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THE EFFECT OF CORTISONE ON EXPERIMENTAL SILICOSIS

BY

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Alterations in cellular and fibrous tissue reaction due to cortisone and A.C.T.H. have been demonstrated in human beings by several workers such as Freeman, Fershing, Wang, and Smith (1950), and by Cavallero, Sala, Amira, and Borasi (1951) in the carbon tetrachloride necrosis of rats' livers. The influence of cortisone on the phagocytic action of the reticulo-endothelial system on carbon particles was studied by Spain, Molomut, and Haber (1950) and on intraperitoneally injected silica particles by Policard and Tuchmann-Duplessis (1951). In both experiments the migration of dust-laden phagocytes was retarded. Kennedy, Pare, Pump, Beck, Johnson, Epstein, Venning, and Browne (1951), working in the Department of Professor J. S. L. Browne of Montreal, treated two patients with chronic beryllium granulomatosis and obtained temporary subjective and objective improvement as judged both by respiratory function studies and *x*-ray films of the lungs. Since chronic berylliosis is characterized by gross fibrosis of the lungs, these workers were encouraged to experiment with the treatment of silicosis by the same means. It is difficult to believe that the densely fibrous nodules which are scattered throughout the lung in silicosis could be influenced to resolve or regress ; and this, indeed, was found to be the case by Kennedy and his colleagues, who were unable to observe any change in the *x*-ray appearance of the lungs of the patient they treated. Nevertheless some relief of his clinical condition was claimed. The cough and sputum disappeared and dyspnoea was relieved.

As a result of the reports of these experiments the Cortisone and A.C.T.H. Sub-Committee of the Industrial Pulmonary Diseases Committee of the Medical Research Council asked us and Professor J. Gough of Cardiff to undertake investigations of the effect of cortisone on the production of experimental silicosis in animals. While, as noted above, it seems improbable that the hormone could have any effect on the established lesions of silicosis, it nevertheless appeared possible that the production of fibrous tissue might be retarded by the administration of cortisone, or that there might be some effect on the movement of dust-laden phagocytes into focal areas of accumulation with a consequent slowing up of the fibrotic response. Professor Gough's results have been reported separately (Magarey and Gough, 1952), and an account of our own investigation is given in the present communication. Whereas we have found the predominant effect of cortisone to be on the movement of dust-laden phagocytes, Schiller (1951, 1952), Magarey and Gough (1952), and Curran (1952) have demonstrated both an inhibition of fibrosis and an alteration in the mobility of macrophages when quartz dust was injected into the peritoneal cavity.

TABLE 1
SIZE DISTRIBUTION OF POWDERED QUARTZ*

Size (μ).	Percentage of Quartz Particles	
	By Number	By Mass
Under 0·45	3·0	0·01
,, 0·64	21·9	1·4
,, 0·9	60·1	9·5
,, 1·3	76·9	19·3
,, 1·8	89·2	41·3
,, 2·6	98·7	86·0
,, 3·6	100·0	100·0

*Prepared from finely ground Belgian glass sand by repeated sedimentation in water and recovered by centrifuging. 99% SiO_2. Microscope count by matching projected diameters against circles of known size using the May (1945) graticule at 950 magnification (oil immersion). Prepared and particle-sized by Dr. G. Nagelschmidt and Mr. J. Cartwright, Safety in Mines Research Establishment, Sheffield.

Method

One hundred and thirteen adult albino male and female rats were used. " Snowit II " quartz (>2μ, Table 1)

was suspended in isotonic saline in a concentration of 50 mg./ml. and was sterilized by autoclaving.

The rats were anaesthetized lightly with ether and the trachea was exposed by dissection. The first 23 animals received 1·5 ml. of the quartz suspension injected intratracheally, but since a small amount was regurgitated the dose was reduced to 1·0 ml. in the remaining rats. During the procedure both the flask and the syringe into which the suspensions were drawn were kept agitated to prevent sedimentation. The injection was done quickly and with a certain amount of force to disperse the dust well into the lung alveoli. The wound was then closed by a single suture. Fifteen rats died during, or soon after, the operation. The remaining 98 rats were divided into two groups of 49 each. One group received no further treatment (control group), the other (test group) received subcutaneous injections of cortisone* acetate suspension. The amount given was 5 mg. daily five days a week for 17 days, and thereafter 5 mg. three days a week. Each week the animals were weighed, eosinophil counts made, and one rat from each group was killed. Lungs and spleens were removed, weighed, fixed, and sectioned. Findings in spontaneous deaths and rats killed when moribund have been separately recorded.

