SILICATES VOL II #56
COPIES OF ARTICLES CITED IN
MONOGRAPH SUMMARY

R26

Silicates-Volume 2 #56 9/28/73

VOLUME 2

GRAS MONOGRAPH SERIES

SILICATES

(COPIES OF ARTICLES CITED IN MONOGRAPH SUMMARY)

prepared for

THE FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION
AND WELFARE

SEPTEMBER 28, 1973

prepared by

Tracor Jitco, Inc.

Bull. Tokyo Med. Dental Univ. 6(3): 383-411, 1959

STUDIES ON THE SILICA IN ANIMAL TISSUE

THE RELATIONSHIP BETWEEN SILICA IN DRINKS AND FOODS AND IN HUMAN BLOOD VESSELS

BY

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INTRODUCTION

At present, it is reported that silica is one of main components in the earth crust, and is generally in the botanical kingdom too, above all, *Gramineae* containing much amount of it. Although silica is also found as a micro component in the animal body, but this has been ignored for long time as a harmless compound to human body in clinical and fundamental medicine. Silicosis, as a well known occupational disease, has been only an object for medical research.

Up to the present, very few biochemical and pathochemical studies on the silica have been reported, but the meaning of its existence in the animal body has not been clarified yet. In recent years, a few attention have come to be focussed upon the silica in the biological materials.[1,2,3,4,5,6].

Silica is contained in natural water, for example, mineral water, hot spring, city water and well-water. In these kinds of water in Japan silica is contained greater in amount than that in other countries, in Europe, America and India. Therefore, the authors supposed that silica may have any effect on the metabolism of animal body as to whether it will be harmfull or useful for body, and performed several experiments.

First, Akiya, one of the authors, analyzed some drinking water of countries in Europe, America and Asia and the sea water in a northern and a central part of Pacific Ocean. The results of the these analyses showed that in general, the drinking water in Japan contained more amount of silica. At the same time, Akiya and Tanimura[7,8] had analyzed the silica contents in the normal and diseased human tissues from corpses as well as the urinary excretion of silica from living human body.

Misawa,[9,10,11,12] one of the authors, have investigated in detail the correlation between silica content in drinking water and the vascular injuries of the central nervous system which have been the first class rate in the Japanese mortality for the past several years, and it was found that one prefecture in where drinking water contained more amount of silica, had higher mortality

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caused by vascular injuries of the central nervous system.

Therefore, it was presumed that silica in the human blood vessels might have been one cause of developing sclerosis of them, besides calcium and cholesterol. So far, there has been only reported on silica in the human aorta by King.[13]

The authors separated the human aorta into the three layers, intima, media and adventitia respectively under strict inspection by the pathologists, and estimated silica content in each layer. From these results, the correlation between silica content and cause of death, or age was discussed.

Materials

1) Drinking water

Drinking water in various districts in Japan, Europe, North and South America, and Southeastern Asia which Akiya sampled on his world travel for the past several years, were used as materials for the analysis. All samples filled in the polyethylene bottles were sent to authors' laboratory.

2) Organs

The normal and diseased human organs from corpses used as materials were the liver, kidney, heart muscle, arteria basilaris and aorta, which were supplied by Departments of Pathology, Anatomy and Legal Medicine, University of Tokyo. The organs of finback whales used as materials were the aorta, heart muscle, kidney, ureter, intestinal mucosa, skin and baleen. These whales were about 49 to 61 ft. in body length and were captured in East China Sea in 1958 by Taiyo Fishery Co.

3) Aortae

The aortae of the Japanese were supplied by Department of Pathology, Tokyo Medical and Dental University, and Tokyo Medical Examiners Office.

The aortae of the German dried after fixing with formalin, which Dr. M. Ichioka (Department of Physiology, Tokyo Medical and Dental University) had sent to the authors from Erlangen University in Germany, and so these aortae could not be separated into three layers.

Experimental Methods

Determination of silica

1) Drying and ashing of samples

It is necessary to destroy the organic material by ashing in the dry way prior to a determination is made on a sample of tissue. The tissue minced was put into a petri dish covering the inside with polyethylene film to protect the sample from being closely in contact with the glass, and the sample was dried in a gas-drier at 100° to 105° to become a definite weight (about 60 hrs.) and further dried in vacuum desiccator for more than 48 hours. 100 to 200 mg of dry tissue was accurately weighed and was put into a platinum crucible. After

ashing in the crucible in an electric muffle furnace, the ash thus obtained was weighed.

In case that the sample was drinking water, it was put into a platinum crucible and evaporated to the dryness on the steam bath following adding every each 1 to 2 ml of the sample succesively, and then the residue obtained was ashed.

2) Preparation of test solution

The ash was boiled with 0.5 to 1.0ml of 60% $HClO_4$ for 15 minutes. As all other constituents of the ash than silica were rendered by this treatment, the reaction solution above obtained could be filtered off leaving the silica behind. After cooling, to that 10 ml. of 1% $HClO_4$ was added, and sucked away through the filter paper on micro glass filter. The residue on the filter paper was washed with 1% HCl and the washings sucked off. The filter paper having silica was put into a platinum crucible and fused with 0.3 g. of anhydrous Na_2CO_3. After cooling, into the crucible N-HCl was added, and the HCl solution transferred from the crucible in a volumetric flask and diluted with H_2O up 50 ml. The suitable pH value of the solution was 3.0 for the colorimetric

Chart 1.

Tissue
↓ Drying, Weighing
Ashing ⟶ Weighing of Ash
↓ Add 0.5-1.0 ml. of 60% $HClO_4$
Heat for 15 min.
↓ Add 10 ml. of 1% $HClO_4$
Filtration

Filtrate

Precipitate
↓ Washing with 2-3 ml. of 1% HCl
Ashing
↓ Add 0.3 g. of Na_2CO_3
Fuse
↓ Add N-HCl and H_2O
Make 50 ml. (pH 3.0) (Test Solution)
↓
25 ml. of Test Solution
↓ Add 2 ml. of N-H_2SO_4 and 1 ml. of 10% $(NH_4)_2MoO_4$
Stand for 15 min.
↓ Add 2ml. of 20% Tartaric Acid
Stand for 2 min.
↓ Add 0.5 ml. of Reducing reagent*
Stand for 20 min.
↓
Measure color at 850 mμ

* Reducing reagent was made as follows: 1 g. of 1, 2, 4-aminonaphthol sulfonic acid was mixed with 60g. $NaHSO_3$, and 7.5g. of this mixture dissolved in 50 ml. of H_2O. This reagent solution must be stored in a brown bottle.

determination and it should be adjusted.

3) Development of color

The principle[14] of this method is based on the developing of molybdenum blue by following reduction of yellow silicomolybdic acid complex with 1, 2, 4-aminonaphthol sulfonic acid. Molybdenum blue was measured at 850 mμ by spectrophotometer. As the co-existence of phosphoric acid in this coloring solution interferes the colorimetric determination of silica, it is necessary to remove the interference of phosphoric acid by addition of the tartaric acid. Tartaric acid develops yellow color of iron tartrate with small amount of Fe ion, but this yellow color is not interfering at 850 mμ.

These procedures of colorimetric determination of silica in tissues, urine and water were shown in Chart 1.

Results and Discussion

1) Silica in drinking water

The authors has determined silica contents in many samples of water. In these experiments, the industrial water of a certain factory in Mie Prefecture in Japan, contained 130 ppm. of silica, which showed the highest in all samples from many countries. In Japan, the drinking water of the lowest silica content was the city water of Kyoto and Osaka area, their fountain was the River Yodo in Kyoto Prefecture, and it was contained about less than 7 ppm. of silica.

In the Tokyo area, the city water from the River Tama contained about 10 to 15 ppm. of silica and the other from the River Edo about 30 to 40 ppm. The well water in this area contained about 30 to 60 ppm. of silica. The well water in the main rice producing districts in Japan, for example Akita, Yamagata, Niigata, Ibaragi, Tochigi and Chiba Prefectures, contained about 40 to 70 ppm. of silica, and in many other prefectures it was contained about 10 to 30 ppm.

The sea water of the Pacific Ocean examined contained only about 0.5 to 3 ppm. of silica, while in general the city water in Europe and America contained about 2 to 7 ppm. of silica. Silica content and hardness of the drinking water in Europe, America and Southeastern Asia were shown in the Table 1.

From those results, it was found that the Japanese have been living on the drinking water which containing larger amount of silica than in the other countries.

2) Relationship between silica content in the drinking water and the mortality caused by the vascular injuries of the central nervous system in Japan.

Akiya has estimated the silica contents in drinking water of several countries in the world, and found that the drinking water in Japan contained larger amount of silica than in the other countries. Misawa has analyzed the silica content in the drinking water in each prefecture in Japan, and then these contents have been compared with the mortality caused by the vascular injuries of the central nervous system in each prefecture.

Table 1. SiO_2 and $CaCO_3$ contents in water of America, Europe and Southeastern Asia.

Place	Country	Sorts of water	Free Si as SiO_2 (ppm)	Total Si as SiO_2 (ppm)	Hardness $CaCO_3$ (ppm)	Remarks
San Francisco	U. S. A.	Drinking water		2.28	32.07	City water
Saõ Paulo	Brazil	〃		2.89	45.82	〃
Campinas	〃	〃		18.45	54 97	〃
Frankfurt	Germany	〃		7.38	32.07	〃
Hamburg	〃	〃		3.94	227.91	〃
Paris	France	〃		4.92	265.71	〃
Basel	Switzerland	〃		4.64	100.79	〃
Teheran	Persia	〃	8.0	19.2	176.0	Well water
Karachi	Pakistan	〃	6.0	8.0	100.0	City water
Bombay	India	〃	7.8	26.4	25.2	Lodging House
〃	〃	〃	17.8	42.4	590.0	Khora-village
〃	〃	〃	19.4	29.2	250.0	〃
Calcutta	〃	Bath water	16.2	34.4	460.0	Grand Hotel
〃	〃	Drinking water	17.2	22.0	430.0	〃
〃	〃	〃	6.8	13,8	72.8	Toyo Menka Co.
New Delhi	〃	〃	3.9	80.0	172.0	City water
Rangoon	Burma	Lake water	5.9	13.8	16.3	Hlawghr Lake
Singapore	Singapore	Drinking water	6.4	9.4	28.5	City water
Jakarta	Indonesia	〃	8.6	21.8	32.6	〃
Bangkok	Thailand	〃	6.4	17.6	36.2	〃
Phnom Pene	Cambodia	〃	13.4	16.6	68.0	〃
Saigon	Vietnam	〃	8.6	16.8	12.0	〃
Manila	Philippine	〃	24.4	29.2	36.0	〃

According to the recent statistics by the Ministry of Welfare of Japan, the mortality caused by the vascular injuries of the central nervous system was the lowest in Osaka Prefecture, and lower in Hyogo, Hokkaido, Tokyo and Kyoto Prefectures and higher in Akita and Nagano Prefectures.

These statistics of the mortality in Japan were shown in the Table 2.

It was considered that the drinking water in the districts having higher mortality caused by the vascular injuries of the central nervous system contained greater amount of silica, and the districts having lower mortality contained smaller amount of silica. Therefore, the relationship between silica content in the drinking water and the mortality caused by the vascular injuries of the central nervous system was investigated statistically, and it was proved that there was the significant linear correlation in both facts.

These results were shown in Fig. 1. Accordingly, it is assumed that the man who lives in the district where silica content in the drinking water is high has a tendency to fall hypertension or apoplexy.

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Tabl 2. Mortality caused by vascular injuries of central nervous system in Japan.

1952		1953		1956	
Prefecture	Mortality per 100,000 population	Prefecture	Mortality per 100,000 population	Prefecture	Mortality per 100,000 population
Akita	194.97	Nagano	194.79	Akita	210.4
Iwate	183.68	Akita	192.55	Nagano	204.7
Yamagata	178.16	Ibaragi	187.33	Yamagata	198.5
Nagano	177.77	Iwate	182.04	Kôchi	193.3
Ibaragi	173.78	Yamagata	181.05	Niigata	191.2
Niigata	171.78	Niigata	174.09	Iwate	190.6
Chiba	163.01	Chiba	172.34	Ibaragi	190.5
Saitama	161.98	Fukushima	170.67	Fukushima	189.5
Fukushima	159.73	Saitama	167.75	Shimane	185.6
Tochigi	159.35	Tochigi	165.98	Chiba	184.6
Kôchi	156.60	Shimane	161.11	Saitama	173.9
Shimane	150.87	Kôchi	160.15	Yamanashi	173.2
Miyagi	149.15	Ishikawa	152.71	Tochigi	171.8
Tottori	147.75	Miyagi	150.83	Tottori	171.4
Aomori	146.62	Aomori	146.19	Okayama	166.1
Gumma	142.20	Gumma	144.92	Ishikawa	163.8
Ôita	140.31	Toyama	141.23	Ôita	163.2
Ishikawa	138.33	Ôita	141.21	Gumma	161.4
Toyama	137.17	Tottori	143.16	Yamaguchi	161.0
Gifu	135.52	Yamanashi	142.95	Gifu	160.6
Okayama	135.16	Kumamoto	141.75	Miyagi	158.1
Yamanashi	134.98	Gifu	140.82	Kumamoto	157.7
Kumamoto	132.78	Kagoshima	139.69	Aomori	157.5
Nara	130.93	Shizuoka	136.78	Toyama	156.7
Yamaguchi	129.93	Shiga	136.64	Shizuoka	154.2
Mean Value	128.39	Okayama	136.08	Nara	153.1
Miyazaki	127.91	Mean Value	133.67	Mie	152.3
Fukui	126.82	Yamaguchi	130.38	Shiga	148.9
Shizuoka	126.33	Saga	129.82	Mean Value	148.2
Saga	124.94	Wakayama	129.18	Fukui	146.0
Aichi	123.83	Tokushima	128.88	Wakayama	143.9
Kagoshima	123.73	Miyazaki	128.03	Kagoshima	143.8
Shiga	123.65	Mie	127.05	Tokushima	142.3
Mie	122.30	Fukui	126.40	Kagawa	141.8
Wakayama	118.59	Aichi	125.70	Saga	141.2
Tokushima	118.28	Hiroshima	125.20	Hiroshima	140.4
Hiroshima	117.85	Nara	123.96	Miyazaki	140.2
Kanagawa	117.56	Kanagawa	117.43	Aichi	135.7
Kagawa	115.97	Kagawa	116.81	Kyoto	131.0
Fukuoka	110.33	Kyoto	114.72	Ehime	130.9
Ehime	108.91	Nagasaki	109.95	Fukuoka	129.2
Nagasaki	106.39	Fukuoka	109.57	Kanagawa	129.0
Kyoto	104.30	Ehime	107.57	Nagasaki	122.3
Tokyo	103.62	Tokyo	105.21	Hyogo	119.1
Hokkaido	101.68	Hokkaido	102.62	Tokyo	116.7
Hyogo	100.73	Hyogo	96.10	Hokkaido	109.0
Osaka	89.23	Osaka	87.77	Osaka	103.0

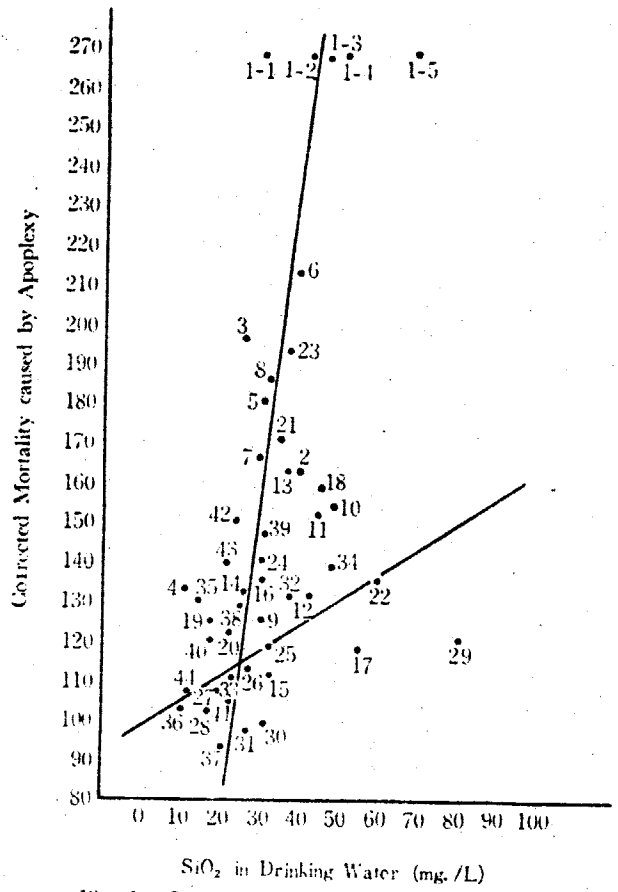

Fig. 1. Correlation between SiO_2 content in drinking water and corrected mortality caused by apoplexy. (Number indicates the name of prefecture.)

No.	Prefecture
1-1	Akita
1-2	Noshiro (Akita)
1-3	Osawago (Akita)
1-4	Takanosu (Akita)
1-5	Oklate (Akita)
2	Nagano
3	Yamagata
4	Kôchi
5	Niigata
6	Iwate
7	Ibaragi
8	Fukushima
9	Shimane
10	Chiba
11	Saitama
12	Yamanashi
13	Tochigi
14	Tottori
15	Okayama
16	Ishikawa
17	Ôita
18	Gumma
19	Yamaguchi
20	Gifu
21	Miyagi
22	Kumamoto
23	Aomori
24	Toyama
25	Nara
26	Mie
27	Fukui
28	Wakayama
29	Kagoshima
30	Tokushima
31	Kagawa
32	Saga
33	Hiroshima
34	Miyazaki
35	Aichi
36	Kyoto
37	Ehime
38	Fukuoka
39	Kanagawa
40	Nagasaki
41	Hyogo
42	Tokyo
43	Hokkaido
44	Osaka

3) *Excretion of silica in urine.*

i) *Relationship between silica content and pH value of the urine.*

The excretion of silica from human bodies was examined. After the definite diet was administered to human body, the pH value and silica content of the urine were measured.

350 g. of buckwheat (Japanese "soba") which contained usually about 35 to 45 mg. of silica was administered as the diet to be made the intake amount of silica constant. 2 to 10 g. of citric acid[15] or $NaHCO_3$ was administered separately or together to elevate the pH value of the urine after taking such diet. The relation between pH value and silica content of the urine was shown in Fig. 2.

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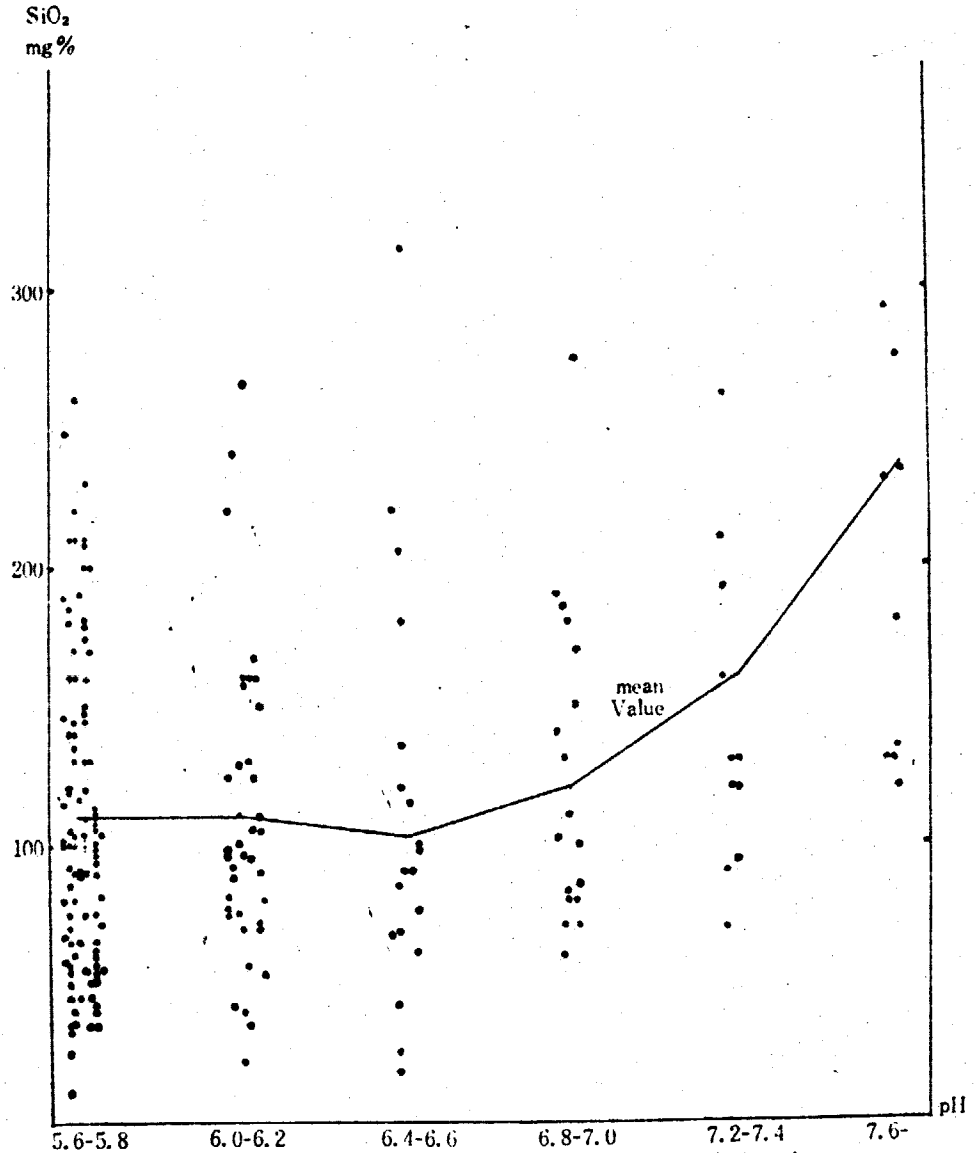
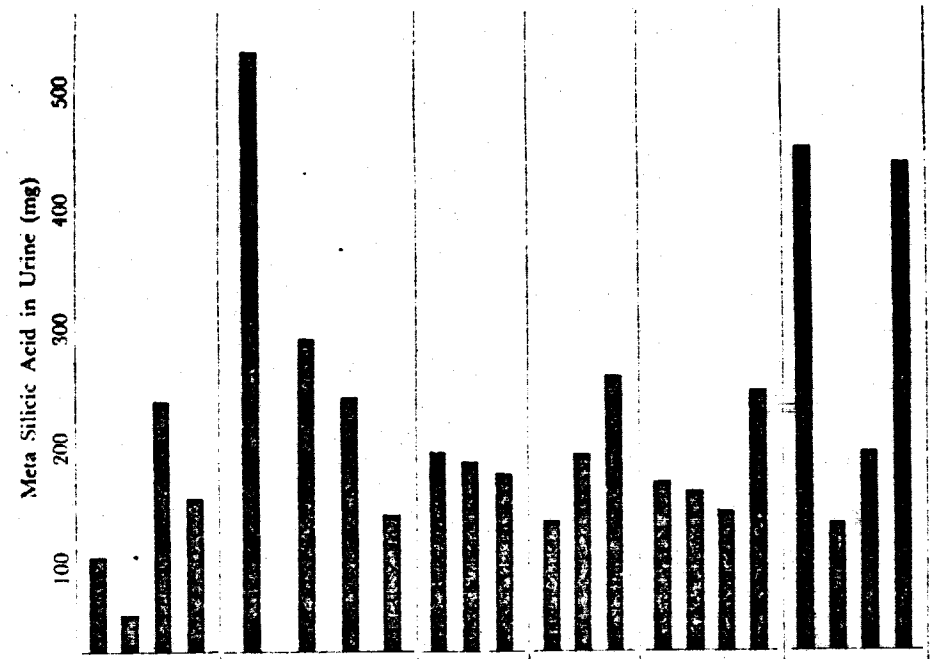


Fig. 2. The relation between pH value and SiO_2 content in urine.

In this experiment, the definite diet was administered only at lunch time, and breakfast and supper were not controlled, so urinary excretion of silica were considerably varied, but in general, the mean value of silica content in the urine increased with elevation of its pH value. The urine which pH value was more than 7.4 contained about twice amount of silica in the urine which pH value was less than 6.0. The single administration of small amount of $NaHCO_3$ without citric acid similarly caused increase of excretion of silica in



Drugs and Foods Administered	Total Urine cc	Specific Gravity	pH	
Usual Diet	1140	1.019	5.60	102.26
Food Cooked by Distilled Water	1280	1.025	6.22	26.62
Potassium Citrate 4.0 g	1220	1.023	5.80	235.63
Potassium Acetate Solution 12.0 g	1250	1.022	6.52	152.75
Potassium Citrate 3.0 g, Potassium Bicarbonate 1.5 g	1160	1.022	6.25	532.32
Sodium Bicarbonate 1.0 g, Potassium Citrate 4.0 g, Malic Acid 0.5 g	770	1.030	5.72	289.29
Sodium Bicarbonate 4.0 g	1150	1.020	6.02	228.74
Potassium Bromide 2.0 g, Sodium Bicarbonate 1.0 g	865	1.025	5.60	128.19
Vitamin C 100 mg	1380	1.020	5.90	181.19
Vitamin C 500 mg	800	1.022	5.76	170.56
Vitamin C 1000 mg	1120	1.019	5.70	168.90
Tangerine 4	1050	1.022	5.50	121.49
Tangerine 6	900	1.026	5.85	177.84
Tangerine 10	1100	1.020	6.10	248.82
Summer Tangerine 3	1850	1.012	5.90	158.73
Shaddock 2	730	1.026	5.70	150.89
Banana 3	765	1.020	5.82	123.32
Apple 3	1500	1.015	6.27	232.05
Navel 3	850	1.024	5.90	447.53
Milk 180 cc	1470	1.021	5.90	112.75
Milk 540 cc	1820	1.012	5.96	182.18
Milk 1080 cc	1560	1.020	6.15	436.02

Fig. 3. Effects of drugs and foods on the excretion of meta silicic acid in urine.

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the urine.

Okumura[16] reported that when silica was orally administered, silica content of each organ increased maximum that is 86% of usual amount. Standing on this fact and authors' experiments, it is considered that pH value of the body fluid has some significance on depositing of administered silica on the tissue, and then its excretion was accelerated at more than 7.0 of pH value of body fluid.

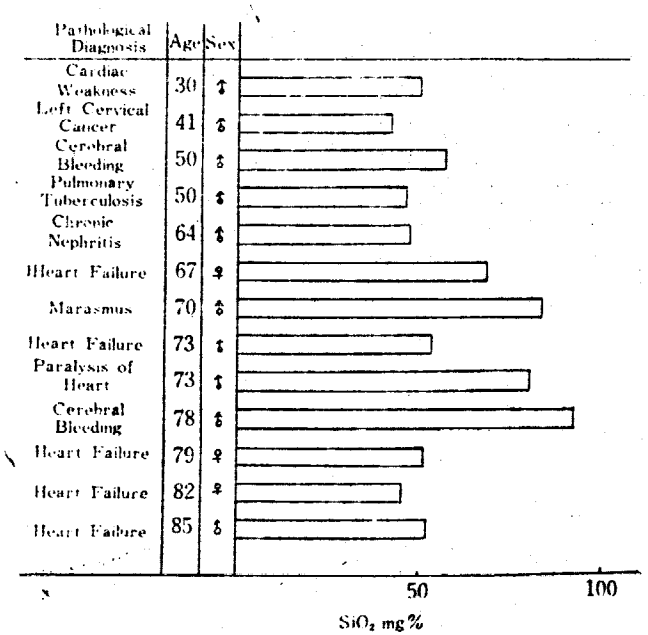
ii) *Effects of drugs and foods on the excretion of meta silicic acid in urine.*

Since the Japanese lives on taking large amount of silica from the drinking water and rice every day, studies on the finding the way of promoting excretion of silica was carried out.

When the usual diet, bicarbonate, salts of organic acids, especially, related to T.C.A. cycle, vitamin C, some fruits and milk were separately or together administered to a healthy human body, and then the amount of metasilicic acid excreted in the urine was measured. The results obtained were shown in Fig. 3.

In the administration of various drugs and foods, generally, potassium and sodium bicarbonate, malic acid, citrus fruits and milk were effective and potassium salts of organic acids were more effective for excreting silica in urine.

The vegetable and fruits contained larger amount of silica than the animal foods, and the acidity of the ash of these foods was alkaline. Therefore, it was considered that the intake of large amount of vegetable or fruits scarcely caused the deposit of silica due to its alkaline, and silica would have been ex-



creted in the urine as alkali salt of meta-silicic acid. On the other hand, as the intake of large amount of animal foods which made the urinary pH value down caused acidosis in human body, led to polymerization of silica in the body, and resulted to its deposit on the tissue, so the excretion of silica in the urine would have decreased.

After all, it is very important that a man should keep the body fluid in alkaline to protect from the deposit of silica.

4) *Silica in organs.*

i) *Silica in human organs.*

Silica contents in normal and diseased human organs were studied. The

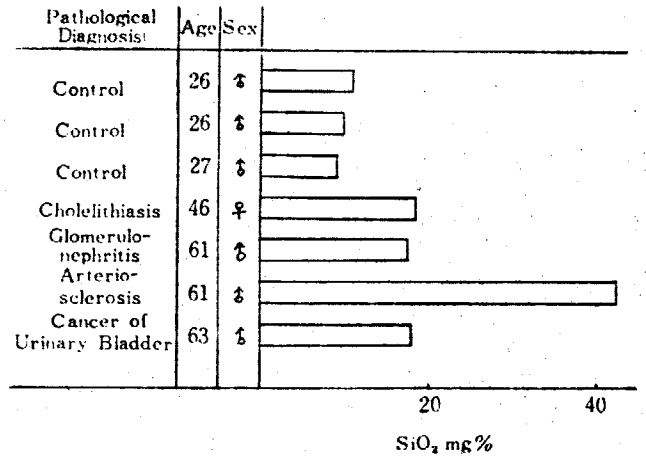


Fig. 5. SiO_2 in heart muscle.

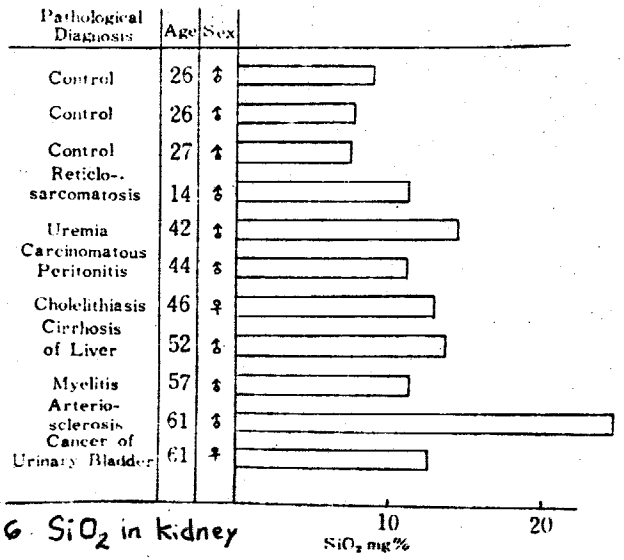


Fig. 6 SiO_2 in kidney

analyzed organs were the arteria basilaris, heart muscle, kidney and liver. Silica in aorta will be explained in detail in another paragraph later. Silica content in the arteria basilaris, age and cause of death were shown in Fig. 4. The largest amount of the silica was observed in a man, 78 years old, who died due to cerebral bleeding.

Silica content in heart muscle was shown in Fig. 5. The largest amount of silica was observed in a man, 61 years old, who died due to the arteriosclerosis. And it was considered that the heart muscle in old age contained larger amount of silica than in yound age. Silica content in the kidney was shown in Fig. 6. The largest amount of silica was observed in the case of death caused arteriosclerosis. The relation between age and silica content was similar to that shown in Fig. 4.

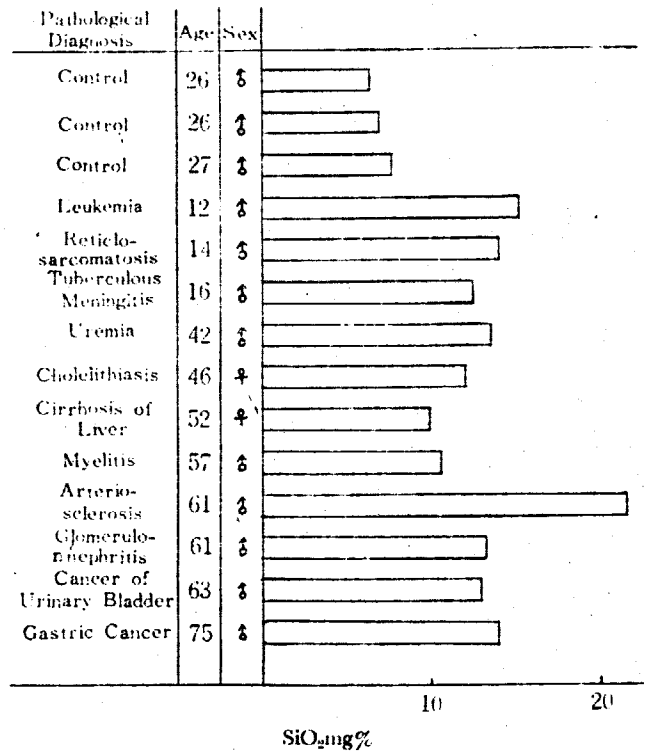


Fig. 7. SiO_2 in liver.

Fig. 7 showed the silica content in the liver. The results observed were similar to that shown in Fig. 5 and 6.

It is presumable that the deposit of silica in a human body depends upon individual environment and kinds of drinks and foods to be taken in his life time as well as genetic factor. Further, it was very difficult to clarify the meaning of silica in the human body, but the following tendencies only were found. Silica contents of human organs increased very often with advance of age, and it decreased in the sequence of the arteria basilaris, aorta, heart muscle, kidney and liver successively. Silica contents in the organs in the case of arteriosclerosis were higher than in another. The relationship between silica content and sex could not be found.

ii) *Silica in whale organs.*

Silica contents in organs of finback whales were studied. Whale organs used as materials were the aorta, heart muscle, kidney, connective tissue of kidney, ureter, intestinal mucosa, skin and baleen. The results (the mean value of five samples) observed were shown in Fig. 8.

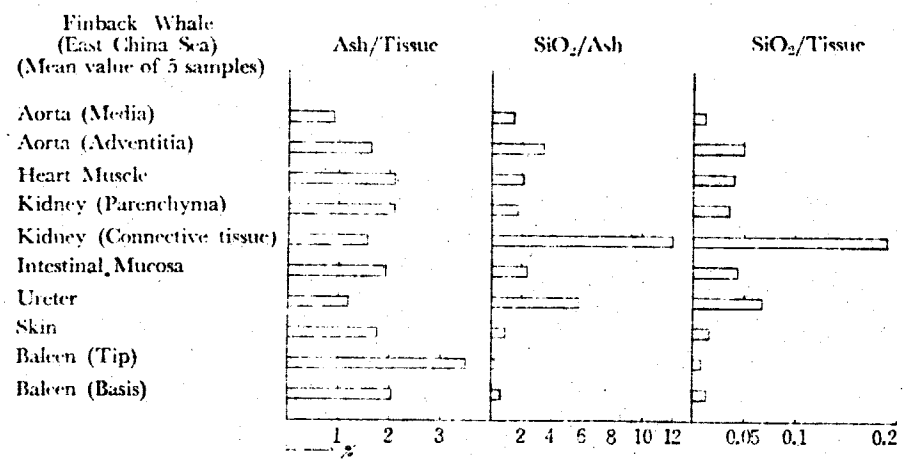


Fig. 8. SiO_2 in whale organs.

The connective tissue of the kidney contained the largest amount of silica in all another organs of finback whales. Then the content of it decreased in the sequence of the ureter und aorta successively. The adventitia of aorta contained larger amount of silica than the media or intima. It was an unexpected result that the baleen contained only a small amount of silica in spite of containing a large amount of mineral substances. But the connective tissue of kidney and the ureter contained a larger amount of silica. Perhaps, these facts would have been caused by the depositing of silica at the time of its excretion. On the other hand, it was presumed that the adventitia of aorta and connective tissue of kidney which were consisted of rather hard tissue would have been needed of silica to keep its form and hardness. This presumption would have been applicable to human body, and it will be described in another report[17] later.

5) *Silica in human aortae*

i) *Silica in aortae of the Japanese.*

89 cases of the aortae of the Japanese fixed with formalin were separated into three layers of the intima, media and adventitia, and silica content of each layer was determined.

In this study, the ratio of ash to tissue, silica to ash and silica to tissue

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Table 3. The age, sex, cause of death and view of aorta of samples.

No.	Autopsy No.	Age	Sex	Cause of Death	View of Aorta
1	1329	3 Mon.	M	Erythroblastosis Fetalis	N.
2	1352	11 Mon.	F	Dystrophia	N.
3	1282	9	M	Acute Myeloic Leukemia	N.
4	1393	9	M	Subarachnoidal Hemorrhage	N.
(5)	16644	15	M	Poisoning of Hypnotics	N.
(6)	16724	16	M	Bacillary Dysentery	N.
(7)	16653	16	M	Drowning	N.
(8)	16671	17	M	Poisoning of Hypnotics	N.
9	1330	20	M	Postoperative Shock	N.
10	1323	20	M	Lung-metastasis of Osteosarcoma	N.
11	1391	20	M	Myeloic Leukemia	N.
12	1264	23	F	Rhabdomyosarcoma	N.
13	1280	24	F	Abdominal Tumor	N.
14	1366	24	M	Leukemia	N.
15	1321	25	M	Esophagus Stenosis	N.
(16)	16632	26	M	Poisoning of Cyanide	N.
17	1221	28	F	Rupture of Ectopic Pregnancy	N.
(18)	16613	28	M	Lobular Pneumonia	N.
19	1284	32	M	Sepsis	N.
20	1317	32	M	Purulent Meningitis	N.
21	1380	32	M	Hepatoma	N.
(22)	16717	34	M	Valvular Disease	N.
23	1342	35	M	Unknown	N.
24	972	35	M	Unknown	N.
25	1348	35	F	Gastric Cancer	N.
26	1370	37	F	Pulmonary Cancer	N.
27	1271	38	M	Neuroblastoma	N.
(28)	16633	38	M	Acute Heart Disease	N.
(29)	16688	38	M	Fracture of Skull	N.
(30)	16694	40	M	Unknown	A(+)
(31)	16696	40	M	Cerebral Bleeding	A(+)
(32)	16629	41	F	Poisoning of Hypnotics	N.
(33)	16624	41	F	Rupture of Tubal Pregnancy	N.
34	878	42	M	Teleangioma of Femoral Bone	N.
35	1260	42	M	Pulmonary Cancer	A(+)
36	1340	42	F	Gastric Cancer	N.
37	1332	43	F	Liposarcoma	N.
(38)	16697	43	M	Acute Cardiac Insufficiency	N.
39	1347	44	M	Aplastic Anemia	N.
40	1296	46	M	Cancer of Maxilla	N.
41	1364	46	M	Pancreatic Cancer	A(+)
42	1316	47	F	Ovarial Cancer	N.
(43)	16710	48	M	Acute Heart Disease	N.
44	1261	49	M	Aortic Aneurysm	A(+++) Ca(+++)
45	1265	49	F	Ovarial Cancer	N.
46	1257	49	M	Gastric Cancer	A(++)
47	1258	49	M	Cerebral Bleeding	A(+++)
48	1383	49	M	Gastric Cancer, Pulmonary Tuberculosis	A(+) Ca(+)
49	1168	50	F	Uterine Cancer	A(+++) Ca(+++)
50	1278	50	M	Gastric Cancer	N.
51	1263	51	M	Esophageal Varix	A(+++)
52	1361	51	M	Auricular Fibrillation	N.
53	1267	51	F	Hepatic Cancer	A(++)
54	1266	52	M	Mesothelioma	N.
55	1262	53	M	Pulmonary Cancer	A(+++) Ca(+)
56	1152	53	M	Liver Cirrhosis with Hepatoma	A(+++) Ca(+)
57	1281	54	F	Arhythmia	A(+++) Ca(++) U.
58	1336	54	M	Pulmonary Cancer	A(++)
59	1313	55	F	Hepatic Cancer	A(++)
60	1304	55	M	Mucoepidermoid Cancer	A(+++) Ca(+++) U.
(61)	16652	55	F	Acute Hemorrhagic Bronchitis	A(+++) Ca(+)
62	1242	56	M	Wilson's Disease	A(++) Ca(+++) U.
63	1285	56	M	Miliary Tuberculosis	N.
(64)	16686	56	M	Coronary Sclerosis	A(+++) Ca(+)
65	1234	57	M	Gastric Ulcer	A(+++) Ca(+++)U.
66	1326	57	F	Aastric Cancer, Renal Tuberculosis	N.
67	1315	58	M	Pulmonary Tuberculosis	A(+++) Ca(+++) U.
68	1374	58	M	Cancer of Mandible	A(++)
69	1290	60	M	Cancer of Maxilla	A(+++) Ca(+++)
70	1289	60	F	Cancer of Rectum	A(+++)
71	1306	62	M	Pulmonary Tuberculosis	A(++)
72	1277	62	M	Unknown	A(+)
73	1358	62	M	Cancer of Colon Transversum	A(+++) Ca(+++) U.
74	1308	62	F	Myocardial Infarction	A(+++) Ca(+++) U.
75	1269	63	M	Prostatic Carcinoma	A(+++) Ca(+++) U.
(76)	16658	63	M	Acute Cardiac Insufficiency	A(+++) Ca(++) U.
77	1104	64	M	Pulmonary Cancer	A(+++)
78	1279	64	F	Abdominal Tumor	A(+++) Ca(+++)
79	1379	64	M	Death after Lobotomy	A(+) Ca(+)
80	1389	65	M	Cancer of Urinary Bladder	A(+++) Ca(+++) U.
(81)	16685	66	F	Acute Coronary Insufficiency	A(+++) Ca(+)
82	1310	67	M	Pulmonary Cancer	N.
(83)	16702	67	F	Acute Pneumonia	A(+++)
84	1355	68	F	Cancer of Colon Descendens	A(+)
85	1372	69	F	Hepatic Cancer	A(+++) Ca(+++) U.
86	1381	69	M	Gastric Cancer	A(+++) Ca(+++) U.
87	1388	70	F	Paralysis of Medullaoblongata	A(+++)
(88)	16700	74	M	Drowning	A(+++) Ca(+++) U.
(89)	16703	74	F	Lobular Pneumonia	A(+++)

Aortae supplied by Tokyo Medical Examiners Office are indicated No. in ( ). Others were supplied by Department of Pathology, Tokyo Medical and Dental University.

View of Aorta N.....Normal, A.....Atherosclerosis, Ca.....Calcification, U.....Ulcer

(+)....slight degree, (++)....middle degree, (+++)....high degree.

were determined. The age, sex, cause of death and view of aorta were summarized and shown in table 3. The photographs of each aorta were shown in the end of this report.

Ash amount of the intima, media and adventitia were shown in Fig. 9. The amount of ash in three layers have a tendency to increase very often with advance of age, but it is not always certain.

Silica content of ash in each three layer was shown in Fig. 10. The ash of the media contained silica in almost the same amounts, and the ash of the

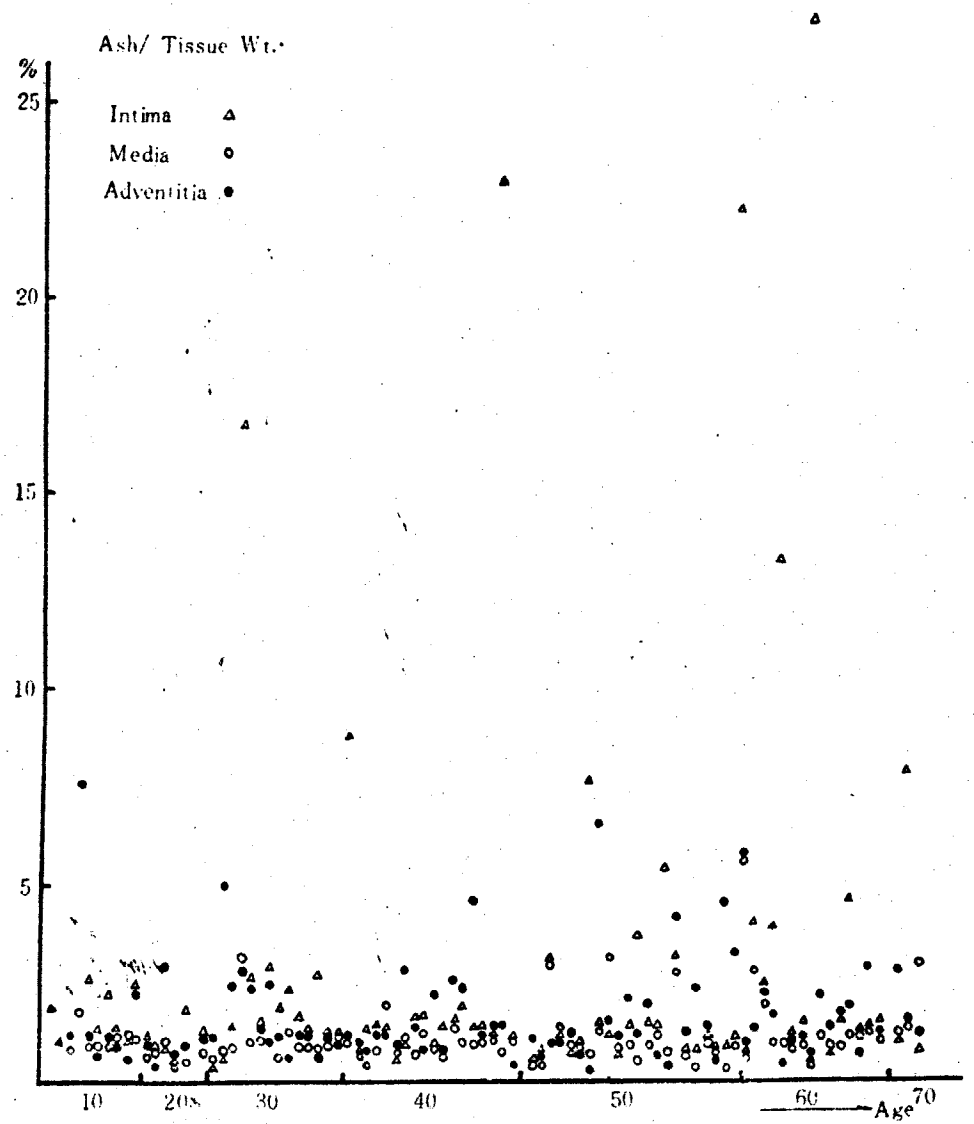

Fig. 9. Ash of aortae (%).

adventitia contained the largest amount of silica but it was not always certain distributed. Then it was considered that the ash of the intima in younger age contained larger amount of silica than older age.

Silica content of tissue was shown in Fig. 11. The media contained silica in almost the same amount. But silica content in the adventitia increased with advance of age, and on the other hand, its content in the intima decreased with advance of age, and at the age of about 40 to 42, the contents of silica in these two layer reversed, that is, much amount of silica was contained in the intima before 40 to 42 years old, but inversely, after this age much amount of silica was contained in the adventitia. It was presumed that silica in the adventitia

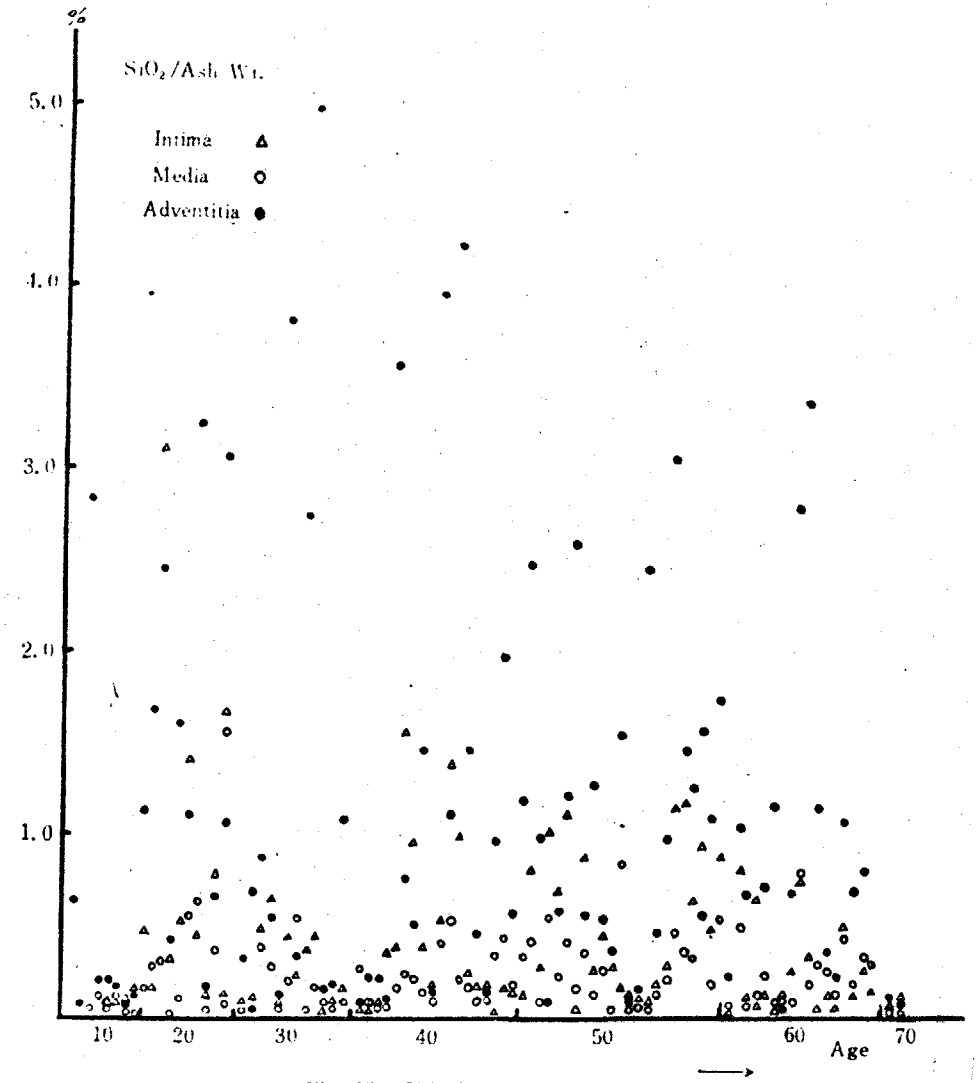

Fig. 10. SiO_2 in ash of aortae (%).

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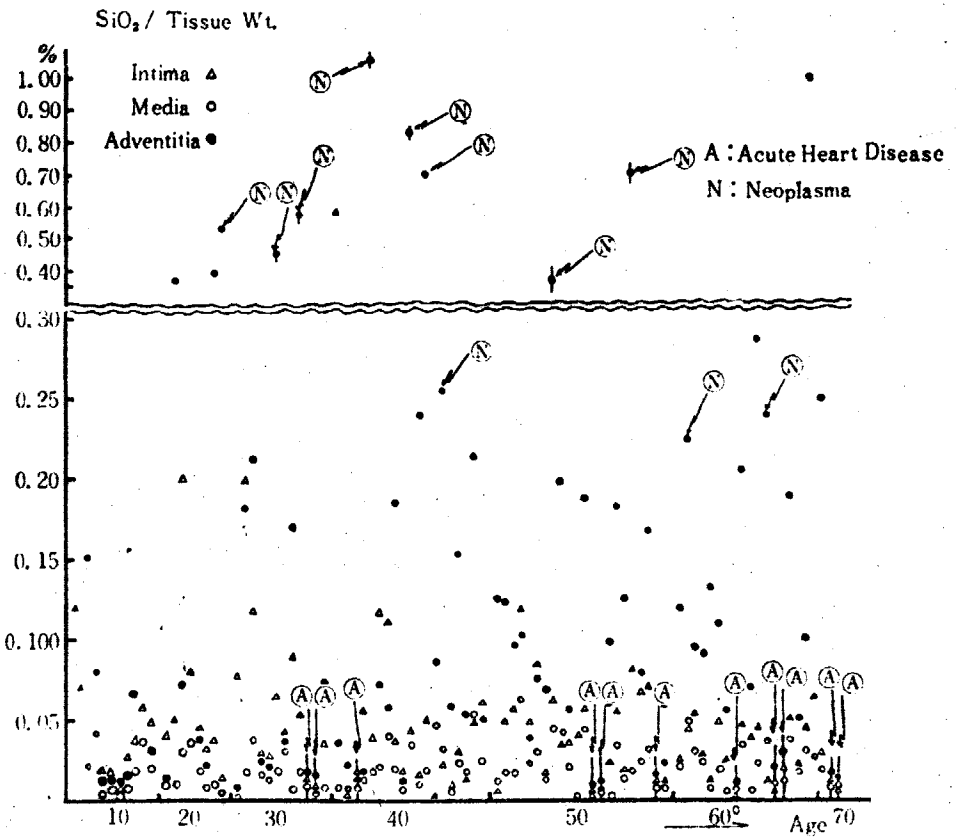

Fig. 11. SiO_2 in aortae (%).

might have any kind of connection with hypertension or the arteriosclerosis which appeared often in the advanced age of more than 40 years old. As it is physiologically said that the human blood vessel grows until 40 years old, in this meaning, this reversing of the silica contents in the intima and adventitia is very interesting.

In Fig. 11, the cases of death by the acute heart disease and neoplasma were especially marked with "→", because the adventitia in the cases of death caused by the neoplasma contained a large amount of silica, and on the other hand, in the cases by the acute heart disease, adventitia contained a small amount of it.

ii) *Silica in aortae of the German.*

Silica contents of aortae and some arteries of the German were determined and the results were shown in Table 4. The aortae could not be separated into three layers, therefore all amounts of ash and silica in three layers together were measured. The aortae of the German contained much smaller amount of silica than in the Japanese, but their contents increased very often with advance of age as the Japanese ones. The foods and drinks in the German

life generally containing a small amount of silica as compared with Japan may be lead to such poor content of silica in aorta of the German. The aortae of the German in the case of the arteriosclerosis were not supplied as materials, so silica content in the arteriosclerosis could not be discussed here.

Table 4. SiO_2 in arteries of the German.

No.	Age	Sex	Pathological Diagnosis	Aorta			Arteria Subclavia			Arteria Femoralis		
				A/T %	S/A %	S/T %	A/T %	S/A %	S/T %	A/T %	S/A %	S/T %
1	15	M	Abdominal Tumor	1.495	0.911	0.013	1.218	0.440	0.005	1.009	0.666	0.006
2	50	F	Brain Tumor	2.270	1.372	0.031	1.600	1.156	0.018	1.036	2.555	0.026
3	57	M	Hepatic Coma	5.193	0.377	0.019	1.344	1.926	0.024	1.360	2.037	0.027
4	64	M	Bronchopneumonia	4.172	0.772	0.032	1.350	2.448	0.033	1.152	2.000	0.023

A : Ash Wt., T : Tissue Wt., S : SiO_2 Wt.

Summary

Silica in the drinking water, the excretion of the silica, the effects of various drugs and foods on its excretion and its contents in various organs and especially in the aortae were studied. And then the statistical relationship between silica in the drinking water and the mortality caused by the apoplexy in Japan, and the relationship between silica in the aortae and the arteriosclerosis were discussed.

The results obtained were as follows:

1) The drinking water in Japan contained larger amount of silica than the any other countries examined.

2) The significant linear correlation was found between silica content in the drinking water and the mortality caused by the vascular injuries of the central nervous system in Japan.

3) The amount of silica excreted in the urine was influenced by the urinary pH value. The urine in alkaline side contained larger amount of silica than in the acidic side.

4) The administration of potassium and sodium bicarbonate, malic acid, citrus fruits and milk were effective and potassium salts of organic acids, especially related to T. C. A. cycle, were more effective for excretion of silica in the urine.

5) Silica contents of human organs increased very often with advance of age. These silica would have been derived from foods and drinking water.

6) Silica content in human body decreased in the sequence of the arteria basilaris, aorta, heart muscle, kidney and liver.

7) The connective tissue of the kidney and the ureter of a whale contained a large amount of silica.

8) The human organs in the case of arteriosclerosis contained larger amount of silica than the other cases.

9) Silica content in the human aorta also had a tendency to increase with

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advance of age, and in the case of the arteriosclerosis its contents was higher than the other cases.

10) In the human aortae, the intima contained a large amount of silica before 40 to 42 years old, but inversely after this age the adventitia contained a large amount of it. Silica contents in the intima and adventitia reversed at the age of 40 to 42, and the media contained constant in amount.

11) Silica should have been considered as one of causes of the sclerosis of blood vessels besides lipids and calcium.

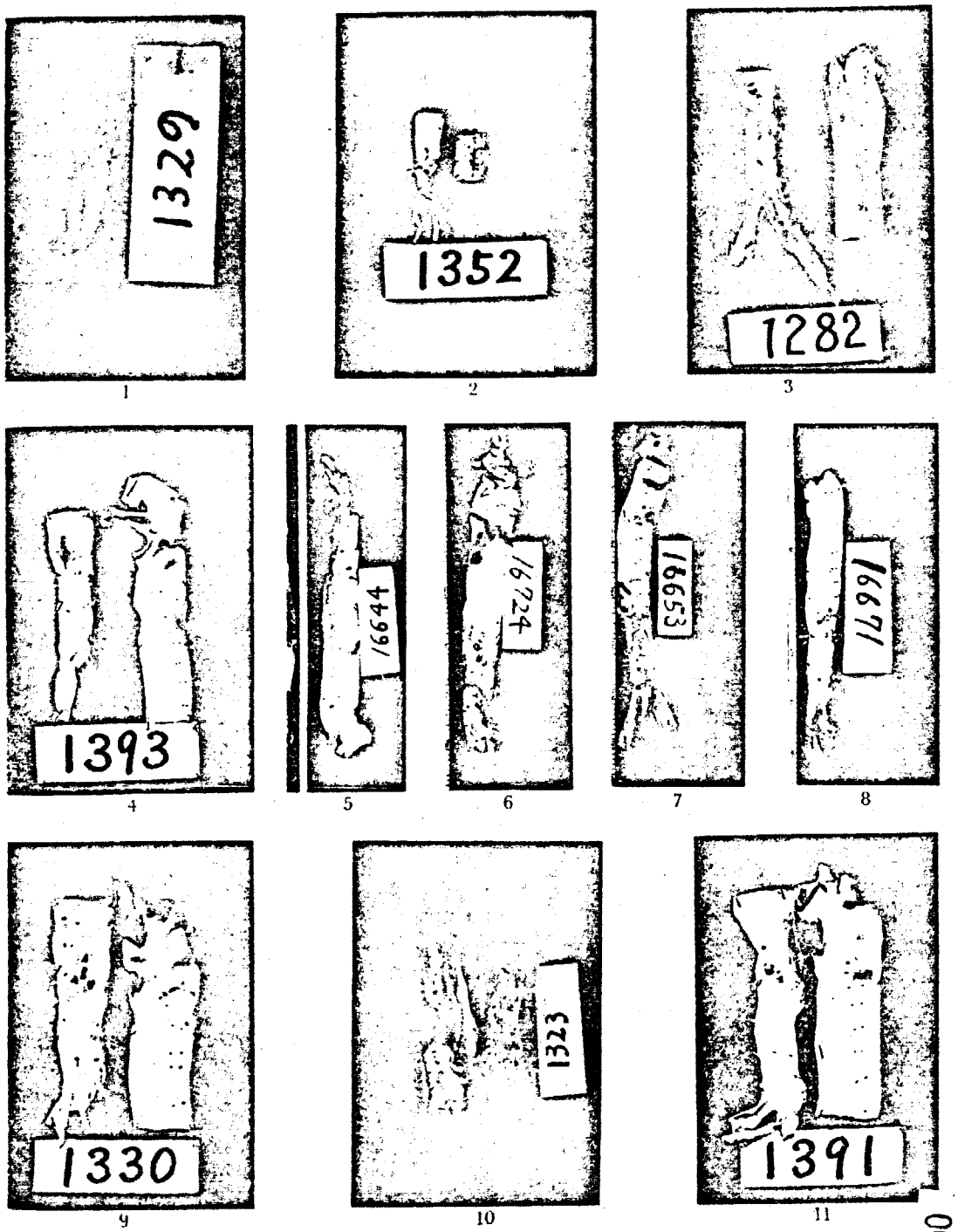
Acknowledgement

The authors wish to express their deepest appreciation to Prof. Dr. K. Muto, Prof. Dr. K. Ohta, Department of Pathology, Tokyo Medical and Dental University and to Prof. Dr. T. Yoshida, Department of Pathology, Prof. Dr. T. Ogawa, Department of Anatomy, Prof. Dr. T. Furuhata, Department of Legal Medicine, University of Tokyo, and Tokyo Medical Examiners Office for their kind advises.

References

1) E. J. King, H. Stantial & M. Dolan: Biochem. J., 33, 1002 (1933).
2) P. Ohlenmeyer & U. Olpp: Z. Physiol. Chem., 281, 203 (1944).
3) L. Holzapfel: Naturwiss., 34, 189 (1947).
4) P. F. Holt & D. M. Yates: Biochem. J., 45, xxxii (1950).
5) P. F. Holt & D. M. Yates: Biochem. J., 48, xliv (1951).
6) P. F. Holt & D. M. Yates: Biochem. J., 54, 300 (1951).
7) S. Akiya & A. Tanimura: Seikagaku (Japan), 26, 430 (1954).
8) S. Akiya & A. Tanimura: Seikagaku (Japan), 28, 635 (1956).
9) T. Misawa, Y. Kitamura, H. Okada, A. Tshuchiya & H. Iizuka: Nihon Iji Shimpo (Japan), 1718, 1719 (1957).
10) T. Misawa, Y. Kitamura, H. Okada, A. Tsuchiya & H. Iizuka: Nihon Naikagaku Zasshi (Japan), 46, No. 5 (1957).
11) T. Misawa: Nihon Eiseigaku Zasshi (Japan), 13, No. 1 (1958).
12) T. Misawa: Tokyo Iji Shinshi (Japan), 75, 453 (1958).
13) E. J. King & Th. H. Belt: Physiol. Rev., 18, 329 (1938).
14) E. J. King & H. Stantial: Biochem. J., 27, 990 (1933).
15) S. Akiya & N. Motohashi: Yakugaku Zasshi (Japan), 76, 111 (1956).
16) Y. Okumura: Nagasaki Igaku Zasshi (Japan), 29, 419 (1954).
17) S. Akiya & T. Niwaguchi: Ochanomizu M. J., in press.

The aortae of the Japanese (I)



The aortae of the Japanese (II)

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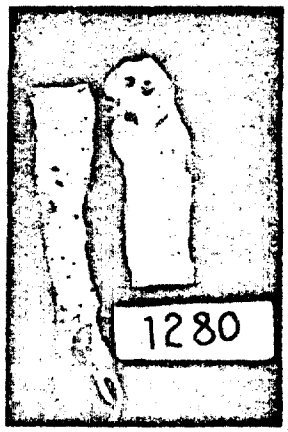

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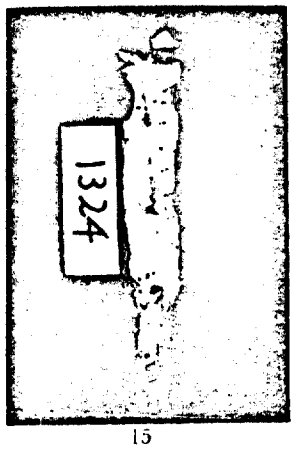

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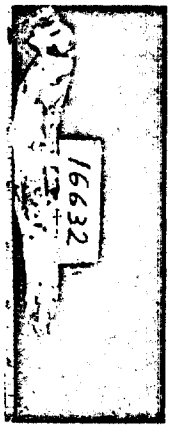

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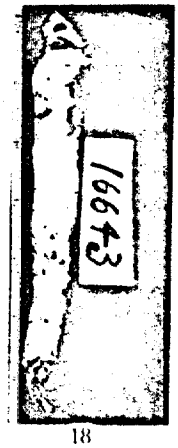

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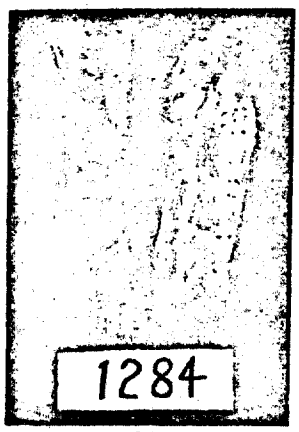

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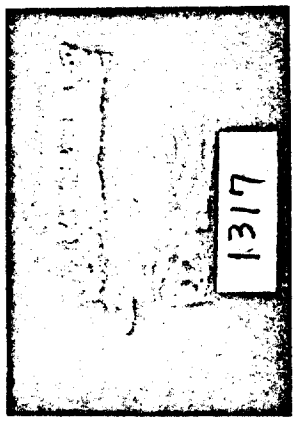

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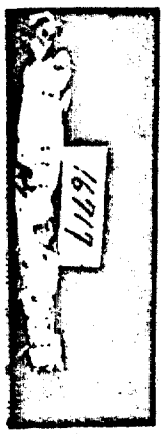
The aortae of the Japanese (III)



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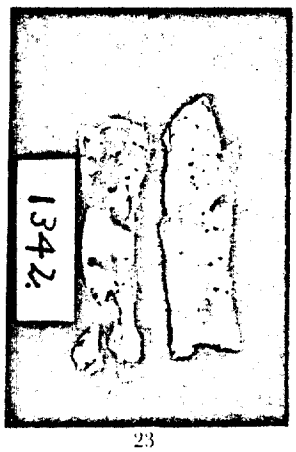

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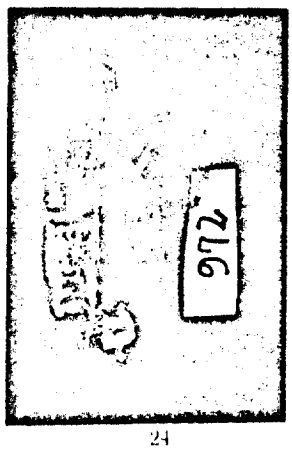

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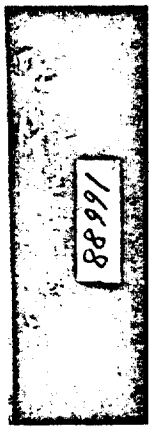

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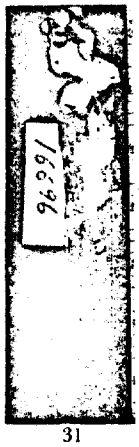

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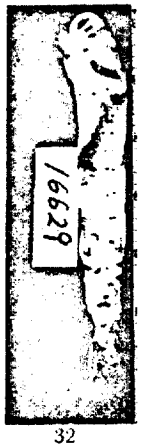

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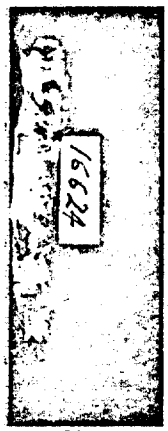

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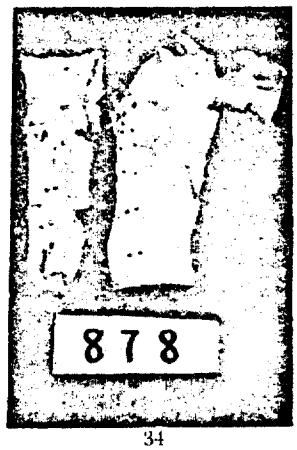

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The aortae of the Japanese (IV)

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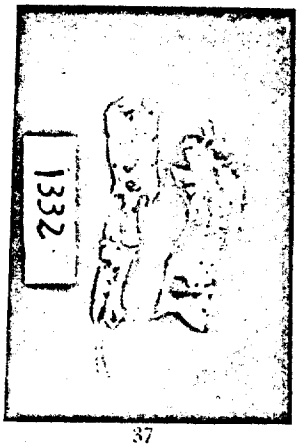

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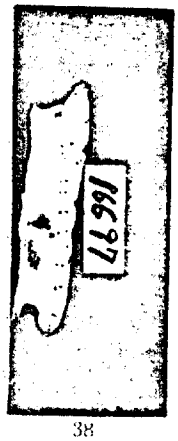

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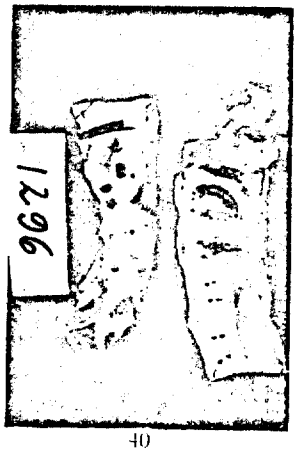

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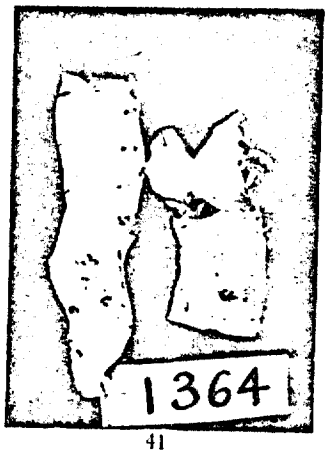

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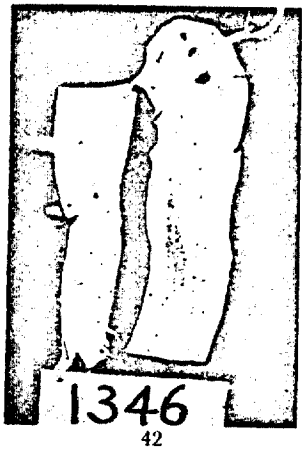

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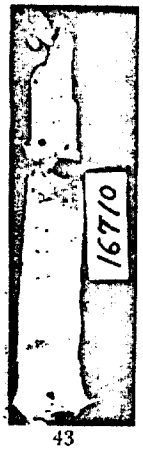

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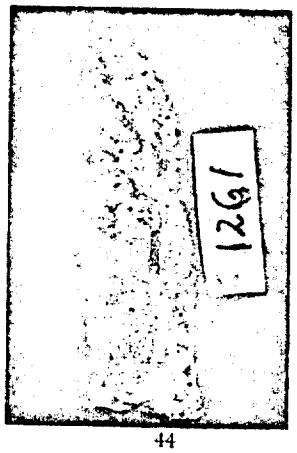

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The aortae of the Japanese (V)

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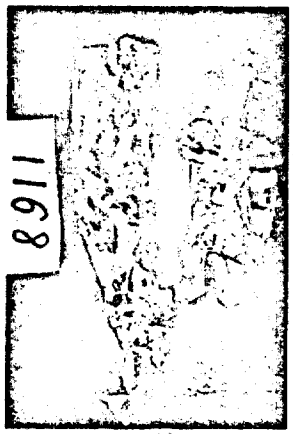

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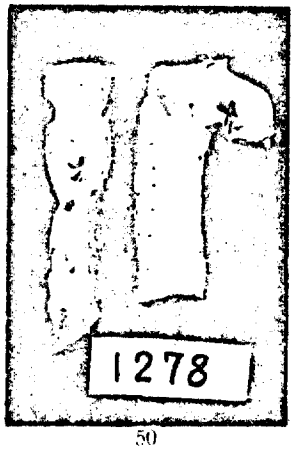

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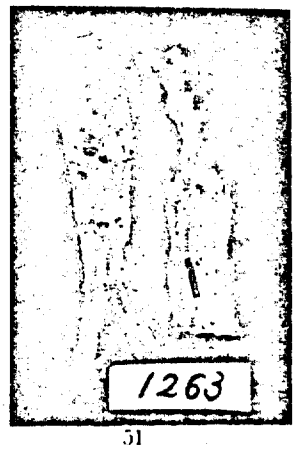

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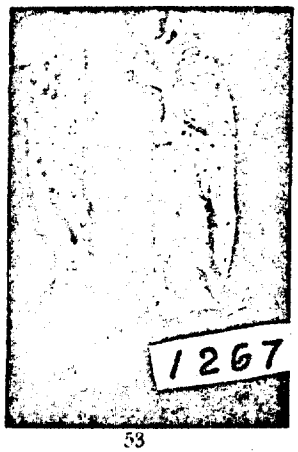

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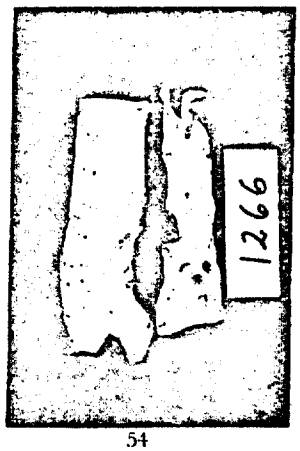

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The aortae of the Japanese (VI)

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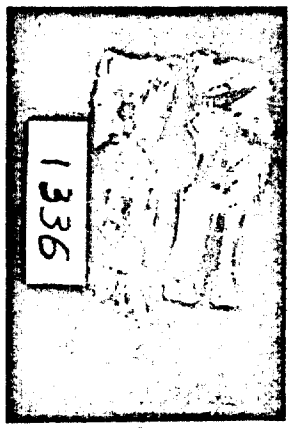

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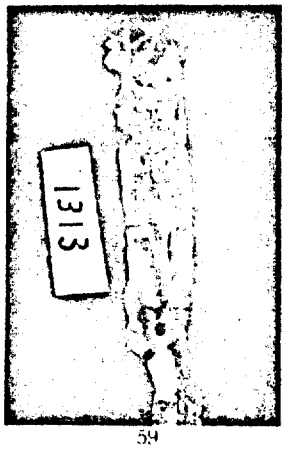

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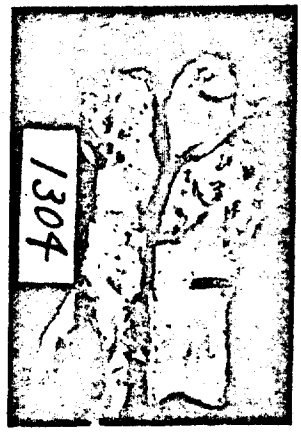

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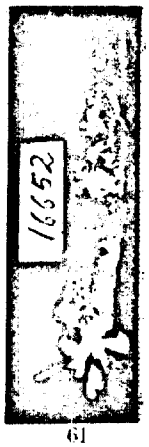

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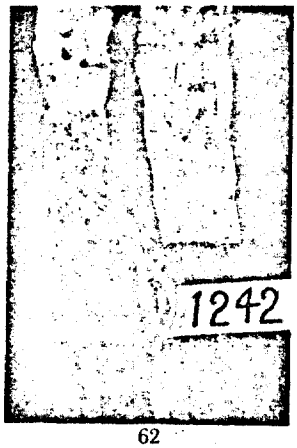

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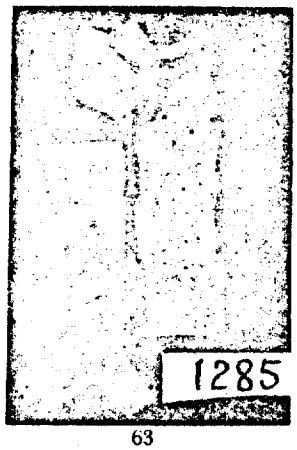

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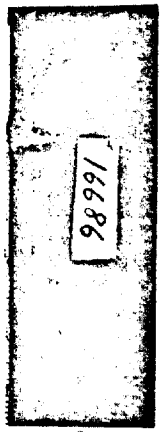

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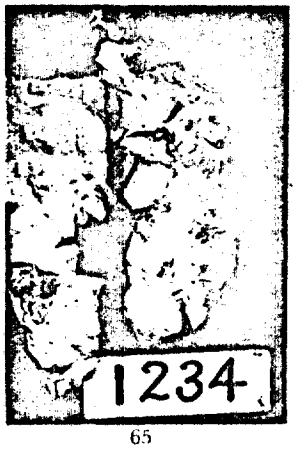

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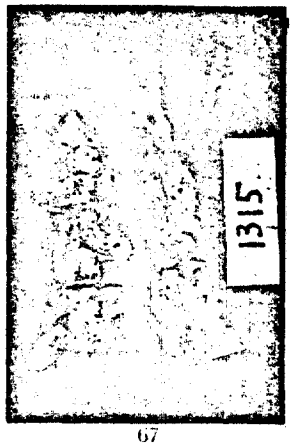

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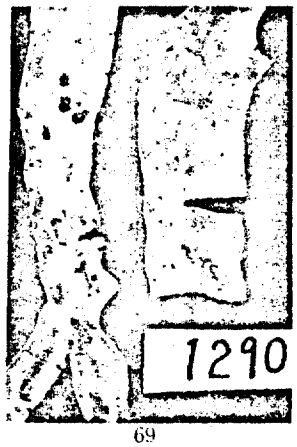

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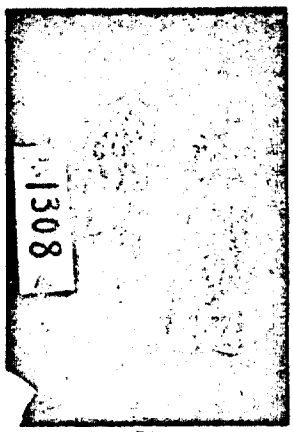

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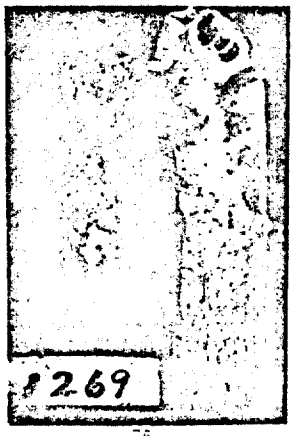

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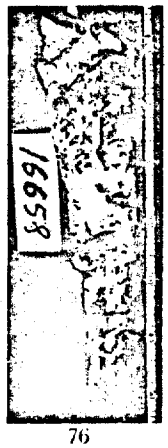

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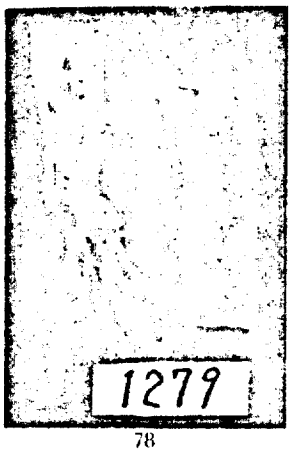

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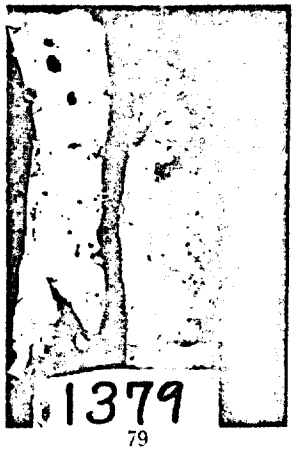

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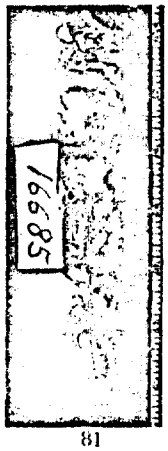

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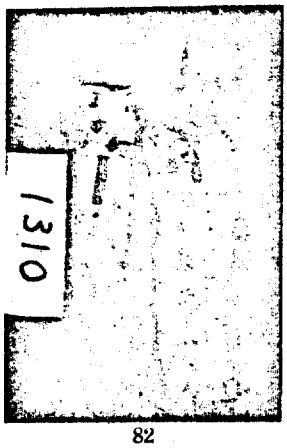

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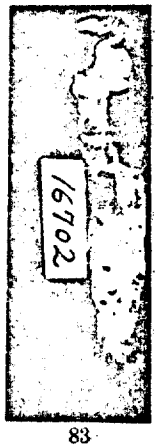

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The aortae of the Japanese (IX)

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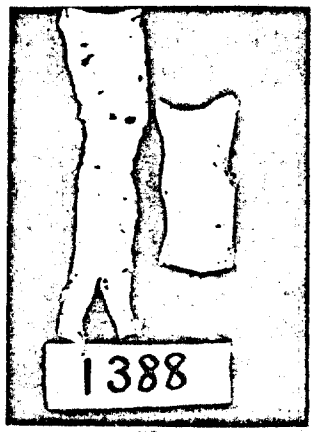

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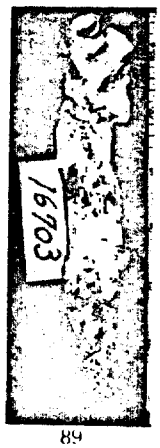

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J. Exp. Med., 124 (2): 141-153, 1966

AN EXAMINATION OF THE CYTOTOXIC EFFECTS OF SILICA ON MACROPHAGES*

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Plates 10 to 13

(Received for publication 9 March 1966)

The fibrogenic effects of silica in man and experimental animals are not yet satisfactorily explained. There is general agreement that in the first instance silica particles deposited in the lung are engulfed by phagocytic cells, which are rapidly destroyed. The cytotoxic effects of silica can be conveniently reproduced in cultures of peritoneal or alveolar macrophages (1), and the relative toxicity of different forms of silica, and of different dusts, on cell cultures agrees with the pathogenicity and fibrogenic potentialities of the dusts in vivo (2, 3).

Silica provides, in fact, a remarkable example of selective toxicity for one cell type by a substance of simple chemical composition and low chemical reactivity. Why particles of silica rapidly kill macrophages whereas other particles of comparable size and surface area (such as carbon particles or diamond dust) are ingested by the cells without harmful effects is a problem of academic interest as well as practical importance in relation to the pathogenesis of silicosis.

Earlier theories of silica toxicity were reviewed by Nagelschmidt (4) and Harington (5). King in his well known "solubility" theory suggested that silicic acid liberated into the tissues from silica particles brings about the deposition of collagen. Later observations did not support this view, as King himself (6) and Nagelschmidt (4) have pointed out. Curran and Rowsell (7) showed that silica particles implanted into the peritoneum in diffusion chambers do not induce any fibrogenic reaction, although silicic acid is liberated from the chambers. Suggestions have also been made that silica dust might damage macrophages as a result of protein adsorption or denaturation, oxidative changes, or interference with enzyme activity such as that of esterases in the cell.

* Preliminary accounts of parts of this investigation were presented at a Symposium on the Pathogenesis of Pulmonary Fibroses (Milan, Italy, December 1964), and at the Second International Symposium on Inhaled Particles and Vapors (Cambridge, England, September 1965).

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None of the evidence in support of these suggestions has been very convincing, and Vigliani and Pernis (8) were led to formulate an autoimmune causation of silicosis. However, extensive investigations have failed to provide any evidence that silica is directly involved in immune reactions, either cellular or humoral, or that an autoimmunity is responsible for the fibrogenesis (9). It now seems likely that if any immune responses occur in silicosis they may be incidental; for example, secondary to infection of the damaged lungs.

Hence the mechanism by which silica particles kill macrophages is still an open question. Recognizing the similarities between the uptake of silica dust and other particles, e.g. bacteria (10), or inert materials, for example Triton WR 1339 (11), we have been led to an alternative explanation of the phenomenon. According to this view, all particles are taken into phagocytic cells in an essentially similar fashion. The cell membrane is invaginated and the particles are enclosed in phagosomes. Primary lysosomes become attached to the phagosomes and discharge their enzymes into them. Provided that the membranes of the secondary lysosomes so formed remain intact, the cells are unharmed. However, if the enclosed particles can react with the lysosomal membranes and make them permeable, enzymes and particles will rapidly escape into the cytoplasm and damage the cells. Thus attention is focused upon interaction of the particles with lipoprotein membranes of phagosomes and of other biological systems. We have elsewhere (12) presented evidence that silica can interact with proteins and phospholipids and lyse red blood cells, whereas nontoxic particles do not have these effects. An analysis of the underlying physicochemical processes was also given.

In this paper cytochemical and electron microscopial observations of macrophages after uptake of silica are described. Conventional biochemical methods involving homogenization and assay of enzymes in particulate and supernatant fractions cannot be applied satisfactorily in the presence of silica. Even if added to normal cells in the cold, silica particles produce damage during the course of homogenization. However, Cohn and Wiener (13) have presented evidence that when lysosomal enzymes are released into the cytoplasm a proportion escapes from the cells into the surrounding medium. We have therefore assayed lysosomal enzymes in the medium of macrophages exposed to silica and other substances, and assume that these reflect, though not quantitatively, release of enzymes from lysosomes.

Material and Methods

Cultures of Macrophages.—Most experiments were carried out with mouse peritoneal macrophages. These were obtained by inoculating 3 ml of culture medium containing 10 IU heparin per ml into mice of strain VSBS/NIMR weighing 18 to 20 g. After brief massage the cells were recovered and dispensed in 1 ml volumes, containing 2×10^6 cells, into tubes with small cover slips. Cells were incubated in culture medium (medium 199, Glaxo Laboratories Ltd., Greenford, England, and 0 to 20% calf serum inactivated at 56°C for 1 hr) overnight before exposure to silica. In some experiments rabbit lung, alveolar macrophages prepared as described by Cohn and Wiener (14) were used.

Silica.—Four types of silica, kindly provided by Dr. I. Bergman of Sheffield were used: (*a*) "Snowit" silica particles (× 9947) of size range 15% 2.5 μ, 49% 2.5 to 5 μ, 30% 5 to 7.5 μ, and 5% 7.5 to 10 μ; (*b*) "Fransil" silica (× 5663), uncompressed, consisting of silica particles of diameter up to 0.3 μ; (*c*) Na-OH-etched Fransil (× 5843 from × 5663) of size range 0.1 to 0.5 μ; and (*d*) silica particles coated with aluminium according to Dale and King (15). All samples were heat-sterilized before being added as fine suspensions to culture media. Living cells were examined by time-lapse, phase-contrast cine-micrography in a warm chamber, and also by dark-ground microscopy.

Control Materials.—(*a*) Diamond dust, particle size 2 to 4 μ (Diamond Products, Sales, Ltd., London). This is not fibrogenic. (*b*) Highly purified carrageenan (Seaplant Corporation, New Bedford, Massachusetts). This sulfated polygalactase is fibrogenic (see reference 16, Williams). Polyvinylpyridine-*N*-oxide (P204, U33) was obtained through the courtesy of Professor H. W. Schlipköter, Düsseldorf.

Histochemical Methods.—Acid phosphatase staining by a modified Gomori method (17) was carried out on unfixed cells and cells fixed in 2% glutaraldehyde buffered to pH 7.0 with 0.1 M cacodylate for 10 to 20 min at 4°C. Controls were treated with sodium fluoride (10^{-2} M) to inhibit the enzyme. Indoxylacetate esterase staining was carried out as described by Holt (18). Fluorescence microscopy of cells (17) was performed after incubation of cells with 1 in 10^5 (w/v) acridine orange in culture medium for (½) hr at 37°C, incubation in fresh medium without acridine for 15 min, mounting in fresh medium, and activation with blue-violet light (460 to 500 mμ). Metachromatic staining for intracellular carrageenan was carried out by washing the cells with saline, fixing in 70% ethanol for 5 min, staining in 0.1% toluidine blue in 30% ethanol for 10 min, and mounting in buffered glycerol.

For histochemical recognition of polyvinylpyridine-*N*-oxide, the method of Jancsó (see reference 47, Pearse) has been used.

Electron Microscopy.—Macrophages were cultured in Petri dishes overnight and then treated with Fransil (100 μg/ml) to avoid difficulty in cutting sections, which was experienced when quartz was used. After time intervals from 2 to 24 hr treated cells and untreated controls were gently scraped off the glass with a silicone rubber "policeman", centrifuged, and fixed in Palade's solution (19) at 4°C. For histochemistry, cells were fixed in 2% cacodylate-buffered glutaraldehyde for 10 min at 4°C, washed, incubated with the Gomori reagent for 15 min at 37°C, washed, and postfixed in Palade's solution. Embedding was in Araldite, and sections were stained with lead according to Karnovsky (20).

Enzyme Determinations in Medium.—Cells were incubated in Petri dishes in standard medium which was replaced by medium lacking serum 2 hr before assay. This can maintain healthy cells for at least 12 hr. Acid phosphatase and β-glucuronidase were measured by conventional techniques (21, 22) using *p*-nitrophenylphosphate and phenolphthalein glucuronide as substrates, incubating 0.2 ml samples of medium in 0.1 M sodium acetate buffer, pH 5.0, at 37°C for 1 hr.

OBSERVATIONS

Light Microscopy.—Macrophages were exposed to silica of various types (10 to 100 μg/ml) in culture media and observed by time-lapse, phase-contrast cine-micrography and dark-ground microscopy. Control cells moved about, preceded by ruffled membranes, and passed freely alongside and sometimes over one another. Phase-contrast dense bodies, identified as lysosomes, moved about in a random fashion in the cytoplasm. Only the larger granules were seen in motion pictures, the movement of the smaller granules being so rapid that it was not recorded. When silica particles were added, they were rapidly engulfed. The particles remained in phagosomes in the cells, often in a paranuclear posi-

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tion but sometimes in the more distal part of the cytoplasm. Cells that were engorged with large amounts of silica remained fully extended and showed apparently normal mobility. Within 2 hr nearly all the lysosomes were attached to phagosomes containing silica, and by 10 hr very few granules were left intact, although cell movement was unimpaired. When moving pictures were made after 15 hr, some cells were already dead, and the events associated with cell death were recorded. Some cells that were moving suddenly stopped and remained immobile for a while before the processes were withdrawn and the cells rounded up. In other cells there were violent threshing movements of the cytoplasm while the cells became rounded and eventually immobile. During the later stages there was a tendency for the cells to stick to another, and occasionally they fused to form giant cells.

Histochemical Changes.—Control, unfixed macrophages showed virtually no phosphatase staining (Fig. 1). This corresponds with previous observations (17, 23) that if the membranes of lysosomes are intact the substrate does not penetrate and give a reaction. In fixed macrophages, numerous lysosomes of varying size showed phosphatase and indoxylacetate esterase in the cytoplasm (Fig. 2). After uptake of silica, the particles were readily seen in the cytoplasm by phase-contrast microscopy or dark-ground illumination (Fig. 3). In unfixed cells within a few hours numerous lysosomes were seen clustered around phagosomes containing the dust (Fig. 4). Later the reaction product accumulated in dense masses around the silica particles, and by 15 to 18 hr enzyme had diffused into the cytoplasm of many cells, which were often rounded up (Fig. 5). This change progressed until by 30 hr virtually all cells were rounded up, diffusely stained, and dead. Similar changes, somewhat less marked, were seen after 100 μg/ml Fransil.

Vital staining with acridine orange confirmed this picture. In normal macrophages intense orange fluorescence of lysosomes of varying size was seen throughout the cytoplasm. These were sensitive to light and readily coalesced into large masses while under observation. 5 hr after uptake of silica, many of the lysosomes were clustered around the phagosomes containing silica, where they still showed orange fluorescence. Thereafter there was progressively less discrete orange fluorescence and more diffuse green fluorescence around the silica particles and later in the cytoplasm of the cells.

Carrageenan (20 to 200 μg/ml) was rapidly taken up, even in the absence of serum or basic protein, and readily demonstrated in the phagosomes by its metachromatic reaction (Fig. 7). After 4 hr the phagosomes in unfixed cells showed discrete granules of phosphatase reaction product, and by 6 hr there was marked and more or less homogeneous staining of the phagosomes. This picture remained unchanged for about 18 hr, most of the cells still being fully extended. By 30 hr many cells had rounded up and showed diffuse phosphatase staining. The cells were often stuck together, sometimes bridged by metachromatic-staining material.

Cells took up aluminium-coated silica or diamond dust in essentially the same fashion as silica, and phosphatase was discharged into phagosomes in the usual way. However, the great majority of the cells remained fully extended and mobile for at least 30 hr (Figs. 10 and 11). The phosphatase reaction product closely enveloped the particles in the phagosomes (Fig. 6) and did not apparently escape into the remainder of the cytoplasm or nucleus.

Electron Microscopy.—Normal macrophages showed the cytoplasmic structures that have been described by others (14, 24). These include short, filamentous mitochondria with well defined cristae and numerous vesicles and cisternae lined by smooth membranes extending outwards from the Golgi region to the periphery of the cell (Fig. 9). Many lysosomes were present, and could be identified by the accumulation of acid phosphatase reaction product, though it was never possible to stain all of them in any cell. The lysosomes varied in size and form, usually being surrounded by single membranes and having fairly dense, granular contents. Occasional multivesicular bodies were also seen. Ribosomes were for the most part free, although some were membrane associated.

After addition of silica particles (Fig. 8) these were seen in invaginations of the cell membranes and in phagosomes lined by single, or occasionally double membranes (Figs. 12 and 13). Lysosomes were seen closely associated with the phagosomes (Fig. 13), and possibly discharging their contents into the phagosomes. At this stage the cytoplasmic detail was well preserved. By 14 hr in a number of cells silica particles were seen apparently free in the cytoplasm and mitochondria were swollen and rounded (Fig. 14). By 25 hr many of the cells were severely damaged, with silica particles widely scattered through the cytoplasm and organelles no longer clearly identifiable (Fig. 15). In one preparation silica particles were seen in the nucleus. It is worth emphasizing that there were considerable differences in the time taken to induce cytotoxic effects. Some cells showed obvious damage by 14 hr, but even at 24 hr some cells remained in good condition.

Appearance of Lysosomal Enzymes in the Culture Medium.—Control cultures released only very small amounts of lysosomal enzymes into the culture medium even after 3 days, whereas the enzyme content of the cells increases in culture. Within 14 hr of uptake of silica particles, release of acid phosphatase and β-glucuronidase into the medium was demonstrable. Much more was released at 24 hr and less at 30 hr. The latter may reflect destruction or inactivation of enzyme. With diamond dust only small amounts of enzyme were released in the medium at 30 hr. With carrageenan there was some enzyme release at 24 hr but more at 30 hr.

Protective Effects of Polyvinyl-Pyridine-N-Oxide (PVPNO).—Schlipköter and his colleagues (25) found that PVPNO diminishes the fibrogenic effects of quartz in animals and protects cultures of L cells against cytotoxic effects of silica. This protection was confirmed in macrophages, using biochemical

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methods of assessing cell integrity (26). We have confirmed that if mouse macrophages are treated with PVPNO considerable protection against cytotoxicity at 24 hr is found. Even if cells are pretreated with 1% PVPNO for 6 hr, followed by exposure to silica in fresh medium lacking PVPNO, considerable protection is seen. The fate of PVPNO and silica in the cells was therefore of interest. Cells were incubated for 6 hr at 37°C with 4% (w/v) PVPNO, washed with fresh medium, and examined with the histochemical technique for PVPNO. Numerous round cytoplasmic bodies containing PVPNO were seen, which suggests that there is uptake of this polymer into macrophage phagosomes in a manner analogous to the previously described uptake of Triton WR 1339 in liver lysosomes (11). If silica was added afterwards in the absence of PVPNO and the cells incubated for a further 2 hr at 37°C, then silica particles were seen in cytoplasmic vacuoles together with PVPNO. Acid

TABLE I

Release of Lysosomal Enzymes from Macrophages into Culture Medium

Measurements of enzyme activity as described in the text at different times after addition of silica or carrageenan.

Time after addition, *hr*	14		24		30	
Enzyme units[1]	Phosphatase	Glucuronidase	Phosphatase	Glucuronidase	Phosphatase	Glucuronidase
Control	0.11	0.04	0.13	0.12	0.16	0.10
Silica, *100 μg/ml*	2.75	2.98	6.58	5.32	1.80	1.13
Silica + PVPNO	0.52	0.33	0.83	0.77	1.34	0.82
Carrageenan, *100 μg/ml*	0.12	0.09	1.03	0.97	3.6	2.76

phosphatase staining of unfixed cells treated with PVPNO alone, or PVPNO and silica, showed staining of the secondary lysosomes, but very little diffuse staining of cytoplasm even at 24 hr. Leakage of lysosomal enzymes into the cytoplasm was also much less marked after exposure to silica and PVPNO than with silica alone (Table I).

DISCUSSION

It is clear that the initial uptake by macrophages of toxic particles or substances (such as silica or carrageenan) is not noticeably different from that of nontoxic particles (diamond dust or aluminium-coated silica). In both cases engulfment and formation of phagosomes, with discharge of lysosomal enzymes into phagosomes, follows essentially the same pattern. However, the subsequent events are markedly different. Cells that have taken up diamond dust remain healthy for at least 30 hr, whereas cells that have taken up silica quite rapidly succumb. Histochemical studies of the cells with engulfed nontoxic particles

show that lysosomal enzymes remain confined within the phagosomes, and only very small amounts of these enzymes are released into the medium. After uptake of silica, diffuse cytoplasmic staining of lysosomal enzymes is seen and the enzymes are detectable in the surrounding medium. Electron micrographs show that silica particles are at first confined within phagosomes whereas later they are distributed throughout the cytoplasm. About the time that silica particles are seen in the cytoplasm, other morphological changes are apparent. Early changes include swelling and rounding of mitochondria and eventually there is complete disintegration of cytoplasmic structure.

These observations suggest that the integrity of the lysosomal membrane plays a large part in determining whether an ingested particle is toxic. Any particle that can react with constituents of the membrane so as to make it permeable may bring about release of lysosomal enzymes into the cytoplasm. There is evidence that other procedures which make lysosomal membranes permeable, such as exposure to bacterial toxins (27) or treatment with visible light after dyes are taken up by lysosomes (28), can have damaging effects on cells. These may be aggravated by direct interaction of the released particles with cytoplasmic constituents.

This interpretation of silica toxicity is supported by observations that silica can react with protein, phospholipid, and artificial or natural biological membrane systems whereas nontoxic particles such as diamond dust do not react in this way. We have recently presented evidence that the main reaction likely to occur under physiological conditions is hydrogen donation from the phenolic hydroxyl groups of silicic acid in the formation of complexes with hydrogen acceptors such as suitable oxygen or nitrogen atoms in living tissues. Such reactions with secondary amide (peptide) groups of proteins are demonstrable experimentally (12), and these could account for interaction of silicic acid with protein films (29) and the denaturation of proteins and rendering them susceptible to tryptic digestion as described by Scheel and his colleagues (30). However, stronger interactions are demonstrable between hydrogen donors and phosphate ester groups of phospholipids (12). The fact that silicic acid and silica particles are hemolytic (12, 31), whereas nontoxic particles are not, shows that silica can induce permeability changes in biological membrane systems, presumably as a result of interaction with phospholipid or protein.

The protective effects of PVPNO are readily explained on the same basis, because PVPNO is a powerful hydrogen acceptor and readily forms hydrogen bonds with silicic acid (12). Silicic acid or silica particles in the presence of PVPNO are much less hemolytic than in the absence of the polymer (12, 31). Hence it is not surprising that if silica particles and polymer are ingested together by macrophages, the cells are protected. When erythrocytes are treated with PVPNO and washed, there is only slight protection against silica hemolysis (12, 31), so the polymer does not remain associated with red cell membranes.

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However, macrophages, unlike red cells, ingest PVPNO, so that the substance can remain in phagosomes which fuse with those containing silica. This is not surprising, since Gordon et al. (32) found that lysosomes prelabeled with colloidal iron fused with others enclosing DNA-protein coacervates labeled with colloidal gold. Likewise, we have observed that if macrophages are exposed first to asbestos and then after some hours to silica, the asbestos and silica particles are demonstrable within the same phagosomes. Thus PVPNO can coat the silica particles before they have time to damage phagosomal membranes. It is worth noting that the silica particles when first ingested are covered with a layer of denatured plasma protein (as can be shown by agglutination with appropriate antisera, see reference 8). Presumably this layer has to be digested away before the silica particles can react with the phagosomal membrane. Silica particles coated with lipid or protein show less interaction with membranes than free particles (31). If silica is added to cells in the presence of high concentrations of serum, its cytotoxic effect is significantly retarded.

The effects of carrageenan will be discussed elsewhere, but it appears that they are similar to those of silica although the cytotoxic effects on macrophages are more slowly exerted than those of silica. The highly acidic groups of the polymer may not be involved in their toxicity (33), but the mechanism of action remains to be fully analyzed.

We have found that silica particles released by death of macrophages have virtually undiminished cytotoxicity when added to fresh cultures of macrophages. Hence in vivo a cyclical killing of these cells would be expected. The relationship between death of macrophages and the fibrogenic effects of silica also remain open to analysis. Suggestive results were obtained by Curran and Clark (34), who implanted agar or egg white into the peritoneum of rats. These brought about death and dissolution of phagocytic cells in the vicinity, and within 4 days there was a well defined fibroblastic reaction around the dying phagocytes with laying down of connective tissue fibres. We have found that repeated subcutaneous injection of frozen and thawed macrophages from the same inbred mouse strain induces local deposition of fibrous tissue. It would thus appear that death of macrophages provides sufficient stimulus for mobilization of fibroblasts and fibrogenesis, although which components of macrophages are involved, and whether there is local transformation of connective tissue cells into fibroblasts or invasion from outside, remain to be determined.

Observations of other investigators, though not primarily concerned with lysosomes, are consistent with our interpretation of silica toxicity. Rilke and Kessel (35) found no detectable change in fixed preparations of acid phosphatase distribution in cells shortly after uptake of silica, whereas Webster et al. (36) found a decrease in macrophages 48 hr after exposure to silica. The methods used would be unsatisfactory for demonstration of redistribution of enzymes such as we have described, and the late changes could be due to loss of enzymes

through intracellular destruction or passage into the medium. Policard et al. (37) following the cellular reaction after injection of silica into rats, showed uptake into phagocytic cells, including neutrophils. They drew attention to disappearance of neutrophil granules in the process. Since neutrophil granules are lysosomes (38), these findings provide additional evidence of lysosomal changes in cells exposed to silica.

There is evidence that acid phosphatase activity increases in vivo in response to silica administration, both acutely in mesenchymal cells of liver and spleen (39) and in developing silicotic nodules (40). The former could be due to lysosomal enzyme activation, the latter to immigration of cells with high acid phosphatase activity and activation.

If lysosomal enzymes play an important part in silica cytotoxicity and the consequent fibrosis, agents which stabilize lysosomal membranes would be expected to diminish these effects. Cortisone and chloroquine are known to stabilize lysosomal membranes and prevent release of lysosomal enzymes under a variety of conditions (41). There is good evidence that cortisone administration diminishes the fibrogenic effects of silica (42, 43) although it is without effect on established lesions (44). Inhibition of the proliferative response to silica by chloroquine has also been described (45).

Thus the available evidence suggests that lysosomes play an important part in silica cytotoxicity and fibrogenesis, and that it is unnecessary to invoke either an immune response by the host or direct stimulation of fibrogenesis by silicic acid to explain the pathogenesis of silicosis. Clearly other factors, such as infection, will often supervene and complicate the pathological picture. The pathological effects of other substances or particles may be explained on a similar basis, although the detailed manifestations and long term effects may be different. We have found, for instance, that the initial fate of asbestos in macrophages is similar to that of silica, and this is one of the substances apart from silica and carrageenan that is fibrogenic. In another situation, the uptake of microcrystals of sodium urate by phagocytic cells, and the inflammatory response so induced, appears to play an important part in the acute exacerbations of gout (46). These inflammatory effects are likewise reduced by prior administration of cortisone or other drugs. Similar amounts of sodium urate in solution do not damage phagocytic cells or induce inflammation and it seems in the case of both silica and urate that having the offending material in particulate form ensures its uptake by phagocytic cells and concentration in a vulnerable region, the lysosome. A great deal of attention has been given to effects of endotoxin and other substances on lysosomes (see reference 41) but relatively little to the importance of these organelles in the pathogenesis of disease by toxic particles or substances. Further work on this aspect of the problem seems to be warranted.

In the case of silica and urate the major uptake is by phagocytic cells (macro-

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phages and polymorphonuclears, respectively), the effects on other cells being secondary and indirect. However, we have indications that other substances such as carrageenan are also taken up by fibroblasts, so that the possibility of direct stimulation of cell multiplication and fibrogenesis, perhaps by a lysosomal mechanism, must be considered. This interpretation is different from the previously held view that silicic acid or other substances act merely as templates on which fibrous tissue is deposited.

SUMMARY

Effects of silica, diamond dust, and carrageenan on mouse macrophages were studied by phase-contrast cine-micrography, electron microscopy, histochemical techniques for lysosomal enzymes and measurements of the release of lysosomal enzymes into the culture medium.

All added materials were rapidly taken up into phagosomes, to which lysosomes became attached. In all cases lysosomal enzymes were discharged into the phagosomes to form secondary lysosomes. Within 24 hr most of the silica particles and enzyme had escaped from the secondary lysosomes and lysosomal enzymes were found in the culture media. Most macrophages were killed by this time. With nontoxic particles (diamond dust, aluminium-coated silica, or silica in the presence of the protective agent polyvinyl-pyridine-N-oxide, PVPNO) ingested particles and lysosomal enzymes were retained within the secondary lysosomes for a much longer time, and cytotoxic effects were considerably delayed or absent altogether.

It is concluded that silica particles are toxic because they are efficiently taken up by macrophages and can then react relatively rapidly with the membranes surrounding the secondary lysosomes. The particles and lytic enzymes can then escape into the cytoplasm, producing general damage, and thence into the culture medium. It is suggested that hydrogen bonding of silicic acid with lipid and protein constituents of the membrane accounts for the induced permeability. Protective agents such as PVPNO are retained in lysosomes and preferentially form hydrogen bonds with silicic acid.

Carrageenan is demonstrable within macrophages by its metachromatic reaction. It brings about release of enzymes from secondary lysosomes, but much more slowly than does silica. Silica released from killed macrophages is as cytotoxic as the original preparation. It is suggested that repeated cycles of macrophage killing in vivo leads to the mobilization of fibroblasts and fibrogenesis characterizing the disease silicosis.

We are grateful to Mr. C. Gilchrist for technical assistance and to Mr. F. Wanless for taking the photomicrographs.

BIBLIOGRAPHY

1. Marks, J., The neutralization of silica toxicity *in vitro*, *Brit. J. Ind. Med.*, 1957, **14**, 81.

2. Marks, J., and Nagelschmidt, G., Study of toxicity of dust with use of in vitro dehydrogenase technique, *Arch. Ind. Health*, 1959, **20**, 383.
3. Vigliani, E. C., Pernis, B., and Monaco, L., A study of the action of quartz particles on macrophages *in vitro*, *in* Inhaled Particles and Vapours, (C. N. Davies, editor) London, Pergamon Press Limited, 1961, 348.
4. Nagelschmidt, G., A review of theories of silicosis, *in* Symposium on Industrial Diseases (E. J. King, and C. M. Fletcher, editors) London, J. and A. Churchill Ltd., 1960, 59.
5. Harington, J. S., Some biological actions of silica: their part in the pathogenesis of silicosis, *S. African Med. J.*, 1963, **37**, 451.
6. King, E. J., Solubility theory of silicosis; critical study, *Occupational Med.*, 1947, **4**, 26.
7. Curran, R. C., and Rowsell, E. V., The application of the diffusion chamber technique to the study of silicosis, *J. Path. and Bact.*, 1958, **76**, 561.
8. Vigliani, E. C., and Pernis, B., Immunological aspects of silicosis, *Advances Tuberc. Research*, 1963, **12**, 230.
9. Webster, I., Palmhert, H., and Sluis-Cremer, G. K., The immunological theory in silicosis, private communication.
10. Cohn, Z. A., The fate of bacteria within phagocytic cells. 1. The degradation of isotopically labeled bacteria by polymorphonuclear leucocytes and macrophages, *J. Exp. Med.*, 1963, **117**, 27.
11. Wattiaux, R., Wibo, M., and Baudhuin, P., Influence of the injection of Triton WR-1339 on the properties of rat liver lysosomes, *Ciba Found. Symp. Lysosomes*, 1963, 176.
12. Nash, T., Allison, A. C., and Harington, J. S., Physico-chemical properties of silica in relation to its toxicity, *Nature*, 1966, in press.
13. Cohn, Z. A., and Wiener, E., The particulate hydrolases of macrophages. II. Biochemical and morphological response to particle ingestion, *J. Exp. Med.*, 1963, **118**, 1009.
14. Cohn, Z. A., and Wiener, E., The particulate hydrolases of macrophages. I. Comparative enzymology, isolation and properties, *J. Exp. Med.*, 1963, **118**, 991.
15. Dale, J. C., and King, E. J., Absorption of dyes, amino acids, proteins, and metal hydroxides on quartz, *Arch. Ind. Hlth. Occupational Med.*, 1953, **7**, 484.
16. Williams, G., A histological study of the connective tissue reaction to carrageenan, *J. Path. and Bact.*, 1957, **73**, 557.
17. Allison, A. C., and Mallucci, L., Histochemical studies of lysosomes and lysosomal enzymes in virus infected cell culture, *J. Exp. Med.*, 1965, **121**, 463.
18. Holt, S. J., Indigogenic staining methods for esterases, *Gen. Cytochem. Methods*, 1958, **1**, 375.
19. Palade, G. E., A study of fixation for electron microscopy, *J. Exp. Med.*, 1952, **95**, 285.
20. Karnovsky, M. J., Simple methods for "Staining with lead" at high pH in electron microscopy, *J. Cell Biol.*, 1961, **11**, 729.
21. Schmidt, G., Acid prostatic phosphatase, in Methods in Enzymology, (S. P. Colawick, and N. O. Kaplan, editors) New York, Academic Press, 1955, **2**, 523.
22. Fishman, W. H., Springer, B., and Brunetti, R., Application of an improved

012

glucuronidase assay method for the study of human blood β-glucuronidase, *J. Biol. Chem.*, 1948, **173**, 449.

23. Bitensky, L., The reversible activation of lysosomes in normal cells and the effects of pathological conditions, *Ciba Found. Symp. Lysosomes* 1963, 362.
24. Pearson, G. R., Freeman, R. A., and Hines, W. D., Thin-section electron micrographs of monocytes infected with *Brucella suis*, *J. Bact.* 1963, **86**, 1123.
25. Schlipköter, H.-W., Dolgner, R., and Brockhaus, A., The treatment of experimental silicosis, *German Med. Monthly*, 1963, **8**, 509.
26. Marchisio, M. A., and Comolli, R., Studio metabolico e morfologico dell' attivita' litico—protettiva della poli-2-vinilpiridina-N-ossido su macrofagi peritoneali trattati con silice, *Med. Lavoro*, 1964, **55**, 401.
27. Hirsch, J. G., Bernheimer, A. W., and Weissmann, K., Motion picture study of the toxic action of streptolysins on leucocytes, *J. Exp. Med.*, 1959, **110**, 223.
28. Allison, A. C., Magnus, I. A., and Young, M. R., The role of lysosomes and of cell membranes in photosensitization, *Nature*, 1966, **209**, 874.
29. Clark, S. G., and Holt, P. F., The interaction of silicic acid with collagen and gelatin monolayers, *Trans. Faraday Soc.*, 1957, **53**, 1509.
30. Scheel, L. D., Smith, B., Van Piper, J., and Fleischer, E., Toxicity of silica, *Arch. Ind. Hyg. and Occupational Med.*, 1964, **9**, 29.
31. Stalder, K., and Stöber, W., Haemolytic activity of suspensions of different silica modifications and inert dusts, *Nature*, 1965, **207**, 874.
32. Gordon, G. B., Miller, L. R., and Bensch, K. G., Studies on the intracellular digestive process in mammalian tissue culture cells, *J. Cell Biol.*, 1965, **25**, 41.
33. McCandless, E. L., Chemical structural requirements for stimulation of connective tissue growth by polysaccharides, *Ann. New York Acad. Sc.*, 1965, **118**, 867.
34. Curran, R. C., and Clark, A. E., Phagocytosis and fibrogenesis in peritoneal implants in the rat, *J. Path. and Bact.*, 1964, **88**, 489.
35. Rilke, F., and Kessel, R. W. I., Histochemical studies into the cytotoxicity of tridymite *in vitro*, *Med. Lavoro.*, 1963, **54**, 161.
36. Webster, I. A., Melamed, M., and Munday, D. E., The effect of silica particles on esterase and acid phosphatase in macrophages, *Med. Lavoro.* 1961, **52**, 481.
37. Policard, A., Collet, A., and Pregermain, S., Etude an microscope electronique des modifications infrastructurales presentees par les polynucleaires neutraphiles dans des paipers inflammatoires aseptiques, *Rev. Hematol.*, 1959, **14**, 97.
38. Cohn, Z. A., and Hirsch, J. G., The isolation and properties of the specific cytoplasmic granules of rabbit polymorphonuclear leucocytes, *J. Exp. Med.*, 1960, **112**, 983.
39. Rilke, F., and Pernis, B., Morphological and histochemical aspects of R.E.S. stimulation induced by crystalline silica particles, *Med. Lavoro*, 1961, **57**, 321.
40. Engelbrecht, F. M., and Paul, R., Enzymes in the development of silicotic fibrosis in the lungs of rats, *Ann. Occupational Hyg.*, 1959, **1**, 135.
41. Weissmann, G., Lysosomes, *New England J. Med.*, 1965, **273**, 1084, 1143.
42. Harrison, C. V., King, E. J., Dale, J. C., and Sichel, R., Effect of cortisone on experimental silicosis, *Brit. J. Ind. Med.*, 1952, **9**, 165.

43. Curran, R. C., Observations on the formation of collagen in quartz lesions, *J. Path. and Bact.*, 1953, **66**, 271.
44. King, E. J., Harrison, C. V., and Attygalle, D., Effect of cortisone on established silicotic fibrosis in lungs of rats, *Brit. J. Ind. Med.*, 1955, **12**, 228.
45. Simon, H., Histochemische Untersuchungen Zur Wirkung von Chlorochindiphosphat, *Naturwissenschaften*, 1961, **48**, 647.
46. Malawista, S. E., and Seegmiller, J. E., The effect of pretreatment with colchicine on the inflammatory response to microcrystalline urate, *Ann. Int. Med.*, 1965, **62**, 648.
47. Pearse, A. G. E., Histochemistry, London, J. and A. Churchill Ltd., 2nd edition, 1960, 948.

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(b)(1), (c)(2), (d)), and under the authority delegated to him by the Secretary of Health, Education, and Welfare (25 F.R. 8625), the Commissioner of Food and Drugs, based on a petition filed by Hoffman-LaRoche, Inc., Nutley, New Jersey, and other relevant material, promulgated an order on April 6, 1963 (28 F.R. 3382), listing β-apo-8'-carotenal as a safe color additive for use in or on foods and exempting it from certification. The act permits a period of 30 days for the filing of objections to an order. Within that period, the Certified Color Industry Committee filed objections to the paragraph exempting the color additive from certification, stating that it would be adversely affected by such an order and specifying its objection.

Having considered the objection filed, the Commissioner has concluded that the person objecting is adversely affected and that the objection is based on reasonable grounds. *Now therefore, it is ordered,* That the effective date of paragraph (e) of § 8.302, exempting β-apo-8'-carotenal from certification, be stayed.

Since the objection related only to the exemption from certification, the remainder of the section became effective on June 5, 1963. Pending resolution of the objection, batches of β-apo-8'-carotenal shall be certified in accordance with the regulations promulgated under Subpart A of this Part 8.

At a later date, the Commissioner will announce in the FEDERAL REGISTER the date and place of a public hearing.

(Secs. 701(e), 706 (b)(1), (c)(2), (d); 52 Stat. 1055 as amended; 74 Stat. 399, 402, 403; 21 U.S.C. 371(e), 376 (b)(1), (c)(2), (d))

Dated: July 24, 1963.

GEO. P. LARRICK,
Commissioner of Food and Drugs.

[F.R. Doc. 63-8034; Filed, July 30, 1963; 8:53 a.m.]

SUBCHAPTER B—FOOD AND FOOD PRODUCTS

PART 42—EGGS AND EGG PRODUCTS; DEFINITIONS AND STANDARDS OF IDENTITY

Dried Eggs, Dried Whole Eggs; Order Amending Standard of Identity

In the matter of amending the standard of identity for dried eggs, dried whole eggs, to provide for the optional use of less than 2 percent of sodium silicoaluminate as an anticaking ingredient:

A notice of proposed rule making in the above-identified matter was published in the FEDERAL REGISTER of June 7, 1963 (28 F.R. 5619). The standard for dried whole eggs, for which the amendment was proposed, provides that the finished food shall contain not less than 92 percent total egg solids. In effect, this provision limits the moisture content to 8 percent. The amendment proposed that the moisture content of dried whole eggs with added anticaking ingredient should be limited to not more than 5 percent. This guards against any substitution of anticaking ingredient for egg solids in the finished food. The notice invited interested persons to comment on the proposal. No comments were received.

In consideration of the information furnished by the petitioner, and other available data, it is concluded that it will promote honesty and fair dealing in the interest of consumers to amend the definition and standard of dried eggs, dried whole eggs as proposed. Therefore, pursuant to the authority vested in the Secretary of Health, Education, and Welfare by the Federal Food, Drug, and Cosmetic Act (secs. 401, 701, 52 Stat. 1046, 1055, as amended 70 Stat. 919, 72 Stat. 948; 21 U.S.C. 341, 371) and delegated to the Commissioner of Food and Drugs by the Secretary (25 F.R. 8625): *It is ordered,* That the definition and standard of identity for dried eggs, dried whole eggs be amended to read as follows:

§ 42.30 Dried eggs, dried whole eggs; identity; label statement of optional ingredients.

(a) Dried eggs, dried whole eggs are prepared by drying liquid eggs. They may be powdered. Sodium silicoaluminate may be added as an optional anticaking ingredient, but the amount used is less than 2 percent by weight of the finished food. The moisture content of the finished food, if the optional anticaking ingredient is used, does not exceed 5 percent by weight; however, if the optional anticaking ingredient is not used the moisture content may exceed 5 percent, but it does not exceed 8 percent. The moisture content is determined by the method prescribed in "Official Methods of Analysis of the Association of Official Agricultural Chemists," Ninth Edition, 1960, sections 16.002–16.003, under "Total Solids."

(b) (1) When the optional anticaking ingredient, as provided in paragraph (a) of this section is used, the label shall bear the statement "Less than 2 percent sodium silicoaluminate added as an anticaking agent."

(2) Wherever the name of the food appears on the label so conspicuously as to be easily seen under customary conditions of purchase, the statement specified in subparagraph (1) of this paragraph, showing the optional ingredient used, shall immediately and conspicuously precede or follow such name, without intervening, written, printed, or graphic matter.

Any person who will be adversely affected by the foregoing order may at any time within 30 days from the date of its publication in the FEDERAL REGISTER file with the Hearing Clerk, Department of Health, Education, and Welfare, Room 5440, 330 Independence Avenue SW., Washington 25, D.C., written objections thereto. Objections shall show wherein the person filing will be adversely affected by the order and specify with particularity the provisions of the order deemed objectionable and the grounds for the objections. If a hearing is requested, the objections must state the issues for the hearing, and such objections must be supported by grounds legally sufficient to justify the relief sought. Objections, preferably in triplicate, may be accompanied by a memorandum or brief in support thereof.

Effective date. This order shall become effective 60 days from the date of its publication in the FEDERAL REGISTER, except as to any provisions that may be stayed by the filing of proper objections. Notice of the filing of objections or lack thereof will be announced by publication in the FEDERAL REGISTER.

(Secs. 401, 701, 52 Stat. 1046, 1055, as amended 70 Stat. 919, 72 Stat. 948; 21 U.S.C. 341, 371)

Dated: July 24, 1963.

GEO. P. LARRICK,
Commissioner of Food and Drugs.

[F.R. Doc. 63-8035; Filed, July 30, 1963; 8:54 a.m.]

PART 121—FOOD ADDITIVES

Subpart D—Food Additives Permitted in Food for Human Consumption

SODIUM NITRATE; SODIUM NITRITE

The Commissioner of Food and Drugs, having evaluated data in a petition filed by Los Angeles Smoking and Curing Company, 778 Kohler Street, Los Angeles 21, California, and other relevant material, has concluded that the food additive regulations should be amended to prescribe further conditions of use of sodium nitrate and sodium nitrite. Therefore, pursuant to the provisions of the Federal Food, Drug, and Cosmetic Act (sec. 409(c)(1), 72 Stat. 1786; 21 U.S.C. 348(c)(1)), and under the authority delegated to the Commissioner by the Secretary of Health, Education, and Welfare (25 F.R. 8625), §§ 121.1063 and 121.1064 are amended in the following respects:

1. Section 121.1063(a)(1) is amended to read:

§ 121.1063 Sodium nitrate.

* * * * *

(a) * * *

(1) As a preservative and color fixative, with or without sodium nitrite, in smoked, cured salmon and smoked, cured shad, so that the level of sodium nitrate does not exceed 500 parts per million (0.05 percent) and the level of sodium nitrite does not exceed 200 parts per million (0.02 percent) in the finished product.

2. Section 121.1064(a)(2) is amended to read:

§ 121.1064 Sodium nitrite.

(a) * * *

(2) As a preservative and color fixative with or without sodium nitrate, in smoked, cured salmon and smoked, cured shad so that the level of sodium nitrite does not exceed 200 parts per million (0.02 percent) and the level of sodium nitrate does not exceed 500 parts per million (0.50 percent) in the finished product.

Any person who will be adversely affected by the foregoing order may at any time within 30 days from the date of its publication in the FEDERAL REGISTER file with the Hearing Clerk, Department of Health, Education, and Welfare, Room 5440, 330 Independence Avenue SW., Washington 25, D.C., written objections thereto. Objections shall show wherein the person filing will be adversely affected by the order and specify with particularity the provisions of the order

deemed objectionable and the grounds for the objections. If a hearing is requested, the objections must state the issues for the hearing. A hearing will be granted if the objections are supported by grounds legally sufficient to justify the relief sought. Objections may be accompanied by a memorandum or brief in support thereof. All documents shall be filed in quintuplicate.

Effective date. This order shall be effective on the date of its publication in the FEDERAL REGISTER.

(Sec. 409(c)(1), 72 Stat. 1786, 21 U.S.C. 348(c)(1))

Dated: July 3, 1963.

GEO. P. LARRICK,
Commissioner of Food and Drugs.

[F.R. Doc. 63-8036; Filed, July 30, 1963; 8:55 a.m.]

PART 121—FOOD ADDITIVES

Subpart D—Food Additives Permitted in Food for Human Consumption

XYLITOL

The Commissioner of Food and Drugs, having evaluated data in a petition filed by Lorenz and Lihn, GmbH., Bad Godesberg-Mehlem, Germany, and other relevant material, has concluded that the food additive regulations should be amended to prescribe further uses of xylitol. Therefore, pursuant to the provisions of the Federal Food, Drug, and Cosmetic Act (sec. 409(c)(1), 72 Stat. 1786; 21 U.S.C. 348(c)(1)), and under the authority delegated to the Commissioner by the Secretary of Health, Education, and Welfare (25 F.R. 8625), § 121.1114 (21 CFR 121.1114; 28 F.R. 968) is amended to read as follows:

§ 121.1114 Xylitol.

Xylitol may be safely used in foods for special dietary uses, provided the amount used is not greater than that required to produce its intended effect.

Any person who will be adversely affected by the foregoing order may at any time within 30 days from the date of its publication in the FEDERAL REGISTER file with the Hearing Clerk, Department of Health, Education, and Welfare, Room 5440, 330 Independence Avenue SW., Washington 25, D.C., written objections thereto. Objections shall show wherein the person filing will be adversely affected by the order and specify with particularity the provisions of the order deemed objectionable and the grounds for the objections. If a hearing is requested, the objections must state the issues for the hearing. A hearing will be granted if the objections are supported by grounds legally sufficient to justify the relief sought. Objections may be accompanied by a memorandum or brief in support thereof. All documents shall be filed in quintuplicate.

Effective date. This order shall be effective on the date of its publication in the FEDERAL REGISTER.

(Sec. 409(c)(1), 72 Stat. 1786; 21 U.S.C. 348(c)(1))

Dated: July 24, 1963.

GEO. P. LARRICK,
Commissioner of Food and Drugs.

[F.R. Doc. 63-8037; Filed, July 30, 1963; 8:56 a.m.]

PART 121—FOOD ADDITIVES

Subpart F—Food Additives Resulting From Contact With Containers or Equipment and Food Additives Otherwise Affecting Food

RESINOUS AND POLYMERIC COATINGS

The Commissioner of Food and Drugs, having evaluated the data submitted in a petition filed by General Electric Company, Silicone Products Department, Waterford, New York, and other relevant material, has concluded that the food additive regulations should be amended to provide for the use of dibutyltin dilaurate as a curing catalyst for silicone polymers employed in the formulation of resinous and polymeric coatings intended for use in contact with food. The Commissioner has also concluded that the use of tin catalysts for silicone polymers should be limited to not more than the amount required to effect optimum cure, which shall not exceed one part of tin per 100 parts of silicone polymer solids. Therefore, pursuant to the provisions of the Federal Food, Drug, and Cosmetic Act (sec. 409(c)(1), 72 Stat. 1786; 21 U.S.C. 348(c)(1)), and under the authority delegated to the Commissioner by the Secretary of Health, Education, and Welfare (25 F.R. 8625), § 121.2514(b)(3)(xxviii) (21 CFR 121.2514; 28 F.R. 3587) is amended by adding a new item to (b). As amended (b) reads as follows:

§ 121.2514 Resinous and polymeric coatings.

* * * * *

(b) * * *

(3) * * *

(xxviii) * * *

(b) Catalysts (the maximum amount of tin catalyst used shall be that required to effect optimum cure but shall not exceed one part of tin per 100 parts of silicone polymer solids):

Dibutyltin dilaurate.
Tin oleate.

Any person who will be adversely affected by the foregoing order may at any time within 30 days from the date of its publication in the FEDERAL REGISTER file with the Hearing Clerk, Department of Health, Education, and Welfare, Room 5440, 330 Independence Avenue SW., Washington 25, D.C., written objections thereto. Objections shall show wherein the person filing will be adversely affected by the order and specify with particularity the provisions of the order deemed objectionable and the grounds for the objections. If a hearing is requested, the objections must state the issues for the hearing. A hearing will be granted if the objections are supported by grounds legally sufficient to justify the relief sought. Objections may be accompanied by a memorandum or brief in support thereof. All documents shall be filed in quintuplicate.

Effective date. This order shall be effective on the date of its publication in the FEDERAL REGISTER.

(Sec. 409(c)(1), 72 Stat. 1786; 21 U.S.C. 348(c)(1))

Dated: July 24, 1963.

GEO. P. LARRICK,
Commissioner of Food and Drugs.

[F.R. Doc. 63-8039; Filed, July 30, 1963; 8:57 a.m.]

PART 121—FOOD ADDITIVES

Subpart F—Food Additives Resulting From Contact With Containers or Equipment and Food Additives Otherwise Affecting Food

ANTIOXIDANTS AND/OR STABILIZERS FOR POLYMERS

The Commissioner of Food and Drugs, having evaluated the data submitted in a petition filed by Farbenfabriken Bayer A.G., Leverkusen, Federal Republic of Germany, and other relevant material, has concluded that the food additive regulations should be amended to provide for the use of *N,N'*-diphenylthiourea as an antioxidant and/or stabilizer in polymers used in the manufacture of articles or components of articles intended for use in contact with food. Therefore, pursuant to the provisions of the Federal Food, Drug, and Cosmetic Act (sec. 409(c)(1), 72 Stat. 1786; 21 U.S.C. 348(c)(1)), and under the authority delegated to the Commissioner by the Secretary of Health, Education, and Welfare (25 F.R. 8625), § 121.2566 (28 F.R. 1796, 5047) is amended by inserting alphabetically in paragraph (b) a new item, as follows:

§ 121.2566 Antioxidants and/or stabilizers for polymers.

* * * * *

(b) List of substances:

	Limitations
* * *	* * *
N,N'-Diphenylthiourea.	For use only: At levels not to exceed 0.5 percent by weight of polyvinyl chloride; or At levels not to exceed 0.5 percent by weight of vinyl chloride-vinyl acetate copolymers containing not more than 20 molar percent of vinyl acetate.
* * *	* * *

Any person who will be adversely affected by the foregoing order may at any time within 30 days from the date of its publication in the FEDERAL REGISTER file with the Hearing Clerk, Department of

1157), and on the basis of these comments, the information contained in the petition filed by Hercules Powder Company, Wilmington, Delaware, and other available and relevant data has concluded that for those foods listed in the notice, where the identity standards are now effective, the amendments should be adopted as proposed. Therefore, the following amendments are ordered, pursuant to the provisions of the Federal Food, Drug, and Cosmetic Act (secs. 401, 701, 52 Stat. 1046, 1055, as amended; 21 U.S.C. 341, 371) and under the authority delegated to the Commissioner by the Secretary of Health, Education, and Welfare (25 F.R. 8625):

1. In § 19.775 *Pasteurized process cheese spread; identity; label statement of optional ingredients*, paragraph (f)(1) is amended to read:

(1) One or any mixture of two or more of the following: Carob bean gum, gum karaya, gum tragacanth, guar gum, gelatin, sodium carboxymethylcellulose (cellulose gum), carrageen, oat gum, algin (sodium alginate), and algin derivative (propylene glycol ester of alginic acid). The total weight of such substances is not more than 0.8 percent of the weight of the finished food.

2. In § 19.782 *Cream cheese with other foods; identity; label statement of optional ingredients*, paragraph (a)(1) is amended to read:

(1) One or any mixture of two or more of the following optional ingredients: Gum karaya, gum tragacanth, carob bean gum, gelatin, guar gum, sodium carboxymethylcellulose (cellulose gum), carrageen, oat gum, algin (sodium alginate), algin derivative (propylene glycol ester of alginic acid). The total quantity of any such substances, including that contained in the cream cheese, is not more than 0.8 percent by weight of the finished food.

3. In § 19.783 *Pasteurized neufchatel cheese spread with other foods; identity; label statement of optional ingredients*, paragraph (b)(1) is amended to read:

(1) One or any mixture of two or more of the following: Gum karaya, gum tragacanth, carob bean gum, gelatin, algin (sodium alginate), algin derivative (propylene glycol ester of alginic acid), guar gum, sodium carboxymethylcellulose (cellulose gum), carrageen, oat gum. The total quantity of any such substances, including that contained in the neufchatel cheese, is not more than 0.8 percent by weight of the finished food.

4. In § 25.2 *French dressing; identity; label statement of optional ingredients*, paragraph (c)(1) is amended to read:

(1) Gum acacia (also called gum arabic), carob bean gum (also called locust bean gum), guar gum, gum karaya, gum tragacanth, extract of Irish moss, pectin, propylene glycol ester of alginic acid, sodium carboxymethylcellulose (cellulose gum), methyl cellulose U.S.P. (methoxy content not less than 27.5 percent and not more than 31.5 percent on a dry-weight basis), hydroxypropyl methylcellulose, or any mixture of two or more of these.

5. In § 25.3 *Salad dressing; identity; label statement of optional ingredients*, paragraph (d) is amended to read:

(d) The optional emulsifying ingredients referred to in paragraph (a) of this section are: Gum acacia (also called gum arabic), carob bean gum (also called locust bean gum), guar gum, gum karaya, gum tragacanth, extract of Irish moss, pectin, propylene glycol ester of alginic acid, sodium carboxymethylcellulose (cellulose gum), methyl cellulose U.S.P. (methoxy content not less than 27.5 percent and not more than 31.5 percent on a dry-weight basis), hydroxypropyl methylcellulose, or any mixture of two or more of these. The quantity used of any such emulsifying ingredient or mixture amounts to not more than 0.75 percent by weight of the finished salad dressing.

The notice in which these amendments were proposed directed attention to the fact that adoption of the proposals would furnish a basis for naming the optional ingredient on labels either as "sodium carboxymethylcellulose" or "cellulose gum."

The standards of identity for pasteurized cheese spread, pasteurized process cheese spread with fruits, vegetables, or meats, and for pasteurized cheese spread with fruits, vegetables, or meats (21 CFR 19.776, 19.780, and 19.781) are so cross-referenced to the standard for pasteurized process cheese spread (21 CFR 19.775) that adopting the amendment proposed for § 19.775 will have the effect of making the same amendment applicable to the standards for the products covered by §§ 19.776, 19.780, and 19.781.

Any person who will be adversely affected by the foregoing order may at any time within 30 days from the date of its publication in the FEDERAL REGISTER file with the Hearing Clerk, Department of Health, Education, and Welfare, Room 5440, 330 Independence Avenue SW., Washington 25, D.C., written objections thereto. Objections shall show wherein the person filing will be adversely affected by the order and specify with particularity the provisions of the order deemed objectionable and the grounds for the objections. If a hearing is requested, the objections must state the issues for the hearing, and such objections must be supported by grounds legally sufficient to justify the relief sought. Objections may be accompanied by a memorandum or brief in support thereof. All documents shall be filed preferably in quintuplicate.

Effective date. This order shall become effective 60 days from the date of its publication in the FEDERAL REGISTER, except as to any provisions that may be stayed by the filing of proper objections. Notice of the filing of objections or lack thereof will be announced by publication in the FEDERAL REGISTER.

(Secs. 401, 701, 52 Stat. 1046, 1055, as amended; 21 U.S.C. 341, 371)

Dated: April 22, 1963.

GEO. P. LARRICK,
Commissioner of Food and Drugs.

[F.R. Doc. 63-4515; Filed, Apr. 26, 1963; 8:48 a.m.]

PART 42—EGGS AND EGG PRODUCTS; DEFINITIONS AND STANDARDS OF IDENTITY

Dried Egg Yolks; Standard of Identity To Provide for Optional Anticaking Ingredient Sodium Silicoaluminate

In the matter of amending the standard of identity for dried egg yolks, dried yolks:

A notice of proposed rule making was published in the FEDERAL REGISTER of February 27, 1963 (28 F.R. 1805), setting forth a proposal by Seymour Foods, Inc., Topeka, Kansas, to amend the definition and standard of identity for dried egg yolks (21 CFR 42.60) to provide for the optional use of less than 2 percent of sodium silicoaluminate as an anticaking ingredient. It was proposed that regardless of whether this anticaking ingredient is used, the finished food should contain not less than 95 percent of egg solids.

Upon consideration of the information furnished by the petitioner, that contained in comments submitted in response to the proposal, and other relevant information, it is concluded that it will promote honesty and fair dealing in the interest of consumers to amend the definition and standard for dried egg yolks as proposed. Therefore, pursuant to the authority vested in the Secretary of Health, Education, and Welfare by the Federal Food, Drug, and Cosmetic Act (secs. 401, 701, 52 Stat. 1046, 1055, as amended 70 Stat. 919, 72 Stat. 948; 21 U.S.C. 341, 371) and delegated to the Commissioner of Food and Drugs by the Secretary (25 F.R. 8625): *It is ordered*, That the amendment in the above-identified matter be adopted without change.

Any person who will be adversely affected by the foregoing order may within 30 days from the date of its publication in the FEDERAL REGISTER file with the Hearing Clerk, Department of Health, Education, and Welfare, Room 5440, 330 Independence Avenue SW., Washington 25, D.C., written objections thereto. Objections shall show wherein the person filing will be adversely affected by the order and specify with particularity the provisions of the order deemed objectionable and the grounds for the objections. If a hearing is requested, the objections must state the issues for the hearing, and such objections must be supported by grounds legally sufficient to justify the relief sought. Objections may be accompanied by a memorandum or brief in support thereof. All documents shall be filed in quintuplicate.

Effective date. This order shall become effective 45 days from the date of its publication in the FEDERAL REGISTER, except as to any provisions that may be stayed by the filing of proper objections. Notice of the filing of objections or lack thereof will be announced by publication in the FEDERAL REGISTER.

(Secs. 401, 701, 52 Stat. 1046, 1055, as amended 70 Stat. 919, 72 Stat. 948; 21 U.S.C. 341, 371)

Dated: April 22, 1963.

GEO. P. LARRICK,
Commissioner of Food and Drugs.

§ 42.60 Dried egg yolks, dried yolks; identity; label statement of optional ingredients.

(a) Dried egg yolks, dried yolks is the food prepared by drying egg yolks. Sodium silicoaluminate may be added as an optional anticaking ingredient, but the amount used is less than 2 percent by weight of the finished food. The moisture content of the finished food, if the optional anticaking ingredient is used, does not exceed 3 percent by weight; however, if the optional anticaking ingredient is not used, the moisture content may exceed 3 percent but it does not exceed 5 percent. The moisture content is determined by the method prescribed in "Official Methods of Analysis of the Association of Official Agricultural Chemists," Ninth Edition, 1960, sections 16.002 and 16.003, under "Total Solids."

(b)(1) When the optional anticaking ingredient sodium silicoaluminate is used, the label shall bear the statement, "Less than 2 percent sodium silicoaluminate added as an anticaking agent."

(2) Whenever the name of the food appears on the label so conspicuously as to be easily seen under customary conditions of purchase, the statement specified in subparagraph (1) of this paragraph, showing the optional ingredient used, shall immediately and conspicuously precede or follow such name, without intervening written, printed, or graphic matter.

[F.R. Doc. 63 4516; Filed, Apr. 26, 1963; 8:48 a.m.]

PART 120—TOLERANCES AND EXEMPTIONS FROM TOLERANCES FOR PESTICIDE CHEMICALS IN OR ON RAW AGRICULTURAL COMMODITIES

Tolerances for Residues of Carbophenothion

A petition was filed with the Food and Drug Administration, by Stauffer Chemical Company, 380 Madison Avenue, New York 17, New York, requesting the establishment of tolerances for residues of the insecticide carbophenothion (*S*-(*p*-chlorophenylthiomethyl) *O,O*-diethyl phosphorodithioate) in or on raw agricultural commodities, as follows:

10 parts per million in or on almond hulls.

5 parts per million in or on alfalfa (fresh), alfalfa (hay), bean straw, clover (fresh), clover (hay), corn forage, and sorghum forage.

2 parts per million in or on sorghum grain.

0.5 part per million in or on corn (kernels plus cob with husks included).

The 10 parts per million tolerance in or on almond hulls represents an increase from the present tolerance of 2 parts per million.

The petition was later amended to change the tolerance requested for corn to 0.2 part per million in or on corn (kernals plus cob with husks removed) and to request a tolerance of 0.1 part per million for residues in the fat of meat of cattle, goats, hogs, and sheep. Data in this petition show that the tolerance of 0.1 part per million in the fat of meat of cattle, goats, hogs, and sheep is adequate to cover residues from use in feeds containing treated almond hulls and treated forage. The data also show that the feeding to dairy animals of treated crops for which tolerances are being established will not result in residues in milk.

The Secretary of Agriculture has certified that this pesticide chemical is useful for the purposes for which tolerances are being established.

After consideration of the data submitted in the petition and other relevant material which show that the tolerances established in this order will protect the public health, and by virtue of the authority vested in the Secretary of Health, Education, and Welfare by the Federal Food, Drug, and Cosmetic Act (sec. 408 (d)(2), 68 Stat. 512; 21 U.S.C. 346a(d)(2)) and delegated to the Commissioner of Food and Drugs by the Secretary (25 F.R. 8625), the regulations for tolerances for pesticide chemicals in or on raw agricultural commodities (21 CFR 120.156) are amended by inserting in § 120.156 new items beginning "10 parts per million," "0.1 part per million," and "zero," by revising the items beginning "5 parts per million," "2 parts per million," and "0.2 part per million." As amended, the affected portions of this section read as follows:

§ 120.156 Carbophenothion; tolerances for residues.

Tolerances for residues of the insecticide carbophenothion (*S*-(*p*-chlorophenylthiomethyl) *O,O*-diethyl phosphorodithioate) in or on raw agricultural commodities are established as follows:

10 parts per million in or on almond hulls.

5 parts per million in or on alfalfa (fresh), alfalfa (hay), bean straw, clover (fresh), clover (hay), corn forage, sorghum forage, sugarbeets (roots), sugarbeets (tops).

2 parts per million in or on grapefruit, lemons, limes, oranges, sorghum grain, tangelos, tangerines.

* * * * *

0.2 part per million in or on corn (kernels plus cob with husks removed), undelinted cottonseed.

0.1 part per million in the fat of meat of cattle, goats, hogs, and sheep.

Zero in milk.

Any person who will be adversely affected by the foregoing order may at any time within thirty days from the date of its publication in the FEDERAL REGISTER file with the Hearing Clerk, Department of Health, Education, and Welfare, Room 5440, 330 Independence Avenue SW., Washington 25, D.C., written objections thereto. Objections shall show wherein the person filing will be adversely affected by the order and specify with particularity the provisions of the order deemed objectionable and the grounds for the objections. If a hearing is requested, the objections must state the issues for the hearing. A hearing will be granted if the objections are supported by grounds legally sufficient to justify the relief sought. Objections may be accompanied by a memorandum or brief in support thereof. All documents shall be filed in quintuplicate.

Effective date. This order shall be effective on the date of its publication in the FEDERAL REGISTER.

(Sec. 408(d)(2), 68 Stat. 512; 21 U.S.C. 346a(d)(2))

Dated: April 22, 1963.

GEO. P. LARRICK,
Commissioner of Food and Drugs.

[F.R. Doc. 63-4522; Filed, Apr. 26, 1963; 8:48 a.m.]

PART 120—TOLERANCES AND EXEMPTIONS FROM TOLERANCES FOR PESTICIDE CHEMICALS IN OR ON RAW AGRICULTURAL COMMODITIES

PART 121—FOOD ADDITIVES

Subpart D—Food Additives Permitted in Food for Human Consumption

ETHION; PESTICIDE AND FOOD ADDITIVE RESIDUES

1. A petition was filed with the Food and Drug Administration by Niagara Chemical Division, FMC Corporation, Middleport, New York, requesting the establishment of tolerances for residues of the insecticide ethion (*O,O,O',O'*-tetraethyl *S,S'*-methylene bisphosphorodithioate) in or on cucumbers and summer squash at 0.5 part per million and proposing an increase from 1 part per million to 2 parts per million in tolerances for residues of ethion in or on apples, grapes, melons, plums, and prunes.

The Secretary of Agriculture has certified that this pesticide chemical is useful for the purposes for which tolerances are being established.

After consideration of the data submitted in the petition and other relevant material which show that the tolerances established in this order will protect the public health, and by virtue of the authority vested in the Secretary of Health, Education, and Welfare by the Federal Food, Drug, and Cosmetic Act (sec. 408(d)(2), 68 Stat. 512 as amended; 21 U.S.C. 346a(d)(2)) and delegated to the Commissioner of Food and Drugs by the Secretary (25 F.R. 8625), the regulations for tolerances for pesticide chemicals in or on raw agricultural commodities (21 CFR 120.173; 27 F.R. 12108) are amended by adding thereto an item read squash, and by increasing the tolerance for ethion on cucumbers and summer squash, and by increasing the tolerances level for residues of this insecticide on apples, grapes, melons, plums, and prunes. To conform with other tolerances established for the commodity, plums and prunes are changed to read "plums (fresh prunes)". As amended, the affected portions of § 120.173 read as follows:

§ 120.173 Ethion; tolerances for residues.

