THE EFFECT ON EXPERIMENTAL SILICOSIS OF HYPERSENSITIVITY INDUCED BY HORSE SERUM

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THE latest theory of silicosis is that it is a form of antigen antibody reaction. A new concept has become necessary because the solubility theory, which has been the most generally accepted during the past decade, is no longer tenable. It had been thought that silica compounds have a toxic action proportional to their degree of solubility, but so many exceptions have been found that this theory has now to be discarded in its simple form. There is evidence that silica can combine with body protein and act as an antigen (Antweiler and Hirsch, 1957). Moreover, silicotic nodules both in man and in experimental animals are not composed entirely of collagen as was formerly believed, but are mixtures of collagen and globulins, including γ-globulins (Ceppellini and Pernis, 1958). The reaction to silica in animals can be greatly modified by the administration of cortisone which is known to affect inflammatory reactions, including hypersensitivity ones. The object of the present experiments was to see whether the silicotic reaction could be modified by rendering animals hypersensitive, that is to say whether they would show the reverse effects from those due to the action of cortisone. Rabbits were given intratracheal injections of silica and then sensitized to horse serum. The resulting lesions were different from those of control animals and showed that there was a more vigorous response to the silica in the hypersensitive animals.

MATERIALS AND METHODS

Powdered quartz (" Snowit II " prepared from Belgian sand) of particle size 0·5-1μ was prepared by Dr. G. Nagelschmidt. This was suspended in normal saline in a concentration of 60 mg./ml. and was autoclaved before use.

Horse serum—Burroughs Wellcome No. 2 (No. E3225A).

Female rabbits of mixed breeds were used. The controls were matched as nearly as possible for breed and weight.

In the test series 8 rabbits were used, and 6 in the control.

Each test animal was given a single intratracheal injection of the silica suspension in a dosage of 1 ml./0·5 kg. body weight, under ether anaesthesia. Apart from one animal in which inadvertently the bulk of the injection was made into the paratracheal tissues, all tolerated this dosage with slight disturbance. Horse serum was given intravenously in a dosage of 10 ml./kg. body weight 7 days later. A state of hypersensitivity was maintained by further intravenous injections in the surviving animals of 1 ml. on the 22nd day ; the original dose on the 23rd ; 2 ml. on the 47th, 2 ml. on the 54th and half the original dose on the 61st and 96th days. During this regime 2 animals died from anaphylaxis. Precipitation titres were not followed throughout the period of the experiment, but all these rabbit sera gave positive precipitin reactions with horse serum.

The 6 control animals received the same dosage of intratracheal silica. All tolerated it well.

The test animals were killed at 27, 37, 57, 75, 96 and 110 days after the silica injection. The other 2 died at 30 and 61 days.

The control animals were killed at 28, 30, 37, 57, 75 and 96 days. All animals were killed by intravenous " Nembutal ".

Post-mortem examination was performed immediately after death. The lungs were inflated with formol-saline before cutting. Paraffin blocks were made and serial sections cut. The lung sections were stained by haematoxylin and eosin, Gomori's Reticulin stain and acid picro-Mallory using Masson's light green solution in place of Soluble Blue for the demonstration of connective tissue. Paraffin sections were also incinerated at 500° for 16 hr. and examined under direct and dark ground illumination.

Haematoxylin and eosin stained sections were prepared in each animal from heart, liver, kidneys, adrenals and spleen.

RESULTS

Both test and control animals showed well developed silicotic lesions in their lungs. The features particularly studied were the size and shape of the silicotic foci, the degree of mature collagen formation, reticulin fibre formation, and the distribution of the quartz particles.

The silicotic lesions in the hypersensitive animal were larger, more clearly demarcated and more localized than in the controls. Fig. 1 and 2 illustrate this at 27 days. The difference was most clearly seen in the animals killed at 27 and 30 days after the silica injection. Thereafter the foci in the controls also began to become more discrete, with some clearing of dust macrophages from the intervening lung parenchyma. However, even at 96 days there was still a difference in the degree of definition and aggregation of the foci (Fig. 3 and 4). The edges of the foci in the test animal were often formed by a collar of lymphocyte-type cells.

Acid picro-Mallory's stain showed that concentric layers of adult collagen were formed sooner and in greater quantity in the test animal (Fig. 5 and 6). Collagen developed more obviously in the controls after this time, but although considerable in amount at 96 days it still did not show the same regularity of concentric layering as in the hypersensitive animal.