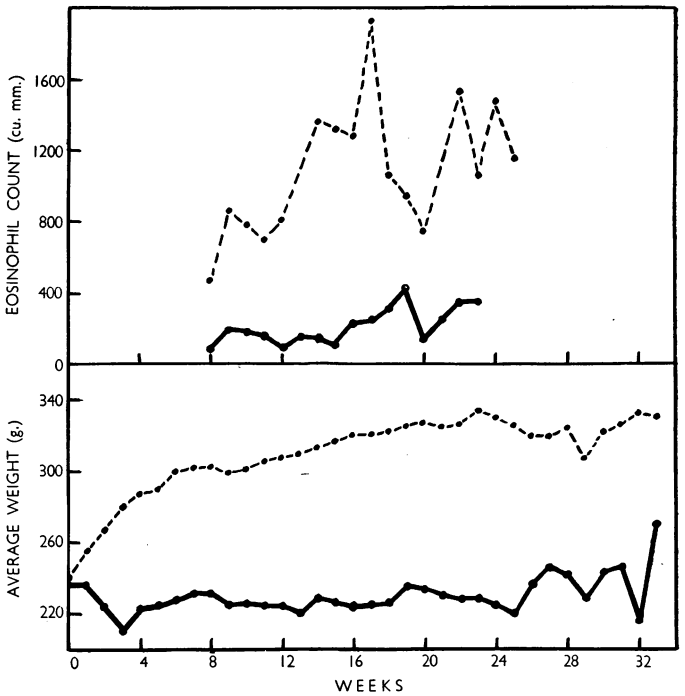

FIG. 1.—Effect of cortisone on the body weight and eosinophil count of rats. ●——● Test group (quartz plus cortisone). ● - - - ● Control group (quartz).

An additional experiment contained two groups of six normal hooded male rats. One group received cortisone in the same dose as given above and the other was used as a control. Each week one rat from each group was killed and lungs and spleens removed for histological examination. No histological abnormalities were observed but the cortisone rats lost weight and the growth of hair was affected (see below).

Pathological Technique

The removal of lungs, injection with formol saline, fixation, embedding, and sectioning were carried out as in former experiments we have reported (Belt and King, 1945). Routine sections across the middle of both lungs and including the hilar lymph glands were cut in all cases, and stained with haematoxylin and eosin, with Gordon and Sweet's (1936) silver impregnation for reticulin, and occasionally with van Giesen's or Masson's stain. Two unstained serial sections were always subjected to micro-incineration (one with HCl treatment) to reveal the presence and disposition of mineral matter in the lungs and in the lesions.

Results

The rats received cortisone for periods extending up to 230 days. After 14 days they showed obvious signs of moulting, and small bare patches appeared where the injections had been given. This was more pronounced in the females. Although the control animals had profuse hair growth over the site of operation in the neck all the test animals showed little or no growth. In all cases the scar healed well.

The effect of cortisone on the average group weight and eosinophil count is shown in the graph. During the first 17 days the weight of the test animals fell, but after the dose was reduced a fairly steady weight level was maintained (Fig. 1). The average spleen weight in both test and control groups was 260 mg. per 100 g. body weight.

Pathological Findings

The control rats developed rounded foci of pulmonary fibrosis similar in type and severity to those which we have found in earlier experiments with similar doses of quartz (Ray, King, and Harrison, 1951). In the cortisone-treated rats (test group) pulmonary fibrosis also developed, but it differed in quantity, in mode of development, and in the character of the final lesions.

* The cortisone used in this work was provided from a generous gift made jointly to the Medical Research Council and the Nuffield Foundation by Merck and Co. Inc.

Quantity of Fibrosis.—To estimate this we compared the silver-impregnated reticulin sections of both lungs of the test rats with those of the control rats killed on the same day. The amount of fibrosis was estimated as the proportion of the lung section replaced by reticulin or collagen. The results were scored as more severe in control or test if the comparison was clear-cut, and as equivocal if there was any uncertainty. In spite of the errors inherent in comparing histological sections, the results were fortunately clear cut (Table 2 and figures). The control rats had more fibrosis in 20 cases, the test rats had more in four cases, and the comparisons were equivocal in five cases.

Development of Lesions.—In the lungs of the control rats the earliest change seen was the presence of intra-alveolar dust cells containing phagocytosed quartz. These cells were diffusely scattered up to 21 days, but after this time they became concentrated in closely packed, rounded masses, around small arteries and small bronchi. This concentration was first visible at 28 days, and proceeded rapidly so that after 42 days free dust cells in the alveoli were scanty, and after 89 days none could be found. In the cortisone-treated rats this process was greatly inhibited. Recognizable perivascular and peribronchial concentration of dust cells was seen as early (28 days) as in the control group, but free intra-alveolar dust cells remained visible as late as 196 days in the cortisone rats (Figs. 6, 7). Presumably as a result of this failure to concentrate the dust, the pattern of fibrosis was altered. In the control rats reticulinosis and later collagenous fibrosis occurred only in the rounded sites of dust concentration leaving the intervening lung free. In the cortisone rats similar perivascular and peribronchial fibrosis also occurred, but where dust cells continued to lie free in alveoli for long periods additional fibrosis also occurred. This took the form of an increase of reticulin fibrils in the alveolar walls and also a net-like formation of fibrils within the alveoli. As this type of fibrosis increased, it caused fibrous replacement of irregular areas of lung tissue (Fig. 12). Often these irregular areas lay adjacent to rounded foci of perivascular and peribronchial dust cells, and the merging of the two produced fibrotic lesions of extremely irregular form.

