Br. J. exp. Path. (1983) **64**, 286

ANGIOTENSIN CONVERTING ENZYME IN THE SERUM OF RATS WITH EXPERIMENTAL SILICOSIS

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Received for publication December 1, 1982

Summary.—The development of silicosis in rats given an intratracheal dose of silica was followed both by normal histological procedures and by measuring the level of angiotensin converting enzyme in the serum. The histological changes demonstrated a variable response due to the uneven deposition of material given by the intratracheal route. While the enzyme activity in the serum increased in the treated group it was not well correlated with the histological changes.

IT has been reported that the activity of the endopeptidase 'angiotensin converting enzyme' (ACE) is elevated in the serum of patients with a variety of pulmonary diseases, particularly those with sarcoidosis (Lieberman, 1975; Ashutosh and Keighly, 1976; Silverstein *et al.*, 1977). The changes in the activity of this enzyme in lung disease have been reviewed recently by Rohatgi (1982). In the present context it is of particular interest that the activity of this enzyme is increased in the serum of patients suffering from asbestosis and silicosis (Gronhagen-Riska *et al.*, 1978) but it must be remembered that such elevations can also occur in some non-pulmonary conditions (see for example Lieberman & Beutler, 1976).

Studies of changes in the activity of this enzyme have been made in experimental animals exposed to a variety of lung damaging insults. Molteni *et al.* (1974) reported that ACE in mouse lung and serum was increased by chronic alveolar hypoxia. Bleomycin treatment of mice can cause a transient increase in serum ACE activity and this occurs before the development of any pulmonary fibrosis (Vats *et al.*, 1977). Newman *et al.* (1980) reported that the enzyme level in rat lung fell after bleomycin treatment, while that in alveolar lavage fluid from such lungs increased at least 30 fold. In contrast, Hollinger *et al.* (1980) found that a dose of thiourea causing acute pulmonary oedema in rats produced an elevation of ACE activity in lung, serum and in pleural effusion fluid.

In this study we investigate the effects of experimental silicosis on serum ACE in laboratory rats in order to explore the possibility that the serum levels of this enzyme could be used to follow the development of the lung lesions.

MATERIALS AND METHODS

Serum ACE was measured using an adaptation of Lieberman's modification of the method of Cushman and Cheung (Lieberman, 1975). Hippuryl-histidyl-leucine was obtained from Calbiochem (Bishops Stortford) and from Sigma Chemical Co. (Poole, Dorset). Rat serum was diluted at either 1:4 or 1:8 with 0·85% w/v NaCl in order to ensure that the enzyme rate was within the linear portion of its activity curve. The reaction between enzyme and substrate was carried out as described by Lieberman (1975) and the liberated hippuric acid extracted with ethyl acetate. This organic extract was dried at 120° for 30 min before the hippuric acid was dissolved in 1M NaCl and estimated in a Cecil 5095 spectrophotometer at 228nM. This drying and dissolving step reduced the formation of chylomicrons in the final aqueous phase and enabled greater reproducibility in the assay.

Animal experiments.—Male Sprague Dawley rats weighing between 150 and 200 g were purchased from Tuck and Son Ltd. (Battlebridge, Essex); these were kept for 3–4 weeks in the animal house before dosing. Silica (DQ12)