Reticulin stains did not show any consistent quantitative difference, but again served to show the greater, more prominent, concentric arrangement in lesions in the test animals (Fig. 7 and 8).

These observed differences are subjective and therefore open to observer error. However, the difference seen in the distribution of dust after micro-incineration is particularly striking and supports the impression gained by histological study. Fig. 9 and 10 show the increased aggregation of dust in the hypersensitive animal at 27 days, with the control at 28 days. This is still clearly present at 75 days (Fig. 11 and 12).

No consistent differences were observed in the nature of the cellular reaction, or in the degree of secondary infection, which was slight and did not interfere in the reaction to quartz.

The heart, liver and kidneys in the test animals gave histological evidence of their state of hypersensitivity.

DISCUSSION

The effect of steroids on the host's response to the administration of silica has been the subject of considerable investigation. Magarey and Gough (1952*a*) demonstrated that cortisone inhibited the development of fibrosis in response to intraperitoneal silica in the mouse. The same authors (1952*b*) claimed that cortisone retarded the further development of concentric fibrosis around already established intraperitoneal silica granulomata in the rabbit. Harrison, King,

Dale and Sichel (1952), experimenting on rats given intratracheal silica injections, found that under the influence of cortisone dust cells remained more loosely scattered throughout the lung. This resulted in a retardation in the development of discrete silicotic nodules. They found little to suggest an inhibition of fibrosis. Marenghi and Rota (1954) exposed rats to clouds of finely dispersed quartz over prolonged periods. The effect of cortisone under these circumstances was to prevent or delay the transformation of reticulin into collagenous fibres, so that the nodules remained smaller in the cortisone group. King, Harrison and Attygale (1955) experimented on the established silicotic lesion in the lungs of rats. Cortisone given under these circumstances had little or no observable histological effect, but chemical tests proved to be more sensitive. These showed that the quantity of collagen found in the cortisone treated animals was less than in the control (Stacy and King, 1954). From these reports it is seen that the principal effects of cortisone on experimental silicosis is a diminution of collagen formation ; a relative failure to aggregate the dust particles ; and the formation of smaller and less well defined nodules. These features are precisely the opposite of those observed in the present experiment. This is in keeping with the known inhibitory effect of cortisone on the hypersensitivity state.

It is debatable whether the intravenous injection of foreign proteins *per se* gives rise to lung changes. A form of local Arthus reaction has been obtained by Opie (1924), Cannon, Walsh and Marshall (1941) and Fried (1933). Rich and Gregory (1943), Hawn and Janeway (1947), Ehrich, Seifter and Forman (1949) describe lung changes in the rabbit after intravenous injection of foreign proteins. However, Germuth (1953), Wissler, Smull and Lesh (1949) could not demonstrate lung changes after single or repeated intravenous injections of foreign protein. It may be concluded that if there are lung changes in the state of experimental serum sickness they are acute in nature and transient. No lung lesions were found in the present experiments solely attributable to the intravenous horse serum.

The mechanism of the modified response to silica found in the hypersensitive animal is uncertain but is probably due to increased activity of the macrophages. Dust is normally taken up by macrophages which migrate to the region of the

EXPLANATION OF PLATES

FIG. 1.—Lung in control animal killed 28 days after silica injection. H. and E. × 45.

FIG. 2.—Lung in hypersensitive animal killed 27 days after silica injection showing the larger, more clearly demarcated lesions. H. and E. × 45.

FIG. 3.—Control animal at 96 days. H. and E. × 88.

FIG. 4.—Test animal showing persistence of the better definition of lesions at 96 days. The collar of lymphocyte-type cells can also be seen. H. and E. × 88.

FIG. 5.—Poorly developed adult collagen tissue in control at 28 days. Acid picro-Mallory. × 88.

FIG. 6.—Well developed adult collagen tissue in hypersensitive animal at 27 days. Acid picro-Mallory. × 88.

FIG. 7.—Reticulin stain in control at 96 days. Gomori's Reticulin. × 88.

