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SILICATES VOL III #56

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VOLUME 3

GRAS MONOGRAPH SERIES

SILICATES

(COPIES OF ARTICLES CITED BUT NOT USED IN MONOGRAPH SUMMARY)

prepared for

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

SEPTEMBER 28, 1973

prepared by

Tracor Jitco, Inc.

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LEWIS, W. H. (1931): *Bull. Johns Hopk. Hosp.* **49**, 17.
METCHNIKOFF, E. (1893): *Lectures on Comparative Pathology of Inflammation;* trans. by F. A. and E. H. Sterling. London: Kegan Paul, French and Trubner.
MUNRO, T. R. (1968): *Exp. Cell Res.* **52**, 392.
STRAUS, W. (1963): *Ciba Foundation Symposium*, p. 151. Ed. A. V. S. de Reuck and Margaret P. Cameron. London: J. & A. Churchill.
THOMAS, L. (1956): *J. exp. Med.* **104**, 245.

EFFECTS OF PARTICLES ON LYSOSOMES*

Dr ANTHONY ALLISON *Clinical Research Centre, Harrow*

IN THE nineteenth century, when advances in microscopy made possible the study of pathology at the cellular level, it became clear that changes in diseased tissue depend on changes in the growth and function of cells. In recent years the electron microscope and the centrifuge have made for increasingly effective investigations at the subcellular level. The aim of the cell biologist is to identify the enzymes that catalyse cellular reactions and find out in what organelles of the cell each reaction takes place. A specific task for the pathologist is to identify and localize the primary error in function that may lead to a secondary disorder in other organelles and systems; in other words, to learn how disease processes may begin with a specific malfunction in an organelle. In certain disorders, for example, the primary damage is to the energy-producing reaction in the mitochondria; in others it is to the protein-synthesizing apparatus of the ribosomes.

When lysosomes were first recognized as distinct organelles, it was clear that they would be of special interest in subcellular pathology. They can digest things that enter the cell; they can also break down part or all of the cell itself or tissues outside the cell. It now appears that in doing so they are implicated in the development and the death of tissues, in diseases such as silicosis and gout, in the immune process and thus perhaps in autoimmune disease and in many other physiological and pathological reactions.

The simplest and the most obvious role of a packet of digestive enzymes within a cell is in necrosis and autolysis, the death and self-dissolution of tissues. The membrane of the primary lysosome simply dissolves, liberating the enzymes to consume the cell. This can follow wounding or other damage to tissue or it can take place naturally in the course of development: when the corpus luteum of the ovary degenerates, for instance, or when a tadpole loses its tail. Any time a tissue or an organ is isolated from its supply of oxygen or nutrients under sterile conditions it breaks down rapidly; the large molecules such as proteins, lipids, nucleic acids and carbohydrates are digested, and there is evidence that enzymes from lysosomes play a part in the process. Rudolf Weber of the University of Berne (see Weber, 1969) found that the concentration of lysosomal enzymes in the tail of an amphibian increases before metamorphosis, and the concentration of these enzymes not consumed in the process of tissue digestion increases as the tail is resorbed.

Soon after lysosomes were first described it was suggested that the release of their enzymes into the cytoplasm or outside the cell might be the primary event that accounted for many other and different types of tissue damage. More detailed scrutiny showed that biochemical changes in other organelles sometimes preceded those that could be demonstrated in lysosomes; the conviction grew that lysosomal release was a secondary effect. It now seems that both situations can prevail. In some cases it is virtually certain that the primary event is an increase in the permeability of the lysosomal membrane, with the consequent release of hydro-

* Text of paper originally entitled *The role of phagocytosis in disease* delivered to Section D (Zoology) on 3 September 1970 at the Durham Meeting of the British Association.

lytic enzymes. In others the lysosomal changes may indeed be secondary to reactions in other systems.

Effects of silica particles on cells

Foreign particles, taken into the human body by inhalation, are usually innocuous, like the carbon particles that remain in alveolar macrophages more or less indefinitely. However, certain particles, such as those of several different crystalline forms of silicon dioxide or of asbestos, stimulate a severe fibrogenic reaction. It is generally accepted that the initial event in silicosis is the phagocytosis of silica particles by alveolar macrophages and consequent death of the cells. The particles so released are taken up by other macrophages which are in turn killed. In this way death of macrophages continues and stimulates collagen synthesis by fibroblasts in the neighbourhood. Analysis of pathogenesis must therefore proceed in two stages: first determining how silica particles kill macrophages and, second, determining how this is related to fibrogenesis.

As Marks (1957) showed, the cytotoxic effects of silica can be conveniently reproduced in cultures of peritoneal or alveolar macrophages, and the relative toxicity of different forms of silica, and of different dusts, on cell cultures agrees with the pathogenicity and fibrogenic activities of the dusts *in vivo* (Marks and Nagelschmidt, 1959; Vigliani, Pernis and Monaco, 1961). When my colleagues (T. Nash, J. Harington and M. Birbeck) and I began working on this problem in 1964, there was no satisfactory explanation of silica toxicity. King in his well-known 'solubility' theory suggested that silicic acid liberated into the tissues from silica particles brings about deposition of collagen. Later observations did not support this interpretation, as King (1947) himself pointed out. Curran and Rowsell (1958) 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. Vigliani and Pernis (1963) formulated an autoimmune theory of silicosis, but several workers were unable to obtain experimental evidence in support of this interpretation.

We therefore made a detailed study of the effects of toxic and non-toxic particles on cultures of macrophages, using time-lapse phase-contrast cine-micrography, histochemistry and electron microscopy (Allison, Harington and Birbeck, 1966). Particles of silica, diamond dust and other materials were rapidly included in phagosomes surrounded by single membranes. Lysosomes became attached to the phagosomes and discharged their enzymes into the phagosomes. So far there was no difference between the toxic and non-toxic particles. After about 18 hours' incubation however, clear differences were apparent. The non-toxic particles and associated enzymes were still enclosed in secondary lysosomes whereas many of the toxic particles and associated lysosomal enzymes had escaped into the cytoplasm. The macrophages that had ingested non-toxic particles were fully extended and moving about freely, whereas many of those exposed to toxic particles were rounded and immobile. Thus it was evident that silica particles, unlike non-toxic particles, can react with lysosomal membranes and make them permeable.

This appears to be a relatively non-specific reaction of silica with a variety of biological membrane systems. The simplest demonstration is provided by mixing washed erythrocytes with suspensions of silica particles or with silicic acid preparations (Stalder and Stöber, 1965; Nash, Allison and Harington, 1966). The erythrocytes are quite rapidly lysed by several fibrogenic forms of crystalline silica, whereas one form which is not fibrogenic (stishovite), and several other types of non-fibrogenic dust of comparable size and surface area, produce very little haemolysis. We have presented reasons (Nash *et al.*, 1966) for believing that the toxicity of silica is due to the fact that the particles are easily ingested and by interation with water form on their surface silicic acid which can act as a powerful hydrogen bonding agent.

There are two classes of hydrogen bonding compounds. The larger class comprises hydrogen acceptors such as ethers and ketones with active lone-pair electrons on oxygen or nitrogen. The smaller class comprises hydrogen donors of which only the phenols are important among organic compounds and silicic and some other very weak acids among inorganic compounds. Compounds of one class interact with those of the other, so that it is not surprising that there is one class (the former) the members of which are compatible in living

lls, sometimes in very high concentrations, ereas those of the other class are damaging.

Model experiments showed that hydrogen nding of phenolic hydroxyl groups, of the type esent in silicic acid, occurs with secondary ide groups of proteins, and this can lead to otein denaturation. However, the interaction th phospholipids is stronger, and we have esented evidence that this is more important interactions with biological membrane systems. vidence in support of the interpretation that ydrogen bonding is important in silica comes om experiments with poly-2-vinylpyridine-*N*-ide (PPNO). Schlipköter, Dolgner and Brockus (1963) found that this substance markedly duces the amount of fibrous tissue formed after travenous injection of silica. The toxic effects of lica on cultures of macrophages are also diminshed in the presence of or after exposure to PNO. We have shown (Allison *et al.*, 1966) that PNO is taken up into lysosomes in much the same ay as dextran, polyvinylpyrrollidine and other olymers (see de Duve and Wattiaux, 1966). However, PPNO is unique in having oxygen atoms hich readily form hydrogen bonds with phenolic ydroxyl groups. Thus PPNO can preferentially nteract with silicic acid on the surface of silica articles before the latter can attack lysosomal embranes. Mr Nash and I synthesized and ested another compound, polyvinylpyridinioacetic acid, which has even greater hydrogen bonding capacity than PPNO and very efficiently protects macrophages from silica toxicity.

These two facts are sufficient to explain why silica is so toxic to macrophages: the particles are taken up into lysosomes and readily damage lysosomal membranes through hydrogen bonding interactions. Various secondary reactions may occur. Thus, Munder, Modolell, Ferber and Fischer (1966) have found a considerable increase in the concentration of lysolecithin, as compared with lecithin, in macrophages damaged by quartz. This could follow activation of the enzyme phospholipase A, which catalyses the reaction lecithin → lysolecithin and which is known to be lysosomal. However, the fact that silica lyses erythrocytes (membranes of which do not contain demonstrable amounts of phospholipase A) shows that this process is unnecessary for interaction of silica with membrane systems, although the formation of surface-active lysolecithin would well accelerate damage induced by silica in macrophages. Suspensions of silica particles readily release enzymes from isolated liver lysosomes *in vitro*, as Stalder's experiments and our own have shown; the relatively low temperature coefficient for this release suggests that physico-chemical rather than enzymic reactions are involved.

Gout and pseudo-gout

Faires and McCarthy (1961) and Seegmiller, Howell and Malawista (1962) produced attacks of gout by injecting microcrystalline monosodium urate into gouty patients and non-gouty volunteers. The crystals are taken up by phagocytic cells, chiefly polymorphs, and the consequent inflammation seems to play an important part in the acute exacerbations of gout. Similar amounts of sodium urate in solution do not damage phagocytic cells or produce inflammation, and it seems that in the case of both silica and urate having the offending material in particulate form ensures its uptake by phagocytic cells and concentration in a vulnerable region, the lysosome.

Colchicine inhibits migration, particle ingestion and degranulation by polymorphonuclear leukocytes, and this may account for its well-known therapeutic effect in gout.

Calcium pyrophosphate dihydrate (CPPD) crystals are sometimes found in joints in association with sodium urate, sometimes alone. Their discovery accounts for the disease pseudo-gout, which can also bring about severe cartilage erosion.

Asbestosis

Inhalation of asbestos particles can produce a fibrogenic reaction (asbestosis). The basic mechanism appears to be similar to that of silicosis, although the association of groups of cells with long asbestos fibres and generation of iron-containing inclusions (ferritin) is characteristic of asbestosis. Small particles of certain types of asbestos (for example, crocidolite, or blue asbestos) also cause tumours of the lung, especially masotheliomas, tumours of the cell layer lining the pleural cavity. This is an important occupational hazard, which is now carefully watched.

Thus study of the effects of particles on lysosomes has been of academic interest and provided information about several types of human disease.

References

ALLISON, A. C., HARINGTON, J. S. and BIRBECK, M. (1966): *J. exp. Med.* **124**, 141.

CURRAN, R. C. and ROWSELL, E. V. (1958): *J. Path. Bact.* **76**, 561.

DE DUVE, C. and WATTIAUX, E. (1966): *Ann. Rev. Physiol.* **28**, 435.

FAIRES, J. S. and MCCARTHY, D. J. (1961): *Clin. Res.* **9**, 329.

KING, E. J. (1947): *Occupational Med.* **4**, 26.

MARKS, J. (1957): *Br. J. ind. Med.* **14**, 81.

MARKS, J. and NAGELSCHMIDT, G. (1959): *A.M.A. Archs ind. Hlth*, **20**, 383.

MUNDER, P. G., MODOLELL, M., FERBER, E. and FISCHER, H. (1966): *Biochem. Z.* **344**, 310.

NASH, T., ALLISON, A. C. and HARINGTON, J. S. (1966): *Nature, Lond.* **210**, 259.

SCHLIPKÖTER, H. W., DOLGNER, R. and BROCKHAUS, A. (1963): *German Med. Monthly*, **8**, 509.

SEEGMILLER, J. G., HOWELL, R. R. and MALAVISTA, S. E. (1962): *J. Am. med. Ass.* **180**, 469.

STALDER, K. and STÖBER, W. (1965): *Nature, Lond.* **207**, 874.

VIGLIANI, E. C. and PERNIS, B. (1963): *Adv. Res.* **12**, 230.

VIGLIANI, E. C., PERNIS, B. and MONACO, L. (1961) In *Inhaled Particles and Vapours*, p. 348. Ed. C. N. Davies. London: Pergamon Press.

WEBER, R. (1969): In *Lysosomes in Biology and Pathology*, vol. II, p. 437. Ed. J. T. Dingle and H. Fell. Amsterdam: North Holland Publishing Co.

THE ROLE OF LYSOSOMES IN ARTHRITIS*

Dr A. J. BARRETT *Strangeways Research Laboratory, Cambridge*

THE EXPERIMENTAL WORK to be described here was done in collaboration with Drs John Dingle, Peter Weston and recently Robin Poole.

In a healthy joint such as the knee joint, the ends of the long bones that move against each other are covered by smooth, resilient layers of *articular cartilage*. The joint space is limited by the *synovial membrane*, and filled with *synovial fluid* which bathes the articulating surfaces. The highly specialized structure of the cartilaginous surfaces allows movement to occur with astonishingly little frictional resistance. In rheumatoid arthritis, however, the synovium first becomes inflamed and later the articular cartilage degenerates so that movement becomes difficult and painful. The basic cause of the primary inflammation is not yet known; some workers believe that there is an immunological reaction in which the body comes to treat the joint tissue as if it were 'foreign', and to reject it, while others have evidence of infection by various micro-organisms. It may be that both infection and immunological abnormalities play their part. Equally, it is not known whether the degeneration of the cartilage matrix is mediated by the cells of the inflamed synovial membrane, or by those of the cartilage itself. The aspect of the disease with which our group is particularly concerned is this degradation of the cartilage, which plays a key role in the loss of joint function.

Cartilage is composed of specialized cells embedded in an extracellular matrix which they synthesize around themselves. The matrix is composed of *collagen*, a protein the fibrils of which contribute to the tensile strength of connective tissues generally, and *proteoglycan* molecules, which are built up from both protein and polysaccharide units, and are sometimes referred to as the protein-polysaccharide complex. The proteoglycan has the property of holding much water, so that cartilage is a very hydrated tissue. Cartilage is far from being metabolically inert: the cells are continually breaking down and replacing the matrix surrounding them, so that the half-life of a proteoglycan molecule is only of the order of one to two weeks.

By the use of specific staining methods, as well as by chemical means, it can be shown that the diseased cartilage in an arthritic joint is deficient in proteoglycan, but not in collagen. The poly-

* Text of a paper delivered to Section D (Zoology) on 3 September 1970 at the Durham Meeting of the British Association.

§ 121.1087 Ethyl cellulose.

The food additive ethyl cellulose may be safely used in food in accordance with the following prescribed conditions:

(a) The food additive is a cellulose ether containing ethoxy (OC_2H_5) groups attached by an ether linkage and containing on an anhydrous basis not more than 2.60 ethoxy groups per anhydroglucose unit.

(b) It is used or intended for use as follows:

(1) As a binder and filler in dry vitamin preparations.

(2) As a component of protective coatings for vitamin and mineral tablets.

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: May 21, 1962.

JOHN L. HARVEY,
Deputy Commissioner of Food and Drugs.

[F.R. Doc. 62-5075; Filed, May 24, 1962; 8:49 a.m.]

PART 121—FOOD ADDITIVES

Subpart D—Food Additives Permitted in Food for Human Consumption

SILICON DIOXIDE

The Commissioner of Food and Drugs, having evaluated the data submitted in petitions filed by Davison Chemical Co., Division of W. R. Grace and Co., Baltimore 3, Maryland, Monsanto Chemical Company, 800 North Lindbergh Boulevard, St. Louis 66, Missouri, and Pennsylvania Glass Sand Corporation, Hancock, West Virginia, and other relevant material, has concluded that the following amendment should issue to § 121.1058 of the food additive regulations for silicon dioxide in specified foods. 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), § 121.1058 (21 CFR 121.1058) is amended by changing the section heading, by providing for other methods of manufacturing silicon dioxide, and by providing for its use in other specified foods. As amended, § 121.1058 reads as follows:

§ 121.1058 Silicon dioxide.

The food additive silicon dioxide may be safely used in specified foods in accordance with the following conditions:

(a) The food additive is manufactured by vapor phase hydrolysis or by other means whereby the particle size is such as to accomplish the intended effect.

(b) It is used or intended for use in food as an anticaking agent as follows:

Food:	Limitations (percent)
Salt and seasoned salt	1
Sodium bicarbonate	1
Vitamin products	0.5
Special dietary products	0.75
Spices	2
Meat curing compounds	2
Flavoring powders	2

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: May 21, 1962.

JOHN L. HARVEY,
Deputy Commissioner of Food and Drugs.

[F.R. Doc. 62-5078; Filed, May 24, 1962; 8:50 a.m.]

SUBCHAPTER C—DRUGS

PART 141d—CHLORAMPHENICOL AND CHLORAMPHENICOL-CONTAINING DRUGS; TESTS AND METHODS OF ASSAY

Chloramphenicol-Paromomycin Ointment; Correction

Effective as of the date of signature of this order, paragraph (a)(1)(ii)(c) of § 141d.316 *Chloramphenicol-paromomycin ointment* is corrected by changing the beginning of the first formula and the second formula, respectively, to read as follows:

Hydrocortisone content (% weight/weight) =
Chloramphenicol content (% weight/weight) =

(Sec. 701(a), 52 Stat. 1055; 21 U.S.C. 371(a))

Dated: May 21, 1962.

JOHN L. HARVEY,
Deputy Commissioner of Food and Drugs.

[F.R. Doc. 62-5074; Filed, May 24, 1962; 8:49 a.m.]

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

Sampling Requirements; Setting of Effective Date

Pursuant to the provisions of the Federal Food, Drug, and Cosmetic Act (sec. 507, 59 Stat. 463, as amended; 21 U.S.C. 357) and in accordance with the authority delegated to the Commissioner of Food and Drugs by the Secretary of Health, Education, and Welfare (25 F.R. 8625), notice is hereby given that no comments were filed on the order published in the FEDERAL REGISTER of March 30, 1962 (27 F.R. 3006), with regard to sampling requirements of penicillin dental cones, suppositories, bougies, and implantation pellets. Accordingly, the amendments promulgated by that order became effective April 29, 1962.

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

Dated: May 21, 1962.

JOHN L. HARVEY,
Deputy Commissioner of Food and Drugs.

[F.R. Doc. 62-5080; Filed, May 24, 1962; 8:50 a.m.]

Title 32—NATIONAL DEFENSE

Chapter V—Department of the Army

SUBCHAPTER B—CLAIMS AND ACCOUNTS

PART 536—CLAIMS AGAINST THE UNITED STATES

Investigating and Processing of Claims

1. Subparagraph (2) of § 536.1(b) is revised to read as follows:

§ 536.1 Purpose and scope.

* * * * * * *

(b) *Scope.* * * *

(2) *Disaster claims.* Disaster claims and claims arising out of an incident involving an aircraft or missile under the control of the Army or the Army National Guard which are properly for settlement under either §§ 536.12–536.23, 536.29, 536.140–536.152, or § 536.26 will be investigated in accordance with standing operating procedures promulgated for that purpose by the commanding general of an Army or comparable command responsible for the area in which the disaster or aircraft or missile incident occurred. Such claims will be

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 C—Food Additives Permitted in Animal Feed or Animal-Feed Supplements

Subpart D—Food Additives Permitted in Food for Human Consumption

CALCIUM SILICATE

The Commissioner of Food and Drugs, having evaluated data in a petition filed by Johns-Manville Products Corporation, 22 East Fortieth Street, New York 16, New York, and other relevant material, has concluded that regulations should issue to prescribe the use of calcium silicate in animal feed and in 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), new sections are added to the food additive regulations, as follows:

§ 121.250 Calcium silicate.

Calcium silicate, including synthetic calcium silicate, may be safely used as an anticaking agent in animal feed, provided that the amount of calcium silicate does not exceed 2 percent.

§ 121.1135 Calcium silicate.

Calcium silicate, including synthetic calcium silicate, may be safely used in food in accordance with the following prescribed conditions:

(a) It is used as an anticaking agent in food in an amount not in excess of that reasonably required to produce its intended effect, and subject to the following limitations:

(1) It is not to be used in infant foods and foods for special dietary use.

(2) It will not exceed 2 percent by weight of the food, except that it may be present up to 5 percent by weight of baking powder.

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 18, 1963.

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

[F.R. Doc. 63-7816; Filed, July 24, 1963; 8:52 a.m.]

Title 24—HOUSING AND HOUSING CREDIT

Chapter II—Federal Housing Administration, Housing and Home Finance Agency

SUBCHAPTER G—HOUSING FOR MODERATE INCOME AND DISPLACED FAMILIES

PART 221—LOW COST AND MODERATE INCOME MORTGAGE INSURANCE

Subpart C—Eligibility Requirements—Moderate Income Projects

Section 221.520 is amended to read as follows:

§ 221.520 Covenant against liens.

(a) The mortgage shall, except as otherwise provided in this section, contain a covenant against the creation by the mortgagor of liens against the property superior or inferior to the lien of the mortgage.

(b) The provisions of paragraph (a) of this section shall not apply where a lien inferior to the lien of the insured mortgage is given in favor of an agency of the United States under such circumstances as may be approved by the Commissioner.

(Sec. 221, 52 Stat. 23; 12 U.S.C. 1715b. Interprets or applies sec. 221, 68 Stat. 590, as amended; 12 U.S.C. 1715l)

Issued at Washington, D.C., July 19, 1963.

PHILIP N. BROWNSTEIN,
Federal Housing Commissioner.

[F.R. Doc. 63-7824; Filed, July 24, 1963; 8:56 a.m.]

Title 28—JUDICIAL ADMINISTRATION

Chapter I—Department of Justice

[Order No. 299-63]

PART 0—ORGANIZATION OF THE DEPARTMENT OF JUSTICE

Assignment of Functions Relating to President's Committee on Equal Employment Opportunity

By virtue of the authority vested in me by Executive Order No. 10925 of March 6, 1961, as amended, and as Attorney General of the United States: *It is hereby ordered, as follows:*

1. Pursuant to section 102(c) of Executive Order No. 10925, I hereby designate John E. Nolan, Jr., Assistant to the Attorney General, as the alternate for the Attorney General on the President's Committee on Equal Employment Opportunity.

2. Subpart O of Part 0 (relating to the organization of the Department of Justice) of Title 28 of the Code of Federal Regulations is hereby amended by redesignating § 0.83 as § 0.84 and by inserting a new § 0.83 as follows:

§ 0.83 Principal Compliance Officer.

Pursuant to section 307 of Executive Order No. 10925 of March 6, 1961, relating to the President's Committee on Equal Employment Opportunity, the Administrative Assistant Attorney General is designated as the Principal Compliance Officer for the Department of Justice, and he is authorized to designate, from time to time, such Deputy Compliance Officers as he may deem to be necessary and appropriate to carry out the provisions of that order.

3. Order No. 242-61 and Order No 268-62 are hereby superseded.

Dated: July 19, 1963.

ROBERT F. KENNEDY,
Attorney General.

[F.R. Doc. 63-7822; Filed, July 24, 1963; 8:53 a.m.]

Title 32—NATIONAL DEFENSE

Chapter VI—Department of the Navy

SUBCHAPTER E—CLAIMS

PART 750—NAVY GENERAL CLAIMS

PART 753—NAVY FOREIGN CLAIMS

Miscellaneous Amendments

Scope and purpose. The amendments deal with the processing of certain types of claims against and in favor of the United States and are intended to update the regulations in accordance with recent Department of Defense notices (28 F.R. 6753 and 7040). Corresponding changes to the Manual of the Judge Advocate General will be distributed to holders of that manual in due course.

1. Section 750.48(a) is revised to read as follows:

§ 750.48 Single service assignment of responsibility for processing of claims.

(a) *List of countries.* The Secretary of Defense (Department of Defense notice, 28 F.R. 6753) has assigned responsibility for the processing of all claims in favor of the United States, or against the United States and cognizable under:

(1) The Foreign Claims Act (10 U.S.C. 2734),

(2) The Military Claims Act (10 U.S.C. 2733), and

(3) 10 U.S.C. 2734a–2734b (formerly the Act of August 31, 1953 (68 Stat. 1006, 31 U.S.C. 224i–2 to 224i–5),

which arise in the following countries to the military departments indicated below in this section:

I. Department of the Army: Belgium, Ethiopia, France, the Federal Republic of Ger-

74 Stat. 404; 21 U.S.C. 376, note), and delegated by the Secretary to the Commissioner of Food and Drugs (21 CFR 2.120; 31 F.R. 3008), § 8.501 *Provisional lists of color additives* is amended in the following respects:

1. Paragraphs (a), (b), and (c) are amended by changing the closing dates of all of the items therein to July 1, 1967.

2. Paragraph (e) is amended by deleting the following items:

Beet juice.
Iron oxides.
Paprika and paprika oleoresin.
Saffron (*Crocus sativus L.*) and its oleoresin.
Turmeric and curcumin.

In order to allow orderly withdrawal from the market of iron oxide and in the absence of information that the continued use of this color additive will adversely affect the health of animals, the Food and Drug Administration will not institute regulatory action against the color additive or the articles in which it has been permitted to be used solely for the reason that it is not provisionally or permanently listed as a color additive for the period ending December 31, 1966.

Notice and public procedure and delayed effective date are unnecessary prerequisites to the promulgation of this order, and I so find, since section 203 (a)(2) of Public Law 86-618 provides for this issuance.

Effective date. This order shall become effective on the date of signature.

(Sec. 203(a)(2), Public Law 86-618, 74 Stat. 404; 21 U.S.C. 376, note)

Dated: June 24, 1966.

JAMES L. GODDARD,
Commissioner of Food and Drugs.

[F.R. Doc. 66-7287; Filed, July 1, 1966; 8:47 a.m.]

SUBCHAPTER B—FOOD AND FOOD PRODUCTS

PART 121—FOOD ADDITIVES

Subpart D—Food Additives Permitted in Food for Human Consumption

SILICON DIOXIDE

The Commissioner of Food and Drugs, having evaluated data in a petition (FAP 6A1958) filed by W. R. Grace & Co., Davison Chemical Division, Baltimore, Md. 21203, and other relevant material, has concluded that the food additive regulations should be amended to provide for wider use of silicon dioxide as an anticaking agent in food consistent with accomplishment of the intended physical effect and in accord with good manufacturing practice.

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 (21 CFR 2.120; 31 F.R. 3008), § 121.1058 is amended by revising the introduction to the section and paragraph (b) to read as follows:

§ 121.1058 Silicon dioxide.

The food additive silicon dioxide may be safely used in food in accordance with the following conditions:

* * * * *

(b) It is used as an anticaking agent, subject to the following conditions:

(1) It is used in only those foods in which the additive has been demonstrated to have an anticaking effect.

(2) It is used in an amount not in excess of that reasonably required to produce its intended effect.

(3) It is not to be used in infant foods except when present as an anticaking agent at a level not to exceed 2 percent in salt and salt substitutes used as components of these foods.

(4) It is used in an amount not to exceed 2 percent by weight of the food.

* * * * *

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, D.C. 20201, written objections thereto, preferably in quintuplicate. 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.

Effective date. This order shall become 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: June 27, 1966.

J. K. KIRK,
Assistant Commissioner for Operations.

[F.R. Doc. 66-7288; Filed, July 1, 1966; 8:47 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 in a petition (FAP 6B1879) filed by Interchemical Corp., Finishes Division, 1255 Broad Street, Clifton, N.J. 07015, and other relevant material, has concluded that the food additive regulations should be amended to provide for the safe use of certain acrylic copolymers as modifiers for epoxy resins used in resinous and polymeric food-contact coatings. 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 (21 CFR 2.120; 31 F.R. 3008), § 121.2514(b)(3)(xx) is amended by inserting alphabetically in the list of acrylic polymers a new item as follows:

§ 121.2514 Resinous and polymeric coatings.

* * * * *

(b) * * *

(3) * * *

(xx) **Acrylics and their copolymers,** as the basic polymer:

* * * * *

2-Ethylhexyl acrylate-methyl methacrylate-acrylic acid copolymers for use only as modifiers for epoxy resins listed in subdivision (viii) of this subparagraph.

* * * * *

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, D.C. 20201, written objections thereto, preferably in quintuplicate. 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.

Effective date. This order shall become 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: June 24, 1966.

J. K. KIRK,
Assistant Commissioner for Operations.

[F.R. Doc. 66-7290; Filed, July 1, 1966; 8:48 a.m.]

PART 121—FOOD ADDITIVES

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

COMPONENTS OF PAPER AND PAPERBOARD

An order was published in the FEDERAL REGISTER of February 25, 1965 (30 F.R. 2430), amending certain food additive regulations with respect to food additives resulting from substances used

Ann. Med. exp. Fenn. 1964:42:132—135.

THE EFFECT OF INTRAVENOUS SILICA ON SERUM ELECTROPHORESIS IN DOG

by

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(Received for publication September 11, 1964)

Summary. The effect of injections of silica on serum proteins was studied in 26 dogs by determining the total protein and performing paper electrophoresis analyses during an observation period ranging from 1 month to 2 years and 9 months.

Albumin decreased and the β- and γ-globulin increased, but the changes did not show any correlation with the length of the observation period and the histologic hepatic changes. The changes established were considered to reflect silica-induced subacute liver damage which could be incipient liver cirrhosis or just a transient phenomenon.

The fundamental difficulty in the production of experimental cirrhosis is the reaction of liver to foreign agents. If the agents employed are too toxic the consequence may be necrosis of the liver leading rapidly to death. This may happen with carbon tetrachloride which has been used to induce cirrhosis of the liver in rats. A point deserving special attention is that different substances vary in their toxic effect on different animal species. On the other hand, the effect of the active agent may be transient and the liver may recover completely thanks to its capacity for regeneration. Chronic sequelae such as cirrhosis are thus rare, especially when the toxins in question are conjugated and detoxicated in the liver or cause hemolysis.

If chronic liver damage ensues the final picture of the histological changes are similar whatever the etiology of the cirrhosis in each individual case. A typical feature in the development of all types of cirrhosis is liver cell necrosis, nodular regeneration, proliferation of connective tissue and destruction of the lobular structure of the liver. Hence there is reason to organise experimental studies in which the agents used to produce cirrhosis are unlikely to cause cirrhosis of the liver in humans. To produce the portal hypertension which develops in connection with cirrhosis Rousselot and Thompson (6) and Volwiler *et al.* (8) injected silica suspension into the circulation of the dog. These experiments were based on the studies conducted by Gye and Purdy (4, 5) and Gardner and Cummings (1) on the hepatic fibrosis caused by parenterally administered colloid silica.

Silica cirrhosis is not generally associated with marked chronic portal hypertension (6, 7, 8) but is accompanied by typical histopathologic changes. Their development, however, requires a long latent period. Volwiler *et al.* (8) established from liver biopsies that during the first 10 months there were few or no signs of fibroblastic proliferation in dogs whereas rats developed extensive fibrosis in 6—12 months.

The process, once it has begun, is progressive and leads gradually to extensive changes in the liver cells, thus offering an opportunity for the observation of the chronic process.

As is well known, the relationship of the structural changes and functional disturbances is often not clear in cirrhosis of the liver.

If there is suffic. normally functioning liver tissue between the fibrotic islets the functional capacity of the liver may be surprisingly normal in spite of the morphologically far-advanced cirrhosis. It is possible that the factor that originally set the cirrhotic process in motion is of importance in this sense. The more diffuse the liver cell damage, the earlier the phase in which functional changes can be expected even if the histologic picture is not serious.

The aim of the present study was to compare paperelectrophoretically the changes in serum protein and the histologic changes caused by experimental silica cirrhosis.

MATERIAL AND METHODS

The material consisted of 26 adult mongrel dogs of both sexes. All the animals received a total of 7–10 g of silica suspension. This was made of powdered Quartz (Bayer) by mixing 3 g of sterilised powder in 100 cc of physiologic sodium chloride immediately before the intravenous injection.

The injections were administered at intervals of about 1 month and each dog received 3–4 injections in all.

Nineteen dogs were given the silica injections into the mesenterial vein in connection with laparotomy, seven dogs received corresponding doses into the vein of the paw. The narcosis was the same in both groups.

Injection of silica:

The dogs were given no food on the day of the injection. The pre-medication was 3–5 cc 3 per cent morphine hydrochloride intramuscularly, followed after some 30 min. by 2–4 cc of Diminal (Astra) intravenously.

The dogs which received the silicate suspension in the vein of the paw were given the injection immediately the anesthesia was sufficiently deep and the respiration was even, in about 15 min., by an ear irrigation syringe.

Laparotomy was performed through an upper median incision, biopsy specimens were taken from the liver and on the dogs which received the injection into the mesenterial vein, the silica suspension was given in the course of c. 15 min. The dogs whose respiration was superficial and irregular were intubated and given oxygen.

Control examinations were made during a period of 1 month to 2.5 years when in addition to the taking of blood samples laparotomy was performed for liver biopsy and to follow the portal pressure.

The control group consisted of seven dogs which were not given silica but treated with anastomosis between the gall bladder and the stomach and which otherwise lived in similar conditions.

Protein analyses:

Total protein was determined by the biuret method (2) and the method reported by Grassman and Hannig (3) was employed in the electrophoresis.

Seven dogs had a fraction between β and γ-globulin which was added up to γ-globulin. As the increase in β- and γ-globulin in liver damage is a concomitant phenomenon and they are difficult to distinguish from each other because of the broadening of the γ-globulin fraction, the β- and γ-globulin fractions were added up in the results and were not treated independently (9).

TABLE 1

	Total Protein	Albumin	α_1	α_2	$\beta + \gamma$	Albumin Globulin
Normal (58)	5.6	51.6	6.3	10.9	31.2	1.1
Dogs given silica (26)	5.8	43.7	6.5	11.8	39.0	0.76
Controls (7)	5.1	47.9	6.6	11.2	31.2	0.9

The means of total proteins (g %) and electrophoresis fractions (%) in the groups of normal dogs, dogs given silica and the controls. The number of dogs is given in brackets.

TABLE 2

No.	Total Protein	Albumin	α_1	α_2	$\beta + \gamma$	Time
4	+ 0.6	− 14.7	+ 6.2	+ 1.1	+ 11.2	3.5 months
8	+ 0.1	+ 9.0	+ 3.4	− 2.1	− 10.4	2 years 9 months
9	+ 0.3	− 10.4	+ 0.1	+ 4.2	+ 6.2	9 months
12	− 0.5	− 7.9	− 0.8	+ 3.4	+ 5.3	1 year 2.5 months
14		− 0.1	− 0.4	− 2.0	+ 2.7	5 months
20	− 1.5	− 4.6	+ 3.1	+ 3.9	+ 13.1	1 year 10 months
24	+ 0.2	− 20.3	− 9.8	− 0.1	+ 17.2	2 years 6 months
25	+ 1.1	− 9.8	+ 0.5	+ 2.7	+ 6.6	3 months
26	0	− 7.1	− 2.6	+ 2.2	+ 7.9	2.5 months
46	+ 0.3	− 1.0	− 0.4	− 0.4	+ 9.7	2.5 months
48		− 0.8	+ 2.9	+ 1.1	− 3.2	2 months
49	− 0.3	+ 2.2	− 0	0	− 1.3	1 month
50	− 1.6	− 8.5	− 3.9	+ 5.6	+ 8.8	2 months
51	− 0.5	+ 4.4	+ 0.8	− 5.0	+ 0.7	3.5 months
52	+ 0.6	− 2.0	+ 1.9	− 3.8	+ 3.6	10 months
53	+ 0.6	− 4.0	− 0.4	+ 2.1	+ 1.7	4 months
56	+ 1.0	− 7.7	+ 1.8	− 1.4	+ 7.2	3.5 months
58	− 0.5	− 10.4	− 3.1	+ 0.9	+ 13.4	3.5 months
60	+ 1.8	− 10.2	+ 2.1	− 1.8	+ 12.2	8 months
61	0	− 31.6	+ 0.6	+ 6.3	+ 21.7	9 months
63		− 5.9	− 1.7	+ 1.3	+ 5.5	10.5 months
64	+ 0.3	− 6.4	− 4.8	+ 4.2	+ 7.9	9 months
65	+ 0.4	− 23.9	+ 1.4	+ 4.0	+ 18.5	10 months
66	+ 0.6	− 20.9	+ 3.5	+ 2.8	+ 14.2	2 months
67	+ 0.1	− 7.2	+ 0.7	− 3.6	+ 10.0	2 months
68	+ 0.2	− 10.9	+ 4.5	0	+ 5.6	1.5 months
Mean	+ 0.2	− 8.1	+ 0.2	+ 0.9	+ 7.7	

Changes in the protein concentrations after injections of silica. + increase, − decrease compared with the initial value.

RESULTS

Table 1 shows the mean electrophoresis values for 58 normal dogs, 26 dogs given silica and 7 controls on which gall bladder to stomach anastomosis was performed. The silica group shows a decline in the albumin fraction and an increase in β- and γ-globulin.

TABLE 3

	Silica via Peripheral vein	Silica via Pportal vein	Controls
Observation period	13 months	6 months	18 months
Decrease in albumin	13 per cent	18 per cent	7 per cent
Increase in $\beta + \gamma$ globulin	16 per cent	28 per cent	

Average observation periods in the different groups, the mean decrease in the albumin concentration from the initial value and the increase in the $\beta + \gamma$-globulin in per cent of the initial value.

	T/		
	Hb	E	L
Before injection of silica	13.5 g	6.3 mill.	7500
After injection of silica	12.4 g	6.0 mill.	7100

Pre-injection blood values (means) for the dogs given silica and values an average of 9.5 months after the silica injections.

In Table 2 the changes in the per cent ratios of the protein fractions of the dogs given silica and the time in each case are given.

Table 3 shows the changes for the groups receiving silica via the peripheral vein and those receiving silica via the mesenteric vein, calculated separately, and the means of the observation periods. The changes were greater when silica was injected into the portal vein, area although the observation time was shorter by half.

Table 4 shows the slight falling tendency in the hemoglobin level and, to a smaller degree, in the erythrocyte count in the group receiving silica.

DISCUSSION

The changes in electrophoresis, as expected, were lowered albumin and elevated globulin values, especially in the β- and γ-fractions. They show that there is no distinct correlation between the changes and the period in which they developed. The changes that occurred in the first few months after the silica injections had actually disappeared after a year. These cases obviously involved a non-specific effect which was mild enough to disappear before any progressive liver change could originate.

A surprising finding was the poor correlation between the electrophoresis changes and the histologic changes. Three dogs (8, 21, 16) of the group receiving silica via the peripheral vein had far-advanced cirrhosis grossly and microscopically (7), but the protein changes were no greater than in the group given silica in the region of the portal vein where the histologic changes were fairly small on the whole and suggestive of a process in the very initial phase. As can be seen from Table 3, the protein changes were more marked in the group receiving silica in the

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portal tract than in the dogs injected via the vein of the paw although the observation period was well over twice as long in the latter group.

The poor correlation of the changes with time and the microscopic picture may mean that the process in question was the detrimental effect of a foreign agent, possibly transient. However, it is not really possible here to speak of protein changes caused by cirrhosis of the liver.

The fact that the group treated with gall bladder and stomach anastomosis and laparotomies but without silica failed to display a corresponding increase in the β- and γ-globulin fractions although there was a tendency towards a lowered albumin fraction argues in favour of the effect of silica and not of the operative procedures in general.