* * * * *

2 parts per million in or on apples, beans, citrus fruits, grapes, melons, plums (fresh prunes), strawberries, tomatoes.

Chapter III—Federal Aviation Agency

SUBCHAPTER C—AIRCRAFT REGULATIONS

[Reg. Docket No. 1970; Amdt. 651]

PART 507—AIRWORTHINESS DIRECTIVES

Certain Lockheed Models Series Aircraft

A proposal to amend Part 507 of the regulations of the Administrator to include an airworthiness directive requiring replacement of all nose landing gear actuating cylinder attach bolts on Lockheed Models 49, 149, 649, 649A, 749, 749A, 1049-54, 1049C, 1049D, 1049E, 1049G and 1049H Series aircraft was published in 28 F.R. 10427.

Interested persons have been afforded an opportunity to participate in the making of the amendment. No objections were received.

In consideration of the foregoing, and pursuant to the authority delegated to me by the Administrator (25 F.R. 6489), § 507.10(a) of Part 507 (14 CFR Part 507), is hereby amended by adding the following new airworthiness directive:

LOCKHEED. Applies to all Models 49, 149, 649, 649A, 749, 749A, 1049-54, 1049C, 1049D, 1049E, 1049G, and 1049H Series aircraft.

Compliance required as indicated.

As a result of general fatigue failures of the nose landing gear actuating cylinder attach bolts, accomplish the following:

(a) For operators maintaining records of landings, within the next 1,000 landings after the effective date of this AD, unless already accomplished within the last 5,000 landings, and thereafter within each 6,000 landings from the last replacement, comply with (c). Where past records of landings are unavailable the number of landings prior to the effective date of this AD may be obtained by substituting one landing for each hour of time in service prior to the effective date of this AD.

(b) For operators not maintaining records of landings, within the next 1,000 hours' time in service after the effective date of this AD, unless already accomplished within the last 5,000 hours' time in service, and thereafter within each 6,000 hours' time in service from the last replacement, comply with (c).

(c) Replace the nose landing gear actuating cylinder bolts as follows:

(1) Replace the two forward bolts, P/N 307403 and P/N 307404, which attach the forward end of the actuating cylinder to the fuselage structure via a universal joint, with new bolts of the same part numbers.

(2) Replace the aft bolt, P/N 307402, which attaches the actuating cylinder rod to the drag strut pivot, with a new bolt of the same part number.

(3) For those aircraft which use P/N 272216-3 and P/N 272238 in lieu of P/N 307402 and P/N 307403, replace these bolts with new bolts of the same part numbers or replace P/N 272216-3 with P/N 307402 and replace P/N 272238 with P/N 307403 in accordance with items 1, 2, 3, 5, 6, and 7 of the section entitled "Description of Change B" of Lockheed Service Bulletin 49/SB-616.

(4) For those aircraft which use P/N 272216-2 in lieu of P/N 307404 replace this bolt with P/N 307404 and accomplish the rework of the section entitled "Description of Change A", Lockheed Service Bulletin 49/SB-616.

This supersedes Amendment 523, 27 F.R. 12828, AD 63-1-2.

This amendment shall become effective December 30, 1963.

(Secs. 313(a), 601, 603; 72 Stat. 752, 775, 776; 49 U.S.C. 1354(a), 1421, 1423)

Issued in Washington, D.C., on November 21, 1963.

W. LLOYD LANE,
Acting Director,
Flight Standards Service.

[F.R. Doc. 63-12398; Filed, Nov. 29, 1963; 8:46 a.m.]

Title 21—FOOD AND DRUGS

Chapter I—Food and Drug Administration, Department of Health, Education, and Welfare

SUBCHAPTER B—FOOD AND FOOD PRODUCTS

PART 121—FOOD ADDITIVES

Subpart D—Food Additives Permitted in Food for Human Consumption

ACRYLAMIDE-ACRYLIC ACID RESIN

The Commissioner of Food and Drugs, having evaluated data in a petition (FAP 1019) filed by The Dow Chemical Company, Midland, Michigan, and other relevant material, has concluded that a regulation should issue to prescribe the conditions of use of acrylamide-acrylic acid resin in the clarification of beet sugar juice and cane sugar juice. Therefore, pursuant to the provisions of the Federal Food, Drug, and Cosmetic Act (sec. 409(c)(1), 72 Stat. 1786; 21 U.S.C. 348(c)(1)), and under the authority delegated to the Commissioner by the Secretary of Health, Education, and Welfare (25 F.R. 8625), the food additive regulations are amended by the addition of a new section to Subpart D, as follows.

§ 121.1092 Acrylamide-acrylic acid resin.

Acrylamide-acrylic acid resin (hydrolyzed polyacrylamide) may be safely used in accordance with the following prescribed conditions.

(a) Acrylamide-acrylic acid resin is produced by the polymerization of acrylamide with partial hydrolysis, or by copolymerization of acrylamide and acrylic acid, with the greater part of the polymer being composed of acrylamide units.

(b) The acrylamide-acrylic acid resin contains not more than 0.05 percent of residual acrylamide.

(c) The acrylamide-acrylic acid resin is used as a flocculent in the clarification of beet sugar juice or cane sugar juice in an amount not to exceed 5 parts per million by weight of the juice.

Any person who will be adversely affected by the foregoing order may at any time within 30 days from the date of its publication in the FEDERAL REGISTER file with the Hearing Clerk, Department of Health, Education, and Welfare, Room 5440, 330 Independence Avenue SW., Washington 25, D.C., written objections thereto. Objections shall show wherein the person filing will be adversely affected by the order and specify with particularity the provisions of the order deemed objectionable and the grounds for the objections. If a hearing is requested, the objections must state the issues for the hearing. A hearing will be granted if the objections are supported by grounds legally sufficient to justify the relief sought. Objections may be accompanied by a memorandum or brief in support thereof. All documents shall be filed in quintuplicate.

Effective date. This order shall be effective on the date of its publication in the FEDERAL REGISTER.

(Sec. 409(c)(1), 72 Stat. 1786; 21 U.S.C. 348(c)(1))

Dated: November 22, 1963.

GEO. P. LARRICK,
Commissioner of Food and Drugs.

[F.R. Doc. 63-12420; Filed, Nov. 29, 1963; 8:49 a.m.]

PART 121—FOOD ADDITIVES

Subpart D—Food Additives Permitted in Food for Human Consumption

CALCIUM SILICATE

Subsequent to the issuance of § 121.1135 *Calcium silicate*, published in the FEDERAL REGISTER of July 25, 1963 (28 F.R. 7559), an objection was filed by Winthrop Laboratories, 1450 Broadway, New York 18, New York, on the ground that the regulation excluded the use of calcium silicate as an anticaking agent in salt substitutes used in special dietary foods. The Commissioner of Food and Drugs has concluded that the objection is valid, and that such use of calcium silicate should not be excluded. Therefore, pursuant to the provisions of the Federal Food, Drug, and Cosmetic Act (secs. 409(c)(1), (h), 72 Stat. 1786, 1787; 21 U.S.C. 348(c)(1), (h)) and under the authority delegated to the Commissioner by the Secretary of Health, Education, and Welfare (25 F.R. 8625), § 121.1135 (a)(1) is amended to read as follows:

§ 121.1135 Calcium silicate.

(a) * * *

(1) It is not to be used in infant foods and foods for special dietary use, except when present as an anticaking agent at a level not to exceed 2.0 percent in salt and salt substitutes used as components of these foods.

* * * * *

Any person who will be adversely affected by the foregoing order may at any time within 30 days from the date of its publication in the FEDERAL REGISTER file with the Hearing Clerk, Department of Health, Education, and Welfare, Room 5440, 330 Independence Avenue SW., Washington 25, D.C., written objections thereto. Objections shall show wherein the person filing will be adversely affected by the order and specify with particularity the provisions of the order deemed objectionable and the grounds for the objections. If a hearing is requested, the objections must state the issues for the hearing. A hearing will be granted if the objections are supported by grounds legally sufficient to justify the relief sought. Objections

may be accompanied by a memorandum or brief in support thereof. All documents shall be filed in quintuplicate.

Effective date. This order shall be effective on the date of its publication in the FEDERAL REGISTER.

(Secs. 409(c)(1), (h), 72 Stat. 1786, 1787; 21 U.S.C. 348(c)(1), (h))

Dated: November 22, 1963.

GEO. P. LARRICK,
Commissioner of Food and Drugs.

[F.R. Doc. 63-12421; Filed, Nov. 29, 1963; 8:50 a.m.]

PART 121—FOOD ADDITIVES

Subpart F—Food Additives Resulting From Contact With Containers or Equipment and Food Additives Otherwise Affecting Food

ETHYLENE-ETHYL ACRYLATE COPOLYMERS

The Commissioner of Food and Drugs, having evaluated the data submitted in a petition (FAP 1169) filed by Union Carbide Corporation, 270 Park Avenue, New York 17, New York, and other relevant material, has concluded that § 121.2554 of the food additive regulations should be amended to provide for the use of ethylene-ethyl acrylate copolymers in coatings complying with § 121.2514 or § 121.2526 in such proportions that the ethyl acrylate content of the coating does not exceed 8 percent by weight of the finished coating. Therefore, pursuant to the provisions of the Federal Food, Drug, and Cosmetic Act (sec. 409(c)(1), 72 Stat. 1786; 21 U.S.C. 348(c)(1)), and under the authority delegated to the Commissioner by the Secretary of Health, Education, and Welfare (25 F.R. 8625), paragraph (b) of § 121.2554 *Ethylene-ethyl acrylate copolymers* is amended to read as follows:

§ 121.2554 Ethylene-ethyl acrylate copolymers.

* * * * *

(b) The ethyl acrylate content of the copolymer does not exceed 8 percent by weight unless it is blended with polyethylene complying with § 121.2510 or with one or more ethylene-alkene-1 copolymers complying with § 121.2508 or with a mixture of polyethylene and one or more ethylene-alkene-1 copolymers, in such proportions that the ethyl acrylate content of the blend does not exceed 8 percent by weight, or unless it is used in a coating complying with § 121.2514 or § 121.2526, in such proportions that the ethyl acrylate content does not exceed 8 percent by weight of the finished coating.

* * * * *

Any person who will be adversely affected by the foregoing order may at any time within 30 days from the date of its publication in the FEDERAL REGISTER file with the Hearing Clerk, Department of Health, Education, and Welfare, Room 5440, 330 Independence Avenue SW., Washington 25, D.C., written objections thereto. Objections shall show wherein the person filing will be adversely affected by the order and specify with particularity the provisions of the order deemed objectionable and the grounds for the objections. If a hearing is requested, the objections must state the issues for the hearing. A hearing will be granted if the objections are supported by grounds legally sufficient to justify the relief sought. Objections may be accompanied by a memorandum or brief in support thereof. All documents shall be filed in quintuplicate.

Effective date. This order shall be effective on the date of its publication in the FEDERAL REGISTER.

(Sec. 409(c)(1), 72 Stat. 1786; 21 U.S.C. 348(c)(1))

Dated: November 22, 1963.

GEO. P. LARRICK,
Commissioner of Food and Drugs.

[F.R. Doc. 63-12422; Filed, Nov. 29, 1963; 8:50 a.m.]

PART 121—FOOD ADDITIVES

Subpart F—Food Additives Resulting From Contact With Containers or Equipment and Food Additives Otherwise Affecting Food

RUBBER ARTICLES INTENDED FOR REPEATED USE

The Commissioner of Food and Drugs, having evaluated the data submitted in a petition (FAP 1C30) filed by Drew Chemical Corporation, 522 Fifth Avenue, New York, New York, 10036, and other relevant material, has concluded that the food additive regulations should be amended to provide for the use of additional substances in the manufacture of rubber articles intended for repeated use in contact with food. Therefore, pursuant to the provisions of the Federal Food, Drug, and Cosmetic Act (sec. 409(c)(1), 72 Stat. 1786; 21 U.S.C. 348(c)(1)), and under the authority delegated to the Commissioner by the Secretary of Health, Education, and Welfare (25 F.R. 8625), paragraph (c)(4) of § 121.2562 *Rubber articles intended for repeated use* (21 CFR 121.2562; 28 F.R. 1222) is amended by inserting alphabetically in subdivision (iv) the following new items:

Triethylene glycol dicaprate.
Triethylene glycol dicaprylate.

Any person who will be adversely affected by the foregoing order may at any time within 30 days from the date of its publication in the FEDERAL REGISTER file with the Hearing Clerk, Department of Health, Education, and Welfare, Room 5440, 330 Independence Avenue SW., Washington 25, D.C., written objections thereto. Objections shall show wherein the person filing will be adversely affected by the order and specify with particularity the provisions of the order deemed objectionable and the grounds for the objections. If a hearing is requested, the objections must state the issues for the hearing. A hearing will be granted if the objections are supported by grounds legally sufficient to justify the relief sought. Objections may be accompanied by a memorandum or brief in support thereof. All documents shall be filed in quintuplicate.

Effective date. This order shall be effective on the date of its publication in the FEDERAL REGISTER.

(Sec. 409(c)(1), 72 Stat. 1786; 21 U.S.C. 348(c)(1))

Dated: November 22, 1963.

GEO. P. LARRICK,
Commissioner of Food and Drugs.

[F.R. Doc. 63-12423; Filed, Nov. 29, 1963; 8:50 a.m.]

SUBCHAPTER C—DRUGS

PART 146a—CERTIFICATION OF PENICILLIN AND PENICILLIN-CONTAINING DRUGS

Phenethicillin Potassium (Potassium α-Phenoxyethyl Penicillin) for Oral Solution; Change of Expiration Date

Under the authority vested in the Secretary of Health, Education, and Welfare by the Federal Food, Drug, and Cosmetic Act (sec. 507, 59 Stat. 463 as amended; 21 U.S.C. 357) and delegated to the Commissioner of Food and Drugs by the Secretary (25 F.R. 8625), the regulations for certification of penicillin and penicillin-containing drugs (21 CFR 146a.18) are amended as follows:

In § 146a.18 *Phenethicillin potassium (potassium α-phenoxyethyl penicillin) for oral solution*, paragraph (c)(1)(ii) is amended by changing the words "or 36 months" to read "36 months, or 42 months".

Notice and public procedure and delayed effective date are not necessary prerequisites to the promulgation of this order, and I so find, since the nature of the change is such that it cannot be applied to any specific product unless and until the manufacturer thereof has supplied adequate data regarding that article.

Effective date. This order shall become effective on the date of its publication in the FEDERAL REGISTER.

(Sec. 507, 59 Stat. 463 as amended; 21 U.S.C. 357)

Dated: November 22, 1963.

GEO. P. LARRICK,
Commissioner of Food and Drugs.

[F.R. Doc. 63-12424; Filed, Nov. 29, 1963; 8:51 a.m.]

PART 147—ANTIBIOTICS INTENDED FOR USE IN THE LABORATORY DIAGNOSIS OF DISEASE

Antibiotic-Dehydrated Media-Triphenyltetrazolium Chloride Sensitivity Discs; Certification Procedure

Under the authority vested in the Secretary of Health, Education, and Welfare by the Federal Food and Drug Act (sec. 507, 59 Stat. 463 as amended; 21 U.S.C. 357) and delegated to the Commissioner of Food and Drugs by the Secretary (25

From the Histochemical Section (Director Dr. F. Timm) and the Cooperative for the Investigation of Silicosis (Director, Prof. Dr. K. Thomas) of the Medical Research Institute of the Max Planck Society of Gottingen

7. On the Behavior of Aerosil in the Animal Body

By: Michael Arnold, Dieter Sasse and Franz-Josef Strecker

Within the framework of investigations with various SiO_2 preparations, Strecker has also examined Aerosil very extensively with respect to its effect upon the tissues. During these experiments, it was noted that in the experimental animals, kidney damage occurred already in the first few days after they had been injected peritoneally with Aerosil. This observation led to the surmise that Aerosil was excreted by the kidneys.

Because of the very small size of the Aerosil particles, however, it was not possible to obtain an unequivocal result, using a light microscope, with respect to either the place or the form of excretion. We have hence attempted to answer these questions by using the electronmicroscope.

Rats served as experimental animals. They were injected intraperitoneally with 1000 mg of Aerosil in a 1% suspension. Sacrifice of the animals took place at various periods following injection. The tissues (peritoneal lymph nodes, kidneys) were fixed in OsO_4 or formalin, both buffered to pH 7.2, and embedded in methacrylate.

The question of whether, in fact, Aerosil is excreted may be most expeditiously decided by observation of a collecting tubule, because it is here that the urine reaches its final concentration.

Fig. 1 shows in cross section such a tubule, whose lumen is filled with strongly contrasting particles. These particles, however, do not

correspond to the primary particles of the injected Aerosil, as becomes apparent in the comparison of the particles of Figures 2 and 3.

There now arises the question of how the Aerosil gets into the collecting tubules. There are the following four possibilities:

(1) In particle form, through the glomerulus.

(2) In particle form, through part of a tubule.

(3) Dissolved, through the glomerulus.

(4) Dissolved, through part of a tubule.

Fig. 3 shows a portion of a glomerule. Since neither with respect to blood nor to urine can any abnormal conditions be determined, no basis can be established for a glomerular excretion of Aerosil particles. It is, however, conceivable that demonstration of the presence of the particles is frustrated only by their extremely slight concentration. Yet, in the region of the proximal tubule also, in which a rise in concentration of Aerosil should be expected on the basis of the reabsorption of water taking place here, we are presented with a clear lumen.

Finally, in Henle's loop, in which 80% of the terminal concentration of urine is attained, there is likewise a clear lumen. The morphological picture corresponds to that of a normal rat kidney.

More distally, however, conspicuous changes now make their appearance, as may be seen in Figures 7-10. In these portions of the nephron, strongly contrasting structures appear within the cells that in the form of bands and chains imitate membranes. In Fig. 8, the section of a cell of the distal tube is reproduced, following fixation in formalin. Since these strongly contrasting structures are also visible after formalin fixation, they cannot be explained by an increased accumulation of osmium, but must bear an immediate relationship to the introduction of Aerosil.

These strongly contrasting masses, present in part as particles, obviously show a preference for attack upon the lamellae enclosing the mitochondria; simultaneously, a vacuolization of the mitochondria occurs; the cristae mitochondriales divaricate. In addition, nucleic pyknoses develop, and finally it comes to a disintegration of the entire tubule cell. Ultimately, all the cell elements are swept into the lumen of the more distally located nephron sections (Fig. 10).

In accordance with these findings, we initially surmised that the Aerosil was excreted through the distal tubule sections in particle form. To substantiate this view, two further test series seemed to us to be necessary.

Using the same test arrangement, instead of 150 m^2/g Aerosil, the following two materials were tested:

(1) Aerosil 0 x 50 (38 m^2/g), about four times coarser, and

(2) Silicic acid gel.

In the first case, the particles occurring in the collecting tubules would have to differ in their size; in the second case, no particles might be expected.

Nevertheless, following injection of Aerosil 0 x 50, in the lumina of the collecting tubules we found particles of the same shape and dimensions as in the first experiment (Fig. 12).

Furthermore, after the use of sililic acid gel, strongly contrasting particles appeared in the collecting tubules; these could be differentiated neither from the particles found after injection of 150 m^2/g Aerosil (Fig. 2), nor from those after Aerosil 0 x 50 (Fig. 12). (See Fig. 13.)

In accordance with these findings, we must assume that, from the surface of the Aerosil, silicic acid enters into solution that polymerizes in the distal nephron sections. Also, the hitherto still open question of whether the dissolved silicic acid reaches the nephron through the glomeruli or the tubules can be answered by the findings following injection of sililic acid gel.

In these animals there occurs already in the lower sections of the proximal tubules an occupation of the brush border with strongly contrasting masses.

This finding we interpret thus: in the case of silicic acid gel, here, polymerous silicic acid is already present that reacts with the portions of the cell facing the lumen. This finding further supports the assumption that the changes visible in the distal tubules are brought about through reabsorption.

In order to gain an overview of the quantitative relationships, the SiO_2 amounts in renal cortex and papilla were determined separately. The results of the analyses are shown in Fig. 15.

Corresponding to the larger surface area, there is produced, in the case of Aerosil with 150 m^2/g, a higher rise of the SiO_2 in the kidney than in the case of Aerosil 0 x 50. The silicic acid gel at first lies in the abdominal cavity as a solid mass; the rise in concentration of SiO_2 in the kidney is correspondingly delayed, but then attains very high values.

The following schematic presentation is intended to summarize our findings once more:

(1) Occupation of the brush border following injection with silicic acid gel points to a glomerular excretion of the silicic acid.

This is confirmed by Baumann's clearance investigations, the results of which have just been reported.

(2) The clear lumina of Henle's loop, on the other hand, are more difficult to interpret. In accordance with the hypothesis of Wirz, of the concentration of urine through a countercurrent principle, as is to be found in Henle's loop, precipitation of the silicic acid should already be anticipated at that point. It is, however, conceivable, that pH conditions, and ion concentration and combination in association with the high flow-through rate in this narrow portion of the nephron effect a stabilization, so that the silicic acid precipitates only farther distalwards.

(3) The findings with regard to the distal tubules are explained by reabsorption of the polymerous silicic acid in this region.

(4) The morphological correspondence of the particles following application of Aerosil 150 m^2/g, Aerosil 0 x 50, and silicic acid gel indicates a glomerular excretion of dissolved silicic acid.

Fig. 1. Section of a collecting tubule, 18 hours after interperitoneal injection with Aerosil. Magnification, 3750 x.

Fig. 2. Section of a collecting tubule, 18 hours after interperitoneal injection with Aerosil. Magnification, 12,500 x.

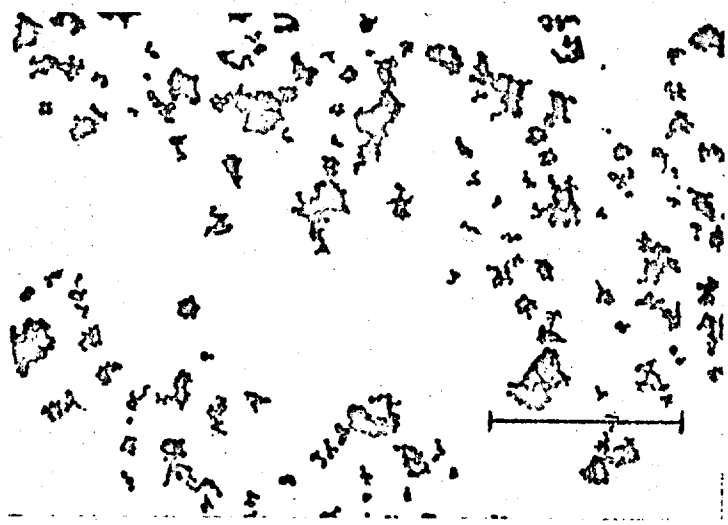


Fig. 3. Electron microscope photograph of Aerosil (applied from aqueous suspension). Magnification, 12,500 x.

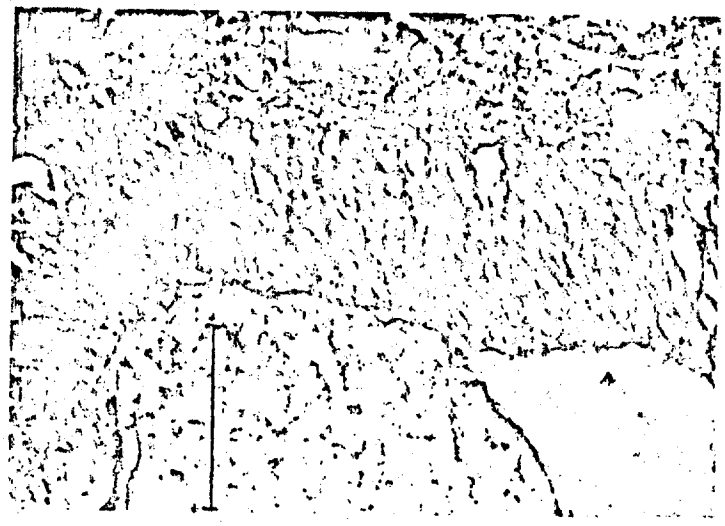


Fig. 4. Section from the glomerulus of a rat kidney 12 hours after interperitoneal injection with Aerosil 150 m^2/g. Magnification, 12,500 x.

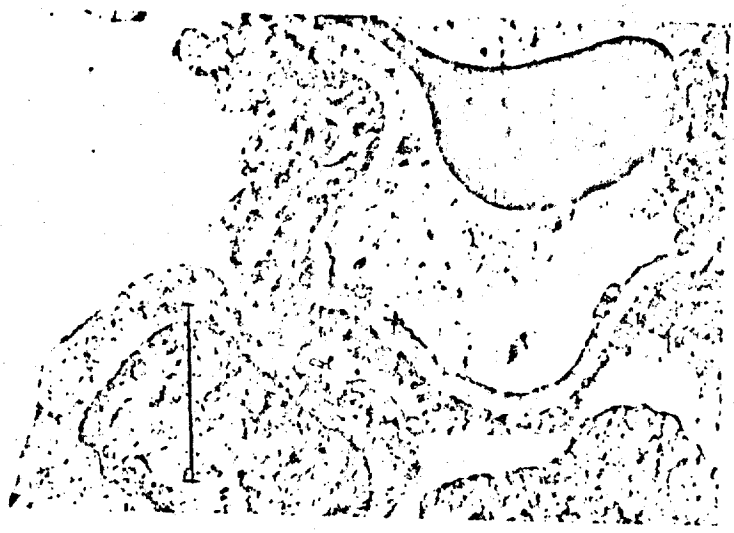


Fig. 5. Section from the proximal tubule of a rat kidney 12 hours after injection with Aerosil 150 m^2/g. Magnification, 12,500 x.

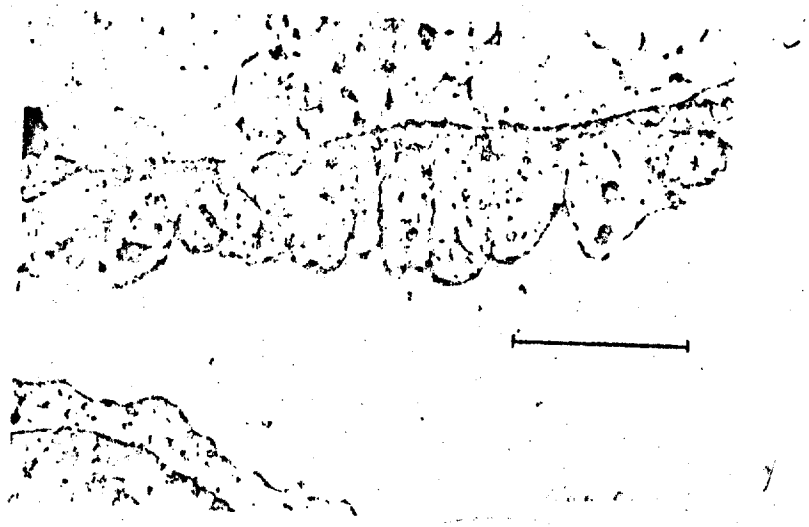


Fig. 6. Section from the thin portion of Henle's loop 12 hours after injection with Aerosil 150 m^2/g. Magnification, 12,500 x.

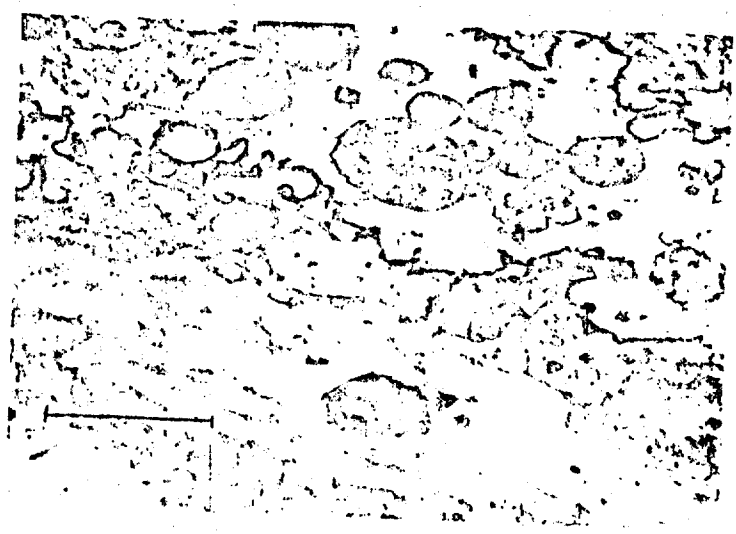


Fig. 7. Section from the cell of a distal tubule 6 hours after injection with Aerosil 150 m^2/g. Magnification, 12,500 x.

Fig. 8. Section from the cell of a distal tubule (fixation in formalin) 12 hours after injection with Aerosil. Magnification, 12,500 x.

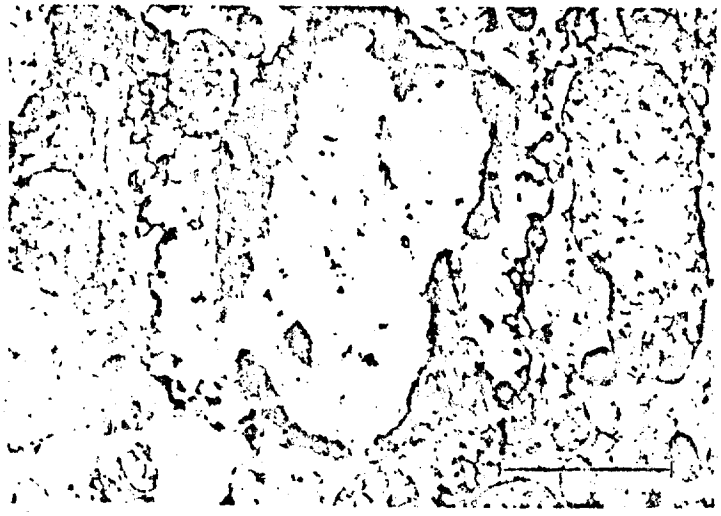


Fig. 9. Section from the cell of a distal tubule 12 hours after injection with Aerosil. Magnification, 12,500 x.

Fig. 10. Distal tubule 12 hours after injection with Aerosil. Magnification, 3750 x.

Fig. 11. Lumen of a collecting tubule with some swollen mitochondria, 18 hours after injection with Aerosil. Magnification, 12,500 x.

Fig. 12. Lumen and wall section of a collecting tubule 4 hours after injection with Aerosil 0 x 50. Magnification, 12,500 x.

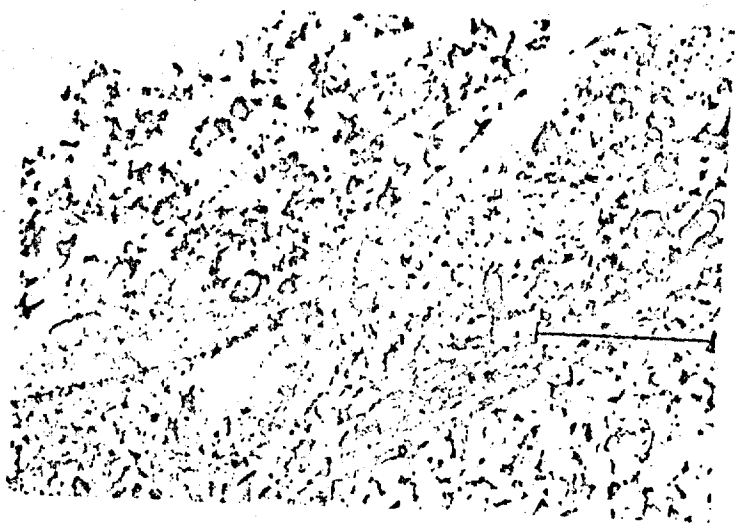


Fig. 13. Lumen and wall section of a collecting tubule cell 4 hours after injection with silicic acid gel. Magnification, 12,500 x.

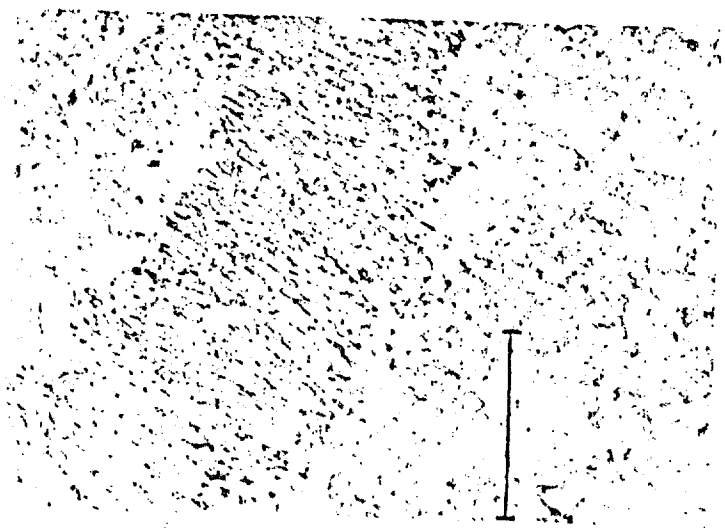


Fig. 14. Section from the cell of a proximal tubule 24 hours after injection with silicic acid gel. Magnification, 12,500 x.

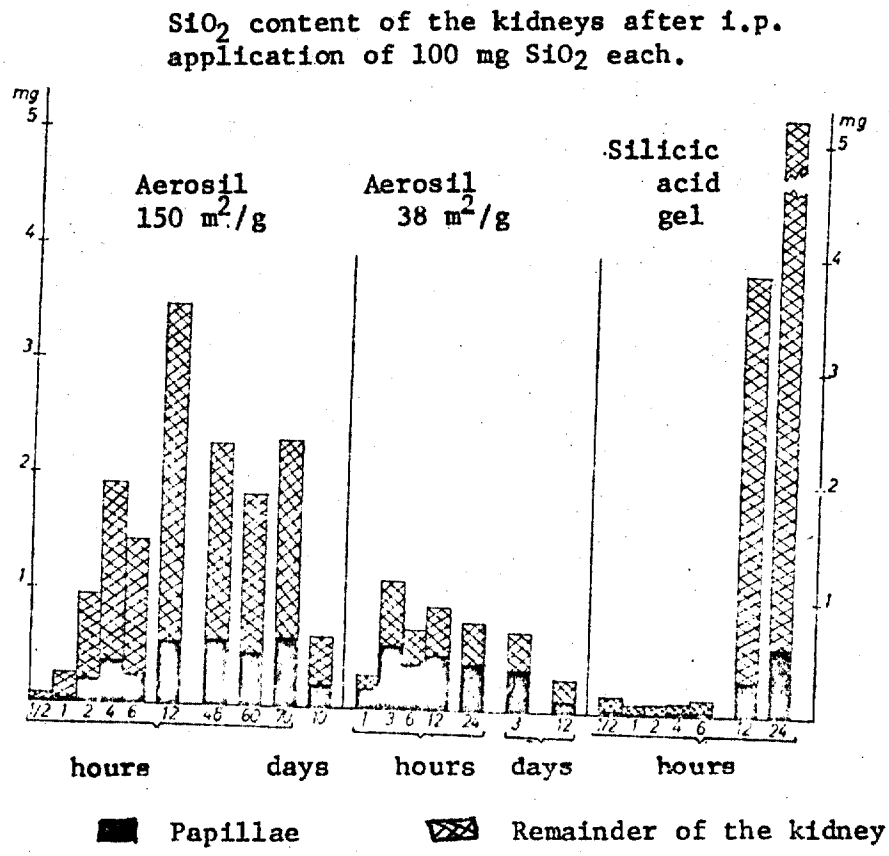


Fig. 15. Quantitative SiO_2 determination in rat kidneys after injection with Aerosil 150 m^2/g, Aerosil 0 x 50, and silicic acid gel.

Nephron (schematic)

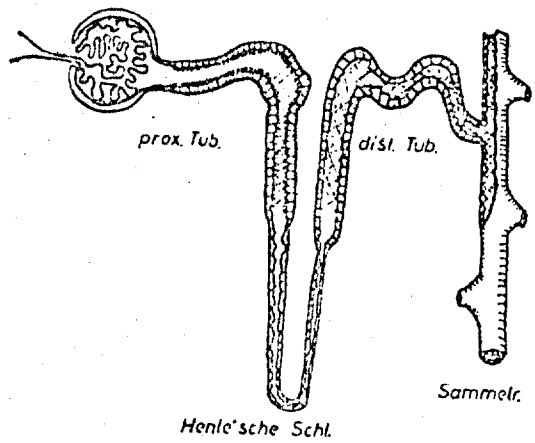


Fig. 16. Schema of the nephron with the changes described in the text.

Beitr. Silikose-Forsch. S-Bd Grundfragen Silikoseforsch. Bd 4 (1960) S 87/97

Aus der histochemischen Abteilung (Leiter: Prof. Dr. Dr. F. Timm) und der Arbeitsgemeinschaft Silikoseforschung (Leiter: Prof. Dr. K. Thomas) der Medizinischen Forschungsanstalt der Max-Planck-Gesellschaft, Göttingen

7. Zum Verhalten des Aerosils im Organismus

von Michael Arnold, Dieter Sasse und Franz-Josef Strecker

Im Rahmen von Untersuchungen mit verschiedenen SiO_2-Modifikationen wurde von Strecker sehr ausgedehnt auch Aerosil auf seine Gewebswirkung hin geprüft. Während dieser Versuche fiel nun auf, daß bei den Tieren schon in den ersten Tagen, nachdem ihnen Aerosil intraperitoneal zugeführt worden war, Nierenschäden auftraten. Dies gab zu der Vermutung Anlaß, daß Aerosil durch die Nieren ausgeschieden wird.

Der Kleinheit der Aerosil-Partikel wegen konnte aber weder über den Ausscheidungsort noch über die Form der Ausscheidung mit Hilfe des Lichtmikroskops eindeutig entschieden werden. Wir haben daher versucht, diese Fragen durch Einsatz des Elektronenmikroskops zu beantworten.

Als Versuchstiere dienten Ratten, denen 1000 mg Aerosil in einer 1%igen Suspension i. p. injiziert wurde. Die Tötung erfolgte zu verschiedenen Zeiten nach der Injektion. Die Gewebe (Peritoneal-Lymphknoten, Nieren) wurden in OsO_4 oder Formol, beide gepuffert auf pH 7,2, fixiert und in Methacrylat eingebettet.

Abb. 1: Abschnitt eines Sammelrohres. 18 Std. nach der i. p. Injektion von Aerosil. Vergrößerung 37501fach.

Ob Aerosil tatsächlich ausgeschieden wird, läßt sich am ehesten durch die Betrachtung eines Sammelrohres entscheiden, da hier der Harn seine endgültige Konzentration erreicht hat.

Abb. 1 zeigt den Querschnitt eines solchen Sammelrohres, dessen Lumen mit kontrastreichen Partikeln ausgefüllt ist. Diese Partikel stimmen jedoch nicht mit den Primärteilchen des zugeführten Aerosils überein, wie beim Vergleich der Partikel der Abb. 2 und 3 hervorgeht.

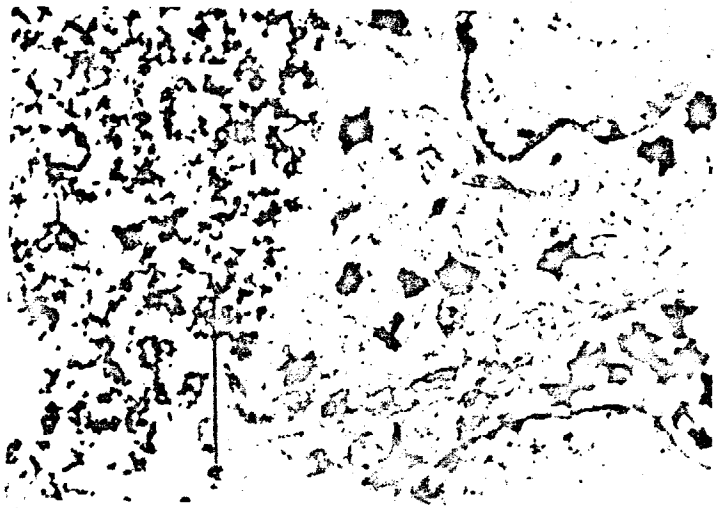


Abb. 2: Ausschnitt eines Sammelrohres, 18 Std. nach der i. p. Injektion von Aerosil. Vergrößerung 12 500fach.

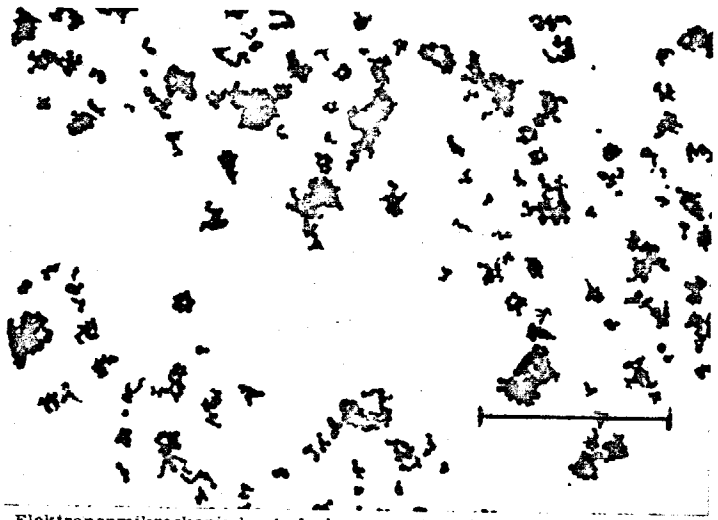


Abb. 3: Elektronenmikroskopische Aufnahme von Aerosil. (Aus wäßriger Suspension aufgetropft.) Vergrößerung 12 500fach.

Es erhebt sich nun die Frage, wie das Aerosil in die Sammelrohre gelangt.

Es bestehen folgende Möglichkeiten:

1. Als Partikel durch das Glomerulum;
2. Als Partikel durch einen Tubulusabschnitt;
3. Gelöst durch das Glomerulum;
4. Gelöst durch einen Tubulusabschnitt.

Die Abb. 3 zeigt einen Glomerulumabschnitt; da weder auf der Blut- noch auf der Harnseite ein von der Norm abweichender Befund zu erheben ist, findet sich kein Anhalt für eine glomeruläre Ausscheidung von Aerosilpartikeln. Denkbar wäre jedoch, daß der Nachweis der Partikel nur durch die extrem geringe

Abb. 4: Ausschnitt aus dem Glomerulum einer Rattenniere 12 Std. nach der i. p. Injektion von Aerosil 150 m²/g. Vergrößerung 12 500fach.

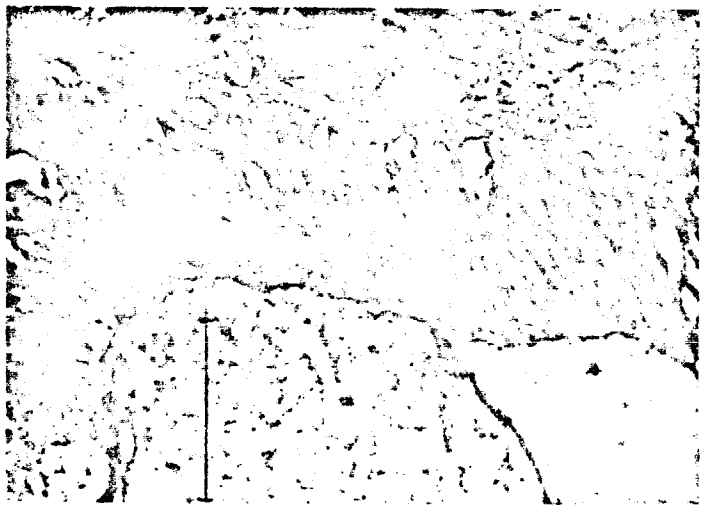


Abb. 5: Ausschnitt aus dem proximalen Tubulus einer Rattenniere 12 Std. p. i. von Aerosil 150 m²/g. Vergrößerung 12 500fach.

Konzentration vereitelt wird. Doch auch im Bereich des proximalen Tubulus, in dem auf Grund der hier stattfindenden Wasserrückresorption ein Konzentrationsanstieg des Aerosils zu erwarten wäre, zeigt sich ein freies Lumen.

Abb. 6: Ausschnitt aus dem dünnen Segment der Henleschen Schleife 12 Std. p. i. von Aerosil 150 m²/g. Vergrößerung 12 500fach.

In der Henleschen Schleife schließlich, in der 80 % der Endkonzentration des Harns erreicht wird, findet sich ebenfalls ein freies Lumen. Das morphologische Bild entspricht dem einer normalen Rattenniere.

Weiter distal aber ergeben sich nun, wie die Abbildungen 7—10 zeigen, auffällige Veränderungen:

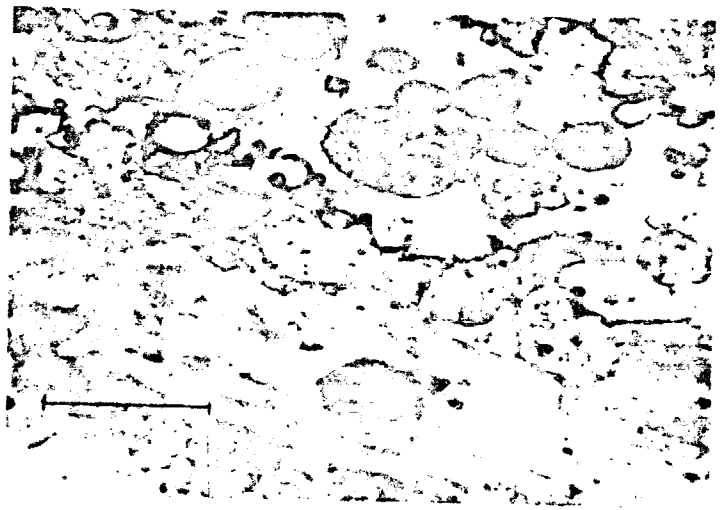


Abb. 7: Ausschnitt aus der Zelle eines distalen Tubulus 6 Std. p. i. von Aerosil 150 m²/g. Vergrößerung 12 500fach.

Abb. 8: Ausschnitt aus der Zelle eines distalen Tubulus (formolfixiert) 12 Std. p. i. von Aerosil. Vergrößerung 12 500fach.

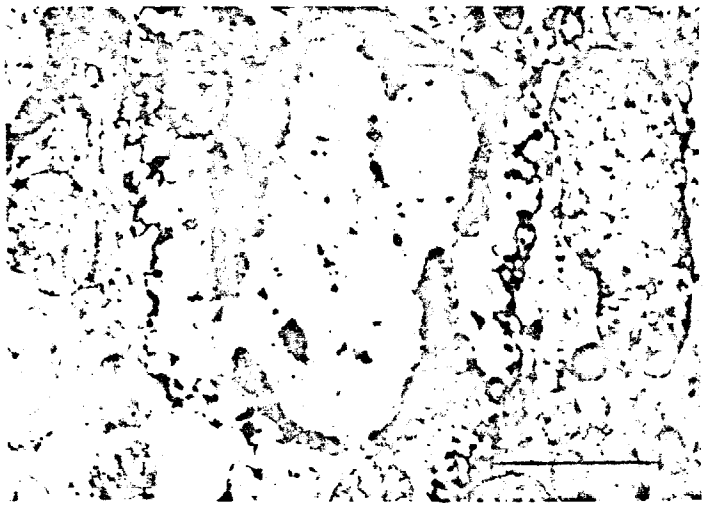


Abb. 9: Ausschnitt aus der Zelle eines distalen Tubulus 12 Std. p. i. von Aerosil. Vergrößerung 12 500fach.

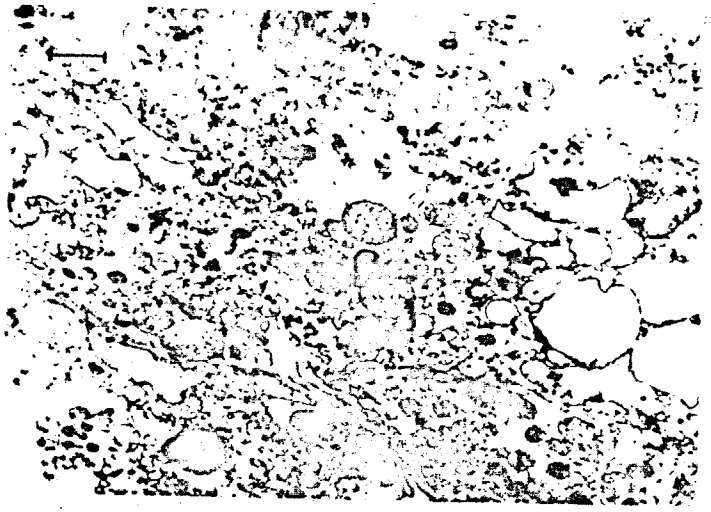


Abb. 10: Distaler Tubulus 12 Std. p. i. von Aerosil. Vergrößerung 3750fach.

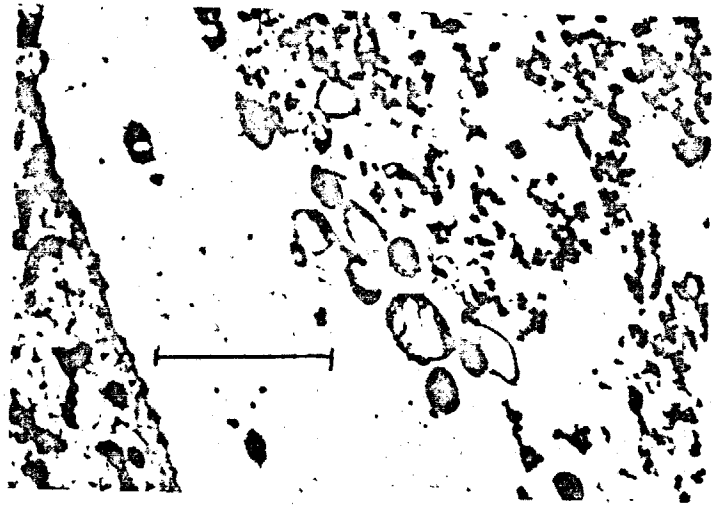


Abb. 11: Lumen eines Sammelrohres mit z. T. gequollenen Mitochondrien 18 Std. p. i. von Aerosil. 12 500fach.

In diesen Nephronabschnitten treten kontrastreiche Strukturen innerhalb der Zellen auf, die in Form von Bändern und Ketten Membranen imitieren. Abb. 8 gibt den Ausschnitt einer Zelle des distalen Tubulus nach Formolfixierung wieder. Da auch nach Formolfixierung diese kontrastreichen Strukturen erscheinen, können diese nicht durch eine verstärkte Osmiumanlagerung erklärt werden, sondern müssen in unmittelbarem Zusammenhang mit der Aerosilzufuhr stehen.

Diese kontrastreichen, z. T. als Partikel vorliegenden Massen gehen offensichtlich bevorzugt an die die Mitochondrien einschließenden Lamellen. Gleichzeitig damit tritt eine Vakuolisierung der Mitochondrien auf; die Cristae mitochondrales weichen auseinander. Zudem kommt es zu Kernpyknosen und schließlich zu einer Auflösung der gesamten Tubuluszelle. Schließlich werden alle Zellelemente in das Lumen der weiter distal gelegenen Nephronabschnitte geschwemmt (Abb. 10).

Nach diesen Befunden vermuteten wir anfangs, daß das Aerosil als Partikel durch die distalen Tubulusabschnitte ausgeschieden wird. Zum Beweis dieser Ansicht schienen uns zwei weitere Versuchsreihen erforderlich:

Bei gleicher Versuchsanordnung wurde statt 150 m²/g Aerosil

1. das ca. 4mal gröbere Aerosil OX 50 (38 m²/g) und
2. Kieselsäuregel geprüft.

Im ersten Falle hätten die im Sammelrohr auftretenden Partikel in ihrer Größe abweichen müssen, im zweiten Falle durfte man keine Partikel erwarten.

Aber auch nach Injektion von Aerosil OX 50 fanden wir Partikel gleicher Form und Größe wie im ersten Versuch in den Lumina der Sammelrohre (Abb. 12).

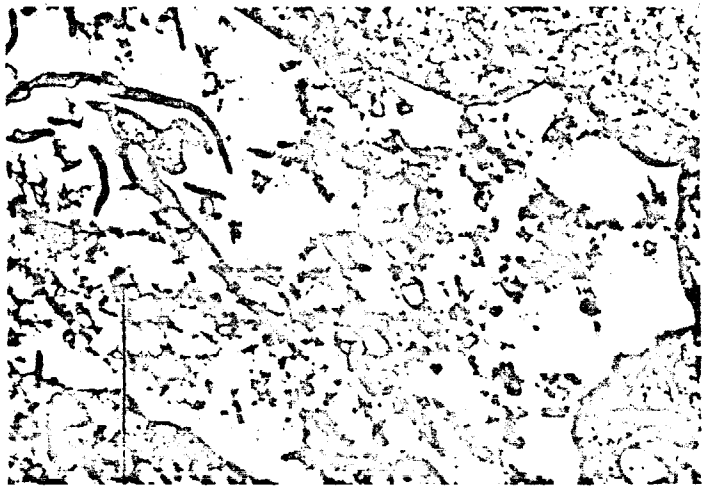


Abb. 12: Lumen und Wandabschnitt eines Sammelrohres 4 Std. p. i. von Aerosil OX 50. Vergrößerung 12 500fach.

Außerdem traten auch nach Kieselsäuregel kontrastreiche Partikel in den Sammelrohren auf, die weder von den Partikeln nach 150 m²/g Aerosil (Abb. 2) noch nach Aerosil OX 50 (Abb. 12) zu unterscheiden waren (Abb. 13).

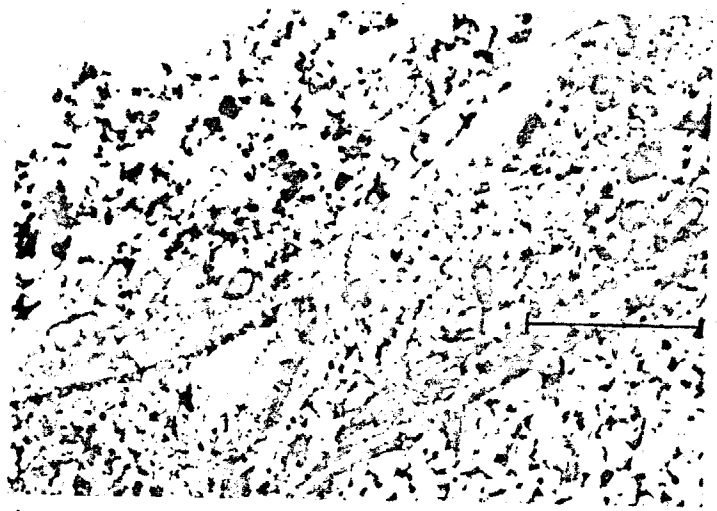


Abb. 13: Lumen und Wandabschnitt einer Sammelrohrzelle 4 Std. p. i. von Kieselsäuregel. Vergrößerung 12 500fach.

Nach diesen Befunden müssen wir annehmen, daß von der Oberfläche des Aerosils Kieselsäure in Lösung geht, die in den distalen Nephronabschnitten polymerisiert. Auch die bis dahin noch offene Frage, ob die gelöste Kieselsäure glomerulär oder tubulär in das Nephron gelangt, kann durch die Befunde nach Injektion von Kieselsäuregel beantwortet werden.

Bei diesen Tieren nämlich findet sich schon in den unteren Abschnitten der proximalen Tubuli eine Besetzung der brush-border mit kontrastreichen Massen.

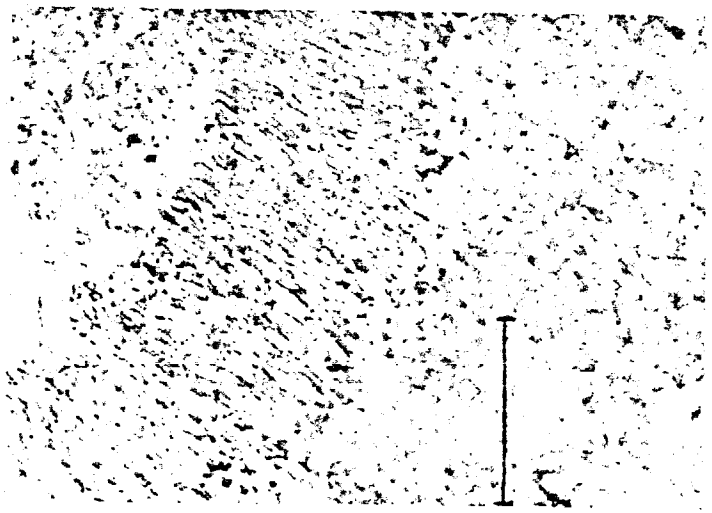


Abb. 14: Ausschnitt aus der Zelle eines proximalen Tubulus 24 Std. p. i. von Kieselsäuregel. Vergrößerung 12 500fach.

Das wird von uns so gedeutet, daß im Falle des Kieselsäuregels schon hier polymere Kieselsäure vorhanden ist, die mit den dem Lumen zugewandten Abschnitten der Zelle reagiert. Dieser Befund stützt weiterhin die Annahme, daß die in den distalen Tubuli sichtbaren Veränderungen durch eine Rückresorption hervorgerufen werden.

Um einen Überblick über die quantitativen Verhältnisse zu erhalten, wurden die SiO_2-Mengen in Nierenrinde und Papille getrennt bestimmt. Abb. 15 gibt die Analysenergebnisse wieder:

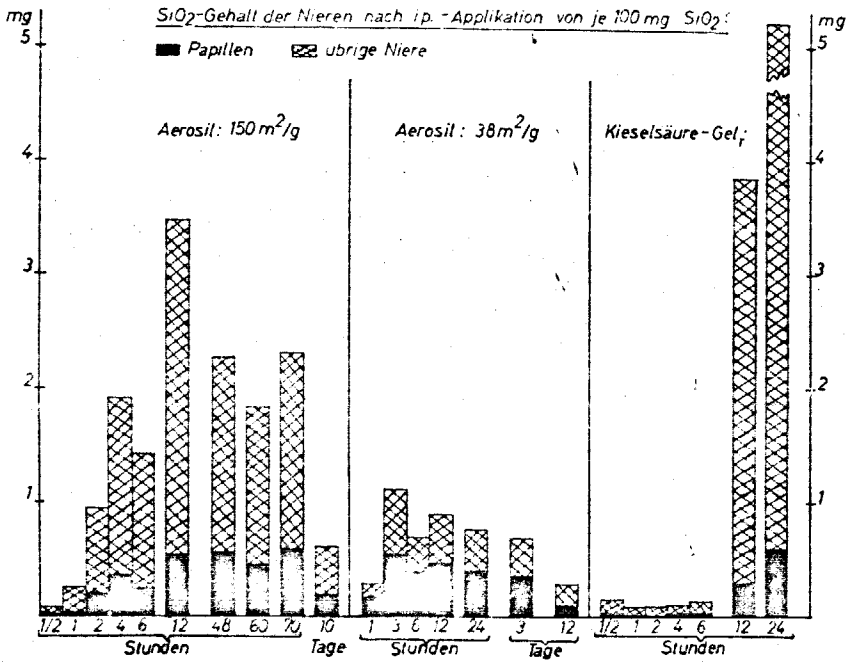


Abb. 15: Quantitative SiO_2-Bestimmung in Rattennieren nach Injektion von Aerosil 150 m²/g, Aerosil OX 50 und Kieselsäuregel.

Entsprechend der größeren Oberfläche kommt es beim Aerosil mit 150 m²/g zu einem höheren Anstieg des SiO_2 in der Niere als beim Aerosil OX 50. Das Kieselsäuregel liegt zuerst als geschlossene Masse in der Bauchhöhle, der Konzentrationsanstieg des SiO_2 in der Niere ist entsprechend verzögert, erreicht dann aber sehr hohe Werte.

Anhand der folgenden schematischen Darstellung sollen die Befunde noch einmal zusammengefaßt werden:

1. Die Besetzung der brush-border nach Injektion von Kieselsäuregel weist auf eine glomeruläre Ausscheidung der Kieselsäure hin. Dies wird durch die von Herrn Baumann soeben mitgeteilten Clearanceuntersuchungen bestätigt.

2. Die freien Lumina der Henleschen Schleife hingegen sind schwerer zu deuten. Nach der von Wirz aufgestellten Hypothese der Harnkonzentrierung durch ein Gegenstromprinzip, wie es in der Henleschen Schleife vorhanden ist, sollte man schon dort die Ausfällung der Kieselsäure erwarten. Man kann sich aber vorstellen, daß pH-Verhältnisse, Ionenkonzentration und -zusammensetzung in Verbindung mit der hohen Durchflußgeschwindigkeit in diesem engen Teil des Nephrons eine Stabilisierung bewirken, so daß die Kieselsäure erst weiter distal ausfällt.

3. Die Befunde an den distalen Tubuli werden durch Rückresorption der polymeren Kieselsäure in diesem Bereich erklärt.

4. Die morphologische Übereinstimmung der Partikel nach Applikation von Aerosil 150 m² g, Aerosil OX 50 und Kieselsäuregel weist auf eine glomeruläre Ausscheidung von gelöster Kieselsäure hin.

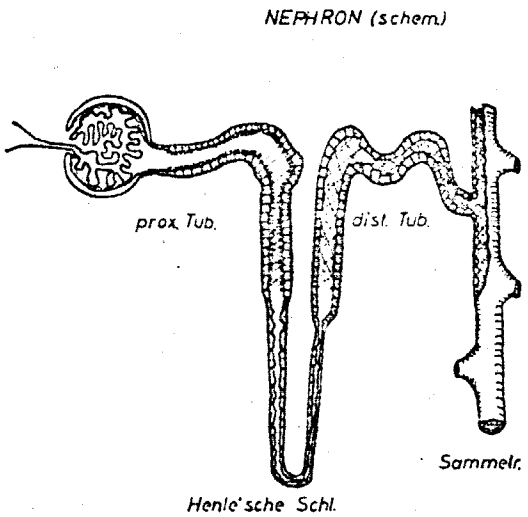


Abb. 16: Schema des Nephrons mit den im Text beschriebenen Veränderungen.

Zusammenfassung:

Die Ausscheidung von Aerosil durch die Rattenniere wurde elektronenmikroskopisch untersucht. Das gleichartige Verhalten von zwei Aerosilproben verschiedener Korngröße und von Kieselsäuregel lassen vermuten, daß von der Aerosiloberfläche Kieselsäure in Lösung geht und diese glomerulär abfiltriert wird.

In den distalen Nephronabschnitten kommt es zu einer durch die starke Wasserrückresorption bedingten Ausfällung von Polykieselsäure. Die am distalen Tubulus beobachteten pathologischen Veränderungen werden als Folgen einer Rückresorption von Polykieselsäure in diesem Bereich erklärt.

Résumé:

L'excrétion d'aérosil par le rein du rat a été étudiée au microscope électronique. Les résultats similaires après l'application de deux échantillons d'aérosil d'un diamètre différent et du gel de l'acide silicique laissent supposer que l'acide silicique est dissolvé de la surface de l'aerosil et que cet-ci est filtré par les glomerules.

Dans les tubes urinaires distals on trouve une précipitation de l'acide polysilicique causée par la réabsorption d'eau intense. Les altérations pathologiques observées aux tubes distals sont expliquées par une réabsorption de l'acide polysilicique dans cette région.

Summary:

The excretion of aerosil through the rat kidney has been examined with the electron microscope. Similar results after the application of two aerosil samples of different particle size and of silicic acid gel led to the suggestion that silicic acid is dissolved from the surface of aerosil and filtered through the glomerules.

Due to the intense reabsorption of water a precipitation of polysilicic acid occurs in the distal part of the nephron. The pathological findings observed at the distal tubules are explained by a reabsorption of polysilicic acid in this part of the nephron.

Literatur

H. Wirz, B. Hargitay und W. Kuhn: Lokalisation des Konzentrierungsprozesses in der Niere durch direkte Kyroskopie. Helv. physiol. pharmacol. Acta **9**, 196 (1951).

H. Wirz: Der osmotische Druck in den corticalen Tubuli der Rattenniere. Helv. physiol. pharmacol. Acta **14**, 353 (1956).

Mit Unterstützung der Deutschen Forschungsgemeinschaft.

Diskussion zum Vortrag 7

Mohn, Homburg/Saar

Nach Verabreichung von ca. 10 mg SiO_2 i. v. an Kaninchen konnte durch Diuretica bisher keine Beschleunigung des Absinkens des SiO_2-Spiegels im Serum erzielt werden (entsprechende Verdünnung der 15%igen Kieselsäurelösung der BASF).

J. Am. Med. Assoc., Vol 219 (1): 49-51
- Jan. 3, 1972

Talc and Cornstarch Emboli in Eyes of Drug Abusers

William E. AtLee, Jr., MD

A characteristic type of retinopathy due to talc and cornstarch emboli was observed in 17 drug addicts following repeated intravenous injections of crushed methylphenidate hydrochloride tablets taken for their stimulatory effects. Ophthalmoscopic examinations revealed tiny glistening crystals largely concentrated in the small vessels about the macula. Two patients with pulmonary hypertension had reduced vision, presumably from retinal edema. Histologic examination confirmed the presence of talc and cornstarch particles in the retina and choroid as well as in the lung and brain. The presence of these particles in the eye is an indication that considerable foreign-body damage has occurred in the lung.

Methylphenidate hydrochloride tablets, crushed and suspended in water, are commonly injected intravenously by drug abusers in Portland to obtain what is called a "mild high." This practice occasionally causes severe pulmonary hypertension which has been fatal to at least four addicts during the past year. The tablet filler, largely talc and cornstarch, has been found, in similar cases reported in the literature, to cause extensive foreign-body granulomas in the lungs and heart valves and to become deposited as minute crystals in the liver, spleen, and kidneys.[1-3] Ocular involvement, although not recorded previously, to my knowledge, is of diagnostic significance. The purpose of this communication is to present a series of patients in whom these embolic particles were visualized ophthalmoscopically; they were also confirmed histologically, both in the retina and the choroid of an addict whose eyes were obtained at autopsy.

From the John E. Weeks Institute of Ophthalmology, University of Oregon Medical School, Portland.

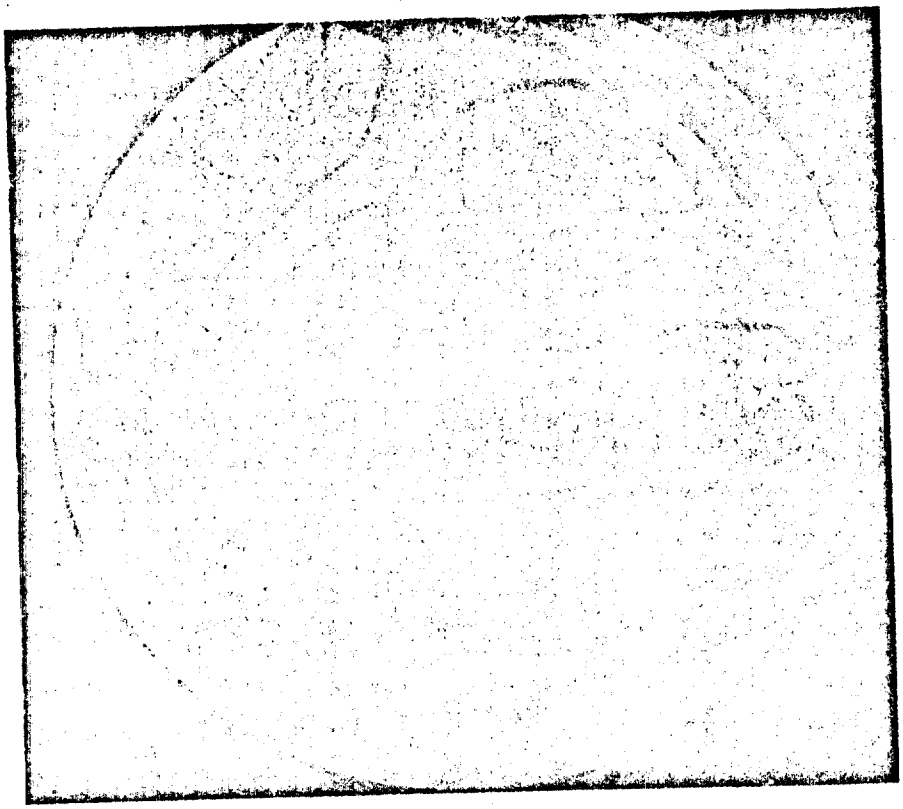
Reprint requests to 221 Eastern Ave, Augusta, Me 04330 (Dr. AtLee).

Report of Cases

CASE 1.—The first patient discovered to have this type of ocular problem was a 23-year-old white woman receiving methadone hydrochloride maintenance for heroin addiction. She was hospitalized with acute abdominal pain and dyspnea following intravenous administration of a 160-mg dose of methylphenidate hydrochloride; she had noticed reduced vision for the two months that she claimed to have been "shooting Ritalin." Examination revealed best corrected visual acuities of 20/200 in the right eye and 20/60 in the left eye with central scotomas in both eyes. There were numerous very tiny, reddish and yellowish, glistening particles scattered throughout the fundi, with the greatest concentration about the maculas. Their linear arrangement in places suggested that they were contained within the smaller vessels, and an occasional particle could be seen within an arteriole or venule. In addition, macular edema, venous engorgement, and several blotch hemorrhages were seen. Two months following recovery from the pulmonary hypertension, the patient's vision remained unchanged in the right eye but improved to 20/25 in the left. The hemorrhages and edema had largely resolved; however, the particulate lesions remained.

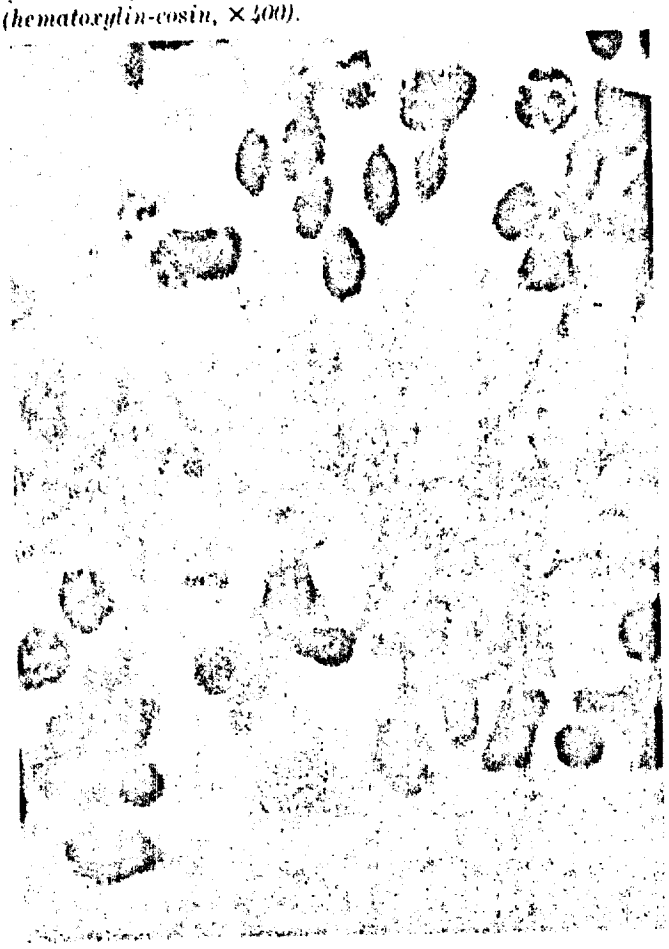
On the assumption that ophthalmoscopic changes could be found in other methylphenidate injectors, 14 of an estimated 60 to 100 local methylphenidate abusers were examined. Their ages ranged from 20 to 39 years. No one in this group had significant visual complaints; however, in all cases the characteristic crystalline particles could readily be seen ophthalmoscopically concentrated in the macular regions. The number of these particles could be roughly correlated with the amount of the drug taken and the duration of its use, which varied from 1 to 100 tablets of 20 mg each daily from two months to three years. Most injections were prepared by dissolving the crushed tablets in water and boiling and then filtering the murky suspension through cotton for intravenous injection. Some preferred the unfiltered preparation to experience what they described as a "talc flash."

The individual (39 years of age) with the most pronounced fundus changes (Fig 1) also was the heaviest user, ie, he had injected approximately 100 tablets of 20 mg each daily for two to three years. Fluorescein sodium angiography of his retinal vessels showed a delayed filling of a few venous branches leaving the macular area and an irregularity in the capillary pattern about the macula. He was totally asymptomatic, and his vi-

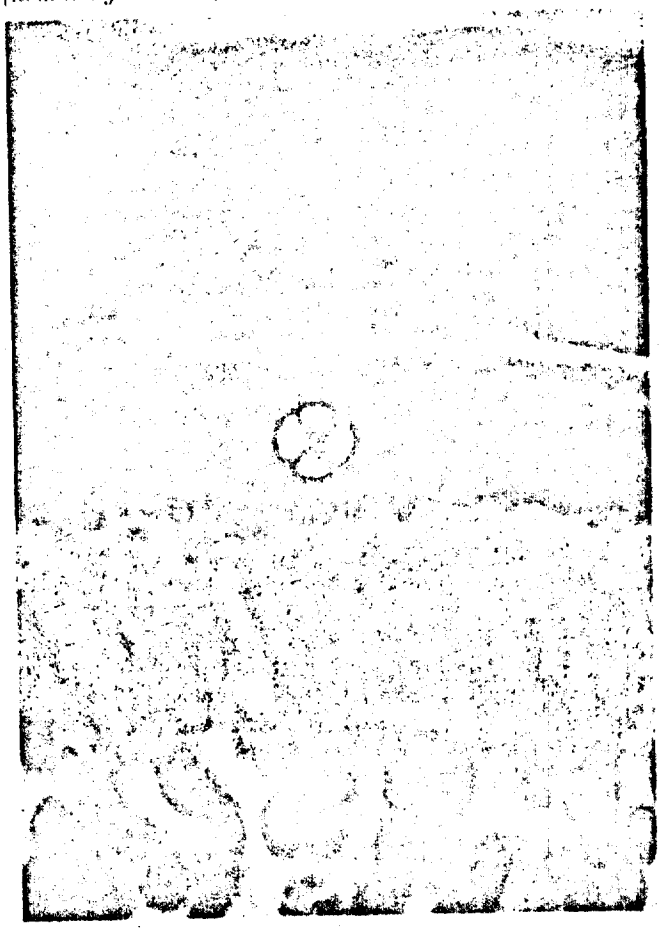


1. Fundus photograph showing characteristic location and appearance of talc and cornstarch crystals about macula. Striate hemorrhage was gone two months later.

2. Polarized light shows birefringent crystals typical of talc in capillary of inner nuclear layer of retina (hematoxylin-eosin, ×400).



3. Cornstarch particle in choriocapillaris with characteristic Maltese cross in polarized light (hematoxylin-eosin, ×400).



sual acuity measured 20/20 in each eye.

Two additional patients (patients 2 and 3), who were hospitalized elsewhere for systemic complications of injecting methylphenidate, showed fundus changes similar to those described in case 1. Patient 2, a 42-year-old Negro man, had, in addition, a cotton wool patch along the inferior temporal vessels in the right eye and also complained of reduced vision; near vision without correction measured 20/20 in the right eye and 20/60 in the left eye.

CASE 3.—A 30-year-old Negro man had several hospital admissions for shortness of breath, vomiting, cough, and weakness over the six months that he had injected methylphenidate. Visual acuity three months prior to his death was 20/20 in both eyes near vision, and he had no visual complaints. The ocular fundi showed venous distension, small blotch hemorrhages, and multiple small particulate emboli about the posterior poles. An x-ray film of the chest was reported to show enlargement of the right side of the heart and pulmonary artery. The white blood cell count was 26,000/cu mm with unexplained 60% eosinophils. He died following an overdose of injected methylphenidate and secobarbital.

Microscopic sections of his eyes were stained with hematoxylin-eosin stain and examined with polarized light. Numerous birefringent particles characteristic of talc were found in the capillaries of the nerve fiber and inner nuclear layers of the retina (Fig 2). An occasional talc crystal, as well as cornstarch particles, identified by their characteristic Maltese cross (Fig 3), could also be seen in the choriocapillaris. A rare cornstarch particle was also seen in the retinal capillaries. The talc particles appeared to lie within the wall of the capillaries and were not associated with a granulomatous reaction. Their nonobstructive nature was suggested by the absence of ischemic damage to adjacent retinal structures. Trypsin digest preparations of the retinal vessels confirmed the presence of talc crystals in the capillaries, small arterioles, and venules. The choroid contained large numbers of eosinophils and may have been the site of their formation. An occasional talc particle could also be seen in a scleral vessel. However, none could be found in the iris or ciliary body.

There were extensive foreign-body granulomas present in the sections of the lung about the innumerable talc and cornstarch particles. Tissue sections of the brain showed only occasional tiny birefringent spicules in the capillaries.

An attempt was made to establish the role of methylphenidate in this process by reproducing this syndrome in rabbits. A 20-mg methylphenidate hydrochloride tablet and a placebo were prepared for intravenous injection by mixing the crushed tablets with water and then boiling the mixture. Two white adult laboratory rabbits tolerated a total of 140 mg each over a period of five days before they died. Talc and cornstarch were found in abundance in the lungs of both rabbits, but no particles were present in the eye or brain tissue examined.

Examination of the ocular fundus while a similar material was injected into the carotid artery of rabbits revealed numerous crystalline particles traversing the retinal and choroidal vessels. Histologic section of these eyes showed talc crystals in both the choroidal and retinal vessels. The methylphenidate, experimentally at least, appeared to play no role in embolization of the filler material.

Comment

In the Portland area, methylphenidate tablets are easily obtainable and are used in the greatest quantity by individuals receiving methadone maintenance for heroin addiction. It is not uncommon for an individual to relate "shooting" a prescription of 100 tablets of 20 mg each over a period of one to two days. More moderate users describe crushing a 20-mg tablet and mixing it with a "spoon" of heroin before injection. Although many relate a transient dyspnea at the time of injection, comparatively few addicts are hospitalized for complications of this form of drug abuse.

The two patients who ascribed visual symptoms to the use of methylphenidate had both experienced acute failure of the right side of the heart and pulmonary hypertension. Presumably, the elevated venous pressure could have caused the retinal hemorrhages and edema which contributed to the reduction in vision. The other two patients with retinal hemorrhages, however, had no visual changes, although one had acute pulmonary hypertension.

The discovery of these characteristic crystals on funduscopic examination should prompt the examiner to inquire about a history of drug abuse and may help in confirming the cause of acute pulmonary hypertension in an uncommunicative patient. Eichner and Aebi[4] found retinal hemorrhages with Roth's spots in drug abusers who had injected a homemade alcoholic beverage intravenously. They also stressed the importance of the ophthalmoscopic examination in acutely ill patients with personality disorders.

The methylphenidate (Ritalin) hydrochloride and placebo used in this investigation were supplied by the Ciba Pharmaceutical Co., Division of Ciba Corp., Summit, NJ.

Paul Blachly, MD, and the Oregon State Methadone Blockade Treatment Program allowed examination of their patients.

References

1. Hahn HH, Schweid A, Beatty H: Complications of injecting dissolved methylphenidate tablets. *Arch Intern Med* 123:656-659, 1969.

2. Hopkins GB, Taylor D: Pulmonary talc granulomatosis: A complication of drug abuse. *Amer Rev Resp Dis* 101:101-104, 1970.

3. Wendt VE, Puro HE, Shapiro J, et al: Angiothrombotic pulmonary hypertension in addicts: "Blue velvet" addiction. *JAMA* 188:755-757, 1964.

4. Eichner HL, Aebi E: Septic retinitis due to injection of a homemade alcoholic beverage *JAMA* 213:1644-1646, 1970.

TOXICOLOGY AND APPLIED PHARMACOLOGY 6, 284-291 (1964)

The Effect of Ingestion of Diatomaceous Earth in White Rats: A Subacute Toxicity Test[1]

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Received June 11, 1963

Diatomaceous earth, the siliceous remains of unicellular and colonial algae, has been studied quite extensively in relation to occupational disease in the diatomite mining and processing industry. Among the first to recognize the problem were Legge and Rosencrantz (1932), who studied pneumoconiosis in a California plant that processed diatomaceous earth. Smart and Anderson (1952) observed that inhalation of diatomaceous earth may produce a distinct lesion characterized by linear fibrosis; furthermore they concluded that diatomaceous earth from freshwater origin appeared to be less toxic than that originating from the marine diatom. Luton *et al.* (1956) observed that amorphous silica was only moderately, if at all, harmful when inhaled. When this amorphous form was heated to approximately 1250 C, cristobalite, a crystalline form, was produced which was as dangerous as quartz. Vigliani and Pecchiani (1949) reported nodular reactions in the peritoneal cavities of guinea pigs injected with calcined diatomite, whereas the peritoneal cavities of guinea pigs injected with crude diatomite showed little change.

Diatomaceous earth is currently being used in insect control, particularly in granaries. The grain so treated will have a residue, which according to the Food and Drug Administration is subject to the Pesticide Amendment to the Federal Food, Drug, and Cosmetic Act. However, little is known as to the tissue damage caused by the mechanical and chemical activity of the diatom particle over a period of time.

The purpose of the toxicity test was to determine the possible damage and to ascertain the maximum concentration that would not produce tissue damage. It was suggested that 5, 3, and 1% diatomaceous earth be used in the diet of test animals and that the test be carried out over a 90-day period. Furthermore, if no damage occurred at the 5% level, only spot checks would be done with animals fed the lower concentration.

The following is a summary of the report submitted to the Division on Pharmacology, Toxicity Branch of the Federal Food and Drug Administration.

MATERIAL AND METHOD

Weanling white rats of the Wistar strain, average weight 50 g, were used. These animals were distributed into four groups, each containing 15 males and 15 females. Group I was designated as the group that would receive 5% diatomaceous earth added to the diet; group II would receive 3%; group III, 1%; and group IV the controls

[1] This research was performed for the Phoenix Gem Co., Inc. through Arizonia State University Foundation.

received the diet without the added test material. The rats were allowed free access to the food throughout the experiment.

The diatomaceous earth[2] was of freshwater origin. Four 50-pound bags were selected at random from the stockpile, and samples were taken from each bag in order to determine the particle size distribution and chemical composition.[3]

To ensure constant concentration, the diatomaceous earth was mixed with water to obtain a thin paste and added, a small quantity at a time, to preweighed feed in a tumbling apparatus. It was possible to obtain uniform distribution of the test substances on the pellets. These pellets were dried prior to use.

Weekly weight changes were recorded for each rat. At the end of the 90 days, the test was terminated and the animals were killed. Autopsies were performed and histologic sections were made from the following organs: stomach, small and large intestines, liver, kidneys, spleen, lung, urinary bladder, adrenal glands, mesenteric lymph nodes, and testes or ovaries. Organ weights were recorded for the livers, kidneys, and spleens; in addition, organ weight:body weight ratios were calculated for each animal. Parts of these same organs were analyzed for residual silica.

For histologic examinations, the tissues were fixed in 10% formalin and stained with Weigert's hematoxylin and eosin stains.

RESULTS

Analysis of Particle Size Distribution

The largest-sized particles in the test material was 0.64 mm. Ninety per cent were smaller than 0.1 mm; of these 55% were smaller than 0.012 mm. The smallest size of the test material was 0.00046 mm.

Chemical Analysis

Spectrographic analysis of the test material is tabulated in Table 1. The major components were in the form of oxides.

Analysis for Residual Silica

Organ analysis for silica in the liver, kidney, and spleen of the 5% level animals and controls are summarized in Table 2. These results are the averages for fifteen animals per group. No analysis were made for the lower two concentrations.

Physical Analysis

Summary of organ weights of livers, kidneys, and spleens are tabulated in Table 3. The average weights for fifteen animals were used in each average grouping. Table 4 reflects the average organ weight to body weight ratio of the 5% level test group and controls. No averages were made on either the 3 or 1% levels.

Weight Changes

The average weekly weight change of animals fed 5% diatomaceous earth over the 90-day period is summarized in Fig. 1. The graph reflects the average weight gain,

[2] Perma Guard, trademark of Phoenix Gems Inc., Phoenix, Arizona. From San Manuel lacustrine deposits.

[3] All chemical analyses were performed by the Arizona Testing Laboratories, Phoenix, Arizona.

TABLE 1

SPECTROGRAPHIC ANALYSIS OF DIATOMACEOUS EARTH USED IN TOXICITY TEST

Components	Percentage
Major elements (oxides)	
Silicon	85.164
Aluminum	3.9
Sodium	5.0
Iron	2.0
Magnesium	1.0
Minor or trace elements	
Boron	0.06
Manganese	0.002
Gallium	0.002
Calcium	0.84
Vanadium	0.002
Copper	0.01
Titanium	2.0
Zirconium	0.01
Strontium	0.01
	100.000

TABLE 2

THE ANALYSIS OF RESIDUAL SILICA FROM TEST ANIMALS AS COMPARED TO CONTROLS

Animal	Per cent silica
Liver	
Control males	0.002
5% males	0.002
Control females	0.002
5% females	0.002
Kidney	
Control males	0.002
5% males	0.002
Control females	0.002
5% females	0.001
Spleen	
Control males	0.002
5% males	0.003
Control females	0.003
5% females	0.003

TABLE 3

COMPARISON OF AVERAGE ORGAN WEIGHTS IN GRAMS OF CONTROL ANIMALS TO THE 5% TEST GROUP (15 ANIMALS PER GROUP)

	Control animals		5% test animals	
Organ	Male	Female	Male	Female
Liver	15.46	9.68	14.60	11.05
Kidney	1.58	1.13	1.43	1.20
Spleen	1.46	1.10	1.42	1.01

TABLE 4

RATIO OF ORGAN WEIGHT TO BODY WEIGHT OF THE 5% TEST ANIMALS AS COMPARED TO THE CONTROL ANIMALS

Animal group	Organ weight : body weight
Control males	0.0480
Liver	0.00544
Kidney	0.00520
Spleen	
5% males	0.0481
Liver	0.00532
Kidney	0.00520
Spleen	
Control females	0.0521
Liver	0.00531
Kidney	0.00542
Spleen	
5% females	0.0520
Liver	0.00538
Kidney	0.00420
Spleen	

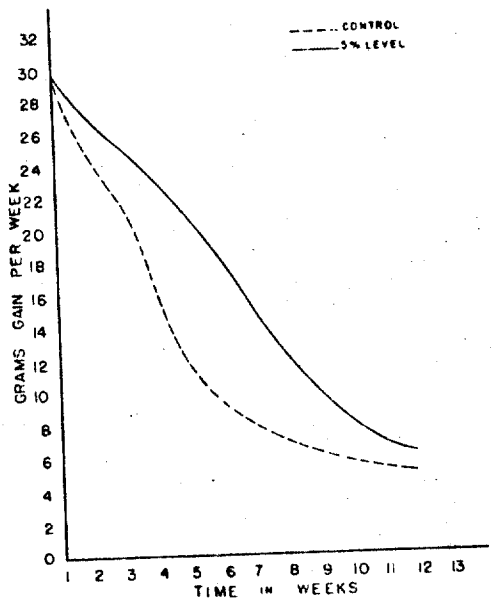

FIG. 1. Average weight gain in grams per week of test animals as compared to controls (30 animals were used in each group).

(combined values for males and females) in grams per week. It was noted that after the first week, the animals fed 5% diatomaceous earth had a higher weight gain than the control animals. The maximum weight gain differential was reached during the sixth week. The following weeks, although the test animals continued to gain more rapidly than the controls, the weight gain differential became progressively smaller

At the end of the test, the test animals were still gaining more than the controls. Projection beyond the 90-day period indicates that the weight gains would possibly be equal sometime between the fourteenth and fifteenth week. No accurate averages were made on the lower two levels; however it suffices to estimate that those animals on the 3% diet had a weight gain similar to that of animals at the higher level, while those animals on the 1% level had weight gains similar to those of controls.

Histologic Analysis

Complete histologic studies were made on the 5% group; in addition, six animals from both the 3% and the 1% groups were examined for comparative purposes.

No differences were observed in sections from the various organs of the 5% group and comparable sections of animals fed no diatomaceous earth (Figs. 2 and 3c). In both the 5% and control groups there were observed an equal number of animals which had a form of lobar pneumonia. In addition, inner zonal fatty degeneration was noted in the livers of five control animals and six animals on the 5% diet.

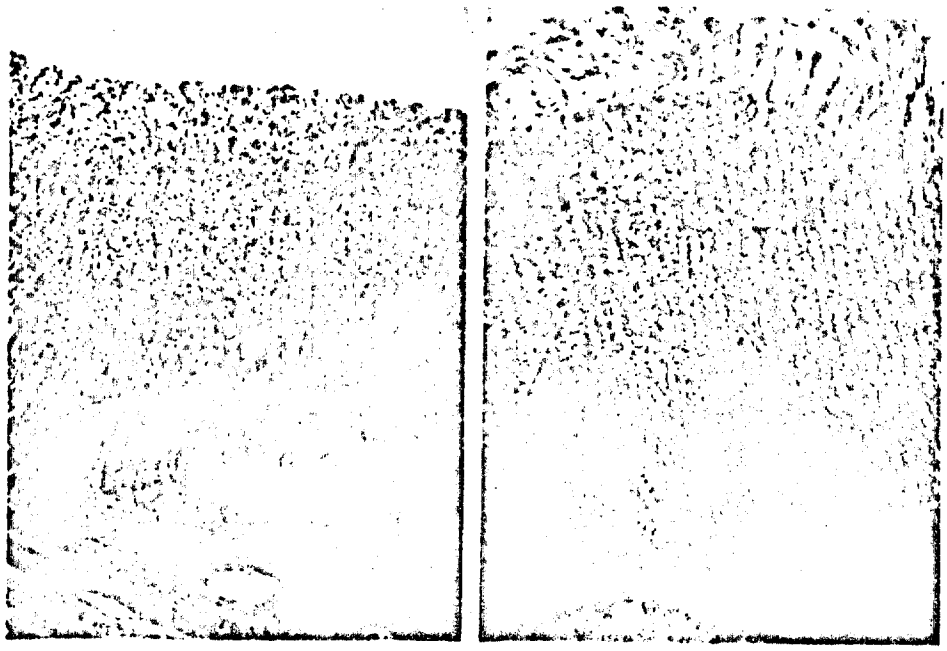

FIG. 2. Fundic region of the stomach of a test animal fed 5% diatomaceous earth (left) compared with comparable area from a control (right). No mechanical damage is evident that is due to the action of the diatomite particles. Hematoxylin and eosin stains. Magnification: × 125.

Of animals fed the lower two concentrations, the sections of the various organs showed no histologic changes when compared with similar areas from the controls.

DISCUSSION

In spite of the hardness of diatomaceous earth, no mechanical damage was observed from histologic sections taken from animals fed 5% of this material in their diet. This is significant in view of the fact that those animals were receiving diato-

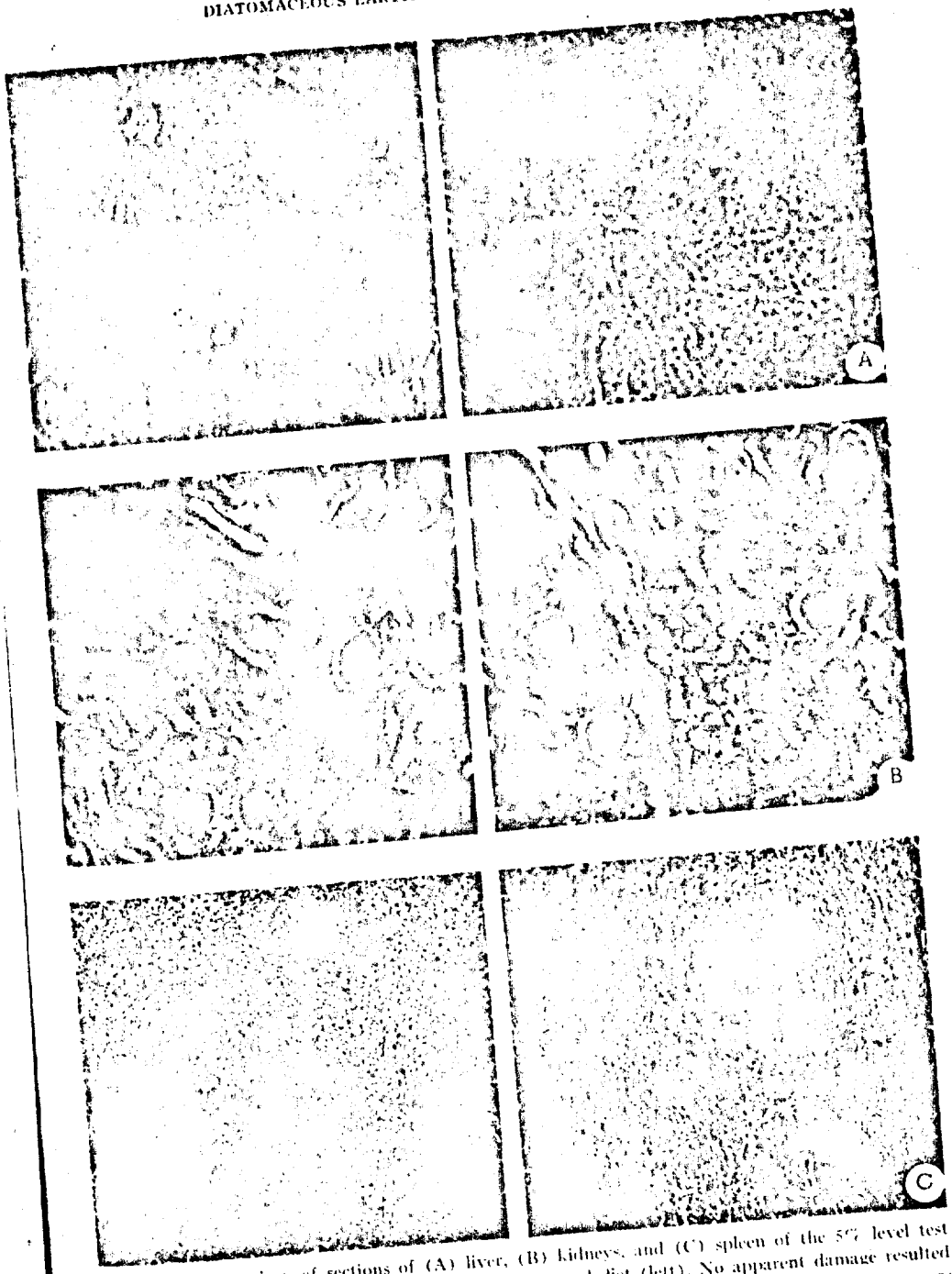

FIG. 3. Comparison of sections of (A) liver, (B) kidneys, and (C) spleen of the 5% level test animals (right) as compared to animals kept on a control diet (left). No apparent damage resulted from the indirect action of diatomaceous earth on these organs. Parts of the same organs were analyzed for residual silicon. Hematoxylin and eosin stains. Magnification: × 125.

maceous earth continually over a 90-day period. The lack of observable lesions (Fig. 2) is believed to be due to several factors. Examination of particle size distribution shows lack of coarse particles that would cause abrasive action. The largest particle size was approximately 0.04 mm; 90% of the remaining distribution was less than 0.12 mm. The second factor was the mode of administration. The diatomite particles were contained on the pellets and the white rats were allowed to feed ad libitum; consequently only a small amount of silica was taken in at any time with the food. Examination of the stomach content showed complete mixing of the test material with the food.

The lack of histologic changes other than at the primary site of action indicated that no toxic chemical absorption had occurred (Fig. 3). It was possible for the silica component, in an alkaline medium such as was present in the small intestines, to be converted into a relatively soluble silicic acid. Even if the amount per day was extremely small, the combined action over a 90-day period could be significant. Once in the circulatory system a drop in pH to 7.4 would precipitate the silica. The precipitated form could be in the crystalline state. If this did occur, necrotizing lesions would be seen because the crystalline form would be as dangerous as quartz. If the precipitate were in the amorphous state no reaction was to be anticipated since no changes occurred in the intestinal tract. To check further on the change in silica count, chemical analyses for residual silica were made from livers, kidneys, and spleen (Table 2). The results indicated no increase in the silica content of these animals. No analyses were made of these organs in the lower two levels.

Analysis of the organ weights and organ weight : body ratios further substantiate the nontoxicity of the diatomite particles (Tables 3 and 4). These animals were in an active growth stage as weanlings. Any toxic material would possibly affect the organ growth of the liver, kidney, and spleen. There was no evidence of atrophy or hypertrophy of these organs.

Aluminum, the next important compound, is apparently nontoxic according to the results of a study made by Campbell *et al.* (1956). The other compounds present were either in too small a concentration or could be utilized by the animal.

It was interesting to note the comparative weight changes. Not only did the test animals gain weight, but from the onset of the test the rate of growth exceeded that for the controls. The maximum rate change occurred during the sixth week of the test. At that time, the average weight change of the test animals over the controls were 45 g (Fig. 1). The weight differential after the sixth week continued, but on a decreasing weekly increment. Had the test been continued beyond 90-days, the weight changes should have been approximately the same for both groups during the fourteenth week.

One can only speculate as to the cause of the increase of weight of the test groups over the controls. One possibility relates to particle size. As particle size decreases, relative surface area increases, resulting in a high surface action. Owing to the adsorbability of diatom particles and the size distribution in this test, the highly active surface could adsorb food, creating not only a further increase in surface area for enzyme reaction, but allowing longer time of action for more complete digestion. Hence, there would be more enzyme product for the animal to utilize. A second possibility is the presence of minor or trace compounds (Table 1). Elements such as

sodium, iron, magnesium, manganese, calcium, and copper are necessary for either general metabolism or special cellular metabolism. The supplement, in conjunction with the high surface area and adsorptive property of diatomaceous earth, may account for this weight change. The graph (Fig. 1) also indicates that the abnormal weight changes are important in the fairly young stage of development. The weight differences were not reflected, however, in an increase of fat deposits.

From the results of this test, there is no evidence of toxic changes due to the ingestion of diatomaceous earth.

Summary

The effects of 3% diatomaceous earth in the diet fed to white rats over a 90-day period indicated no mechanical or chemical damage to the tissues.

There was no significant increase in the percentage of residual silica in liver, kidneys, and spleens of the test animals.

Comparison of weight changes in the 5% group with the control showed a greater weight gain in the 5% test group.

Histologic sections of animals fed 3% and 1% diatomaceous earth also showed no damage.

ACKNOWLEDGMENT

The author wishes to express appreciation to Dr. A. Louis de Lisle, formerly of the Phoenix Gem Company, Inc., and now a Consulting Engineer, for introducing him to the field of silica chemistry and for constant help throughout the experiment.

REFERENCES

Campbell, I. R., Cass, J. S., Cholak, J., and Kehoe, R. A. (1956). Aluminum in the environment of man. *A.M.A. Arch. Ind. Health* **15** (5), 359-448.

Cooper, W. C., and Cralley, L. J. (1948). Pneumoconiosis in diatomite mining and processing. *U.S. Public Health Serv. Bull.* 601.

Legge, R. T., and Rosencrantz, E. (1932). Observation and studies on silicosis by diatomaceous silica. *Am. J. Public Health* **20**, 1055-1060.

Luton, P., Champlix, R. M., and Volland, A. (1956). Recent observation on pneumoconiosis due to diatomaceous earth. *Arch. Maladies Profess. Med. Travail et Securite Sociale* **17**, 125-147.

Smart, R., and Anderson, W. H. (1956). Pneumoconiosis due to diatomaceous earth. *Ind. Med. Surg.* **56**, 214.

Tebbens, B. D., and Beard, R. R. (1957). Experiments on diatomaceous earth pneumoconiosis. I. Natural diatomaceous earth in guinea pigs. *A.M.A. Arch. Ind. Health* **17**, 55-63.

Vigliani, E. E., and Mottura, G. (1948). Diatomaceous earth silicosis. *Brit. J. Ind. Med.* **5**, 148-160.

Vigliani, E. E., and Pecchiani, L. (1949). The etiology of silicosis from diatomaceous earth. *Med. Lavoro* **40**, 33-36. (From Cooper and Cralley, 1948.)

J. Forensic Sci., 15(4): 581-587, Oct. 1970

Disseminated Magnesium and Aluminum Silicate Associated with Paregoric Addiction *

William C. Butz, M.D. **

Multiple foreign body granulomata in the lungs of narcotic addicts have been reported with increasing frequency (1, 2, 3). More recently liver lesions have been recorded in an addict injecting oral medication (4). Paregoric "mainliners" have been reported to have bacterial endocarditis involving the tricuspid valve when the neck veins are used as the site of administration (5). However, the extent to which magnesium and aluminum silicate in addicts using certain preparations containing paregoric can be distributed throughout the viscera is only now being realized.

The purpose of this article is to report a case of widely disseminated aluminum and magnesium silicate crystals in a paregoric addict.

Case Report

A 47-year-old Negro male was admitted to the hospital with the chief complaint of substernal nonradiating dull chest pain. Six hours prior to admission, the patient had noted an inability to open his jaw. This was followed by a stiffness in the neck and back.

Past history: The past history was significant in that for twelve years he had suffered aortic and mitral complications of "rheumatic fever." He had tried "horse" [heroin] in the military service while "just playing around" and admitted that he drank alcoholic beverages fairly regularly, especially on weekends. The patient gave a history of protracted use intravenously and subcutaneously of paregoric in various forms, combinations and concentrations, obtained from proprietary drugs.

* Received for publication June 30, 1970. Accepted for publication July 31, 1970.

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Physical examination: The patient was alert and oriented, but could not open his mouth. The neck was stiff and his head was fixed, resisting any motion. The chest was symmetrical although there were poor respiratory excursions, less laterally than in the anterior posterior; however, the lungs were clear. The pulse was collapsing in type. The point of maximal impulse was in the 5th intercostal space. The rhythm was regular and there was no sternal lift. There was a grade 3/6 holosystolic murmur at the apex transmitted to the neck and axilla.

In the skin of the abdomen multiple circular scars about 1.0 cm in diameter were present. There was involuntary spasm of the rectus abdominis muscles which interfered with palpation of the abdominal organs. There was contraction of the deep voluntary muscles of the back with a striking lordosis of the lumbar spine. The cranial nerves were intact and the deep tendon reflexes were active in the arms but absent in the knees. There were multiple circular scars 1.0 to 2.0 cm in diameter over the entire body. Ulcerated skin abscesses were present on the right calf and right lateral chest.

Laboratory findings: The hematocrit reading was 45% and the hemoglobin was 15.6 gm per 100 ml. The white blood count was 10,350 mm³ with 90 neutrophiles, 9 lymphocytes and 1 monocyte. Gram stains of smears of the abscess on the right calf revealed a mixed bacterial flora, gram positive cocci, gram negative rods and medium-sized gram positive bacilli with a single terminal swelling.

The chest X ray showed left ventricular enlargement.

The patient died from what appeared to be classic tetanus with sudden cessation of breathing eight hours after admission.

Autopsy: At autopsy there were numerous abscesses found involving the skin over the neck, shoulders, upper extremities, chest wall, thighs and lower extremities. The skin sections revealed multiple foreign body granulomata with large birefringent crystals (Fig. 1). The heart weighed 600 gm. There was stenosis of the aortic valve. No vegetations were present. The right and left ventricular walls of the heart were thickened. The lungs weighed 1,550 gm en bloc. There were adhesions over the pleural surface of the left lung. The lungs were boggy and exhibited reduced crepitance.

Microscopic examination of the lungs revealed foreign body granulomata within the pulmonary arterioles (Fig. 2). In the larger pulmonary arteries angiomatoids were present. Birefringent foreign body materials were in the walls of the development as well as within the walls of the venous channels in positions adjacent to the lumina. X-ray diffraction of the material identified it as magnesium silicate. There was extensive pulmonary edema and masses of pigmented histiocytes filled the alveolar spaces.

The liver weighed 1,700 gm. The appearance was grossly normal. On section there was no gross disturbance of the architecture. Microscopically, there was extensive periportal fibrosis. Numerous pigmented macrophages containing double refractile crystals were seen, but no foreign body giant cells were present (Fig. 3). The fibrous area surrounding the portal

triad was infiltrated with numerous lymphocytes, monocytes and polymorphonuclear leukocytes.

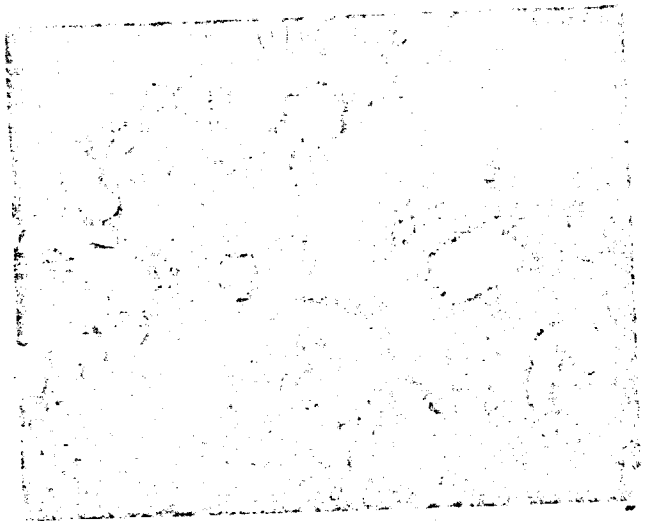

Fig. 1—Section of skin of the leg revealing large crystals of talc (hydrous magnesium silicate) in foreign body giant cells. There is proliferation of dense fibrous tissue surrounding the granuloma.

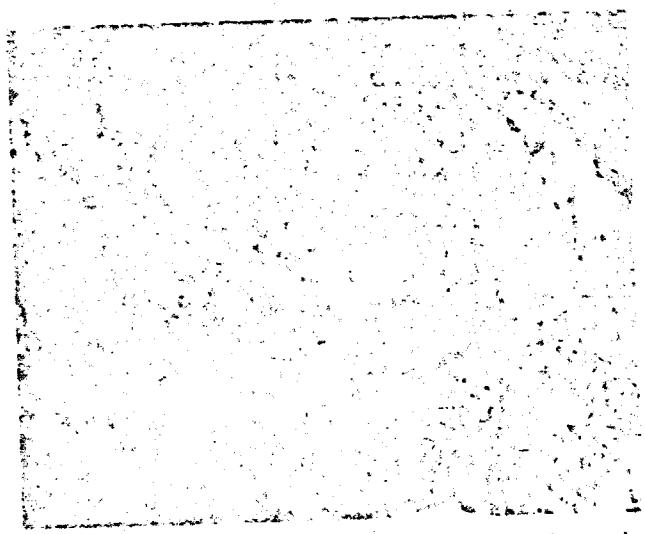

Fig. 2—Lung lesions show angiomatoids with obliterative pulmonary arteritis and recanalization. The talc crystals are in giant cells.

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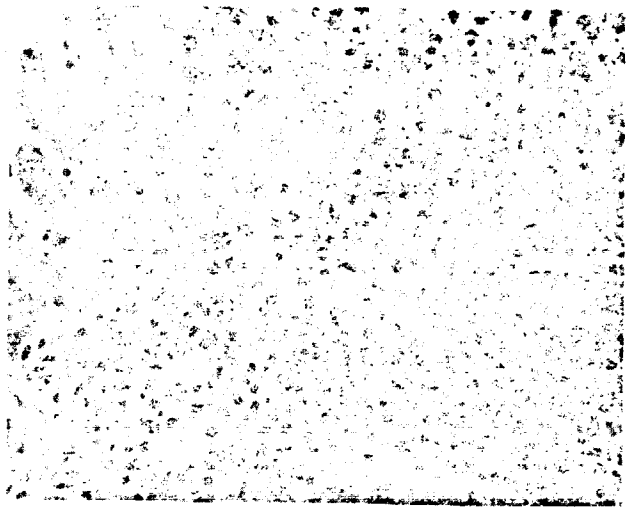

Fig. 3—Section of liver showing crystalline material, i. e., talc, in macrophages in the portal area. No foreign body giant cells are seen.

The spleen weighed 160 gm. The cut surface was unremarkable. Foreign body material was enclosed in macrophages.

The central nervous system disclosed extremely fine, double refractile particles in nerve bundles entering the anterior roots in the cervical region. No reaction was present in these fibers (Fig. 4).

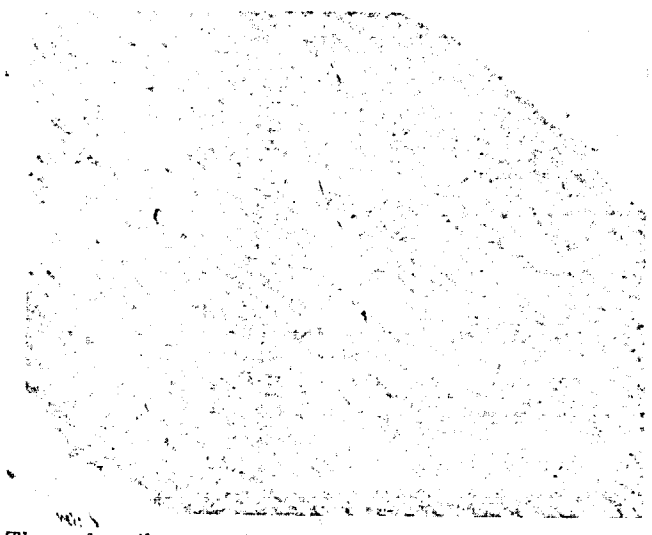

Fig. 4—The refractile crystals in the anterior root fibers of the upper spinal cord are demonstrated.

Discussion

The cause of death in this patient was undoubtedly tetanus. This conclusion is based on the classic clinical symptoms combined with the identification, on smear of an abscess of the right calf, of an organism morphologically characteristic of Clostridium tetani. Tetanus is more common in narcotic addicts using subcutaneous injections ("skin popping") as the method of introducing the chosen narcotic. This, in heroin addicts, is because quinine, which is a frequent adulterant, lowers the reduction oxidation potential of the tissues producing anaerobic conditions which favor the growth of Clostridium tetani (6).

Paregoric is one of the preparations exempted under the Harrison Narcotic Law of 1938. The states may, within their sovereign authority, make the law more restrictive but not less restrictive.

Paregoric, as ordinary camphorated opium tincture, contains 35–46 mg of anhydrous morphine per 100 ml. This drug, in various combinations with amphetamine, secobarbital, glutethimide and tripelennamine has been reported as a major narcotic used in certain parts of the country (7). Proprietary preparations containing paregoric but not requiring a prescription, such as Donnagel and Parepectolin,* are antidiarrheal agents. These preparations are also employed by paregoric users who crudely attempt to separate the paregoric from the kaolin (aluminum silicate).

From the description above, it will become evident that the paregoric user has the possibility of injecting two insoluble native minerals. In the first instance combinations with oral medications, i. e., amphetamine, secobarbital, tripelennamine, etc., in capsule or tablet form, may provide talc (hydrous magnesium silicate).

In the second instance, the addict's inability to separate kaolin allows him to receive aluminum silicate. The very fine crystals of magnesium or aluminum silicate, either through shunts in the lung of the "mainliner" who has an obliterative pulmonary arteritis and angiomatoid formations, or by extrusion from the arterial lumen and transfer to the pulmonary veins allows these crystals to go into the peripheral circulation.

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* Nonproprietary and Trade Name of Drugs: Kaolin, Pectin, Alkaloids and Paregoric—Donnagel; Kaolin, Pectin and Paregoric—Parepectolin.

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The absence of foreign body giant cells from the liver is not understood (4). Perhaps the fine particulate size of the silicate crystals, which get through the lungs into the peripheral circulation favors engulfment by macrophages, while the larger crystals in the lungs generate a foreign body reaction.

The presence of finely particulate birefringent material in the anterior roots of the central nervous system was of considerable interest in a patient whose demise was associated with neurological signs and symptoms. The fact that the level of involvement was high in the cervical region probably means that the insoluble silicate debris came in through the vertebral arteries. In terms of size these particles were the smallest of any seen anywhere within any of the viscera.

The X-ray diffraction patterns were characteristic of talc (hydrous magnesium silicate) and aluminum was found in small quantities.

Conclusions

Proprietary oral compounds injected intravenously for their paregoric content may yield substantial deposits of aluminum and magnesium silicate in organs such as the lungs, liver and central nervous system. The insoluble mineral crystals are progressively filtered so that the size of the particles diminishes as they reach the most distal organs in the peripheral circulation.

Summary

Paregoric, a camphorated tincture of opium, in various combinations with oral drugs is frequently used by narcotic addicts. Since these oral preparations frequently contain hydrous magnesium and aluminum silicate, deposits in organs may ultimately occur with severe damage.

Acknowledgment: The author wishes to thank Dr. Frank Johnson, Armed Forces Institute of Pathology, Washington, D. C., and Capt. Charles C. Whitney, Jr., MSC, Chemistry Department, Toxicology Section, Third United States Army, Ft. McPherson, Georgia, for identification of magnesium silicate (talc) and aluminum in the visceral organs by X-ray diffraction.

REFERENCES

1. Burton, J. F., Zawadeski, E. S., Wetherell, H. R., and T. W. Mayer. Mainliners and Blue Velvet. J. Forensic Sci. 10, 466–472 (1965).

2. Butz, W. C. Pulmonary Arteriole Foreign Body Granuk Associated with Angiomatoids from Intravenous Injestion of Oral Medication, e. g., Propoxyphene Hydrochloride (Darvon). J. Forensic Sci. 14, 317–326 (1969).
3. Hahn, H. H., Schweid, A. J., and H. W. Beaty. Complications of Injecting Dissolved Methylphenidate Tablets. Arch. Intern. Med. 123, 656–659 (1969).
4. Ishak, B. W. and K. G. Ishak. Foreign Body Reaction in the Liver of a Drug Addict. J. Forensic Sci. 14, 515–520 (1969).
5. Certher, J. F., Goodman, J. L., and A. M. Lerner. Infections in Paregoric Addicts. JAMA 190, 683–686 (1964).
6. Wendt, V. E., Puro, H. E., Shapira, J., Mathews, W., and P. L. Wold. Angiothrombotic Pulmonary Hypertension in Addicts. JAMA 188, 755–757 (1964).
7. Hopkins, B. G., and D. G. Taylor. Pulmonary Talc Granulomatosis. Am. Rev. Resp. Disease 101, 101–104 (1970).

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Brit. J. industr. Med., 1961, **18**, 295.

THE TOXICITY OF PRECIPITATED SILICA

BY

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(RECEIVED FOR PUBLICATION APRIL 4, 1961)

The proportion of respirable particles in dust clouds generated from three samples of precipitated silica has been shown to range between one-quarter and one-third by weight.

After a single intratracheal dose of the silicas to rats, chemical analysis shows a progressive disappearance of silica from the lungs, though it is still detectable after 12 months. Some silica appears in the liver and kidneys but in two of the three samples none remains after 12 months.

The nature and duration of the lung lesions produced in rats after a single intratracheal injection are described. A mild degree of fibrosis was observed which showed a steady regression with time and was to some extent influenced by the nature of the silica injected. The lesions showed little resemblance to those arising from quartz and were more akin to those produced by non-fibrogenic dusts.

Recommendations are made for the precautions to be taken during the industrial handling of these dusts.

Amorphous silica has been used for many years in a variety of industries, notably for inclusion into rubber formulations as a filler. Although it appears to have been handled without special precautions, no authenticated case of silicosis or other respiratory disease has been traced which can be attributed to this agent. Nevertheless, the increasing industrial usage of this material has aroused some concern lest hitherto unsuspected toxic properties might emerge.

Amorphous silica is not a clearly defined chemical entity and several methods are employed for its commercial production. Most of the experimental investigations into the toxicity of amorphous silica have been concerned with "flame" silica, prepared by burning a mixture of hydrogen and silicon tetrachloride vapour. It has been shown that this type of silica has a cytotoxic action (Policard and Collet, 1957) and can be lethal when injected intravenously (Swensson, Glomme, and Bloom, 1956), but that when inhaled it produces a reversible lesion in the lungs with only a mild degree of fibrosis and with very little collagen deposition (Schepers, Delahant, Bailey, Gockeler, and Gay, 1957a). Similar effects on the lungs have been shown by intratracheal injections (Klosterkötter and Jötten, 1953).

Another commercially important type of amorphous silica is prepared by acid precipitation from an aqueous solution of sodium silicate, a process similar to that used for the preparation of silica gel. There do not appear to have been any systematic investigations into the toxicity of this precipitated silica, which cannot be assumed to be identical with that of "flame" silica as there is no certainty that the two substances have the same composition. An investigation of precipitated silica from two manufacturers has, therefore, been undertaken, in order to assess the possibility of a silicosis hazard from the industrial use of this material.

Fibrogenic properties of a dust can be determined by studying the cellular reaction to the dust within the lung. Experimentally, dust can be introduced by exposing animals to a dusty atmosphere or injecting dust in some form of suspension directly into the lung. In the former method, the animals automatically select those particles which can enter the respiratory system sufficiently far to be retained (Dautrebande, Beckmann, and Walkenhorst, 1957). In the injection method, the size distribution of the material depends on the degree of dispersion in the suspension administered. Apart from considerations of particle size, the injection method probably provides at least as severe a test of the fibrogenic capacity of a dust as do dusting procedures (Schepers, Durkan, Delahant, Creedon, and Redlin, 1957b; Belt and King, 1945; Kettle and Hilton, 1932).

Amorphous silicas are composed of very fine particles, the average diameter being in the region of 20 mμ, which tend to aggregate loosely in air. The size of these aggregates will determine the respirable fraction of the dust, but will not necessarily determine the effect of the dust once it has entered the body, since the silica may then be exposed to other influences causing a variation in the degree of aggregation.

We have performed two types of experiment with three amorphous silicas. In the first, the proportion of respirable particles in air-borne precipitated silica dust clouds has been measured. In the second we have administered to rats, by intratracheal injection, single doses of the samples, and have studied the distribution and excretion of silica and the nature and duration of the lesions produced.

Samples Investigated

Samples of precipitated silica were obtained from two manufacturers. From one supplier two samples were received; sample A1, the quality normally supplied, and the sample A2 which had the same characteristics when originally manufactured, but which had for reasons not clearly known increased in particle size on ageing. Sample B was obtained from the second supplier.

Sample A1.—The particle size was 19 mμ, calculated by an air permeability method (Pechukas and Gage, 1946).

Sample A2.—When freshly prepared this was shown by electron microscopy to have a particle size of 20 mμ and a specific surface by gas absorption of 110 m.²/g. A later measurement of the specific surface showed that this had decreased to 60 m.²/g. and an air permeability measurement gave a particle size of 60 mμ.

Sample B.—The particle size by an air permeability method was 25 mμ.

The intravenous toxicity of the three samples has been determined on rats. Most deaths occurred within two hours of injection and all rats surviving for 24 hours subsequently recovered. The following LD_{50} values were obtained, with the fiducial limits ($p = 0{\cdot}05$) given in parentheses: sample A1, 35·2 (32-39); sample A2, 41·2 (34·5-42); sample B, 44·4 (40·5-49) mg./kg. These figures are appreciably higher than the values 7-17 mg./kg. obtained on mice with "flame" silica by Swensson *et al.* (1956).

The Respirable Fraction of Precipitated Silica

Theoretical and experimental studies have shown that the ability of dust particles to penetrate into the lungs when inhaled is related to their free falling speed in air and is, therefore, determined by their size and density (Davies, 1952). It is thought that quartz particles with a diameter greater than about 7 μ are trapped in the upper respiratory passages and do not enter the lung alveoli and give rise to silicosis (Wright, 1954). The ultimate particle size of precipitated silica is very much less than this figure, but the particles are associated into loose aggregates whose density will be much lower than that of quartz. The upper size limit for penetration into the lung by these aggregates is uncertain but will be greater than the 7μ quoted for quartz. A classification of a precipitated silica dust cloud by direct measurement of aggregate size would, therefore, have only a limited value as a guide to the pneumoconiosis hazard from the dust, and it would be preferable to base the size analysis on the free falling speed of the particles. A determination of the aggregate size range by trapping the dust in a liquid followed by elutriation would probably be unsuitable as the forces at the solid-liquid interface might change the degree of dispersion. The instrument selected for studying the precipitated silica dust was the Hexhlet Air Elutriator (Casella Ltd.) which collects the fraction of the dust with a free falling speed in air sufficiently low to permit penetration into the lungs (Wright, 1954).

Apparatus

The dust cloud was generated by a modification of the United States Bureau of Mines Apparatus (Schrenk, 1939), in which the dust is filled into a long vertical cylindrical container which is slowly raised at a uniform speed to maintain the surface of the dust in contact with a static head. From this head the dust is aspirated into the air stream by means of a Venturi jet. The original apparatus was designed for a much larger scale than was convenient for these experiments and an attempt to reduce it in size was unsatisfactory as an intermittent dust feed was obtained. This difficulty was overcome by applying an oscillatory rotation to the dust container and a positive flow control to the air supply used to pick up the dust from the static head and introduce it into the main air stream. The apparatus used is shown diagrammatically in Fig. 1. The dust container, A, was constructed from a precision 5 mm. bore glass tube, 20 cm. long, surmounted by a B 14 standard taper socket. A side arm, B, was connected through a flow meter to an air line with flow control. A brass piston, C, 12 mm. long and bearing 12 longitudinal flutes, slid easily into the tube A. A thin-walled stainless steel tube, D, 1·5 mm. diameter and 25 cm. long was fixed into a hole bored centrally in the piston. This tube also passed through a hole drilled in a "Perspex" cone, E, fitted in the B 14 socket and forming an air-tight seal but permitting movement of the tube in the cone. The upper end of the tube was clamped and the tip entered a hole in the base of an open-ended "Perspex" box attached to the end of the Hexhlet apparatus.

The dust container was mounted in a bored rubber stopper in an adaptor on the ram, F, of a slow injection apparatus (C. F. Palmer Ltd.) which had been modified by the makers for a vertical drive. This apparatus was capable of raising the dust container a distance of 4 in. at constant rates which could be varied between 1 in. in 10 to 320 minutes. A horizontal slotted bar, G, was also attached to the rotating head of the ram and in the slot was a vertical pin, H, on an adjustable crank which turned by means of a geared down electric motor at a speed of 30 r.p.m. The vertical pin on the crank was sufficiently long to impart an oscillating angular motion of about 65° to the slotted bar, whatever the position of the ram.

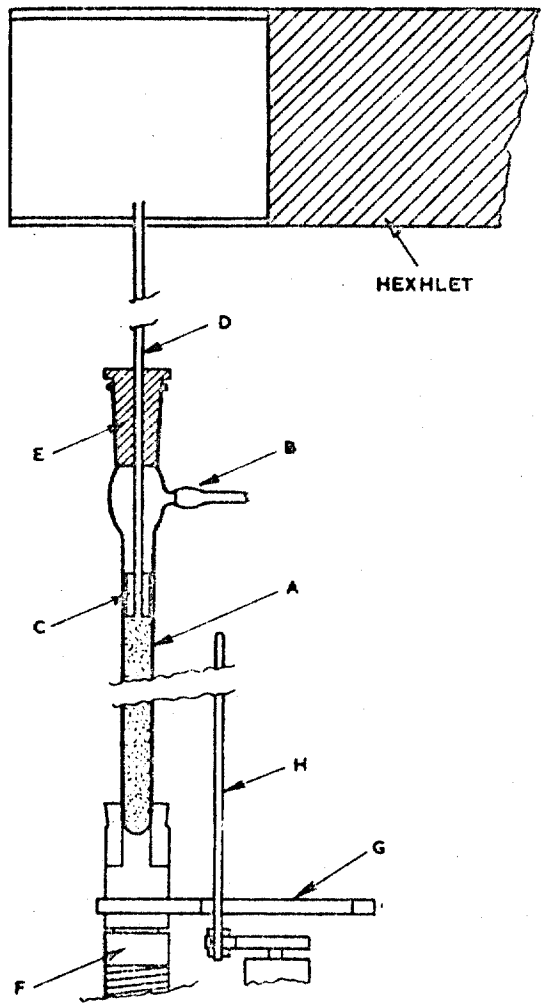

FIG. 1.—Apparatus for the generation of silica dust clouds.
A, dust container; B, entry of air for dust pick-up; C, fluted brass piston; D, stainless steel tube; E, "Perspex" cone; F, ram of slow injection apparatus; G, slotted bar; H, rotating pin.

A weighed amount of the silica sample to be examined was introduced in portions into the dust container, the tube being tapped between each addition. The apparatus was assembled as shown in Fig. 1, and with a soxhlet thimble of known ash weight in the Hexhlet apparatus. The air line to the Hexhlet was turned on, and 0·2 l./min. dry air was passed through the side arm of the dust reservoir. The vertical drive on the dust container was started at a speed of 1 in. in 40 min. and the agitator switched on. By this means the dust was caused to issue at a known rate from the tip of the vertical tube and enter the air stream passing at a rate of 100 l./min. into the Hexhlet.

The run on each sample was continued for two to three hours at the end of which the dust remaining in the container was weighed and hence the total injected into the air stream was calculated. The soxhlet thimble was removed and ashed and the residue weighed and corrected for the ash of the thimble and the measured percentage of loss in weight of the silica sample at the ashing temperature. The results of duplicate experiments on the three samples are shown in Table 1.

The figures in the third column of Table 1 show that the respirable fraction of the precipitated silica samples ranged between 25 and 40%. Both samples from manufacturer A showed a smaller proportion of fine particles than the sample B. The apparently greater aggregate size of sample A2 may be related to the statement that this sample had changed in its physical properties on ageing leading to an increase in the ultimate particle size, and also to the observation made in subsequent experiments that this sample is more difficult to disperse in water. The variation in the atmospheric concentration in duplicate experiments shown in Table 1 must be attributed to differences in the degree of packing of the powder in the dust container; nevertheless, the duplicates on the percentage reaching the filter are intolerably good agreement.

TABLE 1

RESPIRABLE FRACTION OF PRECIPITATED SILICA DUST

Sample	Weight Silica (mg.)		% Silica on Filter	Duration (min.)	Atmospheric Concentration (mg./m.³)
	Injected	Collected			
A1	369	107	29·0	130	28·4
	285	96	33·7	120	23·7
A2	603	148	24·5	170	35·5
	479	129	27·0	170	28·2
B	457	198	43·3	170	26·9
	280	98	35·0	120	23·3

Retention of Precipitated Silica in Lungs

Groups of 50 male and 50 female adult Wistar strain albino rats were injected intratracheally under light ether anaesthesia with 1 ml. of a 2·5% suspension of each of the samples of precipitated silica. The silica was sterilized in hot air at 160° C. for two hours and dispersed in sterile isotonic saline by means of a Griffith's tube. Higher doses than 25 mg./rat caused death within 24 hours. The first injections were made with sample A2 and although initially all rats tolerated the dose well, 12 of the group died within four days with post-mortem signs of consolidation and abscess formation in the lungs. In experiments on the other two samples, which were started several months later, 2,000 units of penicillin were included in the injection and no immediate deaths from lung infection occurred. At three intervals during the 12 months after injection five male and five female rats were killed and the lungs, liver, kidneys, and spleen were taken for silica determinations. Similar groups were killed for a histopathological examination of the lungs.

Silica was determined in the tissues by a modification of the method of King, Stacy, Holt, Yates, and Pickles (1955). A 50% aqueous homogenate of each sample was prepared and 2 g. was fused with 2 g. sodium carbonate in a platinum crucible. Heating was gentle during charring of the tissue and was then applied more strongly until the melt remained white on cooling. The melt was dissolved in water by gently warming and was then cautiously neutralized to Congo Red with 10 N sulphuric acid, and a further 0·2 ml. of the acid added. The solution was diluted to 50 ml. with water and 1 or 2 ml. portions taken for analysis. The subsequent colorimetric determination of silica followed the method described by King *et al.* (1955), the optical density of the blue colour being measured at 810 mμ. The results of these analyses, expressed as μg. SiO_2 per rat, are given in Table 2, together with the average silica content of tissue from normal rats.

TABLE 2

RETENTION OF SILICA BY RAT TISSUES

Sample	Weeks After Injection	Lungs	Liver	Kidneys	Spleen
A1	12	1,570	177	33	0
	24	785	20	12·4	1·4
	52	210	61	9·7	0
A2	12	5,150	249	138	0
	36	1,550	153	44	19
	52	720	153	56	0
B	12	652	234	34	5·8
	24	324	43	8·9	1·4
	52	108	38	12	0
Average silica content of normal rat tissue		28	37	11	5

All rats received an intratracheal dose of 25 mg. precipitated silica. Figures represent μg. SiO_2 per rat.

Effect of Precipitated Silica on Lungs

Groups of five male and five female rats, dosed intratracheally with each of the precipitated silicas as described in a previous section, were taken at three intervals over a period of 12 months for histopathological examination of the lungs. Sections of the whole lungs, cut in the frontal plane, were stained with haematoxylin and eosin, and others impregnated with silver for reticulin fibres. Other sections were micro-incinerated, with and without acid-washing, and a few were stained with Van Giesen.

An examination of the acid-washed incinerated sections showed that the distribution of silica in the lungs was not uniform, but occurred in small areas throughout one or both lungs with occasionally a large amount deposited in one lung segment. The stained sections showed that the three dusts elicited the same type of response in the lungs and that there was no significant difference between the effects produced in male and female rats. The silica particles were contained within macrophages which were aggregated into foci around terminal and respiratory bronchioles. Small numbers of lymphocytes and fibroblasts surrounded these foci and were also intermingled within the macrophages (Figs. 2 and 3), while reticulin fibres were woven through and around the lesion. These general features of the dust lesions were to some extent influenced by the distribution of dust within the lung. Where dust deposition was very heavy the structure of the lung segments was completely obliterated by the presence of the lesions produced. In these areas the basic lesions consisted as before of a group of macrophages with a few fibroblasts and lymphocytes, but they were contiguous and their individuality was only distinguishable by virtue of the peripheral zone of fibroblasts and lymphocytes (Fig. 3). These confluent lesions were maximal 12 weeks after injection; with the passage of time dust was gradually eliminated from them, with some contraction of the lesion resulting in varying degrees of deformity of the lung. The individual lesions also decreased in size and number with time.

The size and number of the lesions was a function of the quantity of dust present in the lungs. For a given dust the lungs with the larger amounts of dust had the larger lesions and with the progressive elimination of the silica from the lungs there was a corresponding decrease in the size of the lesions and, towards the end of the experiment, in their number. The maximal size of the lesions was seen in the first groups of animals examined, 12 weeks after injection. In these sections the reticulin fibres were also most numerous and the amount tended to decrease as the lesions decreased in size during the course of the experiment.

There was little evidence that the lymphatic system was involved in the process of dust elimination. Within the lung itself dust was not often seen in lymphoid tissue. In those sections where paratracheal lymph nodes were present none or only small quantities of dust were seen. The small quantity which could be detected was contained within macrophages lying in sinusoids, and elicited virtually no reaction (Fig. 5). These observations do not preclude the possibility that significant quantities of dust were eliminated through the lymphatics.

The type of dust used affected the nature of the lesion, in the main by virtue of the different rates of excretion of the three samples; inspection of the micro-incinerated sections confirmed the analytical evidence that sample B was the most quickly eliminated from the lung and sample A2 the slowest. There appeared to be slight differences between the dusts in addition to this; sample A2 tended to have slightly

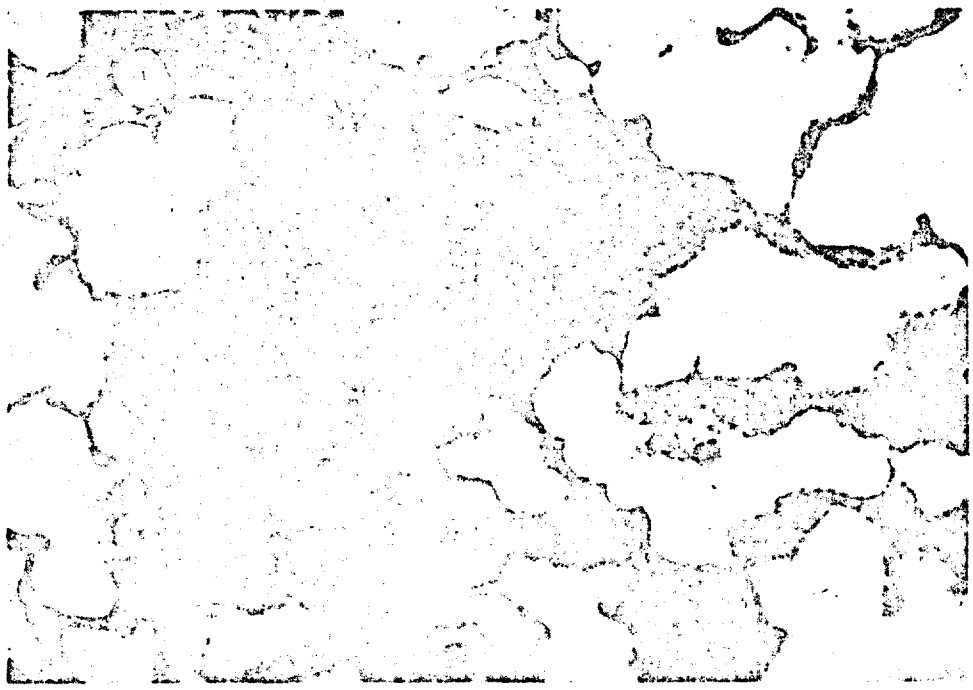

FIG. 2.—Rat lung 12 weeks after an intratracheal injection of 25 mg. of amorphous silica (sample B): an isolated lesion composed principally of macrophages. (H. and E. × 188.)

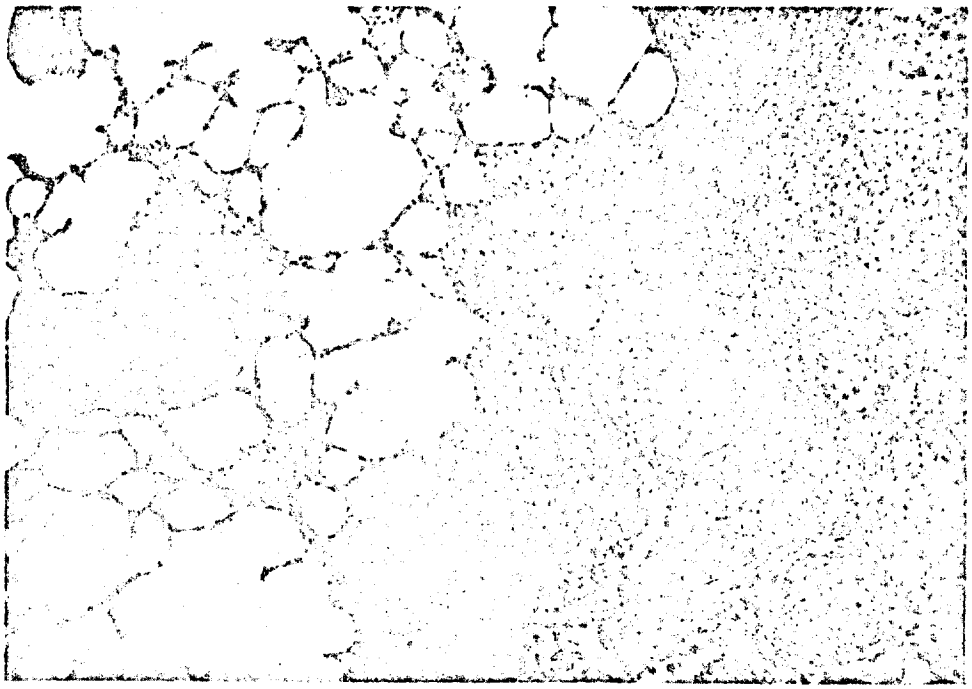

FIG. 3.—Rat lung 12 weeks after an intratracheal injection of 25 mg. of amorphous silica (sample A1): an isolated lesion at the left and confluent lesions to the right. (H. and E. × 100.)

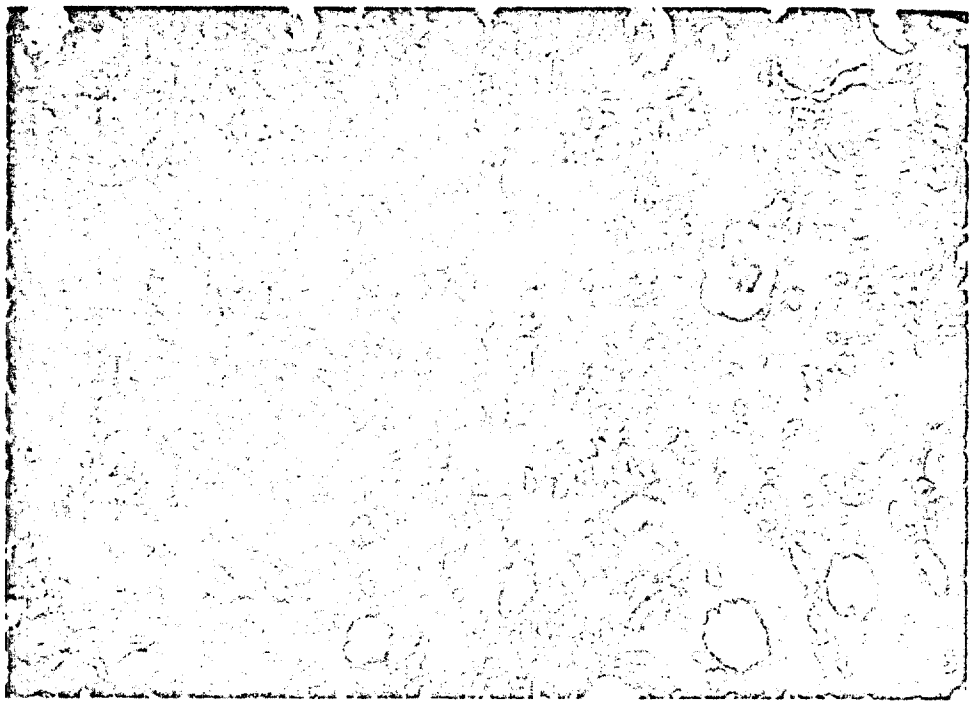

FIG. 4.—Rat lung 52 weeks after an intratracheal injection of 25 mg. of amorphous silica (sample A2): a portion of a confluent lesion showing loss of lung architecture and proliferation of reticulin fibres, with some collagen fibres. (Silver reticulin, neutral red × 150.)

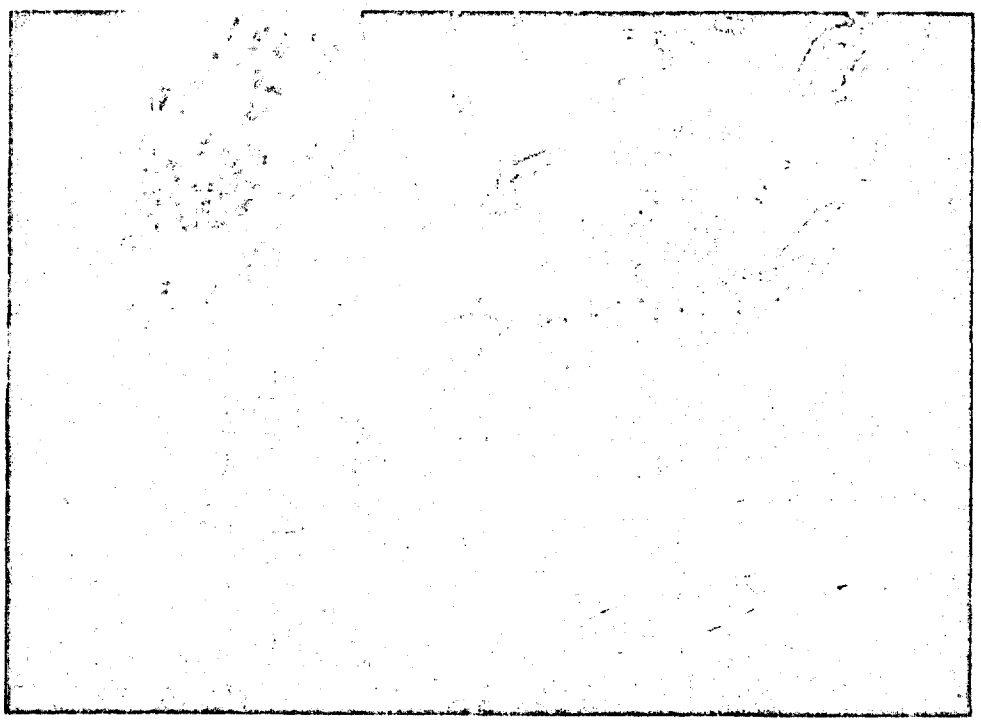

FIG. 5.—Rat mediastinal lymph node (the same animal as Fig. 4), 52 weeks after an intratracheal injection of 25 mg. of amorphous silica (sample A2): sinusoidal collections of macrophages with no associated inflammatory response or fibroblastic proliferation. (H. and E. × 200.)

larger lesions than the others for the same amount of dust, and also to induce a greater amount of fibroblastic proliferation which had the appearance of being more mature. As the lesions from this dust became smaller with time there was some condensation of the reticulin fibres giving rise to a rather more dense distribution at the periphery. In the first batch of animals examined, these lesions showed grade 1, or in one animal grade 2, fibrosis by Belt and King's (1945) classification, and at the termination of the experiment the lesions were graded as 1 minimal to 2 (Fig. 4).

The rats injected with sample A1 showed a slow and steady regression of the isolated lesions with an accompanying reduction of the reticulin fibres and no evidence of their maturation. The first batch examined showed lesions with grade 1 maximal to 1 fibrosis and at the end of the experiments the grades were 1 to 1 minimal; at this point sections from five animals showed no isolated lesions, while in others they were few and small. In areas of heavy dust deposition a rather milder effect was elicited than that from sample A2, and regression went further over the period of observation. The lesions from sample B were initially similar to those from samples A1 (Figs. 2 and 3), but their rate of regression was more rapid and the final sections showed either a complete resolution or small scattered dust foci with few reticulin fibres around or within the lesion (fibrosis grade 1 to 1 minimal). There also remained a few areas of confluent lesions which were small and irregular and showed a rather light reticulin network and a little retraction.

