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SPECIFIC TISSUE REACTION TO PHOSPHOLIPIDS: A SUGGESTED EXPLANATION FOR THE SIMILARITY OF THE LESIONS OF SILICOSIS AND PULMONARY TUBERCULOSIS

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INTRODUCTION

MANY of the pathological lesions of silicosis and pulmonary tuberculosis are identical. Fortunately for the experimental pathologist, the lesions of experimental silicosis and tuberculosis, while not identical with, are similar to those of the actual diseases in most respects. Pathological lesions represent a characteristic tissue response to a definite type of stimulation. The similarity of the lesions in these conditions would indicate that they are produced by substances much more alike in nature than are the tubercle bacillus and quartz. An obvious inference is that inhaled or injected finely particulate quartz powder produces either a physical or chemical change in some substance normally present in the body, and that this altered substance stimulates the formation of the typical pathological lesions found in these conditions.

The microscopic appearance of the early lesions of experimental silicosis[1, 2] suggests that the monocyte is in some way responsible for the later fibrotic reaction. There is an extensive monocytic response to the inhalation or injection of finely powdered quartz with later destruction and disintegration of these cells. Normally, the lung alveolus rids itself of any finely particulate material by phagocytosis and transportation by monocytes. Particles under 5 microns in size, regardless of their nature, are engulfed by monocytes. The monocytes with their load of fine particles either move to the upper air passages and are coughed up or migrate into the lymph spaces, thence to the regional lymphatic nodes, where the particles are disposed of in different ways, depending upon the nature of the material. If the phagocytosed material is not toxic to the monocytes this process goes on indefinitely, as in anthracosis. If the phagocytosed material is toxic it will produce death and disintegration of the monocytes, resulting in an interruption of the normal dust clearance from the alveoli and the formation of certain characteristic pathological lesions.

When large amounts of fine quartz dust are either inhaled by or injected into the lungs of experimental animals there is the usual active monocytic response to particulate material, that is, the phagocytic cells invade the area and become engorged with dust particles. A number of them reach the upper air passages and are coughed up; others reach the lymph spaces and are transported to the lymphatic nodes; still others are held in the alveolar spaces. The silica, either by its physical or chemical action, changes the intracellular material, causing death of the cell with disintegration of the cell membrane and discharge of the intracellular material and the contained quartz particles. There is no evidence to show that the quartz is changed. It still has the ability to stimulate a further monocytic response. According to our hypothesis, the liberated intracellular material is the substance that initiates the typical fibroblastic reaction with formation of nodules.

The different diseases of lipoid metabolism, such as Gaucher's, Neumann-Pick's, Tay-Sach's, Hand-Schiller-Christian's, etc., all show varying degrees of fibrosis. Sabin[3] and her collaborators[4] have shown that of all the fractions of the tubercle bacillus the lipoids alone produce

tubercles. Of their various controls only one material tested acts just like tuberculo-phosphatide, namely, lecithin. These lipoids produce a marked new growth of general connective tissue as well as tubercular granulation tissue and tubercle-like structures. Monocytes are known to contain a considerable amount of lipoid material. The fact that the tissue response to the injection of quartz is mainly a fibroblastic reaction would suggest that the stimulating agent might be lipoidal in nature. For this reason the toxic substance was sought in the lipoid fraction of early silicotic lesions.

Three groups of experiments were performed to determine:

Experiment I.—The presence and nature of the lipoids in early silicotic lesions and the amounts in which they occur.

Experiment II.—The rate and amount of increase in the phospholipids in the lungs of rabbits which had been injected intratracheally with finely particulate silica.

Experiment III. — Whether or not the recovered phospholipid when freed of silica and re-injected into animals will produce tissue reactions comparable to the pathological lesions found in silicosis and tuberculosis.

In order to determine the nature of the lipoids in early silicotic lesions and the amounts in which they occur the following experiments were conducted.

EXPERIMENT I

Eight rabbits were each given 250 mg. of finely powdered quartz (the particles ranging in size from 0.5 to 3 microns) intratracheally, intraperitoneally and subcutaneously. Two rabbits were killed at intervals of 1, 2, 3 and 4 months. The lungs, peritoneal and subcutaneous nodules were removed and extracted separately with ether. The lungs of seven normal rabbits were each extracted in a similar manner as a control on the silica lungs.

