FURTHER STUDIES OF THE NEUTRALIZATION OF SILICA TOXICITY *IN VITRO*

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A previous paper (Marks, 1957) describes work done on a number of substances to ascertain their protective effect on phagocytic cells in tissue culture against injury by silica dust. The most potent of those investigated was 48-80, a drug used in experimental pharmacology to release histamine from mast cells.

In the present paper, several drugs related to compound 48-80 have been examined for their capacity to protect phagocytic cells against the toxic effect of silica dust *in vitro*. It is shown that protection can be dissociated from histamine-releasing activity in these compounds.

Other substances found to neutralize the toxicity of silica dust were certain phenazines, complexes of aluminium with chelating agents, and the histamine-releasing agent L 1935.

Tissue culture offers a convenient means of studying the injury of phagocytic cells by silica dust and their protection by suitable antagonists. In an earlier communication (Marks, 1957), a number of protective agents investigated in this way were described, of which the most potent was compound 48-80, a drug used in experimental pharmacology to release histamine from mast cells. Compound 48-80 was subsequently shown to protect mice against the fibrogenic action of silica dust (Marks, James, and Morris, 1958). Only small amounts of this drug are tolerated by man and therefore certain related compounds have been investigated in order to determine whether the properties of histamine-release and neutralizing action on silica are distinct. The drugs of this series were prepared in the Wellcome Research Laboratories, New York, where their effect on mast cells was assayed by Dr. Stata Norton using her published technique (Norton, 1954). In addition, the histamine-releasing agent L 1935 (Feldberg and Lecomte, 1955) was examined for protective activity.

A number of drugs unrelated to histamine release were included in the present investigations. Phenazine compounds studied in relation to experimental tuberculosis by Barry, Conalty, and Gaffney (1956) and Barry, Belton, Conalty, Denny, Edward, O'Sullivan, Twomey, and Winder (1957) were selected because their structure suggested the possibility of protective activity, and, in addition, three chelates of aluminium with dextran, sequestric acid

and oxytetracycline respectively were prepared and tested.

Materials and Methods

The effect of silica and its antagonists on phagocytic cells was studied in tissue culture chiefly using the dehydrogenase technique described by Marks and James (1959). With this technique cells are incubated for three days with dust and their vitality then determined by their capacity to reduce tetrazolium. The dust used in the present work was a single sample of silica (tridymite) provided by Dr. G. Nagelschmidt, of the Safety in Mines Research Establishment, Sheffield. Its mean volume surface diameter was 1.3 microns and specific surface 2.5 m.²/g. The essential structure of the compounds which were related to 48-80 was as follows:—

(The radicals R_1 and R_2 and values of n are listed in Table 1.)

Compound 46-150 differed from the remainder in that its nitrogen was quaternary. Solutions of these drugs and also L 1935 were made aseptically in Ringer's solution freshly for each experiment.

A quantity of L 1935 was obtained for examination through the courtesy of Dr. B. N. Halpern and Dr.

Dinah M. James. It is a mixture of the following compound and its dehydro form:—

The general structures of the phenazines studied were as follows:—

1

2

(compounds B621 and B654)

The compounds listed below were examined.

Number	R ₁	R ₂	R ₃	R ₄
B283	Phenyl	H	Phenyl	—
B430	Cyclohexyl	Cyclohexyl	H	—
B595	Phenyl	Phenyl	H	—
B629	p-Chlorophenyl	p-Chlorophenyl	H	—
B663	p-Chlorophenyl	CH(CH ₃) ₂	p-Chlorophenyl	—
B673	p-Chlorophenyl	Cyclohexyl	p-Chlorophenyl	—
B740	p-Chlorophenyl	Cycloheptyl	p-Chlorophenyl	—
B778	Phenyl	C ₆ H ₄ OH	Phenyl	—
B782	p-Chlorophenyl	C ₆ H ₄ OH	p-Chlorophenyl	—
B621	Phenyl	—	—	Phenyl
B654	p-Chlorophenyl	—	—	p-Chlorophenyl

An additional compound, B749, with the following structure was also investigated.

