AN EXPERIMENTAL STUDY OF THE PATHOGENESIS OF SILICOTIC GRANULOMA IN THE ANTERIOR EYE CHAMBER IN RABBITS AND CHANGES IN ITS DEVELOPMENT INDUCED BY THE ACTION OF A.C.T.H., INSULIN, COMBINATIONS OF BOTH, AND SALICYLATES

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The contact of quartz with lung tissue and the ensuing inflammatory reaction is the first and most important phase in the development of silicosis. The organism can to some measure influence this reaction by its own neurohumoral regulation, as not everybody falls prey to the disease, and if he does, not to the same degree even under similar exposure. On the other hand, a chronic course of silicosis changes the neurohumoral position of the whole organism (Vyskočil, 1954).

In recent years clinical therapy has been enriched by powerful hormones, especially A.C.T.H. and cortisone, which can inhibit the formation of fibrotic tissue in inflammatory processes. Silicotics clearly do not suffer from any pathological changes of the endocrine glands, even when exhaustion by chronic illness is evident (Vyskočil, 1954), but it has been thought interesting to try and effect a decrease in the fibrosis of the silicotic lung by the administration of therapeutic, not physiological, doses of A.C.T.H. and cortisone. Many authors have used these substances in animal experiments and in man. The results show that a formed silicotic nodule can hardly be influenced by either cortisone or A.C.T.H. (Schiller, 1953; Curran, 1952; Ducommun and Ducommun, 1951; Margarey and Gough, 1952; King, Harrison, and Attygalle, 1955). A more pronounced effect was observed when cortisone or A.C.T.H. was being given from the beginning of the administration of quartz. Cortisone or A.C.T.H. retards the transport of dust to the phagocytes from lung tissue into hilar nodes (Stacy and King, 1954); they enhance quartz dispersion in the whole organism (Policard and Tuchmann-Duplessis, 1951, 1952) which is unfavourable, but on the other hand they retard the growth of the silicotic nodule and the change of reticulin fibres into collagen (Stacy and King, 1954; Ducommun and Ducommun, 1951; Harrison, King, Dale, and Sichel, 1952; Margarey and Gough, 1952; Marenghi and Rota, 1953). The doses were usually large (Michalová, 1954). All these experiments are of more value in understanding pathogenesis than in the clinical therapy of silicosis. The administration of A.C.T.H. and cortisone would have to be prolonged and inhibition of various endocrine organs would be encountered.

We have tried the prolonged administration of very small doses of A.C.T.H. alone or in combination with insulin, which in some respects is an antagonist and in others a synergist of A.C.T.H. Insulin can provoke endogenous A.C.T.H. formation by hypoglycaemia (Bartelheimer and Cabeza, 1942; Knick, 1954) which could be useful as an aid against the possible hypophyseal atrophy in prolonged exogenous A.C.T.H. administration.

Schiller (1953) proved by experiment that depot insulin injected in doses of 0.004 i.u. into white mice can retard fibrotic tissue formation after intraperitoneal quartz administration (10 mg.) in the first 30 days.

It remained to find out how to administer insulin, whether together with A.C.T.H. or on alternate days. To solve this problem we divided the animals into several groups: one group was injected with A.C.T.H. alone, another with A.C.T.H. and insulin, a third one with A.C.T.H. and insulin on alternate days, and a fourth with insulin alone.
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One group of animals received salicylates, as we wanted to determine the relation of A.C.T.H. and salicylates with regard to their effects upon the inflammatory reaction and upon the endocrine organs. The role of salicylates in silicosis has been discussed in another paper (Vyskočil, 1955).

We have found quartz injections into the anterior eye chamber in rabbits a convenient method for following the development of silicotic granuloma. Several interesting conclusions with regard to the pathogenesis of silicosis can be drawn in this manner, the main advantage being that the whole development of the granuloma can be viewed under the transparent cornea and the necessary histological sections can be taken at various intervals.

Methods and Experimental Animals

The injections of quartz dust into the anterior eye chamber were carried out under strictly aseptic conditions. Two days before the injection penicillin drops were put into the conjunctival sac. Granulomas were produced by the injection of 0.05 c.c.m. of a 5% quartz dust suspension; the quartz particles were all under 10μ, most being between 2 and 3μ. The cornea and conjunctival sac were anaesthetized with 4% novocaine in drop form.

