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CONNECTIVE TISSUE CHANGES CAUSED IN GUINEA PIGS BY QUARTZ, QUARTZ-CONTAINING DEAD ROCK, BROWN-COAL AND TALC, AS EXAMINED BY THE METHOD OF MILLER AND SAYERS

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The intraperitoneal test developed in 1936 by Miller and Sayers [7] for studies on the histopathological reactions of industrial dusts has stimulated further, extensive investigations in this field. The numerous investigations carried out during the past two decades clearly proved the significance and simplicity of the intraperitoneal test as compared to the inhalation, intratracheal and other similar techniques. The early trials on dusts by Miller and Sayers [7] were soon extended to tridimite, crystobalite, talc, caoline, aluminium phosphate, carborundum, beryllium oxide, tremolite, graphite, diamond, metallic cobalt, tungsten carbide, titanium carbide, etc. [1, 5, 6, 7, 8, 9, 10, 12]. The investigations in various directions soon clarified the criteria of the method: the choice of the experimental animal, the proper method of treatment, the significance of the particle size, the quantity and chemical nature of the dust injected, the role of the dust depot formed in the abdominal cavity, and the size of the tissue nodule, the best time of testing, the outlooks of gross and microscopic analysis [4, 5, 6, 8, 9].

The pertaining evidence in the literature, favourable [5, 8, 9], or more critical [3, 6, 8], has induced us to regard the method of Miller and Sayers [7] as suitable for histological studies on the fibrogenetic action of various industrial dusts. On the basis of the afore-mentioned considerations we have chosen this method for use in parallel, comparative investigations of the dead rock dust of a coal mine of considerable size in Hungarian relations, on the one hand, and on suitable control dusts, on the other, so as to determine whether the former dust had any fibrogenic effect and eventually to extend the applicability of the method.

Methods

104 normal guinea pigs of both sexes, weighing 350 to 400 g each, were used.

The dusts tested were dead stone from the coal mine in question; chemically pure quartz (Merck); brown coal; and talc, the latter as specified in the Fifth Hungarian Pharmacopoeia. The dead stone contained 77 per cent, the brown coal as little as 0.55 per cent of quartz. The quartz content was estimated by the method of Talvitie [11].

The single test materials were homogenized in a China mortar, separated by the sedimentation method of CUMMINGS [2], and the 1 to 3 micron dust fractions thus obtained were injected intraperitoneally into the animals, giving single doses of 75 mg in 2 ml distilled water. The animals were kept in cages in groups of ten and were fed the usual grain and mixed vegetable diet.

The nodules which had formed in the abdominal peritoneum, alongside the linea alba, were examined 6 and 12 hours, then 1, 3, 5, 7, 10, 14, 20, 24, 30, 60 and 90 days following injection, always simultaneously, in 2 animals. At the proper points of time the animals were taken out unselected and were killed with ether. The abdominal wall from the anterior costal arch to the bony pelvis was removed as completely as possible and was fixed in 4 per cent formaldehyde, stretched on a glass plate with the peritoneal aspect outward. The so-called "typical" nodules (measuring about 4 mm in diameter) were excised, embedded in paraffine-celloidine, cut up in 6—10-micron sections, which were studied after staining with haematoxyline-eosin, by van Gieson's dye, azan, and by the silver impregnation method of PAP, respectively.

Results

General considerations

The findings valid for all the dusts tested were the following.

Capsule. In every case the peritoneum crept as early as 6 hours onto the conglomerate composed of dust, fibrin, and leucocytes. Under the initial perithelial cell layer the young fibrocytes developing in several rows and the acidophilic collagen fibres soon form a capsule around the nodule. As the nodule continues to develop, connective tissue bundles grow toward the centre from the capsule, which increases in width, becomes poorer in cells and richer in collagen fibres of increased thickness.

Cellular elements. Within the capsule the appearance of fibrocytes and fibroblasts is followed by invasions with macrophages, and, subsequently, with foreign body giant cells and giant cells of the Langhans-type. Finally, an increasing number of endothelial cells, vascular buds and capillaries develop.

The macrophages present two forms. One type has a basophilic cytoplasm and a mostly eccentric, small, dark-staining nucleus without nucleoli. The other has a pale pink cytoplasm containing small and medium-sized granules staining with Sudan. The nucleus is bigger than with type 1, is oval in shape, shows a loose chromatin structure and contains a brightly staining nucleolus.