In general the effect of the dust on lung function appeared to be small. There was evidence of mild emphysema in a few lungs but apart from a few large abscesses and foci of bronchiectasis there was little evidence of infection. Pneumonia and bronchopneumonia were infrequent. It does not seem likely that the presence of dust in the lungs greatly influenced the incidence or course of these infections, an opinion that tended to be confirmed by examination of 10 normal adult male and female rat lungs. In these animals microscopic and macroscopic inflammatory lesions were present with a similar overall frequency as in the lungs of the experimental animals.

Conclusions

The presence of particles of respirable size, to the extent of between one-quarter and one-third by weight, in dust clouds generated from the three samples of precipitated silica is evidence that the extent of aggregation is not sufficiently great to cause the dust to be entirely retained in the upper respiratory passages when inhaled and so preclude any cellular reaction in the lungs.

When precipitated silica is injected into rats' lungs a significant amount can still be detected by chemical analysis after a period of 12 months. The rate of elimination is, however, much faster than that observed with quartz and other fibrogenic dusts. Initially some of the silica leaving the lung appeared in the liver and kidneys, but with samples A1 and B no significant amount of silica could be detected in these organs six months after injection. Sample A2 was more persistent and was found in the liver and kidneys 12 months after injection. There is no evidence to indicate the route by which silica is eliminated from the lungs, and whether it is transported in the body as particles or in solution.

The lesions produced by the intratracheal injection of precipitated silica show little resemblance to those arising from quartz and were more akin to the effect of non-fibrogenic dusts. The most marked effect on the lungs was produced by sample A2 where there was a tendency for the lesions to coalesce with a loss of well-defined boundaries, but this in no way resembled the progressive reaction to quartz which produces an expanding lesion with maturation of fibres. Another notable difference resided in the evident regression of lesions during the course of the experiment, corresponding to the disappearance of silica from the lungs.

The severity of the lesions from precipitated silica was to some extent affected by the nature of the dust. Sample A2 produced the largest and most persistent lesions and also a slightly greater deposition of fibres. It seems unlikely that any chemical difference existed between samples A1 and A2, which were initially made by identical processes, and it seems more probable that the greater activity of sample A2 can be attributed to those surface properties which resulted in its greater tendency to aggregate. This tendency could be observed both from the small proportion of fine particles in a dust cloud from this sample, and in the greater difficulty which was encountered in preparing an aqueous dispersion from it.

The single intratracheal dose technique is regarded as an efficient means of detecting the fibrogenic properties of a dust, and the absence of such a cellular reaction when precipitated silica is tested in this manner suggests that silicosis is unlikely to be encountered when materials of this type are used in industry. This does not imply that they are completely innocuous and precautions common to all other dusty materials should be taken to keep atmospheric contamination to a minimum. Where this is impossible, it is desirable that approved respiratory protection should be worn. Workmen exposed to these dusts should be subjected to periodic medical supervision, and should not be allowed to continue

in their employment if there are signs of chronic respiratory disease.

Technical assistance in this investigation was provided by Mr. R. T. Ashmole and Mr. P. Salt.

References

Belt, T. H., and King, E. J. (1945). *Spec. Rep. Ser. med. Res. Coun. (Lond.)*, No. 250. H.M.S.O., London.

Dautrebande, L., Beckmann, H., and Walkenhorst, W. (1957). *Arch. industr. Hlth*, **16**, 179.

Davies, C. N. (1952). *Brit. J. industr. Med.*, **9**, 120.

Kettle, E. H., and Hilton, R. (1932). *Lancet*, **1**, 1190.

King, E. J., Stacy, B. D., Holt, P. F., Yates, D. M., and Fickles, D. (1955). *Analyst*, **80**, 441.

Klosterkötter, W., and Jötten, K. W. (1953). *Arch. Hyg. (Berl.)*, 137, 625.

Pechukas, A., and Gage, F. W. (1946). *Industr. Engng Chem. (Anal. ed.)*, **18**, 370.

Policard, A., and Collet, A. (1957). *Arch. Mal. prof.*, **18**, 50.

Schepers, G. W. H., Delahant, A. B., Bailey, D. A., Gockeler, E. L., and Gay, W. C. (1957a). *Arch. industr. Hlth*, **16**, 499.

——, Durkan, T. M., Delahant, A. B., Creedon, F. T., and Redlin, A. J. (1957b). *Ibid*, **16**, 125.

Schrenk, H. H. (1939). *U.S. Bureau Mines I.C.* 7086.

Swensson, A.; Glomme, J., and Bloom, G. (1956). *A.M.A. Arch. industr. Hlth*, **14**, 482.

Wright, B. M. (1954). *Brit. J. industr. Med.*, **11**, 284.

Gastroenterology, 25(2): 296-299, 1953

TALC GRANULOMATA OCCURRING IN THE PERITONEAL CAVITY

A Case Report

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Numerous reports on the harmfulness of talc as a contaminant in surgical wounds have appeared in the literature. Antopol[1] in 1933 was the first to suggest that silica crystals in talc might be responsible for granulomata occurring in post-operative cases; however, it was not until 1937 when Fienberg[2] suggested the use of the polariscope for identifying silica crystals, that more of these cases were recognized. Talc may be the causative factor in many cases of diffuse postoperative adhesions with obstruction. Tissue specimens of adhesions are not routinely sent to the laboratory unless resection is carried out. Lichtman, et al.[3] at the Mayo Clinic removed tissues routinely from one hundred consecutive cases of secondary operations in which adhesions were present for examination under the polariscope. Silica crystals were found in 21 per cent and extensive deposits of crystals in 6 per cent. German[4] in 1943 presented 50 cases of talc granulomata, 40 of which were intra-abdominal. He stated his belief, that if serial sections were routinely taken from the omentum of patients previously operated upon, granulomata would always be found if talc had been used in the operating room preparation. A number of serious cases with fatalities have been reported by Swingle[5] and by Walker.[6]

CASE REPORT

M. G., a 59 year old female was admitted to St. Luke's Hospital April 13, 1951 with a history of nausea and vomiting of one and a half year's duration. In this interval of time her weight decreased from 140 to 82 pounds, a loss of 58 pounds. She had no abdominal discomfort or pain. Gastrointestinal x-rays had been taken one year before and a repeat thorough work-up at a Clinic in May of 1950 revealed no pathology. She gave a history of a pelvic laparotomy eleven years before at which time adhesions were freed from about the gallbladder. In Oct. 1950 she had been hospitalized for continued weight loss. Neurological examination was essentially negative. R.B.C. count was 3.00 cu. mm. with a Hb. of 11.5 gm. The spinal fluid was clear, negative Pandy, cell count normal, protein 22 mg., sugar 64 mg., complement fixation and colloidal gold tests were negative. There was no free acid in the stomach; after histamine there was 14° free acid and 12° combined. She was placed on therapy for anemia and hydrochloric acid.

In Feb. of 1951 she was again hospitalized because of continued vomiting with dysphagia. Thorough x-ray studies of pharynx, esophagus, gastric cardia and small bowel were negative. At this time her blood count was: R.B.C. 4.22 cu. mm., Hb. 11.5 gm., W.B.C. 5,900 cu. mm., differential was normal. Sedimentation rate 22

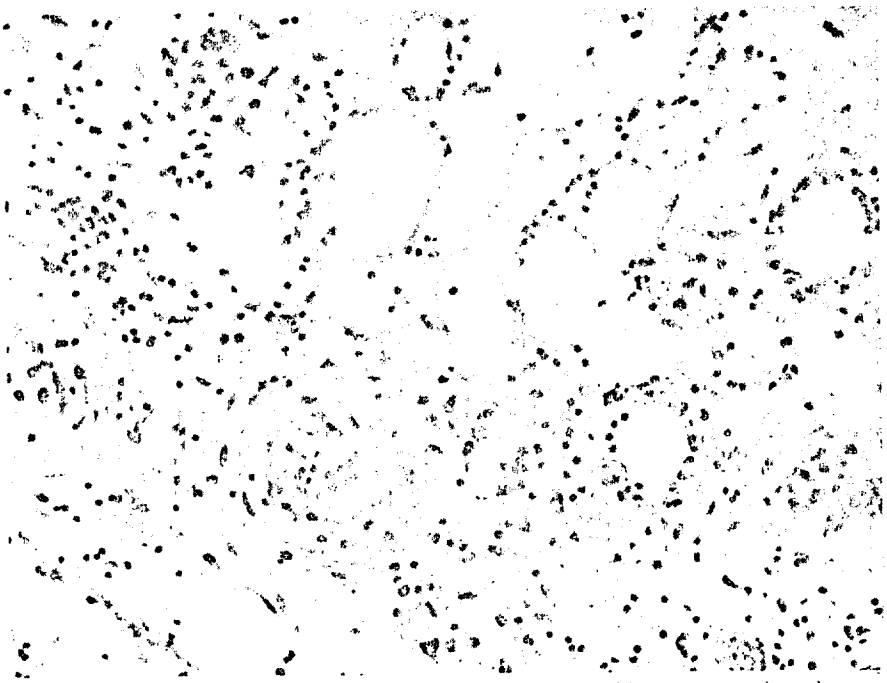

FIG. 1. Photomicrograph. Clusters of foreign body giant cells and large mononuclear phagocytes.

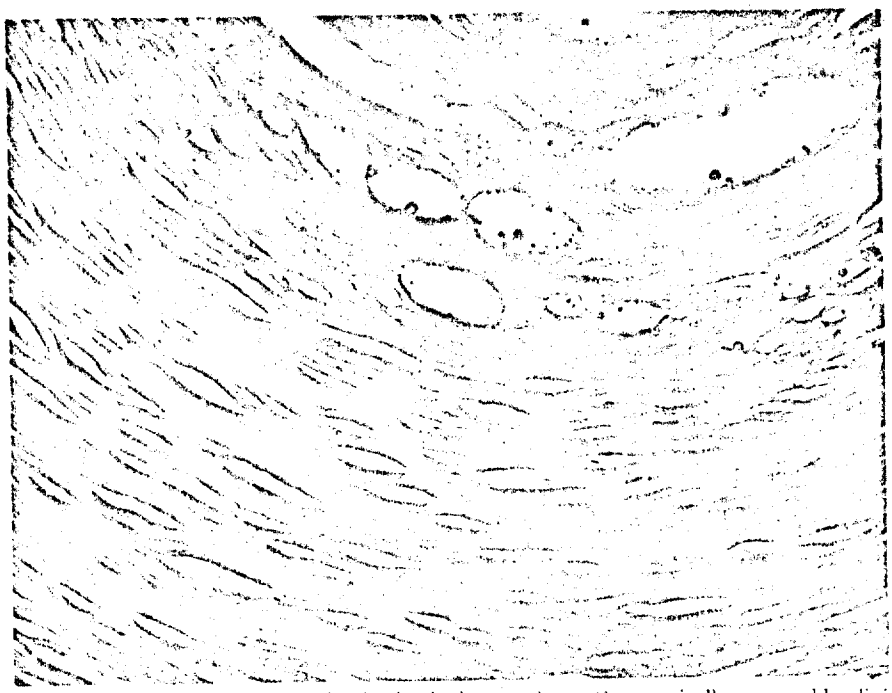

FIG. 2. Photomicrograph. Extensive foreign body granuloma. Concentrically arranged hyalinized fibrous tissue with crevices.

mm. one hour. Blood chemistry: NPN 22 mg., sugar 85 mg., cholesterol 206 mg., sodium 143.5 meq., potassium 5.5 meq., proteins 5.65 mg. per 100 ml. plasma.

Exploratory laparotomy April 17, 1951 revealed numerous small and large grey nodular masses covering the entire peritoneal surfaces and omentum with dense diffuse adhesions throughout the abdomen. There was no ascitic fluid present. The stomach was normal. Nodules were removed for histological examination. Her post-operative course was uncomplicated. She was eating well in 48 hours. There has been no nausea or vomiting since the operation. She has gained 36 pounds in weight.

Histology Report by Dr. Edwin F. Hirsch. "Sections from several levels of the firm portions have a large amount of fibrillar scar tissues that extend into the basic fat. These fibrillar tissues have many large and small vacuoles at whose margins are foreign body giant cells. Scattered in the dense fibrous portions are other small clusters of foreign body giant cells and large mononuclear phagocytes. Some of these giant cells have fragments of refractile material which, examined with the micro-polariscope, showed a needle-like structure with double refractive properties. Another significant structure in one of the histological preparations is a mass of concentrically arranged hyalinized fibrous tissue with small crevices in the center. This is an extensive foreign body granuloma."

DISCUSSION

The pathological picture at the operating table of numerous noncaseous nodules with a dry abdomen suggested tuberculous peritonitis. The histological picture and the identification of numerous silica deposits, however, confirmed the diagnosis of talc granulomata.

On the basis of experimental work, German[4] reported talc in small quantities did not form adhesions unless trauma was a factor. Lichtman[5] believed that infection was an important additional factor. Seelig,[7] was able to produce dense adhesions routinely in mice with intra-peritoneal injections of talc suspensions. Saxin and Tuovinen,[8] Lee and Lehman,[9] Miller, et al.,[10] and Owen[11] concluded the same from their experimental work. The reaction to talc in the peritoneal cavity may be adhesions, granulomata or lupoid-sarcoid tumors of large size as described by German[12] and by Gruenfeld.[13] Lichtman[5] noted extreme variability in the reaction of the host to talc. In some instances it produced a marked inflammatory response, while in others the talc was engulfed by histiocytes with little tissue reaction. It is not known what stimulates a flare-up of the inflammatory reaction after many years of dormancy. Lichtman[5] reported granulomata developed in scars from two months to 36 years after talc was implanted in the skin. The case reported here had a laparotomy 11 years before; Fienberg's[3] patient developed a tumor in the abdominal wall six years after appendectomy. Byron and Welch's[14] case developed five years after hysterectomy, and Lichtman[5] reported a sarcoid of the gall-bladder 11 years after cholecystostomy.

It is postulated that physio-chemical changes in the tissues cause increase in soluble fractions of the talc which stimulates the tissues to reaction. Similar delay of pathological changes in silicosis of the lungs is attributed to slow leaching of the silica.

Ducommun[15] used ACTH in mice in an endeavor to decrease the reaction to talc. If given at the time of the first injection of talc it prevented reaction but had no effect on old cases with granulomatous reactions already present.

SUMMARY

A case of diffuse talc granulomatosis of the abdomen is reported.

REFERENCES

[1] Antopol, W.: Arch. Path. **16:** 326, 1933.
[2] Fienberg, R.: Arch. Path. **24:** 36, 1937.
[3] Lichtman, A. L., McDonald, J. R., Dixon, C. F., and Mann, F. C.: Surg., Gynec. & Obst. **83:** 531, 1946.
[4] German, W.: Surg., Gynec. & Obst. **76:** 501, 1943.
[5] Swingle, A. J.: Ann. Surg. **128:** 144, 1948.
[6] Walker, W.: Brit. M. J. **1:** 1079, 1948.
[7] Seelig, M. G., Verda, D. J., and Kidd, F. H.: J.A.M.A. **123:** 950, 1943.
[8] Saxin, A., Tuovinen, P. I.: Acta chir. Scandinav. **96:** 131, 1947.
[9] Lee, C. M., Jr., and Lehman, E. P.: Surg., Gynec. & Obst. **84:** 689, 1947.
[10] Miller, J. W., Sayers, R. R., and Yant, W. P.: J. A. M. A. **103:** 907, 1934.
[11] Owen, M. A.: Texas State J. Med. **32:** 482, 1936.
[12] German, W.: Am. J. Clin. Path. **10:** 245, 1940.
[13] Gruenfeld, G. E.: Arch. Surg., **59:** 917, 1949.
[14] Byron, F. and Welch, C. S.: Surgery **10:** 766, 1941.
[15] Ducommun, P., and Mach, R. S.: Semaine des Hopitaux **26:** 65, 3173, 1950.

Hygiene & Sanitation 32(5):280-85, 1967

COMMUNICATIONS IN BRIEF

UDC 614.72:661.68]:613.95

SOME DATA CONCERNING THE EFFECT OF LOW ATMOSPHERIC CONCENTRATIONS OF SILICON DIOXIDE ON CHILD HEALTH

(Nekotorye dannye o vliyanii malykh kontsentratsii dvuokisi kremniya v atmosfernom vozdukhe na zdorov'e detskogo naseleniya)

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Silocon alloys have come into extensive use in recent years. Their manufacture is accompanied by the liberation of several substances into the atmosphere. An important place among these is occupied by silicon and its compounds which are liberated into the air in the form of dust due to the condensation of vapor to solid dust particles of silicon dioxide. The effect of this dust has been thoroughly studied by occupational pathologists and hygienists, but the literature does not contain sufficient information on the effect of low concentrations in man and animals. On the other hand, it has been demonstrated by several authors that atmospheric dust containing 10 to 20% of free silica constitutes a certain health hazard (Moskovskaya; Gol'dberg; Malysheva and others).

Investigations carried out in the vicinity of factories manufacturing silicon and its alloys have revealed a fairly high level of atmospheric pollution by the condensation aerosol of silicon dioxide. The pathogenic properties of submicroscopic dust particles of silicon dioxide have as yet been little studied, while relevant experiments indicate the high solubility and considerable toxicity of this dust (Velichkovskii and Ragol'skaya; Velichkovskii, and others).

We attempted the determination of the level of atmospheric pollution by the silica around a factory manufacturing iron alloys and its effect on child health. The factory produces ferrosilicon of 45% and 75%. It is produced in open electric-arc furnaces of 11,000 kW. The furnace gases discharged at the furnace tops consist mainly of the products of sublimation and vaporization of silicon and its compounds. Carbon monoxide is an important gaseous constituent. The furnace gases together with the suction air are discharged into the atmosphere without any treatment through 20 m tall chimney stacks. The sanitary-protective zone contains residential and public buildings (a kindergarten, shops, etc.).

The atmospheric dust content was studied by us at the level of human respiration. Air samples were taken within a radius of 3 km in summer by means of a truck aspirator on filters AFA-18. The samples (136) were tested for total dust concentration, concentration of free silica and the percentage content of the latter in the dust.

The highest dust concentrations (Table 1) were detected on the grounds of the shop; its concentrations (averaged data) were 2 – 3 mg/m³ outside the shop grounds, within a radius of 0.3 – 3 km.

The zonal distribution of free silica obeyed the same law as the overall dust levels in the atmosphere (Table 2).

TABLE 1. Dust concentrations in the atmosphere at different distances from the shop producing ferrosilicon alloys

Distance from the source of atmospheric pollution, km	Number of samples	% of samples found to contain dust	Mean dust concentration found in the samples, mg/m³	% of samples in which the dust concentration exceeded the max. permissible concentration of 0.5 mg/m³
Shop grounds	23	100	4.1	
0.3	25	100	3.1	100
0.5	25	100	2.5	100
1.0	26	100	2.0	100
2.0	24	100	1.75	100
3.0	25	100	3.0	100

TABLE 2. Concentration of free silica and its percentage contents in the dust at different distances from the shop producing ferrosilicon alloys

Distance from the source of atmospheric pollution, km	Number of samples	% of samples found to contain free silica	Mean concentration of free silica in the dust, %	Concentration of free silica in the dust, %	
				mean	maximum
Shop grounds	23	100	0.72	14.0	41.6
0.3	25	100	0.54	16.0	36.0
0.5	25	88	0.30	11.2	31.5
1.0	24	83	0.37	20.0	66.0
2.0	24	87.5	0.19	11.8	32.0
3.0	25	92	0.50	14.7	45.0

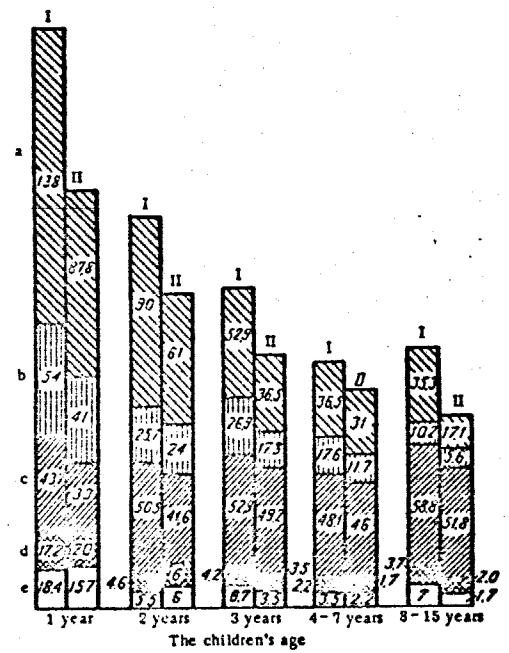
The dust discharged by the furnaces into the atmosphere is of fine dispersion, the dimensions of a majority of particles (70%) being less than 1 micron.

Simultaneously with the determinations of the level of atmospheric polution, studies were made of the effect of industrial discharges on child health. We examined children residing within a radius of 0.2 – 1 km from the shop. The control was provided by a district with relatively pure air,

located at a distance of 6 km from the source of atmospheric discharges of the silicic dust.

The effect of industrial discharges was studied by health statistical analysis of disease incidence in children, based on data of pediatric medical consultations; clinical examination of children and data on their physical development, and a special questionnaire for the population concerned with the effects of the discharges by the shop producing ferrosilicon alloys on health and living conditions.

For comparability of data on the health of children residing in the area with polluted air and in the control district, studies were made of the living and dwelling conditions of the children and the economic means of their families. For this purpose we carried out a special investigation and questioned the adult population. The dwelling and living conditions of the children, the economic status of their families and the medical services proved to be the same for the two areas, and consequently it was possible to compare the health of children residing in the investigated and control areas.



Morbidity per 100 investigated children residing in the area with polluted atmosphere (I) and in the control area (II)

a – ear, nose and throat diseases; b – diseases of the respiratory organs; c – infectious diseases; d – diseases of the gastrointestinal tract; e – other diseases.

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Disease incidence data for the children based on requests for medical pediatric consultations were analyzed in accordance with the children's age for the entire period during which they were attached to the pediatric medical consultation service. We used the histories of children's development which contained chronological records of the children's state of health.

Comparative morbidity data for children residing in the two areas revealed higher overall mobidity in children residing within the radius of influence of the shop manufacturing ferrosilicon alloys (see figure). For instance, the overall children's morbidity was 37 % higher in the investigated area in comparison to the control during their first year of life, 27 % higher during their second year, 30 % during their third year, 16 % during their 4—7th years, and 45 % during their 8—15th years. The ear, throat and nose disorders were most common among the children residing in the investigated area (mainly upper respiratory tract infections). Data of child morbidity based on data of first applications to the pediatric medical consultation service were statistically analyzed, and this analysis confirmed the high statistical significance of disorders of respiratory organs and otorhinolaryngologic disorders in the investigated area (Table 3).

TABLE 3. Morbidity in children in the investigated area (incidence of diseases per 100 investigated children)

Statistical parameter	Overall morbidity	Disorders of respiratory organs	Otorhinolaryngologic disorders	Infectious diseases	Diseases of digestive organs	Other diseases
	Control area					
$M \pm m$	107.5 ± 0.63	15.35 ± 0.80	37.7 ± 1.00	45.9 ± 1.10	4.29 ± 0.45	3.98 ± 0.43
	Area with polluted atmosphere					
$M \pm m$	132.2 ± 2.49 12	21.92 ± 1.40 4.1	54.6 ± 1.69 8.5	51.0 ± 1.70 2.5	4.57 ± 0.70 0.33	6.41 ± 0.83 2.4

The clinical investigation of children included (according to organs and systems) X-ray examination of the chest, anthropometric measurements, and clinical blood tests. The work was carried out with the participation of the physicians working at the Lipetsk children medical consultation center. The investigated children were aged 5 to 15 years and had resided in the investigated areas for at least five years. The number of children investigated in the control area and in the area with polluted atmosphere was 177 and 129, respectively.

Clinical examination of the children also revealed a higher incidence of diseases of the respiratory tract, and especially otorhinolaryngological disorders, in the children residing in the area with the polluted atmosphere. For instance, chronic otitis and tonsillitis were discovered in 18 % of the children as against only 7.8 % of the control children. No notable

differences between children of the two areas were revealed by X-ray examinations of the chest and by the clinical blood tests.

Studies of the children's physical development included measurements of height, weight, and chest circumference. The physical data for each investigated child were compared with the standard for the corresponding age-sex group and evaluated on a 3-"point" system, as medium, below medium and above medium. Among the children residing in the vicinity of the shop manufacturing the ferrosilicon alloys, 71.8 % of boys and 70 % of girls had a medium and above-medium physical development as against 91 and 89.6 %, respectively, in the control area. In the area with polluted atmosphere the weight and chest circumference lagged behind the height in 17.6 % of all boys and 20 % of all girls as against 14.7 and 9 %, respectively, in the control area. In 24 % of investigated boys and 29.8 % of investigated girls some element of physical development was below medium in the area with polluted atmosphere as against 9 and 10.3 %, respectively, in the control area.

Discharges from enterprises manufacturing ferrosilicon alloys also have an unfavorable effect on the sanitary and living conditions of the local population. The inhabitants complained of the wilting of potted plants, the impossibility of airing their rooms or drying their laundry in the open air, etc.

Conclusions

1. In the absence of special installations for the treatment of gases discharged by enterprises manufacturing ferrosilicon alloys, these gases caused considerable atmospheric pollution.
2. Concentrations of finely dispersed dust detected within the radium of 3 km from the shop territory were 1.75 to 3.1 mg/m^3, considerably in excess of the maximum permissible concentration of "nontoxic dust." Some of the determinations revealed the presence of 30—66 % of free silica which is the most toxic component of discharges from enterprises manufacturing ferrosilicon alloys.
3. Atmospheric pollution by the discharges has an unfavorable effect on the health of children, causing an increased overall morbidity and a higher incidence of respiratory infections and otorhinological disorders; moreover, the children's physical development lagged as compared to controls.
4. Industrial discharges have an unfavorable effect on the sanitary and living conditions of the population.
5. Our findings proved the necessity for installations for the treatment of atmospheric discharges from furnaces producing ferrosilicon alloys and for a sanitary-protective zone separating the plants and the residential area.

BIBLIOGRAPHY

Gol'dberg, M.S. — Gig. i San., No. 4, p. 9. 1957.
Malysheva, V.V. — In: Sbornik trudov Kurskogo meditsinskogo instituta, No. 15, p. 102. 1961.

Moskovskaya, K.A. — In: Novye dannye po gigiene detei i podrostkov, p.36, Moskva — Leningrad. 1956.
Velichkovskii, B.T. and F.S. Ragol'skaya. — In: Voprosy gigieny, profpatologii i promyshlennoi toksikologii, p.97, Sverdlovsk. 1959.
Velichkovskii, B.T. — Gig. i San., No.8, p.76. 1961.

Received 11 February 1966

From. FAO/WHO monograph, Toxicological Evaluation of Some Food Colors, Emulsifiers, Stabilizers and Anti-Caking Agents and Certain Other Substances.

ACCEPTABLE DAILY INTAKES (ADI) FOR MAN OF SOME ANTI-CAKING AGENTS

Substance[a]	ADI (mg/kg body weight)
Ferrocyanide, calcium Ferrocyanide, potassium Ferrocyanide, sodium	0 - 0.00125[1]
Phosphate, calcium, tribasic Phosphate, magnesium, tribasic	0 - 30[3]
Salts of Myristic, palmitic and stearic acid with bases accepted for food use	No limit[2]
Silicon Dioxide, Amorphous (includes silica aerogel; hydrated silica; silicic acid; dehydrated silica gel) Aluminium silicate (includes Kaolin) Calcium silicate Magnesium silicate (includes Talc, Magnesium trisilicate) Sodium Alumino silicate	No limit[2]

[a] Specifications will be published for these compounds (see Annex 1, Ref. 19).

[1] Temporary acceptable daily intakes - details on further information required are given in the respective monograph.

[2] Not limited, except by good manufacturing practice.

[3] As total dietary phosphorus intakes from both food and food additives (see Annex 1, Ref. 7).

Note: Other anti-caking agents were considered by the Expert Committee, but no monographs were prepared (see Annex 1, Ref. 18, section 3.3).

SILICON DIOXIDE, AMORPHOUS; ALUMINIUM, CALCIUM, MAGNESIUM, AND SODIUM ALUMINO SILICATES

(includes: silica aerogel, hydrated silica, silicic acid, dehydrated silica gel, Kaolin, Talc and magnesium trisilicate)

Silica, silicic acid and the calcium, magnesium and aluminium salts occur ubiquitously in the environment and some have been used for many years medically. Food contains various amounts of SiO_2, for example: potatoes 10.1, milk 2.1, drinking water 7.1, mineralwater 22.5, beer 131 γSiO_2 per g or cm^3 (Baumann 1960).

Biological Data

Biochemical aspects

Very small amounts of silica are normally present in all body tissues but there is no evidence that they play any physiological role.

Silicic acid is a normal constituent of the urine, where it is found as early as a few days after birth. The amount excreted in the urine, which varies considerably according to the diet, is in the order of 10-20 mg per day (Thomas 1965). The silica content of human tissue varies from 10-200 mg/100 g dry weight (spleen 15 mg, lung 140 mg) (Unilever Research Laboratory 1964). The normal level of silicic acid in human blood is below 1 $\mu g\ SiO_2/cm^3$; the concentration in the corpuscles is practically the same as that in the plasma. Silicic acid is present in plasma in a molybdate reactive form and is not bound to protein or any other substance of high molecular weight. Ingested monomeric silicic acid rapidly penetrates the intestinal wall and becomes distributed throughout the whole extracellular fluid. It enters the blood corpuscles at a slower rate (Baumann 1960).

Silica dust was administered intragestrically to rabbits and dogs leading to a rise in urinary silica output without significant variation in blood silica levels. Considerable absorption took place with peak excretion in dogs occurring between four to eight hours after administration. There appears to be little retention in any organ of the body even if animals ingest large amounts of silicates in their food. Intragastric five per cent. silicic acid administered to dogs leads to considerable absorption and urinary excretion, peak excretion occurring between three to eight hours after dosing. I.V. infusion of neutralized sodium silicate (1 mg/ml) in dogs leads to rapid urinary elimination of about 50 per cent. of the dose (King et al., 1933).

In experiments with two volunteers, it was shown that, after ingestion of 50 mg of monomeric silicic acid in 50 cm^3 liquid, the renal excretion of SiO_2 per time unit was not related to the quantity of urine excreted in the same time unit. Maximum excretion appeared after one to two hours. Even at high concentrations up to more than 700 $\mu g\ SiO_2/cm^3$ urine, the silicic acid was still present in a molybdate reactive form. Silicic acid polymerizes above 100-150 $\gamma\ SiO_2/cm^3$. The speed of polymerization is dependent on pH and concentration. The experiment was designed so as to exclude damage to the urinary tract through precipitation of proteins by polymeric silicic acid formed by polymerization of monomeric silicic acid at high concentrations. If the urine at concentrations in the order of 700 $\gamma SiO_2/cm^3$ was taken at longer intervals, such as two hours, the concentration of monomeric silicic acid was below the total SiO_2 concentration, thus suggesting that some polymerization had taken place. There was no indication of storage breakdown of reabsorption (Baumann 1960).

Administration of 5 g of the siliceous materials listed below in 20 ml of milk by stomach tube to cats showed the following urinary excretion within 120 hours; silicic acid (fresh) 43.3, calcium silicate 37.2, magnesium trisilicate 34.1, silicic acid (moist) 29.0, SiO_2 (quarz) (air sediment very fine) 20.8, magnesium silicates (talc) 9.2, diatomaceous earth 8.8 and calcium silico aluminate hydrate (kaolin) 7.6 mg SiO_2. From this it can be observed that free silica is attacked to a variable extent depending on its physical and chemical condition. Several silicates are apparently unattacked, as non-treated animals excreted an average of 8.6 mg in 120 hours. Some complex silicates appear to suffer partial decomposition by the hydrochloric acid of the stomach, with partial solution of some of the products in the intestine (King et al. 1938).

Oral administration of a single dose of 2.5 g of amorphous polymeric silicon dioxide (99.8 per cent. SiO_2 content of the water free compound) to 12 volunteers caused a slight but statistically insignificant increase in the silicon dioxide level of the urine (Langendorf et al., 1966).

"Quarz water" (water in which silicic acid had been dissoved as a result of contact with quarz powder) given to rats over a prolonged period as the only source of liquid intake did not show any SiO_2 storage in tissues (Klösterkotter 1956).

In vitro investigations on slices of tissue showed that monomeric silicic acid penetrated liver and kidney cells easily, whereas spleen and muscle cells were more or less impenetrable (Kirsch 1980).

Ten γ monomeric SiO_2/cm^3 was shown in vitro to damage the enzymes of isolated mitochondria in rat liver (Hanger et al., 1963).

Acute toxicity

Animal	Route	LD_{50} (mg/kg body-weight)	Reference
Rat	Oral	3.16	Elsea, 1958a

The oral LD_5 in mice of finely ground silicic acid is >5 g/kg body weight (Kimmerle 1968).

Rabbits receiving 3 mg of silicon in their conjunctival sac showed mild irritation for 48 hours (Elsea 1958a).

A single dose of 50 mg of monomeric silicic acid in 50 cm^3 liquid was tolerated by two volunteers. Higher doses should be taken either with more liquid or at intervals of about 20 mins. in order to avoid polymerization of silicic acid in the urine (Baumann 1960). A single dose of 2.5 g of amorphous polymeric silicon dioxide to volunteers did not significantly raise the SiO_2 excretion in the urine thus suggesting poor absorption of the compound (Langendorf 1966).

The probable lethal dose of silica (oral) for man is over 15 g/kg body weight. The probable oral lethal dose of magnesium trisilicate for man is over 15 g/kg body weight. There is some doubt as to whether large doses cause laxation. The probable oral dose for man of sodium silicate lies lower at

between 0.5 and 5 g/kg and may well be due to its alkalinity (Unilever Research Laboratories 1964). Data on inhalation toxicity of silica and silicates are not relevant to consideration of toxic hazard by the oral route.

Short-term studies

Rat. Oral administration of 50 mg amorphous silica to rats for three months did not cause any toxic effects but no experimental details are available (Walten et al., 1964). Micronized silica gel was fed to four groups of ten male rats each in their diet at 0 per cent., 0.2 per cent., 1.0 per cent. and 2.5 per cent. levels for 28 days. No adverse effects on mortality or abnormal gross autopsy findings were discovered. Body weight gain was significantly reduced at the 2.5 per cent. level and nearly significantly at the 1.0 per cent. level. No other parameters were examined (W. R. Grace & Co., 1958).

Fifteen male and 15 female rats received daily 50 mg of amorphous polymeric silicone dioxide (99.8 SiO_2 content of water free compound) by stomach tube for three months. There was no adverse effect on body weight gain and mortality. Pathology of organs not enumerated showed no abnormalities in comparison with the controls (Kuschinsky 1955).

Groups of 15 male and 15 female rats were fed diets containing silica at concentrations of 0.0, 1.0, 3.0 and 5.0 per cent. for 90 days. A fifth positive control group received a diet containing 3.0 per cent. cosmetic talc. No evidence of systemic toxicity caused by silica was found in terms of survival, body weights and food consumption. No appreciable deposition of silicon dioxide was seen in the kidney, livers, spleen, blood and urine in the animals fed at the five per cent. level. No gross or microscopic pathology attributable to silicon were seen (Elsea 1958b).

The same compound was fed to 20 male and 20 female rats at a concentration of 500 mg/kg body weight per day for six months. The same number of animals served as the control. After four and a half months five females were mated. No adverse effects were noted on mortality, body weight gain, haematology (haemoglobin, erthrocyte- and leucocyte-count) and reproductive performance. Histopathology of stomach, intestines, pancreas, liver and kidney of the test group showed no significant difference to that of the control group. Litter size, birth weight and morphological development of the offspring as well as weight gain were normal (Leuschner 1963).

In a similar experiment (see Leuschner 1963) the same results were obtained when feeding a hydrophobic preparation of amorphous polymeric SiO_2 in which some of the silanol groups on the surface reacted with dimethyl-dichlorosilane (98.5 per cent. SiO_2 content of water free compound) (Leuschner 1965).

The compound (see Leuschner 1965) was also fed to groups of five male and five female rats at levels of 0, 500, 1000, 2000 mg/kg body weight for five weeks. The highest level was raised after 14 days to 4000, after 28 days to 8000 and after 42 days to 16 000 mg/kg body weight. When the level was raised to 16 g/kg, all animals lost weight and four animals died. Seven days after the level had been raised to 8000 mg/kg, the animals did not gain weight normally. At 1000 mg/kg two rats out of 10 showed slight changes in the liver epithelia. At higher levels atrophy of the liver epithelia, regression of the basophilic structure and glycogen content were observed. Histopathology of other organs (not enumerated) including the kidney of the animals at all levels showed no significant changes compared with the controls (Leuschner 1964).

Rabbit. The dermal effects of silica was tested on groups consisting of two male and two female rabbits at levels of five and 10 g/kg/day. A negative control group received methyl cellulose solution (0.5 per cent. w/w) and a positive control group received 10 g/kg/day of cosmetic talc. Applications were made five days/week for three weeks. No evidence of systemic toxicity caused by silica was found in terms of body weight, behaviour, silicon content of blood, urine, spleen, liver and kidney. No gross or microscopic pathology was seen in the major organs examined or in the skin (Elsea 1958c).

Man. 60-100 g daily for three to four weeks of 12 per cent. amorphous silicic acid administered orally to patients suffering from gastritis or enteritis were tolerated without adverse effects. Only one thousandth of the substance administered was excreted in the urine (Sarre 1953).

Long-term studies

None available.

Comments

The available data on orally administered silica and silicates, including flumed silicate dioxide, appear to substantiate the biological inertness of these compounds. Any silicate absorbed is excreted by the kidneys without evidence of toxic emulation in the body. Methods for estimating silica in body tissues have been greatly improved in recent years making some of the earlier data somewhat less valuable. A number of short-term studies in two species are available. This information taken together with human clinical experience and the ubiquitous occurrence of these compounds in the environment does not point to any significant toxic effects when these substances are used as food additives.

EVALUATION

Not limited except for good manufacturing practice.

REFERENCES

Baumann, H. (1960) Hoppe-Seylers Z. physiol. Chemie., 320, 11

Elsea, J. R. (1958a) Unpublished report, January 8, from Hazleton Laboratories, Inc.

Elsea, J. R. (1958b) Unpublished report, July 11, from Hazleton Laboratories, Inc.

Elsea, J. R. (1958c) Unpublished report, May 6, from Hazleton Laboratories, Inc.

Hanger, R., Kirsch, K. & Standinger, Hj. (1963) Beitr. Silikose-Forsch. S-Bd Grundfragen Silikoseforsch. Bd. 5, 69

Keller, J. G. (1958) Unpublished report to W. R. Grace & Co.

Kimmerle (1968) Unpublished report submitted by Bayer

King, E. J., Stantial, H. & Dolan, M. (1933) Biochem. J., 27, 1002

King, E. J. & McGeorge, M. (1938) Biochem. J., 32, 426

Kirsch, K. (1960) Beitr. Silikose-Forsch. S-Bd. Grundfragen der Silikoseforsch., 4, 33

Klosterkötter (1956) Diskussionsbemerkung, Beitr. Silikose-Forsch. S-Bd. Grundfragen Silikoseforsch. Bd., 2, 348

Kuschinsky, G. (1955) Unpublished summary report submitted by Degussa

Langendorf, H. von & Lang, K. (1966) Zeitschrift für Ernährungswissenschaft, 8, 27

Leuschner, F. (1963) Unpublished report submitted by Degussa

Leuschner, F. (1964) Unpublished report submitted by Degussa

Leuschner, F. (1965) Unpublished report submitted by Degussa

Malten, K. E. & Zielhuis, R. L. (1964) Industrial Toxicology and Dermatology in the Production and Processing of Plastics, Elsevier, 204

Sarre, H. (1953) Unpublished summary report submitted by Degussa

Thomas, K. (1965) Dtsch. Zeitschr. f. Verdauungs- u. Stoffwechsel-Krankheiten 25, 260

Unilever Research Laboratory (1964) Report No. CH 64888, dated 21 October 1964

Am. J. Pathol. 9(Suppl.): 751-763, 1933

THE REACTION TO FINE AND MEDIUM SIZED QUARTZ AND ALUMINUM OXIDE PARTICLES. SILICOTIC CIRRHOSIS OF THE LIVER.*

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The experiments to be described were performed as part of a general program for study of the reaction of the body to various inorganic dusts. Previous investigation had demonstrated that it is possible to reproduce the typical nodular lesions of silicosis in guinea pigs and rabbits by exposing them for 6 hours daily over a period of several years to the inhalation of fine silica dust.[1] These observations failed to indicate the nature of the injury produced by silica and they did not define the quantity and size of the particles necessary to provoke reaction. The present paper deals with preliminary attempts to evaluate the importance of the factor of size. It also throws further light upon the nature of the injury.

It is well established that the reaction to silica differs from that to any other known type of dust and it has been hypothecated that the biological activity of this substance is due to its solution in the slightly alkaline fluids of the body. This hypothesis is based upon clinical observations of Collis[2] and upon certain experiments of Gye and Purdy.[3] The latter investigators showed that a colloidal suspension of silica is toxic. Intravenous injection of large doses produces almost immediate death; repeated smaller injections injure liver cells and later stimulate proliferation of connective tissue. Subsequent writers have erroneously assumed that the silica sol used by Gye and Purdy was a chemical solution, whereas it was actually a colloidal suspension of exceedingly fine particles. More recently Gardner[4] has suggested that silica is active when freshly fractured because of its molecular structure, which presents numerous unsatisfied oxygen atoms to interact with tissue elements. This hypothesis would demand that the reaction produced by a given quantity of silica be proportional to the size of the particles, since a larger num-

* Received for publication April 13, 1933.

ber of unsatisfied oxygen atoms would be exposed with successive increases in subdivision. Regardless of the mechanism involved all available evidence points to a chemical rather than a physical type of injury by silica.

As originally planned, these experiments were designed to demonstrate the relation of particle size to the amount of reaction that would be produced by a measured quantity of silica. If such reaction were to take place at the surface of the particles it should be proportional to the amount of surface exposed to the body fluids. Since a known quantity of material must be introduced into the body for such a study intravenous injection was substituted for inhalation.

Two series of 8 rabbits each were injected with 1.3 gm. of silica particles. For the first series the particles were approximately the size of leukocytes, being 6 to 12 microns in maximum diameter; for the second, they were about one-tenth as large (1 to 3 microns). To control the action of the silica a third series of 8 rabbits was injected with 1.2 gm. of aluminum oxide particles of the same size as those of the smaller silica fraction (1 to 3 microns). This substance was selected because its chemical properties simulate those of silica more closely than those of any other known substance. The alumina employed was an electric furnace product known as "aloxite." The size of all particles was carefully graded by a process of fractional sedimentation which has been described elsewhere.[5] Two per cent suspensions by weight were made in physiological salt solution and sterilized in the autoclave. It was originally intended to inject each animal with 10 cc. each week of the experiment, but it soon became apparent that this program could not be followed as the fine quartz particles provoked so much reaction in the ears that long periods of rest between injections were necessitated. As a consequence from 1 to 4 months elapsed before the full 1.3 gm., often in doses as small as 3 cc., could be administered. Seven of the 24 animals died of embolism during the course of treatment and 2 more in the group receiving the finer quartz particles died subsequently of silicosis. One animal died during the first injection and showed coccidiosis, but the others were entirely free from any trace of this infection. The remaining members of the three groups were killed from 2 to nearly 3 years after completing the injections.

Experimental Results

Fine Silica Particles (1 to 3 Microns)

This material is irritating and it tends to produce thrombosis in the ear veins, followed by accumulations of the dust in the surrounding tissues.

Ear: In this location the dust provokes a localized necrosis with an acute exudation of polymorphonuclear leukocytes and a small amount of fibrin. The masses of dust have persisted with little decrease in their size throughout the entire period of observation (1067 days). They become surrounded by a zone of mononuclear leukocytes and this in turn is gradually replaced by connective tissue which is at first cellular and later hyaline in character.

Lungs: The particles of this dust fraction are so fine that only a few of the larger ones are retained in the capillaries of the lungs. They are gradually phagocytosed and transported into the perivascular lymph spaces and thence into the pulmonary lymphoid tissues. A small number ultimately reach the tracheobronchial lymph nodes. The amount of silica in any one spot is so small that very little fibrosis results. Of 2 animals killed at the end of the experiment, 1 shows only phagocytes containing dust particles in the intra- and extrapulmonary lymphoid tissues, and the other shows a limited amount of loose, non-cellular, fibrous tissue in the walls of the air spaces adjacent to the lymphoid tissues.

Spleen: The spleen seems to have filtered out and retained most of the silica particles of intermediate size in this fraction. They are found in large, swollen phagocytes with from one to four nuclei. The number of such particles seen in a section of any one cell cut at random rarely exceeds eight to ten. There are also smaller particles, only visible with polarized light, which are collected in great numbers by exceedingly large giant cells. A section of such a cell may exhibit as many as thirty or more nuclei massed in its center or arranged in ring formation about its periphery. They are indistinguishable from the Langhans giant cell of tuberculosis.

Three months after completing the injections compact masses of these cells are found in dilated portions of the sinuses. In another year this these lesions have increased to nearly 1 mm. in diameter and they often extend through the walls of the sinuses into the septa

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and follicles. Blood vessels in the area involved are not destroyed but appear normal at this time. After a year or more the nodules of phagocytes have been replaced by considerably larger nodules of cellular connective tissue exhibiting numerous mitotic figures. They have clearly defined borders and are covered with a thin layer of flat cells, probably sinus endothelium. At their centers the tissue becomes progressively fibrous in character and in the last animal killed a small amount of specific silicotic hyalin has begun to appear. This change is attended by no apparent alteration in the blood vessels.

Liver: The sinusoids of the liver have arrested and retained the major part of the finest particles in this fraction and as these were in great excess, the amount of reaction in this organ is much more pronounced than in any other. No animal was killed sufficiently early to observe the immediate cellular response in this location, but the experiences with larger particles, to be discussed below, would indicate that they are probably phagocytosed by Kupffer cells, which become detached and migrate with their ingested material toward the portal areas. In the first animal killed at 84 days there are numerous multinucleated, dust-containing cells, which are probably still within the sinusoids of the peripheral portions of the lobules. The portal areas are already thickened by proliferating cellular connective tissue and the number of small bile ducts is much more evident than in the normal animal. The lymph vessels in this location are distended with small clumps of dust-containing phagocytes.

The hepatic lymph node shows more marked reaction than the liver at this time. Its medullary sinuses are distended with extremely large giant cells packed with fine dust particles, visible only with polarized light. The afferent lymph vessels are so dilated that their diameter exceeds that of a low power microscopic field. They contain only coagulated lymph.

The next animal, killed at 9 months, shows more dust phagocytes in the sinusoids. Usually they occur in small clusters and are surrounded by a few lymphocytes. The portal connective tissues are now greatly increased in thickness at the expense of the parenchyma, but there is no demonstrable fibrosis within the lobules. At the junction of connective tissue and parenchyma cells there are numerous mononuclear phagocytes but no polymorphonuclear leukocytes. The fibrous tissue is cellular but edematous. The lymphatics are widely dilated and in some places they contain clusters of

dust-filled giant cells. In addition there are compact clusters of mononuclear phagocytes, frequently in mitosis, which are surrounded by concentric strands of compressed cellular fibrous tissue. These foci contain exceedingly large numbers of minute dust particles. In some instances these lesions have already been replaced by compact nodules of loose cellular connective tissue.

The next 2 animals were not killed until the 656th day, by which time the reaction had progressed to extreme limits. The portal connective tissues have increased tremendously and large groups of lobules have been completely destroyed and replaced. Some portions of the parenchyma appear never to have been involved, while others present evidence of regeneration after injury. The connective tissue is generally non-cellular in character and exhibits clusters of nodular whorls of hyaline fibers staining intensely with eosin. Compact masses of minute, doubly refractile dust particles can be found in the centers of such areas. Elsewhere the connective tissue consists of either heavy hyaline bands or loose edematous fibers. Scattered through it are clusters of small bile ducts and fragments of parenchyma varying in size from a few cells to the central half of a lobule. The liver cells are infiltrated with fat. No polymorphonuclear leukocytes are seen in or about them. Traces of lymphatics and sinusoids have disappeared, but in isolated spaces there are multinucleated, deeply eosinophilic cells, which are probably phagocytes as some of them contain dust particles. As evidence of regeneration there are areas of parenchyma in which no suggestion of lobular arrangement or sinusoids can be discovered. The cells in such areas are pale, non-granular and free from visible fat. No dust particles can be found among them. Uninvolved portions of the parenchyma are apparently normal, except for a well marked fatty infiltration rather sharply limited to the central portion of the lobule.

The hepatic lymph nodes in these animals are completely replaced by compact whorls of dense hyaline connective tissue.

The 4 remaining rabbits examined between the 677th and the [illegible]7th day show only minor variations from the picture just described. Apparently the fibrosis continues to progress with increasing amounts of hyalinization, but some of the less dense connective tissue appears to have been replaced again by regenerating liver [illegible]. In its older portions the fibrous tissue is absolutely devoid of [illegible] but where it approaches large blood vessels, in the capsule for

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example, it becomes markedly cellular with numerous mitotic figures. While all trace of sinusoids has disappeared there are occasional areas, apparently unconnected with large blood vessels, which are filled with living blood cells. The large bile ducts are preserved intact but evidence of bile stasis is furnished by scattered phagocytes filled with yellow-green pigment.

The gross picture is that of a coarse nodular cirrhosis with marked distortion of the organ. Areas 2 to 3 cm. in diameter may be composed wholly of white, leather-like scar tissue. Sometimes spherical nodules, 8 to 10 mm. in diameter, are attached to the surface of the organ by strings of fibrous tissue. In the less involved portions bright red areas of granular liver tissue are furrowed by irregular depressed bands of fibrosis. In the animals killed later the regenerated portions of the organ project as smooth, dark red masses elevated above the older, gray, nodular, cirrhotic areas.

Larger Silica Particles (6 to 12 Microns)

The wider variation in the size of the particles used in this group is responsible for involvement of different organs. The larger particles are caught in the pulmonary capillaries; some of the smaller ones are carried to the spleen and liver. Not enough are retained within the latter organ to provoke significant reaction, but they are removed by the lymphatics to the hepatic lymph node near the head of the pancreas.

Lungs: In the lungs large fragments of quartz are phagocytosed by mononuclear and giant cells, which collect within the thickness of the alveolar septa. By 180 days after the last injection they have formed nodules that vary in size from only a few cells to that of a distended alveolus. A few particles are transported to adjacent lymphoid tissues. At this time silver impregnation shows fine reticulum fibrils extending from the surrounding connective tissue between the mass of cells. In the course of years these nodules may increase in size and perhaps in number, but in the majority of cases the progression of such lesions is insignificant. In 1 of 3 animals killed after 3 years the nodules are somewhat larger and considerable numbers of dust-containing phagocytes are seen within the intrapulmonary and tracheobronchial lymphoid tissues. However, [illegible] of the lung nodules has developed any fibrous characteristics [illegible]

phagocytic cells are still round and although the reticulum fibers are more abundant and somewhat thicker no collagen has developed.

Spleen: In the sinuses of the spleen the polarizing microscope shows considerable numbers of smaller quartz particles (6 to 8 microns). They are included within the cytoplasm of extremely large giant cells occurring in compact clusters within the sinuses. These nodules appear to undergo no further change during the maximum period of observation (1130 days).

Liver: In marked contrast to the extreme reaction provoked by the finer sized quartz dust of the first fraction there is practically none in the liver of any animal of this group injected with 6 to 12 micron particles. In gross the organ appeared perfectly normal. In microscopic sections there is an occasional giant cell containing a particle caught in a sinusoid, and very rarely a small cluster of such cells is found in the portal connective tissues. As time has progressed the only additional feature found is the appearance of a small number of lymphocytes about the dust-containing cells.

The hepatic lymph node shows much more extensive reaction. Most of the normal elements are replaced by wide sheets of large phagocytes. Moderately coarse dust particles are visible by polarized light. In one or two areas there are nodular collections of phagocytes surrounded by definite bands of collagen. In such foci there are a greater number of smaller dust particles (6 microns +).

Kidney: In neither group injected with silica has there been evidence of the dust or reaction to it in the kidney. If the foreign body reached this organ it was apparently not retained. In the case of the smaller fraction macroscopic nodules developed in the marrow of the ribs, which progressed to the stage of cellular connective tissue without hyaline formation.

Fine Aluminum Oxide

Eight rabbits were given 1.2 gm. of aluminum oxide particles, 1 to 3 microns in diameter, in from five to seven intravenous injections, during a period of $4\frac{3}{4}$ months. When killed, at intervals during the following 22 months, none of them showed gross evidence of actual disease. The only changes in any way suggestive of lesions were patches of pigmentation where the black dust particles escaped into the areolar tissues of the ears, at the site of injection, and a slightly increased prominence of the lobulation of the liver.

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As in the case of quartz the particles are distributed throughout the various organs according to their size, but aluminum oxide has a very different effect upon the phagocytes and upon connective tissue. A limited number of phagocytes is adequate to care for the alumina injected because each cell ingests an excessive quantity of particles. So many of them accumulate within the cytoplasm that the internal structure of the phagocyte is often completely obscured. Under favorable conditions several nuclei can be made out within a cell hypertrophied to several times its normal size. Migration is apparently impeded as the giant cells remain scattered throughout the tissue and do not collect in masses as in the case of quartz. Furthermore, ingested alumina particles do not seem to injure the cells for there is no accumulation of polymorphonuclear leukocytes or proliferation of mononuclear phagocytes. Evidence of stimulation of fibroblasts has been entirely lacking throughout the entire period of observation. In fact the only further change observed in nearly 2 years after completing the injections has been a slight accumulation of lymphocytes about the alumina-containing cells.

In the *lungs* there are capillary emboli of dust particles surrounded by a few phagocytes. In the *spleen* isolated mononuclear or giant cells packed with black particles are scattered widely throughout the sinuses, follicles and interstitial tissues. In the *liver* there are giant cells in the sinusoids at the periphery of the lobules and in the portal connective tissues. Twenty-two months after completing the injections the only change that has resulted is a moderate degree of lymphocytic infiltration about these depots.*

Discussion

This experiment has provided new data on the biological activity of quartz particles of various sizes. It has also demonstrated the inability of aluminum oxide, a commonly used abrasive material, to provoke a corresponding reaction. To the experimental pathologist these observations are of interest for two reasons: (1) they demonstrate that insoluble foreign particles injected into the blood stream are filtered out by various organs according to their size, and (2) they

* Similar experiments have been made with *diamond dust*. The quantity injected was less than in the case of silica and alumina but 1 of g animals was allowed to survive for 3 years. The distribution and reaction to these extremely hard particles were very respectable to that produced by alumina.

illustrate the capacity of extrapulmonary tissue to react to silica in a characteristic manner. The silicotic cirrhosis produced in the liver is a curious lesion. To the industrial hygienist the contrast between the rapidly progressive reaction produced by very fine quartz particles and the sluggish reaction to coarser quartz and aluminum oxide is of practical significance.

The capacity of the tissues to filter out and localize particles of different sizes in various organs has been the factor determining the distribution of the disease produced by the injected silica. The largest dust particles were apparently retained mechanically in the capillaries of the lungs. Somewhat smaller ones were held back in the spleen while the finest ones were lodged in the liver. The lymphatics of this organ removed as many as possible to its lymph node, situated near the head of the pancreas adjacent to the common bile duct and portal vein. The large number of fine particles provoked such a severe reaction in the node that its function was disturbed; the flow of lymph in the afferent vessels was impeded and the foreign bodies could no longer be eliminated. As a result they accumulated in and about lymph vessels within the liver. This observation constitutes another instance of the importance of the lymphatic system in the development of silicosis. It is of interest to note that whereas in the pulmonary apparatus an obstructive lesion in the tracheobronchial lymph nodes is followed by accumulation of dust in the intrapulmonary lymphoid tissue, in the hepatic system, where there is normally no lymphoid tissue within the viscus, the dust particles accumulate in and about lymphatic vessels. The absence of dust particles and resultant reaction in the kidney is indicative of the lack of a specific mechanism for segregating particulate matter in this organ. These observations also substantiate the conclusions drawn from the inhalation experiments that the reaction to silica is a purely local one about points where particles have been concentrated and that no dissolved silica circulates in the blood stream to damage remote organs. These findings may be of clinical significance because of the disputed contention that the incidence of nephritis is excessive among the victims of silicosis.

A comparison of the reaction to fine quartz with that to aluminum oxide particles of the same size demonstrates the marked difference in the behavior of the phagocytes. The same cell will continue to ingest alumina particles until it becomes so overloaded that its struc-

ture is completely invisible and its power of locomotion is apparently lost. In the case of silica comparatively few particles are usually ingested by any one cell; wherever the number of very fine particles is excessive the cells hypertrophy and form huge giant cells. Either because there is no overloading and no mechanical interference with ameboid activity, or because the irritating particles stimulate migration, the silica-containing cells tend to collect in aggregates. Cells containing alumina, on the other hand, remain more scattered throughout the tissue. This difference in the distribution of the phagocytes ingesting the two types of dust has been maintained throughout the 2 to 3 year observation period of this experiment, and it has been repeatedly observed in other investigations where comparative reactions to quartz and other materials were being studied. It appears to be one of the fundamental factors in determining the outcome of the various forms of pneumonoconiosis.

The importance of the factor of particle size in producing reaction to silica is the chief contribution of this experiment. It has been shown that large particles, 10 to 12 microns in diameter, provoke a foreign body reaction in the lungs which persists without much further change for nearly 3 years. Smaller ones, from 3 to 6 microns in diameter, cause slowly progressive changes in the spleen and hepatic lymph node. Very fine ones of the order of 1 micron in size result in progressive proliferation of connective tissue in the liver.

It might be objected that the number of large particles localizing in any one area was not sufficiently great to provoke reaction, but this objection is met by the findings in the spleens illustrated in Figures 11 and 12. It will be noted that the relatively large number of particles, 6 to 8 microns in diameter, have provoked only a reaction of phagocytes 3 years after their injection, whereas the much smaller number of fine particles, 2 to 3 microns in diameter, have caused an extensive proliferation of connective tissue. It therefore seems justifiable to conclude that fibroblastic proliferation occurs because of the surface area and not because of the mass of silica in a given area.

Space does not permit a discussion of the histogenesis of the silicotic nodule but this subject has already been covered in the previously mentioned paper on silica inhalation.[1] It may be stated here that the original nodule of phagocytes, formed by migrating cells, increases in size as a result of mitotic division. The silica cells

trated in the center of the mass kills many of the cells and this excites a moderate amount of leukocytic infiltration. At the same time fibroblasts are growing into and replacing the original mass, so that it now comes to resemble an actively growing fibrosarcoma. After a year or more this connective tissue becomes fibrous in character and finally a characteristic hyaline swelling takes place at the center, involving one fiber after another. Since such lesions often contain patent blood vessels it is believed that hyaline formation is due to the toxic action of silica rather than to lack of nutrition.

Cirrhosis of the liver developed in the group of animals injected with very fine quartz dust because sufficient quantities of active silica particles were caught and retained in its stroma. No cirrhosis resulted from the larger quartz particles because most of them were removed by the lymphatic system and the few that remained were too large to be active. No cirrhosis was produced by the injection of the same quantity of fine aluminum oxide particles because they are incapable of exciting a progressive fibrosis.

The part played by the phagocytes and the lymphatic system in the concentration of silica particles has already been discussed. The presence of a sufficient quantity of this irritant in the lymphatics of the portal connective tissues set in motion a progressive proliferation of fibroblasts in their immediate vicinity. Increase of the stroma took place at the expense of the parenchyma of the organ until large portions of the functioning elements had been replaced. Whether the liver cells were destroyed wholly as the result of pressure or whether they were first injured by the toxic action of the silica is not clear. There was marked fatty infiltration, but in only one animal, killed on the 980th day, was there any marked infiltration with polymorphonuclear leukocytes. In the inhalation experiments guinea pigs, but not rabbits, exhibited a well marked metastasis of inhaled dust to the liver and in this location there was a well marked leukocytic infiltration. In these animals injected intravenously only mononuclear leukocytes were found between the advancing edge of the fibrous tissue and the parenchyma. Whether leukocytic infiltration is a feature of the process which was not observed because the animals were not killed at proper intervals is not known.

The overgrowth of fibrous tissue between and within the liver lobules resulted in an extreme grade of coarse nodular cirrhosis of [illegible] proportions. It was apparently much more marked

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than the lesion that Gye and Purdy produced with intravenous colloidal silica, but the amount of silica introduced in our experiments was greater and the animals survived considerably longer. The later stages of the picture were further complicated by regeneration of extensive portions of the parenchyma. This furnishes some extremely bizarre gross pathological pictures. Apparently a sufficient portion of the organ continued to function in most instances to maintain the circulation, as only 1 rabbit developed ascites. This was in an animal that died on the 656th day.

Summary and Conclusions

Three series of rabbits were injected intravenously with 1.3 gm. respectively of silica particles 1 to 3 microns in diameter, silica particles 6 to 12 microns in diameter, and aluminum oxide particles 1 to 3 microns in diameter. The injections were given in divided doses and required from 1 to 4 months for their completion.

These particles were segregated in different locations according to their size. The largest ones were caught in the pulmonary capillaries, those of intermediate size in the spleen and hepatic lymph node, and the finest ones in the liver.

Fine silica particles are most active and have produced a progressive, coarsely nodular cirrhosis of the liver attended by extensive destruction of the parenchyma, followed later by regeneration in certain areas. This cirrhosis is the result of a typical, hyaline, nodular, silicotic fibrosis originating in the portal connective tissues.

Course silica particles, 10 to 12 microns in diameter, are much less irritating. They excite a simple foreign body type of reaction which [illegible] very little in practically 3 years time.

Fine aluminum oxide particles of the same size as those of the smaller silica fraction and introduced in essentially the same quantity have been merely phagocytosed and have produced no fibrosis in the stroma of any organ where they were deposited.

These observations support the viewpoint that the injury produced by silica is specific and chemical rather than physical in character.

References

1. Gardner, L. U. Studies on experimental pneumonokoniosis. VIII. Inhalation of quartz dust. *J. Indust. Hyg.*, 1932, **14**, 18–38.
2. Collis, E. L. Industrial pneumonoconioses. With special reference to dust-phthisis. Milroy Lectures, 1915, H. M. Stationery Office, London.
3. Gye, W. E., and Purdy, W. J. The poisonous properties of colloidal silica. I. The effects of the parenteral administration of large doses. *Brit. J. Exper. Path.*, 1922, **3**, 75–94.
4. Heffernan, P. Acute silicosis. *Tubercle*, 1932, **14**, 109–112.
5. Cummings, D. E. Studies on experimental pneumokoniosis. IV. The separation of particulate matter smaller than screen sizes into graded fractions. *J. Indust. Hyg.*, 1929, **11**, 245–256.

Changes in Blood Protein Fractions and Histamine After Exposure to Amorphous and Crystalline Silicon Dioxide: An Experimental Study

I. A. Gel'fon and V. I. Fedorova

According to a number of clinical and experimental studies, the inhalation of dust containing silicon dioxide generally alters the composition of blood proteins by decreasing the albumins and increasing the globulins, chiefly the γ-globulins (I. A. Gel'fon; K. T. Yegorova and T. N. Butkina; R. A. Zaydenverg; M. T. Dmitrenko and V. P. Malinina-Putsenko; B. Barkhad, et al.; Baldi and Boselli). These observations apply mainly to crystalline silicon dioxide (quartz dust). However, the effect of amorphous silicon dioxide on blood proteins has been little studied to date, although exposure to such dust is common under industrial conditions. Accordingly, we decided to run experiments on animals with amorphous silicon dioxide dust - silica gel and "white carbon black".

The effect of silica gel was studied in the first series of experiments. This subject is particularly interesting because the substance is the dried and calcined gel of silicon dioxide and is insoluble in water or organic liquids. The experiments were performed on white male rats weighing 250-300 g. The animals were injected intratracheally with 50 ml of silica gel dust suspended in 1 ml of physiologic saline. For purposes of comparison another group of animals were injected intratracheally with 50 ml of crystalline silicon dioxide dust suspended in 1 ml of physiologic saline. The particles were 1 to 3 μ in diameter. A third group of untreated animals served as the control.

The animals were killed by decapitation at basal metabolism 1, 2, 4, and 7 weeks after injection of the dust. The serum proteins were determined by paper electrophoresis. Histamine in lung tissue was also determined. Morphological changes in the lungs and bifurcation lymph nodes were studied at the same time.

Study of the serum proteins showed a slight increase in total proteins 2 months after exposure to quartz dust and not until 4 months after silica gel (Fig. 1, a). The albumin content changed less the first 2 months after exposure to silica gel than after exposure to quartz. Thereafter the blood albumins of the animals of both groups were almost the same and lower than in the control (Fig. 1, b). As for the globulins, the β- and γ-fractions showed the greatest changes. The sum of these fractions is given in Fig. 1 because the mobility of the β- and γ-globulins appears to be very similar in paper electrophoresis. It is clear that these parameters too changed somewhat more after exposure to quartz dust.

Thus, amorphous silicon dioxide dust induces less pronounced changes in blood proteins than crystalline silicon dioxide and the changes set in later.

One of the earliest lung reactions to quartz dust is known to be an increase in the amount of histamine (V. I. Uspenskiy and I. A. Gel'fon). We therefore decided to investigate the histamine content of the lungs in the same rats after they received silica gel. It increased slightly 2 months later, but much less than after exposure to quartz dust (Fig. 2). After 4 months the amount of histamine in the control was 9.53 γ/g, 15.26 γ/g after silica gel, and 30.83 γ/g after quartz. Only after 7 months

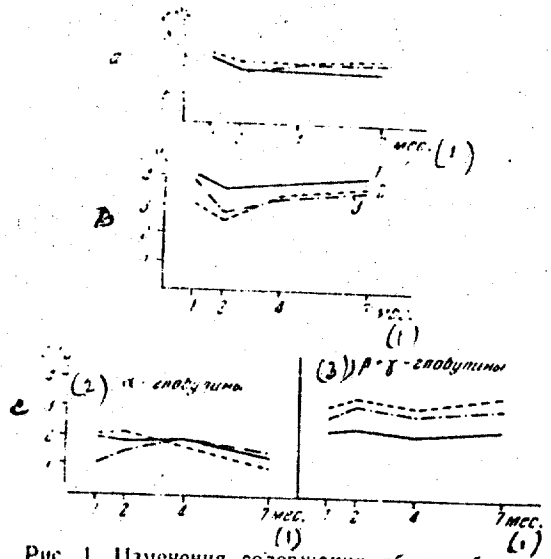

Рис. 1. Изменения содержания общего белка и белковых фракций сыворотки крови белых крыс при воздействии кварцевой пыли и силикагеля.

а — общий белок; *б* — альбумины; *в* — глобулины. *1* — контроль; *2* — введение двуокиси кремния; *3* — введение силикагеля.

Fig. 1. Changes in total protein and protein fractions in white rat serum after exposure to quartz dust and silica gel.

a - total protein; b - albumins; c - globulins

1 - control; 2 - injection of silicon dioxide; 3 - injection of silica gel

(1) months

(2) α-globulins

(3) β and γ-globulins

was the amount of histamine in the animals that received silica gel dust the same as in those given crystalline silicon dioxide.

Histologic examination of the lungs and bifurcation lymph nodes of the animals exposed to quartz dust revealed the changes characteristic of experimental silicosis in white rats. A month after injection of the dust, numerous silicotic nodules consisting chiefly of loosely arranged dust cells could be seen in the alveolar lumens, hyperplastic lymph follicules around the bronchi and blood vessels, and alveolar septa. When impregnated with silver by the Tibor-Pap method, a delicate network of argyrophil fibrils appeared on the periphery of the nodules. Here and there the alveolar septa were considerably thickened owing to multiplication of the histiocytes. The silicotic process progressed by the 2nd and especially 4th month. Precollagenous and collagenous fibers could be clearly seen in the nodules. Interstitial sclerosis and emphysema developed. The lungs of the animals sacrificed 7 months after injection of the dust contained numerous silicotic nodules that frequently coalesced, with distinct signs of sclerosis and even hyalinosis (Fig. 3). Interstitial sclerosis and emphysema also progressed.

Within a month of injection of the dust, the bifurcation lymph nodes contained a substantial number of submiliary and miliary silicotic nodules made up of epithelioid cells which increased both in number and size after 2 to 4 months and exhibited sclerotic changes. By month 7 sclerosis had progressed considerably. In some cases it was accompanied by the formation of coarse bundles of collagenous fibers.

One to 2 months after the injection of silica gel, cellular dust foci consisting of loosely arranged dust cells with a few phagocytized dust

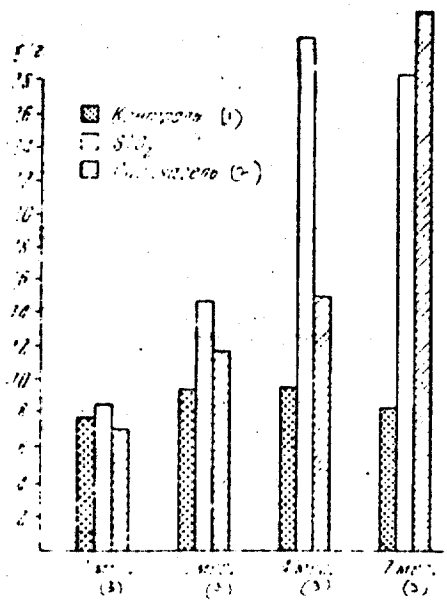

Рис. 2. Содержание гистамина в ткани легких белых крыс при воздействии кварцевой пыли и силикагеля.

Fig. 2. Histamine content of lung tissue in white rats after exposure to quartz dust and silica gel.

(1) Control

(2) Silica gel

(3) months

Рис. 3. Слившиеся силикотические узелки с пучками коллагеновых волокон в легком крысы через 7 месяцев после интратрахеального введения кварцевой пыли. Окраска по ван Гизону. ×56.
Рис. 4. Клеточно-пылевой очажок без явлений склероза в легком крысы через 7 месяцев после введения силикагеля. Окраска гематоксилин-эозином. ×100.

Fig. 3. Coalesced silicotic nodules with bundles of collagenous fibers in the lung of a rat 7 months after intratracheal injection of quartz dust. Stained by van Gieson's method. X 56.

Fig. 4. Cellular dust focus with no signs of sclerosis in the lung of a rat 7 months after injection of silica gel. Stained with hematoxylin-eosin. X 100.

particles in their cytoplasm formed in the alveolar lumens. The cells did not proliferate appreciably in the cellular dust foci. Impregnation with silver or staining by van Gieson's method failed to reveal the presence of sclerosis in the cellular dust foci. The alveolar septa remained thin for the most part.

In some cases there was a slight focal thickening of the septa because of the appearance of dust cells containing particles of various sizes that differed in appearance from the quartz crystals. These particles were dark, irregularly shaped, tiny amorphous grains.

By the 4th and especially 7th month there appeared against a background of virtually unaltered lung tissue miliary and larger cellular dust foci (Fig. 4) differing in structure from the silicotic nodules. They consisted of compact masses of dust cells whose cytoplasm was almost entirely filled with dust particles. There were no signs of sclerosis in the cellular dust foci at this time either. Besides the formation of cellular dust foci in the lungs at this time, there was a slight focal thickening of the septa, focal emphysema, and sclerosis which was indistinct around the bronchi but fairly pronounced around the blood vessels.

After 7 months some of the animals developed inflammatory-desquamative and occasionally purulent bronchitis. The bifurcation lymph nodes contained numerous nodules of epitheloid cells without connective tissue.

Thus, our studies on blood proteins and the pathologoanatomical data showed that silica gel dust is less aggressive than quartz dust. The marked increase in the amount of histamine in the animals' lung tissue 7 months after introduction of the silica gel was apparently caused by the purulent bronchitis that the animals had developed.

Another series of experiments was performed to test the effect of "white carbon black"* injected subcutaneously into male rabbits weighing 3 to 3.5 kg.

The content of albumins decreased appreciably in the rabbits that received quartz dust, but the injection of "white carbon black" had no effect in this respect (Fig. 5). The content of globulins also increased markedly only in response to quartz dust; the injection of "white carbon black" did not alter the total globulins. However, there were definite differences between quartz dust and "white carbon black" (Fig. 5) as far as the globulin fractions were concerned. Within 2 weeks of injection

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*"White carbon black" is an extremely fine white powder. It is so named because it is used in rubber mixtures for the same purpose as carbon black, i.e., as a filler in the preparation of white and black rubber. White carbon black contains 92 to 93% free amorphous silicon dioxide. 50 ml in 1 ml of physiologic saline was administered 4 times at 7-day intervals. For purposes of comparison, another group of animals received under the same conditions 50 ml of quartz dust suspended in 1 ml of physiologic saline. The control rabbits did not receive anything. Blood was taken at basal metabolism from an ear vein 2 or 3 times before exposure to dust and then 1, 2, 3, and 6 months after the first injection. The protein fractions were determined by paper electrophoresis. Blood histamine was also investigated by the biological method in atropinized guinea pig small intestine. Morphological examination followed.

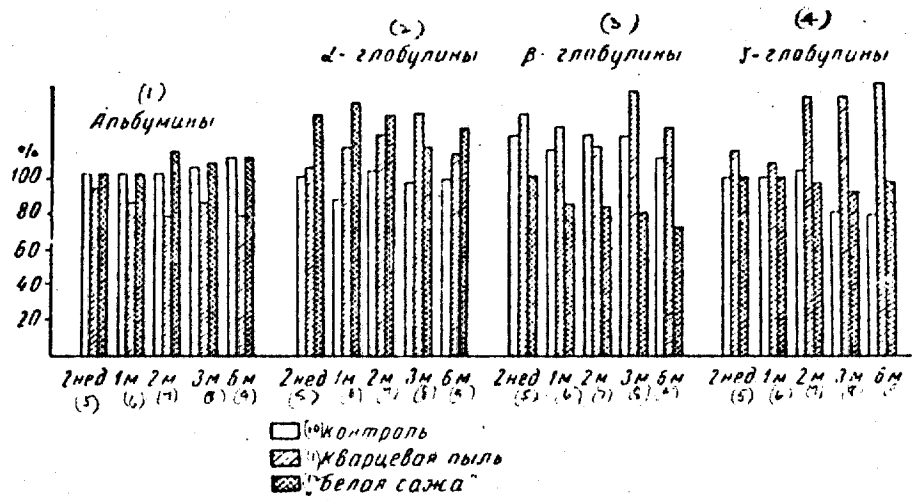

Fig. 5. Changes in serum protein fractions after subcutaneous injection of rabbits with quartz dust and "white carbon black" (in percentages of the original level)

(1) Albumins
(2) α-globulins
(3) β-globulins
(4) γ-globulins
(5) 2nd week
(6) 1st month
(7) 2nd month
(8) 3rd month
(9) 6th month
(10) Control
(11) Quartz dust
(12) "White carbon black"

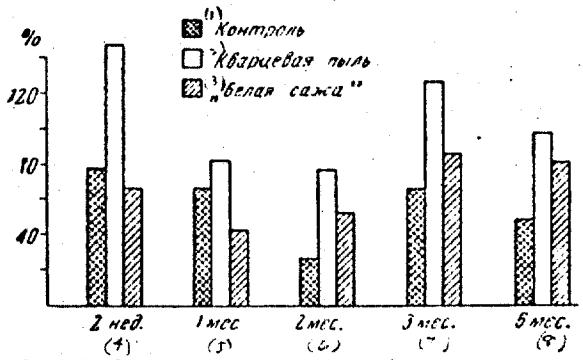

Рис. 6. Содержание гистамина в крови при воздействии кварцевой пыли и «белой сажи» (в процентах к исходному уровню).

Fig. 6. Blood histamine after exposure to quartz dust and "white carbon black" (in percentages of the original level)

(1) Control

(2) Quartz dust

(3) "White carbon black"

(4) 2nd week

(5) 1st month

(6) 2nd month

(7) 3rd month

(8) 5th month

the α-globulins increased and the γ-globulins decreased while the β-globulins remained at the normal level. These changes persisted throughout the observation period (6 months). Injection of quartz dust caused within 2 weeks a slight increase in the β- and γ-globulins. All the globulin fractions increased after a month and remained at a high level. The γ-globulins showed the greatest increase.

The injection of quartz dust caused a substantial increase in blood histamine, especially after 2 weeks (Fig. 6). Two to 4 weeks after exposure to "white carbon black", blood histamine so far from increasing actually fell below the control level, and it was not until month 2 that it increased, although not to the same extent as in the animals that received quartz dust.

Thus, the results of this series of experiments show that "white carbon black" injected subcutaneously causes smaller changes in serum proteins than does crystalline silicon dioxide and they take longer to develop. However, "white carbon black" causes slight changes to arise quickly that differ from those resulting from exposure to quartz dust. For example, it was found that the α-globulins increased while the β-globulins and histamine decreased and the γ-globulins remained unchanged.

Pathologoanatomical examination of the rabbits injected subcutaneously with crystalline silicon dioxide revealed 9 months later the presence of incapsulated solid nodules the size of a large pea at the injection site. In cross section whitish strands could be seen proceeding in different directions. Histological examination showed that the nodules consisted of multiple miliary and larger

coalesced silicotic nodules. Structurally, they were quite typical of the silicotic nodules that appear in subcutaneous tissue after subcutaneous injection of quartz dust (Fig. 7). They were similar to those observed by P. P. Dvishkov, G. A. Kochetkova, and Ye. V. Khukhrina.

The cellular elements in the nodules consisted of large histiocytes with vacuolated protoplasm and eccentrically arranged nucleus. In the protoplasm of these cells could be seen numerous small whitish-brown particles. Among the cells were many fibroblasts and occasional eosinophilic polynuclears. When stained by van Gieson's method, coarse bundles of collagenous fibers proceeding in different directions and large sclerotic areas could be seen in the nodules, especially along the periphery. The walls of the small arteries and arterioles in the sclerotic areas were thickened and hyalinized (Fig. 8). Necrobiotic changes appeared in the center of individual nodules. No changes of a silicotic nature were evident in the lungs, heart, liver, kidneys, spleen, or bifurcation lymph nodes.

In rabbits injected subcutaneously with "white carbon black", no changes were observed 9 months later in the subcutaneous tissue at the injection site. There were interstitial histiocytic infiltrates and sclerosis in the kidneys, faint focal thickening of the alveolar septa, and small accumulations of histiocytes in the myocardium and liver mainly along the course of the blood vessels.

One rabbit exhibited degenerative changes in the kidneys with a marked deposition of amyloid in the glomeruli and walls of the medullary vessels, interstitial sclerosis, and early signs of

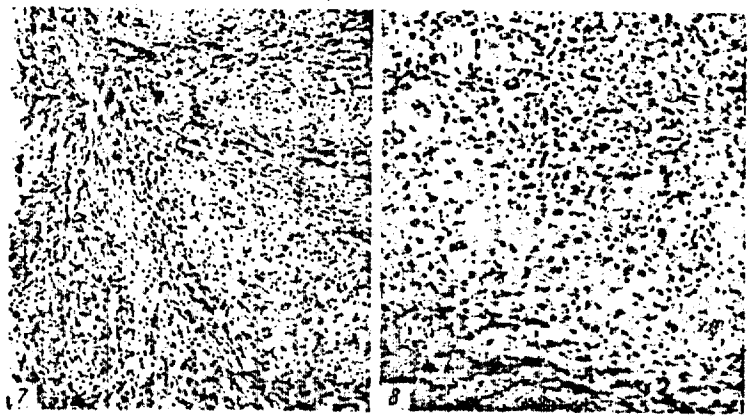

Рис. 7. Силикотические узелки с хорошо выраженным склерозом в подкожной клетчатке кроликов через 9 месяцев после подкожного введения кварцевой пыли.
Окраска гематоксилин-эозином. ×56.
Рис. 8. Тот же препарат. Гиалиноз стенок артериол. ×280.

Fig. 7. Silicotic nodules with pronounced sclerosis of subcutaneous tissue in rabbits 9 months after subcutaneous injection of quartz dust. Stained with hematoxylin-eosin. X 56.

Fig. 8. The same preparation. Hyalinosis of the arteriole walls. X 280.

amyloidosis of the lymph follicles of the spleen. There was a slight focal thickening of the septa in the lungs of this rabbit.

Analysis of the data shows that quartz dust, regardless of the mode of injection (intratracheal in rats or subcutaneous in rabbits) causes histamine to increase in rat lungs and rabbit blood and blood globulins to increase (γ-globulins in rabbits, β- and γ-globulins in rats).

Intratracheal injection of rats with silica gel also increased the amount of histamine in the lungs and β- and γ-globulins in the blood, but not to the same extent as quartz dust.

After subcutaneous injection of rabbits with "white carbon black", the γ-globulins remained within normal limits but the α-globulins increased.

The less pronounced specific changes observed after injection of amorphous dust compared with the crystalline form may have been due, in part, to the fact that amorphous dust has less effect on histamine and serum proteins, γ-globulins in particular, a possible indication of the formation of fewer autoantibodies and of less capacity for adsorption of proteins. The proteins adsorbed on the surface of quartz and the protein decomposition products may be autoantigens which upon combining with antibodies promote the coagulation of proteins, thereby intensifying the pathological process (V. I. Ivanov; Kalman; Pernis, et al.; Scheel, et al.).

We performed experiments on white rats that received intratracheal injections of quartz dust treated beforehand with serum from healthy rats. The experiments showed that changes in the blood protein fractions set in later than when untreated quartz dust was used.

Subcutaneous injection of rabbits with protein coagulated by sodium silicate altered the composition of blood proteins by decreasing the albumins and increasing the γ- and α-globulins. These experiments confirmed the assumption that coagulated proteins have antigenic properties.

It is generally known that among the globulin fractions the γ-globulins are the closest to antibodies. It was therefore important to determine whether an increase in content of γ-globulins affects the development of silicosis. The γ-globulins were increased by repeatedly immunizing white rats with typhoid vaccine injected subcutaneously (1 billion microbial cells in 1 ml of physiologic saline) 3 times at 7-day intervals. A week after the last vaccination the rats were injected intratracheally with 50 mg of quartz dust in 1 ml of physiologic saline. After 2 months the amount of albumin decreased from 4.06 (in the control) to 3.61 (after exposure to quartz dust without immunization) and 3.46 g% (with preliminary immunization). The β- and γ-globulins increased proportionately from 3.32 to 3.53 g% in the nonimmunized animals and to 3.61 g% in the immunized animals. Subsequently (4 to 6 months later) the amount of albumins remained lower than after exposure to quartz alone, while the β- and γ-globulins changed insignificantly compared with the animals given quartz dust or they remained within normal limits.

Thus, the effect of immunization was more perceptible after 2 months. However, the morphological changes in the lungs and bifurcation lymph nodes in these groups of animals were almost identical.

Conclusions

1. Experimental silicosis induced by intratracheal injection of crystalline silicon dioxide dust slightly elevates the total protein level in serum, decreases the albumins, and increases the globulins (mainly the β- and γ-globulin fractions). The amount of histamine increases in lung tissue.

2. The changes in blood proteins and lung histamine in response to intratracheal injection of amorphous silicon dioxide dust - silica gel - are less pronounced and they set in later than when crystalline silicon dioxide is used. The lungs contain cellular dust foci unlike the silicotic nodules typical of experimental silicosis of white rats, focal thickening of the septa, signs of emphysema, and slight peribronchial and perivascular sclerosis.

3. Subcutaneous injection of rabbits with quartz dust lowers the albumin level, and gradually increases the amount of globulins (γ-globulins in particular) and blood histamine. Subcutaneous injection of "white carbon black" has an insignificant effect on the albumins and globulins, but it increases the α-globulins and decreases the β-globulins, with the γ-globulins remaining within normal limits. There is a slight increase in blood histamine.

4. Quartz dust injected into white rats previously immunized with typhoid vaccine causes within 2 months greater changes in the content of serum albumins, β- and γ-globulins than it does in non-immunized animals. However, the morphological changes in the lungs and bifurcation lymph nodes are the same in these groups of animals.

References

Barkhad, B., A. Vlad, and F. Dron, Trudy simpoziuma po probleme pnevmokoniozov (etiologiya i patogenez) (Proceedings of a Symposium on Pneumoconiosis - Etiology and Pathogenesis), Moscow, 1959, p. 38.

Gel'fon, I.A., "Some Biochemical Changes in the Blood in Silicosis and Silicotuberculosis," A candidate's dissertation, Moscow, 1958.

Dvizhkov, P.P., T.A. Kochetkova, and Ye. V. Khurhrina, in the book: Bor'ba s silikozom (Control of Silicosis), Moscow, 1957, Vol. 4, p. 98.

Dmitrenko, M.T., and V.P. Malinina-Putsenko, in the book: Sbornik rabot i avtoreferatov po voprosam gigiyeny truda i profpatologii v gornorudnoy, khimicheskoy i mashinostroitel'noy promyshlennosti (Collection of Articles and Author's Abstracts on Industrial Hygiene and Occupational Diseases in the Mining, Chemical, and Machine Building Industries), Kharkov, 1958, p. 41.

Yegorova, K.T., and T.N. Butkina, Tezisy dokl. 3-y konferentsii Ural'sk. filiala Vsesoyuzn. o-va patofiziologov (Abstracts of Papers Read at the 3rd Conference of the Ural Branch of the All-Union Society of Pathophysiologists), Sverdlovsk, 1954, p. 12.

Zaydenverg, R.A., in the book: Pnevmokonioz (Pneumoconiosis), Kiev, 1954, p. 128.

Ivanov, V.I., same source as in Barkhad, A. above, p. 43.

Uspenskiy, V.I., Arkh. pat. (Archives of Pathology), 1950, No. 6, p. 26.

Uspenskiy, V.I., "Role of Histamine in the Pathogenesis of Experimental Pulmonary Fibrosis." A candidate's dissertation, Moscow, 1951.

Baldi, J., and A. Boselli, Med. Lavoro, 1953, Vol. 44, p. 501.

Kalman, E., Egeszsegtudomany, 1957, No. 1, p. 43.

Pernis, B., and M. Battigelli, Med. Lavoro, 1955, Vol. 46, p. 1.

Scheel, L.D., B. Smith, J. van Riper, et al., Arch. Ind. Hyg., 1954, Vol. 9, p. 29.

J. invest. Derm., 1961, v. 36, p. 337.—Stejskal K., Zbl. Haut u. Geschl.-Kr., 1928, Bd. 26, S. 537.—Szakall A., Arch. klin. exp. Derm., 1955, Bd. 201, S. 331.—Szczesniak A. S., Sherman H., Harris R. S., Science, 1951, v. 113, p. 293.—Treherne J. E., J. Physiol. (Lond.), 1956, v. 133, p. 171.—Unna P., Dtsch. med. Wschr., 1926, Bd. 52, S. 197.—Whitehouse A. G., Ramage H., Proc. roy. Soc. B., 1933, v. 113, p. 42.—White R. P., Occupational Affections of the Skin. London, 1929.—Winsor T., Burch G. E., Arch. intern. Med., 1944, v. 74, p. 428.

SKIN PERMEABILITY FOR NICKEL COMPOUNDS

F. I. Kolpakov

Summary

An apparatus for and a method of skin permeability determination is suggested. Nickel sulfate permeability was studied on cadaver skin. As established, corneal epidermis prevented nickel sulfate penetration through the skin. Malpigian layer, dermis and hypodermis proved to be easily permeable for nickel. The lustrous epidermic layer absorbed nickel sulfate, but this did not serve as a proof of its permeablity for nickel.

ИЗМЕНЕНИЯ БЕЛКОВЫХ ФРАКЦИЙ КРОВИ И ГИСТАМИНА ПОД ВЛИЯНИЕМ АМОРФНОЙ И КРИСТАЛЛИЧЕСКОЙ ДВУОКИСИ КРЕМНИЯ (ЭКСПЕРИМЕНТАЛЬНОЕ ИССЛЕДОВАНИЕ)

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Из патологоанатомической лаборатории (зав.—проф. Н. П. Движков) и биохимической лаборатории (зав. — старший научный сотрудник И. В. Павлова) Института гигиены труда и профзаболеваний (дир. — действительный член АМН СССР проф. А. А. Летавет) АМН СССР

(Поступила в редакцию 12/II 1962 г.)

Вдыхание пыли, содержащей двуокись кремния, на основании клинических и экспериментальных исследований, по данным ряда авторов, большей частью сопровождается сдвигами белкового состава крови в сторону снижения альбуминов и увеличения глобулинов, главным образом γ-глобулинов (И. А. Гельфон; К. Т. Егорова и Т. Н. Буткина; Р. А. Зайденверг; М. Т. Дмитренко и В. П. Малинина-Пуценко; Б. Бархад и др.; Baldi, Boselli). Эти наблюдения относятся главным образом к действию кристаллической формы двуокиси кремния (кварцевая пыль). Влияние же аморфной двуокиси кремния на состав белков крови до сих пор изучено мало, хотя воздействие такой пыли нередко наблюдается в производственных условиях. В связи с этим нами были поставлены на животных опыты с пылью аморфной двуокиси кремния — силикагелем и «белой сажей».

В первой серии опытов исследовали действие силикагеля. Изучение влияния силикагеля особенно интересно потому, что он представляет высушенный и прокаленный гель двуокиси кремния, нерастворимый в воде и органических жидкостях. Опыты проводили на белых крысах-самцах весом 250—300 г, которым интратрахеально вводили 50 мг пыли силикагеля в виде взвеси в 1 мл физиологического раствора. Для сравнения животным другой группы интратрахеально вводили по 50 мг пыли кристаллической двуокиси кремния, взвешенной в 1 мл физиологического раствора. Величина частиц пыли равнялась 1—3 μ. Животные третьей группы были контрольными, им ничего не вводили.

Животных забивали обезглавливанием натощак через 1, 2, 4, 7 месяцев после введения пыли; в сыворотке их крови определяли белковые фракции методом электрофореза на бумаге. Исследовали содержание гистамина в легочной ткани. Одновременно изучали морфологические изменения в легких и бифуркационных лимфатических узлах.

Изучение белков сыворотки показало, что некоторое повышение содержания общего белка обнаруживается при воздействии кварцевой пыли ко 2-му месяцу после запыления, а при воздействии силикагеля — только к 4-му месяцу (рис. 1, *а*). Содержание альбуминов в первые 2 месяца меньше изменяется при воздействии силикагеля, чем при воздействии кварца. В дальнейшем содержание альбуминов в крови животных обеих групп бывает почти одинаковым и оказывается ниже, чем в контрольной группе (рис. 1, *б*). В отношении глобулинов наибольшие изменения отмечаются в β- и γ-фракциях. На рис. 1, *в* представлена сумма этих фракций, так как при электрофорезе на бумаге подвижность β- и γ-глобулинов сыворотки крови крыс очень близка. Как видно, и эти показатели дают несколько большие сдвиги при воздействии кварцевой пыли.

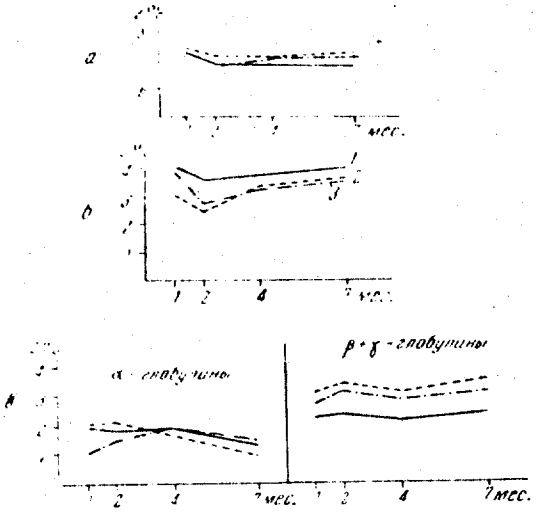

Рис. 1. Изменения содержания общего белка и белковых фракций сыворотки крови белых крыс при воздействии кварцевой пыли и силикагеля.

а — общий белок; *б* — альбумины; *в* — глобулины. *1* — контроль; *2* — введение двуокиси кремния; *3* — введение силикагеля.

Таким образом, пыль аморфной двуокиси кремния по сравнению с кристаллической вызывает менее выраженные изменения белкового состава крови, которые наступают в более поздние сроки.

Известно, что одной из ранних реакций воздействия кварцевой пыли на легкие является повышение в них количества гистамина (В. Н. Успенский; И. А. Гельфон). В связи с этим мы исследовали содержание гистамина в легких и при воздействии силикагеля у тех же крыс.

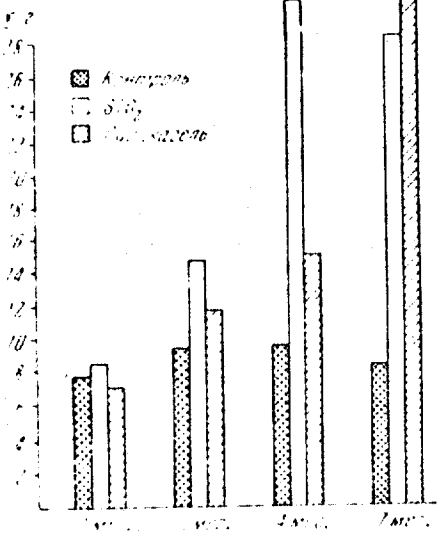

Рис. 2. Содержание гистамина в ткани легких белых крыс при воздействии кварцевой пыли и силикагеля.

Содержание гистамина после введения силикагеля, начиная со 2-го месяца, несколько увеличивается, однако это увеличение бывает значительно меньшим, чем после воздействия кварцевой пыли (рис. 2). Через 4 месяца содержание гистамина у крыс контрольной группы составляло 9,53 γ/г, при воздействии силикагеля — 15,26 γ/г, кварца — 30,83 γ/г. Только через 7 месяцев животных, запылявшихся пылью силикагеля, содержание гистамина стало таким же, как и у крыс, которым вводили кристаллическую двуокись кремния.

При гистологическом исследовании легких и бифуркационных лимфатических узлов у животных, запылявшихся кварцевой пылью, обнаружены изменения, характерные для экспериментального силикоза белых крыс. Через месяц после введения пыли в просветах альвеол, в гиперплазированных лимфатических фолликулах вокруг бронхов и сосудов, альвеолярных перегородках наблюдалось формирование многочисленных силикотических узелков, состоящих преимущественно из рыхло расположенных пылевых клеток. При импрегнации серебром по

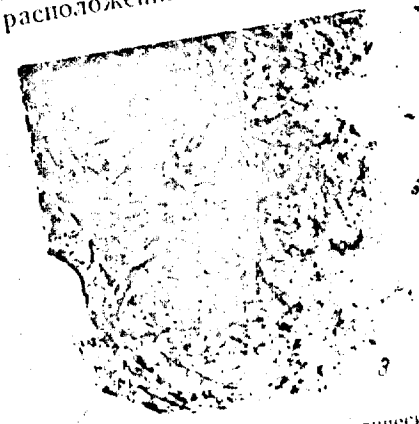

Рис. 3. Слившиеся силикотические узелки с пучками коллагеновых волокон в легком крысы через 7 месяцев после интратрахеального введения кварцевой пыли. Окраска по ван Гизону. ×56.

Рис. 4. Клеточно-пылевой очажок без явлений склероза в легком крысы через 7 месяцев после введения силикагеля. Окраска гематоксилин-эозином. ×100.

Тибору — Папу по периферии узелков выявляется нежная сеть аргирофильных волоконец. Альвеолярные перегородки местами значительно утолщены за счет размножения гистиоцитов. Ко 2-му и особенно к 4-му месяцу отмечается прогрессирование силикотического процесса. В узелках отчетливо выявляются преколлагеновые и коллагеновые волокна, отмечается развитие межуточного склероза и эмфиземы. У животных, забитых через 7 месяцев после введения пыли, в легких обнаруживаются многочисленные силикотические узелки, нередко слившиеся между собой, с хорошо выраженными явлениями склероза и даже гиалиноза (рис. 3). Отмечается также прогрессирование межуточного склероза, эмфиземы.

В бифуркационных лимфатических узлах уже через месяц после введения пыли появляется значительное количество субмилиарных и милиарных силикотических узелков из клеток эпителиоидного типа, которые к 2—4 месяцам увеличиваются в количестве и размерах, в них развиваются склеротические изменения. К 7-му месяцу отмечается значительное прогрессирование склероза с образованием в отдельных случаях грубых пучков коллагеновых волокон.

При введении силикагеля через 1—2 месяца в просветах альвеол обнаруживается формирование клеточно-пылевых очажков, состоящих из рыхло расположенных пылевых клеток с небольшим количеством фагоцитированных пылевых частиц в их цитоплазме. Заметной пролиферации клеток в клеточно-пылевых очажках не наблюдается. При импрегнации серебром и при окраске по ван Гизону развития склероза в клеточно-пылевых очажках не обнаруживается. Альвеолярные перегородки в значительной своей части остаются тонкими.

В ряде случаев определяется небольшое очаговое утолщение перегородок за счет появления в них пылевых клеток, содержащих различ-

ные по величине частички введенной пыли, отличающиеся по своему характеру от кристаллов кварца. Такие частички имеют вид темных, неправильных очертаний, мельчайших аморфных пылинок.

К 4-му и особенно к 7-му месяцу на фоне малоизмененной легочной ткани обнаруживаются милиарные и более крупные клеточно-пылевые очажки (рис. 4), отличающиеся по своему строению от силикотических узелков. Они состоят из компактных скоплений пылевых клеток, цитоплазма которых почти целиком выполнена пылевыми частицами. Развития склероза в клеточно-пылевых очажках и в эти сроки не отмечается. Наряду с образованием клеточно-пылевых очажков в легком в этот период отмечается небольшое очаговое утолщение перегородок, очаговая эмфизема, склероз, слабо выраженный вокруг бронхов и более значительный вокруг кровеносных сосудов.

У ряда животных через 7 месяцев в бронхах отмечается катарально-десквамативный, а иногда и гнойный бронхит. В бифуркационных лимфатических узлах обнаруживаются многочисленные узелки из клеток эпителиоидного типа без развития соединительной ткани.

Таким образом, проведенные исследования по изучению белковых фракций крови и патологоанатомические данные показывают, что пыль силикагеля менее агрессивна, чем кварцевая. Значительное увеличение количества гистамина в легочной ткани крыс через 7 месяцев после введения силикагеля, по-видимому, следует связать с имевшим место у этих животных гнойным бронхитом.

Во второй серии опытов испытывали влияние «белой сажи»[1] при подкожном введении ее кроликам-самцам весом 3—3,5 кг.

Количество альбуминов у кроликов, получивших кварцевую пыль, заметно снизилось, тогда как после введения «белой сажи» оно не изменилось (рис. 5). Содержание глобулинов также значительно увеличилось лишь при воздействии кварцевой пыли, введение «белой сажи» не оказало влияния на суммарное содержание глобулинов. Однако среди глобулиновых фракций имеются определенные различия в воздействии кварцевой пыли и «белой сажи» (см. рис. 5). После введения «белой сажи» уже через 2 недели выявлялось увеличение содержания α-глобулинов и снижение содержания β-глобулинов при нормальном уровне γ-глобулинов. Эти изменения удерживались в течение всего срока наблюдения (6 месяцев). Введение кварцевой пыли через 2 недели привело к небольшому увеличению содержания β- и γ-глобулинов. Через месяц наблюдалось увеличение всех глобулиновых фракций. В дальнейшем фракции оставались увеличенными, причем больше всего повышалось содержание γ-глобулинов.

После введения кварцевой пыли наблюдалось значительное увеличение количества гистамина в крови, причем оно было особенно резким через 2 недели (рис. 6). При воздействии «белой сажи» через 2 недели — 1 месяц количество гистамина в крови не только не увеличилось, а даже снизилось по сравнению с контролем, и лишь со 2-го месяца от-

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1 «Белая сажа» представляет белый порошок чрезвычайно мелкого размола. Название «белая сажа» дано в связи с применением ее в резиновых смесях для тех же целей, что и черной сажи, т. е. в качестве наполнителя для изготовления белой и цветной резины. Белая сажа содержит 92—93% свободной двуокиси кремния в аморфном состоянии. Белую сажу вводили по 50 мг в 1 мл физиологического раствора 4 раза с 7-дневными промежутками. Для сравнения животным другой группы в таких же условиях вводили по 50 мг кварцевой пыли, взвешенной в 1 мл физиологического раствора. Контрольным кроликам ничего не вводили. Кровь брали натощак из краевой вены уха до 2—3 раза до заражения, а затем через 2 недели, 1, 2, 3, 6 месяцев после первого введения пыли. Белковые фракции определяли методом электрофореза на бумаге. Кроме того, исследовали содержание гистамина в крови биологическим методом на атропинизированной гладкой мышце морской свинки. Производили морфологические исследования.

мечалось увеличение количества гистамина, хотя и менее выраженное, чем у животных, которым вводили кварцевую пыль.

Таким образом, результаты этой серии опытов показывают, что «белая сажа» при подкожном введении по сравнению с кристаллической двуокисью кремния вызывает меньшие сдвиги со стороны сы-

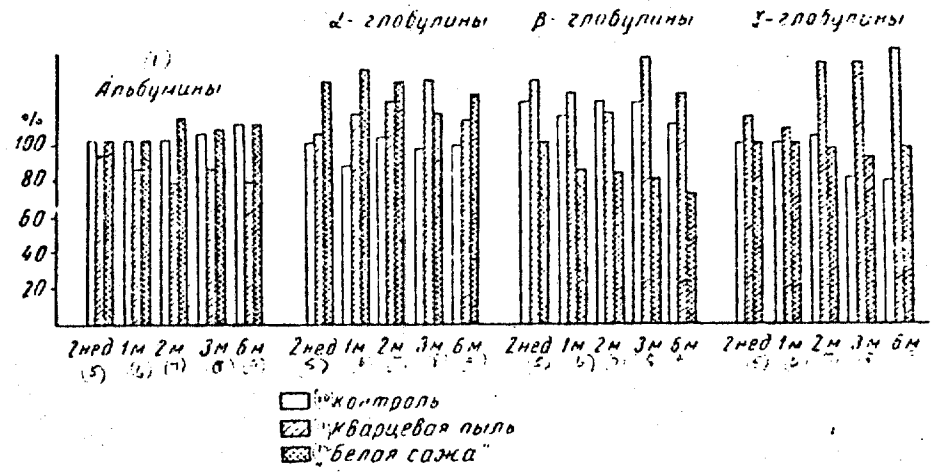

Рис. 5. Изменения белковых фракций сыворотки крови при подкожном введении кроликам кварцевой пыли и «белой сажи» (в процентах к исходному уровню).

вороточных белков и наступают они через больший промежуток времени. Однако при воздействии «белой сажи» в ранние сроки отмечаются некоторые изменения, отличающиеся от тех, которые наблюдаются от действия кварцевой пыли. Так, обнаружено увеличение содержания α-глобулинов, снижение содержания β-глобулинов и гистамина, отсутствие реакции со стороны γ-глобулинов.

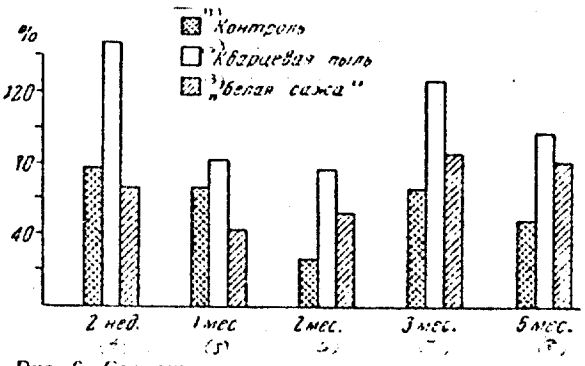

Рис. 6. Содержание гистамина в крови при воздействии кварцевой пыли и «белой сажи» (в процентах к исходному уровню).

При патологоанатомическом исследовании кроликов с подкожным введением кристаллической двуокиси кремния через 9 месяцев на месте введения обнаружены инкапсулированные плотные узловатые образования величиной с крупную фасоль; на их разрезе были видны идущие в различных направлениях тяжи белесоватого цвета. При гистологическом исследовании установлено, что узлы состоят из многочисленных милиарных и более крупных, слившихся между собой силикотических узелков. По своему строению они весьма типичны для силикотических узелков, развивающихся в подкожной клетчатке при подкожном введении кварцевой пыли (рис. 7), и аналогичны наблюдаемым П. П. Движковым, Г. А. Кочетковой, Е. В. Хухриной.

Клеточные элементы в узелках представлены крупными гистиоцитами с вакуолизированной протоплазмой и эксцентрически расположенным ядром. В протоплазме этих клеток видны многочисленные мелкие

пылинки желтовато-бурого цвета. Среди клеток много фибробластов, кое-где видны эозинофильные полинуклеары. При окраске по ван Гизону в узелках, особенно по их периферии, обнаруживаются грубые, идущие в различных направлениях пучки коллагеновых волокон, а также крупные участки склероза. Стенки мелких артерий и артериол в склерозированных участках уплотнены, гиалинизированы (рис. 8). В центральной части отдельных узелков отмечаются некробиотические изменения. Во внутренних органах (легкие, сердце, печень, почки, селезенка) и в бифуркационных лимфатических узлах изменений силикотического характера отмечено не было.

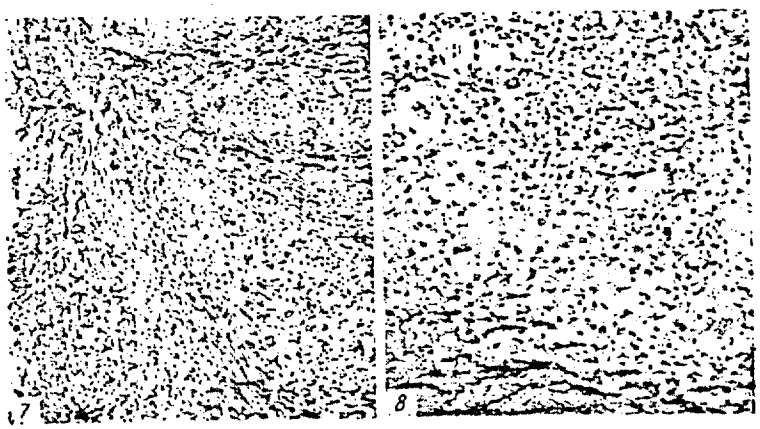

Рис. 7. Силикотические узелки с хорошо выраженным склерозом в подкожной клетчатке кроликов через 9 месяцев после подкожного введения кварцевой пыли.
Окраска гематоксилин-эозином. ×56.
Рис. 8. Тот же препарат. Гиалиноз стенок артериол ×280.

При подкожном введении кроликам «белой сажи» через 9 месяцев изменений в подкожной клетчатке на месте введения не отмечалось. У них обнаруживались межуточные гистиоцитарные инфильтраты и склероз в почках, слабо выраженное очаговое утолщение альвеолярных перегородок, небольшие скопления гистиоцитов в миокарде и печени главным образом по ходу кровеносных сосудов.

У одного кролика наблюдались дистрофические изменения в почках со значительным отложением амилоида в клубочках и стенках сосудов мозгового вещества, межуточный склероз, а также начальные проявления амилоидоза лимфатических фолликулов селезенки. В легких этого кролика отмечено небольшое очаговое утолщение перегородок.

Анализ полученных данных показывает, что кварцевая пыль независимо от способа введения (интратрахеально крысам или подкожно кроликам) вызывает увеличение количества гистамина в легких крыс, крови кроликов и повышение содержания глобулинов в крови (γ-глобулинов у кроликов, β- и γ-глобулинов у крыс).

Интратрахеальное введение силикагеля крысам также сопровождается увеличением содержания гистамина в легких, β- и γ-глобулинов в крови, хотя и в меньшей степени, чем при воздействии кварцевой пыли.

При подкожном введении кроликам «белой сажи» количество γ-глобулинов оставалось в пределах нормы, однако увеличилось содержание α-глобулинов.

Менее выраженные специфические изменения, обнаруженные при воздействии аморфной пыли по сравнению с кристаллической, возмож-

но, в какой-то степени объясняются тем, что аморфная пыль оказывает меньшее влияние на содержание гистамина и белков сыворотки крови, в частности на содержание γ-глобулинов. Последнее может указывать на меньшее образование аутоантител, на меньшую способность к адсорбции белков. Белки, адсорбированные на поверхности кварца, и продукты распада белков могут являться аутоантигенами, которые, соединяясь с антителами, способствуют коагулированию белков и тем самым усугубляют патологический процесс (В. Н. Иванов; Kalman; Pernis и др.; Scheel и др.).

Нами были проведены экспериментальные исследования на белых крысах, которым интратрахеально вводили кварцевую пыль, предварительно обработанную сывороткой крови здоровых крыс. Опыты показали, что сдвиги белковых фракций крови в этих случаях наступали в более поздние сроки, чем при введении необработанной кварцевой пыли.

Подкожное введение кроликам белка, коагулированного под влиянием силиката натрия, вызывает изменения белкового состава крови в сторону уменьшения содержания альбуминов и увеличения γ- и α-глобулинов. Эти опыты подтверждают предположение об антигенных свойствах коагулированных белков.

Известно, что среди глобулиновых фракций ближе всего к антителам стоят γ-глобулины. Поэтому было важно выяснить, влияет ли повышенное содержание γ-глобулинов на развитие силикоза. Увеличения содержания γ-глобулинов достигали путем повторной иммунизации белых крыс брюшнотифозной вакциной, которую вводили подкожно 3 раза с 7-дневными промежутками по 1 млрд. микробных тел в 1 мл физиологического раствора. Через неделю после последнего введения вакцины крысам вводили интратрахеально 50 мг кварцевой пыли в 1 мл физиологического раствора. Через 2 месяца количество альбумина снизилось с 4,06 (у контрольных животных) до 3,61 (при воздействии кварцевой пыли без иммунизации) и 3,46 г% (с предварительной иммунизацией). Соответственно увеличилось содержание β- и γ-глобулинов — с 3,32 до 3,53 г% у неиммунизированных и до 3,61 г% у иммунизированных животных.

В дальнейшем (через 4—6 месяцев) количество альбуминов оставалось сниженным даже в большей степени, чем при действии одного кварца, а содержание β- и γ-глобулинов по сравнению с животными, которым вводили кварцевую пыль, изменялось незначительно или оставалось в пределах нормы.

Таким образом, более заметное влияние иммунизации отмечалось через 2 месяца; однако морфологические изменения в легких и бифуркационных лимфатических узлах у животных этих групп были почти одинаковы.

Выводы

1. При экспериментальном силикозе, вызванном интратрахеальным введением пыли кристаллической двуокиси кремния, в сыворотке крови отмечается некоторое увеличение уровня общего белка, снижение содержания альбуминов и повышение глобулинов (главным образом β- и γ-глобулиновых фракций). В легочной ткани обнаруживается увеличение количества гистамина.

2. При интратрахеальном введении пыли аморфной двуокиси кремния — силикагеля — изменения белкового состава крови и гистамина в легких выражены меньше и наступают в более поздние сроки, чем при воздействии кристаллической двуокиси кремния. В легких обнаруживаются развитие клеточно-пылевых очажков, отличающихся от силикотических узелков, типичных для экспериментального силикоза белых крыс, очаговое утолщение перегородок, эмфизема, небольшой перибронхиальный и периваскулярный склероз.

3. Подкожное введение кроликам кварцевой пыли сопровождается снижением уровня альбуминов, постепенным увеличением количества глобулинов (особенно γ-глобулинов) и повышенным содержанием гистамина в крови. При подкожном введении «белой сажи» сдвиги в содержании альбуминов и глобулинов незначительны, однако наблюдается увеличение содержания α-глобулинов, снижение β-глобулинов при нормальном количестве γ-глобулинов сыворотки. Наряду с этим отмечается незначительное увеличение количества гистамина крови.

4. Кварцевая пыль, введенная белым крысам, предварительно иммунизированным брюшнотифозной вакциной, вызывает у них через 2 месяца несколько бо́льшие сдвиги в содержании альбуминов, β- и γ-глобулинов сыворотки, чем у неиммунизированных животных. Однако морфологические изменения в легких и бифуркационных лимфатических узлах у животных этих групп одинаковые.

ЛИТЕРАТУРА

Бархад Б., Влад А., Дрон Ф. Труды симпозиума по проблеме пневмокониозов (этиология и патогенез). М., 1959, стр. 38.—Гельфон И. А. Некоторые биохимические изменения в крови при силикозе и силикотуберкулезе. Дисс. канд. М., 1958.—Движков П. П., Кочеткова Т. А., Хухрина Е. В. В кн.: Борьба с силикозом. М., 1957, т. 4, стр. 98.—Дмитренко М. Т., Малинина-Пущенко В. П. В кн.: Сборник работ и авторефератов по вопросам гигиены труда и профпатологии в горнорудной, химической и машиностроительной промышленности. Харьков, 1958, стр. 41.—Егорова К. Т., Буткина Т. Н. Тезисы докл. 3-й конференции Уральск. филиала Всесоюзн. о-ва патофизиологов. Свердловск, 1954, стр. 12.—Зайденберг Р. А. В кн.: Пневмокониоз. Киев, 1954, стр. 128.—Иванов В. И. Труды симпозиума по проблеме пневмокониозов (этиология и патогенез). М., 1959, стр. 43.—Успенский В. И. Арх. пат., 1950, в. 6, стр. 26.—Он же. Роль гистамина в патогенезе экспериментального пневмосклероза. Дисс. канд. М., 1951.—Baldi J., Boselli A., Med. Lavoro. 1953, v. 44, p. 501.—Kalman E., Egészségtudomany, 1957, N. 1, стр. 43.—Pernis B., Battigelli M., Med. Lavoro, 1955, v. 46, p. 1.—Scheel L. D., Smith B., van Riper J. et al., Arch. ind. Hyg., 1954, v. 9, p. 29.

CHANGES IN THE BLOOD PROTEIN FRACTIONS AND HISTAMINE UNDER THE EFFECT OF AMORPHOUS AND CRYSTALLINE SILICA

(An experimental investigation)

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In intratracheal administration of quartz dust the following changes are noted in the blood of albino rats: some rise of the total protein, reduction, of the albumin content a rise of globulins and an increase of the histamine content in the pulmonary tissue. Upon silica gel administration the changes in the protein blood composition and histamine in the lungs of rats were less pronounced and occurred at a later period. Cellular-dust foci differing from silicotic nodules, without the development of sclerosis for 7 months observation period occured in the lungs. Subcutaneous administration of quartz dust to rabbits is accompanied by albumin reduction and a rise of glubulins (especially of the gamma-globulins) and a rise of the blood histamine content. In subcutaneous injection of "white soot" the shifts in the albumin content and in the globulin sum were insignificant. However, there was a rise of the alpha-globulins, reduction of the beta-globulins with a normal content of the gamma-globulins. The rise of histamine was less pronounced than after the administration of quartz dust. Greater changes in the beta- and gamma-globulin fraction were provoked by quartz dust administration in the previously immunized than in the nonimmunized rats

AMA Arch. Ind. Health. 17: 204-209, 1958

Studies on the Toxicity of Silicic Acid

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Introduction

Among the more important theories of the developmental mechanism of silicosis, the "solubility theory" has aroused most interest. It was first advanced by Gye and Kettle [1] and has since been discussed mainly by King.[2] As a result of this interest, the chemistry and the biologic effects of silicic acid have received detailed study in recent years. The investigations, however, have been hampered by the difficulty of producing well-defined silicic acids.

It has been the purpose of this investigation to study the toxicity of freshly prepared supersaturated solutions of silicic acid and to follow the change in toxicity when these solutions are permitted to age and colloidal polymerized silica and/or a gel is eventually formed.

Survey of the Literature

It has long been known that silicic acids precipitate protein (Graham [3]). Mylius and Groschuff [4] showed that a solution of silicic acid of low molecular weight (*α-Kieselsäure*), produced by neutralizing a dilute solution of sodium silicate, did not precipitate protein until the solution had aged and higher polymers (*β-Kieselsäure*) had formed. Subsequently the term "oligosilicic acid" was introduced [5-7] for monosilicic and other lower silicic acids, and "polysilicic acid" for the protein-precipitating forms. In the literature relating to the chemical aspects of silicosis, silicic acids of low molecular weight which give the ammonium molybdate reaction to oligoacids are now generally called AMR silica (ammonium molybdate reactive silica). All higher acids which do not show this reaction are called polysilicic acids, or CP silica (colloidal polymerized silica). Weitz, Franck, and Schuchard [8] observed that monosilicic acid reacts completely with molybdic acid within 75 seconds at 20 C, while disilicic acid reacts completely within 10 minutes and higher silicic acids require still more time. It thus seems probable that the chemically inexact designation AMR silica comprises in the first place monosilicic and disilicic acids and, possibly, also trisilicic acid.

That colloidal silica has an acute toxic effect was first demonstrated by Gye and Purdy [9] and has later been confirmed by others.[10-12] Dale and King,[11] summarizing, state that earlier experiments "have seemed to imply that at a certain stage of division of 'colloidal' size silica become intensely toxic, that larger-sized particles (from about 0.1 to 10μ) produce the slow chronic fibrogenic response of silicosis and no acute toxic effect, and that molecularly dispersed silicic acid is freely diffusible through animal tissues and membranes and is nontoxic and readily excreted by the kidney," and that "the acutely toxic pharmacological effect of silica is a matter of particle size and is given only by suspended silica of colloidal dimensions." Scheel, Fleisher and Klemperer [13] found that the toxicity was manifest only if the solid phase was present in the tissue. They also stated that "silicic acid (H_2SiO_3) does not produce a toxic action in the body, does not react with the proteins, and is rapidly excreted from the body via the urine." Klosterkötter and Jötten [14] pointed out "dass die reine molybdataktive Kieselsäure unschädlich ist."

Received for publication May 17, 1957.

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The biologic effect of presumptive monosilicic acid has generally been studied with inexactly defined lower silicic acids (0.10 to 0.17 mg. of SiO_2 per milliliter in saturated equilibrium solution).[12,13,15] As a rule, the solutions were not electrolyte-free and their pH was not always defined. These factors may have influenced the degree of polymerization of silicic acid and may thus explain the highly varying results.

Monosilicic acid solution has never been produced (Her[16]). The dialysis procedure used by previous investigators for obtaining electrolyte-free silicic acid solutions is time-consuming, and most determinations are made after a lapse of time sufficient to change radically the aggregation of the monosilicic acid into higher acids. Now, however, AMR silica of very low polymerization can be produced in electrolyte-free aqueous solution. Alexander[17] described the preparation of a 0.1 M supersaturated solution of almost pure monosilicic acid by the reaction of crystalline sodium metasilicate with the hydrogen form of a cation-exchange resin at about 0 C. The degree of polymerization of the monosilicic acid in the freshly prepared solution was about 1.1 as determined by a cryoscopic method.

Willstätter, Kraut, and Lobinger[7] showed that polyhydric compounds have the capacity to delay considerably the polymerization of silicic acid.

Methods

Preparation of Silicic Acid.—As ion exchanger we used the hydrogen form of Dowex-50 (an ion-exchange resin) (50 to 100 mesh), which had previously been washed with 5% HCl and then carefully washed free from Cl-ions. The resin, about 60 gm. was packed in a standard type column (according to Samuelson[18]) with a diameter of 27 mm. The sodium metasilicate solution was made from pulverized crystalline $Na_2SiO_3 \cdot 9\ H_2O$, Baker analyzed reagent. The silicate solution (usually 250 ml. of 0.1 M sodium silicate solution) was prepared immediately before pouring it through the column, in order to retard the polymerization. The first 50 ml. from the column was discarded and about 150 ml. of the supersaturated silicic acid solution was collected. The pH of the freshly prepared acid was about 4.5 and was found to remain about the same throughout the period of observation. The acid was obtained in the form of a clear solution free from undesired electrolytes. No additions were made to the solution. As a rule the biologic investigation was begun within half an hour after the preparation of the acid.

The silica content in the solution was determined by evaporating a sample of the solution to dryness, heating at 900 C for two hours, weighing and fuming off the silica with hydrofluoric acid, igniting, and reweighing. No potassium could be demonstrated in the solution.

Immediately after preparation, some of the solution was diluted with an aqueous solution of hydroxyethylcellulose, so that the mixture contained 0.44 mg. of cellulose derivative per milliliter. Ten to fifteen minutes after this dilution the mixture became slightly opalescent.

Determination of the Degree of Polymerization.—The silicic acid was prepared at room temperature. Its content of AMR silica was determined by the method of King[19] immediately after preparation and also during the following days.

Determination of Toxicity.—For biologic testing of the toxicity of the silicic acid at various intervals, we used the technique of Dale and King,[11] with fractionated injections into the tail vein of unanesthetized mice until the animals died. The interval between the single injections was one minute. The solution was diluted immediately before the tests so as to produce death after 3 to 5 injections, a method which earlier studies (Swensson, Glömme, and Bloom[20]) had shown to be appropriate.

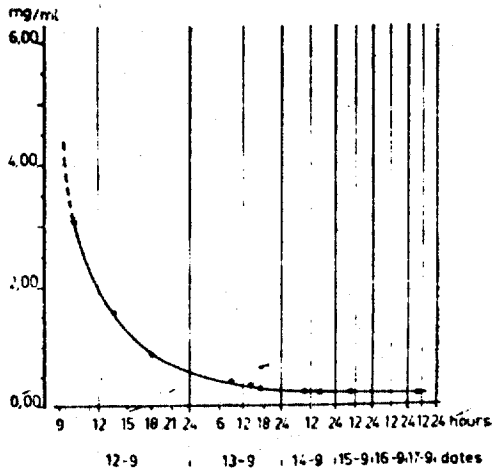

Fig. 1.—Content of AMR silica in the solution at various times after the preparation. Ordinate: milligrams of anhydrous SiO_2 per milliliter of solution.

Results

Degree of Polymerization.—About 10 minutes after preparation of the solution, AMR silica comprised approximately 50% of its total SiO_2 content, but then diminished rapidly (Fig. 1). After 48 hours the content of AMR silica was practically constant and approached the saturation value for silicic acid in water, i. e., about 2 mM. solution (0.120 to 0.140 mg. SiO_2 per milliliter).

The degree of polymerization of CP silica is difficult to determine, and we were unable to make any such assessment. It is probable that mixtures of acids were present which gradually gained in molecular weight.

Toxicity.—The mean lethal dose of the silicic acid within half an hour after the preparation was 5.4 mg. SiO_2 per 30 gm. mouse. The same value was obtained in tests with two solutions of silicic acid prepared on separate occasions and with somewhat dissimilar concentration, viz., 7.44 and 8.48 mg/ml.

As the silicic acid aged, its toxicity showed a fairly rapid increase. After about a month the lethal dose was roughly one-eighth of that originally found. There was then a slow, continuous rise in toxicity throughout the 80 days of the observation period. At the end of that time the lethal dose was about 1/20 of the initial value (Fig. 2). The lethal dose after 80 days was comparable with that found by Swenson, Glönnne, and Bloom[20] for very fine particled (0.01 to 0.02μ) amorphous silica. The toxicity of the latter was approximately 0.20 mg. per 30 gm. mouse, as compared with the maximum of 0.28 mg. per 30 gm. in the present experiments.

When hydroxyethylcellulose was added, the toxicity of the solution rose from 5.4 mg. SiO_2 per 30 gm. mouse immediately before the addition to 3.8 mg. about 15 minutes afterward. This toxicity level and also the appearance of the solution, remained largely unaltered when it was stored at room temperature up to three months.

Comment

The degree of polymerization of the acid used in our experiments initially was higher than that reported by Alexander[21] (1.1 to 1.5). This was probably because our solutions of silicic acid were prepared at room temperature.

During the first two days (3000 minutes) after preparation of the solution, we followed indirectly the course of polymerization by determining the content of AMR silica. The diminution of this acid can be expressed in the equation $1/c - 1/c_0 = kt$, where c = the concentration of AMR silica in milligrams of anhydrous SiO_2 per milliliter of solution, c_0 = the total concentration of

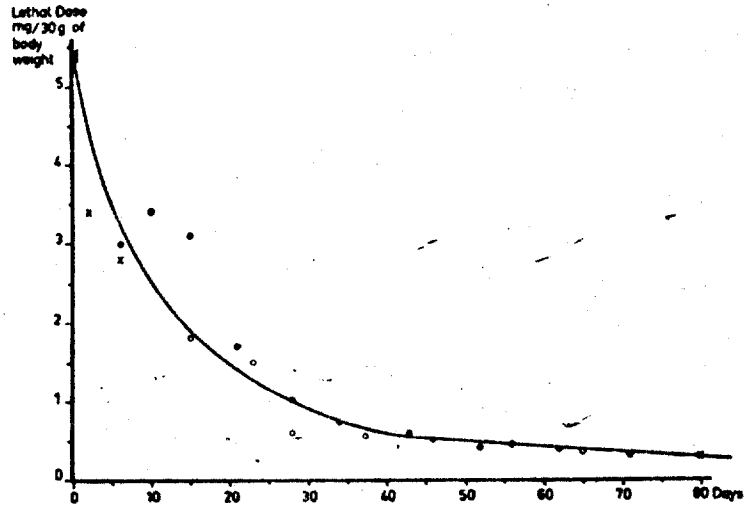

Fig. 2.—Acute toxicity in the mouse of the solutions of silicic acid at various times after the preparation. Lethal dose is given as milligrams of anhydrous SiO_2 per 30 gm. of body weight. Each determination represents the mean of 8-15 mice. The markings (·, O, X) represent solutions of different concentrations, viz., 6.08, 7.44, and 8.48 mg. SiO_2/ml.

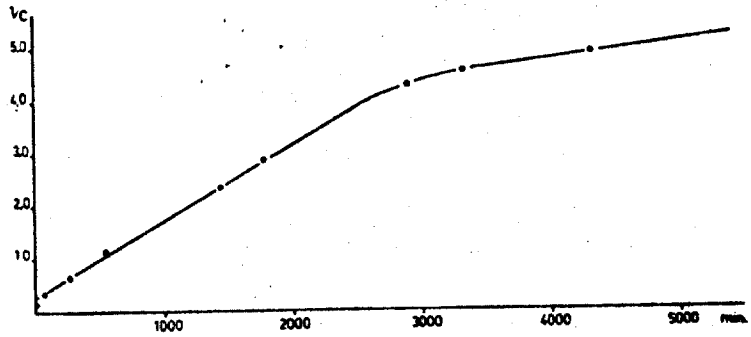

Fig. 3.—Polymerization of AMR silica. The curve shows the diminution of AMR silica with increase of time after preparation of the solution. The curve shows that the polymerization initially follows a second-degree reaction. C = AMR silica as milligrams of anhydrous SiO_2 per milliliter of solution.

SiO_2 in milligrams per milliliter, t = the time in minutes, and k = a constant: $1.6 \cdot 10^{-3}$ ml/min. mg. (Fig. 3). This is in conformity with Alexander's[21] observation that the polymerization of monosilicic acid within the pH region in question is a second-order reaction. After two days the content of AMR silica approached asymptotically the value for the equilibrium concentration.

Schwarz and Knauff[22] prepared a solution of silicic acid containing 12.7 mg. of SiO_2 per milliliter by hydrolysis of an orthosilicate methyl ester and studied it cryoscopically. After three to six days the silicic acid was present mainly as pentasilicic and hexasilicic acids. These writers also assumed the existence of a tetrasilicic acid. Sillén and Lagerström,[23] from E. M. F. (electromotive force) measurements on silica acid equilibrium in a constant ionic medium and subsequent mathematical treatment according to Sillén,[24] also found evidence for a relatively dominant ring-shaped tetrasilicic acid in equilibrium with other complex polynuclear acids. Alexander[21] reported a polymerization degree of about 5 in a 60-hour-old solution of silicic acid at pH 4.36 and at 1.9 C. This corresponds to a molecular weight of approximately 300 on an anhydrous SiO_2 basis.

Willstätter, Kraut, and Lobinger[6] reported a molecular weight of more than 1000 after six days; they considered that most of the unpolymerized acid was present as a disilicic acid. Brintzinger[25] hydrolyzed tetraethyl orthosilicate and found a molecular weight of 290 after 2 days, corresponding to a pentasilicic acid; after 45 days the molecular weight was 8260.

It is thus clear that rising toxicity of silicic acid in animal experiments must be considered against the background of increasing polymerization of the acids and higher molecular weight of the mixture in solution, perhaps up to 1000-10,000. The possibility of the latter weight receives support from the investigations of Baumann,[26] in which molecular weights up to 30,000 were obtained by centrifugation of acids prepared by dissolving Aerosil (a brand of silicic acid).

We made no attempt to hinder appreciably the polymerization of the monosilicic acid. Alexander[21] found that the monosilicic acid in his experiments was most stable in solution at 2 C and pH about 3.2. By preparing and storing our solutions at low temperature and by adjusting the pH to 3.2, we obviously could have increased its content of AMR silica during the first days.

As the silicic acid solutions aged, gelling gradually commenced; it was first discernible after 8 to 14 days. The gelling was progressive. Some of the gel became fixed to the walls of the flask, so that the content in the "solution" was somewhat lower towards the end of the experiment than at its beginning. The observed lethal dose in the terminal experimental period was thus somewhat lower than the figure reported, but this has no essential significance for the results, which showed rising toxicity with the age of the solution and with the resultant polymerization of the silicic acid.

Hydroxyethylcellulose was added to study the toxicologic effect of possible hydrogen bonding, since Iler[27] has stated that such complexes form between some hydrogen bonding agents and silicic acid. We found that addition of hydroxyethylcellulose very rapidly produced a rise in toxicity and some opalescence, which may reasonably be attributed to initially accelerated polymerization. The toxicity subsequently was unaltered. For comparison, it may be mentioned that Alexander[21] demonstrated acceleration of the rate of polymerization by addition of another polyhydric compound, mannitol.

Our findings may thus indicate that a primarily formed hydrogen bonding between the hydroxyethylcellulose and the silicic acid hindered continued polymerization. A similar mechanism may be presumed to have operated in the diminution of toxicity observed by Klosterkötter,[28] when polyvinylpyrrolidone was added to amorphous silica of colloidal dimensions.

Summary

A practically pure silicic acid in supersaturated solution was produced by ion exchange.

The content of low polymerized AMR (ammonium molybdate reactive) silicic acid was observed during the first days after preparation. The concentration fell rapidly, and after two days was almost equal to the equilibrium concentration.

The polymerization and formation of CP (colloidal polymerized) silica continued, and gel formation was observed after about two weeks.

The primarily formed, low molecular silicic acid had a low toxicity; the lethal dose for mouse was 5.4 mg/30 gm. on fractionated injection intravenously.

As polymerization progressed, the toxicity increased, rapidly at first and then more slowly.

After 80 days the lethal dose, 0.28 mg/30 gm. mouse, was similar to the toxicity of very fine synthetic amorphous silica (average particle size about 0.010μ), i.e., 0.2 mg/30 gm.

Addition of hydroxyethylcellulose to delay polymerization gave some immediate rise in toxicity, which was not subsequently increased.

REFERENCES

1. Gye, W. E., and Kettle, E. H.: Silicosis and Miners' Phthisis, Brit. J. Exper. Path. 3:211-25, 1922.

2. King, E. J.: Solubility Theory of Silicosis, Critical Study, Occup. Med. 4:26-49, 1947.

3. Graham, T.: On Liquid Diffusion Applied to Analysis, J. Chem. Soc. 15:216-270, 1862.

4. Mylius, F., and Groschuff, E.: α- und β-Kieselsäure in Lösung, Ber. deutsch. chem. Gesellsch. 39:116-125, 1906.

5. Willstätter, R.; Kraut, H., and Lobinger, K.: Zur Kenntnis der Kieselsäure (IX. Mitteilung über Hydrate und Hydrogele), Ber. deutsch. chem. Gesellsch. 58:2462-2466, 1925.

6. Willstätter, R.; Kraut, H., and Lobinger, K.: Über die einfachsten Kieselsäuren, mit Bemerkungen über Aluminiumhydroxyde (XI. Mitteilung über Hydrate und Hydrogele), Ber. deutsch. chem. Gesellsch. 61:2280-2287, 1928.

7. Willstätter, R.; Kraut, H., and Lobinger, K.: Zur Kenntnis der Monokieselsäure und der Kieselsäure (XII. Mitteilung über Hydrate und Hydrogele), Ber. deutsch. chem. Gesellsch. 62:2027-203[illegible], 1929.

8. Weitz, E.; Franck, H., and Schuchard, M.: Silicic Acid and Silicates, Chem. Ztg. 74:256-25[illegible], 1950.

9. Gye, W. E., and Purdy, W. J.: The Poisonous Properties of Colloidal Silica: I. The Effects of the Parenteral Administration of Large Doses, Brit. J. Exper. Path. 3:75-85, 1922.

10. Modell, W. C., and Salzman, C.: Pharmacology of Silica: Effects and Mechanism of Action of Fatal Intravenous Doses of Silicic Acid, J. Lab. & Clin. Med. 26:774-785, 1941.

11. Dale, J. C., and King, E. J.: Acute Toxicity of Mineral Dusts, A. M. A. Arch. Indust. Hyg. 7:478-483, 1953.

12. Antweiler, H.: Untersuchungen zur Frage der akuten Organwirkung gelöster Oligo- und Poly-Kieselsäuren im Tierexperiment, Beiträge Silikoseforsch. 29:17-52, 1954.

13. Scheel, L. D.; Fleisher, E., and Klemperer, F. W.: Toxicity of Silica: I. Silica Solutions, A. M. A. Arch. Indust. Hyg. 8:564-573, 1953.

14. Klosterkötter, W., and Jötten, K. W.: Die Wirkung verschiedener Kieselsäure-Formen im Tierexperiment, Arch. Hyg. 137:625-636, 1953.

15. Lühning, W.: Über pharmakologische Wirkungen gelöster Kieselsäure mit niedrigem [illegible]

chem Polymerisationsgrad, Beitr. Silikoseforsch. 38:31-49, 1954.

16. Iler, R. K.: The Colloid Chemistry of Silica and Silicates, Ithaca, N. Y., Cornell University Press, 1955.

17. Alexander, G. B.: The Preparation of Monosilicic Acid, J. Am. Chem. Soc. 75:2887-2888, 1953.

18. Samuelson, O.: Ion Exchangers in Analytical Chemistry, New York, John Wiley & Sons, Inc., 1953.

19. King, E. J.: The Colorimetric Determination of Silicon in Micro-Analysis of Biological Material and Mineral Dust, Analyst 80:441-453, 1955.

20. Swensson, A.; Glömme, J., and Bloom, G.: On the Toxicity of Silica Particles, A. M. A. Arch. Indust. Health. 14:482-486, 1956.

21. Alexander, G. B.: The Polymerization of Monosilicic Acid, J. Am. Chem. Soc. 76:2094-2095, 1954.

22. Schwarz, R., and Knauff, K. G.: Über Alkoxysilane und Oligokieselsäuren, Ztschr. anorg. u. allg. Chem. 275:176-192, 1954.

23. Sillén, L.-G., and Lagerström, G.: Personal communication to the authors.

24. Sillén, L.-G.: On Equilibria in Systems with Polynuclear Complex Formation: I. Methods for Deducing the Composition of the Complexes from Experimental Data. "Core + Links" Complexes, Acta chem. scandinav. 8:299-317, 1954; II. Testing Simple Mechanisms Which Give "Core + Links" Complexes of Composition $B(A_cB)_n$, ibid. 8:318-335, 1954.

25. Brintzinger, H., and Brintzinger, W.: Zur Kenntnis der molekulardispers gelösten Kieselsäuren und Titansäuren, Ztschr. ang. Chem. 196: 44-49, 1931.

26. Baumann, H.: Über die Auflösung von SiO_2 in Wasser, Beitr. Silikoseforsch. 37:45-71, 1955.

27. Iler, R. K.: Association Between Polysilicic Acid and Polar Organic Compounds, J. Phys. Chem. 56:673-677, 1952.

28. Klosterkötter, W.: Weitere Untersuchungen über die Gewebswirkung kolloidaler und molekular gelöster Kieselsäure, Arch. Hyg. 137:307-316, 1953.

Br. J. Exp Path: 3 (75-85) 75 - 1922

THE POISONOUS PROPERTIES OF COLLOIDAL SILICA. I: THE EFFECTS OF THE PARENTERAL ADMINISTRATION OF LARGE DOSES.

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Received for publication December 22nd, 1921.

THE starting-point of the investigation described in the present series of papers was an observation made in 1918 by one of the authors (W. E. G.) when working at the subject of anaërobic infections, namely, that intraperitoneal injections of small doses of freshly prepared colloidal silica in mice are lethal.

During the last twenty years a considerable number of medical memoirs on silica have been published; for the most part they are of chemical and therapeutic interest. The majority of the chemical investigations have been merely the mechanical estimation of the percentage of silica in the organs, tissues and fluids of the animal body. The figures published by different observers show great discrepancies, but there is general agreement that silica is a frequent—and possibly a constant—constituent of most of the tissues of the body. Schulz (1903) concludes from the results of a large number of analyses that silica is an essential constituent of connective tissue, and that in young animals the proportion of silica present is greater than in older animals. Kahle's (1921) figures for the analysis of the pancreas do not support this view however. Schulz, Kühn and other investigators believe that silica is metabolised in the body.

In Germany silicic acid, in one form or another, is used in the treatment of pulmonary tuberculosis (Kobert, Kühn (1921), and others). The substance is administered by mouth or by intramuscular injection. The basis of the treatment appears to be, first, that almost immediately following an injection of a small dose there is a considerable increase in the number of leucocytes in the peripheral blood; and secondly, that silica stimulates the production of connective tissue—presumably specifically around the tubercular lesions. A critical examination of the treatment has been made by Roth (1921), whose paper contains a long list of references.

Sodium silicate has been employed by Scheffler, Sartory and Pellissier (1920) and by Kühn (1921) in the treatment of arteriosclerosis.

In spite of the stimulus which the use of a drug in practical medicine gives to laboratory investigations, there has been no systematic and thorough study of the pathological effects which follow the administration of colloidal silica.

Friedenthal (1900), in a footnote to a paper on the passage of colloids through the intestinal wall, states that silicic acid when injected intravenously may kill an animal; he attributes death to the formation of insoluble precipitates of calcium and silica. Kobert utters a warning against the too rapid injection of silicic acid into the blood-stream, pointing out the possibility of the occurrence of embolism. Zuckmayer (1921) quotes an experiment of Siegfried in which a cat died 40 hours after being injected intravenously with 81 mgrm. of silica sol—20 mgrm. per kilo body-weight.

The same author has studied the effects of administering small doses parenterally in dogs. His investigations were stimulated and limited by the consideration of silica as a drug, and give very little information.

Before describing the results obtained by injecting silica sol by different routes, a brief account of the elementary chemistry of silica will be given.

CHEMISTRY OF SILICA.

Silica (SiO_2) occurs abundantly in nature in an amorphous form, and also in a crystalline condition. It is insoluble in water and in all acids except hydrofluoric acid; it can be dissolved by alkalis, *e. g.* by boiling the amorphous powder in a solution of sodium carbonate, to form alkaline silicates, which are easily decomposed by weak acids.

Silica may be obtained in the colloidal state either as a sol or a gel, the form depending upon the extent of hydration of the silica molecule. The sol form, usually known as "orthosilicic acid," to which the formula $Si(OH)_4$ is assigned, may be filtered through a Berkfeld candle, and can be sterilised by boiling or in the autoclave. The gel form, "metasilicic acid," with the formula H_2SiO_3, can be prepared directly by the action of acids on alkaline silicates, or may be obtained by allowing the sol form to gel—a process which is irreversible; that is to say, it is not possible to obtain afresh a solution of colloidal silica from one which has been allowed to gel.

The description of these colloids as acids is not justified by the properties of the substances themselves; it is convenient to regard them as acids in order to provide a satisfactory classification of the mineral silicates. Strictly speaking, however, it is probably an error to put them in the same list as the weak acids (Le Chatelier, 1914). They are practically neutral to litmus, and have a faint acrid taste which persists.

The circulation of silica in nature depends upon the formation and ready decomposition of hydrated silica and of complex-balanced solutions of the alkaline silicates.

When used in this and the following papers the terms "soluble silica" and "silica sol" will refer to orthosilicic acid, and the term "silica gel" will refer to metasilicic acid. The strength of the respective substances will be given as the weight of silica (SiO_2) contained in 100 c.c. of the sol and in 100 grm. of the gel. The silica sol used throughout this investigation was prepared by adding a dilute solution of sodium silicate—"waterglass"—to an excess of strong HCl, and then freeing the mixture of the HCl and NaCl by dialysis. The water-clear sol was then filtered through a Berkfeld candle and stored in test-tubes.

THE EFFECT OF THE INJECTION OF LARGE DOSES OF SOLUBLE SILICA.

Subcutaneous Injections.

When 0·1 c.c. of a 1 per cent. solution of orthosilicic acid is injected under the skin of a mouse an inflammatory reaction occurs (Bullock and Cramer, 1919); the blood-vessels at the site of the injection become dilated, fluid is poured out, large numbers of white cells and a few red blood-corpuscles escape into the œdematous area, which is to the naked eye a flat, colourless, gelatinous swelling from one-half to one square centimetre in size.

Intraperitoneal Injections.

The effects obtained by intraperitoneal injections vary with the degree of dispersion of the silica sol. The freshly-prepared sol is much more toxic than one which has been kept for a long time, *e.g.* three months. The sol always tends to gel; first the viscosity increases, and then, with the lapse of time, definite gelation begins, and proceeds rapidly until all the sol is converted into gel. These changes may be retarded by means of traces of acid or alkali or by a protective colloid such as albumin, but they cannot be prevented. Coincident with this the toxicity in intraperitoneal injections decreases, whilst for intravenous injections the reverse is the case, as will be shown later. It is evident, therefore, that any quantitative statement of poisonous doses must be qualified by a description of the state of the sol. With regard to intraperitoneal injections it may be stated (1) that doses of 1 to 2 mgrm. of freshly-prepared sol kill a mouse, and that 30 mgrm. kill a guinea-pig of 250 grm. weight; (2) that the sol which has been allowed to become opalescent and whose mobility has diminished kills a mouse in doses of 5 mgrm. and a guinea-pig in doses of 50–60 mgrm.; and (3) that the gel does not kill in doses which it is practicable to administer.

There is another difference in action between the highly-dispersed sol and that which has stood for some time. The effects of the former are shown within a few minutes after injection, while the latter produces no symptoms within 12 hours. These points are illustrated in the following protocols.

