CHANGES IN THE TOTAL LIPID CONTENT OF THE LUNGS OF RATS WITH EXPERIMENTAL SILICOSIS

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Strachan and Simson [13], in 1931, first demonstrated an increase in the lipid content of the lungs in miners with silicosis. E. I. Dobrolyubov [1] observed a depression of the lipolytic activity of the blood following inhalation of quartz dust by rabbits, and Sacchitelli and Odaglia [9] made the same observation in human patients with silicosis. A marked lipemia in experimental silicosis was discovered by V. P. Kozak [2]. Marks and Marasas [7] found that the lipid content of the lungs also increased in animals under the influence of quartz dust (in guinea pigs), confirming the observations of Fallon [5] on rabbits. Fallon regarded the increase in the content of phospholipids in the lung tissue in silicosis as a sign of the formation of lipoid "fibrogenic factor," which takes place, it is suggested, in "monocytes" dying under the influence of phagocytosed dust. Fallon's hypothesis has recently received strong support from the adherents of the immunological theory of the pathogenesis of silicosis [6,13].

Regardless of whether the accumulation of lipids in the lungs in silicosis is the result of formation of "fibrogenic factor" or whether it is associated with secondary changes in the lipolytic and (or) lipopectic function of the lungs, affected by the pathological process, or, finally, whether it is due to an associated disturbance of some aspect of the intermediate metabolism of lipids as a result of a primary disturbance of the metabolism of other substances (for example, carbohydrates, as Singh and co-workers, in particular, claim [11] in respect of another dust disease of the lungs—bagassosis), in any case it is essential in the first place to shed light on certain problems relating to the general interpretation of the role and place of these changes in the development of pneumoconiosis. These problems are: 1) does a connection exist between the content of free silica in the dust and the ability of the dust to increase the lipid content in the lungs; 2) at which stage in the formation of the pneumoconiotic changes in the lungs does this increase take place; and 3) is there a correlation between the amount of lipids found in the lungs and the collagen content.

METHOD

Albino rats received a single injection of 50 mg of one of the six dusts encountered in the fireproofing industry, listed in the table, made up as a suspension in physiological saline, into the lungs by means of a bloodless intratracheal method. The animals were then sacrificed in groups at intervals of between 2 weeks and 6 months after the injection. The lipid content of the homogenized tissue of both lungs was determined by the loss of weight of the dry tissue during extraction with ether in a Soxhlet apparatus, and the content of "total" hydroxyproline was estimated by Chvapil's method [4], as an indicator of the intensity of fibrogenesis; the dust content was determined by the method of Stacy and King [12]. In some cases, as will be mentioned when the experimental results are described, the phospholipid content of the lung tissue was also determined by extraction with a mixture of alcohol and ether (3:1) and boiling, by mineralization with a mixture of nitric and perchloric acids (9:1), and by colorimetric estimation of phosphorus by Briggs's method, using the FÉK-M photoelectric colorimeter.

Some of the rats sacrificed at each period were used for the histological verification of the changes produced in the lungs (L. N. El'nichnykh). The material was stained with hematoxylin-eosin, picrofuchsin (Van Gieson's method) and by impregnation with silver (Foot's method). Tissue impressions of the lungs of rats sacrificed 10 days

Absolute Content of Lipids (in mg) in Lungs of Experimental and Control Rats (mean data)

Interval after injection of dust	Control group	Material injected					
		clay	fireclay	dust from fireclay sorting area	quartzite	unused Dinas	used Dinas
14 days	16.7	29.3	34.3	33.6	49.6	42.6	52.7
1 month	15.6	29.3	31.5	30.9	48.4	50.0	31.4
2 months	26.5	33.0	49.9	36.3	71.7	89.5	63.5
4 months	29.2	36.3	57.3	38.4	139.4	116.1	62.4
6 months	32.7	34.3	59.7	53.4	125.6	140.5	85.3

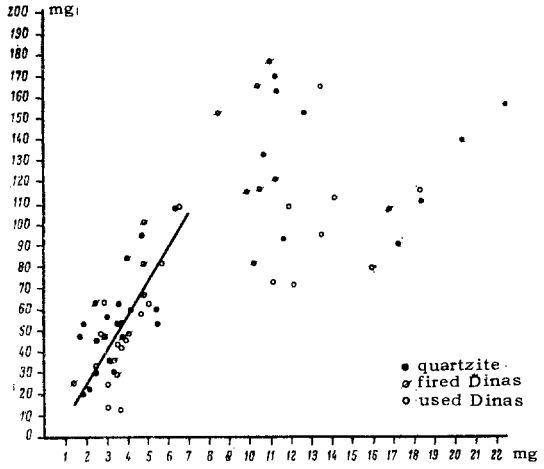

Fig. 1. Correlation between absolute content of lipids (axis of ordinates) and hydroxyproline (axis of abscissas) in lungs of rats injected with quartz-Dinas dust.