The foreign body giant cells contain 8 to 20 round nuclei, which mostly stain dark, are rich in chromatin and contain no nucleolus. The nuclei usually appear crowded in that part of the cytoplasm which contains no foreign substance. The cytoplasm stains dark and contains many dust granules. The giant cells of the Langhans-type have 8 to 20 round nuclei which usually stain slightly, are poor in chromatin and contain nucleoli. The nuclei are usually in the light-staining, marginal part ot the cytoplasm, forming a halo around the incorporated mass of dust particles.

Dust. The granules of dust that at first are situated extracellularly, in the centre of the nodule, appear in ever increasing numbers in the later stages

of the development of the nodule intracellularly, in the macrophages and giant cells characteristic of the single types of dust.

Fibres. In a manner characteristic of the different types of dust, the nodule as a whole is interwoven by argyrophilic reticular fibres, collagen fibres, then by hyalinizing fibres which grow in from the margins and corners.

Specific changes

Within the just described general process, the single types of dust give rise to the following specific histological changes.

Quartz. As the first change taking place at about 3 days after injection, macrophages with acidophilic cytoplasm appear. Their number exceeds throughout that of the basophilic macrophages, which appear later. From the 7th day on, Langhans-type giant cells, and in smaller numbers, foreign body giant cells present themselves. They soon show toxic nuclear lesions and aggregation of the nuclei. After the 14th day increasing numbers of endothelial cells, vascular buds and capillaries can be found. The number of capillaries continues to increase until the 60th to 90th day (Fig. 1).

The reticular fibres appear as early as after 7 days and, growing from the margin toward the centre of the nodule, can be seen as a rich, continuously progressing reticular network in the specimens taken on the 14th, 30th and 60th days. Conversion to collagen starts at the 14th day, from the margins. Collagenization progresses at a considerably slower rate than the formation of the reticular network and thus after 90 days it is still the formation of reticular fibres that dominates the picture (Fig. 5).

Dead rock. Basophilic macrophages appear at 7 days and their number remains higher than that of the acidophilic cells. The foreign body giant cells and (in smaller numbers) the giant cells of the Langhans-type make their appearance at 10 days. From the 14th day on endothelial cells, vascular buds and capillaries are also present, though in considerably smaller numbers than in the case of quartz dust (Fig. 2).

The reticular network develops from the margins, but not before the 10th day. Although it becomes more abundant in 30, 60 and 90 days, at the corresponding points of time it is still less than in the case of quartz dust. In this case, too, collagenization begins from the periphery, after the 14th day, but it is not so abundant as in the case of quartz dust (Fig. 6).

Talc. The first cells to appear (at 7 days) are the eosinophilic macrophages and the giant cells of the Langhans-type. Basophilic macrophages and foreign body giant cells are present in smaller numbers throughout. An abundance of endothelial cells, vascular buds and capillaries can be observed after the 14th day. Their number, however, is smaller than in the quartz and dead rock specimens of corresponding age (Fig. 3).