Extraction of lipoids from the lungs.—The rabbits were anæsthetized with ether and bled from the carotids and jugulars. Each lung was removed at the hylus and as much blood as possible was expressed. They were weighed and this weight taken as "wet weight". Thin sections were taken for histological study. The lung tissue was ground as finely as possible with 40 g. of coarse sand. Five volumes of ether were added and the extraction repeated five times. The ether extract was filtered several times and evaporated. The lung residue was then extracted with absolute alcohol. The alcohol was evaporated. The lipoid obtained by alcohol extraction was redissolved in ether and added to the ether extract. The combined ethereal solutions were evaporated. The residue after evaporation will be referred to as "total lipoid". The extracted lung tissue and sand were dried and weighed. This weight plus the "total lipoid" minus the weight of the sand is called "dry weight" of lungs.

The "total lipoid" was redissolved in ether and filtered into ice-cold acetone. A heavy, white, flocculent precipitate formed. The precipitate was separated by centrifuging and pouring off the supernatant acetone. It was then evaporated until all the remaining acetone was removed. A wax-like, yellowish-brown, viscous, semi-solid material remained.

Results.—The recovered substance on analysis had the physical and chemical properties of a phospholipid. It was then soluble in ether, chloroform and benzene; insoluble in acetone; could be separated into two portions by precipitation in alcohol; formed a fine, even, permanent emulsion with water; could be precipitated from water by acetone; oxidized readily in air, turning brown and having a disagreeable odour. On hydrolysis it yielded fatty acids, phosphoric acid and nitrogen in the proportions found in the phospholipids. It appeared to be a mixture of lecithin and cephalin, or a closely related phospholipid. This material is referred to as "crude phospholipid".

Normal lungs of 9 to 12 g. "wet weight" and 2.5 to 3.8 g. "dry weight" yielded 200 to 290 mg. of lipoid from which an average of 50 mg. of crude phospholipid was obtained (see table). The lungs of rabbits 1 to 4 months after the intratracheal injection of finely powdered quartz yielded 480 to 1,100 mg. of lipoid containing 300 to 420 mg. of crude phospholipid. The peritoneal and subcutaneous nodules also contained considerable amounts of similar material. Pieces of subcutaneous tissue equal in weight to the nodules did not yield any acetone precipitate.

Conclusions.—The greatly increased amounts of phospholipids in the tissues of rabbits after injection of finely particulate quartz suggests that these substances were attracted to the regions by the presence of the quartz. Correlation of the histological picture and chemical findings suggests that the source of the phospholipid was the monocyte. The fact that this substance was found to be increasing with time and the number of monocytes containing quartz particles, seen in histological sections, naturally led to the following group of experiments.

The following experiments were performed to determine the rate and amount of increase in the phospholipids of rabbit lungs injected intratracheally with finely particulate silica. As a control, animals were injected in the same manner with equal amounts of kaolin, the particles of which were of corresponding size. It is an established fact that kaolin alone does not produce fibrosis.

EXPERIMENT II

Fourteen rabbits were injected intratracheally, each receiving 250 mg. of finely particulate quartz (0.5 to 3.0 microns) suspended in 2.5 c.c. of distilled water. Seven rabbits were given similar amounts of kaolin by the same method. Two of the silica-injected rabbits and one kaolin-rabbit were killed 1, 2, 3, 6, 12, 16 and 20 weeks after injection. The phospholipids were extracted from the lungs as described above.

Results.—The material injected, time interval, wet weight, dry weight, total lipoid and crude phospholipid are shown in the accompanying table. The rate and amount of increase in lung phospholipid are shown on the graph.

TABLE I.