The phenazines were prepared for use by dissolving them in hot ethanol at a concentration of 0.1%. Solution was assisted in several cases by acidification with HCl, but in a number a small proportion of the drug remained insoluble. Shortly before use, 1 in 10 dilutions of the ethanolic solutions were made in 25% serum-tyrode solution.

The aluminium chelates were prepared by mixing 1 ml. volumes of 1% AlCl₃·6H₂O with 4.8 ml. of 5% clinical dextran, 1.5 ml. of 1% di-sodium diamino-ethane-tetra-acetic acid (sequestric acid), or 2.5 ml. of 0.1% oxytetracycline. Each mixture was neutralized with 0.1N NaOH and its volume made up to 10 ml. with isotonic saline.

Results

Compounds Related to 48-80.—Compound 46-107, which appears to be relatively harmless to mast cells, proved to be twice as effective as 48-80 in protecting phagocytic cells against the toxic effect of silica dust. Compounds 46-108 and 47-30 were approximately four times less effective than 48-80 and the quaternary compound 46-150 appeared devoid of protective action. The results recorded in Table 1 are representative of those obtained in six experiments in which the dehydrogenase method of assessing cell vitality was used. Table 1 includes details of the structure of the compounds and of their destructive effect on mast cells relative to that of 48-80 which is taken as 100. The control cell cultures had a mean dehydrogenase activity of 50 arbitrary units but cultures exposed to silica dust an activity of only 4 units. The ability of the drugs studied to protect cells against silica injury is shown in Table 1 by the degree to which dehydrogenase activity was main-

tained in their presence. Some of the cultures treated with 46-107 in this experiment gave high readings but the divergence from the mean control was not outside the range of variation met in control cultures nor especially characteristic of the drug. Compound 46-107 was also found to be protective in tissue culture when glucose consumption and morphological criteria of cell damage were employed (Marks, 1957). Despite its histamine-releasing activity, compound 48-80 was tolerated by cells in

TABLE 1

PROTECTION OF PHAGOCYtic CELLS AGAINST EFFECT OF SILICA DUST BY COMPOUNDS RELATED TO 48-80

Drug	Dehydrogenase Activity* of Cultures after Addition of 20 µg. Dust and Stated Quantity of Drug (µg.)					Polymerization and Radicals (see text)			Effect on Mast Cells
	40	20	10	5	2.5	n	R ₁	R ₂	
46-107	—	77	82	69	31	2	CH ₃	CH ₂ N(CH ₃) ₂	0
48-80	55	56	51	27	—	2.4	H	CH ₂ NHCH ₃	100
46-108	43	26	13	—	—	2	H	CH ₂ NHCH ₃	2
47-30	38	29	14	—	—	2.3	H	NHCH ₃	20
46-150	8	6	6	—	—	2	CH ₃	CH ₂ N(CH ₃) ₂	< 5
Nil	Mean dehydrogenase activity = 4 units (untreated dust)								

*Arbitrary units.

The mean dehydrogenase activity of undusted control cultures was 50 units.

somewhat higher concentrations than compound 46-107.

For convenience, drugs were added to the cultures at the same time as the dust in most experiments but in some cells and dust were incubated together for six hours before drugs were added, a period found sufficient to permit phagocytosis. The results in the latter conditions (Table 2) were similar to those in the former, indicating in agreement with previous observations on compound 48-80 (Marks, 1957) that protection of the cells from injury by silica dust was not due to interference with phagocytosis.