Experiment 20 G.—October 10th, 1921: A sample of silica sol, which had been dialysed against distilled water until the chlorides had been removed, was filtered through a Berkfeld candle, and immediately injected intraperitoneally into mice in the doses indicated below. Subsequent analyses of the filtered sol showed the concentrations to be 1 per cent.

Mouse No.	Dose injected.	24 hrs. after.	48 hrs. after.
1	0·1 c.c.	†	—
2	0·1 c.c.	Very ill	†
3	0·1 c.c.	Very ill	Dying. Killed at 52 hrs.
4	0·2 c.c.	Very ill	†
5	0·2 c.c.	†	—
6	0·2 c.c.	Very ill	†

Within five minutes of the injection all the animals became quiet and reluctant to move; later they began to shake and the legs became unsteady. After an hour the mice recovered and then appeared to be quite well.

Post-mortem there was nothing of special importance to note beyond a dilatation of the intestines, the peritoneal surface of which showed here and there fragments of fibrin. Films of the peritoneal exudate contained a large number of lymphocytes, polymorphonuclear leucocytes, endothelial cells and a few red blood-corpuscles. In some of the animals a fair number of bacteria —chiefly a Gram-negative bacillus—were present, in others few or none.

Experiment 21 G.—October 10th, 1921: Six mice were injected intraperitoneally, each with 0·3 c.c. of 1 per cent. silica sol which had been kept for 3 months; the sol was faintly opalescent. The animals remained active and well after the injections. Twenty-four hours afterwards all six mice were ill; 48 hours after the injection 5 had recovered, 1 was dead.

Guinea-pigs are affected in the same way after the injection of larger doses—from 3 to 5 c.c. of 1 per cent. sol, made isotonic with salt immediately before use. When the sol is freshly prepared the injected animals become ill within a few minutes; they often display a curious rhythmical jerking of the head which may persist for half an hour. The legs are spread out in a helpless fashion, the respirations become rapid, and the animals appear moribund. When the animals are disturbed, however, they move away, thus indicating that the distress is temporary. In 30 to 40 minutes they recover and afterwards appear to be quite well. Death may take place in 24 hours or may be delayed for several days.

We are not yet able to state definitely the cause of death. At first we were inclined to attribute it to a septic peritonitis, but this explanation must be rejected. Whilst in a fair proportion of the animals—and especially mice, which are prone to infection—bacteria are found *post-mortem* in large numbers in the peritoneal exudate, in many the numbers found are few or none. In guinea-pigs which die 4 to 7 days after injection the intestines are matted together by fibrinous material in which no organisms are found; the liver and kidneys are pale, and show, when examined microscopically, degenerative changes.

Intravenous Injections.

The results which follow the intravenous injection of silica sol vary with the method of administration, whether, for example, the total dose is administered at once or in fractions at shorter or longer intervals.

An animal may be killed in a few minutes by a single large dose which, for the same sample of sol, is fairly constant, body-weight for body-weight, for rabbits, rats, guinea-pigs and mice. With the freshly prepared sol the lethal dose is roughly 100 mgrm. per kilo body-weight. When the sol has been kept for a long time and gelation has begun the killing dose is less, a mechanical factor probably being added to the toxic. Also when the concentration is high, *e. g.* 3 per cent., the sol is more effective than when the concentration is low. This probably is only another way of stating that gelation reduces the killing dose, since the higher the concentration the more rapidly the sol gels. The cause of death in these cases is intravascular clotting.

When a single lethal dose is administered in fractions at short intervals, *e. g.* every half hour, the animal remains quite well until the last two or three portions are injected. After the last fraction it succumbs.

If the sol be given in large but sublethal doses—40 to 60 mgrm. for a rabbit—at daily intervals, the animal dies after 3 or 4 days, and under these conditions death is due to profound degenerative changes of the liver, kidneys and other organs, together with hæmorrhages in the intestinal mucosa, in the tracheal mucous membrane, in heart-muscle and elsewhere. It is evident that these cases may be separated naturally from those in which death takes place almost immediately after the completion of the injection. We shall, therefore, divide this section into two parts, the first (A) dealing with those cases where death occurs rapidly, and the second (B) with those in which death occurs after several days.

A. *Rapid death.*—The results obtained may be best described by reproducing protocols of experiments.

Experiment 29.—June 21st, 1921: A healthy mouse was injected intravenously with 0·3 c.c. of 1 per cent. silica sol. The solution was injected very slowly. The mouse appeared to be perfectly well when the injection was completed and walked about normally. Two minutes afterwards the animal became unstable on its hind legs, which shook when it attempted to walk; the weakness spread rapidly to the fore limbs, the animal became convulsed and died. The mouse was dead within three minutes from the time of completion of the injection.

A *post-mortem* examination was made at once. The right side of the heart and the large venous trunks were distended with clotted blood; the left ventricle was empty.

The lethal dose varies from 1 to 3 mgrm. When the solution of silica has begun to gel, as is shown by the development of a faint opalescence, the mouse may be killed by a dose of 1 mgrm.; 2 mgrm. of a freshly-prepared solution is usually supported by a mouse of 20 grm. weight. When death occurs immediately it is due to intravascular clotting. Sometimes, and especially after the injection of a dose bordering on the lethal, *e. g.* 2 mgrm. of freshly-prepared solution, an animal may become ill and die in from 2 to 24 hours. The cause of death in these cases is not always easy to determine. The only lesion which we have found microscopically is a necrosis of the convoluted tubules in the kidney.

The next experiment illustrates the effect of a dose which was barely lethal.

Experiment 12 G.—March 14th, 1921: Six mice were injected intravenously, each with 2 mgrm. (in 0·2 c.c. of saline) of soluble silica. On the morning of March 15th two were found dead and were thrown away. One mouse was very ill and was killed with chloroform. *Post-mortem* there was an unusual redness of the subcutaneous tissues; the liver was pale, the gall-bladder distended with dark bile; the intestines were pale, contracted and still; the kidneys were pale, and the bladder was distended. The heart and lungs were normal. Microscopical examination revealed tubular necrosis of the kidney. A fourth mouse was killed on March 17th: this animal also showed necrosis of kidney tubules. The fifth animal died April 9th, 1921, and was discarded.

The last mouse of this series was killed on April 28th, 1921. The kidneys were small and contracted. Microscopic examination showed the replace-

ment by connective tissue of tubules which had been necrosed and had disappeared.

Experiment 28.—June 22nd, 1921: A healthy rabbit weighing 1275 grm. was injected intravenously with a clear 1 per cent. solution of silica, to which was added immediately before use a sufficient volume of saturated salt solution to make the resultant mixture isotonic with blood. Ten cubic centimetres were injected very slowly; the rabbit appearing quite well, a further 9 c.c. was injected. Whilst the last few c.c. were being delivered the animal became rather restless and uneasy. The total dose administered was 190 mgrm. Immediately the injection was completed the rabbit was put on the floor and observed. It began to walk about, but had difficulty with its hind legs; it then lay quiet, then kicked and struggled, rolled over and died. The time which elapsed between completion of the injection and death was 3 minutes.

A *post-mortem* examination was made immediately. The subcutaneous tissues were pallid and no bleeding occurred when skin was cut away. The liver was purple; active peristalsis of the intestines was observed. The vena cava was distended. As the left kidney was cut away a clot came from the renal vein. The lungs were collapsed and firm on section. The right side of the heart was dilated and full of blood-clot, the left side was empty. The pulmonary vessels and the inferior vena cava were filled with clot.

Experiment 35.—June 30th, 1921: A healthy rabbit weighing 1700 grm. was injected intravenously with 2 c.c. of a 3 per cent. silica sol, which was opalescent and less mobile than water. The solution was injected slowly, the injection taking half a minute. The rabbit was restless during the process, and when put on the floor, after the injection was completed, staggered, struggled and died. Death took place two minutes after the beginning of the injection.

The right side of the heart was found to be dilated and filled with blood-clot, the left side almost empty. In this case the dose injected was 60 mgrm.

Experiment 43.—August 14th, 1921: A healthy rabbit weighing 1300 grm. was injected intravenously with 1 c.c. soluble silica of a strength of 1·4 per cent. at 8.35 a.m. and again at 9.10 a.m., 9.50, 10.20, 11.5, 11.40 and 12.20 p.m. The total amount injected was, therefore, 7 c.c., which represented 98 mgrm. of silica.

After the fourth injection the respiration-rate increased. At 11.43 a.m., after the sixth injection, the rabbit was disinclined to move and was panting. At 12.6 p.m. the animal tried to rise, but did not succeed. After the last injection, 12.20 p.m., the animal struggled, took single deep inspirations, rolled on its side and died.

The carcase was examined immediately. The heart was still attempting to beat, the right side was engorged. There was no clot in the right ventricle or inferior vena cava. The blood was dark brown in colour throughout the body.

In a subsequent experiment of the same kind we have found that hæmolysis of the blood occurs, and that the bladder contains urine stained with hæmoglobin.

This and other similar experiments have shown that silica sol when injected in comparatively small doses at short intervals either accumulates

within the blood—that is to say, that excretion is slow—or that lesions that may be caused by small doses are summated. The immediate cause of death in these cases we have not yet elucidated; it is not, however, due to universal clotting. Clot-formation in the smaller vessels we have not excluded.

Our colleague, Dr. Lovatt Evans, very kindly estimated the effect of 100 mgrm. of colloidal silica on the basal metabolism of a full-grown rabbit. The effect was *nil*. It may be concluded, therefore, that a single large dose does not affect, immediately, the metabolic activity of the tissues.

The clotting of the blood which has been described was at first attributed to a direct action of the silica on the blood. This view is easily understood when it is realised how readily a solution of silica gels in the presence of electrolytes. The addition of 2 c.c. of 4 per cent. silica sol to 4 c.c. of broth leads to the formation, in an hour, of a fairly stiff gel, which, when sloped, is suitable as a solid culture medium for bacteria. It is easy to imagine that the colloidal silica injected directly into the blood-stream becomes a gel and that clotting begins round the particles of gel, gradually spreading through the vascular system. This conception is supported by the fact that the sol which has begun to gel acts in smaller doses than the pure sol. When colloidal silica is added to blood *in vitro*, however, the rate of clotting is not accelerated by doses which proportionately are certainly lethal to the animal supplying the blood. The only noticeable effect on the blood is a slight hæmolysis of the red cells. This, of course, was to be expected, since it is known that silicic acid and lecithin together are able to lake red blood-corpuscles.

The rapid production of clot in the blood-vessels of the living animal must, it follows, be due to an action on some other tissue than the blood, and most probably on the vascular endothelium.

The following experiment, one of several, was designed to test this point. It was carried out by Capt. S. R. Douglas (using Wright's method), to whom we desire to express our thanks.

A healthy rabbit, weighing 2·5 kilos, was bled, and the clotting time of the blood taken; this was found to be 6 minutes. The test was repeated in 10 minutes, and the time again found to be 6 minutes. The rabbit was then injected with 50 mgrm. of colloidal silica, made isotonic with blood.

The following observations were then made:

A. 10 minutes afterwards blood was taken: it was very dark in colour; its clotting time was 2 min. 45 sec.

B. 30 minutes afterwards the blood clotted in 2 minutes.

C. 4 hours afterwards the blood clotted in 3¼ minutes.

D. 24 hours afterwards the blood clotted in 8 minutes.

In another experiment, in which the same amount of soluble silica was injected, the clotting time fell from 4 minutes to ¼ minute in 2 hours.

It will be noticed from these experiments that the capacity to clot is rapidly augmented, and that 24 hours later it is diminished. It seems to us impossible to resist the conclusion, which to some extent is supported by the effects of intraperitoneal injections, that colloidal silica acts upon the vascular endothelium.

B. *Slow death.*—In this part of our investigation we have done but few experiments, and the total number are, therefore, given in the following table:

	Wt. of rabbit.	Dose.	No. of doses given.	Result.
1	2100 grm.	24 mgrm.	13	Animal quiet and off its food during the first ten days; tending to recover after 10th day; killed 14th day.
2	1700 ,,	24 ,,	4	† 4 days.
3	1100 ,,	36 ,,	4	† 4 ,,
4	4400 ,,	36 ,,	2	† 3 ,,
5	1050 ,,	60 ,,	3	† 3 ,,
6	2050 ,,	60 ,,	4	† 4 ,,
7	3000 ,,	60 ,,	2	† 4 ,,
8	2850 ,,	60 ,,	2	† 2 ,,
9	2400 ,,	72 ,,	3	† 3 ,,
10	3850 ,,	72 ,,	2	Ill for several days; recovered; killed 14th day.

Thus, of the ten animals used, one alone survived repeated intravenous injections; one, which was injected twice with the largest dose given in the series of experiments, became ill, but was allowed to recover, and was killed later. All the rest died on the third or fourth day. An analysis of the protocols of the separate animals shows that, although there are slight differences clinically in the course of the illness produced—one, for example, showed a conjunctival hæmorrhage, the others did not—and in the *post-mortem* and microscopical findings, the differences are not sufficiently great to warrant a separate description for each animal. A general account of the experiments will be given which is fairly applicable to all.

After the first injection if the dose is 36 mgrm. or more, but after the second injection when the dose is less, the rabbit becomes quiet and listless and loses its appetite. Its weight falls a hundred or even three to four hundred grammes, due probably to the emptying of the intestines and to loss of fluid which is not replaced. There is no regular effect on the temperature of the animal. The condition of debility and lassitude passes on to a pronounced weakness, and the animal dies without exhibiting any premonitory sign that death is imminent. The urine, which at the beginning of the experiment and during the first day contains no albumin, contains, on the third (or fourth) day, an amount which gives a thick cloud on boiling and acidifying. Urine taken from the bladder *post-mortem* contains an enormous number of casts and a few epithelial and red blood-cells.

The most prominent features at *post-mortem* examination are the occurrence of petechial hæmorrhages—in the skin, in the mucosa of the stomach and intestines, the lungs, the thymus, heart muscle and elsewhere—and the evident degeneration of the liver, which is pale and shows a finely mottled surface. The spleen is always enlarged, though to a variable extent, the edges being rounded and the natural depressions and rough markings lost in the general swelling of the organ; the colour varies from a salmon colour to a deep red. The kidney is swollen and pale; on section the larger vessels stand out as deep red points. The lungs are hæmorrhagic. In two animals of the series the ureters showed as red cords, which was found to be due to

hæmorrhages in the submucosa and in the muscular coat. The adrenals are soft and redder than normal; the pancreas, thymus and thyroid are not conspicuously altered. Lymphatic glands, subcutaneous, abdominal and thoracic, are generally swollen and soft and sometimes hæmorrhagic.

Microscopical examination of the tissues confirms these observations. The key to the pathological changes in the organs is found in the destructive action of the silica sol on the endothelial lining of the blood capillaries. This is shown frankly by the occurrence of petechial hæmorrhages, and is demonstrated in sections of organs, especially the liver, in which the vascular endothelium is found in different stages of degeneration, from simple swelling with vacuolation of cytoplasm to desquamation and often complete destruction. We do not wish to draw the conclusion that endothelium alone is poisoned by the sol, and that other pathological effects are merely a consequence of endothelial damage. Other cells are doubtless affected directly by the poison, but from the microscopical findings it is clear that the primary effect is on the vascular endothelium, which is to be expected from the mode of administration. Some of the changes observed are shown in Fig. 1. Large numbers of endothelial cells pass into the general circulation, and are seen in cross sections of blood-vessels. Fig. 2 represents a vein in the kidney, in which 9 of these large cells are seen. The picture is fairly representative of what may be seen in sections of any organ.

The question now arises: Are the lesions observed due to the action of bacteria? In the liver a few Kupfer cells are found containing large numbers of cocci, but the striking feature in respect of bacteria is the occasional occurrence of small masses of cocci—veritably colonies of organisms—in the capillary blood-vessels. They are most frequent in the kidney, liver and spleen, but may be found by persistent searching in most of the organs. The colonies are set in what appears to be altered blood; there is little or no tendency to wander through the capillary walls into or between adjacent cells; and there is no reaction in the tissues round about the masses. With iron hæmatoxylin they stain a more or less uniform black; the individual organisms are best demonstrated by Murray's (1919) nile-blue picrofuchsin method of staining. Fig. 3 represents two glomeruli containing these colonies of bacteria; they must not be taken, however, as typical of the mass of glomeruli in a kidney, the vast majority exhibiting the necrosis of the tuft, or blood and serum in the capsular space, but containing no bacteria. In the case of two animals whose tissues were fixed immediately after death the bacterial masses have not been found.

The formation of these masses of organisms is undoubtedly a terminal phenomenon. The silica sol injected was sterile, and all the usual aseptic precautions were used in performing the operations. Further, the first animal of the series (No. 1 in the table above), after receiving thirteen injections, shows none of these colonies in liver, kidney or spleen.

It has already been stated that the liver and kidney show degenerative changes obvious to the naked eye. The special incidence upon these organs may be due to an attempt on their part to excrete the silica. The liver, in most cases, shows focal necrosis, sometimes to such an extent that more than half the liver parenchyma is dead; in two cases the amount of necrosis was

very small. The distribution of the necrotic areas is not characteristic. It is most frequently in the mid-zone of the lobules, though sometimes the lobule is completely destroyed. There are two kinds of necrosis present, and these may be found side by side. In one the liver-cells are represented by cell *débris* —cell "ghosts"—the columns being widely separated by dilated sinusoids which are packed with altered blood. The general appearance produced is that of a red infarct, but the mode of production of the lesion would appear to be that thrombosis has occurred in the capillaries, with subsequent death of liver-cells.

In the second type of necrosis the liver-cells are not completely destroyed; pyknotic nuclei and vacuolated protoplasm remain. The sinusoids are dilated but contain little or no blood; the endothelial lining is swollen and desquamating. In all cases the areas of necrosis (and in foci in the livers which do not show advanced necrosis) an extreme degree of fatty degeneration is observed. The liver-cells of the normal rabbit show, as a rule, small widely separated particles of fat which are generally situated near the cell boundary; the normal liver endothelium contains fatty particles quite as frequently as the liver-cell proper. Here and there endothelial cells may be found, of which almost the whole cytoplasm stains with sudan. In the degenerated livers of these experiments, however, the stainable fat in the dead and dying liver-cells and in many endothelial cells is enormously increased in quantity and occurs in large globules.

The most conspicuous damage to the kidney is shown by the glomeruli, which are universally affected. The smallest changes observed consist in an extraordinary dilatation of capillaries in the tuft with an exudation of serum into the capsular space; the epithelium lining the capsule is very prominent, often swollen and sometimes desquamated. In the more violent changes the tuft may be completely necrosed, and, as has already been said, colonies of bacteria may be shown in the midst of coagulated blood. Red blood-corpuscles are found in the capsular space and appear in the form of casts in the tubules. The renal epithelium, although showing degeneration here and there, is not so badly affected as would be expected from the violence of the changes in the glomeruli. There is a mild degree of fatty degeneration, which, however, is not so conspicuous in the highly specialised cells of the convoluted tubules as in the cells of the collecting tubules. The ducts of Bellini often show extensive fatty degeneration. Fat is also found in the glomerular tufts. Enormous numbers of casts are found in the tubules, many of which are widely dilated.

The histological changes in the spleen are somewhat variable. In most of the spleens a necrotic effect predominates; in a few an extreme congestion of the organ is most noticeable. In the former type there is an evident disappearance of cells, those remaining being widely separated by a swollen reticulum in which particles of chromatin are found; the lymphoid masses are scanty and often show areas of focal necrosis. In the blood channels large numbers of mononuclear cells with abundant cytoplasm which often contains inclusions are found. In the second type the blood sinuses are distended with blood which contains large numbers of endothelial cells. There is not such evident destruction of cells as in the form already described.

The pathological changes found in the other organs are not sufficiently

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BRITISH JOURNAL OF EXPERIMENTAL PATHOLOGY, VOL. III, NO. 2.

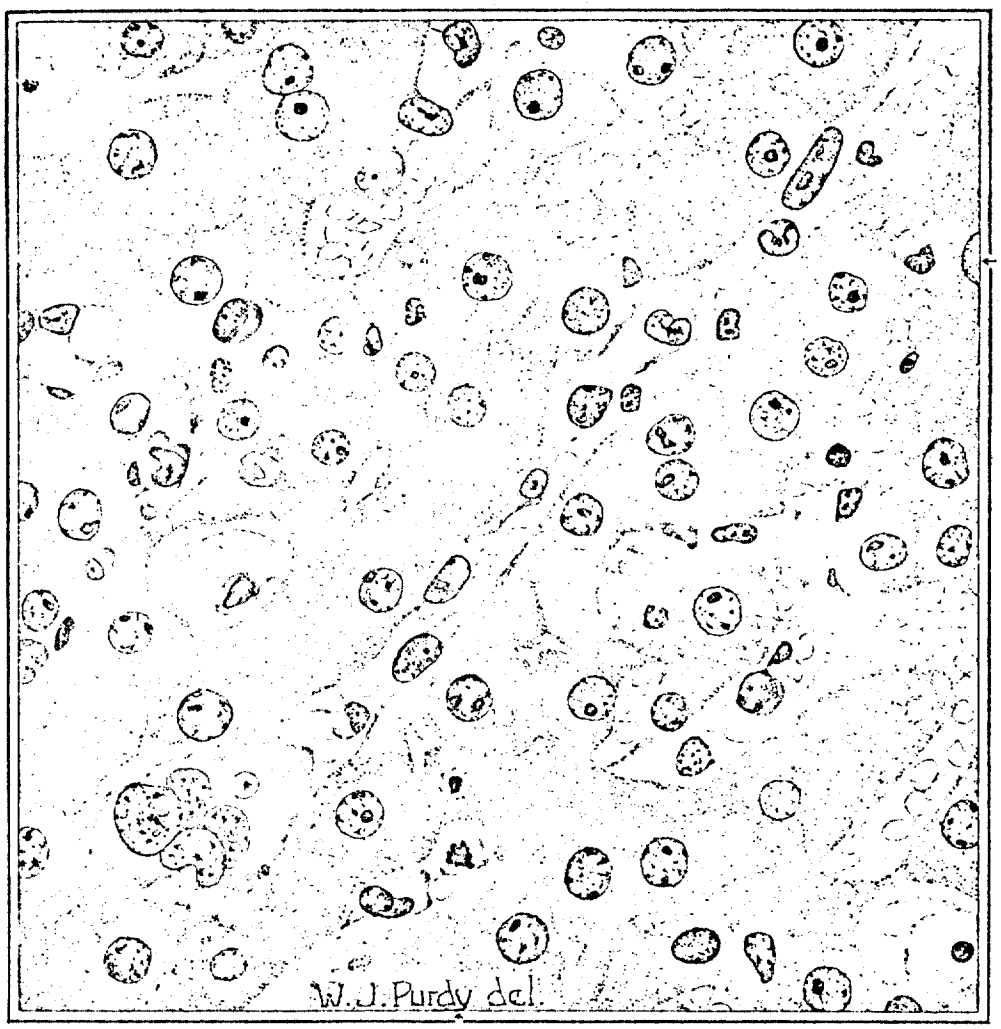

FIG. 1. Scale 830 : 1. — 3

Stained iron-hæmatoxylin and van Gieson.

Liver of Rabbit 76/A which received intravenously for 4 days daily doses of 24 mgrm. of silica. The endothelial cells are increased in number and are considerably changed. They show various degrees of enlargement, vacuolation, and desquamation. Some of those which are free have deeply-staining cytoplasm. Giant-cell formation is seen. Mitotic figures are present in 2 endothelial cells—one opposite each marginal arrow. The liver-cells in the area figured do not show the more extreme changes seen in other parts of the section.

Acta Medica Scandinavica. Vol. 171, fasc. 4, 1962

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Metastatic Carcinoid Tumour Associated with Complicated Silicosis

By

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Since the description by Biörck et al. (1952) and Thorson et al. (1954) of a new clinical syndrome, the carcinoid syndrome or carcinoidosis, numerous reports concerning it have been published. At the present time, when this syndrome has become a clinical entity, the number of reported cases appreciably exceeds one hundred.

The diagnosis of the carcinoid syndrome, which is characterized by diarrhoea, flushing spells, asthmatic attacks, oedema and signs of valvular heart disease, is in most cases not difficult. The diagnosis can be easily verified by demonstrating increased urinary output of 5-hydroxyindolacetic acid, a metabolite of 5-hydroxytryptamine which is released from the tumour tissue. In certain cases, however, a concomitant disease may draw the examiner's attention, and symptoms indicating a carcinoid tumour may be overlooked or interpreted as being caused by the concomitant disease.

In some individuals exposed to silica dust a pneumoconiosis will develop. In others, it either will not develop or will be less severe. Metabolic factors have been suggested as playing a rôle in the development of a pneumoconiosis, but this has not been substantiated (Morrow and Cohen 1959).

The following case presented nearly all the symptoms and clinical manifestations which have been described in connection with metastatic carcinoid tumours. In addition there was a simultaneous complicated pneumoconiosis; this had progressed considerably for several years after the exposure to silica dust had ended.

Case report

The patient was a 53-year-old widow. Starting at the age of 28 she had worked some six years in a china factory. During pregnancy, at the age of 21, she was in poor general condition. She had marked oedema of the legs and face. After a normal delivery the symptoms disappeared, and she was in good health during the following ten years. She had not had other pregnancies, and the menopause occurred at the age of 46. At the age of 31 she lost weight and suffered from fatigue, sweating, palpitation and tremor. A diagnosis of toxic goitre was made and an operation was performed. The symptoms disappeared

Submitted for publication October 9, 1961.

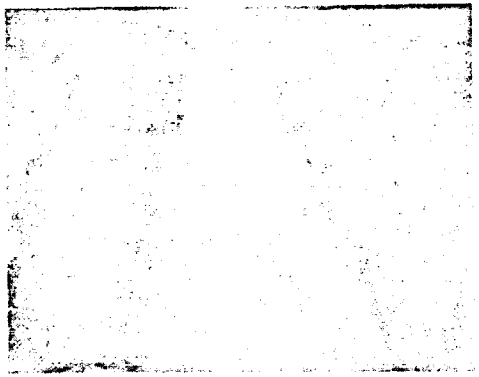

Fig. 1. Chest X-ray showing tumour-like shadows in both lungs.

after the operation. About five years before admission to the hospital she suffered from acute tonsillitis, which was followed by a transient inflammation of the ankle and knee joints. A year later a routine chest X-ray examination revealed a shadow in the left upper lobe near the hilus. A control examination was performed a few months later and a similar shadow was encountered in the right upper lobe. Sputum cultures were negative for tuberculosis. Bronchoscopy did not reveal anything abnormal. For some ten years prior to admission she had had oedema of the legs, especially after longer periods of standing. Two years before admission she developed oedema of her left leg; this was very painful on pressure and was diagnosed as a thrombophlebitis. About one month before her first admission she noticed painful nodules in her left leg and some days later both legs become markedly swollen and tender. About one year prior to admission she developed flushing of the face and neck. The flushing varied very much in frequency and intensity. Sometimes it was accompanied by a burning sensation of the skin. Emotional stress was the only provoking factor which she had noticed. Earlier she had never had flushing, not even during the menopause six years previously. Approximately since the commencement of the flushing she had suffered from asthmatic attacks which were accompanied by respiratory stridor. These attacks were at first unrelated to exertion and appeared usually after midnight, or, in cold and damp weather, during the daytime. The flushing was not usually accompanied by asthmatic attacks. Since October 1958, some three months before her first admission, she had also suffered from exertional dyspnoea, which became gradually worse. Simultaneously with the flushing and asthmatic attacks bouts of diarrhoea started. The bouts at first lasted a day or two and reappeared again every few days. This symptom, too, became gradully worse, and in the few weeks before admission she had several watery evacuations every day. The evacuations were usually more frequent in the forenoon.

She was admitted to the Third Medical Clinic, the University of Helsinki, on December 12th, 1958.

Physical examination

Physical examination on admission revealed a well-developed and well-nourished woman who was in moderately good general condition. There was permanent cyanosis of the lips and multiple teleangiectases of the face. A few *"café au lait"* pigmented patches were seen on the neck and trunk. The skin of the legs was dark and thickened. There was marked oedema of the legs, painful on pressure, and puffiness of the face. Moderate flushing of the face and neck were seen. All flushing observed by us was brief, lasting only a few minutes. An attempt to provoke the flush with a small quantity of alcohol failed.

The apex beat was not palpable. Auscultation of the heart revealed a grade-II systolic murmur, which was best audible at the left lower border of the sternum. At the left sternal edge, in the fourth space, there was audible, in addition to the systolic murmur, a grade-II diastolic murmur, which was accentuated during inspiration. Basal râles were heard over both lung bases. Auscultation of the lungs during the attacks of flushing revealed slight wheezing. This sign was, however, not present every time the patient was auscultated during flushing attacks. The blood pressure varied between 100—120/70—90 mm Hg.

Abdominal examination revealed a palpable hard abdominal mass in the right lower quadrant. The size of the tumour was estimated as that of an egg. The liver was palpable just below the costal margin and was tender. The spleen was not palpable. Ascites could not be verified.

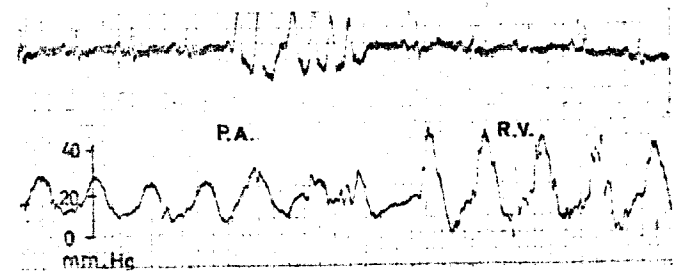

Fig. 2. Pressure tracing from the pulmonary artery and right ventricle. A pressure gradient of about 10 mm Hg across the pulmonary valve.

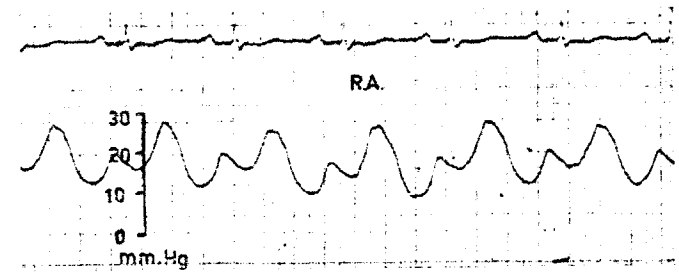

Fig. 3. Right atrial pressure curve.

Gynaecological and neurological examinations were negative. She was mentally submissive and most co-operative at all the examinations.

Laboratory and X-ray studies

The haemoglobin, red-cell count, white-cell count, differential count and thrombocytes were within normal limits. The E. S. R. was 16 mm per hour. The urine contained traces of protein. The reaction was acid and the specific gravity normal. Microscopic examination of the sediment revealed nothing abnormal. Determination of the plasma sodium, potassium, calcium, creatinine and N. P. N. gave normal results. The Wassermann reaction was negative. The serum cholesterol content was 174 mg per 100 ml. The blood sugar level and glucose tolerance tests were within normal limits. The protein amounted to 7.1 g per 100 ml of serum. Paper electrophoresis of the serum proteins showed low albumin and increased α_2-, β- and γ-globulins. The bromsulphalein dye-retention test showed retention of 0.6 mg per 100 ml in 30 min, which is a slightly abnormal value. The value for alkaline phosphatase was 6.0 Bodansky units. The bilirubin content of the serum, the Takata reaction, the thymol turbidity extinction and phenol-sodium chloride extinction were all normal, whereas the zinc sulphate turbidity extinction was slightly elevated. The prothrombin-proconvertin index (P & P) was 68 per cent. The urine contained no biliary pigments. Repeated sputum cultures for tuberculosis were negative. No tumour cells could be demonstrated in the pleural exudate.

A strongly positive screening test for identification of 5-hydroxyindole acetic acid (5-HIAA) in the urine, performed according to the method of Hanson and Serin (1955), strengthened the suspicion of a carcinoid tumour. Several determinations of 5-HIAA in the urine were performed according to the method of Udenfriend et al. (1955). The daily urinary output of 5-HIAA varied from 117 to 266 mg (normal range: 2—8 mg per 24 hours).

A skin biopsy taken from the leg showed thickening and fibrosis of the dermis. An ECG showed low voltage of the QRS-complexes but no other abnormalities.

X-ray examination of the chest revealed that the heart was somewhat enlarged. There was a pleural effusion on the right side. In both lungs close to the hili tumour-like shadows, which extended to the posterior segments of the upper lobes, were seen (fig. 1). The size of these symmetrical tumour shadows was about that of a plum. With the intention of determining the origin of the

Table I

Location	Pressures mm Hg		Mean
	Systolic	Diastolic	
Right atrium	28	7	15
Right ventricle	40	3	—
Pulmonary artery ..	30	11	19
Pulmonary capillary	—	—	11
Brachial artery	98	70	78

shadows, bronchography, bronchoscopy and mediastinoscopy were performed (A. J. Seppälä). None of these examinations explained their quality, however.

X-ray examination of the alimentary canal with contrast media showed a filling defect in the medical margin of the caecum. This filling defect was evidently produced by a tumour projecting into the lumen.

Cardiac catheterization findings

Catheterization of the right heart revealed a pressure gradient of about 10 mm Hg between the pulmonary artery and right ventricle at the pulmonary valve (fig. 2). The right atrial pressure was increased and the x descent in the right atrial pressure curve was replaced by a large regurgitant wave which was fused with the v wave (fig. 3). There was a diastolic pressure gradient of 4 mm Hg across the tricuspid valve. The cardiac output was 5.00 l/min, and the cardiac index 2.80 l/min/m² of the body surface area. The resistances were as follows: the total pulmonary resistance 200 dyne/sec/cm⁻⁵, the pulmonary vascular resistance 120 dyne/sec/cm⁻⁵, and the peripheral resistance 910 dyne/sec/cm⁻⁵. The pressures are given in table I.

Catheterization data indicated valvular pulmonary stenosis, marked tricuspid insufficiency and a slight tricuspid stenosis.

Operative procedure

At the laparatomy (V. Seiro and M. Turunen) the caecum was found to be adherent to the lateral wall of the abdomen. A hard tumour — which was thought to be located in the lumen of the caecum — was palpable. In the middle of the tumour, which was the size of an egg, there was a hollow. In the ileocaecal region there was an area of infiltration which was surrounded by small lymphatic glands. The surface of the liver, the mesentery, parietal peritoneum, and uterus were scattered with yellowish metastases varying in size from that of a pin-point to that of a pea. When the tumour was manipulated a severe bronchoconstriction and convulsive contractions of the small bowel suddenly occurred. The blood pressure fell to shock level. The administration of adrenaline chloride, however, had an immediate effect. The tumour was judged to be inoperable; consequently only samples for microscopic and chemical examination were taken from the metastases, and an ileotransversostomy was made in order to establish the passage. Microscopic examination of the samples revealed the features of a carcinoid tumour.

She was discharged few weeks later because her general condition was still good.

Postoperative course

She was readmitted to the Third Medical Clinic on May 14th. She had developed a pellagra-like dermatitis with hyperkeratosis and pigmentation on both legs. Flushing could no longer be seen. The cardiac silhouette was markedly enlarged since the previous examination. The 5-HIAA excretion was of the same magnitude as before the operation. Gradully a general dropsy developed and at the same time the peripheral cyanosis became deeper. She died on July 10th, 1959.

Postmortem examination

The tumour was located at the end of the ileum. It reached the Bauhin's valve and was adherent to the wall of the caecum and the abdominal wall. The liver weighed 1,800 g. Its surface was nodular. When sections were cut numerous metastatic nodules were found to be scattered throughout the parenchyma. The largest of these nodules, which was located in the left lobe, measured 10 cm in diameter. The remaining part of the liver tissue showed marked fatty degeneration. Metastatic nodules were further seen in the hilum of the spleen, in the mesentery, the omentum, both ovaries and in Douglas' pouch. On microscopic examination of the primary tumour and metastases the cells appeared small and rather uniform. Mitoses could not be seen.

The heart weighed 290 g. The endocardium of the right ventricle appeared pale and fibrotic. The right ventricular wall was slightly hypertrophic, about 7 mm thick. The tricuspid valve cusps were pearly grey, thickened and nodular, and the chordae tendineae were shortened and thickened. The pulmonary valve cusps were thickened, nodular and stiff. The branches of the pulmonary artery were markedly narrowed in the fibrotic areas of the lungs. On the left side of the heart no hypertrophy could be seen, and the mitral and aortic valves appeared normal. Only a few atheromatous patches were seen on the walls of the coronary arteries and aorta.

Microscopic examination showed that the nodules in both valves and in the endocardium near the valves contained, in a clearly basophilic and metachromatic ground substance, a moderate amount of connective tissue cells and delicate fibres; these were stained like collagen. Blood vessels and inflammatory cells were seen in the deeper layers only. Elastic fibres were completely lacking in the pads, and in the elastica-stain a very sharp demarcation was seen from the original cusp tissue, in which the number of elastic fibres was abundant. At this demarcation zone between the nodules and the original cusp tissue increased vascularization, round cell infiltration and areas of homogenous fibrosis could be observed here and there. Mast cells were absent.

The lungs were shrunken and pitted on their surface. In both lungs very wide firm areas were seen, in which the lung tissue was fibrotic throughout and contained coal pigment. The fibrotic areas were attached to the bronchial branches and the surrounding lymphatic glands. The lung structure was only normal in small areas of both the upper and the lower lobes.

Microscopic examination revealed that the lung foci were formed of connective tissue in which the fibres were rather rough. They were partly homogenized and poor in cells. In some areas there were plenty of coal cells and even mononucleated inflammatory cells. In the homogenized areas the number of coal and inflammatory cells was, however, small. When polarized light was used plenty of double-refracting particles were seen in the areas where the coal pigment was seen and also where the macrophage reaction was strongest.

Comments

For the development of silicosis at least 10—15 years' exposure to silica dust is usually required (Marrow and Cohen 1959). It is further known that silicosis may progress even after exposure to silica dust has ended (Gross et al. 1956). Infection is usually held responsible for the complicated pneumoconiosis in which the massive fibrosis is due to conglomeration of the fibrotic nodules. Many of those cases are considered to be tuberculous (Gardner 1949).

The patient considered above had been exposed to silica dust for six years while employed by a china factory. The exposure to silica dust had, however, ended some 15 years before a tumour-like shadow due to complicated silicosis was encountered in her left lung. It is worthy of note that routine chest X-ray examinations are made frequently in the provisions industry firm where she was at work after finishing her job at the china factory.

The carcinoid tumour is a slowly growing tumour, and many patients diagnosed to have a fully developed carcinoid syndrome are known to have been still alive several years later (Thorson 1958). It is perhaps possible that our patient was already suffering from a carcinoid tumour and distant metastases before the fibrotic nodules in the lungs started to coalesce and resulted in massive fibrosis. It is evident that this process advanced rather rapidly. It is perhaps possible that circulating 5-HT was a notable factor in the development of complicated pneumoconiosis in the present case.

This view is supported by the autopsy findings in some cases of carcinoid syndrome. Fabricius et al. (1958) reported a diffuse fibrosis in the thyroid gland,

pancreas, suprarenal gland and kidneys and a pronounced retroperitoneal fibrosis in connection with a carcinoid syndrome. A similar predominantly right-sided fibrosis located around the primary tumour was observed in three other cases of metastatic malignant carcinoid (Cassidy 1930, Hedinger and Gloor 1954, Olesen 1955); and in one case the adhesions of a previous abdominal operation were found to be extraordinarily hard and dense and there was a general increase of collagen, fibrinoid and hyaline material (Bean and Funk 1956).

Confirmatory evidence for the suggested interrelationship between 5-hydroxytryptamine and the aggravated development of the pulmonary silicosis in our case is obtained from animal experiments. Asboe-Hansen and Wegelius (1956) observed, after intraperitoneal injection of 5-hydroxytryptamine, a degranulation of the mast cells and oedema of the connective tissue of the hamster's cheek-pouch. This oedema was suggested to be primary due to the release of water-binding mucopolysaccharides from the cells. The experiments of Feldberg and Smith (1953) and Sparrow and Wilhelm (1957) show that 5-hydroxytryptamine liberates histamine in certain animal species; this substance has known effects on capillary permeability. Rowley and Benditt (1956) observed that 5-hydroxytryptamine itself has a high permeability potency in the rat.

The above observations, though fragmentary, indicate that excessive amounts of 5-hydroxytryptamine, as in a carcinoid syndrome, may enhance the tissue reactions to silica particles and accelerate the conglomeration of nodules into a complicated silicosis. If this concept is correct, it speaks in favour of the biochemical hypothesis for the development of endocardial lesions in the carcinoid syndrome (Thorson et al. 1954, Hedinger and Gloor 1954).

Summary

A well-developed carcinoid syndrome, which exhibited all the typical symptoms and signs of this rather new syndrome, was described. The patient suffered from not only a metastatic carcinoid tumour but also from complicated silicosis, which had considerably progressed several years after the exposure to silica dust had ended.

The possibility of the action of 5-hydroxytryptamine on the development of the silicosis was discussed.

References

Asboe-Hansen, G. & Wegelius, O.: Nature *178:* 262, 1956.

Bean, W. B. & Funk, D.: Trans. Amer. clin. climat. Ass. *68:* 111, 1956.

Björck, G., Axen, O. & Thorson, Å.: Amer. Heart J. *44:* 143, 1952.

Cassidy, M. A.: Quoted by Fabricius et al. 1958.

Fabricius, J., Jensen, K. & Poulsen, H. E.: Dan. med. Bull. *5:* 237, 1958.

Feldberg, W. & Smith, A. N.: Brit. J. Pharmacol. *8:* 406, 1953.

Gardner, L. U.: Med. Clin. N. Amer. *26:* 1239, 1949.

Gross, P., Westrick, M. L. & McNerney, B. S.: Amer. J. Path. *32:* 739, 1956.

Hanson, A. & Serin, F.: Lancet *259:* 1395, 1955.

Hedinger, C. & Gloor, R.: Schweiz. Med. Wschr. *84:* 942, 1954.

Morrow, C. S. & Cohen, A. C.: Med. Clin. N. Amer. *43:* 171, 1959.

Olesen, K. H.: Ugeskr. læg. *117:* 635, 1955.

Rowley, D. A. & Benditt, E. P.: J. exp. Med. *103:* 399, 1956.

Sparrow, E. M. & Wilhelm, D. L.: J. Physiol. *137:* 54, 1957.

Thorson, Å., Björck, G., Björkman, G. & Waldenström, J.: Amer. Heart J. *47:* 795, 1954.

Thorson, Å.: Acta med. scand. suppl. 334, 1958.

Udenfriend, S., Titus, E. & Weissbach, H.: J. Biol. Chem. *216:* 499, 1955.

Biochem J., 27 : 1007-1014, 1933

CXXXI. THE BIOCHEMISTRY OF SILICIC ACID.

III. THE EXCRETION OF ADMINISTERED SILICA.

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(*Received May 30th, 1933.*)

It has already been shown [King, Stantial and Dolan, 1933] that the level of soluble silicates in the urine can be influenced by diet. On continuous injection by the intravenous route of dilute neutralised solutions of sodium silicate, a rapid elimination, continuous during the time of injection and lasting for some hours afterward, has always occurred. Introduction of a suspension of silica dust into the stomach has led to rather smaller increases in the urinary output of silica. Such increases have, however, been definitely significant and point either to a partial dissolution of the silica by the alkaline duodenal juice or possibly to actual passage through the intestinal wall of the finest of the particles.

In the several experiments which have been performed, and in which the urinary excretion of silica has been markedly increased, no significant variations in the blood-silica, other than small temporary increases, have been observed. These facts appear to indicate a very low renal threshold for silicate.

Experimental.

The analytical procedure described by King and Stantial [1933] was followed throughout. Samples of blood and urine were collected in bakelite-coated vessels.

Ingestion of particulate silica. Preliminary experiments with rabbits demonstrated that when small quantities of finely powdered quartz[1] were introduced into the stomach, a significant rise occurred in the level of silica in the urine. This was more marked in dogs where the normal amount of silica in the urine is much smaller and the relative rise therefore greater. When 1 g. of the pulverised quartz, suspended in 50 cc. of water, was given to a dog by stomach tube, the urinary excretion of silica promptly rose from 1 mg. (normal) to 3·9 mg. per 100 cc. at 1 hour and remained high for several hours (4·7 mg. at 8 hours), returning the next day to its original level. Larger amounts of silica dust placed in the stomach appeared to be absorbed to a considerable extent and to be promptly excreted in the urine (Table I).

[1] Kindly prepared for us by Prof. H. E. T. Haultain, of the Department of Mining Engineering of this University, by prolonged grinding of quartz crystals in a steel ball-mill.

Table I. *Excretion of silica in the urine after administration by stomach tube of 5 g. powdered quartz.*

Hours	SiO_2 per 100 cc. urine mg.	SiO_2 per 100 cc. blood mg.
0	2·7	0·8
4	14·3	—
7	14·7	0·7
16	4·8	—
24	3·3	0·7

Ingestion of soluble silica. Owing to the alkalinity of solutions of sodium silicate it was found impossible to administer silica in this way, as the dogs invariably vomited the material. If the solution was neutralised to phenolphthalein with dilute hydrochloric acid just prior to administration by stomach tube, however, it was found that the dogs tolerated it well.

Deposits of gelatinous silicic acid tended to form in 5 % sodium silicate solutions when neutralised with hydrochloric acid, and it is probable that a large part of the silicate administered in this way was precipitated as silicic acid before it had been long in the stomach. The alkaline nature of the juices in the small intestine doubtless caused a partial re-solution of some of the silicic acid, but the amount remaining out of solution was probably a large proportion of the whole. Such portions as were absorbed—and they were considerable, although not necessarily constituting a large percentage of the total administered—appeared to be eliminated very quickly in the urine without any pronounced rise of the level of silica in the blood (Tables II and III). Analyses of the organs of the several dogs which were killed after the conclusion of the experiments failed, moreover, to demonstrate any increased concentration of silica in the tissues.

Table II. *Silica content of the urine after administration of silicic acid by stomach tube.*

Hours	mg. SiO_2 per 100 cc. urine	Hours	mg. SiO_2 per 100 cc. urine
Dog 480: 10 cc. 5 % sodium silicate neutralised by hydrochloric acid		Dog 390: 25 cc. 5 % sodium silicate	
0	1·7	0	2·0
1	4·1	1	17·5
2	23·3	3	49·3
3	14·9	5	56·7
4	22·3	6	43·5
5	25·3		
6	23·8	Dog 495: 25 cc. 5 % sodium silicate	
25	4·8	0	0·7
		2	49·2
		4	22·3
		6	11·9
		22	4·0
		29	2·8
Dog 346: 10 cc. 5 % sodium silicate		Dog 340: 25 cc. 5 % sodium silicate	
0	1·9	0	—
3	72·5	3	55·5
5	37·7	5	43·2
7	15·2	7	45·2
9	21·2	9	32·2
72	1·6	24	12·2
		72	1·4

Table III. *Excretion of silica in the urine after ingestion of silicic acid.*

Hours	mg. SiO_2 per cc. urine	cc. of urine	Total mg. SiO_2 in urine	mg. SiO_2 per 100 cc. blood
Dog 480: 100 mg. SiO_2 as silicic acid introduced into stomach.				
0	0·015			0·8
3	0·480	13·5	6·47	
5	0·367	5·0	1·86	1·5
7	0·357	11·0	3·93	—
9	0·227	6·0	1·36	0·8
13	0·112	6·5	0·73	—
25	0·060	22·4	1·31	—
72	0·026	—	—	—
		Total	15·69	
Dog 390: 250 mg. SiO_2 as silicic acid introduced into stomach.				
0	0·014			0·5
3	0·555	13·0	7·21	1·0
5	0·367	17·0	6·25	—
7	0·357	17·0	6·10	0·8
9	0·247	8·0	1·98	—
13	0·132	5·0	0·66	—
25	0·076	36·0	2·77	
72	0·038	—	—	—
		Total	24·97	

Intravenous injection of silicic acid. Several attempts were made to inject silicic acid in fairly concentrated solution into the ear-veins of rabbits. The solution of 5 % sodium silicate containing the equivalent of 10 mg. of silica per cc., neutralised with hydrochloric acid or by bubbling carbon dioxide into it immediately before using, was injected in quantities of 1 to 5 cc. In almost every case the rabbit died immediately or within a few minutes of the time of administration. The same experience was met with dogs, and it was thought probable that floccules of gelatinous silicic acid forming in the blood-stream caused the animals to die of embolism.

Attention was turned, therefore, to the continuous intravenous injection of solutions containing not more than 1 mg. SiO_2 per cc. over a period of several hours. Fresh solutions were prepared before each experiment.

For the continuous intravenous injections an ordinary hypodermic needle was attached by a fine rubber tube to the nozzle of the barrel of a syringe into which was fitted a rubber stopper carrying a finely pointed glass tube. Rubber tubing connected the glass tube to the tip of a graduated burette and a screw-clamp served to adjust the rate of flow of the solution from the burette. The number of drops falling into the syringe per unit time served as a rough but convenient indication of the rate of flow of the solution. The bottle containing the silicate solution was attached by means of rubber and glass tubing through a two-hole rubber stopper (one hole being plugged with cotton-wool) to the top end of the burette. The solution was delivered into the burette when desired by means of a pinch-cock on the rubber tubing. Before each experiment the whole apparatus was heated in a steriliser and clamped in an upright position to an iron stand which could be placed on the operating table next to the animal.

Female dogs whose urethra was exposed (for catheterisation) by previous operation were employed exclusively for the continuous intravenous injections. The animals were anaesthetised with 0·5 g. of sodium amytal dissolved in a little water. The needle was inserted in the vein of one of the fore-legs and the silicate solution allowed to flow at the desired rate by adjustment of the screw-clamp. A catheter was inserted, tied in place and closed by a pinch cock, so that the contents of the bladder could be withdrawn at intervals. After about 6 hours the animals usually began to show symptoms of recovering from the anaesthesia; a further 0·5 g. of amytal injected into the rubber tube delivering the silicate solution sufficed to retain them under anaesthesia for a further 6 or 8 hours.

In the several experiments conducted in this way quantities of silicic acid from 100 to 200 mg. were injected over periods of 5 or 6 hours. The response in urinary output of silica was prompt, rising from a small initial value to a high value within the first hour and reaching very high concentrations in the urine toward the end of the period of injection.

During this time the level of silica in the blood was considerably raised, although only to a very minor extent when compared with the rise in the urine. The excretion of silica did not keep pace, however, with its introduction into the blood-stream, only half of the total amount administered being recovered in the urine. It is possible that some of the silica was eliminated by way of the faeces, being excreted in a similar way to calcium in the gut, although it is not unlikely that a fairly large proportion was retained in the body. Analysis of the spleen of one of the animals killed after the experiment showed a silica content considerably higher than normal. The presence of albumin in some of the samples of urine was noted in these experiments.

Table IV. *Continuous intravenous injection of silicic acid.*

Hours	cc. of solution injected	mg. SiO_2 per 100 cc. urine	cc. of urine	mg. SiO_2 excreted	mg. SiO_2 per 100 cc. blood
Dog 481: neutralised sodium silicate solution; 1 mg. SiO_2 per cc.					
0	—	1·0	—	—	—
1	10	3·4	11	0·37	0·2
2	14	15·7	7	1·10	2·6
3	20	20·3	6	1·22	2·7
4	30	36·9	7	2·58	2·8
5	30	34·3	6	2·08	1·7
6	65	68·3	4	2·74	2·8
9	—	61·5	30	18·50	2·8
22 (night)		30·7	15	4·61	1·7
28		34·6	33	11·40	1·7
Night		35·8	45	16·10	—
46	Total SiO_2 injected = 169 mg.	15·2	44	6·70	—
73		2·2	860	18·92	1·4
94		3·0	250	7·50	—
120		2·4	—	—	—
166		1·5	—	—	—
			Total	93·82	
Dog 390: neutralised sodium silicate solution; 0·5 mg. SiO_2 per cc.					
0	—	0·6	—	—	0·3
1	50	26·5	3	0·80	—
2	75	96·7	6	5·81	—
3	43	169·2	5	8·46	6·15
4	80	129·2	6	7·76	6·60
5	95	18·7	31	5·81	—
6	50	55·7	11	6·12	—
7	—	26·0	21	5·46	—
8	50 cc saline	3·7	8	0·29	—
9		26·0	78	20·28	—
10		20·2	8	1·62	—
11		7·0	113	7·91	—
26		17·5	152	26·60	—
31		8·0	23	1·84	—
49		3·5	150	5·25	—
55	Total SiO_2 injected = 196 mg.	3·3	16	0·53	—
73		6·5	200	13·00	—
99		5·0	—	—	—
147		4·8	—	—	—
218		2·6	—	—	—
			Total	117·54	

In the several experiments conducted in this way quantities of silicic acid from 100 to 200 mg. were injected over periods of 5 or 6 hours. The response in urinary output of silica was prompt, rising from a small initial value to a high value within the first hour and reaching very high concentrations in the urine toward the end of the period of injection.

During this time the level of silica in the blood was considerably raised, although only to a very minor extent when compared with the rise in the urine. The excretion of silica did not keep pace, however, with its introduction into the blood-stream, only half of the total amount administered being recovered in the urine. It is possible that some of the silica was eliminated by way of the fæces, being excreted in a similar way to calcium in the gut, although it is not unlikely that a fairly large proportion was retained in the body. Analysis of the spleen of one of the animals killed after the experiment showed a silica content considerably higher than normal. The presence of albumin in some of the samples of urine was noted in these experiments.

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Dog 481: neutralised sodium silicate solution; 1 mg. SiO_2 per cc.					
0	—	1·0	—	—	—
1	10	3·4	11	0·37	0·2
2	14	15·7	7	1·10	2·6
3	20	20·3	6	1·22	2·7
4	30	36·9	7	2·58	2·8
5	30	34·3	6	2·08	1·7
6	65	68·3	4	2·74	2·8
9	—	61·5	30	18·50	2·8
22 (night)		30·7	15	4·61	1·7
28		34·6	33	11·40	1·7
Night		35·8	45	16·10	—
46	Total SiO_2 in-	15·2	44	6·70	—
73	jected = 169 mg.	2·2	860	18·92	1·4
91		3·0	250	7·50	—
120		2·4	—	—	—
166		1·5	—	—	—
			Total	93·82	
Dog 390: neutralised sodium silicate solution; 0·5 mg. SiO_2 per cc.					
0	—	0·6	—	—	0·3
1	50	26·5	3	0·80	—
2	75	96·7	6	5·81	—
3	43	169·2	5	8·46	6·15
4	80	129·2	6	7·76	6·60
5	95	18·7	31	5·81	—
6	50	55·7	11	6·12	—
7	—	26·0	21	5·46	—
8	50 cc saline	3·7	8	0·29	—
9		26·0	78	20·28	—
10		20·2	8	1·62	—
11		7·0	113	7·91	—
26		17·5	152	26·60	—
31		8·0	23	1·84	—
49		3·5	150	5·25	—
55	Total SiO_2 in-	3·3	16	0·53	—
73	jected = 196 mg.	6·5	200	13·00	—
99		5·0	—	—	—
147		4·3	—	—	—
218		2·6	—	—	—
			Total	117·54	

Two typical experiments using continuous intravenous injection of neutralised dilute sodium silicate solutions are illustrated in Table IV. The dogs were anaesthetised early in the morning and the catheter left in the bladder until late in the evening, a second administration of anaesthetic being given in the afternoon. The dogs were left in metabolism cages overnight and the urine caught in a metal container. Samples of blood were analysed by both the ashing and deproteinising methods, very little difference being found in the results by the two procedures. The very high silica content of the urine made it appear probable that some precipitation due to the acidity of the urine may have occurred. This appeared to be the case in some of the samples, and an increase in the silica value was obtained on warming the sample of urine with sodium hydroxide on the water-bath. All results for urine given in the Table are for sodium hydroxide-treated samples.

Intravenous administration of particulate silica. The milky suspension obtained by shaking quartz powder with isotonic NaCl was decanted from the precipitate of coarser material and was analysed for silica. The results indicated a SiO_2 content of 0·8 mg. per cc., approximately that of the diluted solutions of silicic acid used for continuous intravenous injection. This suspension was injected as in the experiments described in the previous section, over a period of 3 hours. The urine at this time was very bloody and the injection was discontinued. After 6 hours the dog died with no urine in its bladder, despite the fact that 250 cc. of liquid had been given and only a few cc. recovered during the first 3 hours. The silica content of the urine did not rise very greatly, being considerably less than when powdered quartz was given by mouth. This fact appears to point to dissolution of ingested silica by the duodenal juice. It was thought that the complete stoppage of the kidney secretion was probably due to an accumulation of the particles of silica in the glomeruli, but this was not borne out by histological examination[1]. Analysis of the kidneys did not show an abnormal amount of silica. The total amount given was only 200 mg., and this spread over the several organs of the body could not lead to an appreciable rise in the percentage of silica present. The spleen had a slightly higher than normal content. Results for this experiment are contained in Table V.

Table V. *Intravenous injection of powdered quartz suspension.*

Hours	cc. of suspension	mg. SiO_2 per 100 cc. urine	cc. of urine	mg. SiO_2 per 100 cc. blood
0	—	2·1	—	—
1	100	3·3	14	0·3
3½	150	9·9	19	0·8
6	Total SiO_2 injected = 200 mg.	—	None	0·9

Intravenous administration of organic silicate. "Silistren," a glycol ester of silicic acid which is extensively used as a therapeutic agent in Germany, proved a convenient form of organically bound silica. It is a transparent viscous liquid, readily soluble in water, and contains by analysis 14·7 % SiO_2. When dissolved in water it gives a proportion of blue colour with the colorimetric reagents corresponding to a little less than 5 % SiO_2 on the weight of silistren used. Whether this is due to some uncombined silicic acid, to a partial hydrolysis

[1] Histological examination of the kidney showed generalised degeneration of tubular epithelium. Widespread cytoplasmic degeneration of liver cells may have been a *post mortem* development.

due to the acidity of the test solution (the colour increases at a rate faster than that of a sodium silicate standard) or to a mild action on the reagents by the glycol silicate itself is not clear.

Silistren rapidly yields silicic acid at the temperature of boiling water. At room temperature it breaks down very slowly in watery solution, more rapidly in acid solution and in alkaline solution (Fig. 1).

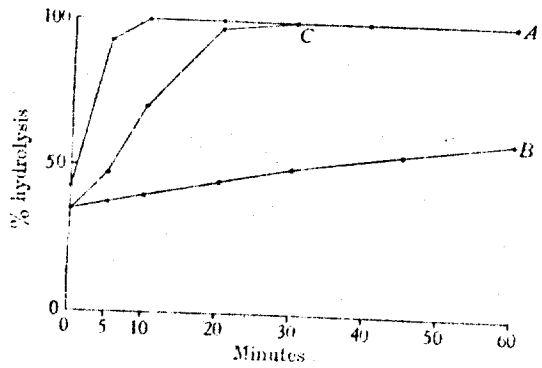

Fig. 1. Hydrolysis of glycol silicate in *A*, boiling water; *B*, $N/10\ H_2SO_4$; *C*, $N/10\ NH_4OH$.

Table VI. *Injection of organic silicate.*

	Dog 387				Dog 388			
	Urine		Blood		Urine		Blood	
Hours	mg. SiO_2 per 100 cc.	Total mg. SiO_2 in urine	mg. SiO_2 per 100 cc.	Total calculated mg. SiO_2 in blood	mg. SiO_2 per 100 cc.	Total mg. SiO_2 in urine	mg. SiO_2 per 100 cc.	Total calculated mg. SiO_2 in blood
0	0·7	—	2·2	22	1·06	—	2·3	27
¼	—	—	14·3	143	—	—	9·4	108
1	No urine		11·5	115	36·1	10·5	—	—
2	2·8	0·3	14·1	141	47·5	8·5	11·3	130
3	—	—	15·0	150	—	—	10·5	121
4	No urine		—	—	35·1	2·2	—	—
5	—	—	12·4	124	27·8	1·9	9·1	105
7	12·5	0·9	9·8	98	25·0	2·3	8·8	101
24	18·7	98·9 (night sample)	4·8	48	17·2	7·2 (night sample)	7·1	82
48	—	—	3·4	34	—	—	6·9	80
72	3·3	—	1·7	17	5·6	—	3·9	45
96	2·7	—	2·4	24	5·5	—	5·2	60
120	0·7	—	—	—	7·3	—	4·3	50

5·68 g. of silistren, corresponding to 0·836 g. of silica, were dissolved in 20 cc. of water in which a faintly opalescent solution was formed. An amount of the solution corresponding to 100 mg. silica was given to each of two dogs by injection into a vein of the fore-leg. Within an hour both dogs appeared ill, showed signs of nausea and later vomited. Samples of blood and urine were taken at regular intervals for analysis.

The urinary silica in one dog reached almost as high a level as that obtained when silicic acid was introduced into the stomach. The increases for the other

dog, which early in the experiment began to show bloody urine and developed anuria, were more moderate.

The blood-silica was raised and maintained at a higher level by using silistren than by any other means tried. It is possible that when present as an organic compound (*i.e.* glycol ester) silicic acid can remain at high concentration in the blood when it is incapable of remaining there as inorganic silicate. From the weight of the animals and the computed amount of blood present (1/13 body weight) it was estimated that at the highest level 150 mg. of silica were circulating in the blood-stream. As 400 mg. were injected and only a small part of the difference was accounted for by what appeared in the urine, it seems that other tissues than that of the kidneys were removing the silica from the blood, though whether as organic or inorganic silicate is, of course, not known. Both dogs died on the fifth day.

Administration of silicic acid by way of the lung. It was observed by Kraut [1931] that the silica level of the blood rose as a result of introducing a silicic acid solution into the lungs of human subjects by means of an ordinary throat spray. In order to determine if the urinary output of silica could be influenced by the animal breathing in silicic acid, two dogs were exposed to a fog of silicic acid solution. A large box with a tight-fitting cover, carrying a glass window, was used to confine the animals and a dilute solution of silicic acid was sprayed in from time to time as the fog appeared to settle. A slow current of air was blown into the box during the time of exposure so that the dogs might not suffer from lack of oxygen. After 2 hours the dogs were removed and catheter specimens of urine used for silica determinations then and at 6 and 24 hours. The results (Table VII) indicated considerable absorption of silica, but it is possible that the absorption took place by way of the intestine from such amounts of the solution as were licked by the dogs from their skin and fur or were expectorated from the trachea and swallowed.

Table VII. *Silicic acid administered by lung.*

2 hours' exposure to fog of neutralised 0·5 % sodium silicate solution.

Dog 340		Dog 390	
Hours	mg. SiO_2 per 100 cc. urine	Hours	mg. SiO_2 per 100 cc. urine
0	—	0	0·5
2	2·4	2	1·3
6	7·2	6	8·6
24	4·1	24	4·6

Table VIII. *Intra-bronchial administration of silicic acid.*

Dog 391: 7 cc. of neutralised 5 % sodium silicate sprayed into lung through bronchoscope.

Hours	mg. SiO_2 per 100 cc. urine
0	0·8
3	7·5
5	10·1
24	4·7
48	1·0
96	0·7

In Table VIII are shown the results obtained when the same silicic acid solution was introduced into the lungs by means of an atomiser used to blow the spray through the barrel of a bronchoscope introduced into the trachea

down to the bifurcation of the bronchus. A markedly increased output of silica was observed in the urine, but it is again not clear that this may not have been due, in part at least, to expectoration and swallowing of some of the solution.

Summary.

Introduction of soluble or of particulate silica into the stomachs of animals leads to marked increases in the output of silicate in the urine without any corresponding increase in the blood.

Moderate increases in the concentration of silica in the blood and enormous increases in the urine were observed following the intravenous injection of silicic acid. Not all the silica injected could be accounted for by that recovered in the urine.

Intravenous injection of a suspension of finely particulate silica caused death of the animal within a few hours with moderate increases in the urinary silica.

The blood appears capable of maintaining a much higher concentration of organic than of inorganic silicate. Injection of a solution of the glycol ester of silicic acid resulted in considerable increases in the "total" silica content of the blood and moderate increases in the urine. The animals died several days subsequent to the administration of the organic silicate.

Spraying of silicic acid into the lungs led to increased urinary output, but it is not certain that this was due to absorption from the lungs.

It is suggested that animals possess a very low renal threshold for silicate.

References.

King and Stantial (1933). *Biochem. J.* **27**, 990.
——— ——— and Dolan (1933). *Biochem. J.* **27**, 1002.
Kraut (1931). *Z. physiol. Chem.* **199**, 81.

PHYSIOLOGICAL REVIEWS

VOL. 18 JULY, 1938 No. 3

THE PHYSIOLOGICAL AND PATHOLOGICAL ASPECTS OF SILICA

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I. DISTRIBUTION OF SILICON. The element silicon occupies the same central position in inorganic nature that carbon occupies in organic nature. Next to oxygen it is the most abundant element. Although it is absent from the air, except as particles of dust, and is only a minor constituent of the hydrosphere, silicon is the element of chief importance in the lithosphere. It has been estimated that about 28 per cent of the solid crust of the earth is silicon. The figure 28 is also the atomic weight of silicon and if the amount of the element be expressed in terms of its oxide (silica-SiO_2), then it is seen that about 60 per cent of all rock is silica. The only important non-siliceous rocks are limestone and dolomite. Quartz and sandstone, gneiss and granite, slate and clay, are all compounds of silicon.

Silicon occurs as the free oxide silica, and in the form of silicates—metallic salts of its acids. Free silica is found in both the amorphous and crystalline state. Opal, tripoli and the deposits of diatomacious earth are composed of amorphous silica, containing a small percentage of water. Flint, chert, jasper and chalcedony are composed of amorphous silica associated with different proportions of crystalline silica. The chief types of crystalline silica are quartz, tridymite, and cristobalite. Quartz is the most common. It may be colourless (rock crystal): milky, owing to the presence of innumerable bubbles of air: smoky, due to carbonaceous material: or coloured, because of dissolved traces of metallic oxides. Tridymite is found less commonly in nature than is quartz. It is formed when quartz is heated a long time at about 1000°. It is formed during the manufacture of, and is found in silica bricks. So, likewise, is cristobalite, which is formed at a still higher temperature. The three kinds of crystalline silica exhibit different

crystal forms. Their specific gravities are: quartz, 2.67, tridymite 2.33, and cristobalite 2.34.

The silicates are the most important constituents of geological formations. Whole mountain ranges are composed of them. Chemically considered, silicates are the combined forms of silica with the oxides of metals, or the salts of bases with silicic acid. Silica is capable of existing in combination with water to form several silicic acids. In association with one molecule of water it exists as meta silicic acid (H_2SiO_3); with two molecules of water as ortho silicic acid (H_4SiO_4). By loss of water from two or more molecules of ortho silicic acid, condensed silicic acids may arise, thus disilicic acid ($H_2Si_2O_7$ from 2 H_4SiO_4) by the loss of 1 molecule of water, and trisilicic acid ($H_4Si_3O_8$ from 3 H_4SiO_4) by the loss of 4 molecules of water. The natural silicates found in rocks correspond to these silicic acids. Wollastonite ($CaSiO_3$) is a meta silicate; Willemite (Zn_2SiO_4) is an ortho silicate; serpentine ($Mg_3Si_2O_7$) a disilicate; and orthoclase ($KAlSi_3O_8$) a trisilicate.

Much of the natural silicate of the earth's crust has suffered a decomposition through weathering during geological time. The carbon dioxide dissolved in natural waters attacks those silicates which contain metals capable of combining with carbonic acid. Orthoclase, for instance, suffers disruption through its potassium being carried away in solution as potassium bicarbonate. Colloidal silica is at the same time liberated, and a residue of aluminium silicate (clay) is left.

II. The determination of silicon. Chemical methods for the estimation of silicon are gravimetric, titrimetric and colorimetric. All gravimetric procedures depend on the decomposition of the siliceous material to liberate silicic acid, which is subsequently dehydrated to the oxide, and isolated and weighed as silica.[1] The silica as isolated often contains small amounts of certain metallic salts which are difficult to eliminate. For this reason it is customary to treat with hydrofluoric acid which completely volatilizes the silica as silicon tetrafluoride (SiF_4). The loss in weight represents the true silica content of the sample.

[1] It is the custom to express the amount of the element in terms of its oxide-silica (SiO_2). This convention takes no account of the different forms in which silicon may exist in the materials under investigation. Only the total amount present is analyzed for and reported. It should, therefore, be borne in mind by those who are not familiar with analytical usage, that a figure for "percentage silica" (per cent SiO_2) gives no information as to how much free oxide of silicon is present. Except where used in some special sense and hence modified accordingly (e.g., "free silica"), the terms "silica" and "total silica" will be used in this article to signify total silicon (calculated as the oxide SiO_2).

Most books on quantitative analysis contain adequate directions for the analysis of various siliceous substances. Hillebrand (67), Treadwell (161), and Scott (141) contain particularly full and valuable discussions of the many procedures which have been described.

The titrimetric determination of silicon (Tananaev and Babko, 156) depends on the reaction represented in the equation:

$$SiO_2 + 6NaF + 4HCl \rightarrow Na_2SiF_6 + 4\ NaCl + 2\ H_2O$$

The method is extremely rapid and easily executed (Koral and Kaluzhskaya 93). A good degree of accuracy can be obtained with fairly large amounts of silica, but in the experience of the authors it is not trustworthy as a micro-method.

Colorimetric methods for the determination of silica were introduced for the purpose of estimating the small amounts of silica in natural waters. The well-known Dienert and Wandenbulche (34) colorimetric procedure is based on the formation of a highly coloured yellow silico-molybdic acid. This reaction was first used for a colorimetric method by Jolles (71) in 1898, and has been variously modified according to the use to which it has been put by Thresh (158), Atkins (4), Thompson and Holton (157), who investigated waters and water supplies, and by King (83) who studied the silica content of animal tissues.

Reduction of the yellow silico-molybdic acid to give a blue complex has been made the basis of other colorimetric procedures. These have been principally used for examining animal tissues and body fluids, whose silica content is often very low, and which consequently require analysis by a very sensitive method. The blue colour developed from a given amount of silica is more intense than the corresponding yellow colour (cf. Roman 134, King and Stantial, 87; De Eds and Eddy, 32).

The quantitative determination of free silica in the presence of silicates presents many difficulties. The problem has been partly solved by utilizing the fact that the amorphous silica set free from many (but not all) silicates by boiling with hydrochloric acid is soluble in aqueous alkalies, whereas crystalline silica (quartz) is little attacked (Shaw, 142). Knopf (94) found that silicofluoric acid (H_2SiF_6) would dissolve the silicate components of rock dust leaving the quartz relatively unattacked. Silicoboric acid was preferred by Line and Aradine (105), who claimed a sharper separation than with silicofluoric. In the absence of an entirely satisfactory method of isolating mineral particles from tissue, these methods have had only a limited use in studying the nature of the dust deposits in lungs.

Spectrography has been applied to the study of the siliceous material in lungs by Policard (126). The x-ray diffraction methods have been applied by Clark and Reynolds (23) and Faber (38). By these means it is possible to determine the percentage of quartz in presence of other mineral substances. Hicks, McElroy and Warga (66) have used both methods for studying the dust deposits in human lung tissues.

III. Silicon in microörganisms. Diatomaceous earth consists of the siliceous skeletons of the microscopic plant life of the sea—the phytoplankton or diatoms. They have been called by Mann (107) "the grass of the sea," and form the most important food of aquatic animal life. In 1904 Richter (131) observed that artificial cultures of these marine organisms thrived in glass bottles but that very poor development took place in paraffined containers. From this it was concluded that silica is essential to their reproduction. Atkins (5) studied silica as a limiting factor in diatom growth in the sea, and Harvey (63), Coupin (28), King and Davidson (85) in artificial cultures. The subject has been reviewed by Harvey (61, 62), Calvert (19) and Cooper (27). Brussoff described a "bac. siliceus" capable of storing and depositing silica (17).

IV. Silicon in plants. Silicon appears to stand very much in relation to the skeletons of some plants as do calcium and phosphorus to that of animals. One family of sponges has a skeleton of almost pure silica. The ash of wheat straw may contain 40 per cent of SiO_2, and that of equisetum (common horse tail) has been shown to consist of up to 80 per cent SiO_2. The silica is usually deposited in the peripheral tissues of the transpiration organs and along the conducting vessels (Frey-Wyssling 43). Silica appears to have been largely superseded, during the evolutionary process, by the development of such stable organic substances as cellulose and lignin, which are capable of forming rigid structures (cf. 144). An extensive literature exists on the rôle of silica in plant growth and nutrition. This is reviewed by Nemec (119), Lemmermann (103) and Sreenivasan (146, 147).

V. Silicon in animal tissues. Silicon appears to be invariably present in animal matter. All tissues and fluids which have been examined contain at least traces, and in many instances the amounts found are of the order of those of other mineral elements which are recognized as being normal tissue constituents. The almost universal distribution of silica makes it probable that silicon will be at least a likely contaminant, if not an essential ingredient, of all protoplasm. All the marine forms examined have contained silica. The eggs of birds (35), milk (31) and the fetuses of mammals have been shown to

have small but appreciable quantities (88). In normal adult animal tissues it is present in somewhat larger amounts.

Schulz (1901) (136) made the first thorough investigation of the presence of silica in human material. He analyzed umbilical cord (137), thyroid glands (139), and pancreases (140), and found some small differences between normal and diseased tissues. Frauenberger (42) criticized some of his results as being too high, but with the analytical procedures then available for estimating the small amounts present in the tissues studied, some disagreement was to be expected. Gonnermann (1917) (47) regarded silicon as being of some importance in the metabolism of man and the higher animals. He greatly extended the analyses of tissues and investigated also the silica content of some important foodstuffs (48). This early work has been reviewed by Kuhn (1926) (99), in whose book will be found the results of a great number of investigations. A good summary of the position of silica in plant and animal physiology is also given by Lemay and Jaloustre (1925) (102). In this article are collected the results of many French investigations which are difficult to trace in the literature.

With the introduction of modern micro-methods of analysis the study of the distribution of silica was greatly facilitated. The use of the microbalance, the colorimeter and the spectrograph has rendered it no longer necessary to analyse large quantities of normal tissue in order to secure a determinable amount of SiO_2. The silica content of biological materials can now be determined easily and accurately on small amounts of tissue, which are preferable because of greater ease of manipulation in the analytical procedures. The results of analyses for a variety of material are collected in table 1. Where variable figures have been obtained by different investigators, the more recent results, or those which in the opinion of the authors, appear most reliable, have been included.

Inspection of the table indicates that the normal range of silica content for most tissues is from 4 mgm. to 40 mgm. per 100 grams dry fetal tissue, and from 10 to 200 mgm. for adult tissues. Lung is amongst the lowest in the fetal tissues, and muscle the highest. The reverse is the case in adult tissues. Normal human lungs and lymph nodes may contain large—in some cases enormous—amounts of silica (10). This is apparently derived from life-long inhalation of ordinary street and house dust, and tends to increase in concentration with age. In contrast is the constant low concentration of silica in other organs and in muscle, which does not appear to vary appreciably during life. Kahane and Antoine (73) have called this the "silice de constitution"

TABLE 1

Silica content of normal tissues

FETAL TISSUES	SiO_2 DRY TISSUE	ADULT TISSUES (HUMAN)	SiO_2 DRY TISSUE
	mgm. per ten grams		*mgm. per ten grams*
Whole mouse[7]	10	Heart: myocardium[1]	11-29
Blood (human)[8]	13	Heart: endocardium[1]	14-28
Brain (human)[7]	22	Ileum[1]	47
Heart (human)[7]	20	Kidney[1]	11-27
Kidney (human)[7]	13	Liver[7,1]	11-17
Kidney (calf)[7]	8-10	Lung[7,8,9,4] (infancy to old age)	10-200
Liver (human)[7]	4-6	Lung[1] (infancy to old age)	14-2000
Liver (calf)[7]	4-10	Lymph glands[1] (peribronchial)	27-5000
Lung (human)[7]	8-10	Mammary gland[1]	12
Lung (calf)[7]	4-10	Milk[6,10]	71
Lung (human)[8]	34	Muscle: pectoral[1]	15-45
Muscle (calf)[7]	24-37	Muscle: psoas[1]	23
Spleen (calf)[7]	11	Nerve: peripheral[1]	11-40
ADULT TISSUES (HUMAN)		Nerve: spinal cord[1]	13-87
Adrenal[1]	14-80	Ovary[1]	10-21
Appendix[1]	68	Oesophagus[1]	8-26
Aorta thoracic[1]	14-34	Pancreas[1,5]	14-31
Artery iliac[1]	14-50	Parathyroid[1]	22-23
Bladder[1]	7-21	Pituitary[1]	27-67
Bone[7,1,2]	12-30	Placenta[8]	29
Brain: dura mater[1]	11-27	Prostate[1]	11
Brain: cortex[1]	12-36	Rectum[1]	15-74
Brain: Cerebellum[1]	16	Skin: scalp[1]	35-270
Brain: pons[1]	13-43	Skin: abdomen[1]	40-130
Brain: pineal[1]	70-90	Skin: sole of foot[1]	11-59
Cecum[1]	20-27	Spleen[1]	15-41
Cartilage: coastal[1]	18-35	Stomach[1]	12-42
Cervix[1]	11	Teeth[2]	6
Diaphragm[1]	11-50	Tendon, Achilles[1]	12-16
Duodenum[1]	10-80	Thymus[1]	81
Gall bladder[1]	31	Thyroid[1]	21-55
		Testes[1]	8-20
		Umbilical cord[1]	33
		Uterus[1]	7-12

[1] Belt et al. (10).
[2] Bowes and Murray (14).
[3] Chapman (22).
[4] Fewweather (41).
[5] Kahle (74).
[6] Kettmann (76).
[7] King et al. (88).
[8] McNally (116).
[9] Sladden (145).
[10] Strohecker et al. (151).

and contrast it with the "silice d'interposition" which comes from inhaled siliceous particles. They have isolated birefringent particles from the blood and from other organs than the lungs, showing that small amounts of particulate matter may find their way from the pulmonary apparatus into the general circulation. This exogenous silica appears as crystalline fragments, leaving a residue after treatment with hydrofluoric acid. In contrast the endogenous silica is amorphous and dissolves completely in hydrofluoric acid. It is likewise soluble in aqueous alkali, whereas Badham and Taylor (7) have pointed out that mineral particles are not so attacked.

The nature of the "silice de constitution" has been a matter of conjecture. Most authors consider it a simple deposit of hydrated silica. The claim of Drechsel (36) to have isolated a cholesterol silicate has not been confirmed (21). Johlin (72 a) deduced evidence for the presence of a silico-organic compound by electro-dialysis of gelatin, tendon, blood and urine. A jelly-like material containing both silicic acid and carbohydrate appeared at the cathode. Colloidal silica, on the other hand, migrates to the anode. Only very small amounts of the substance were obtained and it was not possible to determine its exact nature.

Many claims have been made for the presence of abnormal amounts of silica in diseased tissues, e.g., Kühn (99), Robin (133), Murata and Kageyama (117), Sztankay (154, 155). Anderson and Dible (1) have recently (1938) suggested an etiological relationship between silica and malignancy. A group of cases of pulmonary carcinoma was examined in which the organs contained an excess of silica and exhibited histological evidence of silicotic fibrosis. The elasticity of connective tissue has been held to be associated with its silica content, which, it is claimed, decreases with age (95), and with the concurrent loss of elasticity. This association furnished a rationale for the extensive silicic acid therapy which was practised for many years, particularly in central Europe. Thus A. Kühn (98) treated arteriosclerosis, bronchial asthma and increased blood pressure with injections of silica-containing solutions. Broman (1923, 16) questioned the influence of silica on the elasticity of connective tissue. She failed to find any relation between the SiO_2 content and the tensile strength of tendons of rabbits, or to find any evidence that silica administration strengthened the healing tendon.

The elaborate claims of many authors (e.g., 97, 75, 95) for a beneficial treatment of tuberculosis of the lung with silicic acid preparations are, in the opinion of the writers, devoid of any adequate proof. The whole

question of silica therapy has been critically reviewed by Maver and Wells (108).

VI. SILICON IN BLOOD. In 1857 Witting (168) recorded 0.5 per cent of SiO_2 in the ash of blood. Gonnermann (47) reported 2 per cent in whole blood, 1 per cent in the serum, 3 per cent in the cells, and 16 per cent in the ash of fibrin. Numerous other results are given by Kuhn (99) from the older literature. Kraut's (1931) average figure of 1.8 per cent SiO_2 in the ash of a whole blood for 32 normal persons is in close agreement (96), but Lematte and Kahane's (101) analysis of dried fibrin and blood showed a much smaller content of silica than that given by previous authors.

The custom of recording the silica content as a percentage of the ash makes it difficult to compare many of the recorded results (particularly in the German literature) with those expressed as milligrams per 100 cc. of blood. Only where departures from the normal are claimed can comparisons be made. King et al. (88) found by a colorimetric method (confirmed by gravimetric) that the average silica content of human blood was a little less than 1 mgm. SiO_2 per 100 cc. The blood of several animals yielded very similar figures. Pribyl's results for the blood serum of cattle, sheep, goats and pigs were of the same order (128).

Kraut (96), using a different method of analysis (wet-ashing of 5 cc. of blood, followed by HF treatment of the "sulphate ash" with measurement of the loss in weight due to volatilization—presumably of silica), found a very much higher concentration in the blood. Normal men and women varied between 10 and 30 mgm. SiO_2 per 100 cc., with an average value of 16. Miners with and without silicosis, and tuberculous patients, had considerably more (Böhme and Kraut 13). Reckoned on an ash basis Kraut's figures agree fairly well with the older German results, but they are greatly (at least 10 times) in excess of values determined elsewhere (86, 116, 134). This large discrepancy is probably to be explained on the basis of the technique of analysis used for the silica determinations.

King and Dolan (86) were unable to confirm the finding of an increased blood silica in cases of tuberculosis or silicosis, or in miners exposed to a high concentration of quartz dust in the air. This was in keeping with the findings for animals (89) which were injected with silica and in which no appreciable increase of blood silica could be demonstrated, despite large increases in the amounts in the urine. It was considered that a person exposed to the inhalation of silica dust would, if he dissolved any of it in his lungs, promptly excrete it in his urine.

The claim of Gürsching and Kraut (56) to have found a relationship of the blood silicic acid to the parathyroid glands and the serum calcium could not be confirmed by the authors.

Changes in the silica content of the blood following administration of silicic acid esters have been repeatedly demonstrated (89, 44). It is possible that when present as an organic compound silicic acid can remain at high concentration in the blood when it is incapable of remaining there as inorganic silicate.

VII. ABSORPTION AND EXCRETION OF SILICON. Whether or not silicic acid administration can lead to an increase of the blood silica, there can be no question that absorption of silicic acid takes place, followed by excretion in the urine. Schulz (138) and Salkowski (135) found that silica introduced into the organism with the food is excreted in large measure in the urine, and Gonnermann (49) that the drinking of water rich in silicic acid leads to increased urinary excretion of silica.

The silica content of flours and grains was investigated by Berg (11). In general it may be said that the brans of grains contain more silica than the bolted flours; and hay and straw contain considerable amounts. For this reason herbivorous animals are ingesting much larger quantities of silica than are meat-eating animals. Much of this silica is eliminated in the feces, but a considerable proportion must be absorbed, as is shown by the constant high excretion in the urine. King et al. (88) found that herbivorous animals excrete ten to thirty times as much silica in their urine as do carnivorous animals. The amount of the excretion could be varied at will merely by changing the diet. When the feed of rabbits was changed from oats and hay to white bread and tomato juice, the urinary silica promptly fell to low levels, to be restored again to its previous high level with the restoration of the high silica diet. With human subjects Riessner and Kindt (130) found a similar result when sodium silicate was incorporated in the bread. They claimed (and likewise Kockmann and Maier 92) that a retention of part of the administered silica in the organism took place.

The administration of pure silicic acid, or of neutralized sodium silicate into the stomach leads to an increase in the urinary silica (see fig. 1). Whatever silica enters the blood stream by way of the intestine appears to pass rapidly into the urine, there building up to a high concentration without any significant increase of the level in the blood. By continuous intravenous injection of soluble silica in isotonic sodium chloride (89) it was possible to increase enormously the concentration of silica excreted in the urine (up to 150 times the original), without any very marked increase in the blood. In unpublished experiments on the

absorption and excretion of silicic acid in human subjects King and Brock found large, although variable, amounts of silicic acid to pass from the gut into the urine without demonstrably increasing the level in the blood. These findings seem to indicate that the body possesses a very efficient mechanism for ridding the blood of its dissolved silica. There was also some evidence that parenterally administered silicic acid may be excreted in part by the gut as well as by the kidneys.

VIII. SOLUBILITY OF SILICA. The modern concept of solubility as a figure designating the composition of a liquid phase which is in a state of dynamic equilibrium with a solid phase, constitutes an intelligible

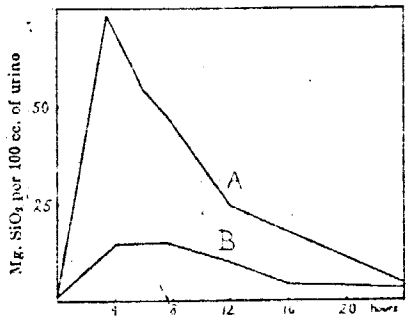

Fig. 1. Effect of administration in the dog's stomach (A) of silicic acid, and (B) of powdered quartz, on the excretion of silica in the urine (after King and Dolan, 86).

picture for systems consisting of gelatinous silicic acid suspended in liquids. But the applicability of this concept to the system represented by a large crystal of quartz in distilled water is not clear. That some silica may have passed from the crystal into the liquid is possible, but the presence of silica in the water is a matter of presumption since there is no means of demonstrating its presence there. It can only be said that the properties of the liquid are approached by those of a silicic acid solution as it becomes more and more diluted by the addition of larger and larger amounts of water. In the limit the presence of silicic acid is certain, but its amount is immeasurable; and its presence in any small portion of the whole is a matter of chance. Some such reasoning is probably the basis of the belief that silica is "insoluble" in water, except in so far as that belief is modified by the conception of all substances having some solubility, be it ever so small.

The "insolubility" of silica, however, does not stand the test of experiment. If the quartz be broken into coarse fragments and suspended in water, the liquid may acquire properties which make it appear that some silica has passed into solution, and if it be reduced to a fine powder then the presence of measurable amounts of silica in the liquid phase becomes determinable. But the measurement of the dissolved silica presents grave technical difficulties because of the difficulty of separating liquid phase from solid. When the solid phase consists of extremely fine particles—less than 1 micron in diameter, then much of it is found to be in a state of more or less permanent suspension. The removal of these fine particles from the liquid defies most technical means which are available. Simple filtration is useless. For this reason the early results on solubility of quartz (e.g. Strücknann 150a) are not a true measure of the silica which is present in a state of molecular dispersion. Lenher and Merrill (1917) recognized this fact in their solubility studies, but had no means of overcoming it (104). Their reported figures are considerably higher than those given by later workers. Only through high-speed centrifugation—at 10,000 to 20,000 r.p.m. (84), or through ultrafiltration—forcing the liquid through a membrane of fine porosity under many atmospheres' pressure (160), can the suspended fine particles be completely removed.

The utilization of a chemical reaction into which only the molecularly dispersed silicic acid will enter furnishes a further means of obtaining a measure of the dissolved silica. Such a reaction is represented in the silico-molybdic acid method which is the basis of most of the colorimetric procedures for silica. Silica solubility figures determined in this way are open to the objection that the reagents may have a solvent effect on particles of undissolved silica, but in point of fact the results so obtained agree very closely with those determined on ultra-centrifugates and ultrafiltrates.

A peculiarity of silica solutions is that they do not appear to become "saturated" except in the presence of a great excess of very finely divided solid. The "solubility" varies, furthermore, with the state of division of the solid (Lucas and Dolan, 106; Briscoe et al., 15). The material used in the solubility experiments (90) illustrated in figure 2 was very finely powdered quartz, prepared from rock crystal which analyzed almost 100 per cent SiO_2. Despite the fact that this specimen of quartz appeared to be a very pure one, it did not obey the simple phase rule criterion of purity which states that the addition of further solid to a saturated solution causes no increase in the amount of dissolved mate-

rial. An equilibrium seems to be reached within a few days between the liquid and any small amount of solid which may be shaken with it. But this apparently saturated solution will dissolve more silica, up to a point, as more quartz is added and shaken with it. It is possible that an additional degree of freedom may exist in the system, viz., there may be another component in the form of hydrated silica existing on the surface of the particles, and it may be this which is the actual material passing into solution. But even this explanation is not satisfactory since it might be expected that as hydrated silica was dissolved from

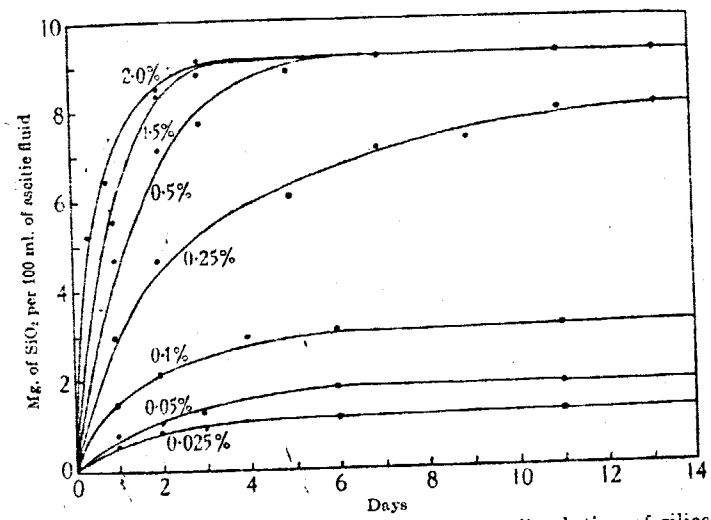

Fig. 2. Effect of concentration of solid on the dissolution of silica. Percentage figures represent grams of rock crystal dust suspended in 100 ml. of ascitic fluid (after King and McGeorge, 90).

the surface of any particle, another layer of it would form. Furthermore, it is found (Lucas and Dolan, 106) that the re-suspension in fresh solvent of the solid material, which has been separated from a "saturated" solution, results in the dissolution of an amount of silica which is equal to that in the first solution. And yet neither solution is saturated in the ordinary sense, since more silica will be dissolved if more solid be added. The total surface of solid exposed probably determines the rate, if not the final amount of silica dissolved, and the whole phenomenon might better be considered as a leaching of material from the surface of particles, than as a simple dissolution.

The vexed question of whether dissolved silicic acid exists in a state of colloidal or molecular dispersion (either in artificial solutions, natural waters, or biological fluids) (6, 166) has been at least partially resolved by combined high-speed centrifugation and ultra-filtration experiments (90). In every instance where an appreciable amount of silica was shown by analysis to be present in the liquid, there was a lower result obtained in the ultrafiltrate than in the centrifugate. The difference was in general greater the larger the amount of dissolved silica. The membranes used were capable of filtering off such colloids as plasma globulin and albumin, and it is probable that the silicic acid removed was present as a colloid of somewhat similar dimensions. It would seem likely, with a feebly ionized substance like silicic acid which is known to polymerise easily that reversible transitions from the molecularly dispersed state to the colloidal are usually taking place in its solutions.

In table 2 are collected the results of several investigators for the amounts of silicic acid dissolved from the different forms of silica. In water and biological fluids most of the reported figures for amorphous silica vary between 5 and 14 mgm. per 100 cc. of solution and for quartz between 4 and 10 mgm. The value for silica solubility recorded by Titus (160) is very much lower, but this was obtained with very much less solid phase than was used by most other workers. The naturally occurring silicates do not appear, in general, to be as soluble as the free forms of silica in neutral or alkaline liquids. In an acid medium some silicates are dissolved to a considerable extent.

Several substances act as depressors of silica solubility. These fall into two classes—certain metals or their oxides, and substances like coal which adsorb silicic acid from solution and hence appear to depress solubility. The metals and metallic oxides (Al, Fe, Ca, Mg, etc.) appear to precipitate silicic acid as fast as it goes into solution as the insoluble silicate of the metal. Aluminium and iron are particularly efficient depressors of solubility (Denny et al., 33). The effect is best seen on the inclusion of a small amount of the finely divided metal with powdered quartz in water. Minute traces only of dissolved silica can be demonstrated in the liquid, even after many days shaking.

IX. Silica in lungs. Since the original demonstrations of Peacock (1860, 121), and of Greenhow (1865, 55 a) of "sand" in human lungs, the study of the accumulations of siliceous material in the tissues has been the subject of numerous investigations. Only a few of them can be mentioned here (for a résumé of the literature see McNally, 115).

TABLE 2

Dissolution of silica in various fluids

FORM OF SILICA		DISS. SiO_2	SOLVENT	EXPOSURE	TEMPERATURE	AUTHOR AND METHOD
	grams solid per 100 cc.	*mgm. per 100 cc.*			°C.	
Amorphous						
Gelatinous	2*	9.0	Serum	1 week	37	Ultra-centrif. (90)
Gelatinous	2	9.1	Ascitic fluid	72 hours	37	Ultra-centrif. (40)
Dry	2.5	10	Water	10 days	37	Colorimetric (3)
Desiccated	2	7.0	Ascitic fluid	8 days	37	Ultra-centrif. (90)
Fresh	2	9.9	Ascitic fluid	8 days	37	Ultra-centrif. (90)
Fresh	2.5	14	Pleural fluid	10 days	37	Colorimetric (3)
Quartz						
	1	3.3	Water	8 days	37	Colorimetric (106)
	4	3.8	Water	8 weeks	20	Colorimetric (15)
	1	5.2	Water	3 hours	100	Colorimetric (15)
	0.01	0.05	Water	2 months	37	Ultra-filtr. (160)
Quartz	1	4.3	Water	10 days	37	Colorimetric (3)
		5	Water			Colorimetric (118)
	3	19	1 per cent $NaHCO_3$	48 hours	37	Colorimetric (161)
	3	18	0.1 N NaOH	24 hours	37	Colorimetric (161)
	1	2	0.1 N HCl	24 hours	37	Colorimetric (161)
"Bull" quartz	2	6.4	Ascitic fluid	8 days	37	Ultra-centrif. (90)
Rock crystal	2	9.1	Ascitic fluid	8 days	37	Ultra-centrif. (90)
	1	4.5	Pleural fluid	10 days	37	Colorimetric (3)
Flint	1	24	Water	3 hours	100	Colorimetric (15)
	1	43	1 per cent $NaHCO_3$	48 hours	37	Colorimetric (161)
	1	18	0.1 N NaOH	27 hours	37	
	0.3	2.7	0.1 N HCl	24 hours	37	Colorimetric (161)
	2	6.5	Ascitic fluid	8 days	37	Ultra-centrif. (90)
Granite	3	5	1 per cent $NaHCO_3$	48 hours	37	Colorimetric (161)
Felspar	1	3.4	Water	3 hours	100	Colorimetric (15)
Orthoclase	1	1.3	Water	7 days	37	Colorimetric (106)
	1	0.8	0.9 per cent NaCl	7 days	37	Colorimetric (106)
	2	0.8	Ascitic fluid	8 days	37	Ultra-centrif. (90)
Sericite	1	2.2	Water	3 hours	100	Colorimetric (15)
	0.01	0.05	Water	2 months	37	Ultra-filtr. (160)
	0.01	1	Serum	2 months	37	Ultra-filtr. (160)
	0.1	0.8	1 per cent $NaHCO_3$	8 days	37	Colorimetric (84)
	2	0.8	Ascitic fluid	8 days	37	Ultra-centrif. (90)
	0.1	0.4	1 per cent $NaHCO_3$	4 hours	100	Colorimetric (84)
Mica	1	0.7	Water	14 days	37	Colorimetric (84)
	1	0.45	1 per cent $NaHCO_3$	14 days	37	Colorimetric (84)
	2	1.8	Ascitic fluid	16 days	37	Colorimetric (90)

* The figures in the first column give the amount of solid phase taken per 100 cc. of solvent.

TABLE 2—*Concluded*

FORM OF SILICA		DISS. SiO_2	SOLVENT	EXPOSURE	TEMPERATURE	AUTHOR AND METHOD
	grams solid per 100 cc.	*mgm. per 100 cc.*			°C.	
Asbestos	1	25	Water	3 hours	100	Colorimetric (15)
	2	1.6	Ascitic fluid	8 days	37	Ultra-centrif. (90)
Fuller's earth	2	1.4	Ascitic fluid	8 days	37	Ultra-centrif. (90)
Kaolin	2	0.4	Ascitic fluid	8 days	37	Ultra-centrif. (90)
		0.3	Ascitic fluid	16 days	37	Colorimetric (84)
Talc	2	0.8	Ascitic fluid	8 days	37	Ultra-centrif. (90)

Kussmaul (1867, 99a) considered that the silica of lung tissue was entirely exogenous and that it only appeared after several months of life. While this view can no longer be held (89, 116), it is true that there is a gradual accumulation of siliceous material in the pulmonary tissues from youth to old age and particularly in persons habitually exposed to the inhalation of siliceous dust. Extensive analyses of lungs of persons with and without dust exposure have been made by McCrae (111), McNally (115), Antoine (2). Workers in dusty trades—miners, stonecutters, pottery workers, grinders, sand-blasters, etc., are apt to suffer an extraordinary accumulation of mineral particles in their lungs; and the lungs of dwellers in the sand-storm regions of the Sahara desert have been shown by Policard and Marrion (125) to have notable deposits of siliceous particles. The amount is usually expressed as a percentage of the total silicon (as SiO_2), contained in the ash or dry tissue. Collins and Dible (24) have pointed out that a figure giving the total silica contained in the lung is a better expression of its mineral content than is a percentage. Antoine (1) found a large degree of variation in the siliceous particle content of different parts of the same lung. The amount is greater in the region of the lower lobe than the upper and is greatest in the region of the hilum. It seems to be concentrated in the root glands whose content may reach 14 times that of the parenchyma.

The average content of silica in the lungs of normal adult persons ranges between about 40 and 200 mgm. of SiO_2 per 100 grams of dry

tissue, i.e., 0.04 to 0.20 per cent. Hackmann (59) considered that in cases of occupational exposure to dust, injuries to the tissues are usually observed when the silica content exceeds 1 per cent. It was held by Sladden (145) that extensive fibrosis is usually found when the silica content is 1 to 1.6 per cent, while an amount in excess of this is said to be almost invariably accompanied by severe fibrosis. These conclusions have been criticised by Belt, Irwin and King (10) (cf. also Fowweather, 41), on the ground that the lungs of non-silicotic miners showed equally as much analyzable silica as the lungs of others who died with massive silicotic fibrosis. In the case of peribronchial lymph glands an accumulation of silica was found to take place with age, which might reach the very high figure of nearly 5 per cent SiO_2 in the dry tissue without any real fibrosis having developed.

The mineralogical nature of the siliceous material in the lungs has concerned many workers. McCrae (111) and Watkins-Pitchford and Moir (165), made a detailed analysis of the ash of silicotic lungs. As might be expected they found not only free silica, but mineral silicates. Jones (72) showed that a high proportion of the mineral particles may consist of sericite, an aluminium potassium silicate of the mica family. Sundius et al. (152, 153) have made detailed analyses of the dust isolated from lungs of workers in dusty trades. In their second paper references are made to many of the investigations of the mineral matter from asbestosis lungs. The subject of mineral dust particles is thoroughly dealt with by Drinker and Hatch in their book, *Industrial Dust* (1936) (37).

It has been frequently postulated that part of the silica tended to become hydrated in the lung to form gelatinous silicic acid—the "occult silica" of Watkins-Pitchford and Moir, 1916, and Belt, 1929 (cf. Heffernan, 65; Irwin, 70). Tideswell (159) and Badham and Taylor (7) attempted to estimate this hydrated silica by chemical means. Its relation to the production of silicotic fibrosis has been a matter for repeated speculation (cf. Faber, 39, who claims x-ray spectrum evidence for the presence of colloidal silica in lung tissue).

X. THE DISSOLUTION OF MINERAL DUSTS IN VIVO. The pathogenicity of silica in relation to silicosis is thought by many people to depend on its solubility. There has accumulated a body of evidence (dealt with in a later section) which seems to imply that dissolved silica has a poisonous effect on the tissues. If the harmful effect of silica in the cells is related to its dissolution, then it might be expected that the silica particles in a silicotic lung would dissolve to some extent in the fluid bathing

them, or at least suffer some leaching of soluble silica from their surfaces. Such a dissolution of silica in the lungs should result in the passing of silicic acid into the blood, followed by its excretion in the urine. The evidence in this connection is conflicting. The inhalation of amorphous silica may lead to a prompt increase in the silicic acid of the urine (91), but inhalation of powdered crystalline silica has led to only minor increases. Only by giving massive doses in intratracheal insufflation experiments with animals (86) was it possible to demonstrate a urinary excretion of extra silicic acid. King and Dolan (86) found the average silica excretion to be higher in a group of gold miners than in a non-mining population, and Bloomfield and Sayers (12) claimed to have shown a relation between the silica dust exposures of miners and the amount of silica in their urines. But it seems likely, on the basis of data at present available, that only a small proportion of the total silica in the urine may come from the lungs. Dietary silica so readily causes variations in the urinary silica, that the food and drink of silicotics would have to be very rigidly controlled before any significant changes due to the dissolution of silica in the lungs could be looked for. The daily differences in urinary silica levels may be quite as great in an ordinary urban population (46) as among miners, or indeed as between individual miners and persons not exposed to the inhalation of excessive dust. An attempt was made to study the 24-hourly excretion of silica in carefully controlled groups of silicotic and nonsilicotic tuberculous patients in a sanitarium (86). No significant differences were observed. But this failure does not preclude the possibility that some of the very finest of the silica particles breathed by a worker in a dusty atmosphere may undergo a fairly prompt dissolution when they come in contact with the fluids of the lung. Larger particles may be dissolved much more slowly—too slowly, in fact, to show any evidence of dissolution other than a slow change in their physical characteristics as revealed by microscopic study.

The increased urinary silica values observed in persons inhaling large amounts of quartz dust may be related to a dissolution of particles in the intestinal tract as well as in the lung. There can be no doubt that large amounts of dust are coughed up (Burke, 18) and swallowed, and it has been shown that the administration of powdered quartz into the stomach of a dog leads to increased excretion of silica (fig. 1). The absorption of silica in the gut from mineral dusts has been studied by Whitehouse (167), who administered to himself samples of powdered rock. These were suspended in distilled water and swallowed. Only

in the case of Scotch whinstone (a rock of mixed silicates) and of very finely divided flint dust was he able to show a significant rise in his urinary output of silicic acid. Working with much larger samples of stone dust King and McGeorge (91) showed that several other siliceous minerals were dissolved to an appreciable extent in the gut and excreted in the urine of cats. Most of the free forms of silica showed this increase and some of the silicates. Those of the latter which appeared to dissolve were silicates which are easily decomposable by acid. They were probably attacked by the hydrochloric acid of the stomach. The resulting amorphous silica would easily be soluble in the alkaline duodenal juice.

Intraperitoneal injection of suspensions of the dusts led to the interesting contrast that whereas the free silicas seemed to be dissolved and excreted, the silicates were attacked to only a minor extent. Those silicates which had led to increased urinary output of silica after oral administration failed to give any comparable result when inoculated into the abdominal cavity. But here the dusts were subjected only to a simple leaching process by the mildly alkaline body fluids, without any preliminary exposure to an acid treatment such as they suffered in passing through the stomach. In all cases where dissolution appeared to have taken place, the excretion of extra silica was only transitory, and only a small proportion of the total silica injected was recovered in the urine.

Although the amounts of mineral dust administered in these experiments (91) were so great as to represent an abnormal exposure, they can probably be taken to indicate that some dissolution of siliceous minerals normally takes place in the body.

XI. EVIDENCE THAT SILICA[2] DUST IS HARMFUL TO MAN is based largely upon epidemiological investigations of miner's phthisis and other allied forms of occupational tuberculosis. Such epidemiological surveys as have been made in this field by Haldane (60), Collis (25), Hoffman (68), Lanza (100), Middleton (112), have strongly suggested an etiological relationship between silica and dust phthisis. On the strength of this evidence the term silicosis has largely supplanted the earlier usage of the term "miner's phthisis."

[2] It is unavoidable that we should have to use the terms silica and siliceous dust more or less interchangeably in a rather loose sense, much in the same way that chemists use the term total silica (see footnote page 330) without trying to indicate a specific compound of silicon (see Belt, Irwin and King 10).

If any one man more than another stands out as the father of the modern conception of silicosis, it is the late Professor J. S. Haldane, whose able investigations into dust hazards are summarised in the Second Report of the Royal Commission on Metalliferous Mines and Quarries, 1914, (60). To this obscure publication the better known and widely quoted Milroy Lectures of Collis, 1915, (25) appear to owe their inspiration. Much of the epidemiological spade work with respect to silicosis which is commonly attributed nowadays to others may be traced to Haldane. The gist of Haldane's thesis was "If in any class a high death-rate from pulmonary tuberculosis is found occurring at a later period of life than is usual for pulmonary tuberculosis and if this high death-rate is associated with a high death-rate from other respiratory diseases, then this class is exposed to the inhalation of injurious dust." The one factor which seemed to be common to all the injurious dusts was silica; therefore the Report concludes "The cause of miners' phthisis is the inhalation of dust of crystalline silica, upon which is superimposed tubercular infection."

Thus, the industrial hazards of siliceous dust first became apparent by virtue of an excessive incidence of pulmonary tuberculosis amongst the affected peoples. To this day, tuberculosis is perhaps still the best index of a harmful dust (Kettle, 1934) (77).

Silicosis may be defined as an industrial or occupational disease of the lungs, sporadic in incidence, insidious in onset, of great chronicity, occurring amongst workers who are exposed for years to the inhalation of siliceous dust. It is characterised essentially by extensive nodular fibrosis and by a marked tendency on the part of its victims to die eventually of pulmonary tuberculosis. "I believe the disease is the same all over the world," said Haldane (60), and most other authorities support this view.

The tissue changes produced in the lung may be and often are of a complex nature, but the essential lesion is a distinctive type of nodular fibrosis, the unit of which is commonly referred to as the silicotic nodule.

XII. THE MORPHOLOGY OF THE SILICOTIC NODULE. The silicotic nodule is a small, firm, rounded, tubercle-like structure varying roughly from 1 to 5 mm. in diameter and distributed unevenly through the lung parenchyma. Under the microscope it appears as a more or less spherical focus of dense, inert, acellular, avascular, collagenous connective tissue. Its component strands of collagen are relatively coarse and tend to be hyalinized. They have a concentric arrangement and often appear to be interwoven like basketwork. They are commonly described as

forming a whorled structure. Some of the American authors have coined an apt simile in comparing the nodule to a miniature ball of twine.

While its morphology is distinctive and, in general, more or less constant, the silicotic nodule has a good many variable features: it is more often conglomerate than single; it may have a necrotic centre or be partly calcified; its outline may be sharply defined or it may be fringed by an irregular mantle of cellular connective tissue; even its dust content lacks a specific constancy (see below).

By the very nature of its variations and by the relative imprecision of histopathological terms it is not possible to phrase an exact definition of the silicotic nodule. It is not surprising, therefore, to find that even amongst the ablest pathologists, there is not strict uniformity of opinion as to the morphology of the lesion. It will be especially important to bear this in mind when we come, presently, to discuss the experimental reproduction of silicosis.

The unparalleled experience of silicosis in the Witwatersrand Gold Mines of South Africa has given rise to some of the most authoritative studies we possess on the pathology of the disease. To such experts in this field as Watkins-Pitchford (163), Mavrogardato (109), Irvine (69) Strachan and Simson (148), we may turn for the most representative illustrations of the silicotic nodule (see plates 1, 2 and 3).

XIII. THE DUST CONTENT OF THE SILICOTIC NODULE. Characteristically, there is some degree of black pigmentation due to particles of anthracotic dust in the interstices of the nodule (see Watkins-Pitchford, 163). This black, carbonaceous dust is probably quite fortuitous in respect of the silicotic nodule, but it represents a convenient indicator of the less obvious, colourless, siliceous dust with which it is usually intimately mixed (see Belt, Irwin and King, 10).

Compared to other parts of the silicotic lung, the nodule itself usually appears to contain relatively little silica; with the polarising microscope there appear to be fewer and smaller siliceous particles within the nodule than in the cellular connective tissue around it (Belt, 8). This appearance is deceptive for the nodule usually contains a characteristic deposit of altered (occult) silica which can only be unmasked by some such process as microincineration (Watkins-Pitchford and Moir, 165).

Microincineration is, at least theoretically, the method of choice for demonstrating silica deposits in tissue sections (see Irwin, 70). By this means Policard and Martin (124) have made a quantitative histochemical study of the ash of silicotic nodules. They calculate that the

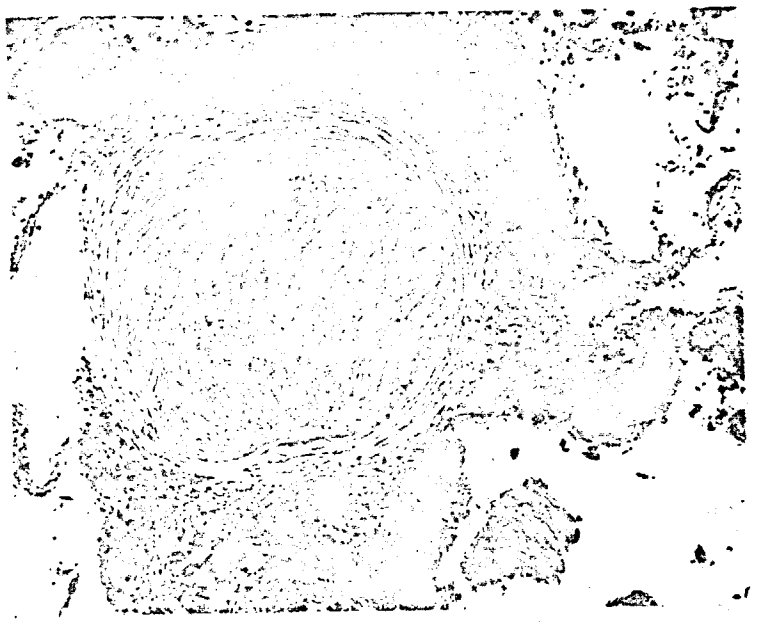

Plate 1. Photomicrograph (magnification about 50). After Simson and Strachan (150). Described as "Fully formed, single, small 'non-infective' silicotic nodule."

Plate 2. Photomicrograph (magnification about 35). After Simson and Strachan (150). Described as "Complete 'non-infective' silicotic nodule."

siliceous residue represents about 5 per cent of the dry weight of the average nodule. This siliceous ash is a white amorphous powder, the like of which is peculiar to the silicotic nodule; it probably derives from inhaled particles which have undergone some chemical transformation in the nodule. Policard (132) believes it has undergone actual chemical

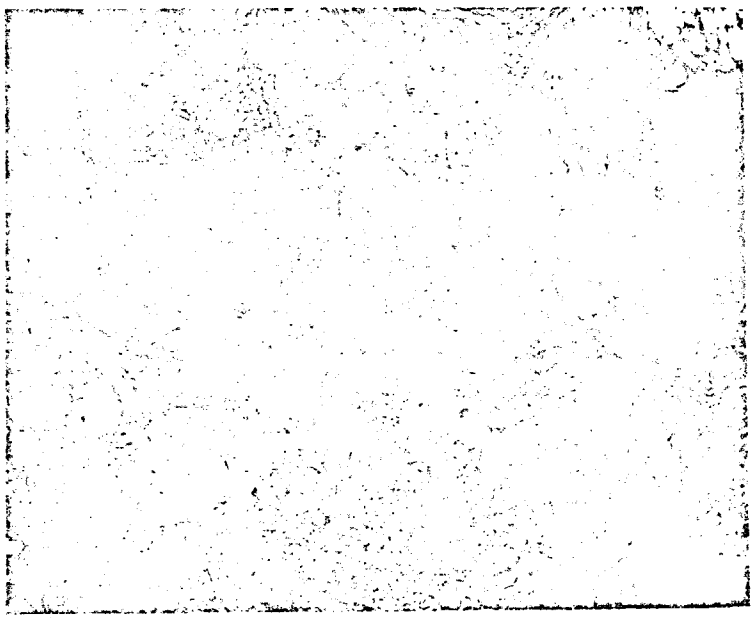

Plate 3. Photomicrograph (magnification about 10). After Simson and Strachan (150). Described as "Silicotic massive fibrosis of 'non-infective' type, composed of numerous contiguous, composite and single silicotic nodules."

combination with the collagen and refers to it as "du type des cendres de constitution, résultant des matières minérales faisant partie de la constitution même du tissu incinéré."

While the quality of the silica deposit appears to be more or less constant from one silicotic nodule to another, the quantity varies considerably (Policard, 124) and probably bears no constant relationship to the size of the nodule. Moreover the dust deposit does not permeate the whole nodule uniformly. Thus there is apparently more fibrosis in the silicotic nodule than could be explained as a simple foreign body reaction. In other words, the silicotic nodule represents a redundant

fibrous reaction, manifesting a bulkier proliferation of fibrous tissue than would be essential for segregating the amount of dust involved.

The size of the harmful particles. By digesting the tissue (or tissue ash) with strong acid and recovering the mineral matter McCrae (111), Watkins-Pitchford and Moir (165), Giese (45), Policard (123) and Jones (72) have studied the size and shape of siliceous particles in the pneumoconiotic lung. There is general agreement that the majority of such particles are of the same order of size as common micro-organisms (Mavrogardato, 109). The average dimension is about 1.μ or less. Particles larger than 12.μ have rarely been observed in the pneumoconiotic lung except in the case of asbestos which, quite unlike other dusts, enters the lung in the form of long fibres, sometimes 100 to 200μ in length. Cooke (26), Gardner (52 a). The small size of the silica particles probably has a good deal to do with their pathogenicity. Gardner and Cummings (54) have shown that particles of quartz under 3μ are much more productive of connective tissue proliferation (liver cirrhosis) than particles of larger size. The smaller the particles, the more available they become for chemical change, and the greater is their relative solubility (Lucas and Dolan, 106; King and McGeorge, 90). The small size of the particles found in the lung is of such an order as to facilitate optimum availability, a factor of great importance since the pathogenicity of silica may be related to its solubility (see p. 338). Middleton (113) has pointed out that the most harmful dusts in industry appear to be those which contain a high proportion of very small particles. From the point of view of an industrial hygienist he believes the greatest damage is caused by particles under 1μ in size.

XIV. THE PATHOGENESIS OF THE SILICOTIC NODULE is related in some way to the presence in the lung of excessive quantities of siliceous dust, but how the lesion develops is still largely a matter of conjecture. Kettle (78) said "Now, in most forms of chronic fibrosis we are able to give some reasonable account of the development and progress of the lesion. By a histological analysis of, say, the various stages of the contracted granular kidney, we can describe pretty well how the condition is produced, but we have a good deal to learn about the development of the pulmonary lesions in silicosis. . . . Experimental investigations might have been expected to help in the elucidation of the process, but for the most part they have not done much to clear the air, and this is not for want of time or trouble."

Surprising as it may seem, it is nonetheless true that great difficulty

has been encountered in tracing the various stages of development of the silicotic nodule in human lungs. The full-blown nodule is well recognised but the early stages are but sketchily defined. This may be due to several factors: 1, not many cases of early silicosis come to autopsy; 2, the nodules in any given case tend to mature synchronously so that there is often little variation from one lesion to another; and 3, the end-stage picture is often so complicated by tuberculous infection that it is difficult to distinguish tuberculous from dust lesions.

The spherical morphology of the nodule indicates a focal origin at a small central point around which the lesion is built up concentrically: but it is difficult to postulate any such focal starting points in the topography of the dust-ridden lung for the dust is usually widely disseminated throughout the pulmonary lymphatic apparatus. In short, the distribution of the dust is more diffuse than focal and therefore provides no obvious clue to the origin of the focal, nodular lesion.

The distribution of the nodules might be expected to offer some indication as to what particular parts of the lung are most vulnerable to silica and perhaps indeed it does. In fact it is generally conceded that the lesions develop in the pulmonary lymphatic apparatus, first in the glands, then along the linear lymph channels which parallel the blood vessels, bronchi, interlobular septa and pleura. But one has only to consult Miller's (114) recent monograph on the anatomy of the lung to realize with what a complicated structure one is dealing in the pulmonary lymphatics and how ill-defined they usually are in ordinary histological preparations of the lung. When one reflects with what difficulty the anatomists themselves have delineated the finer structural details of the normal lung, it is not surprising that pathologists working with the distorted anatomy of the silicotic lung have been puzzled to know in what particular part of the lung lobule the silicotic nodule takes origin. In the absence of better than ordinary histological methods of identifying the smaller lymph channels and lymph nodes of the lung parenchyma, the so-called lymphatic distribution of silicotic nodules is little more than a hypothetical conception.

On the basis of their wide acquaintance with the morbid anatomy of the silicotic lung, Simson and Strachan (149) suggest a constant anatomical relationship between the sites of 1, early aggregation of dust cells, and 2, subsequent nodule development, and that these sites correspond to hypothetical outposts of the pulmonary lymphatic apparatus, namely, small lymph nodes situated at the bifurcation of each terminal bronchiole at the entrance of each lobule. Gardner (53) favours this

view. If such an hypothesis be tenable then there may be said to exist some anatomical basis for the nodular morphology of the silicotic lesion.

These parenchymal outposts of the lymphatic system are visualised as miniature lymph follicles which act as dust traps; the dust-laden phagocytes, migrating from the air sacs towards the hilum or the pleura, collect in these traps, where, presumably, after prolonged residence, small aggregations of siliceous dust provoke their characteristic nodular reaction.

As to the more specific pathogenesis of the silicotic nodule there are two current theories, somewhat at variance, which must occupy our attention. The first of these, which may be called for the sake of convenience, the "silica specificity theory" ascribes the nodule to a specific effect of siliceous dust per se, while the second, which may be styled the "infective theory," invokes a dual etiology, namely, siliceous dust and tubercle bacilli.

1. *The silica specificity theory* regards siliceous dust as the sole etiological agent of the silicotic nodule. Adherents of this theory, who are probably in the majority, have sought to define the modus operandi of silica on a physical or chemical basis. Thus, until comparatively recently it was held generally that silica owed its harmful properties to its physical characters; the hard, sharp particles were supposed to damage the pulmonary tissue, and the whole process was regarded as a kind of microscopic trauma which after prolonged action in minute foci produced redundant fibrosis. Haldane is said to have abandoned this mechanical concept only when Kettle (79) showed that flint particles were rendered innocuous by a thin coating of iron oxide which did not alter their sharpness. Equally convincing is the fact that carborundum dust with particles just as hard and sharp as silica, apparently produces no comparable lesion (Gardner, 50). Thus the theory of mechanical irritation has been replaced by the view that silica becomes soluble in the tissues and exerts a chemical rather than a physical effect. It must not be presumed, however, that the physical presence of silica particles is altogether a matter of indifference to the tissues, for the mere presence of foreign particles always tends to set up a process of phagocytosis and segregation by fixed tissue proliferation, as is well-known in the case of common pigmentations like tattooing, anthracosis and hemochromatosis. In such pigmentations the reaction is of the simplest foreign-body type, and the same reaction necessarily must take part to some degree in the tissue response to siliceous dust, but in the latter instance, as we have seen, the reaction tends to outreach these simple

proportions and become redundant. For the redundant qualities of the silicotic nodule, therefore, some process other than a foreign-body reaction must be called to account.

Simson and Strachan (149) who, in common with most of the other present day investigators in Africa, regard the silicotic nodule as a dust effect pure and simple, trace various histological stages of nodule development in the human lung.

Their statement of what is presumed to take place may fall short of enabling a precise visualisation of the process but it is widely credited as an adequate explanation, namely, that the silicotic nodule springs, tumour-fashion, from a nidus of dust cells by an indolent proliferation of fibroblasts whose growth is in some way initiated and stimulated by a small aggregation of siliceous dust particles. It is agreed generally that silica must undergo dissolution to produce this effect.

Gardner (53), whose unrivalled experience of dusting experiments places him amongst the foremost authorities on the subject, believes that silica, during prolonged residence in the tissue, gives rise to a poisonous solution which causes cell damage, necrosis, lymph stasis and chronic inflammation, and that these changes are obligatory antecedents of the full-blown nodule.

There is experimental evidence for believing that certain forms of soluble silica have a mildly toxic effect upon endothelial and connective tissue cells. Thus Gye and Purdy (58), were able to produce liver cirrhosis in rabbits by the parenteral administration of repeated small doses of colloidal silica, and Gardner and Cummings (54) duplicated this feat with finely ground quartz. Subcutaneous inoculations of silica particles in mice produce what Gye and Kettle (57) called "almost a specific lesion" characterised by acute inflammation and necrosis. Similar results are reported by Fallon and Banting (40). Mavrogardato (109) found evidence for believing that silica had a "mummifying" effect upon the phagocytes which ingested it, and Policard shares this view (122).

On the basis of these experiments and others too numerous to mention it is judged probable that the toxicity of silica may be held accountable for the development of silicosis in man. This hypothesis becomes the more tenable since, as we have indicated in the earlier part of this paper, it is now accepted that silica is appreciably soluble in body fluids (see p. 338). The toxicity theory postulates the following sequence of events in the silicotic lung: silica fragments are transported into pulmonary

tissue spaces by phagocytes and come to rest in small collections throughout the pulmonary lymphatic apparatus; in course of time the phagocyte disintegrates liberating its load of siliceous dust into a connective tissue space; slow chemical change of the silica takes place with the production of a poisonous derivative which produces a local injury; slow proliferation of fibrous tissue is the response to this injury; the nodular morphology of the lesion is explained on the grounds that the dust, in the first instance, tends to collect in spherical foci.

Gardner's (53) dusting experiments have gone a long way to support the silica specificity theory. By causing rabbits and guinea pigs to inhale quartz dust over long periods measurable in years he induced a fibrous nodulation of the lungs comparable to that seen in the human disease and on the basis of this work is generally credited with proving that silicosis can be experimentally reproduced through the action of dust alone. To some morphologists it may seem as if experimentalists in general had taken unwarranted liberties with the generally accepted meaning of the term "silicotic nodule." But if there be any doubt as to the interpretation of the Gardner lesions, then that doubt is likely to be accentuated in respect of other experimental studies (66 a, 113 a, 133 a, 33) which purport to show reproduction of the silicotic nodule. The late Prof. E. H. Kettle, himself a veteran experimentalist in the field of pneumoconiosis, said (78) "Gardner has realised that silicosis is a chronic disease which takes years to develop. He has, therefore, exposed his animals to dusty atmospheres for eight hours a day over periods of two to three years and he has obtained most important results. But most other workers have been content with a total dust exposure of twenty-four to thirty-six hours, and I am not convinced that they have ever produced specific fibrosis; certainly they have produced nothing comparable with what occurs in man."

Whatever may be the merits of the silica specificity hypothesis there are certain important features of silicosis which it apparently does not explain, namely, 1, the great difficulty in reproducing the characteristic lesion in animals. In spite of numerous experiments there is no evidence, for example, to show that silica injected subcutaneously or intraperitoneally ever gives rise to nodular fibrosis comparable to that seen in the human disease. 2. The sporadic incidence of the disease. Watkins-Pitchford (164) points out "The mistake must not be made of supposing that all or even a majority of the miners will develop silicosis. It is only about 3 per cent of those who have worked for ten years or

longer who annually become silicotic in any form." Policard (122), on the basis of a wide experience of silicosis in France, also emphasizes this point.

Sometimes one has the opportunity of examining the lungs of individuals who have worked for years in phthisis-producing dust and have not developed silicosis. The present writers have been much impressed with this apparent immunity to silicosis amongst gold miners in Northern Ontario where silicosis is not uncommon. We have had the opportunity, through the kindness of Dr. J. G. Cunningham, of examining the lungs of several long term miners, who had survived the silica hazard for years and outlived silicotic work-mates of a similar age, to die eventually from some other cause. In such cases, as Mavrogardato (109) has pointed out, one invariably finds large amounts of particulate silica in the pulmonary tissues and one knows that it must have been there for years without provoking any appreciable reaction. This apparent immunity to silica, which must be regarded as of widespread occurrence, is difficult to reconcile with the theory that silica per se is a poisonous substance.

Haldane (60 a), well aware of this common immunity to silica, explained it on his well-known theory of adulterant dusts which postulated that certain relatively innocuous dusts (shale, clay, coal) may forestall the evil effects of silica if inhaled at the same time. Numerous attempts have been made to identify the hypothetical antidote which Haldane visualised (Mavrogardato, 109, 110; Carleton, 20; Haynes, 64; Hefferman, 65 a) but the results have been equivocal (Kettle, 80). Recent work in Sir Frederick Banting's (33) department in Toronto has given promise of allaying the pathogenicity of silica by reducing its solubility through the admixture of aluminium dust, but this conjecture awaits more crucial test. To quote Kettle (78) again "We must have a base line to work on, and in these experiments the base line must be the production of characteristic silicosis in animals. In the absence of this we are not justified in claiming experimental knowledge of how silicosis may be modified by adulterant dusts."

2. *The infective theory* of origin of the silicotic nodule finds its chief support in the fact that silicosis is so often accompanied by tuberculosis and in the fact that the silicotic nodule morphologically resembles the fibroid tubercle.

Towards the end of his long experience of silicosis in the Transvaal, Watkins-Pitchford (164) concluded "that in practically all instances there are two factors pre-requisite to the development of silicosis in its usual form: 1, the condition of latent silicosis, and 2, the access of a tuberculous infection"; and further that "The pathology of almost all cases of what we term simple silicosis now appearing on the Rand is fundamentally the same as that of tuberculosis with silicosis; the development of what appears to be a condition of simple silicosis in these days, is probably a pathologically unique result of a tuberculous infection in a latently silicotic individual."

Kettle's (78) viewpoint is expressed thus "It is true that dust and dust alone may produce disability and death . . . but under modern conditions dust disease, or silicosis, really spells tuberculosis," and again (77) "Pure or simple silicosis is rarely of clinical significance; nearly always silicosis is associated with tuberculosis and my experience leads me to place increasing importance on the infective factor. . . . There has often seemed to me strong evidence that the apparently pneumoconiotic lesions were really infective from the beginning."

Policard (122) suggests that while pure silicosis is a theoretical possibility, it is rarely a practical reality—"En fait, dans l'immense majorité des cas, si non dans tous, le poumon silicotique est toujours aussi un poumon tuberculeux"; and Nicaud (120) writes "On peut donc vraiment se demander si la silicose a une existence propre et si les cas rapportés sous ce nom ne sont pas de simples modalités de tuberculose fibreuse". Rist et al. (132) also comment, "Il devient aussi de plus en plus évident que l'inhalation de poussières siliceuses ne suffit pas à elle seule à créer la silicose . . . c'est que l'intervention d'un autre facteur pathogène est nécessaire. Ce facteur est le plus souvent représenté par la tuberculose, et parfois sans doute par les simples séquelles de tuberculose."

Kettle and Archer (82) have drawn attention to the difficulty sometimes encountered in distinguishing between silicotic nodulation and fibroid tubercles while Nicaud (120) has done much to show a histological similarity between the two.

Kettle (77) by combining dead tubercle bacilli with siliceous dust produced lesions in guinea pigs' lungs comparable to the human silicotic nodule.

Policard and his associates hold strongly to the infective theory. They report inability to duplicate Gardner's experimental reproduction of silicotic nodulation by the action of dust alone. In Policard's prolonged and careful experiments (127), silica per se produced only relatively slight changes, but with the addition of tubercle bacilli (B.C.G.) a result, regarded as comparable to human silicosis, was obtained. Policard regards the silica as having a specific effect which only becomes

apparent in the event of subsequent tuberculous infection. In other words, only on the basis of a preëxisting "silica-granuloma" will the tubercle bacillus give rise to the characteristic fibrous nodule. He believes the silica undergoes some chemical change to produce this effect.

Not without impressive evidence does Policard (122) conclude his treatise on the "Mode of Formation of the Silicotic Nodule" thus: "Le nodule silicotique typique, celui qu'on envisage souvent comme 'pur', ne serait pas autre chose qu'un foyer tuberculeux cicatrisé et guéri, parce qu'il s'est formé au niveau d'un amas de particules siliceuses. La silicose serait une tuberculose pulmonaire devenue fibreuse sous l'influence et au niveau des particules siliceuses. Le nodule silicotique serait une lésion bacillaire développée au niveau d'un amas de poussières siliceuses et devenue tout à fait fibreuse sous leur influence."

Rist, Vallery-Radot and their associates (132) lend the weight of their authority to the view that the silicotic nodule is probably a modified tubercle.

There is of course unanimous agreement that if the silicotic nodule is to be construed as tuberculous then: 1, it represents an anomalous manifestation of tuberculous infection; 2, siliceous dust plays an essential rôle in its development; and 3, silica must modify the tuberculous process in a twofold manner, viz., by offering unusual impetus to fibrosis of the lesions, while at the same time facilitating the progress and spread of the infection.

The infective theory has the advantage of explaining 1, the sporadic incidence of silicosis; 2, the nodular morphology and the distribution of the essential lesion (see Nicaud, 120); 3, the close association of silicosis with tuberculosis; and 4, the difficulty of reproducing the silicotic nodule in animals by the action of silica alone.

Perhaps the most serious objections levelled at the infective theory have been 1, the well-known clinical observation that full-blown cases of silicosis may evince none of the usual signs and symptoms of pulmonary tuberculosis, and 2, the frequent inability to demonstrate tubercle bacilli in silicotic nodules (see Simson and Strachan, 149).

XV. THE INFLUENCE OF SILICA ON THE TUBERCLE BACILLUS. How to explain the tuberculous affinity of the dust-ridden lung has been the subject of intensive study for years. One of the first things to be investigated was the influence of various forms of particulate silica and silicates on the growth of tubercle bacilli in vitro, and in general negative results

seem to have been obtained (Kettle, 78) though Price (129) reported some acceleration of growth with sodium silicate.

The crucial experiments of Gye and Kettle (57) showed a selective localisation and pullulation of tubercle bacilli in subcutaneous silica-lesions in mice, from which these authors suggested that the necrosis induced by silica afforded a protective pabulum favourable to proliferation of the tubercle bacillus. Later, Kettle (81) was able to show that the active growth of the bacilli did not depend merely on the necrosis of the tissue, but was due to the actual presence of the silica. Cummins and Weatherall (29) repeated and confirmed this work but found that the tubercle bacilli tended to disappear in time from the silica lesions, without affecting the survival of the animals.

Vorwald and Landau, (162) working in Doctor Gardner's laboratory at Saranac Lake, also undertook a repetition of the Gye and Kettle experiments. Using white mice they injected various mineral dusts beneath the skin of the abdominal wall. The animals were then infected intravenously with living tubercle bacilli and were killed at different periods after infection. When the subcutaneous dust lesions were examined histologically for the presence of tubercle bacilli it was found that those lesions produced by free silica were more often positive and contained more bacilli than those caused by silicates and non-siliceous dusts. This work, in the main, confirms Kettle's (81) results, but with one reservation, namely, that while silica lesions seem to favour tubercle localisation more than do the lesions of other dusts, yet the latter are not uniformly inimical to the tubercle bacillus. Silicates like sericite, talc and sillimanite and even some non-siliceous powders like fluorspar and aluminium oxide were not without some effect which seemed to favour tubercle localisation.

Gardner has produced reactivation of healing tubercles in guinea pigs' lungs by experimental inhalation of marble, granite, carborundum and quartz dust (51). He also showed that the combined effect of tuberculous infection plus any one of these dusts produced more fibrosis than either agent acting alone (52).

Of special significance are those experiments of Gardner (55) in which he showed that the native resistance of guinea pigs to an attenuated strain of tubercle bacilli (Saranac Lake R1 strain) was altered by silica. In the ordinary course of events guinea pigs infected by inhalation with this organism recover completely, but when dusted with quartz the infection spreads and progresses to fatal proportions. This change is

not due to an increased virulence of the organism, for subinoculations produce merely the characteristic attenuated infection. Gardner believes the silica alters the defence mechanism of the phagocytes by injuring them and perverting their bactericidal activities.

Thus, the laboratory evidence as well as clinical experience all goes to emphasize a notable adjuvant effect of siliceous dust in respect of tuberculous infection. This effect seems to depend upon some intermediary interaction between the dust and the tissues rather than upon the dust itself. Whether the essential intermediary change is humoral as Cummins (30) suggests or cellular as Kettle (78) thought, whether it is lymph stasis, necrosis, fibrosis or incapacitation of phagocytes, remains to be proven, but the available evidence favours the view that the silica-vulnerability of the phagocyte is an important link.

In conclusion it may be said that there has been and there still is much discussion about the relative harmfulness of various siliceous dusts (9). The general tendency has been to regard crystalline silica as the sine qua non of pathogenicity in harmful dusts, but there is reason to believe that some of the silicates may have a like effect. Much work remains to be done before we can satisfactorily gauge the harmfulness of various silicates, but it seems to us not unlikely that future simplification of the silicosis problem may turn more upon further elucidation of the alliance between siliceous dust and the tubercle bacillus than upon assessing the relative pathogenicity per se of various compounds of silicon. There is also the broad and open question of what influence silica may have upon infective organisms other than the tubercle bacillus and to what extent they may be linked with silica in the production of dust fibrosis. Our experience has left us with the impression that what we now know for certain about the pathogenicity of silica is perhaps not very much compared to what we have yet to learn.

REFERENCES

(1) Anderson, C. S. and J. H. Dible. J. Hyg. (in press).
(1a) Antoine, G. Ann. Med. Legale Criminol. Police Sci. **15**: 108, 1935.
(2) Antoine, G. J. Pharm. et Chim. **22**: 412, 1935.
(3) Archer, H. E., quoted by Kettle. J. Path. and Bact. **35**: 395, 1932.
(4) Atkins, W. R. G. J. Marine Biol. Assoc. U. K. **13**: 151, 1923.
(5) Atkins, W. R. G. J. Marine Biol. Assoc. U. K. **15**: 191, 1928.
(6) Aufrere, M. L. Bull. Soc. d'Océanographie France **95**: 1645, 1937.
(7) Badham, C. and H. B. Taylor. M. J. Australia **1**: 511, 1933.
(8) Belt, T. H. Can. Pub. Health J. **20**: 495, 1929.
(9) Belt, T. H. Am. J. Med. Sci. **188**: 418, 1934.
(10) Belt, T. H., D. Irwin and E. J. King. Canadian M. A. J. **34**: 125, 1936.

(11) Berg, R. Biochem. Ztschr. **254**: 329, 1932.
(12) Bloomfield, J. J., R. R. Sayers and F. H. Goldman. U. S. Pub. Health Repts. **50**: 421, 1935.
(13) Böhme, A. and H. Kraut. Arbeitsphysiol. **5**: 269, 1932.
(14) Bowes, J. H. and M. M. Murray. Biochem. J. **29**: 2721, 1935.
(15) Briscoe, H. V. A., J. W. Matthews, P. F. Holt and P. M. Sanderson. Bull. Instn. Min. Metall. Lond., April and June, 1937.
(16) Broman, J. R. Am. Rev. Tuber. **8**: 337, 1923.
(17) Brussoff, A. Arch. Mikrobiol. **5**: 1, 1933.
(18) Burke, H. E. J. Ind. Hyg. **17**: 27, 1935.
(19) Calvert, R. Am. Chem. Soc. Monograph, no. 52, 1930.
(20) Carleton, H. M. J. Hyg. **22**: 438, 1924.
(21) Cerny, C. Ztschr. physiol. Chem. **62**: 296, 1909.
(22) Chapman, E. M. Am. J. Med. Sci. **185**: 171, 1933.
(23) Clark, G. L. and D. H. Reynolds. Univ. of Toronto Studies, Geological Series, **38**: 13, 1935.
(24) Collins, D. H. and J. H. Dible. J. Hyg. **35**: 64, 1935.
(25) Collis, E. L. Milroy Lectures. Public Health **28**: 252, 292; **29**: 11, 37, 1915.
(26) Cooke, W. E.: Brit. Med. J. **2**: 578, 1929.
(27) Cooper, L. H. N. J. Marine Biol. Assoc. U.K. **18**: 697, 1933.
(28) Coupin, H. Comp. Rend. Acad. des Sciences **175**: 1226, 1922.
(29) Cummins, S. L. and C. Weatherall. J. Hyg. **33**: 295, 1933.
(30) Cummins, S. L. Brit. J. Exper. Path. **8**: 237, 1922.
(31) De, N. K. Ind. J. Med. Res. **22**: 499, 1935.
(32) De Eds, F. and C. W. Eddy. J. Biol. Chem. **114**: 667, 1936.
(33) Denny, J. J., W. D. Robson and D. A. Irwin. Canadian M. A. J. **37**: 1 1937.
(34) Dienert, F. and F. Wandenbulche. Bull. Soc. Chim. **33**: 1131, 1923.
(35) Drea, W. F. J. Nutrition **10**: 351, 1935.
(36) Drechsel, E. Zentral f. Physiol. **11**: 361, 1898.
(37) Drinker, P. and T. Hatch. Industrial dust—hygienic significance, measurement and control. New York, 1936.
(38) Faber, O. M. Staub. Halle. **1**: 26, 1936.
(39) Faber, O. M. Staub. Halle. **3**: 372, 1936.
(40) Fallon, J. T. and F. G. Banting. Canadian M. A. J., **33**: 404, 1935.
(41) Fowweather, F. S. Chem. and Ind., 713, 1934.
(42) Frauenberger, F. Ztschr. physiol. Chem. **57**: 17, 1908.
(43) Frey-Wyssling, A. Ber. Deutsch. botan. Ges. **48**: 179, 1930.
(44) Gaubatz, E. Klin. Wchnschr. **14**: 1753, 1935.
(45) Giese, W. Veröffentl. a. d. Gewerbe—u. Konstitutionspath. Jena, Fischer, **7**: 28, 1931.
(46) Goldwater, L. J. J. Ind. Hyg. and Tox. **18**: 163, 1936.
(47) Gonnermann, M. Ztschr. physiol. Chem. **79**: 255, 1917.
(48) Gonnermann, M. Ztschr. physiol. Chem. **88**: 401, 1918.
(49) Gonnermann, M. Ztschr. physiol. Chem. **94**: 163, 1919.
(50) Gardner, L. U. Am. Rev. Tuber. **7**: 344, 1923.
(51) Gardner, L. U. Am. Rev. Tuber. **20**: 833, 1929.

(52) GARDNER, L. U. Am. Rev. Tuber. **4**: 734, 1920; **6**: 163, 1922; **7**: 344, 1923.
(52a) GARDNER, L. U. J. Ind. Hyg. **13**: 65, 1931.
(53) GARDNER, L. U. J. Ind. Hyg. **14**: 18, 1932.
(54) GARDNER, J. U. AND D. E. CUMMINGS. Am. J. Path. **9**: 751, 1933.
(55) GARDNER, L. U. Tubercle **6**: 336, 443, 1924–25.
(55a) GREENHOW, E. H. Trans. Path. Soc. London **16**: 59, 1865.
(56) GURSCHING, J. AND H. KRAUT. Arch. exper. Path. und Pharmakol. **167**: 146, 1932.
(57) GYE, W. E. AND E. H. KETTLE. Brit. J. exper. Path. **3**: 241, 1922.
(58) GYE, W. E. AND W. J. PURDY. Brit. J. exper. Path. **5**: 238, 1924.
(59) HACKMANN, C. Beitr. path. Anat. **90**: 623, 1933.
(60) HALDANE, J. S. Second Report of the Royal Commission on Metalliferous Mines and Quarries. Eyre & Spottiswoode, London, 1914.
(60a) HALDANE, J. S. Chem. Met. and Min. Soc. S. Africa **30**: 54, 59, 1929.
(61) HARVEY, H. W. Biological chemistry and physics of sea water. London, 1928.
(62) HARVEY, H. W., L. H. N. COOPER, M. V. LEBOUR AND F. S. RUSSELL. J. Mar. Biol. Assoc. U. K. **20**: 407, 1935.
(63) HARVEY, H. W. J. Mar. Biol. Assoc. U. K. **19**: 253, 1933.
(64) HAYNES, F. J. Hyg. **25**: 94, 1926; **31**: 96, 1931.
(65) HEFFERNAN, P. AND A. T. GREEN. J. Indus. Hyg. **10**: 272, 1928.
(65a) HEFFERNAN, P. J. Indus. Hyg. **8**: 481, 1926.
(66) HICKS, V., O. MCELROY AND M. E. WARGA. J. Indus. Hyg. and Tox. **19**: 177, 1937.
(66a) HIGGINS, G. M. AND W. S. LEMON. Am. Rev. Tuber. **28**: 471, 1933.
(67) HILLEBRAND, W. F. The analysis of silicate and carbonate rocks. U. S. Geol. Survey Bull. 700, Washington, 1919.
(68) HOFFMAN, F. L. Reports on mortality from tuberculosis in dusty trades. U. S. Nat. Tuber. Assoc. 1918 and 1919.
(69) IRVINE, L. G., A. H. WATT, J. P. JOHNSON AND W. STEWART. Gen. Report of the Miners' Phthisis Prevention Comm. Johannesburg, 1916. App. 6, p. 81.
(70) IRWIN, D. A. Canadian M. A. J. **31**: 135, 140, 1934.
(71) JOLLES, A. AND NEURATH. Ztschr. f. angew. Chem. **11**: 315, 1898.
(72) JONES, W. R. J. Hyg. **33**: 307, 1933.
(72a) JOHLIN, J. M. Proc. Soc. Exper. Biol. Med. **29**: 760, 1932.
(73) KAHANE, E. AND G. ANTOINE. Bull. Soc. Chim. Biol. **18**: 1769, 1936.
(74) KAHLE, H. Beitr. z. Klin. d. Tuber. **47**: 296, 1921.
(75) KESSELER, A. Deutsch. Med. Wchnschr. **46**: 239, 1920.
(76) KETTERMAN, O. Milchw. Forsch. **5**: 73, 1927.
(77) KETTLE, E. H. J. Path. and Bact. **38**: 201, 1934.
(78) KETTLE, E. H. Proc. Roy. Soc. Med. **24**: 1, 1930.
(79) KETTLE, E. H. J. Path. and Bact. **35**: 395, 1932.
(80) KETTLE, E. H. Brit. Med. J. **2**: 281, 1932.
(81) KETTLE, E. H. Brit. J. Exper. Path. **5**: 158, 1924.
(82) KETTLE, E. H. AND H. E. ARCHER. Proc. Roy. Soc. Med. **26**: 15, 1933.
(83) KING, E. J. J. Biol. Chem. **80**: 25, 1928.
(84) KING, E. J. Nature, London **140**: 320, 1937.