TABLE 2

ASSESSMENT OF THE EFFECTS OF QUARTZ AND OF QUARTZ PLUS CORTISONE IN THE LUNGS OF RATS

Duration of Exposure to Quartz (days)	Rats Receiving Quartz Only			Rats Receiving Quartz + Cortisone				Comparison Equivocal
	Diffuse Dust Cells	Grade of Maturity of Fibrosis*	More Severe† Fibrosis	Diffuse Dust Cells	Grade of Maturity of Fibrosis*	Fibrosis in Alveoli	More Severe† Fibrosis	
7	+			+				No lesions present
12	+			+				
21	+			+				
28	+	1	+	+	1			
42	+	1	+	+	1			
49	±	1		+	1			+
56	±	1		+	2	+	+	
61	±	2	+	+	1	+		
68‡	+	2	+	+	1			
75	±	2		+	2	+		+
82	±	2	+	+	2			
89	+	2	+	+	2	+		
96	−	3	+	±	3	+		
103‡	−	3	+	±	3			
110‡	−	3	+	+	3	+		
119	−	4	+	+	2	+		
126	−	3		−	3	+	+	
133	−	5	+	+	4	+		
140	−	5	+	−	3	+		
147	−	3	+	+	3	+		
154‡	−	4	+	+	3	+		
161	−	4		−	4	+	+	
168	−	4		−	3	+		+
175	−	5		−	5	+		+
182	−	4	+	+	3	+		
189	−	5	+	+	3	+		
196	−	4	+	+	3	+		
202‡	−	5	+	−	4	+		
210	−	4		−	4	+		+
216	−	4		−	4	+	+	
223	−	5	+	−	4	+		
230‡	−	5	+	−	4	+		

*Grade of maturity of fibrosis : 1, loose reticulin fibrils with no collagen ; 2, compact reticulin with or without a little collagen ; 3, somewhat cellular but made up mostly of collagen ; 4, wholly composed of collagen fibres and completely acellular ; 5, acellular, collagenous, and confluent.

†Fibrous lesions " more severe " than in the corresponding " quartz + cortisone rat," or " quartz rat," killed on the same day.

‡Lungs photographed.