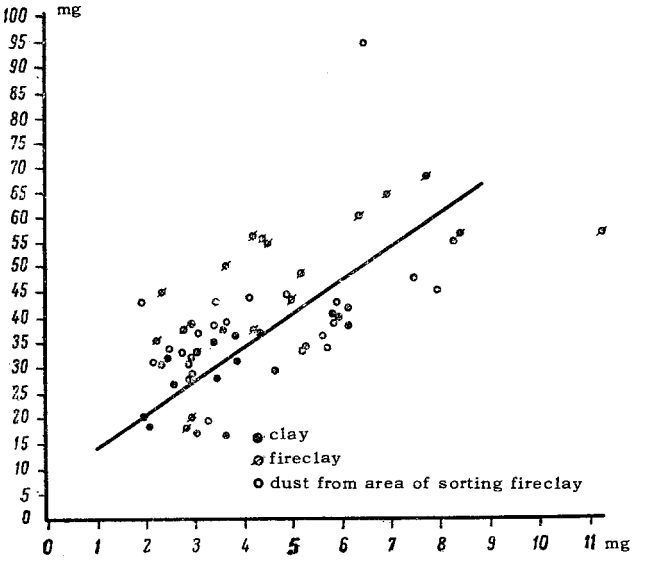

Fig. 2. Correlation between absolute content of lipids (axis of ordinates) and hydroxyproline (axis of abscissas) in lungs of rats injected with aluminosilicate dust.

after intratracheal injection of rock crystal dust, and films made from exudate obtained 24-72 h after intraperitoneal injection of the same dust into mice, were fixed in formalin vapor and stained with Sudan black by the method of Sheehan and Storey [10].

RESULTS

At all periods after the intratracheal injection of dust the relative lipid content in the lungs, expressed as a percentage of the dry weight of tissue, was higher than in the control group. In each experimental group (corresponding to the type of dust used) this index was roughly the same at all periods of the investigation. We therefore show here only the mean values of this index for the groups as a whole (each group included 20-25 rats). The mean lipid content, which amounted to 7.8% in the control group, rose to 8.2% (difference not statistically significant) under the influence of clay dust, containing approximately 12% of free silica; to 10.2% ($P < 0.001$) and to 9.6% ($P < 0.005$) under the influence of two dusts formed from fireclay (up to 30% of free SiO_2); and to 11.0, 12.5, and 13.3% under the influence of three dusts from the Dinas brick industry containing over 90% of free silica (in all three cases the difference from the control value was significant, $P < 0.001$).

Hence, the higher the content of free silica in the dust, i.e., the greater its potential hazard as a cause of silicosis, the more marked the accumulation of lipids in the lungs under its influence. This difference between the dusts was even more obvious when the absolute content of lipids in the lungs was used as the criterion. The results given in the table show that in this case the experimental groups differed not only by the levels attained at the end of the experiment, but also by their dynamics. The higher the silica content of the dust, the more marked the rise

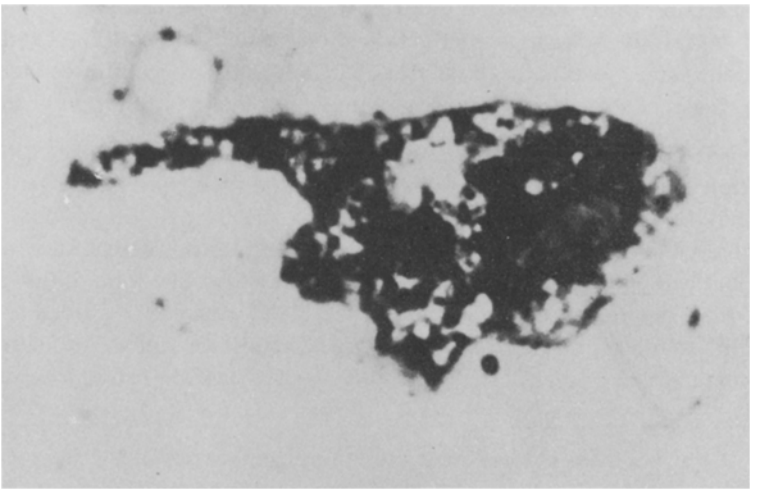

Fig. 3. Macrophage in film of peritoneal exudate 24 h after injection of rock crystal dust. Photomicrograph. Fixation with formalin vapor. Stained with Sudan black followed by azure-eosin. Magnification 15 × 90.

in the absolute content of lipids between the first and last periods of the investigation; in the rats receiving injections of clay, this increase was practically nil, and at the end of the experiment the difference which was undoubtedly present initially had almost disappeared. It should be emphasized that a history of previous working at a Dinas brick oven, which, despite its SiO_2 content which is as high as that of quartzite and unused Dinas, caused a smaller degree of accumulation of lipids in the lungs, occupied in terms of fibrogenicity (hydroxyproline content) an intermediate position between fireclay and quartzite contact.

In a repeat experiment in which 4 of the same 6 dusts were injected into rats for a period of 2 months (the lungs of 13-16 rats in each group were investigated simultaneously), the same pattern was observed. In this case the mean increase in the absolute content of lipids in the lungs (by comparison with the mean control value) could be calculated per milligram of dust found in the lungs of each rat. This index had a value of 7.7 for injection of clay, 14.2 for fireclay, 19.0 for used Dinas, and 26.0 for quartzite.

