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THE *IN VIVO* EFFECT OF QUARTZ AND CARBORUNDUM DUSTS ON THE ACTIVITY OF CYTOCHROME C OXIDASE AND THE DNA CONTENT OF RAT LUNG TISSUE

F. M. ENGELBRECHT AND S. C. BURGER, *CSIR Tissue Damage and Cell Metabolism Research Unit, Department of Physiology, University of Stellenbosch*

In vitro studies have shown that silicous materials inhibit the activity of succinic dehydrogenase,[1,2] succinic oxidase[1,3,4] and cytochrome oxidase.[1] Recently, however, Kilroe-Smith and Breyer[5] showed that quartz dust caused significant increases in the activities of both succinic dehydrogenase and succinic oxidase in lung tissue, whereas no significant change was found in the activity of cytochrome oxidase *in vivo*.

The intratracheal administration of a dust suspension is followed by a foreign-body reaction involving numerous cells of varying types. The enzyme concentration and metabolic rate of these cells might differ widely from those of lung tissue. An increase in the activity of some enzymes may therefore not be due to the stimulating action of a specific dust but rather to the vast number of cells concentrated in the lung as part of the foreign-body reaction.

To assess the specific effect of a particular dust on the cytochrome oxidase activity of lung tissue *in vivo*, the influence of a cytotoxic, fibrogenic quartz dust and a non-fibrogenic carborundum dust was investigated.

MATERIAL AND METHODS

Young male albino rats weighing 200 - 250 G were used. These rats were divided into 3 groups; a control group, a quartz group and a carborundum group.

Dust Samples

Quartz dust was prepared by grinding rock crystal for 60 hours in an electrically driven agate ball-mill until most particles had a size of less than 5 μ.

Abrasive carborundum (FFF) was repeatedly resuspended in distilled water and the supernatant centrifuged until a dust sample was obtained which had a size distribution approximating that of the quartz dust.

Dust suspensions (50 mg./ml. isotonic saline) were prepared, sterilized and administered to the 2 groups of rats via intratracheal injection in doses of 50 mg. per rat.

Investigations

Two rats per group were killed at predetermined intervals (Table I), their lungs removed, weighed and homogenized at 5 - 10°C in a Waring Blendor for three 15-second periods. The following determinations were done on the homogenates:

DNA content. DNA extracts were prepared from the homogenates by a modified method (see below), developed from the technique of Schmidt and Thannauser[6] as described by Logan *et al.*,[7] and the actual DNA content of the different extracts was measured spectrophotometrically:

1. Homogenize lung tissue.
2. Make a 10% homogenate in 0·9% NaCl.
3. Centrifuge 5 ml. of homogenate for 30 min. at 3,500 r.p.m.
4. Add 10 ml. 10% TCA to residue, wash and centrifuge for 5 min.
5. Wash residue 3 times with ethanol/ether (3:1); centrifuge for 10 min. and dry residue in air.
6. Hydrolyse residue with 5 ml. 1 N-KOH for 18 hours at 37°C.
7. Add 5 ml. cold 10% TCA in 1 N-HCl to hydrolysate; centrifuge for 30 min.
8. Wash residue with 5 ml. cold 5% TCA; centrifuge for 10 min.
9. Extract residue with 5 ml. 5% TCA in a waterbath at 90°C for 10 min. (also blank and standard DNA solution of 0·02 mg. DNA/ml. 5% TCA); centrifuge for 10 min. (→supernatant = extract 1).
10. Repeat extraction procedure on residue (step 9) (→supernatant = extract 2).

Extracts 1 and 2 were pooled, diluted, and the DNA content of the resulting extract determined by ultraviolet absorption at 265·5 mμ. The DNA content of the lung tissue is expressed in mg. DNA/G wet tissue.

Cytochrome oxidase activity. The cytochrome oxidase activity of the different homogenates was determined simultaneously and in triplicate in a Warburg apparatus at 38°C over 1 hour, using air as gas phase.

The main compartment of each Warburg flask contained: 1·50 ml. 0·1M $Na_2HPO_4.2\ H_2O$ buffer solution (pH 7·3), 0·35 ml. 4 mM $AlCl_3.6\ H_2O$, 1·00 ml. 0·1 mM reduced cytochrome c (Kilroe-Smith and Breyer[5]), and 0·30 ml. 10% homogenate in 0·9% NaCl. 0·35 ml. sodium ascorbate substrate was placed in the side-arms of the reaction flasks. The Vf was therefore equal to 3·5 ml.

To compensate for auto-oxidation of the suspension medium, 3 flasks contained boiled homogenate. Two flasks contained suspension medium only and acted as thermobarometers to compensate for temperature and barometric changes during the run of the experiment.