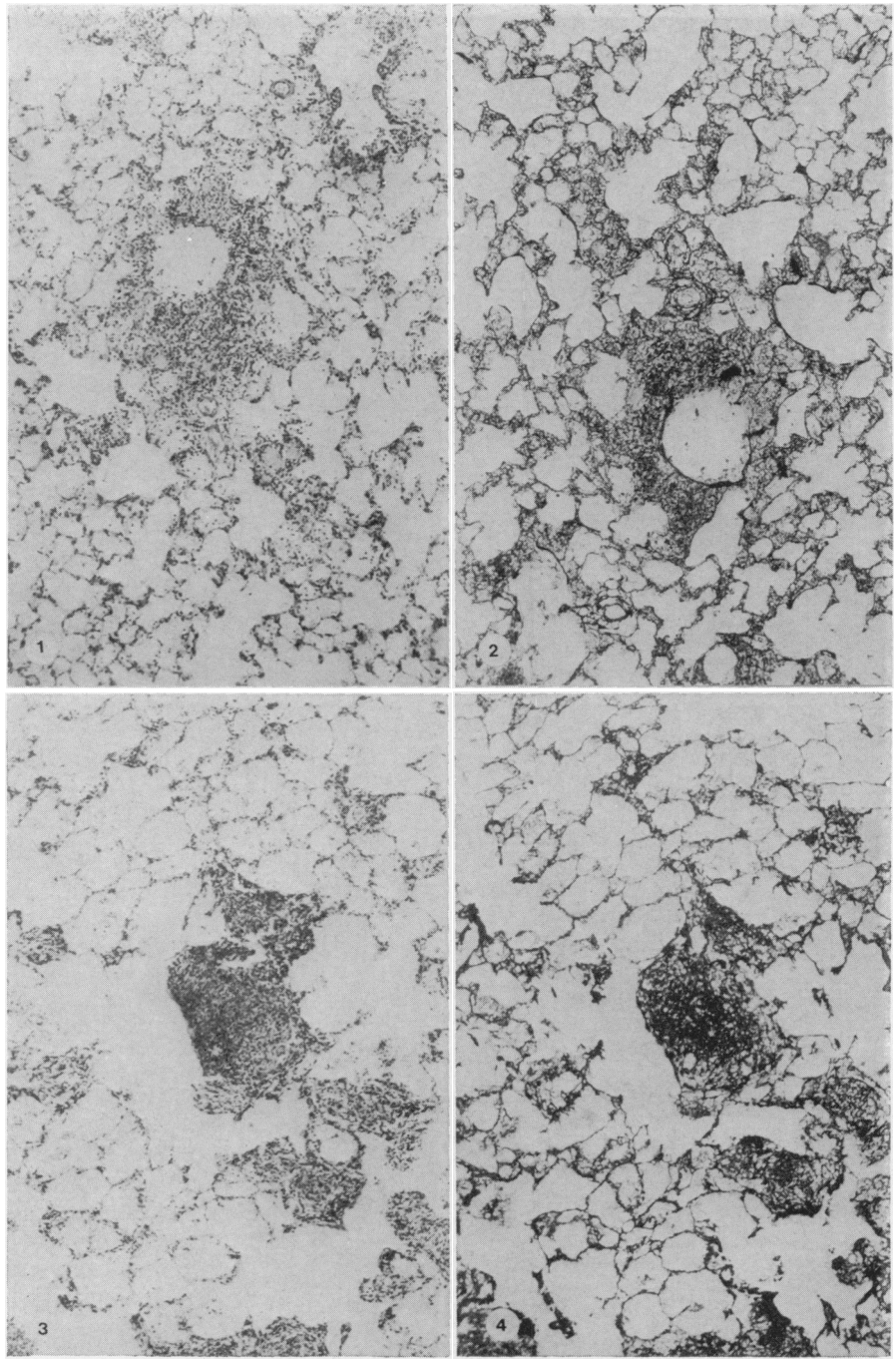

FIG. 1.—A section from the lung of a rat killed 48 h after the intratracheal instillation of 25 ml DQ12. Grade 1 fibrosis. Cellular reaction. H. & E. × 72.

FIG. 2.—As Fig. 1. Shows the presence of increased discrete reticulin fibres in the lesion. Reticulin stain. × 72.

FIG. 3.—A section from the lung of a rat killed 10 weeks after intratracheal instillation of 25 mg DQ 12. Grade 2 fibrosis. Reaction still mostly cellular. H. & E. × 72.

FIG. 4.—As Fig. 3. Reticulin has become more dense. Reticulin stain. × 72.