Rabbit No.	Material injected	Time interval	Wet weight	Dry weight	Total lipoid	Crude phospholipid
Normal lungs	mg.		g.	g.	mg.	mg.
1	–	–	10.5	–	250	30
2	–	–	12.30	3.0	200	28
3	–	–	9.86	3.8	265	93
4	–	–	9.21	3.2	290	71
5	–	–	8.64	2.58	250	24
6	–	–	11.96	2.21	260	41
7	–	–	9.03	2.15	290	48
Pneumonic lungs						
8	–	–	41.34	4.58	480	64
9	–	–	19.67	3.86	280	134
SiO_2 lungs	SiO_2					
32	250 mg.	1 wk.	15.15		430	81
33	" "	1 "	11.50		390	95
34	" "	2 wks.	7.41	2.68	340	107
35	" "	2 "	7.60	2.13	320	55
37	" "	3 "	8.21	2.05	230	130
38	" "	3 "	15.08	5.65	240	200
39	" "	6 "	11.70	3.86	380	180
40	" "	6 "	9.30	3.50	480	380
41	" "	12 "	11.19	4.26	500	373
42	" "	12 "	10.48	3.34	580	352
43	" "	16 "	10.05	2.43	490	397
18 old	" "	16 "	14.56	5.36	720	420
44	" "	20 "	19.21	3.74	790	218
45	" "	20 "	21.18	4.25	580	227
Kaolin lungs	Kaolin					
46	250 mg.	1 wk.	24.80		350	30
47	" "	2 wks.	13.92	4.87	580	89
48	" "	3 "	15.78	6.90	730	160
49	" "	6 "	12.58	4.26	490	110
50	" "	12 "	9.93	3.72	290	121
51 (*pneumonic*)	" "	16 "	15.62	3.15	460	120

Conclusions.—There is a gradual increase in the amount of phospholipid present in the silica lungs for four months. This runs parallel with the histological picture, namely, phagocytosis of the silica, the breaking down of the monocytes, invasion of the area by fibroblasts and the early fibrous tissue reaction. After this there is a falling off in the amount of recoverable phospholipid.

The decreasing amount of phospholipid corresponds in time with the histological picture of fibrosis. The monocytes have disappeared from the central portions of the nodules. They have been replaced by typical fibroblasts. The newly-formed connective tissue is becoming hyalinized. The breaking down of monocytes has become limited to small numbers at the margins of the nodules, where there is still some active fibroblastic reaction. The original large amount of phospholipid has probably, after stimulating the growth of fibrous tissue, been removed or changed by this defense mechanism. The monocytes are now only attracted to the margins of the nodules, where small numbers are broken down and there is a slowly progressing stimulation of fibrous tissue growth. The change in the tissue reaction probably accounts for the decrease in the amount of phospholipid. The amount of phospholipid recovered from the lungs of rabbits appears to correspond with the number of disintegrating monocytes seen in the histological sections.

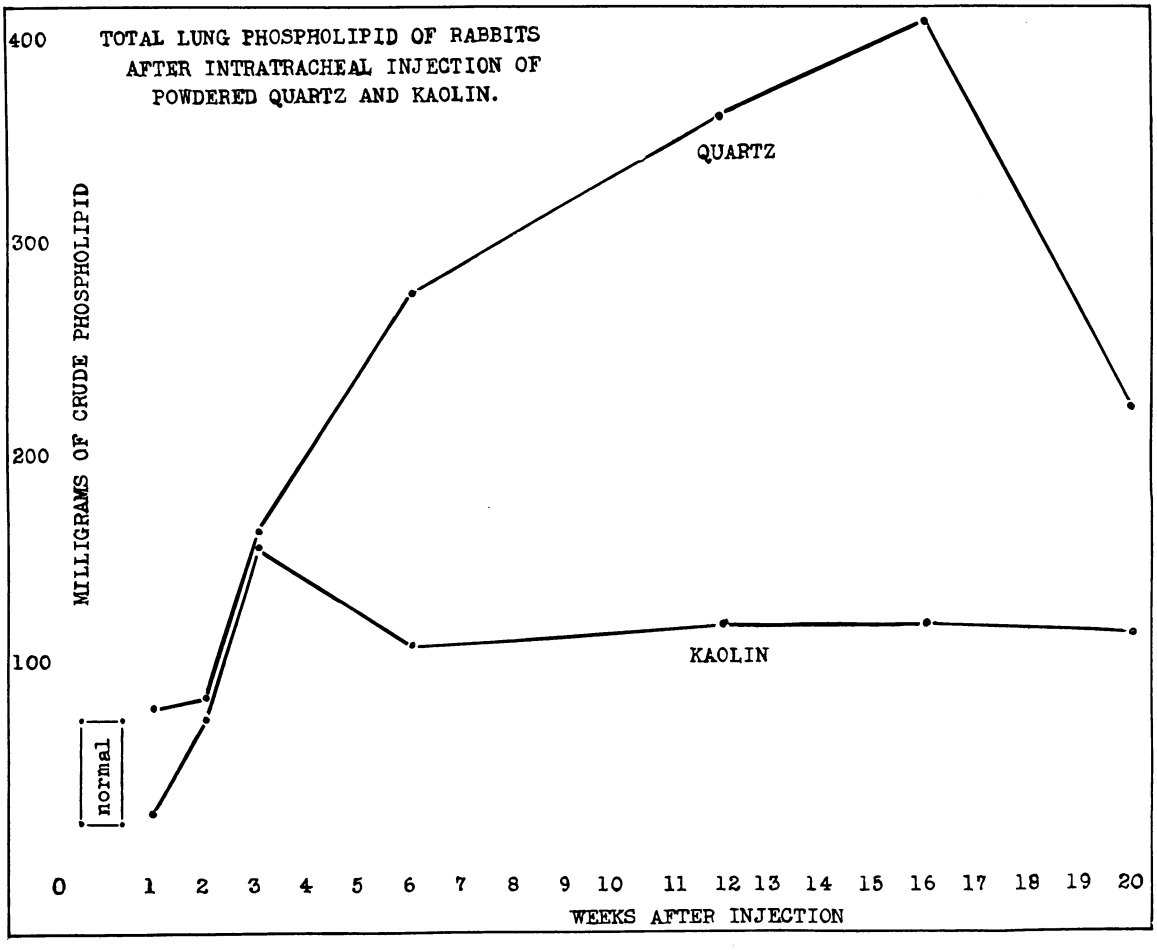
There is a small initial rise in the amount of phospholipid in the kaolin lungs. This is probably accounted for by the mere presence of the monocytes. The curve relating lung-phospholipid with time remains a straight line for months. The maintained slight elevation above the normal phospholipid level is probably accounted for by the engulfing of kaolin by monocytes and foreign-body giant-cells. These cells are seen in microscopic sections to remain in the lungs in increased numbers for long periods of time in a perfectly healthy state.

