THE EFFECT OF CORTISONE ON ESTABLISHED SILICOTIC FIBROSIS IN THE LUNGS OF RATS

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In man, cortisone has not been shown to have any appreciable effect on long-standing fibrosis. Kennedy, Pare, Pump, Beck, Johnson, Epstein, Venning, and Browne (1951) treated a case of pulmonary silicosis with some clinical improvement in the patient's condition, although this was not supported by the x-ray findings; and Kennedy (1954) has recently reported rather similar findings (subjective but no objective improvement) in pneumoconiotic potters and coal-miners.

Harrison, King, Dale, and Sichel (1952) investigated the action of cortisone on developing fibrous tissue in the lungs of rats produced by intratracheal injection of quartz particles. Cortisone appeared to interfere with the migration of dust-laden phagocytes in the lung, where they remained diffusely distributed within alveoli instead of aggregating to form discrete nodules. It also modified the development of pulmonary silicotic nodules in amount and distribution, but did not significantly alter the maturation of fibrous tissue from reticulin to collagen. Marengi and Rota (1954), however, concluded from their experiments with cortisone treatment of quartz-dusted rats that cortisone had very little influence on the initial cellular reaction, but that it delayed or prevented the transformation of reticulin into collagen in the later stages of the fibrotic process.

Curran (1952) showed that cortisone had no effect on existing silicotic lesions in the peritoneum of mice. Magarey and Gough (1952), working with rabbits, were unable to find any reduction of mature fibrous tissue in intraperitoneal silicotic nodules as a result of cortisone treatment, although histological evidence suggested that the administration of cortisone retarded the further proliferation of fibroblasts with consequent retardation of concentric fibrosis in the nodules, preformed collagen remaining unaltered.

It is concluded that cortisone has a modifying effect on developing silicotic fibrosis in the peri-

toneum and lungs of experimental animals, while its action on established silicotic fibrosis is less definite. It seemed desirable, therefore, to study the effect of cortisone on established silicotic fibrosis in the lungs of rats, with particular reference to the histological picture as well as to the collagen and silica content of the lungs.

Plan of Experiment

Thirty-six rats (M.R.C. black-and-white strain, ca. 250 g.) were given intratracheal injections of quartz dust (50 mg.) in order to produce silicotic fibrosis in their lungs. A sufficient period of time (100 days) was allowed for the formation of fully developed collagenous nodules, after which cortisone was administered to some of the animals. Three were lost by cannibalism, and the remaining 33 were divided into two groups; the first group of 11 animals served as controls and received no further treatment, while the 22 animals in the second group were given subcutaneous injections of cortisone acetate suspensions (5 mg. three times a week) over a period of 265 days. Animals from both groups were killed at regular intervals and histological examinations of the lungs carried out. Stacy and King (1954) have reported the collagen and silica contents of the lungs and lymph nodes estimated chemically.

Description of Samples

The dust used was powdered quartz "Snowit II". The sample was made by mixing two previously

TABLE 1
PARTICLE SIZE DISTRIBUTION AND ANALYSIS OF QUARTZ DUST USED FOR INTRATRACHEAL INJECTION

Size in μ	Percentage by No.	Percentage by Mass
0.32-0.45	3.0	—
0.46-0.64	18.9	1.5
0.65-0.9	38.2	7.9
1.0-1.3	16.8	9.6
1.4-1.8	12.3	20.2
1.9-2.6	9.5	43.7
2.7-3.6	1.3	17.1
Specific surface ($m^2/g.$)		1.8
SiO ₂ (%)		98.7
Ignition loss (%)		0.17

prepared fractions from Belgian glass sand. Two-thirds by weight of a fraction of nominal diameter 1 to 2 μ . was mixed with one-third of a fraction 0.5 to 1 μ . in diameter. The resulting size distribution and the data obtained from chemical analyses are shown in Table 1.

The cortisone acetate suspension used in this work was a preparation from Merck & Co. Inc.

Preparation of the Dust Suspension

Quartz dust, 2.5 g., and sterile physiological saline 50 ml. were thoroughly shaken, autoclaved for 20 min. at 15 lb., and kept shaking in a microid flask-shaker till the time of injection. The final concentration of dust was 50 mg./ml. and this amount was injected into each animal.

Experimental Procedure

The animals were lightly anaesthetized with ether and the dust suspensions injected intratracheally via the mouth, according to the technique of Kettle and Hilton (1932), modified by King, Mohanty, Harrison, and Nagelschmidt (1953). There was no regurgitation of dust and no immediate post-operative deaths occurred.

Duration of Experiments

Cortisone injections were begun 100 days after the injection of quartz in the group of test animals. They were continued for 265 days (5 mg. three times a week). The animals were weighed at weekly intervals (Fig. 1).

A pair of animals, one from each group, were killed at fortnightly intervals in the early stages of the experiment. Owing to a number of deaths occurring in the group of cortisone-treated animals the intervals at which the killings were done had to be adjusted in order to extend the experiment over a sufficient period of time and to obtain equal days of survival in both groups. The experiment was concluded 365 days after the injection of quartz.