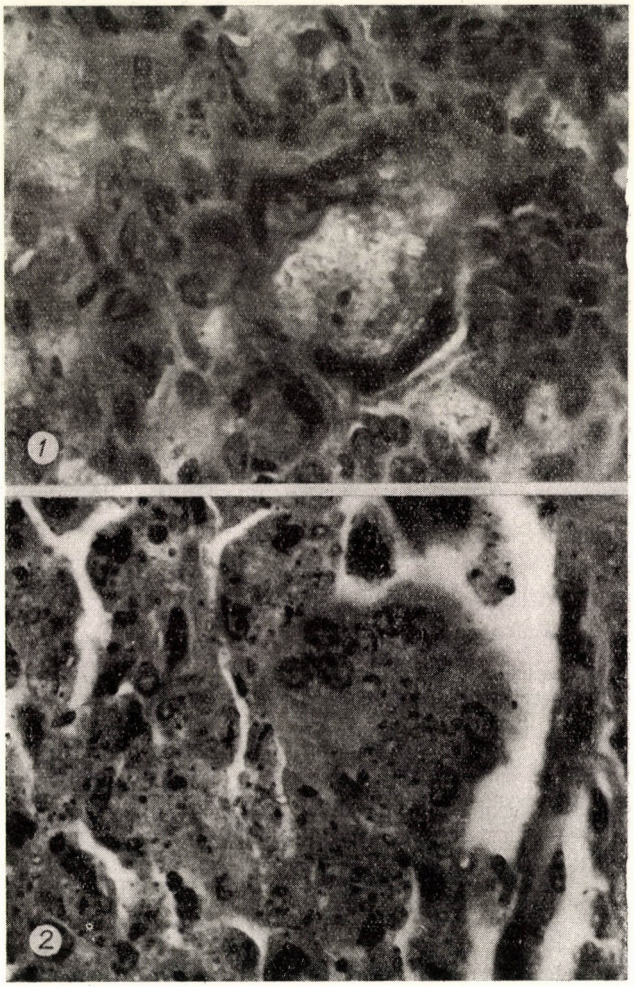

Fig. 1. 30-day tissue nodule of intraperitoneally administered quartz dust. Many Langhans-type giant cells, macrophages and endothelial cells. The grave toxic lesion is indicated by the very marked aggregation of nuclei. Haematoxylin-eosin. × 750

Fig. 2. Nodule 30 days after the intraperitoneal administration of dead rock dust. Many macrophages and, above, a foreign body giant cell. Haematoxylin-eosin. × 750

In the case of talc the formation of reticular fibres starts at 10 days, from the periphery. By the 30th, 60th and 90th days a network with few ramifications, leaving free the site of giant cells, develops. There is no substantial difference in collagenization from the former dusts (Fig. 7).

Brown coal. Macrophages can be observed from the 7th, foreign body giant cells from the 10th day onward. At the same time the number of eosinophilic macrophages and Langhans-type giant cells are small. The

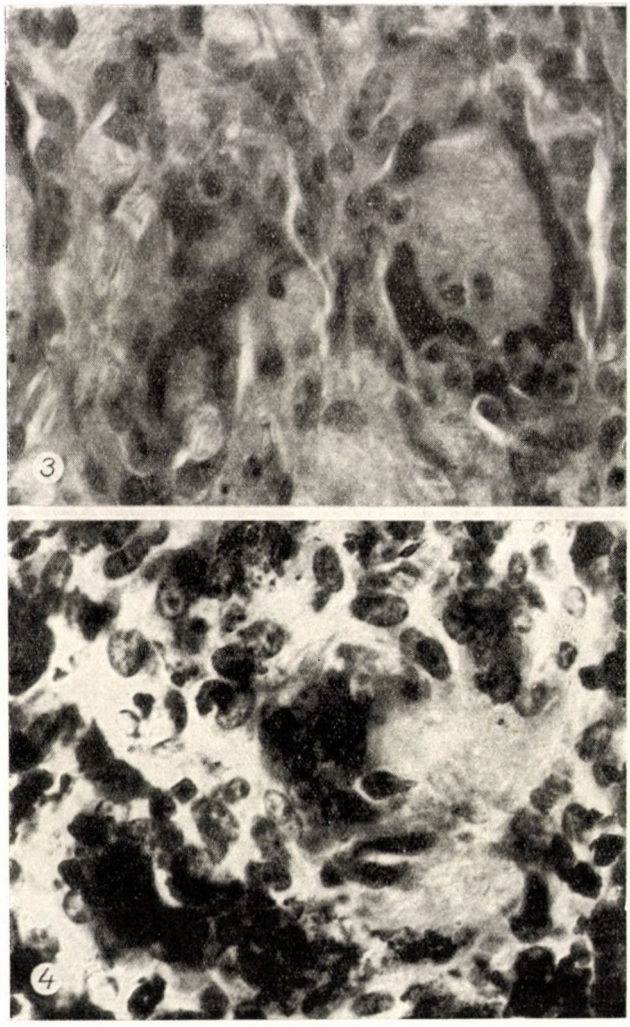

Fig. 3. Nodule 30 days after the intraperitoneal administration of talc. Foreign body giant cells, Langhans-type giant cells and smaller number of macrophages. Haematoxylin-eosin. × 750

Fig. 4. Nodule 30 days after the intraperitoneal administration of coal dust. A foreign body giant cell and many macrophages containing coal granules. Haematoxylin-eosin. × 750

number of macrophages is less and increases at a slower rate than in the case of the former dusts. From the 24th day on a few endothelial cells appear. There are also less vascular buds and capillaries than in the other specimens (Fig. 4).