The bulbus was fixed by grasping the bulbar conjunctiva near the upper external end of the corneal limbus and injecting against this light pull about 1 mm. from the limbus through the cornea and into the anterior chamber. Corneal fluid was not withdrawn; the dust was injected immediately after suspension in a tuberculin syringe. After enucleating the eye the upper bulbar pole was marked so that the vertical meridian could be observed when cutting the histological sections; this is important because the dust particles sink to the floor of the anterior chamber.

We used 50 rabbits of similar weights, all females. They were divided into six groups. The experiment lasted six months, and therapy was started on the first day. Group I contained nine untreated controls. Group II (10 rabbits) received daily for the first two and a half months 0.1 mg./kg. A.C.T.H. and then for three and a half months 0.2 mg./kg. subcutaneously. Group III (six rabbits) was injected with 0.25 i.u./kg. insulin daily subcutaneously. Group IV (six rabbits) received A.C.T.H. (same quantity as group II) and 0.25 i.u./kg. insulin on alternate days. Group V (10 rabbits) received A.C.T.H. (0.1 to 0.25 mg./kg.) and insulin (0.25 i.u./kg.) daily together. Group VI (nine rabbits) received for the first two and a half months 0.250 g./kg. sodium salicylate in a 10% solution by tube or 0.250 g./kg. salicylamid in food; for the remaining three and a half months the dose was lowered to 0.125 g./kg.

These doses of both hormones and salicylates were effective as we observed two- to five-fold increases in circulating pseudo-eosinophils within two hours. Insulin (0.25 i.u./kg.) caused an average decrease of fasting blood sugar of 45% within an hour.

During the first month the inflammatory reaction was observed almost daily, then at monthly intervals. In macroscopic examination we took account of the dilated vessels and their attachment to the granuloma; the turbidity of the chamber liquid, the size of whitish film adhesion above the nodule; the degree of pigmentation round them and the size and deformity of the pupil. By these criteria the inflammatory reaction was classified between 0 and 6 points. All animals were killed after six months.

Histological sections were stained with haematoxylin and eosin, the adrenals with Sudan III for the estimation of lipids, and the hypophysis by the Mallory method for the estimation of chromophobe, eosinophil and basophil cells. All endocrine glands were weighed immediately upon extraction.

Besides these 50 animals in the six groups designed to follow the effect of hormones and salicylates upon the development of the silicotic granuloma, we had in the experiment rabbits into which were injected non-fibrogenic dusts—brick (five rabbits) and scoria (seven rabbits) into one eye and quartz dust into the other eye. Another group of 20 animals was inoculated with quartz dust and left untreated; these animals were killed at various intervals from a fortnight to six months.

Results

Macroscopic examination one day after the quartz injection showed considerable white turbidity in the whole eye chamber and little clouds of white fibrin. This lasted for about three days, then the turbidity began to clear. Round the whole limbus a slight ciliar injection could be seen. As the turbidity decreased, films of fibrin above the quartz deposits could be seen. The relatively calm phase of the reaction occurred about the tenth day, but even then the whitish film above the injected quartz in the iris persisted. Contrasting with this is the fact that the fibrin formed above non-fibrogenic dusts (brick and scoria) was dissolved during these days and finally vanished.

About the tenth day pigment began to migrate around the dust deposits. Anterior synechiae and pupillary deformity sometimes occurred. In the third to fifth weeks after the quartz injection the inflammatory reaction increased, this time without any significant white turbidity in the chamber fluid. Instead we observed dilatation of the iris vessels, increased conjunctival injection, and sometimes vascular dilatation in the posterior corneal wall as well. This increased reaction calmed down within a fortnight with the exception of vascular dilatation which persisted thereafter.

Histological examination of the eyes on the 17th to 30th day revealed nodules of increased histiocytes and sometimes slightly pink, structureless material in the iris and irido-corneal angle. The nodules contained relatively large histiocytes with large vacuolized centres of evidently phagocytic nature.
Conglomerations of pseudo-eosinophils, lymphocytes, and plasma cells were often found. Fibrin films were in some cases encountered above the nodules, but lamellar fibrosis was never seen (Fig. 1).