REFERENCES

1. Gardner, L. U., and Cummings, D. E.: The reaction to fine and medium sized quartz and aluminium oxide particles. Silicotic cirrhosis of the liver. Amer. J. Path. 1933:*9*:751–783.
2. Gornall, A. G., Bardawill, Ch. J., and David, M. M.: Determination of serum proteins by means of the biuret reaction. J. biol. Chem. 1949:*177*:751–766.
3. Grassmann, W., and Hannig, K.: Beitrage zur Methodik der Papierelektrophoretischen Serumanalyse. Klin. Wschr. 1954:*32*:838–871.
4. Gye, W. E., and Purdy, W. J.: The poisonous properties of colloidal silica. II. Brit. J. exp. Path. 1922:*3*:86–101.
5. Gye, W. E., and Purdy, W. J.: The poisonous properties of colloidal silica III. Ibid. 1924:*5*:238–250.
6. Rousselot, L. M., and Thompson, W. P.: Experimental production of congestive splenomegaly. Proc. Soc. exp. Biol. (N.Y.). 1939:*40*:705–714.
7. Seiro, V., Turunen, M., and Actio, L.: On the transposition of spleen into the retroperitoneal space in dog. To be published.
8. Volwiler, W., Grindlay, J. H., and Bollman, J. L.: Chronic portal vein obstruction in dogs from silica cirrhosis. Gastroenterology 1953:*24*:405–416.
9. Wuhrmann, F., and Märki, H. H.: Dysproteinämien und Paraproteinämien. Schaabe & Co Verl. Basel/Stuttgart 1963. p. 235.

Beamer, WM. D., M. H. F. Friedman and *Ph. T. Chu:* Exp. Med. Surg. *11*, 241—247, 1953.

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Influence of Cortisone on Formation of Intraperitoneal Adhesions

By WM. D. BEAMER, M. H. F. FRIEDMAN and PHILIP T. CHU

Introduction

In experimentally produced lesions of the skin, iris, and bone, cortisone has been reported to inhibit various tissue reactions to injury, including fibrin formation, leucocytic invasion, fibroblast proliferation, and granulation tissue formation (1, 2, 3, 4). The present study was undertaken to determine the influence of cortisone on the formation of intraperitoneal adhesions. Since termination of our experiments several other studies (5, 6, 7, 8) on abdominal adhesion formation have been reported but the conclusions drawn have been indefinite or contradictory.

In these experiments introduction of a foreign body into the abdominal cavity was used as a standard technique to induce formation of adhesions. Various sclerosing agents and also cellophane were employed but abandoned for technical reasons in favor of talc. The animals used were adult male and non-pregnant female mice of a known strain bred in this institution for the past twelve years. A standard amount of a sterile suspension of purified talc was introduced by hypodermic needle into the peritoneal cavity. Care was taken to keep the site of injection the same in all animals. The mice were sacrificed at given intervals after the talc injection and both microscopic and macroscopic examinations were made of the viscera.

Results

Control Series. The intraperitoneal injection of more than 65 mg. talc per mouse was not well tolerated and a dose of 85 mg. per animal proved fatal. Nearly all the animals receiving between 50 and 60 mg. showed adhesions. In order, however, to detect possible acceleratory as well as inhibitory effects of cortisone, the dose of talc was reduced to a level which induced adhesions in only 75 per cent of the animals

injected. By trial and error it was found that this incidence was attained in our mice by intraperitoneal injection of 30 to 35 mg. (as 0.35 ml. of a 10 per cent suspension).

By sacrificing two or three animals at varying intervals over a period of 3 weeks, we found in a series of 58 talc-injected mice that adhesions became evident macroscopically in about 12 hours after talc-injection and reached their maximum in numbers and extent within 5 days. Talc particles were found dispersed unevenly in the abdominal cavity. The talc was often seen to be encapsulated and even implanted in the tissues, especially the liver. Although adhesions were found only where there was a focal deposit of talc, it was evident that not all deposits of talc were associated with adhesions.

No gross evidence of spontaneous resorbtion of fibrous bands was obtained. From examination of 58 talc-injected mice it was found that once fully formed, adhesions remained essentially unaltered for the duration of the experiment (6 weeks).

The usual tissue reactions to a foreign body were observed microscopically. Within 24 hours an acute inflammatory reaction consisting of marked polymorphonuclear leucocytic infiltration was found. Dense aggregates of talc crystals were seen diffusely infiltrated by leucocytes of which many were necrotic. By the 48th hour giant cells, lymphocytes and monocytes were noted throughout the granulation tissue.

The granulomatous reactions reached maximum intensity by the end of the fourth day and persisted for at least as long as six weeks (the duration of observation). Well-developed discrete nodules of varying sizes were observed, composed of fibrous connective tissues as well as proliferating endothelial tissue and of talc crystals dispersed within the cytoplasm of giant cells (Fig. 1).

Cortisone Series. A total of 89 talc-injected mice were each given subcutaneously 0.5 mg. cortisone daily from the day of talc injection until they were sacrificed (Table 1). In a second experiment extending over a period of six weeks, an additional 13 mice were given cortisone daily only during the first 10 days and 13 others only during the last 10 days (32nd to 41st days inclusive). In a third experiment 8 mice were pretreated daily with cortisone for 14 days before talc injection and then daily for an additional 7 days.

In comparison with the control talc-injected mice, fewer of the cortisone-treated animals showed intraperitoneal adhesions during the early stages of observation (Table 1). This reduction in the number

Fig. 1. Showing foreign body reaction on 10th day following intraperitoneal injection of talc suspension.

and extent of adhesions, however, represented only a delay rather than a complete suppression of fibrous tissue response. Ultimately adhesions formed in spite of continued daily administration of cortisone. At the end of the sixth week there were no gross differences apparent between the animals of the cortisone and control series (Table 1).

Microscopic examination of the tissues supported the gross findings. The influence exerted by cortisone was negligible during the first 12 to 36 hours, at which time the cortisone-treated animals showed tissue reactions similar to those of the controls. The differences between the two series then rapidly became more apparent and by the fourth day were maximum. This condition was maintained essentially unchanged until about the fourteenth day after the talc injection. Due entirely to increase in adhesion formation in the cortisone-treated animals, the difference after the fourteenth day gradually became less prominent, and was no longer evident by the sixth week (Fig. 2).

In animals sacrificed between the fourth and tenth days, the talc was seen to be dispersed more evenly in the cortisone-treated

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Table 1

Influence of Cortisone on Formation of Intraperitoneal Adhesions Following Injections of Talc

(All mice received 35 mg. talc intraperitoneally. Cortisone dose was 0.5 mg. per day)

Treatment	Duration of Experiment, Days	Daily Cortisone Administration, Days	No. Mice Treated	No. Mice Showing Adhesions	Adhesion Occurrence Per Cent
Control	5	0	33	25	75.8
Cortisone	5	5	21	5	23.8
Control	12	0	44	30	68.2
Cortisone	12	12	15	2	13.3
Control	22	0	46	32	69.5
Cortisone	22	14	27	16	60.0
Control	29	0	34	26	76.5
Cortisone	29	22	12	9	75.0
Cortisone	32	29	14	10	71.4
Control	42	0	13	9	69.2
Cortisone	42	First 10 days	13	8	61.5
Cortisone	42	Last 10 days	13	8	61.5
Cortisone	21	14 days pretreatment + 7 days after talc	8	8	100

series than in the control series. Other than this, there was little difference noted in locale of distributions. During this period discrete nodules were found which contained very little fibrous tissue and no giant cells while polymorphonuclear leucocytic infiltration showed persistently.

About 14 days after the talc injections, large granulomatous nodules occupying the greater part of a low power field were found in the cortisone-treated animals. These contained varying amounts of talc crystals with a prominent fibrous tissue and endothelial proliferation. A moderate number of giant cells, most containing talc crystals within their cytoplasm, were noted. A few chronic inflammatory cells were dispersed throughout the granulation tissue but on the whole there were no considerable differences from the tissue reactions observed in the control group at a comparable period (Fig. 3).

In the mice treated with cortisone for the first ten days as well as those treated for the last ten days the gross and microscopic findings in the peritoneal cavity 6 weeks after talc injection were essentially similar to the findings in the control mice. Similarly, abdominal adhesions found in the mice pretreated with cortisone were numerous and dense in all 8 animals and indistinguishable from those of the control series.

Fig. 2. Reaction on 7th day after talc injection in animal receiving cortisone daily. Note mass of free-lying talc on serosa and only little inflammatory reaction.

Fig. 3. Reaction on 17th day after talc injection in animal receiving cortisone daily. Note intense foreign body reaction of similar degree to that shown in Fig. 1.

Discussion

The present study demonstrated that continued administration of cortisone delayed the onset but did not prevent the ultimate formation of adhesions induced in mice by introduction of talc into the peritoneal cavity. Several factors must be considered to explain our findings in view of the reports of other investigators that healing of skin and bone lesions (14) and other processes involving high mitotic activity (15) are suppressed by cortisone.

With few exceptions, the studies leading to the conclusion that cortisone inhibits fibrous tissue reaction covered only comparatively short periods of observations. A similar conclusion would have been drawn from our own experiments if attention had been paid only to the early stages of cortisone treatment. Whatever the mechanism of inhibition may have been, a cortisone-refractory state developed and the factors reponsible for the suppression of fibrous tissue reaction

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were no longer effective. Although only a small number of mice were used, the results of the experiment with cortisone pretreatment for 14 days also lead to the conclusion that tissues ultimately become refractory to cortisone.

It is probable that the duration of effective inhibition by cortisone varies with the species as well as with the type of tissue. Thus, in the rabbit cortisone had been found repeatedly to retard healing of a variety of lesions, but in the guinea-pig the identical lesions were unaffected by cortisone (12, 13). *Cole* et al. (16) in studies on experimental skin lesion in dogs noted a brief phase of retardation of healing shortly after cortisone administration. *Roberts, Florey* and *Toklik* (15) in their study on liver regeneration found in the second day after hepatectomy, when mitoses were normally frequent, that cortisone greatly reduced their number. At 6 days, however, the difference between control and cortisone-treated animals was no longer marked.

To what degree the suppression of healing found by other investigators may have been due to poor nutritional conditions is not known. A poor nutritional state, as evidenced by subnormal plasma protein values, has long been recognized to prolong healing time. *Findlay* and *Howes* (9) found that debility and inadequate intake of protein augmented in the rabbit the action of cortisone on wound healing. In our experiments both the control and cortisone treated mice were kept in a good nutritional state and the caloric intake in both series was comparable.

The dose of cortisone used in our experiments was only half the dose previously found (10) to be toxic in mice of our strain when given daily over a long period of time. This prevented us from determining whether the ultimate outcome of our experiments would have been influenced by large doses of cortisone. *Taylor* et al. (11) expressed the view that cortisone inhibited the healing of skin lesions in the rat only when given in amounts sufficiently toxic to lead to loss in body weight. The suppression of quartz adhesions by cortisone reported by *Magarey* and *Gough* (7) occurred in mice dying within 17 days as the direct result of intolerance to large (2.5 mg.) daily doses of the drug. *Roberts* et al. (15) also reported that mice did not tolerate 2.25 mg. cortisone per day for longer than 6 days.

Our experiments also showed that intraperitoneal adhesions did not tend towards spontaneous resolution. Furthermore, although cortisone was effective in delaying the onset of fibrous tissue reaction, once

the adhesions had formed cortisone had no influence in promoting their resolution.

Summary

The repeated administration of cortisone only delays and does not suppress the onset of formation of abdominal adhesions due to intraperitoneal injection of talc. Development of a cortisone-refractory state may account for the failure to inhibit the fibrous tissue reaction.

Zusammenfassung

Die wiederholte Verabreichung von Cortison verzögert nur die Bildung abdominaler Verwachsungen nach intraperitonealer Talkum-Injektion. Der Mangel einer Hemmung fibröser Gewebsreaktion mag mit der Ausbildung eines Cortison-refraktären Zustandes zusammenhängen.

Résumé

L'administration répétée de cortisone ralentit seulement mais n'empêche pas la formation d'adhérences abdominales après l'injection intra-péritonéale de talc. Le fait d'une absence d'inhibition d'une réaction tissulaire fibreuse dépend peut-être d'un état réfractaire à la cortisone.

References

1. *Ragan, C., E. L. Howes, C. M. Plotz, K. Meyer* and *J. W. Blunt:* Proc. Soc. exp. Biol., N. Y. *72*, 718, 1949.
2. *Shapiro, R., B. Taylor* and *M. Taubenhaus:* Proc. Soc. exp. Biol., N. Y. *76*, 854, 1951.
3. *Michael, M.* Jr. and *C. M. Whorton:* Proc. Soc. exp. Biol., N. Y. *76*, 754, 1951.
4. *Blunt, J. W., C. M. Plotz, R. Lattes, E. L. Howes, K. Meyer* and *C. Ragan:* Proc. Soc. exp. Biol., N. Y. *73*, 678, 1950.
5. *Sandweiß, D. J., H. C. Saltzstein, S. R. Scheinberg* and *A. Parks:* J. Amer. med. Ass. *144*, 1436, 1950.
6. *Thomas, J. W.* and *J. E. Rhoads:* Clin. Congr. Amer. J. Surg. 36 meet., 1950.
7. *Magarey, F. R.* and *J. Gough:* Brit. J. exp. Path. *33*, 76, 1952.
8. *Curran, R. C.:* Brit. J. exp. Path. *33*, 82, 1952.
9. *Findlay, C. W.* Jr. and *E. L. Howes:* New Engl. J. Med. *246*, 597, 1952.
10. *Friedman, M. H. F.* and *W. D. Beamer:* Unpublished, 1949.
11. *Taylor, F. W., T. L. Dittmer* and *D. O. Porter:* Surgery *683*, 31, 1952.
12. *Bangham, A. D.:* Brit. J. exp. Path. *32*, 77, 1951.
13. *Upton, A. C.* and *W. W. Coon:* Proc. Soc. exp. Biol., N. Y. *77*, 153, 1951.
14. *Dougherty, T. F.* and *G. L. Schneebeli:* Proc. Soc. exp. Biol., N. Y. *75*, 854, 1950.
15. *Roberts, K. B., H. W. Florey* and *W. K. Toklik:* Quart. J. exp. Physiol. *37*, 239, 1952.
16. *Cole, J. W., J. L. Orbison, W. D. Holden, T. J. Hancock* and *J. F. Lindsay:* Surg. Gynec. Obstet. *93*, 321, 1951.

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On the Theory of Silicosis

I. Coesite

HEINRICH BRIEGER, MD, PHILADELPHIA, AND PAUL GROSS, MD, PITTSBURGH

ALL KNOWN fibrogenic modifications of crystalline SiO_2 have the structure of SiO_4 tetrahedrons. After extensive investigations, Thomas and co-workers[1-5] have arrived at the opinion that contact of the cell with SiO_4 tetrahedrons and transferable hydroxyl groups attached to them plays an important role in the development of silicosis.

It appeared interesting to ascertain whether a more recently discovered modification of SiO_4, coesite, would produce silicotic changes. Coesite, a high pressure, high temperature product, was synthesized by Coes[6] in 1952; it also has been found in meteorite craters. The most comprehensive descriptions of its physicochemical properties have been published by Stöber.[7,8] It is less soluble than quartz in hydrofluoric acid and aqueous solutions. In comparison with quartz, coesite has fewer silanol groups on the surface. The density of this material is higher (2.93) than that of quartz (2.65). Coesite, too, consists of SiO_2 tetrahedrons, but the distribution of the hydroxyl groups on the surface is different.

In preliminary experiments Charbonnier et al[9,10] injected synthetic coesite intraperitoneally into rats and concluded that the material behaved like an inert substance, both 4 and 21 days after injection. In a subsequent publication Charbonnier et al[11] reported that, three months after intratracheal injection of 50 mg of coesite (approximately 66% of 1μ to 20μ), only a slight fibrogenic effect was in evidence, while the injection of quartz in comparable size distribution produced typical advanced silicosis with marked collagenization. The authors explained these effects on the basis of the solubility theory. Meanwhile, Strecker[5] had observed that intraperitoneal injection of coesite with a quartz content of 1.5% (particle size < 1μ) caused changes that resembled silicotic granulomata more than simple encapsulations. Brieger and Gross,[12] using the same material as employed by Strecker, have observed that the intratracheal injection of 30 mg led, within one month, to severe fibrogenic reaction with a dense network of reticulin fibers and numerous thick collagen bundles; quartz dust (1μ to 3μ) was given in parallel experiments, and it produced relatively little reticulin and no collagen within the same period of time. In continuation of his initial experiments, Strecker has used four samples of natural coesite in different degrees of purity (absence of quartz) and particle size. The most complete study refers to intraperitoneal injection of "Coesite III" particles having a surface of 8 sq meters/gram. This "Coesite III" had been produced from a coesite sample (14 sq meters/gram) containing no quartz, as checked by x-ray analysis. The sample was repeatedly treated with hydrofluoric acid to exclude any possible trace of quartz and to increase the grain size (about two thirds of the quartz-free "Coesite II" was dissolved during this treatment). The experimental animals (rats) were observed

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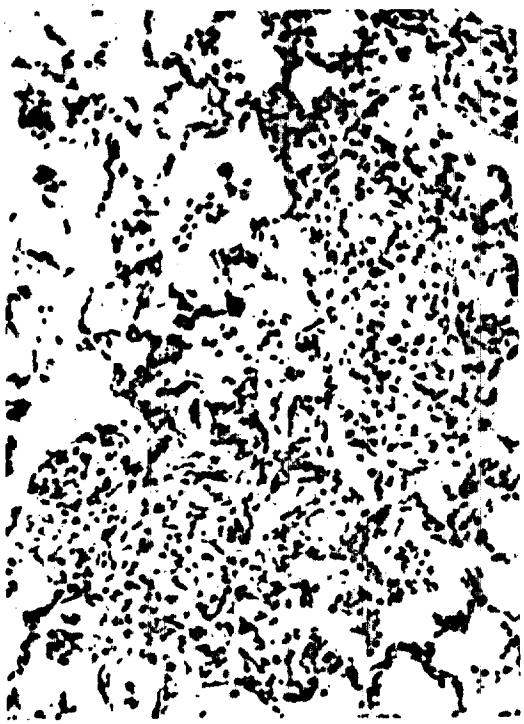


Fig 1.—These are characteristic nodules found four days after coestic dust was administered intratracheally. The nodules are cellular and nearly solid. Adjoining alveolar walls are also cellular and thickened. Macrophages are present in neighboring air spaces (hematoxylin and eosin, × 190).

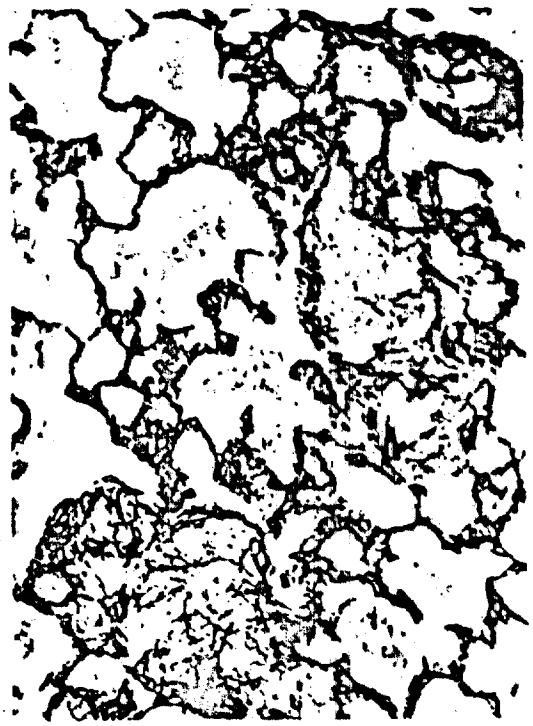


Fig 2.—The same field as in Fig 1 showing the pattern of argyrophilic fibers. It is apparent that the nodules consist of alveolar ducts that have been invaded by networks of delicate argyrophilic fibers. There is mural thickening of alveolar ducts as well as of nearby alveoli by arborescent and interconnected fibers (Gordon and Sweets, × 190).

for a period of five months. Coesite caused a typical silicotic tissue reaction, although more slowly than a comparable quartz sample. From the gradual differences of the tissue reactions, Strecker estimated the silicogenic activity of coesite as one tenth of that of comparable quartz samples. Gross and Brieger[13] have reported that the immediate reaction to pure coesite and the following early tissue changes were the same in rats injected intratracheally with coesite and quartz, respectively.

This paper is concerned with the pulmonary reaction to finely divided, highly purified coesite dust in Wistar-strain rats.

Methods and Materials

Fifty-two rats were injected intratracheally with 30 mg of coesite suspended in 1 ml of isotonic saline solution or water. The coesite was free of quartz as proven by a Debye-Scherrer photograph. The median particle size was 0.14μ (with 4.5% above 0.5μ). The rats were injected, as a rule, in groups of four for each period of survival. They were killed immediately, one, two, three, and four days, one and two weeks, and 1, 2, 4, 6, 8, and 12 months after injection. The same procedure was used in an experiment with pure quartz (0.44μ with 43% above 0.5μ). Control rats (one for each group) were injected intratracheally with 1 ml of isotonic saline solution or water and killed at the same time intervals.

Results

Both coesite and quartz caused in all animals an initial inflammation that tended to be localized in the proximal portion of the acinus or racemus, ie, the respiratory bronchiole and its adjoining ducts. This inflammation, involving the walls of these structures as well as those of the evaginating alveoli, is characterized by the proliferation of alveolar cells and their supporting stroma. In addition, there developed considerable leukocytic exudation within 24 hours following the injection, attributable to the high dosage (in terms of number of dust particles) to which the alveolar membrane had been exposed. However, this leukocytic exudation diminished in the succeeding days so that by the fourth day only small residues of leuko-

Fig 3.—A composite of the ash pattern (dark-field) and the pattern of argyrophilic fibers of the same field as in Fig 1 and 2. The nodules are almost completely permeated by dust. The alveolar macrophages in the neighboring air spaces are laden with dust (× 190).

cytic exudate remained. Also, by the fourth day following the injection of either dust, the proliferating inflammatory tissue had obliterated most of the evaginating alveoli in the proximal portion of the racemus and invaded the lumina of the respiratory bronchioles and alveolar ducts in the form of polypoid occlusive structures. This inflammatory tissue, although rich in plump fibroblast-type cells and reticulin fibers, cannot be designated as "granulation" tissue because it is completely avascular. Whereas many of the inflammatory foci form solid cellular oblong or more rounded structures, there are some in which the lumina are still, at least partially, patent and recognizable as those of alveolar ducts and respiratory bronchioles, particularly in the reticulin (silver-impregnated) preparation. These four-day-old solid, or partially hollow, cellular structures, representing the inflamed proximal portion of the racemi constitute the early developmental stage of silicotic nodules. The inflammatory tissue within these foci was replete with the causative irritant, coesite, or quartz dust (Fig 1-3).

At one week after the injection of either dust, the lumina of nearly all involved structures had disappeared and the dense character of the proliferated reticulin network made identification of earlier existing mural outlines highly uncertain. The exceptions were of two kinds: small round spaces of irregular small slits representing remnants of larger lumina (but of what structures cannot be determined), and lumina of obvious respiratory bronchioles or alveolar ducts in which only a portion of the wall was involved and the inflammation was confined largely to the evaginating alveoli.

At two months a fairly dense network of delicate precolagenous fibers had developed (Fig 4, 5). At six months the collagenization of the nodules was advanced, and by 12 months this process was practically com-

Fig 4.—A nodule two months after the dust was injected intratrachealy shows a high degree of cellularity. It contains a tubular structure lined by cuboidal epithelium. This is a surviving remnant of an alveolar duct. Several small remnants of air spaces lined by cuboidal epithelium are also seen in the lower left portion of the nodule (hematoxylin and eosin, × 190).

Fig 5.—The same field as in Fig 4 following silver impregnation shows a fairly dense network of delicate precollagenous fibers. Only rare collagen fibers are found. Adjoining alveolar walls are delicate and normal (Gordon and Sweets, × 190).

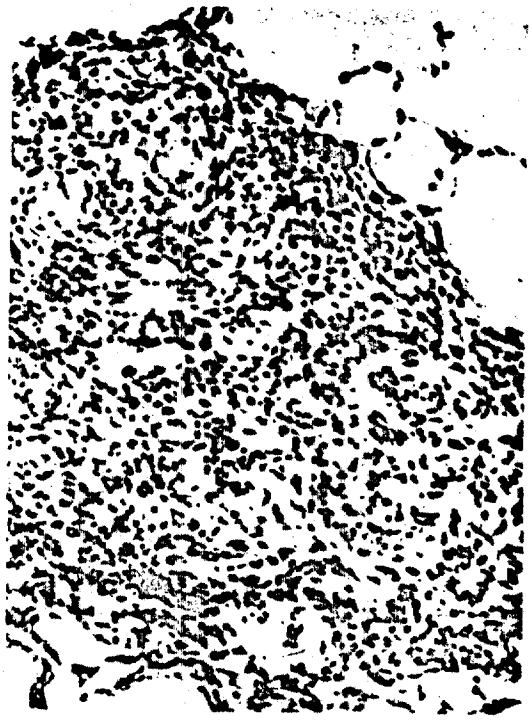


Fig 6.—A nodule 12 months after the coesite dust was injected intratracheally is still cellular. However, the cellularity is noticeably reduced when compared with that of a two-month-old nodule (Fig 4) (hematoxylin and eosin, × 190).

plete (Fig 6, 7). No obvious difference existed between the amount of collagen produced by the coesite dust and that produced by the quartz dust. However, such a judgment is, at best, only a very rough approximation, and it is recognized that quantitative collagen determinations are required to arrive at a reliable comparison.

Concomitant with the development of collagen within the silicotic foci, there was a diminution in the cell population, particularly in the center of the nodule. There, the cells had become separated from one another by a homogeneous, hyaline matrix.

The small spaces observed early within some of the otherwise solid nodules were also present in the lungs of animals killed 12 months after the dust injection. Some of these spaces became lined with metaplastic cuboidal epithelium, whereas others had remained unlined.

Those of the early silicotic foci in which the lumen of the proximal portion of the racemus was largely patent and recognizable apparently remained essentially unchanged except for the maturation of the connective tissue, as far as such partially involved respiratory bronchioles or alveolar ducts could be recognized at the end of the observation period.

The lymph-node enlargement, its increasing content of silica, and the replacement of the lymphoid tissue, first by a dense network of reticulin fibers, followed by collagenization, have also been difficult to compare. It has not been possible to ascertain that one dust is more potent than the other in the production of changes in the lymph nodes.

In summary, the findings may be briefly stated as follows: Given a series of sections from the lungs of rats injected with coesite dust and another series of sections from the lungs of rats injected with quartz dust (both series from animals that had survived the same length of time), it was not possible to determine by any established criteria and with any degree of accuracy which dust produced more extensive silicosis or a greater

Fig 7.—The same field as in Fig 6 after silver impregnation shows a dense stroma composed mostly of coarse, wavy collagen bundles, many tending to arrange themselves in a parallel manner (Gordon and Sweets, ×190).

degree of collagenization. Control animals injected with isotonic saline solution or water did not develop any comparable pulmonary changes.

Comment

The observations recorded here leave no doubt that coesite dust is to be classified as a fibrogenic dust and that its fibrogenic potential is approximately of the same order as that of quartz dust. A relatively minor point that should be mentioned in connection with the evaluation of the effects of the two fibrogenic dusts is the disparity in the average particle size of the two dusts employed, 0.44μ (quartz) and 0.14μ (coesites). King et al [14] have demonstrated that the total surface area of the dust in contact with the alveolar membrane significantly affects the amount of collagen produced, there being a direct relationship between these two parameters.

It has been mentioned that Strecker [5] has found definite silicotic changes following intraperitoneal injection of coesite. These changes developed, however, very slowly compared with the effects of quartz. The results of the experiments described in this paper demonstrate that intratracheal injection of coesite causes rapid development of silicotic changes that cannot be distinguished from those produced by quartz of somewhat larger particle size.

The findings reported in this paper lend support to the theory that the fibrogenic tissue reaction to crystalline SiO_2 is contingent on the tetrahedral configuration of "one or a few Si atoms" [15] within the range of density from 2.22 (tridymite) to 2.93 (coesite). Thomas [4] himself, however, has emphasized that it would be premature to consider this theory as the only alternative.

Summary

Rats were injected intratracheally with 30 mg of pure coesite (median particle size 0.14μ) and with 30 mg of quartz (median particle size 0.44μ), respectively. The animals were killed at intervals ranging from a few minutes after injection to 12 months after injection. The fibrogenic potential of coesite was found to be of the same order as that of quartz. Control animals treated with isotonic saline solution or water were free of any comparable changes.

The results of these experiments support the theory that the tetrahedral configuration of the SiO_2 molecule is a prerequisite for the silicogenic action of crystalline SiO_2.

This work was supported by the Anthracite Health and Welfare Fund, United Mine Workers of America.

The raw material for the coesite originating from the Barringer Meteorite Crater in Arizona was provided by Mr. Brandon Barringer, Barringer Crater Co., Philadelphia. This material was divided and purified in the Medizinische Forschungsanstalt der Max-Planck-Gesellschaft, Göttingen, Arbeitsgemeinschaft Silikoseforschung.

REFERENCES

1. Stöber, W., and Arnold, M.: Zur Frage der Belby-Schicht auf Oberflächen von Quarzpulver, *Beitr Silikoseforsch* 4:73, 1962.

2. Stöber, W.; Liefländer, M.; und Bohn, E.: Topochemische Grenzflächenreaktionen an Oxydoberflächen, *Beitr Silikoseforsch* 4:111, 1962.

3. Strecker, F.J.: Chemisch veränderte SiO_2-Oberflächen im Tierversuch, *Beitr Silikoseforsch*, 4:121, 1962.

4. Thomas, K.: Zur biologischen Wirkungsweise fester SiO_2-Modifikationen, *Beitr Silikoseforsch*, 6:21, 1965.

5. Strecker, F.J.: Histophysiologische Untersuchungen zur "silikotischen Gewebsreaktion" im Intraperitonealtest und zur Gewebswirkung von Coesit und Stischowit, *Beitr Silikoseforsch* **6**:55, 1965.

6. Coes, L., Jr.: A New Dense Silica, *Science* **118**: 131, 1953.

7. Stöber, W.: Physikalisch-chemische Eigenschaften von Coesit und Stischowit im Vergleich zu Quarz, *Beitr Silikoseforsch* **6**:35, 1965.

8. Stöber, W.: Silikotische Wirksamkeit und physikalisch-chemische Eigenschaften verschiedener Siliziumdioxid-Modifikationen, *Beitr Silikoseforsch* **89**:1, 1966.

9. Charbonnier, J., et al: Effets tissulaires d'une silice cristallisée insoluble, *C R Acad Sci* **238**:3068, 1959.

10. Charbonnier, J., et al: Die Beziehungen zwischen Struktur, Löslichkeit und Gewebetoxizität der verschiendenen Minerale, *Beitr Silikoseforsch* **66**:1, 1960.

11. Charbonnier, J., et al: Etude par test trachéal du pouvoir fibrosant d'une coesite synthetique, *Beitr Silikoseforsch* **6**:85, 1965.

12. Brieger, H., and Gross, P.: Lung Lesions Observed One Month After Intratracheal Injections of Not Quartz-Free Coesite (1.5% Quartz), *Beitr Silikoseforsch* **81**:43, 1964.

13. Gross, P., and Brieger, H.: Silicotic Bronchiolitis Obliterans, *Arch Environ Health* **12**:5, 1966.

14. King, E.J., et al: The Action of Flint of Variable Size Injected at a Constant Weight and Constant Surface into Lungs of Rats, *Brit J Industr Med* **10**:76, 1953.

15. Thomas, K.; Einbrodt, H.J.; and Schoedel, W.: Vorwort, *Beitr Silikoseforsch* **6**:13, 1965.

ERRATUM

In the November issue of the ARCHIVES in the article titled "Environment and the Kidney" the word "Gaellic" (line 2 of the third paragraph of the lefthand column) should read "Gallic."

Postscript on an Errant Type

The word was *gallic*, not *gaelic*
(vol 11, p 710, 1965):

Dr. Ed Huth *gaelic?*
As soon St. Patrick *gallic!*
At least it wasn't *garlic*,
Although it might have been.

(So much for not sending
gaelley proof then;
Please send galliy proof now
To just an old galley slave,

J.R.E.)

Compt. rend. soc. biol.,
146:1574-1577, 1953.

Biliary elimination of silica; experimental study in the rabbit

by

A. Collet and H. Moussard

Study of the elimination of silica by the kidney requires prior investigation, for the silica might also be eliminated via other routes, particularly through the bile, which would introduce a cause of error in assessing the analyses. Experiments were carried out to determine the fraction of silica injected intravenously which would be found in the bile.

After dial anesthesia of the rabbit and catheterization of the common bile duct, the bile was collected in polished plexiglass containers, while the biliary output was recorded on a rheograph. The control bile was collected for 30 min. The animal was then given an injection of 1.25 cc/kg of a suspension of highly dispersed silica in distilled water in the saphenous vein (mixture of silicic acid monomers and polymers). A 30 drop sample ("points") was taken out every hour. Meanwhile the bile was collected separately ("sections"). The silica content of the samples was determined by a modified version of the micro-colorimetric method of E.J. King (1).

Seven experiments were made by this method. Six others served as controls, in which the silica injection was replaced by an equal volume of distilled water.

The results are shown in Tables I and II. The excess bile shows the difference between the silica eliminated after the injection and without injection.

The tables clearly show that the quantity of silica eliminated by the bile is very low in relation to the amount injected.

Fig. 1 shows that elimination as a rule increases until the end of the second hour and that the silica content of the bile only slowly resumes its initial value (6 hours). During this period the bile output undergoes diverse variations in different animals: varying decreases, increases or temporary changes.

The control experiments gave the results shown in Table III.

These figures show a stability of the silica content of the bile under our experimental conditions (Fig. 2). As a rule the output decreases evenly.

Under different conditions, E.J. King (2) has observed that urinary elimination accounts for roughly 50% of the injected silica in dogs, and Whitehouse (3) has reported a similar proportion after ingestion by man. Holt et al. (4), after intraperitonetal injection of radioactive silica (Si^{31}), observed that the tagged silica was almost exclusively concentrated in the kidneys.

In summary, the biliary elimination of silica in relation to the quantity injected intravenously is negligible during the 6 hours following the injection, ranging from 0.35 to 1.1%. This does not constitute an appreciable shource of error in the

study of renal elimination, which appears to be the normal principal route of excretion.

Tables.

1. The percentages of silica are expressed in gamma SiO_2 per 1000 gamma dried bile. Key: 1. Date. 2. Control. 3. First hour.

2. Excess eliminated silica. Key: 1. Date. 2. Elimination. 3. In 2 hours, 0.21%. 4. Excess silica in % of injected silica.

3. Key: 1. Date. 2. Control. 3. First hour.

1. Date	2 Témoin	3 1er heure	2e heure	3e heure	4e heure	5e heure	6e heure
6-2-52	0,108	0,18	0,27				
13-2-52	0,17	0,29	0,31	0,37	0,29	0,30	
20-2-52	0,19	0,23	0,26	0,27	0,33	0,29	0,26
21-4-52	0,18	0,21	0,41	0,50	0,35	0,32	0,19
23-4-52	0,10	0,21	0,31	0,34	0,30	0,22	0,19
28-4-52	0,35	0,40	0,44	0,48	0,46	0,44	0,43
30-4-52	0,23	0,27	0,29	0,30	0,36	0,36	0,31

Table I

1 Dates	2 Elimination		
6-2-52	3 En 2 heures	0,21 p. 100	4 Silice excédentaire en p. 100 de la silice injectée
13-2-52	5 »	0,58 p. 100	
20-2-52	6 »	0,59 p. 100	
21-4-52	6 »	1,1 p. 100	
23-4-52	6 »	1,1 p. 100	
28-4-52	6 »	0,35 p. 100	
24-4-52	6 »	0,93 p. 100	

Table II

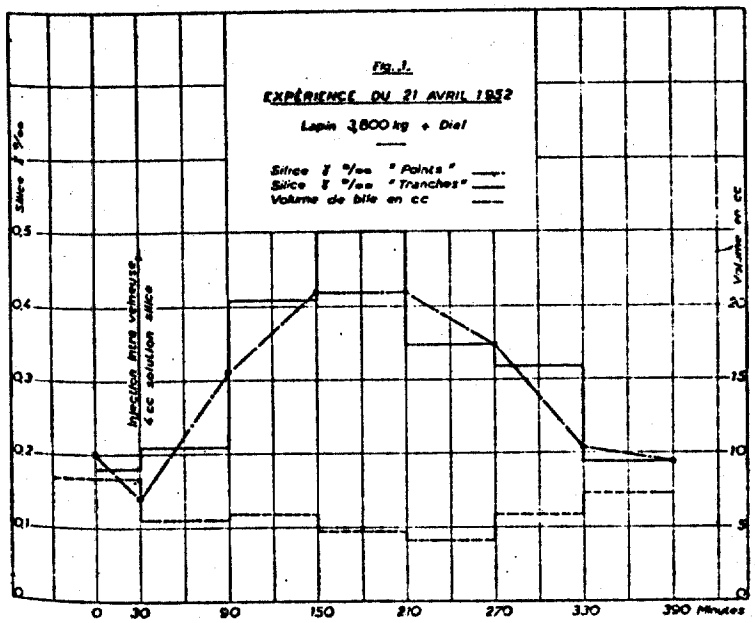
1. Date	2 Témoin	3 1er heure	2e heure	3e heure	4e heure	5e heure	6e heure
1952							
12-3	0,17	0,15	0,15	0,15	0,14	0,15	0,15
17-6	0,32	0,29	0,30	0,31	0,24	0,30	0,26
7-5	0,21	0,21	0,25	0,24	0,24	0,25	0,26
27-5	0,18	0,24	0,18	0,18	0,24	0,24	0,14
10-6	0,25	0,21	0,21	0,21	0,20	0,24	0,14
2-7	0,33	0,32	0,35	0,34	0,29	0,27	0,28

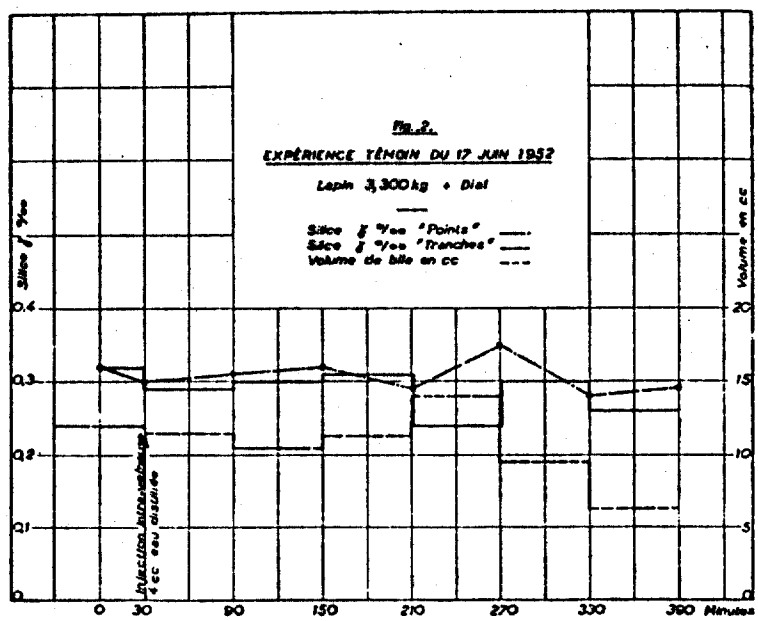
Table III

Figures.

1. Experiment on April 21, 1952. Rabbit, 3.800 kg + Dial. Silica, gamma per thousand, "points", Silica, gamma per thousand, "sections". Volume of bile in cc.

2. Control experiment on June 17th, 1952. Rabbit, 3.300 kg + Dial. Rest as in Fig. 1.





Bibliography

See footnote references.

(From: Center for Carbon Study and Research, France.)

ompt. rend. soc. biol., 146: 1014-1011, 1952

lure cutanée grave mais s'oppose aux manifestations de dommage qui constituent la note neuro-végétative au cours de la réaction d'alarme.

Elimination de silice par la voie biliaire. Etude expérimentale chez le lapin.

par A. Collet et H. Moussard.

L'étude de l'élimination de la silice par le rein nécessite un contrôle préalable, car la silice pourrait également s'éliminer par d'autres émonctoires en particulier la voie biliaire, introduisant une cause d'erreur dans l'estimation du bilan. Des expériences ont été entreprises pour montrer quelle fraction de silice injectée par voie intra-veineuse est susceptible d'être retrouvée dans la bile.

Chez le lapin, après anesthésie au dial et cathétérisme du canal cholédoque, la bile est recueillie dans des récipients vernis au plexiglass pendant que le débit biliaire est enregistré au rhéographe. La bile témoin est récoltée 30 minutes. L'animal reçoit alors une injection par la veine saphène de 1,25 cm³ par kg d'une suspension dans l'eau distillée de silice très dispersée (mélange de monomère et de polymères d'acides siliciques). Toutes les heures, on sépare un prélèvement de 30 gouttes (« points »). Entre temps la bile est recueillie séparément (« tranches »). La teneur en silice des échantillons est déterminée par la micro-méthode colorimétrique de E. J. King (1) modifiée.