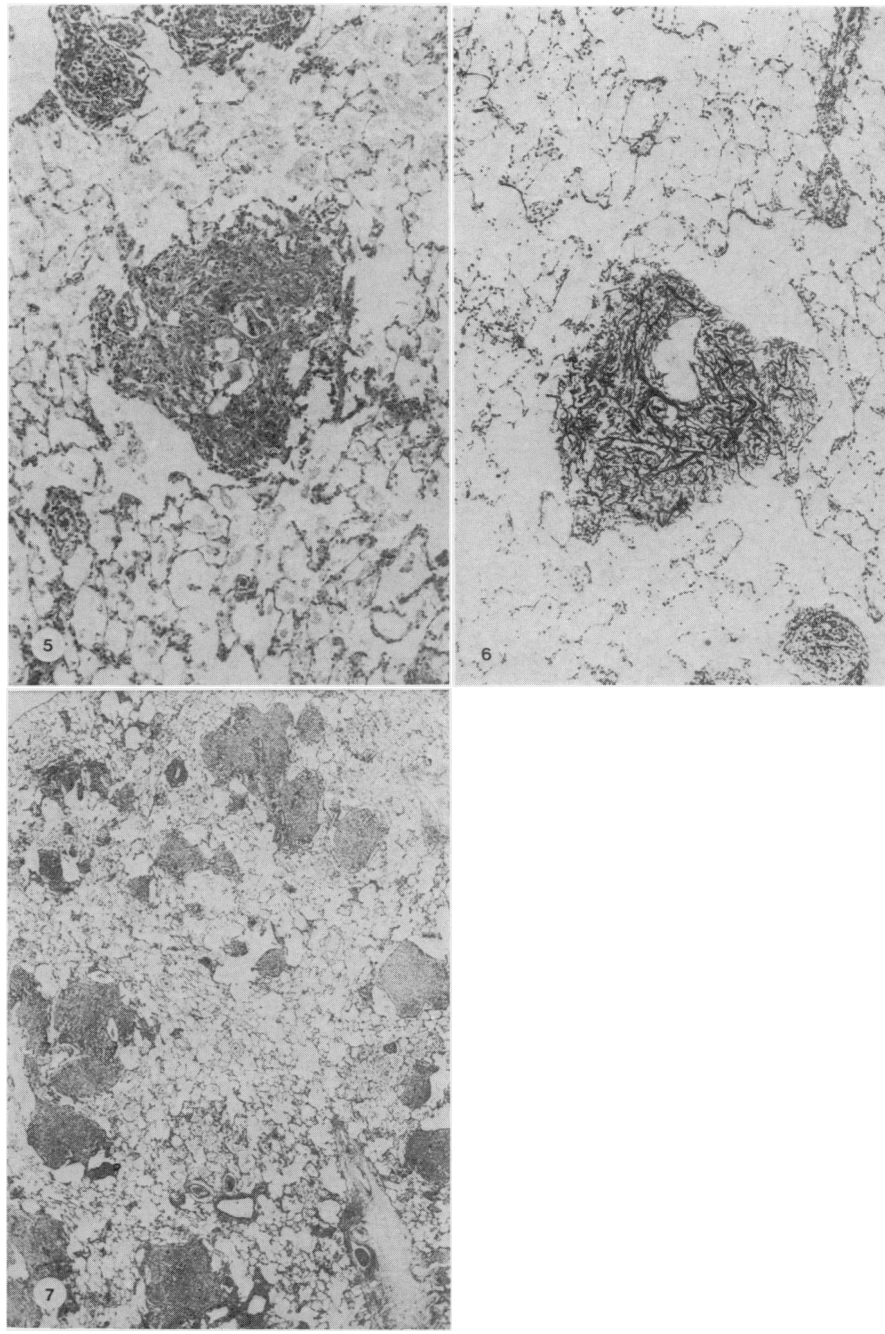

FIG. 5.—A section from the lung of a rat sacrificed 10 weeks after intratracheal instillation of 25 mg DQ12. Grade 3 fibrosis. The lesion has become less cellular. H. & E. × 72.

FIG. 6.—As Fig. 5. The major part of the lesion now consists of collagen fibres. Reticulin stain. × 72.

FIG. 7.—A section from the lung of a rat sacrificed 10 weeks after intratracheal instillation of 25 mg DQ12. Grade 3 fibrosis. Area from a section which in total showed 50% profusion. H. & E. × 18.

haematite and TiO_2 were obtained from Dr I. P. Gormley (Institute of Occupational Medicine, Edinburgh); Cab-o-Sil was obtained from BDH Ltd. Weighed dust samples were autoclaved and a 50 mg/ml suspension prepared in sterile phosphate buffered saline (PBS). The rats were anaesthetized with ether and either 0·5 ml of PBS or 0·5 ml of a dust suspension (diluted if necessary with further PBS) was administered intratracheally. A fibre optic endoscope specially constructed in this laboratory was used for all instillations.

Preparation of serum.—The rats were anaesthetized with sodium amytal, the thorax was opened and the rat bled out from the right ventricle. The blood was transferred to glass tubes and allowed to clot at room temperature, the serum was then separated, clarified by centrifugation, transferred to coded tubes and frozen at $-70°$ until measurement. All of these assays were carried out by a co-worker unaware of the code or origin of the serum. All rats were bled in the late morning to remove any effect of diurnal changes on the serum activity of this enzyme.

