plant this size, during which time 3 reported respiratory fatalities occurred (1 in sulfate and 2 in beryllium copper production). Although a definite decrease is shown for 1944 with only 17 cases reported, the incidence apparently returned to the 1943 level in 1945 and 1946 with 41 and 33 cases reported respectively. During this period, enclosures of various operations (particularly the sulfate) were made by the plant management. The effects of these and an increased medical vigilance by the company health department are reflected in the absence of cases in the beryllium-copper production after 1944 and in the decrease of respiratory cases in the sulfate operations. Beryllium fluoride cases however, accompanying an increase in production, showed a definite increase reaching the proportions of 26 out of 36 reported cases in 1946. The cases attributed to beryllium metal appear only in 1946. (See Table V). The severity of the disease in individual cases decreased during the reported period. This is indicated by the relative ranges of time lost given in Table II.

The range of ages of the workers, also shown in Table II, appeared to be usual for this type of industrial worker with an average range of between 20 and 50 years. The periods of service show that the average exposure for the acute respiratory manifestations were from one to four months. Several of the respiratory cases showed manifestations as early as from 3 to 10 days after

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exposure, indicating possible allergic responses. The dermatologic responses appeared within an average exposure period of from 7 to 10 days with the earliest reported cases appearing in from 2 to 3 days.

Sex differences between the case histories and types of manifestations could not be completely analyzed because records of the number of males and females engaged in each operation were not available. Incomplete records on case histories also prevented an analysis in terms of specific occupations. The types of occupations listed among the case histories include diverse categories as; process operators, furnace tenders, machinists, foremen, construction workers, maintenance help and electricians.

Table III, showing the case frequencies of beryllium poisoning annually at the plant, also presents a partial analysis with respect to sex. The results are given in terms of percentage frequencies and in terms of the rate per million hours of exposure. Since the plant had an exceptionally high labor turnover, the total number of individuals exposed could not be determined. All frequencies are, therefore, based upon average total monthly numbers of employees and total man-hours of exposure determined from payroll records.

For the entire 4-year period of the 136 reported cases of beryllium poisoning, 117 or 86% were males and 19 or 14% were females. The average number of employees during this period was 112.8 per month of which 79.3% were males and 20.7% were females. Examination of the yearly record in Table III shows that, although the above figures indicate no significant difference between the sexes, the female percentage frequency rate was approximately twice that of the male in 1943. For the periods from 1944 through 1946, the male percentage frequency rates were significantly higher

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Table III. Case Frequencies of Beryllium Poisoning
In Plant B

Year	No. Cases			Av. No. Cases Per Month			Av. No. Employees Per Month			Av. Monthly Exposure Man-hours	Case Frequency % Employees			Case Frequencies 10^6 Exposure Hr. Total Yearly Cases
		Sex			Sex			Sex				Sex		
	Total	M	F	Total	M	F	Total	M	F		Total	M	F	
1943	45	33	12	3.75	2.75	1.00	151	126	25	28,276.9	2.48	2.18	4.00	132.6
1944	17	13	4	1.42	1.08	0.33	119	89	30	22,352.7	1.19	1.21	1.10	63.5
1945	41	40	1	3.42	3.33	0.08	113	89	24	19,792.3	3.03	3.74	0.33	172.8
1946	33	31	2	2.75	2.58	0.17	68[a]	54[a]	14[a]	11,548.5[a]	4.04	4.78	1.21	238.1

a. Based on 11-month period (January-November).

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than those of the female. Further study of the 1943 cases indicate the higher female rate to be primarily among sulfate employees showing respiratory manifestations.

During the period studied, the average monthly number of employees show a definite decrease from 151 in 1943 to 68 in 1946. Corresponding with these values was an almost identical decrease in monthly exposure hours of from 28,276.9 in 1943 to 11,548.5 in 1946. Despite the decrease in employees and exposure hours, the case frequencies showed significant increases. The maximal percentage case frequency was 4.04% of the total employees or a maximal value of 238.1 cases per million hours of exposure in 1946. The yearly averages of the frequencies in terms of exposure hours range from 63.5 to 238.1 accidents per million man-hours compared with the standard accepted level of 4.0. Such values characterize a definitely hazardous plant.

Table IV shows the percentage case frequencies of the chief manifestations of the occupational disease at Plant B. For the entire period, the average number of cases per month (1.42) and the total number of cases (68) were the same for both the dermatologic and the respiratory tract disorders. The percentage frequency values show a significant increase in the dermatologic cases during the reported period, with values of 0.72% and 0.56% obtained in 1943 and 1944, and 2.82% obtained in 1946. For the respiratory tract manifestations, only a slight decrease is evident. The highest value of 1.77% was obtained in 1943 corresponding to values of 1.26% and 1.22% in 1945 and 1946. A significantly lower value of 1.63% was obtained in 1944.

Table V shows the case frequencies considered to arise from exposure to the beryllium substances. The results illustrate the points made with respect to these substances under the general discussion of Table II. For the entire

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Table IV. Percentage Case Frequencies of Beryllium Poisoning in Terms of the Principal Occupational Disease Manifestations

Year	D E R M A T O L O G I C			R E S P I R A T O R Y		
	Total No. Cases	Av. No. Cases per month	Case Frequency in % Employees	Total No. Cases	Av. No. Cases per month	Case Frequency in % Employees
1943	13	1.08	0.72	32	2.67	1.77
1944	8	0.67	0.56	9	0.75	0.63
1945	24	2.00	1.77	17	1.42	1.26
1946	23	1.92	2.82	10	0.83	1.22

Table V. Correlation of Case Frequencies of Beryllium Poisoning With Beryllium Compounds

Compound	Year	TOTAL NO. CASES		DERMATOLOGIC		RESPIRATORY	
		Av. No. Cases Per Month	Case Frequency % Employees	Av. No. Cases Per Month	Case Frequency % Employees	Av. No. Cases Per Month	Case Frequency % Employees
Beryllium Sulfate	1943	2.50	1.66	0.67	0.44	1.83	1.22
	1944	1.00	0.84	0.50	0.42	0.50	0.42
	1945	1.33	1.18	0.67	0.59	0.67	0.59
	1946	0.33	0.49	0.33	0.49	0.00	0.00
Beryllium Fluoride	1943	0.92	0.61	0.42	0.28	0.50	0.33
	1944	0.25	0.21	0.17	0.14	0.08	0.07
	1945	2.08	1.84	1.33	1.18	0.75	0.67
	1946	2.17	3.19	1.58	2.32	0.58	0.86
Beryllium Metal	1946	0.25	0.37	0.00	0.00	0.25	0.37
$BeCu$ Alloy	1943	0.33	0.22	0.00	0.00	0.33	0.22
	1944	0.17	0.14	0.00	0.00	0.17	0.14

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period the average monthly percentage frequency indicates the following relative classification of the operations from the most hazardous to the least hazardous: beryllium fluoride, 1.46%; beryllium sulfate, 1.04%; beryllium metal, 0.37%; and the beryllium copper alloy, 0.18%.

Further analysis of respiratory and dermatologic manifestations indicates a difference between those caused by sulfate and fluoride. For the dermatologic manifestations, the average frequency obtained with the fluoride is higher than that obtained for the sulfate. This is similar to the total fluoride and sulfate frequencies with values of 0.98% and 0.49% respectively. In the case of the respiratory manifestations, however, the sulfate frequency was 0.55%, or slightly larger than the fluoride frequency of 0.48%.

Part II. Survey of the Plant Conditions. A survey of the environment of plant B was made by members of the Industrial Hygiene Section, Rochester Atomic Energy Project in December, 1946*. In addition to the primary purpose of providing information for toxicologic work at Rochester, this surwy had several objectives. These included the characterization of known hazardous areas of the beryllium plant in terms of atmospheric dust and fume concentrations and particle size, 2) the procurement of various samples of industrial materials for subsequent development of methods for recovery and analysis and 3) the testing of various types of atmospheric sampling devices for field use and the determination of their relative value when applied to beryllium industrial hygiene problems.

At the plant a preliminary study was first made of the nature of each of the various operations. The most hazardous dust and fume areas in

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* Additional personnel aiding in the collection of samples: Dr. F. Bryan and Mr. R. Wilson, Engineering Department, University of Rochester, Atomic Energy Project.

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operation at the time were selected for sampling. These included the beryllium metal and the beryllium fluoride furnaces. The ore treatment area was also selected, despite the lack of a history of toxic beryllium exposure in this area, because our inspection showed an extremely dusty area and suggested a future potential hazard at this site.

The Dust and Fume Survey. Samples of the atmospheres present in and near the selected areas were taken by means of each of the following methods: the Modified Cascade Impactor, the Filter Paper Dust Sampler, and the U.S.A. Midget Impinger [1]. The samples collected with the Modified Cascade Impactor and the Filter Paper Dust Sampler were taken at a sampling rate of 14 liters per minute. Those collected with the Midget Impinger were taken at a sampling rate of 0.1 ft^3 per minute. Most of the collected samples were analyzed chemically

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(1) Pharmacology and Toxicology of Uranium Compounds, Chapter X, Atomic Energy Project, University of Rochester, Rochester, N.Y., In press.

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for beryllium using the spectrographic method of analysis (Dr. L. Steadman).[2] Recoveries of beryllium from the samples were made by methods developed by Dr. W. Neuman and Mr. M. Cucci[2]. Several of the samples collected in each area were analyzed for fluorine content by Dr. F. Smith [2] using a modification of the Willard and Winter distillation and thorium alizarin titration.

The results obtained for each of the areas surveyed are given in Tables VI-VIII in terms of concentrations of beryllium or fluorine per cubic meter of air. Table IX shows the particle-size mass distributions obtained with the Modified Cascade Impactor at each of the areas.

Beryllium Metal Furnace. As shown in Table VI three operational phases of the beryllium metal furnace were sampled. These included the pouring period, the coke-removal period, and the immediate period after operations. All of the samples taken during the pouring and coke-removal periods except for samples No. 5, 6, and 14 were taken from positions of from 3 to 6 feet in front of the furnace opening. These sampling positions corresponded to the ones in which the men engaged in the operation were working. During the pouring operations, the Filter Paper and the Cascade Impactor samples showed a range of beryllium concentrations varying from 1.43 to 4.71 mg/m^3. The impinger sample corresponding to these samples yielded a significantly lower concentration value of 0.293 mg Be/m^3. The beryllium air concentrations decreased during the coke-removal period as indicated by values of 0.110 mg/m^3 obtained with the Filter Paper Sampler and 0.533 mg/m^3 obtained with the Cascade Impactor. The impinger also showed this decrease and also significantly lower concentrations than the other methods; a range of values of from 0.059 to 0.082 mg/m^3 were found.

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(2) Atomic Energy Project, University of Rochester, Rochester, New York.

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Table VI. Air-Dust Concentrations at Beryllium Metal Furnace of Plant B

Sample No.	Phase of Operation	Sampling Position	Time Hrs. p.m.	Sampling Method*	Sampling Time mins.	Sample Analysis Total in 10^{-3} mg Be	Sample Analysis Total in 10^{-3} mg F	Air Concentration mg/m³ Be	Air Concentration mg/m³ F
1	Touring	3-6 feet in front	9:00 -	Ip	6	5.0	-	0.293	-
2	"	of furnace opening	9:15	Fp	5	100.0	-	1.43	-
3	"	" "	"	Fp	5	330	-	4.71	-
4	"	" "	"	CI	45	328	-	1.56	-
5	"	Over top of the	"	Ip	10	50.0	-	1.76	-
6	"	furnace	"	Ip	4	-	18.7	-	1.65
7	Coke removal	3-6 feet in front							
8	"	of furnace opening	9:15-9:30	Ip	4	0.8	-	0.070	-
9	"	" "	"	Ip	4	0.7	-	0.062	-
10	"	" "	"	Ip	6	1.0	-	0.059	-
11	"	" "	"	Ip	4	0.7	-	0.062	-
12	"	" "	"	Ip	4	-	12.9	-	0.757
13	"	" "	"	Fp	10	1.5	-	0.110	-
14	"	" "	"	CI	15	172	-	0.533	-
15	"	10 ft from door	"	Fp	10	-	54.3	-	0.387
25	After	of furnace in	9:30-10	Fp	25	-	12.8	-	0.062
26	operations	outer room	"	CI	30	2.5	-	0.006	-

* Ip - Midget Impinger
Fp - Filter Paper Dust Sampler
CI - Cascade Impactor

Correspondingly the impinger values analyzed for fluoride showed a comparatively high value of 0.757 mg/m^3. Two impinger samples were taken during the pouring phase in a position over the top of the furnace. Sample No. 5 analyzed for beryllium yielded 1.76 mg/m^3; sample No. 6 analyzed for fluoride 1.65 mg/m^3. A filter paper sample taken during the coke removal from a position 10 feet from the door of the furnace room showed a fluoride concentration in the same range as that obtained within the furnace room (0.387 mg/m^3). A similar sample taken in the immediate period after operations indicated a decrease in fluoride concentration to a value of 0.061 mg/m^3.

All of the particle-size mass distributions obtained within this area showed an atypical form of distribution indicating a mixture of dust and fume sizes. The size-mass medians and 90%-size limits given in Table IX show similar values obtained for the two phases of the operation. Median values of 0.38 micra and 0.84 micra were obtained at the pouring and coke-removal operations. The corresponding 90%-size limits were 1.72 and 1.12 micra respectively. For the period after the operation in the area of the room outside the furnace room, larger particle sizes were found. A mass median of 1.47 micra and a 90%-size limit of 5.8 micra were obtained.

Beryllium Fluoride Furnace. As shown in Table VII , two operational phases of the beryllium fluoride furnace were sampled. These included the pouring period and the period immediately after operations. Starting from positions approximately 3 feet in front of the furnace opening, samples were taken during the pouring period at varying distances extending into the outer alley of the furnace room to approximate the area occupied by the men engaged in this operation. In the case of this area, the impinger samples gave higher values than those collected with the Filter Paper Dust Sampler. Samples collected with the Cascade Impactor were in the approximate range as those collected with the impinger. Concentrations ranging from 0.05% to 0.070 mg Be/m^3 were obtained

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Table VII. Air-Dust Concentrations at Beryllium Fluoride Furnace of Plant B

Sample No.	Phase of Operation	Sampling Position	Time Hrs. p.m.	Sampling Method*	Sampling Time mins.	Sample Analysis Total in 10^{-3} mg Be	Sample Analysis Total in 10^{-3} mg F	Air concentration mg/m³ Be	Air concentration mg/m³ F
1	Pouring	3 feet from furnace opening	20:45 - 11:00	Ip	3	0.6	-	0.070	-
2	"	" "	"	Ip	3	0.5	-	0.059	-
3	"	" "	"	Ip	3	0.7	-	0.082	-
4	"	" "	"	Ip	3	-	499	-	58.2
5	"	6-8 feet from furnace opening	"	Fp	5	0.6	-	0.009	-
6	"	" "	"	Fp	10	0.3	-	0.002	-
7	"	" "	"	Fp	5	-	160.3	-	2.29
8	"	" "	"	CI	15	5.9	-	0.026	-
9	"	Outer alley 15 ft from doorway to furnace room	"	Ip	60	0.4	-	0.023	-
10	"	"	"	Ip	5	0.2	-	0.014	-
11	"	20 feet over top of furnace	"	Ip	6	-	1092	-	64.1
12	"	10 feet from furnace mouth	23:00 - 11:30	CI	30	4.7	-	0.011	-
13	Immediately after pouring	In outer alley	"	CI	30	-	89.6	-	0.32

* Ip - Midget Impinger
Fp - Filter Paper Dust Sampler
CI - Cascade Impactor

at a position 3 feet in front of the furnace opening. These decreased in areas away from this position to value of 0.01^{4} mg Be/m^3 in the outer alley 15 feet from the doorway to the furnace room. The two filter paper samples at positions 6-8 feet from the furnace opening showed very low values of from 0.002 and 0.009 mg/m^3.

The fluoride concentration in this area was of the order of one thousand times that of beryllium. A value of 58.2 mg/m^3 was obtained for an impinger sample collected in the 3-foot position where Be concentrations ranged from 0.059 to 0.082 mg/m^3. Corresponding to the low filter paper beryllium samples at 6 to 8 foot position, the filter paper sample analyzed for fluoride also showed a relatively high fluoride value (2.29 mg/m^3). An impinger sample taken from a position 20 feet above the furnace yielded a value of 64.1 mg fluoride/m^3 which is of the same order as the value obtained in front of the furnace. Sample No. 13 taken in the area of the outer alley immediately after the pouring operation shows the lowest fluoride value obtained. This value of 0.32 mg/m^3 however, is still larger than any of the beryllium concentrations obtained.

Both of the particle-size mass distributions obtained during the pouring operation showed normal types of distributions when analyzed as beryllium. As shown in Table IX similar results were obtained in both cases, the mass medians were 2.60 and 2.32 micra. Corresponding geometric standard deviations were 2.27 and 2.2^{4}. The sample taken immediately after operations and analyzed in terms of fluoride show this material to be present largely in the form of extremely small sizes. An atypical distribution was obtained in which the mass median size indicated a value below 0.1 micra. The 90% size limit for this sample was found to be 1.5 micra.

Beryllium Ore Treatment. The results obtained during the period of operation of the rotary kiln drier are given in Table VIII in terms of beryllium and fluoride-air concentrations. Samples were collected at varying positions in this area ranging from 1½ feet in front of the discharge end of the kiln to the elevated ramp on the far side of the area. Except for sample No. 8, all impinger samples collected in this area indicated lower values of beryllium concentrations than corresponding values obtained with the Cascade Impactor and the Filter Paper Dust Sampler. In terms of fluoride concentration, however, the impinger samples gave higher values than those obtained with the other methods. The beryllium dust concentration showed a variation of from 0.050 to 0.528 mg/m^3 over the entire area with the highest concentration indicated in the region of the elevated ramp. The fluoride-air concentrations were of the same order of magnitude as the beryllium dust concentrations, ranging from 0.031 to 1.17 mg/m^3. The results showed the lowest value present in the immediate vicinity of the rotary kiln increasing in the direction of the elevated ramp.

Normal distributions were obtained for particle-size samples taken in this area. As shown in Table IX the mass median sizes obtained were very large ranging from 2.95 to 10.0 micra, corresponding geometric standard deviations ranged from 2.08 to 2.57.

Analysis of Beryllium Ores. During the plant survey considerable interest was aroused in the group by the fact that one of the varieties of beryl ore used in the plant processes was suspected of containing uranium or other radioactive substances. According to available records, at least three varieties of beryl ore had been used in the period from 1943 to 1946. Approximately 30% of the total ore used during 1946 was of the suspected variety.

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Table VIII. Air-Dust Concentrations at Beryllium Ore Treatment of Plant B

Sample No.	Phase of Operation	Sampling Position	Time Hrs. a.m.	Sampling Method*	Sampling Time Mins.	Sample Analysis Total in 10^{-3} mg Be	Sample Analysis Total in 10^{-3} mg F	Air concentration mg/m^3 Be	Air concentration mg/m^3 F
1	Rotary kiln	1½ ft in front	10-11	Ip	4	1.0	-	0.086	-
2	during operation	of discharge end	"	Ip	15	-	1.3	-	0.037
3	" "	of kiln	"	Fp	5	10.0	-	0.143	-
4	" "	6 ft to side of	"	Ip	4	1.4	-	0.123	-
5	" "	discharge end of	"	Fp	6	15.0	-	0.179	-
6	" "	kiln	"	CI	15	64.6	-	0.307	-
7	" "	6 ft at 45° angle from neck of kiln	"	CI	15	43.5	-	0.207	-
8	" "	12 ft to side of discharge end of kiln	"	CI	15	10.5	-	0.050	-
9	" "	20 ft to side of	"	Ip	15	-	50.0	-	1.37
10	" "	discharge end of kiln	"	Fp	15	-	74.8	-	0.765
11	" "	Elevated ramp	"	Ip	4	2.0	-	0.172	-
12	" "	to far side of room	"	Ip	4	6.0	-	0.526	-

* Ip - Midget Impinger
Fp - Filter Paper Dust Sampler
CI - Cascade Impactor

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Table IX. Particle-Size Mass Distribution* at Plant B

Operation	Phase	Sample No.	Sampling Position	SIZE-MASS DISTRIBUTION				
				Major Component	Median Size	Geom. St'd Deviation	Type of Distrib.	90% Size Limit
					Microns	σ_g		Microns
Beryllium Metal furnace	Pouring	4	3-6 ft in front of furnace	Be metal	0.85	-	Atypical	1.72
	Coke-removal	13	opening	"	0.63	-	"	1.12
	After-operation	16	10 ft from door of furnace in outer room	"	1.47	-	"	5.8
Beryllium fluoride furnace	Pouring	5	6-8 ft from furnace opening;	BeF_2	2.60	2.27	Normal	-
	"	12	10 ft from furnace mouth	"	2.32	2.24	"	-
	Immediately after pouring	15	in outer alley	"	Below 0.1	-	Atypical	1.5
Ore treatment	Rotary kiln during operation	6	5 ft to side of discharge end of kiln	Beryl ore	7.73	2.44	Normal	-
	"	7	5 ft at 45° angle from neck of kiln	"	2.95	2.08	"	-
	"	8	12 ft from discharge end of kiln	"	30.0	2.57	"	-

* Determined with the Modified Cascade Impactor

Spot samples of each of the types of ores were secured for radioactivity and chemical analyses. In order to determine the possible atmospheric contaminants resulting from use of these ores, a quantity of settled dust found in the ore processing area was also removed for analysis.

A preliminary test for radioactivity was made by placing the ore specimens over a sheet of x-ray film protected by a 1/16 inch cardboard envelope. After exposure for 22 hours a definite autoradiograph was produced with the suspected ore. No traces of radiation were detectable in the other ore specimens.

The ore specimens and the settled dust specimen were analyzed for beta activity by Mr. R. Hayes (3). Table X shows the results obtained in terms of percent of uranium by weight necessary to produce equivalent beta activity. Results are also given for spectrochemical analyses of each sample (Dr. L. Steadman(3)

In terms of beta activity the suspected ore type (Beryl ore A) only showed a significant amount of radioactivity. All other samples showed values low enough to be within the background count or error of the analytical method.

The spectrographic analysis consisted of two types; a rough qualitative analysis and an accurate, quantitative analysis of the important constituents. For the qualitative analysis all samples showed the presence of beryllium, aluminum, silicon, and small traces iron. None of the samples showed any detectable amounts of arsenic, cadmium, mercury, or thallium. Although the beryl ore sample A and the settled ore dust both showed the presence of lead, only the ore sample A contained uranium and other radioactive materials.

The quantitative spectrographic analysis of all samples showed the bery-

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(3) Service Division, Department of Radiology, Atomic Energy Project, Rochester.

Table X. Analysis of Beryllium Ore Samples at Plant B

Sample	Description of Sample	% Uranium by weight necessary to produce equivalent beta activity	Spectrographic Analysis
Beryl ore A*	Irregular crushed rock fragments and crystals, light earth brown streaked with yellow "rust".	12.6	Qual. - Be, Al, Si Traces Fe Quant.- 5.0% Be 4.0% U 0.4% Th 0.4% Pb
Beryl ore B	Irregular crushed rock fragments and crystals, pale translucent blue streaked with white quartz (pure beryl)	Less than 0.004%	Qual. - Be, Al, Si Traces Fe Quant.- 4.8% Be
Beryl ore C	Irregular crushed rock fragments and crystals, mixed light earth brown and pale green, infiltrated with quartz and mica	Less than 0.004%	Qual. - Be, Al, Si Traces Fe Quant.- 5.0% Be
Settled ore dust (Processing room)	Light earth brown showing many quartz-like crystals. About 200 mesh size	Less than 0.006%	Qual. - Be, Al, Si Traces Fe Quant.- 4.8% Be 0.03% Pb

* Ore suspected of containing radioactive materials

llium content to be approximately the same 4.5 - 5.0%. The analysis of the beryl ore sample A showed the presence of 4.0% uranium, 0.4% each of thorium and lead. The lead content of the settled ore dust was insignificant with a value of only 0.03%

Discussion

The most hazardous of the three areas surveyed were those of the beryllium metal furnace and the beryllium fluoride furnace. Since these operations represent short exposure periods of the order of 0.5 hour or less, the results obtained in this survey indicate that very low levels of beryllium-air concentrations may be toxic.

The relatively high fluoride concentrations obtained in the surveyed areas are of particular significance since they may represent a hazard by themselves and also suggest a combined action with beryllium. Further study of this factor is required especially in the case of the beryllium fluoride furnace where the relative fluoride concentration was 1000 times that of beryllium.

The results obtained show that for an industrial hygiene survey involving beryllium several types of sampling devices are required. For the insoluble beryllium dust, the Filter Paper Dust Sampler and the Cascade Impactor gave better concentration values than the Midget Impinger. In the case of fluorine concentration and the soluble beryllium fluorides, however, the results indicate better values were obtained with the Midget Impinger. Excellent particle-size results were obtained with the Cascade Impactor; however, several preliminary samples taken on glass slides in the furnace areas indicated the presence of many fume sizes and aggregates of small particles. This indicates the necessity of further study of such areas using an instrument such as the thermal precipitator.

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The presence of radioactive materials showing the high beta activity characteristic of beryl ore Type A indicate a considerable radon and thoron hazard. The absence of this activity in the processed ore dust suggests that the radioactive materials are removed during the first operations (crushing, and preliminary heat treatment) or that ore of the type considered was not being processed during the period of the December , 1946 survey. An additional point of interest is that no specific history of poisoning has been reported in the pre-processing area. These results suggest the possibility of uranium and other radioactive materials acting synergistically or predisposing the cases of acute beryllium poisoning reported. Further study of this aspect of the problem is indicated.

signed Sidney Laskin
Sidney Laskin

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A REVIEW OF ANALYTICAL METHODS FOR BERYLLIUM

William F. Neuman

1947

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A REVIEW OF ANALYTICAL METHODS FOR BERYLLIUM

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ABSTRACT

Procedures for the analysis of biological specimens for content of beryllium are discussed. Two colorimetric, one fluorescent, and the spectrographic methods are presented, together with data on the accuracy, sensitivity, and specificity of each.

The advantages of and experimental procedures for the use of beryllium isotopes are also reviewed.

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A REVIEW OF ANALYTICAL METHODS FOR BERYLLIUM

In this review, analytical procedures for beryllium are to be considered, with emphasis on the analysis of biological specimens. In dealing with biological materials, there are a number of limitations which have to be considered before any analytical procedure can be critically evaluated.