Histopathological Technique

Routine necropsies were carried out on the animals that were killed and on those found dead. Their lungs were removed and fixed in 10% formol-saline. The tracheo-bronchial lymph nodes were dissected out, weighed, and fixed separately. Blocks were taken along the long axes of both lungs through the hila, dehydrated, and embedded in paraffin wax. Serial sections, 5 μ in thickness, were stained by Gordon and Sweets' (1936) silver impregnation method for reticulin, and with

FIG. 1.—The effect of cortisone on the body weight of rats.

Ehrlich's haematoxylin and eosin. An unstained serial section was subjected to micro-incineration and then washed in concentrated HCl and dry-mounted.

Pathological Findings

Twenty-one days after the start of the cortisone injections the animals showed signs of moulting and bare patches appeared at the site of the injections. These patches became larger as the experiment progressed. During the first 42 days the animals lost weight steadily. The protein content in their diet was then increased and a slight improvement was noticed. Towards the end of the experiment they lost weight considerably, particularly during the last 56 days.

Amongst the 11 control animals there was one spontaneous death due to infection, while nine animals from the cortisone-treated group also died from infection. This is statistically significant and may be regarded as a result of cortisone treatment. The animals that died spontaneously, and whose lungs were found to be infected, were not used for histological comparison. Killings of animals from the two groups were adjusted as far as possible to obtain equal days of survival in both. The duration of the experiment, the days of survival of the animals, their mode of death, and the pathological grading of the lesions produced in their lungs, as assessed according to Belt and King (1945), have been summarized in Table 2.

TABLE 2

ASSESSMENT OF FIBROSIS IN LUNGS OF CORTISONE-TREATED AND CONTROL RATS PREVIOUSLY INJECTED WITH QUARTZ DUST

Days of Survival	Control		Cortisone-treated	
	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis
122	D	—	—	—
123	K	4	K	4
124-150	K	5	K D (4)	5
			D	5
151-200	D	5	D (4)	—
			D	5
201-230	K	5	K *	5
231-300	K *	5	K *	5
			D	5
301-330	K	5	D	—
331-365	K (4)	5	K (2) K (4)	5

* Sections from these animals are reproduced in Figs. 2 and 3. K = Killed; D = Dead; Numbers in parentheses indicate the number of animals killed or found dead at the period.

Macroscopic Appearances of the Lungs.—On macroscopic examination there was very little difference between the lungs of the control animals and those which received cortisone.

Control Quartz Dust.—The lesions in the lungs were similar to those found previously in cases of

quartz-induced fibrosis. At 123 days both lungs showed firm, white patches of fibrosis, which appeared to be running together in certain areas. By 150 days there were large areas of confluent fibrosis in the right lung, while in the left lung the fibrosis was mainly patchy. At 230 days the greater part of both lungs was replaced by confluent, white fibrous areas. From this period until the end of the experiment at 365 days, the naked-eye appearances of the lung were similar to those at 230 days, except that the amount of fibrosis in the lungs steadily increased. The lymph nodes were enlarged and firm from the first (123 days) and gradually enlarged further to several times normal as the experiment progressed.

Cortisone-treated Animals.—At 123 days there were firm, white areas of fibrosis in both lungs. They were mainly patchy, but showed a tendency to become confluent dorsally in some areas. The confluent areas had increased in size by 150 days and at 230 days most of the right lung showed areas of confluent fibrosis, while the left lung had a few discrete patches of fibrosis. After this the fibrosis seemed to increase as judged by the confluent areas seen macroscopically, and by 365 days most of the lung tissue was replaced by fibrous areas. The tracheo-bronchial lymph nodes were slightly enlarged and firm; but, although they were larger than the lymph nodes of normal rats, were not as large as those found in the group of animals which had been injected with quartz dust without subsequent cortisone treatment.

Microscopic Appearances.—The pulmonary lesions in the animals which had quartz dust only and those which had subsequent cortisone treatment revealed no significant differences so far as the maturity of the fibrosis was concerned. But when paired lung sections from the two groups (animals killed at the same time) were examined with a hand lens there was a definite difference in the areas of lung tissue involved by the fibrous process. In the cortisone-treated animals, the area of lung involvement appeared to be less than in the controls.

Control Quartz.—The earliest sections examined were at 123 days, when the lungs showed numerous nodular foci typical of quartz fibrosis. The lesions were, for the most part, acellular at the centre, with some cellularity at the periphery. On reticulin staining they were seen to be composed entirely of compact collagen fibres (Grade 4 fibrosis). A few nodules showed a tendency to become confluent, but the majority were discrete. The fibrous process involved approximately one-third of the left lung field and one-half of the right one. By 150 days the lesions were confluent, acellular and collagenous

FIG. 2.—Control quartz rat lung 300 days after an injection of 50 mg. of quartz dust. Confluent silicotic nodule composed of compact collagen (Grade 5 fibrosis). Silver impregnation. $\times 36$.

