THE TREATMENT OF EXPERIMENTAL SILICOSIS WITH COMPOUND 48/80

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To treat established silicosis it would appear to be necessary to inactivate the dust in the lungs in order to prevent further toxic action and permit the repair of what damage is reversible. The drug used would presumably need to reach phagocytic cells which have transported the dust from the alveoli to the interstitial tissue and lymph nodes. In the conditions of experimental silicosis, aluminium has a protective effect (Dworski, 1955) but its administration to man by inhalation has not been proved to benefit the disease when once established (Kennedy, 1956). It is not certain that inhaled aluminium powder has the capacity to reach silica in the tissues and it is possible that soluble antagonists would prove more effective.

In an earlier communication (Marks, 1957), it was reported that compound 48/80, an organic base used experimentally as a histamine liberator, protects phagocytic cells in tissue culture against the toxic effect of silica dust. The degree of protection observed appeared sufficiently high to merit a study of the drug's activity *in vivo*. Mice were used because of their relatively low susceptibility to histamine and because a convenient technique has been described for inducing silicosis in them (Zaidi, King, Harrison, and Nagelschmidt, 1956).

Experimental Methods

Administration of Silica.—Male albino mice were used. They weighed 18 to 20 g. except in experiment 4 in which they weighed 25 to 30 g.

A single batch of tridymite dust (provided by Dr. G. Nagelschmidt, Safety in Mines Research Establishment, Sheffield) was used. It had the following size distribution:

Diameter(μ)	0.06-0.11	0.23	0.45	0.9	1.8	3.6	7.2
No. %	26.7	37.9	30.3	5.0	0.095	0.092	0.009

The dust was normally suspended in pyrogen-free 0.9% NaCl at a concentration of 10 mg. per ml., heated at 100° C. for 15 minutes to sterilize it, and then shaken

mechanically for 20 minutes. A single dose of 0.5 ml. of suspension was given by slow injection into a tail vein. Some mice died within a few minutes of the injection. Such immediate deaths are recorded below in the text but not in the tables. Survivors of the dust injection were allotted to the experimental groups at random.

Administration of Compound 48/80.—Fresh solutions were made daily in sterile saline and given intraperitoneally in a volume of 0.3 ml. or 0.5 ml. according to the following treatment schedules.

Day	High-dose Schedule (μ g.)	Low-dose Schedule (μ g.)
1	10 twice	6 twice
2	20 "	12 "
3	30 "	18 "
4	40 "	24 "
5	50 "	30 "
6	50 once	30 once
7	No treatment	No treatment
8	50 once	30 once
9	55 "	33 "
10	60 "	36 "
11	65 "	39 "
12	72.5 "	43.5 "
13	80 "	48 "
14	No treatment	No treatment

When treatment extended beyond two weeks, mice on the high-dose schedule received 80 μ g. and those on the low-dose schedule, 48 μ g. once each day, omitting Sundays.

In two of the experiments, of which details are given below, short intensive courses of compound 48/80 were used, preceded by mepyramine to protect against the effects of histamine release.

Treatment began on the same day as the dust injections after an interval of a few hours.

Assessment of Severity of Silicosis.—Mice were killed between two and three months after having received dust. The livers were weighed before and after taking approximately 10% for section. The collagen content of the remainder of the liver was estimated by a modification (to be published) of the method of Stacy and King (1954). The collagen values given in the tables represent the content of the whole liver, allowance having been made for the portion taken for histology.

The histological assessment was made on sections

stained with haematoxylin and eosin using the following grades:—

- O No structural abnormality.
- A A few small collections of mononuclear cells (not more than were found in some normal mice).
- B A small to moderate number of early silicotic nodules with little collagen production.
- C A large number of well-developed silicotic nodules which were predominantly cellular although collagen was obvious.
- D Intermediate between C and E in that some lesions were mainly cellular and others mainly collagenous.
- E A large number of nodules in which collagen predominated.
- F A large number of nodules, almost entirely acellular.