In the first place, the LD50 is important in determining the sensitivity required of any analytical procedure. Beryllium administered intravenously is very toxic; the LD50 is approximately 0.36 mg/kg. Thus, for distribution and excretion experiments, not more than 0.2 mg/kg can be administered. At such a dose level, many tissues may be expected to contain 0.1 /^{u}g/g or less. Thus, any procedure, to be useful, must detect with accuracy, much less than one microgram.

Secondly, since there are a host of other elements present in any biological sample, the method must be highly specific and not subject to interference by relatively large proportions of similar and dissimilar elements.

Thirdly, since the high order toxicity of beryllium requires that ultra micro techniques be applied, rigorous precautions must be exercised in all phases of the study to insure against possible contamination.

Preparation of Samples

One of the technical difficulties attending the analysis of beryllium samples is the volatility of the chloride of beryllium. No conceivable chemical method can be expected to give reliable results unless all organic matter is first removed. In our experience, temperatures as high as 600°C

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are required to give complete ashing of most tissues. $BeCl_2$ boils at temperatures over 400°C. Since all tissues contain fair amounts of chloride, varying quantities of beryllium will be lost in ashing. Urine is particularly troublesome in this respect.

Two alternative schemes for ashing are available. Samples may be wet-ashed in sulfuric acid (Kjeldahl procedure); in this method all chlorides are distilled off before the temperature rises above the volatility of $BeCl_2$. Although this procedure has not, as yet, been tested in our laboratory, no technical difficulties are to be anticipated. In general, wet-ashing techniques are tedious. Accordingly, a procedure developed in our laboratory offers some advantages. The sample is placed in a platinum crucible together with 2 g of sodium pyrophosphate, and placed in a muffle furnace. The temperature is raised slowly and left at 400°C until all carbonacious material is burned off. The samples can be dissolved with the aid of heat in small volumes of 2N HCl.

Analytical Methods

Two chemical, one fluorescent, and the spectrographic methods of beryllium have been studied:

1. Colorimetric
   References: Cucci, M., Monthly Reports, 1947, Rochester
   Fairhall, Bull. 181, Nat'l Inst. of Health

The method of Fairhall (NIH Bulletin 181) was studied in detail. Several difficulties were encountered with the procedure as reported by Fairhall. The beryllium sample was mixed with 1,4 dihydroxy anthraquinone, 2 sulfonic acid in ammonium acetate buffer at approximately pH 7.0. A red color is produced in contrast to the yellow blank. pH control was found

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to be critical and, since it is a well-known fact that the ammonium acetate does not buffer at pH 7.0, histidine hydrochloride was substituted. Heating appeared to necessary for full color development. Spectrophotometric analysis of the color of the blank and a beryllium sample showed that maximum sensitivity is obtained if light of a wave length of 575 millimicrons is employed. The limit of detection is of the order of 0.5 /ug. The standard error is 12%. Many substances interfere, among which are F, Fe, PO_4, and Al.

2. Colorimetric

Reference: Kosel, G., and Neuman, W., Final Report M-1965, Rochester

Another procedure, developed in our laboratory, utilizes aluminon (aurin tricarboxyllic acid) as a color reagent. Dr. Rothstein had noticed that beryllium interfered in the determination of aluminon. Further study showed that this reaction could serve as a method with some advantages and some disadvantages, as compared with the Fairhall procedure. Beryllium is mixed with aluminon at pH 7.5 in ammonium chloride buffer. The color produced is read against light of 535 millimicrons wavelength. The limit of detection is 1 /ug. The standard error is about 10%, and again many substances interfere, particularly Fe, Al, Pb, Ca, Cu, and Mn. Po_4 and F in excess (10 or 100 to 1 moles) also interfere.

3. Fluorescent

References: Underwood, A., Carlson, A. and Neuman, W., Final Report M-1951, Rochester.

Fairhall, Bull. 181, Nat'l Inst. of Health

Fairhall (NIH Bulletin 181) also reported a fluorescent procedure for the analysis of beryllium samples. Again it was difficult to duplicate

Fairhall's reported results. Variations in the ratio of dye to beryllium and pH was observed to have pronounced effect on the fluorescence produced by a given quantity of beryllium. Beryllium was mixed with 1 amino, 4 hydroxy anthraquinone in diethyl amine buffer at 11.3. The fluorescence was read on a sensitive fluorimeter built in our laboratory. The limit of detection is about 0.5 /ug. The standard error about 12% and again many substances interfere, principally Ca, Mg, Fe, Cu, and Mn. PO_4, F, and other common anions offered no problem--an advantage of this method.

4. Spectroscopic

Dr. Steadman has investigated the spectrographic method for beryllium analysis. As might be expected, beryllium, having a simple emission spectrum, is an element which is particularly suited to spectrographic analysis. Without the need of special sensitizers, very high sensitivity can be attained. Employing fairly standard techniques--a commercial Bausch and Lomb spectrograph, DC carbon arc with either platinum or iridium as an internal standard--as little as 0.005 /ug can be detected (2348 Å). By shifting to another line (2494) greater amounts can be quantitated, up to 10 /ug. The biggest drawback is error which, for a single determination, probably is of the order of 25%. No chemical separation is necessary since beryllium distills with difficulty and other elements do not materially interfere.

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Neuman, W.

Radioisotope

There are two radioactive isotopes of beryllium which might be useful: Be_{10} and Be_7. The principal difficulty in the use of both of these is that of obtaining samples of sufficient activity. Beryllium has a small "cross-section" and therefore requires long bombardment time. For our purposes, Be_7 was more useful. This isotope is produced principally by the bombardment of Li by protons. Since it is a transmutation reaction, pure isotopic beryllium is obtained and the sample may be diluted with inert carrier beryllium to give the desired Specific Activity. Be_7 is a gamma-emitter. Although no gamma counting tubes are commercially available, almost any type of G. M. tube will register gamma radiation. In our laboratory, the immersion type of tube designed by Bale proved to be more efficient than the popular mica-window type of tube.

In brief, the essential details of the radioactive technique of studying beryllium are as follows:

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1. Li target is bombarded with a proton beam for about 6 hours in the University of Rochester cyclotron.

2. Sample is dissolved in 0.5N HCl, diluted, boiled, and filtered. 0.1 to 10 mg beryllium carrier is added plus 10 mg of zinc. The solution is made alkaline with ammonia at the boiling point. The precipitate which forms is filtered and washed with ammonia. The sample is reprecipitated without zinc, washed, dissolved in dilute HCl, and neutralized to pH 4 or 5. The salt concentration is adjusted to 0.9%.

3. Generally one obtains for injection purposes 50 ml containing 250,000 c/m.

4. A minimum of 50,000 c/m are administered to the experimental animal.

5. Tissues are excised, dry-ashed, dissolved in 2N HCl, and counted.

Only preliminary distribution data were available in time for clearance, therefore we are unable to discuss further the application of this technique.

William F. Neuman
W. F. Neuman

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The Pathological Anatomy of Acute Experimental Beryllium Poisoning

Peripheral Blood Changes Resulting From Intravenous Administration of Beryllium Sulphate

Dr. James K. Scott

This material will be included in a future Pharmacology Report.

Note: Figures 1 - 26 are colored slides which are not included in this copy because of excessive expense.

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Abstract

THE PATHOLOGICAL ANATOMY OF ACUTE EXPERIMENTAL BERYLLIUM POISONING AND PERIPHERAL BLOOD CHANGES RESULTING FROM INTRVENOUS ADMINISTRATION OF BERYLLIUM SULPHATE

The lesions produced in the organs following a single intravenous administration of hydrated beryllium sulphate are described. The lesions of the lungs and eyes of animals exposed to the above compound are reviewed. When the beryllium sulphate is given intravenously, midzonal focal necrosis of the liver cells, necrosis of cells of the distal one-third of the proximal convoluted tubules of the kidney and degenerative changes in the cells of the hemopoietic system are produced. Following exposure of animals to beryllium sulphate dust (100 mg/m^3, 8 hours daily for eleven days), inflammatory pulmonary lesions are produced, which vary in intensity with different species. Pulmonary edema, a terminal bronchitis, and focal atelectasis are the most commonly observed lesions. The eyes of some species exposed to this dust develop conjunctivitis, keratitis, and corneal ulcers.

Following a single intravenous administration of beryllium sulphate, rather sharp changes occur in the elements of the peripheral blood. These consist of a secondary anemia (probably resulting from intravascular lysis of red cells), a leukocytosis, and an increase in the number of circulating platelets.

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THE UNIVERSITY OF ROCHESTER
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The Pathological Anatomy of Acute Experimental Beryllium Poisoning

Introduction

The gross and microscopic descriptions to be given below have to do mostly with the changes occurring in tissues following administration of hydrated beryllium sulphate ($BeSO_4.4H_2O$) by inhalation, or by the intravenous and intraperitoneal routes. Since beryllium sulphate acts locally as an irritant causing an inflammatory reaction and sometimes necrosis (e.g. following subcutaneous administration), one must distinguish between the changes resulting from generalized toxicity and the local response such as occurs in the lungs when the compound is inhaled. Beryllium sulphate has been used almost exclusively in these studies because it can be obtained in rather pure form and because there is little reason to think that the sulphate ion would modify or mask the reactions of the tissues. The rat has been used for most of this work. In general, tissue changes were approximately the same in all the species receiving beryllium sulphate intravenously, although it should be noted that there were considerable differences in the dosages required to kill animals of different species. The intensity and even location of lesions varied somewhat with the dosage; therefore, some statement concerning toxic levels should be made. For white female rats weighing 175 - 200 grams, the intravenous L.D. 50 for beryllium sulphate is around 7.2 mg. per kilo (0.36 mg/kg. of beryllium); a few animals have died at 6.0 mg/kg. and survived at 8.0 mg/kg.

Gross Findings

Little was found grossly in the organs of animals dying following intravenous administration of beryllium sulphate. When large doses (30 - 60 mg/kg.) were given, petechial hemorrhages on the serous surfaces were seen; there was usually a considerable increase in pleural fluid which

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sometimes was bloody, the spleen was enlarged and deep purple in color. Following administration of smaller doses, around the L.D. 50, none of these changes were seen. Jaundice was observed in all rats dying three days or more after a single intravenous administration of beryllium sulphate.

Microscopic Findings

Liver: The most conspicuous pathological feature following a single intravenous administration of beryllium sulphate was a necrosis of liver cells. When large doses were given, the necrosis was found within 16 - 18 hours; with smaller doses (6 mg/kg.), it was not histologically clear until the third day (72 hours). The extent of the necrosis also varied directly with the dosage. Only a few of the rats that received 5 mg/kg. have shown liver necrosis; all that received 6 mg/kg. have shown necrosis. The earliest change was observed in the mid-zonal portion of the liver lobule and consisted of a small focus of cells in which the cytoplasm assumed a homogeneous eosinophilic stain and the nuclei became pyknotic (Figs. 1 and 2). The amount of necrosis may vary from a few scattered small foci consisting of only a few cells to almost complete necrosis with only a layer or two of viable cells around the portal areas (Fig. 3). Usually, there was no inflammatory reaction about these necroses; rarely, small areas of neutrophil infiltration (Salmonella) were observed.

The liquefaction and absorbtion of the necrotic cells proceeds rather slowly, usually requiring two to three days. During this period (from the 3rd to 6th days after a single intravenous administration), one sees varying numbers of necrotic cells and areas in which cells have been absorbed (Fig. 4), leaving foci of closely packed vascular sinusoids and stroma. These foci consist of a stroma of reticulum and collagen in which endothelial cells from

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the sinusoids, phagocytic cells, and a few granulocytes and lymphocytes are seen (Figs. 5, 6, and 7). There seemed to be no increase in collagen and at least following a single intravenous administration, no perceptible permanent scar has been found. There was no evident injury to the collagen or reticulum (Figs. 8 and9). The phagocytic cells mentioned above contained a yellow pigment which remained after the tissues have passed through alcohol, but the same cells contain a finely divided lipid which stains clearly with Sudan IV. Since the sinusoidal cells of the normal rats' liver contain a pigment of similar staining properties, it is impossible to state with certainty whether the pigment was phagocytized from degenerating liver cells or represented a number of closely packed normal sinusoidal cells.

The regeneration of the liver, as indicated by an increased number of mitoses, usually became evident on the 5th to 6th day after a single administration of the beryllium sulphate. This proceeded quite rapidly and by two weeks, and usually in less time, the liver had a normal microscopic appearance.

The behavior of fat, as stained by Sudan IV, is worth mentioning. The necrotic liver cells contained a moderate amount of finely divided brownish staining fat which disappears when the cells disappear (Fig. 10). The liver cells immediately adjacent to the foci of necrosis, which with an ordinary hematoxylin and eosin stain appeared normal, contained a large amount of coarse deeply orange staining droplets (Fig. 11). These remained during the entire period of liver regeneration and disappeared sometime between the 8th and 14th days.

The irregular, nodular, hyperplastic lobules, the large multinucleated hepatic cells and the heavy cords of hepatic cells have not been observed in livers regenerating after this type of injury. Also, we have not observed bile canalicular thrombi, an excess of bile in the interlobular bile ducts or the inflammatory reaction of the portal areas so frequently observed in other types of liver injury.

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Kidney changes: The histological renal changes were not nearly as extensive as those of the liver; however, they occurred at a dosage somewhat lower than that which produced liver necrosis. Thus, 6 mg/kg. of beryllium sulphate has produced definite and constant liver necrosis, whereas 5 mg/kg. produced only minimal liver necrosis in an occasional rat; however, at 5 mg/kg. most rats show definite renal changes on the 4th or 5th day. Also, the amount of damage to the kidneys has not seemed to be proportional to dosage. This may, perhaps, be because with high doses the rats die before the renal damage has reached its maximum. It might be noted also that the most severe renal changes have been observed in rabbits which have inhaled beryllium sulphate as a dust.

The renal changes seen were almost entirely limited to the distal portion of the proximal convoluted tubule; however, in some few animals, changes have been observed to a lesser extent throughout the entire proximal convoluted tubule (Fig. 12). In the case of the rabbits, some changes were seen in the descending loops of Henle and there was considerable dilatation of the collecting tubules. The lesion consisted of a scattered necrosis of the epithelial cells. The first change observed was a change in staining reaction from the ordinary finely stippled pink cytoplasm (eosin stain) to a deeper homogenous reddish color. The nuclei of such cells became pyknotic. Such cells could be found still lining the tubule and apparently resting on the basement membrane. These were soon desquamated and were found in the lumens of the tubules distal to their origin. Epithelial casts are occasionally seen (Figs. 13, 14, and 15).

Hemopoietic System

When beryllium sulphate was administered to rats in large doses (30 to 60 mg/kg.) the spleen became enlarged, engorged and purple in color.

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Microscopically, such a spleen showed extensive engorgement of the sinusoids with blood and fluid, and a marked decrease in the usual number of nucleated cells (Fig. 16). In such an organ, one sees nuclear degenerative changes. The lymphocytes showed a clumping of nuclear chromatin at the periphery and this is probably followed by karyorrhexis, since abundant amounts of nuclear debris could be seen throughout the red pulp. The Malpighian corpuscles seemed to contain many fewer cells and no active germinal centers were observed. In animals receiving doses of beryllium sulphate around the L.D. 50 or less, the only change that has been observed was the presence of nuclear debris in the phagocytic cells; since this is found to some extent in normal rats, it is not thought to be significant.

The bone marrow showed a granulocytic hyperplasia. With the higher doses, there were degenerative changes in the nuclei.

The lymph nodes showed little detectable change, except with high doses some degenerative changes were noted in the nuclei of the lymphocytes.

The effects of beryllium sulphate on the hemopoietic system can be better studied by changes in the peripheral counts and in the marrow (Vide infra).

Local Effects of Beryllium Sulphate on Tissues

We have only had opportunity to study this problem on the eyes and the respiratory tract in animals which inhaled approximately 100 mg/m^3 of a beryllium sulphate dust for eleven days.

The guinea pigs and dogs showed the most extensive and frequent ocular changes. These consisted of a conjunctivitis with the usual microscopic picture of an acute or organizing inflammation. In many of the animals, there developed a keratitis of rather severe degree consisting of vascularization of the cornea, development of granulation tissue, and frequently

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rather marked ulceration (Fig. 17). In some animals, a purulent exudate was found in the anterior chamber, which was accompanied by a mild uveitis (Fig. 18).

The lungs of all the animals showed histological changes; in some species the lesions were few in number and not diffuse; in others the lesions were more widespread and frequently involved most of one or more lobes (Figs. 19 and 20). An inflammatory exudate in the lumens of the terminal bronchi has been found in all species. There was a coagulated exudate in which nuclear debris was found--the latter probably arose from disintegrating granulocytes and bronchial epithelium (Figs. 21, 22, 23). Some superficial ulceration of bronchial epithelium was frequently present which was accompanied by bronchial epithelial proliferation (Fig. 24). Some atelectasis, usually involving only isolated lobules, was seen. In some species, there is a fairly marked pulmonary edema. The granulocytic cellular exudate so frequently found in other inflammatory processes has not been particularly prominent: The alveolar sacs usually contained a few large phagocytic cells and the bronchi contained a few granulocytes.

No pulmonary fibrosis has been observed in any of the animals studied thus far. However, occasionally fibroblasts have been seen growing into the lumens of the bronchi (Fig. 25).

A number of animals receiving beryllium compounds or beryllium metal intratracheally or intraperitoneally have shown peculiar blue staining structures (Fig. 26). The origin, development, and disappearance of these bodies are not understood.

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Fig. 1. Y-2372. Rat sacrificed 3 days after receiving 6 mg/kg. of beryllium sulphate. Shows small midzonal necroses, the earliest liver change noted at this dosage. X 120.

Fig. 2. Y-2372. High power of above. X 400.

Fig. 3. Y-2053. Rat sacrificed 5 days after receiving 21 mg/kg. of beryllium sulphate intraperitoneally. Shows extensive necrosis with only a layer or two of viable liver cells remaining about the portal areas. X 120.

Fig. 4. Y-2374. Rat sacrificed 4 days after receiving 6 mg/kg. of beryllium sulphate intravenously. Shows period during which liquefaction and absorbtion is occurring. X 120.

Fig. 5. Y-2396. Rat sacrificed 5 days after receiving 6 mg/kg. of beryllium sulphate intravenously. Appearance of the liver after liquefaction of necrotic cells and before regeneration has occurred. X 100.

Fig. 6. Y-2396. High power of Fig. 5. Shows cellular structure of closely packed sinusoids after the necrotic hepatic cells have disappeared. X 300.

Fig. 7. Y-2497. Rat sacrificed 8 days after receiving 6 mg/kg. of beryllium sulphate. Regeneration is not complete. X 100.

Fig. 8. Y-2507. Rat sacrificed 8 days after receiving 6 mg/kg. of beryllium sulphate. Wilder reticulum stain. Shows preservation of reticulum. X 100.

Fig. 9. Y-2507. Higher magnification of above. X 400.

Fig. 10. Y-2374. Rat sacrificed 4 days after receiving 6 mg/kg. of beryllium sulphate intravenously. Shows finely divided brownish staining fat in the necrotic cells and large coarse droplets of fat in the surrounding viable cells. Sudan IV. X 120.

Fig. 11. Y-2507. Rat sacrificed 8 days after receiving 6 mg/kg. of beryllium sulphate intravenously. Note the large coarse droplets of fat in the otherwise normal appearing liver cells; also, the finely divided fat in the sinusoidal cells. X 120.

Fig. 12. Y-2057. Rat sacrificed 6 days after receiving 5 mg/kg. of beryllium sulphate intravenously. Section from the inner 1/3 of cortex. Note the isolated cells with pyknotic nuclei and eosinophilic cytoplasm. X 400.

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Fig. 13. Y-2312. Rabbit sacrificed after 11 days' exposure to an atmosphere of 100 mg/m^3 of beryllium sulphate. Note the marked dilatation of collecting tubules. X 100.

Fig. 14. Y-2311. Rabbit sacrificed after 11 days' exposure to an atmosphere of 100 mg/m^3 of beryllium sulphate. Shows necrosis and desquamation of tubular epithelial cells. X 120.

Fig. 15. Y-2311. Higher magnification of Fig. 14. X 400.

Fig. 16. Y-1781. Rat sacrificed 3 days after receiving 60 mg/kg. of beryllium sulphate intravenously. Shows the congestion and edema of the splenic pulp. The margin of a Malpighian corpuscle is seen on the left. Note the aburdance of nuclear debris, some of which has been phagocytized. X 120.

Fig. 17. Y-2316. Guinea pig sacrificed after 11 days' exposure to an atmosphere of 100 mg/m^3 of beryllium sulphate. Shows corneal ulcer and subacute keratitis. X 100.

Fig. 18. Y-2316. Same section as Fig. 17. To show the purulent exudate in the anterior chamber. X 100.

Fig. 19. Y-2314. Guinea pig--same exposure as above. Shows the minimal involvement of the lung observed in this species.

Fig. 20. Y-2311. Rabbit exposed to beryllium sulphate under the same conditions as the animal depicted in Fig. 19. Note the more diffuse and extensive involvement of the lung in this species.

Fig. 21. Y-2314. Guinea pig. Same exposure as animal depicted in Fig. 19. Section is through one of the small foci of reaction in the lung. X 120.

Fig. 22. Y-2311. Rabbit lung. Exposure to beryllium sulphate under same conditions as above. To show type of pulmonary response in this species. X 120.

Fig. 23. Y-2318. Guinea pig. Exposure to beryllium sulphate under same conditions as above. X 180.

Fig. 24. Y-2320. Rat. Exposed to beryllium sulphate under same conditions as above. Shows the bronchial epithelial proliferations. X 120.

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Fig. 25. Y-2326. Rat. Exposed to beryllium sulphate under same conditions as above. Shows organization of bronchial exudate. X 120.

Fig. 26. Y-1750. Rat sacrificed 7 days after receiving 100 mg/kg. of beryllium metal intratracheally. To show peculiar "blue bodies". X 400.

Note: THIS COPY DOES NOT CONTAIN FIGURES 1-26 BECAUSE OF TIME AND EXPENSE

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Peripheral Blood Changes Resulting From Intravenous Administration of Beryllium Sulphate

The interpretation of changes in the blood elements after administration of beryllium sulphate by routes other than the intravenous is difficult or impossible because beryllium acts locally as an irritant. When administered intravenously, there is no local inflammatory reaction and any changes occurring must result from generalized action of the compound which, at least for a period of time, is carried to most of the tissues of the body.

The procedure in this experiment was simply to follow the blood counts, usually daily, for two weeks or longer after a single intravenous injection of beryllium sulphate. The doses of beryllium sulphate tetrahydrate were 7.2, 5.0, 3.0, and 1.5 mg/kg. Eight rats were used at each level. The various charts below indicate the average counts of the different elements from the eight animals at each level.

The red counts at doses of 7.2 and 5.0 mg/kg. showed a slow elevation during the first 3 or 4 days after administration of the beryllium sulphate; this is followed by a precipitous drop in count which returned to normal about 2 weeks after the injection. On about the 4th day after the administration, there is a rapid increase in the percentage of reticulocytes and nucleated red cells. This anemia and reticulocyte response failed to develop in animals receiving less than 5 mg/kg. (Fig. 1).

No definite statement can be made concerning the cause of this anemia. However, it would appear that during the period of 3 to 4 days following administration of the compound there occurred concomitantly a hemoconcentration and an intravascular lysis of red cells; this is followed by a return of the plasma volume to somewhere near normal. The hemolysis with resulting anemia must have begun at the time of administration of the compound since the reticulocyte response occurred on the 4th day.

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There was a marked increase in the total white cell count, reaching a peak on the 2nd to 5th day after administration of the compound. This leukocytosis was a result of an absolute increase of neutrophiles. The number of neutrophiles increased from around a normal of 4000 per cubic mm. to around 22,000 per cubic mm. The count than gradually decreased and on the 9th or 10th days approached normal. This leukocytosis occurred in all animals at the levels of 7.2, 5.0, and 3.0 mg/kg.; at 1.5 mg/kg. only about one half of the animals showed this change (Fig. 2).

The most interesting change occurred in the platelets. At 7.2 mg/kg. a sharp drop in the number of platelets occurred on the 3rd day, the lowest point was reached on the 6th, following which there was a gradual increase which reached normal about 2 weeks after administration of the beryllium sulphate. When doses of 5.0, 3.0, and 1.5 mg. were used, the platelet response was exactly the opposite. At 5.0 mg/kg. there was a slight depression followed by a rise on the 4th day which reached a peak of almost three times the control level on about the 7th day; after this, the counts gradually decreased, approaching control levels after 2 weeks. At levels of 3.0 and 1.5 mg/kg., no initial depression of the count occurred, and the counts began increasing 24 hours after administration of the beryllium sulphate and reached the peak on the 9th and 10th days (Fig. 3).