Date	Témoin	1re heure	2e heure	3e heure	4e heure	5e heure	6e heure
6-2-52	0,108	0,18	0,27				
13-2-52	0,17	0,29	0,31	0,37	0,29	0,30	
20-2-52	0,19	0,23	0,26	0,27	0,33	0,29	0,26
21-4-52	0,18	0,21	0,41	0,50	0,35	0,32	0,19
23-4-52	0,10	0,21	0,31	0,34	0,30	0,22	0,19
28-4-52	0,35	0,40	0,44	0,48	0,46	0,44	0,43
30-4-52	0,23	0,27	0,29	0,30	0,36	0,36	0,31

Tableau I.

Les pourcentages de silice sont exprimés en gamma de SiO_2 pour 1000 gammas de bile desséchée.

Sept expériences ont été réalisées selon cette technique. Six autres ont servi de témoins, dans lesquelles l'injection de silice est remplacée par un égal volume d'eau distillée.

Les résultats sont consignés dans les tableaux I et II. La silice excédentaire représente la différence entre la silice éliminée après injection et celle qui l'aurait été sans injection.

(1) E. J. King, *Biochem. J.*, 1939, t. 33, p. 944.

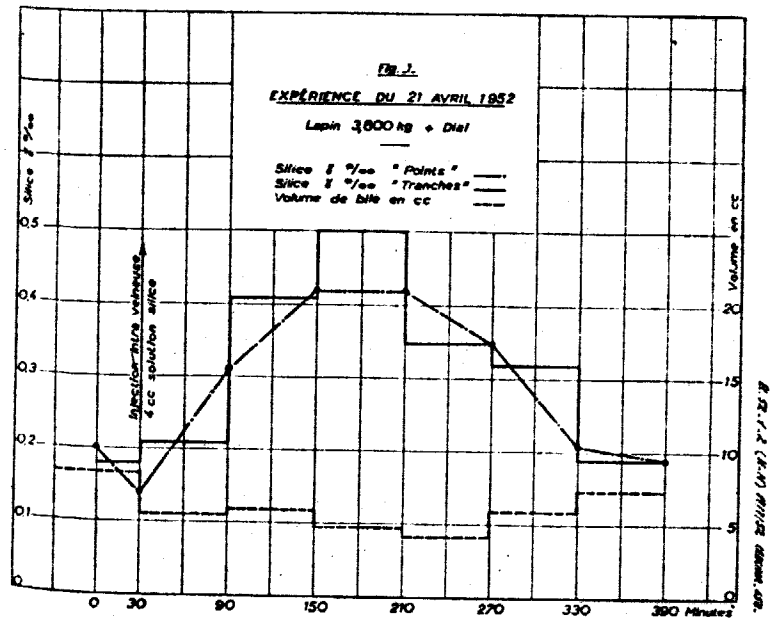
Ces tableaux établissent clairement que la quantité de silice éliminée par la bile est très faible par rapport à celle qui a été introduite.

Dates	Elimination		
6-2-52	En 2 heures	0,21 p. 100	Silice excédentaire en p. 100 de la silice injectée
13-2-52	5 »	0,58 p. 100	
20-2-52	6 »	0,59 p. 100	
21-4-52	6 »	1,1 p. 100	
23-4-52	6 »	1,1 p. 100	
28-4-52	6 »	0,35 p. 100	
24-4-52	6 »	0,93 p. 100	

Tableau II.

Silice excédentaire éliminée.

La figure 1 montre que l'élimination s'accroit jusqu'à la fin de la 2e heure en général et que la teneur de la bile en silice ne retrouve



que lentement sa valeur initiale (6 heures). Pendant ce temps, le débit de bile subit des variations différentes d'un animal à l'autre : réduction, augmentation ou modifications passagères en plus et en moins.

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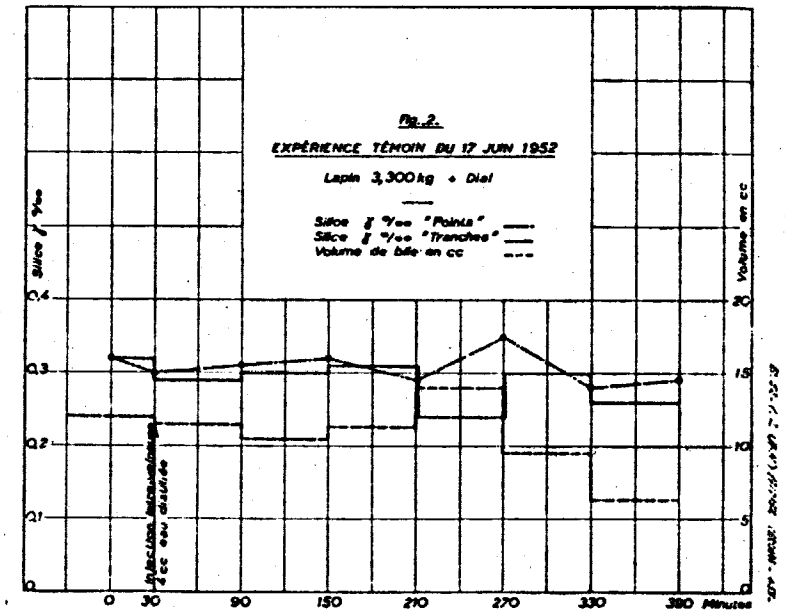
Les expériences témoins ont apporté les résultats consignés dans le tableau III.

Date	Témoin	1re heure	2e heure	3e heure	4e heure	5e heure	6e heure
1952							
12-3	0,17	0,15	0,15	0,15	0,14	0,15	0,15
17-6	0,32	0,29	0,30	0,31	0,24	0,30	0,26
7-5	0,21	0,21	0,25	0,24	0,24	0,25	0,26
27-5	0,18	0,24	0,18	0,18	0,24	0,24	0,14
10-6	0,25	0,21	0,21	0,21	0,20	0,24	0,14
2-7	0,33	0,32	0,35	0,34	0,29	0,27	0,28

Tableau III.

Ces chiffres montrent une bonne stabilité de la teneur en silice de la bile dans nos conditions expérimentales (fig. 2). Le débit diminue en général régulièrement.



Dans des conditions différentes, E. J. King (2) a observé que l'élimination urinaire représente chez le chien environ 50 p. 100 de la silice introduite et Whitehouse (3) relève une proportion voisine

(2) E. J. King, Stantial et M. Dolan, *Biochem. J.*, 1933, t. 27, p. 1007.
(3) A. G. R. Whitehouse, *J. Industr. Hyg. Toxicol.*, 1937, t. 19, p. 590.

après ingestion chez l'homme. Holt et ses collaborateurs (4) avec du silicium radioactif (Si^{31}) constatent, après injection péritonéale, une concentration du silicium marqué presqu'exclusivement au niveau des reins.

En résumé, l'élimination biliaire de silice par rapport à la quantité injectée par la voie veineuse est insignifiante pendant les 6 heures qui suivent l'injection ; de 0,35 à 1,1 p. 100. Il n'y a pas là une cause d'erreur appréciable dans l'étude de l'élimination rénale qui semble constituer la voie normale principale d'excrétion.

(*Centre d'Etudes et Recherches des Charbonnages de France*).

(4) P. F. Holt, D. M. Yates et D. H. Tomlin, *Biochem. J.*, 1951, t. 48, XLIV.

SILICON DIOXIDE

Silica Aerogel; Hydrated Silica

DESCRIPTION

Silica aerogel is a white, fluffy, powdered or granular microcellular silica. *Hydrated silica* is a precipitated, hydrated silicon dioxide occurring as a fine, white, amorphous powder, or as beads or granules. Both forms of silicon dioxide are insoluble in water and in alcohol and other organic solvents, but are soluble in hot phosphoric or hydrofluoric acids and in solutions of alkalies at 80° to 100°.

SPECIFICATIONS

Assay

Silica aerogel: Not less than 90.0 percent of SiO_2.
Hydrated silica: Not less than 89.0 percent of SiO_2, calculated on the dried basis.

Loss on drying. *Hydrated silica:* not more than 6 percent.

Loss on ignition. Not more than 6 percent.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).
Heavy metals (as Pb). Not more than 30 parts per million (0.003 percent).
Lead. Not more than 10 parts per million (0.001 percent).
Soluble ionizable salts (as Na_2SO_4). Not more than 5 percent.

TESTS

Assay. Transfer about 2 grams, accurately weighed, into a tared platinum crucible, ignite at 600° for 2 hours, cool in a desiccator, and weigh. Moisten the residue with 7 or 8 drops of alcohol, add 3 drops of sulfuric acid, and add enough hydrofluoric acid to cover the wetted sample. Evaporate to dryness on a hot plate, using medium heat (95–105°), then add 5 ml. of hydrofluoric acid, swirl the dish carefully to wash down the sides, and again evaporate to dryness. Ignite the dried residue to a red heat over a Meker burner, cool in a desiccator, and weigh. The difference between the total weight loss and the weight loss after ignition at 600° represents the weight of SiO_2 in the sample taken.

Loss on drying, page 931. Dry hydrated silica at 105° for 2 hours.

Loss on ignition. Transfer about 5 grams, accurately weighed, into a suitable tared crucible, and ignite at 600° to constant weight.

Sample Solution for the Determination of Arsenic, Heavy Metals, and Lead. Transfer 10.0 grams of the sample into a 250-ml. beaker, add 50 ml. of 0.5 *N* hydrochloric acid, cover with a watch glass, and heat slowly to boiling. Boil gently for 15 minutes, cool, and let the undissolved material settle. Decant the supernatant liquid through a Whatman No. 3 filter paper, or equivalent, into a 100-ml. volumetric flask, retaining as much as possible of the insoluble material in the beaker. Wash the slurry and beaker with three 10-ml. portions of hot water, decanting each washing through the filter into the flask. Finally, wash the filter paper with 15 ml. of hot water, cool the filtrate to room temperature, dilute to volume with water, and mix.

Arsenic. A 10-ml. portion of the *Sample Solution* meets the requirements of the *Arsenic Test*, page 865.

Heavy metals. Dilute 20.0 ml. of the *Sample Solution* to 30.0 ml. with water. A 10-ml. portion of the dilution meets the requirements of the *Heavy Metals Test*, page 920, using 20 mcg. of lead ion (Pb) in the control (*Solution A*).

Lead. A 10-ml. portion of the *Sample Solution* meets the requirements of the *Lead Limit Test*, page 929, using 10 mcg. of lead ion (Pb) in the control.

Soluble ionizable salts. Weigh accurately 12.5 grams of the sample, and stir it with 240 ml. of water for at least 5 minutes with a high speed mixer. Transfer the mixture into a 250-ml. graduate, and wash the mixer container with water, adding the washings to the graduate to make 250 ml. Stopper the graduate, and invert it several times to mix the slurry. The conductivity of the slurry, determined with a suitable conductance bridge assembly, is not greater than that produced by a control solution containing 750 mg. of anhydrous sodium sulfate in each 250 ml.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Anticaking agent; defoaming agent.

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unt of ACTH have previously been observed[12,13]. BANERJEE and GHOSH[14] have reported the histochemical changes in testes and adrenal glands during scurvy to be corrected on administration of ascorbic acid. DEB and BISWAS[15] have suggested that the inhibition of spermatogenesis in rats produced by high tyrosine diet was possibly due to a reduced secretion of hypophyseal gonadotropin, although they have noted a correction of the said disturbances on vitamin C. The functional activity of the pituitary gland in ascorbic acid deficiency has not been studied in detail. DEB and BANERJEE[16] have noted a fall in alkaline phosphatase in the anterior pituitary during scurvy and postulated it to be possibly due to diminution of some pituitary trophic hormone. Recently, pituitary bioassay revealed that the gonadotropic potency has been increased in C-deficient guinea-pigs (DEB and BISWAS[17]). An increased gonadotropic content and reproductive disturbances also have been reported in B_6 deficient rats[18,19]. The testicular degeneration in ascorbic acid deficiency may, therefore, be caused by failure of the pituitary to release sufficient gonadotropic hormone[20].

Résumé. La dégénération des testicules de cobayes manquant d'acide ascorbique a pu être corrigée par l'administration du sérum gonadotropin.

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Department of Physiology, University College of Science, Calcutta 9 (India), April 8, 1965.

[12] N. W. NOWELL and I. CHESTER JONES, Acta endocr. *26*, 273 (1957).
[13] J. T. SCHARDO, *The Endocrinology of Reproduction* (Oxford University Press, New York 1958), p. 79.
[14] S. BANERJEE and S. K. GHOSH, Proc. Nat. Inst. Sci. India *29*, 225 (1963).
[15] C. DEB and N. M. BISWAS, Exper. *21*, 73 (1965).
[16] C. DEB and S. BANERJEE, Proc. Nat. Inst. Sci. India *23*, 1 (1957).
[17] C. DEB and N. M. BISWAS, unpublished.
[18] E. WOOLEN, M. M. NELSON, M. E. SIMPSON, and H. M. EVANS, Endocrinology *56*, 860 (1955).
[19] M. M. NELSON, W. R. EVOSS, and H. M. EVANS, Endocrinology *48*, 726 (1951).
[20] Acknowledgment: We would like to express our thanks to Dr. S. R. MAITRA, Head of the Department of Physiology, University of Calcutta, for his constant encouragement and S. GUHA ROY for statistical analysis. The work presented in this report has been financed by the Council of Scientific and Industrial Research, Government of India.

Experientia, 21 (9): 543-544, 1965

Effect of Silica on Protein Biosynthesis by Rat Liver Cell-Free Systems

Silica particles engulfed by macrophages in vitro bring about an injurious effect to these cells. The mechanisms of cytotoxic action of silica and their specificity have been widely investigated in recent years on cultured macrophages and on various cells and tissues by several workers[1-8]. Nevertheless, the nature of the toxic effect of silica remains a problem still open to question.

The present report is a further study[9,10] on the same question. It deals with the action of particulate silica on protein biosynthesis at subcellular levels by investigating the amino acid incorporation rate into protein by rat liver cell-free extracts.

The microsomal system of ZAMECNIK and KELLER[11] as described by STEIN and GROSS[12] has been used, and purified preparations of crystalline HF-treated silica (tridymite) and of vitreous HF-treated silica (both in a diameter range $\sim 1\ \mu$) tested. Liver homogenates 1:3 by volume were prepared with teflon pestle homogenizer in 0.25 M sucrose with 0.025 *M* $KHCO_3$, 0.02 *M* potassium phosphate buffer pH 7.8 and 0.01 *M* nicotinamide. Nuclei and cellular debris were removed by centrifugation at 800 *g* for 6 min and the resulting supernate recentrifuged for 15 min at 10,000 *g*. Centrifugations were made in a refrigerated International centrifuge. 1.0 ml of the 10,000 *g* supernate was incubated in Warburg flasks with 20 μM fructose 1-6 diphosphate potassium salt, 2.0 μM ATP potassium salt, 2.0 μM $MgCl_2$ and 0.26 μM DL-leucine-1-C^{14} (specific activity 3.8 $\mu c/\mu M$) in a final volume of 2.0 ml. When present silica was in amount of 15 mg. Incubations were carried out for 1 h at 37°C in a Warburg metabolic shaking incubator with O_2 95% + CO_2 5% as gas phase. The reaction was stopped by TCA and protein

In vitro incorporation of DL-leucine-1-C^{14} into rat liver microsomal protein. Each figure (c.p.m. per mg protein) is the mean ± S.E. of 2 flasks

Experiment	Control	Crystalline silica	Vitreous silica
1	92.5 ± 4.47	52.0 ± 1.25	–
2	80.5 ± 5.48	59.0 ± 5.00	–
3	116.0 ± 3.00	62.0 ± 1.40	–
4	111.0 ± 9.00	65.0 ± 6.00	–
1	81.0 ± 3.00	–	65.5 ± 7.48
2	75.5 ± 10.49	–	43.0 ± 6.00
3	84.0 ± 10.00	–	76.5 ± 3.46
4	80.5 ± 5.48	–	62.5 ± 7.48

The statistical evaluation of data has been made with the analysis of variance test.

[1] E. V. ROWSELL and R. A. LEONARD, Biochem. J. *70*, 57 (1958).
[2] J. MARKS and D. M. JAMES, J. Path. Bact. *77*, 401 (1959).
[3] J. MARKS and G. NAGELSCHMIDT, Arch. indust. Health *20*, 383 (1959).
[4] H. DANIEL-MOUSSARD, C. r. Soc. Biol. *155*, 699 (1961).
[5] R. W. I. KESSEL, L. MONACO, and M. A. MARCHISIO, Brit. J. exp. Path. *44*, 351 (1963).
[6] H. HAUGER, K. KRISCH, and H. STAUDINGER JR., Beitr. Silikose-Forsch., Sbd. *5*, 69 (1964).
[7] B. RASCHE and W. T. ULMER, Int. Arch. Gewerbepath. Gewerbehyg. *21*, 27, 39 (1964).
[8] K. KOSHI, Ind. Health *2*, 19 (1964).
[9] R. COMOLLI and A. PERIN, Proc. Soc. exp. Biol. Med. *113*, 289 (1963).
[10] R. COMOLLI, Boll. Soc. Ital. Biol. sper. *40*, 1075 (1964).
[11] P. C. ZAMECNIK and E. B. KELLER, J. biol. Chem. *209*, 337 (1954).
[12] O. STEIN and J. GROSS, Proc. Soc. exp. Biol. Med. *109*, 817 (1962).

isolated using the procedure of SIEKEVITZ[13]. Calculated portions of dried protein were plated on stainless steel planchets and counted dry with a thin mica end window G.M. counter. Counting error was less than 2%. Protein was estimated after nitrogen determination with Nessler reagent.

From the data given in the Table it can be seen that leucine incorporation into the microsomal protein by rat liver homogenates is depressed by tridymite ($P < 0.001$); conversely, vitreous silica seems to carry out a less definite action in that sense ($P < 0.05$). It is to be noted that there is a large fluctuation in the experimental data. This is the result of the combined biological and analytical errors, a phenomenon which might also obscure the experimental response if data were incorrectly analysed.

The observations here reported are in keeping with those of ZAMECNIK and KELLER[11] as far as the incorporation rate in control cell-free systems is concerned. The observations show the presence of an inhibitory action by silica dusts on protein biosynthesis at subcellular levels. This action is more pronounced in the presence of crystalline than vitreous silica, an effect which might possibly be related with the well-known less toxic, in vitro, and fibrogenic, in vivo, properties of the latter dust. These observations may actually confirm the derangement of protein biosynthesis[9], a possible specific action of silica in tissues.

Particulate silica in the form of quartz is known to adsorb protein material (e.g. serum proteins and enzymes) and amino acids on its surface[14,15] and to have also oxidative and hydroxylative properties on these latter compounds[16,17]. The decreased protein radioactivity found by the experiments here reported might be the consequence of the peculiar surface activity of particulate silica. As a matter of fact, the extent of the observed inhibition of the amino acid incorporation into the microsomal protein seems to be related to the surface structure of the silica particle tested[18].

Riassunto. È stata studiata l'azione di particelle di silice a struttura cristallina e non cristallina sulla incorporazione di leucina-1-C^{14} nelle proteine microsomiche di fegato.

La silice inibisce l'incorporazione, ma con diversa intensità. Ciò appare essere in rapporto con la struttura delle particelle impiegate.

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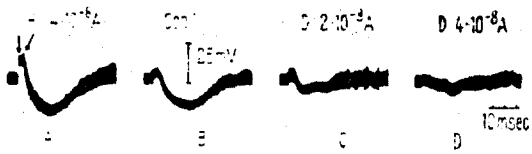
[13] P. SIEKEVITZ, J. biol. Chem. *195*, 549 (1952).
[14] F. M. ENGELBRECHT and R. PAUL, Ann. occup. Hyg. *1*, 135 (1959).
[15] J. S. HARINGTON, South Afr. Med. J. *37*, 451 (1963).
[16] L. W. MARASAS and J. S. HARINGTON, Nature *188*, 1173 (1960).
[17] L. W. MARASAS, Med. Lavoro *55*, 579 (1964).
[18] This investigation has been supported by a grant from the European Community for Coal and Steel (C.E.C.A.).

'Disfacilitation' of Red Nucleus Neurones

It has been reported[1] that the large neurones of the red nucleus (RN) receive powerful excitatory postsynaptic potentials (EPSPs) monosynaptically from the contralateral nucleus interpositus (IP) of the cerebellum. On the other hand, stimulation of the cerebellar cortex provokes inhibitory postsynaptic potentials (IPSPs) monosynaptically in the IP neurones, presumably via the corticofugal axons of cerebellar Purkinje cells[2]. Thus it may be expected that the cerebellar cortex acts upon the RN indirectly by inhibiting the IP. This process of disfacilitation, as it may be called, has indeed been demonstrated in the present work.

Cats were used under light nembutal anaesthesia. The experimental procedures were essentially the same as those employed previously[1]. The RN cells were impaled with glass-microelectrodes containing *2 M* NaCl and having electrical resistance of 10–20 *MΩ*.

In the case of the Figure A–D, the IP region was stimulated with needle electrodes. EPSPs were first induced at latencies of 1.0 and 2.2 msec, respectively (indicated by vertical and oblique arrows), presumably by direct and transsynaptic activation of IP neurones. Thereafter, starting at 0.7 msec a large hyperpolarization occurred, lasting over several ten-milliseconds (Figure B). These observations are in agreement with those by MASSION and ALBE-FESSARD[3,4]. When the membrane potential was displaced by applying hyperpolarizing (Figure A) or depolarizing (Figure C, D) currents, the amplitude of this membrane hyperpolarization was increased or decreased (control in Figure B) in parallel with that of EPSPs. In Figure D the hyperpolarization virtually disappeared together with the EPSPs, indicating that the nul-potential of the hyperpolarization is the same as that of EPSPs. Consequently, the membrane hyperpolarization should not be the IPSP but it should be due to removal of tonically induced EPSPs in RN neurones. Membrane hyperpolarization of the same nature has been seen in ex-



Membrane potential changes produced in a RN neurone by stimulation of IP region. A, during application of hyperpolarizing currents of $4 \cdot 10^{-8}$ A; B, control; C and D, during passage of depolarizing currents of $2 \cdot 10^{-8}$ A and $4 \cdot 10^{-8}$ A, respectively. All records were taken by superposing about 20 faint traces.

[1] N. TSUKAHARA, K. TOYAMA, and K.-KOSAKA, Exper. *20*, 63 (1964).
[2] M. ITO, M. YOSHIDA, and K. OBATA, Exper. *20*, 575 (1964).
[3] J. MASSION and D. ALBE-FESSARD, Electroenceph. clin. Neurophysiol. *15*, 435 (1963).
[4] J. MASSION, *Contribution à l'étude de la régulation cérébelleuse du système extrapyramidal* (Masson et Cie, 1961).

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CYTOTOXICITY OF SILICA AND LIBERATION OF LYSOSOMAL ENZYMES

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Lysis of lysosomal granules occurs in polymorphonuclear and macrophage cells after phagocytosis (Hirsch and Cohn, 1960; Hirsch, 1962; Cohn and Wiener, 1963*b*). This phenomenon, which is known to involve a progressive transfer of the lysosomal hydrolases from the granular bound form to soluble cytoplasmic fractions (Cohn and Hirsch, 1960*b*; Cohn and Wiener, 1963*b*)—a finding clearly indicating the intracellular redistribution of granule contents with discharge into the phagocytic vacuole (Lockwood and Allison, 1963; North and Mackaness, 1963; Zucker-Franklin and Hirsch, 1964)—causes an early appearance in the extracellular medium of increasing amounts of certain of the lysosomal enzymes.

Lysosomes are known to be involved in tissue injury (de Duve, 1963, 1964), possibly in two different ways. In one the injury proceeds independently of significant lysosomal damage (Kerr, 1965; Slater and Greenbaum, 1965; Trump, Goldblatt and Stowell, 1965) and lysosomal enzymes possibly act as post-mortem scavengers, and in the other lysosomal damage occurs early in the course of injury (Allison and Sandelin, 1963; Allison, 1965; Mallucci and Allison, 1965). The latter occurs in injury with streptolysin O (Hirsch, Bernheimer and Weissmann, 1963; Weissmann, Keiser and Bernheimer, 1963; Keiser, Weissmann and Bernheimer, 1964; Weissmann, Becher and Thomas, 1964); this toxin appears to act on phagocytic cells through a primary action on lysosomal membranes, suggesting that lysosomal granules may operate in this case as "suicide bags" according to de Duve (1959), with redistribution of the lysosomal hydrolases and apparent discharge into the cell sap.

Silica particles kill phagocytes only after they have been captured and engulfed by them. Previous histochemical studies by Harington and Allison (1965) on the behaviour of acid phosphatase and indoxylacetate esterase in macrophages cultivated *in vitro* with silica suggested the possibility of a lysosomal mediation in the toxic action of these dusts. It is possible that granule modifications like those described previously occur after ingestion of silica by macrophages, and that these lead to increased extracellular release of hydrolases, a phenomenon depending on the intracellular redistribution of these enzymes after phagosome formation, and possibly also on the toxic action of these dusts on phagosomal membrane, as suggested by Harington and Allison.

In the present paper I report studies on the extracellular release of some lysosomal and non-lysosomal enzymes by rat peritoneal mononuclear (macrophage) cells incubated *in vitro* with different particulate silica preparations and carbon particles.

Materials and methods

General

Cells. Peritoneal macrophages were obtained by the technique of Cohn and Wiener (1963*a*) from rats weighing approximately 450 g.; 4–6 rats were used in each experiment. Sterile paraffin oil (6 ml.) was injected intraperitoneally and 4 days later the rats were killed by cervical dislocation. 30 ml. saline were introduced into the peritoneal cavity, which was then opened to remove the exudate. The exudate was centrifuged at 150*g* for 6 min. in a refrigerated International centrifuge and the supernatant with the upper oil layer discarded. The pellet was washed once with saline, filtered through gauze and recentrifuged. The final pellet was suspended to the appropriate concentration in the following cold balanced salt

Table I

Characteristics of dust particles used

Dust	Percentage frequency of particles of diameter					Dvs (μ) * of dust	Description
	$>5\,\mu$	$5-3\,\mu$	$3-2\,\mu$	$2-1\,\mu$	$1-0{\cdot}5\,\mu$		
Carbon A88M	0·3	5·0	16·0	50·0	28·0	2·6	British anthracite: q. content 0·1 per cent.
Crystalline silica T67–70–72M	0·2	0·4	1·0	20·0	78·0	1·6	Hydrofluoric acid-etched tridymite
Crystalline silica T51M	0·5	2·0	8·0	50·0	40·0	2·3	Unetched tridymite
Vitreous silica VS74M	0·05	0·1	0·2	8·0	92·0	1·0	Fused quartz, etched hydrofluoric acid

* $\text{Dvs} = \frac{\Sigma Nd^3}{\Sigma Nd^2}$, where d is diameter of a particle and N is number of particles having that diam

solution: NaCl 0·127M; KCl 0·005M; $CaCl_2$ 0·0027M; KH_2PO_4 0·0013M; $MgSO_4$ 0·0013M; tris(hydroxymethyl)aminomethane-HCl buffer *p*H 7·4 0·030M. Final osmolarity 0·308M.

Counting and staining of cells. Cells were counted with a Bürker haemacytometer. Only 5–10 per cent. of cells appeared to contain small oil droplets. Cells were stained with May-Grünwald-Giemsa or haematoxylin and eosin. Incubated cells were examined by phase-contrast microscopy.

Dust particles. The dusts used (kindly supplied by the Mineralogy Laboratorium of the Clinica del Lavoro, the University of Milan, Italy) and their characteristics are reported in table I.

Incubation and manometric assays. Incubation was carried out in a Warburg respirometer. The O_2 uptake by cells was measured in parallel in control and experimental conditions, with air as gas phase and temperature 37°C. The main chamber of the flask contained $(1{\cdot}5–3{\cdot}0)\times10^7$ cells in 3 ml. incubation medium; the centre well of the flask contained 30 per cent. KOH and a roll of filter paper. When necessary, dust particles (1 mg. per 10^7 cells) were added to the main chamber of the flask at 4°C ten minutes before the beginning of manometric readings. Zero time was taken as that at which the mixture had reached equilibrium with the bath. At 0, 15, 30, 60 and 120 min. thereafter control and experimental flasks were withdrawn and cooled in an ice-bath, and their contents were quickly centrifuged at 6000*g* for 10 min. in a refrigerated International centrifuge. The resulting cell-free supernates were used for the enzymatic measurements.

Table II

Oxygen uptake of incubated cells at 15 min. intervals

Dust added	Mean oxygen uptake ± its standard error, as $\mu l.O_2$ absorbed by 10^6 cells between							
	0′ and 15′	15′ and 30′	30′ and 45′	45′ and 60′	60′ and 75′	75′ and 90′	90′ and 105′	105′ and 120′
None (28) *	0·29±0·008	0·29±0·013	0·30±0·010	0·28±0·011	0·30±0·012	0·27±0·013	0·26±0·010	0·26±0·009
Carbon (7) (A88M)	0·28±0·017	0·28±0·028	0·27±0·027	0·27±0·028	0·25±0·039	0·24±0·023	0·24±0·023	0·25±0·028
Etched tridymite (8) (T67–70–72M)	0·30±0·014	0·27±0·017	0·28±0·017	0·27±0·021	0·28±0·020	0·22±0·016	0·22±0·033	0·15±0·028
Unetched tridymite (6) (T51M)	0·31±0·014	0·31±0·022	0·29±0·018	0·29±0·020	0·27±0·029	0·29±0·022	0·29±0·025	0·28±0·027
Vitreous silica (7) (VS74M)	0·30±0·017	0·31±0·027	0·29±0·020	0·27±0·027	0·25±0·021	0·24±0·033	0·25±0·031	0·25±0·031

* In this and subsequent tables numbers in brackets are numbers of determinations.

Enzymatic and chemical assays

Acid phosphatase (EC 3.1.3.2*) was determined according to Cohn and Hirsch (1960*a*) with sodium beta-glycerophosphate as substrate. The reaction was allowed to continue for 60 min. at 37°C and was then stopped with trichloracetic acid (TCA) to a final concentration of 10 per cent. Inorganic phosphorus was determined by the method of Fiske and Subbarow (1925).

Acid ribonuclease (EC 2.7.7.16) was measured by the method of Schneider and Hogeboom (1952) as modified by Cohn and Hirsch (1960*a*), with RNA (Bayer, Leverkusen), purified according to Cohn and Wiener (1963*a*), as substrate. Incubation was carried out at 37°C for 30 min. The results are expressed as 260 mμ absorbing units released by the enzyme in 30 min., and referred to 10^6 cells.

Beta-glucuronidase (EC 3.2.1.31) was determined by the method of Talalay, Fishman and Huggins (1946) as modified by Paigen (1961), with phenolphthalein beta-glucuronide (Mann, New York) as substrate. The reaction was allowed to continue for 60 min. at 37°C. The results are expressed as μg. phenolphthalein released by the enzyme in 60 min., and referred to 10^6 cells.

Cathepsin (EC 3.4.4.9) was assayed according to Cohn and Hirsch (1960*a*) with denatured haemoglobin (Serva, Heidelberg) as substrate. The reaction was allowed to continue for 30 min. at 37°C and was stopped by addition of TCA to 2·5 per cent. final concentration. The deproteinised filtrate was assayed for chromogen by the method of Lowry *et al.* (1951). The results are expressed as protein equivalents liberated by the enzyme in 30 min., and referred to 10^6 cells.

Lysozyme (EC 3.2.1.17) was assayed by the procedure of Shugar (1952) as modified by Cohn and Hirsch (1960*a*). *Micrococcus lysodeikticus* agar slants were kindly supplied by the Institute of Microbiology, the University of Milan, Italy. Results are expressed as μg. lysozyme, and referred to 10^6 cells.

Fructosediphosphate aldolase (EC 4.1.2.13) was determined at 340 mμ with 0·056M collidine buffer *p*H 7·4, 0·003M monoiodoacetate and 0·003M fructosediphosphate in the presence of reduced NAD and a glyceraldehyde dehydrogenase triosophosphate isomerase mixture (UV test, Boehringer, Mannheim). Temperature 37°C, incubation time 20 min. Results are expressed as milliunits (I.U.B.) enzyme in the incubation medium, and referred to 10^6 cells.

Isocitrate dehydrogenase (EC 1.1.1.42) was measured at 340 mμ with 0·05M triethanolamine buffer *p*H 7·45, $MgCl_2$ 0·1M, DL-isocitrate trisodium salt 0·04M and NADP (1 mg. per 0·1 ml.). Incubation was carried out at 37°C for 20 min. Results are expressed as milliunits (I.U.B.) enzyme in the incubation medium, and referred to 10^6 cells.

Statistical analyses

The statistical evaluation of data has been made by the analysis of variance. No significance has been attached to differences with $P > 0·05$.

RESULTS

Table II gives the results of the estimations of viability of the incubated cells as determined from their oxygen uptake after every 15 minutes' incubation. It is evident that during the 1st hr of incubation the dust-treated cells show only a slight fall in viability (3·5–10 per cent.) and the control cells none at all. During the 2nd hr of incubation control cells also show small falls (10 per cent.) in O_2 uptake, but dust-treated cells show a severe fall (50 per cent.) in oxygen uptake and cell viability only in the presence of the T67–70-72M silica

* The numbers are those recommended by the Report of the Commission on Enzymes of the International Union of Biochemistry, 1961, *Oxford, London, New York* and *Paris*.

TABLE III

Acid ribonuclease activity in the incubation medium per 10^6 cells incubated

Dust added	260 mμ absorbing units (mean ± its standard error) released from RNA in 30 min. at 37°C by incubation fluid corresponding to 10^6 cells at					P for difference from controls
	0′	15′	30′	60′	120′	
None (10)	0·158±0·0134	0·182±0·0047	0·180±0·0044	0·205±0·0060	0·236±0·0084	...
Carbon (4) (A88M)	0·172±0·0136	0·197±0·0086	0·228±0·0119	0·286±0·0185	0·325±0·0194	<0·001
Etched tridymite (3) (T67–70–72M)	0·205±0·0212	0·248±0·0133	0·260±0·0063	0·393±0·0344	0·623±0·1025	<0·001
Unetched tridymite (5) (T51M)	0·170±0·0124	0·186±0·0043	0·188±0·0087	0·227±0·0088	0·277±0·0299	<0·05
Vitreous silica (3) (VS74M)	0·178±0·0108	0·175±0·0165	0·212±0·0045	0·252±0·0045	0·362±0·0045	<0·01

TABLE IV

Beta-glucuronidase activity in the incubation medium per 10^6 cells incubated

Dust added	μg. Phenolphthalein (mean ± its standard error) released from its glucuronide in 60 min. at 37°C by incubation fluid corresponding to 10^6 cells at					P for difference from controls
	0′	15′	30′	60′	120′	
None (11)	0·89±0·080	1·00±0·047	1·12±0·047	1·28±0·068	1·62±0·112	...
Carbon (3) (A88M)	0·97±0·043	0·93±0·001	1·11±0·002	1·51±0·001	2·16±0·290	n.s.
Etched tridymite (5) (T67–70–72M)	1·06±0·022	1·36±0·063	1·81±0·125	2·70±0·346	5·10±0·674	<0·001
Unetched tridymite (3) (T51M)	0·97±0·069	1·23±0·002	1·28±0·001	1·65±0·110	2·09±0·055	<0·01
Vitreous silica (3) (VS74M)	0·98±0·049	1·41±0·060	1·86±0·345	2·66±0·669	4·77±1·655	<0·05

TABLE V

Cathepsin activity in the incubation medium per 10^6 cells incubated

Dust added	μg. Protein equivalents (mean ± its standard error) released from denatured haemoglobin in 30 min. at 37°C by incubation fluid corresponding to 10^6 cells at					P for difference from controls
	0′	15′	30′	60′	120′	
None (11)	3·06 ± 0·460	3·43 ± 0·038	4·04 ± 0·044	4·27 ± 0·086	4·87 ± 0·338	...
Carbon (3) (A88M)	3·23 ± 0·056	3·83 ± 0·395	3·59 ± 0·437	4·40 ± 0·515	6·07 ± 0·610	n.s.
Etched tridymite (3) (T67–70–72M)	3·45 ± 0·363	5·47 ± 0·501	7·03 ± 0·405	9·86 ± 0·807	15·20 ± 0·791	< 0·001
Unetched tridymite (3) (T51M)	3·54 ± 0·332	4·48 ± 0·607	5·28 ± 0·071	6·59 ± 0·675	10·27 ± 1·152	< 0·01
Vitreous silica (4) (VS74M)	3·59 ± 0·324	4·62 ± 0·343	4·93 ± 0·798	6·53 ± 1·198	13·61 ± 2·351	< 0·001

TABLE VI

Lysozyme activity in the incubation medium per 10^6 cells incubated

Dust added	μg. Lysozyme (mean ± its standard error) in incubation medium corresponding to 10^6 cells at					P for difference from controls
	0′	15′	30′	60′	120′	
None (12)	0·007 ± 0·0001	0·020 ± 0·0027	0·038 ± 0·0035	0·069 ± 0·0080	0·118 ± 0·0134	...
Carbon (2) (A88M)	0·007 ± 0·0001	0·021 ± 0·0009	0·035 ± 0·0019	0·058 ± 0·0047	0·089 ± 0·0049	> 0·05
Etched tridymite (3) (T67–70–72M)	0·007 ± 0·0001	0·020 ± 0·0005	0·061 ± 0·0080	0·099 ± 0·0215	0·138 ± 0·0224	> 0·05
Unetched tridymite (4) (T51M)	0·007 ± 0·0001	0·025 ± 0·0071	0·054 ± 0·0045	0·080 ± 0·0126	0·144 ± 0·0233	> 0·05
Vitreous silica (4) (VS74M)	0·007 ± 0·0001	0·029 ± 0·0054	0·038 ± 0·0045	0·071 ± 0·0054	0·136 ± 0·0134	> 0·05

Less inhibition (17 per cent.) is caused by the VS74M silica, and cell viability with T51M silica and with carbon particles is very similar to that of the incubated control cells.

Tables III–VI show the results of determinations of hydrolase activity in the extracellular medium, namely of acid ribonuclease (table III), beta-glucuronidase (table IV), cathepsin (table V) and lysozyme (table VI). The results for acid phosphatase are not reported in the present paper, as satisfactory reproducibility could not be obtained. It is evident that the enzymes acid ribonuclease, beta-glucuronidase and cathepsin behave similarly in all conditions studied. During incubation, the extracellular activities of these enzymes increase:

TABLE VII

Fructosediphosphate aldolase activity in the incubation medium per 10^6 cells incubated

Dust added	Milliunits fructosediphosphate aldolase activity in incubation medium corresponding to 10^6 cells at			
	0′	15′	60′	120′
None	0·00	0·00	0·00	0·27
Carbon (A88M)	0·00	0·00	0·00	0·16
Etched tridymite (T67–70–72M)	0·78	1·35	2·22	5·94
Unetched tridymite (T51M)	0·00	0·03	0·06	1·32
Vitreous silica (VS74M)	0·36	0·42	2·25	4·86

these increases are of little moment in supernatants from control cells, whereas addition of dusts produces significant increases. At zero time, that is 10 min. after the addition of dust particles, supernatants from dust-treated cells already show small increases in the extracellular activity of these enzymes, as compared with those from controls. The extent of hydrolase release by cells seems to depend largely on the nature of the particle tested. Particles not very toxic to cells, like carbon dusts and the crystalline silica T51M, have only a small effect on enzyme release: more cytotoxic particles (e.g., T67–70–72M and VS74M silicas) have a much greater one. The results with lysozyme are completely different: the extracellular release of this enzyme from dust-treated cells is no greater than that from controls.