Intermediate gradings, denoted by a + sign, were made in some instances. In order to avoid bias in grading, the identities of specimens were not disclosed until assessment was complete.

Results

Experiment 1.—After the dust suspension was shaken, some coarse aggregations remained which were dispersed by gentle grinding in a Griffith's tube. The intravenous injection of the mice in experiment 1 appeared to be less effective than in later experiments when experience had been acquired. For example, there was only one immediate death out of 61 mice injected and only two premature deaths subsequently. Moreover, the incidence of silicosis in the untreated mice killed after two months was low as compared with later experiments. Nevertheless, the results of the initial experiment were sufficiently useful to merit description.

Of the mice surviving the dust injection, groups of six were treated with compound 48/80 for two, four, or eight weeks, using the high-dose schedule or for eight weeks using the low-dose schedule, and 12 mice were given control injections of 0.5 ml. of saline intraperitoneally, timed as in the eight-week course. The other 24 mice given dust were used for a purpose not relevant. One mouse in the low-

dosage group died after 57 days and is included in the table with the two-month series. A mouse which died after 21 days on high dosage was discarded. The majority of the mice were killed two months after their dust injections but two of each treated group and four untreated mice were allowed to survive a further month. Ten control mice were not given dust but were treated with saline intraperitoneally, six being killed after two months and four after three months.

The histological and chemical observations made in experiment 1 are recorded in Table 1, the position of individual mice in the table being the same in both sections. For convenience, mice given silica but no treatment will be designated group S, those given silica and 48/80 as group ST, and controls given neither silica nor treatment, as C. The mice killed after two months afforded little information since silicosis had developed in only a minority of group S. However, all four of the group S mice allowed to survive three months developed silicosis, which was moderately severe in three. In contrast, there was only a single very mild case amongst the eight mice of group ST killed after three months. The treated mice have been assembled for analysis in group ST, irrespective of their course of 48/80, because of their small numbers. The liver collagen estimations agreed with the histological findings in demonstrating the protective effect of 48/80 in the three-month series. The mean liver collagen content after three months was 5.65 mg. in group S, 2.53 mg. in group ST, and 2.25 mg. in group C. The difference between groups S and ST is significant ($t = 5.24$, $t_{0.05} = 2.23$), that between groups ST and C is not significant ($t = 1.23$, $t_{0.05} = 2.23$). A similar analysis for the mice killed after two months shows

TABLE 1
EXPERIMENTAL SILICOSIS TREATED WITH COMPOUND 48/80: EXPERIMENT 1

No. of animals		Histological Grading and Liver Collagen Content					
		Given Silica (5 mg. tridymite intravenously)					No Silica
		No Treatment	48/80 Low Dose 8 Weeks	48/80 High Dose			No Treatment
				2 Weeks	4 Weeks	8 Weeks	
		12	6	6	6	6	10
Histological grading	Killed after 2 months	D B O O	B+ O O O	A O O O	O O O O	B A O O	A O O O
	after 3 months	D+ D	D+ B O	O O O	A O O	A+ O	A O O
	Killed after 2 months	5.2 2.7 2.3 2.0	6.7 2.3 2.2 1.8	2.5 2.4 2.2 1.9	3.0 2.7 2.3 1.8	2.5 2.1 1.9 2.6	2.7 2.6 2.7 2.0
	after 3 months	7.4 6.1	5.7 3.4	2.5 2.4	3.2 1.8	2.8 2.1	2.6 2.3
Liver collagen content (mg.)							

Individual mice are represented in similar places in the two sections of the table. See text for the histological grades.