In the case of the animals receiving intratracheal injections of finely particulate quartz there is a marked increase in the phospholipid of all lungs showing the typical early tissue response. If our hypothesis (that the phospholipid liberated from the monocyte is responsible for the typical cellular reaction) is correct, injection of this phospholipid material obtained from the lungs should produce lesions comparable to those found in experimental silicosis and tuberculosis. The fact that Sabin and others have been able to produce fairly typical tubercles with the phospholipid fraction of tubercle bacilli and with pure lecithin would suggest that the greatly increased amounts of phospholipid present in the lungs after intratracheal injections of silica are responsible for the production of the fibrous nodules which so closely resemble tubercles.

The following experiments were performed to determine the tissue reaction to intraperitoneal injections of recovered, silica-free phospholipid.

EXPERIMENT III

The recovered phospholipid dissolved in ether was filtered to remove any particulate silica. Microincineration of the recovered phospholipid and acid treatment of the ash showed this material to be free from siliceous particles. Sabin's method of injection was followed in order that the lesions produced in our animals could be compared with those which she produced and so clearly described. Sabin, in her production of tubercles with the phospholipids of tubercle bacilli, gave daily intra-



peritoneal injections of 80 and 122 mg. for periods of from 1 to 15 days. The animals were then killed at intervals from twenty-four hours to six months after the last injection.

Experiment.—Daily doses of 18.5 mg. of recovered phospholipid in emulsion in 2.5 c.c. of distilled water were injected into the peritoneal cavities of four rabbits daily for fourteen days. The rabbits were killed 1, 2, 3 and 4 weeks after the last injection.

Results.—The pathological lesions found in the four rabbits injected intraperitoneally with the recovered phospholipid were all very similar, both in the gross appearance and microscopic sections.

Grossly, the peritoneal surfaces were smooth, moist and glistening except for a few fine adhesions between adjacent loops of bowel and a number of discrete nodules either subserosal or adhering to the surfaces of the liver and spleen and extending into these organs. There was a generalized prominence of the milk-spots. The omentum was thickened and contained a number of rounded, firm, grey-pink granular nodules situated between its peritoneal layers. It was not adherent to other structures in any of the animals. The nodules adhering to the peritoneal surfaces and in the subserosa varied in size from 0.1 to 2 cm. A number of the larger nodules on section showed central necrosis. They contained considerable amounts of thick, yellow caseous material. This material did not resemble the pus usually found in rabbits.

