DAMON RUNYON MEMORIAL FUND FOR CANCER RESEARCH

PROGRESS REPORT *** SUPPORT BEYOND SECOND YEAR

Title of Project: ENVIRONMENTAL CANCER — DRIR# 75 C

The capacity of inhaled industrial dust to produce pulmonary cancer in experimental animals.

Amount $9,745.23
FROM December 1 1953 to November 30 1954

Name of Institution The Saranac Laboratory

7 Church Street, Saranac Lake, New York

Dates covered by report Sc. 12/28/53 to 9/15/54

Name of Project Director G. W. H. Schepers, M.D., D.

Title of Project Director Director, Saranac Lab.

Years of support for this Project October 3, 1950 to November 30, 1954

Names and Titles of Technical and Professional participants in projects:

P. C. Pratt, M.D.	Pathologist
E. J. Urban, M.S.E.	Engineer
M. Roberts, M.E.	Engineer (replacing Urban)
D. Bailey	Petrographer
A.S. Delahant	Research Associate
Russel Clark	
Wallace Gay	
Edward Gockeler	
Robert Liddy	Technicians
Miles Moody (replacing J. Marconi)	
Jacob Jacobsen	
Oliver Soula	

Total amount of support from DRIF $73,293

Amount requested for next year $26,510

SUMMARY STATEMENT OF PROGRESS

The study is still in progress. A number of rats remain to be sacrificed in accordance with the original plan and the detailed histological and biochemical analyses of a number of the animals already sacrificed are yet to be completed.

Preliminary observations include the following:

(a) Various types of neoplasmata have been provoked in rats by the inhalation of beryllium sulphate dust for continuous periods of 6 months.

(b) These neoplasmata include papillary adenocarcinoma, mucous forming adenocarcinoma and squamous cell carcinoma.

(c) As many cancers resulted from the current exposure schedule, lasting 6 months only, as were provoked after continuous exposure for 14 months in a previous comparable experiment on rats.

Aims of the Project:

The statistical study of the growth characteristics of transplanted lung cancers. A systematic exploration of the metabolic activity of the tumors and the reaction of the host thereto.

A definitive and progressive investigation of co- and anti-carcinogenic agents.

Expansion of Facilities:

The beryllium dusting phase of the investigation will be conducted by means of another research grant, but the dust chambers built by means of earlier Damon Runyon grants will continue to be used with some modifications. Specific instrumental acquisitions will involve the purchase of electrophoretic apparatus and various microscope accessories.

Changes in personnel comprise the following:

(a) Addition of two pathologists to handle the clinical pathology phase (Olga Dobrogorski, M.D. and J. Schmidt).

(b) One biochemist (L. Scheel, Ph.D.), and one additional technician (E. Gordon).

(c) One statistician (S. Kaszer, M.E., E.E.) in view of the increased significance of this phase. Mr. Kaszer has already been participating in the preceding study.

(d) One senior histologist (F. Creedon) with technical assistant (E. Fear).

(e) One additional animal caretaker (R. Whitelaw).

(f) One part-time typist (G. Nesbitt).

SUMMARY OF PROPOSED BUDGET FOR NEXT YEAR

Personnel	12,500
Permanent equipment	1,300
Consumable supplies	2,500
Other expenses	8,236
Institutional overhead	1,924
Total budget for year	26,510

Remarks: A small surpl
is accumulati
in the existing budget
the status of which wi
be reported on Nov. 30,

Signature of Project Director

Progress Report
on

PROJECT DRIR-75C

ENVIRONMENTAL CANCER: THE CAPACITY OF INHALED INDUSTRIAL DUST TO PRODUCE PULMONARY CANCER IN EXPERIMENTAL ANIMALS

I. INTRODUCTION

The above current experiment at The Saranac Laboratory was designed to evaluate various methods for the experimental induction of pulmonary neoplasm through exposure to atmospheric dusts. The possibility that industrial dusts of various compositions may cause pulmonary neoplasm in industrial workers has for many years received considerable attention in medical and legal circles. Tissue specimens of numerous industrial workers who developed pulmonary carcinoma are constantly submitted to The Saranac Laboratory for review, and consultation about such cases is often sought in respect to claims for compensation based on the alleged carcinogenic influence of various dusts. This state of affairs creates a great and urgent need for definitive evidence concerning carcinogenesis in industry.