TABLE 2
EXPERIMENTAL SILICOSIS TREATED WITH COMPOUND 48/80 : EXPERIMENT 2

	Histological Grading and Liver Collagen Content							No Silica	
	Given Silica								
	No Treatment	Mepyramine + 48/80		48/80 Low Dose 8 Weeks	48/80, High Dose*			No Treatment	
		2 Days	7 Days		2 Weeks	4 Weeks	8 Weeks		
No. of mice	14	6	6	6	19			6	
Deaths after admission to group	10	4	5	4	7			0	
Histological grading	D	D	B	B	C	C	A +	..	
	C +	D		A +	B	B	A +	..	
	C				A +	A	A	..	
	B					O	O		
Liver collagen content (mg.)	7.5	10.3	3.7	4.8	4.2	4.8	3.2	2.8	2.3
	4.4	8.2		4.7	4.0	4.2	3.1	2.2	2.1
	7.6				5.0	4.9	3.0	2.1	2.1
	4.4					3.5	2.8		
							2.7		

*The duration of the experiment was two months.

*The three groups were not distinguished until after two weeks' treatment by which time five of the seven deaths had occurred.

no significant difference between groups S and ST ($t = 1.60$, $t_{0.05} = 2.08$) or between groups ST and C ($t = -1.23$, $t_{0.05} = 2.09$).

Experiment 2.—The silica dust was prepared and administered as in experiment 1. The greater effectiveness of the injection with experience was confirmed by an immediate mortality of seven out of 58 mice given dust, the loss of a further 30 before the experiment ended, and the development of silicosis in two months in all the mice given dust without treatment. Two additional treatment schedules were introduced in experiment 2. The dose of 48/80 used was 50 μ g. intraperitoneally twice daily for two days in one schedule and for seven days in the other, each dose preceded by 100 μ g. of mepyramine given subcutaneously 20 minutes earlier. The purpose of the mepyramine was to diminish the toxicity of 48/80 in short intensive courses.

Deaths were frequent in the mice given dust whatever their group. Most occurred in the first two weeks and especially in the first seven days. There were five deaths amongst 19 mice receiving the high-dosage schedule by the time allotments were made to the two-, four-, and eight-week treatment groups at the end of two weeks' treatment. Four (1), four (0), and six (1) mice were allocated respectively to these three groups, the number of deaths subsequently being given in parenthesis. The total loss of seven mice out of 19 treated with the high-dose schedule compares favourably with the loss of 10 of 14 mice given silica without treatment (group S). The mortality in the other treatment groups was about the same as in group S.

Surviving mice were killed two months after the injections of dust. The results of their examination are given in Table 2. With both methods of assess-

ment it was evident that the mice given the eight-week high-dose schedule of 48/80 (to be called group ST8) were considerably protected against the effects of silica. A slight degree of protection appeared to be given by the same schedule continued for only two or four weeks but survivors in the other treatment groups were too few to be useful. The mean liver collagen content was 5.97 mg. in group S, 2.96 mg. in group ST8, and 2.27 mg. in group C (no silica). The difference between groups S and ST8 is significant ($t = 3.74$, $t_{0.05} = 2.37$). That between groups ST8 and C is also significant ($t = 4.69$, $t_{0.05} = 2.26$), showing that the protection conferred on group ST8 was not complete.

Experiment 3.—For this experiment the dust was lightly ground in saline in a glass tissue grinder ("dewarex" brand) and dextran solution then added to give a final concentration of 4.5% dextran and 10 mg. dust per ml. The modification (suggested by Dr. D. Rivers) was introduced in an attempt to reduce the lethal effect of the dust injections by providing a finely dispersed suspension slow to settle out. Later investigation of tissue grinders of the type used indicated that the toxicity of silica dust ground in them may be reduced by contamination with metal, e.g., iron, used in their manufacture. In the event, the mortality in experiment 3 was somewhat lower than that in experiment 2 which had been carried out concurrently with the same stock of mice. There were three immediate deaths amongst the 52 mice injected with dust and 18 others during the course of the experiment, which was ended after 11 weeks.