(85) KING, E. J. AND V. DAVIDSON. Biochem. J. **27**: 1015, 1933.
(86) KING, E. J. AND M. DOLAN. Canadian M. A. J. **31**: 21, 1934.
(87) KING, E. J. AND H. STANTIAL. Biochem. J. **27**: 990, 1933.
(88) KING, E. J., H. STANTIAL AND M. DOLAN. Biochem. J. **27**: 1002, 1933.
(89) KING, E. J, H. STANTIAL AND M. DOLAN. Biochem. J. **27**: 1007, 1933.
(90) KING, E. J. AND M. MCGEORGE. Biochem. J. **32**: 417, 1938.
(91) KING, E. J. AND M. MCGEORGE. Biochem. J. **32**: 426, 1938.
(92) KOCHMANN, M. AND L. MAIER. Biochem. Ztschr. **223**: 228, 1930.
(93) KOROL, S. S. AND V. M. KOLUZHSKAYA. Zavodskaya Lab. **3**: 908, 1934.
(94) KNOPF, A. U. S. Pub. Health Repts. **48**: 183, 1933.
(95) KOBERT, R. Monograph, Rostock, 1918.
(96) KRAUT, H. Ztschr. physiol. Chem. **199**: 81, 1931.
(97) KÜHN, A. Therapeut. Monatsh. **33**: 201, 1919.
(98) KÜHN, A. Münch. Med. Wchnschr. **68**: 1612, 1921.
(99) KÜHN, A. Die Kieselsäure. Ferdinand Enke, Stuttgart 1926.
(99a) KUSSMAUL, A. Deutsch. Arch. f. klin. Med. **2**: 89, 1867.
(100) LANZA, A. J. J. Pub. Health **6**: 474, 1916.
(101) LEMATTE, L. AND E. KAHANE. Compt. rend. **196**: 575, 1933.
(102) LEMAY, P. AND L. JALOUSTRE. La nature **53**: 180, 1925.
(103) LEMMERMANN, O. Ztschr. Pflanzenernährung, Düngung und Bodenkunde **13A**: 28, 1929.
(104) LENHER, V. AND H. MERRILL. J. Am. Chem. Soc. **37**: 2630, 1917.
(105) LINE, W. R. AND P. W. ARADINE. Ind. Eng. Chem. Anal. Edn. **9**: 60, 1937.
(106) LUCAS, C. C. AND M. DOLAN. Canadian M. A. J. **38**: 1938.
(107) MANN, A. Diatomaceae of North America. Philadelphia, The Comus Press, 1894.
(108) MAYER, M. E. AND H. G. WELLS. Am. Rev. of Tuber. **8**: 318, 1923.
(109) MAVROGARDATO, A. E. Contributions to the study of miners' phthisis. Publ. S. African Inst. for Med. Res. no. 19, 1926.
(110) MAVROGARDATO, A. E. Internat. Conf. on Silicosis, Johannesburg, 1930. Pub. by Internat. Labour Office, Geneva, p. 211.
(111) MCCRAE, J. Pub. South African Inst. Med. Res., March 3, 1913.
(112) MIDDLETON, E. L. Tubercle **1**: 257, 1919.
(113) MIDDLETON, E. L. Milroy Lectures, 1936. Lancet, July 1936, p. 1 and p. 59.
(113a) MILLER, J. W. AND R. R. SAYERS. U. S. Pub. Health Repts. **50**: 1619, 1935.
(114) MILLER, W. S. The lung. Baillière, Tindall and Cox, London, 1937.
(115) MCNALLY, W. D. J. A. M. A. **101**: 584, 1933.
(116) MCNALLY, W. D. AND W. L. BERGMAN. J. Indus. Hyg. **17**: 171, 1935.
(117) MURATA, M. AND K. KAGEYAMA. Trans. Japan. Path. Soc. **22**: 153, 1932.
(118) MYERS, W. M. U. S. Bur. Mines. Rep. Inves. No. 2548, 1923.
(119) NEMEC, A. Biochem. Ztschr. **190**: 42, 1927.
(120) NICAUD, M. P. Bull. Soc. Méd. des Hopitaux de Paris **54**: 250, 1938.
(121) PEACOCK, T. B. Trans. Path. Soc., London **12**: 36, 1861.
(122) POLICARD, A. Presse Médicale **41**: 89, 1933.
(123) POLICARD, A. La Médecine du Travail, no. 5, 171, 1933.

(124) POLICARD, A. AND E. MARTIN. Bull. d'Histologie Appliquée 10: 22, 19
(125) POLICARD, A. AND C. MARRION. Bull. Acad. Méd. 111: 198, 1934.
(126) POLICARD, A. AND A. MOREL. Méd. du Travail 4: 56, 1932.
(127) POLICARD, A. AND A. DUFOURT. Ann. de Méd. 41: 183, 1937.
(128) PRIBYL, E. Klin. Spisy Skoly Zverol Brno. 10: 1, 1934.
(129) PRICE, R. M. Proc. Exper. Biol. and Med. 28: 819, 1931.
(130) RIESSNER, O. AND B. KINDT. Ztschr. physiol. Chem. 174: 40, 1928.
(131) RICHTER, O. Verh. Gesell, deut. Naturf. Arzte. Breslau 2: 249, 1904.
(132) RIST, E., P. VALLERY-RADOT, G. MAURIC, J. LEMANT AND GAUTE VILLARS. Bull. Soc. Méd. des Hopitaux de Paris 54: 214, 1938.
(133) ROBIN, A. Bull. Acad. Med. 87: 128, 1922.
(133a) ROBSON, W. D., D. A. IRWIN AND E. J. KING. Canadian M. A. J. 31: 1934.
(134) ROMAN, W. Biochem. Ztschr. 233: 344, 1931.
(135) SALKOWSKI, E. Ztschr. physiol. Chem. 83: 143, 1913.
(136) SCHULZ, H. Pflüger's Arch. 84: 67, 1901.
(137) SCHULZ, H. Pflüger's Arch. 144: 346, 1912.
(138) SCHULZ, H. Pflüger's Arch. 144: 350, 1912.
(139) SCHULZ, H. Biochem. Ztschr. 46: 376, 1912.
(140) SCHULZ, H. Biochem. Ztschr. 70: 464, 1915.
(141) SCOTT, W. W. Standard methods of chemical analysis. 4th ed., York, 1925.
(142) SHAW, A. The analyst 59: no. 700, 446, 1934.
(143) SHELDON, J. H. Brit. Med. J. 1: 47, 1934.
(144) SIMPSON, E. S. Chem. Eng. Min. Rev. 24: 323, 1932.
(145) SLADDEN, A. F. Lancet, July 15, 123, 1933.
(146) SREENIVASAN, A. Current Sci. 3: 193, 1934.
(147) SREENIVASAN, A. Proc. Ind. Acad. Sci. 3B: 283, 1936.
(148) STRACHAN, A. S. AND F. W. SIMSON. Internat. Conf. on Silicosis, Johan burg, p. 228, 1930. Pub. by Internat. Labour Office, Geneva.
(149) SIMSON, F. W. AND A. S. STRACHAN. Publ. of S. African Inst. Med. R 6: 367, 1935.
(150) SIMSON, F. W. AND A. S. STRACHAN. Proc. Transvaal Mine Med. Offic Assoc. Spec. Suppl. Oct. 23, 1930.
(150a) STRUCKMANN, G. Ann. 94: 337, 1855.
(151) STROHECKER, R., R. VAUBEL AND K. BREIT-WIESER. Ztschr. Untersu Lebensm. 70: 345, 1935.
(152) SUNDIUS, N. AND T. B. BYGDEN. Trans. Cer. Soc. 35: 167, 1936.
(153) SUNDIUS, N. AND T. B. BYGDEN. Arch. f. Gewerbepath. u. Gewerbeh 8: 26, 1937.
(154) SZANKAY, A. v. Pharm. Zentralhalle 74: 33, 1933.
(155) SZANKAY, A. v. J. Trop. Med. Hyg. 39: 46, 1936.
(156) TANANAEV, N. A. AND A. K. BABKO. Ztschr. anal. Chem. 82: 145, 193 1933.
(157) THOMPSON, T. AND H. HOLTON. Indus. and Eng. Chem. Anal. Ed. 5: 4
(158) THRESH, J. C. Analyst 47: 459, 1922.
(159) TIDESWELL, F. V. Refractories J. 10: 185, 1934.

(160) TITUS, A. C. J. Indus. Hyg. and Tox. 19: 138, 1937.
(161) TREADWELL, F. P. AND W. T. HALL. Analytical Chem. 8th ed., 1932. New York. J. Wiley & Sons.
(162) VORWALD, A. J. AND A. LANDAU. Arch. Path. 24: 8, 1937.
(163) WATKINS-PITCHFORD, W. Med. J. S. Africa 9: 196, 1914; 10: 167, 1915.
(164) WATKINS-PITCHFORD, W. J. Ind. Hyg. 9: 109, 1927.
(165) WATKINS-PITCHFORD, W. AND J. MOIR. Pub. S. African Inst. Med. Res. no. 7: 207, 1916.
(166) WATTENBURG, H. Rapports et Procés—Verbaux des Réunions 103: 17, 1937.
(167) WHITEHOUSE, A. G. R. J. Indus. Hyg. and Tox. 19: 590, 1937.
(168) WITTING, E., JR. J. f. prakt. Chem. 73: 121, 1857.

Biochem. Zeitschr.
223:228-238, 1930.

The biochemistry of silicic acid. I. Influence of silicic acid on the body weight of growing rats

by

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(From: Pharmacological Institute of the University of Halle)

Sent in: May 9, 1930.

The simplest experiment to determine whether a substance has any influence upon metabolism appears to consist of establishing the increase in body weight of animals still in the process of growing. This procedure, at any rate, has proved itself in testing the effect of iron[1].

The same procedure was used in the present experiments with silicic acid. A number of not quite fully grown albino rats were fed with a qualitatively identical diet (bread and dried milk) in a quantity chosen by the animals at will. In the first experimental series 5 animals each, in addition to this diet, received 15 cc distilled water or mineral water containing silicic acid. At the request of the Study Group for Scientific Research on mineral waters, the Silvana hotspring was made available to us, whose water has the following composition:

1 kg unfiltered water contains the following:

Cations		Anions	
Potassium	0.0018	Chlorine	0.0014
Sodium	0.0131	Iodine	traces
Lithium	traces	Sulfate	0.0056
Ammonium	traces	Hydrophosphate	0.0110
Calcium	0.0160	Hydroarsenate	traces
Magnesium	0.0115	Hydrocarbonate	0.1399
Ferro iron	0.0083	SiO_2	0.058
Ferri iron	-		
Manganese	0.0006		

Free carbon dioxide 2.439 g.

In a second experimental series the animals were fed with a number of commercial silicic acid preparations, notably Siliquid, a colloid silicic acid solution with 0.25% SiO_2, colloid silicic acid (v. Heyden) with 1% SiO_2, and Silistren, O-silicic acid tetra glycolester with 18 to 20% SiO_2. The quantity was so selected that 15 cc liquid contained roughly the same amount of silicic acid as the same volume of natural mineral water.

It is not necessary to describe the experiments in detail; the results may be taken from the data summarized in the Tables.

The two experimental series cannot, of course, be simply compared with one another, since the growth energy of the smaller, younger rats in the second series was greater than that of the rats with the higher initial weight in the first series. Comparison is only permissible within the same experimental series in which the initial weight was roughly the same.

In the first experimental series the rats fed with mineral water showed a greater weight increase than the control animals,

while on the other hand in the second series the weight increase of the rats fed with silicic acid did not reach that of the control animals, although the animals fed with Siliquid and colloid silicic acid came close to the control animals. Only rats fed with Silistren showed a pronounced lag in body weight which was definitely beyond the limits of error. Thus if the animals that received mineral water grew more rapidly than the control animals - while admittedly the difference is slight - this must have been due to other components of the natural mineral water. We are inclined to believe that it was due to the iron content, even though it too was not very great, and possibly to the traces of arsenic. Possibly the carbon dioxide content was also responsible, since it enhances secretion of digestive juices and absorption and improves the utilization of food. In any event it may be concluded from the results that the metabolism of growing rats was favorably influenced by the admixtures referred to. Silicic acid itself apparently has no appreciable effect on the increase in body weight, in other words on processes of assimilation and dissimilation. This, of course, does not mean that it might not play some role in intermediate metabolism. Special experiments will be required to determine whether this is the case.

Summary

1. Silicic acid has no influence on body weight in the quantities given on still growing rats.

2. The silicic acid present in a special combination in Silistren results in inhibition of the increase in body weight of growing animals.

3. The slight increase in body weight observed after administration of natural mineral water is in all probability due to other admixtures (iron, carbon dioxide, etc.).

Biochem. Zeitschr.
223:231-242, 1930.

The biochemistry of silicic acid. II. Retention of silicic acid by the rat organism

by

M. Kochmann and L. Maier

The rats whose growth following the administration of silicic acid was the subject of the previous study were killed after termination of the diet by chloroform inhalation and their silicic acid content was determined. We wished to establish whether silicic acid retention had taken place in the whole animal when the quantities of SiO_2 were small and corresponded to quantities ingested by man when drinking mineral waters containing silicic acid. The quantities of silicic acid fed to the animals ranged between 5o to 70 mg during a period of experimentation of 57 to 60 days. In relation to man's body weight, this would correspond to a daily ingestion of at least 6 liters of mineral water from the Silvana hot springs which have a relatively high SiO_2 content. The quantities administered to the rats were thus relatively very high.

We have only been able to find one reference in the literature to silicic acid retention in the whole animal. Breest[1] gave mice 90 or 75 mg SiO_2 in the form of silicic acid hydrate or sodium silicate in addition to their basic diet for a period of 15 days. The doses were thus considerably higher than those administered by us. Bearing in mind the body weight, Breest's

mice received almost 20 times more silicic acid within a shorter period than our rats. Breest observed a fairly considerable increase in the silicic acid content of the mouse organism.

We proceeded as follows in our experiments: After killing the animals, four or five animals of one series were ground up in a meat grinder, the fresh substance was weighed, dried over the water bath and rubbed to a fine substance. The water content of this dry substance was determined by further drying at 102°, until weight constancy was reached. Next, in an aliquot portion, the fat was extracted with ether in the Soxleth and quantitatively determined by weighing. In another portion the nitrogen was determined according to Kjeldahl and ashing took place by the very delicate method of Stolte[1], followed by the determination.

Silicic acid was determined in the ash according to the method of H. Schulz[2].

In this manner it was possible to calculate the water content and at the same time the dry substance, the fat content, the nitrogen and silicic acid content of the dry substance or ash from the values found. The results may be seen from the table.

The values show the following:

1. The water content varied between 68 and 72%. Thus there is no appreciable difference between individual animals.

2. The fat content is lower in the older animals of the first series than in the younger ones of the second series.

1 Fütterungsart der Ratten	8 Naßsubstanz g	9 Trockensubstanz in 100 g Naßsubstanz	10 Fett in 100 g Naßsubstanz	11 Wasser in 100 g Naßsubstanz	13 In 100 g reiner Trockensubstanz sind enthalten: Asche/12 I. %	Asche/12 II. %	Asche Mittelwert 14 %	N_2 %	N_2 Mittelwert/15 %	Eiweiß Mittelwert % 16	SiO_2 I. II. %	SiO_2 Mittelwert % 17	In 100 g Asche SiO_2 mg % 18	
2 Silvana, 5 Ratten	850,00	23,40	4,20	72,20	15,52	15,65	15,58	I. 14,95 II. 15,65	15,30	95,63	I. 0,015 II. 0,012	0,014	84	A
3 Kontrolle zu Silvana, 4 Ratten	705,00	21,20	6,60	72,05	17,76	17,89	17,83	I. 13,12 II. 13,12	13,12	82,00	I. 0,0123 II. 0,0167	0,0145	79	
4 Silistren, 4 Ratten	498,00	20,98	9,61	69,40	16,68	16,79	16,74	I. 15,01 II. 15,01	15,01	93,81	I. 0,0379 II. 0,0431	0,041	259	B
5 Siliquid, 5 Ratten	694,00	22,53	9,54	67,94	14,11	15,65	14,88	I. 13,74 II. 13,74	13,74	85,87	I. 0,0204 II. 0,0199	0,0201	196	
6 Kolloidale Kieselsäure, 5 Ratten	676,00	21,04	8,91	70,04	15,49	16,54	16,01	I. 14,66 II. 14,66	14,66	91,63	I. 0,0299 II. 0,0273	0,0286	179	
7 Kontrolle zu Silistren, Siliquid u. kolloidal. Kieselsäure, 5 Ratten	671,00	20,23	8,58	71,78	16,78	16,03	16,41	I. 14,78 II. 15,10	14,94	93,37	I. 0,027 II. 0,023	0,025	144	

M. Kochmann u. L. Maier:

1.Type of feeding which rats received

2.Five rats received water from Silvana mineral springs -Control of

3.Four rats received water from Silvana mineral springs

4.Four rats received Silistren(trade name)

5.Five rats received Siliquid (trade name)

6.Five rats received colloidal silicic acid

7.Five rats received Silistren, Siliquid, and colloidal silicic acid-Control of

8.Wet substance

9.Dry substance in 100 g. wet substance

10.Fat in loog.wet substance

11.Water in 100g. wet substance

12.Ash

13.Contained in 100g drysubstance

14.Average wt. of ash

15.Av. wt. N2

16.Av. wt. protein

17.Av. wt. SiO2

18.In 100g SiO2 ash

3. The fat-free substance varies between 20 and 23%. There appears to be no characteristic difference between larger and smaller animals on the one hand and rats fed with silicic acid and control rats on the other.

4. The nitrogen content of the dry substance, ranging between ca. 13 and 15%, reveals no characteristic features of any sort.

5. The same applies in general to the ash content of the animals, which was between 14.8 and 17.8%. The low value of 14.8% of animals fed with Siliquid is not likely to be due to the preparation, since animals fed with colloid silicic acid, a very similar preparation, had a higher ash content of 16%.

6. The silicic acid content of the dry substance and the ash content was lower in the heavier, older animals than in the lighter, younger animals. If we compare the two series of control animals with one another, the differences of 14 and 25 mg% of the dry substance and 79 and 149mg% of the ash are too great to fall within the limits of error. The finding accords with the results of the analyses of Hugo Schulz[2], who always found larger quantities of SiO_2 in the organs of younger subjects.

7. No difference could be discerned between the silicic acid content of animals fed with SiO_2 (in the form of natural mineral water) in the first experimental series and the control animals. Thus no retention, no accumulation of SiO_2 took place.

8. In the second experimental series the animals fed with Silistren and possibly those fed with colloid silicic acid (von Heyden) showed an accumulation of SiO_2. Why the animals receiving

Siliquid, which had already revealed a low ash content, had a lower SiO_2 content, is not known. The most likely possibility is that colloid silicic acid is not absorbed in the form of Siliquid. In any event the higher silicic acid values, particularly in the case of animals fed with Silistren, are beyond the limits of error.

9. It is noteworthy that even when administering such small quantities of SiO_2 as was the case in our experiments, an accumulation of silicic acid could be achieved in the organism in some circumstances.

Analytical protocols for the control rats of the first series

A.	Wet substance	705.00g
B.	Preliminary dry substance (after drying on the water bath)	240.00 g
C.	Crude dry substance (after ether extraction)	193.00 g
D.	Extraction residue (fat etc.)	47.00 g

I. Determination of dry substance at 102°

Sample: 1. 0.5590 g 2. 0.5387 g crude dry substance

0.1209 g 0.1239 g water loss

= 21.63% = 23.0% Mean value 22.31%

a) Total water content of the wet substance:

705.00 g wet substance

- 240.00 g preliminary dry substance

465.00 g H_2O

In 193.0 g crude dry substance there is still 22.31% H_2O.

= + 43.05 g H_2O

Total 508.05 g water = 72.05%

b) Total dry substance in 705.00 g wet substance

= 193.00 g crude dry substance

- 43.5 g water, dry substance = 149.95 g

+ 21.2%

c) Fat content in 705.00 wet substance

= 47.00 g = 6.6%

Total 99.85%

II. Nitrogen determination according to Kjeldahl

Sample:	1. 0.4740 g	2. 0.452 g crude dry substance
Substance:	50 cc n/10 H_2SO_4	50 cc n/10 H_2SO_4
Titrated:	15 cc n/10 NaOH	16.7 cc n/10 NaOH
Used:	34.7 cc n/10 H_2SO	33.3 cc n/10 H_2SO_4
N_2 =	0.0486 g	0.0467 g
	13.12%	13.12% of the pure dry substance
		mean value 13.12%

III. Determination of ash content

Sample:	1. 9.9985 g	2. 9.9765 g crude dry substance
Ash:	1.3868 g	1.3939 g
	= 17.76%	17.89% of the pure dry substance
		mean value 17.82%

IV. Silicic acid determination

Empty crucible:	1. 16.1461 g	2. 16.1418 g
+ substance:	16.1538 g	16.1541 g
SiO_2 + filtered ash + impurities:	0.0077 g	0.0123 g
- filtered ash:	0.0068 g	0.0110 g
- impurities SiO_2 pure substance:	0.0009 g	0.0013 g
=	0.0123%	0.0167% in the dry substance Mean value 0.0145%

Oxide etc. impurities due to fuming with HF

Crucible + impurities	1. 16.1507 g	2. 16.1505 g
Empty crucible:	16.1461 g	16.1418 g
Impurities:	0.0046 g	0.0087 g

Analytical protocols of rats fed with Sylvana water

A. Wet substance	850.00 g
B. Preliminary dry substance (after drying on water bath)	281.00 g
C. Crude dry substance (after ether extraction)	245.00 g
D. Extraction residue (fat etc.)	36.00 g.

I. Dry determination at 102°

Sample:	1. 0.5650 g	2. 0.5262 g crude dry substance
	0.1022 g	0.0996 g water loss
	= 18.09%	= 18.93% Mean value 18.51%

a) Total water content of the wet substance:

859.00 wet substance

281.00 g preliminary dry substance

569.00 g H_2O

In 245 g crude dry substance there is still 18.51% H_2O

15.35 g H_2O

Total 614.35 g water = 72.2%

b) Total dry substance in 859.00 g wet substance

245.00 g crude dry substance

- 45.35 g water

199.65 g dry substance = 23.4%

c) Fat content in 850.00 g wet substance

36.00 g fat = 4.2%

Total: 99.8%

II. Nitrogen determination according to Kjeldahl

Sample:	1. 0.5288 g	2. 0.6564 g crude dry substance
Applied:	50 cc n/10 H_2SO_4	75cc n/10 H_2SO_4
Titrated:	4 cc n/10 NaOH	15.2 cc n/10 NaOH
Used:	46.0 cc n/10 H_2SO_4	59.8 cc n/10 H_2SO_4
N_2 =	0.064 g	0.084 g
	= 14.95%	= 15.65% of the pure dry substance

Mean value: 15.30%

III. Determination of ash content

Sample:	1. 10 g	2. 10 g
Ash:	1.2653 g	1.2750 g
	= 15.52 %	= 15.65% of the pure dry substance

mean value: 15.58%

IV. Silicic acid determination

Empty crucible:	1. 16.1424 g	2. 16.1424 g
+ substance:	16.1503 g	16.1493 g
SiO_2+ filtered ash + impurities:	0.0079 g	0.0069 g
- filtered ash - impurities:	0.0067 g	0.0059 g
SiO_2 pure substance:	0.012 g	0.0010 g
	= 0.015%	0.012% of pure dry substance

Mean value: 0.014%

Oxide etc. impurities due to fuming with HF.

Crucible + impurities:	1. 16.1461 g	2. 16.1460 g
Empty crucible:	16.1424 g	16.1434 g
Impurities:	0.0037 g	0.0036 g

Analytical protocols of rats fed with Silistren

A. Wet substance	498.00 g
B. Preliminary dry substance (after drying on water bath)	180.00 g
C. Crude dry substance (after ether extraction)	132.12 g
D. Extraction residue[1] (fat etc.)	47.88 g

[1] 50 g crude dry substance extracted with ether weighed 36.7 g = 13.3 g weight loss = 47.88 g fat for 132.12 g crude dry substance, after ether extraction.

I. Dry determination at 102°

Sample: 0.5070 g crude dry substance:

0.1054 g water loss

= 20.79% 20.79%

a) Total water content of the wet substance:

498.00 g wet substance

- 180.00 g preliminary dry substance

318.00 g water

In 132.12 g crude dry substance there is still 20.79% water

= 27.62 g H_2O

Total 345.62 g water = 69.4%

b) Total dry substance in 498.00 g wet substance

= 132.12 g crude dry substance

- 27.62 g water

= 104.50 g dry substance = 20.98%

c) Fat content in 498.00 g wet substance

= 47.88 g fat = 9.61%

Total: 99.99%

II. Nitrogen determination according to Kjeldahl

Sample: 1. 0.2269 g crude dry substance

Applied: 50.5 cc n/10 H_2SO_4

Titrated: 31.2 cc n/10 NaOH

Used: 19.3 cc n/10 H_2SO_4

N_2 = 0.027 g

= 15.01%

Sample: 2. 0.1881 g crude dry substance

Applied: 50.5 cc n/10 H_2SO_4

Titrated: 34.55 cc n/10 NaOH

Used: 15.95 cc n/10 H_2SO_4

N_2 = 0.0223 g

= 15.01% N_2 of the pure dry substance

Mean value: 15.01%

III. Determination of ash content

Sample:	1. 10 g	2. 7.0284 g crude dry substance
Ash:	1.3210 g	0.9363 g
	16.68%	16.79% of the pure dry substance

Mean value: 16.73%

IV. Determination of silicic acid

Empty crucible:	1. 16.1403 g	2. 16.1400 g
+ substance:	16.1484 g	16.1480 g
SiO_2 + filtered ash + Impurities:	0.0081 g	0.0080 g
- filtered ash - impurities:	0.0051	0.0055 g
SiO_2 pure substance:	0.0030 g	0.0025 g
	= 0.0379%	0.0431%

Mean value: 0.0405%

Oxide etc. impurities due to fuming with HF.

Crucible + impurities:	16.1454 g	16.1455 g
Empty crucible:	16.1403 g	16.1400 g
Impurity:	0.0051 g	0.0055 g

Analytical protocols of rats fed with Siliquid

A.	Wet substance	694.00 g
B.	Preliminary dry substance (after drying on water bath)	269100 g
C.	Crude dry substance (after ether extraction)	198.00 g
D.	Extraction residue[1] (fat etc.)	66.25 g

L. Dry determination at 102°

Sample: 0.5392 g crude dry substance

0.1155 g water loss

= 21.45% 21.45%

a) Total water content of the wet substance:

694.00 g wet substance

265.00 g preliminary dry substance

429.00 g water

In 198.00 g crude dry substance there is still 21.45% water

= 42.57 g water

471.57 g water = 67.94%

b) Total dry substance in

694.00 g wet substance

= 198.00 g crude dry substance

- 42.57 g water

= 155.43 g dry substance = 22.3%

c) Fat content in 694.00 g wet substance

= 66.25 g fat = 9.54%

Total: 99.78%

Footnote from p. 238:

[1] 50 g crude dry substance extracted with ether still weighed 37.5 g = 12.5 g weight loss = 66.25 g fat for 198.00 g crude dry substance.

II. Nitrogen determination according to Kjeldahl

Sample: 1. 0.5030 g

Applied: 50.5 cc n/10 H_2SO_4

Titrated: 11.4 cc n/10 NaOH

Used: 39.1 cc n/10 H_2SO_4

N_2 = 0.055 g

= 13.74%

Sample: 2. 0.4637 g crude dry substance

applied: 50. 5 cc n/10 H_2SO_4

Titrated: 14.8 cc n/10 NaOH

Used: 35.7 cc n/10 H_2SO_4

N_2 = 0.050 g

= 13.74% of the pure dry substance

Mean value: 13.74%

III. Determination of ash content

Sample:	1. 10 g	2. 7g crude dry substance
Ash:	1.1095 g	0.8612 g
	= 14.11%	15.65% of the pure dry substance

Mean value: 14.88%

IV. Silicic acid determination

	1.	2.
Empty crucible:	16.1138 g	16.1172 g
+ substance:	16.1188 g	16.1228 g
Silicic acid + filtered ash + impurity:	0.0050 g	0.0056 g
- filtered ash - impurity	0.0034 g	0.0045 g
SiO_2 - pure substance:	0.0016 g	0.0011 g
	0.0204%	0.0199%

Mean value: 0.0201%

Oxide impurities by fuming with HF.

Crucible + impurity:	16.1172 g	16.1217 g
Empty crucible:	16.1138 g	16.1172 g
Impurity:	0.0034 g	0.0045 g

Analytical protocols of rats fed with colloid silicic acid

A.	Wet substance	676.00 g
B.	Preliminary dry substance (after drying on water bath)	245.00 g
C.	Crude dry substance (after ether extraction)	184.73 g
D.	Extraction residue[1] (fat etc.)	60.27 g

[1] 50 g crude dry substance extracted with ether, after extraction weighed 37.7 g = 60.27 g fat in a total crude dry substance of 184.73 g.

1. Dry determination at 102°

Sample: 0.5099 g crude dry substance

0.1169 g water loss

= 22.97 % 22.97%

a) Total water content of the wet substance:

676.00 g wet substance

- 245.00 g preliminary dry substance

431.00 g H_2O

In 184.73 g crude dry substance there is still 22.17% H_2O.

42.46 g H_2O

473.46 g H_2O = 70.04%

b) Total dry substance in 676.00 g wet substance

184.73 g crude dry substance

- 42.46 g H_2O

142.27 g dry substance = 21.04%

c) Fat content in 676.00 g wet substance

60.27 g fat = 8.91%

Total: 99.99%

II Nitrogen determination according to Kjeldahl

Sample: 1. 0.3539 g

Applied: 50.5 cc n/10 H_2SO_4

Titrated: 22.0 cc n/10 NaOH

Used: 28.5 cc n/10 H_2SO_4

N_2 = 0.040 g

= 14.66%

Sample: 2. 0.2726 g crude dry substance

Applied: 50.0 cc n/10 H_2SO_4

Titrated: 28.5 cc n/10 NaOH

Used: 22.0 cc n/10 H_2SO_4

N_2 = 0.031 g

= 14.66% of the pure dry substance

Mean value: 14.66%

III. Determination of ash content

Sample:	1. 10.00 g	2. 7.00 g crude dry substance
Ash:	1.1930 g	0.8916 g
	= 15.49%	= 16.54% of the pure dry substance

Mean value: 16.01%

IV. Determination of silicic acid

Empty crucible:	1. 16.0823 g	2. 18.0825 g
+ substance:	16.0888 g	16.0880 g
SiO_2 + filtered ash + impurity:	0.0065 g	0.0055 g
- filtered ash - impurity:	0.0042 g	0.0040 g
SiO_2 pure substance:	0.0023 g	0.0015 g
	= 0.0299%	0.0273%

Mean value: 0.0286%

Impurity (oxides etc.) due to fuming with HF.

Crucible + impurity:	16.0865 g	16.0865 g
Empty crucible:	16.0823 g	16.0825 g
Impurity:	0.0042 g	0.0040 g

Control rats from second series

A. Wet substance	661.00 g
B. Preliminary dry substance (after drying of the substance on the water bath):	240.00 g
C. Crude dry substance (after ether extraction)	182.40 g
D. Extraction residue[1] (fat etc.)	55.70 g

1. Dry determination at 102°

Sample: 0.5012 g crude dry substance

0.1282 g water loss

= 25.57% 25.57%

a) Total water content of the wet substance:

671.00 g wet substance

- 240.00 g preliminary dry substance

431.00 g H_2O

In 182.40 g crude dry substance there is still 25.27% water.

= 46.64 g

Total: 477.64 g H_2O = 71.78%

b) Total dry substance in 671.00 g wet substance

182.40 g crude dry substance

- 46.64 g water

= 135.76 g dry substance = 20.23%

[1] 50 g crude dry substance extracted with ether weighed 38.0 g after extraction. In 182.40 g crude dry substance there was 57.60 g extract.

c) Fat content in 671.00 g wet substance

57.6 g fat = 8.58%

Total: 100.59%

II. Nitrogen determination

Sample:	1. 0.2933 g	2. 0.2592 g crude dry substance
Sulfuric acid bound to ammonia:	23.03 cc	20.80 cc
Nitrogen:	0.0322 g	0.0291 g
	= 14.78%	= 15.10%

Mean value of the pure dry substance 14.94%

III. Ash determination

Sample:	1. 10.0000 g	2. 7.0000 g crude dry substance
Ash:	1.2488 g	0.8351 g
	= 16.78%	16.03%

Mean value of pure dry substance 16.40%

IV. Silicic acid determination

Empty crucible:	1. 16.1377 g	2. 16.1413 g
+ substance:	16.1433 g	16.1454 g
SiO_2 + filtered ash + impurity:	0.0056 g	0.0051 g
- filtered ash - impurity:	0.0036 g	0.0039 g
SiO_2 pure substance:	0.0020 g	0.0012 g
	0.027%	0.023%

Mean value: 0.025%

Impurity (oxides etc.) due to fuming with HF.

Crucible + impurity:	16.1413 g	16.1452 g
Empty crucible:	16.1377 g	16.1413 g
Impurity:	0.0036 g	0.0039 g.

Biochem Zeitschr.
223:243-248, 1930.

The biochemistry of silicic acid. III. Influence of silicic acid on the elasticity of connective tissue

by

M. Kochmann and L. Maier.

It is known, particularly from the studies of Hugo Schulz[1], but also those of Gonnermann[2], a student of Kobert's, that silicic acid is a normal component of the human organism. Schulz has shown that silicic acid occurs mainly in connective tissue, in larger quantities in young connective tissue than in that of older subjects. He concluded from this that silicic acid is an essential component of connective tissue. So far there have only been conjectures as to the role of silicic acid in connective tissue. The authors cited, as well as Kühn[3], have expressed the view that from the pharmacological point of view silicic acid has roughly the same importance for connective tissue as iron for erythrocytes, in other words is simultaneously a stimulant for the formation of the tissue and itself a structural material.

Whether silicic acid is capable of altering the properties of connective tissue, in other words whether it is capable of exercising detectable pharmacological effects on connective tissue, has not so far been the subject of any investigations to our knowledge.

For this reason we undertook the present investigations, the purpose of which was to show whether one important quality

of connective tissue, namely elasticity, could change under the influence of silicic acid. A colloid silicic acid was used as silicic acid preparation, and the albino mouse was chosen as experimental animal.

In the first experimental series a 4.5 cm long and 1 cm wide piece was cut out of the dorsal skin of the mouse and this was cut longitudinally into two 1/2 cm wide pieces. One piece was introduced into Ringer solution for warm-blooded animals, the other in a 5 mg% solution of the colloid silicic acid, in Ringer solution. Both pieces, the one in Ringer solution and the one in silicic acid-Ringer solution, were examined for their elasticity after 3 hours. For this purpose the skin was suspended longitudinally according to Manus's well known method, in the manner used for the isolated uterus or intestine, i.e. the lower end was attached to a glass tube bent at right angles, while the upper end was attached to a powerful recording lever (for this we used the lever device of Mangold's sclerometer) and the lever was loaded in such a way that the thread was just taut and the weight of the skin was compensated by the load. Next the lever was set horizontally. The skin was in a vessel containing Ringer solution which was bubbled with oxygen from a gasometer. The vessel stood in a water bath whose temperature was adjusted to 37° by a flame and thermoregulator. Next the skin was stretched by loading the recording lever, using different weights in different experiments, varying between 5 and 100 g. The skin was then subjected to stretching by the 5 g weight for

5 min, after which the weight was removed. For the next 5 min the piece of skin was left unloaded, after which it was loaded successively with 10, 15, etc. g, in the same manner, that is loaded for 5 min, then unloaded for 5 min. The changes in length were recorded on a kymograph and yielded very characteristic curves.

By this means it was possible to measure the stretch or stress elasticity which is in direct proportion to the length and inversely proportional to the width. Since these dimensions were always chosen as equal as possible with material as complicated as the skin, these proportions were given no further particular attention. We also refrained from an exact mathematical evaluation of the curves, since we were aware that this would not be entirely possible.

There can be no doubt that the figures obtained from the curves are relatively rough estimates, but in our view they nevertheless suffice to give an idea of the differences between the skin pieces placed in Ringer solution and in silicic acid solution.

It is apparent from the curves that the weights give rise to stretching, according to Triepel[1], to an elastic displacement, which takes place instantaneously, or almost instantaneously. This is followed in the five minutes of loading by an elastic after-effect. Upon removal of the load the curve rises once more, that is the skin "snaps" back together. The curve again shows an almost instantaneous effect of the removal of the weight and

a displacement due to the after-effect. It can be seen from all the curves that after stretching, a permanent change occurs, differing in degree according to the weight. The skin, and therewith the lever, thus do not return to the original position, despite removal of the load.

In the skin pieces immersed in silicic acid solution stretching by the loads is not as pronounced as in the skin pieces placed in Ringer solution. However, it will also be seen that the elastic after-effect does not reach the same degree and that upon removal of the load the curve more closely approaches the initial position. An important factor in assessing elasticity of the tissue is the elasticity modulus, which, according to Triepel, is directly proportional the force and inversely proportional to the change, in other words $E = \frac{\text{force}}{\text{change}}$. The elasticity modulus has clearly increased under the influence of silicic acid; elasticity, although far from being complete, is nevertheless more pronounced than in the skin pieces immersed in Ringer solution. See Tables I and II.

The objection might be raised against these experiments, which show that a relatively brief effect of colloid silicic acid is sufficient to give rise to a measurable change in elasticity, that colloid itself had exercised an effect of its own. Therefore in a second experimental series colloid silicic acid was injected subcutaneously to mice in a quantity of 2.5 mg per day for 8 and 14 days. Next the animals were killed and once again the skin prepared as in the first experimental series. The result was qualitatively the same, but quantitatively even more distinct

than in the first experimental series, as is apparent from the curves and the figures (Tables I and II). A particularly characteristic feature is the considerable increase in the elasticity modulus. The elasticity of the skin of mice treated with silicic acid thus is far closer to complete elasticity than that of the control animals.

Discussion

It was hitherto concluded from the fact that more silicic acid was found in young connective tissue than in that of older subjects that it had some significance for connective tissue. No matter how obvious this conclusion may be, analytical figures in themselves do not furnish definitive proof of this, for it is not entirely impossible that silicic acid accumulates in connective tissue for some unknown reason, but has no importance for function. We believe that the above experiments, admittedly not very numerous, which will be continued on other tissues and with other determinations, furnish the beginnings of proof that silicic acid does have an enhancing effect on a very essential quality of connective tissue, namely on elasticity.

Summary

1. The elasticity, measured on elastic changes upon stretching and release of equal pieces of the dorsal skin of albino mice, is increased by immersion in silicic acid solution. The elasticity modulus is distinctly increased.

2. Equal pieces of the skin of albino mice that had received silicic acid subcutaneously for prolonged periods showed greater elasticity than preparations from the control animals.

3. In both cases the immediate elastic movement and the elastic after-effect are slighter; the immediate elastic shortening and the after-effect of elastic shortening are more pronounced. The elasticity modulus $E = \frac{\text{force}}{\text{change}}$ increases.

A first attempt is made thereby to place the effect of silicic acid on connective tissue on an experimental pharmacological foundation.

Tables

1. Key: 1. Weight. 2. Comparison. 3. Stretch, in cm. 4. Silicic acid in Ringer solution. 5. Silicic acid injected. 6. Average.

2. Key: 1. Comparison. 2. Silicic acid in Ringer solution. 3. Silicic acid injected. 4. Weight. 5. Stretch. 6. Shortening. 7. Remaining stretch. 8. Average

Table I

1 Gewicht g	2 Vergleich: Dehnung cm (3)	2 Vergleich: $E = \frac{K}{V}$	4 Kieselsäure R. L.: Dehnung cm	4 Kieselsäure R. L.: $E = \frac{K}{V}$	5 Kieselsäure injiziert: Dehnung cm	5 Kieselsäure injiziert: $E = \frac{K}{V}$
5	6	0,83	2	2,5	1	5,0
10	15	0,67	5	2,0	3	3,3
15	21	0,71	9	1,67	6	2,5
20	28	0,71	13	1,54	7	2,86
30	35	0,85	24	1,25	14	1,25
50	46	1,09	39	1,28	17	2,94
6 Durchschnitt:						
21,7	25,2	0,71	15,3	1,71	8	2,98

Table II

4 Gewicht g	1 Vergleich: 5 Dehnung %	1 Vergleich: 6 Verkürzung %	1 Vergleich: 7 Dehnungsrückstand	2 Kieselsäure R. L.: Dehnung %	2 Kieselsäure R. L.: Verkürzung %	2 Kieselsäure R. L.: Dehnungsrückstand	3 Kieselsäure injiziert: Dehnung %	3 Kieselsäure injiziert: Verkürzung %	3 Kieselsäure injiziert: Dehnungsrückstand
5	17	11	6	13	11	2	11	10	1
10	31	16	15	22	17	5	20	17	3
15	39	18	21	34	25	9	29	23	6
20	53	22	28	43	30	13	37	30	7
30	62	27	35	59	35	24	48	34	14
50	81	35	46	85	46	39	58	41	17
8 Durchschnitt:									
21,7	46,7	21,5	25,2	42,7	27,3	15,4	33,8	25,8	8,0

Figures

1. The abscissa figures give the load, the ordinate the percentual alteration. a stretch curve under loads, a_1 remaining stretch upon removal of load in the skin pieces of the control rats. b, b_1 the same with skin pieces that had been left for 3 hours in SiO_2, c, c_1 with skin pieces from mice that received SiO_2 subcutaneously. Averages of 15 experiments.

2. Dorsal skin left for 3 hours in Ringer solution.

3. Dorsal skin from the same mouse, left for 3 hours in colloid silicic acid.

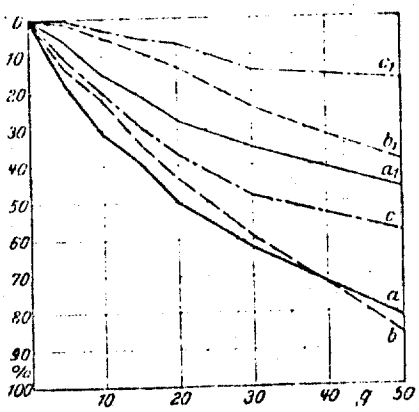

Fig. I

Fig. II

Fig. III

Beiträge zur Biochemie der Kieselsäure.

I. Mitteilung:

Einfluß der Kieselsäure auf das Körpergewicht wachsender Ratten.

Von

M. Kochmann und L. Maier.

(Aus dem Pharmakologischen Institut der Universität Halle.)

(Eingegangen am 9. Mai 1930.)

Der einfachste Versuch, um einen Anhalt dafür zu gewinnen, ob eine Substanz einen Einfluß auf den Stoffwechsel ausüben könne, scheint die Feststellung der Körpergewichtszunahme noch wachsender Tiere zu sein. Jedenfalls hat sich diese Versuchsanordnung bei der Prüfung der Eisenwirkung bewährt[1].

In den vorliegenden Versuchen mit Kieselsäure wurde in der gleichen Weise vorgegangen. Eine größere Anzahl noch nicht ganz ausgewachsener, weißer Ratten wurde mit einem qualitativ gleichen Futter (Brot und Trockenmilch) ernährt, dessen Menge von den Tieren beliebig gewählt werden konnte. In der ersten Versuchsreihe erhielten je fünf Tiere zu dieser Nahrung 15 ccm destilliertes Wasser bzw. kieselsäurehaltiges Mineralwasser. Auf Veranlassung der Arbeitsgemeinschaft für wissenschaftliche Heilquellenforschung wurde der Silvanasprudel von folgender Zusammensetzung zur Verfügung gestellt.

In 1 kg unfiltrierten Wasssers sind enthalten:

Kationen		Anionen	
Kalium	0,0018	Chlor	0,0014
Natrium	0,0131	Jod	Spuren
Lithium	Spuren	Sulfat	0,0056
Ammonium	Spuren	Hydrophosphat	0,0110
Calcium	0,0160	Hydroarsenat	Spuren
Magnesium	0,0115	Hydrocarbonat	0,1399
Ferroeisen	0,0083	SiO_2	0,058
Ferrieisen	—		
Mangan	0,0006		

Freies Kohlendioxyd 2,439 g.

[1] *M. Kochmann* und *H. Seel*, diese Zeitschr. 198, 362, 1928.

In einer zweiten Versuchsreihe wurde eine Anzahl Kieselsäurepräparate des Handels verfüttert, und zwar *Siliquid*, eine kolloide Lösung von Kieselsäure mit 0,25 % SiO_2, *kolloide Kieselsäure (v. Heyden)* mit 1 % SiO_2 und *Silistren*, o-Kieselsäure-Tetraglykolester mit 18 bis 20 % SiO_2. Die Menge wurde so gewählt, daß in 15 ccm Flüssigkeit der annähernd gleiche Gehalt an Kieselsäure vorhanden war wie in dem gleichen Volumen des natürlichen Mineralwassers.

Es erübrigt sich, die Versuche ausführlich wiederzugeben; es sei vielmehr nur eine Zusammenfassung angeführt, aus der das Ergebnis entnommen werden kann.

I. Versuchsreihe von 57tägiger Dauer.

	Durchschnittsgewicht von je 5 Ratten	
	mit 15 ccm H_2O	15 ccm Silvanasprudel
Anfangsgewicht	151 g	141,5 g
Endgewicht	181 g	182 g
Zunahme	30 g = 20 %	40,5 g = 29 %
Tägliche Zunahme	0,53 g	0,71
Verhältnis der Zunahme	100	145
SiO_2 im ganzen		50 mg

II. Versuchsreihe von 60tägiger Dauer.

	Durchschnittsgewicht von je 5 Ratten			
	Vergleichstiere	Siliquid	Koll. SiO_2	Silistren
Anfangsgewicht	85,0	94,0	90,0	92,0
Endgewicht	148,4	157,8	152,8	139,3
Zunahme	63,4 = 74 %	63,8 = 68 %	62,8 = 70 %	47,3 = 51 %
Tägliche Zunahme	1,06	1,06	1,05	0,79
Verhältnis	100	92	95	69
SiO_2 im ganzen		70 mg	70 mg	70 mg

Die beiden Versuchsreihen können selbstverständlich nicht ohne weiteres miteinander verglichen werden, da die Wachstumsenergie der kleineren, jüngeren Ratten der zweiten Versuchsreihe größer ist als die der Ratten mit höherem Anfangsgewicht der Reihe I. Ein Vergleich ist nur innerhalb derselben Versuchsreihe erlaubt, bei denen das Anfangsgewicht annähernd dasselbe ist.

In der ersten Versuchsreihe haben die mit Mineralwasser gefütterten Ratten stärker zugenommen als die Vergleichstiere, in der zweiten Reihe dagegen hat die Gewichtszunahme der Kieselsäuretiere nicht die der Vergleichstiere erreicht, wenn auch die Tiere, denen Siliquid und kolloidale Kieselsäure dargereicht wurde, nahe an die Kontrolltiere herankommen. Nur die Silistrenratten zeigen ein starkes Zurückbleiben

des Körpergewichts, das mit Sicherheit außerhalb der Fehlergrenzen liegt. Wenn nun die Tiere, denen das Mineralwasser gegeben wurde, schneller gewachsen sind als die Vergleichstiere — die Geringfügigkeit des Überschusses zugegeben —, so müssen andere Bestandteile des natürlichen Mineralwassers dafür verantwortlich gemacht werden. Wir sind geneigt, den allerdings auch nicht erheblichen Eisengehalt und möglicherweise die Spuren von Arsen dafür verantwortlich zu machen. Vielleicht ist auch der Kohlensäuregehalt daran schuld, der die Sekretion der Verdauungssäfte und die Resorption sowie Ausnutzung der Nahrung verbessert. Immerhin wird man aus dem Ergebnis schließen dürfen, daß der Stoffwechsel wachsender Ratten gerade durch die genannten Beimengungen eher im günstigen Sinne beeinflußt worden ist. Die Kieselsäure selbst besitzt offenbar keine erhebliche Wirkung auf die Körpergewichtszunahme, also das Verhältnis von Assimilations- und Dissimilationsvorgängen. Damit ist natürlich nicht gesagt, daß sie im intermediären Stoffwechsel keine Rolle spielen könnte. Hier müßten besondere Versuche einsetzen, um diese Frage zu klären.

Zusammenfassung.

1. Die Kieselsäure übt in den verfütterten Mengen bei noch wachsenden Ratten keinen Einfluß auf das Körpergewicht aus.

2. Die im Silistren in besonderer Bindung vorhandene Kieselsäure hat eine Hemmung der Körpergewichtszunahme wachsender Tiere zur Folge.

3. Die geringe Körpergewichtszunahme, die nach Verfütterung eines natürlichen Mineralwassers beobachtet wird, ist mit größter Wahrscheinlichkeit anderen Beimengungen (Eisen, Kohlensäure usw.) zuzuschreiben.

Beiträge zur Biochemie der Kieselsäure.

II. Mitteilung:

Retention der Kieselsäure im Organismus der Ratten.

Von

M. Kochmann und L. Maier.

(Aus dem Pharmakologischen Institut der Universität Halle.)

(Eingegangen am 9. Mai 1930.)

Die Ratten, deren Körperwachstum bei Kieselsäuredarreichung Gegenstand der Untersuchung war, wurden nach Beendigung der Fütterung durch Chloroformeinatmung getötet und auf ihren Kieselsäuregehalt untersucht. Es sollte dabei festgestellt werden, ob im ganzen Tier eine Retention der Kieselsäure stattgefunden habe, wenn die Mengen SiO_2 gering waren und denen entsprachen, die beim Trinken eines kieselsäurehaltigen Mineralwassers vom Menschen aufgenommen werden. Die verfütterten Kieselsäuremengen bewegten sich zwischen 50 und 70 mg bei einer Versuchsdauer von 57 bis 60 Tagen. Dies würde im Verhältnis zum Körpergewicht beim Menschen einer täglichen Aufnahme von mindestens sechs Liter des verhältnismäßig starken SiO_2-haltigen Mineralwassers des Silvanasprudels gleichkommen. Die den Ratten dargereichten Mengen sind also vergleichsweise noch sehr hoch.

In der Literatur haben wir über Kieselsäureretention am ganzen Tiere nur eine Angabe finden können. *Breest*[1] gab Mäusen 15 Tage lang zu einem Grundfutter 90 oder 75 mg SiO_2 als Kieselsäurehydrat bzw. Natriumsilikat. Die Gaben waren also wesentlich größer als die von uns verfütterten. Unter Berücksichtigung des Körpergewichts hätten die Mäuse *Breests* fast 20mal mehr Kieselsäure in kürzerer Zeit erhalten als unsere Ratten. *Breest* konnte eine ziemlich hohe Zunahme des Kieselsäuregehalts des Mäuseorganismus feststellen.

Im einzelnen gingen wir bei unseren Versuchen folgendermaßen vor: Nach Tötung der Tiere wurden die vier oder fünf zu einer Reihe gehörigen Tiere zusammen durch einen Fleischwolf zerkleinert, die Frisch-

[1] *Breest*, diese Zeitschr. 30, 108, 1908.

Tabelle.

Futterungsart der Ratten	Nahrsubstanz g	Trockensubstanz in 100 g Nahrsubstanz	Fett in 100 g Nahrsubstanz	Wasser in 100 g Nahrsubstanz	In 100 g reiner Trockensubstanz sind enthalten: Asche I. ‰	Asche II. %	Asche Mittelwert %	N_2 I. %	N_2 II. %	N_2 Mittelwert %	Eiweiß Mittelwert %	SiO_2 I. II. %	SiO_2 Mittelwert %	In 100 g Asche SiO_2 mg %	
Silvana, 5 Ratten	850,00	23,40	4,20	72,20	15,52	15,65	15,58	I. 14,95 II. 15,65		15,30	95,63	I. 0,015 II. 0,012	0,014	84	A
Kontrolle zu Silvana, 4 Ratten	705,00	21,20	6,60	72,05	17,76	17,89	17,83	I. 13,12 II. 13,12		13,12	82,00	I. 0,0123 II. 0,0167	0,0145	79	
Silistren, 4 Ratten	498,00	20,98	9,61	69,40	16,68	16,79	16,74	I. 15,01 II. 15,01		15,01	93,81	I. 0,0379 II. 0,0431	0,041	259	B
Siliquid, 5 Ratten	694,00	22,53	9,54	67,94	14,11	15,65	14,88	I. 13,74 II. 13,74		13,74	85,87	I. 0,0204 II. 0,0199	0,0201	196	
Kolloidale Kieselsäure, 5 Ratten	676,00	21,04	8,91	70,04	15,49	16,54	16,01	I. 14,66 II. 14,66		14,66	91,63	I. 0,0299 II. 0,0273	0,0286	179	
Kontrolle zu Silistren, Siliquid u. kolloidal. Kieselsäure, 5 Ratten	671,00	20,23	8,58	71,78	16,78	16,03	16,41	I. 14,78 II. 15,10		14,94	93,37	I. 0,027 II. 0,023	0,025	144	

substanz gewogen, auf dem Wasserbade getrocknet und fein zerrieben. In dieser Trockensubstanz wurde der Wassergehalt durch weiteres Trocknen bei 102° bis zur Gewichtskonstanz bestimmt, dann in einem aliquoten Teil das Fett im Soxleth mit Äther extrahiert und quantitativ durch Wägung festgestellt. Dann wurde in einem anderen Teil der Stickstoff nach *Kjedahl* bestimmt und nach der überaus schonenden Methode von *Stolte*[1] die Asche hergestellt und bestimmt.

In der Asche wurde dann die Kieselsäurebestimmung nach den Vorschriften vorgenommen, wie sie von *H. Schulz*[2] gegeben wurden.

Auf diese Weise ließ sich aus den gefundenen Zahlen der Wassergehalt und mithin die Trockensubstanz, der Fettgehalt, der Stickstoff- und der Kieselsäuregehalt der Trockensubstanz bzw. der Asche berechnen. Die Ergebnisse lassen sich aus der Tabelle entnehmen.

Aus diesen Zahlen ergibt sich:

1. Der Wassergehalt schwankt zwischen 68 und 72 %. Ein erheblicher Unterschied zwischen den einzelnen Tieren ist also nicht festgestellt worden.

2. Der Fettgehalt ist bei den älteren Tieren der ersten Reihe niedriger als bei den jüngeren der zweiten Reihe.

3. Die fettfreie Trockensubstanz schwankt zwischen 20 und 23 %. Ein charakteristischer Unterschied zwischen größeren und kleineren Tieren einerseits und kieselsäuregefütterten Ratten und den Vergleichstieren andererseits scheint ebenfalls nicht vorhanden zu sein.

4. Der Stickstoffgehalt der Trockensubstanz, zwischen etwa 13 und 15 % schwankend, läßt in keiner Hinsicht etwas Charakteristisches erkennen.

5. Dasselbe gilt im allgemeinen für den Aschegehalt der Tiere, der in den Grenzen von 14,8 und 17,8 % liegt. Der niedrige Wert von 14,8 der mit Siliquid gefütterten Ratten dürfte kaum auf das Präparat zurückzuführen sein, da die mit kolloidaler Kieselsäure, einem ganz ähnlichen Präparat, gefütterten Tiere einen höheren Aschegehalt von 16 % aufweisen.

6. Der Kieselsäuregehalt der Trockensubstanz und der Aschegehalt ist bei den schwereren, älteren Tieren geringer als bei den leichteren und jüngeren Tieren; wenn man die beiden Reihen der Kontrolltiere miteinander vergleicht, so sind die Unterschiede von 14 und 25 mg-% der Trockensubstanz und 79 und 149 mg-% der Aschе zu groß, als daß sie in die Fehlergrenzen fallen könnten. Der Befund steht im Einklang mit den bekannten analytischen Ergebnissen von *Hugo Schulz*[2], der in den

[1] *Stolte*, diese Zeitschr. 35, 104, 1911.
[2] *H. Schulz*, Pflügers Arch f. d. ges. Phys. 144, 350, 1912.

Organen junger Individuen immer eine größere Menge SiO_2 feststellen konnte.

7. Ein Unterschied im Kieselsäuregehalt der mit SiO_2 (als natürliches Mineralwasser) gefütterter Tiere der ersten Versuchsreihe im Vergleich zu den Kontrollen ist nicht zu erkennen. Es hat also keine Retention, keine Anreicherung der SiO_2 stattgefunden.

8. In der zweiten Versuchsreihe zeigen die mit Silistren und vielleicht auch die mit kolloidaler Kieselsäure (*von Heyden*) behandelten Tiere eine Anreicherung an SiO_2. Warum die Tiere, denen Siliquid dargereicht wurde und die schon einen niedrigen Aschegehalt aufwiesen, einen geringeren SiO_2-Gehalt zeigen, läßt sich nicht entscheiden. Am ehesten würde man daran denken können, daß die kolloidale Kieselsäure in Form des Siliquids nicht resorbiert wurde. Jedenfalls liegen die höheren Zahlen der Kieselsäure besonders bei den mit Silistren gefütterten Tieren außerhalb der Fehlergrenzen.

9. Es ist auf jeden Fall bemerkenswert, daß selbst bei Verfütterung solch geringer Mengen von SiO_2, wie sie in unseren Versuchen zur Verwendung kamen, unter Umständen eine Anreicherung an Kieselsäure im Organismus erzielt werden kann.

Analysenprotokolle der Kontrollratten der ersten Serie.

A. Naßsubstanz . . . . . 705,00 g
B. Vortrockensubstanz (nach Trocknung auf dem Wasserbad) . . . . . 240,00 g
C. Rohtrockensubstanz (nach Ätherextraktion) . . . 193,00 g
D. Extraktionsrückstand (Fett usw.) . . . . . 47,00 g

I. Trockenbestimmung bei 102° C.

Einwage:	1. 0,5590 g	2. 0,5387 g Rohtrockensubstanz	
	0,1209 g	0,1239 g Wasserverlust	
	= 21,63%	= 23,0%	Mittelwert 22,31%

a) Gesamtwassergehalt der Naßsubstanz:

705,00 g Naßsubstanz
− 240,00 g Vortrockensubstanz
465,00 g H_2O

In 193,0 g Rohtrockensubstanz sind noch 22,31% H_2O
= + 43,05 g H_2O
Zusammen 508,05 g Wasser = 72,05%

b) Gesamttrockensubstanz in 705,00 g Naßsubstanz
= 193,00 g Rohtrockensubstanz
− 43,05 g Wasser, Trockensubstanz = 149,95 g
= 21,2%

c) Fettgehalt in 705,00 g Naßsubstanz
= 47,00 g = 6,6%
Zusammen 99,85%

II. Stickstoffbestimmung nach Kjeldahl.

Einwage:	1. 0,4740 g	2. 0,4542 g Rohtrockensubstanz
vorgelegt:	50 ccm n/10 H_2SO_4	50 ccm n/10 H_2SO_4
titriert:	15,3 ccm n/10 NaHO	16,7 ccm n/10 NaOH
verbraucht:	34,7 ccm n/10 H_2SO_4	33,3 ccm n/10 H_2SO_4
N_2 =	0,0486 g	0,0467 g
	= 13,12%	13,12% der reinen Trockensubstanz
		Mittelwert 13,12%

III. Aschegehaltsbestimmung.

Einwage:	1. 9,9985 g	2. 9,9765 g Rohtrockensubstanz
Asche:	1,3868 g	1,3939 g
	= 17,76%	17,89% der reinen Trockensubstanz
		Mittelwert 17,82%

IV. Kieselsäurebestimmung.

Tiegel leer:	1. 16,1461 g	2. 16,1418 g
+ Substanz:	16,1538 g	16,1541 g
SiO_2 + Filterasche + Verunrein.:	0,0077 g	0,0123 g
− Filterasche:	0,0068 g	0,0110 g
− Verunrein. SiO_2 Reinsubstanz:	0,0009 g	0,0013 g
	= 0,0123%	0,0167% in der Trockensubstanz
		Mittelwert 0,0145%

Verunreinigung an Oxyden usw. durch Abrauchen mit HF.

Tiegel + Verunreinigung:	1. 16,1507 g	2. 16,1505 g
Tiegel leer:	16,1461 g	16,1418 g
Verunreinigung:	0,0046 g	0,0087 g

Analysenprotokolle der Silvanaratten.

A. Naßsubstanz . . . . . 850,00 g
B. Vortrockensubstanz (nach Trocknung auf dem Wasserbad) . . . . . 281,00 g
C. Rohtrockensubstanz (nach Ätherextraktion) . . . . 245,00 g
D. Extraktionsrückstand (Fett usw.) . . . . . 36,00 g

I. Trockenbestimmung bei 102° C.

Einwage:	1. 0,5650 g	2. 0,5262 g Rohtrockensubstanz	
	0,1022 g	0,0996 g Wasserverlust	
	= 18,09%	= 18,93%	Mittelwert 18,51%

a) Gesamtwassergehalt der Naßsubstanz:

850,00 g Naßsubstanz
281,00 g Vortrockensubstanz
569,00 g H_2O

In 245 g Rohtrockensubstanz sind noch 18,51% H_2O
= 45,35 g H_2O

Zusammen 614,35 g Wasser 72,2%

b) Gesamttrockensubstanz in 850,00 g Naßsubstanz
245,00 g Rohtrockensubstanz
45,35 g Wasser
199,65 g Trockensubstanz = 23,4%

c) Fettgehalt in 850,00 g Naßsubstanz
36,00 g Fett 4,2%

zusammen: 99,8%

II. Stickstoffbestimmung nach Kjeldahl.

Einwage:	1. 0,5288 g	2. 0,6564 g Rohtrockensubstanz
vorgelegt:	50 ccm n/10 H_2SO_4	75 ccm n/10 H_2SO_4
titriert:	4 ccm n/10 NaOH	15,2 ccm n/10 NaOH
verbraucht:	46,0 ccm n/10 H_2SO_4	59,8 ccm n/10 H_2SO_4
N_2 =	0,064 g	0,084 g
	= 14,95%	= 15,65% der reinen Trockensubstanz

Mittelwert: 15,30%

III. Aschegehaltsbestimmung.

Einwage:	1. 10 g	2. 10 g
Asche:	1,2653 g	1,2750 g
	= 15,52%	15,65% der reinen Trockensubstanz

Mittelwert: 15,58%

IV. Kieselsäurebestimmung.

Tiegel leer:	1. 16,1424 g	2. 16,1424 g
+ Substanz:	16,1503 g	16,1493 g
SiO_2 + Filterasche + Verunreinigung:	0,0079 g	0,0069 g
− Filterasche − Verunreinigung:	0,0067 g	0,0059 g
SiO_2 Reinsubstanz:	0,0012 g	0,0010 g
	= 0,015%	0,012% d. r. Trockensubst.

Mittelwert: 0,014%

Verunreinigungen an Oxyden usw. durch Abrauchen mit HF.

Tiegel + Verunreinigung:	1. 16,1461 g	2. 16,1460 g
Tiegel leer:	16,1424 g	16,1434 g
Verunreinigung:	0,0037 g	0,0026 g

Analysenprotokolle der Silistrenratten.

A. Naßsubstanz . . . . . 498,00 g
B. Vortrockensubstanz (nach Trocknung auf dem Wasserbad) . . . . . 180,00 g
C. Rohtrockensubstanz (nach Ätherextraktion) . . . . 132,12 g
D. Extraktionsrückstand[1] (Fett usw.) . . . . . 47,88 g

I. Trockenbestimmung bei 102°.

Einwage: 0,5070 g Rohtrockensubstanz
0,1054 g Wasserverlust
20,79% 20,79%

a) Gesamtwassergehalt der Naßsubstanz:

498,00 g Naßsubstanz
− 180,00 g Vortrockensubstanz
318,00 g Wasser

In 132,12 g Rohtrockensubstanz sind noch 20,79% Wasser
= 27,62 g H_2O

Zusammen 345,62 g Wasser = 69,4%

b) Gesamttrockensubstanz in 498,00 g Naßsubstanz
= 132,12 g Rohtrockensubstanz
− 27,62 g Wasser
= 104,50 g Trockensubstanz = 20,98%

c) Fettgehalt in 498,00 g Naßsubstanz
= 47,88 g Fett = 9,61%

zusammen: 99,99%

II. Stickstoffbestimmung nach Kjeldahl.

Einwage: 1. 0,2269 g Rohtrockensubstanz
vorgelegt: 50,5 ccm n/10 H_2SO_4
titriert: 31,2 ccm n/10 NaOH
verbraucht: 19,3 ccm n/10 H_2SO_4
N_2 = 0,027 g
= 15,01%

Einwage: 2. 0,1881 g Rohtrockensubstanz
vorgelegt: 50,5 ccm n/10 H_2SO_4
titriert: 34,55 ccm n/10 NaOH
verbraucht: 15,95 ccm n/10 H_2SO_4
N_2 = 0,0223 g
= 15,01% N_2 der reinen Trockensubstanz

Mittelwert: 15,01%.

[1] 50 g Rohtrockensubstanz mit Äther extrahiert wiegen nach Extraktion 36,7 g = 13,3 g Gewichtsverlust = 47,88 g Fett für 132,12 g Rohtrockensubstanz.

III. Aschegehaltsbestimmung.

Einwage:	1. 10 g	2. 7,0284 g Rohtrockensubstanz
Asche:	1,3210 g	0,9363 g
	= 16,68%	16,79% der reinen Trockensubstanz

Mittelwert: 16,73%

IV. Kieselsäurebestimmung.

Tiegel leer:	1. 16,1403 g	2. 16,1400 g
+ Substanz:	16,1484 g	16,1480 g
SiO_2 + Filterasche + Verunreinigung:	0,0081 g	0,0080 g
− Filterasche − Verunreinigung:	0,0051	0,0055 g
SiO_2-Reinsubstanz:	0,0030 g	0,0025 g
	0,0379%	0,0431%

Mittelwert: 0,0405%

Verunreinigung an Oxyden usw. durch Abrauchen mit HF.

Tiegel + Verunreinigung:	16,1454 g	16,1455 g
Tiegel leer:	16,1403 g	16,1400 g
Verunreinigung:	0,0051 g	0,0055 g

Analysenprotokolle der Siliquidratten.

A. Naßsubstanz	694,00 g
B. Vortrockensubstanz (nach Trocknung auf dem Wasserbad)	265,00 g
C. Rohtrockensubstanz (nach Ätherextraktion)	198,00 g
D. Extraktionsrückstand[1] (Fett usw.)	66,25 g

I. Trockenbestimmung bei 102°.

Einwage: 0,5392 g Rohtrockensubstanz
0,1155 g Wasserverlust
= 21,45% — 21,45%

a) Gesamtwassergehalt der Naßsubstanz:
694,00 g Naßsubstanz
− 265,00 g Vortrockensubstanz
429,00 g Wasser

In 198,00 g Rohtrockensubstanz sind noch 21,45% Wasser
− 42,57 g Wasser
471,57 g Wasser — 67,94%

b) Gesamttrockensubstanz in
694,00 g Naßsubstanz
= 198,00 g Rohtrockensubstanz
− 42,57 g Wasser
= 155,43 g Trockensubstanz = 22,3%

c) Fettgehalt in 694,00 g Naßsubstanz
= 66,25 g Fett — 9,54%

Zusammen: 99,78%

[1] 50 g Rohtrockensubstanz mit Äther extrahiert wiegen noch 37,5 g = 12,5 g Gewichtsverlust = 66,25 g Fett für 198,00 g Rohtrockensubstanz.

II. Stickstoffbestimmung nach Kjeldahl.

Einwage: 1. 0,5030 g
vorgelegt: 50,5 ccm n/10 H_2SO_4
titriert: 11,4 ccm n/10 NaOH
verbraucht: 39,1 ccm n/10 H_2SO_4
N_2 = 0,055 g
= 13,74%

Einwage: 2. 0,4637 g Rohtrockensubstanz
vorgelegt: 50,5 ccm n/10 H_2SO_4
titriert: 14,8 ccm n/10 NaOH
verbraucht: 35,7 ccm n/10 H_2SO_4
N_2 = 0,050 g
= 13,74% der reinen Trockensubstanz

Mittelwert: 13,74%

III. Aschegehaltsbestimmung.

Einwage:	1. 10 g	2. 7 g Rohtrockensubstanz
Asche:	1,1095 g	0,8612 g
	= 14,11%	15,65% der reinen Trockensubstanz

Mittelwert: 14,88%

IV. Kieselsäurebestimmung.

Tiegel leer:	1. 16,1138 g	2. 16,1172 g
+ Substanz:	16,1188 g	16,1228 g
Kieselsäure + Filterasche + Verunreinigung:	0,0050 g	0,0056 g
− Filterasche − Verunreinigung	0,0034 g	0,0045 g
SiO_2-Reinsubstanz:	0,0016 g	0,0011 g
	0,0204%	0,0199%

Mittelwert: 0,0201%

Verunreinigung an Oxyden durch Abrauchen mit HF.

Tiegel + Verunreinigung:	16,1172 g	16,1217 g
Tiegel leer:	16,1138 g	16,1172 g
Verunreinigung:	0,0034 g	0,0045 g

Analysenprotokolle der mit kolloidaler Kieselsäure gefütterten Ratten.

A. Naßsubstanz	676,00 g
B. Vortrockensubstanz (nach Trocknung auf dem Wasserbad)	245,00 g
C. Rohtrockensubstanz (nach Ätherextraktion)	184,73 g
D. Extraktionsrückstand[1] (Fett usw.)	60,27 g

I. Trockenbestimmung bei 102° C.

Einwage: 0,5088 g Rohtrockensubstanz
0,1169 g Wasserverlust
= 22,97% — 22,97%

[1] 50 g Rohtrockensubstanz mit Äther extrahiert wiegen nach Extraktion 37,7 g = 60,27 g Fett in der Gesamtrohtrockensubstanz von 184,73 g.

a) Gesamtwassergehalt der Naßsubstanz:

676,00 g Naßsubstanz
− 245,00 g Vortrockensubstanz
431,00 g H_2O

In 184,73 g Rohtrockensubstanz sind noch 22,17% H_2O
42,46 g H_2O
473,46 g H_2O = 70,04%

b) Gesamttrockensubstanz in 676,00 g Naßsubstanz
184,73 g Rohtrockensubstanz
− 42,46 g H_2O
142,27 g Trockensubstanz = 21,04%

c) Fettgehalt in 676,00 g Naßsubstanz
60,27 g Fett = 8,91%
zusammen: 99,99%

II. Stickstoffbestimmung nach Kjeldahl.

Einwage: 1. 0,3539 g
vorgelegt: 50,5 ccm n/10 H_2SO_4
titriert: 22,0 ccm n/10 NaOH
verbraucht: 28,5 ccm n/10 H_2SO_4
N_2 = 0,040 g
= 14,66%

Einwage: 2. 0,2726 g Rohtrockensubstanz
vorgelegt: 50,0 ccm n/10 H_2SO_4
titriert: 28,5 ccm n/10 NaOH
verbraucht: 22,0 ccm n/10 H_2SO_4
N_2 = 0,031 g
= 14,66% der reinen Trockensubstanz

Mittelwert: 14,66%

III. Aschegehaltsbestimmung.

Einwage:	1. 10,00 g	2. 7,00 g Rohtrockensubstanz
Asche:	1,1930 g	0,8916 g
	= 15,49%	= 16,54% der reinen Trockensubstanz

Mittelwert: 16,01%

IV. Kieselsäurebestimmung.

	1.	2.
Tiegel leer:	16,0823 g	18,0825 g
+ Substanz:	16,0888 g	16,0880 g
SiO_2 + Filterasche + Verunreinigung:	0,0065 g	0,0055 g
− Filterasche − Verunreinigung:	0,0042 g	0,0040 g
SiO_2-Reinsubstanz:	0,0023 g	0,0015 g
	= 0,0299%	0,0273%

Mittelwert: 0,0286%

Verunreinigung (Oxyde usw.) durch Abrauchen mit HF.

Tiegel + Verunreinigung:	16,0865 g	16,0865 g
Tiegel leer:	16,0823 g	16,0825 g
Verunreinigung:	0,0042 g	0,0040 g

Kontrollratten der zweiten Serie.

A. Naßsubstanz . . . . . 661,00 g
B. Vortrockensubstanz (nach Trocknung auf dem Wasserbad) . . . . . 240,00 g
C. Rohtrockensubstanz (nach Ätherextraktion) . . . . 182,40 g
D. Extraktionsrückstand[1] (Fett usw.) . . . . . 57,70 g

I. Trockenbestimmung bei 102° C.

Einwage: 0,5012 g Rohtrockensubstanz
0,1282 g Wasserverlust
25,57% 25,57%

a) Gesamtwassergehalt der Naßsubstanz:

671,00 g Naßsubstanz
− 240,00 g Vortrockensubstanz
431,00 g H_2O

In 182,40 g Rohtrockensubstanz sind noch 25,27% Wasser
= 46,64 g
zusammen: 477,64 g H_2O = 71,78%

b) Gesamttrockensubstanz in 671,00 g Naßsubstanz
182,40 g Rohtrockensubstanz
− 46,64 g Wasser
135,76 g Trockensubstanz = 20,23%

c) Fettgehalt in 671,00 g Naßsubstanz
57,6 g Fett = 8,58%
zusammen: 100,59%

II. Stickstoffbestimmung.

	1.	2.
Einwage:	0,2933 g	0,2592 g Rohtrockensubstanz
an Ammoniak gebundene Schwefelsäure:	23,03 ccm	20,80 ccm
Stickstoff:	0,0322 g	0,0291 g
	= 14,78%	= 15,10%

Mittel in der reinen Trockensubstanz 14,94%

III. Aschebestimmung.

Einwage:	1. 10,0000 g	2. 7,0000 g Rohtrockensubstanz
Asche:	1,2488 g	0,8351 g
	= 16,78%	16,03%

Mittelwert in der reinen Trockensubstanz 16,40%

[1] 50 g Rohtrockensubstanz mit Äther extrahiert wiegen nach Extraktion 38,0 g. In 182,40 g Rohtrockensubstanz 57,60 g Extrakt.

IV. Kieselsäurebestimmung.

	1.	2.
Tiegel leer:	16,1377 g	16,1413 g
+ Substanz:	16,1433 g	16,1464 g
SiO_2 + Filterasche + Verunreinigung:	0,0056 g	0,0051 g
+ Filterasche + Verunreinigung:	0,0036 g	0,0039 g
SiO_2-Reinsubstanz:	0,0020 g	0,0012 g
	0,027%	0,023%
		Mittelwert: 0,025%

Verunreinigung (Oxyde usw.) durch Abrauchen mit HF.

Tiegel + Verunreinigung:	16,1413 g	16,1452 g
Tiegel leer:	16,1377 g	16,1413 g
Verunreinigung:	0,0036 g	0,0039 g

Beiträge zur Biochemie der Kieselsäure.

III. Mitteilung:

Einfluß der Kieselsäure auf die Elastizität des Bindegewebes.

Von

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(Eingegangen am 9. Mai 1930.)

Mit 3 Abbildungen im Text.

Besonders durch die Untersuchungen von *Hugo Schulz*[1], aber auch die von *Gommermann*[2], einem Schüler *Koberts*, wissen wir, daß die Kieselsäure ein normaler Bestandteil des menschlichen Organismus ist. *Schulz* hat gezeigt, daß die Kieselsäure besonders im Bindegewebe vorkommt, und zwar im jüngeren Bindegewebe in größerer Menge als in dem älterer Individuen. Er schloß daraus, daß die Kieselsäure ein wesentlicher Bestandteil des Bindegewebes sei. Welche Aufgabe die Kieselsäure im Bindegewebe zu erfüllen habe, darüber sind nur Vermutungen ausgesprochen worden. Von den genannten Autoren und von *Kühn*[3] ist die Ansicht geäußert worden, daß in pharmakologischer Hinsicht die Kieselsäure für das Bindegewebe etwa dieselbe Bedeutung hätte wie das Eisen für die roten Blutkörperchen, also gleichzeitig Reizstoff für die Bildung und Aufbaumaterial des Gewebes darstellt.

Ob die Kieselsäure imstande ist, die Eigenschaften des Bindegewebes zu verändern, oder mit anderen Worten: ob sie faßbare pharmakologische Wirkungen auf das Bindegewebe ausüben könne, ist unseres Wissens bisher noch nicht Gegenstand der Forschung gewesen.

Aus diesem Grunde wurden die vorliegenden Versuche unternommen, die zeigen sollten, ob eine wesentliche Eigenschaft des Bindegewebes,

[1] *H. Schulz*, Pflügers Arch. **89**, 112, 1902; **131**, 447, 1910; **144**, 346, 1912. Münch. med. Wochenschr. 1902, Nr. 11, Sonderdruck.

[2] *M. Gommermann*, Zeitschr. f. physiol. Chem. **99**, 255, 1917; diese Zeitschr. **94**, 165, 1919.

[3] *A. Kühn*, Die Kieselsäure. Stuttgart 1926.

nämlich die Elastizität, sich unter Einwirkung der Kieselsäure ändern könne. Als Kieselsäurepräparat wurde eine kolloidale Kieselsäure und als Versuchstier die weiße Maus gewählt.

In der ersten Versuchsreihe wurde aus der Rückenhaut der Maus ein 4,5 cm langes Stück von 1 cm Breite herausgeschnitten und dieses der Länge nach in zwei Teile von je ½ cm Breite zerschnitten. Das eine Stück wurde nunmehr in Warmblüterringerlösung gebracht, das andere in eine Lösung der kolloidalen Kieselsäure von 5 mg- , in Ringerlösung. Beide Stücke, sowohl die in Ringerlösung wie in Kieselsäureringer-Lösung, wurden nach drei Stunden auf ihre Elastizität untersucht. Zu diesem Zwecke wurde die Haut der Länge nach in Anlehnung an die bekannte *Magnus*sche Versuchsanordnung aufgehängt, wie man einen isolierten Uterus oder Darm zu präparieren pflegt, d. h. das untere Ende wurde an ein rechtwinklig gebogenes Glasrohr befestigt, das obere Ende mit einem kräftigen Schreibhebel verbunden (wir benutzten dazu die Hebelvorrichtung des *Mangold*schen Sklerometers) und der Hebel so belastet, daß der Faden gerade angespannt wurde und das Gewicht der Haut durch die Belastung ausbalanciert war. Darauf wurde der Hebel wagerecht gestellt. Die Haut befand sich in einem Gefäß mit Ringerlösung, durch die aus einem Gasometer Sauerstoff hindurchperlte. Das Gefäß wiederum stand in einem Wasserbad, dessen Temperatur durch Flamme und Thermoregulator auf 37° eingestellt war. Nun wurde die Haut durch Belastung des Schreibhebels gedehnt, und zwar fanden in den einzelnen Versuchen verschiedene Gewichte Anwendung; sie schwankten zwischen 5 und 100 g. Die Haut wurde dann fünf Minuten lang der Dehnung durch das Gewicht von 5 g ausgesetzt und darauf das Gewicht entfernt. In den nächsten fünf Minuten blieb das Hautstück entlastet; dann wurde die Belastung mit 10, 15 g usw. in derselben Weise vorgenommen, also fünf Minuten lang Belastung, fünf Minuten Entlastung. Die Längenveränderungen wurden auf ein Kymographion aufgeschrieben und gaben ganz charakteristische Kurven.

Auf diese Weise ließ sich die Dehnungs- oder Zugelastizität messen, die im geraden Verhältnis zur Länge steht und umgekehrt proportional der Breite ist. Da diese Maße immer so gleich gewählt wurden, wie das bei einem so komplizierten Gebilde wie die Haut nur irgend möglich ist, so wurden diese Verhältnisse nicht mehr besonders berücksichtigt. Wir haben auch auf eine genaue mathematische Auswertung der Kurven verzichtet, da wir uns bewußt waren, daß eine solche doch nicht vollkommen möglich ist.

Es kann gar keinem Zweifel unterliegen, daß die Zahlen, die aus dem Kurvenmaterial gewonnen wurden, verhältnismäßig rohe Angaben sind; sie genügen aber nach unserer Auffassung doch, um einen Überblick über

die Unterschiede zwischen den Hautstücken, die in Ringerlösung bzw. in Kieselsäurelösung sich befanden, festzustellen.

Aus den Kurven läßt sich entnehmen, daß durch die Gewichte eine Dehnung, nach *Triepel*[1] eine elastische Verschiebung, zustande kommt, die augenblicklich oder fast augenblicklich einsetzt. Dieser folgt in den fünf Minuten der Belastung eine elastische Nachwirkung. Bei Entlastung geht die Kurve wieder in die Höhe, d. h. die Haut „schnurrt" zusammen, und zwar kann man an der Kurve wiederum eine fast augenblickliche Wirkung bei Fortnahme des Gewichts und eine Nachwirkungsverschiebung feststellen. Auf allen Kurven ist zu sehen, daß nach der Dehnung je nach dem Gewicht in verschiedenem Grade eine bleibende Änderung zustandekommt. Die Haut und damit der Hebel gehen also trotz Entlastung nicht in die Ausgangsstellung zurück.

Bei den Hautstücken nun, welche in der Kieselsäurelösung gelegen haben, ist einmal die Dehnung durch das Gewicht nicht so groß wie bei den Hautstücken, die sich in Ringerlösung befanden. Des ferneren aber erkennt man, daß die elastische Nachwirkung nicht denselben Grad erreicht und daß bei der Entlastung die Kurve sich der Ausgangsstellung wieder besser nähert. Wesentlich für die Beurteilung der Elastizität des Gewebes ist der Elastizitätsmodul, der nach *Triepel* direkt proportional der Kraft und umgekehrt proportional der Veränderung ist, also $E = \frac{\text{Kraft}}{\text{Veränderung}}$. Der Elastizitätsmodul ist zweifellos unter dem Einfluß der Kieselsäure größer geworden; die Elastizität, weit davon entfernt, eine vollkommene zu sein, hat sich dieser doch mehr genähert als die der Hautstücke, die sich in Ringerlösung befanden. Siehe die Tabellen I und II.)

Tabelle I.

Gewicht	Vergleich		Kieselsäure R. L.		Kieselsäure injiziert	
g	Dehnung cm	$E = \frac{K}{V}$	Dehnung cm	$E = \frac{K}{V}$	Dehnung cm	$E = \frac{K}{V}$
5	6	0,83	2	2,5	1	5,0
10	15	0,67	5	2,0	3	3,3
15	21	0,71	9	1,67	6	2,5
20	28	0,71	13	1,54	7	2,86
30	35	0,85	24	1,25	14	1,25
50	46	1,09	39	1,28	17	2,94
			Durchschnitt:			
21,7	25,2	0,71	15,3	1,71	8	2,98

[1] *H. Triepel*, Einführung in die physikalische Anatomie. Wiesbaden 1902.

Tabelle II.

	Vergleich			Kieselsäure R. L.			Kieselsäure injiziert		
Gewicht	Dehnung	Verkürzung	Dehnungsrückstand	Dehnung	Verkürzung	Dehnungsrückstand	Dehnung	Verkürzung	Dehnungsrückstand
g	%	%		%	%		%	%	
5	17	11	6	13	11	2	11	10	1
10	31	16	15	22	17	5	20	17	3
15	39	18	21	34	25	9	29	23	6
20	53	22	28	43	30	13	37	30	7
30	62	27	35	59	35	24	48	34	14
50	81	35	46	85	46	39	58	41	17
				Durchschnitt:					
21,7	46,7	21,5	25,2	42,7	27,3	15,4	33,8	25,8	8,0

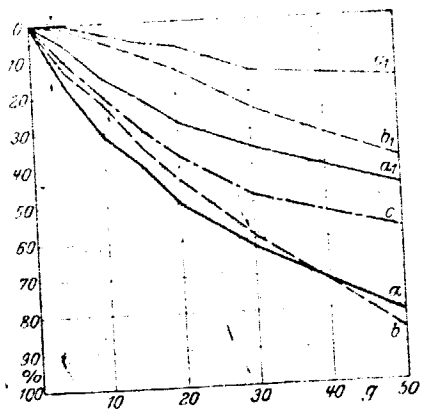

Abb. 1.

Abszissenzahlen geben die Belastung. Ordinate die prozentischen Änderungen an. a Dehnungskurve bei Belastung, a_1 bleibende Dehnung bei Entlastung der Hautstücke der normalen Mäuse, b, b_1 das gleiche von Hautstücken, die in SiO_2 drei Stunden lang gelegen hatten, c, c_1 von Hautstücken der Mäuse, die SiO_2 subkutan erhalten hatten. Durchschnittszahlen von 15 Versuchen.

Gegen diese Versuche, die zeigen, daß eine verhältnismäßig kurze Einwirkung der kolloidalen Kieselsäure genügt, um eine meßbare Elastizitätsveränderung herbeizuführen, könnte man einwenden, daß das Kolloid als solches wirksam gewesen ist. Deshalb wurde in einer zweiten Versuchsreihe Mäusen kolloidale Kieselsäure subkutan injiziert, und zwar 8 und 14 Tage 2,5 mg täglich. Darauf wurden sie getötet und die Haut wiederum so präpariert, wie in der ersten Versuchsreihe auseinandergesetzt ist. Das Ergebnis war qualitativ gleich, quantitativ noch deutlicher als in der ersten Versuchsreihe, wie aus der zusammen-

fassenden Kurve und den Zahlen (Tabelle I und II) hervorgeht. Besonders charakteristisch ist die erhebliche Zunahme des Elastizitätsmoduls. Die Elastizität der Häute der Kieselsäuremäuse nähert sich also bei weitem mehr der vollkommenen Elastizität als die der Vergleichstiere.

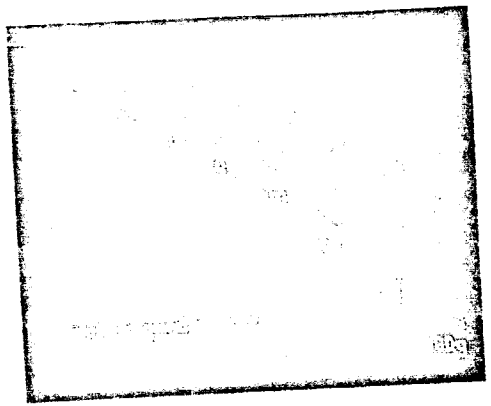

Abb. 2.

Maus 4. Rückenhaut 3 Std. lang in Ringerlösung.

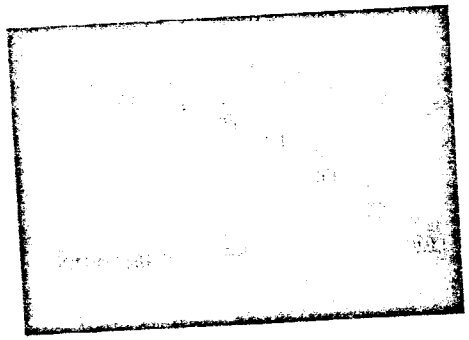

Abb. 3.

Rückenhaut derselben Maus. 3 Std. lang in kolloid. Kieselsäure.

Erörterung.

Bisher wurde aus der Tatsache, daß die Kieselsäure im Bindegewebe und zwar im jungen Bindegewebe mehr als im alten gefunden wurde, der Schluß gezogen, daß sie eine gewisse Bedeutung für das Bindegewebe hätte. So einleuchtend diese Schlußfolgerung ist, so liefern die analytischen Zahlen an und für sich doch noch keinen bündigen Beweis hierfür; denn es wäre nicht vollkommen ausgeschlossen, daß die Kieselsäure aus irgend einem uns nicht näher bekannten Grunde im Bindegewebe

angereichert ist, aber für die Funktion keine Bedeutung besitzt. Wir glauben durch die vorliegenden, noch nicht sehr zahlreichen Versuche, die noch an anderem Gewebe und bei anderer Dosierung fortgesetzt werden sollen, den Anfang eines Beweises geliefert zu haben, daß die Kieselsäure tatsächlich auf eine sehr wesentliche Eigenschaft des Bindegewebes, nämlich auf die Elastizität, einen fördernden Einfluß ausüben könne.

Zusammenfassung.

1. Die Elastizität — gemessen an den elastischen Veränderungen bei Dehnung und Entlastung — gleich großer Stücke der Häute weißer Mäuse wird durch Einlegen in Kieselsäurelösung erhöht. Der Elastizitätsmodul ist deutlich vergrößert.

2. Gleich große Stücke der Haut weißer Mäuse, die Kieselsäure längere Zeit subkutan erhalten hatten, zeigen eine größere Elastizität als die Präparate der Vergleichstiere.

3. In beiden Fällen sind die augenblickliche Elastizitätsverschiebung und die elastische Nachwirkung geringer: die augenblickliche elastische Verkürzung und die elastische Verkürzungsnachwirkung größer. Der Elastizitätsmodul $E = \frac{\text{Kraft}}{\text{Veränderung}}$ nimmt zu.

Dadurch ist ein erster Versuch gemacht worden, die Wirkung der Kieselsäure auf das Bindegewebe auf experimentell pharmakologische Grundlage zu stellen.

URINARY CALCULI CONTAINING SILICA: A CASE REPORT

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Urinary calculi containing silica have rarely been reported. The finding of silica without calcium, magnesium, uric acid, oxalate or phosphate in a calculus alleged to be urinary in origin, might even be taken as *prima facie* evidence that it was not authentic. This was at first considered to be a possible explanation in the case we are reporting here. Only one other 'calculus' containing silica had been previously seen by one of us (B.M.B.), and this was alleged to have been passed *per urethram* by a boy aged 7 years; doubt was however expressed about its urinary origin. A similar view has been taken by other authors.[1,2]

A second possibility is an association with silicosis. Very rarely 'calculi' have been reported to contain silicates in individuals with long exposure to silica.[3] However, our patient was not a miner, had not worked underground, and had no undue exposure to silica-containing dust. The third and most likely cause advanced by one of us (E.L.), followed a report by Lagergren[4] of the development of silica calculi after prolonged oral administration of magnesium trisilicate. Our attention was then drawn to the fact that our patient, too, had taken magnesium trisilicate for some years for a digestive disorder. As magnesium trisilicate is a commonly used antacid, we felt it would be of interest to record this apparently unusual complication, which may, however, be more common than the few published reports suggest.[4-7]

CASE HISTORY

Mr. J.M., aged 62 years, has a hiatus hernia with resultant pyrosis, and has taken magnesium trisilicate for years, 2–4 times daily. He also has coronary artery disease—he has had two, or probably three attacks of coronary thrombosis, and has had anticoagulant therapy. He is hypercholesteraemic and has been taking a diet low in dairy and animal fats and high in fruit and vegetables for some years.

In June 1961 he complained of pain, and a calculus was diagnosed in the left lower ureter with blockage. He passed some calculous fragments *per urethram* in the subsequent few days. In October 1961 he again passed 'gravel', which was found to consist almost entirely of inorganic material. Tests for urates, oxalates and calcium were negative, but there was insufficient material for further analysis. An intravenous pyelogram was normal and no calculi were evident.

In June 1962 prostatectomy was carried out, and 8 days later the patient passed a calculus *per urethram* which was later tested and found to be radiopaque. His pain indicated that the calculus had, like the others, come down the left ureter. Since it was opaque it must have been formed subsequent to the intravenous pyelogram. It was irregularly round, facetted, approximately 0·5 - 1 cm. in diameter and brown in colour. Calcium, magnesium, oxalate, uric acid, cystine and xanthine were not detected. A trace of phosphate and a slight amount of organic matter were present. Tests for silicates gave a positive result.[8] Some of the powdered material was sent to Dr. I. Webster, Director of the Pneumoconiosis Research Unit, who confirmed that a large amount of silica—approximately 50% of the specimen—and traces of iron were present.

DISCUSSION

In 1962 Lagergren[4] reported 5 cases of silica stones in Sweden. One consisted only of silica, 3 also contained small quantities of calcium hydroxyapatite, and 1 contained small quantities of calcium oxalate. He also referred to 2 previous cases of calculi in Sweden, the first reported by Hammarsten and his associates[5] in 1953, and the second by Hammarsten[6] in 1958. A further case was recorded by Herman and Goldberg[7] in 1960. All the patients had taken magnesium trisilicate more or less regularly for some years. A search of the literature has revealed no further cases.

Silicon, as silicates, is present in vegetables and the husks of whole grains. Soluble silicates are easily absorbed from the alimentary tract, but silica, as far as is known, has no physiological role. The blood-plasma level is approximately 1 mg. per 100 ml., and usually does not rise since any excess is rapidly excreted in the urine.[9]

Synthetic magnesium trisilicate ($Mg_2Si_3O_8 . nH_2O$), which is a white insoluble powder, was shown by Mutch[10] not to be absorbed from the intestine and to have absorbent and antacid properties of particular value in the treatment of gastric acidity and chronic peptic ulceration. The value of magnesium trisilicate and the silica resulting from the reaction with gastric hydrochloric acid apparently depends on the fact that both are active hydrated colloids. Although the chemical reaction can be represented as $Mg_2Si_3O_8 . nH_2O + 4\ HCl \rightarrow 2\ MgCl_2 + 3\ SiO_2 + (n+2)\ H_2O$ the conditions of interaction are complex and are not completely understood. Page *et al.*[11] suggested that more complex end-products than silicon dioxide (or silica) were produced, viz. various combinations with water to form different silicic acids and silicates. They considered that these products could include soluble orthosilicic acid (H_4SiO_4, i.e. $SiO_2 + 2\ H_2O$), partially soluble metasilicic acid (H_2SiO_3, i.e. $SiO_2 + H_2O$), partially soluble trisilicic acid ($H_4Si_3O_8$, i.e. $3\ SiO_2 + 2\ H_2O$), and nearly insoluble disilicic acid ($H_2Si_2O_5$, i.e. $2\ SiO_2 + H_2O$). The variation in the hydration of the SiO_2 molecule thus gives a change in solubility, and this factor, as well as the physico-chemical state of the silica—which may remain in solution as a colloidal sol or may partially separate as a gel— probably determine its absorption from the intestine.

Page *et al.*[11] showed that there was a ten-fold increase of the normal silica content of the urine following oral administration of magnesium trisilicate. From a mean daily level of 16·2 mg. of silica before treatment, the daily excretion of silica reached 172, 178 and 162 mg. of silica on the second, third and fourth days of the administration of 5 G. of magnesium trisilicate daily, indicating that about 5% of the silica ingested was excreted in the urine.

The manner in which silica is excreted in the urine after magnesium trisilicate ingestion does not, however, appear to have been studied, nor has the effect of pathological changes in the urinary tract been considered. It is not surprising, therefore, that the pathogenesis of silica calculi remains obscure and that no explanation can be offered for the occurrence of this complication of magnesium trisilicate therapy. Lagergren[4] considered that the structure

J. Path., vol. 103 (1967): 35-40

EXPERIMENTAL CHRONIC INTERSTITIAL NEPHRITIS COMPARED WITH ENDEMIC HUMAN NEPHROPATHY

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Plates XV-XVIII

This paper is a report of the experimental induction of chronic interstitial nephritis by a quartz suspension, and a comparison of the induced renal lesions with the histopathological changes found in endemic nephropathy in man.

Endemic human nephropathy

The prevalence of chronic nephritis in the Balkan Peninsula has been recognised for a long time (Volhard, cited by Milošević, 1959). Many years ago Djordje Joanović suggested that the incidence of chronic nephritis among the rural population was due to bad hygienic conditions in the villages, inadequate clothing, cold and humidity. Immediately before the Second World War Anka Savić warned that nephritis was likely to be endemic in some villages along the River Drina in Serbia.

However, the first publications in the medical literature on the endemic occurrence of this disease came from Bulgaria (Tančev *et al.*, cited by Mušicki and Vasić, 1966). In 1956, Tančev *et al.* reported that the population of about 20 villages along the lower reaches of the River Iskar, in the Mihajlovgrad and Vraca districts in the west of Bulgaria, was afflicted with this disease.

Foci of endemic nephropathy were discovered in villages along the River Kolubara in Serbia in 1957, and at the same time along the lower reaches of the Rivers Drina and Sava, and in Bosnia and Croatia. The prevalence of this disease, affecting up to one-third of the total population of some villages, and the high mortality rate, proved to be a serious health problem.

It was concluded at the Planning Conference on the Endemic Nephropathy of South-Eastern Europe, organised by WHO in Dubrovnik in October 1964, that the Bulgarian, Rumanian and Yugoslav nephropathies were the same disease. It was most prevalent in the villages situated in lowland, flooded areas, close to some rivers, whilst its incidence was considerably lower in the mountains. In its endemic form it was not observed in towns, and because of its clinical features the disease was classified as chronic interstitial nephropathy.

The histology of this disease is distinctive, with primary lesions of the tubules, and a characteristic interstitial inflammatory-proliferative infiltration. Whilst the authors from Yugoslavia and Bulgaria consider that the changes in the glomeruli are secondary to vascular lesions, some Rumanian authors consider them to be primary.

So far as aetiology is concerned it was concluded that it is not of an infectious character, but is caused by chemical agents.

In our papers on this endemic nephropathy (see Marković and Lebedev, 1965*a* and *b*), we reported the first experimental results of inducing chronic interstitial nephritis with a suspension of a complex silicate mineral (granite), and with drinking water from an area of endemic nephropathy (the village Donji Dobrić, within the drainage-basin of the Rivers Jadar and Drina).

Received 16 Jan. 1967; *accepted* 31 July 1968.

This chronic renal disease is prevalent in regions characterised by a silicate geochemical structure, where conditions predispose to the deposition and accumulation of the eroded material along river banks. Unlike the silicates with a completed geochemical cycle, i.e., chemically stable and inactive ones, the silicates originating from magmatic silicate rocks (acid granites) are liable to metamorphic processes, and are the most toxic for human kidneys. The incidence of this disease in endemic proportions in the lower reaches of some large rivers —the Sava, Drina and Kolubara—is attributable to a suitable permeable terrain in these areas (alluvial soil), with consequent diffusion of the eroded silicate material from the rivers into the drinking water of some human settlements.

Endemic nephropathy may be found in mountainous regions where similar conditions of accumulation exist, but it is considerably less frequent.

Crumbled silicate material deposited on such a soil encounters conditions suitable for its further solution, because disintegrated humus matter (humus acids) and CO_2 are present in abundance (Marković and Lebedev, 1965*a* and *b*, 1967). When this is drunk, further solution of these silicates occurs at *p*H 7·2–7·4 (King, 1947).

The silicic acid is polymerised as it is excreted by the kidneys and thus denatures the kidney proteins, producing well-defined pathological effects (Gye and Purdy, 1922; Policard and Collet, 1954; Policard *et al.*, 1960).

EXPERIMENTAL

Materials and methods

Quartz. Concentrations of 50 and 250 mg per litre water of a pure quartz (SiO_2) suspension were used. The quartz, from the mount of Bukulja, situated within the Kolubara drainage-basin, and well above known foci of endemic nephropathy (the villages of Sopić, Petka and Vreoci), was provided by a geologist from the Institute of Nuclear Raw Material in Beograd. It was ground in a mortar and the size of the particles was measured under the microscope. It was added to "clean" drinking water. (The water from the Beograd waterworks, chemically* and bacteriologically† controlled and suitable for human consumption, was used.) In the period from May to October 1955, while the experiments were carried out, the water was bacteriologically safe and did not show any significant change in chemical content, or in *p*H (7·2–7·8). The test animals were given a silicate suspension in clean drinking water under normal physiological conditions for several months.

Animals. Guinea-pigs more than 6 mth old were used. In earlier experiments we have established an interdependence between the time at which inflammatory-proliferative lesions of the kidney occur and the age of the animals: the younger the animal the longer must it be exposed to the noxious agent. We have therefore used animals weighing more than 500 g.

The *control group* of 20 guinea-pigs was fed with oats for the first 5 days in the week and this was supplemented with carrots for the last 2 days of the week. They had "clean" water from the Beograd waterworks for the first 5 days in the week and were deprived of water on the last 2 days of each week.

The two *experimental groups* each consisted of 20 animals.

Group 1. Quartz, 50 mg per litre, finely ground to a particle size of *c.* 1–3 μm, was added to the water from the Beograd waterworks on the same watering days as for the control group.

Group 2. Treatment of these animals was the same as for group 1, except that the quartz was given in a dose of 250 mg per litre of water.

The animals were kept in a dry, warm room, and the quartz suspension, which was shaken beforehand, was provided in shallow porcelain dishes once a day. The animals were deprived

* International standard for drinking water, standard of chemical and physical quality, p. 27, WHO, *Geneva*, 1958.

† Standarde metode za fizicko-hemijsko i bacteriolosko ispitivanje voda, Zavod za zdravstvenu zastitu SR Srbije, *Beograd*, 1961.

EXPERIMENTAL AND HUMAN INTERSTITIAL NEPHRITIS

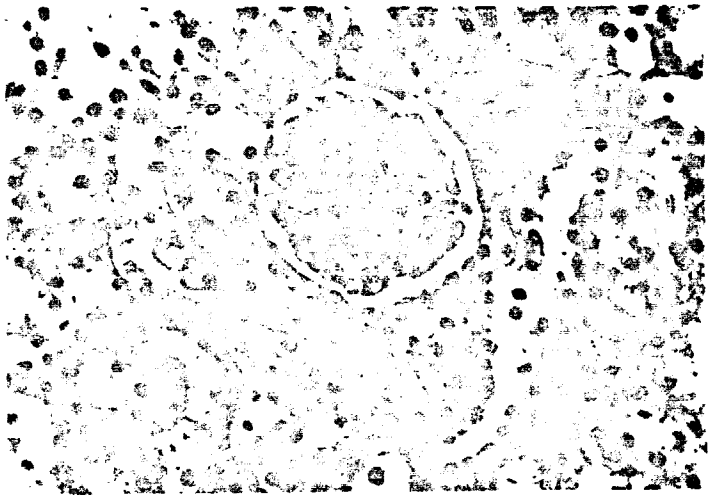

FIG. 1.—Kidney of a control guinea-pig. Haematoxylin and eosin (HE). ×230.

FIG. 2. Guinea-pig kidney. Interstitial infiltration of the outer zone of the cortex stretching from the kidney capsule towards the middle of the cortex. Quartz, 50 mg per litre for 4½ mth. HE. ×35.

FIG. 3. Guinea-pig kidney. Focal interstitial perivascular, periglomerular and peritubular infiltrates with fibrosis and hyalinisation. Bowman's capsule thickened in some glomeruli. Venous blood vessels widened and deformed. Quartz, 50 mg per litre for 6 mth. HE. ×100.

Experimental chronic interstitial nephritis.

EXPERIMENTAL AND HUMAN INTERSTITIAL NEPHRITIS

FIG. 4.—Guinea-pig kidney. Higher magnification of fig. 3. Fibrous, hyaline, thickened Bowman capsule, with periglomerular infiltration and fibrosis. Lobulation of the glomerular capillaries, with reduction in number of endothelial nuclei, and partial hyalinisation of the intercapillary tissue. HE. ×400.

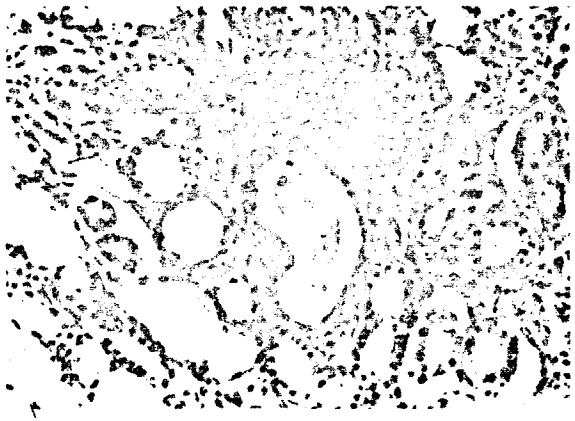

FIG. 5.—Guinea-pig kidney. Peritubular interstitial infiltration and fibrosis with hyalinisation. Tubular lumina deformed, dilated or narrowed. The epithelium of the tubules dystrophic or atrophic. Colloid in lumen of one tubule. Quartz, 50 mg per litre for 6 mth. HE. ×100.

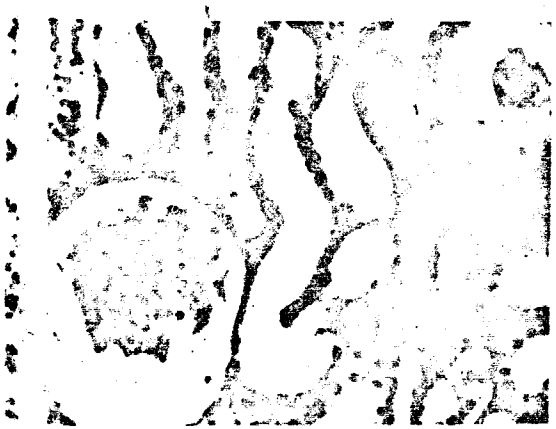

FIG. 6.—Guinea-pig kidney. Atrophic glomeruli with widened subcapsular space. Tubular lumina deformed and dilated. Slight inflammatory-proliferative reaction in some places. Quartz, 250 mg per litre for 6 mth. HE. ×230.

Experimental chronic interstitial nephritis.

EXPERIMENTAL AND HUMAN INTERSTITIAL NEPHRITIS

FIG. 7.—Human kidney. Peritubular interstitial mononuclear infiltration with fibrosis and hyalinisation. Tubular lumina deformed, dilated or narrowed. Tubular epithelium dystrophic. HE. ×230.

FIG. 8.—Human kidney. Perivascular sclerosis. Dilatation of tubules, epithelium dystrophic. Van Gieson. ×230.

FIG. 9.—Human kidney. Periglomerular sclerosis. Bowman capsule shows fibrous and hyaline thickening. Subcapsular space widened. Intercapillary sclerosis. Van Gieson. ×400.

Endemic human nephropathy.

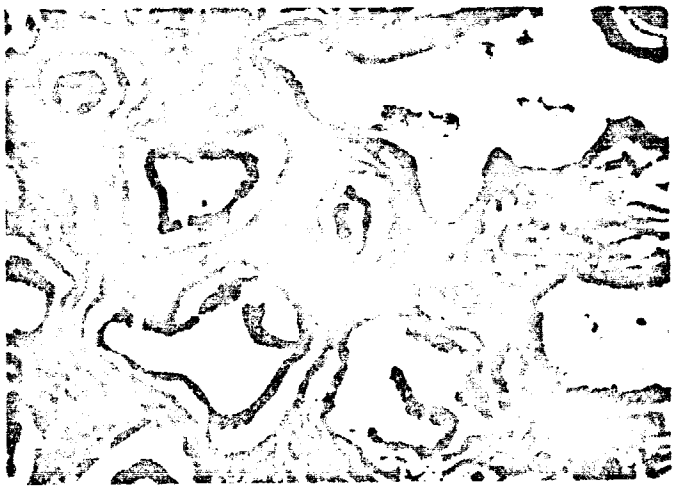

FIG. 10.—Human kidney. Interstitial peritubular sclerosis. Tubular lumina deformed, dystrophic-atrophic epithelium. Van Gieson. 400.

FIG. 11.—Human kidney. Interstitial sclerosis of the outer zone of the cortex. The glomeruli show varying stages of hyalinisation and retraction. HE. ×35.

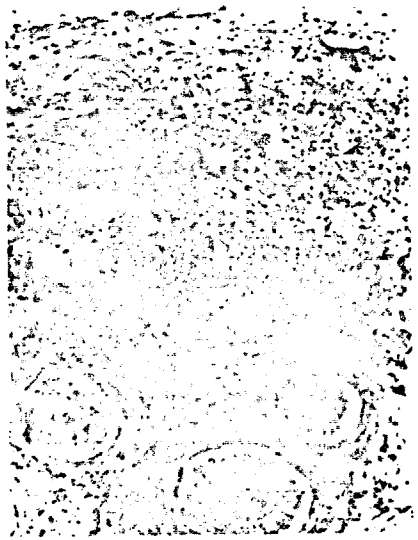

FIG. 12. Human kidney. The glomeruli have fibrous thickened Bowman's capsules and are at varying stages of hyalinisation and retraction. The interstitial tissue is focally infiltrated with mononuclear cells. HE. ×100.

Endemic human nephropathy.

of food for 6 hr before water was provided; they were then allowed to drink water *ad libitum* for 1 hr, and thereafter feeding was continued.

RESULTS

By the 2nd or 3rd mth of the experiment the animals showed clinical signs of the disease: they were losing weight progressively, they were taking less and less food, and their movements became slow and their eyes sunken. The animals were killed at intervals within a period of 6 mth.

Histopathology: experimental and human

Two processes are taking place in the kidneys of the animals given quartz: a diffuse dystrophic-atrophic process in the parenchyma, and a focal inflammatory-proliferative process in the interstitial tissue (see figs. 1 and 2).

Diffuse dystrophic-atrophic changes are observed first in the epithelium of the distal parts of the tubules. Focal interstitial inflammatory-proliferative lesions begin with perivascular lymphocytic and plasma-cell infiltration and this is followed by perivascular fibrosis and hyalinisation with secondary sclerotic changes in the blood vessels (figs. 3, 4 and 8).

Focal periglomerular inflammatory-proliferative infiltration leads to fibrotic thickening of Bowman's capsule (fig. 3). With further progression the hyaline fibrous, thickened Bowman's capsule narrows concentrically, and there is hyalinisation of the glomerular tuft, with proliferation of connective tissue between the capillaries. The process ends with retraction and transformation of the glomerulus into a compact hyaline mass (see figs. 3, 4, 9, 11 and 12).

The focal peritubular inflammatory-proliferative process produces fibrosis and hyalinisation of the stroma, deformation, narrowing and dilatation of the lumen of the tubules, and eventually atrophy of the tubular epithelium (figs. 5, 7 and 10). The lumina of some tubules contain a proteinaceous colloid substance (fig. 5), and some tubules are cystic. In severe cases the changes affect most of the kidney (fig. 6), which is fibrotic and contracted. In some of the glomeruli the subcapsular space is widened; some glomeruli are partially or completely atrophic.

The superficial cortex and the juxtamedullary parts of the cortex are most affected by the inflammatory-proliferative process, and the cortex becomes more and more narrow in relation to the medulla. The occurrence of massive " acellular sclerosis ", with absence of mononuclear infiltration, is characteristic (Dojčinov, 1966). These changes are best accounted for by the observation, in these and in earlier experiments, that the initial localisation of the inflammatory-proliferative infiltrate is characteristically subcapsular, especially along the blood vessels, and extends towards the middle of the cortex (fig. 2).

However, it is impossible for the present to answer the question whether " direct " hyalinisation occurs in some parts of the affected kidneys, without previous inflammatory proliferative infiltration.

In man, the kidneys, under the prolonged influence of quartz, weigh 50 g, or even less. As a rule the contracted kidney retains a smooth surface— " nephrocirrhosis glabra " (fig. 11).

Discussion and general conclusions

It is known that silicates have a nephrotoxic effect and that long exposure to silicic acid produces chronic interstitial nephritis (Policard and Collet, 1954; Policard, Collet and Pregermain, 1957; Policard *et al.*, 1960).

In this study, experiments were done with natural SiO_2 (mineral quartz) under physiological conditions on the basis of King's theory of silicate solubility (King, 1947).

Quartz mechanically ground into microparticles and added to clean drinking water is nephrotoxic; after a longer exposure it brings about chronic renal inflammation of the interstitial type.

The dose of the quartz suspension is important in relation to the period of time necessary to produce the first parenchymatous and mesenchymal lesions. With the dose of 250 mg per litre it is possible to induce parenchymatous lesions even after 2 wk, whilst the minimum time for the induction of inflammatory-proliferative lesions is 4–6 wk, regardless of the dose.

The lowest concentration of the silicate suspension that, under these conditions, induced interstitial nephritis was 50 mg per litre of water; this is approaching the quantity of SiO_2 detectable in the drinking water in areas affected by endemic nephropathy, which varies, however, with the season of the year (Marković and Lebedev, 1965*a*).

We do not know the minimum dose of the silicate suspension that would cause definite pathological effects in the kidneys experimentally, but the human kidney (Houssay, 1955) is much more sensitive to silicate than the kidneys of herbivorous animals (Dukes, 1954) because of the acid *p*H of the urine.

The dose of silicate suspension is also important in determining the severity of the experimental lesion.

Comparing the results of the experiments, at the two dose levels of 50 mg and 250 mg per litre of the silicate suspension, it is clear that a more intense inflammatory-proliferative effect is produced by the former, whereas with 250 mg per litre atrophic changes are dominant. The *p*H of the urine, the age of the test animals and the period of exposure also play a part and all these factors are involved in the endemic nephropathy in man. In men who have spent a sufficient time in areas affected by endemic nephropathy, the disease may become clinically manifest after they have moved elsewhere, and they may die only after some years.

On the basis of this study it may be concluded that pure SiO_2 (quartz) is nephrotoxic under certain experimental conditions. Its nephrotoxicity is caused by disintegration of quartz particles with the release of silicic acid, which under certain biochemical conditions in the kidneys becomes toxic and produces the pathological changes we have described.

One more question needs answering—What is the effect of ingested silicate compounds and of other minerals, light and heavy metals, etc., on other organs (Lambrev, Atanasov and Petrinska-Venkovska, 1963; Petković, Tomić and Mutavdžić, 1966; Pukhlev, 1966; Šijivić, 1966)?

Summary

This paper describes the experimental induction in guinea-pigs of chronic interstitial nephritis by pure silica (SiO_2). Quartz was ground mechanically into microparticles about 1–3 μm in diameter, added to "clean" drinking water, and given to animals to drink over several months. Under these experimental conditions the guinea-pigs developed renal pathological changes similar to those found in man in Yugoslavia—the endemic nephropathy caused by rock erosion in village communities on the banks of the lower reaches of large rivers.

The renal lesion is caused by release of silicic acid, which affects the kidneys chemically.

The silicate causes two processes in the kidneys: a dystrophic-atrophic lesion in the parenchyma, and a proliferative-inflammatory one in the interstitial tissue.

The dose of silicate suspension determines the pathogenic effects produced. The concentration of the silicate suspension, the *p*H of the urine, the age of the animal and the period of exposure are important factors. In the course of time the kidneys became atrophic and fibrotic.

We express our thanks to Dr Ljubomir Jovanovic, Head of the Department of Bacteriology at the Republic Institute for Health Protection in Belgrade, for professional help in this study. We are also indebted to Mr Rade Minic, laboratory technician in the Pathological Unit of the 2nd Clinic of Surgery in Belgrade, for his help in preparing histopathological slides, to Mr Toma Stefanovic for the photomicrographs and Mrs Smiljana Naumovic for the English translation.

REFERENCES

Dojčinov, D. 1966. *In* Proceedings of a symposium on endemic nephropathy, Niš, pp. 114–133.

Dukes, H. H. 1954. The physiology of domestic animals, 6th ed., *Ithaca, N.Y.*

Gye, W. E., and Purdy, W. J. 1922. The poisonous properties of colloidal silica. I. The effects of parenteral administration of large doses. *Br. J. Exp. Path.*, **3**, 75.

Houssay, B. A. 1955. Human physiology, 2nd ed., *New York.*

King, E. J. 1947. Solubility theory of silicosis: a critical study. *Occup. Med.*, **4**, 26.

Lambrev, S., Atanasov, N., and Petrinska-Venkovska, S. 1963. Proceedings of an international conference on endemic nephropathy, Sofia, pp. 186–191.

Marković, B., and Lebedev, S. 1965*a*. Rôle étiopathogénique des silicates érosifs dans la néphrite endémique. *Presse méd.*, **73**, 401.

Marković, B., and Lebedev, S. 1965*b*. Proceedings of a Geochemical Symposium, Beograd, pp. 251–256.

Marković, B., and Lebedev, S. 1967. Endemic nephropathy in Jugoslavia. *Klin. Med., Mosk.*, **1**, 91 (in Russian).

Milošević, M. 1959. Utisci o endemskom nefritisu. *Glasn. Zav. zdrav. zašt., Beograd*, **3**, 57.

Mušicki, B., and Vasić, V. 1966. *In* Proceedings of a symposium on endemic nephropathy, Niš, pp. 51–58.

Petković, S., Tomić, M., and Mutavdžić, M. 1966. Quelques considérations sur l'étiologie et la clinique du cancer du bassinet. *J. Urol. Nephrol.*, **72**, 429.

Policard, A., and Collet, A. 1954. Étude expérimental des lésions rénales provoquées par élimination de la silice. *J. Urol. méd. chir.*, **60**, 164.

Policard, A., Collet, A., Daniel-Moussard, H., and Pregermain, S. 1960. On the first stages of experimental changes in the kidney caused by silicic acid. Study with electron microscope. *J. Urol. méd. chir.*, **66**, 505 (in French).

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POLICARD, A., COLLET, A., AND PREGERMAIN, S. 1957. Sur quelques points de la cytopathologie des néphrites toxiques étudiées au microscope electronique. *Presse méd.*, 65, 1585.

PUKHLEV, A. 1966. Proceedings of a symposium on endemic nephropathy, Niš, pp. 31–40.

ŠUJIVIĆ, B. 1966. *In* Proceedings of a symposium on endemic nephropathy, Niš, pp. 205–210.

WHO REGIONAL OFFICE FOR EUROPE 1964. Planning Conference on the endemic nephropathy of South Eastern Europe, Dubrovnik.

Science, Vol. 171: 1141-1142, Sept. 1971

Talc-Treated Rice and Japanese Stomach Cancer

Abstract. *The Japanese prefer talc-dusted rice in their diet. The incidence of stomach cancer in Japan is unusually high. Most talc has some asbestos contaminants. Epidemiologic evidence is presented that the asbestos-contaminated talc on rice in the diet is the carcinogen or cocarcinogen responsible for the high incidence of Japanese stomach cancer.*

The high incidence of stomach cancer in Japanese men represents a challenging problem in cancer epidemiology. Study of age-adjusted rates of mortality from stomach cancer in 24 countries showed that from 1962 to 1963 Japanese men had the highest rate of all nations; the rate, 67.96 per 100,000 in the male population, was seven times that for men in the United States (*1*). Since talc frequently contains asbestos (*2*), and since workmen exposed to asbestos show a marked increase in gastrointestinal cancer, especially stomach cancer (*3*), the hypothesis that the increased rate of cancer of the stomach in Japan is due to ingestion of rice contaminated with asbestos seems attractive and has been recently suggested in a brief note (*4*). This report presents evidence for this relationship at somewhat greater length.

Rice grown in California and meant for the American consumer is milled mechanically. Rice is prepared differently for the Japanese consumer. It is milled and treated with glucose, and then talc is added. The talc is held to the surface of the grain and is said to preserve its flavor better. Japanese consider this rice more tasty, both here and in Japan. Two percent of all talc produced in California from 1959 to 1963 was used as a rice additive, primarily for export to Japan (*5*). Rice containers in Los Angeles markets intended for the Japanese customer usually bear the legend: "Coated with glucose and talc." Optical microscopy of the ash of such store-bought rice showed 3.7×10^6 asbestos-form fibers per gram (Fig. 1).

Talc, the mineral, is a hydrous silicate of magnesium. So are some forms of asbestos. The difference between these two minerals is not in their chemical compositions, but rather in their structure, asbestos being fibrous and mineral, talc being flaky or granular. However, commercial talc is composed not only of the pure mineral talc, but also of fibrous silicates, which are in part classifiable as asbestos. There is no firm mineralogic definition of commercial talc, and in most areas the mineral talc forms much less than half of commercial talc. The composition of commercial talc varies with the deposit. Vermont mines produce a platy or granular type of mineral relatively free of asbestos minerals (*6*). New York and California tales contain more fibrous silicates, including anthophyllite and tremolite (*2, 5*).

Fig. 1. Photomicrograph of the ash of a specimen of rice intended for Japanese consumption and purchased in a Los Angeles market. Arrows indicate asbestos-form fibers. The specimen contained 3.7×10^6 such fibers per gram.

Respired asbestos and talc dust cause similar disorders in workmen. Older talc workers show more than four times the expected incidence of lung and pleural cancer (*7*). Talc or talcose minerals, including contaminating asbestos, are apparently the responsible carcinogens since, where confirmatory evidence could be obtained, all those talc workers with pleural and lung cancer also had talc pneumoconiosis. Autopsies on six talc workers with talc pneumoconiosis showed the presence of the characteristic "asbestos body" in the lungs of every one (*8*). When talc is examined by phase contrast microscopy, from 8 to 30 percent of the particles are fibrous silicates. Identifiable tremolite, anthophyllite, and chrysotile are also seen (*9*). The large talc deposits of New York and California regularly contain the amphibole asbestos minerals, tremolite and anthophyllite. These are normal ingredients of commercial tales from these areas. Chrysotile asbestos occurs in veins in talcose mineral deposits and may be then mined with the talc and create a contaminant (*9*).

Asbestos is an established carcinogen. Not only does it cause lung cancer, but it is associated with a substantial increase of morbidity from stomach cancer (*3*). Although chrysotile is the most important commercial fiber of asbestos, there is no reason to regard the amphibole asbestos minerals as safe. To the contrary in one study an amphibole asbestos mineral predominated in asbestos-bodies in the sputum of a group of men exposed to asbestos and having a high incidence of mesotheliomata (*10*). The fact that talc workers exposed to fibrous talc, which contains amphibole asbestos, develop more pulmonary injury than do workers exposed only to tales relatively free of fibrous silicates or amphiboles (*11*), indicates the pathogenicity of amphibole asbestos.

The Japanese preference for talc-coated rice means that rice eaters of that nation are exposed to the hazard of ingestion of amphibole asbestos, as

well as the unpredictable ingestion of chrysotile in areas where talc contains these carcinogenic materials.

Examination of the known epidemiologic characteristics of stomach cancer in Japanese strongly suggests that the causative agent is an additive to rice. Not only is the incidence of stomach cancer high in rice-eating Japanese, but inside Japan stomach cancer is most prevalent in those prefectures where rice is the main staple; it is less so in prefectures where rice is supplemented with cereal (*12*) and where the population eats heavily of the soybean preparations shoyo and miso (*13*). The morbidity from stomach tumors is greater among Japanese who eat rice with every meal than it is among Japanese who eat it less often; among Japanese men who like milk and vegetables there is a lesser morbidity from stomach neoplasms (*14*). Experimental consumption of polished rice will cause ulceropapillomata of the forestomach of rats (*15*).

These observations suggest a relationship between rice consumption and stomach cancer. However, the agent is not the rice itself, since in other oriental countries where talc-coated rice is not popular the incidence of stomach cancer is not unduly high.

A final indication of the environmental causes of stomach cancer in Japanese is provided by statistics concerning its prevalence in Japanese immigrants to western countries. The incidence in Japanese males in Japan in 1962 to 1964 was 77.9 per 100,000 (*16*). Japanese men in Hawaii had an incidence of only 45 per 100,000. Even this lowered rate was high compared to that for Chinese and Caucasian men of Hawaii who had incidences of 9.2 and 8.3, respectively. Native-born Japanese have a higher incidence of stomach cancer than Japanese born in Hawaii or continental North America (*17*). Dietetic studies on immigrant Japanese show that from 50 to 70 percent of their diet is Japanese-type food (*18*). The longer the immigrant has been in the West the more western his diet becomes; this increase in western food in the diet coincides with a decrease in the incidence of stomach cancer.

The hypothesis that the carcinogenic agent causing the high incidence of Japanese stomach cancer is asbestos-containing talc on rice appears therefore to satisfy the characteristics of the known epidemiology of that form of cancer in Japan. However, standardized mortality rates are also high for Chile, Finland, and Iceland, where rice is not a major dietary staple (*12, 19*). In view of the evidence for the Japanese, the presence of asbestos-contaminated talc as an additive to some nationally popular food should be suspected in these countries. Further studies of the total contamination of the common diet by talc and asbestos in these nations with high incidences of stomach cancer are needed.

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References and Notes

1. T. Hirohata and M. Kuratsune, *Br. J. Cancer*, 23, 465 (1969).
2. L. A. Wright, *Talc and Soapstone* (California Division of Mines Publications, Bulletin 176, San Francisco, 1957).
3. I. Selikoff, J. Churg, E. Hammond, *J. Am. Med. Assoc.* 188, 22 (1964).
4. R. Merliss, *ibid.* 216, 2144 (1971).
5. J. R. Wells, in *Mineral Facts and Problems* (U.S. Bureau of Mines, Washington, D.C., 1965).
6. B. Weiss and E. Boehner, *Arch. Environ. Health* 14, 30[illegible] (1967).
7. M. Kleinfeld, J. Messite, O. Kooyman, M. Zaki, *ibid.*, p. 663.
8. M. Kleinfeld, C. Giel, J. Majeranowski, J. Messite, *ibid.* 7, 101 (1963).
9. L. Cralley, M. Key, D. Groth, W. Lainhart, R. Ligo, *Am. Ind. Hyg. Assoc. J.* 29, 3[illegible] (1968).
10. J. Stumphuis and P. Meyer, *Ann. Occup. Hyg.* 11, 283 (1968).
11. J. Messite, G. Reddin, M. Kleinfeld, *Am. Med. Assoc. Arch. Ind. Health* 20, 468 (1959).
12. M. Segi and M. Kurihara, *Tohuku J. Exp. Med.* 72, 169 (1960).
13. M. Segi, L. Fukushima, S. Fujisaku, M. Kurihara, S. Saito, K. Asano, M. Kamoi, *Gann* 48 (Suppl.), 1063 (1957).
14. I. Hirayama, in *Proceedings of the International Conference on Gastric Cancer* (Maruzen, Tokyo, 1968).
15. A. Braunschwig and R. A. Rasmussen, *Cancer Res.* 1, 371 (1941).
16. R. Doll, P. Payne, J. Waterhouse, *Cancer Incidence in Five Continents* (Springer-Verlag, New York, 1966).
17. W. B. Quisenberry, *Acta Unio Int. Contra Cancrum* 17, 858 (1961).
18. W. MacDonald, *Canadian Cancer Conference 6th* (1966), pp. 451–459.
19. N. Dungal and J. Siaurjonsson, *Br. J. Cancer* 21, 270 (1967).

7 June 1971

J. Lab. Clin. Med., 26: 774-785, 1941

THE PHARMACOLOGY OF SILICA*

Effects and Mechanism of Action of Fatal Intravenous Doses of Silicic Acid

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THE study presented here is part of a larger project on the nature of silicosis. The exact form of silica which produces silicosis is not known, but there is evidence that silicic acid may play a role as an intermediary chemical agent in the development of the disease.[1] We shall deal here with the results of an investigation of the pharmacology of silicic acid† and give special consideration to the following: I. Toxic and lethal dose. II. Systemic effects. III. Mechanism: (a) role of blood clot formation and (b) site of action. IV. Significance of the colloidal nature of silicic acid.

METHOD

Cats were used in our experiments. Silicic acid sol was the only form of colloidal silica examined. It was prepared by dissolving suitable amounts of sodium silicate in water and treating with an excess of hydrochloric acid. This was dialyzed against water until free of chlorides and then concentrated to somewhat more than 1 per cent by evaporation with heat under reduced pressure. The silicon dioxide content was determined gravimetrically by drying and blast burning. The solution was then diluted to 1 per cent‡ and sufficient sodium chloride was added to make an 0.85 per cent saline concentration. A small amount of hydrochloric acid was added to retard the development of gel. The solutions were stored in a refrigerator when not in use. All solutions were slightly opalescent at the outset but no gel was visible macroscopically. The pH of each batch was determined electrometrically. This varied with each specimen. The same batch of silicic acid was used in each series of experiments.

The cats were anesthetized with sodium amytal by the intravenous or the intraperitoneal route, usually the former in doses of 40 mg. per kilogram.§ In most experiments kymographic records were made of the effects of silicic acid on blood pressure, heart rate, and respiration, although some observations were made on intact unanesthetized animals. Electrocardiographic records were made

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This study was made under the provisions of a Works Progress Administration Project sponsored by the Department of Health of New York City, and carried out by the Cornell University Medical College, New York Hospital, and the Industrial Hygiene Committee of the New York Tuberculosis and Health Association acting as cooperating agencies.

Received for publication, December 5, 1939.

†The terms silicic acid and colloidal silica will be used interchangeably.

‡Batches 1 and 6 were made up as 0.75 per cent and 2.0 per cent, respectively. The amount of silicic acid is always expressed in terms of silicon dioxide content.

§All doses are considered in relation to body weight, but for purposes of convenience the phrase, "per kilogram," will be omitted.

in some experiments. Silicic acid was always administered intravenously. In order to prevent the formation of intravascular clots and clots in the recording system, an anticoagulant dye was used in all but a few stated experiments. This procedure is discussed later.

All animals were examined after death. Sections of tissue were preserved for microscopic examination, but the results of this examination will form part of another report.

EXPERIMENTAL

I. Toxic and Lethal Dose.—The lethal dose of silicic acid was determined by continuous intravenous injection. The injection was stopped when blood pressure fell to about a level of 100 mm. Hg, since such falls were almost always fatal. However, more silicic acid was administered if necessary. The animals were anesthetized and the blood pressure was recorded from the carotid artery. The rate of intravenous injection was varied to determine what role the rate of administration might play in the toxicity of silicic acid.

Great variability of the fatal dose of silicic acid within each batch and between batches was a prominent feature. The average lethal dose of batches varied from 15 mg. to 193 mg. The smallest single fatal dose was 5 mg. and the largest was 284 mg. The lethal dose within each batch, however, remained in the same general range. Thus, in the batch in which 5 mg. was lethal in one case, the largest dose was 20 mg., while in the batch in which 284 mg. was the largest dose, the smallest dose was 142 mg. Lethal doses of the same specimen of silicic acid varied as much as 100 per cent on the same day, ruling out the possibility that a change in the silicic acid had occurred. The toxic dose (i.e. the dose necessary to produce a perceptible fall in blood pressure) was as variable as the lethal dose; generally it was about 60 per cent of the lethal dose.

TABLE I

TABULATION OF pH OF SPECIMENS OF SILICIC ACID AND THE LETHAL DOSE

BATCH NO.	pH	AVG. L. D. MG./KG.	NO. CATS
4	1.53	165	8
1	1.55	15	2
7	1.60	48	5
7a	1.60	53	9
2	1.78	15	3
3	1.87	30	2
6	2.40	152	10
5	2.65	193	14

The pH of the different batches of silicic acid varied between 1.53 and 2.65. Table I indicates the absence of correlation between the lethal dose and the pH. In addition, preliminary experiments with salt solutions, to which hydrochloric acid had been added to produce the same pH as that of the silicic acid specimens used in our experiments, indicated that the acidity itself was without any effect in the amounts used.

From the results in Table II it appears that the more rapid injections required larger amounts of silicic acid to produce death, but this may be due to a latent period in the development of the effects of silicic acid, so that the end

TABLE II

TABULATION OF AVERAGE RATE OF INJECTION AND THE LETHAL DOSE

BATCH NO.	AVG. RATE INJECTION MG./KG./SEC.	AVG. L. D. MG./KG.	RANGE OF RATE INJECTION MG./KG./SEC.	NO. CATS
7	0.25	48	0.19-0.37	5
3	0.26	30	0.17-0.35	2
1	0.44	15	0.33-0.54	2
2	0.53	15	0.20-0.83	3
4	1.33	165	0.06-3.91	8
5	1.40	193	0.47-2.37	14
7a	2.03	53	0.96-3.33	9
6	2.53	152	0.70-4.00	10

TABLE III

TABULATION WITHIN BATCHES OF SILICIC ACID OF THE RATE OF INJECTION AND THE LETHAL DOSE

RATE INJECTION MG./KG./SEC.	LETHAL DOSE MG./KG.
Batch 6—pH 2.40	
0.7	85
1.6	146
1.6	190
1.8	106
1.8	211
2.6	220
3.6	134
3.6	180
4.0	88
4.0	159
Batch 7—pH 1.60	
0.19	69
0.21	37
0.24	58
0.25	30
0.37	44
Batch 7a—pH 1.60	
0.96	43
1.26	63
1.28	41
1.33	56
1.54	54
2.34	82
2.92	35
3.27	49
3.33	50

Note: Batches 7 and 7a are the same specimen of silicic acid. The division into groups represents slow and rapid injections.

point was passed in the titration. In Table III, however, in which data for the lethal dose and the rate of administration for all experiments with two batches of silicic acid are given, there appears to be no significant correlation with the injection rate.

II. Systemic Effects.—The effects produced by all batches of silicic acid were qualitatively the same. In unanesthetized animals it produced an acceleration of respiration, which in the case of fatal doses was followed by progressive weakness and finally clonic convulsions. In the fatal cases the entire sequence of events usually occurred within five minutes after the injection.

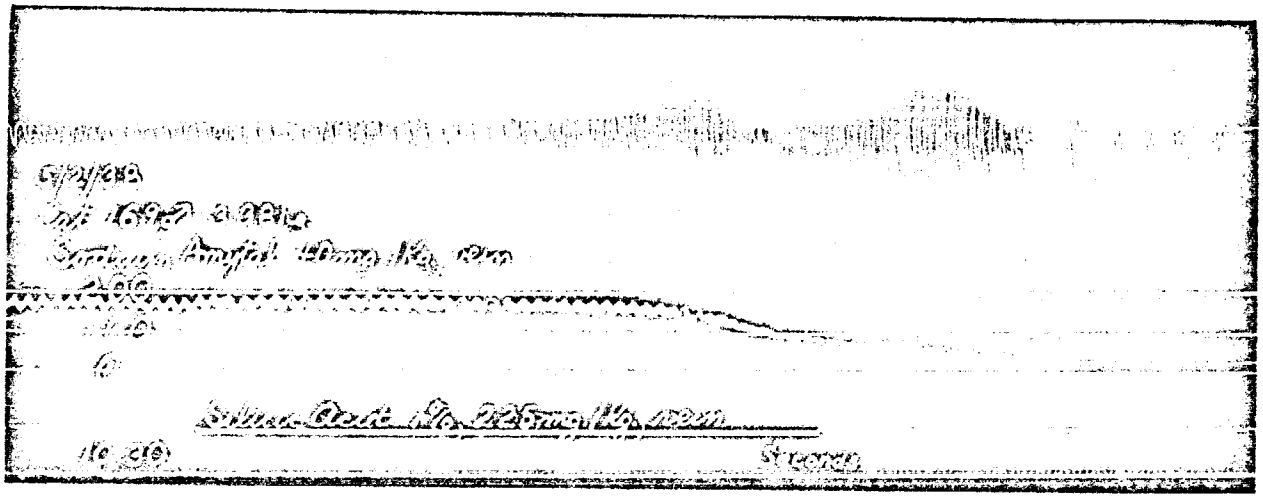

Fig. 1.—The effect of a massive intravenous dose of silicic acid on respiration, blood pressure, and heart rate. The cardiac pulsations toward the end of the tracing are too feeble to be visible in the reproduction.

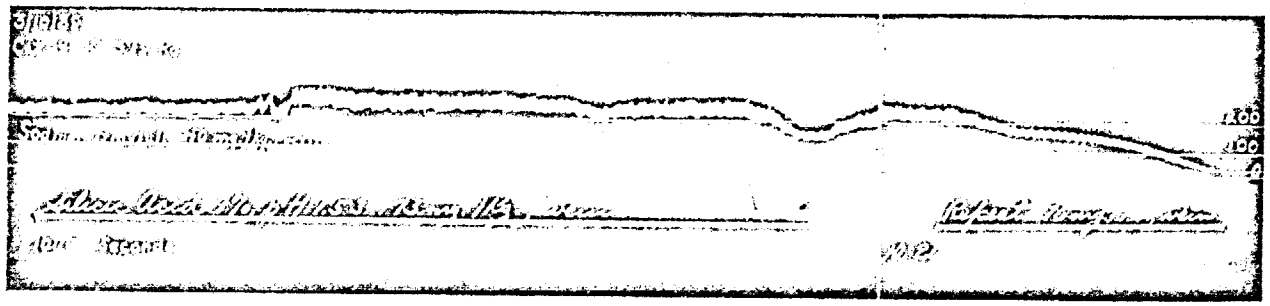

Fig. 2.—Complete recovery from an insufficient dose of silicic acid, with death after an additional dose.

In anesthetized cats records were made of the blood pressure, heart rate, and respiration (Fig. 1). In almost all cases there was an acceleration of respiration (from an average of 28 to an average of 48 per minute). In all cases there was a sharp fall in blood pressure (from an average of 180 to an average of 75 mm. Hg) and then a slow decline to zero. This was accompanied by slowing of the heart. In most cases respiratory stimulation occurred simultaneously with or soon after the fall in blood pressure. This was always followed by slowing, and finally cessation, of respiration. Cardiac pulsations persisted for several minutes after respiration had ceased. Convulsions were generally seen even in the presence of the anesthesia after the blood pressure had fallen.

A marked drop in blood pressure (about 75 to 100 mm. Hg) was rarely followed by recovery (Fig. 2). When the drop was less than 75 mm. and the injection was discontinued, recovery was usually prompt and often complete. However, in two instances after a drop there was a spontaneous but temporary recovery, and five and twenty-five minutes later, there was a second and fatal fall in blood pressure. This delayed effect was seen only twice during the entire study, although many experiments were conducted under similar conditions.

Records were also made of intrapulmonary blood pressure by cannulation of the left pulmonary artery (Fig. 3). When silicic acid was injected, the first effect seen was a rise in the systolic level of the intrapulmonary pressure which was followed by a rise in the diastolic level if more silicic acid was administered. These effects occurred before any change in systemic blood pressure was observed. It was only after there was a rise in intrapulmonic blood pressure that the systemic pressure began to fall. When the systemic pressure fell to about 50 mm. Hg, even under artificial respiration, diaphragmatic respiratory movements were accelerated and were soon followed by convulsions. When electrocardiograms were taken, they showed no change until after the blood pressure fell. The changes which then followed were of the type usually found in asphyxia.

An examination of the heart at the termination of all experiments in which silicic acid proved fatal revealed that the right side of the heart and the great vessels were distended with blood while the left side was essentially empty. Although the systemic blood pressure was at zero, the heart was usually contracting vigorously.

III. Mechanism: (a) Role of Blood Clot Formation.—It was noted by Gye and Purdy[2] that when silicic acid is administered intravenously, it produces massive intravascular clots. Since clots may produce systemic changes mechanically and mask the action of silicic acid, an anticoagulant dye, Chlorazol Fast Pink BKS,[3] was used to prevent the formation of clots. The dye produces no systemic effects in the amounts used.

Silicic acid was administered intravenously to two groups of six cats, only one group receiving Chlorazol. In both groups the symptoms preceding death, as well as the lethal dose, were the same. Examination after death in those treated with the Chlorazol revealed no clots.

(b) Site of Action.—The distention of the right side of the heart and the great veins, and the rise in intrapulmonic pressure after the intravenous administration of silicic acid may be explained by either failure of the right ven-

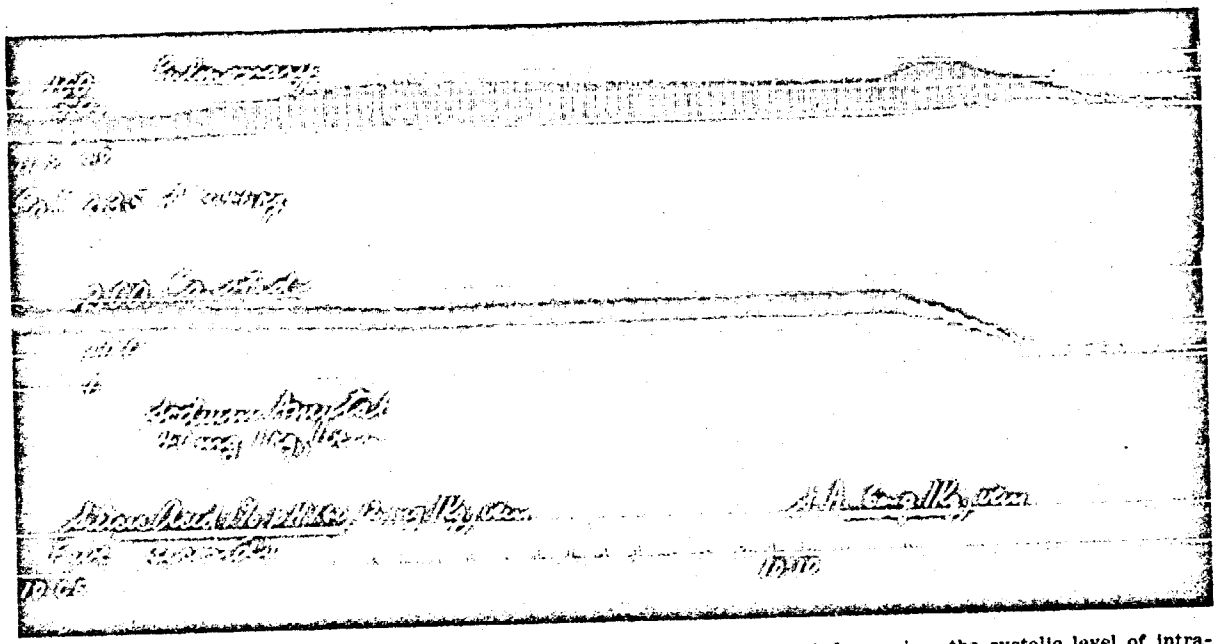

Fig. 3.—Effect of silicic acid on intrapulmonary blood pressure. The first dose raises the systolic level of intrapulmonary blood pressure and causes a slight drop in systemic blood pressure. The second dose causes a rise in the diastolic level of intrapulmonic blood pressure and a sharp fall in systemic blood pressure.