Histological examination after four to six months revealed granulomas in the cedematous iris tissue, in the irido-corneal angle, and in the corpus ciliare. The main granuloma constituents were histiocytes, fibroblasts, lymphocytes, pseudo-eosinophils, histiocytes with phagocytosed pigment liberated from the migrating pigment epithelia, and extracellular pigment; sometimes also disintegrated sclera. This phase was marked by the finding of massive lamellar fibrosis. We want to emphasize that these firm, slightly cellular fibrotic lamellae composed of collagen, eventually of hyalin (equal to third to fifth grade by the Belt and King classification, 1945), were only formed above the nodules in the free irido-corneal angle lying against the posterior corneal wall, and never on the opposite part of the nodule in the iris stroma or the irido-corneal junction or corpus ciliare which contain a rather dense fibroblastic net even under normal conditions (Figs. 2 and 3). What we see here is evidently mostly fibrotic organization of plasma exudate.

The group which was injected with non-fibrogenic dusts also had whitish turbidity and fibrotic films in the first days after the inoculation, but these were smaller and vanished more quickly, within the first six days. The vessel reaction in the following three to five weeks, found with quartz dust, did not appear. Also the deposits contained a smaller amount of pigment. Histological examination did not reveal any fibrotic lamellae above the nodule in the free irido-corneal angle.

Group I: Quartz Alone (Control).—The control group of nine untreated animals with which other groups (II—VI) were compared had the greatest amount of whitish turbidity in the anterior eye chamber in the first three days. This was classified as 3-2 points. The reaction slowed down until on the tenth to seventeenth day it began to increase again, this time mostly by vascular dilatation. There was no secondary infection. After six months the macroscopic reaction was classified as 2-7 points.

Histological examination revealed silicotic granulomas as described above in the iris stroma, corpus ciliare, and the irido-corneal angle. Fibrotic lamellae, slightly cellular and almost collagenous up to completely acellular in the free irido-corneal angle, were found in 66% of the animals (Figs. 4 and 5).

The average weight increase was 15 dkg. (the animals are less at the beginning because of the trauma). Average weights of the endocrine glands are to be found in Table I.

Group II: Quartz and Daily A.C.T.H. Administration.—Macroscopic reaction was smaller in the first days than in the control group (2-5 points). Two animals showed secondary infection in the eye chamber and conjunctiva during the first month which is understandable with A.C.T.H. administration. After six months the silicotic granuloma in animals with no infection was classified as 1 point.

Histological examination revealed silicotic granulomas with identical cellular elements as in the control group, but the granulomas seemed to be somewhat smaller and contained less pigment in histiocytes and extracellularly. In two animals (25%) only could fibrosis in the corneal angle be found (Fig. 6a). One animal had several layers of fibroblasts localized between the histiocye infiltrates in the iris and the Descement membrane. There was neither collagen nor hyalinization (Fig. 6).

The average weight increase was 23 dkg. The adrenals registered a statistically significant weight increase when compared with that of the control group (P < 0.01). Histological examination revealed a slightly enlarged adrenal cortex, mainly in the zona glomerulosa and the zona fasciculata, and an enlarged adrenal medulla with dilated sinusoids.

### Table I

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Animals</th>
<th>Average Weight (kg.)</th>
<th>Incidence of Secondary Infection (%)</th>
<th>Macroscopic Classification</th>
<th>Incidence of Lamellar Fibrosis (%)</th>
<th>Glucose Fall (% after 0.25 u/kg Insulin after 6 Months)</th>
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Statistical evaluation with regard to the control group gave* = P < 0.01; ** = P < 0.02; *** = P < 0.05.

† Excluding animals with secondary infection.
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Fig. 1.—Silicotic granuloma in the anterior eye chamber in rabbit after 18 days. Stained with haematoxylin and eosin. × 240.

Fig. 2.—Silicotic granuloma in the anterior eye chamber in rabbit after six months. From the left: iris with incorporated quartz dust and liberated pigment; fibrotic lamellae; cornea. Stained with haematoxylin and eosin. × 240.