Histological investigation showed that even when quartzite-Dinas dusts were injected, collagen sclerosis of the silicotic nodules (and not in every case) could not be observed until 6 months after injection. Meanwhile, an obvious increase in both the relative and the absolute content of lipids was observed only 2 weeks after injection of even the low-silica fireclay dusts. In 2 of the 4 rats sacrificed only 5 days after injection of quartzite it was much higher than in any of the 25 rats of the control group, although the relative content of hydroxyproline still showed no difference from the control value. Hence, the changes in the lipid metabolism of the lungs evidently preceded the development of fibrosis, and enabled the dusts to be distinguished much sooner than the histological study of the reaction of the lungs to their injection and, as our subsequent investigations showed, sooner than the estimation of hydroxyproline.

The answer to the third of the questions raised in the introduction to this paper is more complicated. Even if the theory of the "lipoid fibrogenic factor" is accepted as a working hypothesis, there are no grounds for postulating the presence of a constant quantitative correlation between the lipids and collagen in the lungs, since the increase in the content of these substances is regarded as a manifestation of two processes differing in their biochemical nature and in their cellular localization, one of which precedes the other. It is not surprising, therefore, that the almost linear relationship between the absolute contents of lipids and hydroxyproline in the lungs should be so clearly defined, although with considerable scatter of the points,* only at relatively low concentrations of hydroxyproline,

*Subsequent investigations showed that the correlation between the lipid and hydroxyproline contents appears higher and statistically significant if these values are determined in the left and right lungs individually, for after intratracheal injection of dust, its distribution in the lungs is not, as a rule, uniform and the mixing of their tissue evens out the changes caused by the dust.

corresponding (for quartzite-Dinas dusts) to the initial stages of the process (Figs. 1 and 2). However, with an increase in fibrosis to the relatively high degrees obtained only after the injection of quartzite and Dinas, any correlation between the compared indices practically disappears. This fact also demonstrates that the increase in the lipid content was not the result of the silicotic pneumosclerosis, but the manifestation of some preceding process.

Are there grounds for localizing this process in the macrophages of the lungs, in accordance with the views described above? Impressions of lungs from animals exposed to rock crystal dust, stained with Sudan black, in fact revealed the presence of large numbers of macrophages with abundant lipid inclusions in their cytoplasm. There were far fewer of these cells in impressions of the lungs of control rats. Cells of the same character (Fig. 3) were also found in large numbers in films of peritoneal exudate caused by injection of this dust. The connection between the macrophages of the exudate and the monocytes of the blood cannot be regarded as certain, but it is interesting to note that, according to reports in the literature [3], sudanophilic inclusions are extremely few in number in the monocytes. The cytochemical findings cited are insufficient to confirm Fallon's hypothesis, but neither do they contradict it.

The problem of the phospholipid nature of the "fibrinogen factor" is a different matter. We determined the phosphorus content of the phospholipids in the lungs of rats sacrificed 2 and 6 months after receiving an injection of fireclay dust (group A) or dust of quartzite or unused Dinas in a kiln (group B), i.e. two groups of dust differing greatly in their ability to cause silicosis. Six months later the phosphorus content of the phospholipids, which was 1.592 mg in the controls, amounted to 2.064 mg in group A and 4.156 mg in group B (differences between the groups are significant, $P<<0.001$). The same index, calculated in terms of dry weight of tissue, was 0.344% for group A and 0.455% for group B ($P<0.05$), and in this case only group B differed from the control value. Meanwhile, when calculated per milligram of total lipids, the phosphorus content of the phospholipids was actually lowered (although not significantly) by comparison with the controls. In the rats sacrificed 2 months after the injection, the general character of the changes was the same, but in this case the degree of lag of the increase in the phospholipid content behind the increase in the total lipid content was still more marked, so that in group B, in which the absolute content of lipid phosphorus was also higher at this period than in group A (3.37 mg compared with 2.13 mg; $P<<0.001$), a completely opposite relationship was obtained when the calculation was made per milligram of total lipids (0.298 mg compared with 0.398 mg; $P<0.05$). Consequently, if the accumulation of phospholipids in the lung tissue plays the important role ascribed to it by Fallon in the development of silicosis, in any case the increase in the content of total lipids is far more marked in the quantitative respect 2 months after the injection of dust, and the divergence between these indices is wider, the higher the rick of silicosis developing from the dust. We have already pointed to the possibility of a different interpretation of the nature of the observed changes, and at this stage of our investigations there is little to be gained from expressing preference for either point of view. However, the fact that a very early accumulation of lipids takes place in the lungs during the deposition of dust in these organs, and the clear relationship between this accumulation and the degree of its silicosis-producing power, appear to be well established. These facts are interesting, not only from the point of view of the study of the pathogenesis of silicosis, but also in connection with the development of methods of comparative biological evaluation of industrial dusts.

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