Tables VII and VIII show the results of typical experiments on the effect of dust particles on the extracellular release of two non-lysosomal enzymes, fructosediphosphate aldolase, a structure-bound cytoplasmic enzyme (Amberson, Roisen and Bauer, 1965: Green *et al.*, 1965), and isocitrate dehydrogenase, an enzyme found predominantly in the cell sap (Judah, Ahmed and McLean, 1964). The supernatants from control cells and from cells incubated with carbon particles have these enzymatic activities only at the end of incubation. Supernatants from

cells incubated with silica show enzymatic activity earlier: measurable amounts of these enzymes are found at zero time when the more toxic

TABLE VIII

Isocitrate dehydrogenase activity in the incubation medium per 10^6 cells incubated

Dust added	Milliunits isocitrate dehydrogenase activity in incubation medium corresponding to 10^6 cells at			
	0′	15′	60′	120′
None	0·00	0·00	0·00	0·11
Carbon (A88M)	0·00	0·00	0·00	0·13
Etched tridymite (T67–70–72M)	0·11	0·33	0·72	1·12
Unetched tridymite (T51M)	0·00	0·11	0·33	0·33
Vitreous silica (VS74M)	0·10	0·00	0·33	0·92

particles are used. As compared with control values, increases in these enzymes are several times higher than in lysosomal hydrolases.

Table IX summarises the experimental results at the end of incubation as percentage increases over control values at the same time.

TABLE IX

Changes in experimental values as percentage of control values after 2 hours' incubation

Value estimated	Change in value, as percentage of control value, when dust added was			
	carbon (A88M)	HF-etched tridymite (T67–70–72M)	unetched tridymite (T51M)	HF-etched fused quartz (VS74M)
Oxygen uptake	−1	−40	0	−8
Enzymatic leakage				
acid ribonuclease	+38	+164	+17	+53
beta-glucuronidase	+33	+215	+29	+194
cathepsin	+25	+212	+110	+180
lysozyme	0	+17	+22	+15
FDP aldolase	0	+2200	+500	+1800
isocitrate dehydrogenase	+20	+1000	+300	+800

DISCUSSION

In these studies of the changes in the hydrolase activities of dust-treated macrophages I have used throughout the "indirect" method, in which lysosomal hydrolases are assayed in the supernatants from incubated cells, and any leakage is assumed to be due to "activation" and intracellular redistribution of the hydrolases (Cohn and Wiener, 1963*b*). The more "direct" method of determining lysosomal hydrolase distribution in subcellular fractions from homogenised cells suffers from the unavoidable mechanical damage produced by dust particles during homogenisation. Cellular integrity during incubation has been checked by determination of the extracellular leakage of two non-lysosomal enzymes whose release is dependent on the state of permeability of the plasma membrane, as suggested from studies on the efflux of enzymes *in vitro* under various conditions (Pellegrino and Bonucci, 1962; Pellegrino and Bibbiani, 1964; Dawson, 1966).

The behaviour of control macrophages during incubation deserves some consideration. In these cells the extracellular release of the lysosomal hydrolases appears to be an early phenomenon. This might be due to lysis of the cells, diffusion of the enzymes from the cells due to unphysiological conditions of incubation or to de-novo synthesis of the enzymes (Cohn and Benson, 1965*c*), and possibly to vacuole formation. All these processes may operate simultaneously, but I have no evidence of any significant lysis of control cells during incubation. As the experiments are performed without synthetic substrate or serum, a loss of granule constituents might occur (see Cohn and Benson, 1965*b*). It is also possible that autophagic vacuoles are formed to provide breakdown products for utilisation in continued maintenance of metabolic processes, and so cause some extracellular hydrolase release. Bertini, Brandes and Buetow (1965) state that starvation *in vitro* brings about increased acid hydrolase activity with formation of autophagic vacuoles.

The addition of dust particles to the incubated cells increases the extracellular release of some of the acid hydrolases studied. Similar observations have been made by Cohn and Hirsch (1960*b*) and Cohn and Wiener (1963*b*) on phagocytic cells that have ingested micro-organisms, and the changes are probably, at least in part, the consequence of the intracellular redistribution of the acid hydrolases after particle ingestion. In-vivo as well as in-vitro endocytosis occurs with silica and carbon dusts. During incubation it is common for dust particles to be adsorbed by the cellular membrane, as is readily observed by phase-contrast microscopy; this is probably the natural prerequisite for particle engulfment, a phenomenon less clearly assessed with these techniques. The modifications of cell metabolism usually implied by phagocytosis (Oren *et al.*, 1963), were not observed in the present study or in previous investigations (Comolli and Perin, 1963*a* and *b*; Munder and Fischer, 1963; Marchisio and Comolli, 1964); as Carr (1962) suggests, the process should involve little energy exchange, since it depends mainly on the surface tension of the cell for its accomplishment.

Though dust-treated cells release increased amounts of acid ribonuclease, beta-glucuronidase and cathepsin into the medium, they release little more lysozyme than the control cells. This failure of leakage of lysozyme which is also a feature of cellular anoxia (unpublished observations), is probably due to the different granule localisation by

this enzyme, which is not manufactured by the phagocytic cell but taken up from the outside (Cohn and Benson, 1965*a* and *b*), and to the lower susceptibility of the lysozyme granule to damage, as Leake and Myrvik (1964) have shown for alveolar macrophages. Acid phosphatase determinations have not been reported in the present paper because reproducible results could not be obtained from replicate experiments. If the possibility of technical errors is ruled out, the inhomogeneity of results found with this enzyme should suggest that no acid phosphatase or minimal amounts of it are released by cells, the bulk of the enzyme being retained inside them; previous observations with leucocidin-treated polymorphonuclears (Woodin and Wieneke, 1964) indicate that alkaline and acid phosphatases in these cells behave similarly. In this connexion, observations by de Duve (1965) seem to indicate a large inhomogeneity among the various hydrolase-carrying particles.

It seems evident that the extent of hydrolase release by the incubated dust-treated macrophages depends very much on the nature, structure and surface activity of the dust tested, etching with hydrofluoric acid (HF) being of paramount importance. Previous studies carried out in this laboratory, in which it has been shown that the addition of silica to cells and cell-free systems deranges protein biosynthesis (Comolli and Perin, 1963*b*; Comolli, 1965), support this concept. Present results indicate that a vitreous HF-etched silica (VS74M) has more hydrolase-releasing effect than a crystalline unetched one (T51M). In this connexion, it is important to note that in-vivo experiments with T51M silica have demonstrated the low fibrogenicity of this dust when it is introduced into the bronchial tree of rats (Clerici, Mocarelli and Bairati, 1965). By contrast, the crystalline HF-etched silica T67–70–72M has the highest toxic effect on incubated macrophages. Carbon particles have little or no effect on the extracellular release of acid hydrolases, acid ribonuclease excepted.

Taking the results as a whole, it appears very probable that the slightly increased release of acid hydrolases by macrophages incubated with inert carbon dusts is the consequence of the intracellular redistribution of the lysosomal hydrolases after particle ingestion and phagosome formation. Presumably acid hydrolases are "activated" by particle ingestion, and this activation precedes and is possibly one of the main causes of death of cells that have engulfed dusts. In the presence of silica, hydrolase release is greater and its level is closely associated with the cytotoxicity of the various silica preparations. In these conditions, the extracellular release of non-lysosomal enzymes is relatively greater, as compared with controls, than that of the acid hydrolases (see table IX). This suggests that one of the main characteristics of silica cytotoxicity is the ability of this dust to act on the plasma membrane of incubated macrophages. The increased hydrolase release in the presence of silica might then be the straightforward consequence of increased plasma and lysosome membrane permeability caused by

a loss of cellular osmotic control. These suggestions may be in accordance with the electron-microscope observations by Löblich (1961) on the early changes in phagocytic cells in the silicotic granuloma, where there is evidence of loss of osmotic control by these cells with vesiculation of the tubular profiles of the endoplasmic reticulum, changes very similar to those found in liver cells subjected to ischaemia, where changes in the plasma membrane occur soon after interruption of the blood supply (Bassi and Bernelli-Zazzera, 1964).

Increased hydrolase release by macrophages incubated in the presence of silica dusts may therefore be a result only of cellular damage rather than the mechanism by which silica damages the phagocytic cells, as supposed by Harington and Allison (1965). The changes in leucocidin- (Woodin and Wieneke) and streptolysin-treated polymorphonuclear leucocytes (Zucker-Franklin, 1965) are similar.

Recent observations by Stalder and Stöber (1965), extending those by Harley and Margolis (1961) with colloidal silica, show that different fibrogenic silica modifications lyse erythrocytes, possibly by adsorption on membrane lipids. This suggests that one of the earliest actions of silica on incubating macrophages may be on the ultrastructural constituents of the plasma membrane, after adsorption of dust on this membrane has taken place.

Summary

When rat peritoneal macrophages are incubated *in vitro*, some lysosomal enzymes—acid ribonuclease, beta-glucuronidase and cathepsin leak through apparently intact plasma membranes into the suspending fluid. Addition of carbon dust, unetched tridymite or vitreous silica to the incubated macrophages slightly increases the leakage, which is greatest when etched tridymite is used. With carbon there is no significant increase in plasma membrane permeability, as judged from leakage of membrane-bound and cell-sap enzymes, but with silica the increased plasma membrane permeability runs parallel with lysosomal enzyme leakage and both run parallel with the cytotoxicity of the silica. It is therefore suggested that an important factor in the cytotoxic effect of silica is its action on the plasma membrane.

Addendum

Since this paper was submitted for publication, Munder *et al.* (1966) and Nash, Allison and Harington (1966) have published studies on the effect of polymeric silicic acid and of tridymite on membrane phospholipids. The former study demonstrates haemolysis of red cells by polymeric silicic acid, acting possibly as a hydrogen donor in the formation of hydrogen-bonded complexes with active groups (quaternary or phosphate ester groups) of phospholipids or, to a lesser extent, with secondary peptide groups of membrane proteins. The

paper by Munder *et al.* presents evidence of a very rapid phospholipid-splitting action by tridymite on macrophages, possibly through phospholipase-A activation, as suggested by control experiments with red cells.

An important paper on a similar subject has recently been published by A. C. Allison, J. S. Harington and M. Birbeck (1966, *J. Exp. Med.*, **124**, 141). Their investigations with cultured peritoneal macrophages point to a primary reaction between silica and the membrane of the phagolysome containing the dust. They show that, in the conditions they used, *p*-nitrophenylphosphatase and β-glucuronidase are released from cells into the medium.

REFERENCES

ALLISON, A. C. . . . . 1965. *Nature, Lond.*, **205**, 141.
ALLISON, A. C., AND SANDELIN, K. . 1963. *J. Exp. Med.*, **117**, 879.
AMBERSON, W. R., ROISEN, F. J., AND BAUER, ADELIA C. 1965. *J. Cell. Comp. Physiol.*, **66**, 71.
BASSI, MARIA, AND BERNELLI-ZAZZERA, A. 1964. *Expl Molec. Path.*, **3**, 332.
BERTINI, F., BRANDES, D., AND BUETOW, D. E. 1965. *Biochim. biophys. Acta*, **107**, 171.
CARR, I. . . . . 1962. This *Journal*, **83**, 443.
CLERICI, E., MOCARELLI, P., AND BAIRATI, A., JR 1965. *Archs Path.*, **80**, 9.
COHN, Z. A., AND BENSON, BELINDA 1965*a*. *J. Exp. Med.*, **121**, 153.
" " " " 1965*b*. *Ibid.*, **121**, 835.
" " " " 1965*c*. *Ibid.*, **122**, 455.
COHN, Z. A., AND HIRSCH, J. G. . 1960*a*. *Ibid.*, **112**, 983.
" " " " . 1960*b*. *Ibid.*, **112**, 1015.
COHN, Z. A., AND WIENER, EDITH . 1963*a*. *Ibid.*, **118**, 991.
" " " " . 1963*b*. *Ibid.*, **118**, 1009.
COMOLLI, R. . . . . 1965. *Experientia*, **21**, 543.
COMOLLI, R., AND PERIN, A. . . 1963*a*. *Med. Lavoro*, **54**, 329.
" " " . . . 1963*b*. *Proc. Soc. Exp. Biol. Med.*, **113**, 289.
DAWSON, D. M. . . . . 1966. *Biochim. biophys. Acta*, **113**, 144.
DE DUVE, C. . . . . 1959. *In* Subcellular particles, ed. by T. Hayashi, *New York*, p. 128.
" . . . . . 1963. *In* Lysosomes (Ciba Foundation Symposium), ed. by A. V. S. de Reuck and Margaret P. Cameron, *London*, p. 1.
" . . . . . 1964. *In* International Symposium on Injury, inflammation and immunity, ed. by L. Thomas, J. W. Uhr and L. Grant, *Baltimore*, p. 283.
" . . . . . 1965. *Harvey Lect.*, **59**, 49.
FISKE, C. H., AND SUBBAROW, Y. . 1925. *J. Biol. Chem.*, **66**, 375.
GREEN, D. E., MURER, E., HULTIN, H. O., RICHARDSON, S. H., SALMON, B., BRIERLEY, G. P., AND BAUM, H. 1965. *Archs Biochem. Biophys.*, **112**, 635
HARINGTON, J. S., AND ALLISON, A. C. 1965. *Med. Lavoro*, **56**, 471.

HARLEY, J. D., AND MARGOLIS, J. . 1961. *Nature, Lond.*, **189**, 1010.
HIRSCH, J. G. . . . . 1962. *J. Exp. Med.*, **116**, 827.
HIRSCH, J. G., BERNHEIMER, A. W., AND WEISSMANN, G. 1963. *Ibid.*, **118**, 223.
HIRSCH, J. G., AND COHN, Z. A. . 1960. *Ibid.*, **112**, 1005.
JUDAH, J. D., AHMED, K., AND MCLEAN, A. E. M. 1964. *In* Cellular injury (Ciba Foundation Symposium), ed. by A. V. S. de Reuck and Julie Knight, *London*, p. 187.
KEISER, H., WEISSMANN, G., AND BERNHEIMER, A. W. 1964. *J. Cell Biol.*, **22**, 101.
KERR, J. F. R. . . . . 1965. This *Journal*, **90**, 419.
LEAKE, EVA S., AND MYRVIK, Q. N. 1964. *Brit. J. Exp. Path.*, **45**, 384.
LÖBLICH, H. J. . . . . 1961. *Beitr. Silikose-Forsch.*, **71**, 33.
LOCKWOOD, W. R., AND ALLISON, F. 1963. *Brit. J. Exp. Path.*, **44**, 593.
LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., AND RANDALL, ROSE J. 1951. *J. Biol. Chem.*, **193**, 265.
MALLUCCI, L., AND ALLISON, A. C. 1965. *J. Exp. Med.*, **121**, 477.
MARCHISIO, MARIA A., AND COMOLLI, R. 1964. *Med. Lavoro*, **55**, 401.
MUNDER, P. G., AND FISCHER, H. . 1963. *Beitr. Silikose-Forsch.*, Sbd **5**, 21.
MUNDER, P. G., MODOLELL, M., FERBER, E., AND FISCHER, H. 1966. *Biochem. Z.*, **344**, 310.
NASH, T., ALLISON, A. C., AND HARINGTON, J. S. 1966. *Nature, Lond.*, **210**, 259.
NORTH, R. J., AND MACKANESS, G. B. 1963. *Brit. J. Exp. Path.*, **44**, 601.
OREN, RACHEL, FARNHAM, ANN E., SAITO, K., MILOFSKY, EVA, AND KARNOVSKY, M. L. 1963. *J. Cell Biol.*, **17**, 487.
PAIGEN, K. . . . . 1961. *Expl Cell Res.*, **25**, 286.
PELLEGRINO, C., AND BIBBIANI, CECILIA 1964. *Nature, Lond.*, **204**, 483.
PELLEGRINO, C., AND BONUCCI, E. . 1962. *Sperimentale*, **112**, 247.
SCHNEIDER, W. C., AND HOGEBOOM, G. H. 1952. *J. Biol. Chem.*, **198**, 155.
SHUGAR, D. . . . . 1952. *Biochim. biophys. Acta*, **8**, 302.
SLATER, T. F., AND GREENBAUM, A. L. 1965. *Biochem. J.*, **96**, 484.
STALDER, K., AND STÖBER, W. . . 1965. *Nature, Lond.*, **207**, 874.
TALALAY, P., FISHMAN, W. H., AND HUGGINS, C. 1946. *J. Biol. Chem.*, **166**, 757.
TRUMP, B. F., GOLDBLATT, P. J., AND STOWELL, R. E. 1965. *Lab. Invest.*, **14**, 1946.
WEISSMANN, G., BECHER, B., AND THOMAS, L. 1964. *J. Cell Biol.*, **22**, 115.
WEISSMANN, G., KEISER, H., AND BERNHEIMER, A. W. 1963. *J. Exp. Med.*, **118**, 205.
WOODIN, A. M., AND WIENEKE, A. A. 1964. *In* Cellular injury (Ciba Foundation Symposium), ed. by A. V. S. de Reuck and Julie Knight, *London*, p. 30.
ZUCKER-FRANKLIN, DOROTHEA . . 1965. *Amer. J. Path.*, **47**, 419.
ZUCKER-FRANKLIN, DOROTHEA, AND HIRSCH, J. G. 1964. *J. Exp. Med.*, **120**, 569.

Brit. J. Exp. Path. 12: 239-244, Aug. 1931

THE RETARDATION OF LYTIC PROCESSES BY COLLOIDAL SILICA SOLUTION.

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SILICA, in colloidal solution, is known to possess curious and, at first sight, anomalous properties in relation to the lysis of red blood-corpuscles. On the one hand, Landsteiner and Jagic (1904) have shown that silica sol may act like a hæmolytic "amboceptor," so altering erythrocytes that they dissolve on the subsequent addition of fresh active rabbit serum; and, on the other, Landsteiner and Stankovic (1906) have proved that a mixture of colloidal silica and ox serum inhibits the complementary action of a hæmolytic system.

One of us (S. L. C.) showed (1922) that colloidal silica completely protects typhoid bacilli from the bactericidal action of fresh human serum, and, in attempting to explain the mechanism of this protection, carried out experiments which suggested that colloidal silica solution prevented specific hæmolysis by interfering with the action of complement, and drew the conclusion that its anti-bactericidal action is due to the same property. It seems probable that the factor at work in all these experiments is the fine precipitate produced by the addition of silica sol to serum—the "kieselsaureeiweissfällung" of Landsteiner and Stankovic.

As a preliminary to further investigations, it seemed desirable to study more closely the anti-bactericidal properties of colloidal silica solution in regard to relatively serum-soluble organisms on the one hand, and relatively serum-fast organisms on the other.

The technique adopted was that used in the earlier experiments with typhoid bacilli in 1922, and need not be described again here. It suffices to say that our more recent experiments, on precisely similar lines to those of 1922, have proved conclusively that the addition of colloidal silica solution, under the condition of our experiments, completely prevents the lysis of *B. typhosus* (Rawlings), *B. coli communis* and *B. dysenteriæ* (Flexner) by normal human blood or fresh human serum.

On the other hand, we have found that relatively serum-resistant organisms, including staphylococci, streptococci and tubercle bacilli, are just as numerous after exposure to the action of blood without admixture of silica, as after exposure to a mixture of blood and silica sol. These germs, being naturally resistant against the solvent action of blood and of blood-serum, derive no advantage from the presence of a substance capable of inhibiting "complement."

Another series of experiments with *B. typhosus*, in which the blood was left out and a similar volume of nutrient broth substituted, served to show that colloidal silica solution possesses no growth-stimulating properties *in vitro*.

These preliminary tests confirmed the conclusion already formed that the action of colloidal silica solution, in preventing the destruction of serum-soluble bacteria, depends upon an interference with the lytic action of "complement."

Earlier unpublished observations on tryptic digestion had, however, suggested that silica sol might possess anti-lytic powers apart from this interference with complement, and so we decided to examine its effects upon the lysis of pneumococcus in ox bile—a reaction in which complement is not known to play a part.

Experiment I.

A fairly strong suspension of Type I pneumococcus culture in sterile normal saline emulsion was prepared and a supply of fresh ox bile obtained. The solution of colloidal silica employed was prepared by Dr. Tytler from water-glass (Boots), according to the method described by Gye and Purdy (1922). This solution, used throughout all the following experiments, was found to give a residue of 0·878 per cent. when dried, and its activity was proved by its power to agglutinate erythrocytes in salt solution, and its antibactericidal action in relation to typhoid bacilli.

The following mixtures of the above reagents were set up in small test-tubes:

Mixture I:

Pneumococcus emulsion	5 parts.
Normal saline	4 ,,
Ox bile	1 part.

Mixture II:

Pneumococcus emulsion	5 parts.
Colloidal silica solution	4 ,,
Ox bile	1 part.

Mixture III:

Pneumococcus emulsion	5 parts.
Normal saline	5 ,,

All three mixtures were placed in the incubator at 37° C. for one hour, after which they were removed and examined.

Naked-eye appearances:

Mixture I: Clear.
Mixtures II and III: Turbid.

Hanging drop:

Mixture I: Very few pneumococci visible.
Mixtures II and III: Very many pneumococci seen.

Stained films (Gram's stain):

Mixture I: Hardly any pneumococci to be found. Much granular *débris.*
Mixtures II and III: Numerous pneumococci staining normally and showing well-marked "negative" capsules.

From this experiment it was clear that the presence of silica sol interfered to a marked extent with the lysis of pneumococci by ox bile.

This finding, which suggested that colloidal silica solution was capable of interfering with a lytic process regarded as being independent of the presence of complement, led us back to the earlier unpublished experiments on the interference of silica sol with tryptic digestion.

An attempt was now made to repeat these as follows:

Experiment II.

Allen & Hanbury's trypsin solution ("Bacteriological in Ampoules") was used in a 1·8 per cent. solution of sodium phosphate as the digestive fluid. To this, in final concentrations of 1/20 and 1/40, slices of coagulated egg-white 3 mm. square, and cut so thin as to be almost transparent, were added, with and without 0·5 c.c. silica sol. It was found that, especially with the weaker solutions of trypsin, the digestion of egg-white was markedly retarded.

Experiment III.

This experiment was carried out with freshly prepared fibrin. About 1 c.c. of freshly drawn human blood was stirred with a thin glass rod during clotting. The fibrin, collected on the end of the glass rod, was washed in running water until nearly free from colour. Two masses of fibrin, so prepared, and of approximately equal size, were added to trypsin diluted 1/25 with phosphate, with and without an equal volume of silica sol.

The result, after two hours at 37° C., showed that whereas the control without silica was digested, with silica the fibrin was merely broken up.

These experiments indicated that tryptic digestion was interfered with to a considerable extent by silica sol, but suggested that the action was a retardation rather than an actual inhibition of lysis, the time factor being of importance. Similar conclusions were drawn from experiments in which the degree of tryptic digestion was estimated with Congo-red fibrin and also in the case of peptic digestion.

In view of the known effects of inhaled silica dust in leading to lung fibrosis and lymphatic obstruction in those exposed to it for sufficient periods of time, and in view of the theory enunciated by Gye and Kettle (1922), and now generally accepted, that "the fibrosis brought about by finely divided silica" is "due to the slow formation in the tissues of silica sol," these anti-lytic properties appertaining to or associated with colloidal silica solution appear worthy of consideration in connection with the pathology of silicosis.

All those who have examined sections from silicotic lungs are familiar with the collections of dust-cells to be seen packed tightly into, and apparently blocking up, the perivascular and peribronchial lymphatics. These cell collections may be seen months or years after the miner has ceased to be exposed to dust, and the question arises as to how the cells come to persist.

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Mavrogordato (1922) suggests that "there are two reactions characteristic of free silica:

"A. That it exerts a preservative action on the cells that take it up.

"B. That cells laden with free silica tend to flock together and are preserved *en masse*, *i. e.* tend to agglutinate and form pseudo-tubercles."

To explain this preservation he suggests the following hypothesis: "That the fine particles of free silica are slightly soluble in the alkaline tissue juices and that there is a drift of alkaline silicate through the invaded cells, the principle being similar to that of the preservation of eggs by water-glass. Tissue death is associated with acid autolysis and the alkaline silicate will be precipitated as silica acid, thus, perhaps, preserving the cells as 'mummies' by impregnation."

The experiments above described appear to add significance to the suggestion of Mavrogordato as to the possibility of a prevention of acid autolysis of dust-cells by silica in solution.

As to the agglutination of these cells into masses, another curious property of colloidal silica comes to mind. Landsteiner and Jagic (1904) called attention to the agglutination of red blood-corpuscles by silica sol in the presence of salt in appropriate amount. One of us (S. L. C.) (1922, *b*) independently observed and reported the same phenomenon, and he takes this opportunity of expressing his regret that, being at that time unaware of the earlier work of Landsteiner, he failed to refer to it in his paper.

In view of these anti-lytic and cell-agglutination properties of colloidal silica, the following experiment, designed to throw further light on its effects upon a suspension of leucocytes and dust-cells, was carried out:

Experiment IV.

A heavy suspension of silica dust was made in sterile saline solution. The dust used was part of a sample given us by Dr. Mavrogordato, and represented the insoluble residue recovered from the lungs of a gold miner dying from silicosis on the Rand.

Two c.c. of this suspension were inoculated intraperitoneally into guinea-pig No. 1936 on January 6th, 1931. This animal was killed next day, and a sample of peritoneal fluid removed with sterile precautions, and mixed with an equal volume of sterile 3·8 per cent. solution of sodium citrate to prevent clotting.

Mononuclear cells larger than the usual blood monocytes were a conspicuous feature in "wet" preparations. Examined under "crossed Nichols," many of these large cells were seen to contain doubly refracting particles, presumably phagocyted silica dust.

This suspension of leucocytes and dust-cells was divided into two portions, one, labelled A, being mixed with an equal volume of sterile normal saline solution, and the other, labelled B, with an equal volume of colloidal silica solution. These mixtures were incubated in sealed capillaries, and pairs of these were examined from time to time.

The results were as follows:

Examination of Fixed Films.

8.1.31	Series A	Cells evenly distributed and well preserved.
	,, B	Cells agglutinated into irregular masses: the individual cells being well preserved and each mass apparently held together in a gelatinous matrix.
15.1.31	,, A	Cells rare and degenerated.
	,, B	Cells in agglutinated masses as before: many well preserved but some degenerated.
29.1.31	,, A	Cells very rare.
	,, B	Many cells in masses.
5.2.31	,, A	Cells degenerate and ill-stained.
	,, B	Many cells well preserved and in masses, but some are ill-stained and irregular in outline.
19.2.31	,, A	Cells all appear degenerate.
	,, B	Many cells still in masses and well preserved, though others are indistinct in outline.

Cell enumeration in Füchs and Rosenthal Cytometer.

	Series A.	Series B.
8.1.31	117 cells per c.mm.	Cells uncountable.
15.1.31	16 ,, ,,	Uncountable.
29.1.31	4 ,, ,,	29 per c.mm. ("degenerates" omitted).
5.2.31	11 ,, ,,	132 per c.mm. (estimate only).
19.2.31	13 ,, ,,	67 per c.mm.

The "counts" in Series B were always difficult owing to the large number of cells present and their agglutination in clumps. This agglutination seemed to become rather less marked from January 29th, 1931, onwards, but the counts were never satisfactory.

This experiment, by indicating that the cells were better preserved in Series B than in Series A, suggested that it was the added colloidal silica solution rather than the phagocyted silica particles which had led to the interference with the normal cytolysis observed. It seemed probable that the cells lost their vitality shortly after removal from the body, and that the tendency to slow solution of silica particles in phagocytes, postulated by various workers to occur *in vivo*, was not operative under the conditions of our experiment.

To obtain further information on this point, the following experiment was now carried out:

Experiment V.

Two guinea-pigs were inoculated intraperitoneally, one with anthracite dust in saline and the other with silica dust.

Both these animals were killed in 24 hours, and their peritoneal fluid pipetted off with sterile precautions, citrated, and the fluid from each guinea-pig put up in two series, Series A being without and Series B with added colloidal silica solution.

The results in both animals were similar to those obtained in Experiment IV, showing that it was the added colloidal silica solution which was operative in agglutinating and preserving the cells, rather than any silica which might have been dissolved from the quartz dust used to produce the cellular exudate.

It was, of course, improbable that the time allowed in these experiments would suffice to allow of the slow solution of silica particles which appears to take place in the silicotic lung. It was for this reason that we depended on the added silica sol to obtain the effects noted.

CONCLUSIONS.

1. Following upon an earlier observation that the addition of colloidal silica solution preserves typhoid bacilli from lysis in human blood or serum, we have shown that this property extends to other serum-soluble bacteria.

2. We have obtained evidence that colloidal silica solution is also able to preserve Type I pneumococci from lysis in ox bile.

3. Our experiments show that both tryptic and peptic digestion of albuminous substances is markedly retarded by the presence of colloidal silica solution.

4. We find that the addition of colloidal silica solution to a cellular exudate produced by the intraperitoneal injection of dust particles leads to the agglutination of the leucocytes and dust-cells and to the preservation of a large proportion of these cells, intact though probably dead, for a period of two months and upwards, beyond which time our observations have not been extended.

5. We think it probable that these anti-lytic and agglutinating properties of colloidal silica solution may, through interference with cytolysis, be factors in the production of the lymphatic obstruction associated with pulmonary silicosis in man.

We desire to express our thanks to Dr. W. H. Tytler for help and advice in the biochemical problems involved in our experiments, and the Medical Research Council for a grant which has facilitated our work.

REFERENCES.

CUMMINS, S. L. (1922, *a*) *Brit. J. Exp. Path.*, **3**, 237; (1922, *b*) *ibid.*, **3**, 260.
GYE, W. E., AND PURDY, W. J. (1922) *Ibid.*, **3**, 75.
GYE, W. E., AND KETTLE, E. H. (1922) *Ibid.*, **3**, 241.
LANDSTEINER, K., AND JAGIC, N. (1904) *Munch. med. Wschr.*, No. 27.
LANDSTEINER, K., AND STANKOVIC, R. (1906) *Zbl. Bakt.*, **42**, 353.
MAVROGORDATO, A. (1922) *Rep. No. 15 S. Afr. Inst. Med. Res.*, p. 121.

Brit. J. Exp. Path., 12: 245-249, 1931

EFFECTS OF COLLOIDAL SILICA UPON THE GROWTH OF TUBERCLE BACILLI IN BLOOD.

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GYE and Kettle (1922, *a*) have described experiments which point to a marked adjuvant action of silica in establishing the growth of tubercle bacilli in animal tissues. These authors consider that, when tubercle bacilli are introduced into the subcutaneous tissue of mice along with silica, "a small dose of bacilli becomes a dose of considerable magnitude and, inasmuch as an important factor in determining an infectious process is the number of organisms introduced, a simple explanation is forthcoming of the effect of silica upon such tuberculous lesions as we have described."

The same authors (1922, *b*), in a second paper published in the same year, investigated the effects of silica sol on the course of a general tuberculosis in susceptible animals. They found that "silica sol circulating in the blood-stream does not materially influence a general tuberculosis. It is apparently only when the poison is sufficently concentrated upon local areas of tissue destruction that the growth of the tubercle bacillus is promoted." Kettle (1924), further to this communication, concluded that "tubercle bacilli injected into the circulation of rabbits and mice, tend to settle in localized subcutaneous lesions produced by various agents which bring about increased vascularity and tissue necrosis. . . . Inasmuch as similar results are not obtained with the provocative agents, silica appears to have some specific action on the growth of the tubercle bacillus in the tissues."

Gye and Kettle, in the light of their experiments, concluded that "the abnormal prevalence of tuberculosis among men exposed to fine silica dust may be explained by (1) the destructive action of silica on cells whereby foci of necrosis are produced in which tubercle bacilli can multiply, and (2) the disorganization of the lymphatic drainage of the lung"; and they expressed the view that "the actual cell poison is a soluble derivative of silica."

It was the enunciation of these views, in which so much stress was laid upon the importance of silica in the form of a colloidal solution, that led one of us (S. L. C., 1922) to investigate the inhibitory effects of silica sol upon the bactericidal action of human blood. It seemed likely that the presence of silica as a colloidal solution in a restricted area of tissue might favour the growth of pathogenic bacteria by preventing or diminishing the bactericidal action of the cells and the humours; and this question appeared to be capable of submission to experiment *in vitro*.

As a preliminary, it was decided to work with a germ markedly susceptible

to the bactericidal action of normal blood. An old laboratory strain of *B. typhosus* (B. T. Rawlings) was therefore used for the investigation. Experiments carried out with this organism proved that the presence of silica sol was sufficient to abolish the bactericidal action of human blood or blood-serum on *B. typhosus*. Further, it was demonstrated by haemolytic tests on similar lines that this action of colloidal silica depended upon an interference with the lytic action of "complement."

Further experiments since performed and described elsewhere (p. 239) have shown that this antibacteriolytic action of colloidal silica is only demonstrable for serum-soluble organisms such as *B. typhosus*, *B. coli* and *B. dysenteriæ*. No such action can be demonstrated in the case of relatively serum-fast bacteria such as staphylococci and streptococci; nor is the technique employed for experiments with *B. typhosus* well calculated to bring to light any form of anti-bactericidal activity not depending on the inhibition of lytic processes.

In working with *B. typhosus* and other serum-soluble bacteria, it is necessary to restrict to 1 hour 40 minutes the time of exposure of the bacillary emulsion to the action of blood, in order to avoid the possibility of confusion of results through the rapid multiplication of a few bacteria surviving after the lysis of the majority.

In the case of a slow-growing organism such as the tubercle bacillus, however, it appeared safe to allow of a much longer time. We therefore decided to leave our cultures for a week or two instead of a few hours, and to rely, for the reading of results, not only on sub-cultures, as in the previous experiments, but on microscopic examination and the rough enumeration of tubercle bacilli or colonies in stained films.

Wright (1924) proved by special methods of culture that tubercle bacilli can grow freely in blood-clots, and at the same time afforded evidence that the aggregation of leucocytes around masses of bacilli in such preparations has a definite anti-bacterial effect. This latter point appeared of much interest in view of the suggestion by Gye and Kettle that the adjuvant action of silica upon the growth of tubercle bacilli in the tissues depends upon its being a cell-poison. Our experiments with staphylococci and streptococci had shown that there was little chance of demonstrating an anti-bacteriolytic action of silica in the protection of a germ so serum-fast as the tubercle bacillus in contact with blood; but there remained the interesting question whether the bacilli might, by this means, be protected from the anti-bacterial action of the leucocytes through the poisoning of these cells by silica.

Attempts to employ Wright's technique of growth in clots for our experiments failed, because the addition of silica sol interfered with the normal process of coagulation. It was obvious that we must devise a new technique. Here it will suffice to describe that finally selected. One of the main difficulties in work of this sort lies in the liability to air-borne contaminations. Another difficulty is to ensure sufficient aëration of the surface of the culture. This, as pointed out by Wright in his work on the growth of tubercle bacilli in clots in capillary glass tubes, is a matter of considerable importance.

For reasons already given we abandoned attempts to work with clot-cultures, and our aim was to employ instead small volumes of fluid blood.

Preparation of Tubercle Bacillary Emulsion.

From a 4-weeks culture of tubercle bacilli on egg-medium a sufficient amount of growth was removed on a platinum spatula and introduced into a thick-walled tube. Using the rounded end of a glass rod, "hooded" with a piece of cardboard to afford a shield against air contaminations, masses of tubercle culture were slowly ground up into a paste with drops of sterile 3·8 per cent. citrate solution. Finally, sufficient citrate solution was added to make a thin grey emulsion, which was then "spun" for a few minutes to remove clumps.

Preparation of Cultures.

Four 0·01 c.c. volumes of sterile blood were drawn up from the finger into a pipette, and followed by one volume of citrated tubercle bacillary emulsion, and five volumes of either sterile saline solution or of sterile colloidal silica solution.

The whole, amounting to a volume of 0·1 c.c., was now blown out into a Dreyer's tube and gently mixed by means of the pipette. The outer portion of the plug was snipped off with a sterile scissors and the remainder pushed half-way down the tube. On to this inner plug of cotton-wool a little sterile water was dropped, and the tube closed with a small rubber stopper and sealed with paraffin wax. The cultures so prepared were incubated for ten to twelve days in a sloped position.

Spreading and "Counting" of the Cultures.

The contents of each culture tube were drawn up into a sterile pipette and gently mixed on a glass slide. From this mixture a standard loopful was spread evenly over a circular area 15 mm. in diameter on a microscope slide, along with a similar loopful of a standard staphylococcus emulsion. The latter was added to provide an indication as to whether the films were evenly spread. It should be noted that the cultures containing silica tended to be rather more viscid, and to contain more *débris* than those containing saline only. All the films, made as above, were fixed by heat, stained with Ziehl and methylene-blue.

The tubercle cultures used consisted of 4-weeks-old growths on egg medium and were of origin as follows:

(1) T.B. Constantanides. Virulent strain. Isolated at Cardiff.
(2) T.B. "R. 1." From Trudeau Laboratories, Saranac (non-virulent).
(3) T.B. A. W. Jones. Strain of low virulence. Isolated at Cardiff.

The counting of the cultures was carried out as follows:

Using a $\frac{1}{6}$-in. objective and a No. 3 ocular with a square obturator, the visible "colonies" were counted in successive fields from top to bottom across the diameter of the circle. This meant that about 20 fields were counted, including the thinner central and the thicker peripheral parts of the film. By using this rather low magnification only growing "colonies" of tubercle bacilli were seen, the individual bacilli, which might have merely survived from the originally implanted emulsion, being invisible. The colonies of tubercle bacilli were found to vary much in size and consisted of wavy whorls of growth.

In order to take the size as well as the numbers of colonies into account we classified the colonies into five groups, the smallest being included in Group A, the largest in Group E, and the intermediate ones in Groups B, C and D according to size. In comparing the growths we adopted the convention of allotting 1 mark for each colony in Group A, 2 for each in Group B, and so on up to 5 marks for each colony in Group E. The total of marks for all the colonies added together was recorded as the "value" for each film. Examination of a series of fields across the same diameter with the $\frac{1}{12}$-in. oil-immersion lens and a smaller "obturator" assured us that the cocci were evenly spread, indicating that the films were roughly comparable in this respect. The results of a "count" on these lines are given in Table I:

TABLE I.—*"Colony" Counts in Blood-Cultures of Tubercle Bacilli.*

Film No.	T.B. strain. Source.	Blood source.	Saline or silica.	Total colonies.	"Value" in marks.	Average number of cocci per field.
1.	Constantanides	C. W—*	Saline	59	158	47·1
2.	Ditto	C. W—	Silica	49	114	54·0
3.	R. 1	C. W—	Saline	47	129	47·3
4.	Ditto	C. W—	Silica	151	372	64·3
5.	Jones	C. W—	Saline	110	269	39·5
6.	Ditto	C. W—	Silica	78	167	30·6
7.	R. 1	K. S—†	Saline	142	353	30·9
8.	Ditto	K. S—	Silica	64	139	42·8
9.	Jones	K. S—	Saline	56	109	38·3
10.	Ditto	K. S—	Silica	5‡	5‡	32·0‡

* A healthy individual.
† An arrested case of pulmonary tuberculosis.
‡ Film 10 was not countable across the diameter of the circle as the central part washed off in staining. Only the peripheral part was visible, but this contained very few colonies.

These "counts" show that all the cultures contained growing colonies of tubercle bacilli. The numbers of colonies and the "colony value" was greater in the case of the "saline" cultures than in the "silica" cultures except in Film 4, which, as indicated by the staphylococcus count, was an unusually thick film. The differences, however, are not sufficient to be significant, and it is clear, from this and other similar experiments, that the growth of tubercle bacilli in blood *in vitro* is neither favoured nor inhibited by the presence of colloidal silica solution.

From a similar series of preparations grown at 37° C. for 12 days as above, cultures on egg medium were made and incubated. The growths, after five weeks' incubation of the egg media, were well marked in all, being, however, rather more profuse in those tubes inoculated from the "blood-saline" than in those from the "blood-silica" preparations.

It is interesting to note that, in the blood of K. S—, an "arrested" case of pulmonary tuberculosis, there appeared to be more profuse growth of tubercle bacilli in the "saline" than in the "silica" mixtures. Had the silica possessed any considerable anti-bactericidal power, it might have been expected to show

it here by affording a greater protection, and leading to a more marked growth of tubercle bacilli in a blood presumably fairly active against the tubercle bacillus.

Our experiments "*in vitro*," all being variations of the general scheme of investigation above described, lead us to the conclusion that silica sol does not in any way further the pullulation of tubercle bacilli in human blood outside the body.

These experiments have been facilitated by a grant from the Medical Research Council, for which we desire to express our thanks.

REFERENCES.

CUMMINS, S. L.—(1922) *Brit. J. Exp. Path.*, **3**, 237.
GYE, W. E., AND KETTLE, E. H.—(1922, *a*) *Ibid.*, **3**, 211; (1922, *b*) *Lancet*, **2**, 855.
KETTLE, E. H.—(1921) *Brit. J. Exp. Path.*, **5**, 158.
WRIGHT, SIR A. E.—(1921) *Lancet*, **1**, 218.