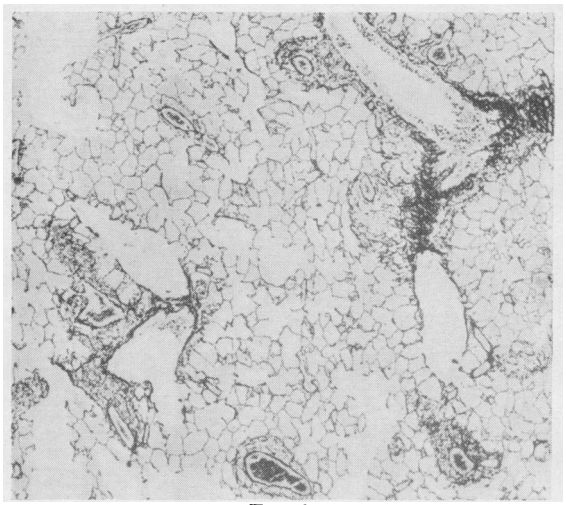

FIG. 2

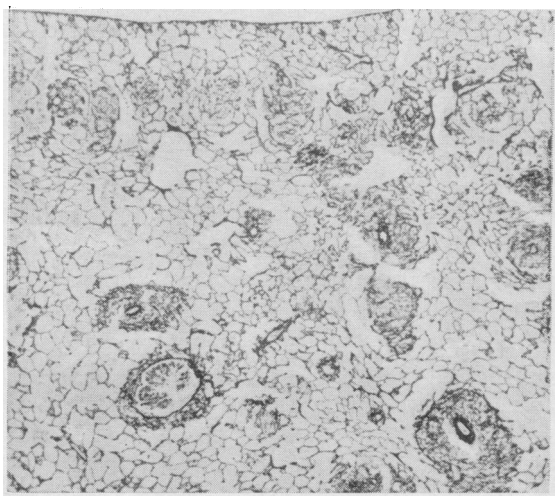

FIG. 3

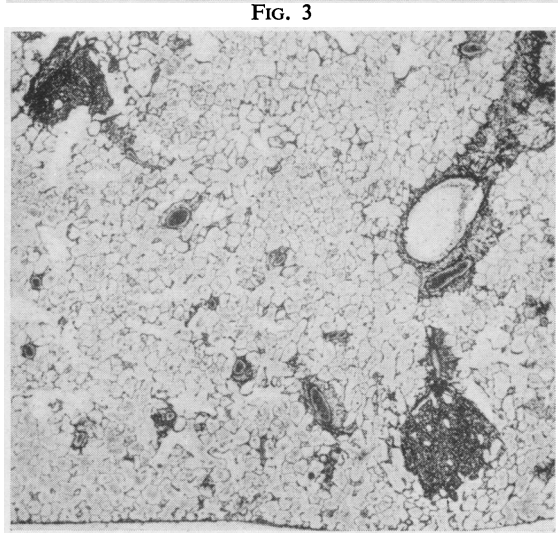

FIG. 4

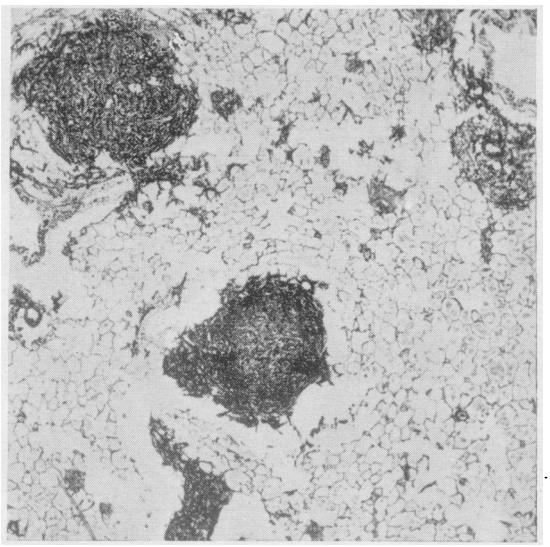

FIG. 5

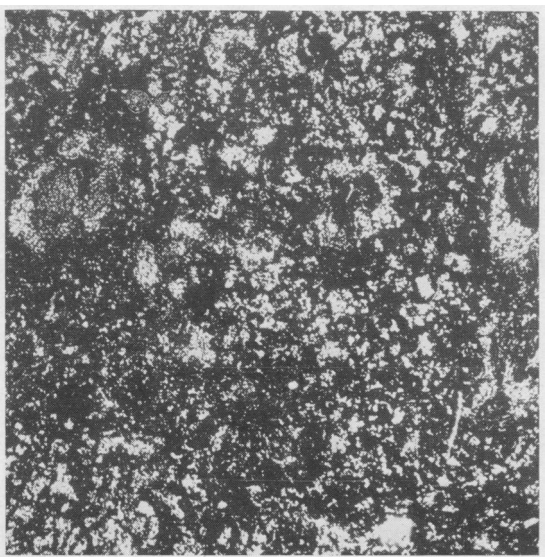

FIG. 6

FIG. 2.—Cortisone-treated quartz rat; 68 days. Slight focal reticulinosis. (Silver impregnation × 36.)

FIG. 3.—Quartz rat; 68 days. Focal reticulinosis slightly more severe than in Fig. 9. (Silver impregnation × 36.)

FIG. 4.—Cortisone-treated quartz rat; 103 days. Focal fibrosis around vessels. (Silver impregnation × 27.)

FIG. 5.—Quartz rat; 103 days. Focal fibrosis more severe than in Fig. 4. (Silver impregnation × 27.)

FIG. 6.—Cortisone-treated quartz rat; 110 days. Micro-incinerated preparation showing quartz lying in alveoli, and very little concentrated around vessels or bronchi. (Dark ground illumination × 24.)