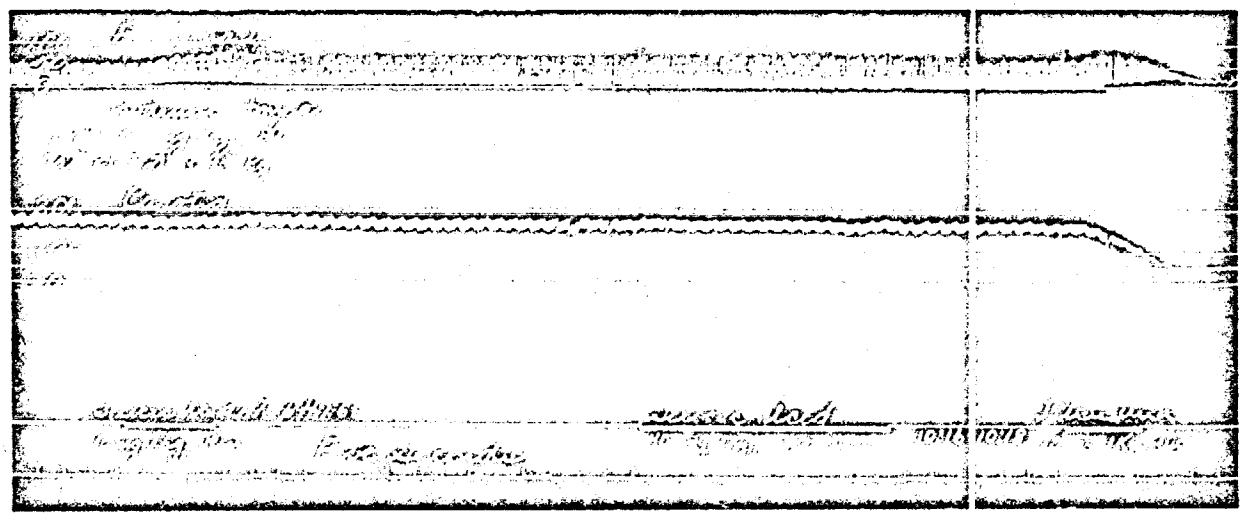

Fig. 4.—Effect of silicic acid when injected into the left auricle. The first dose administered intravenously produces a rise in both systolic and diastolic levels of intrapulmonic blood pressure, while a subsequent dose, four times as great, injected into the left auricle produces only an almost imperceptible rise in the systolic level of intrapulmonic blood pressure. The third dose injected intravenously, the same size as the first, is fatal.

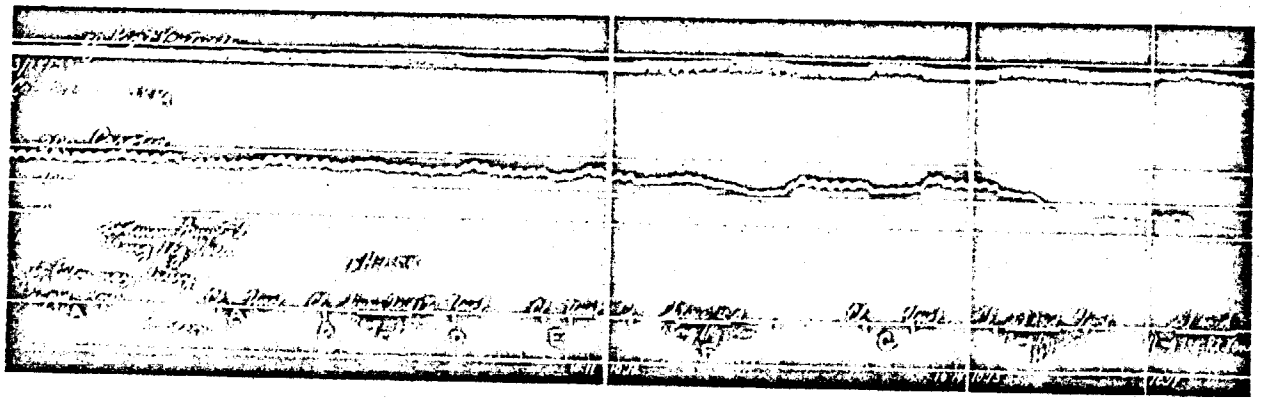

Fig. 5.—Effect of protection of lung from silicic acid. *Cl*, closing of left branch of pulmonary artery; *Uncl.*, opening of left branch of pulmonary artery; *A*, control showing effect of closing and opening of left branch of pulmonary artery before any silicic acid is injected. Closing produces very slight rise in pulmonic blood pressure and slight fall in systemic blood pressure. Opening restores to original level. *B*, closing of left pulmonary artery; *C*, injection of silicic acid with left lung out of circulation produces rise in pulmonic blood pressure and fall in systemic blood pressure; *D*, opening of left pulmonary artery. Blood pressure levels return to normal. *E*, closing of left pulmonary artery now produces greater rise in pulmonic blood pressure and fall in systemic blood pressure than in control (*A*); *F*, additional dose of silicic acid with left pulmonary artery closed produces more marked effect than before (as in *C*); *G*, now closing of pulmonary artery produces more pronounced effects on blood pressure than before, as in *A* and *E*; *H*, still more pronounced effects from another dose of silicic acid, with only slight recovery after opening of pulmonary artery.

tricle or block in the pulmonary circulation. The following observations were made in experiments designed to shed light on this question:

When silicic acid is injected intravenously, it reaches the right ventricle in relatively high concentration. It is conceivable, therefore, that the right ventricular muscle is damaged by the concentrated acid reaching it. Injections of silicic acid, therefore, were made into the left auricle, so that the acid reached the left ventricle before it circulated through the body. Such injections produced the same effects as the intravenous injections; namely, distention of the right side of the heart, rise in intrapulmonic blood pressure, and fall in systemic blood pressure. The only noticeable difference was that considerably more silicic acid was required to produce the same effects than was required with intravenous injections. Four times the dose which when injected intravenously produced marked changes in intrapulmonary pressure, was without effect when injected into the left auricle (Fig. 4).

It was also noted in a series of experiments that silicic acid was 25 per cent more toxic when the circulation of the left lung was ligated than when both lungs were intact. In another type of experiment (Fig. 5) the circulation of the left lung was constricted while silicic acid was being injected, and was re-opened after the injection had been completed. Thus the left lung was temporarily protected from the effects of silicic acid. The injection produced a rise in intrapulmonary pressure (recorded from the right ventricle) and a fall in systemic pressure. As soon as the protected lung was restored to the lesser circulation, both the intrapulmonary and systemic blood pressures tended toward normal. This protection against the effects of silicic acid was in evidence until massive (fatal) doses were given, when the protection afforded was only temporary. Control observations indicated that the constriction of the circulation to the left lung was itself without appreciable effect, whereas after the administration of silicic acid this procedure produced a definite rise in intrapulmonary and a fall in systemic blood pressures.

IV. Significance of the Colloidal Nature of Silicic Acid.—Inasmuch as silicic acid is a colloid and it has been demonstrated by Hanzlik[4] that colloids because of their physical nature can exert systemic effects, it was decided to determine whether the colloidal nature of silicic acid played any role in the effects observed.

Cats were injected with solutions of egg albumen, agar-agar, and acacia. Sufficiently large doses of these substances produced a fall in systemic pressure and an acceleration or respiration. However, in experiments with egg albumen and acacia, in which intrapulmonary blood pressure was determined, the initial effect was a rise in systemic pressure and not a rise in intrapulmonary pressure (Fig. 6).

Egg albumen does, however, exert a synergistic action on silicic acid. An initial dose of egg albumen (300 mg.), which produced only a transient fall in systemic blood pressure, considerably increased the toxicity of silicic acid administered two to three minutes later. In 8 cats the lethal dose of silicic acid averaged 165 mg., whereas after egg albumen the lethal dose of the same specimen for 7 cats averaged only 45 mg. In another series the control lethal dose of

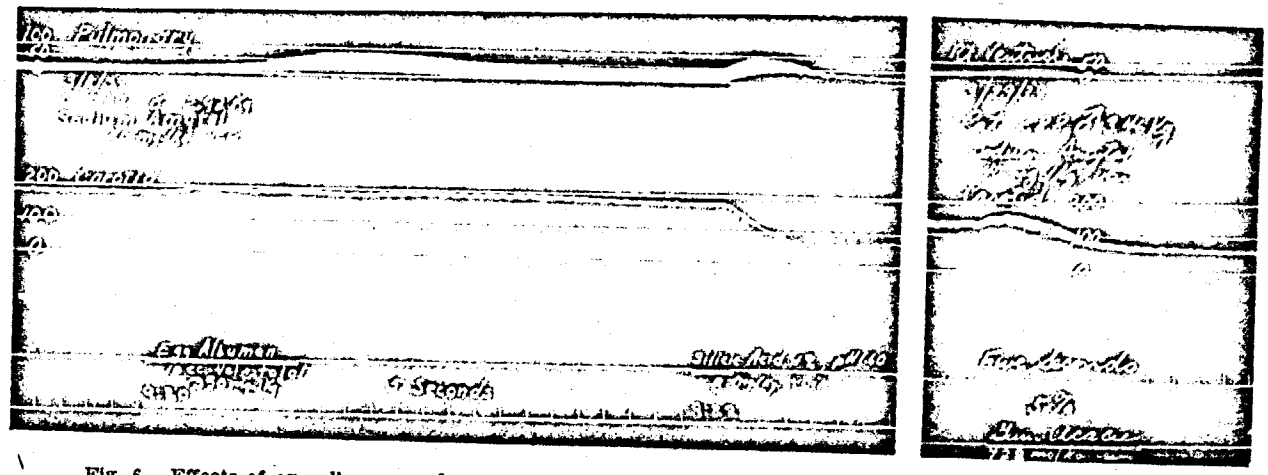

Fig. 6.—Effects of egg albumen and gum acacia on systemic blood pressure and pulmonary blood pressure.

silicic acid was 193 mg., and after egg albumen it was 110 mg. In both series the effects following the combination were the same as those following silicic acid alone.

DISCUSSION

The physical and chemical relationship between silicic acid and silica is very close; it has been claimed that silicic acid is hydrated silica,[5] and x-ray diffraction observations indicate that the atomic mosaic of both is very similar if not identical.[6] Gye and Purdy[2] have shown that silicic acid may, if administered over a long period of time, produce anatomical changes in organs other than the lungs, which are in many respects similar to those seen in the lungs in silicosis.

The lethal dose of silicic acid was very variable. There is no evidence that either the pH of the solutions used or the rate of administration of silicic acid played any role in the variable toxicity.

In the studies by Gye and Purdy it was demonstrated that death after intravenous injections of silicic acid was accompanied by massive intravascular clotting. These clots filled the chambers of the heart and many of the large veins. On the basis of other experiments these workers concluded that while clotting was the cause of death, it was secondary to endothelial damage. In our experiments it was demonstrated that the lethal dose and the toxic effects of silicic acid are not affected when the formation of clots is prevented by the use of an anticoagulant. It may be concluded, therefore, that while intravascular clotting may cause or contribute to the fatal outcome, there is another more direct action of silicic acid which may cause death.

The picture produced by ligation of the pulmonary artery, namely, distention of the right side of the heart, empty left side, rise in pulmonary blood pressure, and fall in systemic blood pressure, is exactly reproduced by the intravenous administration of silicic acid. The best explanation available for the production of the toxic effects of silicic acid is that it constricts the blood vessels of the lungs.

The question whether the effects of silicic acid are due to its chemical or physical nature is still an open one. Experiments by Hanzlik[4] indicate that colloids in general may produce marked changes in systemic blood pressure, but no colloid other than silicic acid has been demonstrated to produce an initial rise in intrapulmonary blood pressure. However, the synergism between silicic acid and egg albumen is suggestive of some common action.

SUMMARY AND CONCLUSIONS

Silicic acid solutions, prepared according to a standard procedure, differ markedly in their toxicity when injected intravenously into cats. The average lethal dose of different batches varied from 15 to 193 mg. per kilogram, and was not appreciably affected by wide range in pH or rate of injection.

Immediately following injection there was a rise in intrapulmonary arterial pressure and a fall in systemic blood pressure, with an accompanying engorgement of the right side of the heart and an empty left side. Similar changes followed when silicic acid first passed through the systemic capillary bed, as was the case when injection was made directly into the left auricle.

The effects on the lung circulation were not influenced by the use of an anticoagulant, and, therefore, they were not caused by intravascular clotting. Nor is it likely, in view of the selective action on the lung capillaries and the failure of

other colloidal substances (egg albumen, agar-agar, and acacia) to produce similar changes, that they represent a nonspecific colloidal effect of silicic acid.

It is concluded that silicic acid exerts a selective action on the capillary bed of the lungs which results in an increased resistance to blood flow and accounts for the toxic symptoms and death.

REFERENCES

1. Kettle, E. H.: Experimental Silicosis, J. Indust. Hyg. & Toxicol. 8: 491, 1926.
   Koppenhofer, G. F.: Untersuchungen zur Pathogenese silikotischer Gewebsveränderungen. IV. Mitteilung. Über die geweblichen Veränderungen nach experimenteller Zufuhr von kolloidaler Kieselsaüre, Virchows Arch. f. path. Anat. 297: 271, 1936.
   Hefferman, P.: Some Notes on the Biophysics of Silica and the Etiology of Silicosis, Brit. M. J. 2: 489, 1929.
2. Gye, W. E., and Purdy, W. J.: Poisonous Properties of Colloidal Silica. I. Effects of the Parenteral Administration of Large Doses, Brit. J. Exper. Path. 3: 75, 1922; Poisonous Properties of Colloidal Silica. II. Effects of Repeated Intravenous Injections on Rabbits; Fibrosis of the Liver, Ibid. 3: 86, 1922; Poisonous Properties of Colloidal Silica. III. Ibid. 5: 238, 1924.
3. Huggett, A. StG., and Rowe, F. M.: Azo-Dyes as Anti-Coagulants, J. Physiol., proc. 78: 25, 1933.
   Modell, W.: Chlorazol Fast Pink BKS as an Anti-Coagulant, Science 89: 349, 1939.
4. Hanzlik, P. J., and Karsner, H. T.: Anaphylactoid Phenomena From the Intravenous Administration of Various Colloids, Arsenicals, and Other Agents, J. Pharmacol. & Exper. Therap. 14: 379, 1920; A Comparison of the Prophylactic Effects of Atropine and Epinephrine in Anaphylactic Shock and Anaphylactoid Phenomena From Various Colloids and Arsphenamine, Ibid. 14: 425, 1920; Further Observations on Anaphylactoid Phenomena From Various Agents Injected Intravenously, Ibid. 23: 173, 1924.
   Hanzlik, P. J.: Basis of Allergic Phenomena, J. A. M. A. 82: 2001, 1924.
   Hanzlik, P. J., De Eds, F., and Tainter, M. L.: Blood and Symptomatic Changes Following the Intravenous Administration of a Variety of Agents and Solutions, Arch. Int. Med. 36: 447, 1925.
5. Weiser, H. B.: Inorganic Colloidal Chemistry, John Wiley & Sons, Inc., New York, 1935, Vol. 2, pages 199, 200, 226.
6. Krejci, L., and Ott, E.: Study of Silica Gel (X-ray Study), J. Physical. Chem. 35: 2061, 1931.

TOXIC HEPATITIS DUE TO SULFANILAMIDE*

Report of a Fatal Case With Histopathologic Findings in the Liver

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THAT sulfanilamide has toxic effects is generally known. That it may cause hepatitis has been stressed by several observers. Hageman and Blake[1] have observed a case of toxic hepatitis, presumably due to sulfanilamide, in an alcoholic person. Saphirstein[2] recorded a severe but nonfatal case of hepatitis. Long[3] encountered jaundice with marked decrease in liver function on one occasion. Bannick, Brown, and Foster[4] reported two fatal cases of hepatitis in which they believed pre-existent liver damage a possibility. Garvin[5] recorded a series of five cases of hepatitis, one of which proved fatal, but no autopsy was obtained. Cline[6] was the first to place on record a case of yellow atrophy of the liver with autopsy findings. Fitzgibbon and Silver,[7] in a series of 200 cases showing toxic

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Received for publication, December 16, 1939.

266: 36-37 (Jan 4) 1962. Jan. 4,

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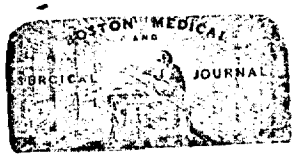

FATAL ASPIRATION OF TALCUM POWDER BY A CHILD*

Report of a Case

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THE talcum-powder container, often a favorite plaything of small children, may be a source of tragedy in the home. The case reported below demonstrates a rapidly fatal course in a twenty-two-month-old child, who aspirated talcum powder while playing with such a container.

Aspiration of baby powder containing stearate of zinc was reported relatively often in the past, and fatal cases due to aspiration pneumonia have been recorded.[1] Since talcum has largely replaced zinc stearate as a dusting powder for babies it was anticipated that accidents caused by its inhalation would have been described. It was surprising, therefore, to find in the literature only 1 case report of fatal talcum aspiration in a child.[2]

CASE REPORT

A 22-month-old Negro boy was admitted to the Bronx Municipal Hospital Center 30 minutes after aspiration of talcum powder. The incident was brought to the mother's attention when she heard the child choking. On entering his room, she found him with talcum powder in his mouth, breathing heavily and coughing. An open can of talcum powder was beside him. The mother immediately gave him a mixture of vinegar and milk, which failed to induce emesis.

Physical examination showed a well developed and well nourished boy in moderately severe respiratory distress, and with perioral cyanosis. The mouth and pharynx were clear. There were bilateral coarse rhonchi in the chest, with occasional grunting. Examination of the heart was within normal limits.

The temperature was 98°F., the pulse 140, and the respirations 70.

The hemogram showed a hemoglobin of 9.0 gm. per 100 ml. and a white-cell count of 23,500, with 73 per cent neutrophils, 7 per cent band forms, 16 per cent lymphocytes and 4 per cent monocytes. A roentgenogram of the chest was normal.

The patient was given 600,000 units of penicillin and 175 mg. of chloramphenicol¶ intramuscularly and placed in an oxygen tent with cool mist. About 7 hours after admission the respiratory distress increased, with the rate reaching 90 per minute. The heart rate was 190 per minute. Examination of the chest at this time revealed bila moist rales and rhonchi throughout both lung fields. liver edge was palpated 4 to 5 cm. below the right c margin. An electrocardiogram was unremarkable. The tient was digitalized rapidly with 360 microgm. of la side C‖ intravenously (30 microgm. per kilogram of b weight) and was given 2 mg. of morphine and 0.3 m meralluride** intramuscularly. There was transient impr ment after this therapy, but intractable cardiopulmo failure gradually developed and he died 20 hours a aspiration of the talcum powder.

At autopsy the oral cavity, pharynx, trachea and esoph were free of any foreign material. The mucous membr of the larynx, trachea and main bronchi were covered viscid, transparent mucus and were slightly injected. lungs were voluminous, feathery on palpation and pin with dark-red segments. The pleural surfaces were sm and shiny and free of any nodules or exudate. On the surface of the lung several branches of medium-sized small bronchi were filled with greenish-yellow materia creamy consistence. Alternating atelectatic and em sematous segments were present in both lungs. The at tatic areas were solid, and with pressure some edema could be squeezed from their surface. The heart was of mal size, with prominent dilatation of the pulmonary co Marked congestion was found in all the organs.

On microscopical examination all sections of the showed focal areas of edema and emphysema, with

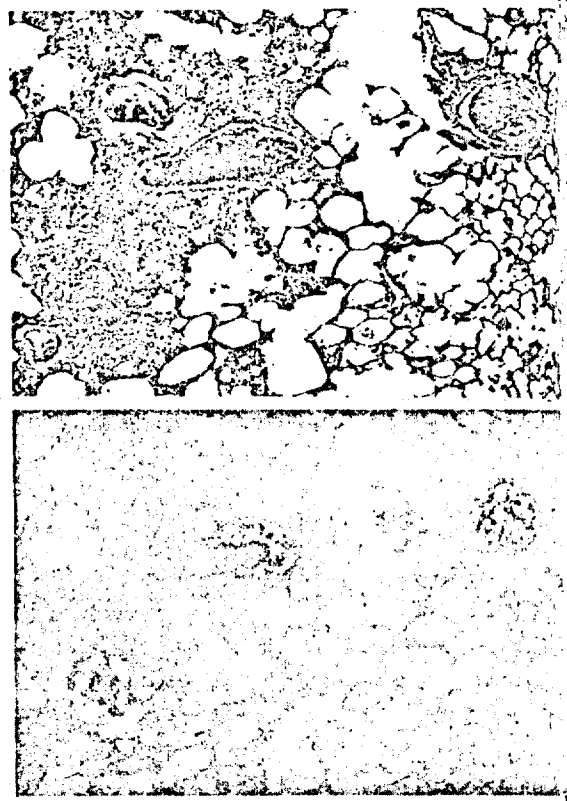

FIGURE 1. *Photomicrograph (X40), Showing Oblite Small Bronchi in Emphysematous and Edematous (Upper Half) and the Same Area Examined in Pola Light, Showing the Birefringent Component of the Oblit ing Masses (Lower Half).*

characteristic lesions involving the bronchial tree (Fig Most of the bronchial lumens were virtually obliterate a slightly basophilic, somewhat granular mass. Gene a slitlike space separated the mass from the bron epithelium. Leukocytes, epithelial fragments and refr particles were embedded in a homogeneous matrix. epithelium was completely desquamated in many br

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¶In the form of Chloromycetin, Parke, Davis and Company, Detroit, Michigan.

‖In the form of Cedilanid, Sandoz Pharmaceuticals, Hanover, Jersey.

**In the form of Mercuhydrin, Lakeside Laboratories, Incorp Milwaukee, Wisconsin.

or showed various grades of edema, hyperplasia and squamous metaplasia. The submucosa was swollen and often infiltrated with polymorphonuclear leukocytes and refractile particles. There was no significant fibrosis of the submucosa. The muscular sheath was essentially normal. There was a moderate peribronchial accumulation of leukocytes. The intrabronchial mass did not give any sign of organization. In some places the intraluminal mass extended through the respiratory bronchioles, filling the adjacent alveoli (Fig. 2). Marked leukocytic reaction was present in such areas.

FIGURE 2. *Photomicrograph (X140), Showing the Destruction of a Terminal Bronchiole by the Aspirated Mass and Polymorphonuclear Leukocytes, with Involvement of the Adjacent Alveoli (Upper Half), and the Same Area Examined in Polarized Light (Lower Half), in Which the Shape, Size and Distribution of Talc Particles Are Clearly Visible.*

The examination of the sections in polarized light clearly demonstrated the distribution of the aspirated material. The brilliant birefringence of the irregular, sharply angulated talc particles revealed their presence in minute quantities (Fig. 1 and 2). Their size varied from 2 to 50 microns in length and 1 to 10 microns in thickness. Intracytoplasmic particles could be detected in scattered leukocytes and macrophages with this technic. With Prussian blue reaction the particles were negative. With reticulum and trichrome stains the pulmonary framework seemed to be intact. No pleural reaction was seen. Penetration of the particles through capillaries or larger vessels could not be demonstrated. The post-mortem diagnosis was acute bronchitis and bronchiolitis due to aspiration of talcum powder.

DISCUSSION

Commercial talcum powder is a mixture. In the case described it consisted of 93 per cent of talc, 5 per cent magnesium carbonate and 2 per cent of other ingredients. Talc is a hydrous magnesium silicate ($3MgO.4SiO_2.H_2O$). It is primarily marketed in ground form as a white powder. Pulmonary disease due to inhalation of talc has recently been emphasized in occupational medicine.[3] The pathological changes in chronic talcosis are similar to those of asbestosis, but are unlike those described here after acute aspiration.

The acute bronchitis and bronchiolitis found at pathological examination has been known as the earliest change in bronchiolitis obliterans.[4] This rare condition usually follows inhalation of irritant substances, aspiration of foreign bodies or certain viral infections of the respiratory tract. If the patients survive the first twenty-four to forty-eight hours, organization of the intraluminal exudates will begin with active fibroblastic proliferation.

The short survival period in the present case, just as in the case of Cless and Anger,[2] did not permit organization of the bronchial exudate to develop. The almost identical clinical and pathological features of the 2 cases suggest that the condition is reproducible experimentally.[1]

The potential danger of talcum powder is not appreciated. The aim of this report is to create an awareness of the hazard of talcum aspiration. To avoid additional deaths it is urged that physicians emphasize to parents the importance of storing talcum-powder containers out of the reach of children.

SUMMARY

A case of fatal aspiration of talcum powder in a twenty-two-month-old child is reported. Only 1 other case has been recorded in the literature. Attention is called to the hazard of exposure to this substance.

REFERENCES

1. Heiman, H., and Aschner, P. W. Aspiration of stearate of zinc in infancy: clinical and experimental study. *Am. J. Dis. Child.* **23**: 503-510, 1922.
2. Cless, D., and Anger, R. Erstickungstod durch Aspiration von Kinderpuder. *Kinderärztl. Praxis* **22**:506-508, 1954.
3. Johnstone, R. T., and Miller, S. E. *Occupational Diseases and Industrial Medicine.* 482 pp. Philadelphia: Saunders, 1960. P. 216.
4. LaDue, J. S. Bronchiolitis fibrosa obliterans: report of case. *Arch. Int. Med.* **68**:663-673, 1941.

Arch. Mal. Prof. Med. Trav. Secur. Soc.
24(6):503-521, June, 1963.

Experimental silicosis of the rabbit induced by Intravenous administration of silica[1)]

by

M. Mosinger, J. Jouglard-Duplay, A. Versino and C. Granier.

In earlier research carried out in this institute, a systematic study was made on rats, guinea pigs and dogs of local reactions, the general syndrome, the anatomic-pathologic aspects, the biochemical aspects and the metabolism of silica following intraperitoneal or intratracheal administration or chronic dusting. These studies were reported in a group of publications and in the theses of El Feki (1957)(9) and Cartouzon (1960)(3). They led to the proposal of a pathogenic concept of silicosis, in the manner of a mesenchymatosis, that is to say a collagen disease (7,8,30,31,32,33,35,36).

In silicosis there are histopathologic, biochemical, immunologic and endocrine factors analogous to those encountered in other collagen diseases (4).

At this point it seemed of interest to study the overall general and localized histopathologic changes, as well as the biochemical and serologic alterations observed in animals

1) Work financed by the High Authorities of the European Community for Coal and Steel.

treated with intravenous injections of silica. It is anticipated that in these animals the general changes will be particularly marked and instructive.

Our experiments were carried out on rabbits treated with repeated intravenous injections of silica with a particle size of 3 microns.

Background

Gye and Purdy (1923)(11) appear to have been the first to use the intravenous route in rabbits. These authors injected colloid silica and thus obtained cirrhosis. This discovery seems to us particularly important, for it shows that as early as 1922 it was demonstrated that colloid silica can give rise to sclerosis in certain organs, in the same manner as crystalline silica. It will be recalled that according to observations made at our institute, colloid silica and silicic acid upon repeated administration give rise to peritoneal sclerosis with hyalinization.

Siegmund (who has studied silicosis since 1922) and other authors in 1934 (28,29,42,43) published the histologic results of their first intravenous experiments on rabbits. Siegmund has demonstrated the production of giant histiocytic cells in the liver, and sclerosis and granulomas in the periportal-biliary tissue. He also found cellular silicotic granulomas in the spleen, lymph nodes, kidney glomeruli and bone marrow.

In 1935 Giese (12, 13) confirmed these results and also described pulmonary reactions with the presence of cells with dust in the interalveolar septa and the alveoli, and pulmonary

edema with histiocytosis. These lesions ended in sclerosis containing nodules of reticulin fibers.

At the same time other authors only observed liver fibrosis (2, 21). Thus in 1940 Simon and Strachan (44) injected quartz intravenously and obtained liver cirrhosis.

In 1944 Gardner, Dworski and Delahaut (10) showed that the intravenous injection of 500 mg silica measuring 203 μ gave rise to massive silicotic cirrhosis within one year. They also noted that the same quartz administered in a 0.33% suspension of a colloid gel of aluminum silicate only gave rise to an accumulation of phagocytes in the porto-biliary spaces.

In 1945 Haythorn and Taylor (15) showed that the intravenous administration of the ash of lungs of silicotic subjects caused silicotic granulomas.

In 1950 Rattner (39) demonstrated liver fibrosis obtained by injection of 80 mg quartz suspended in water. In 1952 Meesen (27) produced silicotic granulomas of the liver by intravenous injection of silica.

Roemer (38) in 1953 showed cell masses rich in giant cells in the liver, notably in the periportal tissue. He also noted sparse giant cells in the sinuses of the spleen.

Purpose of our investigations

Our object was to make a detailed study of the histologic lesions caused by the intravenous injection of silica and a comparative biochemical study on initially healthy animals that were subsequently rendered silicotic.

For this study we used the rabbit, which, owing to its size, allowed repeated blood samples to be drawn from the same animal, and thus allowed us to follow the development of the silicosis.

The results obtained cover a period of 18 months.

On the basis of the biologic and histologic study of these "silicotic" rabbits, we sought to find an answer to the following questions:

1. What is the route followed by the silica administered intravenously and where is it deposited? To find an answer to this question, silica was determined in the blood of live rabbits. In the autopsy their organs were removed for silica determinations.

2. What protein disturbances are revealed by electrophoresis? On this subject several proteinograms, glucidograms and lipidograms were made on the same animal in order to study the effects of the administration of silica on the blood.

3. What hormonal disturbances have been observed in the silicotic rabbit? In reply to this, 17-ketosteroid determinations were made on the urine.

4. What organs are injured by the intravenous injection of silica and what is the nature of these lesions? Macroscopic and microscopic examinations were made of all the organs of all animals killed. This histologic study was supplemented by liver biopsies, which allowed us to study the histologic development of silicosis.

5. Are there any characteristic hematologic reactions in the silicotic rabbit?

6. Are there serologic changes of an immunologic nature in the silicotic rabbit? To reply to this question, certain serologic tests were made, notably a search for the rheumatoid factor.

The last two investigations will be dealt with in a later report.

Experimental method

The silica injected into our animals was a silica of known particle size ranging from 1-5 μ, with a ca. 80% proportion of 3 μ particles.

The silica was prepared by us from crystalline R.L.B. silica graded by sedimentation in water. The silica was next dried and sterilized, and before use it was once again checked under the microscope.

The rabbits used for our experimentation were of the same strain (tawny Bourgogne) aged at least 3 months and weighing 1,200 to 1,500 g. It was established that despite their treatment with intravenous injections of silica, these animals had a normal weight development.

The silica suspended in physiological fluid can easily be injected intravenously into the marginal ear vein. This injection was always preceded by the drawing of a blood sample for the various laboratory tests. When the animals were killed, much larger quantities of blood were removed by cardiac puncture.

One hundred mg was administered per injection. The total dose, spaced over 10 to 15 months of treatment, varied between 500 and 600 mg.

Different authors have reported very variable doses injected: thus Ruttner (39) obtained cirrhosis with a total dose of 80 mg. Gardner, Dworski and Delahant (10) injected 500 mg. Worwald, Durkan and Pratt (49) have experimented with doses up to 1 g of silica.

Destiny of the injected silica

Silica determination in the blood took place according to a modified version of King's method (23, 25).

The principle of this method is based on the specificity of the reduction of silicomolybdate at a pH and oxide-reducing potential such that only the silicomolybdate is reduced.

In an acid medium silica forms a yellow silicomolybdate complex with ammonium molybdate. Phosphorus also yields a yellow phosphomolybdate complex (16, 46); its presence is thus particularly disturbing in laboratory analyses. However, silica can be determined in the presence of phosphorus by reducing the silicomolybdate complex with sodium bisulfate at a low pH of around 2, at which phosphomolybdate is not reduced. By this means a molybdene blue is obtained which can be photometrically measured (at 700 mμ). A calibration curve is made from pure silica, solubilized by alkaline fusion. The reagents are all prepared with double distilled water and kept in polyethylene flasks.

Systematic silica determinations were made on the blood of normal rabbits so as to obtain the normal average value of blood silica in the rabbit. Fifteen results were obtained varying from 0.305 to 0.770 mg per 100 ml blood. Attention is drawn to the

high average (0.454 mg/100 ml blood) of these results. In man, the values obtained with our method are included between 0.320 and 0.360 mg/100 ml blood (34). Beyond 0.400 mg, in man, values are considered to be abnormally high.

We attribute this difference in the normal values in man and rabbit to the exclusively vegetable diet of the latter.

Twenty-two examinations were made on 13 silicotic rabbits. The values obtained were very high: 9 were higher than 1 g per 100 ml blood. These values show the silica impregnation of the circulating blood. We have plotted a diagram of the silica in the blood as a function of the time elapsed since the last injection. A decreasing curve was obtained which showed that beyond a period of 2 months after the last silica injection the silica content of the blood reverts to normal in the majority of cases. It is thus reasonable to suppose that the silica injected intravenously will be localized in the receptor organs, since thereafter no trace of the silica injection remains in the blood.

Which are the organs in which the injected silica tends most to accumulate?

In the autopsies the whole organs were weighed, after which silica determinations were made. The organs primarily involved were the liver, kidneys, spleen and lungs. The organs were carefully crushed in a mortar. Parallel with the removal of samples for the silica determinations made in platinum crucibles, samples were also placed on watch glasses. These had been dried in the incubator at 40°C until weight constancy. Thus

we established the percentage of water, which varied from single to double when an organ was left longer in the freezer at -20°.

The following table shows the silica content found by different authors in the organs of normal rabbits.

Dosage chez le lapin (en mg de silicium par kg ou l de tissu ou de sang frais).

	Hamel [37]	Isaacs [17]	King [22]	King et Stantial [24]
1 Cerveau.....	0	8,8 à 18	—	—
2 Sang	12	—	—	3,4 à 5,8
3 Foie	35	0 à 5,6	150-212	96 — 57
4 Rein	28	5 - 17,5	115-157	67 — 76
5 Poumon	270	3,9 - 12	125-139	85

Table: Determination in the rabbit (in mg silica per kg or l tissue or fresh blood). Key: 1. Brain. 2. Blood. 3. Liver. 4. kidney. 5. Lungs.

This table shows the extent of variability of the results according to the method used. In order to obtain comparative values we followed a strict procedure (9), using purified, redistilled reagents, a procedure which was first used on normal rabbits.

The determination was made according to King and Isaacs (17, 22).

The principle of the determination includes three stages: 1) Destruction of the organic matter in the form of borosilicate; 2) dissolution of silica with normal and heated soda; 3. Colorimetric determination of silica based on the same principle as that described above for blood silica.

This method allows easy determination of considerable quantities of silica of around 20 to 80 g in one sample. Thus

the method had to be adapted for silica determinations in the organs of normal rabbits by doubling the samples, and, obviously, the quantities of mineralizing reagents.

To make our results more readily comparable, we have omitted the variations due to the percentage of water of 30 to 70% as a function of the time of stay in the freezer. We therefore express our values in mg silica per 100 g dry tissue.

Normal organs	mg silica per 100 g dry tissue
Liver	0 to 13
Kidneys	1 to 3.4
Lungs	1 to 4
Spleen	0 to 2

The results obtained in our silicotic rabbits are very high compared to these normal values and we were obliged to change our samples for the determinations.

The particularly high silica content of the liver (8.8 mg to 435 mg per 100 g dry tissue) and the lungs (2.6 mg to 278 mg) must be stressed, on the one hand manifesting the first mechanical stoppage of the silica particles in the pulmonary capillaries, and on the other the process of liver detoxication. The spleen is also loaded with silica (up to 200 mg), while the kidneys do not accumulate such large quantities as the other organs in their parenchyma (maximum of 37.7 mg).

Protein disturbances revealed by electrophoresis in our silicotic rabbits

In view of the small quantity of blood required for its use, electrophoresis seemed to us to be the method of choice for

following protein disturbances caused by the intravenous administration of silica in our experimental rabbits. Thus by drawing repeated blood samples we have been able to follow the development of the changes throughout the lifetime of our animals.

We used the usual methods of paper electrophoresis in sodium veronal buffer at pH 8.3 and an ionic force of 0.1 M, with a migration of 5.4 V/cm and 0.34 mA/cm in 16 hours. The optimal quantity of proteins deposited was 0.56 µg. Thus we established a curve which gave us the quantity in µl serum deposited according to the total value of blood proteins. This was determined according to the densimetric method of Philips and van Slyke. Five times more serum was deposited for the glycoproteinogram and 6 times more for the lipidogram.

The proteinogram was visualized with amido black 10 B, the lipoproteinogram with cerol black (Swann's method)(45). The glycoproteinogram fractions were detected by means of the Schiff reaction, using p-rosalinine (47, 48).

We had some difficulty in recovering the globulin fractions in animals.

In order to determine the electrophoretic mobility of these fractions, we wished to take into account the electroendosmotic flux. For this, a colored, uncharged substance was made to migrate parallel with them, namely dinitrophenyl-ethanolamine, prepared according to Sanger's method (20, 26, 40).

In the literature we have found normal values of protein fractions in rabbits, obtained in 1945 by Deutsch and Goodloe (5,6),

in 1954 by Geinitz (9, 11) and in 1955 by Scheiffarth (41). Considerable divergences were observed in the data of these different authors.

For this reason we preferred to follow the development of the electrophoretic fractions in the same animal. However, with the aid of the first electrophoreses carried out on the healthy rabbits we were able to make a statistical analysis of the results obtained, which yielded physiological values (to be published later).

Our electrophoreses (numbering 103) have led us to conclude the following: the first observation is the imbalance in the albumin-globulin ratio, with a rise in globulins and drop in albumins of around 20-30% between the 7th and 8th month of treatment. The α-globulins reveal no uniformity in their variations.

The δ and γ-globulins are clearly increased in all cases. The δ-globulins increase less than the γ-globulins and reach a 90% increase roughly in the 7th month. The γ - globulins increase more quickly than the δ-globulins, their % increase is less uniform, but is always much higher (around 130%).

The glucidogram shows an average increase of the α-2-globulins of 30%.

The lipidogram shows a 30 to 50% increase of the δ-lipoproteins.

In a later report we will give all our electrophoretic results calculated statistically.

Results of total 17-ketosteroid and urinary creatinine determinations

The 17-ketosteroids were determined in 24 hour urines collected in the cages specially constructed in our laboratory so as to avoid loss by evaporation to the maximum possible extent. The urine was collected on concentrated hydrochloric acid or on a merseptyl solution in norbutanol.

Several animals were made to urinate simultaneously in different cages, always in the same room, whose temperature varied very little, to minimize losses due to evaporation.

The 17-ketosteroid determinations were only carried out when diuresis was more than 100 ml. When it is lower than this, inconstant and usually reduced levels were found. To avoid this drawback and to obtain dilute and not highly pigmented urine, the rabbits were fed on lettuce during this test. Furthermore, in order to follow diuresis, creatinine determinations were made on each of our samples.

For the 17-ketosteroid determination we used the method of Jayle et al. (18, 19).

An aliquot portion of fresh urine brought to a pH of ca. 1 was subjected to acid hydrolysis at 100°.

The hydrolysate was extracted several times with ethylester in vertical cylindrical tubes. After washing with sodium hydroxide, which eliminates the phenol steroids, and washing with water, the ether was evaporated until dry.

After taking up the extracts in ethanol, Zimmermann's color reaction with m-dinitrobenzene was carried out in an alcoholic potassium medium. The reference scale was established from a pregnant solution of dehydro-epiandrosterone. Two scales and two controls were always made. After being placed in a water bath at 37° the reaction was arrested by immersing the tubes in iced water. Next, after the addition of 30% alcohol, the color was extracted with pure anesthetic ethylether. A rose-violet supernatant was thus obtained which was measured photometrically with Allen's correction (1).

Allen's correction is mandatory in this case, since we are dealing with very small quantities of 17-ketosteroids and fairly strongly pigmented urine.

With urine that was not much pigmented and containing larger quantities of 17-ketosteroids (human urine) we observed a perfect concordance between the results obtained with or without Allen's correction, while with very strongly pigmented urine, ca. 50% of the value obtained without correction was actually due to a parasitic color.

When dehydroepiandrosterone is added after hydrolysis, the percentages recovered are around 75 to 95%. Since we had neither sulfate, nor DHEA glucuronate, we were unable to test our hydrolysis, which was carried out according to the specifications of Jayle et al.

Creatinine was determined parallel on the same urines. The addition of picric acid in a basic medium gives rise to

an orange color which allows photocolorimetric determination at 530 mμ. The reference scale was established from creatinine hydrochloride.

In normal female rabbits, after repeated urinations, reproducible values were found 1 1/2 months after the first determination. The same was found for the creatinine levels.

During the first stage a few averages were established on normal rabbits. In normal female rabbits we obtained a level of 0.33 mg per 24 hours and in normal male rabbits a level of 0.28 mg per 24 hours.

In the silicotic rabbits determinations repeated at 15 day intervals yielded reproducible results. The average level obtained for females was 0.16 mg and for males 0.13 mg per 24 hours. Thus there is a reduction of ca. 50% in both sexes. This drop becomes manifest at the beginning of treatment (from the 1st month after the 1st injection of 100 mg) and becomes more marked as the silicosis develops.

Histologic lesions observed in our silicotic rabbits

Macroscopically only liver and spleen lesions were observed. The liver became cirrhotic after 4 to 5 months. It is important to note that the sclerosis was very variable in different lobes studied. The spigelian lobe was always the first affected. It could be completely sclerotic, while in the rest of the liver no cirrhosis was yet detectable.

Macroscopic examination also invariably revealed signs of degenerative hepatosis, in some cases with a picture of steatosis.

Lesions of the spleen were macroscopically manifested by splenomegaly, sclerotic thickening of the capsule of the spleen, silicotic nodules and diffuse sclerosis.

In the lungs, which, as we shall see, there were histologic silicotic lesions, no silicotic nodules could be macroscopically detected. However, the lungs did present a picture of emphysema.

The cervical and mediastinal lymph nodes were hypertrophied in some animals.

From the histologic standpoint four types of lesions could be detected:

1. Liver cirrhosis of the insular and annular type, or the diffuse type, with production of large, for the most part hyalinized sclerotic zones. It is interesting to note that the different lobes of the liver are unequally affected. In particular the spigelian lobe is invariably that which reveals the most advanced lesions. This experimental cirrhosis is of an essentially non-specific appearance, developing in the manner of cirrhoses produced by nutritional deficiencies or toxic processes. In almost all the animals the hepatic lesions begin with large degenerative epithelial lesions and periporto-biliary reactions consisting of inflammatory infiltration with resulting sclerosis. The reactive infiltration is largely made up of histiocytes, lymphocytes and sparse plasmocytes. An unusual feature is that in most animals the Kupffer cells show no particular reaction.

In some animals, however, in addition to the non-specific cirrhosis, there are typical silicotic lesions characterized

by the production of intralobular reticulo-histiocytic follicles which secondarily undergo collagenous-fibroblastic sclerosis and hyalinization. In the same animals the Kupffer cells show a diffuse reaction.

Consequently, under the influence of intravenous silica, the liver on the one hand reveals a non-specific cirrhotic process, and on the other, silicotic phenomena with production of sclero-hyaline nodules.

Sometimes there are concentric fibrillar formations in certain extensive sclerotic zones, recalling silicotic nodules.

The portal vessels are often considerably dilated.

Another feature of considerable interest is an intense biliary proliferation, and in some animals, a veritable state of multiple biliary adenomatosis. This is an important feature in view of the tumorigenic capacity of silica under some experimental conditions.

We should also note the scarcity of plasmocytes in the silicotic liver, despite the pronounced hyalinization, and the similar appearance of silicotic livers to those in certain cases of hepatic syphilis.

2. In other animals specific silicotic lesions were observed in the spleen, the lymph nodes and the lungs.

Silicosis of the spleen starts with reticulohistiocytic nodules in the white pulp or the red pulp. These nodules are numerous and undergo sclero-hyaline transformation. They often merge, producing extensive sclerotic areas.

In the lymph nodes the silicotic nodules are generally found in the cortical substance, more rarely in the medullary zone. As in the spleen, they may be multiple and, by merging, give rise to extensive sclerotic zones.

The pulmonary silicotic nodules are characterized by the fact that they invariably have a septal topography and are always found around pulmonary capillaries. As in the other organs, they secondarily undergo sclerosis and hyalinization. It is interesting to note that the intraseptal nodules can give rise to foci of perilobular emphysema. Pulmonary plasmocytes are sparse. There is also diffuse reticular pneumonia, emphysema, alveolitis and exudative bronchitis. In some animals who died within a few days after the intravenous injection of silica, diffuse bronchopneumonia is observed, with large or giant cell alveolitis.

3. In the kidneys, glomerulitis and tubular nephritis is invariably observed. In our experiments we found no glomerular silicotic nodules analogous to those described by Siegmund (42, 43). But as will be discussed later, in one animal there was glomerular and interstitial amyloidosis coexisting with intense nephritis.

4. In the silicotic rabbit we observed a general intense, mesenchymal, visceral and neuro-endocrine reactive syndrome analogous to that described by this institute in the rat, guinea pig and dog. It is pointed out that in the rabbit diffuse reactive plasmocytosis is relatively slight.

5. The one case of diffuse amyloidosis is particularly interesting, because amyloidosis and para-amyloidosis are often

both regarded as conditions of immunologic origin. In the animals of this experimental series amyloid deposits were observed in the kidneys, notably in the Malpighian corpuscles and the interstitial renal connective tissue, in the spleen, lymph nodes and the myocardium. In the latter there were also diffuse sclerotic zones.

It should be noted that in the same animal the periglomerular basal membranes in the kidneys showed a considerable thickening, detected by the method of MacManus-Hotchkiss, while mastocytes did not appear to be increased.

6. In this animal considerable calcium deposts were found in many of the silicotic foci.

7. In another animal an unusual aortic lesional process was observed, characterized by multiple and voluminous foci in the intima and the media, containing homogeneous or fibrillar PAS-positive substance, lipid droplets and calcium granulations. These lesions and the amyloidosis will be the subject of a separate study[1].

Summary and conclusions

1. Repeated intravenous injections of 1-5 μ silica in 100 mg doses were given to rabbits. The total dose, spread over a period of 10-15 months of treatment, varied between 500-600 mg.

2. The silica level in the blood, which is normally 0.54 mg

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1) This work is the subject of a doctoral thesis in pharmacy presented before the Marseille Faculty of Medicine and Pharmacy on June 1, 1962 by Jacqueline Duplay.

per 100 ml blood, rose up to more than 1 g per 100 ml blood in animals receiving the intravenous treatment. The level invariably decreased within a period of 2 months after the last injection.

3. The silica administered accumulates mostly in the liver, lungs and spleen, while the amount of silica found in the kidneys remains practically unchanged.

4. So far as the changes in the proteinogram are concerned, they reveal essentially an imbalance in the albumin-globulin ratio in favor of the globulins. There is furthermore a distinct increase in all cases in the δ and γ-globulins. An increase in the α-2-glycoglobulins and δ-lipoproteins is also observed.

5. From the hormonal standpoint, a roughly 50% reduction was observed in urinary 17-ketosteroids in both male and female animals.

6. In the liver a non-specific cirrhotic process is observed on the one hand, and on the other, nodular silicosis. These processes may be accompanied by multiple biliary adenomatosis.

7. Specific silicotic lesions are observed in the spleen, the lymphnodes and the lungs.

8. The pulmonary silicotic nodules invariably have a septal topography and always develop around pulmonary capillaries. They are accompanied by pulmonary emphysema.

9. In the kidneys there is always glomerulitis and tubular nephritis.

10. One case of diffuse amyloidosis was observed, as well as diffuse calcinosis.

11. An unusual aortic lesional process was also observed.

12. In the silicotic rabbit, as in other species, there is a general mesenchymal, visceral and neuro-endocrine reactive syndrome.

13. Immunologic reactions observed in our animals will be dealt with in a separate work..

Figures

1. Rabbit no. 12354. Liver cirrhosis. The left lobe is almost completely sclerotic.

2. Rabbit 12340. Insular and annular liver cirrhosis (non-specific).

3. Rabbit 12344. Non-specific cirrhosis. Enlarged and sclerotic periporto-biliary space.

4. Rabbit 12361. Hepatic epithelial reactions (non-specific) and histiocytic nodules of various sizes (specific).

5. Histiocytic follicle in the same animal with stronger magnification.

6. Rabbit 12340. Intralobular silicotic nodule.

7. Rabbit 12340. Sclero-hyaline silicotic nodule.

8. Concentric fibrillar formations in a diffuse sclerotic zone.

9. Rabbit 12354. Calcium deposit in a zone of liver sclerosis.

10. Rabbit 12344. Proliferative epithelial nodules (right).

11. Rabbit 12344. Biliary adenomatosis in a large sclerotic area.

12. Rabbit 12354. Nodular silicosis of the spleen.

13. Rabbit 12354. Pulmonary silicotic nodules.

14. Rabbit 12354. Renal glomerular amyloidosis.

15. Rabbit 12354. Thickening of the peritubular basal membranes (kidney).

16. Rabbit 12354. Myocardial amyloidosis.

17. Rabbit 12340. Non-hyalinized pulmonary silicotic nodule with perinodular emphysema.

18. Aorta of animal 12345. Presence in the intima and the media of a nodule of chondroid appearance with calcification. Edema of the intima.

Bibliography

See pp. 520-521.

Archives des Maladies Professionnelles (Paris), 1963, **24**, n° 6, Juin (pp. 503-521).

MÉMOIRES ORIGINAUX

Sur la silicose expérimentale du lapin obtenue par l'administration intraveineuse de silice (1).

Par

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(Marseille).

Dans des recherches antérieures réalisées à notre Institut, nous avons fait l'étude systématique, chez le rat, le cobaye et le chien, des réactions locales, du syndrome général, anatomo-pathologique et biochimique et du métabolisme de la silice, à la suite de l'administration intrapéritonéale ou intratrachéale ou par l'empoussiérage chronique. Ces recherches ont donné lieu à un ensemble de publications et aux thèses de El Feki (1957) [9] et de Cartouzou (1960) [3]. Elles ont permis de proposer une conception pathogénique de la maladie silicotique, considérée comme une mésenchymatose, c'est-à-dire une maladie de collagène [7-8-30-31-32-33-35-36].

Effectivement, on relève, dans la silicose, des incidences histopathologiques, biochimiques, immunologiques et endocriniennes analogues à celles qui existent dans les autres maladies du collagène [4].

Il était intéressant, dans le cadre de cette conjoncture, d'étudier l'ensemble des modifications histo pathologiques générales et localisées et des altérations biochimiques et sérologiques pouvant être observées chez des animaux traités par des injections intraveineuses de silice. Il faut s'attendre, *a priori*, à ce que chez ces animaux les modifications générales soient particulièrement marquées et instructives.

Notre expérimentation a porté sur le lapin traité par des injections intraveineuses répétées de silice de dimensions particulaires de 3 microns.

Historique.

Gye et Purdy (1923) [14] semblent avoir été les premiers à utiliser la voie intraveineuse, chez le lapin. Ces auteurs injectaient de la silice colloïdale et ont ainsi obtenu de la cirrhose. Cette découverte nous serait particulièrement importante, car elle montre que dès 1922, il avait été démontré que la silice colloïdale peut déterminer, dans certains organes, de la sclérose au même titre que la silice cristalline. Nous rappellerons que d'après les constatations faites à notre Institut, la silice colloïdale et l'acide silicique entraînent, en administration répétée, de la sclérose péritonéale avec hyalinisation.

Siegmund (qui depuis 1922 étudiait la maladie silicotique) et d'autres auteurs, publient, en 1934 [28, 29, 12, 13] les résultats histologiques de leurs premières expérimentations par voie intraveineuse sur le

(1) Travail financé par la Haute Autorité de la Communauté Européenne du Charbon et de l'Acier.

lapin. Siegmund met en évidence la production de cellules géantes histiocytaires dans le foie, de sclérose et de granulomes dans le tissu péri-porto-biliaire. Il trouve des granulomes silicotiques cellulaires également dans la rate, les ganglions lymphatiques, les glomérules rénaux et la moelle osseuse.

En 1935, Giese [12, 13] confirme ces résultats et décrit, en outre, des réactions pulmonaires avec présence de cellules à poussières dans les cloisons interalvéolaires et les alvéoles et d'un œdème pulmonaire avec histiocytose. Ces lésions aboutissent à une sclérose contenant des nodules à fibres de réticuline.

A la même époque, d'autres auteurs ne relèvent qu'une fibrose hépatique [2, 21]. Ainsi, en 1940, Simson et Strachan [41] injectent du quartz intraveineux et mettent en évidence une cirrhose hépatique.

En 1944, Gardner, Dworski et Delahant [10] montrent que l'injection intraveineuse de 500 mg de silice de 1 à 3 μ donne, au bout d'un an, une cirrhose silicotique massive. Ils remarquent aussi que le même quartz administré en suspension à 0,33 p. 100 dans du silicate d'alumine colloïdal gélatineux, ne donne au bout d'un an qu'une accumulation de phagocytes dans les espaces porto-biliaires.

Haythorn et Taylor montrent, en 1945 [15] que l'administration intraveineuse de cendres de poumons de silicotiques donne des granulomes silicotiques.

Rattner [39], en 1950, met en évidence la fibrose hépatique obtenue par injection de 80 mg de quartz en suspension dans de l'eau. En 1952, Meesen [27] obtient, à son tour, des granulomes silicotiques hépatiques, par injection intraveineuse de silice.

Romer [38], en 1953, met en évidence dans le foie, des amas cellulaires riches en cellules géantes notamment dans le tissu périportal. Dans la rate, il note de rares cellules géantes dans les sinus spléniques.

Buts de nos recherches.

Nous nous sommes proposé de faire une étude détaillée des lésions histologiques causées par l'injection intraveineuse de silice, ainsi qu'une étude biochimique comparative entreprise chez les animaux sains puis secondairement rendus silicotiques.

Pour cette étude, nous avons utilisé le lapin qui, par sa taille, nous permet d'effectuer des prises de sang répétées chez le même animal et, de ce fait, nous permet de suivre l'évolution de la maladie silicotique.

Nous avons un recul de 18 mois pour juger les résultats obtenus.

Par une exploration biologique et histologique de ces lapins « silicotiques » nous avons tenté de répondre aux questions suivantes :

1° Quel est le chemin suivi par la silice administrée par voie intraveineuse et où se dépose-t-elle? Pour résoudre ce problème, nous avons effectué des dosages de la silicémie chez nos lapins vivants. A l'autopsie, nous avons recueilli leurs organes afin d'y doser la silice.

2° Quels sont les désordres protéiques révélés par l'électrophorèse? — A ce sujet, nous avons fait des protidogrammes, des glucidogrammes et des lipidogrammes à plusieurs reprises chez le même animal afin d'étudier les répercussions sériques de l'administration de silice.

3° Quels sont les désordres hormonaux que nous pouvons mettre en évidence chez le lapin silicotique? — Nous avons pratiqué, à cet égard, le dosage des 17-cétostéroïdes urinaires.

4° Quels sont les organes lésés par cette injection intraveineuse de silice et quelle est la nature de ces lésions? — Nous avons pratiqué l'examen macroscopique et microscopique de tous les organes chez tous les animaux sacrifiés. Cette étude histologique a été complétée par des biopsies hépatiques, nous permettant ainsi d'étudier l'évolution histologique de la maladie silicotique.

5° Existe-t-il des réactions hématologiques caractéristiques chez le lapin silicotique?

6° Existe-t-il des modifications sérologiques d'ordre immunologique, chez le lapin silicotique? — Nous avons pratiqué, pour répondre à cette question, certaines réactions sérologiques, notamment la recherche du facteur rhumatismal.

Ces deux dernières explorations feront l'objet d'une prochaine publication.

Technique expérimentale.

La silice que nous avons injectée à nos animaux, est une silice de dimension particulaire comprise de 1 à 5 μ, avec une proportion de 80 p. 100 environ de particules à 3 μ.

Nous la préparons nous-mêmes à partir de la silice cristalline B.L.B. que nous trions par sédimentation dans l'eau. La silice est ensuite séchée et stérilisée puis subit, avant emploi, un nouvel examen microscopique de contrôle.

D'autre part, les lapins que nous avons employés pour nos expérimentations sont des lapins de race blanche de Bourgogne âgés de 3 mois au moins, et pesant 1 200 à 1 500 g. Nous avons constaté que ces animaux, quoique traités par des administrations intraveineuses de silice, ont un développement pondéral normal.

L'injection intraveineuse de la silice, mise en suspension dans du sérum physiologique, se pratique commodément dans la veine marginale de l'oreille. Nous faisons précéder systématiquement cette injection d'un prélèvement de sang pour les divers examens biologiques. Lors du sacrifice, nous prélevons une quantité beaucoup plus importante de sang par ponction cardiaque.

Les doses administrées sont de 100 mg par injection. La dose totale, étalée sur 10 à 15 mois de traitement, varie entre 500 et 600 mg.

Chez les différents auteurs, nous avons pu relever des doses très variables : ainsi Buttner [39] obtient une cirrhose avec une dose totale de 80 mg. Gardner, Dworski et Delahant [10] injectent 500 mg. Worwald, Durkan et Pratt [49] ont expérimenté des doses allant jusqu'à 1 g de silice.

Destinée de la silice injectée.

Le dosage de la silice dans le sang est effectué par la méthode de King modifiée [23, 25].

Le principe de cette méthode est basé sur la spécificité de la réduction du silicomolybdate à un *p*H et à un potentiel oxydoréducteur tels que, seul, le silicomolybdate soit réduit.

En effet, la silice forme, en milieu acide, avec le molybdate d'ammonium, un complexe silicomolybdique de couleur jaune. D'autre part, le phosphore donne également un complexe phosphomolybdique jaune [16, 46]; sa présence est donc particulièrement gênante pour les analyses biologiques. Mais le dosage de la silice en présence de phosphore est possible par réduction du complexe silicomolybdique par le bisulfite de sodium à un *p*H bas; de l'ordre de 2. *p*H auquel le phosphomolybdate n'est pas réduit. On obtient alors un bleu de molybdène photométrable (à 700 millimicrons). On construit une courbe d'étalonnage à partir de silice pure, solubilisée par fusion alcaline. Les réactifs sont tous préparés avec de l'eau bidistillée et conservés en flacons de polythène.

Nous avons pratiqué des silicémies systématiques sur des lapins normaux afin d'obtenir une valeur normale moyenne de la silicémie chez le lapin. Nous avons 15 résultats variant de 0,305 à 0,770 mg pour 100 ml de sang. Nous insisterons sur la moyenne élevée (0,454 mg/100 ml de sang) de ces résultats. En effet, chez l'homme, les valeurs que nous avons obtenues avec notre méthode sont comprises entre 0,320 et 0,360 mg/100 ml de sang [34]. Au delà de 0,400 mg nous considérons les chiffres comme anormalement élevés chez l'homme.

Nous attribuons cette différence des valeurs normales chez l'homme et chez le lapin à la nourriture exclusivement végétale de ce dernier.

Chez 13 lapins silicotiques, nous avons pratiqué 22 examens. Les chiffres que nous avons obtenus sont très élevés : 9 résultats sont supérieurs à 1 g pour 100 ml de sang. Ces chiffres révèlent l'imprégnation logique en silice du sang circulant. D'autre part, nous avons rapporté sur un diagramme les silicémies en fonction du temps écoulé depuis la dernière injection. Nous obtenons une courbe décroissante montrant qu'au delà d'un délai de 2 mois après la dernière injection de silice, la teneur en silice du sang redevient normale dans la majeure partie des cas. Il est logique alors de penser que la silice injectée par voie intraveineuse va se localiser dans les organes récepteurs puisqu'ensuite il ne reste plus de trace sanguine de l'injection de silice.

Quels sont les organes qui vont accumuler préférentiellement la silice injectée?

Lors des autopsies des animaux, nous avons pesé les organes entiers puis pratiqué des dosages de silice. Les organes principalement intéressés ont été le foie, les reins, la rate et les poumons. Les organes sont soigneusement broyés dans un mortier. Parallèlement aux prélèvements pour le dosage de la silice effectués dans des creusets de platine, nous faisons des prélèvements sur verres de montre, prélèvements desséchés à l'étuve à 40° et pesés jusqu'à poids constant. Nous établissons ainsi le pourcentage d'eau qui varie du simple au double lorsque l'on augmente le séjour d'un organe au congélateur à −20°.

Nous rappellerons dans le tableau suivant les teneurs en silice retrouvées dans les organes de lapins normaux par différents auteurs.

Dosage chez le lapin (en mg de silicium par kg ou l de tissu ou de sang frais).

	Ramel [37]	Isaacs [17]	King [22]	King et Stantial [24]
Cerveau	0	8,8 à 18	—	—
Sang	12	—	—	3,4 à 5,8
Foie	35	0 à 5,6	150-212	96 — 57
Rein	28	5 — 17,5	115-157	67 — 76
Poumon	270	3,9 — 12	125-139	85

D'après ce tableau, on voit à quel point les résultats sont variables suivant les techniques utilisées. C'est pour avoir des chiffres de comparaison que nous avons suivi un mode opératoire strict [9] utilisant des réactifs purifiés, redistillés et que nous avons appliqué tout d'abord cette méthode à des lapins normaux.

Le dosage est effectué suivant la technique de King et Isaacs [17] [22].

Le principe de ce dosage comprend les trois temps classiques : 1) la destruction de la matière organique sous forme de borosilicate ; 2) la mise en solution de la silice par la soude normale et à chaud ; 3) le dosage colorimétrique de la silice suivant le même principe que celui indiqué précédemment pour la silicémie.

Cette méthode permet de doser commodément des quantités assez importantes de silice, de l'ordre de 20 à 80 *g* dans une prise d'essai. C'est pour cela que cette méthode a dû être adaptée aux dosages de silice dans les organes de lapins normaux en doublant les prises d'essai et, bien sûr, les quantités de réactifs minéralisants.

Afin de rendre nos résultats plus facilement comparables, nous avons éliminé les variations dues au pourcentage d'eau variant de 30 à 70 p. 100 en fonction du temps de séjour au congélateur ; c'est pourquoi nous exprimons nos valeurs normales en milligrammes de silice pour 100 g de tissu sec.

Organes normaux	mg de silice pour 100 g de tissu sec
Foie	0 à 13
Reins	1 à 3,1
Poumons	1 à 4
Rate	0 à 2

Les résultats obtenus chez nos lapins silicotiques sont très élevés par rapport à ces valeurs normales et nous ont obligé à modifier nos prises d'essai lors des dosages.

Nous devons souligner la teneur particulièrement importante de la silice dans le foie (de 8,8 mg à 435 mg pour 100 g de tissu sec) et les poumons (2,6 mg à 278 mg) traduisant d'une part le premier arrêt mécanique des particules de silice au niveau des capillaires pulmonaires, et d'autre part, le processus de détoxication hépatique. La rate se trouve également chargée en silice (jusqu'à 200 mg), les reins n'accumulent pas dans leur parenchyme d'aussi fortes quantités de silice que les autres organes (37,7 mg au maximum).

Désordres protéiques révélés par l'électrophorèse chez nos lapins silicotiques.

Par la petite quantité de sang que réclame son exécution, l'électrophorèse nous est apparue comme une méthode de choix pour suivre les désordres protidiques engendrés par l'administration de silice intraveineuse chez nos lapins d'expérience. De ce fait, par des prises de sang répétées, nous avons pu suivre l'évolution des modifications tout au long de la vie de nos animaux.

Nous avons utilisé les méthodes classiques d'électrophorèse sur papier, en tampon véronal sodique de *p*H 8,6 et de force ionique 0,1 M avec une migration à 5,4 volts/cm et 0,34 mA/cm pendant 16 heures. La quantité optima de protéines à déposer est de 0,56 µg. Nous avons donc établi une courbe nous donnant la quan-

Fig. 1 (*Lapin* 12351). — *Cirrhose hépatique.* Le lobe gauche est presque complètement sclérosé.

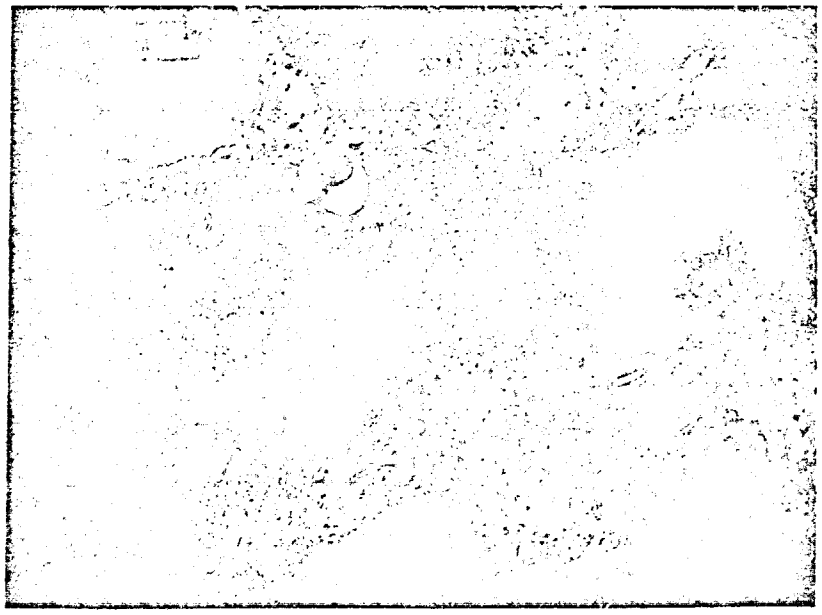

Fig. 2. (*Lapin* 12340). — Cirrhose hépatique insulaire et annulaire (aspect non spécifique).

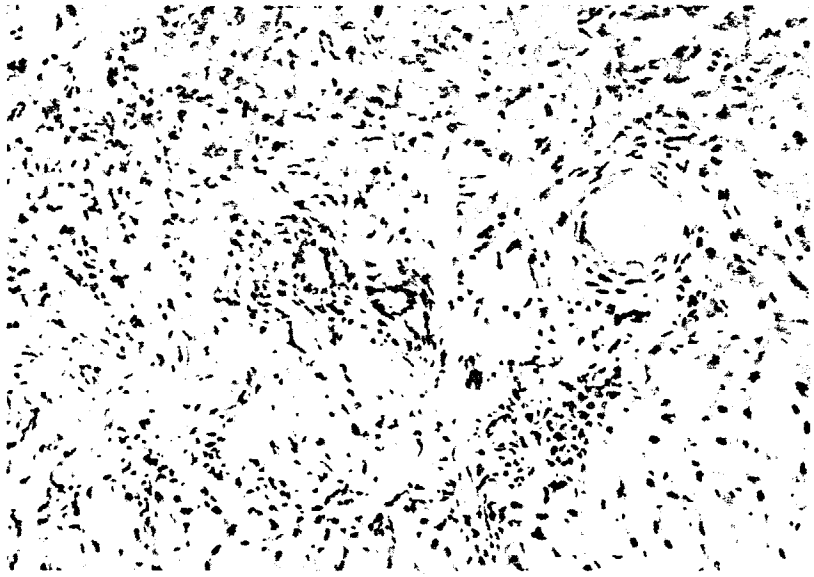

Fig. 3 (*Lapin* 12311). — Cirrhose non spécifique. Espace périporto-biliaire élargi et scléreux.

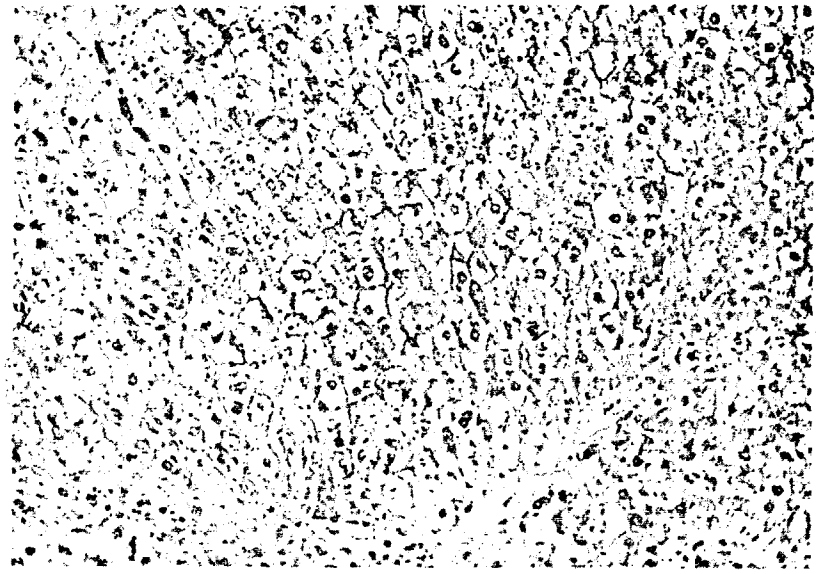

Fig. 4 (*Lapin* 12361). — Réactions épithéliales hépatiques (aspect non spécifique) et présence de nodules histiocytaires de taille variable (aspect spécifique).

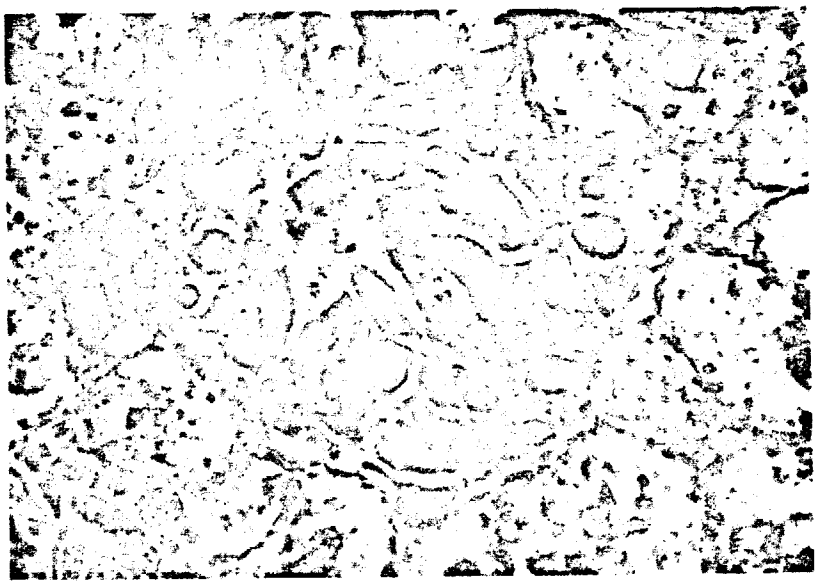

Fig. 5. — *Follicule histiocytaire* chez le même animal à un plus fort grossissement.

Fig. 6 (*Lapin* 12340). — Nodule silicotique intra-lobulaire.

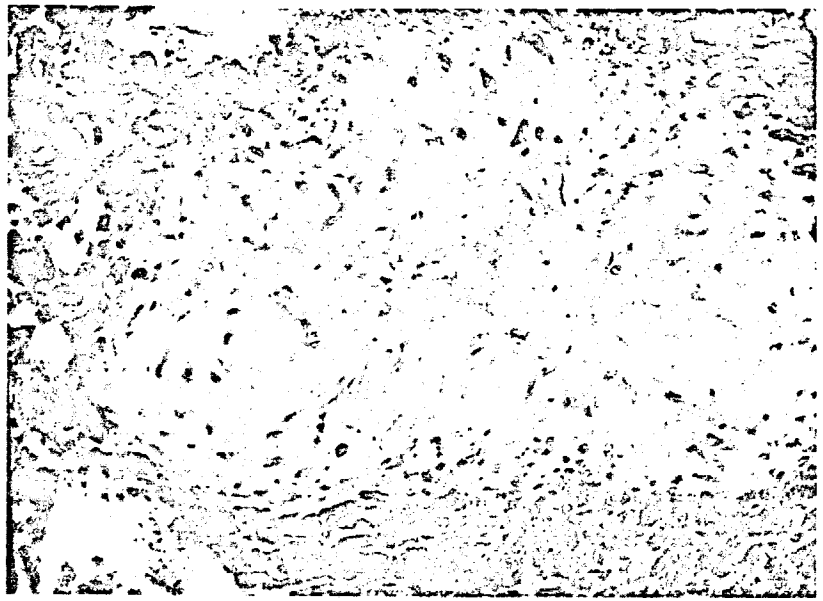

Fig. 7 (*Lapin* 12340). — Nodule silicotique sclero-hyalin.

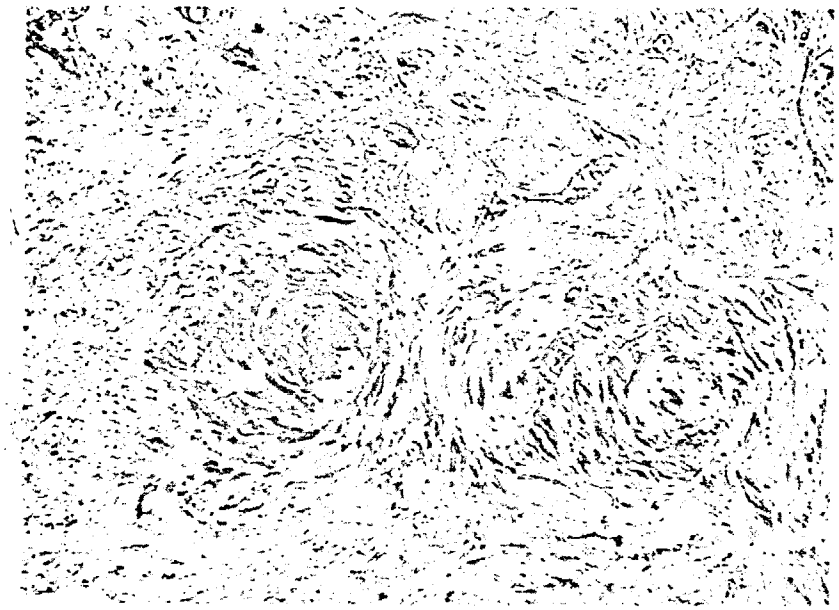

Fig. 8 (*Lapin* 12345). — Formations concentriques fibrillaires dans une plage de sclérose diffuse.

Fig. 9 (*Lapin* 12354). — Dépôt calcaire dans une plage de sclérose hépatique.

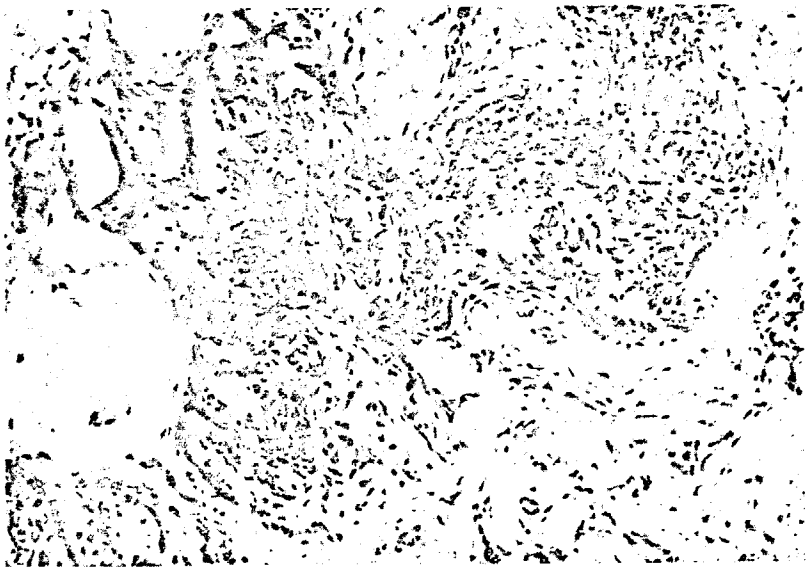

Fig. 10 (*Lapin* 12344). — Nodules épithéliaux prolifératifs (à droite).

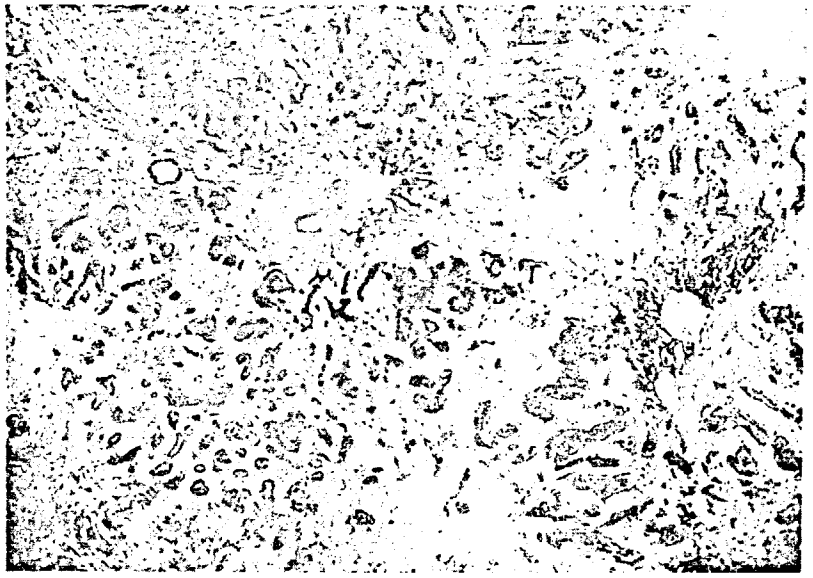

Fig. 11 (*Lapin* 12344). — Adénomatose biliaire dans une vaste plage de sclérose.

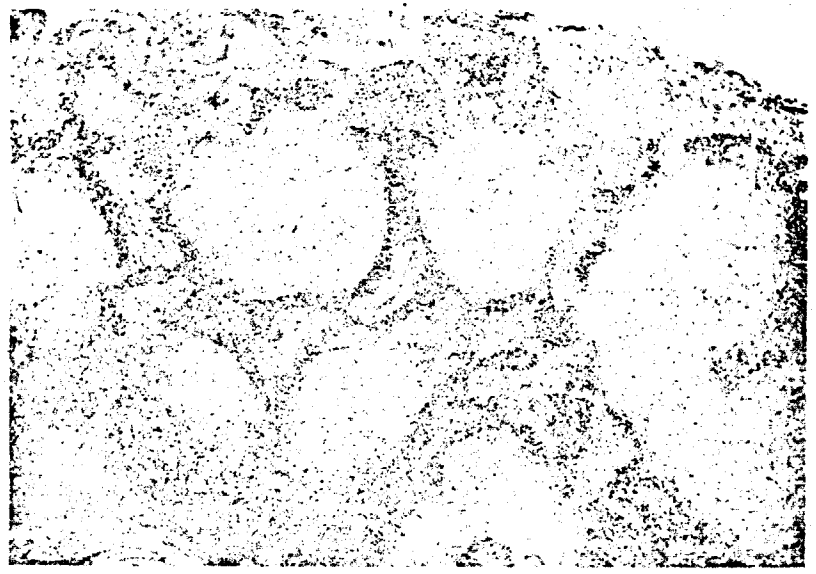

Fig. 12 (*Lapin* 12354). — Silicose splénique nodulaire.

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Fig. 13 (*Lapin* 12354). — Nodules silicotiques pulmonaires.

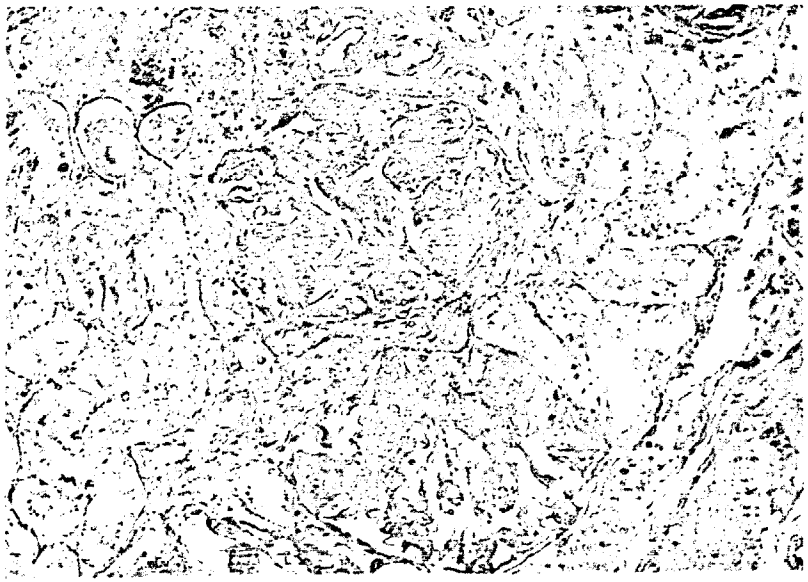

Fig. 14 (*Lapin* 12354). — Amyloïdose glomérulaire rénale.

Fig. 15 (*Lapin* 12354). — Épaississement des membranes basales péri-tubuleuses (rein).

Fig. 16 (*Lapin* 12354). — Amyloïdose myocardique.

Fig. 17 (*Lapin* 12340). — Nodule silicotique pulmonaire non hyalinisé avec emphysème périnodulaire.

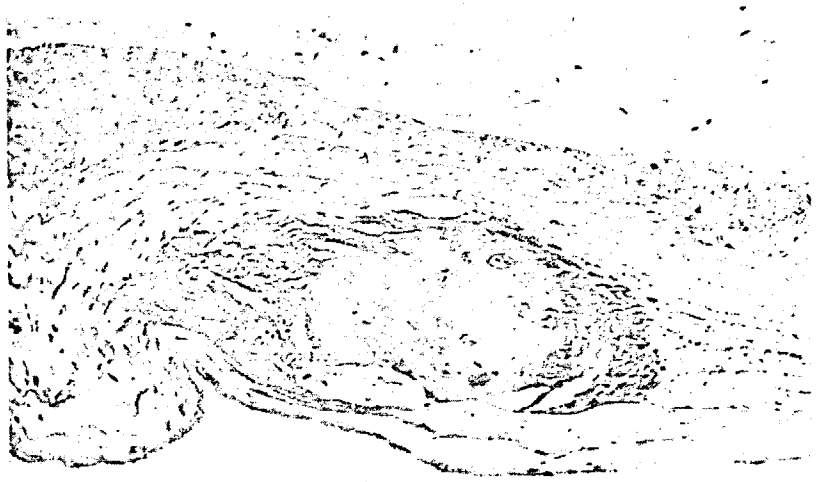

Fig. 18 (*Aorte de l'animal* 12345). — Présence dans l'intima et dans la média d'un nodule d'aspect chondroïde avec calcification. Œdème de l'intima.

en µl de sérum à déposer selon le chiffre de protidémie totale. Celle-ci est déterminée par la méthode densimétrique de Philips et Van Slyke. Nous déposons cinq fois plus de sérum pour le glycoprotidogramme et six fois plus pour le lipidogramme.

La révélation du protidogramme est faite avec l'amidoschwarz 10 B. Celle des lipoprotidogrammes par le noir cérol (technique de Swahn) [45], enfin la mise en évidence des fractions des glycoprotidogrammes se fait par réaction de Schiff en utilisant la p-rosaniline [47, 48].

Cependant, chez l'animal nous éprouvons parfois quelques difficultés à repérer les fractions globuliniques.

Afin d'apprécier la mobilité électrophorétique de ces fractions, nous avons voulu tenir compte du flux électroendosmotique. Pour cela, nous avons fait migrer parallèlement, une substance colorée, dépourvue de charge qui est la dinitro-phényl-éthanolamine que nous avons préparée par la méthode de Sanger [20, 26, 40].

Nous avons relevé dans la littérature des valeurs normales des fractions protéiques chez le lapin établies en 1945 par Deutsch et Goodloe [5, 6], en 1954 par Geinitz [9, 11] et en 1955 par Scheiffarth [41]. On trouve chez ces différents auteurs des divergences notables.

De ce fait, nous avons préféré suivre l'évolution des fractions électrophorétiques chez le même animal. Cependant, à l'aide de toutes les premières électrophorèses pratiquées chez nos lapins sains, nous avons pu effectuer une analyse statistique des résultats obtenus qui nous a permis d'établir des valeurs physiologiques (publication à paraître).

Toutes nos électrophorèses (au nombre de 103) nous ont permis d'établir les conclusions suivantes : la première notion évidente est le déséquilibre du rapport albumine-globuline, avec augmentation des globulines et chute des albumines de l'ordre de 20 à 30 p. 100 entre le 7e et le 8e mois de traitement. Les α-globulines ne présentent pas d'homogénéité dans leurs variations.

Les δ et les γ-globulines augmentent nettement dans tous les cas. Les δ-globulines augmentent moins que les γ et atteignent 90 p. 100 d'augmentation au 7e mois environ. Les γ-globulines augmentent plus rapidement que les δ-globulines, leur pourcentage d'augmentation est moins régulier, mais il est toujours bien plus élevé (de l'ordre de 130 p. 100).

Le glucidogramme montre une augmentation moyenne de 30 p. 100 des α-2-globulines.

Le lipidogramme montre une augmentation de 30 à 50 p. 100 des δ-lipo-protéines.

Nous présenterons dans un mémoire ultérieur tous nos résultats électrophorétiques traités par méthode statistique.

Résultat des dosages des 17-cétostéroïdes totaux et des créatinines urinaires.

Les dosages des 17-*cétostéroïdes* ont été effectués sur les urines de 24 heures recueillies dans des cages construites spécialement dans notre laboratoire pour éviter au maximum les pertes par évaporation. Les urines sont recueillies sur de l'acide chlorhydrique concentré ou sur une solution de merseptyl dans le norbutanol.

Nous faisons uriner plusieurs animaux à la fois, dans différentes cages, et nous les plaçons toujours dans la même pièce, dont la température ne varie que très peu, pour pallier aux pertes par évaporation.

Nous ne pratiquons les dosages des 17-cétostéroïdes que lorsque la diurèse est supérieure à 100 ml. Nous avons constaté, lorsqu'elle est inférieure à ce chiffre, des taux inconstants le plus souvent diminués. C'est pour pallier à cet inconvénient et obtenir des urines diluées et peu pigmentées, que nous nourrissons les lapins avec de la salade pendant cette épreuve. De plus, afin de contrôler la diurèse, nous effectuons sur chacun de nos prélèvements, des dosages de créatinine.

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Nous utilisons pour le dosage des 17-cétostéroïdes la technique de Jayle et Coll. [18, 19].

Nous faisons subir une hydrolyse acide à 100° à une portion aliquote de ces urines fraîches amenée à pH aux environs de 1.

Nous extrayons plusieurs fois l'hydrolysat par de l'éther éthylique dans des allonges cylindriques verticales. Après lavage à la soude, qui élimine les phénol-stéroïdes, et lavage à l'eau, nous évaporons l'éther à siccité.

Après avoir repris les extraits secs par de l'éthanol, nous effectuons la réaction colorée de Zimmermann au m-dinitrobenzène, en milieu potasse alcoolique. La gamme de référence est constituée à partir d'une solution mère de déhydro-épiandrostérone. Nous faisons deux gammes et deux témoins systématiquement. Après séjour dans un bain-marie à 37°, nous arrêtons la réaction en plongeant les tubes dans de l'eau glacée. Puis, après adjonction d'alcool à 30 p. 100, nous extrayons la coloration par de l'éther éthylique pur anesthésique. Nous obtenons alors un surnageant rose violacé qui est photométré avec correction d'Allen (1).

La correction d'Allen est obligatoire dans ce cas, car nous avons affaire à des quantités très peu importantes de 17-cétostéroïdes et à des urines assez fortement pigmentées.

Nous avons constaté avec des urines peu pigmentées et contenant des quantités plus importantes de 17-cétostéroïdes (urines humaines), une parfaite concordance entre les résultats obtenus avec et sans correction d'Allen. Alors que pour des urines très fortement pigmentées, environ 50 p. 100 de la valeur obtenue sans correction est due en fait à une coloration parasite.

Lorsque la déhydroépiandrostérone est ajoutée après l'hydrolyse, les pourcentages de récupération sont de l'ordre de 75 à 95 p. 100. Comme nous ne possédions ni sulfate, ni glycuronate de D.H.E.A., nous n'avons pas pu tester notre hydrolyse, que nous avons faite selon les conditions décrites dans la méthode de Jayle et Coll.

Le dosage de la créatinine est effectué parallèlement sur ces mêmes urines; l'adjonction d'acide picrique en milieu basique développe une couleur orangée qui permet un dosage photocolorimétrique à 530 millimicrons. La gamme de référence est constituée à partir de chlorhydrate de créatinine.

Chez les lapins femelles normales, nous avons constaté des chiffres reproductibles en faisant uriner à plusieurs reprises ces animaux, un mois 1/2 après le premier dosage. Il en est de même du taux de la créatinine.

Dans un premier temps, nous avons établi quelques moyennes sur des lapins normaux. Nous avons obtenu chez des lapins femelles normales un taux de 0,33 mg par 24 heures et pour les lapins mâles normaux un taux de 0,28 mg par 24 heures.

Chez nos lapins silicotiques, des dosages répétés à 15 jours d'intervalle nous ont donné des chiffres reproductibles ; les taux moyens obtenus sont pour les femelles de 0,16 mg et pour les mâles 0,13 mg par 24 heures. Nous avons donc des diminutions de l'ordre de 50 p. 100 dans les deux sexes. Cet abaissement se manifeste dès le début du traitement (dès le 1er mois après la première injection de 100 mg) et s'accentue au cours de l'évolution de la maladie silicotique.

Les lésions histologiques observées chez nos lapins silicotiques.

Du point de vue macroscopique, il convient de signaler que seules les lésions hépatiques et spléniques ont été apparentes.

La glande hépatique, en effet, a présenté manifestement une atteinte cirrhotique au bout de 4 à 5 mois. Il est important de noter que la sclérose présente une intensité très variable suivant les lobes étudiés. Le lobe de Spiegel est constamment frappé le premier. Il peut être complètement sclérosé lorsque, dans le restant de la glande, on n'est pas encore en mesure de mettre en évidence des phénomènes cirrhotiques.

(1) Do Allen = Do 520 — $\frac{\text{Do 440} + \text{Do 600}}{2}$.

L'examen macroscopique permet également de relever constamment des signes d'hépatose dégénérative avec, parfois, un aspect stéatosique.

Les lésions spléniques se manifestent macroscopiquement par de la splénomégalie, un épaississement scléreux de la capsule de l'organe, la présence de nodules silicotiques et une sclérose diffuse.

Au niveau des poumons qui présentent, comme nous le verrons, des lésions de silicose histologique, nous n'avons pas pu mettre en évidence macroscopiquement, de nodules silicotiques. Un aspect emphysémateux cependant a pu être relevé.

Les ganglions lymphatiques cervicaux et médiastinaux ont présenté, chez certains animaux, de l'hypertrophie.

Du point de vue histologique, nous avons pu mettre en évidence quatre types de lésions :

1° Une cirrhose hépatique du type insulaire et annulaire ou du type diffus avec production de vastes zones scléreuses en grande partie hyalinisées. Il est intéressant de noter que les différents lobes hépatiques sont inégalement atteints. En particulier, le lobe de Spiegel est celui qui présente constamment les lésions les plus avancées. Cette cirrhose expérimentale présente essentiellement un aspect non spécifique, car elle évolue comme les cirrhoses obtenues par des régimes carentiels ou par des procédés toxiques. En effet, chez presque tous les animaux, le processus lésionnel hépatique commence par d'importantes lésions dégénératives épithéliales et par des réactions péri-portobiliaires consistant en infiltration inflammatoire avec sclérose consécutive. L'infiltration réactionnelle est constituée essentiellement d'histiocytes, de lymphocytes et d'assez rares plasmocytes. Il est curieux de noter que le système kuppférien chez la plupart des animaux ne présente pas d'importantes réactions.

Cependant, chez certains animaux, on relève, outre le processus cirrhotique d'allure non spécifique, des lésions silicotiques typiques caractérisées par la production de follicules réticulo-histiocytaires intra-lobulaires qui subissent secondairement la sclérose collagéno-fibroblastique et l'hyalinisation. Chez les mêmes animaux, les cellules de Kuppfer sont réactionnelles de manière diffuse.

Par conséquent, la glande hépatique présente, sous l'influence de la silice intraveineuse, d'une part un processus de cirrhose non spécifique, d'autre part des phénomènes de silicose avec production de nodules scléro-hyalins.

Parfois également on peut relever dans certaines plages de sclérose étendue, des aspects fibrillaires concentriques rappelant les nodules silicotiques.

Nous insistons également sur l'importance fréquente de la distension des vaisseaux portiux.

Enfin, fait d'un grand intérêt, on observe fréquemment une intense prolifération biliaire, et, chez certains animaux, un véritable état d'adénomatose biliaire multiple. Ce fait est important à signaler en raison de la capacité tumorigène de la silice, dans certaines conditions expérimentales.

Il convient aussi de signaler la rareté des plasmocytes dans le foie silicotique, malgré l'importance de l'hyalinisation et les aspects de foie silex analogues à ceux qui s'observent dans certaines syphilis hépatiques.

2° Des lésions silicotiques spécifiques s'observent, chez nos animaux, dans la rate, dans les ganglions lymphatiques et au niveau des poumons.

La silicose splénique débute par des nodules réticulo-histiocytaires siégeant dans la pulpe blanche ou dans la pulpe rouge. Ces nodules sont nombreux et subissent la transformation scléro-hyaline. Ils deviennent souvent confluents avec production de plages étendues de sclérose.

Au niveau des ganglions lymphatiques, les nodules silicotiques siègent généralement dans la substance corticale et plus rarement dans la zone médullaire. Comme

dans la rate, ils peuvent être multiples et déterminer par confluence, des zones sclérotiques étendues.

Les nodules silicotiques pulmonaires sont caractérisés par le fait qu'ils présentent une topographie septale constante et se développent toujours autour de capillaires pulmonaires. Ils subissent secondairement, comme dans les autres organes, la sclérose et l'hyalinisation. Il est intéressant de noter que les nodules intra-septaux peuvent entraîner des foyers d'emphysème péri-lobulaires. Les plasmocytes pulmonaires sont peu nombreux. On relève, en outre, dans le poumon, de manière diffuse, de la pneumonie réticulée, de l'emphysème, des phénomènes d'alvéolite et de bronchite exsudative. Chez certains animaux qui meurent dans les premiers jours consécutifs à l'injection intraveineuse de silice, on peut observer une broncho-pneumonie diffuse avec alvéolite à cellules volumineuses ou géantes.

3° Au niveau du rein, on note constamment de la glomérulite et de la néphrose tubuleuse. Nous n'avons pas relevé, dans nos expériences, de nodules silicotiques glomérulaires analogues à ceux décrits par Siegmund [42, 43]. Mais, comme nous y insistons plus loin, il existait chez un animal une amyloïdose glomérulaire et interstitielle coexistant avec une néphrose intense.

4° Nous avons relevé chez le lapin silicotique un syndrome général réactionnel intense mésenchymateux, viscéral et neuro-endocrinien analogue à celui qui a été décrit, à notre Institut, chez le rat, le cobaye et le chien. Nous insistons sur le fait que chez le lapin, la plasmocytose réactionnelle diffuse est relativement peu marquée.

5° Le cas d'amyloïdose diffuse observée est particulièrement intéressant en raison du fait que l'amyloïdose et les para-amyloïdoses sont souvent interprétées l'une et l'autre comme des affections d'origine immunologique. Chez l'animal de cette série expérimentale, les dépôts amyloïdiques s'observaient au niveau des reins, notamment dans les glomérules de Malpighi et le tissu conjonctif interstitiel rénal, au niveau de la rate, des ganglions lymphatiques et du myocarde. Dans celui-ci, il existait également des zones de sclérose diffuse.

Nous signalerons que, chez le même animal, les membranes basales péri-glomérulaires et péri-tubuleuses, au niveau des reins, présentaient un épaississement considérable mis en évidence par la méthode de Mac Manus-Hotchkiss, tandis que les mastocytes ne paraissent pas augmentés.

6° Chez le même animal, on relève des dépôts calcaires considérables dans de nombreux foyers silicotiques.

7° Chez un autre animal, nous avons relevé un processus lésionnel aortique particulier, caractérisé par des foyers multiples et volumineux, dans l'intima et la média, contenant de la substance PyA.S.-positive homogène ou fibrillaire, des gouttelettes lipidiques et des granulations calcaires. Ces lésions et l'amyloïdose feront l'objet d'une étude à part (1).

Résumé — Conclusions.

1° Nous avons pratiqué, chez le lapin, des injections intraveineuses répétées de silice, de 1 à 5 μ, par doses de 100 mg. La dose totale étalée sur 10 à 15 mois de traitement, varie de 500 à 600 mg.

2° Le taux de la silicémie, qui est normalement de 0,54 mg pour 100 ml de sang, s'élève jusqu'à plus de 1 g pour 100 ml de sang chez l'animal soumis au traitement intraveineux. Cependant, ce taux décroît régulièrement à partir d'un délai de 2 mois après la dernière injection.

(1) Ce travail a fait l'objet d'une thèse de Doctorat en Pharmacie, soutenue devant la Faculté de Médecine et de Pharmacie de Marseille le 1er juin 1962, présentée par Jacqueline Duplay.

3° La silice administrée s'accumule de manière préférentielle dans le foie, les poumons et la rate alors que le taux de la silice trouvé dans les reins reste pratiquement inchangé.

4° En ce qui concerne les modifications du protidogramme, on relève essentiellement un déséquilibre du rapport albumine/globulines au profit des globulines. On relève, en outre, une augmentation nette, dans tous les cas, des β et des γ-globulines. On observe, en outre, une augmentation des α-2-glycoglobulines et des β-lipoprotéines.

5° Du point de vue hormonal, nous avons constaté une diminution de l'ordre de 50 p. 100, chez les animaux mâles et femelles, des 17-*céloslérоïdes* urinaires.

6° Au niveau de la glande hépatique, on observe d'une part un processus de cirrhose non spécifique, d'autre part de la silicose nodulaire. Ces processus peuvent s'accompagner d'adénomatose biliaire multiple.

7° Des lésions silicotiques spécifiques s'observent dans la rate, les ganglions lymphatiques et les poumons.

8° Les nodules silicotiques pulmonaires présentent une topographie septale constante et se développent toujours autour des capillaires pulmonaires. Ils s'accompagnent d'emphysème pulmonaire.

9° Au niveau des reins, il existe constamment de la glomérulite et de la néphrose tubuleuse.

10° Un cas d'amyloïdose diffuse a été observé ainsi que de la calcinose diffuse.

11° Un processus lésionnel aortique particulier a été également observé.

12° Le lapin silicotique présente, comme les autres espèces, un syndrome réactionnel général mésenchymateux, viscéral et neuro-endocrinien.

13° Les réactions immunologiques observées chez nos animaux feront l'objet d'un travail séparé.

Bibliographie.

[1] Allen, W. M. : *The Journal of Clinical Endocrinology*, 1950, **10**, 71-83.
[2] Banting, F. G. : Silicosis Research. *Canad. Med. Ass. J.*, 1936, **35**, 289-293.
[3] Carpouzou, G. : Thèse de Doctorat en pharmacie. Marseille, juin 1960.
[4] Charbonnier, J., Collet, A., Lebouffant, L., Daniel-Moussard, H. et Durif, S. : *C. R. Acad. Sc.*, 1959, **248**, 3066-3068.
[5] Deutsch, H. F. et Goodloe, M. B. : *J. Biol. Chem.*, 1945, **161**, 1.
[6] Deutsch, H. F. et Mac Chan, W. H. : *J. Biol. Chem.*, 1949, **180**, 219.
[7] Duplay, J. et Versino, A. : L'électrophorèse sur papier chez les animaux de laboratoire normaux et silicotiques (lapin, chien, cobaye, rat). Marseille, 1961.
[8] Duplay, J. : La silicose expérimentale intraveineuse chez le lapin (étude biochimique comparative avec le lapin normal). *Thèse*, Marseille, 1er juin 1962.
[9] El Feki : Thèse de Doctorat en pharmacie. Marseille, 23 octobre 1957.
[10] Gardner, L. V., Dworsky, M. et Delahant, A. B. : *J. Industr. Hyg.*, 1944, **26**, 211-223.
[11] Geinitz, W. : *Klin. Wschr.*, 1954, **32**, 1108-1111.
[12] Giese, W. : *Beitr. Path. Anat. Jena*, 1935, **94**, 442-190.
[13] Giese, W. : *Verh. Dstch. Ges. Inn. Med.*, 1936, **48**, 107-125.
[14] Gye, W. E. et Purdy, W. Y. : *Brit. J. Exper. Path.*, 1922, **86**, 3.
[15] Haythorn, S. R. et Taylor, F. A. : *Amer. J. Path.*, 1945, **21**, 123-145.
[16] Hure, J. et Orthus, Th. : *Bull. Soc. Chim. Fr.*, 1949, 834-835.
[17] Isaacs, M. L. : *Bull. Soc. Chim. Biol.*, 1924, **6**, 157-168.
[18] Jayle, M. F. et Crepy, O. : *An. Biol. Clin.*, 1953, **11**, 233.
[19] Jayle, M. F., Crepy, O. et Scholler, R. : *S.H.P. (Arch. Biol. Méd.). Fiche de Technique Biologique*, 1957, **4**, 33.
[20] Justizz, M., Privat, M., Suquet, M. et Fromageot, C. : *Bull. Soc. Chim. Biol.*, 1954, **36**, 117.
[21] Kettle, E. H. et Hilton, R. : *Lancet*, 1930, **24**, 78-94.
[22] King, E. J. : *J. of Biol. Chem.*, 1928, **80**, 25-31.

35

[23] King, E. J. : *Bull. Soc. Chem. Biol.*, 1939, **12**, 903-909.
[24] King, E. J., Stantial, H. et Dolan, M. : *Biochem., J.*, 1933, **27**, 1002-1014.
[25] King, E. J., Stacy, B. D. et Weedon, W. : *Biochem. J.*, 1954, **56**, 11-16.
[26] Lederer, E. et Lederer, M. : Chromatographie en chimie organique et biologique. 1960, **2**, 279.
[27] Meesen, H. : *Thieme* 1952. Stuttgart, **7**, 153-155.
[28] Miller, J. W. et Sayers, R. R. : *Publ. Health. Rep. Wash.* 1934, **49**, 80-89.
[29] Miller, J. W., Sayers, R. R. et Yant, W. P. : *J. Amer. Med. Ass.*, 1934, **103**, 907-912.
[30] Molitor, L., Mosinger, M., Barthel, J., Henari, H. et Perinet, G. : Recherches sur les poussières de lamenoirs et leur capacité pathogène. *Bulletin de la Société des Sciences Médicales du Grand-Duché de Luxembourg*, août 1957.
[31] Mosinger, M. : Une conception pathogénique de la silicose. Le rôle du système neuro-ergonal. *Arch. Mal. Prof.*, 1948, **19**, 4, 356-372.
[32] Mosinger, M. : Sur la silicose ganglionnaire, microamyloïdose. *Arch. Mal. Prof.*, 1957, **18**, 6, 797-80.
[33] Mosinger, M. : Sur la pathologie et la pathogénie de la silicose, mésenchymatose sclérosante. Le rôle du système neuro-ergonal. Influence de certains facteurs agressifs. *Arch. Mal. Prof.*, 1957, **18**, 361-411.
[34] Mosinger, M., Aynaud, R., Fiorentini, H., El Feki, M., Cartouzou, G., de Bisschop, G. et Boucson, M. *Revue Européenne de Médecine*, 1959, **8**, 1375.
[35] Mosinger, M., Fiorentini, H., El Feki, M., Cartouzou, G. et Gras, A. : Sur la pathogénie de la silicose. Une conception de synthèse neuro-ergonale. Recherches expérimentales, cliniques et pathologiques. *Die Staubeingenerkrankungen*, Band 3, 1957, 256-270.
[36] Mosinger, M., Fiorentini, H., Gras, A., Garcin, G., El Feki, M. et Cartouzou, G. : La silicose expérimentale du chien. *Arch. Mal. Prof.*, 1961, **22**, 1, 2, 5 39.
[37] Ramel, P. : Thèse de Doctorat en pharmacie, Lyon, 1954.
[38] Remer, D. : *Aufendorf*, 1953, **3**, 360-362.
[39] Rutiner, J. R. : *Schweiz. Zschr. Allg. Path.*, 1950, **13**, 812-816.
[40] Sanger, F. : *Biochem. J.*, 1945, **39**, 507.
[41] Scheitfarth, Kimbel et Berg. : Naturwissenschaften, 1955, **42**, 214-215.
[42] Siegmund, H. : *Aerztl. Sachverts. Ztg.*, 1934, **40**, 285-289.
[43] Siegmund, H. : *Verg. Dtsch. Path. Ges.*, 1934, **27**, 262-271.
[44] Simson, F. W. et Strachan, A. S. : *Publ. S. Afr. Inst. Med. Res.*, 1940, **9**, 95-122.
[45] Swahn, B. : *Scand. J. Clin. Lab. Invest.*, 1954, **1**, 114.
[46] Thayer, L. A. : *Ind. Eng. Chem. Anal. Ed.*, 1939, **2**, 276-283.
[47] Van Oss, G. J., Annicolas, D. et Labil, C. : *Bull. Soc. Chim. Biol.*, 1959, **11**, 1711.
[48] Vogel : A Text book of pratical organic chemistry. Loggmans, Green and C° 2 Ed. 1951.
[49] Vorwald, A. J., Durkan, T. M. et Pratt, P. C. : A.M.A. *Arch. Industr. Hyg.*, 1951, **3**, **1**, 43.

Proceedings of the National Academy of Sciences
Vol. 65, No. 4, pp. 872–875, April 1970

Renal Damage Associated with Silicon Compounds in Dogs*

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Communicated by Robert A. Alberty, January 19, 1970

Abstract. A number of oral preparations of various forms of silicon were fed to young adult Beagle dogs and young rats of both sexes for a period of four weeks. During the test period the animals were observed for clinical symptoms and urine and blood measurements were made. At the end of the experimental period all animals were sacrificed and subjected to a complete necropsy and histopathologic study. Polydipsia, polyuria, and soft stools in some animals fed sodium silicate and magnesium trisilicate were the only untoward clinical signs observed; all clinical tests on blood and urine were within normal limits. Gross and microscopic renal lesions were observed in dogs fed sodium silicate and magnesium trisilicate but no changes were seen in those animals fed silicon dioxide or aluminum silicate. Lesions were not observed in any of the rats. In view of the large number of commercial preparations which contain sodium silicate and magnesium trisilicate used in human medicine, these compounds deserve further study.

During the course of investigations into the effects of a number of metals on the kidney of the dog and rat, we observed subcapsular hemorrhage and tubular alterations along with moderate to severe reactive lesions of the renal interstitium in dogs fed relatively large doses of magnesium trisilicate and sodium silicate (unpublished data). Similar effects were not observed in rats. Since these two silicon compounds were the only ones in the group tested which were associated with renal lesions of any significance, experiments were repeated in which a number of oral preparations of various forms of silicon were fed to Beagle dogs and rats of both sexes. These included silicon dioxide, aluminum silicate, sodium silicate, and magnesium trisilicate. The results of these studies form the basis for this report.

Materials and Methods. Purebred Beagles of both sexes about 6 months of age and weighing 7–9 kg were purchased from a licensed breeder, vaccinated against leptospirosis, canine distemper, and hepatitis, and conditioned for 2 weeks in our laboratory. Since a preliminary experiment had shown that feeding or intubation resulted in an equivalent intake of compound and in equivalent renal lesions, for 4 weeks the dogs were fed a highly palatable diet[1] in which the daily dose of the respective silicon compound was incorporated (Table 1). The doses administered provided in each case an approximately equivalent amount of silicon dioxide as the end product (0.8 gm/kg/day).

All animals were examined daily and weighed weekly. Urinary specific gravity and protein and glucose concentrations were measured before the tests began and at weekly intervals, along with total and differential white cell counts, packed cell volume, pro-

TABLE 1. *Renal lesions in dogs fed silicon compounds for four weeks.*

Treatment	Dose (gm/kg/day)	Sex	Incidence of renal lesions
Control	0	M	0/6
		F	0/6
Silicon dioxide	0.8	M	0/9
		F	0/8
Aluminum silicate	1.3	M	0/6
		F	0/7
Sodium silicate	2.4	M	8/8
		F	7/8
Magnesium trisilicate	1.8	M	9/9
		F	9/9

thrombin time, serum concentrations of hemoglobin and urea-nitrogen. At the end of the four weeks, the dogs were anesthetized with sodium pentobarbital, exsanguinated, and necropsied. Organ weights were determined, and a complete set of tissues preserved in formaldehyde solution for histopathologic examination.

Rats of both sexes of the Charles River Cesarean-Derived (CD) strain, weighing 80–100 gm, were fed the respective silicon compounds incorporated into a semisynthetic diet.[2] Fifteen rats of each sex were fed each compound for 4 weeks in amounts equivalent to those fed to dogs. Clinical evaluations, necropsy procedures, and histopathologic examinations were the same as those for dogs.

Results and Discussion. The only significant clinical abnormalities exhibited by the dogs at any point during the four-week period were polydipsia and polyuria, observed in a few animals fed sodium silicate and magnesium trisilicate. Soft feces, discolored by unabsorbed compound, were seen occasionally in most treated dogs. Body weight, food intake, and urinary and blood measurements were essentially normal in all animals.

The only clinical symptoms observed in the rats were polydipsia, polyuria, and soft stools, seen intermittently in a few animals fed magnesium trisilicate or sodium silicate. All clinical chemical tests were within normal limits.

Gross cortical lesions of the kidney were observed in all male dogs, and in all but one female dog fed sodium silicate (Figs. 1 and 2). The lesions appeared to be focal, subcapsular hemorrhages but, on the cut surface, they suggested cortical infarcts. Similar lesions were seen in all animals of both sexes fed magnesium

FIG. 1.—Gross lesions of kidney typical of those observed in dogs fed sodium silicate or magnesium trisilicate. The lesions appeared to be focal, subcapsular hemorrhages when viewed from the unsectioned cortical surface of the kidney.

FIG. 2. Gross appearance of sectioned lesion of the renal cortex characteristic in those seen in dogs fed sodium silicate or magnesium trisilicate. The appearance of the cut surface suggested cortical infarcts.

trisilicate but they were not observed in animals fed aluminum silicate or silicon dioxide.

Histopathologic studies revealed characteristic lesions in the kidneys of all dogs fed sodium silicate or magnesium trisilicate but none in any of the other groups. The nature of the lesion was the same in all cases, but severity varied from one animal to another and from one area to another within a kidney. Selected tubules were affected and were often in juxtaposition to normal ones (Fig. 3). Hypertrophy of tubular epithelium, with or without degenerative changes, inflammatory cell infiltration into the interstitium, and dilatation of some and collapse of other tubules were all observed to varying degrees within localized areas of the kidney (Fig. 4). Occasionally, deposits of crystalline material,

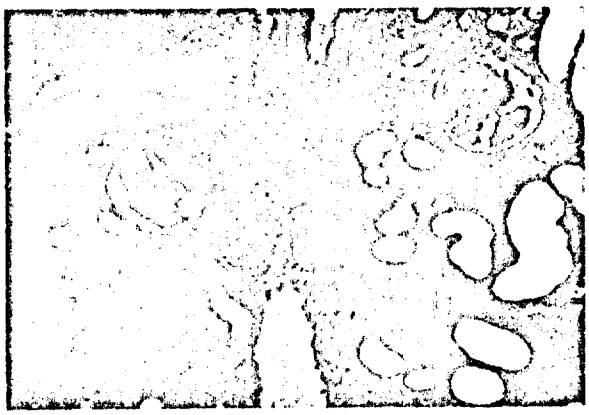

FIG. 3.—Microscopic appearance of affected area of kidney of dog fed magnesium trisilicate. Affected tubule and interstitial infiltration of inflammatory cells are in juxtaposition to normal-appearing nephrons. Glomeruli appeared to have been spared in all cases. Hematoxylin and eosin stain ×210.

FIG. 4.—Section of kidney from dog fed magnesium trisilicate illustrating degenerating and regenerating renal tubule epithelium, dilatation of tubules, and infiltration of inflammatory cells into interstitium. Hematoxylin and eosin stain ×460.

characteristic of mineralization, were observed in degenerated tubular epithelium but such alterations were not common. Glomeruli did not appear to be damaged. The tubular lesions appeared to be reactive and were not considered to result from mechanical blockage, although an occasional tubule was apparently partially blocked by the hypertrophy and proliferation of regenerating epithelium. The general impression was one of irritation of tubular epithelium followed by degenerative and regenerative changes; these alterations were accompanied by inflammatory cell infiltration into the interstitium.

Despite the presence of extensive renal damage, impairment of renal function was not detected by any of the clinical tests conducted on serum or urine. Apparently, renal reserve was adequate for normal functional demands. Perhaps longer exposure to the compounds or examination of the animals after a longer period of time following termination of exposure would have revealed impairment of renal function associated with more advanced renal lesions.

There were no drug-related lesions in any of the rats. The only departure from normal observed in an occasional rat from each group was an isolated hyaline tubular cast.

We can only speculate on the mechanism of action of the two silicon compounds associated with renal lesions in the dog. Both of them were apparently absorbed from the gut and damaged the kidney as they or their metabolic products were excreted into the urine. Although the earlier reports of Mutch[3] and Kraemer and Aaron[4] stated that neither magnesium trisilicate nor the hydrated silica (SiO_2) could be absorbed, the work of Page *et al.*[5] showed conclusively that either magnesium trisilicate or one of its silica compounds is absorbed by human subjects and excreted in the urine as silica. Furthermore, Lagergren[6] has reported urinary calculi, consisting mainly of silica, in five human patients, all of whom had been ingesting magnesium trisilicate tablets over several years. In contrast to the sparse information on urologic problems in man associated with silica, it is well known that calculi composed chiefly of silica are common in some animals and constitute a serious economic problem among cattle in many areas.[7, 8]

Although the rat was unaffected in these experiments, the unusual lesions in the kidney of the dog suggest a basic defect in the ability of this species to metabolize or excrete these compounds and present an interesting pathologic lesion for further study. The species difference observed in our studies in response to the silicates is interesting, and the rat data agree with similar negative observations in rats and mice reported earlier by Mutch.[9] On the other hand, Settle and Sauer[10] have shown that siliceous deposits form in the kidneys of guinea pigs given large doses of soluble silica orally or intraperitoneally. In view of the widespread use of various forms of silicon in products consumed by man,[11, 12] it would appear fruitful to investigate these compounds further in a broader spectrum of animal species.

* This work was supported in part by Department of Army Contract DA-49-193-MD2560.

[1] Newberne, P., *Federation Proc.*, **25**, 1701 (1966).
[2] Newberne, P., and G. Williams, *Arch. Environ. Health*, **19**, 489 (1969).
[3] Mutch, N., *Brit. Med. J.*, **1**, 254 (1936).
[4] Kraemer, M., and B. Aaron, *Am. J. Digest. Diseases*, **7**, 57 (1940).
[5] Page, R. C., R. R. Hefner, and A. Frey, *Am. J. Digest. Diseases*, **8**, 13 (1941).
[6] Lagergren, C., *J. Urol.*, **87**, 994 (1962).
[7] Whiting, F., R. Conell, and S. A. Forman, *Can. J. Comp. Med.*, **22**, 332 (1958).
[8] Swingle, K. F., *Am. J. Vet. Res.*, **14**, 493 (1953).
[9] Mutch, N., *Brit. J. Med.*, **1**, 205 (1936).
[10] Settle, W. R., and F. Sauer, *Am. J. Vet. Res.*, **21**, 709 (1960).
[11] *Chemical Rubber Company Handbook of Food Additives*, ed. T. E. Furia, The (Cleveland, Ohio) Chemical Rubber Co.
[12] *Physicians Desk Reference to Pharmaceutical Specialties and Biologicals*, 23rd edition, (Oradell, New Jersey) Medical Economics, Inc.

NAS/NRC COMPREHENSIVE GRAS SURVEY REPORT

(Pertinent tables reproduced in monograph)

Arch. Ind. Hyg. Occup. Med. 9:389-395, 1954

TOXIC AND FIBROSING ACTION OF SUBMICROSCOPIC PARTICLES OF AMORPHOUS SILICA

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AND
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VAPOR given off from strongly heated silica condenses into a fine powder made up of spherical submicroscopic particles less than 0.1 μ in size. This silica is pure, noncrystalline, and very toxic. Intravenous injections will kill animals almost instantaneously (Gardner [1] and King [2]). Small doses injected into the lung or the peritoneal cavity are usually fatal (Jötten and Klosterkötter [3] and Gärtner [4]). On the other hand this type of silica will produce only very little fibrosis (Lundgren and Swensson [5]).

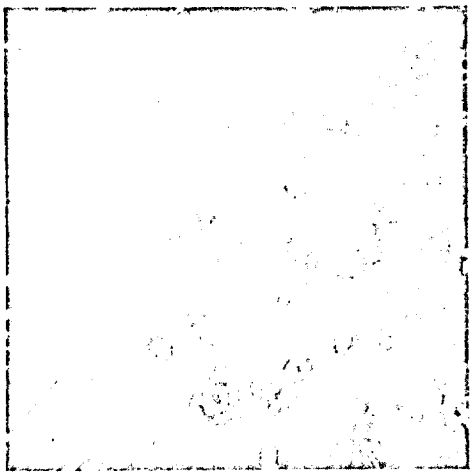

Fig. 1.—Electron micrograph of a sample of silica condensate (× 22,000).

We have studied this silica condensate using intratracheal and intraperitoneal injections in rats (Miller and Sayers' test [6]). The object of intraperitoneal injection was to obtain a less violent effect so that the animals would survive. The fibrosing action of this silica could then be assessed, which is impossible in animals dying too quickly after intravenous or intrapulmonary injections. Following our work on the peritoneal test,[7] we recorded only the changes in the omentum and mediastinal glands and excluded the larger nodules seen in the abdominal walls, which are valueless for test purposes.

Honorary Professor, Medical Faculty of the University of Lyon (Dr. Policard).
From the French Collieries Study and Research Center, Paris and Verneuil (Oise).

EXPERIMENTAL TECHNIQUE

The silica condensate used was collected in aspiration tubes in factories where quartz is melted.

The distribution of particle sizes was determined by the electron microscope, as shown in Figure 1. This distribution was as follows:

Particle Sizes, Mμ	Particles, %
< 14	0.1
< 20	2.3
< 25	26.4
< 33	61.6
< 50	79.9
< 100	96.5
< 150	99.9

X-ray diffraction, with the use of Debye and Scherrer's method, has not demonstrated according to Figure 2 any crystalline structure in this silica. Spectrographic analysis has shown the silica content to be 99.9%, the impurities being Ca, Na, and K.

Rats of the Wistar strain, with an average weight of 200 to 250 gm., received intratracheal and intraperitoneal injections of this silica suspended in sterilized

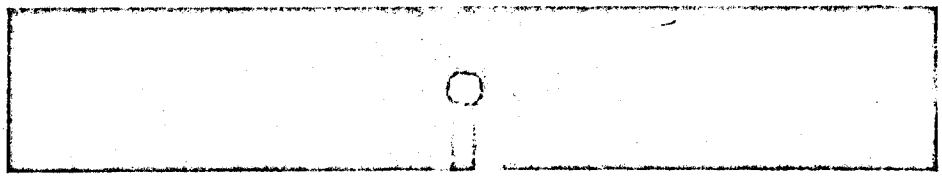

Fig. 2.—Diffraction pattern of Debye and Scherrer.

isotonic saline. Injections of 100, 50, and 30 mg. per kilogram were given. Some experiments were made on rabbits.

The animals that did not die at once were killed after 4, 8, 20, 30, and 60 days, and an autopsy was performed. The miliary nodules in the omentum and the mesenteric and tracheobronchial lymph nodes were studied in those receiving intraperitoneal injections, the lungs and the tracheobronchial lymph nodes in those receiving intratracheal injections. The sections were stained with hemalum-eosin-orange, Masson's trichrome modification of Mallory's methyl blue stain, and Gordon and Sweets's silver stain.

RESULTS

Two effects have to be considered—the general toxic effect and the local fibrosing effect.

I. General Toxic Effect.—Submicroscopic silica particles have a widespread toxic effect. Intravenous injections in the rabbit are nearly always fatal. Intratracheal injections of 30 and 50 mg. in the rat are fatal either immediately or in a few hours in 80 or 90% of the animals. Death results from acute pulmonary edema, and often there are alveolar hemorrhages and dilatation of the pulmonary blood vessels.

Intraperitoneal injection is less harmful. After receiving 100 mg. of silica, only 20 to 30% of the animals die quickly, and with doses of 50 mg. all survive. One can study the fibrosing effects in this last group.

Acute general poisoning has also been studied in animals following intraperitoneal injections. Physical signs appear during the first four days and consist of loss of weight, diarrhea, and prostration. If these signs get worse, death ensues, and postmortem examination gives the following results.

Most of the histiocytes in the peritoneal cavity and omentum show changes. Vacuoles appear in the cytoplasm, and the nuclei are fragmented or destroyed. Areas can be seen with centers made up of damaged cells and with normal or slightly altered histiocytes at the periphery. These areas are always edematous, sometimes intensely so, though the edema diminishes with the passage of time.

The lymph nodes are greatly enlarged. They contain groups of large histiocytes in varying stages of degeneration. The lymphocytes are less numerous and are of a young type. The medullary sinuses are packed with clear cells.

The thymus is atrophied and inverted in appearance, the cortex being clearer than the medulla.

The spleen is hypertrophied and altered in appearance. The malpighian corpuscles have almost completely disappeared, and the zones of blood sinusoids are disorganized.

The liver is generally enlarged and contains many fat cells and clusters of cells, mainly constituted of histiocytes.

The adrenals are frequently enlarged, and there is a 50 to 97% increase in weight. The lipids in the cortex, stained with Sudan red after formol fixation, show a change in their usual distribution. Normally there are few lipids between the glomerular and the fascicular zones and few in the reticular zone. Now there is either a general increase of lipid cells throughout the cortex or, more rarely, a widespread diminution. In nearly every case, the lipid-free area between the glomerular and the fascicular zones has disappeared.

These observations permit the conclusion that a "stress reaction" has been produced by the intraperitoneal injection of silica condensate. Such a reaction is characteristic of the general effect of silica. It confirms the action of the breakdown products of this silica in that they behave like all poisons.

II. Local Fibrosing Action.—Physical signs are always less severe in those animals resistant to early poisoning. When the animals are killed after 8, 20, 30, and 60 days, peritoneal edema progressively diminishes and then disappears. Small spherical nodules, 1.5 to 2 mm. in size, appear on the omentum. They are hard and sometimes confluent and look like frog spawn. The mesenteric and tracheobronchial lymph nodes are two to four times their normal size. These lesions resemble those caused by quartz, but they form earlier and are more accentuated.

Microscopically the peritoneal lesion is a granuloma, the center of which is at first occupied by degenerating and dead cells, then by altered histiocytes of different types (Fig. 3). The outer edge of the granuloma is made up of histiocytes, whose cytoplasm is basophile in reaction and whose nuclei are large, clear, and irregular. Specific staining of the connective tissue shows a fibrous reaction. The latter is poor at first but gradually increases. After 20 to 30 days the center of the granuloma consists of a few cells and irregular, thickened collagen fibers, lying in all directions. At the periphery there is a packed system of reticular fibers filling the intercellular spaces and surrounding the cells. This network is thicker toward the center, where

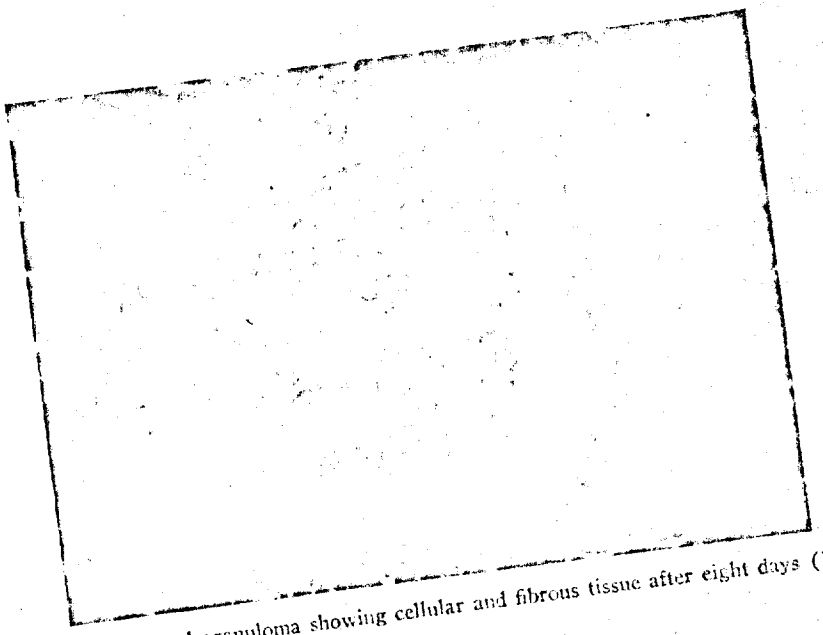

Fig. 3.—Peritoneal granuloma showing cellular and fibrous tissue after eight days (Masson's trichrome stain; × 300).

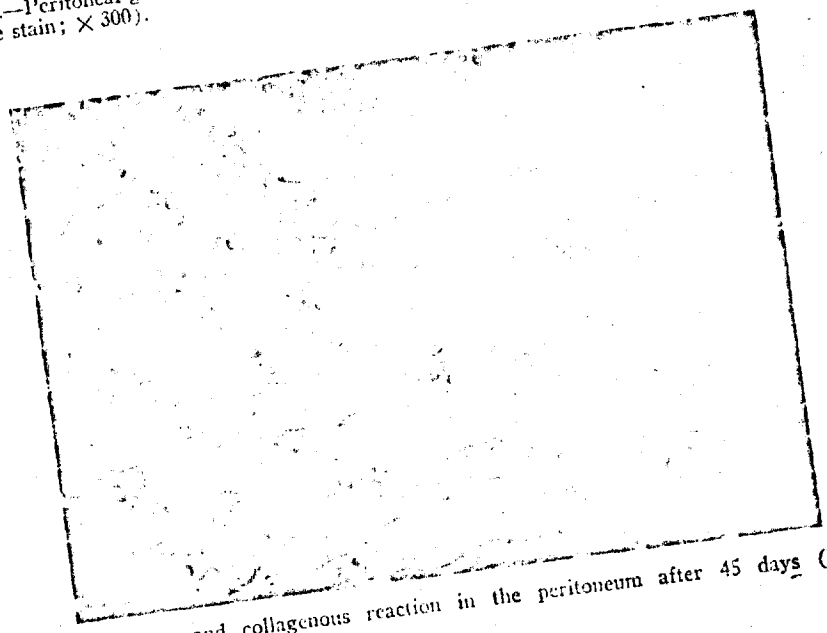

Fig. 4.—Fibrous and collagenous reaction in the peritoneum after 45 days (Masson's trichrome stain; × 300).

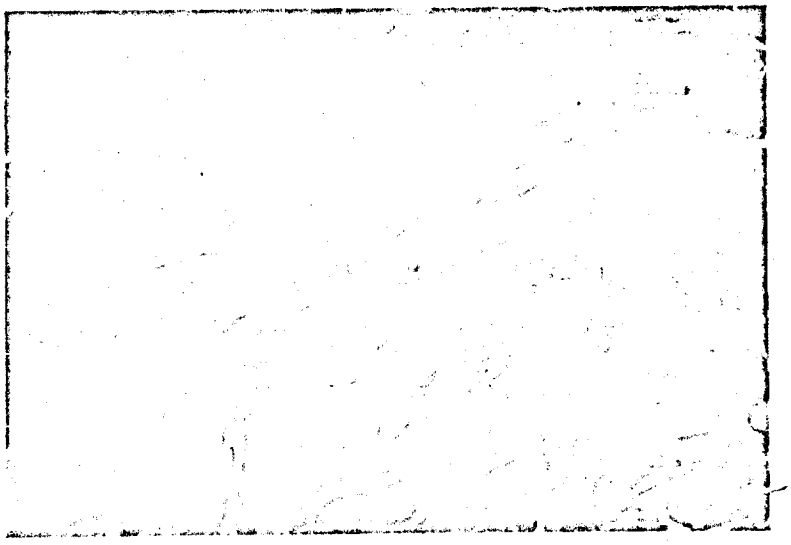

Fig. 5.—Another view of the fibrous reaction after 45 days (Masson's trichrome stain; × 500).

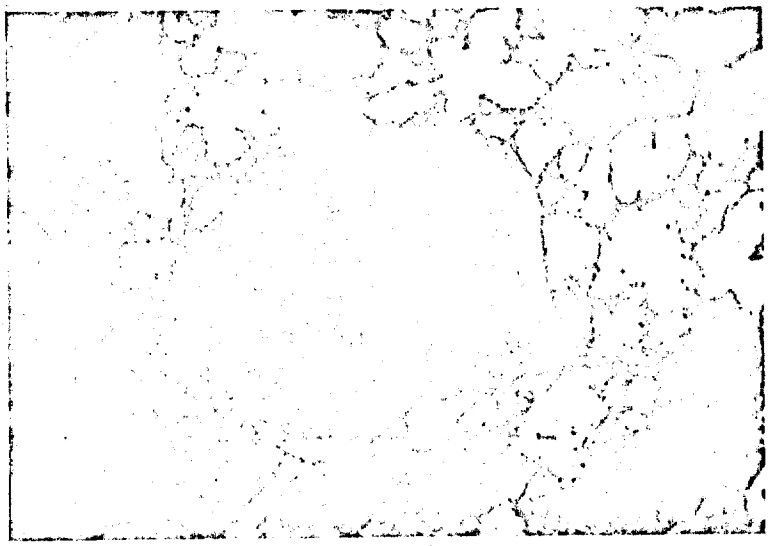

Fig. 6.—Pulmonary nodule 60 days after intratracheal injection (Gordon and Sweets's silver stain; × 70).

it joins the collagen fibers. This fibrosis gradually increases, and after 30 to 60 days one can see extensive fibrous areas which are almost acellular (Figs. 4 and 5). A similar process occurs in the lymph nodes, though it is less rapid than in the omental nodes. The zones of clear cells are considerably altered, like those in the omentum. Networks of reticular fibers form, become more dense, and change to collagen in the same way as in the peritoneal granulomata.

A small proportion of the rats survived intratracheal injections of silica condensate. Changes similar to those described above have been seen, namely, formation of confluent nodules of histiocytes, with subsequent fibrosis due first to reticular fibers and then to collagen (Fig. 6). The histology of the pulmonary and that of the peritoneal lesions correspond exactly. This work shows the value of the peritoneal test for assessing the toxicity of mineral dusts.

The lesions produced by silica condensate are comparable to those produced by crystalline silica (quartz), except that the former occur more quickly and are grosser. After the injection of silica condensate, fibroblasts which can be stained by Mallory's methyl blue method are visible four to five hours after injection.

COMMENT

Experiments previous to ours have shown the general intense toxic effects of submicroscopic particles of silica, and we have confirmed these results. On the other hand, they have not in the main demonstrated the fibrosing effects. What is the reason for this discrepancy?

There is one possible explanation. Electron microscopy reveals that many of the submicroscopic silica particles used in other experiments ("aerosol," for example) are finer than the silica particles used by us. This property renders the substance so toxic that the animals die before fibrosis can develop. This explanation does not apply, however, to the experiments of Lundgren and Swensson.[5] On the contrary, one can recall the much smaller doses that were used (6 to 60 mg. per kilogram, instead of 150 to 500 mg. in our experiments). Perhaps one also recalls an inadequate interpretation of the reactions produced by the intraperitoneal test.

CONCLUSIONS

The results of our experiments would seem to allow the following conclusions:

1. The end-product of silica condensate is sufficiently toxic to cause a "stress reaction" (as shown by changes in the adrenals and thymus). When a sufficient amount is injected locally, this substance affects cells, characteristically causing vacuoles in the cytoplasm and fragmentation of nuclei.

2. Amorphous silica in submicroscopic particles possesses a distinctive fibrosing action, qualitatively similar to that possessed by crystalline silica (quartz) but quantitatively more powerful.

3. This generalized toxic quality and the local fibrosing quality cannot be separated, as Giese[9] has done, since one finds both these qualities in the same specimen of silica condensate. The fibrosing effect must be considered together with the constant cell changes shown in the living cell by phase-contrast microcinematography[8] and in tissue cultures.[10]

4. Our results show that noncrystalline amorphous silica really does have a fibrosing action. This fact was brought forward first by Gye and Purdy[11] and

then more clearly by Campbell [12] using precipitated silica. Recently King and his co-workers obtained an experimental pulmonary fibrosis with fused silica, but this fibrosis is different from that caused by crystalline silica.[13]

REFERENCES

1. Gardner, L. U., in Silicosis and Asbestosis, edited by A. J. Lanza, New York, Oxford University Press, 1938.

2. King, E. J.: Solubility Theory of Silicosis, Occup. Med. **4**:26-49, 1947.

3. Jötten, K. W., and Klosterkötter, W.: Die Bedeutung der Löslichkeit der Kieselsäure für das Zustandekommen der Pneumonokoniosen, Arch. Hyg. **136**:1-4, 1952.

4. Gärtner, H.: Untersuchungen über die Wirkung feinkörniger, amorpher Kieselsäure in der Lunge von Kaninchen, Arch. Hyg. **136**:451-467, 1952.

5. Lundgren, K. D., and Swensson, A.: Experimental Investigations on the Significance of the Size of the Particles in the Reaction of the Peritoneum to Amorphous Silica, Acta med. scandinav. **145**:84-90, 1953.

6. Miller, J. W., and Sayers, R. R.: Microscopic Appearance of Experimentally Produced Dust Nodules in the Peritoneum, Pub. Health Rep. **50**:1619-1628, 1935.

7. Policard, A., and Collet, A.: Comptes Rendus du Congrès international sur les pneumoconioses, Münster, 1953, to be published.

8. Giese, W.: Experimentelle Untersuchungen zur Staublungenfrage, Beitr. path. Anat. **94**:442, 1935; cited by Jötten, D. W., in Sitzungsber. Bundesarbeitbl. **10**:220, 1951.

9. Policard, A., and Collet, A.: Étude par microcinématographie en contraste de phase de l'action des particules de charbon sur le comportement des phagocytes, Compt. rend. Acad. sc. **235**:680-681, 1952.

10. Ceelen, W.: Zum Staublungenproblem, Beitr. Silikoseforsch. No. 13, pp. 3-15, 1951.

11. Gye, W. E., and Purdy, W. J.: Poisonous Properties of Colloidal Silica: Effects of Repeated Intravenous Injections on Rabbits; Fibrosis of Liver, Brit. J. Exper. Path. **3**:86-93, 1922.

12. Campbell, J. A.: Fibrotic Nodules in the Tracheo-Bronchial Lymph Glands of the Mouse Produced by Breathing Precipitated Silica, Brit. J. Exper. Path. **22**:76-80, 1941.

13. King, E. J.; Mohanty, G. P.; Harrison, C. V., and Nagelschmidt, G.: Action of Different Forms of Pure Silica on Lungs of Rats, Brit. J. Indust. Med. **10**:9-17, 1953.

J. d'Urol. med. chir.
60:164-71, 1954.

Experimental study of renal lesions caused by the elimination of silica

by

A. Policard and A. Collet.

The toxicity of certain forms of silica is no longer disputed today. Its importance in the production of occupational pulmonary fibrosis (silicosis) has led to numerous experimental studies. This toxicity, however, involves not only the lungs. Other organs, notably the kidneys, may be subject to its effects.

In the course of experimental research on the pathogenesis of silicosis we have had occasion to assemble a number of histopathologic data on renal lesions produced experimentally by silicosis in rats. The object of this report is to give an account of these findings.

Position of the problem

Numerous authors have studied the elimination by the kidney of silica administered by different routes.

The first of these, Gye and Purdy (1), induced renal lesions by parenteral administration of silicic acid. The product used was sodium silicate with HCl added until neutrality. In rabbits, intravenous doses of 30 to 70 mg per day caused

death within 2 to 4 days. In these cases intense glomerular lesions were observed in the kidneys (necrosis of the capillary tufts, hyperemia, exudation of plasma into the glomerular spaces), numerous cylinders and a very slight involvement of the tubular epithelium.

Strong doses injected into the peritoneal cavity of mice are invariably fatal. In these cases there have been no reports of anatomico-pathologic examinations.

With intravenous injections of lower daily doses to rabbits, of 0.25 to 10 mg, and weekly doses of 30 mg, the animals can be kept alive for 18 to 28 weeks. In those cases lesions are observed almost exclusively in the glomeruli, initially with hemorrhages or necroses of the capillary tuft, and later a thickening of the capsular membrane which may go as far as total fibrosis of the glomerulus. There is also intertubular fibrosis and the appearance of cylinders.

Following these works many authors have studied the urinary elimination of silica administered by various routes. Contrary to the results of earlier studies, no renal lesions were reported. King et al. (2), using dogs, administered silica orally or intravenously and quickly found roughly half the administered silica again. Holt (3), using rats, has studied urinary excretion by means of a radioactive silicon isotope. Quite recently Scheel et al. (4) have followed the elimination of urinary silica chemically following the intraperitoneal injection of silica gel and they found that the silica was rapidly eliminated in the form of alkaline silicate. The latter authors found

diuresis to be double in volume during the first days; the silica excreted was not found to be toxic.

In the clinical aspects of silicosis, the question of renal lesions has only been the subject of one single work. Saita et al. (5) have observed slight impairment of renal function. These authors do not attribute these changes directly to the silicosis. In truth, the small quantity of silica discussed in connection with the silica particles in the lungs explains the absence of toxic effects on the kidneys.

We asked ourselves whether the toxicity of silica in dispersed form might not be more a question of concentration. By intravenous injection, too strong a dose immediately kills the animals. Non-lethal doses appear to be easily eliminated. Until the present only Gye and Purdy have given daily intravenous injections of a product that caused alterations in the vessels, This may explain their unique results. We therefore sought a route of administration and a form of silica that would yield high concentrations of silica without being fatal. This led us to inject strong concentrations of fresh silica gel into the peritoneal cavity of albino rats.

Experimental methods

The silica gel was prepared by electrolysis of sodium silicate (8), which yields a very pure product with silica concentrations close to 5%. This is a polymer of silicic acid SiO 3112, which has the consistency of a semi-solid gel. This gel was sterilized with ultraviolet rays and chemically analyzed

to establish a volume:weight ratio of silica (SiO_2). The gel can then be introduced aseptically either by means of an abdominal incision or by intraperitoneal injection by syringe with a large needle. Forty-five to 90 mg silica has been administered in one dose by this means.

The animals were killed or died spontaneously after variable periods ranging from 16 hours to 20 days. Different viscera were removed for histologic examination after fixation in Bouin-Hollande. We will consider only the kidneys here, which were studied in paraffin sections after trichrome staining according to P. Masson and silver impregnation of the collagen and reticulin according to Gordon and Sweets (7).

Results

Without being too arbitrary, the results can be classified into 4 groups as a function of the time elapsed since the injection. This appears to be the essential factor. The silica dose, within the limits of our experiments, has little effect on the appearance of the lesions.

The groups were as follows:

a) Time since the injection of 16-19 hours

b) 3-5 days

c) 7-8 days

d) 20 days.

a) Time since the injection of 16-19 hours

With the naked eye the kidneys appeared normal, possibly somewhat soft upon cutting. On the other hand under the micro-

scope an acute tubular necrosis was observed, mainly involving the convoluted tubules (segments with rods and with striated border). The necrosis was distributed over a varying number of areas of various sizes in different animals, but it was generally severe. The cytoplasm had sometimes disappeared, or revealed a few sparse fragments on the basal layer. The remaining nuclei were fragmented or pyknotic. The destruction involved certain glomerulo-tubular systems, while adjacent ones appeared completely normal. One detail which will assume greater importance later was that the stainability of the cytoplasm of the necrotized tubules persisted; even cell fragments stained readily with scarlet. Numerous hyaline cylinders were observed, either in destroyed tubules, or in the corresponding right tubules, the latter being on the whole normal. The glomeruli were not involved.

Thus we were confronted by an acute toxic epithelial nephritis, which recalled acute mercury nephritis. In the 5 animals examined at this stage no infection was observed. One of the kidneys was normal.

b) Time since injection of 3-5 days.

Macroscopically the kidneys were either normal, or pale, of soft consistency. Histologic examination showed the development of acute epithelial nephritis with tubular necrosis and hyaline cylinders, the latter being the predominant lesions. Cortical areas of the convoluted tubules were observed whose cytoplasm had lost its stainability with scarlet. The most striking

feature was the shape of the tubules, which was well preserved, and that of the cell nuclei. The glomeruli were generally normal. Occasionally there was what appeared to be fibrinous fluid in fairly large quantities in the glomerular spaces. At this stage there was no infection. It should be noted that out of 6 animals examined at this period, 3 died spontaneously of acute pulmonary edema. It is not known whether there was any relationship between the silica injection and the pulmonary edema. There may have been a disease of viral origin.

c) Time since injection of 7-8 days.

At this stage chamois-colored spots were often observed on the surface of the kidneys, giving them a marbelized appearance, or colored purple against a paler than normal background.

Microscopically at this stage there were surprisingly numerous cases of acute ascending pyelonephritis. Bellini's tubules and the right and left convoluted tubules were dilated, with thinned or altered walls and lumina filled with intact or altered polynuclears. The glomeruli were not affected. The frequency of this renal infection is unusual, for it is hardly ever observed in the normal rat. What connection is there between the silica and the infection? Since these lesions were discovered by histologic examination, the bladders were not removed, yet this is where the origin of the infection could have been sought. Can an ascending infection be enhanced by disturbances in urinary excretion? Or by the initial dilatation of the urinary tubules? This question remains unsolved.

In addition to the inflammatory lesions, or mixed with them, there were lesions apparently directly caused by the elimination of the silica. These gave the preparations a fairly schematic appearance throughout: a triangular topography with a subcapsular base, very variable in number and size, penetrating in wedges towards the medulla, often without reaching it. The tubules enclosed in these triangles could not be stained with scarlet. While sometimes of normal appearance, at other times there was fusion of their cytoplasm involving the nuclei. The glomeruli were usually normal.

This period of seven days appears to be a period of transition. Sometimes necrotized tubules were still observed, as in the first days, while at other times light areas of interstitial fibrosis were observed, accompanied by a slight thickening of the parietal layer of Bowman's capsule of the glomerulus.

d) Time since the injection of 20 days

With the naked eye the kidneys were either normal or they had a granular, sometimes somewhat wrinkled surface. Under the microscope, infections were rarely observed: in 4 out of 15 rats. The dominant feature was the picture of interstitial nephritis, even glomerulonephritis, with fibrosis between the altered, often dilated tubules.

These alterations, as before, were in the form of triangles on a capsular base. Their expanse and number could be very considerable. Once again we were struck by the loss of stainability of the cytoplasm with scarlet. In these zones the split

of the tubules was considerable, some tubules being reduced to 1-2 nuclei surrounded by a fine cytoplasmic halo. The nuclei were suprisingly little altered. There were scarcely any pyknoses or fragmentations. Staining of the reticulin by silver impregnation showed alteration of the normal reticular stroma of the kidney in these kidneys. The peritubular fibrils underwent a kind of increase, which densified them while multiplying them. Mallory's blue also revealed tubules tightly pressed by a thick fiber sheath. The parietal portion of Bowman's capsule was frequently thickened, showing glomerular involvement.