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Chart 1 **Reticulocytes and Red Blood Count for 4 Groups of Beryllium-Exposed Rats**
By Days after Exposure

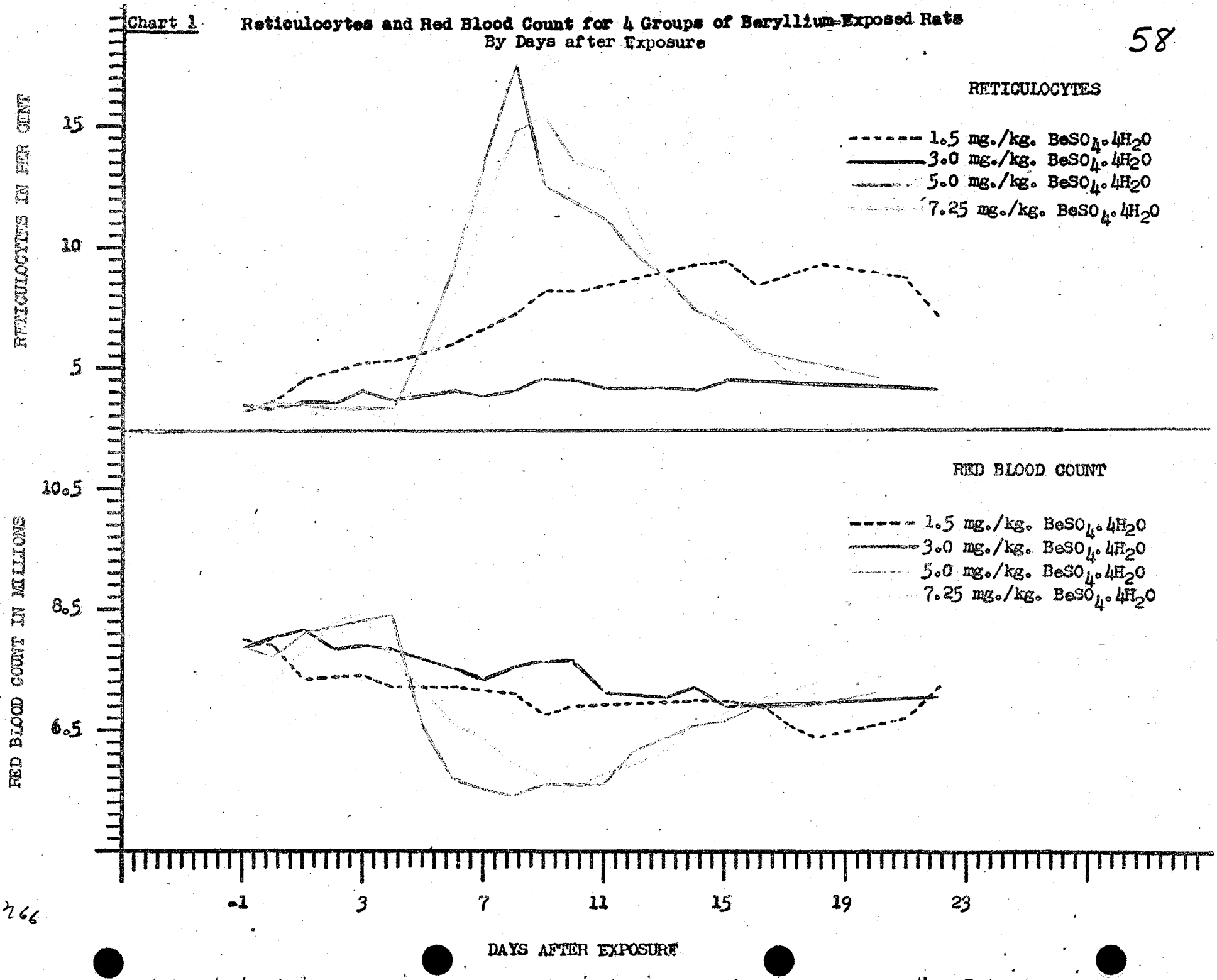

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Chart 2 Absolute Neutrophils for 4 Groups of Beryllium-Exposed Rats
By Days after Exposure

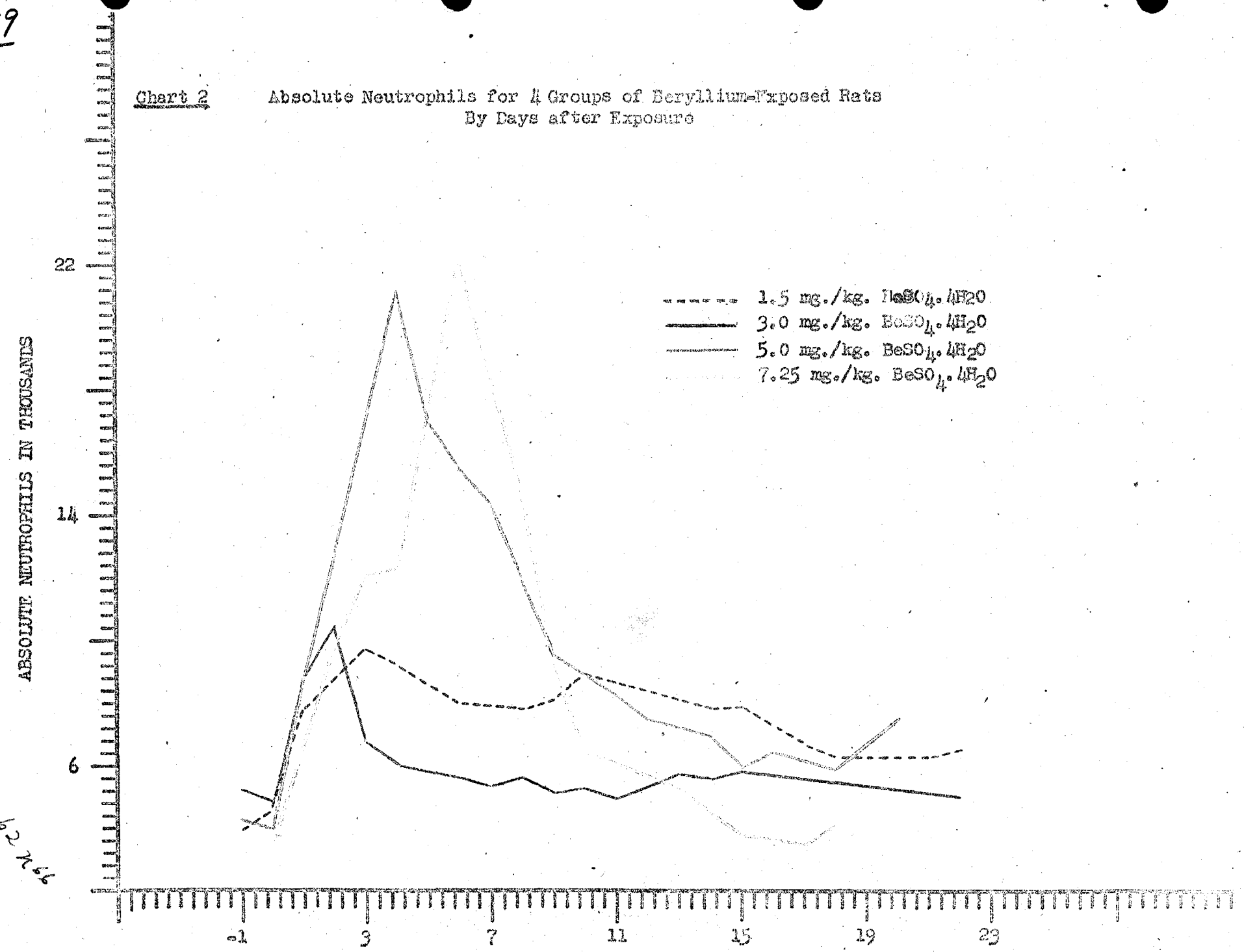

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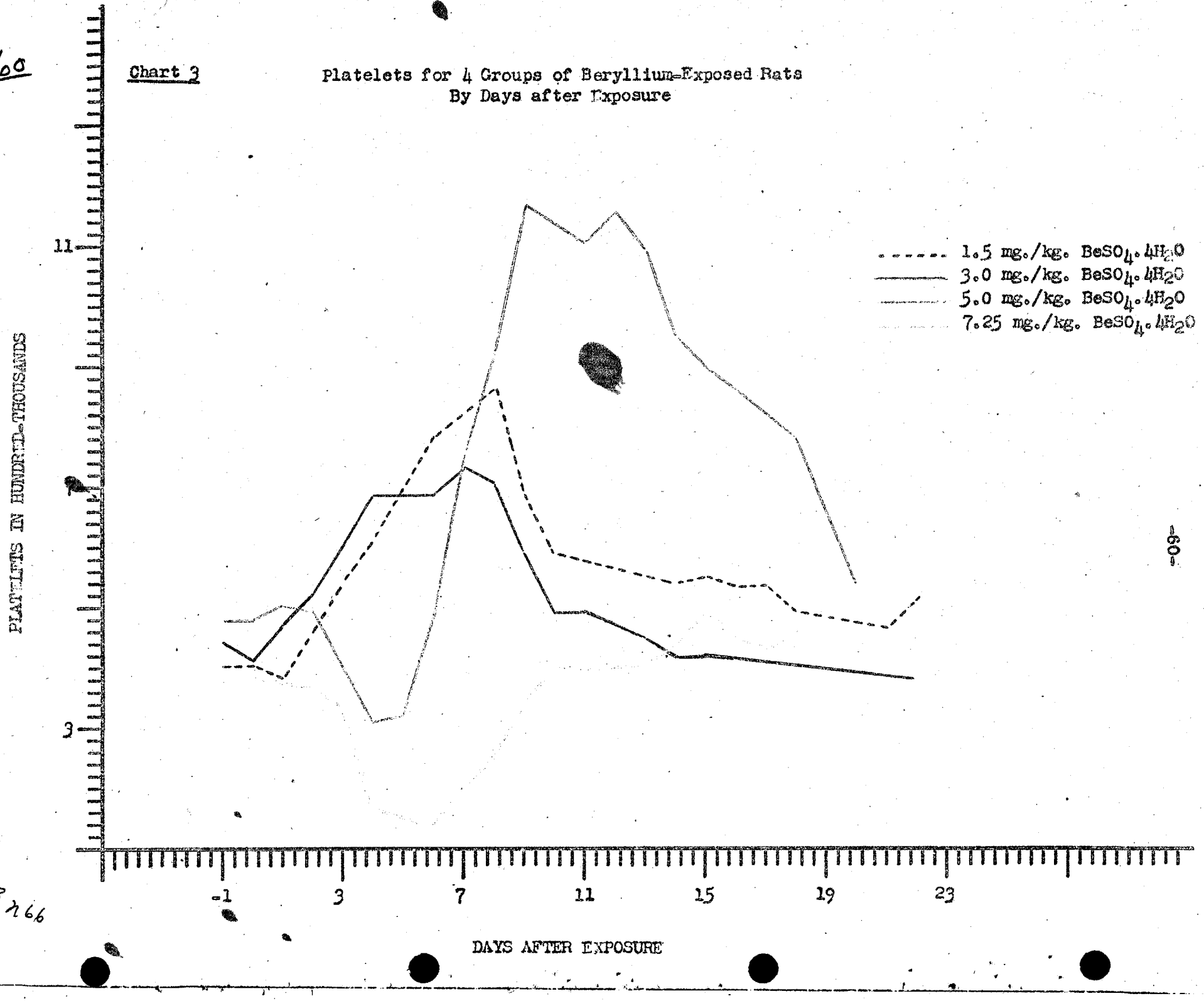
Chart 3

Platelets for 4 Groups of Beryllium-Exposed Rats
By Days after Exposure



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CERTAIN ASPECTS OF THE ACUTE TOXICITY OF BERYLLIUM FOLLOWING INTRAPERITONEAL INJECTION

Elliott A. Maynard, William L. Downs, Harold C. Hodge

This material has been included in Pharmacology Monthly Reports from October 1946 through February 1947 inclusive (M-1923, M-1929, M-1936, M-1946, M-1955). (Rochester)

September 1946 - February 1947

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CERTAIN ASPECTS OF THE ACUTE TOXICITY OF BERYLLIUM FOLLOWING INTRAPERITONEAL INJECTION

ABSTRACT

Following intraperitoneal injection in rats and mice, the LD50 dose based on 24-hour mortalities was determined for various Be compounds. A few rabbits and guinea pigs were also injected. The soluble Be salts gave indication of toxicity of varying degree; the insoluble salts were practically non-toxic. Saline solutions were less toxic than aqueous solutions. Young (weanling) rats and mice were more resistant to Be toxicity than were older animals.

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65266

CERTAIN ASPECTS OF THE ACUTE TOXICITY OF BERYLLIUM FOLLOWING INTRAPERITONEAL INJECTION

LD50 dose determinations based on 24-hour mortality were made for various Beryllium compounds by intraperitoneal injections in rats and mice. A few injections were also made in rabbits and guinea pigs. The results are given in the following tables:

Table I. Rats (25 per group) Injected Intraperitoneally with various Be compounds.

Cmpd.	Solution Saline (S) or Aqueous (A)	Sex	Age	LD50 mg/kg.
Be oxalate	(A)	♂	Mature	5
Be oxyfluoride	(A)	♀	Mature	13
Be oxyfluoride	(A)	♀	Weanling	42
Be sulfate-anhydrous	(A)	♂	Mature	50
Be perchlorate	(A)	♂	Mature	60
Be sulfate-hydrate	(A)	♂	Mature	110
Be sulfate-hydrate	(S)	♂	Mature	200
Be metal	(S)	♂	Mature	(no 24-hr. effect from 500)
Be carbonate	(A)	♂	Mature	(no 24-hr. effect from 500)
Be oxide	(A)	♂	Mature	(no 24-hr. effect from 1000)

Table II. Mice (50 per group) Injected Intraperitoneally with various Be compounds.

Be oxyfluoride	(A)	♀	Mature	33

(Injection of the LD50 dose of 33 mg/kg. gave mortality of 57% in mature; 22% in weanling mice).

Be sulfate-hydrate	(A)	♀	Mature	200
Be sulfate-hydrate	(S)	♀	Mature	300

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Table III. Rabbits - Injected Intraperitoneally with various Be compounds.

Compound		Dose (mg/kg.) and Effect
Be sulfate	(S)	2.6 - no effect over prolonged period; 4.3 and 9.0, died 60 hrs.; 27, died 60 hrs.
Be sulfate	(A)	2.6, died 6 days; 4.3, died 5 days; 9.0 died 4 days.
Be oxide	(A)	770, no effect over prolonged period.
Be metal	(S)	1000, no effect over prolonged period.
Be oxyfluoride	(A)	1, died 4 weeks; 3, died 6 days; 4, died 3 days.

Table IV. Guinea Pigs - Injected Intraperitoneally with various Be compounds.

Compound		Dose (mg/kg.) and Effect (Mortality)
Be sulfate	(S)	5.1, 1/1, 22 days; 10.2, 1/6, 22 days; 20.4, 2/2, 4 days.
Be oxide	(A)	1000, no effect over prolonged period.
Be metal	(S)	1000, no effect over prolonged period.

From the above data it may be seen that the soluble salts of Beryllium are toxic to various degrees when injected intraperitoneally and that the insoluble salts are practically non-toxic when so administered. For some unexplained reason saline solutions were less toxic than aqueous solutions. Young (weanling) rats and mice were more resistant to the toxicity of Be compounds than were mature animals.

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PRELIMINARY DATA ON RAT FEEDING WITH BERYLLIUM

Elliott A. Maynard, William L. Downs, Harold C. Hodge

This material has been included in Pharmacology Monthly Reports from October 1946 through June 1947 inclusive (M-1923, M-1929, M-1936, M-1946, M-1955, M-1961, M-1969, M-1974, M-1986). (Rochester)

October 1946 - June 1947

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PRELIMINARY DATA ON RAT FEEDING WITH BERYLLIUM

ABSTRACT

At a dietary level of 5%, Be sulfate and Be carbonate completely inhibited growth of rats and caused some mortality. A rickets-like condition occurred in these rats. Food intake was reduced by about 50% but this did not account for all growth inhibition as shown by a paired feeding experiment. Return to stock diet after a prolonged period of ingestion of Be salt allowed rapid but not complete growth recovery. Be sulfate was more toxic to old rats than to weanlings, but with Be carbonate mature rats seemed to be more resistent than weanlings. Be carbonate at a level of 2.3% of the diet caused some weight depression in rats. Be metal at a 10% dietary level, and Be oxide at a 5% dietary level did not effect the growth of rats.

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THE UNIVERSITY OF ROCHESTER
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PRELIMINARY DATA ON RAT FEEDING WITH BERYLLIUM

In order to obtain some preliminary information on the effects of the ingestion of Beryllium, albino rats of the Wistar strain raised in our own colony, were fed various dietary levels of Beryllium metal and certain Beryllium salts. Because of the limited amount of available laboratory space where proper precautions could be taken to prevent exposure to Beryllium of other experimental animals, as well as personnel, only two rats per dietary group were fed at one time.

Be sulfate (hydrate).

Effect on growth. Two male rats (28 days old; average body weight 62 g.) were fed a diet containing 5.0% Be sulfate for a period of 172 days at which time one of the animals died and the other was sacrificed for histopathological studies. As may be seen in the accompanying growth curves (Fig. 1) a marked depression of growth was noticeable from the very beginning of the experiment. At the termination of the experiment the average body weight of the rats on the special diet was 306 g. less than that of the control rats. Fig. 1.

In a repeat experiment with 2 male weanling rats (age 28 days; average body weight 62 g.) one animal died at the end of 71 days on the diet and the second one was sacrificed. At that time the average weight of the experimental rats was 234 g. less than that of the control rats. Fig. 1.

Effect on Food Consumption. Records of food consumption were kept for 2 male weanling rats (4 weeks old) on a diet containing 5.0% Be sulfate for a period of 180 days. The average food ingested by these rats daily was about 7 g. as compared with about 15 g. for the control rats. Fig. 2 shows growth curves and food consumption curves for these animals.

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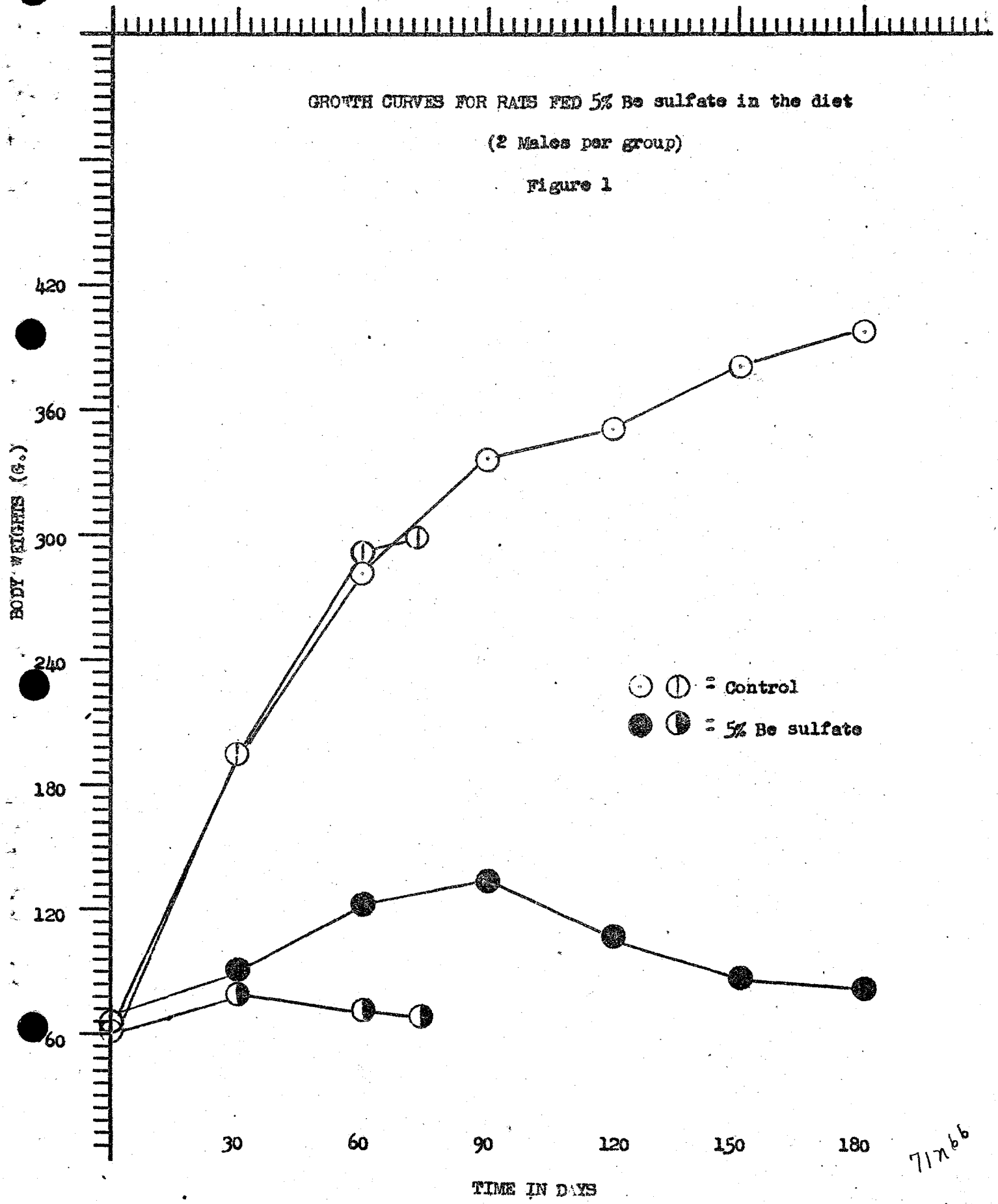
GROWTH CURVES FOR RATS FED 5% Be sulfate in the diet

(2 Males per group)

Figure 1



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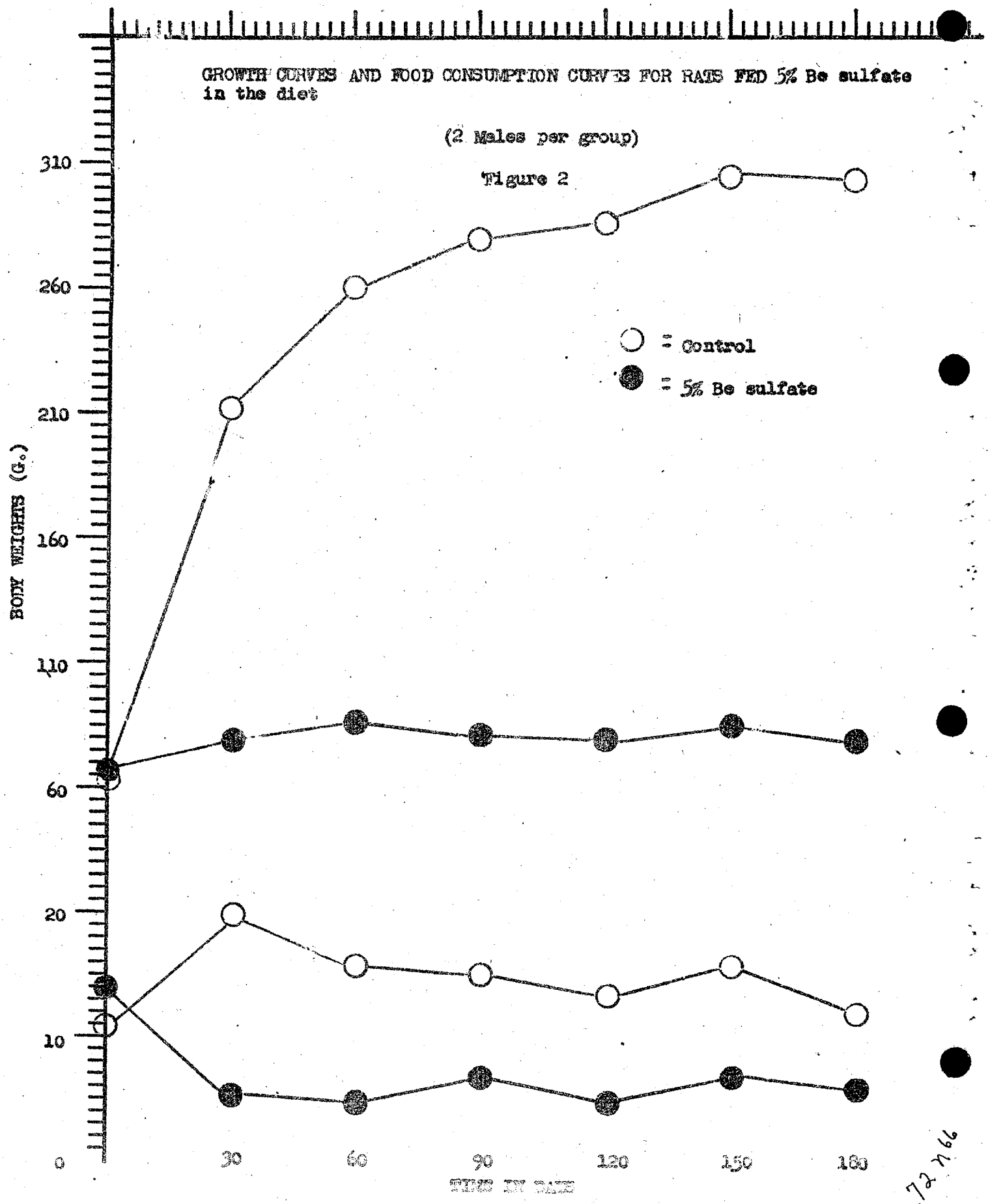
GROWTH CURVES AND FOOD CONSUMPTION CURVES FOR RATS FED 5% Be sulfate in the diet

(2 Males per group)

Figure 2



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Paired Feeding. In order to determine if the depression in growth of the rats fed a diet containing 5.0% Be sulfate was due entirely to reduced food intake, two control rats were fed each day an amount of stock diet equal in weight to the experimental (5% Be sulfate) diet consumed the day previous by their litter-mate brothers. This regimen was continued for a period of 68 days. At that time the rats receiving 5.0% Be sulfate in the diet had average body weights of 91 g. as compared with 133 g. for the controls; a difference of 42 g. Growth curves are shown in Fig. 3. Radiographs of the experimental rats showed a rickets-like condition of the tibial head during the latter part of the experiment. (This condition was principally indicated by an enlargement of the metaphyseal space). This would seem to indicate a definite toxic effect following the ingestion of 5.0% Be sulfate by rats.

Recovery following ingestion of Be sulfate. In order to determine the extent of recovery possible following the ingestion of Be sulfate, the two rats from the above experiment were returned to a stock diet at the end of 68 days. Fig. 3 shows that after 52 days on a stock diet the average body weight of the experimental rats was 47 g. less than that of the controls. Radiographs showed healing of the tibial head at that time. Fig. 4 shows growth curves for two other rats that were returned to a stock diet after receiving 5.0% Be sulfate for a period of 40 days. At the end of 100 days on the stock diet these 2 rats had average body weights 93 g. less than the controls.