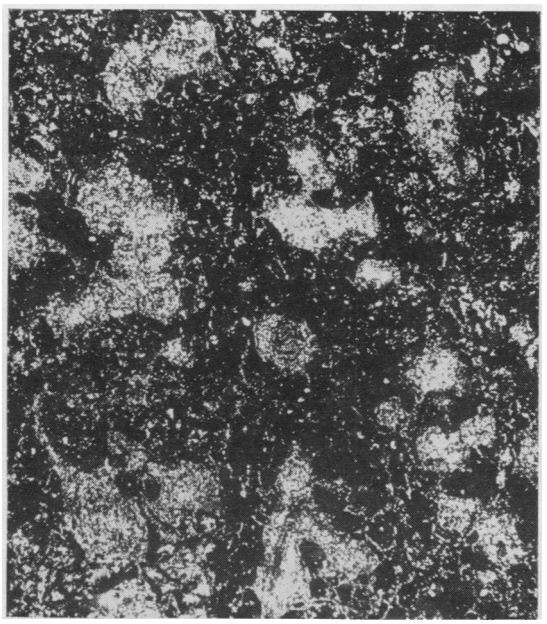

FIG. 7

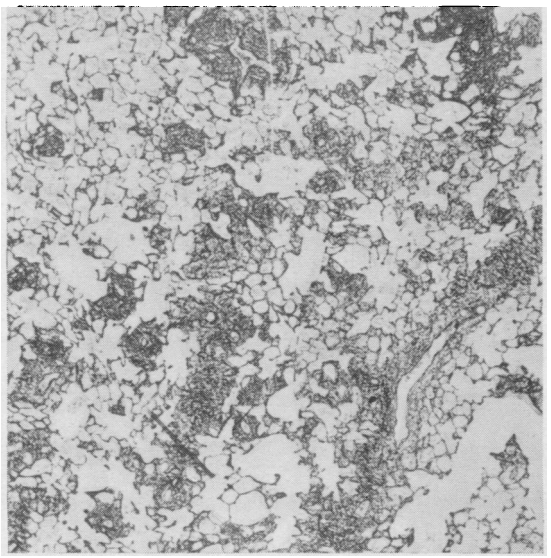

FIG. 8

FIG. 7.—Quartz rat; 110 days. Micro-incinerated preparation showing the majority of the dust concentrated around vessels or bronchi. (Dark ground illumination × 24.)

FIG. 8.—Cortisone-treated quartz rat; 154 days. Some focal fibrosis, but also much fibrosis within lung parenchyma. (Silver impregnation × 36.)

FIG. 9.—Quartz rat; 154 days. Fibrosis entirely focal, denser and more severe than in Fig. 8. (Silver impregnation × 36)

FIG. 10.—Same animal as Fig. 8. Micro-incinerated preparation showing quartz lying in alveoli. (Dark ground illumination × 27.)

FIG. 11.—Same animal as Fig. 9. Micro-incinerated preparation showing quartz almost entirely concentrated around vessels and bronchi. (Dark ground illumination × 27.)