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ACUTE TOXICITY OF MINERAL DUSTS

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GYE AND PURDY[1] showed that there was massive intravascular clotting in rabbits receiving intravenously administered injections of silicic acid. Although this clotting was secondary to endothelial damage, it was the cause of death. Modell and Salzman,[2] using cats, found that even when an anticoagulant was used death still occurred. Their silicic acid was prepared by hydrolyzing sodium silicate, and the lethal dose was determined by continuous intravenous injection. They were able to show that there was no correlation between toxicity and either the acidity of the solution or the rate of injection. The systemic effects included accelerated respiration, which occurred simultaneously with or soon after a marked drop in blood pressure, progressive weakness, and finally clonic convulsions. A marked drop in blood pressure was rarely followed by recovery. In fatal cases the right side of the heart and the great vessels were distended with blood, while the left side was essentially empty. It was suggested that the silicic acid exerts its toxic effect by constricting the blood vessels of the lung; Filley, Hawley, and Wright[3] have shown that colloidal silica produces a specific bronchoconstriction.

Irwin and Gibson[4] made an experimental study of 14 types of quartz by injecting suspensions subcutaneously into rabbits. The tissue response produced at the site of injection was studied at intervals up to two years. All samples gave rise to a nodular fibrosis that was essentially the same in extent and degree, but there were no toxic deaths of the type described above. It is, of course, well known that the interstitial, intraperitoneal, and intratracheal injection of particulate silica produces only a slow chronic silicotic fibrosis and no acute toxic reaction. If given in divided doses over several days, the same particulate quartz can be injected into the blood stream in large amount, whence it is caught by the reticuloendothelial system and

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1. Gye, W. E., and Purdy, W. J.: The Poisonous Properties of Colloidal Silica: The Effects of Repeated Intravenous Injections on Rabbits; Fibrosis of the Liver, Brit. J. Exper. Path. 3:86-94, 1922.

2. 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. 23:774-785, 1941.

3. Filley, G. F.; Hawley, J. G., and Wright, G. W.: Toxic Properties of Silica: Bronchoconstrictor Effect of Colloidal Silica in Isolated Perfused Guinea Pig Lungs, J. Indust. Hyg. & Toxicol. 27:37-46, 1945.

4. Irwin, D. A., and Gibson, C. S.: Experimental Study of Toxicity of Various Types of Quartz, Canad. M. A. J. 39:349-352, 1938.

deposited in the liver and spleen to produce silicotic nodules, and no acute toxic effects are observed. On the other hand, both intratracheal and intravenous injections of very finely divided silica of colloidal dimensions (20 A.) into rats (16 mg. per a 250 gm. rat) produced rapid shock-like death (King [5]); Koch [6] found that large doses of colloidal silica caused prompt death. Rabbits and dogs died quickly after receiving intravenously administered injections of a fairly concentrated solution of silicic acid (freshly neutralized sodium silicate 5%, containing 10 mg. of silica per milliliter) in amounts equivalent to about 10 mg. per kilogram, whereas the same and larger amounts of silicic acid (e. g., 169 and 196 mg. of SiO_2 injected into two dogs weighing 10 kg. each) were tolerated and were rapidly excreted in the urine when large volumes of a much more dilute solution (1 mg. of SiO_2 per milliliter) were used (King, Stantial, and Dolan [7]). The difference was probably due to the formation of colloidal particles and floccules of silicic acid in the concentrated solution and to their absence or near absence from the dilute solution whose silicic acid remained in a state of low molecular size, diffusible and capable of being excreted by the kidney. Very finely particulate quartz (200 mg.) of practically colloidal dimensions (the faintly milky supernatant from fine quartz powder left to settle for several days in isotonic saline, all under 0.1 μ and containing 0.8 mg. of SiO_2 per milliliter) caused hematuria, anuria, and death of the dogs when injected in the same way as the dilute (1 mg. per milliliter) silicic acid solution. Organic silica as the glycol ester of silicic acid is hydrolyzed slowly in water to silicic acid, partly disperse and partly colloidal; injections of this in amounts corresponding to 400 mg. of silica caused the dogs to become ill (nausea and vomiting), to acquire hematuria and later anuria, and to die within a few days.

All these 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 10 μ) 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. Most biological work with silica has been done as long-term experiments to test chronic effects. Only a few investigations have been concerned with immediate toxic effects, and it has seemed to us interesting to conduct further experiments on the injection of a variety of dusts of different composition and particle size, with some of them coated with firmly adsorbed material.

PREPARATION OF MATERIALS FOR INJECTION

Mineral Dusts.—Two varieties of quartz were used. These included rock crystal quartz, a finely powdered preparation mostly under 5μ in size but with numerous particles up to 10μ, and sized quartz, prepared for us by Dr. G. Nagelschmidt from a very pure Belgian glass sand and provided in several size fractions. Kaolin was also prepared by Dr. Nagelschmidt, the particles being practically all between 0.2 and 5μ in size. Ammonford C was a South Wales shale of about the same size. The aluminum dust was the black McIntyre powdered metal used

5. King, E. J.: Solubility Theory of Silicosis: Critical Study, Occup. Med. 4:26-49, 1947.

6. Koch, W.: Action of Different Forms of Silica in Sensitized Animals, Frankfurt. Ztschr. Path. 60:58-96, 1949.

7. King, E. J.; Stantial, H., and Dolan, M.: Biochemistry of Silicic Acid: The Excretion of Administered Silica, Biochem. J. 27:1007-1014, 1933.

for the prevention of silicosis: it was almost entirely 0.05 to 4μ in size. The aluminum hydrate (HX 1010), likewise used to prevent silicosis, was a light white powder, the particles of which consisted of aggregates of lath-like crystals of 0.02 to 0.04 μ in length. The corundum smoke was the condensate from an electrolytic furnace in which bauxite was heated with coke and some iron scrap. It was about half silica, half alumina in composition, and its spherical particles were about 0.02 to 0.5 μ in diameter. The coal dusts (anthracite and bituminous) were prepared by Dr. Nagelschmidt and were both of particle size 0.1 to 2 μ. The 20 A. silica was a condensate of very fine particles averaging about 20 A. (0.002 μ) and was dispersed easily into a faintly milky solution in water or saline.

Aluminum-Coated Quartz.—Four hundred milligrams of quartz (sized less than 2μ) and 421 mg. of $AlCl_3.6H_2O$ was mixed with 10 ml. of water and 10 ml. of imidazole buffer, pH 7.4, and shaken occasionally at room temperature for two weeks. After being centrifuged and washed with water, the mixture was suspended in 8 ml. of isotonic saline (0.9% NaCl) for injection. This adsorption of aluminum on the quartz powder reduced its silica solubility from 4.4 mg. of SiO_2 per 100 ml. to 0.1 mg.

Quartz Coated with Crystal Violet and Quinacrine Hydrochloride.—The quartz was shaken in an aqueous solution of crystal violet, 2.5 mg./100 ml., and quinacrine hydrochloride (Atabrine), 10 mg./ml., at 37 C. for 48 hours, then centrifuged, washed and resuspended in isotonic saline. The quartz remained visibly stained with adsorbed dye.

20A. Silica.—This was similarly adsorbed with aluminum, crystal violet, and quinacrine hydrochloride.

Silica with Adsorbed Protein (from Serum).—One and five-tenths milliliters of serum obtained aseptically from the blood of six mice was diluted with an equal volume of isotonic saline and added to 15 mg. of 20 A. silica. The tube was "pasteurized" at 56 C. for three-fourths of an hour, then kept at 37 C. with occasional shaking for 48 hours.

Colloidal Silica from $Na_2SiO_3.8H_2O$.—$Na_2SiO_3.8H_2$, 2.217 gm., was dissolved in water, neutralized to methyl orange with HCl, made to 25 ml., and dialyzed until free from chloride. After fast mechanical stirring, the larger particles were allowed to settle and the supernatant decanted. This was found to contain 15 mg. of SiO_2 per milliliter and was diluted with NaCl solution to give a final concentration of 5 mg. per milliliter in isotonic saline.

Colloidal Silica from $Si(OC_2H_5)_4$.—$Si(OC_2H_5)_4$, 1.733 gm., was heated with 25 ml. of 0.001N HCl for two days on a water bath. The gel that formed was stirred mechanically, and after the larger particles had been allowed to sink, the silica was estimated in the supernatant. This was 5.7 mg. per milliliter and was diluted to give 5 mg. per milliliter in isotonic saline.

INJECTION

All dusts were shaken through a 300 mesh sieve and with the use of an agate mortar were lightly ground in the isotonic saline until a smooth suspension was obtained. With many dusts, e. g., aluminum and coal, slow rubbing in a mortar with increasing amounts of liquid was the only sure way of bringing them into suspension. The flask containing the suspension was kept agitated to prevent sedimentation. White female adult mice were used, the tail vein being dilated with warm water. With the use of a No. 20 hypodermic needle, the suspension was injected in 0.1 ml. volumes at one-minute intervals until the animal became very weak, then it usually convulsed and died. When suspensions of large particles were used, the syringe was filled with 0.1 ml. only so that settling of the particles would be minimized, and the entire contents were injected. The needle was then left in the vein and the syringe refilled.

RESULTS

The animal response reported by Modell and Salzman in cats was similarly shown in mice in this experiment. Respiration rate increased, and the progressive weakness led to clonic convulsions which were always followed by death. The Table shows the toxicity of the materials used.

Lethal Mouse Doses of Various Mineral Dusts

Dust	Conc. of Suspension, Mg./Ml.	No. of Animals Used	Wt. of Dust Required to Kill 20 Gm. Mouse, Average Mg.	Minimum and Maximum Lethal Dose, Mg.
Rock crystal quartz	50	..	..	....
Freshly prepared	..	3	19	4-38
After 24 hr.	..	3	28	20-30
Rock crystal quartz + 2% aluminum	50	..	..	....
Freshly prepared	..	3	13	12-14
After 48 hr.	..	3	32	31-33
After 96 hr.	..	3	28	26-43
Rock crystal quartz + quinacrine hydrochloride	50	..	..	....
Freshly prepared	..	3	31	8-45
After 48 hr.	..	3	45 mg. did not kill	
Sized quartz (< 2μ)	50	..	..	....
Freshly prepared	..	11	25	7-47
After 48 hr.	..	3	32	29-36
Sized quartz (< 2μ) + 2% aluminum	50	..	..	....
Freshly prepared	..	3	25	19-33
After 48 hr.	..	3	20	11-26
Sized quartz (< 2μ) + $AlCl_3 \cdot 6H_2O$	50	6	20	17-36
Sized quartz (0.2-0.5μ)	50	5	15	3-36
Sized quartz (0.5-1μ)	50	5	30	20-39
Sized quartz (1-2μ)	50	4	32	30-34
Sized quartz (2-4μ)	50	5	17	8-22
Sized quartz (4-8μ)	50	5	17	12-22
Sized quartz (> 8μ)	50	3	14	12-16
Flint (< 0.5μ)	50	4	28	25-33
Kaolin	50	4	6.3	5-12
Aluminum	50	4	29	10-25
Aluminum hydrate	50	4	22	15-28
Aluminum phosphate	50	3	24	23-25
Shale	50	4	28	20-43
Anthracite (< 2μ)	50	3	12	11-13
Bituminous (< 2μ)	50	3	8	7.5-8.5
India ink	110	3	22	20-24
Corundum smoke	50	3	11	9-12
Colloidal silica				
From $Na_2SiO_3 \cdot 8H_2O$	5	4	1.3	0.9-1.7
From $Si(OC_2H_5)_4$	5	4	2.2	1.5-2.7
Silica dust 20 A.	5	..	..	....
Freshly prepared	..	12	1.6	0.1-3.1
Adsorbed crystal violet	..	6	1.5	0.9-3.1
With 2% aluminum	..	6	1.6	1.0-3.2
Adsorbed quinacrine hydrochloride	..	5	0.9	0.1-1.4
Adsorbed $AlCl_3 \cdot 6H_2O$	..	6	1.3	1.1-1.6
Adsorbed serum protein	..	4	1.7	1.5-2.1

COMMENT

Colloidal silica is more than 10 times as toxic as quartz of larger dimensions. Quartz varying in size from 0.2 to 8 μ shows no marked difference in toxicity, nor do any other of the siliceous materials, with the exception of kaolin and corundum smoke, both of which are two to three times more toxic. Bituminous and anthracite coal are also twice as toxic as the quartz. But because of the few animals used for each test, the recorded minimum lethal doses cannot be considered to be very precise, and the various quartzes, flint, kaolin, aluminums, and coals, all of particle sizes from about 0.1 to 10 μ, appear therefore to be rather similar in the amounts of them required to kill. There is, however, an apparent break between their required amounts and those of 20 A. (0.002 μ) silica and colloidal silicic acid. With the big drop in size of particles, the silica has become very much more lethal, and it seems probable that the acutely toxic "pharmacological" effect of silica is a matter of particle size and is given only by suspended silica of colloidal dimensions. The much

larger amounts of larger particulate silica required to kill may well contain some colloidal matter in their suspensions (Clelland, Cumming, and Ritchie [8]).

It is apparent, of course, that not all foreign colloidal matter is toxic in this way; colloidal carbon (India ink), various colloidal dyes, and colloidally dispersed metals and oxides of metals can, as is well known, be tolerated in large amounts. Thus, King and Dolan [9] injected 3 ml. per kilogram of Thorotrast (609 mg. of thorium dioxide) intravenously into rabbits without any apparent effect, either acute or chronic; we have injected India ink into mice in amounts of 0.2 ml. (equivalent to 22 mg. of carbon) before signs of weakness appeared. Silica may not be unique, but we do not know of any other substance which is highly toxic only in the colloidal state, while remaining relatively very much less toxic at a larger size and apparently nontoxic when absorbed in a molecularly dispersed state, as is done constantly in large amounts by all herbivorous animals which ingest silica in their food, absorb much of it, and promptly excrete it in the urine (King and Stantial [7]). The reverse is, of course, quite common; e. g., dissolved arsenic is lethal, while colloidal arsenic can be tolerated in considerable amount.

It could, of course, be argued that the phenomenon is due entirely to surface area; i. e., that colloidal silica has a much greater area than particulate silica of "respirable" dimensions. But in the absence of any knowledge of what "surface" of silica particles implies in the way of special properties, this seems to us to beg the question. It may be that particles of silica, particularly those of the crystalline varieties, have special surface properties which may cause biological effects independent of any silicic acid that may dissolve away from them. It could, for instance, be argued that the prevention of silicosis by coating quartz particles with aluminum can be explained in this way, although the prevention of any silica going into solution because of the coating with aluminum seems a more probable explanation.

It is interesting that substances which adsorb strongly on quartz dust, e. g., aluminum, dyes, and proteins, have no effect on the acute toxic properties of colloidal or 20 A. silica as well as none on quartz itself, although one of them (aluminum) so markedly lessens the fibrogenic activity of quartz.

Although these results have no direct bearing on silicosis, they do suggest a possible mechanism of silicosis production. Fine quartz particles release silicic acid into solution in a liquid or in the liquid contents of a cell. Some of the silicic acid escapes into the blood and is carried away to be excreted; other silicic acid molecules polymerize to colloidal dimensions and become fixed on the protoplasm of the cell. Colloidal silicic acid, like colloidal alumina, is a protein precipitant (Rappaport and Reifer [10]), as is a mixture of them, and its action in precipitating some of the protein of the engulfing cell may be the cause of the typical fibrotic response which is always caused by the presence of free silica particles in tissues. The quartz particles, however, must be of fairly definite size range. They must not be too small, like 20 A. silica, or they will produce an acute toxic effect. If they are too big, no

8. Clelland, D. W.; Cumming, W. M., and Ritchie, P. D.: Physicochemical Studies on Dusts: A high-Solubility Layer on Silicious Dust Surfaces, J. Appl. Chem. 2:31-41, 1952.

9. King, E. J., and Dolan, M.: Unpublished data.

10. Rappaport, F., and Reifer, I.: New Methods of Deproteinization, Mikrochemica Acta. 1:220-225, 1937.

[illegible] dissolution will take place. The formation of silicotic nodules is a slow [illegible] phenomenon. It appears that to produce them silica particles must be small [illegible] to liberate slowly and over a long time soluble silica into solution from their [illegible] in sufficient amount to produce a chronic local irritative effect; they must [illegible] so small that they will quickly and completely dissolve and either be carried [illegible] or produce only an acute local effect which will lack the continuous stimulus [illegible] constantly released colloidal silicic acid. It may be this prolonged irritative or [illegible] effect of constantly released colloidal silica which is responsible for the [illegible] of the fibrous scar tissue of a silicotic nodule (Jötten and Gärtner's [11] [illegible] contains recent similar ideas).

[illegible] [12] described the structure of the silicotic nodule as a core of densely packed [illegible] containing numerous doubly refractive particles of silica surrounded by a [illegible] of fibers (ball of twine structure). Microincineration revealed a dense halo [illegible] silica at the periphery but a space almost vacant of silica between the periphery [illegible] fibrous sheath and the core. This structure strongly suggests a diffusion [illegible] from the central deposit of particles of silicic acid, much of which becomes [illegible] on protoplasmic constituents in a zone slightly outside and surrounding the [illegible], where microincineration reveals it as a multitude of very fine amorphous [illegible], easily seen in dark-field illumination but not visible with the refracting [illegible].

One of us (King [5]) has suggested that particles of quartz in the lung release silicic acid which has special biological properties. This biologically active "nascent" silica may be nothing other than polymerized silicic acid or colloidal silica.

SUMMARY

Intravenously administered injections of colloidal silica caused rapid death in mice. Particulate silica of "respirable" dimensions (0.1 to 5 μ) and other dusts of similar size were tolerated in similar amount, although large doses also caused death. The acutely toxic effect of colloidal silica was not abolished by the addition of aluminum hydroxide, dyes, or protein, substances which are strongly adsorbed on silica of larger particle size. It is suggested that the silicic acid which dissolves from the surface of quartz particles in the tissues becomes in part colloidal and that this colloidal silicic acid may in some way be related to the formation of silicotic lesions.

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11. Jötten, K. W., and Gärtner, H.: Research into the Etiology of Pneumoconiosis, Med. Welt 20:1367-1371, 1951.

12. Belt, T. H.: Silicosis of the Spleen: A Study of the Silicotic Nodule, J. Path. & Bact. 49:39-44, 1939. Belt, T. H.; Ferris, A. A., and King, E. J.: The Silicotic Nodule in Human and Experimental Silicosis: A Comparative Study, J. Path. & Bact. 51:253-267, 1940.

Rev. Franç. Études Clin. et Biol.
5(3):291-293, 1960.

Influence of chelating agents on the development of experimental silica fibrosis in rat

by

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The determination of the effect on tissues produced by an agent such as a harmful mineral particle could theoretically be approached in two ways. On the one hand one could seek to inhibit or reduce the actual tissue reactions elicited by the permanent poison (this is the case with corticoids). On the other, an attempt can be made to "detoxify" the particle itself, that is, render it harmless.

Chelating agents, or cation chelators, could act by chemical isolation of the metals necessary either for the metabolic activity of enzymatic chains involved in tissue reactions, or for the adsorption of proteins onto the particle surfaces. An example of the latter mode of action has been reported by some authors (Caeys and Quinot, 2), who claim that a copper"bridge" is required for binding proteins to quartz.

If the copper were rendered inactive by chelation, the binding of proteins and the possible changes in structure

resulting from this binding would be prevented. In this manner the reactivity of the quartz would be indirectly modified. Unfortunately it has not so far been possible to chelate silica itself, which would represent a genuine biologic neutralization.

From the clinical standpoint, some works have shown that versenate could influence fibroses of different origins: scleroderma (8, 15) and silicosis (Jury, 7, Thieblot, Champeix, Lavarenne and Terlade, 14).

It thus seemed worthwhile to carry out quantitative experimentation to determine the effects of chelating agents on silica fibrogenesis. This is the object of the present work.

Material and Methods

As fibrogenic agent we used crystalline silica dust (quartz) with particles smaller than 3 μ. This silica was injected into the peritoneal cavity of rats in an isotonic saline suspension in a dose of 100 mg per animal weighing ca. 200 g. According to the method of the quantative peritoneal test developed by us (4), after 14 and 22 days of development we determined the fresh weight of the lesions, their collagen content, established by colorimetric determination of hydroxyproline (11), and the collagen:silica ratio. In each case the results were comparable, in relation to untreated animals who received the same silica suspension, the injection being regularly alternated between two control and two treated animals. The injections of chelating agents studied began half a day preceding

the administration of quartz.

The effect of disodium versenate, monocalcium versenate (ethylene-diamine tetraacetate) was examined in this manner in 2 daily 5 mg intraperitoneal injections (2 groups of 22 rats) and 10 mg (1 group of 22 rats). We also studied the effect of B.A.L. (British Anti-Lewisite) or 2-3-dimercaptopropanol in 2 subcutaneous injections (group of 22 rats). Finally we studied 1-hydrazinophthalazine chlorhydrate (Apresoline, CIBA), said by Meier and Desaulles (9) to have anti-inflammatory properties, in a dose of 2 mg per day the first week and 1.5 mg thereafter. This substance proved to be a cation chelating agent (Jacques, Tripod, Meier, 6, Meier, Schuler, 10, Tanner, 13).

Results.

The results are summarized in Table 1, which shows the duration of the development of the fibrogenic granuloma, the number of rats, the doses administered, the route of administration and the % variation compared to the corresponding controls, the latter relating on the one hand to weight of fresh lesions and on the other to the collagen weight per rat in mg. The collagen:silica ratios are listed next to the control values calculated for each group.

It should be recalled that it was concluded from an earlier statistical analysis (4) that for a variation in weight of lesions or collagen content to be significant under our experimental

conditions it had to be at least 25%.

The results reported here scarcely require comment. It is apparent that in doses that are non-toxic for the animals the chelating agents used proved to be incapable of changing experimental silicotic fibrogenesis appreciably. In general, if the general condition of the animals was too much affected by the pharmacodynamic substance, the rats were found to be incapable of forming a granuloma or collagen. This inhibiting effect was not specific and would appear to involve another type of problem, that of the influence of nutrition on fibrogenesis. This problem has recently been dealt with in the case of silica by Chvapil (1), and it also extends to the area of resistance to infections (Dubos and Schaedler, 12, 5).

It should be noted, however, that versenate caused certain variations in the results: twice in the form of an increase of collagen (one significant) and once in the form of a decrease. It will be seen that as a rule the weight of collagen runs parallel with that of the lesions, so much so that in the case of a known material, collagen determinations could be dispensed with. In our case there is a fairly exceptional divergence. On the other hand there is no dose-response proportionality: -18% collagen with 50 mg/kg and +13% with 100 mg/kg. There is a slight increase in the collagen:silica ratio, which suggests a stimulating action of versenate on fibrogenesis. But it seems to us that we are dealing here only with a reduction in the

silica held back in the lesions. Thus with the doses used, our results agree with Claeys and Quinot (3).

In the case of dimercaptopropanol (B.A.L.) the results are uniformly negative, regardless of whether 10 or 20 mg/kg was administered.

With Apresoline the same divergence was found as with versenate; in this case there is a reduction in fresh weight, admittedly non-significant, accompanied by an absence of change in the collagen weight. Here again, the conclusion is a zero effect.

On this point we disagree with the Swiss authors who used cotton pellets (9), but it must be recalled that the quartz granuloma has generally proved very resistant even to the us pharmacodynamic influences.

The purpose of the present work was merely to obtain chemical determinations. Parallel with this, samples were taken for histologic examinations. The results of these investigations will be reported later.

Summary

The effect: have been observed of three chelating agents on silica induced fibro-granulomata in the peritoneal cavity of rats: disodium monocalcium versenate, dimercaptopropanol (B.A.L.) and hydrazinophtalazine (Apresoline). In non toxic doses no significant effect on the granuloma or collagen formation could be demonstrated.

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Table 1.

Substances. 1	Durée d'évolution en jours. 2	Nombre de rats*. 3	Voie d'injection. 4	Doses en mg/kg. 5	6 Pourcentage de variation par rapport aux témoins.		9 Rapport. 12 Collagène/silice	
					7 Poids frais en g.	8 Collagène par rat en mg.	10 Témoins.	11 Traités.
Versénate ..	15	21	IP	50	0	+ 44	2,1	3,6
Versénate ..	14	21	IP	50	— 3	— 18	3,1	2,2
Versénate ..	14	22	IP	100	+ 8	+ 43	3,1	3,5
BAL	20	19	SC	10	— 6	+ 3,4	3,3	3,7
BAL	20	18	SC	20	— 4	0	3,5	3,1
Apresoline ..	22	19	SC	10	— 16	0	3,6	4,7

13 IP : intrapéritonéale.
14 SC : sous-cutanée.
15 * 22 rats injectés au début du traitement.

Key: 1. Substance. 2. Duration of development in days. 3. Number of rats*. 4. Route of injection. 5. Doses in mg/kg. 6. % variation in relation to controls. 7. Fresh weight in g. 8. Collagen per rat in mg. 9. Ratio. 9. Controls. 11. Treated. 12. Collagen:silica. 13. IP: intraperitoneal. 14. SC: subcutaneous. 15. *22 rats injected at the beginning of treatment.

Bibliography

1. Chvapil, M. Influence of undernourishment andof diets of different compositions on the quantity of scleroproteins contained in the rat lungs and liver in experimental silicosis. Arch. f. Gewerbepathol. u. Gewerbehyg., 15:377, 1957.

2. Claeys, C. and Quinot, E. Binding of proteins and fatty acids to quartz. Bull. Soc. Chim. Biol. 38:1063, 1956.

3. Claeys, C. and Quinot, E. Immunologic research on silicosis. C.R. Journées Franç. de Pathol. Minière, Paris, 1958, p. 303.

2a. Claeys, C. and Quinot, E. On the biology of silicosis. Rev. Médicale Minière, 11:15, 1958.

4. Collet, A. Daniel, H. and Soule, J.L. Quantitative assessments of the fibrosing power of industrial dusts by means of the peritoneal test. Arch. Mal. Prof. 18:655, 1957.

6. Jacques, R. Tripod, J. and Meier, R. Reciprocal effects between heavy metal salts,particularly copper salts, and various drugs on the coronary vessels of the isolated heart. Arch. f. exper. Pathol. u. Pharmakol. 230:26, 1957.

7. Jury, R. Attempt at treatment of silicosis with a chelating agent. Th. Médecine, Clermont-Ferrand, 1958.

9. Meier, R. and Desaulles, P.A. Importance of the effect of various groups of anti-inflammatory, antihistaminic, corticoid and hypotensive substance on the mesenchyma in the interpretation of their pharmacodynamic action. J. Physiol. 49:607, 1957.

10. Meier, R. and Schuler, W. Effect of hypotensive hydrazines on metal-catalyzed oxidation of biogenic amines. Helv. Physiol. et Pharmacol. Acta, 15:284, 1957.

13. Tanner, E. Differentiation of metal effects in metal-catalyzed oxidation of various types of auto-oxidizeable compounds and the specificity of inhibiting effects. Inaugural dissertation, Bern, 1958. (Vol. 1, Basel, Apel Ed.).

14. Thieblot, L. Champeix, J., Lavarenne, J. and Teulade, R. Attempt at treatment of silicosis with calcium EDTA. Preliminary report. Arch. Mal. Prof. 20:151, 1959.

15. Thiers, H., Colomb, D., Favolle, J., Moulin, G. and Taine, B. Action of decalcifying chelating agents in a case of scleroderma with predominance of sclerodactylia. Lyon Médical, 25:610, 1957.

INFLUENCE D'AGENTS COMPLEXANTS SUR LE DÉVELOPPEMENT DE LA FIBROSE EXPÉRIMENTALE PAR SILICE CHEZ LE RAT

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Le contrôle des effets tissulaires produits par un agresseur tel qu'une particule minérale nocive peut s'envisager théoriquement de deux manières. D'une part, on peut chercher à inhiber ou à réduire les réactions tissulaires elles-mêmes, provoquées par le toxique permanent (c'est le cas des corticoïdes). D'autre part, on peut tenter de « détoxiquer » la particule elle-même, donc la rendre inoffensive.

Des agents complexants ou chélateurs de cations pourraient intervenir en isolant chimiquement des métaux nécessaires soit à l'activité métabolique de chaînes enzymatiques impliquées dans la réaction tissulaire, soit à l'adsorption de protéines à la surface des particules. Un exemple de ce dernier mode d'action est fourni par certains auteurs (Claeys et Quinot — 2), pour qui un « pont » cuivre serait nécessaire à la liaison des protéines au quartz.

Si l'on rendait le cuivre inactif par chélation, on empêcherait la fixation des protéines et les modifications éventuelles de structure consécutives à cette fixation. Par ailleurs, on modifierait ainsi indirectement la réactivité du quartz. Il n'est malheureusement pas possible actuellement de complexer la silice elle-même, ce qui constituerait une véritable neutralisation biologique.

Par ailleurs, venant de la clinique, certains travaux ont montré que le versénate pouvait avoir une influence sur des fibroses d'origine diverse : sclérodermie (8, 15) et silicose [Jury (7), Thiéblot, Champeix, Lavarenne et Teulade (14)].

Il était donc utile de procéder à une expérimentation quantitative pour estimer les effets de complexants sur la fibrogénèse par silice. C'est le but du présent travail.

MATÉRIEL ET MÉTHODES

Nous avons utilisé comme agent fibrogène de la poussière de silice cristallisée (quartz) avec des particules inférieures à trois microns. Cette silice, en suspension dans l'eau salée isotonique, est injectée dans la cavité péritonéale du rat, à la dose de 100 mg par animal de 200 g environ. Selon la technique du test péritonéal quantitatif que nous avons mise au point (4), nous estimons parallèlement, après quatorze à vingt-deux jours d'évolution, le poids frais des lésions, leur teneur en collagène appréciée par dosage colorimétrique de l'hydroxyproline (11) et le rapport collagène sur silice. Dans chaque cas, les résultats sont comparatifs, par rapport à des animaux non traités recevant la même suspension de silice, l'injection intercalant régulièrement deux animaux témoins et deux animaux traités. Les injections des agents complexants étudiés commencent la demi-journée qui précède l'administration de quartz.

De cette manière, nous avons examiné les effets du *versénate disodique, monocalcique* (sel de l'acide éthylène-diamine-tétra-acétique), à raison de deux injections intrapéritonéales quotidiennes de 5 mg (deux séries de vingt-deux rats), d'une part, et de 10 mg (une série de vingt-deux rats) d'autre part. Nous avons également estimé l'action du *B. A. L.*, ou British Anti-Lewisite ou encore 2-3-dimercaptopropanol, à raison de deux injections sous-cutanées de 1 mg par jour (série de vingt-deux rats) et de deux injections quotidiennes de 2 mg (série de vingt-deux rats). Enfin nous avons étudié le chlorhydrate de 1-*hydrazinophtalazine* (aprésoline CIBA), donné comme étant doué de propriétés anti-inflammatoires par Meier et Desaulles (9), à raison de 2 mg quotidiens la première semaine et 1,5 mg ensuite. Cette substance s'est révélée être par ailleurs un agent complexant de cations [Jaques, Tripod, Meier (6), Meier, Schuler (10), Tanner (13)].

RÉSULTATS

Ils sont rassemblés dans le tableau n° 1, qui précise la durée de l'évolution du granulome fibrogénétique, le nombre de rats, les doses administrées, la voie d'injection et le pourcentage de variations par rapport aux témoins correspondants, ce dernier, d'une part, pour les poids frais de lésions, d'autre part, pour le poids de collagène par rat en milligrammes. Enfin on a rassemblé les valeurs des rapports collagène sur silice, en face des valeurs témoins calculées pour chacune des séries.

Nous rappelons que, d'une analyse statistique précédente (4), nous avions conclu que, pour qu'une variation de poids de lésions ou de teneur en collagène soit significative, dans nos conditions expérimentales, elle devait être au moins égale à 25 p. 100.

Les résultats rapportés ici se passent à peu près

TABLEAU I

Substances.	Durée d'évolution en jours.	Nombre de rats*.	Voie d'injection.	Doses en mg/kg.	Pourcentage de variation par rapport aux témoins.		Rapport. Collagène/silice.	
					Poids frais en g.	Collagène par rat en mg.	Témoins.	Traités.
Versénate . .	15	21	IP	50	0	+ 44	2,1	3,6
Versénate . .	14	21	IP	50	— 3	— 18	3,1	2,2
Versénate . .	14	22	IP	100	+ 8	+ 13	3,1	3,5
BAL	20	19	SC	10	0	+ 34	3,5	3,7
BAL	20	18	SC	20	— 4	0	3,5	4,1
Apresoline .	22	19	SC	10	— 16	0	3,6	4,7

IP : intrapéritonéale.
SC : sous-cutanée.
* 22 rats injectés au début du traitement.

de commentaires. Il apparaît à l'évidence que, à des doses non toxiques pour l'animal, les agents complexants utilisés se révèlent incapables de modifier de façon sensible la fibrogenèse silicotique expérimentale. Le plus souvent, si l'état général des animaux est trop atteint par la substance pharmacodynamique, on constate une incapacité des rats à former un granulome et du collagène. Cet effet inhibiteur n'est pas spécifique et l'on doit toucher là un autre ordre de problèmes, ceux de l'influence de la nutrition sur la fibrogenèse. Ce problème a été récemment abordé pour la silice [Chvapil (1)] et s'étend également au domaine de la résistance aux infections [Dubos et Schaedler (12 et 5)].

Nous remarquons cependant que le versénate a provoqué un certain nombre d'oscillations dans les résultats : deux fois par un accroissement du collagène (dont une significative) et une fois par une réduction. On notera qu'habituellement le poids de collagène est parallèle au poids des lésions, au point que l'on pourrait se passer des dosages de collagène pour un matériel connu. Ici, il y a un désaccord qui est assez exceptionnel. D'autre part, il n'y a pas proportionnalité entre la dose et l'effet : — 18 p. 100 de collagène avec 50 mg/kg et + 13 p. 100 avec 100 mg/kg. Il y a eu par ailleurs une légère hausse du rapport collagène sur silice, ce qui tendrait à montrer une action stimulante du versénate sur la fibrogenèse. Mais il nous semble ici qu'il s'agit seulement d'une diminution de la silice retenue au niveau des lésions. Ainsi, pour les doses utilisées, nous sommes en accord avec Claeys et Quinot (3).

Pour le dimercaptopropanol (B. A. L.), les résultats sont uniformément négatifs, que l'on ait appliqué 10 ou 20 mg par kilogramme.

Avec l'aprésoline, enfin, on retrouve la même divergence que pour le versénate ; ici, c'est une réduction de poids frais, non significative il est vrai, accompagnée d'une absence de modification du poids de collagène. Là encore, la conclusion est celle d'un effet nul.

Nous sommes en désaccord sur ce point avec les auteurs suisses qui ont utilisé les pellets de coton (9) ; mais nous devons rappeler que le granulome à quartz s'est montré de façon générale très résistant aux influences pharmacodynamiques, même classiques.

Il n'a été question dans ce travail que des résultats des dosages chimiques. Parallèlement, nous avons effectué des prélèvements pour examens histologiques. Les résultats de ces observations seront présentés plus tard.

RÉSUMÉ

Par test péritonéal quantitatif chez le rat, on a estimé l'effet, sur un granulome fibrogénétique expérimental par silice, de trois agents complexants : le versénate disodique monocalcique, le dimercaptopropanol (B. A. L.) et la 1-hydrazinophtalazine (aprésoline). A des doses non toxiques pour les animaux, nous n'avons pu mettre en évidence aucun effet significatif de ces substances sur le développement de la lésion et sur la formation de collagène.

SUMMARY

The effects have been observed of three chelating agents on silica induced fibro-granulomata in the peritoneal cavity of rats : disodium monocalcium versenate, dimercaptopropanol (B.A.L.) and hydrazinophtalazine (Apresoline). In non toxic doses no significant effect on the granuloma or collagen formation could be demonstrated.

[Travail du Centre d'Études et Recherches des Charbonnages de France, Verneuil-en-Halatte (Oise), groupe Poussières-Silicose.]

Nous devons remercier ici les laboratoires Ciba, de Bâle, et Delalande pour l'amabilité avec laquelle ils ont mis à notre disposition des informations et des échantillons.

BIBLIOGRAPHIE

1. CHVAPIL (M.). Der Einfluss von Unternährung sowie von Diäten verschiedener Zusammensetzung auf die in der Rattenlunge und -leber enthaltener Menge von Skleroproteinen bei experimenteller Silikose (Arch. f. Gewerbepathol. u. Gewerbehyg., 1957, *15*, 377).
2. CLAEYS (C.) et QUINOT (E.). Fixation des protéines et des acides gras sur le quartz (Bull. Soc. Chim. Biol., 1956, *38*, 1063).
2 *bis*. CLAEYS (C.) et QUINOT (E.). Considérations sur la biologie de la silicose (Rev. Médicale Minière, 1958, *11*, 15).
3. CLAEYS (C.) et QUINOT (E.). Recherches immunologiques sur la Silicose (C. R. Journées Franç. de Pathol. Minière, Paris, 1956, p. 303).
4. COLLET (A.), DANIEL (H.) et SOULE (J. L.). Estimation quantitative du pouvoir fibrosant des poussières industrielles par test péritonéal (Arch. Mal. Prof., 1957, *18*, 655).
5. DUBOS (R. J.) et SCHEDLER (R. W.). Effect of nutrition on the resistance of mice to endotoxins and of the bactericidal power of their tissues (J. Exper. Med., 1950, *110*, 935).
6. JAQUES (R.), TEIPOD (J.) et MEIER (R.). Wechselwirkungen zwischen Schwermettalsalzen, insbesondere Kupfersalzen, und verschiedenen Pharmaka an den Coronargefässen des isolierten Herzens (Arch. f. exper. Pathol. u. Pharmakol., 1957, *230*, 20).
7. JUPY (R.). Essai d'un traitement de la silicose par un agent chélateur (Th. Médecine, Clermont Ferrand, 1958).
8. KLEIN (R.) et HARRIS (S. B.). Treatment of scleroderma, sclerodactylia and calcinosis by chelation (EDTA) (Am. J. Med., 1955, *19*, 708).
9. MEIER (R.) et DESAULLES (P. A.). Importance des effets sur le mésenchyme de différents groupes de substances anti-inflammatoires, antihistaminiques, corticoïdes et hypotenseurs pour l'interprétation de leur action pharmacodynamique (J. Physiol., 1957, *49*, 667).
10. MEIER (R.) et SCHULER (W.). Wirkung blutdrucksenkender Hydrazine auf die metallkatalysierte Oxydation biogener Amine (Helv. Physiol. et Pharmacol. Acta, 1957, *15*, 284).
11. NEUMANN (R. E.) et LOGAN (M. A.). The determination of hydroxyproline (J. Biol. Chem., 1950, *184*, 299).
12. SCHÆDLER (R. W.) et DUBOS (R. J.). Effect of dietary proteins and amino-acids on the susceptibility of mice to bacterial infection (J. Exper. Med., 1950, *110*, 621).
13. TANNER (E.). Die Differenzierung der Metallwirkung bei der metallkatalysierten Oxydation verschiedenartiger Typen autoxydabler Verbindungen und die Spezifizität von Hemmwirkungen. Inaug. Diss. Berne, 1958 (1 vol., Bâle, Apel, éd.).
14. THÉBLOT (L.), CHAMPEIX (J.), LAVARENNE (J.) et TEULADE (R.). Essai de traitement de la silicose par EDTA calcique. Note préliminaire (Arch. Mal. Prof., 1950, *20*, 151).
15. THIERS (H.), COLOMB (D.), FAYOLLE (J.), MOULIN (G.) et TAINE (B.). Action des chélateurs décalcifiants dans un cas de sclerodermie avec sclerodactylie prédominante (Lyon Médical, 1957, *25*, 610).