Fig. 3.—Massive lamellar fibrotic composition stained after Gomori (iron-silver). In the right upper corner white stripe—the Descemet membrane. × 240.
Group III: Quartz and Daily Insulin Administration.—The inflammatory reaction in these animals was not very much different from that in the control group during the first three days and received 3·2 points. Further development was also similar to that in the control group. Secondary infection of the eye chamber was found in 17% of the animals. After six months the reaction by macroscopic examination was classified as 2·2 points.

Histological examination revealed the same cellular composition of silicotic granulomas as in the control group. Sixty-six per cent had a massive fibrotic reaction in the form of slightly cellular parallel fibrotic lamellae. The granulomas contained more pigment (Fig. 8) than in the control group. This group was the weakest of all. Weight increase was only 16 dkg., though in the first two months it
Corneal. One animal had commencing calcification in the lamellar fibrotic structure (Fig. 9).

The average weight increase was 4 dkg., and of the endocrine glands, that of the hypophysis was increased (P < 0.02). Histological examination revealed dilated sinusoids in the normal adrenal medulla and atrophied islets of Langerhans.

Group V: Daily Combined A.C.T.H. and Insulin Administration.—The inflammatory reaction in the first three days was not remarkably influenced, and a change for the better was only observed after the first months. Secondary infection was seen in 10% of the animals. Silicotic granuloma after six months was classified with 1-8 points.

Histological examination revealed granulomas of a composition similar to that in the control group, but fewer fibrotic lamellae in fewer animals could be found (30%). An example of a silicotic granuloma without massive fibrosis in the free irido-corneal angle can be seen in Fig. 7.

The average weight increase was 49 dkg., the largest of all, and of the endocrine glands, an increase in weight was found in the adrenals, and ovaries, and in the uterus. Histological changes consisted of an increased adrenal medulla with dilated sinusoids and slightly enlarged islets of Langerhans.

Group VI: Daily Salicylates.—The first reaction was marked with 2-6 points. It was smaller than in the control group. Secondary infection affected 22% of the animals. After six months the inflammatory reaction received 1-3 points.

Histological examination revealed granulomas with histiocytes, pseudo-eosinophil, and giant cells. The pigment content seemed to be smaller. Massive fibrotic lamellae could only be found in one case which also had secondary infection in the anterior eye chamber and so was not included in the final classification. One animal had in the posterior corneal surface only lamellar fibroblast layers without collagen fibres and hyalinization. Weight decrease averaged 4 dkg. (the gastric mucosa suffering particularly from the high salicylate dosage). Weighing the endocrine glands revealed a slight increase in the weight of the adrenal (P < 0.05). Histological examination revealed a constricted zona glomerulosa in the adrenal cortex (by Na salicylate), diluted sinusoids in a slightly increased adrenal medulla, and decreased eosinophilic cells in the adeno-hypophysis.

Conclusions

Our experiments show that quartz dust, like other dusts, produces in the tissues at first a normal inflammatory reaction, but it is capable of adsorbing strongly plasma, tissue, and cellular proteins on its
surface, on account of its physical properties there (Jäger, 1950; Bohun, 1954). Later on elution of silica seems to take place and its reaction with the surrounding substances.

The adsorbed proteins become denatured and provoke an increased delayed reaction which we observed in the anterior eye chamber during the third to fifth weeks. We saw massive fibrotic lamellae only in the free angle of the anterior eye chamber between the iris and the cornea, where there was exudation from dilated vessels and fibrin adhesion. Plasma proteins were invaded with cellular elements, especially macrophages, which later changed into fibroblasts.

Policard and Rollet (1931), who injected quartz into rabbits intracorneally, could not find the typical lamellar fibrosis round the deposits. The cornea contains few vessels and its firm consistency does not allow production of larger exudate. Our experience in histological examinations of quartz deposits in the cornea was similar: the dust which remained in the injection channel descended between the corneal layers to the lower limbus pole without forming the typical lamellar fibrotic structures and only a small chronic inflammatory infiltrate with histiocytes could be found.