Histological preparation and assessment.—Following collection of blood, the lungs were removed intact and inflated, through the trachea, with 10% neutral formalin. Tissue was also taken from the liver, spleen and kidney and immersion fixed in the same fixative. Blocks from both lungs and other tissue were taken and 5 μm paraffin sections were prepared. These were stained with haematoxylin and eosin, haematoxylin and Van Gieson and with Gordon and Sweet's reticulin stain.

The sections were then assessed for severity of fibrosis using the Belt and King (1945) scale that is:

Grade 1. loose reticulin fibrils with no collagen;

Grade 2. compact reticulin with or without a little collagen;

Grade 3. somewhat cellular but made up mostly of collagen;

Grade 4. wholly composed of collagen fibres and completely acellular;

Grade 5. acellular, collagenous and confluent.

Examples of grades 1, 2 and 3 are illustrated in Figs 1–6.

The extent of the fibrosis was assessed by simple observation whereby the involved area was estimated and expressed as a percentage of the total individual section area. An example of this assessment is shown in Fig. 7.

RESULTS

In a preliminary experiment 18 rats each received an intratracheal instillation of either 0·5 ml PBS or quartz DQ12. Dose levels of 2·5, 10 or 25 mg of silica were used, each suspended in 0·5 ml PBS. Three rats from each group were killed at 1, 3, 7, 14, 30 and 56 days. The serum was collected and the lungs of one rat from each group examined histologically. The results were very variable; the ACE level only rose in the group receiving 25 mg and only at times up to 30 days. The histological results were equally varied and it was therefore decided that to overcome this variability it would be essential to examine the lungs of all the rats in subsequent experiments, not merely those from a sample of the animals. As all the tissue would therefore be fixed for histology no attempt was made to measure the ACE activity of the lung tissue itself.

On the basis of these preliminary results 36 rats were given 25 mg DQ12 and 40 PBS alone. A number of early fatalities occurred and so from each group it was possible to kill and examine 10 rats after both 1 and 2 weeks, 8 after 4 weeks and only 5 treated and 7 controls after 10 weeks. Serum ACE levels were measured and the results are shown in Fig. 8. Similarly a summary of the histological

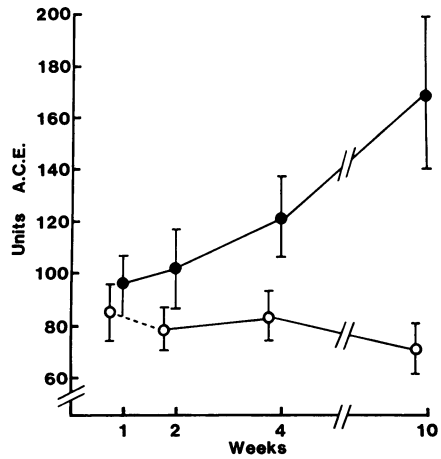

FIG. 8.—The level of angiotensin converting enzyme is shown for rats treated by intratracheal instillation with 25 mg quartz DQ12 (filled circles) or with phosphate buffered saline as a control (open circles). The mean and standard deviation is shown for each group at each time.

TABLE I.—*The extent and grade of fibrosis was determined as described in the text. The number in brackets in the first column is the number of rats, the total ranges are given as any other measure of dispersion would assume a particular mathematical distribution for the data and in the present case this would not be justified.*

	Mean severity	Mean extents and ranges			
		LL	RUL	RML	RLL
1 week (10)	2·2	75 (10–90)	5 (5–20)	6·5 (5–40)	12 (5–60)
2 weeks (10)	2·7	72 (0–90)	1·1 (0–5)	5·5 (0–50)	11 (0–50)
4 weeks (8)	3·1	73 (20–100)	23 (0–70)	16 (0–80)	35 (0–100)
10 weeks (5)	3·5	89 (80–90)	44 (10–70)		50 (20–80)

findings are given in Table I. As can be seen both serum ACE and fibrosis (severity and extent) have increased in the silica treated rats. It can be seen in Table I that there is a profound difference between the development of fibrosis in the left and right lungs. The left lungs showed a large (approximately 70%) involvement at the time of the first sacrifice and this increased only a little during the course of the experiment. The involvement of the right lungs was only around 10% at 1 week but this increased to a mean of over 50% at 10 weeks.