The control mice (group C) were common to the concurrent experiment 2 but will not be further

TABLE 3
48/80 : EXPERIMENT 3

	Histological Grading and Liver Collagen Content							
	No Treatment		48/80 High Dose*					
			2 Weeks		4 Weeks		8 Weeks	
No. of mice	14		12		12		11	
Deaths after admission to group	4		5		5		4	
Histological grading	E+	E	E	E	C+	C	C	B+
	E	E	D	C+	C	C	A	A
	D+	D	C	C	B+	B+	O	O
	D	C+	B	C	A	O	O	O
	C+	C						
Liver collagen content (mg.)	10.2	23.1	13.1	10.1	5.6	6.4	3.7	6.1
	14.3	19.3	7.9	8.3	6.3	5.6	5.0	4.4
	12.1	15.1	7.6	6.9	4.6	4.1	5.8	5.0
	12.2	14.6	4.1		2.8		4.1	
	10.1	5.7						

All mice represented in the table received silica.

*The duration of the experiment was 11 weeks.

considered as they were killed after only two months.

The incidence of deaths, the histological findings, and the collagen values in experiment 3 are recorded in Table 3. It is apparent that 48/80 offered no protection against the lethal effect of the silica. Histologically, protection by 48/80 was clearly evident with the four- and eight-week courses (groups ST4 and ST8 respectively) but was not

obvious with the two-week course (group ST2). However, all three treated groups had lower mean liver collagen values than the untreated mice given silica (group S), the differences observed being significant ($t = 4.65, 4.49$ and 2.61 for comparisons of group S with groups ST8, ST4, and ST2 respectively, $t_{0.05}$ being 2.13). The collagen values for the livers of mice given silica in experiment 3 (and also in experiment 4) were somewhat higher than those for livers with comparable histological gradings in experiments 1 and 2, although the technique of examination was the same. Whatever the cause of the disparity it does not affect comparisons made between the groups in each experiment.

Fig. 1 is a section of the liver of an untreated mouse (group S) histologically graded E+ (collagen 10.2 mg.). It was stained by Mallory's trichrome method, the collagen showing as lighter areas. Fig. 2 is a similarly stained section of the liver of a mouse treated for eight weeks (group ST8) histologically graded O (collagen 4.1 mg.). Fig. 3 is a section of a liver graded E (collagen 19.3 mg.) from group S, stained by Van Gieson's method, the darker areas representing collagen, and Fig. 4 is a section similarly stained of a liver graded O (collagen 5.8 mg.) from group ST8. Fig. 5 is a composite one of silver-impregnated sections of all livers in group S, showing reticulin formation. From left to right their

FIG. 1.—Experiment 3: silicosis of liver in tridymite-injected mouse given no treatment. Mallory's trichrome stain, blue-sensitive plate. $\times 60$.

FIG. 2.—Experiment 3: liver of tridymite-injected mouse treated with a high-dosage schedule of 48/80 for eight weeks. Stained as for Fig. 1. $\times 60$.

FIG. 3.—Experiment 3: silicosis in liver of tridymite-injected mouse given no treatment. Van Gieson's stain. $\times 55$.

FIG. 4.—Experiment 3: liver of tridymite-injected mouse treated with a high-dosage schedule of 48/80 for eight weeks. Van Gieson's stain. $\times 55$.

FIG. 5.—Experiment 3: livers of the 10 tridymite-injected mice given no treatment. Silver impregnation for reticulin. $\times 55$.

TABLE 4
EXPERIMENTAL SILICOSIS TREATED WITH COMPOUND 48/80: EXPERIMENT 4

	Histological Grading and Liver Collagen Content						
	Given Silica					No Silica	
	No Treatment	Mepyramine and 48/80 3 Days	48/80, High Dose			No Treatment	
			2 Weeks	4 Weeks	8 Weeks		
No. of mice	25	13	11	12	11	6	
Deaths after admission to group	19*	6	4	5	3	0	
Histological grading	F	C+	D	D	B+	..	
	D	C	C	C	B	..	
	D	B	C	B	A+	..	
	C	O	B	A	A	..	
Liver collagen content (mg.)	11.9	10.8	8.2	5.0	7.7	3.0	2.5
	17.0	4.6	9.3	5.9	3.9	2.3	2.3
	15.2	10.5	7.5	5.7	3.5	2.3	lost
	12.9	3.8	4.2	5.4	3.9		

The duration of the experiment was two months.