After an equilibration period of 5 minutes the sodium ascorbate was tipped from the side-arm containers into the main

TABLE I. CYTOCHROME OXIDASE ACTIVITY AND DNA-CONTENT OF LUNG TISSUE OVER A PERIOD OF 90 DAYS FOLLOWING INTRATRACHEAL ADMINISTRATION OF DUST SUSPENSIONS OF CARBORUNDUM AND QUARTZ

	Cytochrome oxidase activity (Q_{O_2}/mg. wet tissue)					*mg./DNA/G wet lung tissue*		
Days	*Control*	*Carborundum*	*% deviation from control*	*Quartz*	*% deviation from control*	*Control*	*Carborundum*	*Quartz*
2	5·72	10·07	+76·0	7·36	+28·7	7·1	7·9	6·2
4	4·48	4·79	+6·9	4·52	+0·89	6·9	6·2	5·5
8	4·92	6·05	+22·9	4·89	−0·6	7·6	6·6	5·9
12	5·40	5·90	+9·3	4·65	−13·9	7·5	8·1	6·0
16	5·17	5·45	+5·4	4·66	−9·9	7·5	7·2	5·0
20	5·14	4·98	−3·1	4·32	−15·95	7·3	6·9	5·7
30	6·16	6·05	−1·8	4·89	−20·6	7·3	7·6	5·5
60	4·31	5·31	+23·2	3·06	−29·0	6·4	8·2	5·6
90	5·38	6·22	+15·6	2·74	−49·1	6·6	8·1	3·9

Control—carborundum $P < 0·001$
Control—quartz $P < 0·001$
Carborundum—quartz $P < 0·001$

compartments of the flasks and readings were taken after a further equilibration period of 10 minutes. The cytochrome oxidase activity of each homogenate was calculated in terms of oxygen uptake (1) per mg. wet tissue per hour (QO_2) and (2) per mg. DNA (QO_2/mg. DNA).

RESULTS AND DISCUSSION

The lung tissue of both the quartz- and carborundum-treated animals showed an initial elevation in cytochrome oxidase activity (Table I, Fig. 1). This increase was more prominent in the lungs of animals injected with carborundum dust. Except on 2 occasions (20th and 30th day) the cytochrome oxidase activity of the lung tissue of the carborundum group was significantly higher over a period of 90 days than that of the control group.

The cytochrome oxidase activity of the quartz lungs decreased gradually up to 90 days after an initial increase over the first 4 days. Quartz, a highly fibrogenic dust compared with inert carborundum, therefore caused a significant over-all decrease in enzyme activity.

The DNA content of the control group remained relatively constant throughout the experiment. The DNA values of the carborundum lungs showed a similar tendency to the cytochrome oxidase activity and was significantly higher than the control values except on a few occasions. The DNA content of the quartz lungs was significantly lower than that of both the carborundum and control lungs (Fig. 2). At 90 days after administration of quartz the DNA content of lung tissue was only half of the normal control value.

When the cytochrome oxidase activity is expressed as QO_2/mg. DNA (Table II, Fig. 3) both quartz and carborundum caused a relative increase in the cytochrome oxidase activity over the first 20 days followed by a slight non-significant decrease. In terms of the DNA content, the effect of quartz and carborundum on the cytochrome oxidase activity of lung tissue was very similar.

TABLE II. CYTOCHROME OXIDASE ACTIVITY OF LUNG TISSUE EXPRESSED AS QO_2/mg. DNA

Days	*Control*	*Carborundum*	*% deviation from control*	*Quartz*	*% deviation from control*
2	806	1,275	+58·19	1,189	+47·5
4	649	755	+16·33	825	+27·12
8	648	916	+43·36	828	+27·8
12	720	728	+1·1	759	+5·4
16	689	757	+9·9	932	+35·3
20	704	721	+2·4	759	+7·8
30	844	796	−5·7	889	+5·3
60	673	648	−3·7	546	−18·9
90	815	769	−5·6	702	−13·9

Control—carborundum $P < 0{\cdot}001$
Control—quartz $P < 0{\cdot}001$
Carborundum—quartz $P < 0{\cdot}01$

The DNA content of a tissue is only a reflection of the relative number of cells, while the cytochrome oxidase activity is an indication of the metabolic rate of the cells. Carborundum caused an increase in DNA content and cytochrome oxidase activity and therefore apparently an increase in the number of cells and their respiration rate. Quartz, on the other hand, had a similar initial effect but subsequently the DNA content as well as the cytochrome oxidase activity was gradually lowered, indicating that the number of cells was eventually reduced and replaced by ground substance and fibrous tissue.

It is well known that the cytochrome oxidase activity of different cell types in relation to their DNA content differs widely. In the light of this, the pronounced increase in the cytochrome oxidase activity over the first 20 days after intratracheal administration of both dusts (Fig. 3) might be due to foreign phagocytic cells, with relatively small nuclei and a large amount of cytoplasm, concentrated in the lung, and not necessarily to the direct stimulating effect of the dusts on the respiration rate of lung cells. Whatever the case may be, the increased cytochrome oxidase activity during the acute inflammatory phase could not be concerned with the proliferation of connective tissue because, of the 2 dusts, carborundum had a more marked stimulating effect but did not produce fibrosis in the lungs of rats.

