

## CHANGES IN ACTIVITIES OF RESPIRATORY ENZYMES IN LUNGS OF GUINEA-PIGS EXPOSED TO SILICA DUST

### II. Comparison of the Effects of Quartz Dust and Lampblack on the Succinate Oxidase System

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Kilroe-Smith and Breyer (1963) reported that in the early stages of silicosis in guinea-pigs exposed to the inhalation of quartz dust, before the formation of collagen, there were increases in the specific activities of the complete succinate oxidase system and succinate dehydrogenase. The effects on these enzymes of quartz dust have now been compared with the effects of the fibrogenically 'inert' lampblack. Lampblack causes a slight increase in the specific activities of these enzymes but the effects are small compared to those caused by quartz. Lampblack also causes a much smaller increase in lung weight than quartz, thus the enzyme increases are roughly parallel to the rise in lung weight. It appears that the effects observed on the enzymes are part of the general pattern associated with the early stages of the development of silicosis.

In the early stages of silicosis, even before collagen formation is observed, the activities of the complete succinate oxidase system and of succinate: (acceptor) oxidoreductase (E.C.—1.3.99.1) (succinate dehydrogenase) are increased in the lungs of guinea-pigs exposed to the inhalation of quartz dust (Kilroe-Smith and Breyer, 1963). The observed effects of the enzymes need not necessarily be related to the specific effects of quartz on lungs in the development of silicosis. It is possible that the same effects of the enzymes could be produced by a fibrogenically 'inert' material such as lampblack. This aspect has therefore been investigated.

#### Experimental

Three groups each containing six male guinea-pigs were randomly selected. One group was exposed to the inhalation of quartz dust, a second group to the inhalation of lampblack, and the third was used as a control group.

All animals used in these tests were 6 months old at the start of dusting. All assays were done on sets of three animals on any particular day, one from each group, to eliminate variations due to methodology, enabling the

Scheff method of multiple comparisons to be used. Histological examinations were done on sections of the lower right lobe of the lung from each guinea-pig.

**Method of Dusting Guinea-pigs.**—The animals were dusted in Rabson boxes (Rabson, 1961),  $2 \times 2 \times 2$  ft. ( $0.227 \text{ m.}^3$ ) inside measurements, with baffles so arranged that the dusty air is blown through evenly from the bottom. The animals are placed in wire cages (six per cage) which just fit into the Rabson boxes above the baffles. The dust is dispersed by means of a Wright's disperser (Wright, 1950). Dusting was done for seven and a quarter hours per day, generally for five days per week.

The animals were in the dustboxes for a total of 100 days, quartz or lampblack being blown in during 68 of these days. All animals were then removed from the boxes, and enzyme assays on the lungs were evenly distributed over a period of eight days. For the lampblack the concentration was 70,000 to 80,000 particles per cc. For the quartz dust the concentration was 60,000 particles per cc., mainly in the range 0.1 to  $1.0 \mu$  diameter and containing 94 to 96% silica.

**Materials.**—Lung homogenates were prepared as previously described (Kilroe-Smith and Breyer, 1960, 1963).

Neotetrazolium chloride and cytochrome c were obtained from the Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A.

**Ash-free Dry Tissue.**—Samples of the lungs were dried to constant weight at 100°C. to determine percentage moisture. The percentage ash was then determined in the usual way by incineration, and the percentage ash-free dry tissue was calculated by subtraction of moisture and ash.

**Enzyme Assays.**—The methods have been previously described (Kilroe-Smith and Breyer, 1963).

The nomenclature of enzymes and the units suggested by the Commission on Enzymes of the International Union of Biochemistry (1961) have been adopted.

The relation between the units used in the previous papers and those used in the present one is as follows:—

For succinate dehydrogenase, 1000 $\gamma$  diformazan/15 min. = 0.232 unit.

For succinate oxidase, 1000 $\mu$ l. O<sub>2</sub>/hr. = 1.49 units.

### Results and Discussion

In the table the effect of lampblack is compared with that of quartz dust. The figures are averages from six animals and duplicate assays on each animal. It is clear that the effect of quartz dust is to raise the specific activities of the succinate dehydrogenase and of the succinate oxidase, confirming our previous findings (Kilroe-Smith and Breyer, 1963). The effect of lampblack is not so clear-cut. There is a small increase in the specific activities of the enzyme per mg. ash-free dry tissue which is statistically significant ( $P < 0.05$ ) for succinate dehydrogenase.

The same trends are seen in the ash-free dry weight of the lungs. The lampblack causes a significant increase, but this is only about 20% of that caused by dusting with quartz. There is thus a very

definite difference in degree in the effects of the lampblack and the quartz dust.

The simultaneous increase in lung weight and of the two enzyme specific activities emphasizes the need for caution in an interpretation of the results. The dual increase results in a very much greater increase in the total enzyme activity in the lung since this is the product of two variables, *i.e.* total activity in lungs = specific activity  $\times$  ash-free dry weight of lungs in grammes. It is therefore safer to draw conclusions from the specific activities of the enzymes than from the total activities of the whole lung, since this enables one to determine the changes in enzyme activity without involving the concurrent changes in lung weight.

The lungs at this stage had not advanced beyond the formation of reticulin, as shown by histological examination, and no abnormality in the lungs was revealed by histological examination apart from the effects due to dusting with quartz or lampblack.

Histological examination of the lungs of animals exposed to the inhalation of quartz dust showed a moderate diffuse cellular infiltration into the walls of the air spaces. The cellular infiltration consisted mainly of histiocytes and lymphocytes. Dust-laden phagocytes were present in the air spaces. There was a slight to moderate increase of reticulin fibres but not of collagen.

The lungs of guinea-pigs exposed to the inhalation of lampblack showed a moderate diffuse cellular infiltration, mainly histiocytes and lymphocytes, into the walls of the air spaces and deposition of carbon particles. There was no significant increase of reticulin fibres or collagen.

The lungs of the control animals also showed a slight cellular infiltration, mainly of histiocytes and lymphocytes, into the walls of the air spaces, probably due to age, but no increase of either

TABLE  
A COMPARISON OF THE EFFECTS OF THE INHALATION OF QUARTZ DUST AND LAMPBLACK ON TWO ENZYME SYSTEMS IN THE LUNGS OF GUINEA-PIGS

Treatment of Animals	Ash-free Dry Weight of Lungs (mg.)	Succinate Dehydrogenase		Succinate Oxidase	
		Units/g. A.F.D.T	Total Units per Lung	Units/g. A.F.D.T.	Total Units per Lung
Quartz	1,730 (1,420-2,110)	1.89	3.27	17.6	30.4
Lampblack	890 (740-970)	1.38	1.23	14.9	13.3
Control	680 (590-750)	1.16	0.79	13.2	9.0
Quartz minus control	1,050	0.73†	2.48	4.4*	21.4
Lampblack minus control	210	0.22*	0.44	1.7‡	4.3

Figures in brackets = range on which averages are based.

A.F.D.T. = Ash-free dry tissue.

\* Significant increase ( $P < 0.05$ ).

† Significant increase ( $P < 0.025$ ).

‡ No significant increase ( $P > 0.10$ ).

reticulin or collagen. In two of the control animals the reaction was slightly more marked.

It thus appears that the observed effects on the enzymes are part of the general pattern of the development of silicosis. The causes of the observed increases in the specific activities of the enzymes are not yet known, nor is it known how these increases affect the general metabolism of the lung, but it is clear that quartz is much more effective than lamp-black in bringing about these increases.

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