(Grade 5 fibrosis). About one-half of the areas of both lung fields was involved. At 230 days the histological picture was essentially the same as at 150 days, with about two-thirds of the left lung field and three-quarters of the right one replaced by confluent fibrotic lesions. From this period onwards the area of lung tissue involved increased, and at 300 days about three-quarters of both lung fields were replaced by confluent, acellular, fully collagenous lesions (Grade 5 fibrosis, Fig. 2). A few patent alveoli and some new-formed blood vessels were seen within the confluent areas.

Cortisone-treated Group.—The histological appearances of the lungs at 123 days were similar to those in the control animals. There were numerous nodular lesions throughout the lungs, involving about one-half the area of the right lung field and a little over one-third of the left lung. The lesions were discrete, mainly acellular, with cellularity only at the periphery, and on reticulin staining were completely collagenous (Grade 4 fibrosis). At 150 days many of the lesions were confluent, acellular,

and fully collagenous (Grade 5), but the area of lung involved was about the same as at 123 days. The lesions at 230 days were no different from those at 150 days. By 300 days two-thirds of the area of the right lung field and one-third of the left were replaced by confluent, acellular, collagenous nodules (Grade 5 fibrosis, Fig. 3). An occasional discrete nodule was seen in some areas. Within the confluent fibrous lesions a few patent alveoli were seen. At 365 days the lesions were similar but the areas of lung involved were approximately one-half to two-thirds of one lung field and two-thirds to three-quarters of the other.

Discussion

In our experiments cortisone was administered to rats 100 days after intratracheal injection of quartz, when silicotic nodules were well developed. The histological lesions studied 123 days after the introduction of the dust—when one group of animals had had cortisone for 23 days—were very

FIG. 3.—Cortisone-treated quartz rat lung 300 days after the injection of 50 mg. of quartz followed by subcutaneous injections of cortisone (begun at 100 days and continued thrice weekly). Confluent collagenous lesions (Grade 5 fibrosis). Silver impregnation. $\times 36$.

similar in both the control and cortisone-treated animals, there being fully collagenous nodules in both. At the end of the experiment, 265 days after the start of cortisone treatment, there was still no difference in the grade of fibrosis between the pulmonary lesions of the two groups, both being fully collagenous. Morphologically, the lesions in the control and cortisone-treated animals were identical; there were the same central collections of dust surrounded by dense collagen fibres. At the periphery of some lesions a few cells were seen, but this again was a common finding in the two groups.

Although there was no difference in the maturity of the fibrous tissue in the silicotic nodules of control rats and those treated with cortisone, there seemed to be a slight variation in the actual amount of lung tissue involved when paired sections from the two groups were compared. The cortisone-treated animals showed somewhat less involvement of lung tissue by the fibrous process, as compared with untreated animals, particularly in the later stages of the experiment. This was borne out by chemical estimations of collagen, which was found in smaller amounts in the lungs of cortisone-treated animals (Stacy and King, 1954).

These findings suggest that although cortisone has no effect on the quality of fibrous tissue it may affect the quantity formed. Presumably, the silicotic nodules which were present in the lungs at the time the cortisone treatment was begun were generally prevented from enlarging without any actual resolution of the fibrous tissue taking place.

This aspect of our present finding is in keeping with the results of Marengi and Rota (1954) who also found a great difference in the total degree of pulmonary fibrosis between their cortisone-treated and control animals, though in their experiment the difference was more striking than in ours.

From these results it is evident* that cortisone has no beneficial effects on pulmonary silicosis in animals when administered after the formation of mature silicotic nodules. Harrison and others (1952) were unable to prevent the development of quartz-induced pulmonary fibrosis in rats by the administration of cortisone, even though the treatment was begun almost with the introduction of the fibrogenic agent and pushed to the limit of tolerance.

Although it is not justifiable to apply these findings to man, nevertheless, it seems unlikely that

cortisone would have any beneficial effect on human silicosis.

Summary

The effect of cortisone on established silicotic fibrosis in the lungs of rats was studied by the intratracheal injection of 50 mg. of quartz dust in rats, followed 100 days later by the administration of 3 mg. cortisone acetate three times a week for 265 days.

Cortisone did not alter the pattern of the fibrosis or bring about any regression of existing silicotic nodules. Mature collagenous lesions were present in the control and cortisone-treated animals at all stages of the experiment. The actual amount of lung tissue involved by the fibrotic process was slightly less in the cortisone-treated animals when paired histological preparations from the two groups were compared.

It is concluded that cortisone does not have any appreciable effect on established silicosis as far as the grade of fibrosis is concerned, although a slight reduction in the actual amount of fibrous tissue may be found.

Cortisone retards the phagocytic transport of dust from the lungs to the lymph nodes. There was more silica remaining in the lungs of the cortisone-treated animals than in the controls, while their lymph nodes contained less than the control nodes (Stacy and King, 1954).

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