This interim report summarizes the experimental study of animals which has now been in progress for 16 months. This study was undertaken after completion of a previous study in which it was found that quartz dust and iron-oxide dust did not influence the incidence of pulmonary tumor in mice. That earlier study involved mice of two strains, strain C and strain C57 black, having a high and low spontaneous pulmonary tumor incidence, respectively, and on completion of the experiment it was possible to state to the Damon Runyon Memorial Fund, in a report dated October 20, 1952, that "Neither of these dusts is capable of eliciting tumors in mice of a strain highly susceptible to tumor development or of initiating tumor formation in mice of a strain of low spontaneous tumor incidence".

In the proposal to the Damon Runyon Fund Committee dated October 29, 1952, in which the present experiment was outlined, it was stated that if either quartz dust or iron-oxide dust does influence the incidence of pulmonary carcinoma in man, as some observers suspect, the mouse is valueless for investigation of the relative potency, mechanism of action, or prophylaxis of that effect. It was reasoned further that efforts to identify an experimental technic for the investigation of these aspects of pulmonary carcinogenesis should be continued. Discussion was introduced concerning various possible approaches to the problem and it was concluded that the best avenue of approach would be one involving "preliminary preparation of animals with a standard carcinogenic product followed by their exposure to the substance to be tested".

The study now underway involves this type of approach to the experimental study of pulmonary carcinogenesis. It has been found that rats exposed to beryllium compounds for 10 to 15 months develop true pulmonary carcinomas of the alveolar cell adenocarcinoma type. Since these lesions are true anaplastic malignant tumors of a type sometimes seen in human lungs, they seem more promising for the study of pulmonary carcinogenesis than the peculiar non-anaplastic, usually benign tumors occurring spontaneously in certain strains of mice. The use of animals prepared by exposure to beryllium for the study of other agents as carcinogens is analogous with the fact that in using tumor-susceptible strains of mice, a "neoplasio-genic" factor is present, whether genetic or infectious in nature, so that any experimental agent, even in the mouse, is being studied as a co-carcinogen. This is in further conformity with the belief of most observers that multiple factors probably combine in carcinogenesis in man so that any single implicated agent should probably be regarded as acting as a co-carcinogen with one or more other agents. Beryllium has the added advantage that it undergoes no metabo lic change in animal tissue, so that as a co-carcinogen its effects are precisely definable.

II. METHOD

A period of 6 months exposure to beryllium compounds has been found to be a sufficient stimulus for the subsequent development of pulmonary carcinoma, during a period from about 5 to 10 months after the cessation of the exposure. The current experiment is designed to reveal whether or not quartz or iron-oxide dusts introduced subsequently to the phase of beryllium exposure will alter the latent period or the incidence of such tumors.

The experiment was started with a total of 200 albino rats which were exposed for 6 months in two special dust chambers to an aerosol suspension of beryllium sulphate. An atmospheric concentration of about 12 micrograms of $BeSO_4$ (equivalent to 1 microgram Be) per cu. ft. of air was maintained and the dust was disseminated for 8 hours per day, 5½ days per week. It was expected that during this period of exposure to beryllium sulphate some rats would die in addition to those which were to be sacrificed. The total number of deaths from all causes was 65, somewhat more than had been anticipated. These deaths occurred in two episodes; the first shortly after introduction to the dust chambers, and the second towards the end of the dusting phase. These remaining 135 animals were divided into three groups of 45 animals each. Two of these groups were exposed in separate dust chambers to quartz dust and to iron-oxide dust in a concentration of approximately 300 million particles per cubic foot of air. The third group was reserved for residence in normal air as a control.

Animals from each of the above groups have been and are being sacrificed in samples of four at intervals of three months from the end of exposure to beryllium. It was originally planned that if it appeared at any sacrificing

period that there were differences between the incidence of carcinoma among the groups, a larger number of animals would be sacrificed. Studies of the tissues of the animals included gross and microscopic examination of the lungs, lymph nodes, spleen, liver, and kidneys.

It was planned that if at any time there are differences in the incidence of pulmonary tumors, the lungs of the experimental animals and of the controls would be analyzed for beryllium. This procedure had to be carried out because of the possibility that the inhalation of quartz or of iron-oxide dust might interfere with the excretion of previously accumulated beryllium. If excessive retention should thus occur, it would interfere with the interpretation of the experiment and would necessitate a revision of the technic and repitition of the overall study to ensure that the tissue content of beryllium will be comparable in each group. One important precaution has been taken against this possibility. The animals were kept in normal air for one week between the end of beryllium sulphate exposure and the beginning of the quartz dust and iron-oxide dust exposure, for the reason that during a period of but one week the greater portion of the recently deposited beryllium would be eliminated while in subsequent months but little more beryllium would be excreted.