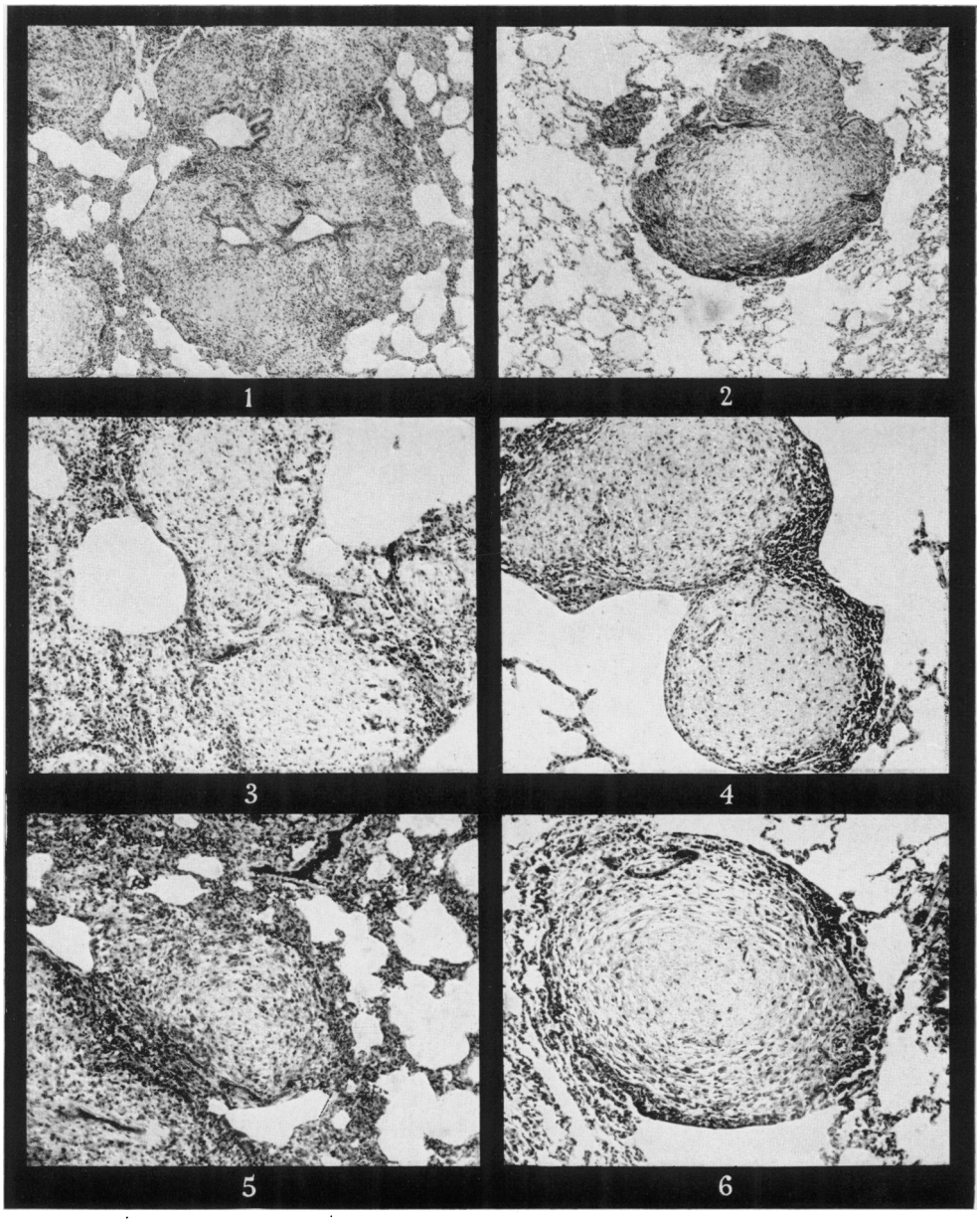
FIG. 8.—More prominent concentric arrangement of reticulin in the hypersensitive animal at 96 days. Gomori's Reticulin. × 88.

FIG. 9.—Distribution of dust in control at 28 days—after incineration. × 88.

FIG. 10.—Distribution of dust in hypersensitive at 27 days—after incineration. The dust is more compact. × 88.

FIG. 11.—Distribution of dust in control at 75 days—after incineration. × 45.

FIG. 12.—Distribution of dust in hypersensitive after incineration. The dust still shows better aggregation at 75 days. × 45.