ÉTUDE DE L'EFFET BRIDGE PAR LES TESTS COMBINÉS DE THROMBOPLASTINOFORMATION ET DE THROMBINOFORMATION

ACTION DES THROMBOPLASTINES PARTIELLES (INCOMPLÈTES) SUR DES SUBSTRATS MANQUANT D'UN FACTEUR THROMBOPLASTIQUE

par G. MAYER, S. MAYER et R. WAITZ,

avec l'assistance technique de E. RIBS* et D. GERHARD

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Lorsqu'une thromboplastine partielle (incomplète), hémophile, Christmas (hémophile B) ou parahémophile (d'hypoproaccélérinémie [Seibert et al. (10), Soulier et al. (11)]) est testée successivement sur un plasma normal et sur un plasma dépourvu du même facteur qu'elle, le temps de coagulation minimum du substrat pathologique dépasse largement celui du substrat normal. C'est là l'effet Bridge

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THE TOXIC PROPERTIES OF SILICA*

I. BRONCHOCONSTRICTOR EFFECT OF COLLOIDAL SILICA IN ISOLATED PERFUSED GUINEA PIG LUNGS

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THE primary objective of this study was to investigate the pharmacological action of silica as part of a general program to elucidate the factors responsible for the symptomatology of silicosis. It was also our hope that the data obtained might have a bearing on the mechanism of the alleged prompt relief of symptoms observed in silicotics following the inhalation of aluminum powder as reported by Crombie et al (1) and Hannon (2).

The respiratory disability in human silicosis is sometimes more severe than one would expect from the evidence of pulmonary involvement as observed in the roentgenogram. Wright (3) has noted "wheezing" when such subjects perform controlled, moderately heavy exercise and has also obtained a measurable increase in the maximum breathing capacity (4) of these men following a subcutaneous injection of 0.5 cc. of 1:1000 epinephrine. Such evidence strongly suggests that bronchial spasm is responsible to a demonstrable degree for a part of the respiratory disability. It is of interest to note that this phenomenon is observed more often in men working underground than on the surface, and that they speak of losing their "wheeze" several months after they return to the surface.

The hypothesis that silica might directly cause the bronchial spasm in human beings was suggested by the observation of Gardner and Dworski (5) that in deeply anesthesized guinea pigs intratracheal instillation of colloidal silica produced symptoms pointing to vigorous contraction of the smaller bronchi. That this effect is specifically due to the colloid and not to the volume of instilled fluid was clearly shown by the complete lack of response when an equal volume of normal saline was introduced. Their work has been amply confirmed by Hawley (6) in a report to be published subsequently.

The observation that bronchial spasm decreases or disappears in miners transferred to the surface implicates some agent (perhaps silica) that is peculiar to their working environment. Furthermore, the reported (1, 2) amelioration of symptoms following the inhalation of aluminum dust might conceivably be due in part to the relaxation of bronchial spasm brought about either by direct action on the bronchial muscle or by the neutralization of irritating quartz with aluminum. We had hoped to study the physiological action of aluminum as an inhibitor of silica and to discover whether it might have any direct effect upon the bronchioles, but it can be stated at once that the technique used precluded such investigation. Other methods must be devised for these purposes.

That quartz can produce acute toxic reactions as well as fibrosis has been amply demonstrated. Denny, Robson, and Irwin (7) attribute all these effects to the action of minute amounts of soluble silica dissolved in the body fluids. They conclude that aluminum protects the organism against silica toxicity by coating the quartz particle and reducing its solubility. On the other hand, Gardner and his associates (8) have been unable to demonstrate any toxic action of silica in its soluble form and maintain that the toxic action of finely divided quartz is confined to the surface of the particles where the

* Received for publication November 12, 1944.
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action of body fluids liberates a layer of highly irritating colloidal silica. They regard the silica in true solution as an inert by-product of the reaction. The protective action of aluminum receives a different interpretation by the latter investigators. The method used in this study afforded us the opportunity to obtain data bearing upon these questions.

the tracheobronchial tree. The method depends on the principle that at a constant head of pressure the rate of intratracheal perfusion varies with the caliber of the tracheobronchial tree.

Guinea pigs 250 grams in weight were used throughout. Nembutal 0.4 cc. (veterinary; 1 cc. contains 1 grain) was injected intraperitoneally ten minutes before the lungs together with the

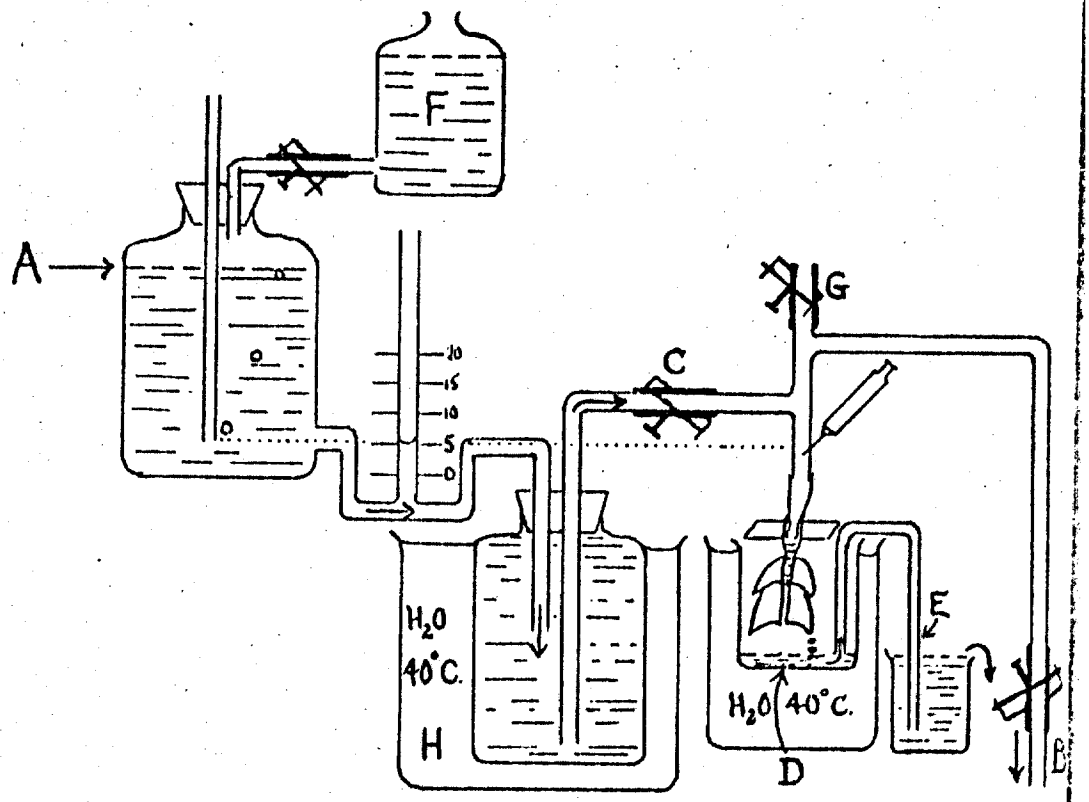


FIG. 1. Perfusion apparatus. For description see text

METHODS

It was not feasible to introduce the dry test substances quantitatively into the lung, hence an aqueous suspension in measured doses was injected directly into the trachea. The perfusion technique devised by Sollman and von Oettingen (9) was used with certain modifications. Briefly the procedure is as follows: The heart and lungs of an anesthetized animal are removed *en bloc* and hung on a perfusion cannula inserted into the trachea. The lungs are distended with fluid and their pleural surfaces are then uniformly pricked with sharp needles to permit the fluid to escape from all parts of the lungs. Changes in the rate of flow of perfusion fluid through such a preparation reflect the effect of test substances upon the patency of

heart were removed for intratracheal perfusion. The early onset of lung rigidity with intense bronchoconstriction noted by other workers (10, 11) occurred less frequently after nembutal than following other methods of anesthesia. Such bronchoconstriction was abolished by injection of 0.1 cc. of epinephrine 1:1000 into the perfusion fluid. The use of this drug interfered in no way with the experiments (Fig. 3).

As soon as the lungs were removed from the animal they were hung on a perfusion cannula, and perfusion fluid (12)[2] at 38°C. pH 7.3–7.5 and 5 cm.

[2] Ion concentrations in millimols per liter of solution

Na	144.0	Cl	122.0
K	6.2	HCO_3	30.0
Mg	1.0	H_2PO_4 }	1.0
Ca	0.53	HPO_4 }	

of pressure was allowed to run into the trachea (Fig. 1). Gentle massage of the lungs and intermittent use of the syphon system at *B* for five to ten minutes aided in the displacement of air and bronchial secretions from within the preparation. The syphon system was operated by clamping at

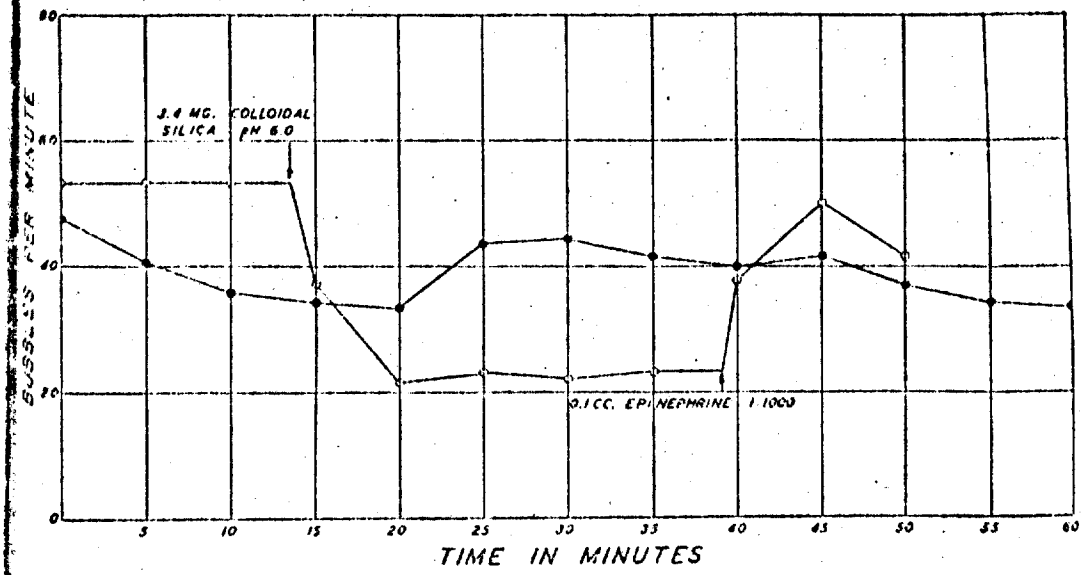


FIG. 2. Curves showing the contrast between normal fluctuations in flow (solid circles) and the effect of colloidal silica and epinephrine (open circles) in a pharmacologically reactive guinea-pig lung.

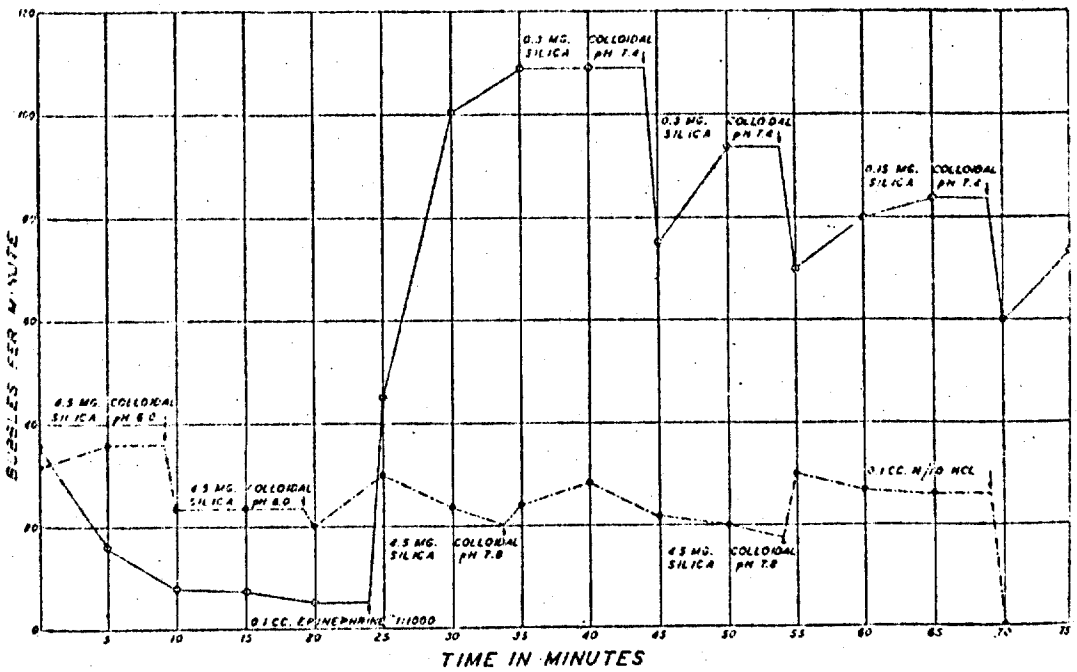


FIG. 3. The upper curve (open circles) illustrates the diminution of flow which sometimes occurred at the start of an experiment, the release of this bronchospasm by epinephrine and the effect of small doses of colloidal silica even at the height of an epinephrine bronchodilatation.

The broken line (solid circles) represents the rate of flow through a "mechanical lung" (see text). Colloidal silica affects the rate slightly at different pHs, but does not produce physical blocking of the preparation. HCl produces a complete cessation of flow due to the release of gas bubbles formed by reaction with the perfusion fluid.

C and removing the clamp at *B*, so that suction was applied to the lung by way of the trachea. When the lungs were well ballooned out, flow of fluid was established through the bronchial tree by thoroughly pricking the pleura with sharp needles over its entire surface until it was uniformly perforated. The outflow from the lungs as it dripped into the covered warming chamber *D*, was siphoned off by another system at *E*, so that fluid did not accumulate around the lung. The warming chamber was covered with a loosely fitted top to keep the temperature within it as nearly constant as possible. The rate of flow through the lung was measured by the number of air bubbles per minute appearing in the Mariotte bottle *A*. This device, though it keeps the pressure relatively constant, allows for error unless the fluid level is always at the same point when a count is made. The bubbles become larger and less frequent as the level falls. Counts were made therefore at five minute intervals with the level at *A* for each count, the level being maintained by running in new fluid when needed from the reservoir at *F*. The temperature of the fluid as it entered the lungs was kept at 38 to 39°C. by varying the temperature of the water bath *H*. This procedure was necessary because of the tendency for cooling of perfusion fluid in the tubing at slow rates of flow. With this technique the lungs remained reactive to epinephrine and colloidal silica for about three hours. When aqueous test substances were introduced in volumes greater than 1 cc., they were added in equal volume to a double strength perfusion fluid so that the ionic concentration of perfusion salts remained unchanged. Such test mixtures were run into the lungs at 5 cm. of pressure and 38°C. from a separate Mariotte system. All test substances were introduced one minute before a count was taken.

The perfusion fluid used had the disadvantage of liberating CO_2 gas, especially if the pH was lowered by the injection of acid solutions. Because of the gradual accumulation of gas bubbles within the lung during perfusion, it was necessary to flush the preparation every 5 minutes by syphoning at *B* for 10 seconds. This procedure eliminated the slowing of flow which otherwise took place due to physical blocking of the tracheobronchial system by gas bubbles. A perfusion fluid buffered by other means than by CO_2 should be investigated for future perfusion experiments. The clamp at *G* was opened at the end of an experiment to allow the escape of accumulated gas from the apparatus.

In order to demonstrate the influence of gas bubble formation and to study the possible blocking effect of test substances, a "mechanical" lung was devised by injecting a perfused guinea pig lung with 0.5 cc. of 40 per cent Formaldehyde. Within 10 minutes this preparation became pharmacologically unresponsive, but retains the gross physical properties of a reactive lung. In such a "mechanical" lung one may readily demonstrate changes in the rate of flow due to gas bubble formation and explore the physical effects of various test substances to be used in a pharmacologically active lung.

Celloidin casts of the tracheobronchial tree were prepared by the method of Hanzlik (13) with slight modifications. Parlodion 15 gms. and camphor 12 gms. were dissolved in 100 cc. of acetone and the solution was colored with 1 cc. each of 5 per cent gentian violet and 5 per cent brilliant green in acetone. The viscid solution was forced into the trachea at 110 mm. Hg for 15 minutes immediately after a perfusion experiment was completed. After 24 hours in distilled water, the injected specimen was placed for another 24 hours in HCl (6 per cent of the concentrated acid) to digest the lung tissue from the bronchial cast. The remnants of pulmonary tissue were removed with a fine stream of water and the cast was preserved in distilled water to which a few drops of formalin had been added.

Microscopic sections were made from small slices of lung tissue which were cut from a preparation during perfusion. These slices were placed at once in Zenker's solution and transfer to running water was made within 6 hours. The sections were stained with hematoxylin-eosin.

Materials to be Tested

1. *Colloidal Silica.* This was prepared by dialysis from commercial waterglass (Rutland) diluted to a specific gravity of 1.060-1.090 at 20°C. Fifteen cc. of the diluted waterglass were added dropwise to 5 cc. of concentrated HCl in 1[illegible] cc. of distilled water. The resultant mixture was dialysed in a cellophane bag against running tap water until the $AgNO_3$ test for chloride was faint to negative. Colloidal silica of pH 5-7 and [illegible] concentrations of 1-1.5 per cent (reported as total SiO_2 by gravimetric analysis) can be thus prepared.

and if kept on ice will not gel for at least a month. Over 90 per cent of the silica in such a preparation is in the form of a hydrosol, a small amount being "soluble silica" as shown by a comparison of the total silica determined gravimetrically and the "soluble silica" found present by the colorimetric molybdate test (14). The pH of the colloid can be raised to 7.8 without gel formation by adding N/1 NaOH dropwise.

2. *"Soluble Silica"*. Three solutions were prepared by allowing 1 per cent aqueous suspensions of finely powdered quartz crystal to stand for prolonged periods at 37°C. The particles were then removed by centrifugation at 20,000 revolutions a minute for fifteen minutes. "McIntyre" quartz (BQ 722)[3] standing for 66 hours, dissolved to a concentration of 74.2 parts per million (pH 7.4). Pure silex (99.9 per cent quartz) particles, 0.5μ and under, one preparation standing for 89 hours and another for 64 days dissolved to concentrations of 51.2 and 108.5 parts per million respectively. The pH of the latter two solutions was 7.0.

3. *Gold Sol*. A sol containing 0.5 per cent Au was prepared at pH 7.8 by the method of de Bollini (15).

4. *Particulate Silica*. Three 1 per cent aqueous suspensions of silex with average particle diameters of 1 to 3μ, 0.5μ and under[4] and 20Å[5] respectively were prepared. The pH of these suspensions was 7.0.

5. *Aluminum Hydroxide (XH-1010)*. A 1 per cent aqueous suspension of the powdered material was prepared. This form, chiefly amorphous, with small amounts of crystalline alpha-monohydrate has proven an effective inhibitor of quartz in animal experiments reported by Gardner, Dworski, and Delahant (16).

Results

1. *Control Preparation*. The rate of flow of perfusion fluid through the tracheobronchial tree usually showed spontaneous fluctuations at the beginning of an experiment. Within 20 minutes after perfusion was begun, however, these fluctuations diminished so that a fairly constant rate of flow was maintained, the maximum change being 10 bubbles per minute over a 5 minute interval. A typical normal curve is illustrated in Figure 2.

Microscopic examination of lung tissue from such a control preparation revealed the bronchi to be of normal caliber for the most part; a few were slightly dilated and occasionally one was constricted. The microphotographs (Fig. 5A and 5B) illustrate typical bronchi in the control preparation.

When celloidin was injected into the trachea of an untreated lung immediately after removal from the perfusion apparatus, the injection mass was found to fill the tracheobronchial tree quite completely and to extend to the finer divisions as shown in Fig. 6.

2. *Effect of Colloidal Silica*. Forty-four of 45 injections of colloidal silica, in doses of 0.1 to 6.0 mgm. (0.01 cc. to 0.6 cc. of a 1 per cent solution) retarded the rate of flow by 20 to 60 bubbles per minute, a decrease which was outside the range of normal fluctuations. The minimal effective dose was approximately 0.1 mgm. The effect was the same when the colloid was injected in concentrated form (2 per cent) or diluted in warmed perfusion fluid to 60 parts per million and introduced without change of temperature or pressure. The slowing was always evident within one minute after introduction, but did not usually reach a maximum until the 6th minute. In a majority of instances a recovery was observed, and if the injection was repeated, a typical effect was again obtained (Fig. 3). Epinephrine was found to be capable of counteracting the slowing effect of silica sol (Fig. 2). Conversely, colloidal silica could produce slowing at the height of an epinephrine effect (Fig. 3).

Histological sections made from lung tissue affected by colloidal silica showed (Fig. 5C and 5D) that both the large and small bronchi were contracted. The difference between them and the bronchioles of an untreated control preparation is obvious in Figure 5.

Examination of the celloidin casts (Fig. 6) of the tracheobronchial tree of lungs treated with colloidal silica shows that the celloidin mass failed to extend beyond the major divisions of the bronchi. These casts were similar to those prepared from untreated lungs which show spontaneous broncho-

[3] An impure variety used extensively by Irwin in many of his experiments.

[4] Finely divided Silex crystal was separated by fractional sedimentation and decantation from aqueous suspensions into graded particle sizes.

[5] "20A Silica" is a vitreous silica of exceedingly small particle size prepared by discharging a jet of gas at high pressure into a stream of molten quartz.

constriction and were in marked contrast to casts of the control lung with fully patent bronchi.

3. *Effect of "Soluble Silica"*. The same procedures were repeated to test the action of silica in soluble form prepared from quartz crystals. Three different preparations of "soluble silica" in doses ranging from 0.48 to 1.0 mgm. failed to produce any significant effect. Such amounts are 5–10 times the minimal effective dose of colloidal silica.

5. *Effect of Aluminum Hydroxide (XH-1010)*. When this substance was injected into the perfusion tubing in doses as small as 0.5 mgm. (0.05 cc. of a 1 per cent suspension), the resulting mixture entering the lung produced an immediate reduction in flow which was not followed by a period of recovery. As this response occurred in both the reactive and the "mechanical" lungs, it was evidently due to physical blocking of the tracheobronchial tree[6].

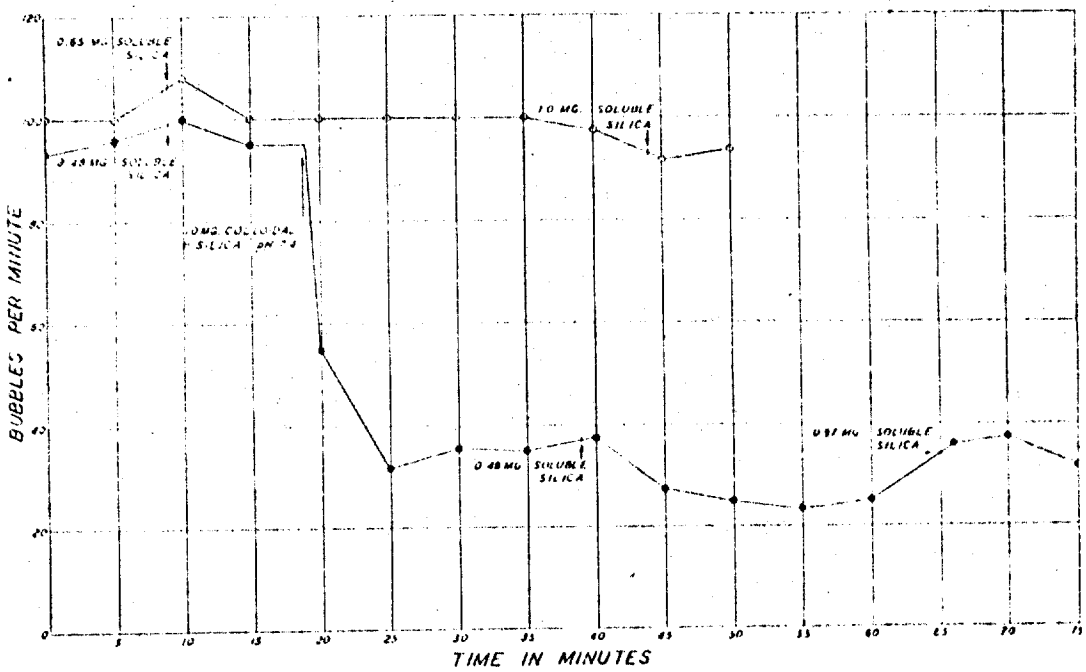


FIG. 4. "Soluble silica" from "McIntyre" quartz (open circles) has no influence on flow in a reactive lung. The lower curve (solid circles) shows the contrast between the effects of colloidal silica and soluble silica prepared from Silex, 0.5μ and under.

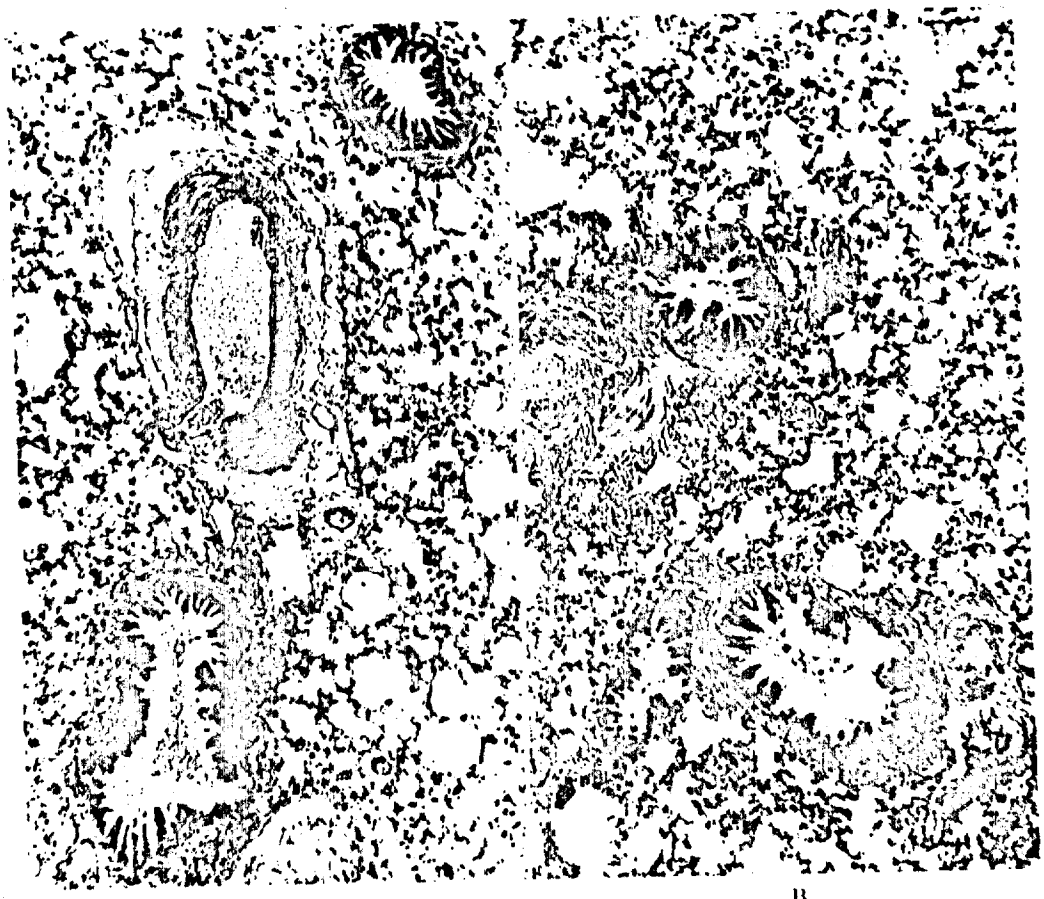
As shown in Figure 4, where the two forms of silica were tested in the same preparation, the colloid markedly slowed the rate of flow while the "soluble silica" had no appreciable action.

4. *Effect of Particulate Silicas and Gold Sol*. The introduction of three different suspensions of quartz particles, with diameters averaging 1.3μ, 0.5μ and under and 20Å respectively, resulted in an immediate slowing of perfusion flow. As the same degree of retardation could also be reproduced in the formalinized lung, the effect was evidently due to mechanical obstruction in the tracheobronchial tree. The introduction of non-siliceous particles of colloidal size (gold sol) produced no effect whatever on the rate of flow through either a reactive or a "mechanical" lung.

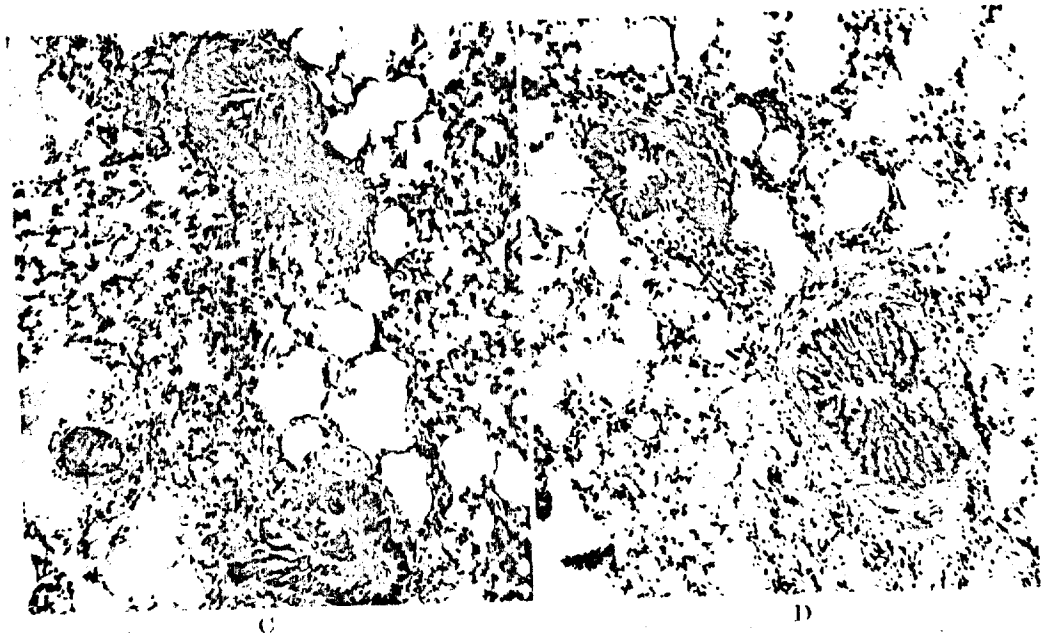
6. *Effect of pH*. When the pH of the perfusion fluid entering the preparation was lowered, CO_2 gas was liberated and the resultant bubbles impeded flow. Figure 3 illustrates the effect of 0.1 cc. of N/10 HCl in producing complete cessation of flow in the "mechanical" lung. In this instance perfusion could be reestablished after the lung was massaged and flushed free of accumulate gas bubbles within it.

With the exception of colloidal silica, the solutions and suspensions tested were either neutral or

[6] Finely powdered metallic aluminum, freshly suspended in perfusion fluid, also produced an immediate reduction in flow which was not followed by recovery. Although this substance was not tested in the "mechanical lung" we assumed the mechanism to be that of physical blockage of the tracheobronchial passages.



A B



C D

FIG. 5. A. and B. Microphotographs of sections taken from perfused lungs in which the flow was over 50 bubbles per minute. The large and small bronchioles of normal caliber or slightly dilated. C. and D. Similar sections taken 6 minutes after injection of 3.4 mg. of colloidal silica. Flow 20 bubbles per minute. The large and small bronchioles show constriction. No evidence of gel or precipitate is seen in the lumen.

so weakly acid or alkaline that the buffering power of the perfusion fluid brought the pH to 7.3–7.5 by the time the test substance entered the trachea. In the case of colloidal silica, the effect of pH was demonstrable though slight. Figure 3 illustrates the changes in flow produced by acid and alkaline colloidal silica in the "mechanical" lung. These effects were negligible compared to the pharmacological action of the colloid in the reactive lung (see Figures 2, 3 and 4).

Table 1 summarizes the effects of the various substances tested.

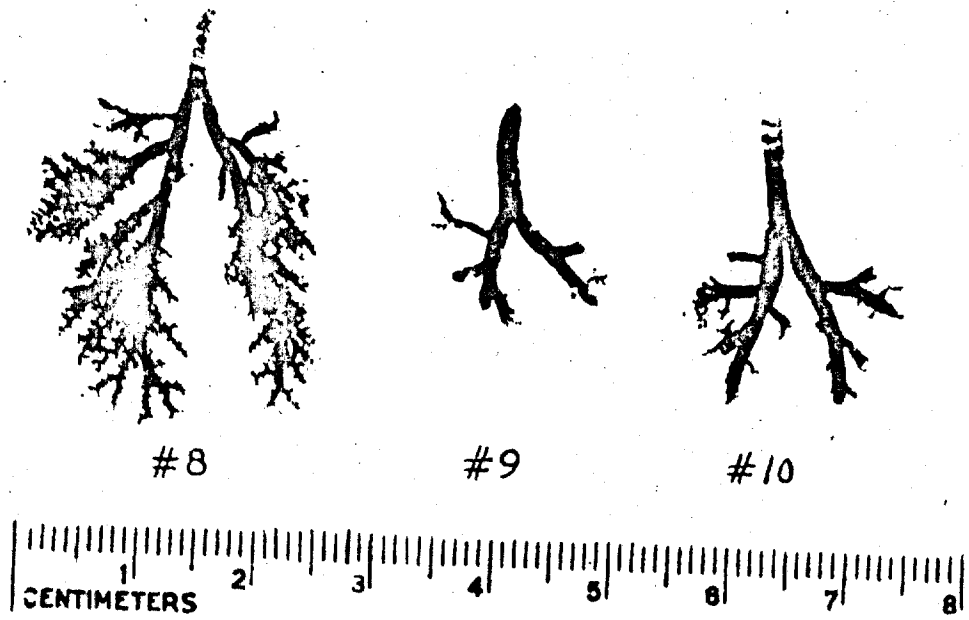


FIG. 6. #8 Celloidin cast of a control, untreated perfused lung. #9 Cast of lung which showed spontaneous bronchoconstriction. #10 Cast of a perfused lung treated 6 minutes previously with 3.4 mg. of colloidal silica.

TABLE 1

TEST SUBSTANCE	EFFECT ON RATE OF FLOW		BRONCHIAL EFFECT
	Reactive lung	Mechanical lung	
Epinephrine	increase	0	dilatation
Colloidal silica	reduction	0	constriction
Soluble silica	0	0	0
Gold sol	0	0	0
Particulate SiO_2	reduction	reduction	mechanical block
Aluminum hydroxide	reduction	reduction	mechanical block
Metallic aluminum	reduction	—	mechanical block

INTERPRETATIONS

It is customary to interpret a reduction of flow in this type of pharmacological preparation as due to bronchial constriction. The peculiar nature of the substances we are testing, however, requires us to show that reduction in flow is due to true constriction of the tracheobronchial tree and not to mechanical obstruction in this structure.

It is conceivable that colloidal silica might mechanically retard the rate of flow for this substance tends to gel in the presence of sufficient concentrations of electrolyte. However, in this study the perfusion fluid apparently had no effect on the physical properties of the colloid for gel formation was never observed either *in vitro* or after it entered and passed through the lung. In the "mechanical" formalized preparation (Fig. 3) successive large doses of colloidal silica had no influence on the general slope of the flow curve, show-

ing again that no obstruction was produced as a result of the *physical properties* of the colloid. In the reactive lung two features strongly suggest true pharmacological action rather than mechanical obstruction. The maximum response to moderate doses required six minutes to develop and it was followed by a period of recovery. If the retardation had resulted from mechanical blocking it would have occurred immediately; if it were due to gelation there could have been no recovery for gel formation is an irreversible reaction. Furthermore, as noted by Gardner and Dworski (5), the pharmacological reaction to colloidal silica would appear to be specific and not merely a response to a colloidal solution for gold sol was entirely without effect.

Sections of lung tissue (Fig. 5) taken during perfusion with colloidal silica indicate that bronchoconstriction is not uniform throughout the lung. Presumably not all the bronchioles are perfused equally. The narrowing is apparently due to constriction of the muscle fibers since the sections show definite thickening of these structures. The distribution of the constriction appears to be widespread since it involves both large and small bronchioles deep within the pulmonary parenchyma. The resistance created by such constriction is considerable; when celloidin is forced at a pressure of 110 mm. Hg into a lung effected by colloidal silica, it does not penetrate beyond the major divisions of the tracheobronchial tree, and the resulting cast (Fig. 6) resembles that made from a lung in which marked bronchospasm had occurred spontaneously.

Because particulate silica, aluminum hydroxide and metallic aluminum blocked the perfused lung mechanically, their pharmacologic action on the bronchi, either alone or in combination could not be evaluated.

Comments

That silica in soluble form has no effect on the bronchial caliber of guinea pig lungs (Fig. 4), and hence appears to be pharmacologically inert in this respect, constitutes a further contribution to current theories of the irritant action of quartz. We have confirmed the earlier observations of Gardner and Dworski (5) that colloidal silica produced symptoms suggestive of bronchial spasm in anaesthetized guinea pigs and that so-called "soluble silica", demonstrated colorimetrically by the molybdate method, does not. In the present experiments a physiological preparation was used which was sensitive to 0.1 mgm. of colloidal silica. The fact that it would not respond to 10 times the same amount of silica in soluble form indicates that the latter is probably not the irritating substance liberated in the solution of quartz within the body.

Although colloidal silica has been proved to be a specific irritant to the smooth muscle of the bronchioles, similar effects upon other cells may or may not play a part in causing the fibrous lesions of silicosis. Available evidence suggests, that on contact with body fluids the surface of finely divided quartz may be converted to highly active colloidal silica and that in this process a certain amount of inert soluble silica is liberated into the tissues as a by-product. Whether or not this hypothesis proves to be correct, the fact that silica in the colloidal state is highly toxic indicates that surface phenomena are operative.

From this investigation no definite conclusions can be drawn concerning the cause of bronchospasm in human silicosis because technical interference precluded a study of the action of particulate silica and aluminum hydroxide. However, the evidence obtained from the reaction to colloidal and to soluble silica does strongly suggest that the latter is not the agent involved.

Summary and Conclusions

1. A modified lung perfusion technique is described which is a sensitive method for the assay of substances influencing bronchial caliber if the factors producing mechanical blocking are analyzed and controlled.

2. Colloidal silica produces a specific pharmacologic bronchoconstriction in isolated perfused guinea pig lungs apparently due to constriction of the smooth muscle fibers. The minimal dose for this response is approximately 0.1 mgm.

3. Silica in soluble form in doses as high as 1.0 mgm. produces no response under the same conditions.

4. Particulate silica produces mechanical obstruction in the tracheobronchial tree of perfused lungs which obscures any pharmacologic action it may have.

5. Aluminum hydroxide likewise causes mechanical obstruction in isolated lungs and hence its effects could not be evaluated.

6. The relation of the bronchoconstrictor effect of colloidal silica to the action of quartz in producing silicosis and to the apparent bronchospasm in patients with silicosis is discussed.

BIBLIOGRAPHY

(1) Crombie, D. W., Blaisdell, J. L., and MacPherson, G.: The treatment of silicosis by aluminum powder. Canad. M. A. J., *50:* 318, 1944.

(2) Hannon, J. W. G.: Private communication, 1943.

(3) Wright, G. W.: Unpublished data. E. L. Trudeau Foundation, Saranac Lake, N. Y., 1942.

(4) Hermannsen, J.: Untersuchungen über die maximale Ventilationsgrosse (Atemgrenzwert). Ztschr. f. d. ges. exper. Med., *90:* 130, 1933.

(5) Gardner, L. U., and Dworski, M.: Private communication, 1943.

(6) Hawley, J. G.: Unpublished data. E. L. Trudeau Foundation, Saranac Lake, N. Y., 1944.

(7) Denny, J. J., Robson, W. D., and Irwin, D. A.: Prevention of silicosis by aluminum; preliminary report. Canad. M. A. J., *37:* 1, 1937.

(8) Gardner, L. U.: Recent developments in relation to silicosis. Industrial Med., *9:* 45, 1940.

(9) Sollman, T., and von Oettingen, W. F.: Bronchial perfusion of isolated lung as method for studying pharmacologic reactions of bronchiolar muscle. Proc. Soc. Exper. Biol. and Med., *25:* 692, 1928.

(10) Petrovskaia, B.: Broncho- and vaso-motor responses of guinea-pig lungs. Quart. J. Exp. Physiol., *29:* 121, 1939.

(11) Hebb, C. O.: Bronchomotor responses to stimulation of ganglia and to injection of acetylcholine in isolated perfused guinea-pig lungs. J. Physiology, *99:* 57, 1940.