There was no simple correlation (within the treated group) between the severity or the extent of fibrosis and serum ACE. The rat with the lowest ACE level at 10 weeks had a less extensive fibrosis in the right upper lobe (10% compared with scores of 30, 40, 70 and 70%) for the others in that group. The lowest scoring animals at 4 weeks, however, were no different from the highest, and it is particularly interesting to note that the lowest ACE level was found in the rat with the most extensive fibrosis.

A further experiment was set up to investigate the effects of other dusts on the ACE level in rat serum. Ten rats each were dosed with either PBS alone, haematite or titanium dioxide and 8 with DQ12, all the dusts being given at a dose of 25 mg. An attempt was also made to give a group of rats the amorphous silica "Cab-o-Sil" but 4 animals given 25 mg died without recovering from the anaesthetic. The dose was therefore reduced to 12·5 mg and 8 rats dosed, but even at that level only 4 animals survived for 24 hours.

All the surviving animals were killed after 3 months, serum ACE measured and the lungs examined. The results of these examinations are given in Table II. In those rats treated with DQ12 fibrosis was not as extensive as in the previous experiment, and although increased the difference between the ACE levels in the control and treated animals failed to reach statistical significance. The Cab-o-Sil dosed animals developed a marked fibrosis but no elevation in ACE. There was only a mild reaction to haematite and titanium dioxide with again no elevation in ACE.

DISCUSSION

The intratracheal instillation of quartz (DQ12) produced fibrosis and an increase in the serum level of ACE. The changes in the activity of this enzyme were variable and only poorly correlated with the observed histological changes. The effects of instillation were very variable; within individual animals the left lungs were affected more than the right and the right lower lobes more than the upper. This suggests that the distribution of the silica was extremely uneven. Similarly a great deal of variability occurred between animals and between separate experiments, in the third experiment the increase in ACE failed to reach a significant level in animals treated either with DQ12 or Cab-o-Sil, although both of these substances produced significant fibrosis.

TABLE II.—*As for Table I*

	Angiotensin converting enzyme mean ± s.e. mean	Mean severity	Mean extents and ranges LL	RUL	RML	RLL
Control	90·0 ± 3·9	0·3	1 (0–5)	0·5 (0–5)	0·5 (0–5)	1 (0–5)
DQ12	102·9 ± 8·2	3·4	49 (20–70)	36 (5–70)	40 (5–80)	41 (10–80)
Haematite	85·2 ± 5·6	1·0	4 (0–5)	4 (0–5)	4 (0–5)	4 (0–5)
TiO_2	79·0 ± 6·7	1·0	5 (0–5)	5 (0–5)	5 (0–5)	5 (0–5)
Cab-o-Sil	66·0 ± 7·5	2·5	9 (5–10)	8 (5–10)	5 (5)	5 (5)

The amorphous silica "Cab-o-Sil" produced a reasonably severe reaction but this involved only a small area of the lung. This patchy deposition of the dust has always been a major drawback to the use of the intratracheal method of exposure.

It seems that the serum level of ACE is not a useful parameter in monitoring the response of individual rats to lung damaging agents. The activity in the serum of a sufficiently large population or even in lung tissue or alveolar lavage fluid might, however, be able to indicate developing lung damage.

The normal source of this enzyme is believed to be the endothelial cells in the pulmonary capillary bed, it is a membrane bound glycoprotein and has been shown to occur in pinocytic vesicles of the capillary endothelial cells (Ryan *et al.*, 1976). In sarcoidosis the lymph nodes contain excess levels of ACE (Silverstein *et al.*, 1976) and within these nodes the source of the enzyme seems to be the epithelioid and giant cells of the sarcoid granulomata (see review by Rohatgi, 1982). The source of the increased enzyme in the present case is probably the macrophages surrounding the accretions of silica in the developing fibrotic lesions. The lymph nodes could also be involved or it may even be that the dust cleared from the lung is having an extrapulmonary effect. Indeed histological examination of the livers from the rats given DQ12 showed the presence of occasional granulomata, this possibility could be investigated further by exposing some animals to silica by intravenous injection.

Which ever tissue is the origin of this enzyme it is most likely to be derived from cells of the monocyte-macrophage system particularly as these have been induced to synthesize large amounts of ACE in culture (Freidland *et al.*, 1977, 1978). It is planned to treat such cells *in vitro* with sub-lethal doses of silica and investigate any changes in the synthesis of this enzyme.

The authors wish to thank Dr J. H. Edwards for carrying out the instillations and Mr F. Mason for his assistance in animal care.

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