*A mouse which died one day before the end has been included (histology grade F, collagen 11.9 mg.).

histological gradings on haematoxylin-and-eosin-stained preparations were C+, E, C, E, C+, E, E+, D, D, D+ and collagen values 14.6, 23.1, 5.7, 14.3, 10.1, 19.3, 10.2, 12.2, 15.1, 12.1 mg.

Experiment 4.—The dose and preparation of the dust were the same as in experiment 1 but larger mice (25 to 30 g.) were used in an attempt to reduce their mortality. Nevertheless, there were 11 immediate deaths amongst 83 mice injected with dust. Five others were very sick and were discarded, and, of the remainder, 37 died during the course of the experiment which lasted two months. Four groups of the mice given dust were treated with 48/80, three receiving the high-dose schedule for two, four, or eight weeks and the fourth, 50 µg. doses twice daily for three days. Mepyramine, 200 µg., was given subcutaneously 20 minutes before each dose of the three-day course of 48/80. Six control mice were given neither silica nor 48/80.

The incidence of deaths and the histological and chemical findings are shown in Table 4. The lethal effect of silica appeared to be reduced by treatment with 48/80 and it was clear that, given at the high dosage level for eight weeks, the drug conferred considerable protection against silicosis. Shorter courses of treatment were less effective but afforded some protection even with the period limited to three days. The collagen values for each of the treated groups were significantly lower than for the mice left untreated after receiving silica, as the following results show:—

Treatment	Nil	Three Days	Two Weeks	Four Weeks	Eight Weeks
Mean collagen (mg.)	15.22	7.96	7.80	5.66	5.11
t (comparison with untreated group)	—	4.85	5.34	8.61	9.52
t _{0.05}	—	2.20	2.20	2.20	2.18

The eight-week course of 48/80 treatment was not completely protective, however, either by histological or chemical criteria. Collagen values for mice on this course were significantly higher than for the controls ($t = 4.05$, $t_{0.05} = 2.20$).

Discussion

The chief value of the observations described above would appear to lie in their demonstration that it is possible for a drug given systemically to reach and inactivate toxic silica dust in the tissues of an animal. Jäger and Müller (1956) have claimed a similar effect for a protective agent but give no account of its chemical nature and the method they used to assess protection lacked precision. Although compound 48/80 has been administered to man (Nordlander, 1957), its toxicity precludes consideration of its use in the treatment of silicosis. Investigation into the relation between silica neutralization by organic bases and their histamine-releasing activity is needed in order to ascertain whether any can be found which are suitable for therapeutic trial.

Although the experiments described have clearly demonstrated the protective power of compound 48/80 against silicosis, it appears in retrospect that the dose of tridymite used may have been somewhat greater than the optimum for the display of the antagonists' activity. In experiments 2 and 4 the dose of silica used resulted in a very high death rate in the untreated mice, although in compensation the protective effect of 48/80 against the lethal effect of the dust was well displayed. The results presented in the tables show broad agreement between the histological and biochemical methods used to assess the degree of liver silicosis although there were discrepancies in some individual cases.

Information is not available at present as to the rate at which 48/80 is destroyed or excreted. In further work on screening drugs for use in silicosis it would perhaps be advantageous to select for examination compounds which are not readily metabolized, because the toxic action of silica appears to be maintained *in vivo* for a considerable time if not permanently.

Summary

Mice were given silica dust (tridymite) intravenously and the amount of silicosis induced in the liver assessed by histological examination and by estimation of the liver collagen content. A considerable degree of protection against the effect of the dust was obtained by treating the mice with

compound 48/80, an organic base previously shown to be effective *in vitro*.

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