(12) Van Dyke, H. B. and Hastings, A. B.: Response of smooth muscle in different ionic environments. Am. J. Physiol., *83:* 563, 1928.

(13) Hanzlick, P. J.: Demonstration of bronchial constriction and compression from physiological and other causes. Am. J. Physiol., *72:* 55[illegible], 1925.

(14) King, E. J., and Stantial, H.: Biochemistry of silicic acid; micro-determination of silica. Biochem. J., *27:* 990, 1933.

(15) de Bollini, L. S. V.: Langes reaction. A new reducing agent for the preparation of colloidal gold sols. Rev. facultad. cienc. quim., *16:* 10[illegible], 1941.

(16) Gardner, L. U., Dworski, M. and Delahant, A. B.: Experimental study of inhibitory effects of aluminum compounds in silicosis. Bull. of the Am. Ceramic Soc., *20:* 283, 1941.

Brit. J. industr. Med., 1966, 23, 71

Intratracheal Injection into Rats of Size-graded Silica Particles

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An experiment is described in which suspensions of size-graded silica particles of less than 1 μ, 1 to 3 μ, and 2 to 5 μ nominal size ranges and of equal surface area (600 sq. cm./ml.) were injected intratracheally into rats. After four months the rats were killed and the lungs were examined histologically for the grade of fibrosis and chemically for the silica and collagen content.

The results were analysed statistically and it was found that, under the experimental conditions described, the degree of fibrosis produced by the two large size fractions was more than that produced by the under 1 μ particles and was apparently related to the quantity (by weight) of the silica injected or the size of the particles and not to the surface area.

In this experiment suspensions of silica particles of three different size ranges but of equal total surface area and hence containing different weights of silica were injected intratracheally into rats. After four months the lungs were examined histologically for the grade of fibrosis and chemically for the silica and collagen content.

Experimental Procedure

Dust Samples The suspensions of silica dust were prepared by the Transvaal and Orange Free State Chamber of Mines Dust and Ventilation Laboratory, Johannesburg and were of the size ranges less than 1 μ, 1 to 3 μ, and 2 to 5 μ, of which 13·99 mg., 46·1 mg., and 92·7 mg. were suspended in each ml. saline respectively. These concentrations were calculated to give a surface area of silica of 600 sq. cm./ml. for each of the size ranges. Counts were done by means of the light and electron microscopes, and the details are given in Tables I to III, together with the *x*-ray and chemical analysis of the silica content. The *p*H of the suspensions was 6·7, 6·8, and 7·0 for the 2 to 5 μ, 1 to 3 μ, and under 1 μ particles, respectively.

Using Cu K alpha radiation, comparisons were made with the *x*-ray quartz standard, using the 101 reflection. The results are summarized in Table III.

They indicate that considerable peak broadening was present in both samples, no. 2 more than no. 1. This would almost certainly be due to the crystallite size being smaller than that of the *x*-ray standard. Both samples would appear to be highly crystalline, the percentages probably being somewhat greater than the indicated values of 95% for sample 1 and 94% for sample 2, as the peaks were assessed over 2 degrees (2θ), whereas the tails, although small, are thought to extend some considerable distance further. (The analysis was carried out by the Transvaal and Orange Free State Chamber of Mines Physical Science Laboratory.)

Animals Male white rats approximately 3 months old were divided into three groups by random selection. Thirty-five animals from each group were then given 1 ml. of the respective silica suspensions by the intratracheal method. Previous work (Goldstein, Webster, and Sichel, 1962) had shown that 25 to 30 animals from each group could be expected to produce statistically significant results and, to allow for deaths, five extra animals were used. One rat from each group was injected in rotation to avoid variation in technique favouring any particular group.

A number of animals died during the course of the experiment and the survivors, a total of 66 rats, were killed after four months. The body weight of each animal was recorded and then the lungs were removed. After ascertaining the wet weights of the right and left lungs separately, they were fixed in 10% formol-saline, and histological sections were cut at 5 μ and stained with haematoxylin and eosin and for reticulin. Without knowing to which group they belonged, the sections were examined for the

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TABLE I

SIZE FREQUENCY DISTRIBUTION OF VARIOUS SIZE FRACTIONS OF THE DUST: ELECTRON MICROSCOPE RESULTS

Size (microns)	*Diameter*[1]	< 1 μ			1 to 3 μ			2 to 5 μ		
		No.	*%*	*% Area*	*No.*	*%*	*% Area*	*No.*	*%*	*% Area*
0·02-0·04	0·0009	10	5·1	0·03						
0·04-0·06	0·0025	14	7·1	0·1						
0·06-0·1	0·0064	25	12·8	0·6	0					
0·1 -0·2	0·0225	30	15·3	2·4	2	1·5				
0·2 -0·3	0·0625	34	17·4	7·5	0	0·0	0			
0·3 -0·4	0·1225	30	15·3	12·9	4	3·1	0·1	0		
0·4 -0·5	0·2025	22	11·2	15·6	0	0·0	0	1	0·7	
0·5 -1	0·5625	31	15·8	61·0	2	1·5	0·2	0	0·0	0
1 -1·5	1·5625	0			8	6·2	1·7	2	1·3	0·16
1·5 -2	3·0625				37	28·5	15·8	3	2·0	0·49
2 -3	6·25				60	46·2	52·1	47	31·3	15·69
3 -4	12·25				16	12·3	27·2	59	39·3	38·60
4 -5	20·25				1	0·8	2·9	33	22·0	35·75
5 -6	30·25				0			4	2·7	6·55
6 -8	49·00							1	0·7	2·75
> 8								0		
		196	100·0	100·1	130	100·1	100·0	150	100·0	99·99

Notes

1. In column 1 the size refers to the diameters of circles of the same projected areas as the particles being sized.
2. The main headings of columns 3 to 11 refer to the nominal size ranges of the dust samples.
3. Under the heading 'No.' is given the actual number of particles counted for each size interval. This is followed by the number expressed as a percentage of the total number counted. These percentages are all given to one decimal place, but when using them for calculations, the number of significant figures given in the result should be determined from the actual number of particles counted for the particular size range.
4. In the size fraction less than one micron, particles smaller than 0·02 μ were not counted.
5. % Area = Diameter[2] × number %.

TABLE II

X-RAY ASSESSMENT OF QUARTZ

Sample	*Response (%)*
2-5 μ	100
1-3 μ	100
Under 1 μ	92
	Chemical Analysis
2-5 μ	99·85% silica
1-3 μ	99·85% silica
Under 1 μ	99·80% silica

TABLE III

COMPARISON OF THE DUST WITH A STANDARD QUARTZ POWDER

	X-ray Standard	*Sample 1*[1]	*Sample 2*[2]
Relative intensity using peak heights (%)	100	90	86
Relative intensity using peak areas (%)	100	95	94

[1]Quartz powder from Dr. Nagelschmidt (London).
[2]Quartz powder, P.R.U. standard (Table I).

presence of dust lesions, and these were graded by the number of lesions and the degree of fibrosis, according to the grading of Belt and King (1945), modified by omitting grade 5 and taking into consideration the number of lesions present (Goldstein, 1960). All the trimmings from the cutting were preserved and then dewaxed with the appropriate blocks, after which the tissue from the individual lungs was utilized for the chemical estimation of collagen and silica by the methods of Neuman and Logan (1950) and King, Stacy, Holt, Yates, and Pickles (1955) respectively. The results were then analysed statistically.

The association between particle size and the histological grading of the degree of fibrosis is shown in the contingency tables (Table IV).

Statistical Analysis

The following data were submitted for analysis: body weight of the animals; wet weight of the lungs; weight of silica in the lungs; weight of collagen in the lungs; and histological grading.

The analysis consisted of (1) a comparison of the three groups with respect to the different variables; (2) a comparison of the right and left lungs with

TABLE IV.

NUMBER OF LUNGS CLASSIFIED ACCORDING TO NUMBER OF LESIONS AND DEGREE OF FIBROSIS

Particle Size	*Histological Grading according to Number of Lesions*					
	No Histological Lesions or Destroyed by Abscess	*Occasional Nodules*	*Few Nodules*	*Numerous Nodules*	*Confluent Nodules*	*Total*
Under 1 μ	9	4	22	15	—	50
1 3 μ	9	—	4	9	26	48
2 5 μ	5	—	1	4	24	34
Total	23	4	27	28	50	132

Particle Size	*Histological Grading according to Degree of Fibrosis*					
	No Histological Lesions or Dstroyed by Abscess	*Grade 1*	*Grade 2*	*Grade 3*	*Grade 4*	*Total*
Under 1 μ	9	18	20	3	—	50
1-3 μ	9	—	2	5	32	48
2-5 μ	5	—	—	2	27	34
Total	23	18	22	10	59	132

respect to the different variables; and (3) a correlation between the different variables.

Comparison between the Three Groups

Body Weight of the Animals The average body weights of the three groups were found to be not significantly different from each other (95% level of significance).

Wet Weight of Lungs per 100 g. Body Weight The average values for the three groups showed that at the 5% level there was no significant difference between the two larger particle sizes, but both these values were significantly higher than for the under 1 μ size. (The average values were 2·23 g. (under 1 μ), 6·40 g. (1 to 3 μ), and 7·61 g. (2 to 5 μ).) It therefore appears that the relative increase in the wet weights of the lungs is dependent upon the size and/or weight of the dust injected.

Weight of Silica in Lungs From the average percentage recovery (41%, 29%, and 15% for the under 1 μ, 1 to 3 μ, and 2 to 5 μ groups respectively), it appears that the smaller particles are retained in the lungs more readily than the larger particles.

Weight of Collagen in Lungs The averages for the three groups were significantly different from each other and were 14·5 mg., 33·2 mg., 47·2 mg. for the under 1 μ, 1 to 3 μ, and 2 to 5 μ particles respectively, with the weights of right and left lungs combined. This indicates a relation between the quantity or size of silica injected and the amount of collagen formed, but this relation is not directly proportional.

Histological Grading Contingency tables (Table IV) showed that the fibrosis caused by the 1 to 3 μ and 2 to 5 μ particles was much more severe than that of the under 1 μ particles. The difference between the 1 to 3 μ and 2 to 5 μ particles was, however, not pronounced.

Comparison of and Correlation between Right and Left Lungs with Respect to the Different Variables

Wet Weight of Lungs It was shown that the injection of silica dust into the lungs can greatly increase the weight of the lung which received the most silica.

Weight of Silica It was shown, as in the previous experiment (Goldstein *et al.*, 1962), that the silica injected is seldom shared equally by the two lungs.

Weight of Collagen The quantities of collagen were usually larger in the right than in the left lungs for the under 1 μ and 1 to 3 μ particles, but this bias was not present in the other group.

Correlation between the Variables, Wet Weight of Lungs, Weight of Silica, and Weight of Collagen No significant correlations (at the 5% level) were found in the under 1μ group, and the correlations which are significant at the 5% level for the other two groups are shown in Table V.

TABLE V

CORRELATIONS SIGNIFICANT AT 5% LEVEL

Variables	*Size Fraction* (μ)	*n*	*Correlation Coefficient*
Wet weight of lungs/Silica	1-3	24	0·44
Silica/Collagen	1-3	24	0·44
Silica/Wet weight per 100 g. body weight	1-3	24	0·50
Collagen/Wet weight per 100 g. body weight	1-3	24	0·41
Wet weight of lungs/Silica	2-5	16	0·46

Discussion

It was found that the two larger sized fractions of silica produced more fibrosis (histologically) than the under 1 μ particles and also produced a more marked increase of the wet weight of the lungs and the collagen content.

As the surface areas of the samples injected were the same for all the size-fractions, it appears that the reaction which occurs in the lungs is possibly related to the quantity (by weight) of the silica injected or the size of the particles and not to the total surface area.

A further experiment should now be undertaken to determine whether injections of equal weights of different sized particles produce similar degrees of fibrosis.

That the quantity of dust injected plays an important part in the production of fibrosis is further borne out by an experiment in which the under 1 μ particles were injected into animals in a higher concentration of 50 mg. per animal and the degree of fibrosis obtained was much more marked than when the surface area of 600 sq. cm. per animal was used with only 13·99 mg. silica in each dose.

It is also interesting to note that, although 41% of under 1 μ particles injected was recovered as against 15% of the 2 to 5 μ particles, the latter produced much more fibrosis. However, 41% of the under 1 μ particles would be equivalent to an average retention of 5·7 mg., while the 15% of the 2 to 5 μ particles represents 13·9 mg. This could indicate that the more silica is retained, the greater the degree of fibrosis.

The differences between the two larger size fractions were not as marked as between these two sizes and the under 1 μ particles. It may be that the under 1 μ particles behave in a different manner from particles above 1 μ, in which case comparison of the fibrogenic properties of dusts that are smaller and larger than 1 μ may have to be re-assessed.

The silica recoveries in this experiment were lower than in the previous experiments (Goldstein *et al.*, 1962). This could be due to a combination of increased loss by regurgitation over the longer duration of the experiment and dissolution within the body.

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REFERENCES

Belt, T. H., and King, E. J. (1945). In *Spec. Rep. Ser. Med. Res. Coun. (Lond.)*, No. 250, p. 29.

Goldstein, B. (1960). In *Proceedings of the Pneumoconiosis Conference, Johannesburg*, 1959, p. 94. A. J. Orenstein, Ed. Churchill, London.

——, Webster, I., and Sichel, H. S. (1962). *Brit. J. exp. Path.*, **43**, 38.

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

Neuman, R. E., and Logan, M. A. (1950). *J. biol. Chem.*, **184**, 299.

Compt. Rend. Soc. Biol.
131:1005-1008, 1939.

Intraperitoneal sclerosis induced upon contact with kieselgur in rats suffering from vitamin A deficiency

by

Norbert Grelet

The in vivo mechanism of the development of the collagen substance of connective tissue has been the subject of experimental studies on scorbutic guinea pigs (1) and on rats kept on a diet deficient in lysine (2) or cystine (3). The method by which sclerosis could be induced in these cases and which was also used by us is as follows: intraperitoneal injection of 1 cc of a kieselgur suspension in Ringer solution leads to the formation of granulomas the progressive organization of which was followed.

Our aim was to investigate the effects of Vitamin A on the development of intraperitoneal granulomas in young rats, limiting ourselves to the initial stages of 2, 4, 7 and 10 days, that is to say the initial processes of connective tissue histogenesis.

Diet: Our experiments were carried out on 3 categories of rats: deficient, normal and loaded.

1. Male 22 day old rats weighing 25-30 g and just weaned were placed on a diet (4) deprived of vitamin A. The deficiency

(4) The composition of the diet was given to us by Mrs. L. Randoin: dextrine Byla 63 g; muscle peptone, Byla 17 g; peanut oil deprived of vitamin A by heating to 100° in an air jet for 24 hours 12 g;

yeast Byla 4 g; salts (Terroine mixture) 4 g; total 100 g. Vitamin D was given in the form of Irrasterine Byla, added to the oil in a quantity of 100 drops per liter.

always became manifest between the 38th and the 45th day of the diet, with xerophthalmia and a levelling off of weight around 90 g.

2. Control rats received the same deficient diet, but supplemented each day by ingestion of 10 mg of a freshly prepared mixture consisting of 200 parts peanut oil added to a 3.5% vitamin A solution in fish liver oil (product A 313) (5). Thus in theory the animals received 1.75γ vitamin A per day, which is sufficient to protect them against this deficiency.

3. Some rats kept on a deficient diet received 20 mg A 313 or 700γ vitamin A orally 64 and 40 hours before they were killed. These rats, which had 2, 4, 7 and 10 day old granulomas, were thus suddenly loaded with vitamin during the last 2 days of the development of the granuloma.

Histological examination: We were not able to demonstrate any difference between granulomas of the same age in deficient, normal or loaded animals. In all cases the 2-day granulomas merely revealed a fibrinous coagulum, agglomerating the kieselgur in a mass infiltrated to a varying depth by polynuclear cells. The 4-day old granulomas always revealed the existence of newly

(5)
Chabre Brothers Pharmaceutical Laboratories, Toulon.

formed collagen, in sinuous, fine fibers forming the periphery of a sheath of varying thickness, which replaced the almost adherent fibrin sheath of the 2-day granuloma. The appearance of the granuloma differs somewhat according to the organism which supports it and according to the thickness of the kieselgur mass: in some cases the polynuclear invasion only reached as far as the center of the mass, while in others the secondary invasion of histiocytes penetrated the entire mass and the diatomic fragments may be seen surrounded by macrophages, sometimes forming giant multinuclear cells at the edge of the granuloma in the vicinity of the collagen fibers of the sheath.

In the seven and ten-day granulomas from deficient animals collagen developed normally. At various times according to the position of the granuloma and its volume, all the diatomic fragments are engulfed by macrophages or enveloped by giant cells and the collagen fibers penetrate throughout the mass.

Thus it seems to us that a vitamin A deficiency has no effect either on the inflammatory reaction (fibrinous coagulum, mobilization of polynuclears and then histiocytes) or on the production of fibrils.

By injecting a few cc of alcurone boullion to deficient or control rats it was found that the deficiency had no influence on the inflammatory reaction. The proportion of the various categories of cells in the peritoneal fluid developed in analogous fashion in the deficient and the normal animals: the

number of polynuclears increased considerably, reaching 50% of the total number of cells on the first day; they then decreased to 10% on the fourth day. The remaining cells were made up of mononuclear histiocytes and a few mast cells. These results accord with those of Webb obtained in his very exhaustive work on peritoneal reactions of the albino rat (4).

Conclusions

The studies of Chevallier, Escarras and Paillas on the "influence of vitamin A reserves of the liver on pleural reactions in the guinea pig" (5) led them to investigate the humoral effect of vitamin A on the genesis of connective tissue, by a method other than dietary deficiency. After intrapleural injection of 1 cc of a kieselgur suspension these authors observed a proliferation of connective tissue which was much more pronounced in the guinea pigs with vitamin reserves than in those without reserves.

Thus our results do not confirm the hypothesis advanced by the work of Chevallier, Escarras and Paillas, but the differences in material and approach between their investigations and ours must be taken into account.

Their studies dealt with guinea pigs from two different strains, one having hepatic reserves living with an excess vitamin load, the other without hepatic reserves, living with a bare sufficiency of vitamins.

We, on the other hand, compared rats of the same sex from the same litters. Some were kept in their normal state of vitamin sufficiency, while others were brought into a pathologic state

of deficiency. Our experiments show that this pathologic state has no effect on the first stages of the histiogenesis of intraperitoneal granulomas formed upon contact with kieselgur.

Bibliography

See footnote references.

From: Cytology Laboratory of the Applied School of Higher Studies.

inoculation sous-cutanée ou intra-péritonéale, pourrait, peut-être, fournir des résultats différents.

Conclusions. — 1° Chez l'homme, inoculé par le virus chorio-méningitique, l'examen de sang décèle, pendant la première phase fébrile pure et pendant l'apyrexie secondaire, des variations systématiques, retrouvées dans 14 cas sur 16, et qui commencent à disparaître pendant la seconde phase fébrile et méningée : leucopénie, granulopénie, puis lymphocytose et mononucléose, enfin éosinophilie relative avec, parfois, polynucléose transitoire.

2° Chez le singe et la souris, on ne constate, inconstamment, qu'une polynucléose transitoire ; mais ces animaux, qu'il faut inoculer par voie intra-cérébrale, ne font pas de phase fébrile préalable, mais présentent d'emblée la méningo-encéphalite.

3° Les variations leucocytaires de l'homme traduisent probablement une réaction des organes leucopoïétiques à l'agression directe du virus chorio-méningitique pendant le stade septicémique initial de l'affection ; ce stade étant ordinairement méconnu dans les cas spontanés de la maladie, les examens hématologiques des auteurs n'ont été pratiqués qu'au stade méningé, alors que la formule sanguine tend à redevenir normale.

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Scléroses intrapéritonéales provoquées au contact de Kieselgur chez des Rats carencés en vitamine A,

par Norbert Grelet.

Le mécanisme de l'élaboration *in vivo* de la substance collagène et des structures conjonctives a fait l'objet d'études expérimentales chez des Cobayes scorbutiques (1) et chez des Rats soumis à un régime déficient en lysine (2) ou en cystine (3). La technique qui a permis d'obtenir les scléroses dans ces cas, et que nous avons utilisée ici, est la suivante : l'injection intrapéritonéale de 1 c.c. d'une suspension de Kieselgur dans le liquide de Ringer conduit à la formation de granulomes dont on suit l'organisation progressive.

Nous nous sommes proposé de rechercher chez de jeunes Rats

(1) L. Randoin et H. Mazoué, *C. R. de la Soc. de biol.*, 1936, t. *122*, p. 1184.
(2) H. Mazoué, *C. R. de la Soc. de biol.*, 1938, t. *127*, p. 1043.
(3) H. Mazoué et B. Nataf, *C. R. de la Soc. de biol.*, 1938, t. *128*, p. 825.

les effets de la carence en vitamine A sur l'évolution de granulomes intrapéritonéaux, en nous limitant aux premiers stades : 2, 4, 7, et 10 jours, c'est-à-dire aux processus initiaux de l'histogenèse conjonctive.

Régimes alimentaires. — Nous avons fait porter nos expériences sur trois catégories de Rats : carencés, complétés et chargés.

1° Des Rats mâles, âgés de 22 jours environ, pesant 25 à 30 gr. venant d'être sevrés, ont été mis à un régime privé de vitamine A. La carence s'est régulièrement manifestée entre le 38e et le 45e jour de régime, par l'apparition de la xérophtalmie et le palier de poids vers 90 gr.

2° Des Rats témoins ont eu le même régime de carence, complété chaque jour par l'ingestion de 10 mgr. d'un mélange fraîchement préparé, constitué par 200 parties d'huile d'Arachide pour une de solution à 3,5 p. 100 de vitamine A dans une huile de foie de Poisson (produit A 313) (5). On donne ainsi théoriquement 1,75 γ de vitamine A par jour, ce qui suffit à protéger les Rats contre la carence.

3° Quelques Rats carencés ont reçu par ingestion buccale 20 mgr. de A 313, soit 700 γ de vitamine A, 64 et 40 heures avant d'être sacrifiés. Ces Rats, porteurs de granulomes de 2, 4, 7, 10 jours ont donc été brusquement chargés en vitamine durant les deux derniers jours de l'évolution du granulome.

Examen histologique. — Nous n'avons pu mettre en évidence aucune différence entre les granulomes de même âge d'animaux carencés, ou complétés ou chargés. Dans tous les cas, les granulomes de deux jours n'ont montré que des coagulum fibrineux, agglomérant le Kieselgur en un amas que les polynucléaires infiltrent plus ou moins profondément. Les granulomes de 4 jours ont toujours montré l'existence de collagène néoformé, en fibres flexueuses et fines formant à la périphérie une gaine plus ou moins épaisse, qui s'est substituée à la gaine de fibrine presque anhiste du granulome de 2 jours. L'aspect du granulome est d'ailleurs un peu différent selon l'organe qui lui sert de support et l'épaisseur de la masse de Kieselgur : parfois l'invasion des polynucléaires vient seulement d'atteindre le centre de l'amas ;

(4) La composition du régime nous a été indiquée par Mme L. Randoin : Dextrine Byla, 63 gr. ; Peptone de muscle Byla, 17 gr. ; Huile d'arachide privée de vitamine A par chauffage à 120° dans un courant d'air pendant 24 heures, 12 gr. ; Levure Byla, 4 gr. ; Sels (mélange de Terroine), 4 gr. ; total, 100 gr.

La vitamine D est apportée sous forme d'Irrastérine Byla, ajoutée à l'huile à raison de 100 gouttes par litre.

(5) Laboratoires pharmaceutique Chabre frères, Toulon.

parfois, au contraire, l'invasion secondaire des histiocytes a déjà pénétré toute la masse, et à la périphérie du granulome, au voisinage des fibres collagènes de la gaine, on commence à voir les débris de Diatomées englobés par des macrophages, qui se constituent parfois en cellules géantes multinucléées.

Dans les granulomes de 7, 10 jours, recueillis sur des animaux carencés, le développement du collagène s'effectue normalement. Plus ou moins tôt, suivant la position du granulome et son volume, tous les débris de Diatomées sont englobés dans des macrophages ou enveloppés par des cellules géantes ; et les fibres de collagène parcourent toute la masse.

Ainsi, la carence en vitamine A nous a semblé n'avoir d'influence ni sur la réaction inflammatoire (caillot fibrineux, mobilisation des polynucléaires puis des histiocytes), ni sur la fibrillogenèse.

Nous avons confirmé que la carence n'avait pas d'influence sur la réaction inflammatoire, en injectant à des Rats carencés ou témoins quelques c.c. de bouillon d'aleurone. La proportion des diverses catégories de cellules dans le liquide péritonéal a évolué de façon analogue chez les animaux carencés et normaux : le nombre des polynucléaires augmente considérablement pour atteindre au premier jour 50 p. 100 du nombre total de cellules ; il diminue ensuite jusqu'à 10 p. 100 le quatrième jour : le reste des cellules est constitué par des histiocytes mononucléaires et quelques mast-cells. Ces résultats sont en accord avec ceux que Webb a obtenus dans son travail très complet sur les réactions péritonéales chez le Rat blanc (4).

Conclusions. — Les recherches de Chevallier, Escarras et Paillas relatives à l' « influence de la réserve hépatique en vitamine A sur les réactions pleurales chez le Cobaye » (5) les ont conduits à aborder, autrement que par l'établissement d'une carence alimentaire, l'étude de l'action humorale de la vitamine A sur la genèse du tissu conjonctif. Ces auteurs constatent, après injection intrapleurale de 10 mmc. d'une suspension de Kieselgur, une prolifération de tissu conjonctif considérablement plus forte chez des Cobayes avec réserve que chez des Cobayes sans réserve.

Nos résultats ne vérifient donc pas l'hypothèse que faisait naître le travail de Chevallier, Escarras et Paillas ; mais il faut noter la différence de matériel et de point de vue entre leurs recherches et les nôtres.

En effet, leurs expériences ont porté sur des Cobayes appartenant à deux races différentes : les uns, qui ont une réserve hépatique, vivent dans un état normal de surcharge vitaminique ; les autres, qui n'ont pas de réserve hépatique, vivent dans un état normal de stricte suffisance vitaminique.

(4) R.-L. Webb, *The Amer. Journ. of Anat.*, 1931, t. *49*, p. 283.

(5) Chevallier, Escarras et Paillas, *C. R. de la Soc. de biol.*, 1938, t. *128*, p. 916.

Nous avons, au contraire, comparé des Rats de même sexe pris dans les mêmes portées : les uns sont maintenus dans leur état normal de suffisance vitaminique ; les autres sont portés à un état pathologique de carence. Nos expériences montrent que cet état pathologique n'exerce aucune répercussion sur les premiers stades de l'histiogénèse de granulomes intrapéritonéaux formés au contact du Kieselgur.

(*Laboratoire de cytologie de l'Ecole pratique des Hautes Etudes.*)

L'ÉLIMINATION BILIAIRE DE BASES AMINÉES CHEZ L'HOMME,

par MAURICE LOEPER et ANDRÉ LESURE.

L'élimination de la tyramine par la bile et les résultats de l'expérimentation chez l'animal nous ont amenés à étudier chez un certain nombre de sujets sains et malades la richesse en tyramine de la bile recueillie par tubage duodénal. Chez les sujets normaux, nous trouvons, pour 1000, 10 et 13 mgr. de tyramine dans la bile du cholédoque et 14 environ dans la bile de la vésicule.

Chez les sujets pathologiques, les résultats sont assez différents et presque toujours en augmentation sur les normaux.

		Bile Tyramine en mgr. p. 1.000
G...	intoxication digestive	23,2
C...	intoxication par les moules	36
D...	ictère, période d'état	18 à 22,4
	terminaison	27 à 31
D...	hépatite chronique	11
C...	néphrite, urémie	39
A...	migraine	32 et 34
B...	migraine	23

Il semble donc que l'élimination de la tyramine s'accroisse dans les intoxications digestives, surtout dans l'intoxication par les moules, qu'elle diminue dans les processus hépatiques aigus ou chroniques, au moins momentanément, pour remonter au-dessus de la normale à la crise, que l'élimination de tyramine est assez abondante au cours des migraines, que l'augmentation

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LYSOSOMAL ENZYMES IN RELATION TO THE TOXICITY OF SILICA

J. S. HARINGTON A. C. ALLISON

INTRODUCTION

Several studies have shown that damage to the macrophage by silica is a pre-requisite for the subsequent fibrosis caused by this dust (MARKS, MASON and NAGELSCHMIDT, 1956; CURRAN and ROWSELL, 1958; MARKS and NAGELSCHMIDT, 1959; VIGLIANI and PERNIS, 1959; MARKS and JAMES, 1959; VIGLIANI, PERNIS and MONACO, 1961). In addition, the relative toxicity of different forms of silica in cell culture agrees with their pathogenicity and fibrogenic qualities *in vivo* (MARKS, MASON and NAGELSCHMIDT, 1956; VIGLIANI, PERNIS and MONACO, 1961; MARKS and NAGELSCHMIDT, 1960). The last named authors have also pointed out (1959) that the correspondence between toxicity and fibrogenic activity supports the hypothesis that the primary dust lesion in animals is the injury to the phagocytic cells which take up the dust.

However, the nature of the primary damage to the macrophage and the subsequent steps leading to fibrosis remain undetermined. KING, in his « solubility » theory of silicosis, suggested in 1947 that silicic acid liberated into the tissues from silica particles caused the fibrous reaction, though KING himself and his colleagues were later to point out (1953) some of the defects inherent in such a concept. Theories involving immunologisal reactions in which silica dust migh participate have been extensively investigated by VIGLIANI and PERNIS (1958; 1962), and others. Also, suggestions have been made that silica dust might damage macrophages as a result of protein adsorption or denaturation, oxidative changes, interference with intracellular enzyme activity such as that of esterases in the cell, or by other mechanisms (see HARINGTON, 1963, for references). Interesting accounts of the different concepts of the pathogenesis of silicosis may be found in papers by VIGLIANI and PERNIS (1958), NAGELSCHMIDT (1960) and ANTWEILER (1963).

It has recently been pointed out (HARINGTON, 1963) that two separate stages may occur in the pathogenesis of silicosis: (1) a first stage, during which the macrophage is killed by the silica dust, and (2) a second stage, in which materials released from the damaged and dead cells are responsible for the ensuing fibrosis. The importance of materials shed from the dying macrophages had been emphasized earlier (MARKS and NAGELSCHMIDT, 1959) and attention was subsequently drawn to the possible participation of lipolysaccharides with some properties of endotoxins (PERNIS, VIGLIANI and

MONACO, 1960), or of lipids and lipoproteins in such an action (HARINGTON, 1963).

Concerning the mode of action of silica in the first stage, the experimental evidence at present available does not allow any firm conclusions to be drawn in favour of either silicic acid or the peculiar surface properties of the silica particle being responsible for the primary lesion. The biological activity of silica seems to be due to either the combination of an essential cell constituent with the surface of the particles or from the liberation into the cytoplasm of a toxic substance, presumably silicic acid, or to both (MARKS and NAGELSCHMIDT, 1959; NAGELSCHMIDT, 1960). The rapid toxic action of silica particles in cell culture suggested that the former mechanism was the more likely one (see also KESSEL, MONACO and MARCHISIO, 1963). POLICARD, COLLET and REY (1959) found that the toxic action of silica particles appeared to be related to their intracellular presence following phagocytosis and that the solubility of the particles seemed to be of importance only after the latter had made contact with the cytoplasm.

Silicic acid *per se* is not fibrogenic though there is little doubt that it can produce pharmacological effects of a particular kind, such as vasoconstriction, bronchoconstriction, protein precipitation and changes in membrane permeability (see HARINGTON, 1963; HARINGTON and SUTTON, 1963, and CURRAN and ROWSELL, 1958, for references). Colloidal silica at low concentration causes acute haemolysis (HARLEY and MARGOLIS, 1961; MAC NAB, in HARINGTON and SUTTON, 1963) which HARLEY and MARGOLIS thought to be the result of rapid adsorption of the particles of colloidal silica by the cell surface with subsequent protein denaturation and lysis of the cell membrane. Polymerized silicates are toxic and directly haemolytic to erythrocytes (CHARACHE, MAC LEOD and WHITE, 1962).

The present study concerns the behaviour of silica particles and inert materials within macrophages in cell culture, as determined by histochemical methods. It is now known that silica and certain inert materials (as this investigation and a parallel one, using electron microscopy, show), asbestos (DOURMASHKIN and DOUGHERTY, 1961; DAVIS, 1963 *a*, *b*), bacteria (COHN, HIRSCH and WIENER, 1963) and non-ionic detergents, for example, Triton WR-1339 and polyvinylpyrrollidone (MAUER and WILLHAGEN, 1963; DE DUVE, 1963), are taken up into phagosomes (phagocytic vacuoles) in the cytoplasm of the macrophage. But whereas inert materials, for instance, diamond dust and carbon, have a negligible effect on the phagosomal membrane, silica, either directly (by surface action) or by the release of silicic acid in a confined situation, damages the membrane of the phagosome in such a way that the hydrolytic enzymes secreted into it by lysosomes during phagocytosis are released into the cytoplasm of the cells. This results in autolysis and the eventual death of the cell.

The damage to the phagosomal membrane may be due to protein precipitation or changes in permeability, and it is this damage specifically which is believed here to be the primary site of damage to the cell by silica.

THE LYSOSOME CONCEPT

The lysosome concept developed from the work of DE DUVE and his colleagues (1955) who showed that in rat liver a series of hydrolytic enzymes,

including acid phosphatase, acid deoxyribonuclease, acid ribonuclease and acid protease, are present in a group of organelles of similar size to mitochondria but distinct from them. These « primary lysosomes » are found in nearly all the cells of higher organisms that have been studied. The hydrolytic enzymes are stored in the lysosomes in an inactive form; they are released under a variety of conditions, and can then be recovered from the supernatant instead of the particulate fraction of cell homogenates (see DE DUVE, 1963).

Lysosomes are especially numerous in polymorphonuclear leucocytes (the neutrophil granules are lysosomes) and in macrophages, and the role of lysosomal enzymes in digestion of bacteria and other particles taken into these cells by phagocytosis has been studied in detail by COHN, HIRSCH and their colleagues (see COHN, HIRSCH and WIENER, 1963: COHN and WIENER, 1963).

The uptake of silica particles by macrophages is a very similar process, and is shown diagrammatically in Figure 1.

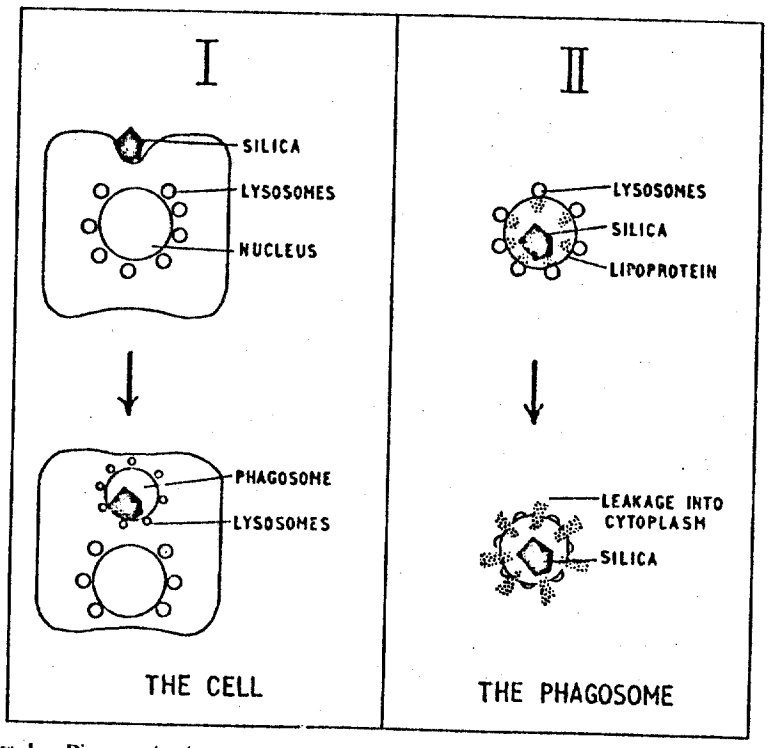

Figure 1 - Diagram showing uptake of silica particle into a phagosome (I), release of lysosomal enzymes into the phagosome and subsequent leakage of these enzymes into the cytoplasm (II).

The bacteria or silica particles are engulfed and taken into phagocytic vacuoles or phagosomes. The membrane around the phagosome (originally part of the cell membrane) is modified in some way, so that lysosomes adhere to and fuse with it, discharging their enzymes into the phagosome.

Under normal conditions, bacteria are more or less completely digested by the lysosomal enzymes. Provided that the enzymes are confined within a phagosome, the cell is not damaged. However, under certain conditions lysosomes can be disrupted, when they discharge their enzymes directly into the cytoplasm, and this leads rapidly to cell death. Such lysosomal disruption has been observed when cells are exposed to the bacterial toxins, streptolysin S or O (WEISSMANN, KEISER and BERNHEIMER, 1963; HIRSCH, BERNHEIMER and WEISSMANN, 1963). It is also seen when macrophages and other cells are exposed to anthracene or porphyrin, which are selectively taken up by lysosomes, and the cells are then illuminated by monochromatic light of wavelength efficiently adsorbed by the photosensitizing agent (ALLISON and MAGNUS, 1965). The membranes of the lysosomes immediately become permeable, and release of lysosomal enzymes rapidly brings about cell death.

Useful information about lysosomes can be obtained by histochemical methods for the enzymes they contain. Convenient techniques are available for acid phosphatase and 5-bromo-4-chloroindoxylacetate esterase (HOLT, 1958). If the Gomori reaction for acid phosphatase is carried out on normal, unfixed cells, little or no staining is observed (Fig. 4). As DE DUVE (19[illegible]) and BITENSKY (1963) have shown, the membranes of intact, healthy lysosomes are impermeable to the β-glycerophosphate substrate. However, if the cells are fixed in a suitable medium which does not inactivate the enzyme, and then stained for acid phosphatase, numerous discrete lysosomes containing enzyme are revealed (Fig. 2). Lysosomes can also be shown by vital staining with neutral red and some other basic dyes (Fig. 3). Moreover, if cells are damaged in any way so that the membranes of the lysosomes become permeable, the lysosomes are stained in unfixed cells.

MATERIALS AND METHODS

Cultures of macrophages. The term « macrophage » in this context refers to mononuclear cells obtained from peritoneal washings, without prior stimulation, and which become attached to the glass substrate in culture. Nearly all the cells take up large amounts of carbon or particulate dyes soon after the cultures are set up, so that they can be considered as belonging to the rather miscellaneous group of cells of macrophage type. The cells were obtained by inoculating 3 ml of culture medium (solution 199, Glaxo, and 20 % calf serum inactivated at 56° for ½ hour) containing 10 i.u. heparin per ml into mice of strain VSBS/NIMR, weighing 18-20 g. After brief massage the cells were recovered and dispensed in 1 ml volumes (containing 2 x 10^5 cells) into tubes containing small coverslips. Cells were incubated overnight before exposure to silica.

Silica. Four types of silica, kindly provided by Dr. I. BERGMAN of Sheffield, were used: (1) « Snowit » silica particles (X9947) of size range 15 % < 2.5 μ, 49 % 2.5-5 μ, 30 % 5-7.5 μ and 5 % 7.5-10 μ; (2) « Fransil » silica (X5663), uncompressed, consisting of vitreous silica particles of diameter up to 0.3 μ; (3) NaOH - etched « Fransil » (X5843 from X5663) of size range 0.1 μ to 0.5 μ; (4) Aluminium-coated silica (DALE and KING, 1953). All samples were first sterilised before being added as fine suspensions to the culture media.

Control materials. (1) Diamond dust, particle size 2-4 μ (Diamond Products (Sales) Ltd., London); (2) Highly purified carageenan (Seaplant Chemical Corporation, Massachusetts). Polyvinylpyridine-N-oxide (P204, U33) was obtained through the courtesy of Professor H. W. SCHLIPKÖTER, Düsseldorf.

Figure 2 - Macrophages fixed in glutaraldehyde and stained for acid phosphatase, showing numerous lysosomes containing the enzyme. × 1140.