As no indication had arisen at the time of the 9 months sacrificing subsequently to the cessation of beryllium dusting that either the quartz or the iron-oxide exposure was exerting any material influence upon the induction period of carcinogenesis or the numbers of pulmonary tumors being produced, and as the animals had already been under experiment for a total of 15 months and could not be continued on this status indefinitely, it was decided to sacrifice half of the remainder in each group. This was the only material departure from the course of the experiment as originally projected.

The observations made at the various sacrificial periods are assembled in Tables I to IV and in Figure I. It must be recorded that this interim report is incomplete insofar that it has not as yet been possible to process all microscopic preparations or biochemical analyses on the tissues of the recent large group of sacrificed animals. Therefore, the results presented, especially as they pertain to these latter animals, are based on gross observation only and must be considered to be tentative.

The inferences which may be drawn from the tables are that neither quartz nor iron-oxide inhalation has affected the latent period or overall incidence of pulmonary carcinomata. Comprehensive microscopic study of all the tissues will allow further evaluation of the results in terms of certain additional criteria, such as size, type, and location of tumors. It has already, however, been noted that various types of tumor have occurred, including papillary adenocarcinoma, mucous-forming adenocarcinoma, and squamous cell carcinoma. The ratios in which these tumor types occur in the various animal sub-groups of this experiment yet remain to be determined.

Table I shows the macroscopic observations in each of the groups. The vertical "Duration of Exposure" column at the left is self-explanatory. The column marked "Number of Animals Examined" records the number of animals examined at appropriate time intervals from each of the types of dust exposure, namely, beryllium sulphate followed by quartz, beryllium sulphate followed by iron oxide, and beryllium sulphate followed by normal air. It shows also the totals of animals from all groups examined at each interval. The column marked "Number of Tumors Seen" presents figures for the number of tumors observed in each animal examined. Thus, in the second line (6 months $BeSO_4$ and three months time lapse) there were 6, 4, and 3 animals observed in the quartz, iron, and normal air groups, respectively; and under "Number of Tumors" there are groups of 6, 4, and 3 zeros to show that no tumors were observed in these animals at that time.

Table II summarizes the pooled data from Table I and reveals a remarkably sharp increase in the rate of incidence of tumors between nine and ten months. Presumably this is an indication that following a six-month exposure to beryllium sulphate dust the induction period before the development of tumors is comparatively consistent and is approximately nine months in duration. The peak onset of tumor production, therefore, commences approximately 15 months from the date of onset of beryllium sulphate dust exposure. It is of interest to note that in an earlier experiment in which exposure to beryllium was continuous the peak rate of appearance of pulmonary carcinomata also occurred at about 14 months from the date of commencement of the dusting experiment. This phenomenon is graphically emphasized in Figure I.

Table III presents the comparative data by type of exposure for the occurrence of tumors in the groups of animals killed after 10 months' exposure to quartz, to iron-oxide, and to normal air , following the preparatory exposure to beryllium sulphate dust. The data are broken down as indicated in the left hand column for the purpose of other comparisons which are presented in Table IV. In Table III the important figures to note are the totals. In the column headed "Number of Tumors per Animal" it is apparent that exposure of the rats to quartz or iron-oxide dust after the preparatory exposure to beryllium sulphate dust has not altered the incidence of tumors to a material degree.

Table IV presents the results of combining the data garnered thus far in this experiment in various ways other than by type of exposure. Thus, two strains of animals were used and the first set of comparative data in Table IV shows that there is a slight, but immaterial difference in tumor incidence between the two strains. The original 200 rats were exposed to the beryllium dust in two separate dusting chambers, and the beryllium dust in two separate dusting chambers, and the second set of data compares the incidence of the resultant tumors. Again there is no significant difference. The third set of data compares the incidence of tumors in male and female rats and once more no significant difference is apparent.

In a previous comparable study on mice conducted by means of a Damon Runyon grant, neither quartz nor iron-oxide dust appeared to influence the incidence of spontaneous pulmonary tumors. Evidence now exists that at least for rodents of these two genera, neither quartz nor iron-oxide dusts act as co-carcinogens with beryllium sulphate or any other primary carcinogen. In view of the large numbers of human beings who are constantly being exposed to either quartz or iron dusts of various kinds, this observation may have a certain measure of significance.