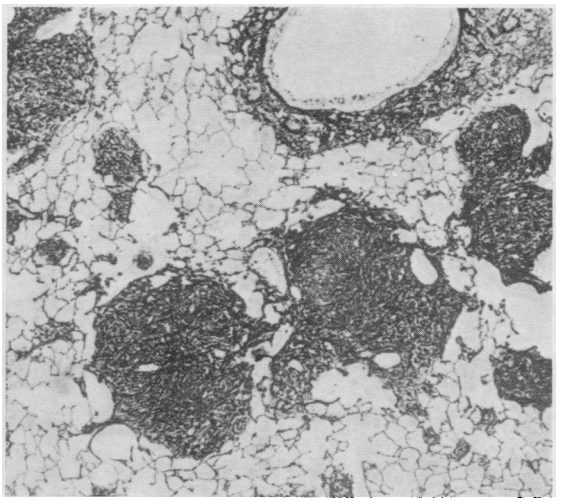

FIG. 9

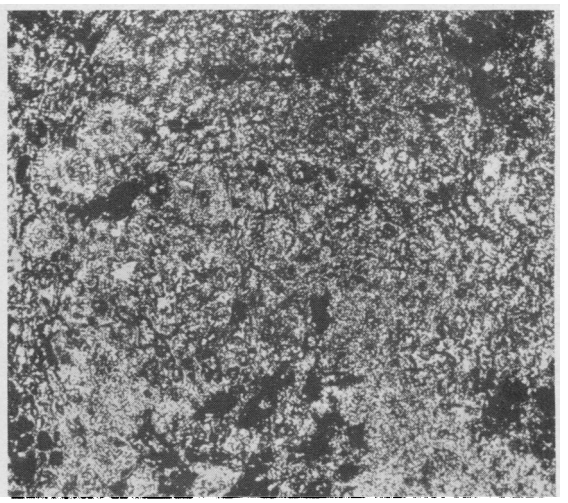

FIG. 10

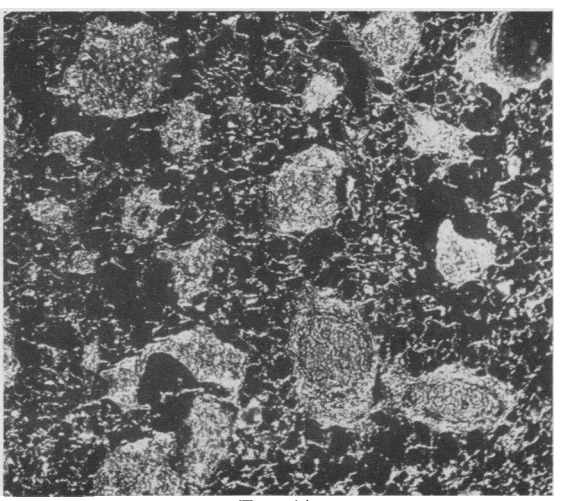

FIG. 11

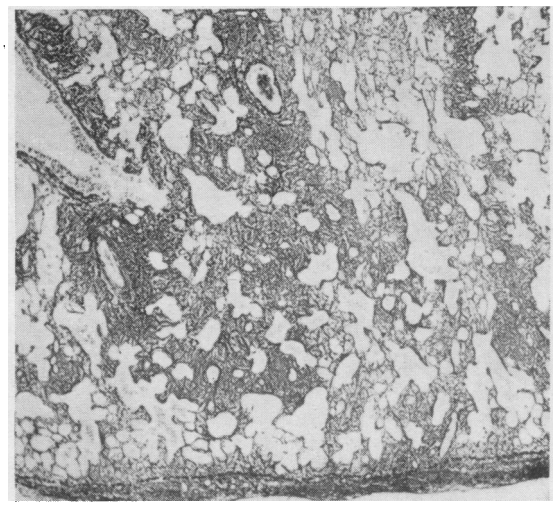

FIG. 12

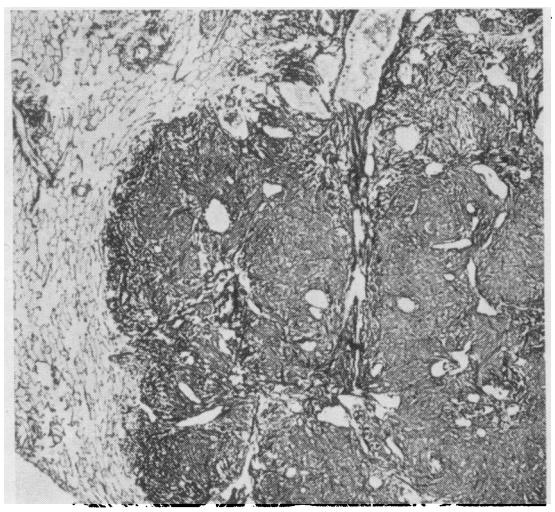

FIG. 13

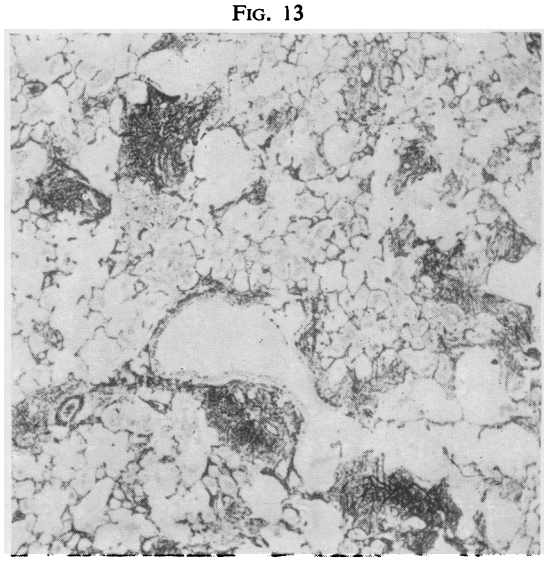

FIG. 14

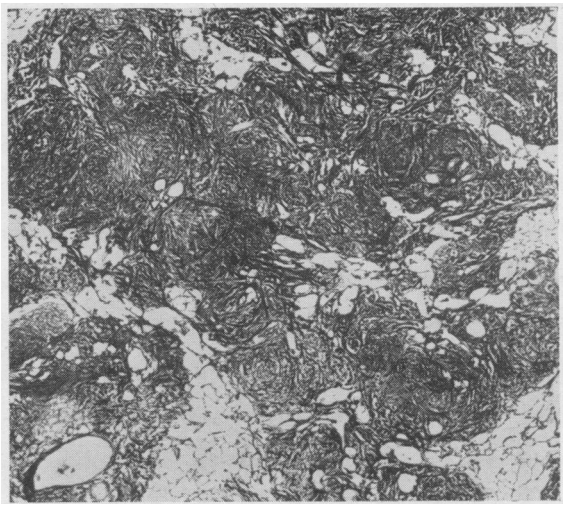

FIG. 15

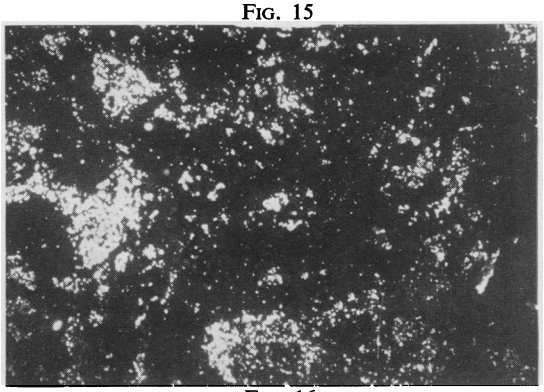

FIG. 16

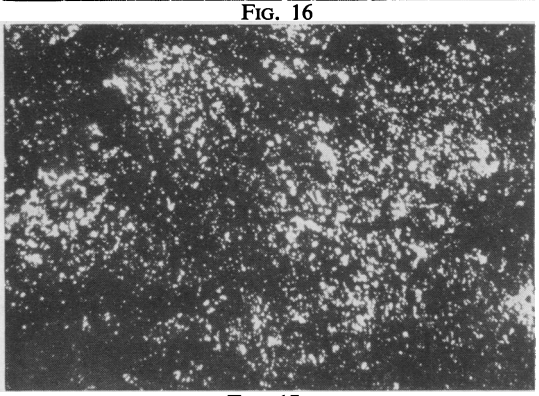

FIG. 17

FIG. 12.—Cortisone-treated quartz rat; 202 days. Fibrosis is almost entirely irregular and involves lung parenchyma. (Silver impregnation × 36.)

FIG. 13.—Quartz rat; 202 days. Severe coalescent nodular fibrosis. (Silver impregnation × 36.)

FIG. 14.—Cortisone-treated quartz rat; 230 days. Relatively slight fibrosis, partly focal and partly in parenchyma. (Silver impregnation × 30.)

FIG. 15.—Quartz rat; 230 days. Severe coalescent nodular fibrosis. (Silver impregnation × 30.)

FIG. 16.—Same part of lung as Fig. 14. Micro-incinerated preparation showing large quantity of quartz. (Dark ground illumination × 54.)

FIG. 17.—Same part of lung as Fig. 15. Micro-incinerated preparation showing roughly as much quartz as Fig. 15 in spite of the different degrees of fibrosis. (Dark ground illumination × 56.)

In earlier papers we have used a system of numbers to denote the type of fibrosis found in experimental silicosis, the figure 1 being used to indicate a loose meshwork of reticulin, 4 for dense acellular collagen, and 5 for coalescent fibrosis. In the present experiment we have found that although the quantity and the distribution of fibrosis was different in the two sets of rats, the maturity of the fibrosis, as denoted by this numbering system, was not significantly different.