Fibre formation is restricted throughout to collagen which forms the capsule and the wall of minor compartments. There is no appreciable reticular fibre formation (Fig. 8).

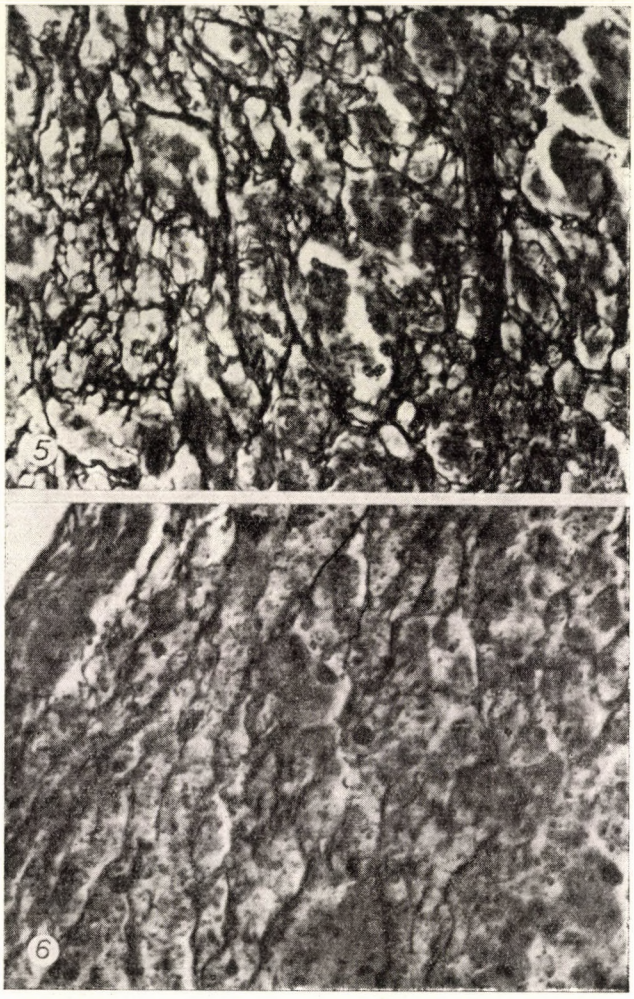

Fig. 5. Very rich, reticular network demonstrated by silver impregnation in a 30-day nodule of quartz dust. *Pap*'s silver impregnation. × 350

Fig. 6. Scarce reticular network in a 30-day nodule of dead rock dust. *Pap*'s silver impregnation. × 350

Histological phases

The tissue nodules formed in response to the dusts tested show 3 histologically different phases.

Phase 1 (the phase of non-specific inflammation caused by the foreign body) lasts from 6 hours until 4 days. In this phase no definite differences can be detected between the four dusts, except that in the case of quartz dust the macrophages appear as early as at 3 days. Thus, phase 1, which ends on