III. TRANSPLANTATION OF LUNG TUMORS

A major result of this series of studies on environmental cancer has been to demonstrate that beryllium dust is a reliable experimental tool by means of which lung cancer can be provoked in rats. It had not yet been established whether the presence of the inhaled beryllium was necessary for the survival of the neoplasm, i.e., whether the cancer cells used the beryllium during some or other phase of abnormal metabolism or whether the new growth could continue in the absence of the beryllium, i.e., whether some genetic change had been effected, changing the cancerous cell to a parasite rather than merely maintaining it as a diseased cell. This point could not be proved readily on the tumors in the animal lungs because the progressive expansion of the tumor and the occurrence of multiple tumors rapidly killed the rats.

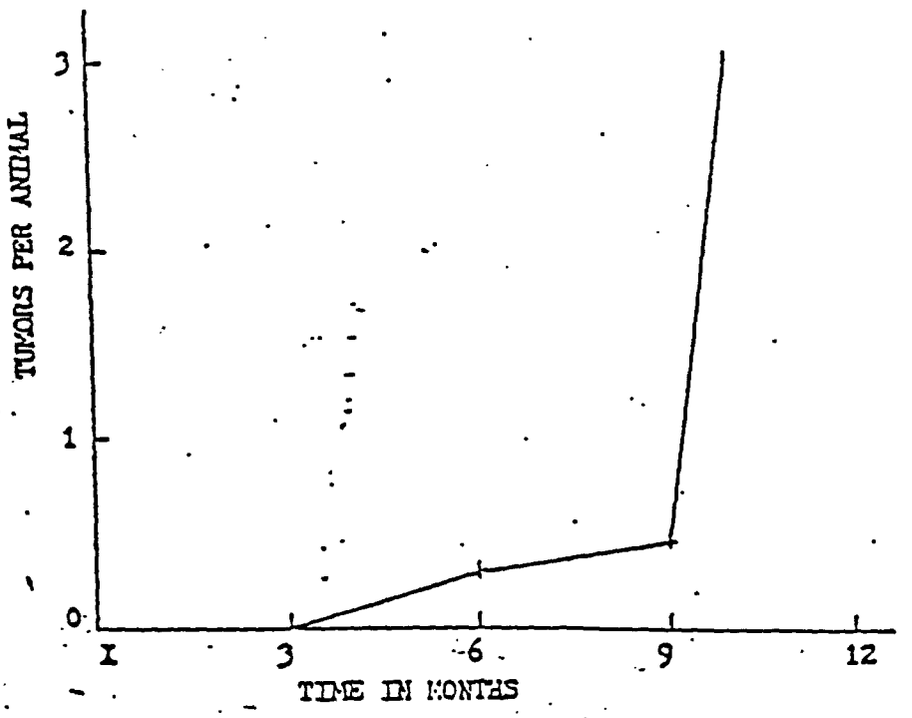
The extension of the biochemical study of the tumor could only be pursued therefore if the tumor could be induced to grow more slowly or in another accessible tissue.

Nor would any attempt at therapeusis prove meaningful until the experimental cancers could be proved by histological methods prior to the institution of any proposed method of treatment. This could not be attempted in animals such as the rat unless the tumors could be transplanted.

Fortunately, it was discovered that the beryllium-induced neoplasmata possess such biological vigor that they readily survive on subcutaneous

FIGURE I

INCIDENCE OF TUMORS AT VARIOUS INTERVALS AFTER EXPOSURE FOR 6 MONTHS TO $BeSO_4$ (from TABLE II)



X = DENOTES 6 MONTHS OF $BeSO_4$

TABLE I

DATA FOR OCCURRENCE OF TUMORS
arranged according to time and type of exposure

Duration of Exposure		Number of animals examined				Number of tumors seen			
To $BeSO_4$	To Quartz or Fe_2O_3 or Normal Air	Quartz group	Fe_2O_3 group	Normal air group	Total	Quartz group	Fe_2O_3 group	Normal air group	Total
months	months								
6	0	-	-	6	6	-	-	0 00000	0
6	3	6	4	3	13	0 00000	0000	000	0
6	6	7	4	5	16	00 00001	0011	00011	5
6	9	9	4	6	19	0000 01111	0001	0 00211	9
6	10	11	17	15	43	*36	*51	*43	130