Thus one may speak of glomerulonephritis with tubular alterations and interstitial fibrosis, succeeding the initial acute epithelial lesions.

The right tubules were little affected by these processes. Their alterations for practical purposes were manifested only by ascending infection and pyelonephritis. Examination of the blood vessels revealed no lesions. The kidney capsule was normal.

Discussion

What connection is there between these various types of alterations? The regression of the initial acute toxic nephritis appears to be very quick and complete, as is generally the rule in cases of acute toxic nephritides. We have been unable to discern the course of this regression. Can these later lesions with tubular splitting and interstitial fibrosis be considered as points of cicatrization? This does not seem probable in view of the difference in topography compared to the intial acute

lesions. The latter are distributed completely at random in the cortex, while the wedge-shaped chromophobic areas are generally under the capsule.

Is there then a connection between the pyelonephritis and the interstitial nephritis? This does not seem likely either, for in certain cases areas that have lost their affinity for scarlet coexist with infected zones, without covering them. The pyelonephritis appears either to lead to death of the animal towards the 7th day, or to subside more or less completely (low frequency at 20 days). Finally, it is not invariably present.

The pathogenic explanation of these lesions still remains hypothetical. Acute toxic nephritis may be linked to the silica concentration in the convoluted tubules, or to changes in pH which alter the physicochemical nature of the silica (alkaline silica or colloid silica). The concentration would seem to be the most likely phenomenon, if we refer, on the one hand, to the works of Scheel et al. (4), who have shown that urinary silica is in the form of an alkaline silicate, and on the other, to those of Holt and Yates (8), who have stated that renal silica is a mineral silicate which cannot be extracted with organic solvents.

The pyelonephritis appears while the acute nephritis develops or subsides, possibly due to changes in urinary output. We believe that the infection is not linked to this third type of lesion.

These lesions are difficult to interpret. They may be due to a slower silica poisoning in which the cytoplasm of the tubular cells loses its affinity for scarlet and which secondarily causes the proliferation of the reticulin fibers under the basal layer and thus splitting of the tubules. Or, the silica might act directly on the interstitial connective tissue of the kidney, manifesting the fibrotic effect well known in silicosis. It would be of great interest to establish this point. Its solution would be of primary importance in explaining the mechanism of silicosis.

The resemblance between such pictures and those recently described by J. Oliver (9) is striking. These demonstrate the restoration of the nephrons, but without regeneration of their characteristic cytoplasmic arrangement (mitochondrial rods, brush border).

In summary, from the standpoint of general pathology of the kidney, a mineral poison, after an ordinary stage of acute nephritis, appears to give rise to progressive interstitial glomerulonephritis. Later investigations will be aimed at determining the more long-term development of these lesions, particularly to establish whether in the absence of the wholly absorbed poison the interstitial fibrosis can nevertheless continue to develop.

Summary

The single intraperitoneal administration of silica gel in

a dose of 45 to 90 mg to rats causes almost invariable renal lesions. These lesions develop in the course of time and may be divided into 3 phases.

1. Phase of typical acute toxic epithelial nephritis, recalling mercury nephritis, without glomerular involvement, detectable 16 hours after the injection.

2. Transitional period towards the 7th day, during which the toxic nephritis subsides and foci of interstitial nephritis appear. As a rule this period is marked by a large number of inflammatory lesions of the pyelonephritic type, involving highly dilated tubules.

3. Phase of progressive tubular atrophy with interstitial fibrosis and glomerular alterations, which may be designated as interstitial glomerulonephritis.

The connections between the three types of lesions are discussed, without arriving at objective conclusions. The problem is raised whether silica first gives rise to epithelial or interstitial damage in connection with late lesions, a problem which is of considerable importance for the understanding of silicosis. Finally the following experimental finding is underscored: The development of a chronic experimental nephritis occurring after an acute toxic lesion caused by a mineral compound.

Figures

1. Acute tubular necrosis 19 hours after intraperitoneal injection of silica gel. Masson trichrome staining, 460x.

2. A different picture of tubular necrosis after a period of 4 days. Same technical conditions as in fig. 1.

3. Subacute infection of the urinary tubules 7 days after an injection of silica gel. Same technical conditions as in Fig. 1.

4. Atrophy and loss of differentiation of the convoluted tubules. Interstitial sclerosis. Thickening of the Bowman capsule. Same technical conditions as in Fig. 1.

Bibliography

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5. G. Saita and O. Zavaglia. Renal function in silicotic subjects. Med. de Lavoro, 42:41-48, 1951.

6. Renault National Mine Bureau. Solutions of concentrated silicic acid. F.P. 968, 361, June 25, 1948.

From: Center for Carbon Study and Research France, Paris-Verneuil, Oise).

ÉTUDE EXPÉRIMENTALE DES LÉSIONS RÉNALES PROVOQUÉES PAR ÉLIMINATION DE LA SILICE

Par A. POLICARD et A. COLLET

La toxicité de certaines formes de silice n'est plus discutée aujourd'hui. Son importance dans la genèse des fibroses pulmonaires professionnelles (silicose) a donné lieu à de nombreuses études expérimentales. Cette toxicité, cependant, ne s'exerce pas seulement sur le poumon. D'autres organes, et en particulier le rein, peuvent subir cette action.

Au cours de recherches expérimentales sur la pathogénie de la silicose, nous avons pu réunir un certain nombre de documents histopathologiques sur les lésions rénales provoquées expérimentalement par la silice chez le rat. Le but de cet exposé est de donner une relation de ces faits.

Position du problème.

De nombreux auteurs jusqu'ici ont étudié l'élimination par le rein de la silice administrée par des voies diverses.

Les premiers, Gye et Purdy [1] ont déterminé des lésions rénales par administration parentérale d'acide silicique. Le produit utilisé était du silicate de Na additionné d'HCl jusqu'à neutralité. Chez le lapin, des doses intraveineuses de 30 à 70 mg par jour provoquent une issue fatale en deux à quatre jours. On observe alors au niveau des reins des lésions glomérulaires intenses (nécrose du flocule capillaire, hyperhémie, exsudation plasmatique dans la chambre glomérulaire), de nombreux cylindres hématiques et atteinte épithéliale tubulaire très modérée.

De fortes doses injectées dans la cavité péritonéale chez la souris sont toutes mortelles. Les auteurs ne rapportent pas d'examens anatomo-pathologiques dans ces cas.

Des injections intraveineuses chez le lapin de doses plus modérées, quotidiennes, de 0,25 à 10 mg et hebdomadaires, de 30 mg permettent de conserver les animaux dix-huit à vingt-huit semaines. Les auteurs observent alors au niveau des reins des lésions presqu'exclusivement glomérulaires avec, au début, des hémorragies ou des nécroses du flocule capillaire et, plus tardivement, un épaississement de la mebrane capsulaire qui va jusqu'à la fibrose totale du glomérule. Il y a, d'autre part, une fibrose interstitiale et des cylindres.

A la suite de ces travaux, de nombreux auteurs ont étudié l'élimination urinaire de la silice administrée par des voies diverses. Contrairement aux résultats des premières recherches, il n'a pas été signalé de lésions rénales. King et coll. [2] chez le chien injectent la silice par voie digestive ou veineuse et retrouvent rapidement la moitié environ de la silice introduite. Holt [3], chez le rat, étudie l'excrétion urinaire à l'aide d'un isotope radioactif du Silicium. Tout récemment, Scheel et coll. [4] suivent chimiquement l'élimination de la silice urinaire après injection intra-péritonéale de gel de silice et constatent que cette silice s'élimine rapidement sous forme de silicate alcalin. Ces derniers auteurs constatent seulement une diurèse

doublée de volume les premiers jours ; la silice ainsi excrétée ne serait pas toxique.

Cliniquement dans la silicose la question des lésions rénales a fait l'objet d'un seul travail. Saita et coll. [5] observent de légères atteintes de la fonction rénale. Ces auteurs n'attribuent pas directement à la silice les modifications constatées. A la vérité, la faible quantité de silice dissoute à partir des particules de silice pulmonaires permet de comprendre l'absence d'effets toxiques rénaux.

Nous nous sommes demandé si la toxicité de la silice sous forme dispersée n'était pas une question de concentration. En effet, par injections intraveineuses une dose trop forte tue immédiatement les animaux. Les doses non létales semblent aisément éliminées. Jusqu'à présent, seuls, Gye et Purdy ont pu réaliser des injections intraveineuses quotidiennes avec un produit altérant les vaisseaux ; cela explique peut-être leur résultat isolé. Nous avons alors cherché une voie d'administration et une forme de silice capables de réaliser de hautes concentrations de silice sans être mortelles. Cela nous a conduit à injecter dans la cavité péritonéale de rats blancs de la gelée de silice fraîche à forte concentration.

Méthodes expérimentales.

La gelée de silice est préparée par électrolyse de silicate de sodium [6], technique permettant d'obtenir des produits très purs à des concentrations voisines de 5 p. 100 en silice. On est alors en présence d'un polymère de l'acide silicique SiO_3H_2, possédant la consistance d'une gelée mi-solide. Cette gelée est stérilisée par les rayons ultra-violets puis dosée chimiquement de manière à établir une relation volume-poids de silice (SiO_2). On peut alors (aseptiquement) soit introduire la gelée par une incision abdominale, soit pratiquer une injection péritonéale à l'aide d'une seringue et d'une grosse aiguille. On administre ainsi 45 à 90 mg de silice en une fois.

Les animaux sont sacrifiés, ou meurent spontanément, après des délais, variables s'étageant de seize heures à vingt jours. Différents viscères sont prélevés pour examens histologiques après fixation par le Bouin-Hollande. Nous aurons en vue ici seulement les reins, examinés sur des coupes à la paraffine après coloration au trichrome de P. Masson et imprégnation argentique du collagène et de la réticuline d'après Gordon et Sweets [7].

Résultats.

Les résultats peuvent sans trop d'arbitraire être classés en 4 groupes en fonction du temps écoulé depuis l'injection. Ce semble être là le facteur essentiel. La dose de silice, dans les limites de notre expérience, intervient peu sur l'aspect des lésions.

Ces groupes sont les suivants :

a) délais de seize à dix-neuf heures ;

b) de trois à 5 jours ;

c) de sept à huit jours ;

d) de vingt jours.

a) *Délais de seize à dix-neuf heures.* — A l'œil nu, les reins paraissent normaux, un peu mous peut-être à la coupe. Au microscope, au contraire, on observe une nécrose tubulaire aiguë portant avant tout sur les tubes contournés (segments à bâtonnets et à bordure striée). Cette nécrose est répartie en plages, de nombre et d'étendue variables suivant les animaux, mais généralement importantes. Le cytoplasme a parfois disparu ou a laissé

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quelques rares débris contre la basale. Les noyaux restants sont fragmentés ou pycnotiques. La destruction touche effectivement certains systèmes glomérulo-tubulaires, leurs voisins paraissent tout à fait normaux. Détail qui prendra plus loin sa valeur, la colorabilité du cytoplasma des tubes nécrosés est conservée ; même les fragments de cellule se teignent par le ponceau. Par ailleurs on observe de nombreux cylindres hyalins, soit dans les tubes détruits, soit dans les tubes droits correspondants, ceux-ci étant normaux dans l'ensemble. Les glomérules ne sont pas atteints.

On est donc en présence d'une néphrite épithéliale aiguë du type toxique

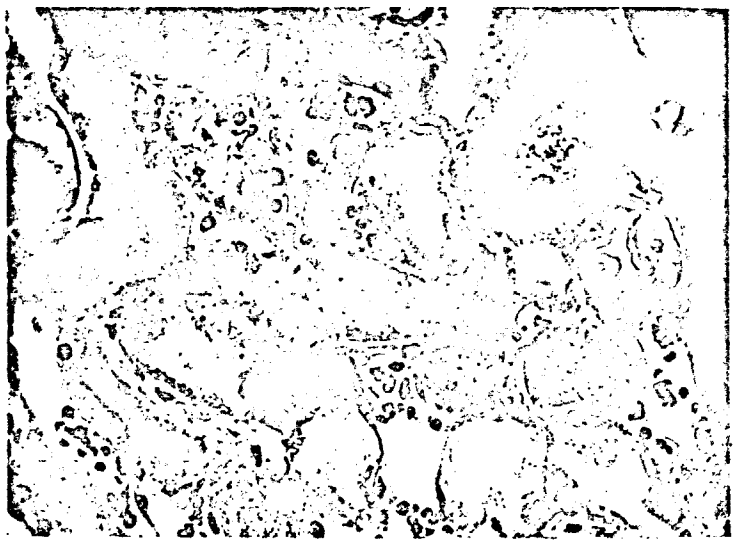

Fig. 1. — Nécrose tubulaire aiguë dix-neuf heures après l'injection intrapéritonéale de gel de silice. — Trichrome de Masson gross. 460 fois.

qui rappelle la néphrite aiguë mercurielle. Chez les 5 animaux examinés à ce stade, on n'a constaté aucune infection. Un des reins était normal.

b) *Délais de trois à cinq jours.* — Macroscopiquement les reins sont ou normaux ou pâles, avec une consistance molle. L'examen histologique montre l'évolution de la néphrite épithéliale aiguë avec nécrose tubulaire et cylindres hyalins, qui sont les lésions prédominantes. On relève par ailleurs des plages corticales de tubes contournés dont le cytoplasme a perdu sa colorabilité par le ponceau. Ce qui frappe est le plus souvent la forme des tubes, bien conservée, ainsi que celle des noyaux cellulaires. Les glomérules sont habituellement normaux. On observe parfois la présence dans la chambre glomérulaire de liquide apparemment fibrineux en quantité assez grande. Il n'y a pas d'infection à ce stade. Notons que sur 6 animaux examinés à cette période, 3 sont morts spontanément d'œdème pulmonaire

aigu. On ne peut dire s'il y a une relation entre l'injection de silice et l'œdème pulmonaire, il peut s'agir d'une affection d'origine virale.

c) *Délais de sept à huit jours.* — On observe alors fréquemment des taches de teinte chamois à la surface des reins, leur donnant un aspect marbré ou bien pigmenté lie de vin sur un fond plus pâle que normalement.

Microscopiquement à cette période, on observe avec une fréquence surprenante de la pyélonéphrite aiguë du type ascendant. Les tubes de Bellini, les tubes droits et les tubes contournés sont dilatés, à paroi amincie

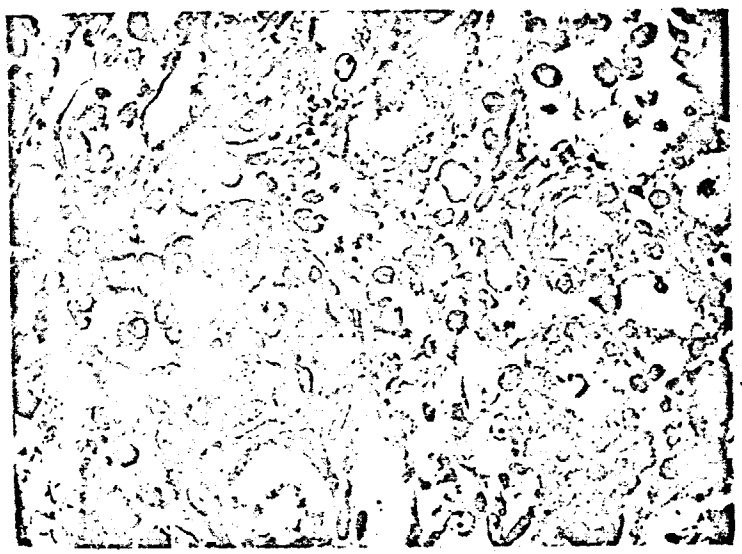

Fig. 2. — Autre aspect de nécrose tubulaire après un délai de quatre jours. Mêmes conditions techniques que fig. 1.

ou altérée, avec une lumière remplie de polynucléaires intacts ou altérés. Les glomérules ne sont pas atteints. La fréquence d'une telle infection rénale est singulière car elle ne s'observe pratiquement jamais chez le rat normal. Or, sur 11 animaux expérimentés, 8 présentaient de l'infection urinaire. Quelle relation unit la silice et l'infection ? Ces lésions ayant été une découverte d'examens histologiques, aucun prélèvement de vessie n'a été fait qui eût permis d'y rechercher l'origine de l'infection. Une infection ascendante est-elle favorisée par des troubles de l'excrétion urinaire ? Ou bien par la dilatation initiale des tubes urinaires ? La question n'a pas été résolue.

A côté des lésions inflammatoires ou mélangées avec elles, on observe des lésions semblant dues directement à l'élimination de la silice. Elles prennent un aspect assez schématique retrouvé partout : topographie en

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triangles à base sous-capsulaire, de taille et de nombre très variables s'enfonçant en coin en direction de la médullaire, très souvent sans l'atteindre. On note aussi l'absence de colorabilité par le ponceau des tubes compris dans ces triangles. Souvent d'aspect normal, ils montrent d'autres fois une fonte cytoplasmique respectant longtemps les noyaux. Les glomérules sont habituellement normaux.

Cette période de sept jours doit être une période de transition. On observe parfois encore des tubes nécrosés, comme dans les premiers jours, et d'autres fois l'apparition dans les plages claires d'une fibrose interstitielle

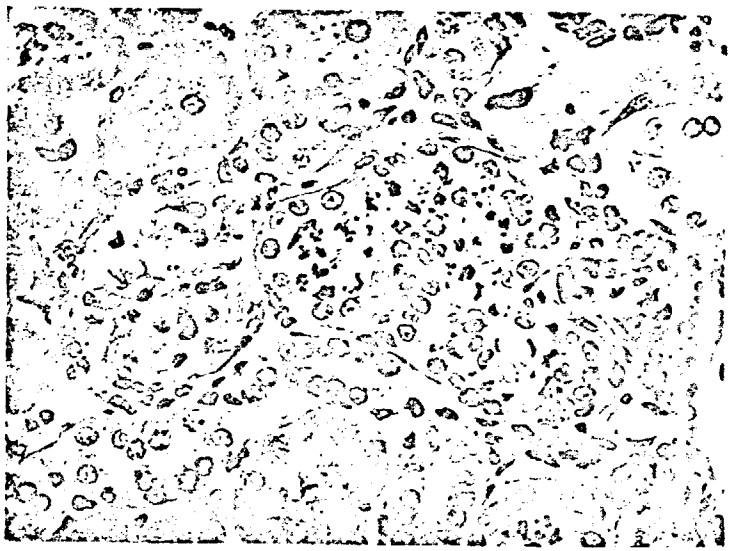

Fig. 3. — Infection subaiguë des tubes urinaires, sept jours après une injection de silice gélatineuse. — Mêmes conditions techniques que fig. 1.

s'accompagnant alors d'un léger épaississement du feuillet pariétal de la capsule de Bowmann du glomérule.

d) *Délais de vingt jours.* — A l'œil nu, les reins sont ou normaux ou avec une surface grenue, un peu ridée parfois. Au microscope, l'infection est rare : quatre fois sur 14 rats. Ce qui domine est l'aspect de néphrite interstitielle, glomérulo-néphrite même, avec fibrose s'installant entre des tubes altérés, fréquemment dilatés.

Ces altérations se présentent comme précédemment, sous la forme de triangles à base capsulaire. Leur étendue et leur nombre peuvent alors être très grands. Là encore, on est frappé par la perte d'affinité des cytoplasmes pour le ponceau. Dans ces zones, la fonte tubulaire est importante, certains tubes étant réduits à un ou deux noyaux entourés d'une fine auréole cytoplasmique. Les noyaux sont étonnamment peu altérés. On découvre avec

peine quelques pycnoses ou fragmentations. La coloration de la réticuline par imprégnation argentique montre dans ces reins une altération de la trame réticulaire régulière du rein. Les fibrilles péritubulaires subissent une sorte de foisonnement qui les densifie en les multipliant. Le bleu de Mallory montre également des tubes étroitement serrés par une gaine épaisse de fibres. De même, la partie pariétale de la capsule de Bowmann est fréquemment épaissie, révélant une atteinte glomérulaire.

On peut donc parler de glomérulo-néphrite avec altération tubulaire et fibrose interstitielle, ayant succédé aux lésions épithéliales aiguës du début.

Fig. 4. — Atrophie et perte de la différenciation des tubes contournés. Sclérose interstitielle. Epaississement de la capsule de Bowman. — Mêmes conditions techniques que fig. 1.

Les tubes droits sont peu touchés dans ces processus. Leurs altérations ne se manifestent pratiquement qu'avec l'infection urinaire ascendante et les pyélo-néphrites. L'examen des vaisseaux sanguins n'a pas révélé de lésion. La capsule de l'organe est normale.

Discussion.

Quels sont les rapports respectifs de ces divers types d'altérations ? La régression de la néphrite aiguë toxique du début semble très rapide et complète comme il est de règle pour les néphrites aiguës toxiques. Nous n'avons pas pu saisir la marche de cette régression. Pouvons-nous considérer les lésions plus tardives de fonte tubulaire avec fibrose interstitielle comme

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des points de cicatrisation ? Cela paraît peu vraisemblable en raison de leur différence de topographie avec les lésions aiguës du début. Celles-ci sont distribuées tout à fait au hasard dans la corticale, tandis que les plages chromophobes en forme de coin, sont le plus souvent sous-capsulaires.

Y a-t-il alors relation entre pyélonéphrite et néphrite interstitielle ? Il ne semble pas non plus, car, dans certains cas, les plages ayant perdu leur affinité pour le ponceau coexistent avec les zones infectées, sans les recouvrir. La pyélonéphrite paraît soit entraîner la mort de l'animal vers le septième jour, soit régresser plus ou moins complètement (faible fréquence à vingt jours). Enfin, elle n'est pas constante.

L'explication pathogénique de ces lésions demeure encore d'ordre hypothétique. La néphrite aiguë toxique peut être rattachée à la concentration de la silice au niveau des tubes contournés ou à des modifications de *pH* qui changent la forme physico-chimique de cette silice (silicate alcalin ou silice colloïdale). La concentration paraît être le phénomène le plus probable si l'on se réfère, d'une part, aux travaux de Sheel et coll. [4] qui prouvent que la silice urinaire est sous forme d'un silicate alcalin et, d'autre part, à ceux de Holt et Yates [8] pour qui le Si rénal est un silicate minéral non extractible par les solvants organiques.

L'infection pyélique apparaît alors que la néphrite aiguë évolue ou régresse, peut-être à la faveur de modifications du débit urinaire. Nous ne pensons pas que cette infection soit liée au troisième type de lésions.

Celles-ci sont difficiles à interpréter. Il peut s'agir d'une intoxication plus lente par la silice faisant perdre au cytoplasme des cellules des tubes urinaires leur affinité pour le ponceau et déterminant secondairement la prolifération des fibres de réticuline sous la basale et, par là, la fonte tubulaire. Ou bien, la silice peut agir directement sur le conjonctif interstitiel du rein pour manifester l'effet fibrosant bien connu pour la silice particulaire. Il serait particulièrement intéressant de trancher ce point. Sa démonstration serait capitale pour l'explication du mécanisme de la silicose.

On est frappé de la ressemblance de tels aspects avec ceux que J. Oliver [9] a récemment décrits et qui démontrent la réparation des néphrons atteints mais sans régénération de leurs dispositifs cytoplasmiques caractéristiques (bâtonnets mitochondriaux, bordure en brosse).

En résumé, du point de vue de la pathologie générale rénale, un toxique minéral paraît provoquer, après un stade de néphrite aiguë banale, une glomérulo-néphrite interstitielle évolutive. Des recherches ultérieures viseront à préciser l'évolution plus lointaine de ces lésions, en particulier pour savoir si, en l'absence du toxique totalement résorbé, l'édification de fibrose interstitielle peut cependant se poursuivre.

Résumé.

L'administration en une fois par voie péritonéale de silice gélifiée à la dose de 45 à 90 mg détermine chez le rat des lésions rénales pratiquement constantes. Ces lésions évoluent avec le temps et l'on peut schématiquement distinguer 3 périodes :

1° Phase de néphrite épithéliale aiguë toxique typque, rappelant la néphrite mercurielle, sans atteinte glomérulaire, décelable seize heures après injection ;

2° Période de transition vers le septième jour pendant laquelle la néphrite toxique régresse et des foyers de néphrite interstitielle se dessinent. Habituellement, cette période est marquée par une fréquence élevée de lésions inflammatoires du type pyélonéphrite atteignant des tubes très dilatés ;

3° Phase d'atrophie tubulaire progressive avec fibrose interstitielle et altération glomérulaire que l'on peut qualifier de glomérulo-néphrite interstitielle.

Les rapports des trois types de lésions sont discutés sans que l'on puisse aboutir à une interprétation objective. On soulève le problème d'une atteinte par la silice d'abord épithéliale ou d'abord interstitielle, à propos des lésions tardives, problème présentant pour la compréhension de la silicose un intérêt considérable. On souligne enfin le fait expérimental suivant : constitution d'une néphrite chronique expérimentale survenant après une lésion aiguë toxique par composé minéral.

(Centre d'Etudes et Recherches des Charbonnages de France, Paris-Verneuil, Oise.)

BIBLIOGRAPHIE

[1] W. E. Gye et W. J. Purdy. *Toxic properties of colloidal silica, Brit. J. Exp. Pathol.*, 1922, **3**, 75, et 1924, **5**, 28.

[2] E. J. King, H. Stantial et M. Dolan. The biochemistry of silicic acid. CXXXI. The excretion of administred silica, *Biochem. J.*, 1933, **27**, 1 007-1 014.

[3] P. F. Holt, D. M. Yates et D. H. Tomlin. Distribution of injected Si 31 in rats, *Biochem. J.*, 1951, **48**, xliv.

[4] L. D. Shell, E. Fitzgerald et F. W. Xlemperer. *Toxicity of silica. I. Silica solutions, Arch. Industr. Hyg. Occup. Med.*, 1953, **8**, 564-573.

[5] G. Saita et O. Zavaglia. La funzionalita renale nei silicotici, *Med. del Lavoro*, 1951, **42**, 41-48.

[6] Régie nationale des usines Renault. Solutions d'acide silicique concentrées. *F. P.* 908, 361 du 25 juin 1948.

[7] H. Gordon et H. A. Sweets. A simple method for the silver impregnation of reticulum, *Amer. J. Path.*, 1936, **12**, 545.

[8] P. F. Holt et D. M. Yates. Tissue Silicon : a sutudy of the ethanol soluble fraction using Si 31, *Biochem. J.*, 1953, **54**, 500-505.

[9] J. Oliver. Correlation of structure and function and mechanisms of recovery in acute tubular necrosis, *Amer. J. Medicine*, 1953, **15**, 535-557.

Compt. Rend. Acad. Sci.
251:314-315, 1960.

Silica deposits in the mitochondria of the urinary tubule*

by

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*
Meeting of July 11, 1960.

(1)
With the technical assistance of Christiane Renet.

As has recently been reported in this journal[(2)], oligomeric silicic acid appears to be capable of penetrating by diffusion into the mitochondria of the renal epithelium of experimental animals (rats) having received intraperitoneal silica gel some hours previously. Inside the mitochondria, under the influence of a different equilibrium of the medium, the silica is polymerized and becomes visible in the high resolution electron microscope, in the form of very fine very dense granulations measuring some 40 Å. These granulations are stuck together, either directly or through the agency of a lighter substance of protein appearance. They thus form very electron-dense heterogenous granular masses of a size varying between ca. 200 and 500 Å. They are always situated between the widely spaced internal membranes of the mitochondria. The purpose of the present note is to determine the development of mitochondria thus loaded with silica and their role in renal filtration.

The mitochondria containing these silica deposits do not remain normal. They reveal two kinds of pathologic development, which are easy to detect in the electron microscope.

In the first type, which is the more common, the mitochondrium undergoes pronounced swelling. Its internal membranes, widely scattered, become progressively fainter. The silica masses become increasingly visible in the mitochondrial contents which now appear clear. They appear to continue to increase, which may be explained by the arrival of more oligomeric silica brought in by the water penetrating into the mitochondrium and producing its swelling. Gradually the outline of the mitochondrium blurs. It appears to break up in the ambiant cytoplasmic milieu which is much altered by edema. The masses of silica are then free in the protein magma resulting from the degeneration of the renal epithelial cells. This magma occupies the lumen of the tubules and constitutes the well-known "granular cylinders".

The swelling revealed by the mitochondria containing silica masses does not appear to be directly dependent upon these masses. In fact adjacent mitochondria without silica masses reveal identical alterations. The swelling of mitochondria appears more to be linked to a certain condition of the ambient cytoplasmic milieu, whether or not they contain silica masses. This condition may be the diffusible oligomeric silica in the cytoplasm distinguished by chemical analysis (high silica content of the kidney). The alteration of the mitochondria is certainly

not directly linked to the silica precipitates which they sometimes contain.

In the second type, instead of being swollen, the mitochondria become increasingly densified without changing their dimensions. Inside their dense interior there are silica masses similar to those in the swollen mitochondria, but difficult to discern owing to the absence of contrast. The cause of this increased density of the mitochondria and that of the frequent densification of cytoplasmic areas remains obscure. Is it due to the formation of a dense silico-protein complex similar to that formed around the submicroscopic particles of silica phagocytized by macrophages? This acceptable hypothesis requires confirmation. In any event, the fact of the densification of certain mitochondria and cyoplasmic areas is indisputable.

Generally speaking, light and swollen mitochondria and densified mitochondria are not mixed together. In certain zones of degenerated epithelium the light mitochondria predominate, while in other immediately adjacent zones the dense mitochondria predominate. The reason for this common but not invariable arrangement is not known.

In any event, the final destiny of these two types of mitochondria is identical. Together with the silica masses which they contain, they reappear in the cylinders that occupy the lumen of the injured urinary tubules. For the most part, as is known, these cylinders are borne along by the glomerular urine and are thus excreted. The urinary tubule freed in this

manner may then undergo the well-known process of regenerating a new epithelium. Thus, by means of a complex but efficient circuit, the silica introduced into the kidney is eliminated. This mode of kidney purification, in some way based on cellular degeneration, is somewhat analogous to the purification of the lungs. Here again, through the action of degenerated alveolar cells progressively eliminated through the bronchial passages, the lungs succeed in ridding themselves of silica particles that may have entered them. In both cases, however, the process of purification cannot be entirely complete. Because they are attached to the connective stroma of the kidneys or lungs, some silica deposits may escape elimination through the bronchi or the urinary tubules. They are the cause of persistent and often progressive sclerotic foci both in the lungs and in the kidneys (pneumoconiosis or sclerous silicotic nephritis). In their general course these two processes are comparable.

Bibliography

See footnote reference, p. 315.

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CYTOPATHOLOGIE INFRASTRUCTURALE. — *Dépôt de silice dans les mitochondries du tube urinaire. Rôle dans l'épuration rénale.* Note (*) de MM. **Albert Policard**, **André Collet**, Mme **Henriette Daniel-Moussard** et Mlle **Simone Pregermain** (1):

Dans la néphrose expérimentale par gel de silice intrapéritonéal, SiO_2 oligomère se polymérise dans les mitochondries rénales. Celles-ci dégénérant, elle est entraînée par les cylindres urinaires et excrétée avec eux.

Comme il a été exposé récemment ici même (2), de l'acide silicique oligomère semble capable de pénétrer par diffusion dans l'intérieur des mitochondries de l'épithélium rénal chez l'animal d'expérience (Rat) ayant reçu quelques heures auparavant de la gelée de silice dans le péritoine. Dans l'intérieur de la mitochondrie, sous l'influence de nouvelles conditions d'équilibre dans le milieu, la silice se polymérise et devient visible, par microscopie électronique à haut pouvoir de résolution, sous forme de très fines granulations très denses d'une quarantaine d'angströms environ. Ces granulations s'accolent les unes aux autres, soit directement, soit par l'intermédiaire d'une substance plus claire d'apparence protéique. Elles forment ainsi des masses granuleuses hétérogènes très denses aux électrons et d'une taille variable de 200 à 500 Å environ. Elles sont toujours logées entre les membranes internes largement espacées des mitochondries. La présente Note a pour objet de préciser l'évolution des mitochondries ainsi chargées de silice et leur rôle dans l'épuration du rein.

Les mitochondries renfermant ces dépôts siliceux ne demeurent pas normales. Elles montrent deux types d'évolution pathologique, faciles à caractériser au microscope électronique.

Dans un premier type, le plus fréquent, la mitochondrie subit un gonflement accentué. Ses membranes internes, fortement écartées, s'effacent progressivement. Dans un contenu mitochondrial devenu clair, les masses siliceuses sont de plus en plus apparentes. Elles semblent continuer à s'accroître, ce qu'explique vraisemblablement l'arrivée de nouvelle silice oligomère entraînée par l'eau qui pénètre dans la mitochondrie et provoque son gonflement. Peu à peu le contour de la mitochondrie s'efface. Elle semble se disloquer dans le milieu cytoplasmique ambiant très altéré par l'œdème. Les amas siliceux deviennent alors libres dans le magma protéique résultant de la dégénérescence des cellules épithéliales rénales. Ce magma occupe la lumière des tubes et constitue les « cylindres granuleux » bien connus.

Le gonflement montré par les mitochondries avec amas siliceux ne paraît pas dépendre directement de ceux-ci. En effet, des mitochondries voisines sans amas de silice peuvent présenter des altérations identiques. Le gonflement des mitochondries paraît être plutôt en rapport avec une certaine condition du milieu cytoplasmique ambiant, qu'elles renferment ou non des

amas siliceux. Cette condition est peut-être la silice oligomère diffusible qui se trouve dans le cytoplasma et que l'analyse chimique caractérise (teneur élevée du rein en silice). L'altération des mitochondries n'est certainement pas liée directement aux précipités de silice qu'elles peuvent renfermer.

Dans un second type, au lieu d'être gonflées, les mitochondries sont devenues de plus en plus denses sans changer de dimensions. Dans leur intérieur densifié s'observent des amas siliceux semblables à ceux des mitochondries gonflées, mais difficiles à discerner en raison de l'absence de contraste. L'origine de l'augmentation de la densité des mitochondries, aussi bien que celle de la densification fréquente de territoires cytoplasmiques, est encore imprécisée. Est-elle due à la formation d'un complexe silicoprotéique dense analogue à celui formé autour des particules submicroscopiques de silice phagocytées par des macrophages ? Cette hypothèse acceptable demande confirmation. En tous cas, le fait de la densification de certaines mitochondries et de territoires cytoplasmiques est incontestable.

D'une façon assez générale, les mitochondries claires et gonflées et les densifiées ne sont pas mélangées. Dans certaines zones d'épithélium dégénéré, les mitochondries claires sont dominantes, dans d'autres zones immédiatement voisines, ce sont les mitochondries denses. La raison de cette disposition, fréquente mais non constante, n'est pas précisée.

En tous cas, le sort final de ces deux types de mitochondries est identique. Avec les amas siliceux qu'elles renferment, elles se retrouvent dans les cylindres occupant la lumière des tubes urinaires lésés. Pour la plupart, on le sait, ces cylindres sont entraînés par l'urine glomérulaire et sont ainsi excrétés. Dans le tube urinaire ainsi libéré, peut alors s'opérer le processus bien connu de la régénération d'un épithélium nouveau. Ainsi se trouve éliminée, par un circuit complexe mais efficient, la silice apportée dans le rein. Ce mode d'épuration rénale reposant en quelque sorte sur des dégénérescences cellulaires, n'est pas sans analogie avec l'épuration pulmonaire. Dans ce cas aussi, par le jeu de cellules alvéolaires dégénérées et progressivement éliminées par les voies bronchiques, le poumon arrive à se débarrasser des particules de silice qui ont pu y entrer. Dans les deux cas, toutefois ce processus d'épuration peut ne pas être tout à fait complet. Parce qu'ils sont fixés dans le stroma conjonctif du rein ou du poumon, certains dépôts de silice peuvent échapper à l'élimination par les bronches ou par les tubes urinaires. Ils sont à l'origine de foyers persistants et souvent progressifs de sclérose, dans le poumon comme dans le rein (pneumoconiose ou néphrite scléreuse par silice). Dans leur marche générale, ces deux processus sont comparables.

(*) Séance du 11 juillet 1960.

(1) Avec la collaboration technique de Christiane Reuet.

(2) A. Policard, A. Collet, H. Daniel-Moussard et S. Prégermain, *Comptes rendus*, 251, 1960, p. 17.

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J. Ind. Hyg. Toxicol. 24(4):75-79, 1969

COMMERCIAL TALC
ANIMAL AND MINERALOGICAL STUDIES*

R. Z. SCHULZ[1] AND CHARLES R. WILLIAMS[2]

THE possible pathological effects of exposure to talc dust in industry have been discussed to a certain extent in the literature. It is a well-known fact that wherever talc is used as a dusting agent, workers may be exposed to large amounts of talc dust. Dreessen and Dalla-Valle (1) (2) after studying the effects of exposure to dust in talc mines and mills in Georgia and Vermont arrived at the conclusion that talc does not produce a disabling pneumoconiosis, but does result in diffuse fibrosis, which may be seen in roentgenograms. Experimental work by Gardner (3 and 4) indicates that the reaction caused by talc produced some inflammation which showed no tendency to progress in 2 years. There is, however, one important factor which has not been investigated, and that is the effects of variations in composition of commercial talcs. The term "talc" as used industrially generally refers to a substance which meets certain physical requirements rather than one which has a definite chemical composition. The mineral talc, is a specific substance, a hydrous magnesium silicate ($H_2Mg_3(SiO_3)_4$). Several minerals may accompany it as it occurs in nature and some of them may be quantitatively more important than the talc. The most prominent minerals are serpentine, (hydrous magnesium silicate, $H_4Mg_3Si_2O_9$), dolomite (calcium magnesium carbonate), and tremolite (calcium magnesium silicate). Variations in composition of "talcs" used in industry (in cosmetics, as fillers in paper, paints, lineoleum, as a dusting agent in the rubber industry, etc.) are extreme (5).

For this reason, the writers deemed it advisable to study the effects on guinea pigs of several different types of so-called "talcs" used industrially.

A series of 7 commercial "talcs" with widely-different mineralogical compositions were used in the experiments. They were picked from the group of 51 analyzed talcs (Table 1) to represent the whole range of composition found. The mineralogical analyses of the experimental samples and the silicate-carbonate ratios are shown in Table 2. These ratios are given since we have assumed in the past that a "talc" with a low proportion of silicates would be safest to use. The numbers, 814-6A; 814-11C, etc., will be used to identify these talcs in subsequent discussions.

* Received for publication February 18, 1942

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METHOD

The samples were prepared for injection by suspending 5 gm of a given "talc" in 100 cc of physiological saline, autoclaving for 30 minutes at 20 lbs pressure following the technic used by Sayers and Miller (6).

Guinea pigs weighing 250 gm were given a single intraperitoneal injection of 4 cc of the suspension which had the equivalent of 200 mgm of "talc". Each "talc" was injected into a group of 10 animals. One animal from each group which had received one of the above-named "talcs" was sacrificed at intervals ranging from 10 days to 15 months.

Tissues removed were fixed in 10% formalin and sections were cut from paraffin or celloidan blocks. One section from each was mounted unstained in Canada balsam. Others were stained with Hematoxylin-eosin. Special stains were used to bring out connective tissue.

RESULTS

Mineralogical

Unstained sections mounted in Canada balsam were studied petrographically to determine mineralogical changes which had taken place. The results are shown in tables 3 to 9.

"Talc" 814-6A is one which has a high carbonate content (a low silicate ratio). The most notable change which took place was a sharp drop in the amount of carbonate present. This occurred during the first few weeks and then leveled off, although at the end of 15 months there was still a small amount of carbonate left, but mostly as very coarse particles (larger than 20 μ). There is also a tendency for the serpentine to decrease, as indicated by the marked change in the proportions between "talc" and serpentine in the original sample, compared with the samples which had

TABLE 1

SAMPLE NUMBER	WT. % SOLUBLE	% LESS THAN 10 μ BY COUNT	% BY COUNT LESS THAN 10 μ						USE
			Talc	Serpentine	Carbonate	Quartz	Tremolite	Others	
1	49.18	97	4	6	82	—	—	8	Dusting rubber
2	46.14	95	9	5	83	tr.	—	3	Dusting rubber
3	45.53	95	21	33	32	tr.	tr.	14	Dusting rubber
4	43.88	90	12	6	75	—	tr.	7	Dusting rubber
5	43.03	95	14	10	63	—	9	4	Dusting rubber
6	41.73	95	6	5	86	—	—	3	Dusting rubber
7	41.52	95	8	6	70	—	12	4	Dusting rubber
8	39.23	95	5	6	72	—	12	5	Dusting leather
9	38.27	95	15	28	45	tr.	—	12	Dusting rubber
10	36.28	95	10	8	67	tr.	6	9	Filler in felt
11	35.56	90	12	35	45	tr.	tr.	8	Dusting rubber
12	34.18	95	12	5	58	—	16	14	Packaging for redistribution
13	32.41	85	5	13	79	—	—	3	Dusting rubber
14	32.09	90	16	22	57	—	—	5	Dusting rubber
15	29.86	95	15	9	68	—	3	5	Dusting rubber
16	28.78	95	16	12	64	tr.	—	8	Dusting rubber
17	27.94	95	14	12	67	—	tr.	7	Dusting rubber
18	26.65	85	16	11	58	—	9	6	Talc mill (Gs.)
19	25.27	90	6	8	82	—	—	4	Dusting rubber
20	24.79	90	4	3	88	—	—	5	Dusting rubber
21	24.78	96	6	14	77	—	tr.	3	Talcum powder
22	24.43	95	14	11	67	tr.	—	8	Dusting asphalt
23	24.08	95	17	10	68	—	—	5	Dusting rubber
24	23.54	90	11	6	78	—	—	5	Lubricated mechanical packing
25	22.94	90	16	26	47	tr.	—	11	Dusting rubber
26	22.83	90	4	6	80	3	—	7	Dusting rubber
27	22.14	97	47	29	12	—	—	12	Talcum powder
28	20.94	95	11	14	66	tr.	5	4	Dusting rubber
29	20.09	75	24	55	4	—	11*	6	Dusting rubber
30	19.98	90	14	38	21	tr.	24	3	Filler in soap
31	19.14	70	20	46	26	tr.	—	8	Dusting rubber
32	17.22	90	29	7	34	tr.	21	9	Dusting rubber
33	14.68	90	24	30	32	tr.	8	6	Dusting in shoe industry
34	12.52	90	3	3	8	—	82	4	Dusting rubber
35	10.20	85	36	33	20	tr.	—	11	Dusting rubber
36	9.96	80	51	27	7	—	—	15	Dusting rubber
37	9.14	75	15	22	17	tr.	31	5	Dusting rubber
38	9.02	65	35	49	12	—	—	4	Talcum powder
39	8.51	90	41	54	tr.	tr.	—	5	Talc mill
40	7.95	90	32	49	9	tr.	tr.	10	Dusting rubber
41	6.00	65	25	51	19	—	—	5	Talcum powder
42	5.38	90	37	52	5	tr.	—	5	Talc mill
43	5.08	80	27	57	9	tr.	2	5	Dusting rubber
44	4.39	85	40	47	5	tr.	—	8	Talcum powder
45	4.20	75	4	6	3	tr.	79	8	Dusting rubber
46	4.02	90	31	23	38	—	—	8	Talcum powder
47	3.85	90	3	5	8	—	80	4	Filler in paper
48	3.56	75	38	50	8	tr.	—	4	Dusting rubber
49	1.85	40	52	34	8	tr.	—	6	Talc mine
50	1.13	50	52	39	—	tr.	—	9	Filler in paper
51	0.93	35	44	52	tr.	tr.	—	4	Talc mine

* Organic.

been in the animals for the full period of the experiment. It is also interesting to note that toward the end of the experiment there was a marked parallelism of the "talc" needles. This may be the result of the purely mechanical factor of movement in the abdominal wall of the animal.

This material (814-11C) contained about 50% dolomite when injected. As in the first case, it had disappeared at the end of 15 months. Serpentine, likewise, showed a relative decrease.

TABLE 2

TALC SAMPLES

	WT. % SOLUBLE	TALC	SERP.	CARB.	QTZ.	TREM.	OTHERS	$Si:CO_2$ RATIOS
		%	%	%	%	%	%	
1. 814-6A	41.73	6	5	86	—	—	3	15:85
2. 814-11C	35.56	12	35	45	tr	tr	8	55:45
3. 814-25G	22.94	16	26	47	tr	tr	11	53:47
4. 814-33	14.68	24	30	32	tr	8	6	68:32
5. 814-40B	7.95	32	49	9	tr	tr	10	91:9
6. 814-50	1.13	52	39	—	—	—	9	100:0
7. 814-34	12.52	3	3	8	tr	82	4	92:8

TABLE 3

814-6A

	RAW MAT'L.	A*	B	C	D	E
	%	%	%	%	%	%
Talc	6	45	48	55	70	69
Serpentine	5	16	17	15	18	20
Carbonate	86	31	28	22	6	4
Others	3	8	7	8	6	7
Max. size in μ	—	150	100	100	120	80
% $<10\mu$	—	97	90	90	80	70

* The letters A to E refer to animals killed at times progressing from 10 days to 15 months after injection of "Talc."

Table 5 shows a "talc" which also contains about 50% carbonate. During the first few months there was a marked decrease in the amount of carbonate, which leveled off to a residue of coarse particles persisting throughout the remainder of the experiment. Here again, there was also an apparent loss of serpentine. The "talc" is the one constituent which is unaffected.

In this case (814-33), the same general trend was found, that is, an increase in "talc" with a decrease in carbonate. The outstanding characteristic of this "talc" is its fine grain with 80 μ as the maximum size and 90–95% of the grains below 10 μ.

TABLE 4

814-11C

	RAW MAT'L.	A	B	C	D	E
	%	%	%	%	%	%
Talc	12	32	34	36	45	49
Serpentine	35	34	30	34	37	42
Carbonate	45	27	28	20	10	tr
Quartz	tr	tr	tr	tr	tr	tr
Tremolite	tr	tr	tr	tr	tr	tr
Others	8	7	8	10	8	9
Max. size in μ	—	80	80	80	75	70
% $<10\mu$	—	90	90	90	90	85

TABLE 5

814-25G

	RAW MAT'L.	A	B	C	D	E
	%	%	%	%	%	%
Talc	16	39	42	46	44	50
Serpentine	26	24	28	20	22	38
Carbonate	47	28	22	24	25	4
Quartz	tr	tr	tr	tr	tr	tr
Tremolite	tr	tr	tr	tr	tr	tr
Others	11	9	8	10	9	8
Max. size in μ	—	60	70	70	80	60
% $<10\mu$	—	85	85	80	75	80

TABLE 6

814-33

	RAW MAT'L.	A	B
	%	%	%
Talc	24	32	36
Serpentine	30	40	42
Carbonate	32	15	10
Quartz	tr	tr	tr
Tremolite	8	6	5
Others	6	7	7
Max. size in μ	—	80	80
% $<10\mu$	—	90	95

This sample (814-40B) contains about 10% dolomite at the start, but this had disappeared at the end of 4 months. As shown in table 7, serpentine also decreased during the course of the experiment.

This "talc" (814-50) contained no dolomite. It is the nearest to mineralogically-pure talc that was encountered in all of the analyzed samples. It contained about 50% of the mineral talc. There was very little change in composition throughout the 15 months of the experiment as table 8 shows.

TABLE 7

814-40B

	RAW MAT'L.	A	B	C	D
	%	%	%	%	%
Talc	32	44	52	61	64
Serpentine	49	45	38	32	26
Carbonate	9	3	2	tr	tr
Quartz	tr	tr	tr	tr	tr
Tremolite	tr	tr	tr	tr	tr
Others	10	8	8	7	9
Max. size in μ	—	80	70	80	80
% < 10μ	—	90	90	90	90

TABLE 8

814-50

	RAW MAT'L.	A	B	C	D
	%	%	%	%	%
Talc	52	54	56	52	62
Serpentine	39	38	36	42	30
Others	9	8	8	6	8
Max. size in μ	—	75	70	80	75
% < 10μ	—	90	90	85	90

TABLE 9

814-34

	RAW MAT'L.	A	B	C	D
Talc	3	4	3	3	2
Serpentine	3	3	2	tr	tr
Carbonate	8	—	—	—	—
Quartz	tr	tr	tr	tr	tr
Tremolite	82	88	90	93	92
Others	4	5	5	4	6
Max. size in μ	—	100	100	150	140
% < 10μ	—	95	95	95	95

Tremolite is occasionally used as a "talc" where a more brittle type of fiber is required. Such a material is shown in table 9. The composition remained practically the same during the experiment.

From the mineralogical point of view, two facts stand out as a result of these experiments. First, most of the dolomite disappeared in 12 to 15 months with only the coarsest particles remaining; second, in "talcs" which contained carbonate, the serpentine was also removed, but to a lesser degree. As an interesting sidelight, it was also noted that after a period of about 8 to 12 months there was a tendency of the talc needles to align themselves parallel to the abdominal wall of the animal.

Gross Findings

At the time the animals were sacrificed, they appeared to be in good health and nutrition. At post-mortem, the organs appeared to be essentially normal, the talc having accumulated for the most part on the ventral parietal surface of the peritoneum, although smaller nodules were also found on other peritoneal surfaces. The nodules at first were large, of greyish buff color and moderately firm. Even at the end of 12 days, they were not particularly fragile. In the course of time, the lesions became more dense and firm. In talcs 814-6A and 814-11C the nodules at the end of 15 months were smaller than those in animals sacrificed at earlier periods. There was no apparent increase in the size of the nodules, except what could be expected in the variations in the amount of talc locally deposited.

Microscopic Findings

The histological study of tissue changes reveals that the material is mostly taken up and held by macrophages and giant cells. In talcs 814-33 and 814-25G, the macrophages predominated throughout the entire period of time. In the remaining talcs, large giant cells were more numerous and prominent. These frequently were packed with particles leaving only a rim of cytoplasm containing the nuclei. In all cases, the fibrous tissue was of minimal amount and in no instance was there evidence of a progressive proliferation of the fibrous tissue similar to that found in the silicotic nodule. The histological end results are those of storage of foreign material, primarily in phagocytic cells, with sufficient accompanying fibrous tissue to hold these cells in place, thereby storing the foreign material permanently in such a way that it might well be called a "storage fibrosis".

The greatest amount of fibrous tissue was pre-

duced by talcs 814-34 and 814-50. In the latter, fibroblastic proliferation was most pronounced during the early phases, although the amount of connective tissue formed was not prominent at the end of the experiment. Because of this tendency to produce more fibrous tissue than the others, they should be placed more or less in a class by themselves. The permissible concentration of dust should be kept at a lower level than in the other "talcs" examined. The animals, however, did not attain the fibrosis found in active silica.

Discussion

The organs of the body showed no lesions, with the exception of the places where the "talc" had been deposited. The pathological histology of all of the "talcs" tested had in common the holding of the particles in a local area by phagocytic cells of the macrophage and giant cell type. The kind of cell which would ultimately predominate was in large part determined by the size of the particle. In addition an early slight fibrous tissue formation occurred with "talcs" 814-34, 814-50. The early fibrous tissue proliferation appears to be the result of an acute early reaction which was later lost. Progressive fibrosis, such as produced by crystalline-free silica, did not occur in the talcs studied. The fibrosis which does occur is only enough to hold and limit the space for storing the foreign material. Such localization tends to restrict the zone of possible hazard. While inhalation of this material in large quantities could probably be tolerated without symptomatic effect, the material, the cells in which it is stored, and the fibrous tissue holding these cells and "talc" particles, increase the rigidity of the tissues involved.

The lung, particularly the terminal portions or alveoli, whose very essence of function is that of being stretched and compressed with each respiration, would be impeded in its movement by the accumulation of these particles and the resulting reaction. The degree of this impediment would depend upon the extent of involvement. Fortunately, the reserve capacity of a normal lung is sufficiently great to offset a considerable impairment without affecting the vital requirement. However we wish to emphasize that while the degree of impairment is not great, it cannot be assumed that there is no hazard.

Summary

Petrographic analysis showed that in "talcs" 814-6A, 814-11C, 814-25G, and 814-33 carbonates and serpentine decreased as the experiment progressed. Such "talcs" are to be preferred from the health aspect, inasmuch as a smaller amount of material is more or less permanently stored. The greatest amount of fibrous tissue was developed in the presence of the two "talcs" (814-34, 814-50) which contained the least carbonate. "Talcs" of this type should be avoided wherever possible, but when these are essential, methods of controlling the dust should be employed.

BIBLIOGRAPHY

1. Dreessen, W. C.: Effects of certain silicate dusts on the lungs. This J., *15:* 68–78 (March, 1933).
2. ——— and DallaValle, J. M.: The effects of exposure to dust in two Georgia talc mills and mines. U. S. Pub. Health Report, *50:* 131–143 (February, 1935).
3. Gardner, L. U.: Reaction of the living body to different types of mineral dusts with and without complicating infection. Am. Inst. Min. & Metallurgical Engineers., Tech. Pub. 929, 15 p. (1938).
4. ——— Etiology of pneumoconiosis. J. A. M. A., *111:* 1925–1936 (Nov. 1938).
5. Williams, C. R.: Talc dust in the rubber industry. Safety Engineering, *73:* 49–51 (April, 1937).
6. Sayers, R. R. and Miller, J. W.: The physiological response of the peritoneal tissue to dusts introduced as foreign bodies. U. S. P. H. Report, *49:* 80–89 (Jan. 19, 1939).

Cancer Res., 2(11): 770-775, 1942

The Relation of Diet to the Development of Gastric Lesions in the Rat

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(Received for publication June 3, 1942)

Cancer of the stomach in man is one of the most common of all tumors (34), but in animals it is a rare finding (63).

It has long been suspected that gastric cancer is caused by chronic irritation and unbalanced diets. Fibiger (18) in 1913 succeeded in producing lesions in the forestomach of rats, which he believed to be squamous cell carcinoma, by infesting them with the parasite *Gongylonema neoplasticum*. He thought that the tumors were elicited by the parasites, but, in a paper published in 1920 (20), emphasized the fact that the animals were kept on a diet consisting only of white bread and water. Passey (42) suggested that such a diet must have been deficient in vitamins, and on the basis of this assumption carried out an experiment in which he showed that vitamin A deficiency, in the absence of the *Gongylonema*, led to papillomatosis of the forestomach of the rat. Cramer (12), however, found that warts or papillomas might occur in the forestomach of old rats which had received an adequate amount of vitamins.

Stimulated by Fibiger's observations, many workers have made attempts to produce gastric cancer in different species of animals by feeding parasites, artificial and deficient diets, and chemicals known or suspected to be carcinogenic. The results obtained from these studies are summarized in Table I, which presents, though admittedly in uncritical form, all the published observations. It is hoped that this will be a convenient supplement to the critical review by Klein and Palmer (32, 33). Since the publication of this review Braunschweig and Rasmussen (5) have described ulceropapillomas in the stomach of rats fed a variety of unbalanced diets such as high fat (lard), high carbohydrate (rice), high protein (casein), with and without vitamins A, B_1, B_2, C, and D. The lesions did not appear to be the direct result of deprivation of the vitamins mentioned above, or caused by heated fats, but are referable to a certain degree of malnutrition or possibly to a deficiency of some vitamin as yet unidentified.

The most interesting and best established observations revealed by all these data are that the incidence of the gastric lesions is definitely related to dietary deficiency and that they are found generally in the rumen (forestomach).

Certain facts should be pointed out concerning these attempts to produce cancer of the stomach. First, rats have been used almost exclusively, since guinea pigs, mice, and rabbits do not develop gastric tumors. Therefore, any conclusions drawn must be restricted to the rat. Second, the tumors produced in the forestomach of rats have practically all been described as papilloma and only occasionally as carcinoma. In the glandular portion cancer is still more rare. Third, animals have often died long before the expiration of 14 months, the average induction period for both benign and malignant squamous cell tumors of the forestomach with methylcholanthrene. The time factor appears to be very important.

The object of the present study was to find whether or not a combination of an irritant and a deficient diet could produce squamous cell carcinoma in the forestomach, or adenocarcinoma in the glandular portion of the stomach, in rats.

EXPERIMENTAL

In this investigation polished rice was selected for the production of gastric lesions in rats, because young rats fed on polished rice will develop ulceropapillomas in the forestomach in the course of 30 to 45 days (5). As a mechanical irritant kieselguhr was selected.

Young albino rats of the Sherman stock, from 30 to 75 days old and weighing from 60 to 125 gm., were segregated from stock animals (which were fed upon a regular diet of Purina laboratory chow) and placed on the mixture of polished rice, 95 per cent, and kieselguhr, 5 per cent. Unlimited water was allowed.

As a control a number of young rats were maintained upon polished rice alone. Others were kept on a diet of unpolished rice (brown rice) with or without carrot.

Feeding on the respective diets was continued until the death of the animals, at which time their stomachs were examined in the gross and microscopically.

The results (Table II) show clearly that the daily oral administration of kieselguhr had a definite stimulating effect upon the production of lesions in the forestomach. Of 21 rats in the kieselguhr group, 20, or 95 per cent, showed lesions (2 to 32). In the non-kieselguhr group, 29 out of 51 rats, or 57 per cent, had lesions. They appeared like umbilicated warts, measuring 2 to 4 mm. in diameter, and were elevated above the surface 1 to 2 mm. (Fig. 1). These small nodules had punctate hemorrhages in the center. They proved on microscopic examination to be epithelial hyperplasia and hyperkeratosis; there were no evidences of malignant change (Fig. 2). Occasionally ulceration of the epithelium was noted. The glandular portion was free from the hyperplastic lesions but quite frequently diffuse hemorrhages were observed.

FIG. 1.—Stomach of 30 day old rat fed polished rice and kiesselguhr for 86 days. Numerous lesions in the forestomach and hemorrhage in the glandular region of the stomach can be seen.

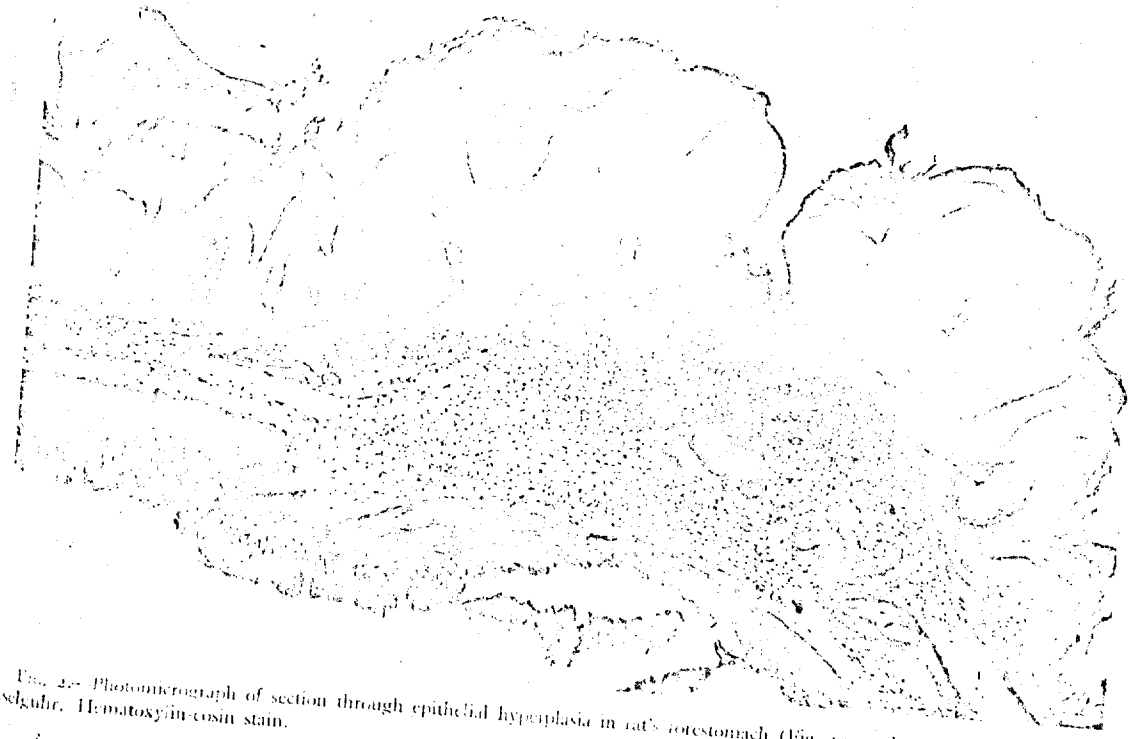

FIG. 2.—Photomicrograph of section through epithelial hyperplasia in rat's forestomach (Fig. 1) produced by polished rice and kieselguhr. Hematoxylin-eosin stain.

TABLE I: EXPERIMENTAL PRODUCTION OF GASTRIC LESIONS IN ANIMALS

Year	Author	Animal	Carcinogenic agent	Diet	Lesion in forestomach	Authors' comments
1913	Fibiger (18)	Rat	*Gongylonema neoplasticum*	White bread	Papilloma, carcinoma	Infection
1913	Singer (53)	Rat, mouse	*Spiroptera*	White bread	Ulceropapilloma	Infection
1914	Fibiger (19)	Rat	*Gongylonema neoplasticum*	White bread	Papilloma, carcinoma	Infection
1918	Bullock and Rohdenburg (7)	Rat	Scarlet red, pine tar, or pig's bristles	Bread, turnips, carrots, and greens	Papilloma	Chemical or mechanical irritants with a deficient diet
1919	McCarrison (36)	Monkey		Autoclaved rice, butter, and onion	Carcinoma	Vitamin B deficiency
1920	Fibiger (20)	Rat, mouse	*Gongylonema neoplasticum*	White bread	Papilloma, carcinoma	Infection
1921	Ishibashi and Ohtani (29)	Rabbit	Coal tar *per os*	Not mentioned	Adenoma	Chemical
1923	Buschke and Peiser (9)	Rat	Thallium chloride *per os*	Not mentioned	Papilloma	Chemical
1923	Buschke and Langer (8)	Rat	Coal tar *per os*	Not mentioned	Ulceropapilloma	Chemical
1923	Buschke and Langer (8)	Mouse	Methylcholanthrene *per os*	Not mentioned	Benign squamous cell papilloma	Chemical
1924	Pappenheimer and Larimore (41)	Rat	Wood shavings and hair	White flour and salt mixture	Keratosis, papilloma	Dietary deficiency and trauma
1924	Yokogawa (64)	Rat	*Gongylonema orientale*	Not mentioned	Epithelioma	Infection
1926	Fujimaki (23)	Rat		Casein, dextrin, olive oil, salt mixture, and yeast	Hyperkeratosis, papilloma, carcinoma	Vitamin A deficiency
1926	Uyeyama (58)	Rat		Rice and butter	Papilloma	Excessive fat and hunger
1927	Bonne (3)	Mouse	Tar painting	Not mentioned	Papilloma	Chemical
1928	Erdmann (16)	Rat		Casein, dextrin, salt mixture, butter, yeast, and cod liver oil	Papilloma	Overrich in vitamins B and D
1928	Findlay (21)	Rat		Casein, rice starch, salt mixture, and cod liver oil	Papilloma	Vitamin B_2 deficiency
1928	Fujimaki *et al.* (24)	Rat		Casein, dextrin, salt mixture, butterfat, and yeast	Hyperkeratosis, papilloma, carcinoma	Excessive butterfat
1928	Büchner *et al.* (6)	Rat	Histamine injection	Not mentioned	Ulcer	Starvation and chemical
1928	Erdmann and Haagen (17)	Rat		Casein, dextrin, salt mixture, olive oil, and yeast	Papilloma	Vitamin A deficiency
1928	Cramer (12)	Rat		Adequate diet	Papilloma	Not caused by vitamin deficiency
1929	Voronoff and Alexandrescu (60)	Rat	Coal tar, wood fat, aniline oil, toluylenediamine *per os*	Not mentioned	Adenocarcinoma of the glandular stomach	Chemical
1930	Sugiura and Benedict (56)	Rat		Casein, dextrin, salt mixture, olive oil, and yeast	Keratinization	Unbalanced diet
1931	Bisceglie (2)	Rat		Casein, dextrin, salt mixture, olive oil, and yeast	Papilloma	Vitamin A deficiency
1931	McCarrison (37)	Rat		Tapioca or rice with chilies, tamarind, and fish	Ulcer	
1932	Dalldorf and Kellogg (14)	Rat		Casein, cornstarch, salt mixture, cod liver oil, and butterfat	Ulceropapilloma	Vitamin B_1 deficiency
1932	Twort and Bottomley (57)	Rat, mouse	Coal tar painting	Not mentioned	Papilloma	Chemical
1932	Hoelzel and Da Costa (25)	Rat		White bread	Ulceropapilloma	Starvation and protein restriction
1934	Yoshida (65)	Rat	o-Aminoazotoluene *per os*	Polished rice and greens	Papilloma	Chemical
1934	Passey (42)	Rat	*Gongylonema neoplasticum*	White bread	Papilloma	Infection and vitamin A deficiency
1935	Passey *et al.* (43)	Rat	*Gongylonema neoplasticum*	White bread	Hyperkeratinization, papilloma	Infection and vitamin A deficiency
1935	Otsuka (40)	Mouse	Aminoazobenzene *per os*	Unpolished rice and greens	Papilloma	Chemical
1936	Sharpless (49)	Rat		Casein, starch, salt mixture, butterfat, and yeast	Ulceropapilloma	Protein deficiency
1936	Howes and Vivier (27)	Rat		White flour and salt mixture	Hyperkeratinization, papilloma	Dietary inadequacy

1936	Kimura (30)	Rat	Ethanolic [illegible] *per os*	[illegible]	[illegible]	[illegible]
1936	Waterman (61)	Mouse	Cholanthrene *per os*	Not mentioned	Carcinoma, adenocarcinoma	Chemical
1936	Waterman (61)	Mouse	Benzpyrene in lard *per os*	Not mentioned	Squamous cell carcinoma	Chemical
1936	Reinhard and Candee (34)	Mouse	Dibenzanthracene in butter *per os*	Dog biscuit, cocoanut, butter, wheat, Klim, CaCO.	Negative results	
1936	Bloch (28)	Dog	Dibenzanthracene in cholesterol pellets by injection	Not mentioned	Negative results	
1936	Bloch (28)	Ferret	Benzpyrene in cholesterol pellets by injection	Not mentioned	Negative results	
1936	Branch (4)	Mouse	Dibenzanthracene in benzene *per os*	Dog biscuit and vegetables	Negative results	
1936	Oberling *et al.* (39)	Mouse	3,4-Benzpyrene in lard *per os*	Not mentioned	Benign hyperplasia	Chemical
1937	Hedzel and DaCosta (26)	Rat, mouse		White bread and flour	Ulceropapilloma	Starvation and protein restriction
1937	Cramer (13)	Rat		Boiled maize, boiled rice, bread, cod liver oil, and marmite	Papilloma	Not to any specific dietary deficiency
1937	Cox (11)	Rat		Casein, starch, salt mixture, lard, and yeast	Papilloma	Vitamin A deficiency
1937	Lock *et al.* (10)	Rat	Dibenzanthracene in lard *per os*	Not mentioned	Negative results	
1937	Waterman (62)	Mouse	1,2-Benzpyrene in lard *per os*	Not mentioned	Carcinoma	Chemical
1937	Sharpless (50)	Rat		Casein, cornstarch, dextrin, salt mixture, butterfat, and yeast	Papilloma	Protein deficiency
1938	Rofo (43)	Rat	Heated animal fats	Bread, milk, and greens	Ulceropapilloma	Fats
1938	Rofo (43)	Rat	Irradiated cholesterol *per os*	Bread, milk, and greens	Carcinoma	Sterol
1938	Lognoli (11)	Mouse		Rice and olive oil, then regular diet, alternating	Papilloma, adenocarcinoma of the glandular stomach	Fats
1938	Meixner *et al.* (38)	Rat	Pepsin HCl by injection	Yellow maize, milk powder, linseed oil meal, casein, salt mixture, and greens	Ulcer	Low protein
1938	Kimura and Harada (31)	Rat	4-Oxyazobenzene *per os*	Unpolished rice, dried fish, and greens	Papilloma	Chemical
1939	Von Prohaska *et al.* (59)	Mouse	Dibenzanthracene in lard *per os*	Not mentioned	Squamous epithelial papilloma	Chemical
1939	Sharpless (51)	Rat		White flour, salt mixture, and butterfat	Ulceropapilloma	Protein deficiency
1939	Sharpless (52)	Rat		White flour, salt mixture, butterfat, thiamin, riboflavin, pyridoxine, and nicotinic acid	Ulceropapilloma	Protein deficiency
1939	Fridericia *et al.* (22)	Rat		Casein, rice starch, salt mixture, agar agar, lard, and yeast	Papilloma	Vitamin A deficiency
1940	Rusch *et al.* (48)	Rat, mouse	3,4-Benzpyrene, 1,2,5,6-dibenzanthracene by injection	Not mentioned	Adenocarcinoma of the glandular stomach	Chemical
1940	Stewart (54)	Mouse	20-Methylcholanthrene in petrolatum by injection	Purina dog chow	Papilloma, squamous cell carcinoma	Chemical
1940	Lorenz and Stewart (35)	Mouse	20-Methylcholanthrene, 1,2,5,6-dibenzanthracene *per os*	Purina dog chow	Squamous cell carcinoma	Chemical
1941	Braunschweig and Rasmussen (7)	Rat		Boiled rice alone, or casein, or heated lard	Ulceropapilloma	Unbalanced diet
1941	Stewart and Lorenz (55)	Mouse	20-Methylcholanthrene by injection	Purina dog chow	Adenocarcinoma of the glandular stomach	Chemical
1941	Peck and Peacock (1)	Mouse	Heated fats *per os*	White bread, whole oats, milk, and green vegetables	Ulceropapilloma	Fats
1941	Rofo (36)	Rat	Tobacco tar *per os*	Bread, milk, and greens	Squamous cell carcinoma	Hydrocarbons
1941	Rofo (47)	Rat	Fuel oil distillate *per os*	Bread, milk, and greens	Papilloma, adenocarcinoma of the glandular stomach	Chemical

TABLE II. SUMMARY OF INCIDENCE OF LESIONS OBSERVED IN THE FORESTOMACH OF RATS FED RICE ALONE OR RICE AND KIESELGUHR

Experiment No.	Diet	Number of rats used	Age of rats, days	Duration of feeding, days	Number of animals with lesions	Number of animals without lesions
1	White rice and kieselguhr	10	30	49-91	9	1
2	White rice and kieselguhr	11	75	98-116	11	0
3	White rice	27	30	54-148	13	14
4	White rice	24	75	65-156	16	8
5	Brown rice	12	30	54-75	0	12
6	Brown rice and carrot	30	75	88-200	0	30

Young rats, maintained on the diet of unpolished rice (brown rice) alone or unpolished rice plus fresh carrot, and surviving in fair health for periods of 75 to 200 days, showed no abnormalities in their stomachs. Certainly no new growths could be detected with the naked eye. Apparently the outer coating (bran) of the *rice contained substances which inhibited the development of gastric lesions*, since white rice without kieselguhr frequently produced tumors.

SUMMARY

1. Rats fed polished rice developed areas of epithelial hyperplasia in the forestomach and diffuse hemorrhage in the glandular portion in about 60 days.

2. A deficient diet combined with kieselguhr produced more definite changes than could be elicited by polished rice alone.

3. Rats fed unpolished rice with or without carrot did not develop any abnormality in the stomach. No hyperplasia and no hemorrhage could be detected with the naked eye.

The author wishes to express his appreciation to Dr. C. P. Rhoads for his interest and valuable advice, and to Dr. Fred W. Stewart of the Department of Pathology for interpretation of the histological sections.

REFERENCES

1. Beck, S., and Pearce, F. R. Gastro-Papillomatosis Due to Vitamin A Deficiency Induced by Heated Fats. Brit. M. J., **2**:85-85. 1941.
2. [illegible] V. Vitamin [illegible] Tumori. Le [illegible] alimentari con dieta squilibrata [illegible] Tumori, **5**:167-[illegible]. 1931.
3. [illegible] Tierversuchen. Ztschr. f. Krebsforsch., **25**:22. 1927.
4. [illegible] Tumors in Controlled Studies of Mice. Am. J. Cancer, **20**:[illegible]. 1934.
5. [illegible] R. A. The Relation of [illegible] Rats. [illegible] Research, **1**:[illegible]. 1935.
6. [illegible] Mäuse. Z. f. [illegible] Gewebe der Ratten [illegible] Arch. f. path. Anat. u. z. allg. Path., **81**:[illegible]. 1928.
7. [illegible] Experimental [illegible] of Animals and Their Relation to Human Malignant Tumors. J. Cancer Research, **3**:227-[illegible]. 1918.
8. [illegible] Schleimhaut [illegible] Ratten infolge von [illegible]. Ztschr. f. Krebsforsch., **21**:[illegible]. 1923.
9. Buschke, A., and Pfeifer, B. Epithelwucherungen am Vormagen der Ratten durch experimentelle Thalliumwirkung. Ztschr. f. Krebsforsch., **21**:11-18. 1923.
10. Cook, J. W., Haslewood, G. A. D., Hewett, C. L., Hieger, I., Kennaway, E. L., and Maynebord, W. V. Chemical Compounds as Carcinogenic Agents. Am. J. Cancer, **29**:219-259. 1937.
11. Cox, A. Magenveränderungen bei Ratten bei A-Avitaminose. Beitr. z. path. Anat. u. z. allg. Path., **98**:362-374. 1937.
12. Cramer, W. *26th Scientific Report, Imperial Cancer Research Fund*, England, p. 8. 1928.
13. Cramer, W. Papillomatosis in the Forestomach of the Rat and Its Bearing on the Work of Fibiger. Am. J. Cancer, **31**:537-555. 1937.
14. Dalldorf, G., and Kellogg, M. Incidence of Gastric Ulcer in Albino Rats Fed Diets Deficient in Vitamin B (B_1). J. Exper. Med., **56**:391-398. 1932.
15. Domack, G. Weitere experimentelle Untersuchungen über die Ursachen des Krebses. Ztschr. f. Krebsforsch., **48**:283-297. 1938.
16. Erdmann, R. Production of Tumours in Rats by a Faulty Metabolism. British Empire Cancer Campaign, Report of the International Conference on Cancer, London, p. 39. 1928.
17. Erdmann, R., and Hagen, E. Der Einfluss von Vitaminschäden auf die Entstehung bösartiger Neubildungen. Ztschr. f. Krebsforsch., **26**:333-347. 1928.
18. Fibiger, J. Untersuchungen über eine Nematode (Spiroptera sp. n.) und deren Fähigkeit, papillomatöse und carcinomatöse Geschwulstbildungen im Magen der Ratte hervorzurufen. Ztschr. f. Krebsforsch., **13**:217-280. 1913.
19. Fibiger, J. Weitere Untersuchungen über das Spiropteracarcinom der Ratte. Ztschr. f. Krebsforsch., **14**:295-326. 1914.
20. Fibiger, J. Untersuchungen über das Spiropterakarzinom der Ratte und der Maus. Ztschr. f. Krebsforsch., **17**:1-79. 1920.
21. Findlay, G. M. Pellagra-Like Lesions Associated with Deficiency of Vitamin B_2 in the Rat. J. Path. & Bact., **31**:553-564. 1928.
22. Fridericia, L. S., Gudjonsson, S., Vinterup, B., Clemmesen, S., and Clemmesen, J. Stomach Lesions in Rats Kept on Diets Deficient in Vitamin A. Am. J. Cancer, **39**:51-60. 1940.
23. Fujimaki, Y. Formation of Gastric Carcinoma in Albino Rats Fed on Deficient Diets. J. Cancer Research, **10**:469-477. 1926.
24. Fujimaki, Y., Okabe, K., Kimura, T., Wada, Y., and Hirayasu, T. On the Morphological Changes of the Forestomach of Albino Rats Due to Feeding upon Specific Diets. Report III. Relationship between Morphological Changes in the Forestomach of Rats Fed on Special Diets and the Constituents of the Diets. Tr. Soc. path. jap., **18**:538-553. 1928.

35. Helzel, F., and Da Costa, E. Production of Ulcers in the Prostomach of Rats by Protein Restriction. Proc. Soc. Exper. Biol. & Med., **29**:382-384. 1932.

36. Helzel, F., and Da Costa, E. Production of Peptic Ulcers in Rats and Mice by Diets Deficient in Protein. Am. J. Digest. Dis. & Nutrition, **4**:325-331. 1937.

37. Hoers, E. L., and Vivier, P. J. The Relation of Diet to the Occurrence of Gastric Lesions in the Rat. Am. J. Path., **12**:689-700. 1936.

38. Hueper, W. The Experimental Production of Visceral Tumors with Hydrocarbons. Am. J. Cancer, **26**:743-753. 1936.

39. Ishikawa, M., and Ohkami, S. Die Künstliche Erzeugung des papillären Adenoms am Kaninchen-Magen. Gann, **15**:2-5. 1921.

40. Kinosita, R. Researches on the Cancerogenesis of the Various Chemical Substances. Gann, **30**:423-426. 1936.

41. Kinosita, R., and Hirata, M. Production of Multiple Papillomas in the Stomach of the Rat by the Oral Administration of 4-Oxyazobenzene. Gann, **32**:225-229. 1938.

42. Kline, A. J., and Palmer, W. L. Experimental Gastric Carcinoma. A Critical Review with Comments on the Criteria of Induced Malignancy. Arch. Path., **29**:814-831. 1940.

43. Kline, A. J., and Palmer, W. L. Experimental Gastric Carcinoma. A Critical Review with Comments on the Criteria of Induced Malignancy. J. Nat. Cancer Inst., **1**:579-583. 1941.

44. Livingston, E. M., and Pack, G. T. Late Results in the Treatment of Gastric Cancer. Pp. 1-179. New York: Paul B. Hoeber, Inc. 1940.

45. Lorenz, E., and Stewart, H. L. Squamous Cell Carcinoma and Other Lesions of Forestomach in Mice, Following Oral Administration of 2-Methylcholanthrene and 1,2,5,6-Dibenzanthracene (Preliminary Report). J. Nat. Cancer Inst., **1**:273-276. 1940.

46. McCarrison, R. The Pathogenesis of Deficiency Disease. IX. On the Occurrence of Recently Developed Cancer of the Stomach in a Monkey Fed on Food Deficient in Vitamines. Indian J. M. Research, **7**:342-345. 1919.

47. McCarrison, R. Experimental Production of Gastric Ulcer in Albino Rats. A Preliminary Report. Indian J. M. Research, **19**:61-66. 1931.

48. Mayzlis, M. I., Windwer, C., and Sorel, A. E. The Role of Protein in Prevention of Experimental Gastric Ulcers. Am. J. Digest. Dis. & Nutrition, **5**:36-38. 1938.

49. Orsatti, C., Sannié, C., Guérin, M., and Guérin, P. Recherches sur l'action cancérigène du 1,2-benzopyrène. Bull. Assoc. franç. p. l'étude du cancer, **25**:156-186. 1936.

50. Ottsen, I. Über die experimentelle Papillomerzeugung im Vormagen der Mäusen durch Diazoaminobenzol. Gann, **29**:209-212. 1935.

51. Pappenheimer, A. M., and Larrabee, L. D. The Occurrence of Gastric Lesions in Rats. Their Relation to Lactar Deficiency and Their Ingestion. J. Exper. Med., **40**:719-732. 1924.

52. Passey, R. D. British Empire Cancer Campaign. 11th Ann. Rep., pp. 68-78. 1934; 12th Ann. Rep., pp. 87-89. 1935.

53. Passey, R. D., Leist, A., and Knox, J. C. Spinocell Cancer and Diet Deficiency. J. Path. & Bact., **40**:198-199. 1935.

54. Rusthard, M. C., and Candee, C. F. Development of Multiple Tumors in Mice: Part III. Results of Ingestion of Carcinogenic Agents: Preliminary Report. Am. J. Cancer, **26**:552-553. 1936.

45. Rotto, A. Tumores malignos desarrollados en el aparato digestivo por la ingestión de grasas oxidadas por calentamiento. Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, **15**:407-521. 1938.

46. Rotto, A. H. Cancerización gástrica por ingestión de alquitrán tabáquico. Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, **18**:39-68. 1941.

47. Rotto, A. H. Cancerización gástrica experimental por ingestión de destilado de fuel-oil. Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, **18**:487-558. 1941.

48. Rusch, H. P., Baumann, C. A., and Mason, G. L. Production of Internal Tumors with Chemical Carcinogens. Arch. Path., **29**:8-19. 1940.

49. Shafflless, G. R. Neoplastic Hyperplasia of Gastric Mucosa in Rats. Proc. Soc. Exper. Biol. & Med., **34**:684-685. 1936.

50. Shafflless, G. R. Malignant Changes in the Forestomach of Rats Related to Low Protein (Casein) Diet and Prevented with Cystine. Ann. Surg., **106**:562-567. 1937.

51. Shafflless, G. R. Choline and Epithelial Hyperplasia in the Forestomach of Rats. Proc. Soc. Exper. Biol. & Med., **45**:487-488. 1940.

52. Shafflless, G. R. Prevention of Hyperplasia in the Forestomach Epithelium of Rats Fed White Flour. J. Nutrition, **19**:31-37. 1940.

53. Sinclair, C. The Production of Ulcer of the Stomach in the Rat. Lancet, **2**:279-281. 1913.

54. Stewart, H. L. Induction of Gastric Tumors in Strain A Mice by Methylcholanthrene. Arch. Path., **29**:153-162. 1940.

55. Stewart, H. L., and Lorenz, E. Induction of Adenocarcinoma of the Pyloric Stomach in Mice by Methylcholanthrene. J. Nat. Cancer Inst., **2**:193-196. 1941.

56. Sugiura, K., and Benedict, S. R. A Critical Study of Vitamin A and Carcinogenesis. J. Cancer Research, **14**:306-310. 1930.

57. Twort, C. C., and Bottomley, A. C. The Aetiology of Breast Cancer. Lancet, **2**:776-780. 1932.

58. Uehyama, Y. Über die Epithelwucherung im Vormagen bei den mit Butter gefütterten weissen Ratten. Tr. Soc. path. jap., **16**:209-211. 1926.

59. Van Prohaska, J., Brunschwig, A., and Wilson, H. Oral Administration of Methylcholanthrene to Mice. Arch. Surg., **38**:328-333. 1939.

60. Volkonoff, S., and Alexandrescu, G. Tumeurs abdominales multiples chez le rat blanc expérimentalement produites par l'ingestion longtemps répétée d'un mélange à base de goudron. Néoplasmes, **8**:129-151. 1929.

61. Waterman, N. Experimental Production of Carcinoma in the Stomach of Mice. Acta Cancerologica, **2**:375-388. 1936.

62. Waterman, N. Production of Carcinoma of the Stomach in Mice by 1,2-Benzopyrene. Acta brev. Neerland., **7**:18-20. 1937; abstr., Am. J. Cancer, **30**:370. 1937.

63. Wells, H. G., Seal, M., and Holmes, H. F. Comparative Pathology of Cancer of the Alimentary Canal, with Report of Cases in Mice. Studies in the Incidence and Inheritability of Spontaneous Tumors in Mice: 34th Report. Am. J. Cancer, **33**:223-238. 1938.

64. Yokogawa, S. On the Cancroid Growths Caused by Gongylonema orientale n. sp. in the Rat. Gann, **18**:28-69. 1924.

65. Yoshida, T. Studien über die Entwicklung des experimentellen Hepatoms durch o-Amidoazotoluol, besonders die stufenweise Verfolgung der Leberveränderung bis zur Carcinomentstehung. Tr. Soc. path. jap., **24**:523-530. 1934.

Arch. Fiziol. 24: 1-23, 1926

Pharmacological Research on Colloidal Silicic Acid

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Very little research has been done on the biological effects of silicon; the first, that goes back to Rabuteau and Papillon (1), refers to the anti-fermentative effect of sodium silicate in the alcoholic fermentation of grape juice, of urea in urine, of milk, and of bitter almonds. Picot (2) was the first to take the study of it into the toxicological field and observe that sodium silicate, in doses smaller than 2.5 g was indifferently tolerated by rabbits, while in doses of 2.5-7.5 g it produced, in normal rabbits and in those which septicemia was caused, by an injection of decomposed blood into the circulatory system, toxic phenomena characterized by anorexia, diarrhea, fever, tachypnea, changes in the blood crasis and death by hemorrhagic gastro-enteritis. Spica and Chirone (3) observed the same transient toxic phenomena in rabbits whose blood had been injected with 15-45 mgr. of colloidal silicic acid and in dogs where they observed a change in the reaction of the urine from acid to alkaline. From the use of silicic waters in the treatment of herpetism, varicose ulcers, chronic eczema, utero-vaginal catarrhs and other disease symptoms connected with uric diathesis, Beranger, Bonjean, Petrequin, Socquet (4) observed that these waters acted to profoundly modify the material balance. Astolfoni (5) studied, under glass, (in vitro) the anti-fermentative effect of colloidal silicic acid, in contrast to alkaline silicates, which exert a notable anti-fermentative

effect, hardly influences the proteolytic power of the pepsin, the saccharifying power of the trypsin and does not influence at all the oxidases (oxidation enzymes), even if administered in high doses. In live studies, he observed in frogs, with doses larger than 10 mgr., toxic phenomena on the muscles of the skeleton, stoppage of respiration, and death; in dogs, that tolerated this substance better, lowering of the blood pressure and an increase in the urine of products of the material balance. Alessandrini and Scala (6), injecting colloidal silicic acid subcutaneously in guinea pigs, rabbits, and dogs caused after a certain time, a grave intoxication characterized by loss of strength, falling out of the fur, spastic pareses, paralysis, serious anemia, a disease picture very similar to that of pellagra, whence they expressed the opinion that this disease ought to be placed in relation with the use of silicic waters rather than with the pellagra-causing diet, supporting their hypothesis with the observation of sufferers from pellagra in areas where these waters are found. They observe, furthermore, in dogs subjected for a long time to the effect of colloidal silicic acid, modifications of the material balance consisting of the elimination of small quantities of urine with low specific gravity, poor in extractive substances, salts especially chlorides, and acidosis of the blood probably related to an excessive release of acids following upon a high retention of mineral salts.

For the last few years silicic preparations have been introduced into human therapy: the colloidal silicic acid Erba subcutaneously, Bayer's silisteren orally in tuberculosis (Robin, Volrath, Rossle, and Kahle) (7) and in inoperable cancer (Giordano and Valtorto) (8) and silicate of soda endovenously in arteriosclerosis, in arterial

hypertension, in cardio-renal disease forms with azotemia and acloruria (Scheffer, Sartory, Pellissier) (9).

The experimental research carried out up to now on colloidal silicic acid is not very extensive nor is it consistent in results; in this research little account has been taken of the toxicity of this substance even if administered in small doses over a long time, and of the local irritating effect, for which reason it is still to be considered daring to apply it in human therapy.

Colloidal silicic acid was prepared by me chemically, dissolving 112 g of sodium silicate in 300-400 cc. of distilled water, adding 67 g of HCl (187 g of HCl with a density of 1.18) diluted in a half-liter of distilled water and bringing the total volume to one liter (10). After being dialysized, it was sterilized at 100°C.in an autoclave. The colloid obtained is in the form of a clear, colorless, odorless liquid with a weakly acid reaction and which remains stable for a long time. With an ultra-microscope, it appears to be formed of numerous granules with a slight Brownian movement, showing Tyndall's phenomenon (11).

The silicic acid content was determined (12) in the form of SiO_2 evaporating, in a porcelain dish with a water bath, a definite quantity of colloid, that first coagulates in the form of gelatin and is then reduced to an amorphous white powder that is treated with concentrated HCl and is left for 20 minutes at room temperature to change into chlorides the basic salts and the oxides formed during the steaming off and the drying process. After the addition of distilled water and heating to the boiling point, the silicic acid is deposited at the bottom of the dish and the liquid remaining above is poured off

through a filter with known ashes. The residue, after being washed 3-4 times by decantation with hot water, is carried over the filter and is washed until disappearance of the chlorine reaction. Finally, the filter is burned in a platinum crucible, it is put in a dryer with sulfuric acid and is weighed. With this process in 25 cc. of colloid prepared, there were weighed 0.2494 g of SiO_2 instead of 0.25 g. The small difference is to be considered in relation to the small losses caused by the passage of the silicic acid in the filtrate, losses that can be avoided by repeating the process described several times on different filtrates. It can therefore be deducted that the prepared colloid contained .1 g of colloidal silicic acid per cubic centimeter.

Local effect (13)--Colloidal silicic acid is to be considered among the chemical substances that, without any intervention by micro-organisms, are capable of causing aseptic suppuration as was observed by Astolfoni, Scala, and Alessandrini. In a first and second dog, the subcutaneous injection of colloidal silicic acid, carried out with strict asepsis, rapidly caused, i.e., in less than 24 hours, an abscess at the site of injection without however those general symptoms that are so important in suppurations caused by micro-organisms. In contrast to the latter no metastasis was observed and the cure was rapidly effected. Pus from the abscess drawn out with a sterile syringe and reinoculated into another dog did not turn out to cause a virulent reaction. In stained preparations numerous muco-purulent corpuscles and a few extra-cellular cocci were observed microscopically.

Toxic effect--Colloidal silicic acid, administered by mouth with a gastric bougie to three dogs, with respective doses of 50-30-25 cc.,

rapidly produced acute poisoning. The animals, a short time after the ingestion of the substance, began to show a general indisposition, tachypnea, on the second day they refused food and had 2-3 diarrheic discharges mixed with blood, and on the third day they died. At the necroscopic examination, the gastric and intestinal mucus membrane were strongly hyperemic and that of the stomach had hemorrhagic spots scattered in various places. The lungs, the heart, the liver, the spleen, and the kidneys were very congested and showed spots of fatty degeneration.

Chronic poisoning was manifest in one dog after about one month with doses of 1.62 g of which 1.55 g were given by mouth and .7 g endovenously, and in another dog after about one month with doses of .8 g given endovenously. In the period of observation, that lasted five months for the first animal and a month and a half for the second, no disease symptoms worthy of mention were observed; the animals rarely refused their food, the first animal had diarrhea a single time. Sometime after administration of the substance the examination of the urine revealed nephritis and examination of the blood revealed azotemia as is shown in the experiments that will be described below.

The histological preparations of the organs examined after the death of the animal, stained with acid hematosilin and eosin provided this microscopic evidence.

The Stomach and Intestines--The superficial epithelium was entirely desquamated and the inter-tubular connective was increased. In the lumen of the glandular tubes, there were found accumulations of cells that filled the cavity while the glandular epithelia were stained (colored) diffusely and at some point were detached from

the wall of the organs and fell into the lumen of the tube. The blood vessels were full of blood.

Liver--No increase of the interstitial connective was noted. The branches of the vena cortae are rich in blood. The hepatic cells of the periphery of the acinus showed a slight biliar infiltration. The vessels of the acinus were narrowed (compressed) by the enlargement of the hepatic cells that showed a slight degree of turbid swelling and adipose degeneration.

Pancreas--The glandular cells completely fill the lumen of the acini, because they are very much swollen and with granular protoplasma, with very evident granulations. Nothing notable is seen in the interstitial gland.

Spleen--The capsule (covering) is a little thickened and in it there are dividing connective partitions that are somewhat thickened. Malpighi's corpuscles are a little enlarged and show a clear center because of scantiness of the cellular elements while the peripheral zone is more obvious through dilation of the peripheral capillaries. The pulp is atrophic and many cells filled with pigment are found in it.

Kidney--Malpighi's glomerules seem richer in nuclei, but in Bowmann's capsule there is no abnormal content. In the convoluted tubules, the epithelium, where it exists, presents a turbid swelling, hydropic degeneration and is generally desquamated, and in the lumen beside the cells there is seen an albuminous exudate with hyaline appearance and colloid in some points. In many tubules of the white matter there are also masses cellular in part and in part formed by a homogeneous hyaline and colloid exudate. The connective tissue of

the cortex is in some points increased in the form of parvi-cellular infiltration to the point of forming small nodules.

Because of the local irritant effect and because of the toxicity of the doses used by other experimenters, I administered colloidal silicic acid orally and into the blood stream in small doses in my research on the balance, on the physical-chemical contents of the blood, and on the uric acid of the blood and of the urine.

Research on the Material Balance

For the experiments, I selected robust, healthy adult dogs that I kept at rest and accustomed to eating a constant nutritional ration, well-tolerated, of relatively fixed composition, consisting of 300 g daily of white bread, a nutritious food rich in carbohydrates, not well provided with protein substances and salts, very poor in fats. With this type of ration, the animal received about 803 calories (14) and 5.44 g of nitrogen, an average of three measurements made in the course of the experiments on various specimens of the same quality of fresh bread.

This figure agrees with the one reported by Coronedi and Luzzatto (15), who found that 100 g of dry bread contained 2.72 g of nitrogen and is higher than the figure that is usually read in the analysis of bread according to which 300 g of bread would contain 3.36 g of nitrogen, calculating on an average in the bread 7% of proteins and in the proteins 16% of nitrogen.

Water was administered in a constant quantity.

Of the products of metabolism of the organic nutritive substances eliminated with the organic excretions, I limited myself to determining

the elimination of nitrogen and of the urinary salts. The course of the experiments was divided into three periods.

First Period: During which the animal was accustomed to the ration indicated up to the point of maintaining the body weight and to obtaining analytical data with regard to the urine, data that were constant in order to be taken into consideration;

Second Period: In which colloidal silicic acid is administered;

Third Period: In which the silicic treatment was suspended.

The duration of each experiment was 24 hours during which the animal was kept in a cage. After this time, the urine was collected either by letting the animal urinate spontaneously or by catheterizing it.

The total nitrogen was determined with Kjeldahl's method (17).

The ureic nitrogen was calculated, by dividing 2.14 the figure for the amount of urea determined according to the sodium hypobromite method with Dannecy's ureometer. The chloride was determined by Mohr's method with the titrated solution of silver nitrate, using potassium chromate as an indicator. The phosphoric acid was determined in the form of P_2O_5 with Neubauer's volumetric method with a solution of uranium acetate, using potassium ferrocyanide as an indicator. The total sulfuric acid in the form of barium sulfate with P. Mohr's method modified by H. Shultz.

DATA	N alimentare introdotto gr.	quantità di urina cm³	Peso specifico	N orinario totale gr.	N ureico gr.	Cl orinario gr.	P_2O_5 totale gr.	H_2SO_4 totale gr.	Peso corporeo Kgr.
7 febbraio	5.44	100	1039	2.39	1.33	3.10	0.33	0.70	7.700
8-9 (¹)	—	—	—	—	—	—	—	—	
10 »	5.44	240	1032	3.63	2.10	2.99	0.40	0.39	
11-12 (¹)	—	—	—	—	—	—	—	—	
13 »	5.44	150	1054	3.02	2.04	3.39	0.54	0.54	
Media	5.44	168	1041	3.01	1.82	3.16	0.42	0.54	
				1.79%	1.08%	1.88%	0.25%	0.32%	

N introdotto gr.	N eliminato gr.	N bilancio gr.
5.44	3.01	+ 2.43

Dog No. 1, brought into the laboratory in January, 1925 with body weight of 7.250 kg. Feeding with 300 g of bread and water is begun immediately.

(1) N in food given

(2) Quantity of urine

(3) specific gravity

(4) Total N in the urine

(5) Ureic N

(6) Urinary Cl

(7) P_2O_5 total

(8) H_2SO_4 total

(9) Body weight

(10) February

(11) Average

(12) N introduced

(13) N eliminated

(14) N balance

(15) Allowed to run about with continuation of the same feeding throughout.

(16) Allowed to run about.

Second Period

This begins on February 14 with the administration of colloidal silicic acid by mouth with a daily dose of 15 cc. on the first day and 20 cc. on three days following. In the aggregate there was introduced into the animal during this period 75 cc. of colloidal

silicic acid, equal to 0.75 g of SiO_2, the animal remaining in good health and having on the fourth day of administration of the substance an abundant diarrheic discharge while on the other days defecation is normal. On February 19, the animal is kept in a cage and the urine collected yeilds these analytical data:

DATA	N alimentare introdotto gr. (1)	Quantità di urina cm³ (2)	Peso specifico (3)	N orinario totale gr. (4)	N ureico gr. (5)	Cl orinario gr. (6)	P_2O_5 totale gr. (7)	H_2SO_4 totale gr. (8)	Peso corporeo Kgr. (9)
19 febb. (*) (10) (13)	5.44	100	1045	1.94	1.25	2.30	0.36	0.30	7.800
21 febb. (*) (14)	5.44	125	1035	2.18	1.56	3.50	0.27	0.30	
23 febb. (*) (15)	5.44	225	1017	2.23	2.17	3.36	0.40	0.45	
25 febb.	5.44	250	1018	2.48	2.16	4.00	0.32	0.44	
Media (11)	5.44	200	1028	2.20	1.78	3.29	0.34	0.37	
				1.10 %	0.89 %	1.64 %	0.17 %	0.18 %	
Bilancio: (12)	N +3.24	Cl −0.13	P_2O_5 +0.08	H_2SO_4 +0.17					

(1) N in food given

(2) Quantity of urine

(3) Specific gravity

(4) Total N in urine

(5) Ureic N

(6) Cl in urine

(7) P_2O_5 total

(8) H_2SO_4 total

(9) Body weight

(10) February

(11) Average

(12) Balance

(13) On February 19 and 20, the animal is allowed to run free and he is given in the aggregate another 40 cc. of colloidal silicic acid, i.e., 0.40 g of SiO_2.

(14) On February 21 and 22, the animal is again allowed to run free and is given in the aggregate another 40 cc. of colloidal silicic acid.

(15) On February 23 and 24, the animal runs free and is given in the aggregate 40 cc. of colloidal silicic acid.

Third Period

There is a period of thirteen days in which the administration of the colloidal silicic acid is suspended, after which the analysis of the urine shows that the animal has returned almost to the figures of the first period for all the elements included in the examination with the exception of the total H_2SO_4 that continues to decrease.

DATA	(1) N alimentare introdotto gr.	(2) quantità di urina cm³	(3) Peso specifico	(4) N urinario totale gr.	(5) N ureico gr.	(6) Cl urinario gr.	(7) P_2O_5 totale gr.	(8) H_2SO_4 totale gr.	(9) Peso corporeo Kgr.	(10) Osservazioni
(11) 1 marzo	5.44	200	1025	3.02 1.51 %	2.44 1.22 %	3.20 1.60%	0.47 0.23%	0.28 0.14%	7.800	

(1) N in food given

(2) Quantity of urine

(3) Specific gravity

(4) Total N in urine

(5) Ureic N

(6) Cl in urine

(7) P_2O_5 total

(8) H_2SO_4 total

(9) Body weight

(10) Observations

(11) March

Fourth Period

This begins on March 12 and lasts 7 days, i.e., until March 19, in which the administration is resumed of colloidal silicic acid endovenously with an aggregate injection of 13 cc. equal to 0.13 g.

DATA	N alimentare introdotto gr. (1)	quantità di urina cm³ (2)	Peso specifico (3)	N urinario totale gr. (4)	N ureico gr. (5)	Cl urinario gr. (6)	P_2O_5 totale gr. (7)	H_2SO_4 totale gr. (8)	Peso corporeo Kgr. (9)	Osservazioni (10)
(11) 20 marzo	5.44	190	1018	1.40	1.17	1.37	0.41	0.74		L'esame dell'urina (13) rivela nefrite
				0.73%	0.61%	0.72%	0.21%	0.38%		
(12) Bilancio:		N	Cl	P_2O_5	H_2SO_4					
		+ 4.01	+ 1.79	+ 0.01	− 0.20					

(1) N in food given

(2) Quantity of urine

(3) Specific gravity

(4) Total N in urine

(5) Ureic N

(6) Cl in urine

(7) P_2O_5 total

(8) H_2SO_4 total

(9) Body weight

(10) Observations

(11) March

(12) Balance

(13) Examination of the urine gives evidence of nephritis.

After these seven endovenous injections, the animal no longer returns to the normal figures as the examination of the data collected after a long period allows us to observe, in which period administration of the drug was suspended.

DATA	N alimentare introdotto gr. (1)	quantità di urina cm³ (2)	Peso specifico (3)	N urinario totale gr. (4)	N ureico gr. (5)	Cl urinario gr. (6)	P_2O_5 totale gr. (7)	H_2SO_4 totale gr. (8)	Peso corporeo Kgr. (9)	Osservazioni (10)
(11) 23 aprile	5.44	150	1020	1.09	1.02	2.70	0.12	0.15		
(12) 3 magg.	5.44	100	1035	1.59	0.89	2.94	0.22	0.19		
(13) 18 magg.	5.44	80	1036	0.70	0.55	0.92	0.03	0.12		
(14) 22 magg.	5.44	100	1030	1.25	0.77	2.52	0.06	0.13	7.750	
(13) Media	5.44	107	1030	1.16	0.80	2.27	0.10	0.14		
				1.08%	0.74%	2.12%	0.09%	0.12%		
(14) Bilancio:		N	Cl	P_2O_5	H_2SO_4					
		+ 3.28	+ 0.89	+ 0.32	+ 0.40					

(1) N in food given

(2) Quantity of urine

(3) Specific gravity

(4) Total N in urine

(5) Ureic N

(6) Cl in urine

(7) P_2O_5 total

(8) H_2SO_4 total

(9) Body weight

(10) Observations

(11) April

(12) May

(13) Average

(14) Balance

The animal is killed and upon autopsy there is noted an extensive fatty degeneration of the kidney and degenerative spots (scabs) in the outer organs.

Dog No. 2, with a weight of 6.3 kgr. brought into the laboratory in April 1925, fed with 300 g of bread and water.

First Period

DATA	N alimentare introdotto gr.	quantità di urina cm³	Peso specifico	N urinario totale gr.	N ureico gr.	Cl urinario gr.	P_2O_5 totale gr.	H_2SO_4 totale gr.	Peso corporeo Kgr.
6 maggio (1)	5.44	280	1018	2.00	1.82	3.69	0.20	0.28	6.500
22 maggio	5.44	140	1035	2.30	1.31	2.55	0.21	0.31	
Media	5.44	210	1026	2.15 1.02 %	1.56 0.02 %	3.12 1.48 %	0.20 0.09 %	0.29 0.13 %	
		N introdotto gr.		N eliminato		N bilancio			
		5.44		2.15		+ 3.29			
				2° periodo					
25 maggio (1)	5.44	160	1016	1.30 0.81 %	1.20 0.75 %	2.72 1.29 %	0.20 0.09 %	0.31 0.19 %	
31 maggio	5.44	90	1010	1.90	0.45	1.89	0.18	0.32	
Media	5.44	125	1028	1.60 1.28 %	0.82 0.65 %	2.30 1.84 %	0.19 0.15 %	0.31 0.24 %	
Bilancio:	N + 3.81	Cl + 0.42	P_2O_5 + 0.01	H_2SO_4 − 0.02					

Translation on following page

(1) N in food given

(2) Quantity of urine

(3) Specific gravity

(4) Total N in urine

(5) Ureic N

(6) Cl in urine

(7) P_2O_5 total

(8) H_2SO_4 total

(9) Body weight

(10) May

(11) Average

(12) N introduced

(13) N eliminated

(14) N balance

(15) From May 6 to 21 the animal runs loose and is always kept on the same food. From May 22 to 25 an aggregate of 3 cc. of colloidal silicic acid, equal to .3 g of SiO_2 is injected endovenously. From May 25 to 30 the endovenous injections are repeated with 2 cc. daily, an aggregate of 10 cc., equal to 1 g of SiO_2.

(16) May, average

(17) Balance

(18) Same as 15.

When the endovenous injections are suspended, the animal no longer returns to normal and, when killed, shows fatty degeneration of the various organs and principally of the kidney.

The figures collected in these charts represent the results obtained in the elimination of nitrogen and urinary salts within a 24-hour period. The figures of the first period are the normal figures, those of the second period the figures obtained after the administration of colloidal silicic acid orally and endovenously, those of the third period, in the first dog, are the figures obtained after suspension of administration of the drug, those of the fourth period are the figures of the period that we shall call the toxic period, because the elimination of the said principal substances no longer returns to normal but remains persistently scanty. Under normal conditions and by the effect of the type of feeding adopted, the animals never regained a balance of nitrogen and salts in an absolute sense but they

did regain the capacity for almost constant elimination of these substances as is shown by the figures obtained, which differ very little from each other.

In this first period, having determined only the nitrogen introduced with food and not the salts, we can assert that the animal consumed 5.44 g of N, eliminated 3.01 g of it, retained 2.43 g of it, and eliminated 3.16 g of NaCl, 0.42 g of P_2O_5 and 0.54 g of H_2SO_4. In the second period, common to both dogs in the experiment, the administration of colloidal silicic acid determines a lesser elimination of N, that, in relation to the constant quantity introduced and the animals always having consumed their whole ration, is reduced in the first dog to 2.20 g, the retention being accentuated to 3.24 g, and in the second dog being reduced to 1.30 g, the retention being accentuated to 4.14 g in the first period and to 3.84 in the second period of administration of the drug. The elimination of the phosphoric acid and the urinary sulfuric acid, from 0.42 g and 0.54 g, is reduced in the first dog to 0.34 g and 0.37 respectively, with a retention of 0.08 g and 0.17 g; in the second dog, however, the quantity of phosphoric acid eliminated does not undergo variations in the first period of endovenous injections of colloidal silicic acid, being maintained constantly at 0.20 g, and diminishing to 0.18 g in the second period; the total sulfuric acid, on the contrary, in both periods of injections is always increased by the normal quantity of 0.29 g to 0.31-0.32 g. The quantity of Cl, administered in the form of NaCl, after silicic treatment increases in the first dog by 1.3 g and diminishes in the second dog by 8.2 g. The figures for the third period, with respect to the first dog show that when

the administration of the substance is suspended, the animal tends to return to the figures of the first period, with the effect of the colloidal silicic acid in the organism being presumably exhausted. The figures of the first period cited for reasons of brevity only for the first animal, agreeing in fact with the figures for the second animal, show finally that the last administration of the substance endovenously, in a dose of 1.3 g in seven consecutive days, i.e., at a dose of about 0.0026 g per kg of weight produces toxic symptoms, as is demonstrated by the chemical and microscopic examination of the urine, by the examination of the renal permeability and by the macroscopic and histological examination of the organs removed from the animal after death as I have described above. The examination of the urine of the two animals in this period shows a decrease in quantity, high specific gravity, a coloring tending to red, a decrease of the solid organic and inorganic material, the presence perhaps of albumen in a not notable quantity, of casts, of renal epithelial cells, of leukocytes and of red blood corpuscles. With adapted experiments I tried to determine whether the scanty elimination of N was in ratio to the decrease of the oxidative processes or in ratio to azotemia. For the study of the organic oxidated processes, I studied the oxidative ferments in the blood with Schultz's method (18). According to this method, preparations of the blood after drying out are fixed in a solution of 4% formalin, for a minute, then washed with running water, stained for one minute in an aqueous solution of 1% of α-naphthol and for another minute in an aqueous solution of 1% dimethyl-p-phenylenedinine. When observed under the microscope, after fixing in water or glycerine, the oxidizing

ferments contained in the leukocytes are shown with a blue coloring because of the formation of a leuko-product caused by a reaction between dimenthyl-p-phenylenedinine and the α-naphthol. The study made on the canine blood under normal conditions showed that 140 leukocytes counted, 18 presented blue-colored granules, i.e., 12%. In the same dog, after administration of colloidal silicic acid orally and endovenously for several days consecutively, the number of granules colored blue increased to 86%, there being found oxidizing ferment in 52 leukocytes of 64 counted in the preparations. We must state from this study that the decreased elimination of N is not to be placed in relationship with the absorption of the oxidases, caused by the colloidal silicic acid, since the preparations of drug examined showed instead an increase of these ferments.

The dosage with Kjeldahl's method of N in the blood in another dog showed that the colloidal silicic acid determined the increase of N in the blood. In 5 cc. of blood before administration of the drug there were measured 0.07196 g of N, i.e., 1.439%, after administration there were counted, however, 0.188720 g, i.e., 3.774%.

Renal permeability has been studied in a small dog with healthy kidneys, with the selection from the urophane (urethane) substances (19) of methylene blue in a 5% aqueous solution. After the emptying of the bladder by catheterization, 1 cc. of methylene blue solution was injected subcutaneously, the elimination of which began after 1 hour and 20 minutes and was completed after three days. The injection of 6 cc. of colloidal silicic acid quickly produced renal alterations, there being found hematuric albuminuria and casts in the urine. The methylene test, repeated one day later, showed that the elimination

began three hours after injection of 1 cc. of the substance and lasted through three consecutive days. Continuing the endovenous injections of colloidal silicic acid in a dosage of 1 cc. per day for another eight consecutive days and repeating the methylene blue test with the same dosage, the elimination of this substance began after 1 hour and 26 minutes and lasted another three days. In fact, the methylene blue test did not yield different results for healthy kidneys and diseased kidneys. It can be seen from the aggregate of the data collected in the proceding tables that colloidal acid has an obvious effect on the N balance and the balance of the urinary salts. The elimination of the N is constantly decreased in all the experiments. The elimination of the salts shows different results: in the first dog, P_2O_5 and H_2SO_4 diminish and Cl increases, in the second dog, P_2O_5 at the first moment shows no modification and at the second moment decreases; H_2SO_4 always increases.

Although the mechanism of the effect of the colloidal silicic acid is not very clear in the balance of N and of the urinary salts, it can be asserted from the results obtained that at first this substance acts to determine a lowering of the functional power of the tissue cells, and then in the toxic period acts to determine alterations of the blood crasis, of the nutritive material that gets to the cells themselves and to cause destructive lesions of the organs.

In demonstration of the functional alterations in some organs, I refer to the results obtained on the gastric, salivary, and biliary secretion by the effect of colloidal silicic acid. In a large and robust dog, operated on for gastric fistula by Cl. Bernard (20), the examination of the gastric juice under normal conditions gave these

results with Toepfer's method (21), that represents the average of two experiments. The quantity collected in two hours, 85 cc., total acidity 80%, formed by 50% free HCl, by 13% combined HCl and by 17% organic acids. For three consecutive days the dog was given an aggregate of 45 cc. orally and for an additional 4 days 8cc. of the substance in the blood stream. When the gastric juice was collected, after two hours the quantity was 17cc., and only after 6 hours was there a measurement of 100 cc.'s. The chemical examination showed an absence of free HCl with Gunzburg's reaction and with Boas-Danilewski's reaction (22); the total acidity 80%, formed with regard to 56% from combined HCl and with regard to 24% from organic acids. The absence of free HCl in this animal, which this was demonstrated prior to silicic treatment, is a sign of defective secretion. For this reason and inasmuch as it has been demonstrated by Astolfoni, with regard to the decreased proteolytic power of the pepsin, it is to be thought that because of the effect of colloidal silicic acid, digestion is not carried out completely.

In the second dog, operated on for fistula of Wharton's duct (23), 15 drops of saliva per minute were counted normally; after the injection of 5 cc. of colloidal silicic acid, the drops were reduced to 12 minutes, five minutes after injection, and to 6 after ten minutes. After two minutes the injection of .1 g of pilocarpine caused an increase of the quantity of saliva to 32 drops, and the injection of another 5 cc. of colloidal silicic acid again caused a decrease in the saliva to 6 drops. The effect lasted for 3/4 hour and after this time the quantity of saliva progressively increased to 18 drops

per minute. This experiment thus shows that colloidal silicic acid is capable of modifying the salivary secretion.

A third dog, operated on for biliary fistula and fed for the entire experimental period with a uniform ration composed of 300 g lean meat, 400 g white bread, and water, always fed at the same time, yielded the results collected in the following table:

(1) Giorno di esperimento	(2) Quantità di bile cm³	(3) Peso specifico a + 15° C.	(4) Acqua %	(5) Residuo secco %	(6) Residuo secco totale	(7) Ceneri %	(8) Viscosità η 37° C	(9) Δ	(10) Conduttività elettrica a γ 37° C.
(11) 10 luglio	26	1.0116	95.802	4.198	1.0914	1.31	1.41	-0°.510	$238 \cdot 10^{-4}$
16 »	16	1.0142	95.600	4.400	0.7043	1.43	1.35	-0°.550	191×10^{-4}

(1) Day of experiment

(2) Quantity of bile, cc.

(3) Specific gravity, a + 15% C.

(4) Water, %

(5) Dry residue, %

(6) Dry residue, total

(7) Ashes, %

(8) Viscosity, η 37°C

(9) Delta (Δ)

(10) Electrical conductivity at γ 37°C

(11) July

From this, we have the result that colloidal silicic acid, injected into the blood for seven consecutive days in a dosage of .2 g per day caused these modifications. The quantity of bile secreted, the electric resistance and viscosity decreased; however, the percentual quantity of the residue and of the ashes, and the freezing point increased.

The results obtained in these experiments lead to the following conclusions:

1. Colloidal silicic acid has a local irritant effect which counter-indicates its use through subcutaneous injections.

2. When it is introduced into the organism orally or into the blood stream, it modifies the balance of N and of the urinary salts, decreasing the elimination of nitrogen and causing modifications in the elimination of the salts, which are not always in the same direction.

3. Small doses even if given for a short time, produce immediate and persistent toxic symptoms.

The decreased elimination of N and of salts, demonstrated in the preceding experiments, the decreased elimination of uric acid and the actual increase in hemoalkalinity demonstrated in studies that will be published subsequently, have induced me to study the immediate and later modifications of the physical-chemical constants of the defibrinated blood and of the serum by the effect of the colloidal silicic acid. There is nothing on this subject in the literature, and therefore I have considered important, the studies carried out to show whether Si+ is capable of causing modifications in the physical-chemical balance of the organism.

For the experiments I chose healthy and robust dogs, always fed with the same ration, made up of white bread and water that was fed at the same hour. The colloidal silicic acid was administered orally and into the blood stream. The blood required for examinations (80cc.) was taken by blood-letting from an artery (carotid or femoral) and partially collected in a sterilized Erlenmeyer, containing small glass

spheres for the defibrination, and collected partially in a glass cylinder that was kept in a refrigerator for the rapid separation of the serum. The freezing point, the viscosity, the electrical conductivity, and the surface tension of the defibrinated blood and of the serum were determined. For each of these studies three examinations were made, the result being taken as the average of the three. The viscosity of the serum and of the defibrinated blood was determined at 37°C always with the same quantity of liquid (4cc.) with Ostwald's viscosimeter with vertical capillary provided with a toluene thermo-regulator and with a stirrer (beater), and the value was calculated with Mayer's formula (24) ($\eta = tx/tH_2O$), that is by dividing the time of flow (discharge) of the liquid being examined by the time of flow of the distilled water, the times being measured with the same viscosimeter. The cryoscopic value was measured using 10 cc. of the liquid examined, with Beckmann's apparatus, determining the zero of the thermometer with bi-distilled water at the beginning and at the end of each series of determinations. The electrical conductivity was determined with Kohlrausch's method, first determining the resistance capacity of Ostwald's Cell (celletta) immersed in a thermostat at 37°C. Making use of a solution of 0.1 n of KCl, we find a capacity equal to 0.388. The surface tension was determined by counting the number of drops of distilled water, of defibrinated blood and of serum that at the temperature of 20°C flowed from Traube's stalagmometer. For the distilled water, the number of drops flowing from the stalagmometer was 18.8.

I report the results of the experiments carried out:

Experiment 1--April 22, 1925, Dog weighing 5.500 kg; at 10 a.m., a blood-letting from the left femoral, taking 80 cc. of blood.

	(1) Coefficiente di viscosità η 37°	(2) Valore crioscopico Δ	(3) Conducibilità elettrica specifica $K^{37} \times 10^{-4}$	(4) Tensione superficiale goccie
(5) Sangue defibrinato	3.40	-0°.550	73×10^{-4}	24.4
(6) Siero	1.40	-0°.515	113×10^{-4}	22

(1) Coefficient of viscosity
(2) Cryoscopic value
(3) Specific electrical conductability
(4) Surface tension drops
(5) Defibrinated blood
(6) Serum

From April 22 to 27, 2 g were injected daily endovenously, an aggregate of 1.2 g of colloidal silicic acid, tolerated well by the animal.

April 27; 10 a.m. blood-letting from the right femoral taking 80 cc.

	(1) Coefficiente di viscosità η 37°	(2) Valore crioscopico Δ	(3) Conducibilità elettrica specifica $K^{37} \times 10^{-4}$	(4) Tensione superficiale goccie
(5) Sangue defibrinato	2.91	-0°.580	81×10^{-4}	22.5
(6) Siero	1.40	-0°.520	120×10^{-4}	21

(1) Coefficient of viscosity
(2) Cryoscopic value
(3) Specific electrical conductability
(4) Surface tension drops
(5) Defibrinated blood
(6) Serum

Experiment II--April 30, 1925, Dog weighing 4.500 kg; at 10 a.m., blood-letting from the right femoral, taking 80 cc. of blood.

	(1) Coefficiente di viscosità η 37°	(2) Valore crioscopico Δ	(3) Conducibilità elettrica specifica $K^{37} \times 10^{-4}$	(4) Tensione superficiale goccie
(5) Sangue defibrinato	3.548	-0°.604	72×10^{-4}	22.8
(6) Siero	1.387	-0°.560	154×10^{-4}	21.8

(1) Coefficient of viscosity

(2) Cryoscopic value

(3) Specific electrical conductability

(4) Surface tension drops

(5) Defibrinated blood

(6) Serum

From April 30 to May 4, .1 g of colloidal silicic acid was injected daily, a total of .6 g.

May 4, 1925; at 10 a.m. blood-letting from the left femoral artery taking 80 cc. of blood.

	(1) Coefficiente di viscosità η 37°	(2) Valore crioscopico Δ	(3) Conducibilità elettrica specifica $K^{37} \times 10^{-4}$	(4) Tensione superficiale goccie
(5) Sangue defibrinato	2.87	-0°.500	84×10^{-4}	22
(6) Siero	1.41	-0°.530	154×10^{-4}	21.4

(1) Coefficient of viscosity

(2) Cryoscopic value

(3) Specific electrical conductability

(4) Surface tension drops

(5) Defibrinated blood

(6) Serum

The injections of colloidal silicic acid are suspended.

May 12, 1925; at 10 a.m. blood-letting from the left carotid taking 80 cc. of blood.

	(1) Coefficiente di viscosità η 37° C.	(2) Valore crioscopico Δ	(3) Conducibilità elettrica specifica $K^{37} \times 10^{-4}$	(4) Tensione superficiale goccie
(5) Sangue defibrinato	2.67	-0°.560	99×10^{-4}	22.3
(6) Siero	1.41	-0°.530	157×10^{-4}	21

(1) Coefficient viscosity

(2) Cryoscopic value

(3) Specific electrical conductability

(4) Surface tension drops

(5) Defibrinated blood

(6) Serum

Experiment III--May 20, 1925; Dog weighing 15.500 kg; at 10 a.m., blood-letting from the left femoral, taking 80 cc.

	(1) Coefficiente di viscosità η 37°	(2) Valore crioscopico Δ	(3) Conducibilità elettrica specifica $K^{37} \times 10^{-4}$	(4) Tensione superficiale goccie
(5) Sangue defibrinato	3.161	-0°.560	71×10^{-4}	22.9
(6) Siero	1.483	-0°.540	153×10^{-4}	21

(1) Coefficient of viscosity

(2) Cryoscopic value

(3) Specific electrical conductivity

(4) Surface tension drops

(5) Defibrinated blood

(6) Serum

From May 20 to 28, 20 cc. of colloidal silicic acid are administered orally at a time, in the aggregate 180 cc. (1.80 g of colloidal silicic acid) that are well tolerated by the animal.

May 28, 1925; blood-letting from the right femoral artery, taking 80 cc. of blood.

	(1) Coefficiente di viscosità η 37° C.	(2) Valore crioscopico Δ	(3) Conducibilità elettrica specifica $K^{18} \times 10^{-4}$	(4) Tensione superficiale goccie
(5) Sangue defibrinato	2.58	-0°.500	74×10^{-4}	22
(6) Siero	1.48	-0°.555	164×10^{-4}	21

(1) Coefficient of viscosity
(2) Cryoscopic value
(3) Specific electrical conductability
(4) Surface tension drops
(5) Defibrinated blood
(6) Serum

When the administration of colloidal silicic acid is suspended, the blood-letting is repeated taking 80 cc. of blood at 10 a.m., June 4, 1925.

	(1) Coefficiente di viscosità η 37° C.	(2) Valore crioscopico Δ	(3) Conducibilità elettrica specifica $K^{18} \times 10^{-4}$	(4) Tensione superficiale goccie
(5) Sangue defibrinato	1.61	-0°.560	112×10^{-4}	22
(6) Siero	1.61	-0°.530	162×10^{-4}	18

(1) Coefficient of viscosity
(2) Cryoscopic value
(3) Specific electrical conductability
(4) Surface tension drops
(5) Defibrinated blood
(6) Serum

Summarizing the results of the experiments, we can make these statements.

Viscosity of the defibrinated blood

This is decreased and does not return to normal after suspension of administration of the colloidal silicic acid. This decrease is not to be attributed to oligocythemia as was demonstrated by the examinations of blood in the third dog. In this animal under physiological conditions, i.e., prior to administration fo the colloidal silicic acid, the number of erythrocytes was on the order of 5,175,000, during the silicic treatment it was maintained at 5,160,000 and after suspension of administration of the drug it was maintained at 5,185,000. Nor can we think of a decrease in the viscosity of the red corpuscles, since we had to acknowledge rather a swelling of them through the absorption of water from the blood; if it is legitimate to suppose that the colloidal silicic acid causes a decrease in many organic secretions (salivary, gastric, biliary, and urinary), as was demonstrated above, it can cause in the organism almost a state of hydremia. One hypothesis that can give us a probable explanation is the numerical decrease of the leukocytes, demonstrated in the experiments on the uric acid of the blood. A second hypothesis, even more possible, is that the viscosity is decreased by the increase of the hemo-alkalinity in relation to the increase of uric acid in the blood. In this case, it can be supposed that, by the introduction of colloidal silicic acid into the blood and by the production of acids in the organism, the tension of the carbonic acid is increased, and excites the respiratory center to a greater pulmonary ventilation and therefore to a greater elimination of carbonic acid. This decrease in carbonic

acid, as was already demonstrated by Ferrai (25) in Bottazzi's laboratory, is capable of modifying the viscosity of blood, making it decrease. A last hypothesis, that is however not very satisfactory, could be the effect of the blood-letting which, as was demonstrated by Burton-Opitz (26), which causes an irregular decrease in blood viscosity by penetration of lymph into the bloodstream, but the results I obtained show variations of viscosity that are much smaller than those observed in the prior experiments. In a normal dog, under the same experimental conditions as the previous ones, without administration of colloidal silicic acid, the blood viscosity after repeated blood-lettings at the same interval of time observed for the other dogs under the effect of colloidal silicic acid, yielded these results:

July 9	η	37°C 3.55
July 15	η	37°C 3.35
July 21	η	37°C 3.16

This decreased viscosity of the blood, that ought to move in the organism through the vessels and capillary spaces, signifying a decrease in the work of the heart and therefore a lowering of the blood pressure, can justify the use of colloidal silicic acid in arteriosclerosis.

The viscosity of the serum undergoes minimal increases that can be placed in relation to the increase in concentration of the blood caused by products circulating in the blood (Si) and retained (uric acid, etc.).

Cryoscopic Value

This value for defibrinated blood in the first experiment increases by about 5%, in the second experiment it decreases by about 7%, and remains unchanged in the third experiment. The molecular concentration of serum increases in the first experiment by about 1.1%, decreases in the second experiment by about 5%, and increases in the third experiment by about 3%. In general, an increase of the Δ prevails, i.e., the value of the osmotic concentration is for the most part increased and this increase of the Δ is to be placed in relationship with the retention of osmotically active molecules not eliminated by the kidneys, that are the most important apparati responsible for the regulation of osmotic pressure of organic fluids.

It is legitimate to suppose therefore, in accordance with the data selected regarding the material balance, that the effect of the administration of colloidal silicic acid is to increase the osmotic concentration of the defibrinated blood and of the serum and that, if in some experiments the cryoscopic measurements speak in a contrary sense, that may depend on the blood-letting that, putting lymph into the blood stream, causes a dilution phenomenon. The superimposing of the two contrary phenomena can explain how, though having in general an increase of the Δ, the latter decreases now and then.

Specific Electrical Conductability

This is increased in all the experiments in regard to the defibrinated blood, however, with regard to serum, it is increased in the second and fourth experiment, is unchanged in the first and third experiments and is increased in the third experiment after

suspension of administration of the substance. In fact, even the specific electrical conductivity is increased for the most part, and this increase normally caused by inorganic substances, mostly NaCl, in a lesser degree sodium carbonate, and in the least degree by other salts as well, in our experiments can be traced to Si+ and to the increase of the alkalis. It is to be observed, however, that the percentage increases of conductivity have always been notably greater than the increases in Δ and in some experiments the cryoscopic values are rather decreased. And since all the dissolved substances contribute to the value of Δ, whether dissociated or not, and only the dissociated substances contribute to the value of K, thus at first sight we could think that there has been an increase in the concentration of the electrolytes, concomitantly with the decrease of the analectrolytes. It is to be observed, however, that in the serum there are always found very small variations of viscosity, and the variations in conductivity have been at a very low percentage and therefore not very apparent, while in the defibrinated blood there have been, on the contrary, percentage increases in conductivity that are greater. This diversity of behavior, that may seem strange at first sight, is not so when it is considered that, with regard to defibrinated blood, there have always been great decreases in viscosity, that signify an increase in the mobility of the ions and consequently an increase in conductivity even when the concentration of the ions has remained unchanged. For these reasons it is asserted that the behavior of the defibrinated blood is not different from that of the serum as would appear at first sight.

Surface Tension

The surface tension of the blood and of the serum presents generally very small variations with a slight tendency to an increase. Now since it is seen that there is a slight tendency to an increase in the concentration of the electrolytes, even at the expense of the concentration of the analectrolytes, and since it is known that the former increase the surface tension while the latter decrease it, as is observed in general and as has been demonstrated in urine by H. Frenkel and J. Gluzet (27), even these results agree with those previously described.

BIBLIOGRAPHY

(1) Rabiteau and Papillon. Studies on the anti-ferment properties of sodium silicate. "Comptes Rendus de L. Acc., de Sciences", 1872-1873.

(2) Picot. Regarding the anti-ferment properties of sodium silicate. "Comptes Rendus de L. Acc., de Sciences", 1875, results also reported by Lewin. Toxicologie.

(3) Spica and Chirone. "Medico-pharmaceutical chemistry." "Chimica Medico-Farmaceutica, Vol. I, p. 671, Feltre 1896.

(4) Citations from F. Felix. The antiseptic and therapeutic effect of the alkaline silicates and of the silicated natural mineral waters. "La Revue Med.", June 15, 1898, p. 185.

(5) Astolfoni. Regarding the biological effect of colloidal silica. "Archivio di Farmacologia e Terapeutica.", Vol. XII, 1906.

(6) Alessandrini and Scala. New contribution to the etiology and pathogenesis of pellagra. "Annali di Igiene sperimentale." Vol. XXIV, 1914.

(7) Citations from E. Levi. "Gazzetta degli Ospedali e delle Cliniche." May 11, 1924.

(8) Citations in the "Revista semestrale" of Dr. Zambelletti, No. 4, June 15, 1921.

(9) A paper read in the session of August 17, 1920 to the Academy of Sciences in Paris, reported in the "Rivista" of Dr. Zambelletti, No. 4, June 15, 1921.

(10) D. Vitali. Inorganic pharmaceutical chemistry and toxicology. Unione Editrice Tip. Torinese.

(11) Kopaczewski, W. Theory and practice of the colloids in biology and in medicine. Vigot Freres, Publishers, 1923.

(12) Treadwell. "Analytical chemistry. Quantitative analysis." C.E.F. Vallardi.

(13) Lustig, A., and G. Galeotti. "General pathology." C.E. Libraria Milanese.

(14) Bottazzi, F., and G. Jappelli. "A treatise on social medicine (The physiology of diet)." C.E.F. Vallardi.

(15) Coronedi, G., and R. Luzzato. The nutritional balance in feeding with fatty allogenates. A paper presented at the VI Congress of Applied Chemistry, Rome, 1906, and reported in "Archivio di Famacologia e Terapeutica", Vol. XII, 1906.

(16) Tigerstedt, R. "Treatise on the physiology of man." C.E. Spa "Sapientia" Pasquale Martinucci, Napoli; V. De Giaxa. Manual of Hygiene, F. Vallardi.

(17) Reale, E. "Manuale di Chimica Clinica." IV Edition, C.E.F. Vallardi.

(18) Citations from A. Ferrata. Le emopatie. Vol. I, p. 78-79, Societa Editrice Libraria, Milano.

(19) Ferrannini, L. Semeiologia Medica. C.E.V. Idelson, Naples.

(20) A citation from Charles Livon. "Manual de visisections." Librarie J.B. Bailliere et fils, Parusm, 1882. Paris, 1882.

(21) A citation from H. Deaunis and V. Aducco. "Fisiologia Umana," Vol. III, p. 78.

(22) Citation from H. Deaunis and V. Aducco. "Fisiologia Umana." Vol. III.

(23) Livon, Ch., Loco citao.

(24) Jones, Harry C. "Trattato di Chimica Fisica" a cura di M. Giua, U. Hoepli, 1923.

(25) A citation from F. Bottazzi. "Principii di Fisiologia. Elementi di Chimica Fisica," Vol. I,,p. 357, Societa Editrice Libraria, Milano.

(26) A citation from F. Bottazzi. Loco citato, p. 856.

(27) A citation from F. Bottazzi. Loco citato, p. 369.

I.

RICERCHE FARMACOLOGICHE SULL'ACIDO SILICICO COLLOIDALE.

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Poche ricerche sono state fatte sulle azioni biologiche del silicio; le prime, che rimontano a RABUTEAU e PAPILLON (1), si riferiscono all'azione antifermentativa del silicato di sodio nella fermentazione alcoolica del mosto, dell'urea nell'urina, del latte e delle mandorle amare. PICOT (2) fu il primo a portarne lo studio nel campo tossicologico ed osservò che il silicato di sodio, a dosi inferiori a 25 cgr. era tollerato indifferentemente dai conigli, mentre a dosi di 25-75 cgr. produceva nei conigli normali e in quelli nei quali era stata provocata setticemia, con sangue putrefatto iniettato per via sanguigna, fenomeni tossici caratterizzati da anoressia, diarrea, elevazione termica, tachipnea, alterazioni della crasi sanguigna e morte per gastro-enterite emorragica. SPICA e CHIRONE (3) osservarono gli stessi fenomeni tossici transitori nei conigli ai quali iniettarono nel sangue 15-45 mgr. di acido silicico colloidale e nei cani cambiamento della reazione dell'orina da acida in alcalina. Dall'impiego delle acque siliciche nella cura dell'erpetismo, delle ulceri varicose, dell'eczema cronico, dei catarri utero-vaginali e di altre manifestazioni morbose legate alla diatesi urica, BÉRANGER, BONJEAN, PETREQUIN e SOCQUET (4) ammisero che esse agissero modificando profondamente il ricambio materiale. ASTOLFONI (5) studiò in vitro l'azione antifermentativa dell'acido silicico colloidale e dalle sue esperienze risulta che l'acido silicico colloidale, a differenza dei silicati alcalini, i quali esercitano notevole azione antifermentativa, influenza scarsamente il potere proteolitico della pepsina, il potere saccarificante della tripsina e non influenza affatto le ossidasi, anche se somministrato a dosi elevate. In vivo, egli osservò nelle rane, per dosi superiori a 10 mgr., fenomeni tossici sui muscoli dello scheletro, arresto della respirazione e morte, nei cani, che tollerano meglio questa sostanza, abbassamento della pressione sanguigna e aumento nell'urina dei prodotti del ricambio materiale. ALESSANDRINI e SCALA (6), iniettando sotto cute alle cavie, ai conigli e ai cani acido silicico colloidale, provocarono dopo un certo tempo un'intossicazione grave caratterizzata da deperimento, caduta dei peli, paresi spastiche, paralisi, anemia grave, quadro morboso molto simile a quello della pellagra, onde essi opinarono che questa malattia dovesse mettersi in rapporto all'uso di acque siliciche anzichè all'alimentazione maidica, avvalorando la loro ipotesi con la constatazione di pellagrosi in località dove si rinvengono queste acque. Inoltre essi osservarono, nei cani sottoposti per lungo tempo all'azione dell'acido silicico colloidale, modificazioni del ricambio materiale consistenti nell'eliminazione di piccole quantità di urina con basso peso specifico, povere di sostanze estrattive, di sali, specie cloruri, e acidosi del sangue probabilmente in rapporto ad un'eccessiva liberazione di acidi consecutiva ad una forte ritenzione di sali minerali.

Da pochi anni sono stati introdotti nella terapia umana dei preparati silicici: l'acido silicico colloidale Erba per via sottocutanea, il silistren Bayer per via orale nella tubercolosi (ROBIN, VOLRATH, ROSSLE e KAHLE) (7) e nel cancro inoperabile (GIORDANO e VALTORTO) (8) e il silicato di soda per via endovenosa nell'arteriosclerosi, nell'ipertensione arteriosa, nelle forme morbose cardiorenali con azotemia e acloruria (SCHEFFER, SARTORY e PELLISSIER) (9).

Le ricerche sperimentali finora compiute sull'acido silicico colloidale sono scarse e discordi, in esse poco s'è tenuto conto della tossicità di questa sostanza, anche se somministrata a piccole dosi per lungo tempo, e dell'azione locale irritante, ond'è da considerare ancora audace l'applicazione alla terapia umana.

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L'acido silicico colloidale è stato da me preparato per via chimica, sciogliendo cioè gr. 112 di silicato di sodio in 300-400 cm^3 di acqua distillata, aggiungendovi gr. 67 di HCl (gr. 187 di HCl della

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densità di 1,18) diluito in mezzo litro di acqua distillata e portando il volume a un litro (10). Dopo dializzato è stato sterilizzato a 100° C. in autoclave. Il colloide ottenuto si presenta sotto forma di liquido limpido, incolore, inodoro, di reazione debolmente acida e si conserva stabile per lungo tempo. All'ultramicroscopio appare costituito di numerosi granuli dotati di scarso movimento browniano, mostranti il fenomeno di TYNDALL (11).

Il contenuto di acido silicico è stato determinato (12) sotto forma di SiO_2 evaporando cioè in una capsula di porcellana a bagnomaria una determinata quantità del colloide, che coagula dapprima sotto forma di gelatina e si riduce poi in una polvere bianca amorfa, che si tratta con HCl concentrato e si lascia per 20 minuti a temperatura ambiente per trasformare in cloruri i sali basici e gli ossidi formatisi durante lo svaporamento e l'essiccamento. Aggiungendo acqua distillata e riscaldando alla ebollizione, l'acido silicico si deposita nel fondo della capsula e il liquido soprastante si decanta attraverso un filtro a ceneri note. Il residuo lavato 3-4 volte per decantazione con acqua calda, si trasporta sul filtro e si lava fino a scomparsa della reazione del cloro. Infine si brucia il filtro nel crogiuolo di platino, si mette in essiccatore ad acido solforico e si pesa. Con questo procedimento, in 25 cm^3 del colloide preparato si pesarono gr. 0,2494 di SiO_2 invece di gr. 0,25. La piccola differenza è da mettersi in rapporto a lievi perdite, causate da passaggio di acido silicico nel filtrato, che si possono evitare ripetendo più volte sui diversi filtrati il procedimento esposto. Si può ritenere quindi che il colloide preparato conteneva 1 cgr. di acido silicico colloidale per cm^3.

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Azione locale (13). — L'acido silicico colloidale è da considerarsi tra le sostanze chimiche che, senza nessun intervento di microrganismi, sono capaci di procurare suppurazione asettica, come osservarono già ASTOLFONI, SCALA e ALESSANDRINI. In un primo e in un secondo cane l'iniezione sottocutanea di acido silicico colloidale, fatta con rigorosa asepsi, causò rapidamente, cioè in meno di 24 ore, un ascesso nel sito dell'iniezione, senza però quei fenomeni generali, che sono così considerevoli nelle suppurazioni microbiche. A differenza di queste non si osservò metastasi e la guarigione si effettuò facilmente. Il pus dell'ascesso, aspirato con siringa sterile e

rienoculato in un altro cane, non riuscì virulento. Nei preparati colorati si osservarono al microscopio numerosi corpuscoli muco-purulenti e scarsissimi cocchi extra-cellulari.

Azione tossica. — L'acido silicico colloidale, somministrato per bocca con sonda gastrica a tre cani, rispettivamente alla dose di 50-30-25 cm^3, produsse rapidamente avvelenamento acuto. Gli animali, poco dopo l'ingestione della sostanza, cominciarono ad accusare malessere generale, tachipnea, al secondo giorno rifiutarono il cibo ed ebbero 2-3 scariche diarroiche miste a sangue e al terzo giorno morirono. All'esame necroscopico la mucosa gastrica e intestinale erano fortemente iperemiche e quella dello stomaco si presentava in diversi tratti cosparsa di chiazze emorragiche. I polmoni, il cuore, il fegato, la milza e i reni erano fortemente congestionati e mostravano chiazze di degenerazione grassa.

L'avvelenamento cronico si manifestò in un cane dopo circa un mese per la dose di gr. 1.62, di cui gr. 1.55 dati per bocca e cgr. 7 per via endovenosa e in un altro cane dopo circa un mese per la dose di 8 cgr. dati per via endovenosa. Nel periodo di osservazione, che per il primo animale durò 5 mesi e per il secondo un mese e mezzo, non si osservarono fenomeni morbosi degni di rilievo; rare volte gli animali rifiutarono il cibo, una volta sola il 1° animale ebbe diarrea. Dopo parecchio tempo dalla somministrazione della sostanza l'esame delle urine svelò nefrite e l'esame del sangue azotemia, com'è dimostrato negli esperimenti che saranno esposti in seguito.

I preparati istologici degli organi esaminati dopo la morte degli animali, colorati con ematossilina acida ed eosina hanno fornito questi reperti microscopici.

Stomaco e intestino. — L'epitelio superficiale è tutto desquamato e il connettivo intertubolare è aumentato. Nel lume dei tuboli glandolari si trovano ammassi di cellule che riempiono la cavità, mentre gli epiteli glandolari si colorano in un modo diffuso e in qualche punto si staccano dalla parete e cadono nel lume del tubolo. I vasi sanguigni sono ripieni di sangue.

Fegato. — Non si nota aumento del connettivo interstiziale. I rami della vena porta sono ricchi di sangue. Le cellule epatiche della periferia dell'acino mostrano un leggiero infiltramento biliare. I vasi dell'acino sono ristretti per ingrandimento delle cellule epatiche che mostrano un leggiero grado di rigonfiamento torbido e degenerazione adiposa.

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Pancreas. — Le cellule glandolari riempiono completamente il lume degli acini, perchè fortemente rigonfie e con protoplasma granuloso, con granulazioni molto evidenti. Non si vede niente di notevole nella glandola interstiziale.

Milza. — La capsula è un poco ispessita e in essa partono dei tramezzi connettivali alquanto spessi. I corpuscoli di Malpighi sono un poco ingranditi e presentano un centro chiaro per scarsezza degli elementi cellulari, mentre la zona periferica è più evidente per dilatazione dei capillari periferici. La polpa è atrofica e vi si trovano molte cellule ripiene di pigmento.

Rene. — I glomeruli di Malpighi sembrano più ricchi di nuclei, ma nella capsula di Bowmann non v'è alcun contenuto anormale. Nei tuboli contorti l'epitelio, dove esiste, presenta rigonfiamento torbido, degenerazione idropica ed è generalmente desquamato e nel lume si vedono oltre alle cellule un essudato albuminoso di apparenza ialina e in qualche punto colloideo. In molti tuboli della sostanza midollare si trovano egualmente delle masse in parte cellulari e in parte costituite da un essudato omogeneo ialino e colloideo. Il tessuto connettivo della sostanza corticale è in qualche punto aumentato sotto forma di infiltramento parvicellulare fino a formare dei piccoli noduli.

Per l'azione irritante locale e per la tossicità delle dosi usate da altri sperimentatori, ho somministrato l'acido silicico colloidale per via orale e per via sanguigna a dosi piccole nelle ricerche sul ricambio, sulle costanti fisico-chimiche del sangue e sull'acido urico del sangue e delle orine.

Ricerche sul ricambio materiale.

Per gli esperimenti scelsi dei cani adulti robusti e sani, che tenni a riposo e abituai a cibarsi d'una razione alimentare costante, ben tollerata, di composizione relativamente fissa, essendo costituita di 300 gr. giornalieri di pane bianco, alimento nutritivo, essendo ricco di idrati di carbonio, mediocremente provvisto di sostanze proteiche e di sali, poverissimo di grassi. Con questo tipo di razione alimentare l'animale riceveva circa 803 calorie (14) e introduceva gr. 5,44 di azoto, media di tre determinazioni fatte nel corso degli esperimenti su diversi campioni della stessa qualità di pane fresco.

Questa cifra concorda con quella riportata da Coronedi e Luzzatto (15), i quali trovarono che 100 gr. di pane secco contengono

gr. 2,72 di azoto ed è superiore a quella che si legge comunemente nelle analisi del pane, secondo le quali 300 gr. di pane conterrebbero invece gr. 3.36 di azoto, calcolandosi in media nel pane il 7% di proteine e in queste il 16% di azoto.

L'acqua era somministrata in quantità costante.

Dei prodotti del metabolismo delle sostanze nutritive organiche eliminate cogli escreti organici, mi sono limitato a determinare l'eliminazione dell'azoto e dei sali urinari. Il corso delle esperienze è stato diviso in tre periodi.

1° periodo, durante il quale l'animale è stato abituato alla razione alimentare indicata fino a stabilire l'equilibrio di peso corporeo e ad ottenere dati analitici relativi all'orina costanti per poter essere presi in considerazione;

2° periodo, nel quale si somministra acido silicico colloidale;

3° periodo, nel quale era sospeso il trattamento silicico.

La durata di ogni esperimento era di 24 ore, nelle quali l'animale stava rinchiuso in gabbia. Dopo questo tempo si raccoglievano le orine o facendo urinare spontaneamente l'animale o cateterizzandolo.

L'azoto totale fu determinato col metodo di Kjeldahl (17).