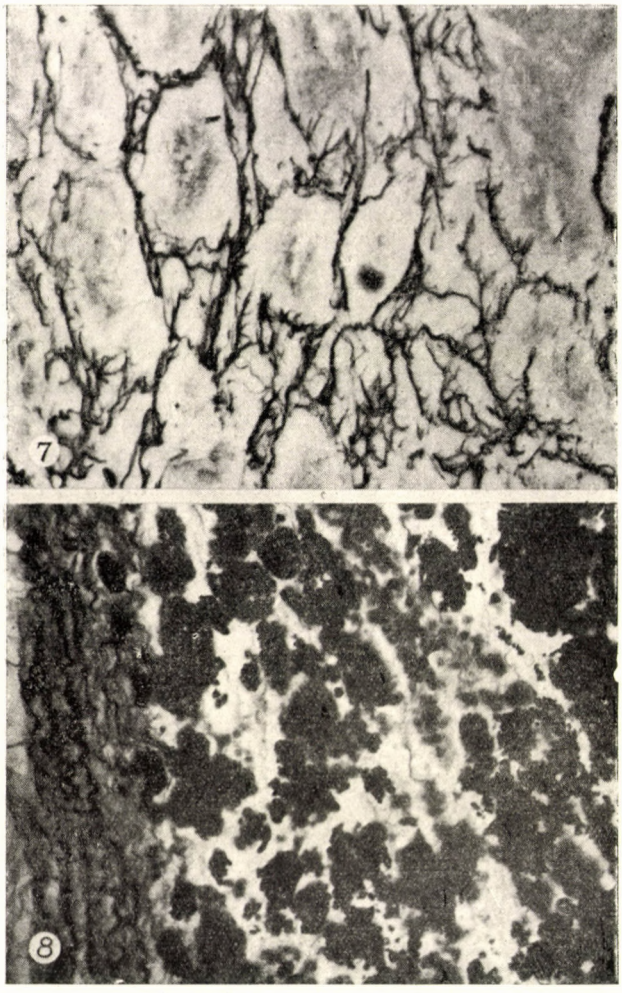

Fig. 7. 30-day nodule of talc, showing argyrophilic fibres of "storing character". *Pap*'s silver impregnation. × 350

Fig. 8. No appreciable reticular fibre formation can be seen in the 30-day old nodule of coal dust. *Pap*'s silver impregnation. × 350

the 4th day, does not facilitate a classification of different dusts on the basis of their fibrogenic properties.

Phase 2 (the histioid phase of nodule formation) lasts from the 5th day until the 20th. In this phase the quartz dust nodule shows the earliest appearance, the greatest number and the greatest accumulation of mononuclear and polynuclear phagocytes. The time of appearance, distribution and relation to one another of the macrophages foreign body giant cells and Langhans-type giant cells exhibit specific features characteristic the different dusts and

may thus serve as the basis of distinction. Reticular fibre formation and the course of collagenization also show well appreciable differences.

The formation of reticular fibres begins the earliest in the case of quartz dust, at 7 days. It gradually increases and at 14 days it is definitely more intensive than in the dead rock or talc specimens of comparable age. In the latter two cases reticular fibres do not appear until the 10th day and even then their formation is less intense than in the case of quartz dust. At the same time, only encapsulating, dividing collagen fibres can be seen in the nodule produced by coal dust, with a very small amount of reticular fibres among them.

Phase 3 (phase of collagenization) begins with the 20th day. In this phase the connective tissue involved in encapsulation becomes more and more poor in cells. The multiplying and thickening collagen fibres enclos and divide into an increasing number of compartments the cellrich nodule. Inside the nodule the connective tissue cells, the endothelial cells, the vascular buds, and capillaries, as well as the typical mononuclear and polynuclear phagocytes dominate. The latter have mostly incorporated the particles of dust.

Throughout the observation period reticular fibre formation was the most intensive and most progressing in the case of quartz dust. Though it was considerable also in the case of dead rock, it lagged behind the former both quantitatively and in the rate of development. In the case of talc, fibre formation was of a peculiar "storing"-type, different from what occurred with the other dusts. On the other hand, the nodules of the brown coal dust did not show any appreciable reticular fibre formation in this phase, either. Thus, phase 3 offers an even more definite possibility to distinguish the different kinds of dust on grounds of their fibrogenic property.

Discussion

The findings suggest that the detailed histologic study of the nodules formed in the peritoneum of guinea pigs present a reliable method for the experimental demonstration of differences in the fibrogenic properties of different dusts.

Histological analysis has revealed that the study at one single point of time of cellular components, the collagen and hyalinizing fibres of the single nodules furnished a rather unreliable and not too convincing basis for distinction. On the other hand, we advanced considerably when attention was devoted also to the different macrophages among the cellular components and when the reticular element among the fibres was included in the study. However, experience has shown that a fully convincing distinction between the single test dusts could not be made until the nodules were studied in their development, as units. This was achieved by studying systematically

in comparison with suitable control dusts the time of appearance, topography, peculiar qualitative and quantitative changes and the relation to one another of all the cellular and fibrous elements contained in the nodules.