* For the final period (6 months plus 10 months) the number of tumors per animal was as follows:

	Number of Animals	Number of Tumors In Each Animal	Total
Quartz group	11	4 1 5 4 7 3 0 4 0 6 2	36
Fe_2O_3 group	17	6 4 1 5 3 2 3 3 2 5 5 6 1 0 0 2 3	51
Normal air group	15	0 6 1 2 2 4 5 3 5 3 3 3 2 3 1	43

TABLE II

SUMMARY OF DATA OF TABLE I
(All observations pooled at each time interval)

Duration of Exposure		Number of Animals examined	Number of Tumors (total)	Number of Tumors per animal
To $BeSO_4$	To Fe_2O_3 or to Quartz			
months	months			
6	0	6	0	0
6	3	13	0	0
6	6	16	5	0.31
6	9	19	9	0.47
6	10	43	130	3.02

TABLE III

COMPARISON OF TUMOR INCIDENCE AT 10 MONTHS ONLY

IN RATS OF EACH OF THE GROUPS EXPOSED TO

$BeSO_4$, THEN TO QUARTZ
$BeSO_4$, THEN TO Fe_2O_3
$BeSO_4$, THEN TO NORMAL AIR

Data of exposure: Strain of animal	Sex	Chamber used	Number of animals examined	Number of animals with tumors	Number of tumors	Number of tumors per animal
Experiment #1236 $BeSO_4$ for 6 months followed by quartz for 10 months						
Sherman	F	#10	3	3	9	
Sherman	F	11	2	2	10	
Sherman	M	11	1	0	0	
Sherman	M	10	3	3	7	
Sar. Lab.	F	10-11	2	2	8	
		Total	11	10	34	3.08
Experiment #1237 $BeSO_4$ for 6 months followed by Fe_2O_3 for 10 months						
Sherman	F	#10	4	4	16	
Sherman	F	11	2	2	3	
Sherman	M	10	6	6	26	
Sar. Lab.	F	10-11	5	3	6	
		Total	17	15	51	3.00
Experiment #1238 $BeSO_4$ for 6 months followed by normal air for 10 month						
Sherman	F	#10	4	3	9	
Sherman	F	11	3	3	10	
Sherman	M	10	5	5	19	
Sar. Lab.	F	10-11	3	3	5	
		Total	15	14	43	2.87

TABLE IV

COMPARISON OF POOLED DATA FROM 10-MONTH SACRIFICINGS

TO PROBE THE VALIDITY OF THE STUDY

Factors compared	Number of animals examined				Number of tumors seen (probable or definite tumors)				Number of tumors per animal
	Quartz group	Fe_2O_3 group	Normal air group	Total	Quartz group	Fe_2O_3 group	Normal air	Total	
Sherman strain	9	12	12	33	26	45	38	109	3.3
vs.									
Saranac Lab. strain	2	5	3	10	8	6	5	19	1.9
Chamber 11 (Sherman)	3	2	3	8	10	3	10	23	2.9
vs.									
Chamber 10 (Sherman)	6	10	9	25	16	42	28	86	3.4
Male animals	4	6	5	15	7	26	19	52	3.5
vs.									
Female animals	7	11	10	28	27	25	24	76	2.7

Total number of animals examined 43
Total number of tumors 128
Tumor per animal (overall average) 2.98

September 1, 1954

MEMORANDUM:

Does Silicosis Protect Against Carcinoma of the Lung?

Reference: Zentr. Allchem. Pathol. u. Pathol. Anat., Vol. 89, p 187, 1952. S. Spoerlein

At the University of Wurzburg, 383 cases of silicosis were determined from 24,571 necropsies performed during the period 1930 to 1947. There was evidence of bronchial carcinoma in only 7 of these cases. This gives an incidence of carcinoma a fifth to a sixth of that which could have been calculated had silicosis been without effect on the lung in respect of carcinoma. Moreover, the greatest incidence of carcinoma was amongst the cases with the least amount of silicosis. Thus, there were 5 cases of bronchial carcinoma amongst 9 subjects with slight silicosis, and 2 amongst 105 with moderate silicosis, and none amongst 137 with severe silicosis. The inference is made that silica or silitic acid, instead of pre-disposing to the development of bronchial carcinoma, decidedly protects against it.

cc: Damon Runyon Fund experiment

CHRS:gn