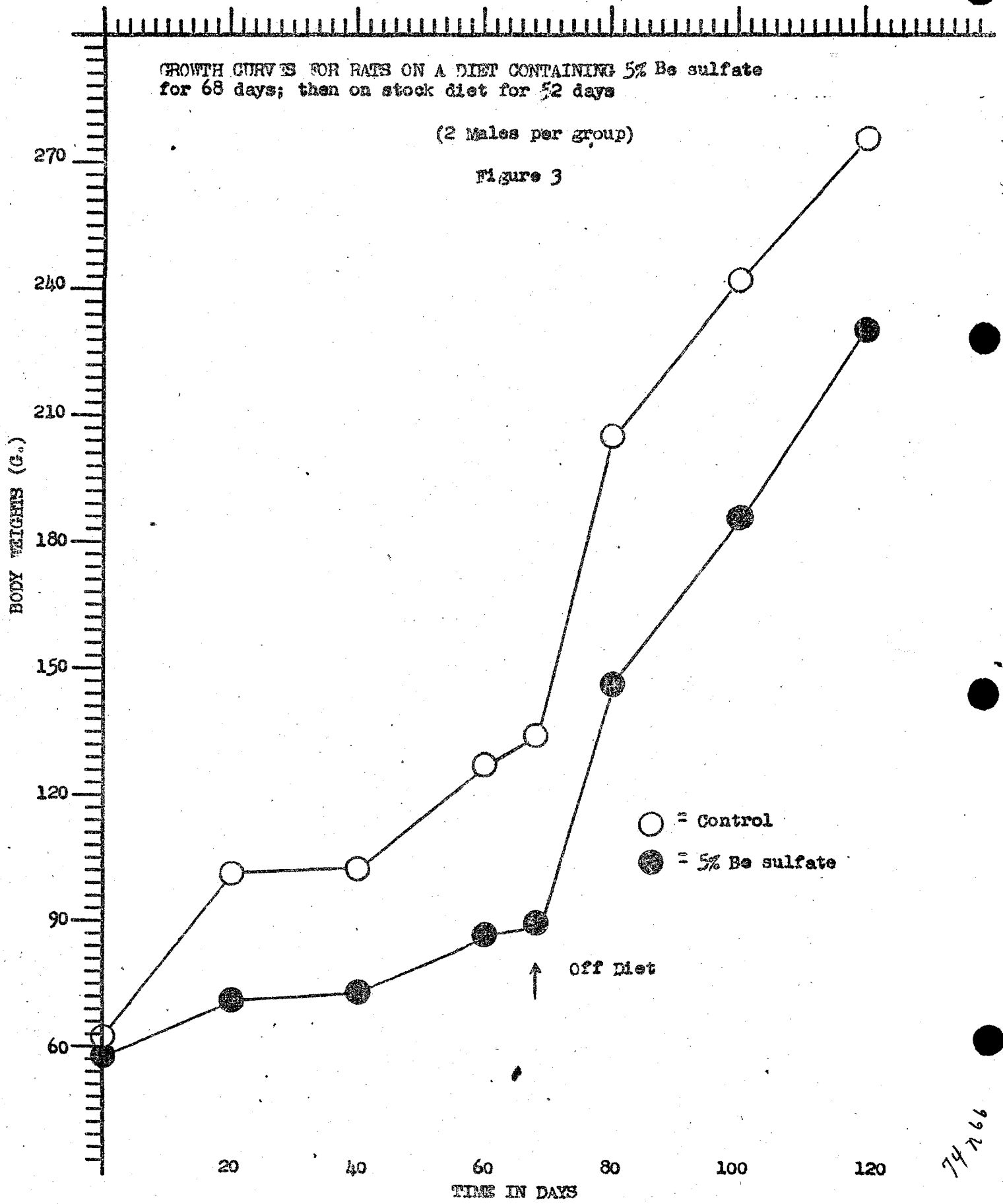
Effect of single dose (24 hr.) feeding of 5.0% Be sulfate. Fig. 5 shows there was no noticeable effect on food consumption or growth of two weanling rats which were fed 5.0% Be sulfate in the diet for a 24-hour period.

Age as a factor in the toxicity of Be sulfate in rats. Two male rats (age 173 days) and two male rats (age 81 days) were fed a diet containing 5.0%

GROWTH CURVES FOR RATS ON A DIET CONTAINING 5% Be sulfate
for 68 days; then on stock diet for 52 days

(2 Males per group)

Figure 3



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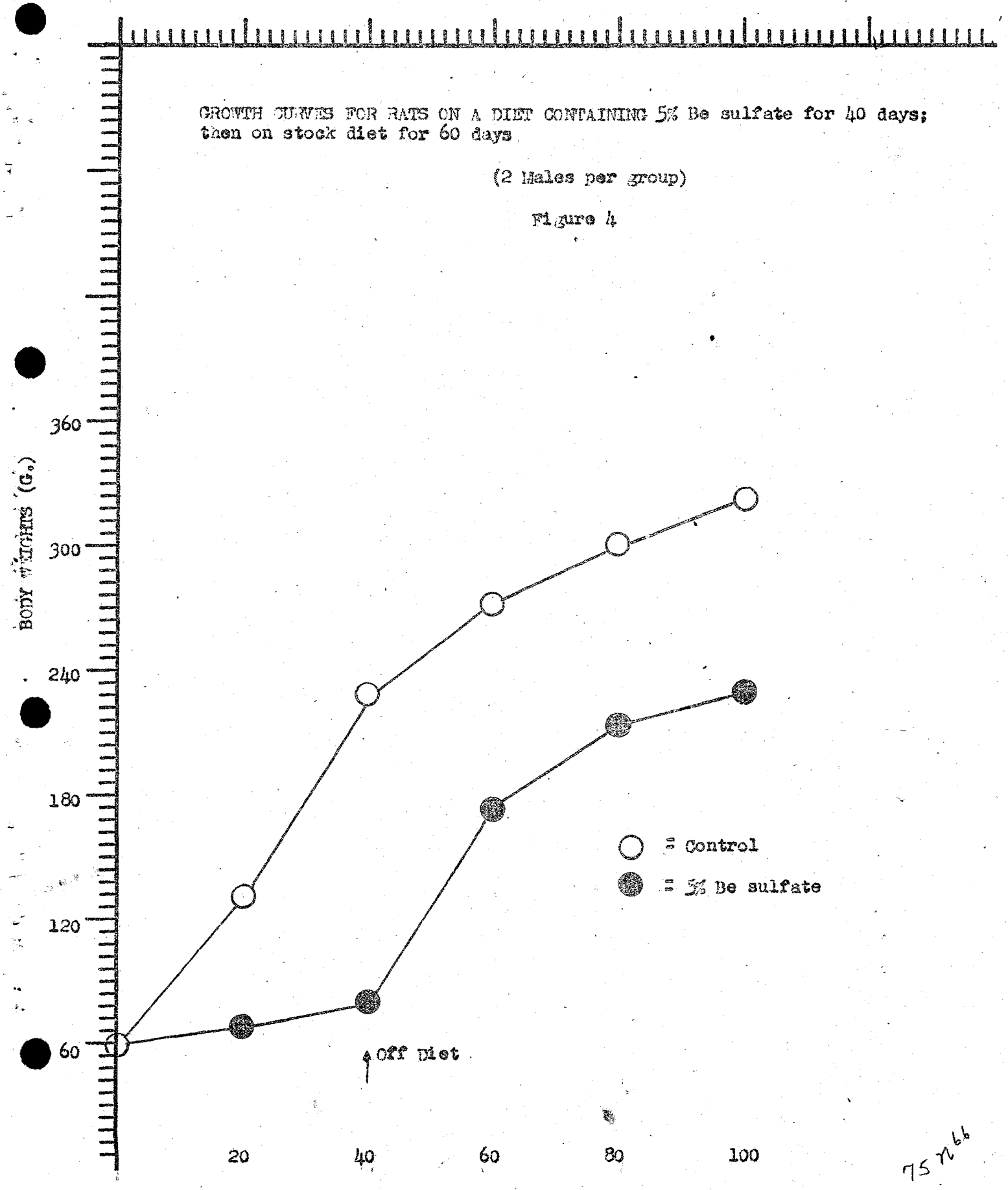
GROWTH CURVES FOR RATS ON A DIET CONTAINING 5% Be sulfate for 40 days; then on stock diet for 60 days

(2 Males per group)

Figure 4



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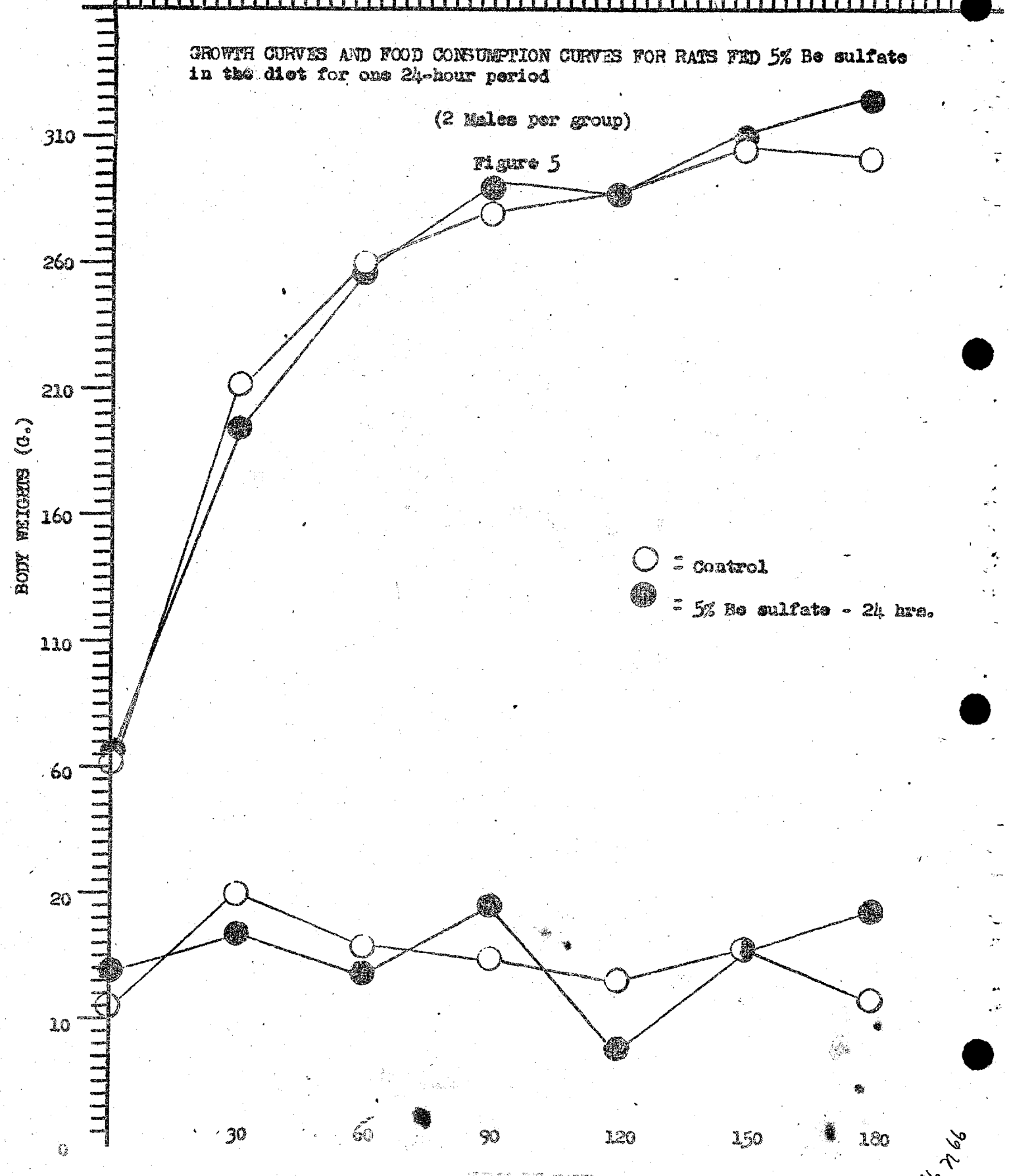
GROWTH CURVES AND FOOD CONSUMPTION CURVES FOR RATS FED 5% Be sulfate in the diet for one 24-hour period

(2 Males per group)

Figure 5



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Be sulfate. Fig. 6 shows growth curves for these animals. At the end of 60 days one of the older rats died. At that time the average body weight of the two animals was 222 g. as compared with an average initial weight of 399 g.; an average loss of 177 g. In the sixty day period the 81 day old rats decreased from an average initial body weight of 330 g. to 175 g.; a loss of 155 g. Thus, it seems that Be sulfate is more toxic to older rats than to weanlings (4 weeks old).

Table I. Rats of various ages (2 per group) Fed 5.0% Be sulfate in the diet.

Average Starting Age	Av. Bd. Wt.	Average Weight Change	Mortality
28 days	62 g.	+ 20 g.	172 days; sacrificed
28 days	62 g.	+ 8 g.	71 days; sacrificed
81 days	330 g.	-181 g.	94 days; alive
173 days	399 g.	-177 g.	60 days; alive

Be carbonate:

5.0% Dietary Level.

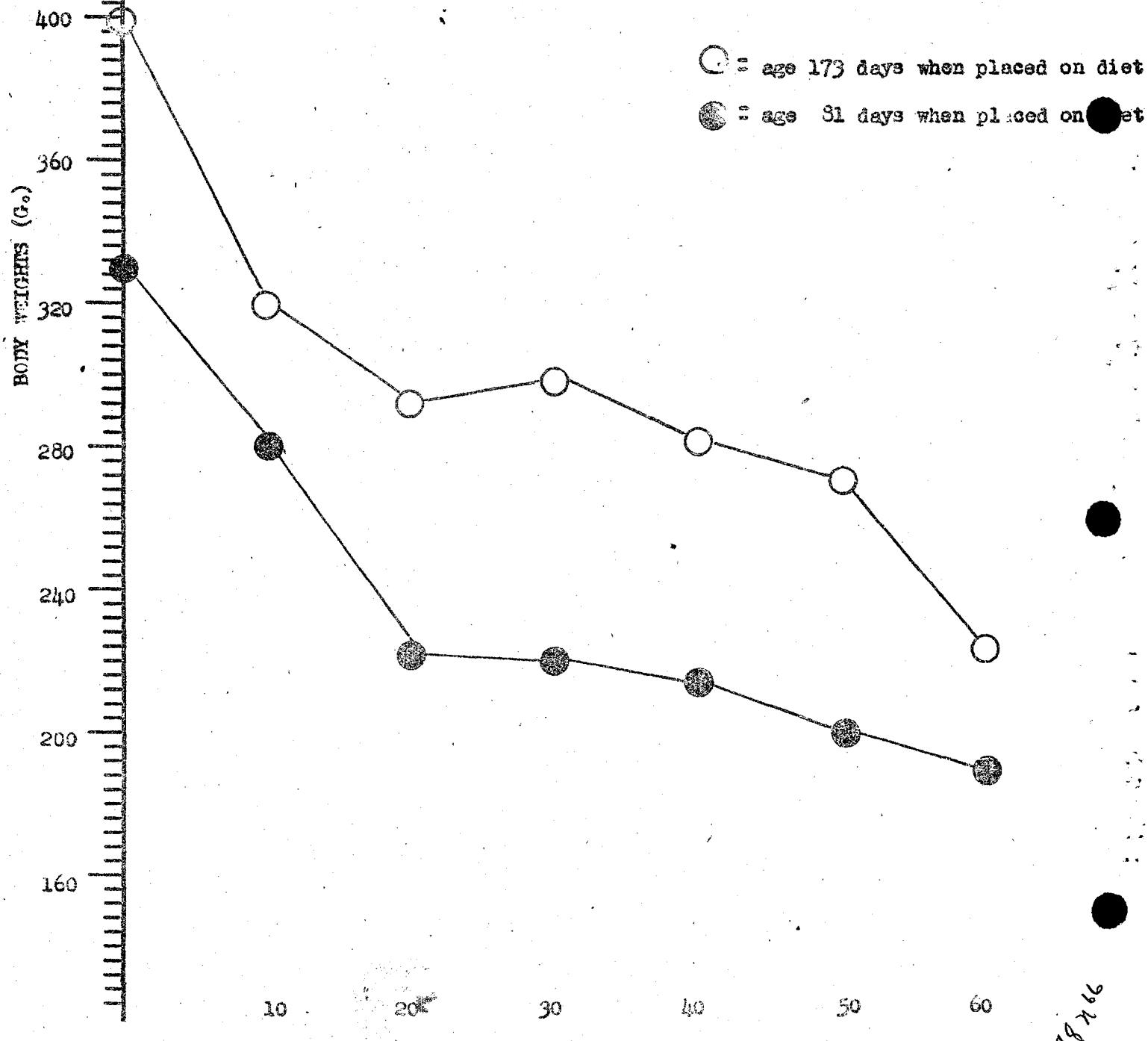
Effect on growth. Two weanling male rats (age 30 days; average body weight 64 g.) were fed 5.0% Be carbonate in the diet. These animals lost weight steadily and died in 39 and 59 days. Figure 7. Radiographs of the tibial head showed the same rickets-like condition in these rats and in the 130 g. rats (next paragraph) as was reported above for rats fed 5.0% Be sulfate.

Age as a factor in the toxicity of Be carbonate in rats. Two male rats (130 g. average initial weight; age 49 days) were fed a diet containing 5.0% Be carbonate. Growth curves (Fig. 8) show that these rats lost weight steadily and died after 19 and 33 days. The two control rats were then (age 99 days) placed on a diet containing 5.0% Be carbonate for a period of 65 days during which time they sustained an average weight loss of about 5 g. Figure 8. In Table II it may be seen that in direct contrast to the effect of Be sulfate as

GROWTH CURVES FOR MATURE RATS FED 5% Be sulfate in the diet

(2 Males per group)

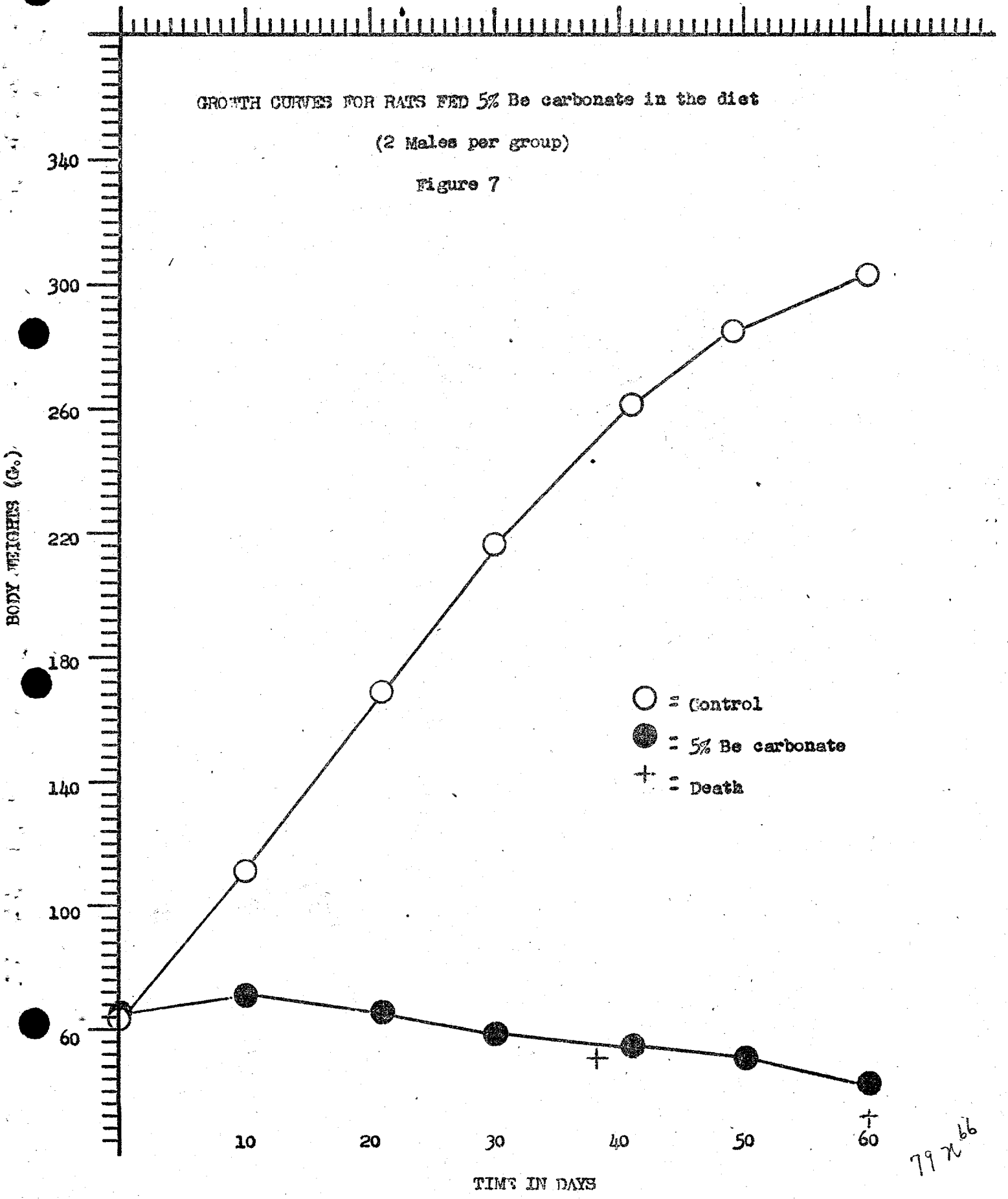
Figure 6



GROWTH CURVES FOR RATS FED 5% Be carbonate in the diet

(2 Males per group)

Figure 7



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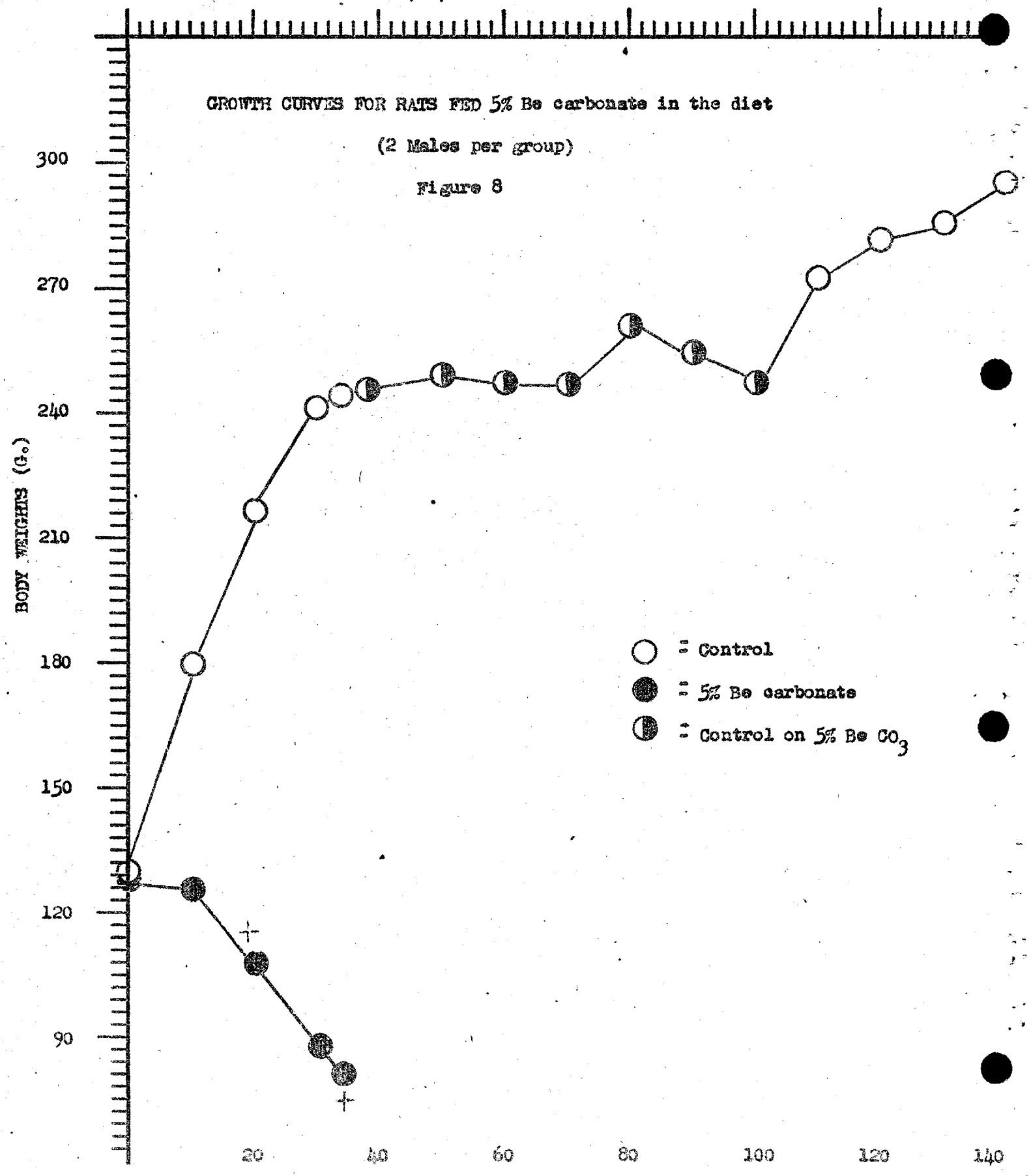
GROWTH CURVES FOR RATS FED 5% Be carbonate in the diet

(2 Males per group)

Figure 8



80 rats

given in table I, mature rats are somewhat more resistant to Be carbonate in the diet than are weanlings.

Table II. Rats of various ages (2 per group) Fed 5.0% Be carbonate in the diet.

Average Starting Age	Av. Bd. Wt.	Average Weight Change	Mortality
30 days	64 g.	-10 g.	39 days; 59 days
49 days	130 g.	-50 g.	19 days; 33 days
99 days	248 g.	+ 8 g.	none / 65 days

Recovery following ingestion of Be carbonate. The two mature rats (age 99 days) were returned to a stock diet after 70 days on a diet containing 5.0% Be carbonate. These rats (now 164 days old) immediately made a spurt in growth and at the end of 48 days had made an average weight gain of 40 grams and appeared to be normal animals. Figure 8.

2.3% Dietary Level.

Effect on growth. Two male rats (130 g. initial weight; age 46 days) were fed a diet containing 2.3% Be carbonate for a period of 102 days at the end of which time these animals had average weight depressions of 53 g. when compared with the controls. Figure 9.

Be metal:

Two weanling male rats (age 27 days; average body weight 58 g.) were fed a diet containing 10% Be metal for a period of 75 days. Fig. 10 shows that the experimental rats have grown equally as well as the controls.

Be oxide:

Two weanling male rats (age 26 days; average body weight 54 g.) were fed a diet containing 5% Be oxide for a period of 33 days. Fig. 11 shows that there was no effect on the growth of these rats when compared with the controls.