Microscopic sections of the different nodules, stained with hæmatoxylin and eosin, showed pathological changes varying from small clusters of foreign-body giant-cells and monocytes filled with lipoid granules and surrounded by lymphocytes and fibroblasts to dense masses of connective tissue. Fairly typical tubercle formation is seen in many of these foci. The general picture is one of a central aggregation of epithelioid cells, monocytes and foreign body giant-cells filled with lipoid material, interspersed with lymphocytes and surrounded by layers of epithelioid cells and fibroblasts. Langhan's giant-cells in varying numbers are seen in some of these nodules. A number of the larger nodules show varying amounts of amorphous, acidophilic debris. In numerous foci the fibroblasts tend to be arranged in whorls closely resembling the lesions of tuberculosis, silicosis and experimental silicosis.

Conclusions.—The pathological findings were similar to those in the animals treated with particulate silica and comparable to those produced by Sabin with her tuberculo-phospholipids and brain lecithin.

The accompanying photomicrographs are taken from several typical microscopic fields.

COMMENT AND CONCLUSIONS ON EXPERIMENTS I, II AND III

A phospholipid has been extracted from early experimental silicotic lesions. The amount increases in proportion to the extent of the early cellular reaction. This material was also present in very slightly increased amounts in kaolin-produced lesions. This material, when free of

silica and re-injected into the peritoneal cavities of rabbits, produces a fibrotic reaction similar to that produced by the injection of finely particulate quartz. These lesions are also very similar to those produced by Sabin with the phospholipid fractions of tubercle bacilli. The recovered phospholipid, in much smaller amounts than those used by Sabin, produces fairly extensive lesions. It is suggested that the characteristic tissue reactions of silicosis and tuberculosis are foreign-body reactions to phospholipids. This accounts for the identical reac-