Other Effects of Cortisone.—Amongst the control rats there were three spontaneous deaths due to infection. In a similar number of cortisone-treated rats there were 17 deaths due to infection. This difference is statistically significant and it is likely that it was related to the cortisone treatment. Those rats which died spontaneously were not used for comparison, but their lungs were sectioned and examined, and it was noted that as a general rule they seemed to have less pulmonary fibrosis than their fellows. In order to test this we compared the lungs of each rat that died spontaneously with those of the two cortisone-treated rats killed immediately before and immediately after it, that is, the two cortisone-treated rats exposed to quartz for approximately the same length of time. The results of these comparisons are set out in Table 3, and it will be seen that in 11 comparisons the rat that died spontaneously had less silicosis, that in three comparisons there was no difference, and in only one comparison was there more silicosis in the rat that died.

In contrast to this it is noteworthy that in the three control rats that died spontaneously two had more fibrosis than their fellows and the other one was equivocal.

TABLE 3
COMPARISON OF CORTISONE-TREATED RATS DYING SPONTANEOUSLY WITH THOSE KILLED

Duration of Exposure to Quartz (days)		Result of Comparison : More Fibrosis in Rats
Died	Killed	
34	28 and 42	Killed
47	42 and 49	Killed
62	61 and 68	Killed
64	61 and 68	Killed
74	68 and 75	Killed
90	89 and 96	Killed
93	89 and 96	Killed
109	103 and 110	Equivocal
111	110 and 119	Killed
121	119 and 126	Equivocal
127	126 and 133	Died
155	154 and 161	Killed
161	161 and 168	Equivocal
165	161 and 168	Killed
173	168 and 175	Killed

Discussion

The administration of cortisone to quartz-treated rats has modified considerably the development of silicosis both in respect of the amount of fibrosis and its distribution. What is not so clear is whether these are two separate effects or different aspects of a single action.