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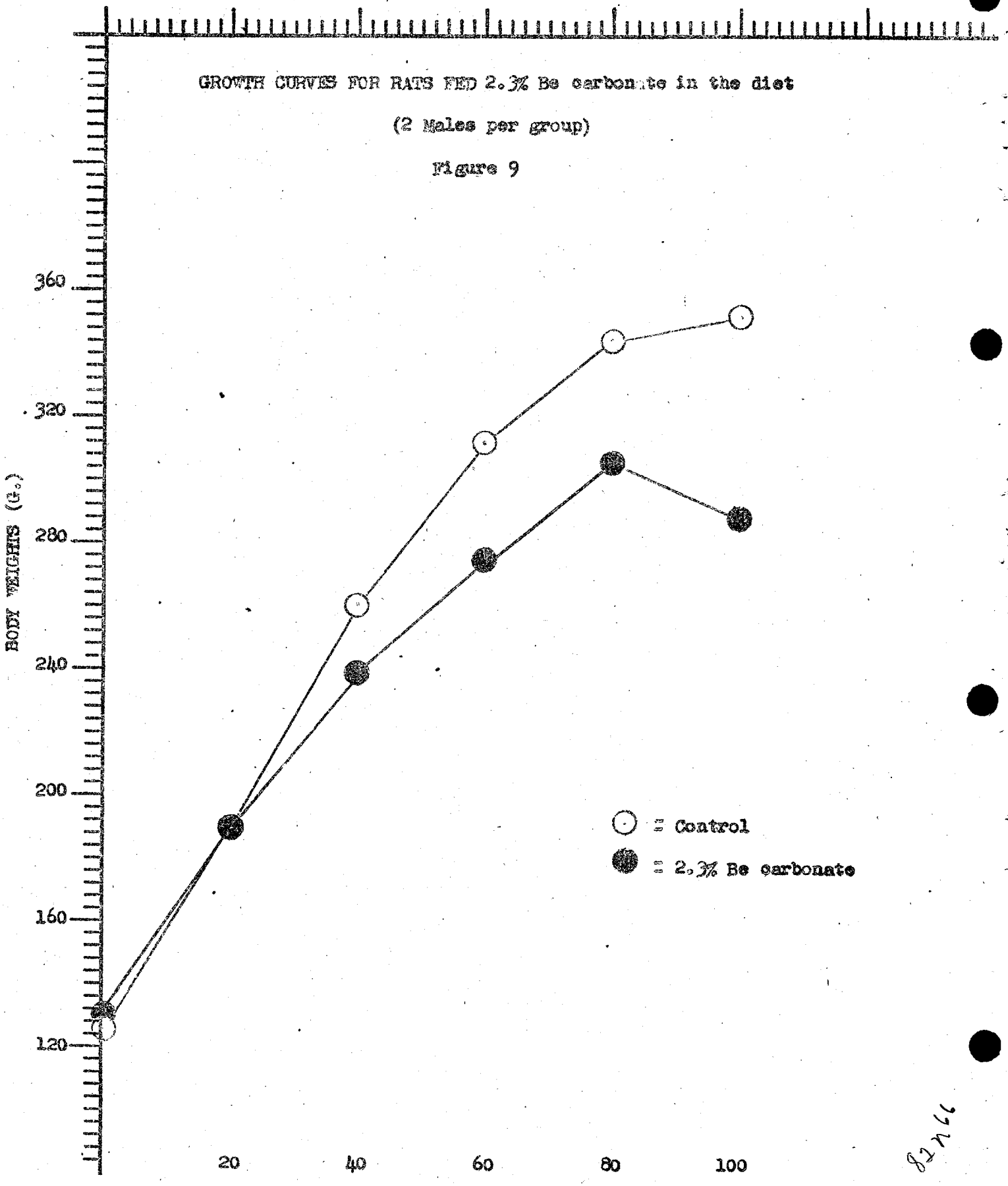
GROWTH CURVES FOR RATS FED 2.3% Be carbonate in the diet

(2 Males per group)

Figure 9



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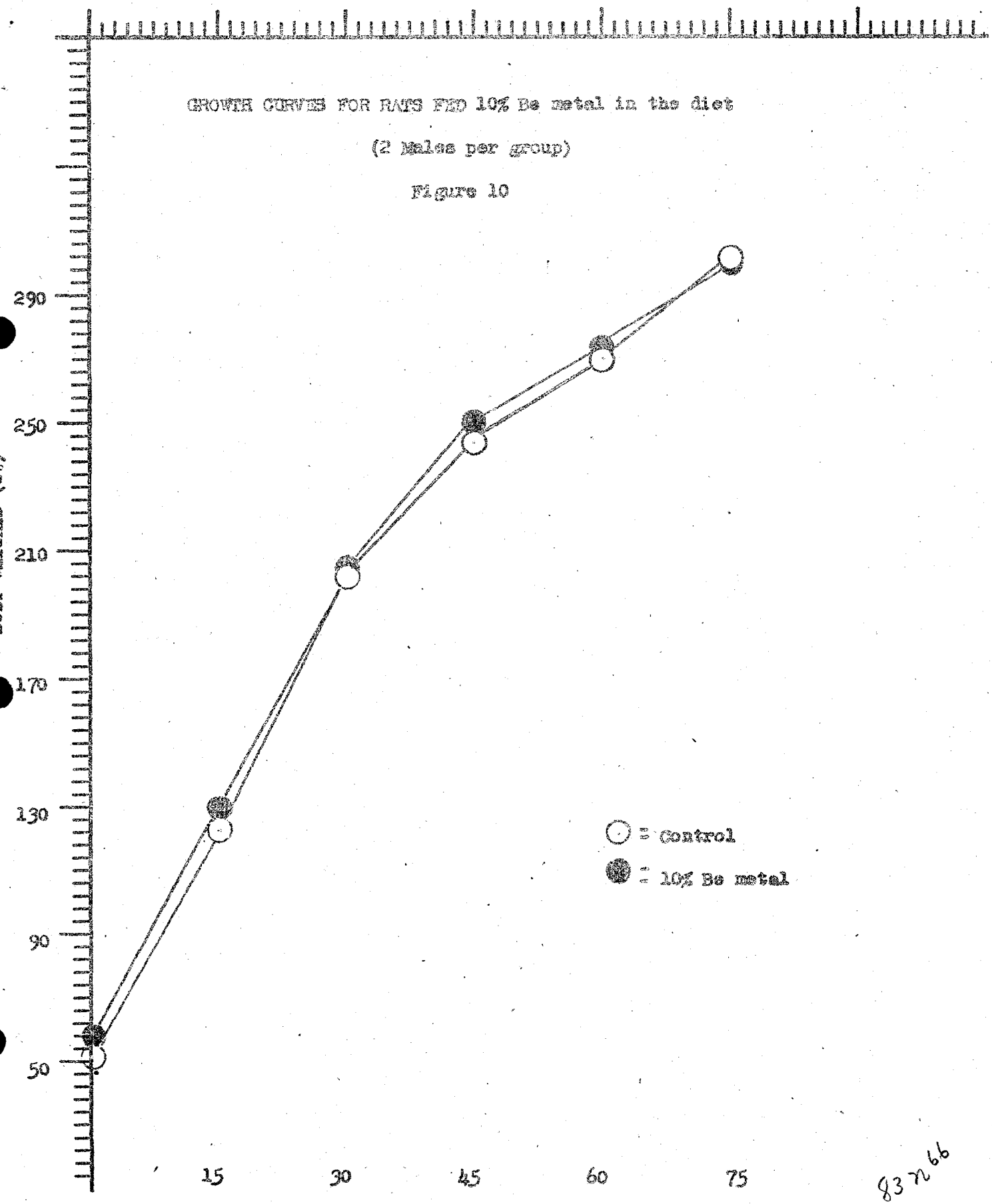
GROWTH CURVES FOR RATS FED 10% Be metal in the diet

(2 Males per group)

Figure 10



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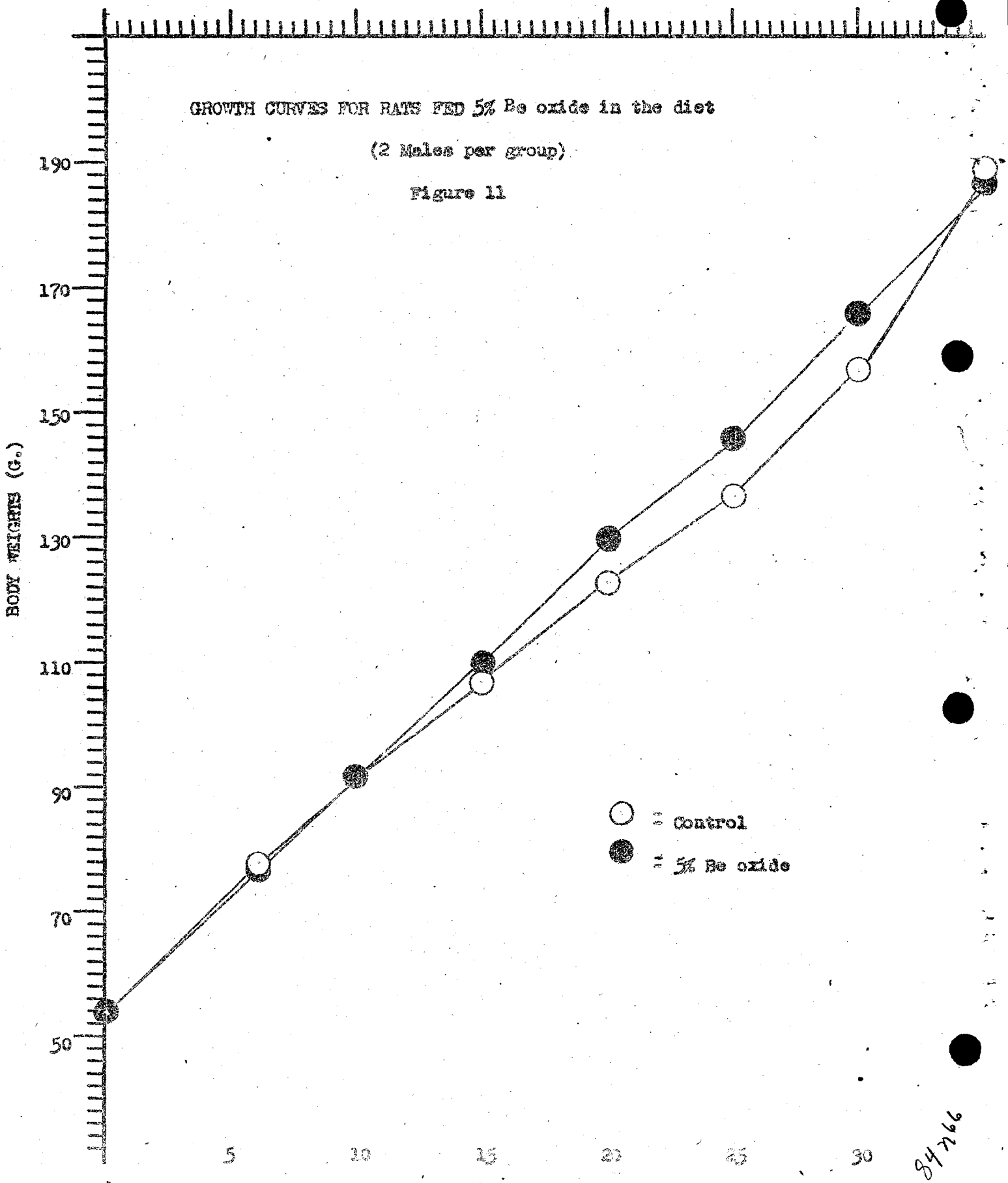
GROWTH CURVES FOR RATS FED 5% Be oxide in the diet

(2 Males per group)

Figure 11



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PRELIMINARY STUDIES IN THE TOXICITY OF BERYLLIUM

THE EFFECT OF INTRATRACHEAL INJECTION OF BERYLLIUM IN EXPERIMENTAL ANIMALS

Charles W. LaBelle

and

Martha Reid Cucci

This material is included in the following Monthly Reports: #M-1923, M-1929, M-1936, M-1946, M-1955, M-1961, M-1969, M-1974

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ABSTRACT

Beryllium and certain of its industrially important compounds have been administered to rats by the intratracheal route as an experimental introduction to the field of inhalation toxicity of beryllium dusts. The tracheal route permits known amounts of toxic materials to be introduced directly into the lungs of animals and allows a more rapid appraisal of pulmonary toxicity than is possible by the more cumbersome inhalation studies.

In the absence of information on the nature of beryllium poisoning in animals via the lung, the tracheal studies were designed to supply information on: 1) the relative toxicities (MLD) of insoluble beryllium dust suspensions and certain soluble beryllium compounds, 2) the type of physiological response, whether acute or chronic, 3) the variables which might offer a means of appraising beryllium toxicity.

The 48-hour MLD for beryllium metal dust and the insoluble beryllium compounds, the oxide, the carbonate, and the carbide, were all greater than 100 mg of material per kg of body weight of rat; that of the soluble compounds, beryllium fluoride, sulfate, and oxyfluoride, was 15, 10 and 2 mg of salt per kg of body weight, respectively. These values were comparable to the MLD via the tracheal route of such acidic salts of heavy metals as copper and zinc sulfate, and mercuric chloride. It has been concluded accordingly, because of the similarity in response, that the deaths from soluble compounds were caused by direct mechanical interference with normal lung function rather than by any specific toxic effect of beryllium

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per se.

The type of response was distinct for the soluble and insoluble beryllium-materials. The soluble beryllium compounds produced acute toxic death. On the other hand, the insoluble beryllium materials produced effects of a chronic nature. Deaths from the latter group of materials did not occur before the 100th day following exposure and became most prominent between this period and the 300th day.

In addition to decreased growth response, hematologic changes gave greatest promise of the indicating current injury from beryllium. Changes in the blood picture were confined chiefly to increases in the polymorphonuclear leukocytes. These findings were by no means uniform as was characteristic of beryllium poisoning in general in animals. At death pathological changes in the lungs of rats given beryllium metal dust consisted of an inflammatory reaction and in some instances necrosis.

At present, no animal host has been found nor method of producing the pulmonary type of injury in animals identical with that of men, despite the fact that several animal species have been investigated and numerous methods of modifying the pulmonary exposure have been tried.

signed Charles W. LaBelle
Charles W. LaBelle M.P.C

signed Martha Reid Cucci
Martha Reid Cucci

THE UNIVERSITY OF ROCHESTER
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Charles W. LaBelle

and

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INTRODUCTION

The experiments to be described in the following pages were intended to provide approximate answers to a number of general questions on the toxicology of beryllium, such as 1) the relative toxicity of certain industrially important compounds of beryllium, 2) the physiological response of the animal body to beryllium, 3) whether this response is an acute response to be studied in short-term experiments, or 4) a chronic type of response requiring long-term studies, and 5) what physiological variables may be measured in experimental animals which will reflect this response. This latter is especially important in a program of small animal research, since the literature relating to cases of suspected beryllium toxicity in human beings abounds with references to such clinical symptoms as pain, malaise, decreased vital capacity, fatigue, and respiratory sounds, all of which symptoms are difficult if not impossible to measure in experimental animals and which must be replaced by other tests more applicable to experimental animals.

The technique employed for most of these studies involved the introduction of solutions or suspensions of the materials to be studied into the lungs of white rats by injection into the lumen of the trachea. This method has the advantage of localizing the material in the lung which is

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the organ which most often serves as the portal of entry into the body in human exposures without requiring the engineering studies prerequisite to the preparation of dusty atmospheres for inhalation studies. The method also permits the measurement of exact dosage with a precision impossible to attain by inhalation techniques.

The Measurement of Minimal Lethal Dosage. When the action of a foreign material on the living body is to be studied, it is essential on the one hand to administer sufficient material to insure the production of a physiological response, and equally essential on the other hand that the dosage be sufficiently small that the animal does not die before the response may be measured. It was, therefore, necessary to determine the approximate value of the minimal lethal dose for each compound studied. The general procedure was as follows: for soluble salts, a solution was prepared approximately isotonic with physiological saline solution. One and one-half milliliters of this solution per kilogram of body weight was injected intratracheally into groups of two rats each. If either rat died, the solution was diluted 1:3 with physiological saline, and one and one-half milliliter per kilogram of the more dilute solution injected into two more rats. This process was repeated using successive dilutions of 1:3, 1:10, 1:30, 1:100, etc., until a concentration was reached which killed neither animal within 48 hours. For insoluble powders the material was suspended in saline at any convenient concentration and the suspensions diluted where necessary with physiological saline. The number of animals dying within 48 hours is shown in Table 1. The effects of 3 other acidic salts are given for comparison. The results may be summarized as follows:

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Approximate Minimal Lethal Dose

	Mg salt/kilogram body weight	Mg metal/kilogram body weight
Metallic beryllium	over 200	over 200
Beryllium oxide	over 200	over 75
Beryllium carbonate	over 100	over 10
Beryllium carbide	over 100	over 30
Beryllium fluoride	15	8
Beryllium sulfate	10	1
Beryllium oxyfluoride(a)	2	0.7
Zinc sulfate	10	5
Copper sulfate	2	1
Mercuric chloride	1	0.7

(a) Assuming a formula of $2BeO \cdot 5BeF_2$ and which closely approximated analytically determined values for Be.

The insoluble beryllium materials, beryllium metal, the oxide, carbonate and carbide thus exhibit a very low acute toxicity in the lung, while the soluble beryllium salts, the fluoride, oxyfluoride, and the sulfate, are comparable in toxicity to the heavy metal salts. The deaths caused by the soluble beryllium salts appear to be the result of direct mechanical interference with normal lung function. Therefore, any specific, toxic effect a particular beryllium compound might have would be obscured by such mechanical effects.

Thirty-Day Studies on Insoluble Compounds. Each of the insoluble beryllium materials was injected into groups of five rats at a level of 100 mg substance per kilogram body weight. Since an insoluble compound may require weeks or months before it is eliminated completely from the lungs, the animals were followed for thirty days. Weights were recorded daily, and white blood counts made at intervals of 1 or 2 days.

The growth curves, each representing the mean values for a group of five animals, are shown in Figure 1. The only compound which shows definite

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Table 1. Mortality Following Intratracheal Injections of Beryllium Compounds. Number of Animals Dying Within 48 Hours

	DOSE = TOTAL SALT INJECTED										
Dose mg/kg	Metal	Oxide	Carbonate	Carbide	Fume	Fluoride	Oxyfluoride	Sulfate	Copper sulfate	Zinc sulfate	Mercuric Chloride
200	0/5	0/2			0/2						
175											
150	0/2										
125											
100	0/5	0/5	0/5	0/5							
75										2/2	1/1
50							2/2		2/2		
40								2/2			
30											
20									2/2	0/2	2/2
15						2/2	2/4	2/3			
10											
7.5						1/2				1/2	2/2
5.0							2/3		2/2		
4.0						0/2		0/2			
2.5						0/2			1/2	0/2	
2.0											0/2
1.50							2/4				
1.25								0/2			
1.00									0/2		
0.75						0/2					0/2
0.50							0/2	0/1	0/2		
0.25						0/2			0/2		
0.10							0/2	0/1			
0.075						0/2					
0.050											
0.025											
0.010											

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Table 1. continued

Dose mg/kg	DOSE = TOTAL METAL INJECTED										
	Metal	Oxide	Carbonate	Carbide	Fume	Fluoride	Oxyfluoride	Sulfate	Copper sulfate	Zinc sulfate	Mercuric Chloride
200	0/5										
175											
150	0/2										
125											
100	0/5										
75		0/2									1/1
50											
40		0/2									
30				0/5						2/2	
20									2/2		
15							2/2				2/2
10			0/5							0/2	
7.5						2/2			2/2		
5.0											2/2
4.0						1/2	2/4				
2.5								2/2		1/2	
2.0						0/2			2/2		
1.50											0/2
1.25						0/2	2/3				
1.00									1/2	0/2	
0.75								1/3			
0.50						0/2	2/4		0/2		0/2
0.25								0/2	0/2		
0.10						0/2	0/2		0/2		
0.075								0/2			
0.050						0/2	0/2				
0.025								0/1			
0.010						0/1		0/1			

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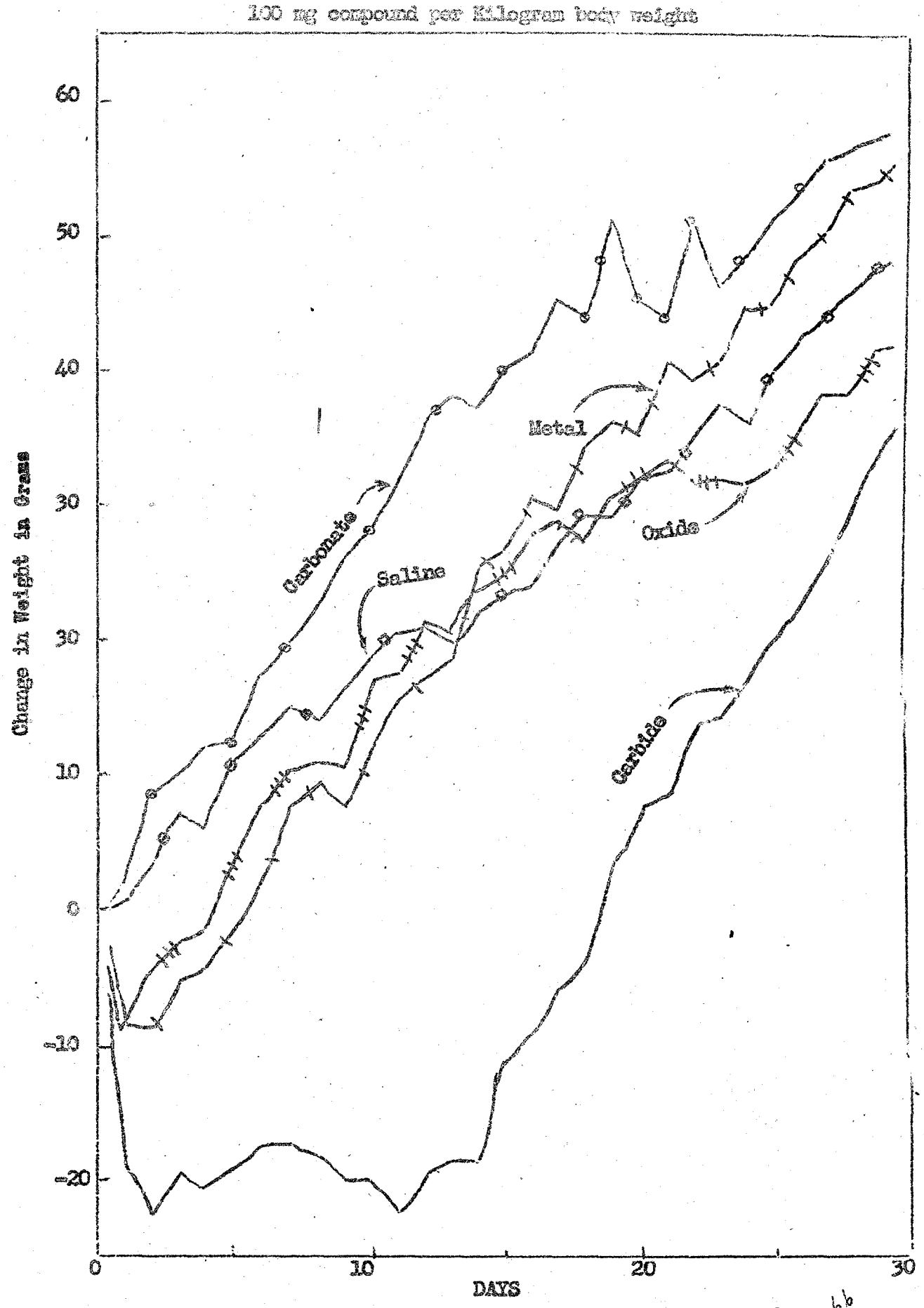
Figure 1. Growth of Rats Following Intratracheal Injection of Beryllium and Its Compounds.



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evidence of a toxic reaction is beryllium carbide; further examination of this compound showed that it decomposes slowly in water to yield acetylene, in a manner analogous to the decomposition of calcium carbide* The odor of acetylene could be detected readily in the exhalations of the rats injected with beryllium carbide, and it is probable that the response visible in the growth curve is related more directly to this reaction than to any more specific property of beryllium per se.

The curves representing the white blood counts for these same groups are shown in Figure 2. The animals treated with beryllium carbide again show some evidence of abnormality, the mean leukocyte count rising at one point to twenty-two thousand cells per cubic millimeter. Somewhat curiously, this peak occurs at a time when the weight curve would indicate that the animals had returned to a normal growth rate; no explanation is offered for this fact.

In addition, the animals injected with metal powder show unusually high leukocyte counts, peaks occurring at ten days and at thirty days. Re-examination of the data and several repetitions of the experiment revealed that the peaks are produced by one or two animals rising to extremely high values, raising the mean for the group significantly. Thus, in a group of twenty animals being studied daily, two reached maxima of 39,000 and 47,000 cells respectively at five days, a third reached 34,000 at ten days, while seven others followed daily for forty days never exceeded 21,000

* Small amounts of beryllium carbide in water in a closed test tube release sufficient acetylene over 2-3 days to produce a flame when ignited; the odor of acetylene is likewise pronounced.

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Table 2A. Means and Standard Deviations of Hemogology Variables for a Group of Control and Beryllium Injected Rats

Variable	Group	N	Mean	σ	Fiducial Limits (1%)	
WBC	Control	132	13.2	4.26	25.98	0.42
	Treated	261	13.9	4.49	27.37	0.43
% Polymorphs	Control	132	28	8.5	53.3	2.5
	Treated	261	37	13.1	76.3	0
% Eosinophils	Control	132	3	2.5	10.5	0
	Treated	261	4	3.7	15.1	0
% Lymphocytes	Control	132	69	8.7	95.1	42.9
	Treated	261	59	12.8	97.4	20.6
Absolute Polymorphs	Control	132	3.8	1.76	9.08	0
	Treated	261	5.3	3.17	14.81	0
Absolute Eosinophils	Control	132	0.35	0.332	1.346	0
	Treated	261	0.51	0.513	2.049	0
Absolute Lymphocytes	Control	132	9.1	3.14	18.52	0
	Treated	261	8.1	2.69	16.17	0.03

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Table 2B. Animals Intratracheally Injected with Beryllium. Hematological Changes in Relation to Other Signs of Toxic Damage

Group	No. of Det'ns	WBC	% Polymorphs	% Eosinophils	% Lymphocytes	Absolute Polymorphs	Absolute Eosinophils	Absolute Lymphocytes
Controls	132	13.2	28	3	69	3.8	0.35	9.1
All rats exposed to beryllium	261	13.9	37	4	59	5.3	0.51	8.1
Exposed rats during periods of normal growth	65	12.20	29.4	2.8	63.2	3.61	0.33	8.30
Exposed rats during periods of weight loss	23	14.98	49.2	0.52	50.2	7.17	0.10	7.55
Exposed rats during final week before death	20	15.89	48.8	0.70	50.1	7.47	0.14	8.36

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morphonuclears of the blood. The fact that this rise may or may not be sufficiently great to affect the total white cell count undoubtedly explains some of the inconsistencies observed in the white cell counts described in the preceding section.

Figure 3 shows the growth and mortality curves for a period of 250 days for a series of twelve beryllium-injected rats and ten control animals injected intratracheally with saline solution. Growth appears to be essentially normal, at least for the first two hundred days; the fluctuations in the last fifty days are the unavoidable effect of the loss of animals from a small residual group. The mortality curve indicates that 75% of the exposed group has died, compared with none of the control group. Since these rats were less than a year old at the end of the period described, and since normal mortality from "old age" is from 9-12% at one year for rats in our colony, this high mortality rate cannot be ascribed to "old age". Several other series which have been similarly treated with beryllium, but for shorter time intervals, are confirming the earlier portions of this mortality curve.