By this procedure (in which we compared the dusts to quartz dust) the following evidence has been obtained. Although the dust of the dead rock of the coal mine in question could be markedly differentiated from quartz dust, it was very closely similar to, and in fundamental features it was identical with, quartz, as far as the quantitative and qualitative aspects of the tissue reaction are concerned. For this reason this dust had to be qualified as dangerous as quartz dust from the point of view of fibrogenic effect and prevention. At the same time, talc produced a fibrosis markedly different from that produced by the other two kinds of dust. Finally, the brown coal dust tested differed completely from the other test dusts in its fibrogenic property and elicited merely a "foreign body" connective tissue response without any so-called fibrosis.

Summary

1. 104 adult guinea pigs of both sexes were tested for response to chemically pure quartz (Merck), to dead rock dust containing 77 per cent quartz obtained from a Hungarian coal mine, to quartz-free brown coal dust, and to talc, respectively. The method of testing was that described by MILLER and SAYERS.

2. 1 to 3-micron fractions of the test dusts were injected intraperitoneally into guinea pigs under sterile conditions, giving single doses of 75 mg suspended in 2 ml of distilled water.

3. The typical nodules developing in the abdominal peritoneum, alongside the linea alba, have been studied histologically 6 and 12 hours, as well as 1, 3, 5, 7, 10, 14, 20, 24, 30, 60 and 90 days following injection.

4. The histological study of the nodules appears to be suitable for the demonstration of the fibrogenic properties peculiar to different dusts, if the various cellular and fibrous elements (mainly the macrophages, as well as the reticular fibres) are examined as inseparable parts of a unit, in the process of their development.

5. The quartz-containing dead rock dust from the coal mine proved to be a fibrogenic agent similar to quartz. Talc proved to produce a fibrosis of "storing" nature, different from that caused by quartz. The quartz-free brown coal dust elicited merely a "foreign body" connective tissue response.

6. The intraperitoneal test is recommended as a valuable procedure for the evaluation of the fibrogenic properties of industrial dusts.

REFERENCES

1. BOVET, P. (1952): Die Wirkung von Graphit und anderen Kohlenstoffmodifikationen im Tierversuch; zugleich ein Beitrag zur experimentellen Silikoseforschung. Schweiz. Ztschr. allg. Path. Bakt. *15*, 548. — 2. CUMMINGS, D. E. (1929): Studies on Experimental Pneumoconiosis. IV. The Separation of Particulate Matter Smaller than Screen Sizes. J. Indust. Hyg. Toxicol. *11*, 245. — 3. GRANDJEAN, E., TURRIAN., and NICOD. J. L. (1956): The Fibrogenic Action of Quartz Dusts. Arch. Indust. Health *14*, 426. — 4. KING, E. J. und NAGELSCHMIDT, G. (1954): Die pathologische Wirkung verschiedener Mineralstaube im Tierversuch. In die Staublungenerkrankungen, edited by Jötten, K. W., Klosterkötter, W. and Pfefferkorn, G., Steinkopf, Darmstadt, *2*, 84. — 5. LUNDGREEN, K. D., and SWENSSON, A. (1953): Experimental Investigations on the Significance of the Size of the Particles in the Reaction of the Peritoneum to Amorph Silica. Acta med. Scand. *145*, 84. — 6. MARWYCK, G. H. (1954): Der Peritonealtest. In: Die Staublungenerkrankungen, edited by Jötten K. W., Kloserktötter, W. and Pfefferkorn, G., Steinkopf, Darmstadt, *2*, 95. — 7. MILLER, J. W., and SAYERS, R. R. (1953): Microscopic Appearance of Experimentally Produced Dust Nodules in the Peritoneum. Pub. Health Rep. *50*, 1619. — 8. POLICARD, A., COLLET, A.

(1954): Etude experimentale des effects pathologiques du kaolin. Schweiz. Ztschr. allg. Path. Bakt. 17. 320. — (1954): Condition et valeur pratique du test péritoneal de nocivité des poussières. Die Staublungenerkrankungen *2*, 111. — 9. RÜTTNER, R. J. (1950): Grossfollikuläres Lymphoblastom mit Ausgang in Reticulosarkom. Schweiz. Ztschr. allg. Path. Bakt. *13*, 92. — (1954): Die Gewebsreaktionen auf Mineralstäube im Peritonealtest an der Maus. Die Staublungenerkrankungen *2*, 106. — 10. SCHULZ, R. Z., and WILLIAMS, R. (1942): Commercial Talc Animal and Mineralogical Studies. J. Indust. Hyg. Toxicol. *24*, 75. — 11. TALVITIE, N. A. (1951): Determination of Quartz in Presence of Silicates Using Phosphoric Acid. Analyt. Chem. *23*, 623. — 12. WEBER, R.: Die Wirkung von Kaolin im intraperitonealen Mäuseversuch. Inaug. Diss. Cit. Marwyck.