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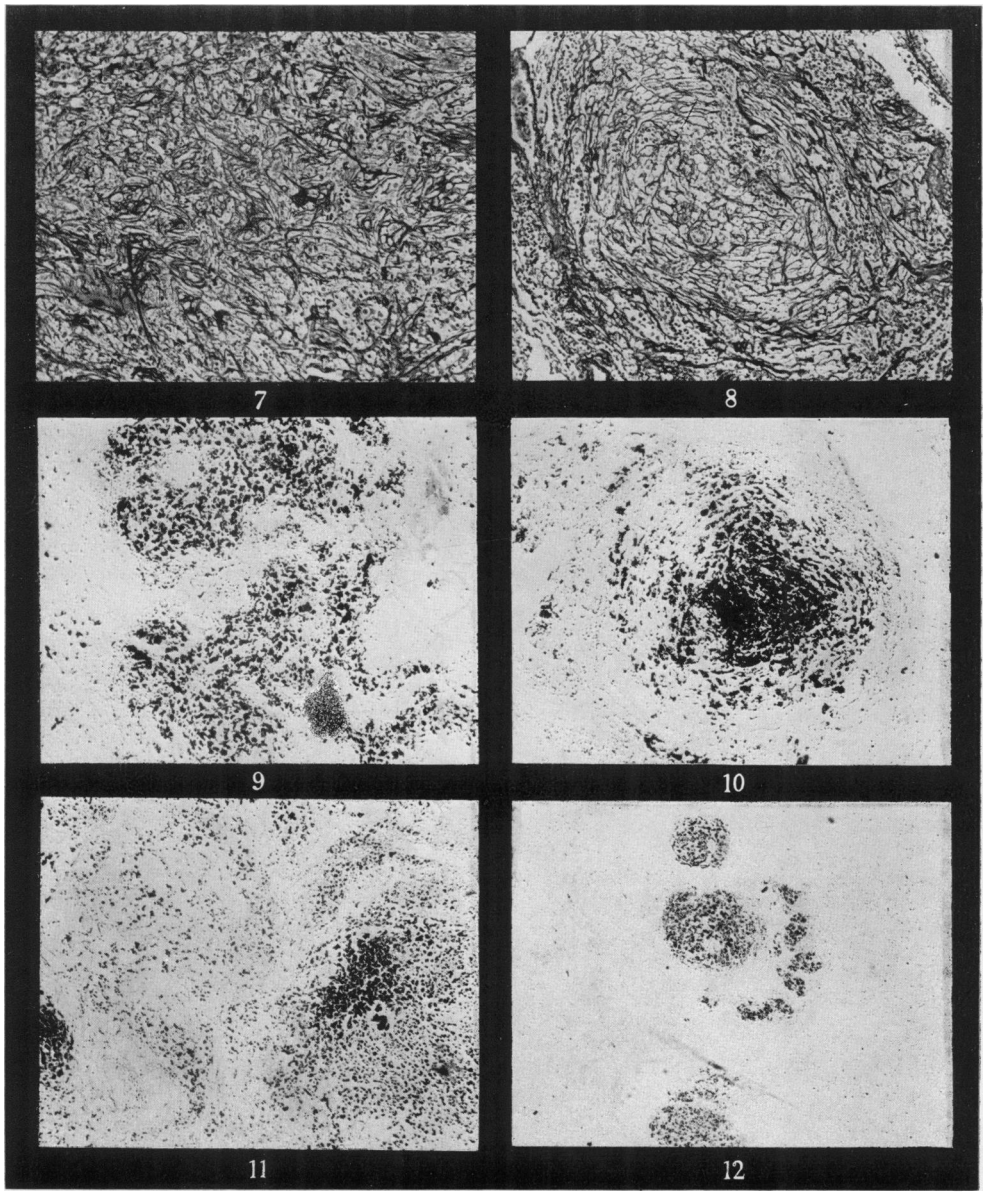
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respiratory bronchioles and accumulate there. This process of migration is retarded by cortisone but seems speeded up by hypersensitivity—this can be judged on the rate of clearing of the lung in between the foci of fibrosis. The extent and nature of the modification must necessarily be variable—as variable as is the response of the individual experimental animals to the intravenous injection of foreign proteins. The timing of silica and serum injections are also probably important variables. The influence on the silicotic lesion of steroids in the one direction and non-related antigen-antibody reactions in the other, may have a bearing on the explanation of the modification of clinical pneumoconiosis found, for example in association with rheumatoid arthritis (Caplan, 1953). They also afford a possible explanation of the variable reaction to silica in different individuals working under the same conditions.

SUMMARY

Experiments on rabbits showed that hypersensitivity produced by horse serum modified the reaction to quartz injected into the lungs.

The lesions were larger and more clearly demarcated. More collagen was found and the concentric arrangement was much more distinct.

Incinerated sections showed that the dust was accumulated into more compact foci.

These effects are the reverse of those due to cortisone.

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