A limited amount of pathological data is available for these animals up to about 100 days*. When injected into the lungs of rats, beryllium metal induces an inflammatory reaction sufficiently severe to lead to necrosis of tissue. The metal is gradually removed from the lung parenchyma and appears largely in the peribronchial lymphatics, some reaching the tracheobronchial lymph nodes (where its presence has been confirmed spectroscopically). No permanent scarring, fibrosis, or granulomatous lesions in the lung have been produced up to 100 days and no changes in other organs of the body were

* For this we are indebted to Dr. James Scott of the Pathology Department of the Atomic Energy Project in Rochester.

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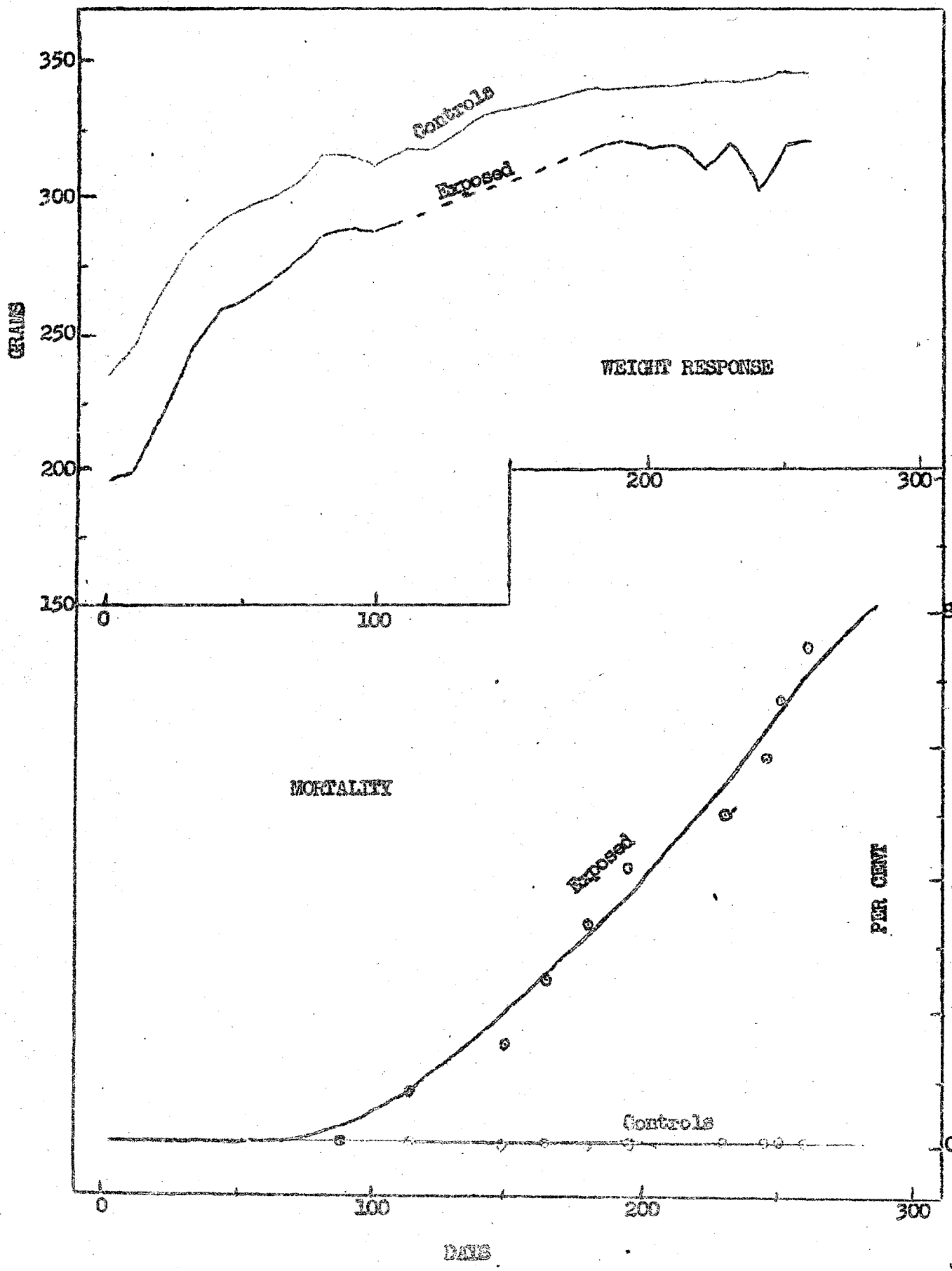
Figure 3. Growth and Mortality in Rats Following Intratracheal Injection of Metallic Beryllium at 100 mg Be/kg

12 Animals



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demonstrable. The ultimate fate of the injected metal is still uncertain f from the autopsy material which is so far available.

The deaths that occurred in this series of animals appeared to follow a reasonably consistent pattern. The terminal weights for some of the dying animals is shown in Figure 4 where the individual animal weights are plotted for the thirty days preceding death. It will be seen that although the group as a whole increased in weight (Figure 3), the individual animals lost weight consistently for the month preceding death. The relative and absolute polymorphonuclear counts for the same period are shown in Figure 5. The black circles for the exposed animals represent counts made at the times indicated, while the red circles represent control counts made at approximately the same calendar dates. While the separation between exposed and control animals is not without some overlapping, the tendency toward high polymorphonuclear counts in these animals is well marked.

Concurrent with these changes, the animals tended to develop dyspnea, severe rales, and an increased irritability. Normal activity was replaced by hyperexcitability during handling. The most consistent gross post mortem finding was a severe lung damage involving various combinations and degrees of edema, hemorrhage, consolidation, and ulceration.

The Effect of Secondary Factors on Beryllium Toxicity. Since the time intervals involved in the preceding study represent so large a fraction of a rat's total life span, a series of short-term experiments have been carried out to determine whether a combination of beryllium exposure with other common debilitating agents might cause a more rapid development of the damage described above. Among the secondary agencies studies were enforced activity; daily exposure to high temperatures (110°-120° F);

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Figure 4. Terminal Effects: Weight changes in exposed animals during month preceding death.

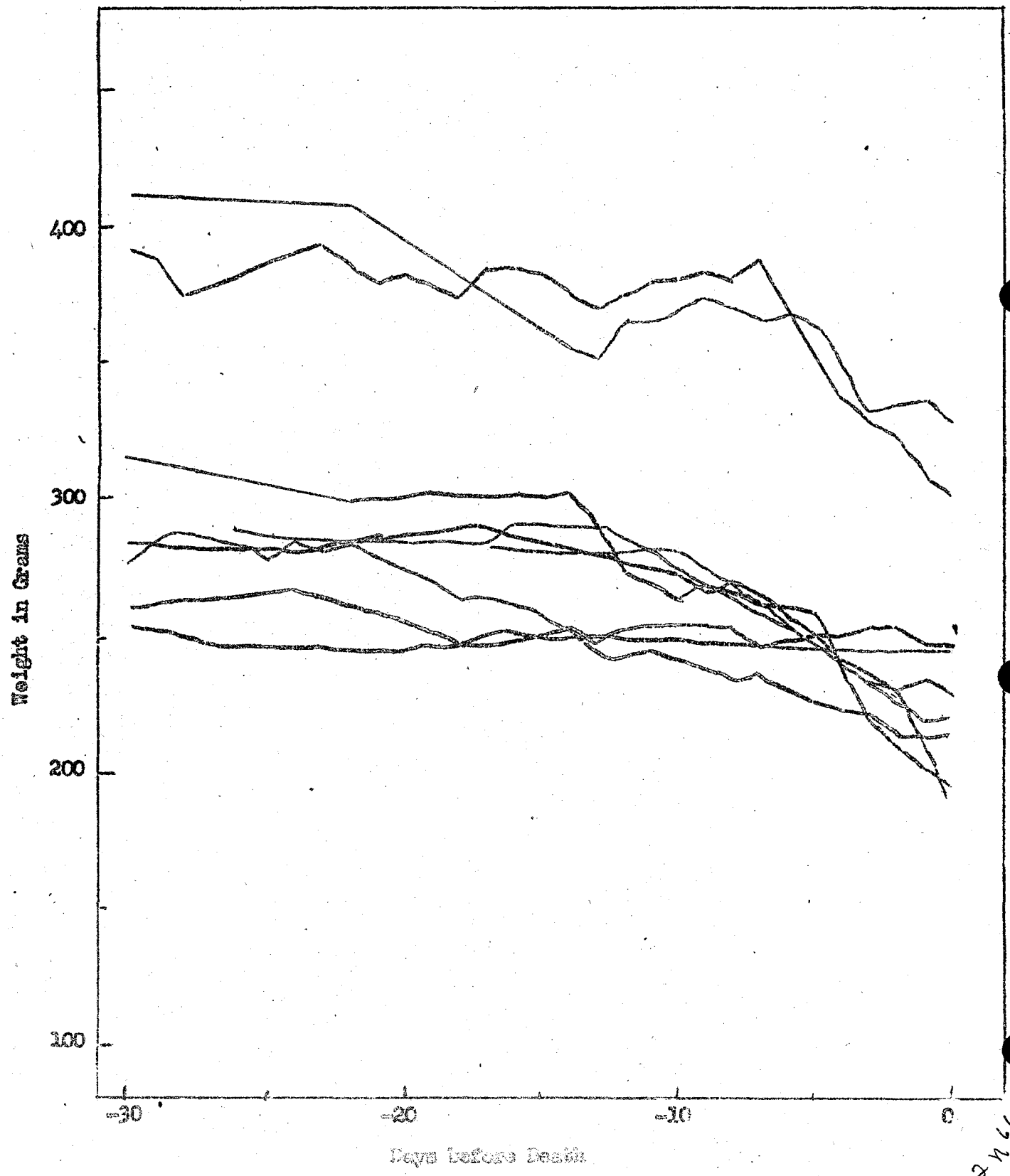
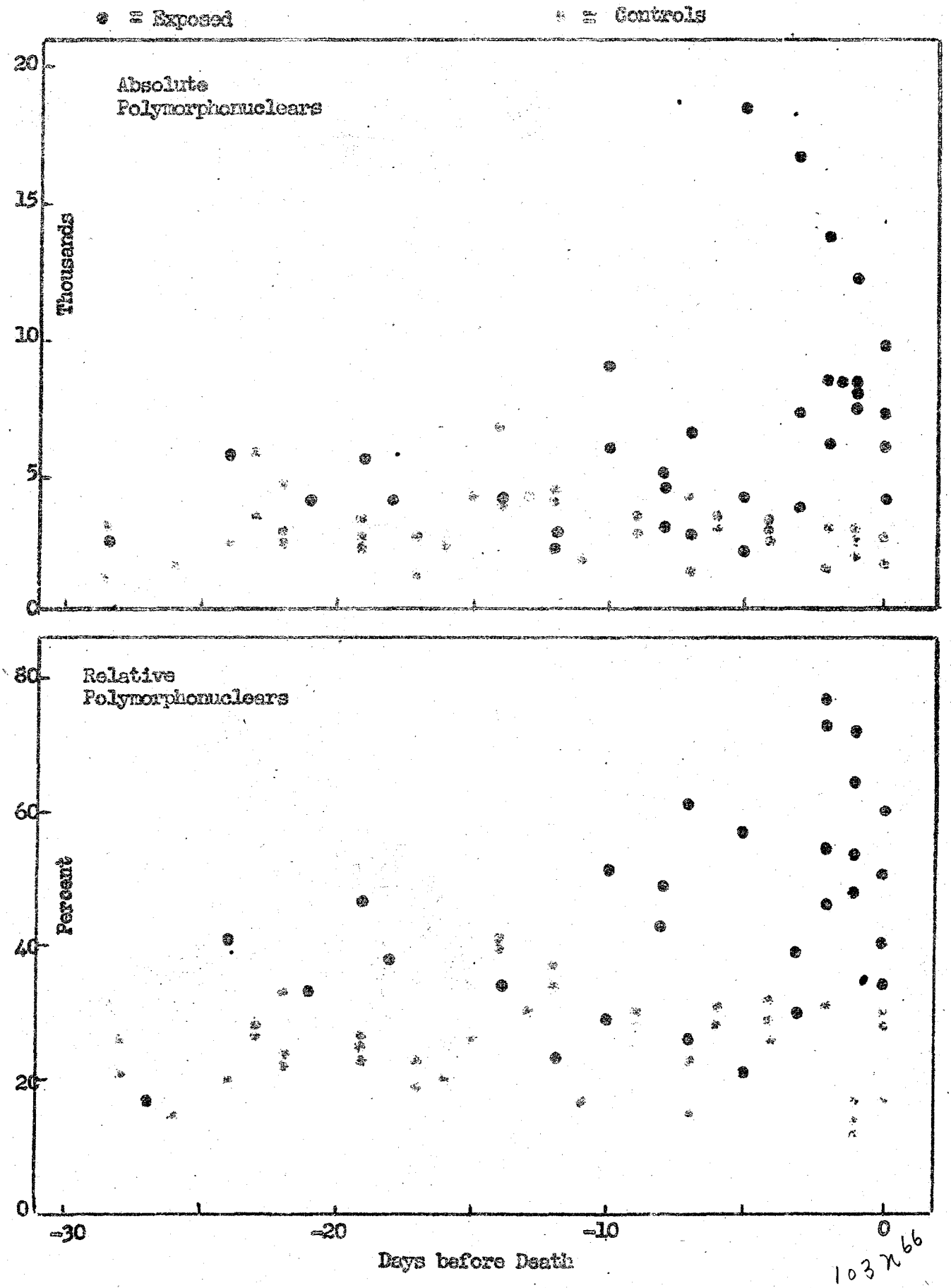


Figure 5. Terminal Effects: Relative and absolute polymorphonuclear counts made during month preceding death.



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daily exposure to low temperatures (20°-40° F); infection with various pathogens such as salmonella, pneumococcus, and pertussis; exposure to uranium dusts; exposure to hydrogen fluoride vapors; repetitive exposure to beryllium in an attempt to produce anaphylaxis; and the use of oils rather than saline as a carrier for the metal powder. Only a half-dozen deaths resulted from the entire study, comprising nearly 150 animals, and to date in no case was unequivocal evidence obtained which would indicate a specific relationship of any magnitude between any of these agencies and the toxic effects of beryllium itself.

CONCLUSIONS

Soluble beryllium salts, when introduced into the lungs of rats, exhibit lethal properties comparable to those of the heavy metals such as copper, zinc and mercury. Insoluble beryllium compounds are much less toxic when so introduced. Metallic beryllium and possibly other beryllium compounds as well produces a more chronic type of lung damage of an apparently nonspecific type which eventually leads to the death of the animal. The lung damage is apparently not identical with the human types of damage. No method has been found by which this chronic process may be appreciably hastened.

signed Charles W La Belle
Charles W. LaBelle M.R.C.

signed Martha Reid Cucci
Martha Reid Cucci

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INITIAL STUDIES OF THE INHALATION TOXICITY OF BERYLLIUM SULFATE AND BERYLLIUM METAL FUME

Geo. F. Sprague, Chas. W. LaBelle, Alton G. Pettengill, Herbert E. Stokinger

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INITIAL STUDIES OF THE INHALATION TOXICITY OF BERYLLIUM SULFATE AND BERYLLIUM METAL FUME*

Geo. F. Sprague, Chas. W. LaBelle, Alton G. Pettengill, Herbert E. Stokinger

ABSTRACT

Beryllium Sulfate. Data from 56 animals were collected to supply information relevant to the poisonous effects, including the organs injured and the route of the damage, caused by the inhalation of beryllium sulfate tetrahydrate dust at a concentration approximating 90 mg of the salt per cubic meter of air. Six criteria of toxicity were utilized in evaluating the information obtained from the animals which comprised 2 dogs, 3 rabbits, 10 rats, 14 guinea pigs, 20 mice and 7 hamsters. The animals were exposed 6 hours daily for a two-week period, a total of 66 hours, to a dust of this soluble beryllium salt in a small inhalation exposure chamber.

Mortality for all species dying as a result of exposure was 43% of the 56 exposed animals or 20 of 20 mice, 2 of 10 rats, and 2 of 7 hamsters. The mice died from the 3rd to the 11th calendar day with an LD_{50} being attained on the 7th day following the start of exposure. No deaths occurred among the dogs, rabbits, or guinea pigs. Weight response data showed that all species save the guinea pig were adversely affected. The rabbit lost 5% in weight, whereas the rat and hamster lost 11%. Clinical chemical values indicated renal impairment and some subsequent regeneration in the rabbits. Much less serious kidney damage and hepatic injury in dogs was indicated from many chemical tests which included frequent analyses of the blood for sugar, NPN, urea nitrogen, amino acid nitrogen, bromsulfalein and serum

* Acknowledgement is gratefully made to Dr. Harold C. Hodge, Head of the Division of Pharmacology, Atomic Energy Project and Professor of Pharmacology, School of Medicine and Dentistry, University of Rochester for his helpful assistance and guidance in these studies.

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protein. All of the urinary variables including amino acid nitrogen/creatinine ratio, sugar and protein were normal at all times. Hematologic results showed definite upward trends in the leukocytic count, notably the absolute neutrophil counts of the dogs, rats and rabbits during the second week of exposure. The other cellular blood elements gave no characteristic trend for any of the species. External symptoms varied somewhat with the species. Ocular opacity developed in the guinea pig, mouse and dog following 12 hours of exposure. Cutaneous lesions of varying size developed over the body of the dog, while the rats were found to have râles. The hamsters and rabbits manifested no external signs of beryllium damage. The histological findings showed the effect of absorption of beryllium sulfate tetrahydrate through the respiratory tract of the mouse, rabbit and rat to be pulmonary damage especially edema. This was not the type of injury, however, found in man. Additional lung lesions observed included inflammatory exudate in the lumina of the terminal bronchi and some foci of atelectasis of the rabbit, hemorrhage of the alveolar sacs of the hamster, and infiltration of neutrophils and monocytes in the lungs of rat. Hepatic injury was observed in mice dying during the first 9 days, but no lesions were noted thereafter, indicating rapid regeneration. Renal changes were found in the rabbit and rat but some regeneration of the tubular epithelium was observed in the rabbit kidneys.

Marked species variation, but unusually good conformity within each species, was thus observed in the response to the inhalation of $BeSO_4 \cdot 4H_2O$. The sulfate ion, conferring a high acidity upon the molecule as a whole (with an approximate pH of 1.0 for the saturated solution), was felt to contribute significantly to the toxicity of the beryllium salt.

Beryllium Metal Fume. Rats have been exposed by inhalation to beryllium metal fume at an approximate concentration of 800 mg/m^3 of air under a variety of exposure schedules to determine the character of the toxic response. Seven of 10 rats died of pulmonary hemorrhage following exposure to 24,000 CT units of fume administered as eight exposures of from 3 to 4 minutes each over a period of two hours. When the exposure was one-half this value, or 12,000 CT units, no rats died or showed other untoward response. Moreover, rats were found to tolerate 200,000 CT units or nearly 10 times the lethal dosage if administration of the fume was made at the rate of 4,000 units daily for a period of 60 days. Some reduction in growth rate, however, was noted on this schedule.

It was, therefore, concluded that beryllium metal fume acts as a primary irritant which, when administered rapidly in high dosage, may be fatal but which is tolerated relatively well as long as no single dose exceeds 12,000 CT units. No cumulative poisonous effects were seen.

signed Geo. F. Sprague
Geo. F. Sprague

signed Chas. W. La Belle
Chas. W. LaBelle

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INITIAL STUDIES OF THE INHALATION TOXICITY OF BERYLLIUM SULFATE AND BERYLLIUM METAL FUME

Geo. F. Sprague, Chas. W. LaBelle, Alton G. Pettengill, Herbert E. Stokinger

BERYLLIUM SULFATE STUDY

An inhalation study of the toxic effects of a soluble beryllium salt, the sulfate tetrahydrate, has been performed on 56 animals representing six species. Following a conditioning period of two weeks, the animals were exposed six hours daily in a dust inhalation chamber for a period of two weeks, a total of 66 exposure hours. The concentration of dust approximated 90 mg of $BeSO_4 \cdot 4H_2O$ per cubic meter of air; expressed as the cation, 4.5 mg Be/m^3 or 0.5 mM Be/m^3.

For reasons of chamber space limitations, not all the animals could be exposed concurrently. Three individual exposures were made under similar conditions of time and dust concentration. Mortality, weight response and histologic changes were measures of toxicity employed in one study; cellular blood counts and biochemical findings in addition to the above tests were employed in another study. In the third study, in which two dogs only were used, several biochemical tests for detecting possible beryllium injury were employed as well as thorough hematologic studies, in an effort to establish critical diagnostic aids for beryllium poisoning as well as the route and site of beryllium damage.

MATERIALS AND METHODS

The chamber in which the animals were exposed was a 31" transite-lined cube with a capacity of 17.8 cu ft (505 liters). The animals shown in the chamber in Figure 1 occupied a volume of approximately 19.7 L or 3.9% of the chamber space. Also visible is the type of cage employed for exposure of the

Figure 1, photograph of exposure chamber, is not included in this copy because of time and expense.

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animals. Whenever practicable, partitioned cages were used to insure individual exposure of the animals. The photograph, taken at the conclusion of a day's operation shows a considerable deposit of the sulfate on the cages, around the opening of the doorway (the door has been removed), and on the rear observation window.

Figure 1 also shows the majority of the valves and manometers used in the control of the chamber dust concentration. The valve shown at the right center of the open doorway is used to adjust the air velocity during sampling of the dusty atmosphere. The manometer to the right of the valve indicates the hydrostatic pressure of the sampling line. At the middle right of the unit is one of the ports where the sampling line may be inserted. Below the sampling port is an inclined manometer to indicate the rate of air flowing through the chamber at any given time. The vertical manometer at the extreme right of the chamber is used to indicate the rate of nitrogen flow through the dust feed. Air from the centrally located filtering and refrigeration unit is introduced into the chamber at the base of the unit at the rate of 18 cu ft per minute thus permitting one air change per minute. A fan, to aid in the even distribution of the air and dust in the atmosphere, is located in the ceiling of the unit. The air-evacuation flues are located on the side walls near the ceiling. Before being drawn into the central rotoclone, the exhausted air is drawn through a scrubber (the cone-shaped portion of which is visible above the unit) using water as the scrubbing medium. Scrubbing traps the beryllium sulfate and prevents its liberation into the air in or around the building.

The type of dust feed found most satisfactory in producing the desired concentration was the rotating barrel feed of 3" diameter (shown below the

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unit in Figure 1). A 1/20th horsepower motor rotated the barrel on its horizontal axis so that the dust came to the apex of the conical cylinder. Here it was forced into the feed line by a stream of dry nitrogen and carried up into the chamber. At the base of the chamber the air-intake line and feed line converged, thus blowing the finely divided dust into the chamber. A constant check of the concentration was maintained by taking frequent samples (from 9 to 15 per day) of the chamber atmosphere. The concentration in terms of milligrams of $BeSO_4 \cdot 4H_2O$ per cubic meter of air was determined gravimetrically.

Because of the somewhat hygroscopic nature of this beryllium compound, considerable difficulty was encountered in preparing a dust whose median particle size was no greater than 2 micra. The technique developed involved drying the beryllium salt for at least 24 hours in a vacuum desiccator, before passing it twice through a micropulverizer. Only the dust that passed a 230-mesh sieve was ultimately used in the feed, which was filled twice daily with vacuum-dried dust. Between each step of the dust preparation, the material was kept in evacuated desiccators. Beryllium sulfate tetrahydrate, left exposed to the air, completely dissolves in the absorbed water, yielding a saturated solution of approximately pH 1.0.

It was impossible to maintain at all times the desired concentration of 90 mg of the salt per cubic meter of air in the inhalation chamber. A weighted mean concentration determined gravimetrically from 137 samples was 84.3 mg/m^3 with a standard deviation of 36.6. The extreme values were 13.2 and 267.0 mg/m^3. Most, 94, of the concentration values were between 50 and 135 mg/m^3.

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Toxicity Criteria. The types of data collected and analyzed (Figure 2) included mortality, weight response, micropathologic and hematologic findings and biochemical changes. The blood determinations included nonprotein nitrogen, urea nitrogen, amino acid nitrogen, serum protein, sugar, albumin-globulin ratio, bromsulfalein and fibrinogen levels. The urinary studies included sugar, protein, and amino acid nitrogen/creatinine ratio. Figure 2 lists the number of animals of each species used in each of the toxicologic observations. Obviously, the animals of the various groups, particularly the dogs, were used for more than one test.

RESULTS

Mortality. Figure 3 is a graphic representation of the mortality rate for the three species in which deaths occurred. It shows that all of the 20 mice exposed in the chamber died before the termination of the experiment, an ID 50 occurring on the 7th calendar day. Because of the high mortality rate observed in this species, 10 additional mice were exposed for two days before the termination of the study. Two of these ten mice died following the second day of exposure. Two of ten rats died and two of seven hamsters died as a result of the exposure. The broken lines on the graph indicate the time from the start of exposure until the first death occurred; the broken line indicating the uncertainty in the length of time of exposure required to cause the first death. There were no deaths among the dogs, rabbits, or guinea pigs.