In studies of fibrosis in the silicotic lung all authors insist upon the formation of fibrotic tissue in the interstitial free space. Our experiments show that in the lung, coniophage and liberated quartz conglomerations probably appear not only in the interstitial tissue but in the alveolar lumen as well, and that here the fibrotic exudate organizes. The lung alveolus with open spaces and a rich blood supply is therefore well adapted for the formation of massive fibrosis.

We found retarded fibrinolysis above the quartz deposits; this shows where the silicotic lung process might be similar to the tuberculous. In the tuberculous lung an increased antifibrinolysin level may be found (Rosenmann, 1937).

In our experiments, aimed at influencing the development of the silicotic granuloma, the results show that salicylates, A.C.T.H. alone and in combination with insulin, when administered simultaneously, have the most pronounced effect upon the growth of the silicotic granuloma. Both macroscopic and histological evaluations revealed quantitative changes, never qualitative, and the whole effect was only slight.

The A.C.T.H. dosage was low and did not cause any deterioration in the general state of health of the animals. Nor did we find any significant weight or histological changes in the endocrine organs, with the exception of the adrenal. Histological examination showed that salicylates in large doses were the most influential agent. When comparing the action of salicylates with that of A.C.T.H. administered in very small doses, we saw smaller enlargement of the adrenal and smaller histological changes so we suggest that salicylates have their own specific effect in the periphery. Salicylates stimulate the system: the central nervous system and the hypophysis but the adrenals only secondarily (Vyskočil, in the press).

Insulin had no favourable influence upon the silicotic granuloma. This is in contrast to the findings of Schiller (1953), but this author administered depot insulin and apparently the comparison was difficult because he used white mice, gave smaller amounts of insulin, and only followed the response to quartz in the peritoneal cavity for 30 days.

The assumption that endogenous A.C.T.H. production stimulated by insulin would have a favourable effect upon the fibrosis was not confirmed. The animals deteriorated after insulin, while the inflammatory reaction itself did not benefit, even in the first days. Insulin administered on alternate days with A.C.T.H. did not prove our assumption that it would stimulate the hypophysis to A.C.T.H. production and so prevent hypophyseal atrophy after the organism had been overloaded with exogenous A.C.T.H. This group of animals also deteriorated after insulin and the reaction to quartz was the same as in the control group. Insulin apparently had a greater effect than A.C.T.H. Both these groups of animals had slightly heavier hypophyses, dilated sinusoids in the adrenal medulla, and atrophied islets of Langerhans due to adaptation to insulin. All these changes were more pronounced in the insulin group than in the combined insulin-A.C.T.H. group and, besides, the insulin group had smaller ovaries and uteri and fewer eosinophil cells in the adenohypophysis.

In contrast to this, the group receiving A.C.T.H. and insulin daily together had a smaller fibrotic reaction than the control group and their general state of health was the best of all. All animals put on weight and had enlarged ovaries and uteri and even slightly enlarged islets of Langerhans.

The mutual relation of A.C.T.H. and insulin and questions connected with their therapeutic administration and histological findings in endocrine organs are discussed elsewhere (Vyskočil and Dufka, 1956).

In conclusion we may say that our experimental study has contributed more to our knowledge of the pathogenesis than to either the therapy or prophylaxis of silicosis. The results in animals do
not point towards anti-inflammatory hormone administration in man. The formation of fibrotic nodules could still be observed, though in a smaller number of animals. Though the group with combined A.C.T.H. and insulin administration had no large changes in endocrine function, the danger of disturbing this function still exists. But we should not overlook the favourable effects of salicylates on secondary inflammations in the silicotic lung (Vyskočil, 1955), as we may thus prevent the development of more massive fibrotic lesions. Our endeavours in research on clinical silicosis prevention must be directed toward prevention of inflammatory lung disease, especially tuberculosis.

Summary
Massive lamellar fibrosis after quartz inoculation was only produced in rabbits in the free space of the irido-corneal angle and mainly by organization of the plasma exudate.

The development of silicotic granuloma was reduced by salicylates, by A.C.T.H. alone, and by A.C.T.H. given with insulin and at the same time in that order.

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