ИССЛЕДОВАНИЕ МЕТОДОМ МИЛЛЕРА И САЙЕРСА ДЕЙСТВИЯ КВАРЦА, ДРОБЛЕННОГО ПЕСКА С СОДЕРЖАНИЕМ КВАРЦА, БУРОГО УГЛЯ И ТАЛЬКА НА СОЕДИНИТЕЛЬНУЮ ТКАНЬ МОРСКИХ СВИНОК

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Авторы проводили исследования по методу Миллера и Сайерса над 104 взрослыми морскими свинками обоих полов для определения действия кварца фирмы Мерка, глухой породы из одной каменноугольной копи в Венгрии с 77%-ым содержанием кварца, бескварцового бурого угля и талькового порошка. Фракции в 1—3 микронов исследуемых порошков взвешивались в количестве 75 милиграммов на животное в 2 мл дестиллированной воды и в стерильном состоянии внутрибрюшинно впрыскивались животным. Развивавшиеся на перпетальной брюшине типичные тканевые узлы микроскопически исследовались на 6. и 12. часы, а также на 1., 3., 5., 7., 10., 14., 20., 24., 30., 60. и 90. дни после обработки. Гистологическое исследование оказалось — при рассмотрении образования макрофагов и волокнистых элементов в процессе развитии во всей свой совокупности — пригодным методом для экспериментального выявления фиброгенетических свойств исследованных порошков. Из исследованных порошков глухая порода с содержанием кварца из каменноугольной копи оказалась — подобно чистому кварцу — вызывающим фиброз веществом. Тальк вызвал так наз. накопляющий фиброз, а бескварцевый бурый уголь обуславливал лишь соединительно-тканевую реакцию «инородного тела». Авторы того мнения, что значение внутрибрюшинной пробы для выявления фиброгенетических свойств промышленных порошков нашло путем их исследований новое подтверждение.

UNTERSUCHUNGEN DER WIRKUNG VON QUARZ, QUARZHALTIGEM REIBSAND, BRAUNKOHLE UND TALK AUF DAS BINDEGEWEBE VON MEERSCHWEINCHEN MIT DER MILLER-SAYERSSCHEN METHODE

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Die Wirkung von Merckschem Quarz, eines 77% Quarz enthaltenden tauben Gesteins aus einer ungarischen Steinkohlengrube, von quarzfreier Braunkohle und Talkpulver würde an 104 erwachsenen Meerschweinchen beiderlei Geschlechts mittels der Methode von Miller und Sayers untersucht. 75 mg pro Tier der 1—3 μ Fraktionen der Pulver wurden in 2 ml destilliertem Wasser suspendiert steril intraperitoneal eingespritzt. Die am Peritoneum entstehenden typischen Gewebsknoten wurden in der 6., 12. Stunde, bzw. am 1., 3., 5., 7., 10., 14., 20., 24., 30., 60. und 90. Tage nach der Behandlung mikroskopisch untersucht. Die histologische Untersuchung — die Entstehung der Makrophagen und von Fasernelementen als zusammenhängendes Ganzes im Prozess der Entwicklung betrachtet — erweis sich als geeignetes Mittel zur Feststellung der fibrogenetischen Eigenschaften der untersuchten Pulver. Aus den untersuchten Pulvern erweis sich das quarzhaltige taube Gestein der Steinkohlengrube fibrogenetisch, ähnlich dem reinen Quarz. Talk verursachte eine sog. Speicherungsfibrose, während quarzfreie Braunkohle bloss eine »Fremdkörper«-Bindegewebsreaktion hervorrief. Die Bedeutung der zur Feststellung der fibrogenetischen Eigenschaft von industriellen Pulvern gebräuchlichen intraperitonealen Methode wurde dadurch von neuem bestätigt.

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