The outward symptoms and reactions to the beryllium dust differed markedly for each of the species. At the conclusion of the first 6-hour day of exposure, the eyes of the mice were irritated but were improved by morning. The mice, while undergoing treatment, were hyperirritable, more

Figure 2. Distribution of Animals for Toxicity Criteria

Criteria Studied	SPECIES					
	Dog	Rabbit	Guinea Pig	Mouse	Hamster	Rat
Mortality	2	3	14	20	7	10
Weight Response	2	3	14		7	10
Biochemistry						
Blood						
NPN	2	3				
Urea N	2					
AAN	2					
Sugar	2					
Serum Protein	2					
A/G Ratio	2					
Urine						
Sugar	2					
Protein	2	3				
AAN/Creatinine	2					
Hematology	2	3				10
Pathology						
Terminal	2	3	14		5	8
Dying Animals				20	1	2
Liver Function Tests						
Fibrinogen Levels	2					
Bromsulfalein Retention	2					

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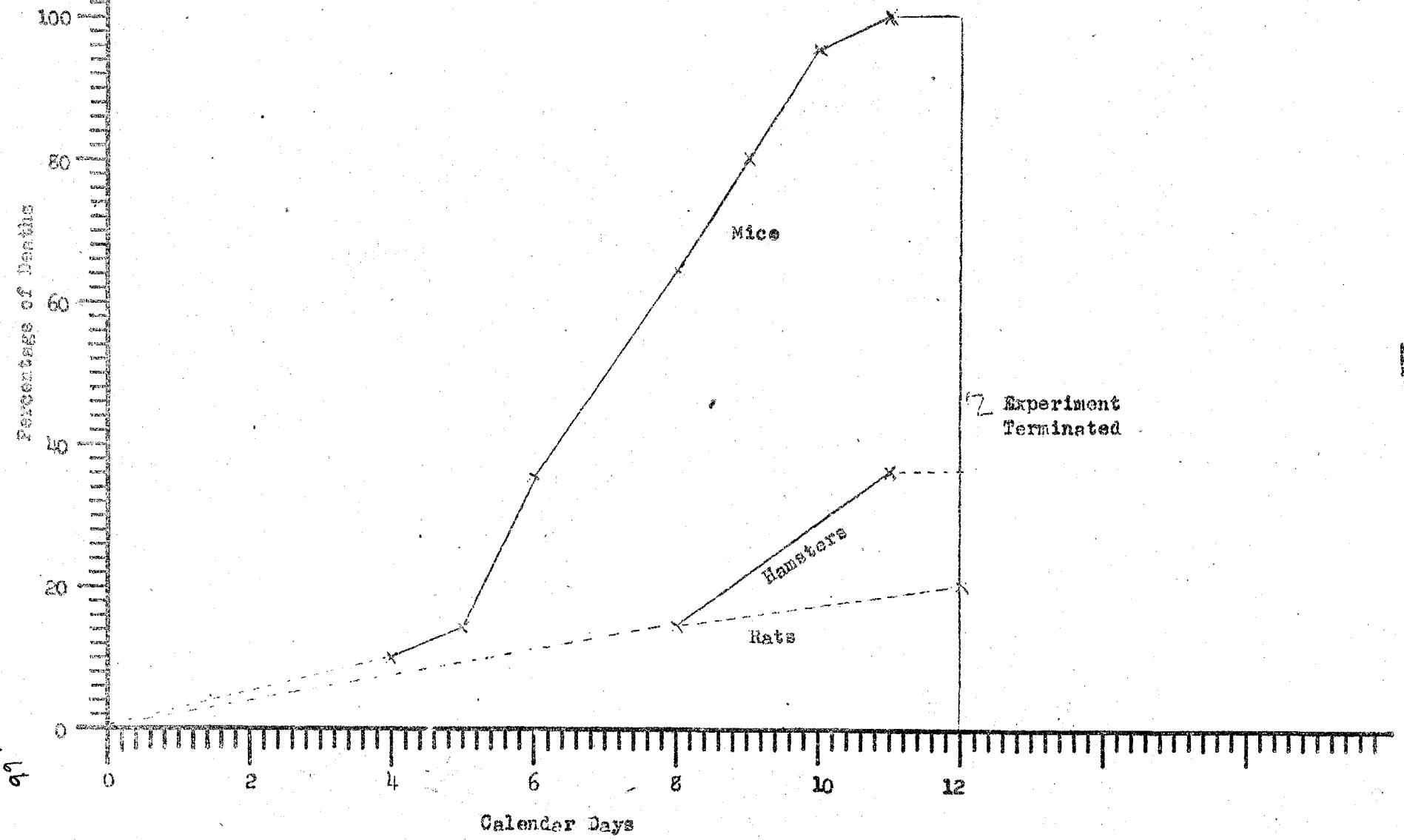
Figure 3. Mortality Curves of Mice
Hamsters and Rats Exposed to 90 mg/m^3 of Beryllium Sulfate



active than normal, and before death usually went into convulsive spasms. The eyes of 6 of the 14 exposed guinea pigs showed definite signs of cataract formation. The ocular opacity of these animals developed after two days or 12 hours of exposure. The eyes of a few of the rats appeared hemorrhagic and the breathing of some indicated râles.

It was originally planned to expose the two dogs for two weeks and then hold the animals for a year in order to follow the effects of the beryllium exposure; however, such serious ocular injury and periorbital lesions occurred (Figure 4) that it appeared probable that at least one of the dogs would die from subsequent infection. These lesions were aggravated by the animal scratching its eyes with its paws and also by rubbing its head and eyes against the metallic mesh of the cages. This action caused the left eye to protrude ½ inch while the ulcer about the right eye, at autopsy, burst upon the application of slight pressure. Other less extensive (Figure 5) but well-defined ulcerative lesions developed over the legs and body of the second dog. A small cataract was noted on the left eye of this animal. The highly acidic character of this beryllium compound, no doubt, was responsible for the start of ulcerative lesions observed in these exposed animals.

The beryllium sulfate caused fewer discernible external symptoms in the rabbit and hamster, than in the other species. A hypersensitivity and nervousness was noted in the hamster, but no outward changes were observed in any of the three rabbits.

The <u>weight response</u> data showed that all of the animals, with the exception of the guinea pigs (Figure 6), were adversely affected. There was less fluctuation in the weight of the guinea pigs during the exposure

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Figures 4 and 5, photographs of the dogs showing ulcerous lesions, are not included in this copy because of time and expense.

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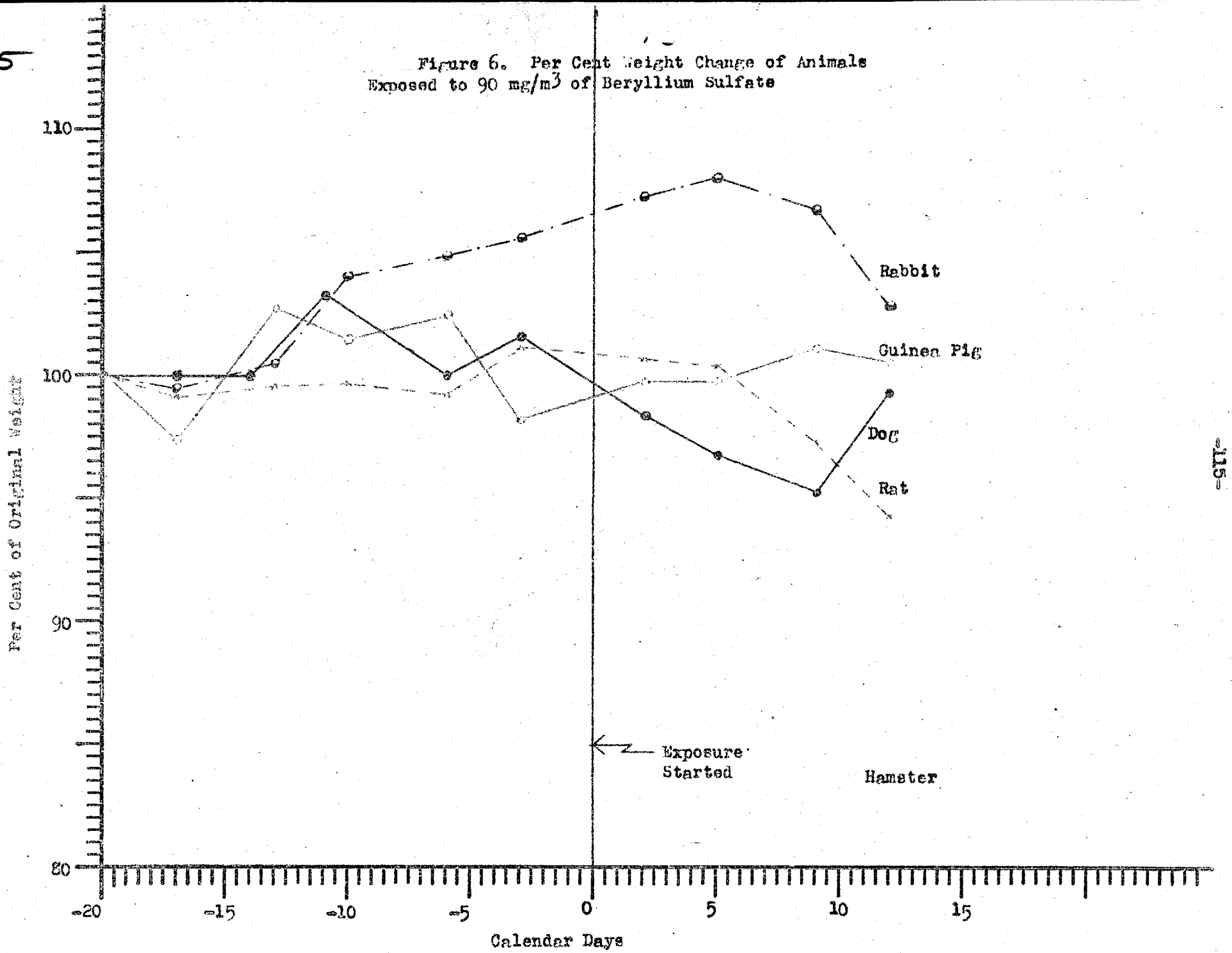
Figure 6. Per Cent Weight Change of Animals Exposed to 90 mg/m^3 of Beryllium Sulfate



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period than during the two-week conditioning interval. The rabbits lost 5% in weight while the hamster and the rat lost 11% of their weight during the period of exposure. The dogs, as shown in Figure 6 were 5% below their original weight but regained this by the time the exposure terminated. Since no mice survived, complete weight data are not available, but it was noted that the mean weight declined for the mice surviving until the 9th calendar day.

Of 9 hematologic blood variables studied in the dog, rabbit and rat, (Figures 7 & 8) only the leukocyte and the absolute neutrophils* showed any significant change. This constituent showed a marked rise for the three species between the 9th and 12th day of the experiment; however, values from normal animals have occasionally been recorded that were higher than any noted in this study. The red blood count showed little change.

The clinical chemical values indicated that renal damage occurred in the rabbits during the second week of the experiment, (Figure 9). During the 2-week conditioning period and the first week of dust exposure, the mean NPN values were unusually constant for rabbits; but in the second week the values rose to 78 mg% and were over 70 mg% at the termination. The rabbit urinary protein values showed much the same trend with the first positive value being recorded on the 6th day and a high mean of 350 mg% on the 10th day. The final protein value was 80 mg%. This decreased proteinuria may be an indication of regeneration of the renal cortical tubules.

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* Obtained by multiplying the percentage of neutrophils by the number of white blood cells.

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Figure 7. White Blood Count of Rats and Rabbits Exposed to 90 mg/m3 of Beryllium Sulfate

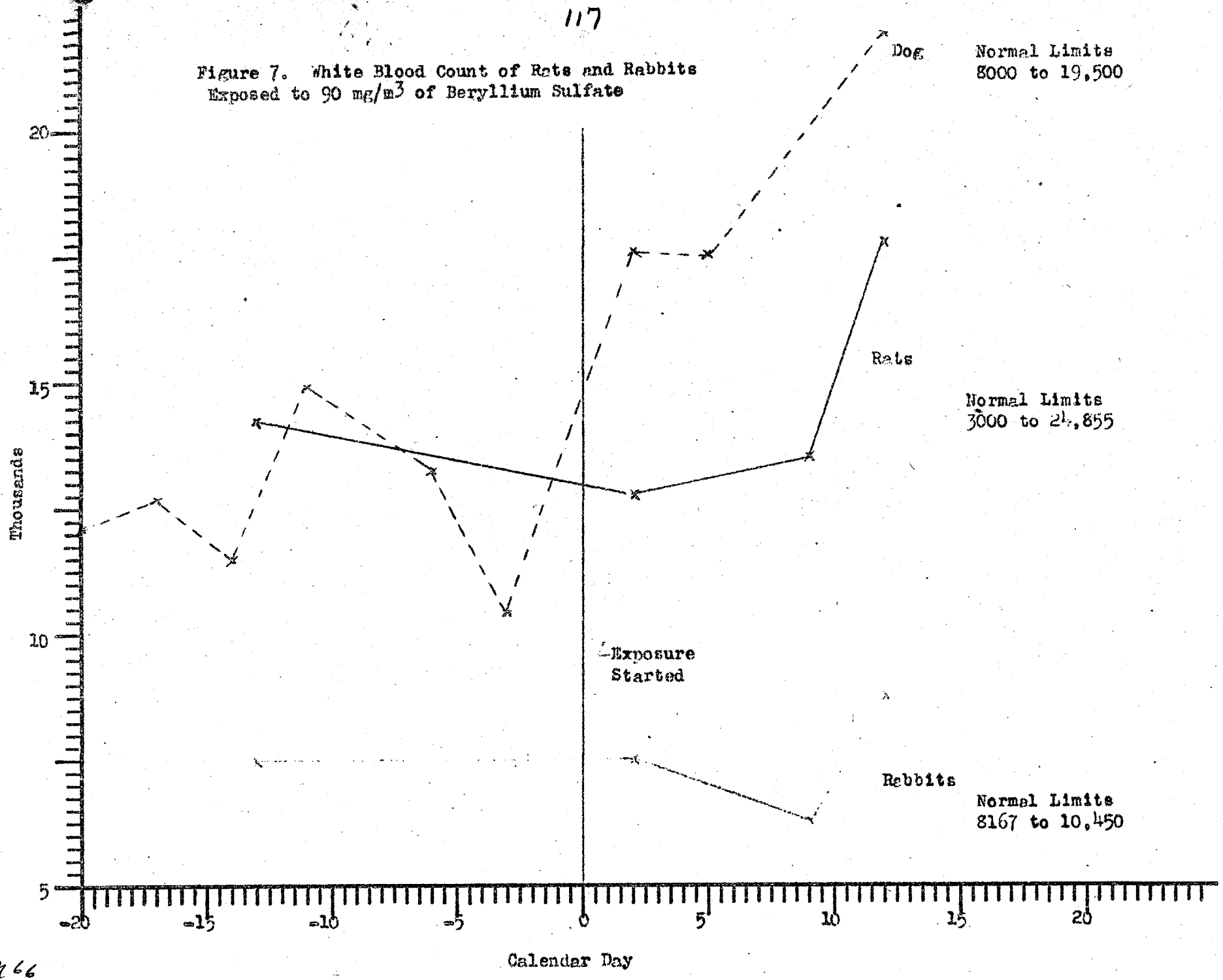

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Figure 8. Absolute Neutrophils of Rats and Rabbits Exposed to 90 mg/m3 of Beryllium Sulfate

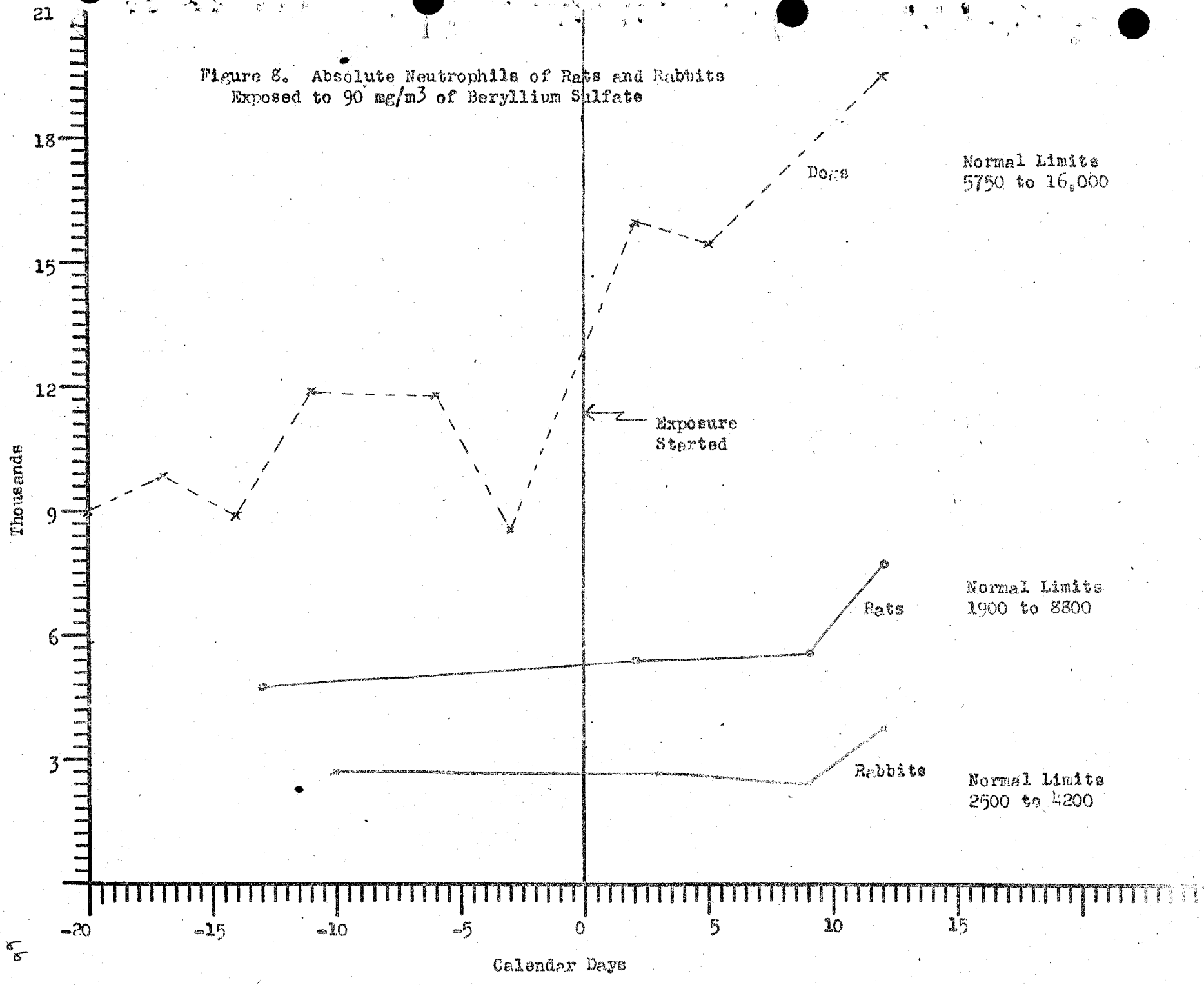
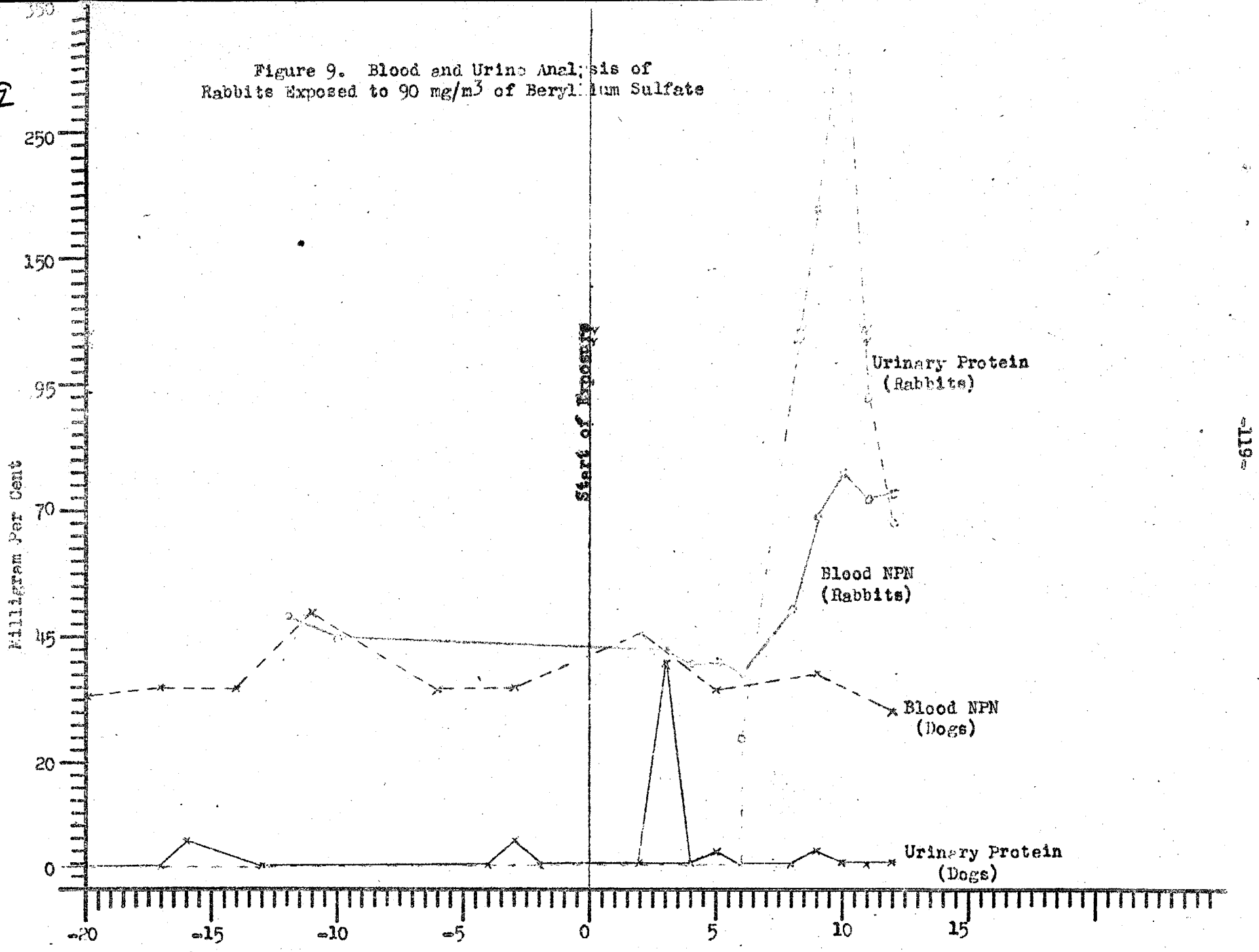


Figure 9. Blood and Urine Analysis of Rabbits Exposed to 90 mg/m^3 of Beryllium Sulfate



The biochemical findings in the dog failed to reveal any indication of renal or hepatic injury during the course of exposure. As seen in Figure 9, a higher NPN value was obtained for the dogs during the conditioning exposure than was found at any time after the dust was introduced into the chamber. The high urinary protein value found on the second day of exposure was probably caused by contamination of the specimen. The other urinary constituents failed to show any results that were indicative of kidney impairment. All of the amino acid nitrogen/creatinine ratios and urinary sugar values were definitely normal.

Biochemical evidence of a negative nature was obtained from all of the analyses of the blood of the dogs for sugar, urea nitrogen, amino acid nitrogen bromsulfalein and serum protein as all of these values were within normal limits and showed no trends from a time-study viewpoint. The albumin/globulin ratio results showed an upward trend toward the end of the experiment. The rise was caused by a decrease in the globulin fraction of one of the dogs with a corresponding decrease being noted in the fibrinogen level, which is a globulin protein, of the blood.

The <u>micropathologic</u> results, (Dr. James K. Scott) like the data obtained by means of the other toxicologic criteria, showed a marked species differentiation yet reasonable similarity within each of the species. Seventeen of the 20 dying mice were autopsied and microscopic sections of the organs made. Since considerable autolysis had occurred between the time the animal died and before the sections could be taken, the lung and kidney sections, two organs of importance in beryllium sulphate poisoning, were of little value so far as this species was concerned. The liver of the mouse showed definite lesions that were comparable to those seen when lethal doses of beryllium

sulfate was administered intravenously or intraperitoneally. The lesion was a central or midzonal necrosis of liver cells, with the amount of cellular necrosis varying with the time of death of the animal. The last four mice to die had no lesions of this type indicating complete regeneration of the liver had occurred by the 9th calendar day.

The rabbits showed the most severe and widespread pulmonary lesions of the species used in this experiment. These lesions consisted of a fairly widespread edema, some foci of atelectasis, and some inflammatory exudate in the lumina of the terminal bronchi. The kidneys of the rabbits showed considerable necrosis of the tubular epithelium, particularly in the distal portion of the proximal convoluted tubule and in the descending loop of Henle. Regeneration and necrosis can be seen occurring in the same kidney. No lesions were found in the glomeruli. The other organs of the rabbit, including the eye and liver, failed to show any remarkable changes.

The guinea pigs showed the fewest histological changes of any of the species the lesions being limited to the lung and the eye. The lung lesions were similar to those noted in the rabbit but were much less extensive and less severe. The eyes of most of the guinea pigs showed changes consisting of conjunctivitis and keratitis. All of these animals showed an inflammatory reaction at the limbus.

The injury to the hamster was almost entirely limited to the lung, consisting chiefly of edema accompanied by varying amounts of hemorrhage of the alveolar sacs. None of the other organs of the hamster had any changes that could be attributed to beryllium poisoning.

Two rats died on the final day of the experiment. Whereas the liver sections showed no necrosis, the cells lining the kidney tubules showed

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numerous lesions. The most extensive changes found in the sacrificed animals occurred in the lungs and kidneys. The pulmonary change most prominently seen was a rather marked edema which was accompanied by a moderate infiltration of neutrophils and monocytes. The kidney damage seen in the rat was much like that noted in the rabbit. There was no liver damage in this species.

The last figure (10) summarizes the histological findings in the various species. In this table the higher numbers represent the greater amount of tissue damage. It is evident that the guinea pig and hamster seem to be the most resistant species and the rabbit and mouse the most sensitive. No explanation is offered for the lack of finding of hepatic injury in species other than the mouse although hepatic injury was observed in mice that died early in the exposure.

The histologic findings for the two dogs are not yet available; however, the gross pathologic examination failed to show any marked damage by the $BeSO_4 \cdot 4H_2O$.

SUMMARY

Data have been collected and evaluated on 2 dogs, 10 rats, 3 rabbits, 14 guinea pigs, 20 mice and 7 hamsters exposed to a high concentration of $BeSO_4 \cdot 4H_2O$.

The most striking feature of the study was the marked species difference with respect to most of the toxicologic criteria employed. Thus, all of the 20 mice died before the termination of the experiment; the histological findings showed marked pulmonary damage in all of the species, hepatic damage in the mice, ocular lesions in the guinea pig and dog, renal necrosis in the rabbit and rat. The micropathological findings confirmed the biochemical observation that renal impairment had occurred in the rabbit;

Figure 10. The Effects of $BeSO_4 \cdot 4H_2O$ Upon The Tissues of Exposed Animals

	Mouse	Rabbit	Guinea Pig	Hamster	Rat
Lung	?	4+	1+	2+	3+
Liver	4+	0	0	0	0
Kidney	?	4+	0	0	4+
Eye	0	0	4+	0	0

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however, the clinical chemical observations failed to reveal any significant indication of renal or hepatic injury in the dog.

The only consistent result observed in all species was the marked rise of the leukocyte and absolute neutrophil count in the dog, rabbit, and rat. The erythrocyte count for the three species was most constant throughout the period of exposure.

The highly acidic nature of the beryllium sulfate tetrahydrate was the primary cause of the ulcerative lesions that were observed in the eyes and on the skin of the dogs, guinea pigs and mice.

At present, no animal host has been found by means of the inhalation techniques capable of producing the type of pulmonary lesion identical with that of man.