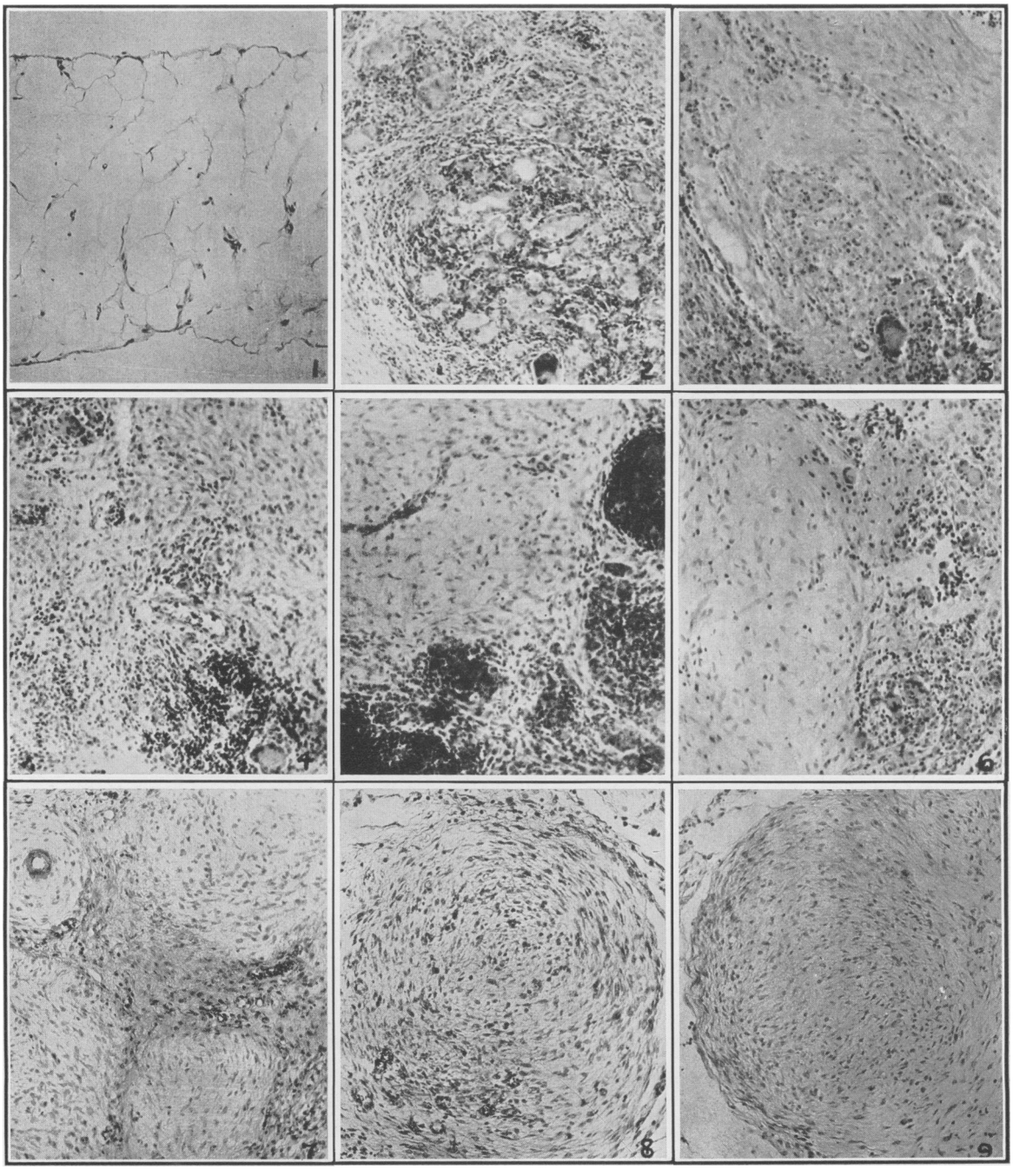


Fig. 1.—Normal rabbit omentum. **Fig. 2.**—Early lesions in rabbit omentum produced by intraperitoneal injection of silica-free phospholipid recovered from the lungs of rabbits with experimental silicosis. The section shows the phospholipid in monocytes and foreign-body giant-cells, lymphocytic infiltration and one Langhan's giant-cell. **Figs. 3, 4, 5 and 6.**—Sections from rabbit omentum showing a tubercle-like reaction consisting of zones of lymphocytes and fibroblasts surrounding phospholipid deposits with occasional endothelial giant-cells. **Figs. 7, 8 and 9.**—Sections of rabbit omentum showing rounded aggregations of fibroblasts which resemble the lesions in experimental silicosis.

tion to inhaled quartz dust and the tubercle bacillus in silicosis and pulmonary tuberculosis.

Silicates, for example, kaolin, stimulate an early monocytic response similar to that of quartz, but the subsequent chemical and histological findings are different. The silicates are taken up by monocytes and foreign-body giant-cells and remain apparently inert in these cells. The kaolin lungs used as a control showed a slightly increased phospholipid content throughout the experiment. This may probably be accounted for by the small increase in number of this type of cell. The reason that the silicates do not produce fibrosis may be that they have no effect, or only a minimal one, upon the metabolic processes of the monocytes.

It is evident from the work of Sabin and others that either the phospholipids of the tubercle bacilli alone, or in combination with the lipoids of numbers of disintegrated monocytes, produce the typical pathological lesions of tuberculosis. Early typical lesions of tuberculosis will disappear when the process is checked. This is not the case in the lesions of silicosis. The difference is probably due to the fact that when the tubercle bacilli are all destroyed the toxic phospholipids are gradually phagocytosed and removed, with the later removal of the scar tissue. In silicosis the monocytes become loaded with quartz particles; there is a physico-chemical change in the monocyte that causes disintegration of the cell and discharge of its contents; the liberated silica, which is practically unchanged, can now stimulate a further monocytic response with further disintegration and so on, indefinitely. The extent of the reaction depends upon the amount of inhaled silica which is imprisoned in the lung.

SUMMARY

1. The amount of phospholipid in the lungs of rabbits increases rapidly after intratracheal injection of finely particulate quartz.

2. The increasing amount of phospholipid corresponds with the type and intensity of the cellular reaction.

3. Re-injection of the recovered (silica-free) phospholipid stimulates a fibrous tissue reaction similar to that produced by the injection of finely particulate quartz.

4. A reason for the similarity of the lesions of silicosis and pulmonary tuberculosis is suggested.

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HISTOLOGICAL VARIATIONS IN FETAL CALVES' THYROIDS AND A COMPARISON WITH MATERNAL THYROIDS*

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IN recent publications,[1, 2] we have demonstrated that the normal thyroid varies a great deal in its histological appearance and that these variations are to be considered normal. Marine originally described the normal thyroid as a mass of rounded acini, varying slightly in size, and with walls formed of uniform low cubical cells with small, darkly-staining nuclei. The cells are arranged in a single layer. The stainable colloid is dense and homogeneous, and, staining uniformly, it sharply abuts upon the epithelial cells. Marine believed that a columnar cell is pathological. Wilson,[3] on the other hand, states that the normal epithelium is almost entirely columnar in type and that the low cuboidal form is usually found only in pathological states. In contradistinction to this we found that the thyroid gland must be given a much wider range of physiological variation, depending on

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