L'azoto ureico fu calcolato, dividendo per 2.14 la cifra dell'urea determinata secondo il metodo dell'ipobromito sodico con l'ureometro di Dannecy. Il cloro fu determinato secondo il metodo di Mohr con la soluzione titolata di nitrato di argento, usando come indicatore il cromato di potassio. L'acido fosforico fu determinato sotto forma di P^2O^5 col metodo volumetrico di Neubauer con la soluzione di acetato di uranio, usando per indicatore il ferrocianuro di potassio. L'acido solforico totale sotto forma di solfato di bario col metodo di P. Mohr modificato da H. Schultz.

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Cane n. 1, entrato in laboratorio nel gennaio 1925 con peso corporeo di kg. 7,250. Si incomincia subito l'alimentazione con pane gr. 300 e acqua.

1° periodo.

DATA	N alimentare introdotto gr.	quantità di urina cm³	Peso specifico	N orinario totale gr.	N ureico gr.	Cl orinario gr.	P_2O_5 totale gr.	H_2SO_4 totale gr.	Peso corporeo Kgr.
7 febbraio	5.44	100	1039	2.39	1.33	3.10	0.33	0.70	7.700
8-9 (1) »	—	—	—	—	—	—	—	—	
10 »	5.44	240	1032	3.63	2.10	2.99	0.40	0.39	
11-12 (2) »	—	—	—	—	—	—	—	—	
13 »	5.44	150	1054	3.02	2.04	3.39	0.54	0.54	
Media	5.44	168	1041	3.01	1.82	3.16	0.42	0.54	
				1.79 %	1.08 %	1.88 %	0.25 %	0.32 %	

N introdotto gr.	N eliminato gr.	N bilancio gr.
5.44	3.01	+ 2.43

(1) È rimesso in libertà continuando sempre la stessa alimentazione.

(2) In libertà.

2° periodo.

Comincia il 14 febbraio con la somministrazione dell'acido silicico colloidale per bocca, alla dose giornaliera di cm³ 15 il primo giorno e di cm³ 20 negli altri tre giorni consecutivi. Complessivamente l'animale ha introdotto durante questo periodo cm³ 75 di acido silicico colloidale, pari a gr. 0.75 di SiO_2, mantenendosi in buone condizioni di salute ed avendo al quarto giorno di somministrazione della sostanza un'abbondante scarica diarroica, mentre negli altri giorni la defecazione è normale. Il 19 febbraio viene rinchiuso in gabbia e nelle urine raccolte si ottengono questi dati analitici:

DATA	N alimentare introdotto gr.	Quantità di urina cm³	Peso specifico	N orinario totale gr.	N ureico gr.	Cl orinario gr.	P_2O_5 totale gr.	H_2SO_4 totale gr.	Peso corporeo Kgr.
19 febb. (1)	5.44	100	1045	1.94	1.25	2.30	0.36	0.30	7.800
21 febb. (2)	5.44	125	1035	2.18	1.56	3.50	0.27	0.30	
23 febb. (3)	5.44	225	1017	2.23	2.17	3.36	0.40	0.45	
25 febb.	5.44	250	1018	2.48	2.16	4.00	0.32	0.44	
Media	5.44	200	1028	2.20	1.78	3.29	0.34	0.37	
				1.10 %	0.89 %	1.64 %	0.17 %	0.18 %	
Bilancio:	N	Cl	P_2O_5	H_2SO_4					
	+3.24	−0.13	+0.08	+0.17					

3° periodo.

Durà tredici giorni nei quali viene sospesa la somministrazione dell'acido silicico colloidale, dopo i quali l'analisi dell'urina dimostra che l'animale è tornato quasi alle cifre del primo periodo per tutti

(1) Il 19 e il 20 l'animale è tenuto in libertà e gli si somministrano complessivamente altri 40 cm³ di acido silicico colloidale, cioè gr. 0.40 di $Si O_2$.

(2) Il 21 e il 22 l'animale è rimesso in libertà e gli si somministrano complessivamente altri 40 cm³ di acido silicico colloidale.

(3) Il 23 e il 24 animale in libertà e somministrazione complessiva di 40 cm³ di acido silicico colloidale.

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gli elementi presi in esame, ad eccezione dell' H^2SO_4 totale che continua a diminuire.

DATA	N alimentare introdotto gr.	quantità di urina cm³	Peso specifico	N orinario totale gr.	N ureico gr.	Cl orinario gr.	P_2O_5 totale gr.	H_2SO_4 totale gr.	Peso corporeo Kgr.	Osservazioni
1 marzo	5.44	200	1025	3.02 1.51 %	2.44 1.22 %	3.20 1.60 %	0.47 0.23 %	0.28 0.14 %	7.800	

4° periodo.

Comincia il 12 marzo e dura 7 giorni, fino cioè al 19, nei quali si riprende la somministrazione di acido silicico colloidale per via endovenosa iniettandone complessivamente 13 cm³ pari a gr. 0,13.

20 marzo	5.44	190	1018	1.40 0.73 %	1.17 0.61 %	1.37 0.72 %	0.41 0.21 %	0.74 0.38 %		L'esame dell'urina fa rilevare nefrite
Bilancio:		N + 4.01	Cl + 1.79	P_2O_5 + 0.01	H_2SO_4 — 0.20					

Dopo queste 7 iniezioni endovenose l'animale non torna più alle cifre normali, come è dato osservare dall'esame dei dati raccolti dopo un lungo periodo nel quale fu sospesa la somministrazione del farmaco.

23 aprile	5.44	150	1020	1.09	1.02	2.70	0.12	0.15		
3 magg.	5.44	100	1035	1.59	0.89	2.94	0.22	0.19		
18 magg.	5.44	80	1036	0.70	0.55	0.92	0.03	0.12		
22 magg.	5.44	100	1030	1.25	0.77	2.52	0.06	0.13	7.750	
Media	5.44	107	1030	1.16 1.08 %	0.80 0.74 %	2.27 2.12 %	0.10 0.09 %	0.14 0.12 %		
Bilancio:		N + 3.28	Cl + 0.89	P_2O_5 + 0.32	H_2SO_4 + 0.40					

L'animale viene sacrificato e all'autopsia si nota un'estesa degenerazione grassa del rene e chiazze degenerative negli altri organi.

CANE n. 2, del peso di kgr. 6.300 entrato in laboratorio nell'aprile del 1925 alimentato con 300 gr. di pane e acqua.

1° periodo

DATA	N alimentare introdotto gr.	quantità di urina cm³	Peso specifico	N orinario totale gr.	N ureico gr.	Cl orinario gr.	P_2O_5 totale gr.	H_2SO_4 totale gr.	Peso corporeo Kgr.
6 maggio (1)	5.44	280	1018	2.00	1.82	3.69	0.20	0.28	6.500
22 maggio	5.44	140	1035	2.30	1.31	2.55	0.21	0.31	
Media	5.44	210	1026	2.15 1.02 %	1.56 0.02 %	3.12 1.48 %	0.20 0.09 %	0.29 0.13 %	
		N introdotto gr.		N eliminato		N bilancio			
		5.44		2.15		+ 3.29			
				2° periodo					
25 maggio (1)	5.44	160	1016	1.30 0.81 %	1.20 0.75 %	2.72 1.29 %	0.20 0.09 %	0.31 0.19 %	
31 maggio	5.44	90	1040	1.90	0.45	1.89	0.18	0.32	
Media	5.44	125	1028	1.60 1.28 %	0.82 0.65 %	2.30 1.84 %	0.19 0.15 %	0.31 0.24 %	
Bilancio:	N + 3.84	Cl + 0.42	P_2O_5 + 0.01	H_2SO_4 — 0.02					

Sospese le iniezioni endovenose l'animale non ritorna più al normale e sacrificato presenta degenerazione grassa dei diversi organi e del rene principalmente.

(1) Dal 6 al 21 maggio è messo in libertà e tenuto sempre alla stessa alimentazione.
Dal 22 al 25 maggio si iniettano per via endovenosa complessivamente 3 cm³ di acido silicico colloidale, pari a 3 cgr. di SiO_2.
Dal 25 al 30 maggio si ripetono le iniezioni endovenose di cm³ 2, pro die, complessivamente 10 cm³, pari a cgr. 10 di SiO_2.

Le cifre raccolte in questi quadri rappresentano i risultati ottenuti nell'eliminazione dell'azoto e dei sali urinari delle 24 ore. Quelle del 1° periodo sono le cifre normali, quelle del 2° periodo le cifre ottenute dopo la somministrazione di acido silicico colloidale per via orale e per via endovenosa, quelle del 3° periodo, nel primo cane, sono le cifre ottenute dopo sospesa la somministrazione del farmaco, quelle del 4° periodo sono le cifre del periodo che chiameremo tossico, perchè l'eliminazione di detti principii non ritorna più al normale, ma rimane persistentemente scarsa. In condizioni normali, e cioè per effetto del tipo di alimentazione adottato, gli animali non raggiunsero mai un equilibrio d'azoto e di sali in senso assoluto, ma un'eliminazione di queste sostanze pressochè costante, come dimostrano le cifre ottenute, le quali si differenziano di poco tra loro.

In questo primo periodo, avendo determinato solo l'azoto introdotto con l'alimentazione e non i sali, possiamo ritenere che l'animale introduceva gr. 5.44 di N, ne eliminava gr. 3.01, ne risparmiava gr. 2.43 ed eliminava gr. 3.16 di NaCl, gr. 0.42 di P_2O_5 e gr. 0.54 di H^2SO_4. Nel secondo periodo, comune ad entrambi i cani in esperimento, la somministrazione di acido silicico colloidale, determina una minore eliminazione di N, che, in rapporto alla quantità costante introdotta, avendo sempre gli animali consumata intera la razione, si riduce nel primo cane a gr. 2.20, accentuandosi il risparmio a gr.3.24, e nel secondo cane a gr. 1.30, accentuandosi il risparmio a gr. 4,14 nel primo periodo e a gr. 3,84 nel secondo periodo di somministrazione del farmaco. L'eliminazione dell'acido fosforico e dell'acido solforico urinario da gr. 0,42 e 0,54 si riducono nel primo cane rispettivamente a gr. 0,34 e 0,37, con un risparmio cioè di gr. 0,08 e 0,17, e nel 2° cane invece la quantità di acido fosforico eliminata non subisce variazioni nel 1° periodo di iniezioni endovenose di acido silicico colloidale, mantenendosi costantemente a gr. 0,20, nel secondo periodo diminuisce a gr. 0,18, l'acido solforico totale al contrario in entrambi i periodi delle iniezioni è sempre aumentato dalla quantità normale di gr. 0,29 a gr.0,31-0,32. La quantità di Cl, dosato sotto forma di NaCl, dopo il trattamento silicico aumenta nel primo cane di 13 cgr. e diminuisce nel secondo cane di 82 cgr. Le cifre del terzo periodo, riferibili al primo cane, dimostrano che, sospesa la somministrazione della sostanza, l'animale tende a tornare alle cifre del primo periodo, essendosi presumibilmente esaurita l'azione dell'acido silicico colloidale nell'organismo. Quelle del primo periodo, citate per brevità solo pel

primo animale, essendo affatto concordi per il secondo animale, dimostrano infine che l'ulteriore somministrazione della sostanza per via endovenosa, alla dose di 13 cgr. in 7 giorni di seguito e cioè alla dose di circa gr. 0,0026 per kg. di peso produce fenomeni tossici, come dimostrano l'esame chimico e microscopico delle urine, l'esame della permeabilità renale e l'esame macroscopico e istologico degli organi prelevati dopo la morte dell'animale, come ho esposto innanzi. L'esame delle urine dei due animali in questo periodo fa osservare diminuzione delle quantità, alto peso specifico, colorito tendente al rosso, diminuzione del materiale solido organico ed anorganico, presenza talvolta di albumina in quantità non molto notevole, di cilindri, di cellule epiteliali renali, di leucociti e di emazie. Con adatte esperienze ho cercato di stabilire se la scarsa eliminazione di N fosse in rapporto a diminuzione dei processi ossidativi o ad azotemia. Per lo studio dei processi ossidativi organici ho ricercato i fermenti ossidativi nel sangue col metodo di Schultz (18). Secondo questo metodo i preparati di sangue, dopo essiccamento, sono stati fissati in una soluzione di formalina al 4 % per un minuto primo, quindi lavati con acqua corrente, colorati per un minuto in una soluzione acquosa all'1 % di α -naftolo e per un altro minuto in una soluzione acquosa all'1% di dimetil -p- fenilendiamina. Osservati al microscopio, dopo montaggio in acqua o glicerina, i fermenti ossidanti contenuti nei leucociti si mostravano colorati in bleu per la formazione di un leuco-prodotto dovuto ad una reazione tra la dimetil -p-fenilendiamina e l'α -naftolo. La ricerca fatta sul sangue di cane in condizioni normali dimostrò che su 140 leucociti contati, 18 presentavano granuli colorati in bleu e cioè il 12 %. Nello stesso cane dopo la somministrazione di acido silicico colloidale per via orale e per via endovenosa per alcuni giorni consecutivi, il numero di essi aumentò all'86 %, essendosi riscontrati i fermenti ossidanti in 52 leucociti su 64 contati nei preparati. Da questa ricerca bisogna ammettere che la diminuita eliminazione di N non è da mettersi in rapporto a inibizione delle ossidasi, causata dall'acido silicico colloidale, avendo mostrato invece i preparati di sangue esaminati aumento di questi fermenti.

Il dosaggio col metodo di Kjeldahl dell'N nel sangue in un altro cane ha dimostrato che l'acido silicico colloidale determina aumento di N nel sangue. In 5 cm³ di sangue prima della somministrazione del farmaco furono dosati gr. 0.07196 di N, cioè l'1.439%,

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dopo la somministrazione ne furono dosati invece gr. 0,188720, cioè il 3,774 %.

La permeabilità renale è stata studiata in un piccolo cane a rene sano, scegliendo tra le sostanze urofane (19), il bleu di metilene in soluzione acquosa al 5 %. Dopo vuotamento della vescica, mediante cateterismo, fu iniettato sotto cute 1 cm³ della soluzione di bleu di metilene la cui eliminazione cominciò dopo un'ora e 20 minuti e finì dopo 3 giorni. L'iniezione di 6 cm³ di acido silicico colloidale produsse in breve alterazioni renali, riscontrandosi nelle urine albuminuria ematuria e cilindruria. La prova del bleu di metilene, ripetuta il giorno dopo, fece osservare che l'eliminazione cominciò dopo 3 ore dall'iniezione di 1 cm³ della sostanza e si protrasse per 3 giorni consecutivi. Continuando le iniezioni endovenose di acido silicico colloidale alla dose di 1 cm³ pro die per altri otto giorni consecutivi e ripetendo la prova del bleu di metilene alla stessa dose, l'eliminazione di questa sostanza cominciò dopo un'ora e ventisei minuti e durò tre giorni di seguito. In verità la prova del bleu di metilene non ha dato risultati differenti a rene sano e a rene leso. Dall'insieme dei dati raccolti nelle precedenti tabelle risulta che l'acido silicico colloidale ha azione evidente sul ricambio dell'N e dei sali orinari. L'eliminazione dell'N è costantemente diminuita in tutti gli esperimenti. La eliminazione dei sali mostra risultati differenti: nel primo cane P_2O_5 e H_2SO_4 diminuiscono e il Cl invece aumenta, nel secondo cane al contrario il Cl aumenta, P_2O_5 in un primo momento non presenta alcuna modificazione, in un secondo momento diminuisce, H_2SO_4 aumenta sempre.

Per quanto il meccanismo d'azione dell'acido silicico colloidale non sia ben chiaro nel ricambio dell'N e dei sali orinari, tuttavia si può, da questi risultati ottenuti, emettere l'ipotesi che dapprima questa sostanza agisca determinando abbassamento del potere funzionale delle cellule dei tessuti e in un secondo tempo nel periodo tossico cioè, determinando alterazioni della crasi sanguigna, del materiale nutritivo cioè che arriva alle cellule stesse, e lesioni distruttive degli organi.

A dimostrazione delle alterazioni funzionali di alcuni organi riferisco i risultati ottenuti sulla secrezione gastrica, salivare e biliare sotto l'azione dell'acido silicico colloidale. In un cane grande e robusto, operato di fistola gastrica alla Cl. Bernard (20), l'esame del succo gastrico in condizioni normali dette questi risultati col metodo di Toepfer (21), che rappresentano la media di due esperimenti.

Quantità raccolta in 2 ore cm³ 85, acidità totale 80 %, costituita dal 50 % HCl libero, dal 13 % di HCl combinato e dal 17 % di acidi organici. Per tre giorni consecutivi gli furono somministrati per bocca complessivamente 45 cm³ e per altri quattro giorni 8 cm³ per via sanguigna della sostanza. Raccolto il succo gastrico, dopo due ore la quantità fu di 17 cm³ e solo dopo 6 ore se ne misurarono 100 cm³. L'esame chimico dimostrò assenza di HCl libero con la reazione di Günzburg e con quella di Boas-Danilewski (22); l'acidità totale 80 %, costituita pel 56 % da HCl combinato e pel 24 % da acidi organici. L'assenza di HCl libero in questo animale, nel quale prima del trattamento silicico era dimostrabile, è segno di difettosa secrezione. Per questa ragione e per quanto è stato dimostrato da Astolfoni, circa il diminuito potere proteolitico della pepsina, è da pensare che sotto l'azione dell'acido silicico colloidale la digestione non si effettui completamente.

In un secondo cane, operato di fistola del dotto di Wharton (23), si contarono normalmente 15 goccie di saliva al minuto, dopo l'iniezione di 5 cm³ di acido silicico colloidale le goccie si ridussero a 12 dopo 5 minuti dall'iniezione e a 6 dopo 10 minuti. L'iniezione di 1 cgr. di pilocarpina fece aumentare dopo 2 minuti la quantità di saliva a 32 goccie e l'iniezione di altri 5 cm³ di acido silicico colloidale tornò a far diminuire a 6 goccie la saliva. L'azione durò ³/₄ d'ora e dopo questo tempo la quantità di saliva aumentò progressivamente a 18 goccie al minuto. Questo esperimento dimostra dunque che l'acido silicico colloidale è capace di modificare la secrezione salivare.

Un terzo cane, operato di fistola biliare e alimentato per l'intera durata del periodo sperimentale con una razione uniforme composta di carne magra gr. 300, pane bianco gr. 400 e acqua, somministrata sempre alla stessa ora, fornì i risultati raccolti nella seguente tabella:

Giorno di esperimento	Quantità di bile cm³	Peso specifico a + 15° C.	Acqua %	Residuo secco %	Residuo secco totale	Ceneri %	Viscosità η 37° C	Δ	Conduttività elettrica a η 37° C.
10 luglio	26	1.0116	95.802	4.198	1.0914	1.31	1.41	-0°.510	238×10^{-4}
16 »	16	1.0142	95.600	4.400	0.7043	1.43	1.35	-0°.550	191×10^{-4}

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Da questa risulta che l'acido silicico colloidale, iniettato nel sangue per 7 giorni di seguito, alla dose di 2 cgr. al giorno dette luogo a queste modificazioni. La quantità di bile emessa, la resistenza elettrica e la viscosità diminuirono, invece aumentarono la quantità percentuale del residuo e delle ceneri e il punto di congelazione.

CONCLUSIONI.

I risultati ottenuti in queste esperienze conducono alle seguenti conclusioni:

1. — L'acido silicico colloidale è dotato di azione irritante locale che ne controindica l'uso per iniezioni sottocutanee.

2. — Introdotto nell'organismo per bocca o per via sanguigna modifica il ricambio dell'N e dei sali orinari, diminuendo l'eliminazione dell'azoto e determinando modificazioni nell'eliminazione dei sali, le quali non sono sempre nello stesso senso.

3. — Le piccole dosi, anche se somministrate per breve tempo, producono fenomeni tossici immediati e persistenti.

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La diminuita eliminazione di N e di sali, dimostrata nei precedenti esperimenti, la diminuita eliminazione di acido urico e l'aumentata emoalcalinità attuale dimostrata in ricerche che verranno pubblicate in seguito, m'hanno indotto a studiare infine le modificazioni immediate e a distanza delle costanti fisico-chimiche del sangue defibrinato e del siero per azione dell'acido silicico colloidale. Nella letteratura non v'è nulla al riguardo e perciò ho ritenuto non trascurabili le ricerche eseguite per dimostrare se $\overset{+}{Si}$ sia capace di provocare modificazioni nell'equilibrio fisico-chimico dell'organismo.

Per gli esperimenti ho scelto cani sani e robusti, alimentati sempre con la stessa razione, costituita di pane bianco e acqua, che veniva somministrata alla stessa ora. L'acido silicico colloidale è stato somministrato per via orale e per via sanguigna. Il sangue occorrente per le determinazioni (80 cm³) veniva estratto per salasso da un'arteria (carotide o femorale) e raccolto in parte in un Erlenmeyer sterilizzato, contenente palline di vetro per la defibrinazione, e in parte in un cilindro di vetro che veniva tenuto in ghiacciaia

per la separazione rapida del siero. Del sangue defibrinato e del siero sono stati determinati il punto di congelamento, la viscosità, la conduttività elettrica e la tensione superficiale. Per ognuna di queste ricerche sono state fatte tre determinazioni, prendendo per risultato la media di esse. La viscosità del siero e del sangue defibrinato è stata determinata alla temperatura di 37° C sempre sulla stessa quantità di liquido (cm³ 4) con un viscosimetro Ostwald a capillare verticale, provvisto di termoregolatore a toluolo ed agitatore, e il valore è stato calcolato con la formula di Mayer (24) ($\eta = tx/tH^2O$), dividendo cioè il tempo di deflusso del liquido in esame per il tempo di deflusso dell'acqua distillata misurati con lo stesso viscosimetro. Il valore crioscopico è stato misurato, su 10 cm³ di liquido in esame, con l'apparecchio di Beckmann, determinando lo zero del termometro con acqua bidistillata al principio e alla fine di ogni serie di determinazioni. La conduttività elettrica è stata determinata col metodo di Kohlrausch, determinando in precedenza la capacità di resistenza della celletta di Ostwald immersa in termostato alla temperatura di 37° C. Avvalendosi di una soluzione 0,1 n di KCl, si è trovata una capacità eguale a 0,388. La tensione superficiale è stata determinata, contando il numero delle goccie di acqua distillata, di sangue defibrinato e di siero che alla temperatura di 20° C defluivano dallo stalagmometro di Traube. Per l'acqua distillata il numero delle goccie fluenti dallo stalagmometro usato era di 18,8.

Riporto i risultati delle esperienze eseguite:

Esperienza I. — 22 aprile 1925. Cane di kg. 5,500; alle ore 10 salasso dalla femorale sinistra di 80 cm³ di sangue.

	Coefficiente di viscosità η 37°	Valore crioscopico Δ	Conducibilità elettrica specifica $K^{37} \times 10^{-4}$	Tensione superficiale goccie
Sangue defibrinato	3.40	-0°.550	73×10^{-4}	24.4
Siero	1.40	-0°.515	113×10^{-4}	22

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Dal 22 al 27 aprile si iniettano per via endovenosa 2 cgr. al giorno, complessivamente 12 cgr. di acido silicico colloidale, che l'animale tollera bene.

27 aprile; ore 10 salasso dalla femorale destra di 80 cm³.

	Coefficiente di viscosità η 37°	Valore crioscopico Δ	Conducibilità elettrica specifica $K^{37} \times 10^{-4}$	Tensione superficiale goccie
Sangue defibrinato	2.91	-0°.580	81×10⁻⁴	22.5
Siero	1.40	-0°.520	120×10⁻⁴	21

Esperienza II. — 30 aprile 1925. Cane di kg. 4,500; alle ore 10 salasso dalla femorale destra di 80 cm³ di sangue.

	Coefficiente di viscosità η 37°.	Valore crioscopico Δ	Conducibilità elettrica specifica $K^{37} \times 10^{-4}$	Tensione superficiale goccie
Sangue defibrinato	3.548	-0°.604	72×10⁻⁴	22.8
Siero	1.387	-0°.560	154×10⁻⁴	21.8

Dal 30 aprile al 4 maggio si inietta 1 cgr. di acido silicico colloidale al giorno, in tutto 6 cgr.

4 maggio 1925; ore 10 salasso dalla arteria femorale sinistra di 80 cm³ di sangue.

	Coefficiente di viscosità η 37°	Valore crioscopico Δ	Conducibilità elettrica specifica $K^{37} \times 10^{-4}$	Tensione superficiale goccie
Sangue defibrinato	2.87	-0°.560	84×10⁻⁴	22
Siero	1.41	-0°.530	154×10⁻⁴	21.4

Si sospendono le iniezioni di acido silicico colloidale.

12 maggio 1925; ore 10 salasso dalla carotide sinistra di 80 cm³ di sangue.

	Coefficiente di viscosità η 37° C.	Valore crioscopico Δ	Conducibilità elettrica specifica $K^{37} \times 10^{-4}$	Tensione superficiale goccie
Sangue defibrinato	2.67	-0°.560	99×10⁻⁴	22.3
Siero	1.41	-0°.530	157×10⁻⁴	21

Esperienza III. — 20 maggio 1925. Cane del peso di kg. 15,500; ore 10 salasso dalla femorale sinistra di 80 cm³.

	Coefficiente di viscosità η 37°	Valore crioscopico Δ	Conducibilità elettrica specifica $K^{37} \times 10^{-4}$	Tensione superficiale goccie
Sangue defibrinato	3.161	-0°.560	71×10⁻⁴	22.9
Siero	1.483	-0°.540	153×10⁻⁴	21

Dal 20 al 28 maggio si somministrano per bocca 20 cm³ di acido silicico colloidale per volta, in complesso 180 cm³ (gr. 1,80 di acido silicico colloidale) che sono bene tollerati dall'animale.

28 maggio 1925. Salasso dall'arteria femorale destra di 80 cm³ di sangue.

	Coefficiente di viscosità η 37° C.	Valore crioscopico Δ	Conducibilità elettrica specifica $K^{37} \times 10^{-4}$	Tensione superficiale goccie
Sangue defibrinato	2.58	-0°.560	74×10⁻⁴	22
Siero	1.48	-0°.555	164×10⁻⁴	21

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Dal 22 al 27 aprile si iniettano per via endovenosa 2 cgr. al giorno, complessivamente 12 cgr. di acido silicico colloidale, che l'animale tollera bene.

27 aprile; ore 10 salasso dalla femorale destra di 80 cm³.

	Coefficiente di viscosità η 37°	Valore crioscopico Δ	Conducibilità elettrica specifica $K^{37} \times 10^{-4}$	Tensione superficiale goccie
Sangue defibrinato	2.91	-0°.580	81×10^{-4}	22.5
Siero	1.40	-0°.520	120×10^{-4}	21

Esperienza II. — 30 aprile 1925. Cane di kg. 4,500; alle ore 10 salasso dalla femorale destra di 80 cm³ di sangue.

	Coefficiente di viscosità η 37°	Valore crioscopico Δ	Conducibilità elettrica specifica $K^{37} \times 10^{-4}$	Tensione superficiale goccie
Sangue defibrinato	3.548	-0°.604	72×10^{-4}	22.8
Siero	1.387	-0°.560	154×10^{-4}	21.8

Dal 30 aprile al 4 maggio si inietta 1 cgr. di acido silicico colloidale al giorno, in tutto 6 cgr.

4 maggio 1925; ore 10 salasso dalla arteria femorale sinistra di 80 cm³ di sangue.

	Coefficiente di viscosità η 37°	Valore crioscopico Δ	Conducibilità elettrica specifica $K^{37} \times 10^{-4}$	Tensione superficiale goccie
Sangue defibrinato	2.87	-0°.560	84×10^{-4}	22
Siero	1.41	-0°.530	154×10^{-4}	21.4

Si sospendono le iniezioni di acido silicico colloidale.

12 maggio 1925; ore 10 salasso dalla carotide sinistra di 80 cm³ di sangue.

	Coefficiente di viscosità η 37° C.	Valore crioscopico Δ	Conducibilità elettrica specifica $K^{37} \times 10^{-4}$	Tensione superficiale goccie
Sangue defibrinato	2.67	-0°.560	99×10^{-4}	22.3
Siero	1.41	-0°.530	157×10^{-4}	21

Esperienza III. — 20 maggio 1925. Cane del peso di kg. 15,500; ore 10 salasso dalla femorale sinistra di 80 cm³.

	Coefficiente di viscosità η 37°	Valore crioscopico Δ	Conducibilità elettrica specifica $K^{37} \times 10^{-4}$	Tensione superficiale goccie
Sangue defibrinato	3.161	-0°.560	71×10^{-4}	22.9
Siero	1.483	-0°.540	153×10^{-4}	21

Dal 20 al 28 maggio si somministrano per bocca 20 cm³ di acido silicico colloidale per volta, in complesso 180 cm³ (gr. 1,80 di acido silicico colloidale) che sono bene tollerati dall'animale.

28 maggio 1925. Salasso dall'arteria femorale destra di 80 cm³ di sangue.

	Coefficiente di viscosità η 37° C.	Valore crioscopico Δ	Conducibilità elettrica specifica $K^{37} \times 10^{-4}$	Tensione superficiale goccie
Sangue defibrinato	2.58	-0°.560	74×10^{-4}	22
Siero	1.48	-0°.555	164×10^{-4}	21

Sospesa la somministrazione di acido silicico colloidale si ripete il salasso di 80 cm³ di sangue alle dieci del 4 giugno 1925.

	Coefficiente di viscosità η 37° C.	Valore crioscopico Δ	Conducibilità elettrica specifica $K^{\infty} \times 10^{-4}$	Tensione superficiale goccie
Sangue defibrinato	1.64	-0°.560	112×10^{-4}	22
Siero	1.61	-0°.530	162×10^{-4}	18

Riassumendo i risultati degli esperimenti si possono fare queste considerazioni.

Viscosità del sangue defibrinato:

Essa è diminuita e non ritorna al normale dopo sospesa la somministrazione di acido silicico colloidale. Questa diminuzione non è da attribuirsi ad oligocitemia, come hanno dimostrato gli esami di sangue nel 3° cane. In questo animale in condizioni fisiologiche, prima cioè della somministrazione di acido silicico colloidale, il numero degli eritrociti era di 5.175.000, durante il trattamento silicico si mantenne a 5.160.000 e dopo sospesa la somministrazione del farmaco si mantenne a 5.185.000. Neppure si può pensare a diminuzione della viscosità dei corpuscoli rossi, dovendosi ammettere anzi un rigonfiamento di essi per assunzione di acqua dal sangue se è lecito supporre che l'acido silicico colloidale, facendo diminuire molte secrezioni organiche (salivare, gastrica, biliare e orinaria), come innanzi è stato dimostrato, possa determinare nell'organismo quasi uno stato di idremia. Un'ipotesi che può darci una probabile spiegazione è la diminuzione numerica dei leucociti, dimostrata negli esperimenti sull'acido urico del sangue. Una seconda ipotesi, ancora più plausibile, si è che la viscosità sia diminuita per l'aumento dell'emoalcalinità in rapporto all'aumento di acido urico nel sangue. In questo caso si può ammettere che, per l'introduzione di acido silicico colloidale nel sangue e per la produzione di acidi nell'organismo, aumenti la tensione dell'acido carbonico, che eccita il centro respiratorio ad una maggiore ventilazione polmonare e quindi ad una maggiore eliminazione

di acido carbonico. Questa diminuzione di acido carbonico, come ha già dimostrato Ferrai (25) nel laboratorio di Bottazzi, è capace di modificare la viscosità del sangue, facendola diminuire. Un'ultima ipotesi, che però è poco soddisfacente, potrebbe essere l'azione del salasso, il quale, come ha dimostrato Burton-Opitz (26), fa diminuire irregolarmente la viscosità del sangue per penetrazione di linfa nel torrente circolatorio, ma i risultati da me ottenuti mostrano variazioni della viscosità molto più piccole di quelle osservate nei precedenti esperimenti. In un cane normale, nelle stesse condizioni sperimentali dei precedenti, senza somministrazione di acido silicico colloidale, la viscosità del sangue dopo i salassi, ripetuti con lo stesso intervallo di tempo osservato per gli altri cani sotto l'azione dell'acido silicico colloidale, dette questi risultati:

9 luglio η 37° C. 3.55
15 » » 3.35
21 » » 3.16

Questa diminuita viscosità del sangue, che deve muoversi nell'organismo per vasi e spazi capillari, importando una diminuzione del lavoro del cuore e quindi un'abbassamento della pressione sanguigna, può dare ragione dell'impiego dell'acido silicico colloidale nell'arteriosclerosi.

La viscosità del siero subisce aumenti minimi che possono essere messi in rapporto ad aumento della concentrazione del sangue dovuta a prodotti in esso circolanti (Si) e ritenuti (acido urico, ecc.).

Valore crioscopico:

Questo per il sangue defibrinato nella 1ª esperienza aumenta di circa il 5 %, nella 2ª diminuisce di circa il 7 % rimane immutato nella 3ª. La concentrazione molecolare del siero aumenta nella 1ª esperienza di circa l'1 %, diminuisce nella 2ª di circa il 5 % ed aumenta nella 3ª del 3 %. In generale prevale un aumento del Δ, cioè il valore della concentrazione osmotica è per lo più aumentato e questo aumento del Δ è da mettersi in rapporto a ritenzione di molecole osmoticamente attive non eliminate dai reni, che sono i più importanti apparecchi deputati alla regolazione della pressione osmotica dei liquidi organici.

È lecito supporre quindi, in armonia con i dati raccolti sul ricambio materiale, che per effetto della somministrazione di acido silicico colloidale aumenti la concentrazione osmotica del sangue defibrinato e del siero e che se in alcuni esperimenti le misure criosco-

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piche parlano in senso opposto, ciò può dipendere dal salasso che, immettendo linfa nel torrente circolatorio, determina un fenomeno di diluizione. La sovrapposizione dei due fenomeni contrari può spiegare come, pure avendosi in generale un aumento del Δ, questo diminuisca talvolta.

Conducibilità elettrica specifica.

Questa è per il sangue defibrinato in tutte le esperienze aumentata, per il siero invece è aumentata nella 2ª e 4ª esperienza, è invariata nella 1ª e 3ª ed è aumentata nella 3ª dopo sospesa la somministrazione della sostanza. In realtà anche la conduttività elettrica specifica è per lo più aumentata e questo aumento, dovuto normalmente alle sostanze anorganiche, in massima parte al NaCl, in parte minore al carbonato sodico e in parte minima anche ad altri sali, nei nostri esperimenti può riferirsi a Si^{+} ed allo aumento degli alcali. È da osservare però che gli aumenti percentuali della conduttività sono sempre stati notevolmente maggiori degli aumenti di Δ e in qualche esperienza i valori crioscopici sono anzi diminuiti. E poichè al valore di Δ contribuiscono tutte le sostanze disciolte, dissociate o non, e al valore di K contribuiscono solo le sostanze dissociate, così a prima vista si potrebbe pensare che si sia avuto un aumento della concentrazione degli elettroliti concomitantemente alla diminuzione degli analettroliti. È da osservare però che nel siero si sono riscontrate sempre piccolissime variazioni di viscosità e le variazioni di conduttività sono state di poche unità per cento, e quindi poco appariscenti, mentre che nel sangue defibrinato si sono avuti invece aumenti percentuali più forti della conduttività. Questa diversità di comportamento, che può sembrare strana a prima vista, non lo è quando si consideri che per il sangue defibrinato si sono avute, sempre forti diminuzioni di viscosità, le quali importano un aumento della mobilità degli joni e conseguentemente un aumento della conduttività anche quando la concentrazione degli joni sia rimasta immutata. Per tali ragioni è da ritenere che il comportamento del sangue defibrinato non è discordante con quello del siero, come sembrerebbe a prima vista.

Tensione superficiale.

La tensione superficiale del sangue e del siero presenta variazioni in generale piccolissime con lieve tendenza all'aumento. Ora poichè si è visto che c'è lieve tendenza all'aumento della concentrazione degli elettroliti, magari a spese della concentrazione degli analettroliti, e poichè è noto che i primi aumentano la tensione

superficiale mentre i secondi la diminuiscono, come si osserva in generale e come hanno dimostrato nell'orina H. Frenkel e J. Cluzet (27), anche questi risultati concordano con quelli precedentemente esposti.

BIBLIOGRAFIA.

(1) Rabuteau e Papillon. Récherches sur les proprietés antifermentiscibles du silicate de soude. « Comptes Rendus de l'Acc. de Sciences », 1872-1873.

(2) Picot. Sur le proprietés antifermentiscibles du silicate de soude. « Comptes Rendus de l'Acc. de Sciences », 1875, risultati riportati pure da Lewin. Toxicologie.

(3) Spica e Chirone. « Chimica medico-farmaceutica », vol. I, pag. 671, Feltre 1896.

(4) Citati da F. Felix. Action antiseptique et thérapeutiques des silicatées alcalins et des eaux minerales naturelles silicatéy. « La Revue med. », 15 Juin 1898, pag. 185.

(5) Astolfoni. Intorno all'azione biologica della silice colloidale. « Archivio di Farmacologia e Terapeutica », vol. XII, anno 1906.

(6) Alessandrini e Scala. Contributo nuovo all'etiologia e patogenesi della pellagra. « Annali di Igiene sperimentale », vol. XXIV, 1914.

(7) Citati da E. Levi. « Gazzetta degli Ospedali e delle Cliniche », 11 maggio 1924.

(8) Citati nella Rivista semestrale del Dr. Zambelletti, n. 4, 15 giugno 1921.

(9) Comunicazione fatta nella Seduta del 17 agosto 1920 all'Accadémie des Sciences di Parigi, riportata nella Rivista del Dott. Zambelletti, n. 4, 15 giugno 1921.

(10) D. Vitali. Chimica inorganica farmaceutica e tossicologia. Unione Editrice Tip. Torinese.

(11) Kopaczewski W. Theorie e Pratique des colloides, en biologie et en Médecine. Vigot Frères. Editeurs. 1923.

(12) Treadwell. Chimica analitica. Analisi quantitativa C. E. F. Vallardi.

(13) A. Lustig e G. Galeotti. Patologia generale. C. E. Libraria Milanese.

(14) F. Bottazzi e G. Jappelli. Trattato di medicina sociale (Fisiologia dell'alimentazione) C. E. F. Vallardi.

(15) G. Ceronti e R. Luzzatto. Bilancio nutritivo nell'alimentazione con grassi alogenati. Comunicazione presentata al VI Congresso di Chimica applicata, Roma, 1906 e riportata nell'a Archivio di Farmacologia e Terapeutica », vol. XII, 1906.

(16) R. Tigerstedt. Trattato di Fisiologia dell'uomo, C. E. « Sapientia », Pasquale Martinucci, Napoli; V. De Giaxa. Manuale di Igiene. Casa Editrice F. Vallardi.

(17) E. Reale. « Manuale di Chimica Clinica », IV ediz. C. E. F. Vallardi.

(18) Citato da A. Ferrata. Le emopatie. Vol. I, pagg. 78-79, Società Editrice Libraria, Milano.

(19) L. Ferrannini. Semeiologia Medica. C. E. V. Idelson. Napoli.

(20) Citato da Charles Livon. Manuel de vivisections. Librairie J. B. Baillière et fils, Paris, 1882.

(21) Citato da H. Beaunis e V. Abucco. Fisiologia Umana, vol. III, pag. 78.

(22) Citato da H. Beaunis e V. Abucco. Fisiologia Umana, vol. III.

(23) Ch. Livon. Loco citato.

(24) Harry C. Jones. Trattato di Chimica Fisica a cura di M. Giua, U. Hoepli, 1922.

(25) Citato da F. Bottazzi. Principii di Fisiologia. Elementi di Chimica Fisica, vol. I, pag. 357, Società Editrice Libraria, Milano.

(26) Citato da F. Bottazzi. Loco citato pag. 356.

(27) Citato da F. Bottazzi. Loco citato pag. 360.

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No. 3, 1961
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EFFECT OF SILICIC ACID AND SILICON DIOXIDE ON BLOOD LIPIDS

I. Szabo, J. Mody, and J. Szekely

Our earlier studies showed that the β-lipoprotein fraction increases in the blood of silicosis patients. We decided to run some experiments on animals to determine the nature of these changes in more detail. Twelve rabbits were injected subcutaneously 5 times in a period of 11 days with 0.5 ml of an 0.5% solution of colloidal silicic acid. Besides silicic acid, the animals received daily 20 ml of cortisone intramuscularly. Serum proteins and lipoproteins were determined before and one and two weeks after the above substances were first injected. Every animal developed severe dysproteinemia. The blood lipid level rose and the relative and absolute amounts of the β-lipoproteins increased as did the β/α ratio (Table 1).

6 to 20 mg of cortisone had no effect on these changes.

Heparin (400 units) restored the normal blood lipid [redacted] and β-lipoprotein levels to normal (decrease from 7.6.6 to 323 mg% and from 76.2 to 59.8 mg%, respectively).

Table 1

	(1) Общие липиды сыворотки (в мг%)	(2) Липопротеидные фракции			
		(3) в граммах на 100 г липопротеидов		(4) в миллиграммах на 100 г сыворотки	
		α	β	α	β
(5) До введения кремневой кислоты	391±55,0	36,5±1,63	63,5±1,63	141,5±22,1	246,5±33,6
(6) После введения кремневой кислоты . . .	550±41,3	24,8±3,3	75,2±3,3	143,5±11,1	416,5±46,5
t	4,77	3,76	3,76	3,46	6,52
P<	0,01	0,02	0,02	0,02	0,01

1 - Total serum lipids (in mg %)
2 - Lipoprotein fractions
3 - in grams/100 g of lipoproteins

4 - in milligrams/100 g of serum

5 - Before injection of silicic acid

6 - After injection of silicic acid

In another series of experiments, 14 dogs were injected intravenously every 2 or 3 days for 30 days with 2 ml/kg of an 0.5 solution of colloidal silicic acid. Serum proteins and lipoproteins were determined before the injections and every 10 days thereafter. The amount of lipoproteins was high in all cases and the β/α ratio markedly increased (Table 2).

Table 2

	(1) Общие липиды сыворотки (в мг%)	(2) Липопротеидные фракции			
		(3) в граммах на 100 г липопротеидов		(4) в миллиграммах на 100 г сыворотки	
		x_1	x_2	x_1	x_2
(5) До введения кремневой кислоты	452,4±18,2	58,2±2,8	41,8±2,8	263,4±19,9	189±16,0
(6) После введения кремневой кислоты . . .	736,1±19,7	47,3±2,5	52,7±2,5	350,1±19,6	386±29,2
t = ...	8,8	8,25	8,25	6	5,8
p <	0,001	0,001	0,001	0,001	0,001

1-6 - Same as in Table 1

We also investigated the effect of silicon on the serum cholesterol level in 26 guinea pigs. It averaged 84.7±2.4 mg % in the control. Four weeks after a single intraperitoneal injection of 50 mg of silicon dust or 10 mg of amorphous silicon dioxide, the blood cholesterol level rose significantly on the average to 118.1±8.7 or to 109.6±9.3 mg % ($t = 3.04$ and 2.72, respectively; $p < 0.02$)

Thus, the injection of animals with colloidal silicic acid, amorphous or crystalline silicon dioxide induced dysproteinemia and at the same time markedly altered the content of blood lipids and lipoproteins.

сохранилось долго. Наблюдавшееся снижение резервной щелочности при острой лучевой болезни статистически достоверно. Вероятность достоверности по отдельным тем заболеваниям лежит в пределах 95—99,7%.

Анализ общей картины заболевания, гематологических и патоморфологических данных показал, что уменьшение резервной щелочности тесно связано с тяжестью течения лучевой болезни и нередко с последующей гибелью животных.

Во второй серии опытов на 12 облученных кроликах параллельно с изучением сдвигов резервной щелочности определяли активность карбоангидразы крови.

Через 5 часов после облучения у 9 из 12 животных наряду с понижением резервной щелочности отмечалось и угнетение активности карбоангидразы крови, однако менее выраженное, чем снижение резервной щелочности. На 4-й день лучевой болезни активность карбоангидразы понизилась у 11 из 12 кроликов, а резервная щелочность уменьшилась у всех животных. Наиболее выраженные сдвиги активности карбоангидразы отмечены в разгар заболевания, когда и изменения резервной щелочности принимали столь же характер. В дальнейшем восстановление карбоангидразы, так же как и резервной щелочности крови, несмотря на внешнее улучшение общего состояния животных, происходило крайне медленно. К концу срока наблюдения (30-й день) активность карбоангидразы достигла исходных величин лишь у одного кролика из 9.

Средние значения активности карбоангидразы и резервной щелочности во время лучевой болезни при определении их у одних и тех же животных представлены на рисунке.

Наряду с изменением активности карбоангидразы крови в отдельных органах и тканях (головной мозг, слизистая желудка, легкие, печень, почки, сердце, надпочечники, мышца бедра) активность карбоангидразы в разгар лучевой болезни снижалась на 26—44%.

Механизм изменения кислотно-щелочных соотношений при лучевых поражениях в настоящее время во многом еще не ясен. На наш взгляд, наряду с другими факторами определенное значение в снижении уровня резервной щелочности, особенно в разгар лучевой болезни, имеет угнетение активности карбоангидразы крови и органов (прежде всего почек), так как инактивация карбоангидразы в организме приводит, как известно, к сдвигу кислотно-щелочного равновесия в сторону ацидоза с изменением в ряде случаев реакции крови [1—4].

ЛИТЕРАТУРА

1. Генкин А. М., Генкина Б. Л. Клин. мед., 1945, № 3, стр. 45. — 2. Крепс Е. М. Успехи совр. биол., 1944, т. 17, № 2, стр. 149. — 3. Maren T. H., Mayer E., Wadsworth B. C., Bull. Johns Hopk. Hosp., 1954, v. 95, p. 199. — 4. Nadell J., J. clin. Invest., 1953, v. 32, p. 622.

Поступила 11 VI 1960 г.

ВЛИЯНИЕ КРЕМНЕВОЙ КИСЛОТЫ И ДВУОКИСИ КРЕМНИЯ НА ЛИПОИДЫ КРОВИ

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В предыдущих работах нами было установлено увеличение фракции β-липопротеидов в кровяной сыворотке.

Для более детального выяснения характера этих изменений мы решили поставить ряд опытов на животных. Двенадцати кроликам пятикратно в течение 11 дней вводили внутривенно по 5 мл 0,5% раствора коллоидальной кремневой кислоты. Помимо кремневой кислоты 6 кроликам вводили ежедневно по 20 мг кортизона внутримышечно. До и через одну и две недели после начала введения этих веществ определяли белок и липопротеиды сыворотки. У каждого животного развивалась сильная липопротеидемия, уровень липоидов крови, а также относительное и абсолютное количество β-липопротеидов было повышены, соотношение β и α фракций липопротеидов увеличивалось (табл. 1).

Кортизон в количестве 6—20 мг не оказал никакого действия на эти сдвиги.

Гепарин (150 единиц) в течение 2—4 часов нормализовал уровень липопротеидов крови (снижение с 716,6 до 323 мг%) и β-липопротеидов (с 76,2 до 59,8 мг%).

Таблица 1

	Общие липиды сыворотки (в мг%)	Липопротеидные фракции: в граммах на 100 г липопротеидов		Липопротеидные фракции: в миллиграммах на 100 г сыворотки	
		β_1	α	β	α
До введения кремневой кислоты	391±55,0	36,5±1,63	63,5±1,63	144,5±22,1	246,5±33,6
После введения кремневой кислоты	550±41,3	24,8±3,3	75,2±3,3	133,5±14,1	416,5±46,5
t	4,77	3,76	3,76	3,36	6,52
p<	0,01	0,02	0,02	0,02	0,01

В другой серии опытов 14 собакам через каждые 2—3 дня вводили внутривенно по 2 мл/кг 0,5% раствора коллоидальной кремневой кислоты в течение 30 дней. До начала и в период его проведения через каждые 10 дней определяли белки и липопротеиды сыворотки. Во всех случаях количество липопротеидов было повышено и отношение β/α фракций липопротеидов заметно возросло (табл. 2).

Таблица 2

	Общие липиды сыворотки (в мг%)	Липопротеидные фракции: в граммах на 100 г липопротеидов		Липопротеидные фракции: в миллиграммах на 100 г сыворотки	
		β_1	α	β	α
До введения кремневой кислоты	452,4±18,2	58,2±2,8	41,8±2,8	263,4±19,9	189±16,6
После введения кремневой кислоты	736,1±19,7	47,3±2,5	52,7±2,5	350,1±19,6	386±29,2
t	8,8	8,25	8,25	6	5,8
p<	0,001	0,001	0,001	0,001	0,001

Мы исследовали также действие кремния на уровень холестерина сыворотки у 26 морских свинок. У контрольных животных в среднем было 84,7±2,4 мг%. Через 1 недели после однократного введения 50 мг кремневой пыли или 10 мг аморфной двуокиси кремния внутрибрюшинно уровень холестерина в крови был значительно повышен, в среднем до 118,1±8,7 или же до 109,6±9,3 мг% ($t=3,04$ и 2,72 соответственно; $p<0,02$).

Таким образом, введенные в организм животных коллоидальная кремневая кислота, аморфная и кристаллическая двуокись кремния наряду с диспротеинемией приводят к значительным изменениям в содержании липоидов и липопротеидов крови.

Поступило 15 X 1960 г.

Poli Clinico (Sez Prat) 47(3):87-88, 91-92, 95 (1940)

Notes and Contributions

The Anti-Goitre Provencial Committee of AOSTA

The Goitre Condition, the Siliceous Theory and Iodine Prophylaxis

Dr. Giovanni A. Trikurakis

In spite of the repeated protests of optimists -- who would like to affirm that by now the goitre condition, as an endemic phenomenon, has already disappeared and lost that social importance that we attributed to it in the past -- a greater and interesting development of the study of goitre has been developing for some time both with regard to the etiology and the pathogenesis and treatment of the phenomenon under consideration.

The geological theory of the genesis of the goitre condition is again returning to the limelight with several authorities upholding it. Among the various theses, that of Buzzi da Sondrio -- who was already the first, at that distant first international congress of Milan for the study of goitre, to propose amid general incredulity of the participants, the adoption of iodized salt for the prophylaxis and collective treatment of the phenomenon -- is, also with regard to pathogenesis, the theory that is the most convincing and attractive for us, since it appears to me to explain the various manifestations of this complex phenomenon in the simplest way, a phenomenon which up to now was not successfully explained, with regard to its etiology, its pathogenesis and its treatment and natural prophylaxis, more simply and rationally.

It would seem to be a question of a silicic intoxication*; a customarily slow intoxication, with elective effect, according to Buzzi on the connectives in general and especially on the connectives of the embryo or fetus or on the perithyroid endothelial reticulum for goitre, or on the cortical connective of the neuroglia or on the mesocephalic connective for the cretin.

This ingenous pathogenetic theory awaits experimental confirmation, which we expect to be affirmative; -- I do not declare that I am a priori enthusiastic -- either because it does not contradict my own observations or because of the numerous arguments that are in its favor.

It is indisputable that the phenomenon is intimately and consistently connected to a location, thus it is probable that the nature of the soil is a contributing factor; -- since it is spread over more or less vast areas, in the whole surface of the earth -- and especially in mountainous and volcanic regions where silicon (silicium) is the most widespread and predominant element -- it is easy to explain its wide diffusion. The phenomenon is most intensively present in the lowest parts of mountainous slopes and less in the higher areas since, water being the most common way of introduction into our organism, the latter i.e. water, is all the richer in such elements as the soils that it crosses are rich in them and they

*Not to be confused with the bronchial silicosis of miners, this being a local phlogistic -- traumatic disease caused by the inhalation of quartz dust.

are especially abundant and widespread if these soils are precipitous (crumbling) dissolved, and uncovered, i.e. bare, and clearly and prevalently containing siliceous compounds at the base, and the water cannot have deposited them elsewhere before being consumed by the inhabitants**.

**According to data, furnished to me by Eng. E.A. Gaillard of Barcellona, the maximum solubility of siliceous soils (in a solution carbonate at 1% and at 37°C) would have the finest dust; there follow in order of solubility: quartz, porphyry, granite, sandstone, cerite, the sandy dusts. The addition of clay dust reduces the said solubility by a quarter, while chalk (gypsum) reduces it by 78% and carbon dust by 4 to 71% according to the various cases. Calcium carbonate has no effect.

If one considers that in landslides there always exist waste matter (detritus) from vegetable matter that decomposes with the possible formation of potassium carbonate or the ammonium carbonates it is very probable -- according to the above indications -- that a notable dissolution of silicon contained in these landslides occurs while there would not be such dissolution except in the slightest degree, in compact silicated soils, without landslides and therefore without traces of organic substances. The above seems to demonstrate that we ought to find more silicon dissolved in waters that cross soils subject to landslides (crumbling soils) in comparison with the waters that remain in contact with the compact soils of siliceous rocks or also containing clay or chalk.

The nature of the soil has an influence on the chemical composition of vegetation and therefore also of animals including man. It is therefore no cause for wonder if the whole connective system (which seems to have a greater affinity for siliceous compounds) of man and of animals that live on a soil rich in silicon, is more developed in comparison with the connective system of man and animals that live, for example, on calcareous or dolomite soil; and that therefore the thyroid of the inhabitants of prevalently siliceous regions is naturally more developed (enlargement of the thyroid gland) than those who live in regions that in contrast are poorer in silicon.

This hypothesis is shown to us and it would succeed, if confirmed, in explaining the whole complex of symptoms to which the name of goitre condition has been given.

In justification of my belief in the siliceous theory of Buzzi, I refer to what I wrote in this regard in 1931-32; (On the anti-goitre activity in the province of Aosta during the year 1931-32. Scritti Biologici VIII, 1933) and that is: "for me, the chemical composition of water can have great importance in the genesis of goitre" and further; "I believe that the primary hypertrophy of the thyroid is the effect of a lack of nutritional chemical balance".

We know that silicon normally enters to make up part of our tissues. The bones, especially the enamel of teeth and many parts of the eye and especially Warthon's Gelatin that forms the cover and the support of the communicating cells of the umbilical cord in fetuses and newborn babies, are rich in silicates. The latter appear to have a predilection for the connective tissue of the brain in process of formation (the fetal period)

or of the thyroid in its period of formation, its development and greatest activity (childhood and puberty), and probably for the connective tissue as well of the other endocrine glands and of organs among the most important glands and organs of the organism, etc.

It might be a matter at first of a simple connective hyperplasia that later can cause the pressure reaction with strangling (throttling), cirrhosis and thereafter the destruction (in direct effect) of the nerve cells in the brain or the destruction of the nerve cells of the glandular tissue proper of the thyroid and the other glands.

This theory -- without excluding the common pathogenetic relationship and perhaps the evolutive interrelation of goitre and cretinism -- would render inexact the aphorism that goitre is the first symptom of a disease of which the last stage is cretinism; since according to the siliceous theory, cretinism is not for the most part, a consequence of the altered functioning of the thyroid but also one of the primary direct symptoms, caused by the quid that also causes goitre, deaf-mutism, stammering, dwarfism and perhaps also some spasmophilias, rickets, etc.

And thus the concept is negated of the diseased participation of the other endocrine glands in endemic cretinism, a theory upheld by Schonemann, by Syon, by Wegelin, by Marcovecchio and especially by Pende, who expresses himself thus; "In the study of this disease, it is necessary to insist on the alterations of all the endocrine glands and not only of the thyroid; since even more than for myxedema, I believe that for endemic cretinism, the concept is admissable of a diffuse lesion of the endocrine system with the thyroid lesions almost always dominant, the endocrine lesions being otherwise in combination with other alterations of the organism dependent directly on the factor of cretinism".

Thus, this thesis would explain for us the reason for the frequency, in endemic zones, of the various forms of meningo-encephalic diseases, epilepsy, chorea, and teratologies of arrested development; it would explain how the introduction of good potable water in certain locations has attenuated and even wiped out the phenomenon; why during pregnancy and the menstrual period, through the inevitable introduction of a greater quantity of water, we witness an exaggerated enlargement of the thyroid; why the goitre condition is more frequent among poor people and peasants than among workmen and bourgeois, more frequent in animals that run about and get tired in the mountains, such as hunting and sheep dogs, burden-bearing mules that necessarily drink a great deal of water, in swine that drink more of everything and root about with their snouts in the mud and thus the reason for the rapid beginning of thyroid hypertrophia in children because of their running about playing and drinking large quantities of water; why certain foods that contain many siliceous compounds (cabbages, chestnuts with their interior skin, black rye bread, that usually contains a large quantity of bran, etc.) favor the development of goitre; it furthermore explains to us the importance of cleanliness for keeping away from the hands fruit, vegetables, bread, etc. of earth and dust (that contain soil elements) and the slightest introduction into our body of these elements; and in general, the importance of sanitation both in regard to living quarters and drinking water.

Thus, this hypothesis satisfies the upholders of the hydric theory and the upholders of the geological or earth theory; thus is explained the theory of Bojer (a Swede) (the inclination, sloping, and extension of the earth on slopes above; the depth, mass and velocity of water) and

in a certain way satisfies the theory of iodine deficiency, in as much as iodine, according to Buzzi, would serve as an antidote to siliceous intoxication as a natural opponent.

The theories of infections -- aside from that of Balp, who talks of re-infections and slow and prolonged infective toxins, therefore more properly to be included among the intoxications and therefore in harmony with the present thesis -- no longer have many upholders. And in fact, if it were a question of an infection, the phenomenon would have been spread in zones which were formerly free of it, which has never happened; nor would it decrease with the removal of the carrier into a non-endemic zone, as has often happened. Furthermore, there never has been found or identified a special germ nor has a culture been made that, having been inoculated, produced the disease, nor has a part of a goitre inoculated into tissue or thyroid produced goitre.

There remains furthermore the radioactivity theory which, however, still presents many questionable points and is less satisfactory. After all, radioactivity is almost always present only in eruptive and volcanic siliceous rocks, but while the silicon seems to follow goitre and goitre and silicon are very widespread in the world, radioactivity, on the other hand, is not very widespread.

With regard to cretinism and the various distrophies of predilection, even with the siliceous theory, the constitutional and hereditary factor does not, according to my opinion, lose its importance as the qualitative nutritional factor does not lose importance for goitre (therefore poverty is also included).

Up to now I have dealt with the arguments in favor of the Buzzi theory without naturally being able to demonstrate that it is indeed a

question of a silicon intoxication (silicon being the most widespread metal on our planet) and in what form*, or if it is rather an intoxication

*Buzzi is inclined to believe that, among the infinite compounds of silicon (there are already known 250 siliceous minerals), the colloidal compounds and silicon compose alone more than all the other simple bodies together and if iron limonite and aluminum are colloidal, these are usually in combination with silicon in their compounds. And among the silicon compounds, Buzzi is partial to the colloidal siliceous acids, ortho-metaldehyde, and trisilicon and to the silicates of magnesium, lime, soda, iron, and aluminum.

Buzzi inculpates silicon because it is a part of the carbon group and that is of the base of organic bodies and it is quadrivalent, very diffuse, transparent, and hard as diamond and having colloidal compounds like the animal body and especially connective tissue. Attention should be given to the strange and frequent resemblance of certain siliceous compounds to certain animal tissues, between the fibrous and reticular asbestos and the sinews and aponeuroses, between the fluid glass, sodium silicate and potassium silicate and carpenter's glue (from boiled hides and bones) or the gelatin from bones, between the transparent and lamellosity siliceous mica and of the glass and the gelatin, the vitreous humor, cornea, the synovial membranes, white of egg, etc., between the concoid fracture of pumice stone, of quartz and glass and the fracture of glue and dry gelatin and of hardened white of egg, of spun glass or glass wool with the wool hairs and connective fibrils and the other several resemblances between siliceous compounds and animal tissues.

by another element whatever and that it is not a question for example, of radiation or deficiency; a subject that will be able to form the object of our coming studies and experiments. I should not however pass over in silence the objection that I have made to the intelligent originator of this theory, and worthy specialist in goitre condition, Dr. Buzzi, regarding the manner of reconciling the above theory with the result of the experiments made in Val Bisagno by Prof. Cerletti of Rome, whose competence and authority do not allow doubts; according to these experiments in an endemic area it was possible to cause thyroid hypertrophy in white rats closed hermedically in cylinders and carried -- if I remember properly -- periodically to Genoa for eating, a change of air and cleansing. If the closure was really hermetic, this should have prevented the penetration of air and thus of atmospheric fine dust; and then with the siliceous theory, the thyroid hypertrophy of the rats would not be explained; if, on the other hand, this closure allowed the possibility of penetration by air and fine dust, the experiment would not be in opposition with Buzzi's theory.

Prophylaxis and Treatment

The oldest treatment, which has up to now yielded the most satisfactory and definite results, is the iodine treatment. There is general recognition of this, based on long experience and repeated tests and confirmation. Iodized salt -- which was proposed for the first time in 1909 by the originator himself of the siliceous theory for the pathogenesis of the goitre condition, the Italian Buzzi, and tried out with success first in Switzerland and Austria and then through the praise-worthy and benign interests of the Inspector of public health, Ambrosi, in Valtellina beginning in 1925, and in Bresscia, Varese, Como, Bergamo, and the high

valley (Alta Valle) of Aosta beginning in 1934 -- in addition to being not very expensive and of unequaled practicability, has yielded results sufficient to give credence to its true curative effectiveness and particularly its prophylactic effectiveness in the collective treatment.

It has been said by many specialists with regards to the results obtained in Valtellina and Switzerland, all of the improvement encountered must not be attributed to iodized salt; since the goitre condition becomes attenuated spontaneously as well with progress and sanitation as in fact, has been noticed even after only 15 or 20 years. That however does not mean that iodine treatments, either individual or collective, have not contributed to it or that iodized salt has not brought about a clear and decisive transformation. It was therefore that we in Valle d'Aosta, wished to experiment with it in a single zone, the western zone or Alta Valle d'Aosta, leaving the contiguous eastern zone as a region for comparison. It was obvious that, after a certain period after introduction of iodized salt, a substantial difference was discovered in the progress of the phenomenon in the two zones; this difference would have been cited exclusively in favor of the effectiveness of iodized salt, since progress and sanitary improvements -- which the present regime is providencially effecting in our province as well -- have not favored the zone subject to prophylaxis to a greater degree.

Since the last year, visiting in the company of other sanitation officials some schools of the Alta Valle d'Aosta (the zone with iodized salt prophylaxis), we noted, to my pleasure, the absence of notable and obvious hypertrophies in the thyroid glands of the pupils, to such an extent that some of us asked ourselves: Isn't endemic goitre condition

possibly something imagined? The children of the Valle d'Aosta in fact, did not appear to be in a condition -- with regard to their thyroid glands or thyroid disfunctions -- inferior to those of all the other regions of Italy. Quite a different observation, however, was made when we visited later some schools of the Aosta zone which was not subject to prophylaxis and of Canavese, also without prophylaxis. The difference in the endemic picture was obvious since in these latter regions the thyroid hypertrophies were still frequent and very obvious.

This has also been the result of the investigation made during the first months of the current year, five (5) years after the last government investigation, that preceded the beginning of distribution of iodized salt, an investigation carried out as previously by the sanitation officials in all the elementary schools of our province and with data gathered according to the same norms and with the same methods already adopted in the previous investigation.

As appears from the summary included (see table), it results that in the zone subject to prophylaxis, thyroid hypertrophies were reduced to one-third, while they remain almost unchanged in the zones not subject to prophylaxis. The contribution of iodized salt can thus not be placed in doubt.

A new question has recently been raised at various times by some specialists; this concerns the percentage of iodate (?) or K (iodide) to be added to the salt for prophylatic purposes, a percentage that according to some (Danielopolu of Rumania, Buzzi, Marcovecchio, etc.) ought to be increased.

I do not share the opinion of the latter. In the first place, because if the present percentage (1 gram of IK per quintal of kitchen salt) does not represent any danger to the community, and increase might lead to obvious symptoms of hyperthyroidism of which many hidden forms are frequently found in endemic areas. In the second place we have many years of tests behind us, that show a sufficient effectiveness of the present proportion of iodized salt.

The goitre condition is not an acute disease, but rather a chronic condition that develops slowly for the most part; it should not be said therefore, that the best treatment is an intensive treatment; the treatment ought to be arranged in the lowest dosage sufficient to serve only to neutralize the quantity of the goitre-causing quid, that -- in the same period of time -- acts to harm the organism.

The treatment and prophylaxis therefore, ought not to be excessive, above all in order not to harm in a possibly not less grave manner.

According to the results already described by the various authors (Ambrosi, Muggia, Eggemberger, etc.) with regard to the tests carried out up to now, iodized salt in the proportions used at present, satisfactorially prevents the disease and corrects the common symptoms of the goitre condition in less time than the symptoms might have had to develop; it would therefore not represent an insufficient percentage of iodate of K. On the other hand, it is absurd to subject a whole population to intensive treatment when it is a question simply of dealing with a relatively low percentage of although very important percentage of individuals with permanent organic changes.

I do not believe on the other hand -- as I had mentioned several times -- that the specific endemic agent (whether it is silicic intoxication or something else) can lead to serious forms of goitre, cretinism, deaf-mutism, dwarfism, etc., if other factors, for the most part constitutional, do not cooperate with it. Since, if the improved condition of life has contributed everywhere to the attenuation of the phenomenon -- or rather to decrease the frequency of the saddest symptoms -- and since the specific quid has not been removed, this means that it is not very probable that we will come, with it alone, to the serious forms of the goitre condition without the association of third factors that might be endogenous (hereditary, constitution, etc.) or exogenous (food deficiency, alcoholism, poor sanitation, etc.).

In order to prevent and extirpate as well the serious forms of the endemia, it is necessary, beside specific prophylaxis (iodine), to maintain a rigorous application of all those sanitary provisions imposed by the civilization of our century and to continue to raise the condition of life of the populations subject to the endemia.

Summary

The author, dealing with the pathogenesis of the goitre condition, upholds the theory of silicic intoxication advanced by Dr. Buzzi di Sondrio, that he analyzes and elucidates comparing it with his personal observations. He therefore mentions the iodine treatment and prophylaxis and reports the brilliant results obtained from the distribution of iodized salt during the last five years in the high valley of Aosta, comparing these results with the stationary situation of the goitre condition in the contiguous zone not subject to prophylaxis of the Valle of Aosta. Finally, he states his opposition to an increase in the percentage of IK in the said prophylactic salt.

				Persons visited	Normal throats N	Normal throats %	Full throats N	Full throats %	Large throats N	Large throats %	Small goitres N.	Small goitres %
Valle d'Aosta	Zone subject to prophylaxis		1934	4996		35,61		41,59		22,15		0.65
			1939	5705	3930	68,80	1524	26,71	248	4,35	3	0,05
	Zone not subject to prophylaxis		1934	2810		68,95		26,65		4,23		0.17
			1939	3599	2437	67,71	920	25,56	224	6,23	18	0,50
	Canavese	1939	1934	12854		61,74		29,29		7,92		1,05
			Total	13019	(*)	77,70		17,50		4,08		0,71
			High zone	2505	1929	77,04	398	15,86	105	4,19	73	2,91
			Low zone	10514	8187	77,86	1880	17,88	427	4,06	20	0,19
	Provincia d'Aosta	1939	1934	20658		50,04		36,75		12,37		0,84
			Total	22323	16483	73,87	4722	21,14	1004	4,49	114	0,50
			M.	11633	8497	73,05	2567	22,06	513	4,41	56	0,48
			F.	10690	7936	74,70	2155	20,16	491	4,60	58	0,54

*It should be remembered that some areas of Canavese have continued the distribution in schools of iodized chocolates.

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beneficati e si danneggerebbero molto quelli ai quali la cura mista può giovare.

4) Nella blenorragia cronica la sulfamido-piridina ha spesso azione risolutiva.

5) Nella epididimite acuta questo medicamento è spesso ottimo; appena cessata la infiammazione, subito iniziare cura locale nell'uretra.

6) Nelle altre complicazioni (prostatiti, littiti, ecc.) l'azione è in molti casi ottima. Consiglio però fare anche subito cure locali nell'uretra.

7) Nelle vulvo-vaginiti gonococciche delle bambine, la sulfamido-piridina si è dimostrata ottimo rimedio. Non tralasciare anche qui di fare cure locali.

RIASSUNTO.

L'A. ha sperimentato la sulfamido-piridina nella blenorragia maschile acuta, cronica e complicata e nella vulvo-vaginite blenorragica delle bambine.

Le dosi impiegate furono di 18 grammi in 9 giorni. I risultati ottenuti ottimi, tanto che ritiene doverosa la somministrazione di questo farmaco a tutti i blenorragici.

NOTE E CONTRIBUTI

COMITATO PROVINCIALE ANTISTRUMICO DI AOSTA.

Gozzismo, teoria silicea e profilassi iodica.

Dott. GIOVANNI A. TRIKURAKIS.

Malgrado le reiterate proteste degli ottimisti — i quali vorrebbero sostenere che oramai il gozzismo, quale fenomeno endemico, sia già scomparso e abbia perduto quell'importanza sociale che gli si volle attribuire in passato — si sta delineando, da un po' di tempo, un maggiore ed interessante sviluppo dello studio, sia dell'eziologia che della patogenesi e della cura del fenomeno in parola.

La teoria geologica sulla genesi del gozzismo ritorna nuovamente alla ribalta con parecchi autorevoli sostenitori. Fra le varie tesi, quella del Buzzi da Sondrio — che fu già il primo, in quel lontano I Congresso internazionale di Milano per lo studio del gozzo, a proporre, fra la generale incredulità dei partecipanti, l'adozione del sale iodurato per la profilassi e la cura collettiva del fenomeno — e, anche sulla patogenesi, la teoria che più ci convince e ci attrae, poichè secondo me riesce, in maniera molto semplice, a spiegare le varie manifestazioni di questo complesso fenomeno, che non riusciva finora a trovare una spiegazione soddisfacente alla sua etiologia, alla sua patogenesi ed alla cura e profilassi naturale più semplice e razionale.

Si tratterebbe di una *intossicazione* silicica (*); intossicazione solitamente lenta, con azione elettiva, secondo il Buzzi, sui connettivi in genere, e massime sull'embrionale o fetale ossia sul reticolo endoteliale peritiroideo pel gozzo, o sul corticale della nevroglia o sul mesocefalo pel cretino.

Questa ingegnosa teoria patogenetica attende la sua conferma sperimentale, che auguriamo positiva; — io tuttavia non esito a dichiararmene a priori entusiasta — sia perchè essa non contrasta con le mie osservazioni, sia per le numerose argomentazioni che stanno in suo favore.

È indiscutibile che il fenomeno è legato intimamente e costantemente al luogo, quindi è verosimile che la natura del terreno vi contribuisca; — essendo poi esso diffuso su aree più o meno vaste, in tutta la superficie terrestre — e specialmente nelle regioni montuose e vulcaniche, dove il silicio è l'elemento più diffuso e predominante — è facile spiegarne la grande diffusione. Esso si presenta più intenso nelle parti più basse dei declivi montani e meno in alto, poichè, essendo la via più comune d'introduzione nel nostro organismo l'acqua, questa è tanto più ricca di tali elementi quanto più i terreni che attraversa ne sono ricchi e sono abbondanti e diffusi specialmente se essi siano franosi, sciolti e scoperti cioè nudi ed a base prettamente o prevalentemente di composti silicei, e l'acqua non abbia potuto depositarli prima di essere consumata dagli abitanti (**).

(*) Da non confondersi con la silicosi bronchiale dei minatori, affezione questa flogistico-traumatica locale causata da inspirazione di *polvere* di quarzo.

(**) Secondo i dati fornitimi dall'ing. E. A. Gaillard di Barcellona, la solubilità massima dei terreni silicei (in soluz. di carbonato di sodio all'1 % ed a 37° C.) l'avrebbe la finissima polvere; seguono, per ordine di solubilità: il quarzo, il porfido, il granito, il gres, la cerite, le polveri sabbiose. L'aggiunta di polvere d'argilla riduce di un quarto detta solubilità, mentre il gesso la riduce del 78 % e la polvere di carbone da 4 a 71 % a seconda dei casi. Il carbonato di calcio non ha alcun effetto.

Se si considera che nelle frane esistono sempre dei detriti di elementi vegetali che si decompongono con possibile formazione di carbonato di potassio o di carbonati ammoniacali, è molto probabile — secondo le sovrasposte indicazioni — che avvenga uno scioglimento notevole di silicio contenuto in dette frane, mentre tale scioglimento non avverrebbe, che in minima misura, nei terreni silicati compatti, senza frane e quindi senza traccie di materie organiche. Quanto sopra sembra

La natura del terreno influisce sulla composizione chimica dei vegetali e quindi anche degli animali, compreso l'uomo. Non c'è perciò da meravigliarsi se tutto il sistema connettivale (che sembra avere maggiore affinità coi composti silicei) dell'uomo e degli animali che vivono su di un terreno ricco di silicio, sia più sviluppato, in confronto a quello dell'uomo e degli animali che vivono per es. su terreno calcareo o dolomitico; e che quindi la tiroide degli abitanti di regioni a prevalenza silicea sia naturalmente più sviluppata (*tireomegalia*) di quelli che vivono in regioni, in confronto, povere di silicio.

È un'ipotesi questa che ci si prospetta, e che riuscirebbe, se comprovata, a spiegare tutto il complesso delle manifestazioni a cui finora si è dato il nome di gozzismo.

A giustificazione poi della mia adesione alla teoria silicea del Buzzi, mi riporto a quanto già nel 1931-32 scrissi in proposito: (Sull'attività antistrumica in provincia d'Aosta durante l'anno 1931-32. Scritti Biologici VIII, 1933) e cioè: « per me, la composizione chimica dell'acqua può avere molta importanza nella *genesi del gozzo* », e più oltre: « penso che l'*ipertrofia primaria* della tiroide, sia l'effetto di uno *squilibrio chimico alimentare* ».

Noi sappiamo che il silicio normalmente entra a far parte dei nostri tessuti. Le ossa, specialmente lo smalto dei denti e molte parti dell'occhio e massime la gelatina di Warthon che forma l'involucro ed il sostegno dei vasi del funicolo nei feti e neonati sono ricche di silicati. Essi pare abbiano una predilezione per il tessuto connettivo del cervello in formazione (periodo fetale) o della tiroide nel suo periodo di formazione, sviluppo e maggiore attività (puerizia e pubertà), e probabilmente per il tessuto connettivo anche delle altre ghiandole endocrine e di organi fra i più importanti dell'organismo, ecc.

Potrebbe in un primo tempo trattarsi di una semplice iperplasia connettivale, che più tardi può causare la reazione da pressione con strozzamento, cirrosi ed in seguito la distruzione azione indiretta) delle cellule nervose per il cervello o di quelle del tessuto ghiandolare propriamente detto per la tiroide e le altre ghiandole.

Questa teoria — senza escludere la relazione comune patogenetica, e talvalta, la interrelazione evolutiva del gozzo e del cretinismo — renderebbe inesatto l'aforisma, che il gozzo sia il primo sintomo di una malattia di cui l'ultimo gradino sarebbe rappresentato dal cretinismo; poichè, secondo la teoria silicea, il cretinismo non sarebbe, per lo più, una conseguenza dell'alterata funzionalità della tiroide, ma anche una delle manifestazioni primarie dirette, causate dallo stesso quid che porta pure al gozzo, al sordomutismo, alla balbuzie, al nanismo e forse anche ad alcune spasmofilie, al rachitismo, ecc.

Ed ecco anche appagato il concetto della compartecipazione morbosa delle altre ghiandole endocrine nel cretinismo endemico, sostenuta dallo Schönemann, dal Cyon, dal Wegelin, dal Marcovecchio e specialmente dal Pende, il quale così si esprime: « nello studio di questa malattia, occorre insistere sulle alterazioni di tutte le ghiandole endocrine e non soltanto su quella della tiroide; poichè più che per il mixedema, io credo ammissibile per il cretinismo endemico il concetto di una lesione diffusa del sistema endocrino con lesioni tiroidee quasi sempre dominanti, lesioni endocrine d'altronde associate ad altre alterazioni dell'organismo, dipendenti direttamente dal *fattore* del cretinismo ».

Così questa tesi ci spiegherebbe il perchè della frequenza, nelle zone endemiche, delle varie forme di affezioni meningo-encefaliche, epilessia, corea e teratologie da arresto di sviluppo; ci spiegherebbe perchè l'introduzione di buona acqua potabile in certi luoghi abbia attenuato ed anche distrutto il fenomeno: perchè durante la gravidanza ed il periodo mestruale, per la inevitabile introduzione di una maggiore quantità di acqua, assistiamo ad un esagerato ingrossamento della tiroide; perchè il gozzismo è più frequente nei poveri e nei contadini che negli operai e cittadini, più negli animali che correndo e faticando in montagna, come cani da caccia e pastori, muli da soma in salita, bevono per necessità molte acque, nei suini infine che bevono più di tutti e frugano col muso nella fanghiglia e così dell'inizio rapido dell'ipertrofia tiroidea nei fanciulli pel gran correre, giuocare e bere smodatamente: perchè certi determinati cibi, che contengono molti composti silicei (cavoli, castagne con la buccia interna, pane nero di segala, che abitualmente contiene anche una buona quantità di crusca, ecc.), favoriscano il gozzo; ci spiega inoltre l'importanza della pulizia, per l'allontanamento dalle mani, dalle frutta, dalla verdura, dal pane, ecc., della terra e della polvere (che contiene gli elementi del terreno) e la minore introduzione nel nostro corpo di questi elementi; ed in genere, l'importanza dell'igiene e con essa dell'abitazione e dell'acqua potabile.

Ecco, con questa ipotesi accontentati i sostenitori della teoria idrica e quelli della teoria geologica o tellurica; ecco spiegata la teoria Hojer (svedese) (inclinazione pendenza ed estensione del terreno sovrastante; profondità, massa e velocità dell'acqua) ed in un certo senso soddisfatta la teoria da carenza iodica, in quanto l'iodio, secondo il Buzzi, servirebbe da antidoto all'intossicazione silicea come antagonista naturale.

Le teorie da infezioni — all'infuori in parte di quella di Balp, che parla di reinfezioni e tossine infettive lente e prolungate, perciò più propriamente da annoverare fra le intossicazioni, e quindi in armonia alla presente tesi — non hanno più molti sostenitori. Ed infatti, se si fosse trattato di un'infezione, il fenomeno si sarebbe propagato in zone altre volte indenni, ciò che non è mai risultato; nè diminuirebbe coll'allontanamento del portatore, in una zona indenne, come spesso si è verificato. D'altronde mai è stato trovato ed individuato uno speciale germe, nè formata coltura, che, innestata, abbia prodotto il male, nè innestata parte di gozzo in tessuto o nella tiroide abbia prodotto il gozzo.

Rimane inoltre la teoria da radioattività, la quale però presenta ancora molti punti interrogativi e soddisfa meno. Essa dopo tutto è quasi sempre presente solo nelle roccie silicee eruttive e vulcaniche, ma, mentre il silicio sembra seguire il gozzo e gozzo e silicio sono assai diffusi nel mondo, lo è invece poco la radioattività.

Per quanto riguarda il cretinismo e le varie distrofie da predilezione, anche con la teoria silicea, il fattore costituzionale ed ereditario non perde, secondo me, la sua importanza; come non la perde il fattore alimentare qualitativo (quindi anche l'indigenza) per il gozzo.

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Fin qui ho trattato degli argomenti in favore della teoria Buzzi, senza naturalmente poter dimostrare che si tratti effettivamente di una intossicazione da silicio (che è il metallo più diffuso nella nostra terra) e sotto quale forma (*), o sia piuttosto un'intossicazione da un altro elemento qualunque e che non si tratti per es. di radiazione o di carenza; argomento che potrà formare oggetto dei nostri prossimi studi ed esperimenti. Non vorrei però tacere l'obbiezione che io prospettai all'intelligente ideatore di questa teoria, e benemerito del gozzismo, dott. Buzzi, circa il modo di conciliare la suddetta teoria con il risultato delle esperienze fatte in Val Bisagno dal prof. Cerletti di Roma, la cui competenza ed autorità non permettono dubbi; secondo tali esperienze in paese endemico si è potuto avere l'ipertrofia tiroidea nei ratti bianchi, chiusi ermeticamente in bombole e portati — se ben ricordo — periodicamente a Genova per l'alimentazione, il cambiamento dell'aria e la pulizia. Se la chiusura era realmente ermetica, essa doveva impedire la penetrazione dell'aria, e con essa del pulviscolo atmosferico; ed allora con la teoria silicea non si spiegherebbe l'ipertrofia tiroidea dei ratti; se invece questa chiusura lasciava possibile la penetrazione dell'aria e del pulviscolo, in tal caso l'esperienza non contrasterebbe con la teoria del Buzzi.

Profilassi e cura.

La cura più antica, quella che finora ha dato più soddisfacenti e più concreti risultati, è la cura iodica. È un riconoscimento generale, basato su una lunga esperienza e su ripetute prove e conferme. Il sale iodurato — che fu proposto per la prima volta nel 1909 dallo stesso ideatore della teoria silicea per la patogenesi del gozzismo, l'italiano Buzzi, ed esperimentato con successo, prima in Isvizzera ed Austria, e poi, per il lodevole e benefico interes-

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dimostrare che si deve trovare più silicio disciolto nelle acque che attraversano i terreni franosi, in confronto a quelle che rimangono a contatto di terreni compatti di rocce silicee od anche contenenti argilla o gesso.

(*) Il Buzzi inclina a credere che siano fra gli infiniti composti del silicio (sono già noti 250 minerali silicei) i composti *colloidali* ed il silicio ne fa da solo più di tutti gli altri corpi semplici assieme e se la limonite di ferro e l'alluminio sono colloidali essi sono per solito associati al silicio nei suoi composti. E fra i composti del silicio il Buzzi propende per gli acidi silicei colloidali orto meta e trisilicio e poi silicati di magnesio, calce, soda, ferro, alluminio.

Il Buzzi incolpa il silicio perchè fa parte del gruppo del carbonio e cioè della base dei corpi organici e cioè è tetravalente, diffusissimo, trasparente e duro come il diamante ed avente composti colloidali come il corpo animale e specie il tessuto connettivo. Si ponga mente alla strana e frequente somiglianza fra certi composti silicei e certi tessuti animali, tra il fibroso e reticolare amianto ed i tendini ed aponeurosi, tra il vetro liquido silicato di sodio e potassio e la colla da falegname (da pelle ed ossa cotte) o la gelatina delle ossa, tra la trasparenza e lamellosità della mica silicea e del vetro e la gelatina, umor vitreo, cornea, membrane sinoviali, albume d'uovo ecc., tra la frattura concoide della pietra pomice, del quarzo e vetro e la frattura della colla e gelatina secca e del chiaro d'uovo indurito, del vetro filato o lana di vetro con la lana, capelli e fibrille connettivali, ed altre parecchie somiglianze tra composti silicei e tessuti animali.

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samento dell'Ispettore della Sanità Pubblica Ambrosi, in Valtellina dal 1925, ed a Brescia, Varese, Como, Bergamo ed Alta Valle d'Aosta dal 1934 — oltre ad essere poco costoso e di una praticità senza pari, ha dato dei risultati sufficienti per credere alla sua reale efficacia curativa e specialmente profilattica nella collettività.

Da più studiosi si era detto finora, per ciò che riguarda i risultati ottenuti in Valtellina ed in Isvizzera, che non bisogna attribuire al sale iodurato tutto il miglioramento riscontrato; poichè il gozzismo va attenuandosi anche spontaneamente col progresso e l'igiene, come in realtà si constata anche a distanza di soli 15 o 20 anni. Ciò però non vuol dire che le cure iodiche, private o collettive, non vi abbiano contribuito o che il sale iodurato non vi abbia apportato una netta e decisiva trasformazione. Fu perciò che noi in Valle d'Aosta abbiamo voluto esperimentarlo in una sola zona, la zona occidentale od Alta Valle d'Aosta, lasciando la zona attigua orientale come termine di confronto. Era evidente che, dopo un certo periodo dall'introduzione del sale iodurato, se si riscontrava una differenza sostanziale nell'andamento del fenomeno nelle due zone, questa differenza avrebbe parlato esclusivamente in favore dell'efficacia del sale iodurato, poichè il progresso ed i miglioramenti igienici — che l'attuale regime sta provvidenzialmente attuando anche nella nostra provincia — non hanno favorito maggiormente la zona soggetta a profilassi.

Già dallo scorso anno, visitando in compagnia di altri sanitari alcune scuole dell'Alta Valle d'Aosta (zona profilassata col sale iodurato) notammo con mia compiaciuta meraviglia, l'assenza d'ipertrofie notevoli ed evidenti nelle tiroidi degli alunni, tanto che qualcuno di noi si è chiesto: Il gozzismo endemico non è forse una montatura? I bambini Valdostani infatti non apparivano in condizioni inferiori — sia come tiroidi, sia come disfunzini tiroidee — da quelli di tutte le altre regioni d'Italia. Ben diversa constatazione invece si è fatta visitando più tardi alcune scuole della zona Aostana non profilassata e del Canavese, pure non profilassato. La differenza del quadro endemico era palese poichè in queste ultime le ipertrofie tiroidee erano ancora frequenti e ben manifeste.

Questo fatto è risultato anche dall'inchiesta fatta nei primi mesi del corrente anno, a distanza di 5 anni dall'ultima inchiesta governativa, che precedette l'inizio della distribuzione del sale iodurato, inchiesta eseguita, come allora, dagli Ufficiali Sanitari in tutte le scuole elementari della nostra provincia, e raccolta sugli stessi moduli e con le medesime modalità già adottate nell'inchiesta precedente.

Da essa, come appare dallo specchietto annesso (vedi tavola), risulta che nella zona profilassata le ipertrofie tiroidee si sono ridotte *ad un terzo*, mentre rimasero pressochè invariate nelle zone non profilassate. Il contributo del sale iodurato non può essere messo così in dubbio.

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Un quesito nuovo si è più volte sollevato ultimamente da taluni studiosi; esso concerne la percentuale di ioduro di K da aggiungere al sale a scopo profilattico, percentuale che secondo alcuni (Danielopolu, della Romania, Buzzi, Marcovecchio, ecc.) dovrebbe venire aumentata.

Io non sono del parere di questi ultimi. In primo luogo, perchè se l'attuale percentuale (gr. 1 di JK per q.le di sale da cucina) non rappresenta un pericolo alla collettività, un aumento potrebbe condurre a manifestazioni palesi d'ipertiroidismo, di cui molte forme larvate si riscontrano con frequenza nei paesi ad endemia. In secondo luogo, noi abbiamo molti anni di prove, che ci dimostrano una sufficiente efficacia dell'attuale proporzione del sale iodurato.

Il gozzismo non è una malattia acuta, ma un'affezione cronica che si sviluppa, per lo più molto lentamente; non è detto perciò che la cura migliore sia quella intensiva; essa dovrebbe essere dosata in quantità anche minima, tale da servire soltanto a *neutralizzare la quantità del quid* gozzigeno, che — nella stessa misura di tempo — agisce dannosamente sull'organismo.

La cura e profilassi non dove essere perciò eccessiva, innanzitutto per non nuocere in un modo forse non meno funesto.

Secondo i già descritti dai vari autori (Ambrosi, Muggia, Eggemberger, ecc.) risultati delle prove finora eseguite, il sale iodurato, nelle proporzioni attualmente usate, previene soddisfacentemente la malattia e corregge le comuni manifestazioni del gozzismo in minor tempo di quanto forse esse abbiano impiegato per svilupparsi: non potrebbe quindi rappresentare una percentuale insufficiente di ioduro di K. D'altra parte è assurdo sottoporre a cure intensive tutta una popolazione, quando si tratta semplicemente di agire su una percentuale relativamente bassa, sebbene importantissima, di individui con alterazioni organiche permanenti.

Io non credo d'altronde — come già più volte ho esposto — che l'agente specifico dell'endemia (sia esso l'intossicazione silicica od altro) possa portare alle forme gravi dello struma, del cretinismo, sordo-mutismo, nanismo, ecc., se altri fattori, per lo più costituzionali, non vi cooperino. Poichè se il migliorato tenor di vita ha contribuito finora ovunque ad attenuare il fenomeno — ossia a diminuire la frequenza delle manifestazioni più tristi — e siccome il quid specifico non è stato tolto, vuol dire che è poco probabile arrivare con esso alle forme gravi del gozzismo senza la concomitanza di terzi fattori, che potrebbero essere endogeni (ereditarietà, costituzione, ecc.) od esogeni (deficiente alimentazione, alcoolismo, scarsa igiene, ecc.).

Per prevenire quindi ed estirpare anche le forme gravi dell'endemia occorre, accanto alla profilassi specifica (iodica), una rigorosa applicazione di tutti quei provvedimenti igienici imposti dalla civiltà del nostro secolo ed un'elevazione sempre maggiore del tenore di vita delle popolazioni colpite.

Gignod (Aosta).

				Visitati	gole normali		gole piene		gole grosse		gozzetti	
					N.	%	N.	%	N.	%	N.	%
Valle d'Aosta	Zona profilassata		1934	4996		35,61		41,59		22,15		0,65
			1939	5705	3930	68,89	1524	26,71	248	4,35	3	0,05
	Zona non profilassata		1934	2810		68,95		26,65		4,23		0,17
			1939	3599	2437	67,71	920	25,56	224	6,23	18	0,50
Canavese			1934	12854		61,74		29,29		7,92		1,05
		1939	Totale	13019	(*)	77,70		17,50		4,98		0,71
			zona alta	2505	1929	77,04	398	15,86	105	4,19	73	2,91
			zona bassa	10514	8187	77,86	1880	17,88	427	4,06	20	0,19
Provincia d'Aosta			1934	20658		50,04		36,75		12,37		0,84
		1939	Totale	22328	16183	73,87	4722	21,14	1004	4,49	114	0,50
			M.	11633	8497	73,05	2567	22,06	513	4,41	56	0,48
			F.	10690	7986	74,70	2155	20,16	491	4,60	58	0,54

(*) Si fa presente che alcuni paesi del Canavese hanno continuato la distribuzione nelle scuole dei cioccolatini iodati.

RIASSUNTO.

L'A., trattando della patogenesi del gozzismo si sofferma sulla teoria da intossicazione silicica, ammessa dal dott. Buzzi di Sondrio, che condivide ed illustra, mettendola in relazione alle sue personali osservazioni. Accenna quindi alla cura e profilassi iodica e riporta i risultati brillanti ottenuti dalla distribuzione del sale iodurato durante l'ultimo quinquennio nell'alta Valle d'Aosta, confrontandoli con l'andamento stazionario del gozzismo nella zona attigua non profilassata della Valle d'Aosta. Infine si dichiara contrario ad un aumento della percentuale di IK in detto sale profilattico.

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JAMA, 198 (13): 120-122, Dec. 26, 1966

Talc Granulomas of the Skin

Murray J. Tye, MD, Ken Hashimoto, MD, and Frank Fox, MD

SURGICAL GLOVE TALC can cause granulomas in various intestinal and pelvic organs.[1] In the skin, localized silica granulomas due to the glove talc have been observed in surgical scars[2,3] and as the result of the impregnation of the skin by silicious particles, such as glass or sand, following an injury.[4,5] Extensive talc granulomas of the skin have not been reported, to our knowledge.

Report of a Case

A 45-year-old woman was admitted to the hospital because of the development of increasing numbers of lumps on her shoulders, buttocks, and thighs. These nodules had been developing for the past two years and were at sites where she has had as many as 500 boils over the past eight to ten years. Many of these boils were treated by incision and drainage. The nodules have not appeared on areas of the body where she has not had furuncles. She also has had many injections of staphylococcal vaccines and of cyanocobalamin (vitamin B_{12}) into sites over the upper arms and buttocks.

It has been her practice over the past several years to take one or two baths daily, followed by the liberal dusting of her entire body and even her pillow with powdered talc.

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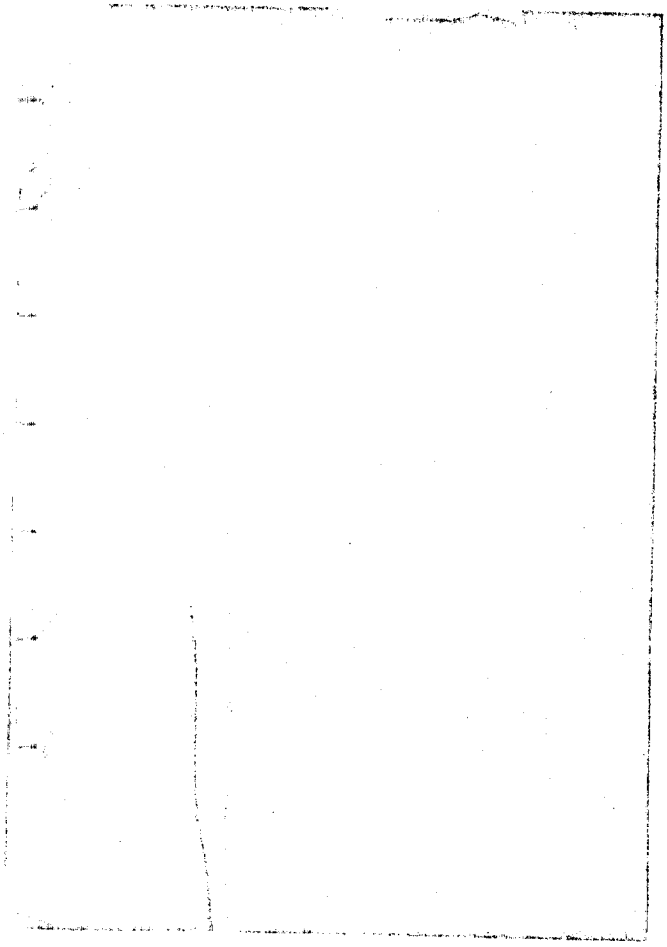
...eds of hard, slightly raised, flesh-colored nodules, ...ng in size from 2 mm to 2 cm, were scattered over the posterior area of the shoulders, upper part of the ...ck, the buttocks, and anterior and posterior aspects of the thighs. The nodules were especially dense about the margins of the many linear and irregular surgical scars in these areas (Fig 1).

Other physical and laboratory findings were not remarkable. The x-ray films of the indurated areas did not reveal calcification of the soft tissues.

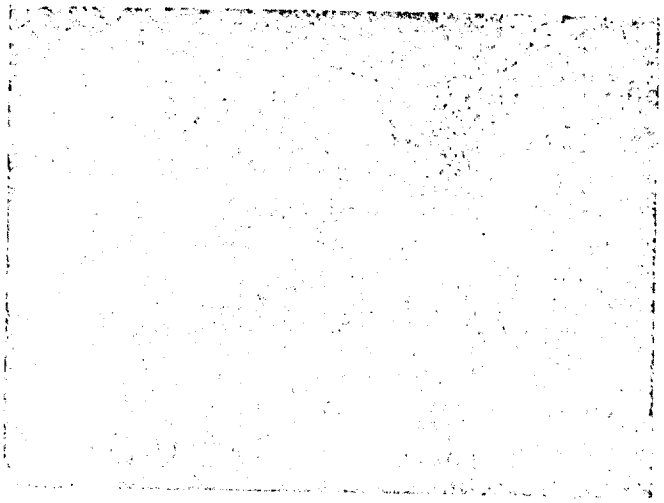
Methods of Examination.—One of the large subcutaneous nodules overlying the right deltoid muscle was excised with the patient under local anesthesia and divided into four parts. One was immediately frozen, sectioned in a cryostat at 8μ, and stained for acid phosphatase[6] and for β-glucuronidase.[7] The remaining tissue was fixed in 10% formaldehyde solution, prepared for paraffin sections, and stained with hematoxylin and eosin.

A second portion was cut into pieces 1 to 5 mm in length, and fixed in 1% osmic acid buffered to pH 7.4 with veronal buffer and adjusted to physiological osmolarity with sucrose. After fixation for one hour, tissue blocks were dehydrated with alcohol and propylene oxide and then imbedded into epoxy resin (Araldite). Thin sections were cut with a diamond knife in an ultra-microtome. Sections were stained first with Reynolds lead citrate[8] and then either with 1% phosphotungstic acid in 50% alcohol or with 1% uranyl acetate in distilled water. An electron microscope was used at 50 kv to observe these stained sections.

A third portion was analyzed for oxalic acid.[9] A thin sliver of the tissue was cut and rinsed in distilled water, blotted on filter paper, and allowed to dry in air for about five hours. It was then mounted on a 7.16-cm radius power camera and a three-hour exposure was taken with filtered copper radiation. A comparison diffraction pattern, with the same camera and factors, was made on a sample of commercial talcum powder mounted in a thin-walled glass tube.



2. Subcutaneous 2-mm to 2-cm nodules on thigh and buttock. Nodules more dense about linear and irregular scars, sites of previous furuncles or incisions.



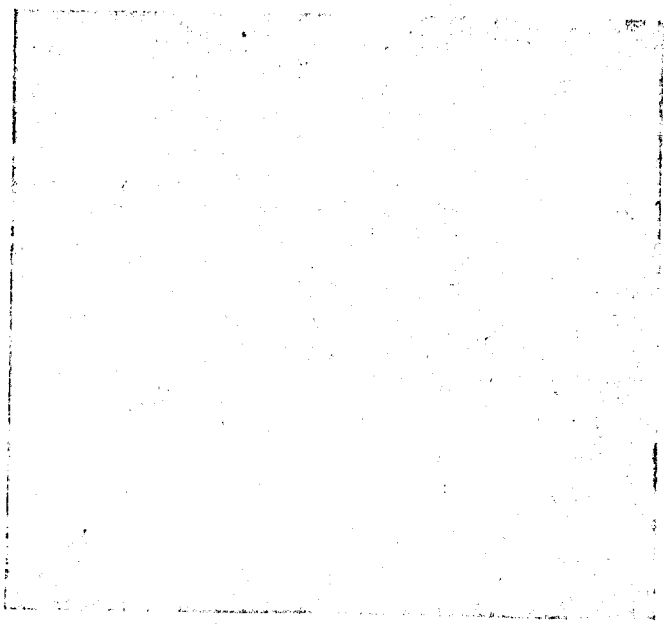
2. Granulomatous lesion observed by polarized light showing large histiocytes, epithelioid cells, and giant cells. One giant cell contains an asteroid body (arrow). Birefringent particles are present (hematoxylin and eosin, × 100).

Talc and crystals of cyanocobalamin dissolved in distilled water were placed on glass slides and observed with polarized light and were also placed on the parlodion-coated copper grid and directly observed with the electron microscope.

Several experimental x-ray plates were taken to determine the radiopaqueness of varying quantities of talc in different thicknesses of tissue. (1) Six 12×3 cm gelatin capsules containing varying amounts of talc were inserted into the center of an 8-cm thick piece of beef. (2) Two cuts were made into a 2-cm thick piece of beef and a small amount of talc was placed into one cut. Talc was thickly packed into the second insert. Roentgenograms were taken.

Histological and Histochemical Findings.—The lesion was composed of islands of granulomas with numerous foreign body cells (Fig 2). With polarized light, numerous *white* birefringent particles were found in histiocytes, epithelioid cells, and in some of the giant cells. Structures similar to asteroid bodies as described in sarcoid were also noted in some giant cells. These cells also showed a strong reaction with both acid phosphatase and β-glucuronidase stains. The crystals of cyanocobalamin gave a strong red birefringence.

Electron Microscopic Findings.—The most striking finding with lower powers was the presence of strongly electron-dense lamellar structures. These ranged widely in dimension from 100 mμ to several micra and were located in large phagocytic cells, corresponding to histiocytes or epithelioid cells with light microscopy, and also in perivascular cells (Fig 3). When the lamellae were not displaced from their original location, electron-dense membranes were often observed surrounding them. Endothelial cells and vessel wall cells, a type of phagocytic cell, also contained the lamellar substances in abundance. Giant cells, on the other hand, contained only small amounts of lamellar substance but numerous fine filaments. These filaments converged on many dense lines in the cytoplasm in much the same manner as tonofilaments converge toward desmosomes. These converging centers were often straight, although rectangular and other forms were found.



3. Electron-dense lamellar structures in endothelial cell (*E*) and perithelial cells (*P*) (× 14,500).

The lamellar substance in epithelioid cells appeared identical to talc in size, density, and general architecture. Cyanocobalamin crystals were different.

Chemical Analysis for Oxalates.—No abnormal amount of oxalates was found in the tissue or in the urine.

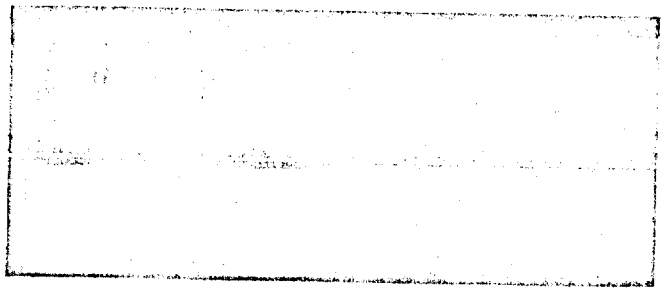
X-ray Diffraction Studies.—The pattern was quite strong and identical to that of talc (magnesium silicate) (Fig 4). There was no evidence of any other crystalline phase.

Roentgenograms of Talc in Tissue.—Studies with talc in the 7-cm thick beef tissue did not demonstrate radiopacities. When thickly packed in 2 cm of tissue, the talc was slightly visible.

Comment

The open, draining furuncles were probably the portals of entry for the talc which the patient had been applying liberally daily, as a dusting powder. The fact that the sites of these nodules were also in proximity to the scars where many furuncles had been incised further corroborates this hypothesis.

The histological findings of polarized white birefringent particles implicated the talc as the casual factor of the foreign body granuloma formation, eliminating cyanocobalamin which had been injected at many of the sites where lesions were found. X-ray diffraction studies confirmed talc as the crystal particle.



4. X-ray diffraction patterns. *Top*, Commercial talcum powder mounted in thin-walled glass tube. *Bottom*, Tissue section from granuloma shows similar pattern.

Electron microscopic findings demonstrated the presence of lamellae in the epithelioid cells identical to talc in size, density and architecture. It also appeared as though some of these lamellae and ferritin particles, which are derived from the hemosiderin of extravasated erythrocytes, were being phagocytized in lysosomes of epithelioid cells. It is not difficult to understand that talc, being a foreign substance, was also trapped in lysosomes together with hemosiderin and ferritin.[10]

Oxalosis had been once suspected because of the widespread distribution of the crystals in the hemotoxylin and eosin stained sections, and birefringence of the particles in the granuloma. Cases of widespread extrarenal involvement in adults have been reported by Edwards[11]. The urinary excretion and skin content of oxalates were not increased in this patient.

Roentgenograms of the tissue in which talc was suspected of being imbedded would not be useful unless the talc was present in large amounts.

Summary

Hundreds of talc granulomas of the skin had developed in a 45-year-old woman. It is postulated that the talcum powder gained entrance through the skin at the sites of draining or incised furuncles. Electron microscopic studies showed the electron-dense lamellae to be morphologically identical to that of talc in many phagocytic cells. X-ray diffraction studies confirmed the foreign crystal to be talc.

This investigation was supported in part by research grant GM-10259 and training grant T1-AM-5220 from the National Institute of Arthritis and Metabolic Diseases.

X-ray diffraction studies were done by Robert L. Collin, PhD, Cancer Research Institute, New England Deaconess Hospital; tissue and urine analyses for oxalic acid by Harry Powers, MD, Tufts University School of Medicine; and x-ray studies by Joseph Stull, MD, Haverhill Municipal Hospital.

Cyanocobalamin was supplied by Merck, Sharp & Dohme, West Point, Pa.

References

1. German, W.M.: Dusting Powder Granulomas Following Surgery. *J Surg Gynec Obstet* **76**:501-507 (April) 1943.
2. Fienberg, R.: Talcum Powder Granuloma, *Arch Path* **24**:36-42 (July) 1937.
3. Gardner, L.U.: The Similarity of the Lesions Produced by Silica and by the Tubercle Bacillus, *Amer J Path* **13**:15-25 (Jan) 1937.
4. Crossland, P.M.: Silicon Granuloma of the Skin, *Arch Derm* **71**:457-461 (April) 1955.
5. Epstein, E.: Silica Granulomas of Skin, *Arch Derm* **71**:24-35 (Jan) 1955.
6. Barka, T., and Anderson, P.J.: Histochemical Methods for Acid Phosphatase Using Hexazonium Pararosanilin as Coupler, *J Histochem Cytochem* **10**:741 (Nov) 1962.
7. Hayashi, M.; Nakajima, Y.; and Fishman, W.H.: The Cytologic Demonstration of B-glucuronidase Employing Naphthol AS-BI Glucuronide and Hexazonium Pararosanilin. A Preliminary Report, *J Histochem Cytochem* **12**:293 (April) 1964.
8. Reynolds, E.S.: The Use of Lead Citrate at High pH as an Electron Opaque Stain in Electron Microscopy, *J Cell Biol* **17**:208-212 (April) 1963.
9. Powers, H.H., and Levatin, P.: Method of Determination of Oxalic Acid in Urine, *J Biol Chem* **154**:207-214 (June) 1944.
10. Hashimoto, K., and Lever, W.F.: Kaposi's Sarcoma, Histochemical and Electron Microscopic Studies, *J Invest Derm* **43**:535-549 (Dec) 1964.
11. Edwards, D.L.: Idiopathic Familial Oxalosis, *Arch Path* **64**:546-555 (Nov) 1957.

THE JOURNAL OF
INDUSTRIAL HYGIENE
AND TOXICOLOGY

VOLUME 24 MARCH, 1942 NUMBER 3

ABSORPTION, ACCUMULATION AND EXCRETION OF INGESTED SILICA*

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CATARACT in India was shown by Burge (1) to be associated with a high silica content of the lens but in American cases no such correlation was found. Analysis of table salt from India demonstrated the presence of silica in considerable amounts and Burge postulated that the ingestion of this silica might well be the etiologic factor for cataract in that region. The present work was undertaken to test this possibility. Simultaneously analyses for silica were made on several organs and the absorption and excretion of silica were investigated.

METHOD

4 series of rats were given silica (Braun's Air-Float, 325 mesh) in their food for 10 months; each series contained 5 animals. The amount of silica given in the food was as follows:

Series I	control
Series II	0.01% SiO_2
Series III	0.1% SiO_2
Series IV	1.0% SiO_2

Checks on their weight were kept at weekly intervals over this time.

At the end of this time the rats were killed by decapitation and the organs to be analyzed were removed and weighed on filter paper. The tissues were then dried for several hours in an electric oven and analyzed for silica. The filter paper, which was silica-free, was analyzed with the tissue because of the absorption of tissue fluid by the paper.

For the determination of silica a micro method was used, modified from the procedure of King and Stantial (2). This method involves the transformation of the silica to silicate and its subsequent determination, after removal of iron and phosphate, colorimetrically by reduction with 1,2,4-aminonaphtholsulfonic acid of a silicomolybdic acid complex to a blue compound.

The complete removal of iron and phosphate is essential. This is done by precipitating the iron as phosphate and the removal of the excess phosphate, along with the original tissue phosphate, by precipitation of the calcium salt. To ensure completeness these precipitations must be carried out at the correct pH. Modification of the procedure of King and Stantial (2) involved only the adjusting of the pH at which these precipitations were made. They advocated the neutralization of the solution from the sodium carbonate ashing; however, when this was done, either by indicator or titration, the removal of phosphate was not complete in all cases. Numerous runs without tissues were made, without added silica, using various proportions of H_2SO_4 and NH_4OH. The results are summarized in table 1. We, therefore, used the empirical proportions of 1.4 ml of H_2SO_4

* Received for publication November 15, 1941.

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and 1.0 ml NH_4OH to control the pH of the precipitation in all the determinations.

The relationship between the amount of silica present, the duration of the ashing and the amount of sodium carbonate used was thoroughly investigated and it was found that all the quantities of silica that might be present could be transformed by ashing for 20 minutes at red heat with 300 mg of sodium carbonate.

Several curves were made from standard silica solutions using three different forms of preparation. As these all gave concordant results they were all combined into one curve and the results read from this. Quantitative data could be obtained down to 30 μgm of SiO_2 per sample.

TABLE 1

DETERMINATION OF OPTIMAL pH

2M-H_2SO_4 (ML.)	1M-NH_4OH (ML.)	COLORIMETRIC READING
1.4	0.5	99.4
1.4	1.0	99.6
1.4	2.0	96.8
1.6	0.5	96.8
1.6	1.0	97.0
1.6	2.0	97.6
1.6	3.0	98.3
1.6	4.0	97.8
1.7	2.0	97.4
1.7	3.0	99.6
1.8	0.5	93.2
1.8	1.0	95.0
1.8	2.0	96.4

TABLE 2

SILICA CONTENT OF TISSUES

ORGAN	SERIES I	SERIES II	SERIES III	SERIES IV
Liver	19.0*	22.7	25.0	40.5
Spleen	10.4	16.8	42.5	59.5
Testis	17.7	18.1	51.8	47.3
Kidney	22.7	16.3	25.0	22.3
Lung	34.1	22.2	37.0	25.9
Eye	64.2	49.6	58.8	57.1
Blood	14.1	17.8	13.3	15.1
Average	19.8	20.6	32.4	35.1

* Mgm. SiO_2 per 100 gm. of fresh tissue.

RESULTS

The silica concentrations for the various organs in each series are given in milligrams of SiO_2 per 100 gm of fresh tissue in table 2. The average values for each series given in the last row were calculated, taking into account the relative weights of the various organs. We may assume that these values approximate the silica concentrations in the total animal and are thus of interest in comparing the various series.

DISCUSSION

Eyes of all the rats were examined by means of the opthalmoscope by Dr. Rodman Irvine of the Division of Opthalmology. None showed any pathological change in the lens or other media in transmitting or refracting light. Indeed there were no detrimental changes observed in the animals during the course of the experiment.

TABLE 3

SILICA ACCUMULATION

	AVERAGE WEIGHT	AVERAGE SILICA CONC.	TOTAL SiO_2
	gm.	mg./100 gm.	mg.
Before feeding	100	15.0	15
After feeding (control)	250	19.8	50
After feeding (III)	250	32.4	81

TABLE 4

ACCUMULATION OF INGESTED SILICA

	%
Amount of added silica ingested	49
Silica accumulated (control)	[illegible]
Silica accumulated (III)	[illegible]
Added silica accumulated (III—control)	[illegible]

The growth rates of the rats were identical in each of the four series.

The concentrations of silica found in the various organs are in general accord with results obtained by others on human tissues, as summarized in the review article of King and Belt (3). It is seen that ingested silica can be accumulated in two organs, the liver and the spleen; the testis gives some evidence of accumulation and the other organs none at all. Since silica is generally supposed to circulate in the blood in colloidal form it is not surprising that it is picked up predominantly by those organs containing a large amount of reticulo-endothelial tissue. It is noteworthy that the eye contains the highest concentration of silica of any organ investigated; however, it seems to be a normal constituent and the added silica does not accumulate here. We can, in accord with the terminology of Kahn

and Antoine (4), say that most tissues possess a relatively constant "silice de constitution," apparently an integral part of the cell, while a few tissues in addition to this can contain a "silice d'interposition," which depends on the amount of silica intake from food and inhalation.

By making several assumptions one can calculate the fraction of the ingested silica that is absorbed through the gastro-intestinal tract and accumulated in the tissues. These calculations are given in tables 3 and 4. Thus when silica was added to the food to the extent of 0.1% the amount accumulated by the tissues represents 0.8% of the amount ingested.

Silica analyses were made on urine and feces of rats kept for 1 week on the series-III diet, the urine and the feces being pooled from 3 days' collection. The results showed that of the silica excreted, 4% occurred in the urine and 96% in the feces. In conjunction with the results shown above for accumulation we may conclude that of the added ingested silica approximately 95% was excreted in the feces, 4% was excreted in the urine and 1% was accumulated in the tissues, when the concentration of the added silica in the food was 0.1%.

The excretion of ingested silica in the urine has been noted by several workers (Schulz (5), Salkowski (6), Gonnermann (7), Riessner and Kindt (8), Kochmann and Maier (9), King, Stantial and Dolan (10) and others). However, there has been found no simultaneous increase in the blood level of silica (King, Stantial and Dolan, (11)). These results have been confirmed in the present work. The kidneys possess a far greater power of removing silica from the blood than any other organ, but this is entirely excreted and not accumulated within the kidney tissue.

The relative proportion of silica excreted in the feces and urine depends on two factors: the amount of silica ingested and the form in which it is administered. As the concentration of silica in the food goes up the fraction accumulated becomes less. Likewise, the fraction excreted in the urine becomes less. When the only source of silica was that naturally occurring in the food, some 10-15% was excreted in the urine. With sodium silicate there is a more rapid absorption through the intestinal wall and a corresponding rapid rise in urinary silica (King and Dolan, (12)); when colloidal silica or silica built into plant tissues is fed, the absorption is slower and less complete.

Summary

Ingested silica causes no signs of cataract or other pathological changes in the rat, nor does it have any influence on their growth rates.

A certain fraction of the ingested silica can be absorbed from the gastrointestinal tract; most of this is excreted in the urine but a small amount is accumulated in the tissues. The liver and spleen are the most active in this regard.

BIBLIOGRAPHY

1. Burge, W. E.: The role of silicate in cataract production. Am. J. Physiol., *136:* 451 (1939).
2. King, E. J., and Stantial, H.: The biochemistry of silicic acid. I. The microdetermination of silica. Biochem. J., *27:* 950 (1933).
3. ——— and Belt, T. H.: The physiological and pathological aspects of silica. Physiol. Rev., *18:* 329 (1938).
4. Kahane, E., and Antoine, G.: Sur la nature de la silice chez les êtres vivants. Silice de constitution et silice d'interposition. Bull. Soc. chim. biol., *18:* 1769 (1936).
5. Schulz, H.: Die quantitative Ausscheidung der Kieselsäure durch den menschlichen Harn. Pflüger's Arch. f. d. ges. Physiol., *144:* 350 (1912).
6. Salkowski, E.: Kleinere Mitteilungen. I. Über den Nachweis der Kieselsäure im Harn ohne Veraschung desselben. Ztschr. f. physiol. Chem., *83:* 143 (1913).
7. Gonnermann, M.: Beitrage zur Kenntnis der Biochemie der Kieselsäure. Ibid., *99:* 255 (1917).
8. Riesser, O., and Kindt, B.: Versuch am Menschen über Kieselsäureausscheidung und Kieselsäurebilanz nach Aufnahme von Natriumsilicat. Ibid., *174:* 40 (1928).
9. Kochmann, M., and Maier, L.: Beitrage zur Biochemie der Kieselsäure. I. Einfluss der Kieselsäure auf das Körpergewicht wachsender Ratten. Biochem. Ztschr., *223:* 228 (1930).
10. King, E. J., Stantial, H., and Dolan, M.: The biochemistry of silicic acid. II. The presence of silica in the tissues. Biochem. J., *27:* 1002 (1933).
11. ———, Stantial, H., and Dolan, M.: The biochemistry of silicic acid. III. The excretion of administered silica. Ibid., *27:* 1007 (1933).
12. ——— and Dolan, M.: Silicosis and the metabolism of silica. Canadian Med. Ass. J., *31:* 21 (1934).

Proc. Soc. Exp. Biol. Med. 124 (2): 524-532, 1967

A Study of Teratogenic Effects of Particulate Compounds in the Amniotic Cavity of Chick Embryos.* (31780)

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In previous studies a significant percentage of gross defects occurred after thalidomide was injected into the amniotic cavity of 3-to 5-day-old chick embryos. The defects included eye defects, encephalocele, beak defects, retarded feathering and retarded ventral closure. However, similar defects were produced by other chemically unrelated compounds, the only obvious characteristic possessed by all being a high degree of insolubility in ordinary diluents and in the amniotc fluid(1). Two compounds of the series tested were colloidal and these produced, in addition, severe distortions of the body axis and limbs. To investigate further the nature of the teratogenic action of the particles several other compounds were studied. Carbon black, easily identified in tissue sections, was used to determine the physical relationship of the particles to the defects produced. Included also was a colloidal silica of known particle size. Several preparations of polystyrene latex balls of graded size were tested in an attempt to determine the relationship of particle size to production of defects. The latex balls agglutinated in the amniotic fluid and thus were not suitable for the purpose, although they did produce the characteristic defects. Due to similarities in the effects of all the compounds, the most obvious supposition is that the developmental anomalies are related to the physical rather than to the chemical nature of the materials. There is, however, no evidence that the compounds may not also produce changes related to their chemical activities. For instance, silica particles, which are etiologically associated with silicosis are known to have specific toxic effects for macrophages which engulf them(2) whereas other particles, including those of alumina and carbon, do not have these toxic effects. In chick embryos the colloidal silica produced the same defects as colloidal alumina with certain slight dissimilarities.

Methods and materials. Materials used. Reagent grade, sugar carbon from Fischer Scientific Co. had maximum impurities of 1% sulfates (calcium, magnesium, sodium, etc.) and 1% ash (alkali metal). For injection it was ground in a tissue grinder and suspended in distilled water or saline buffered at pH 7.2. A suspension of colloidal silica (Ludox®) from Du Pont Co. contained 15% SiO_2, 1% Na_2O (titratable alkali), 0.001% NaCl and 0.003% Na_2SO_4. The silica particles were dense, nonporous spheres approximately 7 mμ in diameter. They remained dis-

* Supported by USPHS Grants HD-00098 and HD-01916 from Inst. of Child Health and Human Development.

TABLE I. Survival and Incidence of Abnormal Development in Chick Embryos Inoculated with Carbon Suspensions into the Amniotic Cavity at 5 Days Incubation.

mg contained in 0.05 ml diluent per egg	Total eggs	Embryos surviving to incubation day: 8	15	Total abnormal of those surviving 8 days or more: No.	(%)
Carbon 0.1	73	38	10	19	(50)
" 0.01	52	30	13	5	(17)
" 0.001	51	29	9	1	(3)
Control	72	51	27	0	

crete and maintained particle size on dilution in water or saline. The pH was 8.5 with minimum stability range at pH 5-6. For injection the "Ludox" was diluted in water or saline to the required concentration. Broth cultures were made of all materials inoculated. The preparations rarely showed bacterial contamination even though sterilization procedures and addition of antibiotics were generally avoided to ensure stability and purity of the preparations. In some experiments carbon suspensions were autoclaved but results were the same whether or not this procedure was followed.

Preparation and inoculation of eggs. Fertile eggs from White Leghorn flocks or from Meatline hens mated to New Hampshire roosters were incubated in a forced draft incubator at 37.5°C. Materials were injected, according to methods previously described(1) into the amniotic cavity of eggs incubated for 5 days. Each received 0.05 mg of the diluent containing calculated amounts, ranging from 0.001 mg to 0.25 mg, of the experimental materials. Controls from the same incubation setting received saline or distilled water with the pH adjusted to that of the experimental material.

Harvesting and examination of embryos. In the initial experiments with carbon and silica, the embryos were injected, then observed daily through the shell openings and the dead embryos removed for gross examination. Surviving embryos were sacrificed at 15 days of incubation. Incidence and description of defects were tabulated for all embryos surviving to the 8th day of incubation or longer. In another series of experiments, embryos were injected with carbon suspensions and sacrificed at intervals of 1, 3 and 7 hours and thereafter at daily intervals up to the 8th day after injection. These were examined grossly, then processed by standard histological techniques for microscopic study. Sections were stained with hematoxylin and eosin, or in some instances, the periodic acid-Schiff stain.

Results. Observation of gross defects. Embryos receiving carbon suspensions showed a significant percentage of striking defects of the types previously observed with thalidomide (Table I). In order of incidence, the defects included retarded lid development or exophthalmia, encephalocele or exencephaly, external edematous blebs, ectopic viscera, retarded feathering and crossed or short upper beak. All abnormal embryos had eye defect or encephalocele or both, predominantly on the left side. This asymmetry is attributed to the fact that carbon aggregates sink to the bottom of the amniotic cavity where the left side of the head lies at the stages injected.

The colloidal silica showed no indication of clumping or settling in the amniotic fluid. It also produced a significant percentage of defective embryos at dosages indicated in Table II. The types and incidence of defects were similar to those observed with the carbon but with the addition of severe axial distortions and correlated limb distortions similar to those previously observed with colloidal alumina(1). The silica-treated embryos frequently showed fusion of the appendages to the body wall with concomitant malformations of the affected limbs. These fusions had not been observed with the colloidal alumina.

Microscopic study of carbon-induced defects. In this series of experiments, eggs were injected at 4 to 5 days with amounts ranging from 0.1 to 0.25 mg of carbon per egg, the larger amounts making it easier to locate particles in tissues without extensive serial sectioning. A total of 53 embryos were studied,

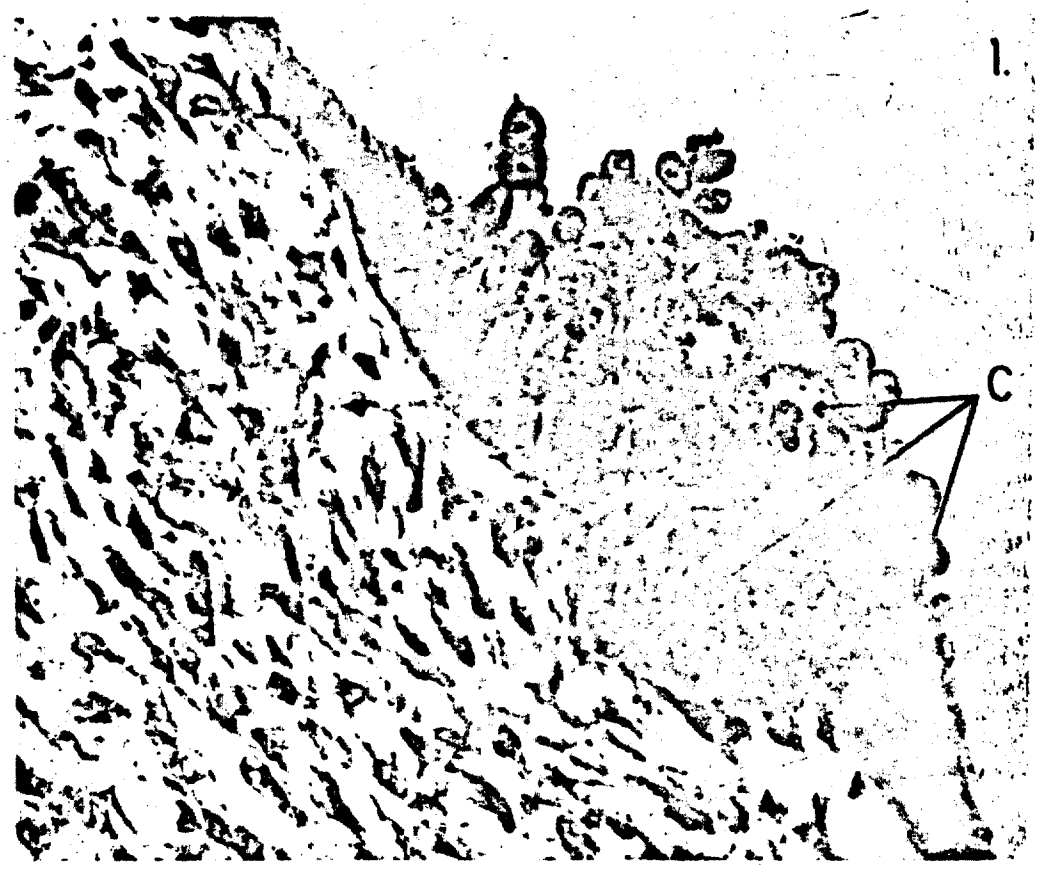
TABLE II. Survival and Incidence of Abnormal Development in Chick Embryos Inoculated with Colloidal Silica into the Amniotic Cavity at 5 Days Incubation.

mg contained in 0.05 ml diluent per egg	Total eggs	Embryos surviving to incubation day: 8	15	Total abnormal of those surviving 8 days or more No.	(%)
Silica 0.82	44	25	14	10	(40)
" 0.082	164	114	64	59	(52)
" 0.0082	44	30	20	5	(17)
Control	110	100	78	0	0

3 to 6 embryos sacrificed at each time interval after injection.

On gross examination the carbon was at first observed floating in the amniotic fluid but within a few hours it began to adhere to tissues and, as the days passed, it disappeared from view in most embryos. Microscopic examination showed that carbon began to enter tissues of the amnion and ectoderm of the embryo by 7 hours after injection. By 1 day small particles of carbon had been taken up by cells of the amnion and aggregates of carbon had been incorporated into the amniotic tissue by outgrowth of cells. Carbon was also present in the integumental ectoderm of the embryo itself.

The mechanism by which the carbon entered embryonic tissues is not clear but phagocytosis *per se* did not appear to be a factor. Particles were sometimes located within ecto-



Microscopic sections of chick embryos injected into amniotic cavity at 5 days incubation with 0.1-0.25 mg of carbon (C).

FIG. 1. Area of localized hyperplasia in cephalic ectoderm, 4 days after injection. × 460.

FIG. 2. Hyperplasia and sloughing of vacuolated tissue of ectoderm over the optic lobe, 7 days after injection. × 230.

dermal cells but were most commonly observed within large tissue spaces, among damaged cells and sometimes between cells which appeared normal. Localized reactions of ectodermal tissues in the vicinity of carbon included: a) sloughing of outer ectodermal cells, some with and some without carbon attached; b) damage to some cells in the vicinity of carbon as indicated by fading of cell membranes and nuclei; c) marked localized hyperplasia of the ectoderm with consequent disorganization of normal cellular relationships (Fig. 1); d) appearance of large clear spaces in the tissues (Fig. 2); e) changes in cell characteristics such as increased size, changes in shape and increased affinity for eosin. Necrosis and sloughing of the ectoderm resulted in its complete destruction in some areas where the outer cells of the mesoderm then joined to form a covering for the subjacent tissues. Although the edges of such areas were usually bounded by thickening of the remaining ectoderm, there was no evidence during the period of observation that the ectodermal cells migrated to cover the exposed mesoderm. In some areas fusions occurred between amniotic membrane and the integument.

At 1 to 2 days after injection, the carbon began to enter the mesoderm. Although carbon could presumably enter through the denuded areas, it was, apparently, also able to pass through ectoderm into the mesoderm. In many sections carbon was observed in undestroyed body ectoderm and in the underlying mesoderm. No characteristic reaction of the mesoderm to the carbon was observed but the tissues in the affected areas always become thinner with carbon located at pro-

FIG. 3. Nasal epithelium in area of superior concha shows carbon particles in sloughing cells, nasal epithelium and subjacent mesenchyme, 5 days after injection. × 460.

gressively deeper levels within the tissues. No inflammatory reaction occurred in the early days after injection, no walling off of carbon by connective tissue and no obvious invasion of the blood stream. There were occasional degenerating cells in the vicinity of carbon particles and rarely a cell with phagocytosed carbon was evident. Some areas of hemorrhage and edema were seen.

Initially it was considered that damage to the body ectoderm might be due to friction of particles against the cells as the embryo moved about in the amniotic cavity. However, sections through the nasal cavities, which are protected against such frictional movements, provided evidence against this explanation. Carbon particles were frequently observed within the nasal epithelium where the effects were much the same as those in the integumental ectoderm, with necrosis and sloughing of cells, disorganization of normal cellular arrangements and passage of carbon through the nasal epithelium (which is much thicker than the integumental epithelium) into the underlying mesoderm (Fig. 3).

Wherever sufficient carbon was present in the tissues, the structures developing in or near such tissues were abnormal. Thus, sclerotic cartilages developed irregularly in the vicinity of carbon, some areas being interrupted by undifferentiated mesenchyme while others appeared thicker than normal (Fig. 4). Feather follicles were frequently absent in the cephalic region (Figs. 5, 6, lids and nictitating membrane were absent or retarded and the cornea was thin or absent. In affected areas of mesoderm, the frontal and squamosal bones were retarded in growth, failing to extend over the cranium (Fig. 6).

Eventually most of the carbon was located

FIG. 4. Shows accumulation of carbon in mesenchyme between left eye and cerebral hemisphere, with abnormalities of the sclerotic cartilage 7 days after injection. × 45.

deep within mesodermal tissues, particularly between the cerebral hemispheres and the interorbital septum, adjacent to or within sclerotic cartilages (Fig. 4), mesenchyme of the ventral abdominal wall and in tissues of extraembryonic membranes including amnion, chorion and yolk sac. Within the experimental period, carbon was not found in tissues of brain, lens, retina or in the visceral organs. However, during the latest stages examined, phagocytosis of carbon particles was increasingly seen in cells within the body cavities and occasionally in cells of the mesoderm over the brain.

Discussion. The similarity of teratogenic effects of colloidal silica to those of colloidal alumina (Du Pont's "Baymal") calls for a comparison of some of the physicochemical properties of the two compounds. The particles of the colloidal alumina were filamentous, ranging from 100 mμ to 300 mμ in length compared to the spherical silica particles only 7 mμ in diameter. Silica releases a small amount of silicic acid which is believed to be involved in its ability to destroy macrophages(2). Recent studies also indicate that silica can interact with proteins and phospholipids and lyse red blood cells (3). In contrast, the colloidal alumina does not have these effects and has been shown to be highly nontoxic in animal studies (Du Pont's manual on "Baymal"). It should not have been too surprising to find the silica preparation causing teratogenic effects but there is no ready explanation for the severe damage to cells and tissues by such materials as alumina or carbon. In fact carbon has been used as a marker in cells of early chick blastoderms to study cell movements. In some of these studies the investigators did report a

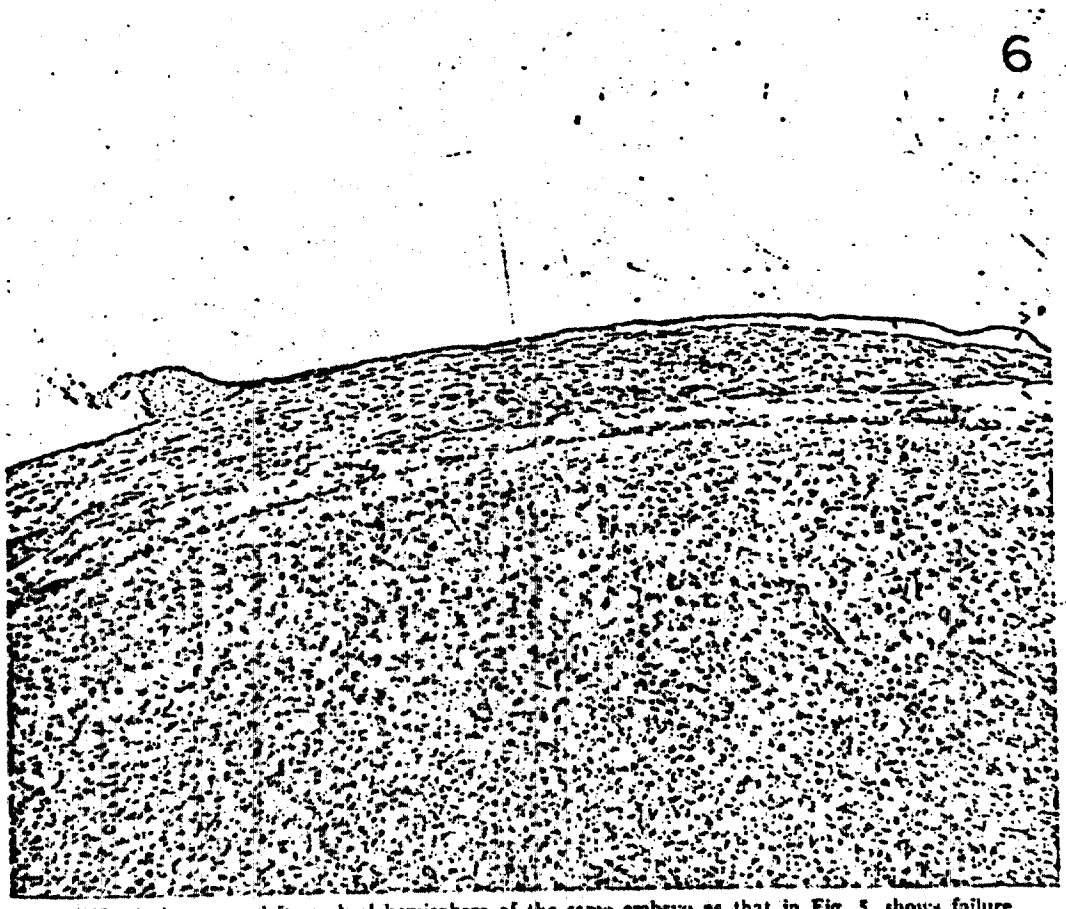


FIG. 6. Area over left cerebral hemisphere of the same embryo as that in Fig. 5, shows failure of normal development of skin and frontal bone and absence of feather follicles. Particles of carbon in epithelium and mesenchyme are too small to identify at this magnification. × 90.

orrhage and edema, but it seems possible that the foreign particles themselves could also interfere with development. Since it has been repeatedly demonstrated that interaction between tissues is necessary for normal development(8) the mechanisms involved in these defects may be quite complex.

Distribution of the carbon particles in tissues with resulting defects can probably be correlated with similar defects caused by other particulate materials and with thalidomide injected into the chick embryo under similar circumstances(1). There is, of course, no evidence that these phenomena can be related to thalidomide-induced defects in the human or with other birth defects. However, the severe defects observed with all the particulate materials and the ability of the carbon to pass from one tissue to another by means other than the circulation or transportation within cells, suggest that it might be worthwhile to consider whether foreign particles could, under naturally occurring conditions, gain access to embryonic tissues. Whether chemical compounds may change under *in vivo* conditions to become deposited in crystalline form in embryonic tissues is not known. During investigations of the mechanisms of teratogenic action, it seems reasonable to consider this possibility along with studies of biological activities involved.

Summary. Silica, which is known to have certain toxic properties, was injected in colloidal suspension of minute particle size into

the amniotic cavity of chick embryos. It produced defects very similar to those previously observed with the highly nontoxic colloidal alumina. Carbon particles also produced striking defects of the same types as those produced by thalidomide and other noncolloidal but insoluble compounds which have been studied previously. Microscopic studies of tissues from chick embryos inoculated with carbon particles indicated that the particles entered the integumental ectoderm where they caused severe necrosis and sloughing of cells as well as marked localized hyperplasia. Following this the carbon entered the mesoderm, either through the denuded areas or, as observed in many instances, by passing through the ectoderm into the subjacent mesoderm. In the mesoderm the particles became located at progressively deeper levels, in some cases penetrating cartilagenous structures. Studies of the distribution of the carbon within the embryonic tissues indicated that defects occurred in structures developing in or near tissues in which the particles were located. Continual destruction of surface epithelium of the cephalic region which plays an important role in the development of skin, feather follicles, eyelids, nictitating membrane and cornea, resulted in defective development of these structures. When carbon was present in areas of the mesoderm from which bone or cartilage developed these structures were retarded or abnormal. Since the greater amount of carbon accumulated in the cephalic region (due to the tendency of carbon aggregates to gravitate toward this region) the frontal and squamosal bones and the sclerotic cartilages were chiefly affected.

1. Williamson, A. P., Blattner, R. J., Lutz, H., Proc. Soc. Exp. Biol. and Med., 1963, v112, 102[illegible]
2. Allison, A. C., Harington, J. S., Birbeck, M., J. Exp. Med., 1966, v124, 141.
3. Nash, T., Allison, A. C., Harington, J. S., Nature, 1966, v210, 259.
4. Hinsch, G. W., Hamilton, H. L., Anat. Rec., 1956, v125, 225.
5. Heine, F., Roux. Arch. EntwMech. Organ, 1936, v134, 283.
6. Dabrowska, K., Bull. Int. Acad. Cracovie, (Acad. Pol. Sci.), 1950, Ser. B, 341.
7. Grabowski, C. T., J. Exp. Zool., 1956, v157, 307.
8. Ebert, J. D., Interacting Systems in Development, Holt, Rinehart and Winston, Inc., 1965.

Received October 27, 1966. P.S.E.B.M., 1967, v124.

HUMAN PATHOLOGY—VOLUME 1, NUMBER 2 *June 1970*

FATAL TALC EMBOLISM IN A DRUG ADDICT

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The syndrome of embolic pulmonary hypertension and acute cor pulmonale following the intravenous use of various drugs, compounded for oral therapy, is now well established.[1-7] The pulmonary findings are well known to experts in forensic pathology and perhaps to pathologists in large metropolitan municipal hospitals, but are likely unfamiliar to most other pathologists.

We recently performed a necropsy on a 31 year old female who had been a heroin addict since the age of 16. She was brought to the Emergency Department by police ambu-

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la ... acute respiratory failure, expiring ... afterward. There were numerous needle puncture marks over the long veins of her arms and legs. The lungs were heavy (combined weight 1800 gm.) and stiff and did not collapse when removed from the chest. Multiple hard white nodules, 0.5 to 3 mm. in diameter, were scattered evenly throughout the lung parenchyma (Fig. 1) and in areas became confluent to form larger masses. The heart weighed 290 gm., and there was dilatation and slight hypertrophy of the right ventricle. Microscopically, the lung showed numerous talc particles, easily identified by polarized light. These were present both in the pulmonary arteries and in the immediately surrounding interstitial tissues and caused foreign body granulomas (Figs. 2, 3). There was thickening of the walls of the pulmonary arteries together with formation of angiomatoid lesions. Organizing thrombi related to talc particles were present as well as marked intimal thickening, often containing talc crystals.

Interestingly, similar particles were found in other tissues, notably liver, spleen, and kidneys. In these tissues the particles were present in small arteries or arterioles and had again elicited foreign body granulomatous ...ions. In the kidneys these lesions were ...panied by small focal areas of cortical ...hy. This suggests that some talc had escaped from the lung filter and caused emboli in the systemic circulation. The lesions are similar to naturally occurring nephrosclerosis[8] and closely resemble those seen in experimental microembolic renal ischemia, caused by platelet aggregate emboli.[9]

Figure 2. Occlusion and recanalization of a medium size pulmonary artery. Note the needle-like talc particles with giant cell reaction. (Hematoxylin and eosin stain. × 80.)

Figure 1. Gross appearance of lung showing multiple small granulomas. (× 1, approximately.)

It has long been known that massive pulmonary fibrosis can result from inhalation of talc (magnesium silicate),[10] but the effects of intravenous administration of talc have been recognized only recently. Talc is introduced into the blood of "main liners" when they crush tablets containing talc and inject the suspension intravenously.[11, 12]

The syndrome has been described in addicts to "blue velvet" (paregoric and tripelennamine hydrochloride tablets).[2–5] "Red devil" addiction consists of injecting the contents of Seconal (secobarbital) capsules intravenously. Starch spherules from the secobarbital capsules lodge in the pulmonary vessels.[2, 4, 5] In other instances the drug may be "cut" by unscrupulous dealers and the diluent is often talc. We do not know the exact source of the talc emboli in our case, but suspect that

Figure 3. Numerous talc particles with foreign body giant cells are seen in and around a small blood vessel. (Hematoxylin and eosin stain. × 80.)

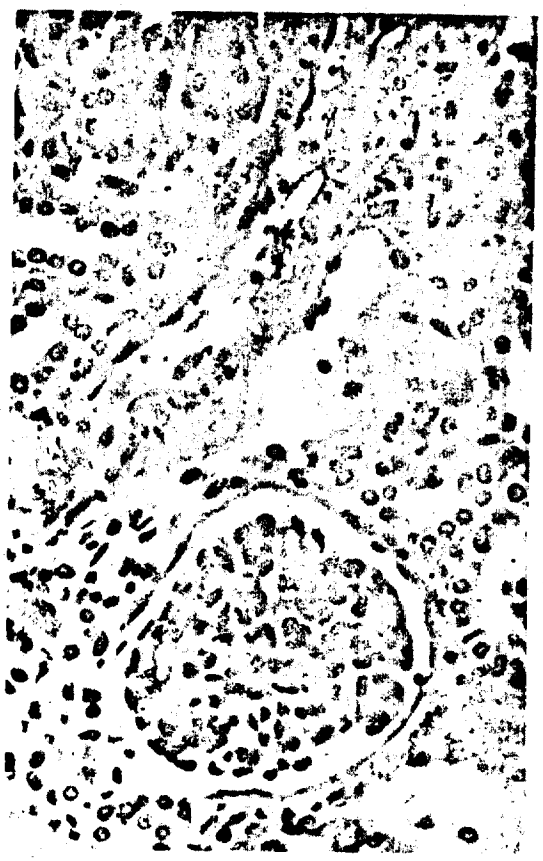

Figure 4. Talc is seen in the intima of an intertubular arteriole and in a glomerulus. (Polarized light. Hematoxylin and eosin stain. × 100.)

it was derived from methadone tablets, injected in suspension intravenously.

References

1. Krainer, L., Berman, E., and Wishnick, S. D.: Parenteral talcum granulomatosis: A complication of narcotic addiction. Lab. Invest., *11*:671, 1962.
2. Wendt, V. E., Puro, H. E., Shapiro, J., Mathews, W., and Wolf, P. L.: Angiothrombotic pulmonary hypertension in addicts. J.A.M.A., *188*:755-757, 1964.
3. Burton, J. F., Zawadzki, E. S., Wetherell, H. R., and Moy, T. W.: Mainliners and blue velvet. Forensic Sci., *10*:466-472, 1965.
4. Puro, H., Long, R., Vazquez, J., and Wolf, P. L.: Production of the human "blue velvet" and "red devil" addict lesions in rabbits. Amer. J. Path., *44*:571, 1965.
5. Puro, H., Wolf, P. L., Skirgavdas, J., and Vazquez, J.: Experimental production of human "blue velvet" and "red devil" lesions. J.A.M.A., *197*:1100-1102, 1966.
6. Lerner, A. M., and Oerther, F. J.: Characteristics and sequelae of paregoric abuse. Ann. Int. Med., *10*:1019, 1966.
7. Dole, V. P., and Nyswander, M. E.: The use of methadone for narcotic blockade. Brit. J. Addict., *63*:55-57, 1968.
8. Moore, S.: The relation of superficial cortical scars of the kidney to aortic arteriosclerosis: Hypothesis of renal ischemia. J. Path. Bact., *88*:471-478, 1964.
9. Moore, S., and Mersereau, W. A.: Microembolic renal ischemia, hypertension and nephrosclerosis. Arch. Path., *85*:623-630, 1968.
10. Hunt, A. C.: Massive pulmonary fibrosis from the inhalation of talc. Thorax, *11*:287-291, 1956.
11. Helpern, M., and Yong-Myun, R.: Death from narcotism in New York City. New York J. Med., *11*:2391-2408, 1966.
12. Sapira, J. D.: The narcotic addict as a medical patient. Amer. J. Med., *45*:555-588, 1968.

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