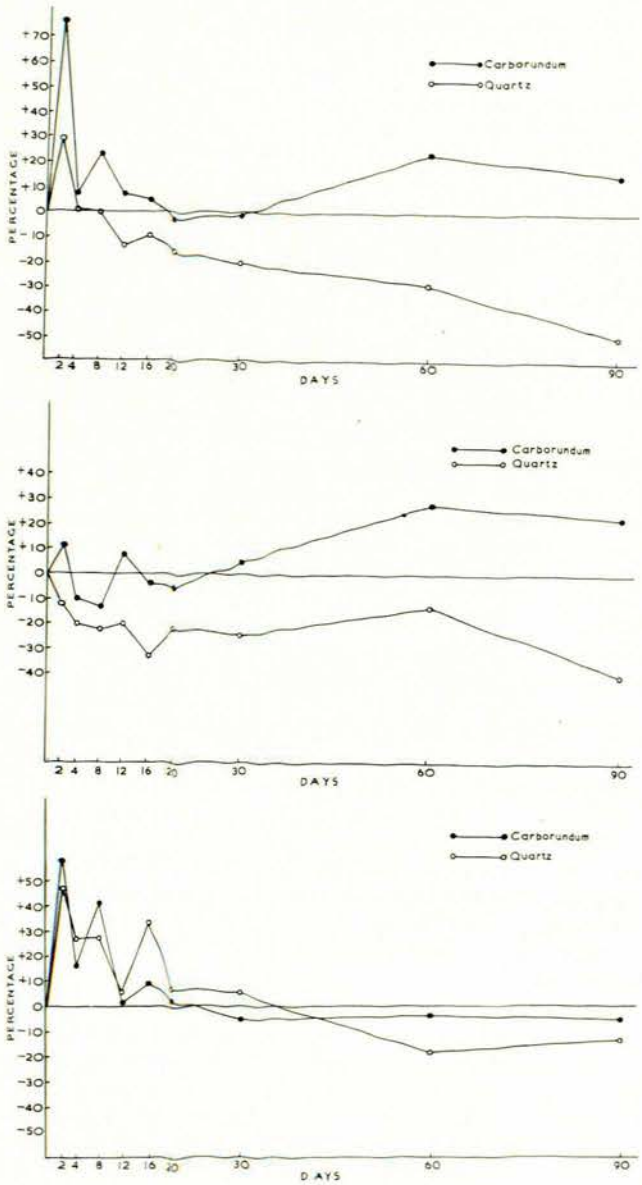

Fig. 1 (*Top*). Cytochrome oxidase activity of lung tissue (QO_2/mg. wet tissue).
Fig. 2 (*Middle*). DNA content of lung tissue (mg. DNA/G wet tissue).
Fig. 3 (*Bottom*). Cytochrome oxidase activity of lung tissue (QO_2/mg. DNA).

Considering these findings, the results of Kilroe-Smith and Breyer,[5] that the cytochrome oxidase activity of lung tissue of animals dusted with quartz was unaltered, but that the succinic dehydrogenase and succinic oxidase activity increased, are difficult to evaluate.

Our results indicate in general that quartz dust markedly reduced the DNA content and the cytochrome oxidase activity of lung tissue and therefore also the number of cells. The mechanism of this cell destruction is not clear but in the light of the findings of Engelbrecht and Burger[9] it might be due to the cytotoxic action of silicic acid inducing cell hypoxia. These workers found that polymerized silicic acid inhibited cytochrome oxidase activity *in vitro*, both in liver homogenates and Keilin and Hartree preparations.

SUMMARY

The effects of quartz and carborundum dusts on the activity of cytochrome oxidase of rat lung tissue were investigated over a period of 90 days after intratracheal administration (50 mg./ml. suspension).

The results indicate that the non-fibrogenic carborundum dust caused an increase of the cytochrome oxidase activity per unit weight of tissue, while quartz, a very fibrogenic dust, lowered the activity of this enzyme significantly. The DNA content of the carborundum and quartz lungs followed the same trend as the cytochrome oxidase activity. When the cytochrome oxidase activity is expressed in terms of QO_2/mg. DNA, both dusts caused an initial stimulation of the cytochrome oxidase system over the first 20 days, followed by a non-significant decrease. The initial stimulatory effects of these dusts could not be concerned with the proliferation of fibrous tissue.

Judged by the effects of a highly fibrogenic quartz dust on the cytochrome oxidase activity and the DNA content of lung tissue, it would appear that fibrogenesis due to pneumoconiotic dusts is preceded by an excessive cell destruction. The cytotoxic action of fibrogenic dusts *in vivo* might primarily be due to their influence on the cytochrome oxidase system of the cells.

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