BERYLLIUM METAL FUME STUDY

A small chamber (6 x 6 x 8") was constructed in which groups of rats were exposed to the fumes produced by striking an arc between a carbon electrode and one made of beryllium. This chamber was used in the following pilot studies.

Fume Concentration. Exposures were regulated by changing the length of time during which the arc was operated, as it was not found possible to regulate the rate at which the fume was produced by the arc. Exposure concentrations maintained for periods up to one minute were estimated by means of a filter paper sampler; the concentration obtained during longer exposures could not be measured directly because the increased amount of fume tended to seal the pores of the filter paper and shut off the flow of air through the filter paper sampler. The output of the arc was accordingly determined for a series of one-minute exposures and was found to give an approximately

constant value of 8.2 ± 0.2 milligrams fume per minute. In the small exposure unit employed, this yielded a concentration of 800 mg/m^3 and all exposures have been calculated as CT values (mg mins/m^3) on the basis of 800 CT units per minute of exposure.

Acute Mortality. Five rats were placed in the chamber and exposed to 24,000 CT units (equivalent to 30 minutes of arcing). The total exposure was subdivided into eight exposures of 3 - 4 minutes each given at 15 - minute intervals so as to avoid excessive temperature rises in the chamber. As a control, five rats were exposed in the same manner to a total of 30 minutes of arcing using two carbon electrodes. Four of the beryllium--exposed animals died, two during the exposure and two within 1½ hours after removal from the chamber; none of the carbon-exposed rats died.

Autopsies were performed on the four exposed rats which died, and the five control rats. The fifth exposed rat which survived the exposure was sacrificed after four days during which time growth appeared normal. The results obtained are shown in Table I.

It would appear from the above that beryllium fume acts at these levels of exposure as an irritant rather than as a systemic poison. The primary cause of death appears to be asphyxia resulting from the destruction of lung tissue or lung function. When this destruction is insufficient to cause a lethal asphyxia, the animal remains alive and recovers rapidly when the exposure is terminated.

To test the above hypothesis, three additional groups of five rats each were exposed. One group was exposed to 24,000 CT units in a single day as above. Three animals died during the exposure as compared with four dying in the preceding group; the post-mortem findings likewise agreed with those shown in Table IA, the lung weights being 1.9, 1.4, and 1.4 per cent of the body weight. Another group of five rats was exposed to one half

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Table I. Post-Mortem Findings Immediately Following Exposure to Fume

Rat No.	Cyanosis	Condition of Lung	$\frac{\text{Lung Wt.}}{\text{Body Wt.}} \times 100$
			per cent
A. Rats succumbing to 24,000 CT units of beryllium fume			
379	Moderate	Hemorrhagic, spotted	0.70
380	Very Severe	Very hemorrhagic	2.41
382	Severe	Hemorrhagic	0.96
378	Severe	Hemorrhagic	0.72
		Average	1.20 ± 0.61
B. Rats surviving exposure to carbon arc			
383	Absent	Pink, normal	0.46
384	"	" "	0.43
385	"	" "	0.50
386	"	" "	0.47
387	"	" "	0.51
		Average	0.48 ± 0.03
C. Rat surviving exposure to 24,000 CT units of fume			
381	Absent	Pink, normal	0.51

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this quantity of fume, or 12,000 CT units; no animals died during this exposure. The third group was exposed to carbon fumes for 30 minutes; no animals died. The weights of all the survivors were followed for 60 days. The results are shown in Figure 1. No reduction in growth rate is demonstrable in any of these curves, even in the case of the two animals surviving exposure at a level approximating an LD 50.

As an additional confirmation of the effect of beryllium fume on growth, four groups of five rats each were selected whose mean body weights were 50 g, 150 g, 200 g, and 375 g respectively. Each of these groups was exposed to 12,000 CT units, and daily weights subsequently recorded. The results are shown in Figure 2; again there is no perceptible after-effect of the exposure visible in the growth curves. At the end of the 60-day period, autopsies were made on each group with the results given in Table II.

Effect of Continuous Exposure. Whereas beryllium fume at high levels may act as an irritant, the possibility remains that this effect may serve to mask more specific reactions. In order to test this hypothesis, four groups of five rats each were exposed in the following manner. The first group received 50,000 CT units given as 2 exposures of 25,000 units each month, each exposure being distributed over two successive days, to insure the survival of all animals. The second group was exposed to 50,000 CT units given as 8 weekly exposures of 6,000 units each. The third group received 50,000 CT units given at a rate of 800 units per day for 60 days. The fourth group received five times this total dosage, or 250,000 CT units, given as 4,000 units per day for 60 days.

None of the animals died during any of the experiments. The weight response during the exposure is shown in Figure 3. It will be seen that the three groups receiving a total of 50,000 CT units each show no evidence of a

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Table II. Post-Mortem Findings in Rats 60 Days after Exposure to 12,000 CT Units of Beryllium Fume

Rat Group	Condition of Lungs	$\frac{\text{Lung Wt.}}{\text{Body Wt.}} \times 100$	S.D.
grams		per cent	
150	Pink, normal, 5/5	0.58	± 0.06
200	Pink, normal, 5/5	0.51	0.08
375	Slight hemorrhage, 2/5	0.52	0.06
200 (controls)	Pink, normal, 5/5	0.50	0.09

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Figure 1. Growth of Rats Following a Single Exposure to Beryllium Fume

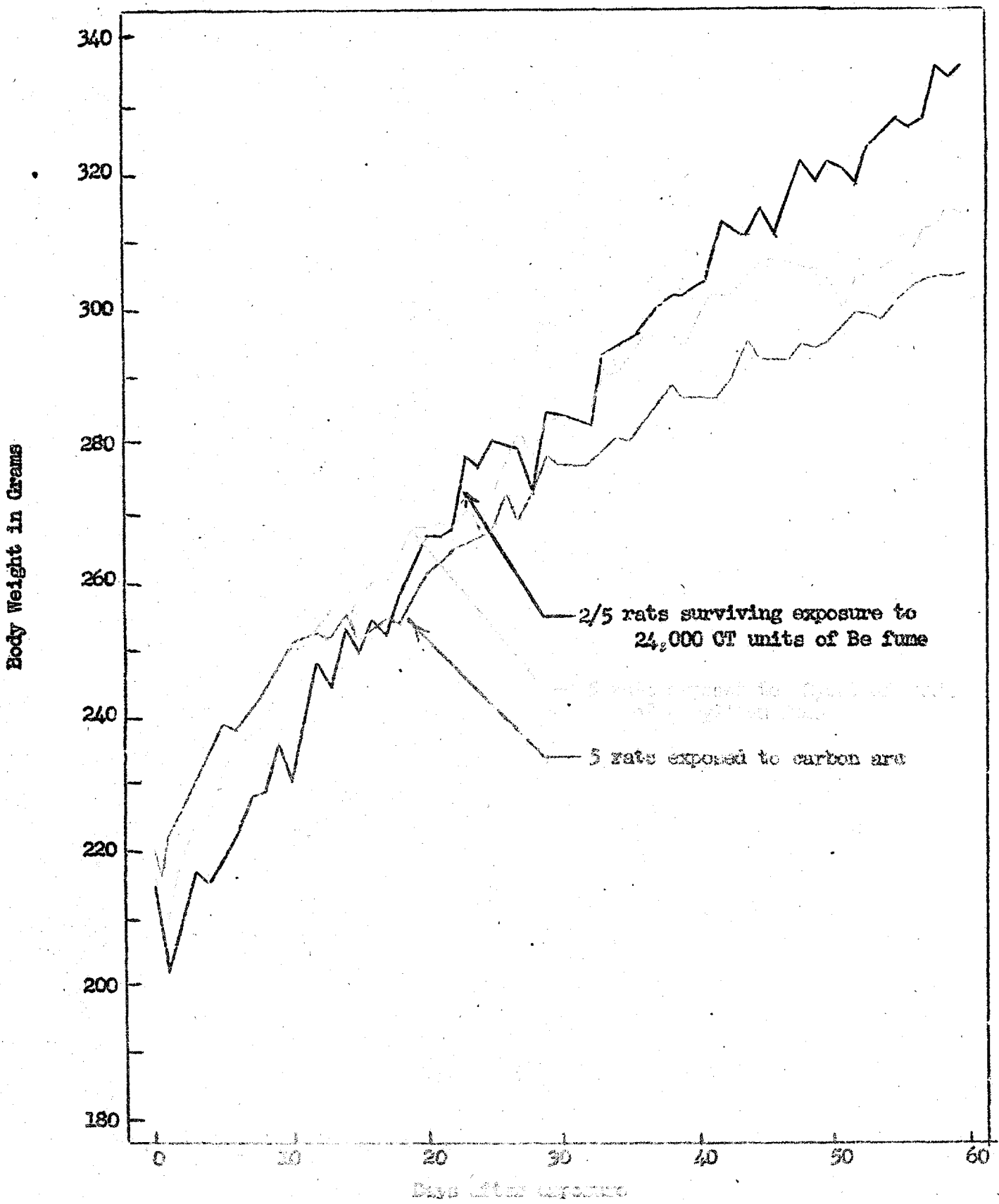

Figure 2. Growth of Rats of Four Different Age Groups Following A Single Exposure to 12,000 CT Units of Beryllium Fumes

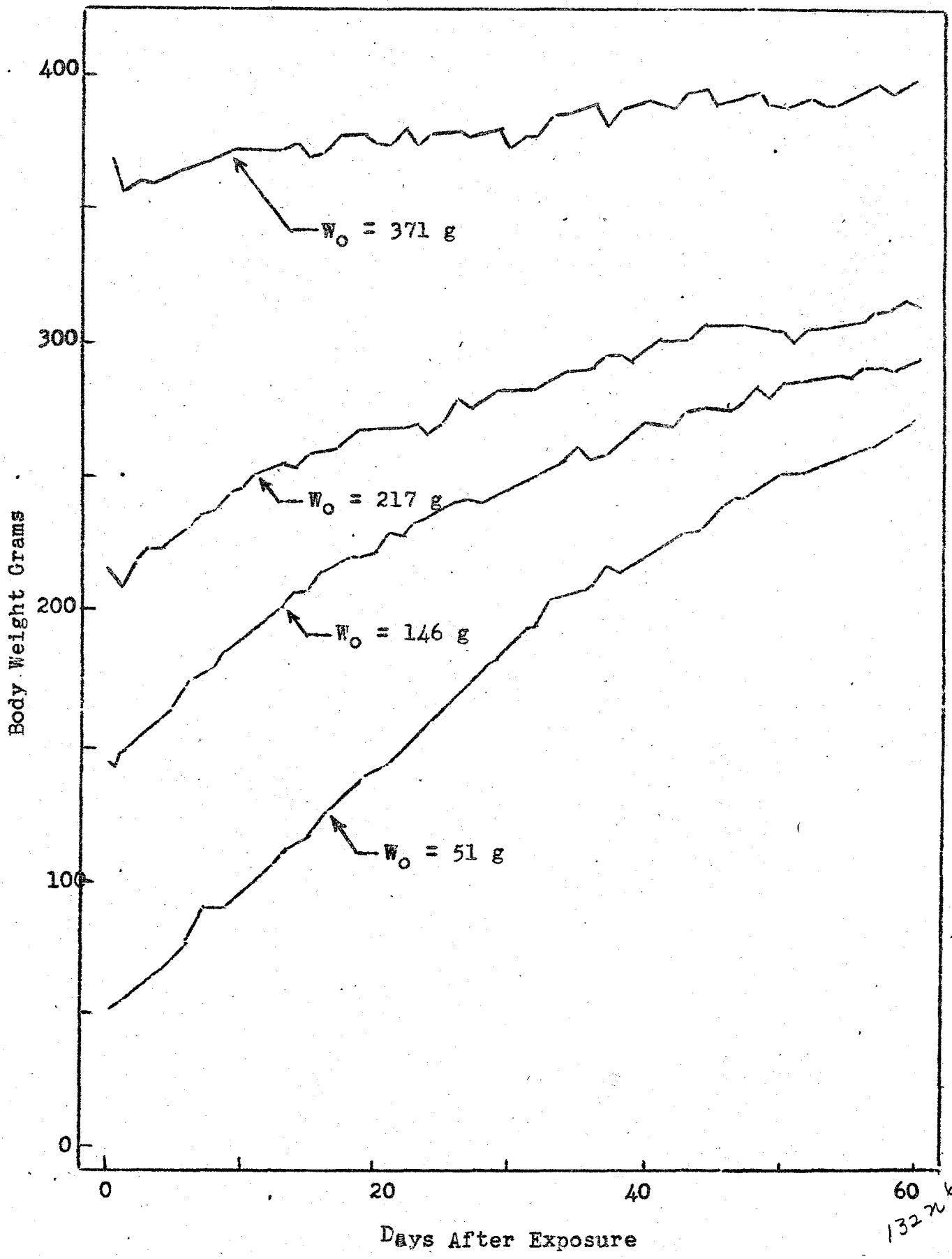

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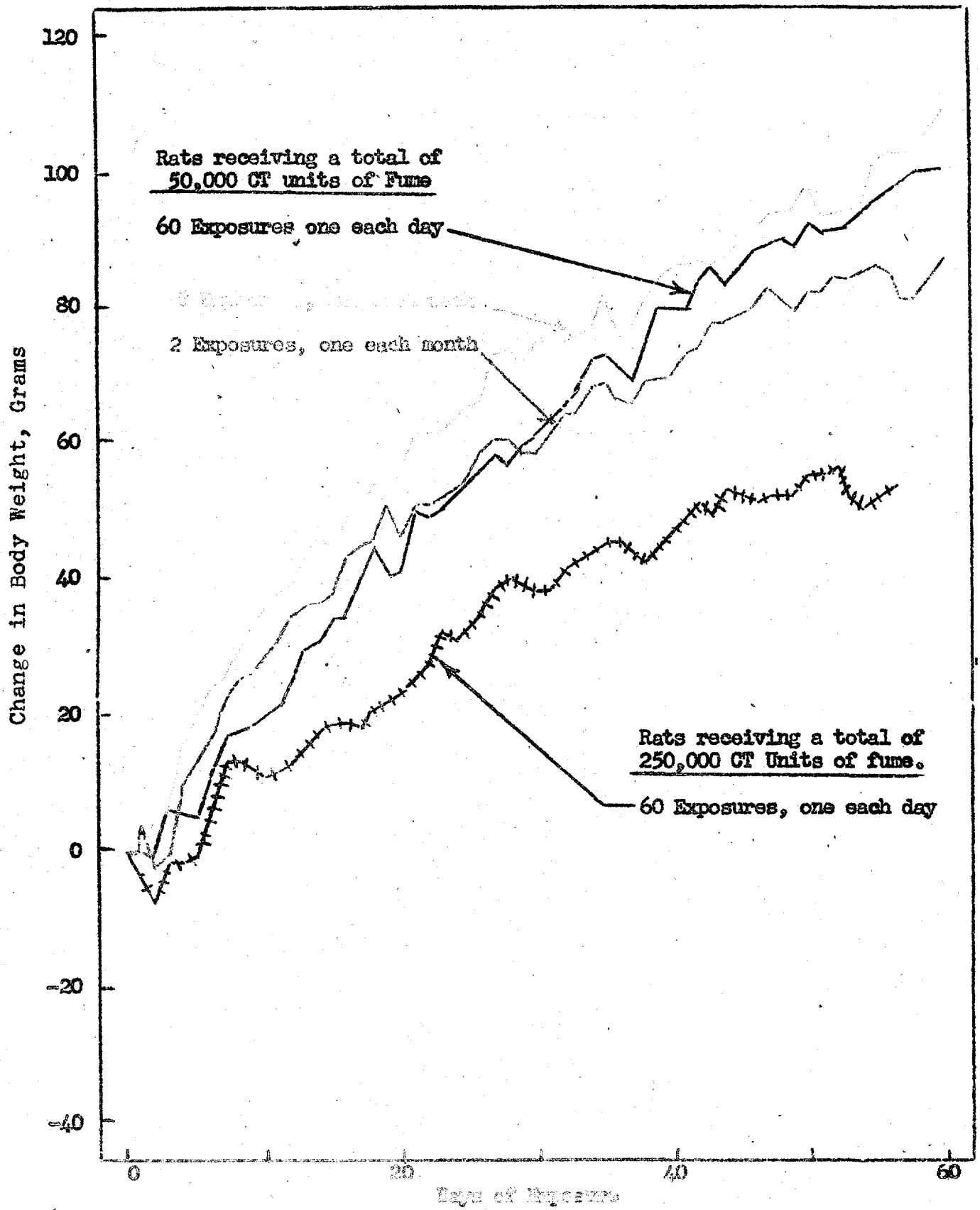
Figure 3. Growth of Rats During Intermittent and Continuous Exposure to Beryllium Fume.



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reduction in growth rate in spite of the fact that this level represents a total exposure to fume twice as great as the single exposure which killed four animals out of five.

The fourth group, receiving five times as great an exposure as the first three groups, and ten times the lethal exposure, shows only a slight reduction in growth rate, increasing in weight by 50 g while the first three groups increased 90 g, 100 g, and 110 g respectively.

The hematologic findings for the two groups receiving daily exposures are shown in Figures 4A and 4B. It will be seen that while there is a slight tendency toward a progressive eosinophilia and neutrophilia, more marked in the rats that received the greater exposure, the values do not at any time depart widely from the control range.

Autopsies of the animals in each of the groups exposed to 50,000 CT units at the end of the exposure revealed the gross findings shown in Table III.

The damage appears to occur chiefly in the lung and to a smaller extent in the kidney. In neither case is the damage so serious as to interfere with normal growth.

Conclusions. The fumes produced by arcing metallic beryllium against a carbon electrode developing high intensities of exposure of 25,000 CT units in a single day appear to act primarily as an irritant. This effect does not appear to be cumulative, for it was found that rats tolerated far greater total exposures as long as no single dose exceeded 12,000 units (equivalent to one hour at 200 mg/m^3). Only a slight response, exhibited by a reduced growth rate, was obtained after daily exposure to 4,000 CT units per day, equivalent to 33 hours at 100 mg/m^3.

Signed George F. Sprague
George F. Sprague

Signed Charles W. LaBelle
Charles W. LaBelle

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Table III. Post-Mortem Findings in Rats Exposed to 25,000 CT Units of Beryllium Fume

Type of damage and fraction of group in which it occurred

Type of Exposure	Lung	Liver	Kidney	Spleen
Daily	Slight hemorrhage, 4/5	Normal, 5/5	Hemorrhage, 2/5	Enlarged, 3/5
Weekly	Slight hemorrhage, 5/5	Fatty, 1/5	Hemorrhage, 1/5	Normal, 5/5
Monthly	Slight hemorrhage, 3/5	Normal, 5/5	Petichiae, 1/5	Enlarged, 2/5

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Figure 4a. Hematologic Changes During 60-Day Exposure to Beryllium Fume; Erythrocytes and Leukocytes. Solid Line = Rats Exposed to 800 CI Units per Day; Broken Line = Rats Exposed to 4,000 CT Units per Day; Shaded Area = Range of Controls.

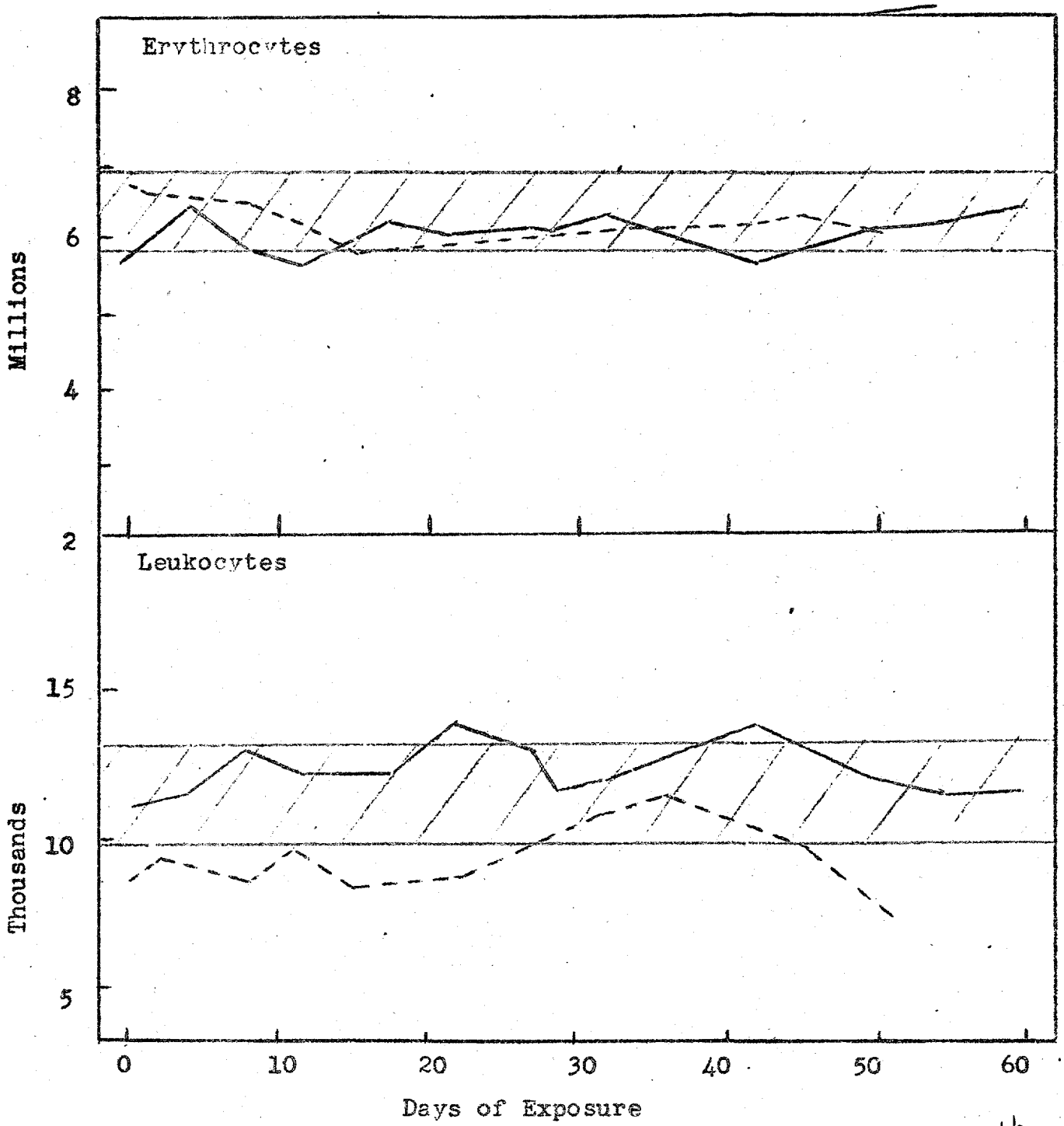
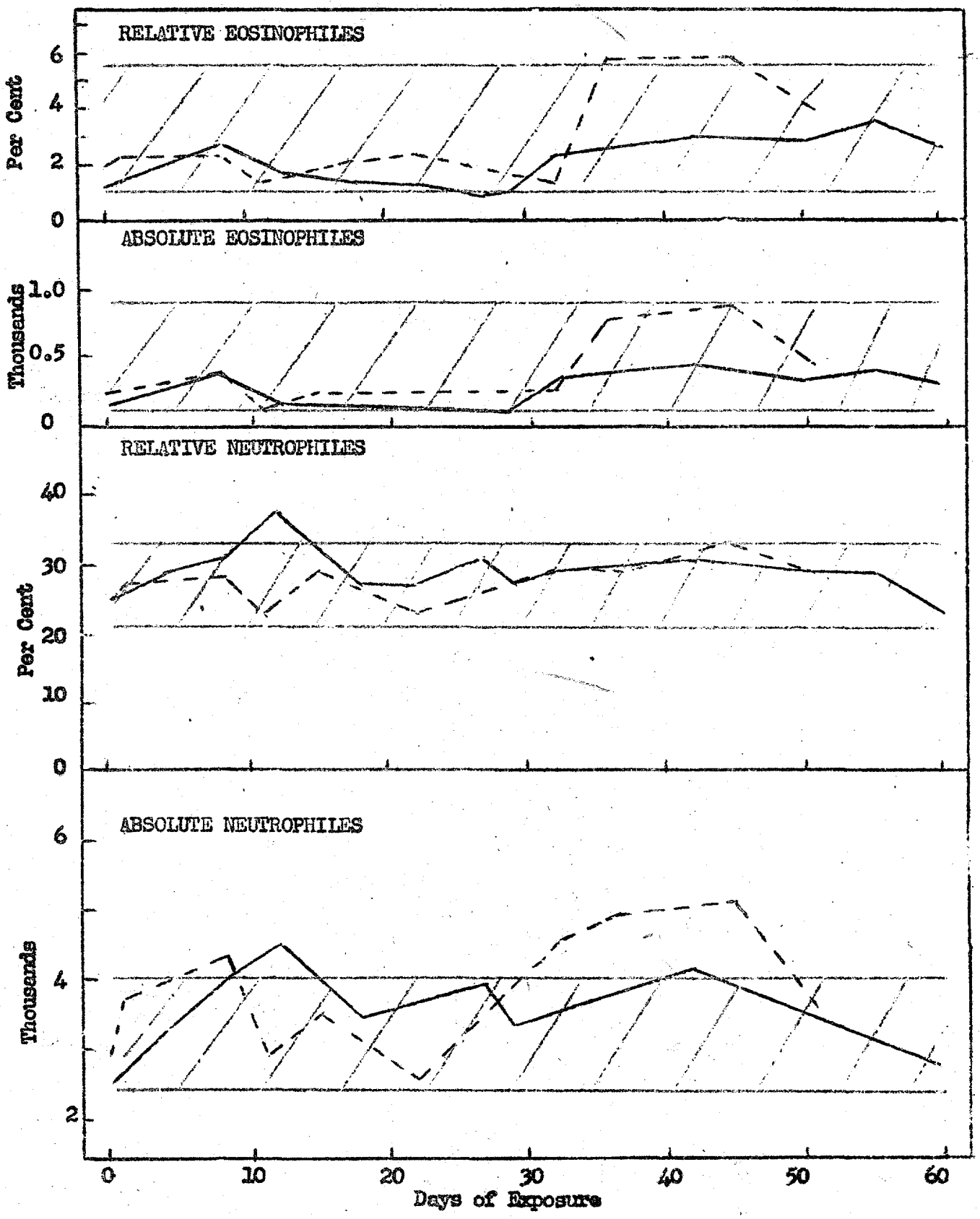


Figure 4b. Haematologic Changes During 60-Day Exposure to Beryllium Fume; Eosinophils and Neutrophils. Sold line = rats exposed to 800 CT units per day; Broken line = rats exposed to 4,000 CT units per day; Shaded Area = range of controls



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