It is obvious that the cortisone interfered with the migration of dust cells and prevented them accumulating in closely packed aggregates. We have found in earlier work that the amount of fibrosis produced in a rat's lung depends on the amount of silica present, and that the more silica there is the more fibrosis there will be and the sooner it will develop. It seems reasonable to suggest from this that the more silica there is, concentrated in any one part of the lung, the more fibrosis will occur there. If this is true, then a given amount of silica concentrated in a few points should produce more fibrosis than the same amount of silica evenly scattered over the whole lung. On this assumption, the difference in the amount of fibrosis between the cortisone-treated and the control rats could be explained solely in terms of phagocyte mobility, and there would be no need to postulate that cortisone has any direct effect on the development of fibrosis. There is, however, some evidence that cortisone may have such an effect. In the control rats that survived for long periods, lesions that had been focal coalesced to form solid masses of collagen replacing large areas of the lung (Figs. 13, 15). In the corresponding cortisone-treated rats (Figs. 12, 14) the amount of fibrosis was substantially less, yet the amount of silica demonstrable in these areas by micro-incineration was essentially the same (Figs. 16, 17). The occurrence of differences of this sort in a significant number of cases seems to us to suggest that cortisone may directly retard fibrosis, quite apart from any effect it has on the distribution of the dust.

In this experiment there is no question of cortisone preventing the development of pulmonary fibrosis. Although the dose of cortisone was pushed to the limit of tolerance, every rat that survived more than 40 days had some pulmonary fibrosis. The amount was less, but this was only appreciable by a careful comparison of paired rats subjected to the same amount of silica for the same length of time.

Whether these results have any bearing on human silicosis is questionable. Different animals vary in their response to cortisone. Although the rat is relatively susceptible, cortisone may have a still greater effect in man, and our experiment cannot justify any conclusion regarding the possibility of cortisone preventing human silicosis. With respect to the therapeutic effect observed by Kennedy and others (1951), it should be remembered that

beryllium differs from silica in producing in man a well marked, sarcoid-like cellular granuloma in addition to fibrosis, and it is possible that cortisone might affect such a granuloma without having any effect on formed fibrous tissue. Indeed, this was their finding in a patient with silicosis in whom they were unable to observe any *x*-ray evidence of change in the lung fibrosis during or after treatment with A.C.T.H., and our experiments, too, give no indication that any useful effect might be expected from the administration of cortisone to cases of silicosis.

Summary

The administration of 5 mg. of cortisone thrice weekly to rats, which had received intracheal injection of quartz into the lungs, modified the development of experimental silicosis, both in respect of its amount and its distribution.

The principal effect of the cortisone appeared to be on the migration of dust cells, which remained much more loosely scattered throughout the lung, mainly in the alveoli, than in animals which received the same amount of quartz but no cortisone.

This interference with the accumulation of quartz particles into focal aggregates retarded the development of discrete silicotic nodules. Whether in addition there was a direct inhibition of fibrosis is uncertain, but there is some evidence that this was so. The maturation of fibrous tissue (reticulin to collagen) was not, however, much affected.

We are grateful to the Medical Research Council for a grant to defray the expenses of this investigation. Mr. B. C. S. Hollands, Mr. W. Weedon, and Miss V. Pash rendered valuable technical assistance, and Mr. E. V. Willmot prepared the photomicrographs. Dr. G. Nagelschmidt kindly gave us the powdered quartz, and the cortisone and A.C.T.H. Sub-Committee the cortisone. To its Chairman, Professor R. V. Christie, we are grateful for advice; and to Dr. R. J. W. Rees and Dr. P. D'Arcy Hart, of the National Institute for Medical Research, for much help with the dosage of cortisone and management of the animals.

References

Belt, T. H., and King, E. J. (1945). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 250, p. 29.
Cavallero, C., Sala, G., Amira, A., and Borasi, M. (1951). *Lancet*, **1**, 55.
Curran, R. C. (1952). *Brit. J. exp. Path.*, **33**, 82.
Freeman, S., Fershing, J., Wang, C. C., and Smith, L. C. (1950). *Proc. 1st Clin. A.C.T.H. Conf.*, Chicago, 1949, ed. Mote, J. R. p. 509. London.
Gordon, H., and Sweets, H. H. (1936). *Amer. J. Path.*, **12**, 545.
Kennedy, B. J., Pare, J. A. P., Pump, K. K., Beck, J. C., Johnson, L. G., Epstein, N. B., Venning, E. H., and Browne, J. S. L. (1951). *Amer. J. Med.*, **10**, 134.
Magarey, F. R., and Gough, J. (1952). *Brit. J. exp. Path.*, **33**, 76.
May, K. R. (1945). *J. sci. Instrum.*, **22**, 187.
Policard, A., and Tuchmann-Duplessis, H. (1951). *C.R. Acad. Sci., Paris*, **232**, 1888.
Ray, S. C., King, E. J., and Harrison, C. V. (1951). *British Journal of Industrial Medicine*, **8**, 62.
Spain, D. M., Molomut, N., and Haber, A. (1950). *Science*, **112**, 335.
Schiller, E. (1951). *Experientia*, **7**, 464.
——(1952). *Verh. anat. Ges.*, **49**. In the press.