TABLE 2

PROTECTION OF CELLS AGAINST EFFECT OF SILICA DUST BY ANTAGONISTS ADDED AFTER AN INTERVAL

Drug Preparation	Amount of Dust per Culture (µg.)	Dehydrogenase Activity with Stated Dose of Drug Preparation (µl.)		
		80	40	20
B283 phenazine, 0.01%	20	14	24	20
B673 phenazine, 0.01%	20	21	16	12
B749 phenazine, 0.01%	20	26	40	20
B778 phenazine, 0.01%	20	42	28	26
Oxytetracycline chelate of aluminium (0.011% aluminium)	20	41	38	36
Compound 46-107, 0.05%	20	45	39	47
Nil	20	Mean activity = 1 unit (untreated dust)		
Nil	Nil	Mean activity = 37 units (controls)		

Cells and dust were incubated together for six hours at 37°C. before antagonists were added. Readings were made after a further three days' incubation.

Compound L 1935.—A comparison was made between this drug and the 48-80 series in four dehydrogenase experiments. It was found to be approximately half as active as 48-80 in protecting cells against the effect of silica dust.

Phenazines.—Of the phenazines examined, only B283, B673, B749, and B778 exhibited appreciable protective activity, the last two being the most effective. A comparison of their effect with that of compound 46-107 and an aluminium complex is recorded in Table 2. Protection by the phenazines

appeared more obvious with the dehydrogenase technique than with the two other methods referred to above. An artefact appeared to be excluded, however, by the observation that phenazines were without effect when added at the same time as tetrazolium to cultures previously incubated with dust.

Aluminium Complexes.—The capacity of these compounds to protect cells against silica dusts was very similar to that of inorganic aluminium salts in equivalent concentration. The oxytetracycline complex was the most effective and the sequestric acid complex the least. A comparison made in a representative experiment is recorded in Table 3.

TABLE 3

PROTECTION OF CELLS AGAINST EFFECT OF SILICA DUST BY DIFFERENT ALUMINIUM COMPOUNDS

Compound Added	Amount of Dust per Culture (µg.)	Dehydrogenase Activity with Stated Dose of Aluminium (µg. metal per culture)		
		8.8	2.2	0.55
Aluminium chloride	40	27	17	10
Al-sequestric acid complex	40	25	13	6
Al-dextran complex	40	32	10	8
Al-oxytetracycline complex	40	44	26	7
Nil	40	Mean activity = 2.5 units (untreated dust)		
Nil	Nil	Mean activity = 44 units (controls)		

Discussion

Tissue culture techniques offer the advantages of convenience and speed in screening drugs for potential neutralizing action on silica dust. It is unlikely that they would fail to detect an agent of therapeutic value. However, the protective activity of a drug *in vitro* is not always reproduced *in vivo*. There are many possible reasons for such failures, for example, the agent may be destroyed, excreted, modified, or sequestered in the intact animal before it reaches the dust-laden phagocyte in an effective form. From the results obtained with compound

48-80 (Marks *et al.*, 1958) and incomplete animal experiments on the drugs described above, it appears that some at least of the compounds selected for their activity *in vitro* offer the possibility of arresting the pathological processes of silicosis. The chief requirement now for progress in this field is a programme of synthesis of compounds suitable for study.

Experience with compound 46-107 has shown that histamine-release and neutralizing activity for silica can be dissociated. It is possible, therefore, that the 48-80 series of compounds may provide a drug of practical value. The protective activity of the phenazines is less well established since it appeared to be much greater with the dehydrogenase technique than with others. Although no artefact was detected, suspicion must remain that part at least of the apparent protection was spurious. Aluminium by inhalation has been used for a considerable period for the prevention and treatment of silicosis. The complexes of aluminium studied in the present work were prepared for possible systemic treatment with the object of reaching dust-laden cells not accessible to inhaled aluminium dust. Aluminium therapy is attractive in that its effect on silica would probably

be more lasting than that of a wholly organic compound which would presumably be liable to chemical alteration. However, preliminary results suggest that the aluminium complexes are less effective than organic compounds in short-term animal experiments and are not innocuous themselves. (It is hoped to report later on the use in mice of the more important of the compounds described in the present work.)

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