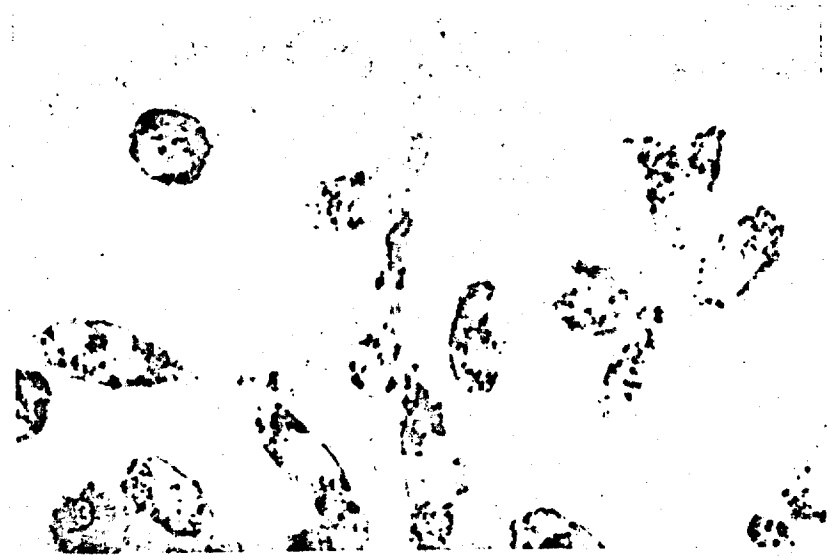

Figure 3 - Macrophages vitally stained with neutral red, which is taken up into the lysosomes. × 1140.

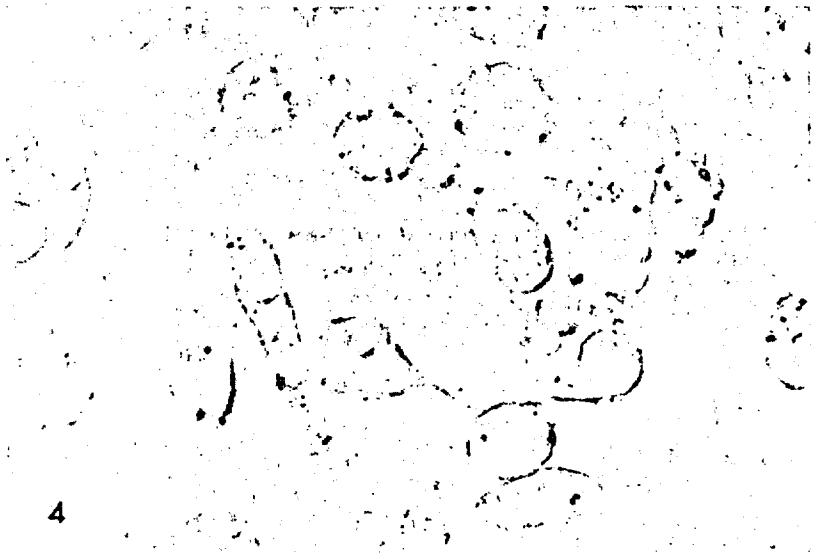

Figure 4 - Unfixed macrophages, stained for acid phosphatase. No enzyme is demonstrated because the substrate does not penetrate into the lysosomal membranes. × 1140.

Figure 5 - Dark-ground photomicrographs of macrophages 4 hours after taking up silica particles, which are shown by arrows. Nuclei are marked (N). × 1140.

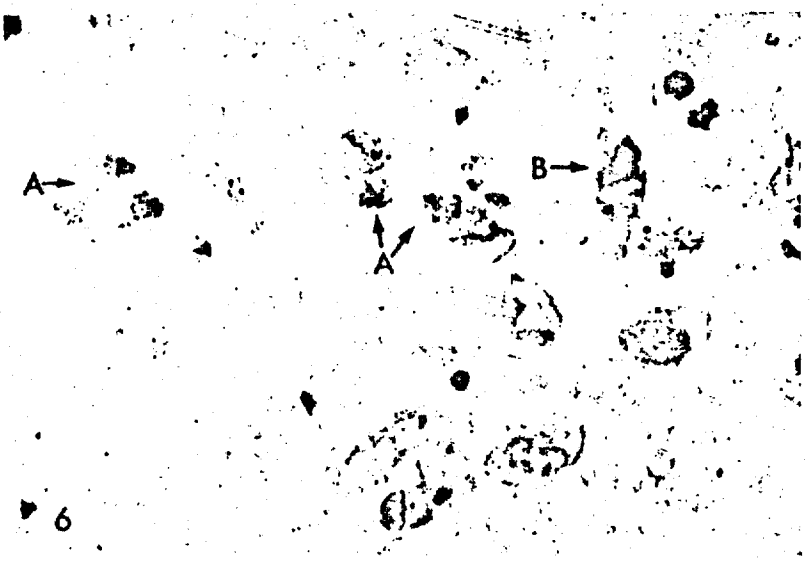

Figure 6 - Unfixed macrophages stained for acid phosphatase 4 hours after ingestion of silica particles. In cells marked (A), discrete lysosomes are attached to phagosomes containing silica. In cells marked (B), phagosomes show accumulation of enzyme. × 1140.

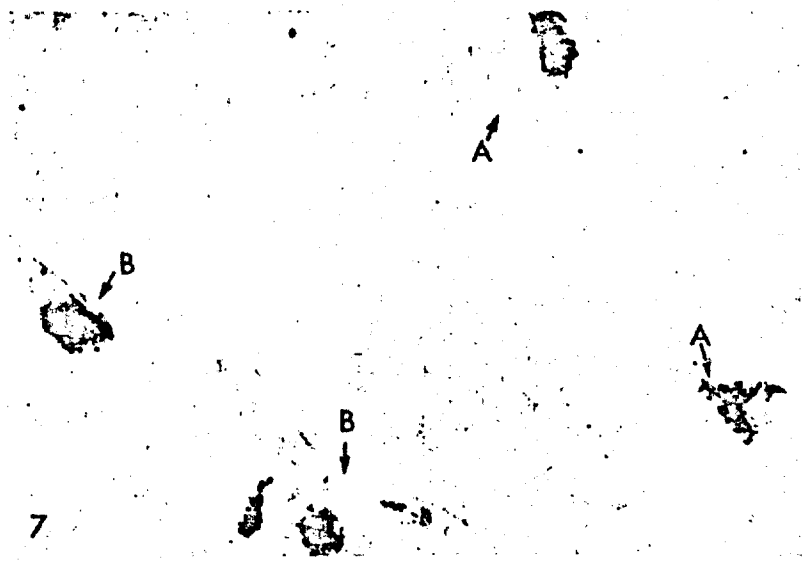

Figure 7 - Unfixed macrophages 8 hours after ingestion of silica, acid phosphatase reaction. There is marked enzyme activity in phagosomes, with some discrete staining in cells marked (A), and general staining in cells marked (B). × 1140.

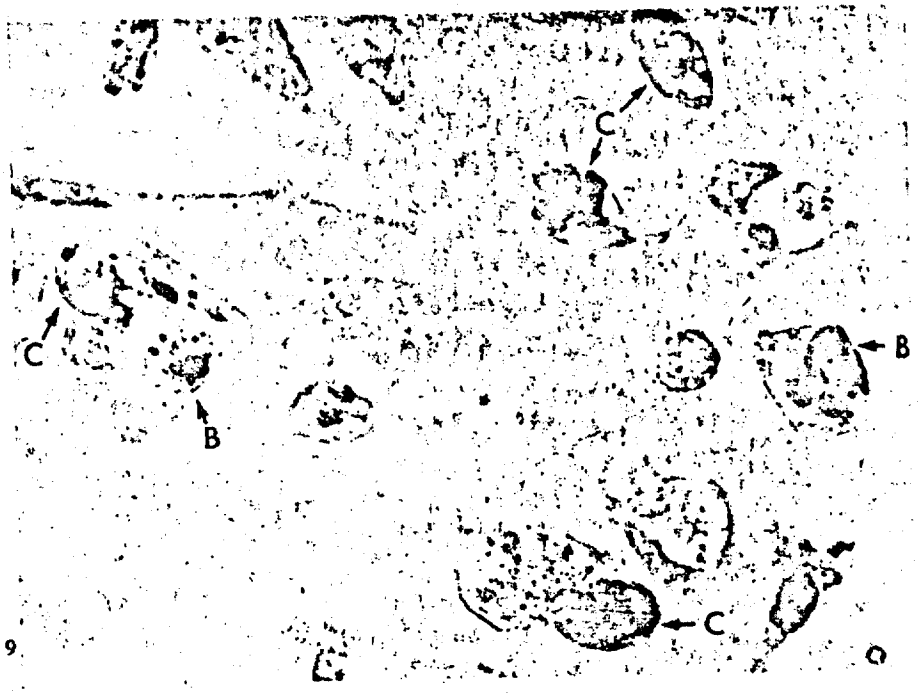

Figure 8-9 - Unfixed macrophages 18 hours after ingestion of silica, acid phosphatase reaction. Marked phagosomal staining is shown in cells marked (B), and diffuse cytoplasmic staining, with rounding of cells, is marked (C). × 1140.

Histochemical methods. Acid phosphatase staining, by a modified Gomori method (ALLISON and MALLUCCI, 1965), was carried out on unfixed cells and on cells fixed in 2 %, buffered glutaraldehyde for 10 min. at 4°. Indoxylacetate esterase staining was carried out as described by HOLT (1958).

Viability tests. Cells were treated with eosin in saline for 5 minutes, and counts of eosin staining in 1000 cells were made.

OBSERVATIONS

Effects of silica on macrophages. Macrophages were exposed to silica of various types (10-100 µg/ml) in the culture media, and observed by phase-contrast, time-lapse cinemicrography, interference microscopy and dark-ground microscopy. Silica particles rapidly became attached to the cell membrane and were engulfed by the cells. They remained in large phagosomes within the cells, where they were most easily shown by dark-ground microscopy (Fig. 5). Lysosomes are relatively refractile particles that can be identified by phase-contrast microscopy. They were seen travelling through the cytoplasm by Brownian movement and becoming attached to the walls of the phagosomes. They were also visible in association with the phagosomes after acid-phosphatase staining (Fig. 6-9). Numerous discrete, enzyme-containing granules were clustered around the silica particles. Later, the enzyme was discharged into the phagosomes so that large, diffuse pools of enzyme enveloped the silica particles (Figs. 7-8). At various times thereafter, diffuse staining of the cytoplasm was observed, and the cells rounded up and became immobile (Fig. 9). Staining with indoxylacetate esterase gave comparable results, so that the behaviour of the two enzymes can probably be taken as representative of that of the other lysosomal enzymes. The results obtained with « Fransil » silica and carageenan were very similar to those obtained with « Snowit » silica, although the intracellular « Fransil » was less easily recognised. Intracellular carageenan was readily seen in fixed preparations by its metachromatic staining with toluidine blue. The behaviour of carageenan is of particular interest since this substance, an acid mucopolysaccharide related to heparin, is a powerful fibrogenic agent (ROBERTSON and SCHWARTZ, 1953; JACKSON, 1956, 1957; SLACK, 1957; WILLIAMS, 1957). In all cases the proportion of moribund or dead cells at 36 hours was high.

Protective effect of aluminium and polyvinylpyridine-N-oxide.

Cells exposed to aluminium-coated silica took up the particles in the normal way and lysosomes discharged their enzymes into the phagosomes, though apparently to a lesser extent than with untreated silica. However, very little escape of enzymes into the surrounding cytoplasm took place, and few of the cells rounded up and showed eosin staining at 36 hours.

Macrophages were incubated overnight in medium containing 0.5 % polyvinylpyridine-N-oxide (P204) and silica (50 µg/ml) was added in the presence of the former. Silica was taken up in the normal way, and lysosomal enzymes were discharged into phagosomes, although to a lesser extent than in the absence of polyvinylpyridine-N-oxide. Again, little cytoplasmic enzyme staining, rounding of cells and cell death was observed, and eosin staining showed considerable protection of cells at 36 hours.

Diamond dust. This was added to macrophage culture media at a concentration of 50 µg/ml. It was taken up by cells in the normal way, with readily detectable discharge of lysosomal enzymes into the phagosomes so formed. Again, little or no release of enzyme into the surrounding cytoplasm, rounding of cells and cell death were observed.

COMMENT

It is clear that the uptake by macrophages of toxic particles or substances (silica and carageenan) is not noticeably different from that of non-toxic particles (diamond dust and aluminium-coated silica). In either case engulfment and formation of phagosomes with discharge of lysosomal enzymes into the phagosomes, follow essentially the same pattern, although the accumulation of the enzymes does seem to be less in the case of non-toxic materials. However, the subsequent events are markedly different. Cells that have taken up diamond dust or amorphous carbon remain healthy for long periods, whereas cells that have taken up silica or carageenan rapidly succumb.

In considering possible mechanisms of toxicity, it is reasonable to start where the offending substance is located, that is to say, the phagosome containing the silica or carageenan. Evidently some reaction initiated by the presence of the silica or carageenan occurs. Evidently, too, the anionic groups of the silica are involved, since coating of the particles with aluminium, or the presence of the weakly basic polyvinylpyridine-N-oxide, markedly diminish the toxic effects of silica. The most likely explanation of the initial toxic event is that silicic acid — either present on the surface of the particles or in the contents of the phagosome — reacts with the lipoprotein membrane surrounding the phagosome and makes it permeable to the hydrolytic enzymes enclosed within the phagosome. The release of these enzymes into the cytoplasm rapidily leads to cell death, in a way analogous to the release of lysosomal enzymes directly from lysosomes into the cytoplasm by streptolysin or photosensitization. There is no need at this stage to specify the sequence of events leading to cell death; many different damaging effects could be brought about by released lysosomal enzymes.

That silica can induce permeability in lipoprotein membranes systems is shown by the well-known lytic effect of colloidal silica on erythrocytes (HARLEY and MARGOLIS, 1961). Quite low concentrations of silicic acid are effective in bringing about this haemolysis, and we have found that prior incubation of erythrocytes with polyvinylpyridine-N-oxide markedly reduces the haemolytic effect of colloidal silica.

Interaction of silicic acid or its polymer with lipoprotein monolayers and protein films has been shown in many investigations (for example, SCHULMAN and RIDEAL, 1937; CLARK, HOLT and WENT, 1957; CLARK and HOLT, 1957). The anionic groups of silicic acid could form electrovalent links with charged groups of protein or lipid, and CLARK et al. have provided evidence that hydrogen bonds might be formed between keto-imino groups of proteins and hydroxyl groups of silicic acid. That interactions of this type can increase permeability of biological membranes is demonstrated by the haemolytic effect of colloidal silica. There is thus good reason for supposing that silicic acid could increase the permeability of phagosomal membranes. Polyvinylpyridine-N-oxide would be expected to form hydrogen bonds with hydroxyl groups of silicic acid and therefore prevent interaction of the latter with biological membranes. In this connection, MARCHISIO and COMOLLI have pointed out (1964) that polyvinylpyridine-N-oxide, after pinocytosis by macrophages, protects these cells either by shielding cellular con-

stituents from the engulfed silica or by reacting directly with the surface of the latter. In the opinion of these authors, this last possibility seemed the more likely one.

Having presented our views on the mechanism of silica toxicity, it is worth discussing how the experimental results of others fit into the scheme. The toxicity of silica to peritoneal and alveolar macrophages obtained from several experimental animals has been described by many investigators, including MARKS (1957, 1959), PERNIS, VIGLIANI and MONACO (1960) and KESSEL (1962). KESSEL has stated that the toxicity is specific for macrophages; in his hands phagocytosis of tridymite dust by neutrophils, chick embryo fibroblasts or liver cells, HeLa, KB or monkey kidney cells led to no demonstrable cytotoxicity. However, BECK, BRUCH and BROCKHAUS (1963) and SCHLIPKÖTER, DAGNER and BROCKHAUS (1963) have found that silica is toxic to the L-strain of mouse fibroblasts, though after a longer delay than with macrophages. SCHLIPKÖTER and his colleagues have used the L-cell system to demonstrate protective effects of polyvinylpyridine-N-oxide in tissue cultures as well as in whole animals, and MARKS (1959) had previously used macrophages cultures for analysis of the protective effects of aluminium derivatives and various other chemical compounds against silica toxicity. Recently, MARCHISIO and COMOLLI have obtained (1964) a clear-cut protection by polyvinylpyridine-N-oxide against the toxicity of tridymite and vitreous silica to macrophages.

Some histochemical work has already been carried out in attempts to test various theories of silica toxicity. PERNIS, VIGLIANI and MONACO (1960) suggested that lysis of macrophages after phagocytosis of silica particles was caused either by the activation of serum complement or activation of non-specific esterase - possibly by interference with an esterase inhibitor. However, WEBSTER, MELAMED and MUNDAY (1961) found by histochemical analysis of fixed preparations a decreased esterase and acid phosphatase activity in macrophages 48 hours after incubation with silica. On the outher hand, RILKE and KESSEL (1963) found no detectable change in acid phosphatase and some other enzymes in macrophages up to three hours after ingestion of silica. The histochemical methods used by these authors, which would demonstrate acid phosphatase activity in lysosomes and phagosomes alike with rather poor intracellular resolution, would be unsatisfactory for demonstration of redistribution of enzymes such as we have described in this paper. Moreover, the experiments of RILKE and KESSEL were carried out very early after silica ingestion, when changes in enzyme distribution, though important, are not conspicuous by microscopy, while the experiments of WEBSTER and his colleagues were carried out very late, when an overall decrease of acid phosphatase through leakage into the culture medium, and possibly intracellular destruction, would be expected. The alpha naphthyl acetate esterase studied by WEBSTER et al. is not lysosomal, whereas the indoxylacetate esterase we have studied is lysosomal.

POLICARD, COLLET and PRÉGERMAIN (1959), in examining the cellular reaction after the injection of silica into rats, observed uptake into phagocytic cells, including neutrophils. They drew attention to the disappearance of neutrophil granules during the process. Since these granules are lysosomes (COHN et al., 1963), these findings provide further evidence that lysosomal changes occur in cells exposed to silica.

There is good evidence that acid phosphatase activity increases *in vivo* in response to silica, both acutely in the mesenchymal cells of liver and spleen (RILKE and PERNIS, 1961) and in developing silicotic nodules (ENGELBRECHT and PAUL, 1959). The former could be due to lysosomal enzyme activation, the latter to the combined effects of immigration of cells with high acid phosphatase activity and activation.

If lysosomal enzymes play an important part in silica toxicity and the consequent fibrosis, agents which stabilize lysosomes would be expected to diminish these effects. Cortisone is known to stabilize lysosomal membranes and prevent release of lysosomal enzymes under a variety of conditions (WEISSMANN and DINGLE, 1962). There is good evidence that cortisone administration diminishes the fibrogenic effects of silica (HARRISON, KING, DALE and SICHEL, 1952), although it has no effect on established lesions (KING HARRISON and ATTYGALLE, 1955). Hence our interpretation of silica toxicity appears to fit in quite well with other experimental results and emphasizes the importance of lysosomal enzymes in the initial action of silica in the cell. Much remains to be learnt about the sequence of events between macrophage death and the development of the established silicotic nodule. It is clear that silica particles are capable of setting in motion a cycle of cellular destruction since particles of silica would be released following autolysis of the cells by which they were first taken up. The continued release of other materials from autolysing cells may provide a sufficient stimulus for collagen deposition and the formation of the ultimate lesion in silicosis.

Acknowledgement - One of us (J.S.H.) wishes to thank the Anna Fuller Fund for full financial support at the Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, and the Wellcome Trust, London, for travel and subsistence expenses.

SUMMARY

Several studies have shown that damage to the macrophage by silica is a prerequisite for the subsequent fibrosis caused by this dust. The present study concerns the nature of the primary damage to the cell by silica and emphasizes the importance of lysosomes and phagosomes (or phagocytic vacuoles) in this process.

The experimental evidence indicates that silica particles are taken up into phagosomes in the cytoplasm of the macrophage. Once taken up, the silica, either directly (by surface action) or by the release of silicic acid in a confined situation, damages the membrane of the phagosome in such a way that the hydrolytic enzymes secreted into it by lysosomes during phagocytosis are released into the cytoplasm of the cells. This results in autolysis and the eventual death of the cell.

The damage to the phagosomal membrane is believed here to be the primary site of damage to the cell by silica.

RIASSUNTO

HARINGTON J. S., ALLISON A. C. — *Enzimi lisosomiali in rapporto con la tossicità della silice.*

Numerosi studi hanno mostrato che l'azione tossica della silice sui macrofagi è un fenomeno essenziale per la evoluzione della successiva reazione fibrotica. La ricerca attuale è indirizzata a studiare la natura del primitivo danno cellulare indotto dalla silice e mette in evidenza l'importanza dei lisosomi e dei fagosomi in questo processo.

I rilievi sperimentali indicano che le particelle di silice vengono inglobate dai fagosomi situati nel citoplasma dei macrofagi. Una volta fagocitata, la silice, sia per azione

di superficie, che per effetto, della liberazione di acido silicico, danneggia la membrana del fagosoma in modo tale da liberare nel citoplasma gli enzimi litici secreti all'interno di esso dai lisosomi.

Ne deriva l'autolisi della cellula. L'alterazione della membrana fagosomica sembra essere il punto d'attacco primario della silice.

ZUSAMMENFASSUNG

HARINGTON J. S., ALLISON A. C. — *Die Enzyme der Lysosomen in ihrer Beziehung zur Toxizität der freien Kieselsäure.*

Zahlreiche Forschungen haben gezeigt, dass die toxische Wirkung der Kieselsäure auf die Makrophagen eine wesentliche Phase in der Entwicklung der fibrotischen Gewebsreaktion darstellt. Die vorliegende Untersuchung hatte das Ziel, das Wesen der primären Zellschädigung infolge von Kieselsäure aufzuklären und es wurde die Bedeutung der Lysosomen und der Phagosomen bei diesem Vorgang nachgewiesen. Die experimentellen Befunde haben ergeben, dass die Kieselsäureteilchen von den Phagosomen aufgenommen werden, welche sich im Zytoplasma der Makrophagen befinden. Wenn die Kieselsäure erst einmal phagozytiert ist, dann schädigt sie teils durch Oberflächenwirkung, teils durch die Löslichkeit der Kieselsäure die Membran des Phagosoms so, dass die Enzyme, welche im Inneren des Lysosoms erzeugt werden, ins Zytoplasma übertreten.

Dadurch kommt es zu einer Autolyse der Zelle. In der Schädigung der Membran der Phagosomen scheint demnach der primäre Angriffspunkt der Kieselsäure zu liegen.

REFERENCES

ALLISON A. C. and MAGNUS I. A. (1965): Nature, Lond. (in the press). — ALLISON A. C. and MALLUCCI L. (1965): J. exp. Med. (in the press). — ANTWEILER H. (1963): Arb. Med., *4*, 125. — BECK E. G., BRUCH J. and BROCKHAUS A. (1963): Ztschr. für Zellforsch., *59*, 568. — BITENSKY L. (1963): In Ciba Foundation Symposium on Lysosomes (J. V. S. de Reuck and M. P. Cameron, editors), London, Churchill, pp. 362. — CHARACHE P., MAC LEOD C. M. and WHITE P. (1962): J. Gen. Physiol., *45*, 1117-1143. — CLARK S. G. and HOLT P. F. (1957): Trans. Faraday Soc., *53*, 1509. — CLARK S. G., HOLT P. F. and WENT C. W. (1957): Trans. Faraday Soc., *53*, 1500. — COHN Z. A., HIRSCH J. G. and WIENER E. (1963): In Ciba Foundation Symposium on Lysosomes. (J. V. S. de Reuck and M. P. Cameron, editors). London, Churchill, pp. 126. — COHN Z. A. and WIENER E. (1963): J. exp. Med., *118*, 1009. — CURRAN R. C. and ROWSELL E. V. (1958): J. Path. Bact., *76*, 561. — DALE J. C. and KING E. J. (1953): Arch. Indust. Hlth. Occ. Med., *7*, 484. — DAVIS J. M. G. (1963 *a*): Brit. J. exp. Path., *44*, 454; (1963 *b*): Brit. J. exp. Path., *54*, 568. — DE DUVE C. (1959): Lysosomes, a new group of subcellular particles. In *Subcellular Particles*. (T. Hayashi, editor) New York, Ronald Press, p. 129. — DE DUVE C. (1963): In Ciba Foundation Symposium on *Lysosomes*. (J. V. S. de Reuck and M. P. Cameron, editors) London, Churchill, pp. 1. — DE DUVE C., PRESSMAN B. C., GIANETTO R., WATTIAUX R. and APPELMANS F. (1965): Biochem. J., *60*, 604. — DOURMASHKIN R. R. and DOUGHERTY R. M. (1961): Exp. Cell. Res., *25*, 480. — ENGELBRECHT F. M. and PAUL R. (1959): Ann. Occup. Hyg., *1*, 135. — HARINGTON J. S. (1963): S. Afr. Med. J., *37*, 451. — HARINGTON J. S. and SUTTON D. A. (1963): Med. Lavoro, *54*, 701. — HARINGTON J. S. (1963): ibid., *54*, 769. — HARLEY J. D. and MARGOLIS J. (1961): Nature, *189*, 1010. — HARRISON C. V., KING E. J., DALE J. C. and SICHEL R. (1952): Brit. J. indust. Med., *9*, 165. — HIRSCH J. G., BERNHEIMER A. W. and WEISSMANN G. (1963): J. exp. Med., *118*, 223. — HOLT S. J. (1958): In General Cytochemical Methods. (J. F. Danielli, editor) New York, Academic Press, *1*, 375. — JACKSON D. S. (1956): Biochem. J., *62*, 25P; (1957): Biochem. J., *65*, 277-284. — KESSEL R. W. (1962): Proc. IV. Intern. Congr. Silicosis, Münster. — KESSEL R. W., MONACO L. and MARCHISIO M. A. (1963): Brit. J. exp. Path., *44*, 351. — KING E. J. (1947): Occup. Med., *4*, 26. — KING E. J., HARRISON C. V. and ATTYGALLE D. (1755): Brit. J. indust. Med., *12*, 228. — KING E. J., MOHANTY G. P., HARRISON C. V. and NAGELSCHMIDT G. (1953): Brit. J. industr. Med., *10*, 9. — MARCHISIO M. A. and COMOLLI R. (1964): Med. Lavoro, *55*, 401. — MARKS J., MASON A. M. and NAGELSCHMIDT G.

(1956): Brit. J. indust. Med., *13*, 187. — MARKS J. (1957): Brit. J. indust. Med., *14*, 81. — MARKS J. (1959): Brit. J. indust. Med., *16*, 166. — MARKS J. and JAMES D. M. (1959): J. Path. Bact., *77*, 401. — MARKS J. and NAGELSCHMIDT G. (1959): Arch. Indust. Hlth., *20*, 383. — MARKS J. and NAGELSCHMIDT G. (1960): Safety in Mines Research Establishment, Research Rep., 186, 1. — MEIJER A. E. F. H. and WILLIHAGEN R. G. J. (1963): Biochem. Pharmacol., *12*, 973. — NAGELSCHMIDT G. (1960): Symposium on Industrial Diseases, eds. King E. J. and Fletcher C. M., London, Churchill, p. 59-68. — PERNIS B., VIGLIANI E. C. and MONACO L. (1960): Med. Lavoro, *51*, 3. — POLICARD A., COLLET A. and PRÉGERMAIN S. (1959): Rev. Hématol., *14*, 97. — POLICARD A., COLLET A. and REY L. (1959): Compt. Rend., *249*, 16. — RILKE F. and KESSEL R. W. I. (1963): Med. Lavoro, *54*, 161. — RILKE F. and PERNIS R. (1961): Med. Lavoro, *52*, 321. — ROBERTSON W. van B. and SCHWARTZ B. (1953): J. biol. Chem., *201*, 689. — SCHLIPKÖTER H. W., DOLGNER R. and BROCKHAUS A. (1963): German Medical Monthly, *8*, 509. — SCHULMAN J. H. and RIDEAL E. K. (1937): Proc. Roy. Soc., *122B*, 46. — SLACK H. G. B. (1957): Biochem. J., *65*, 459. — VIGLIANI E. C. and PERNIS B. (1958): Brit. J. industr. Med., *15*, 8. — VIGLIANI E. C. and PERNIS B. (1959): J. Occup. Med., *1*, 319. — VIGLIANI E. C., PERNIS B. and MONACO L. (196[illegible]). In Inhaled Particles and Vapours. Editor: Davies C. N., Pergamon Press, London, 3[illegible]3. — VIGLIANI E. C. and PERNIS B. (1962): Internat. Arch. Gewerbepath. u. Gewerbehy[illegible], *19*, 507. — WEBSTER I., MELAMED M. and MUNDAY D. E. (1961): Med. Lavoro, 52. — WEISSMANN G. and DINGLE J. T. (1962): Exp. Cell Res., *25*, 207. — WEISSMANN G., KEISER H. and BERNHEIMER A. W. (1963): J. exp. Med., *118*, 205. — WILLIAMS G. (1957): J. Path. Bact., *73*, 557.

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The ultimate particle size of the colloid varies with the conditions of preparation and storage. Most of the investigators quoted have observed that aged sols have far greater biological effects than freshly prepared silicic acid solutions[2-6]. The difference is presumably due to the spontaneous growth of the particles on ageing. Using modern methods it is now possible to make silica dispersions of almost any desired particle size and thus evaluate quantitatively the differences between their biological activity. Such observations have been reported by one of us in relation to clotting of blood[6]. In the present work we have studied the effects of various types of colloidal silica on intact red cells.

To obtain silicic acid, 1–5 per cent solutions of sodium silicate were passed through a column of cation exchange resin ('Zeo-Karb 225'). According to the concentration, *p*H and subsequent heating of the material, a series of sols of particle size from 2 mμ to 6 mμ were obtained (cf. Iler[9]). In addition we used commercial preparations of silica ('Ludox *SM*', Du Pont: 7 mμ and 'Syton W20', Monsanto: about 30 mμ).

Human red cells were washed three times with buffered isotonic saline (phosphate or bicarbonate buffer, *p*H 7·4) and suspended in saline. One-tenth volumes of serial dilutions of silica were then added to a series of tubes with the red cell suspensions and the contents were incubated at 37° C. with occasional gentle agitation. After centrifuging, the supernatant hæmoglobin was measured and expressed as a percentage of the total.

Fig. 1 shows the result of a typical experiment with a 2 per cent cell suspension incubated with silica for 30 min. It is obvious that colloidal silica can be a very potent hæmolytic agent. For example, 1 μgm./ml. of the 30-mμ particles lyses about 50 per cent of the cells. Since the surface area of this silica is somewhat less than 1 cm.²/μgm. while that of 2 per cent cell suspension is about 150 cm.²/ml., it follows that contact of silica with a very small portion of the red cell surface is sufficient to produce hæmolysis. The hæmolytic activity decreases with the particle size

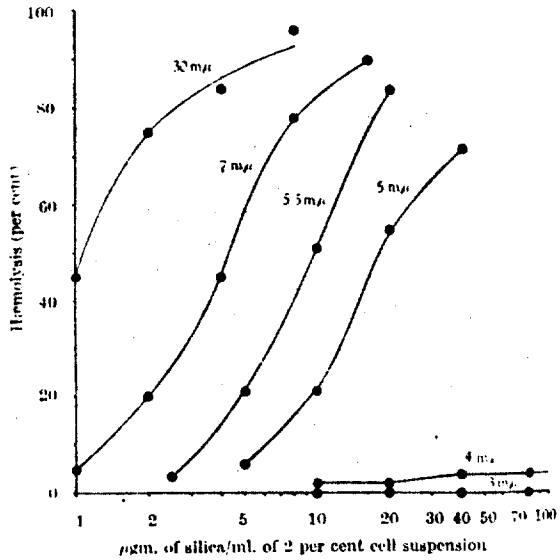

Fig. 1. Hæmolysis of 2 per cent red cell suspension incubated with colloidal silica for 30 min. at 37° C. The figures above the curves indicate the mean particle diameter of the colloid

Hæmolytic Activity of Colloidal Silica

COLLOIDAL silica is highly toxic when administered parenterally[1,2]. In isolated biological systems silica has been shown to interfere with some enzymes[3,4], activate blood clotting and pharmacologically active substances in plasma[5] and interfere with the red cell envelope[6]. Most of these effects are probably due to the adsorption and partial denaturation of proteins at the surface of silica particles[5,7].

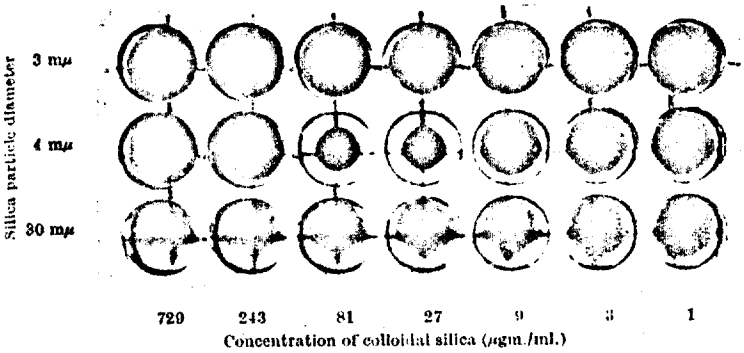

Fig. 2. Hæmagglutination plate viewed from above. 2 per cent red cell suspension was incubated with colloidal silica. Black lines were drawn on the reverse side to emphasize the transparency of the hæmolysed samples. Top row, no visible change; second row, zonal agglutination; bottom row, hæmolysis

of the colloid until it becomes negligible at about 4 mμ diameter. We have also observed that particles in the range of 3·5–7 mμ have a powerful agglutinating effect on the red cells, with a well-marked prozone (Fig. 2). Silica of 3-mμ or smaller particle size has no such activity but protects the cells against the agglutination and hæmolysis by the larger particles. This 'blocking' effect occurs only when the smaller particles are introduced into the cell suspension before the larger ones and it persists after repeated washing with saline, which probably indicates an irreversible adsorption of silica on to the erythrocyte surface. Such 'coated' red cells have other properties in addition to their resistance to the larger particles of silica. Thus they are not hæmolysed by streptolysin *O* and the saponin fragility is somewhat decreased. On the other hand, these cells become more susceptible to immune (rat) hæmolysins and to primaquine. The osmotic fragility is not altered, nor is there any obvious abnormality in appearance except that the cells do not become spherocytic when examined between glass slides and coverslips as do normal washed erythrocytes[10].

In all these experiments it is essential to use well-washed cells because even small traces of plasma markedly inhibit the action of silica. The addition of 1 : 200 plasma to the system as in Fig. 1 completely prevents hæmolysis. The plasma must, however, come in contact with silica before the latter is adsorbed by the red cells. Even a few seconds delay suffices to abolish the protective effect of the plasma. This phenomenon is evidently due to the coating of the silica by certain plasma components. The substances in question are non-dialysable and can be recovered in alkaline eluates from powdered silica which has been exposed to plasma or serum. Such eluates retain their activity after heating to 100° C.

Our observations on the agglutinating and blocking activity of silica agree with the qualitative findings of Renton and Hancock[6], who suggested that these effects may be analogous to those of agglutinating and incomplete antibodies, respectively. The present results extend this model to cover hæmolysis as well.

Although our present knowledge of the red cell structure is still fragmentary, the reaction between silica and the cell membrane may be visualized in general terms as a two-stage process: first, a rapid adsorption of the particles by the cell surface, due mainly to the ionic attraction between silica and the amino-groups at the cell surface and further reinforced by hydrogen bonds and van der Waals forces[7]; secondly, protein denaturation, the extent of which may depend purely on the geometrical relation between the colloidal particles and the protein molecules, as was previously suggested in connexion with blood clotting[5,8]. Individual silica particles of less than 3·5 mμ may not provide sufficient contiguous surface to permit significant unfolding of the much larger protein molecules. Contact with larger particles will, however, allow such unfolding (denaturation), and the resulting interference with the integrity of the cell membrane then leads to agglutination or hæmolysis according to the degree of denaturation. The geometrical picture may, of course, be oversimplified and other physical differences between the large and the small colloidal units may be contributory factors[11]. Despite these reservations, colloidal silica may prove a useful tool in the elucidation of the red cell structure.

We wish to thank Miss Helen Robin for technical assistance.

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[1] Gye, E. W., and Purdy, W. J., *Brit. J. Exp. Path.*, **3**, 75 (1922).
[2] Glömme, J., Holmquist, C. E., and Swensson, A., *Amer. Med. Assoc. Arch. Indust. Health*, **17**, 204 (1958).
[3] King, E. J., Schmidt, E., Roman, W., and Kind, P. R. N., *Enzymologia*, **17**, 341 (1956).
[4] Rowsell, E. V., and Leonard, A. R., *Biochem. J.*, **70**, 57 (1958).
[5] Margolis, J., *Hemophilia and Other Hemorrhagic States*, edit. K. M. Brinkhous, Chapel Hill, 208 (1959).
[6] Renton, P. H., and Hancock, J. A., *Vox Sanguinis*, **2**, 117 (1957).
[7] Holt, P. F., and Bowcott, J. E. L., *Biochem. J.*, **57**, 471 (1954).
[8] Margolis, J., *Eighth International Congress of Haematology*, Tokyo, 1960 (in the press).
[9] Iler, R. K., *The Colloid Chemistry of Silica and Silicates* (Cornell, 1955).
[10] Ponder, E., *Hemolysis and Related Phenomena* (Grune and Stratton, New York, 1948).
[11] Young, G. J., and Bursh, T. P., *Colloid Sci.*, **15**, 361 (1960).

Biochem J. 48. XIIV. 1951

bicarbonate layer deposited a crystalline Na salt of a polybasic acid (II). Subsequent evaporation of the remaining ether layer and steam distillation of the residue gave anisaldehyde.

Acid I, m.p. 285–290° (decomp.), $[\alpha]^{20°}_{5461} + 8{\cdot}2$ (*c*, 5·1) in pyridine, has the probable empirical formula, $C_{30}H_{46}O_4$. It forms a neutral monomethyl ester, m.p. 192–193°, and a diacetate m.p. 212–214° (decomp.), $[\alpha]^{23°}_{5461} + 4{\cdot}2$ (*c*, 6·0) in $CHCl_3$. The diacetate gives formaldehyde on ozonization in $CHCl_3$. Acid I is therefore presumably a dihydroxymonocarboxylic acid with a terminal methylene group. It bears a strong resemblance in properties and colour reactions to the triterpenoid polyporenic acid C, isolated from the natural sporophore of *Polyporus betulinus* Fr. (Cross, Eliot, Heilbron & Jones, 1940; Cross & Jones, 1940). A mixed m.p. determination on the methyl esters of (I) and of acid C (m.p. 190–191°; kindly supplied by Prof. E. R. H. Jones and Dr T. G. Halsall) showed no depression.

Acid II. The Na salt on acidification gives rise to a new crystalline product, $C_{22}H_{38}O_6$, m.p. 78–80°, for which the name *ungulinic acid* is proposed. The acid forms a liquid *dimethyl ester*, $C_{24}H_{42}O_6$, and a *trihydrazide*, $C_{22}H_{44}O_3N_6$, m.p. 190–195° (decomp.). Under different experimental conditions, acidification of the Na salt gives an *anhydride*, $C_{22}H_{36}O_5$, m.p. 53–54·5°. KOH fusion of the Na salt affords stearic, acetic and oxalic acids. Ungulinic acid gives colour reactions which are identical with those described for agaricic acid, $C_{22}H_{40}O_7$. These, and other properties, suggest that ungulinic acid is a lactone of a hydroxytricarboxylic acid.

REFERENCES

Cross, L. C., Eliot, C. G., Heilbron, I. M. & Jones, E. R. H. (1940). *J. chem. Soc.* p. 632.

Cross, L. C. & Jones, E. R. H. (1940). *J. chem. Soc.* p. 1491.

Distribution of Injected ^{31}Si in Rats. By P. F. Holt, D. M. Yates and D. H. Tomlin. (*University of Reading*)

The distribution of silica in tissues has been studied by several workers but particularly by King, Stantial & Dolan (1933), who also measured the excretion rate when silicic acid was injected into a rabbit. He found a very rapid excretion rate, but he was unable to recover all the silicic acid which was injected.

We have investigated the distribution of labelled silicon in a number of tissues after intraperitoneal injection of silicate solutions containing the radioactive isotope ^{31}Si into rats.

There is rapid absorption of ^{31}Si from the peritoneal cavity and analyses of the separate tissues have shown no evidence of accumulation in any of the organs investigated other than the kidney. Concentration in the kidney is presumably due to the tubular re-absorption of water and non-absorption of silicate from the glomerular filtrate. This suggestion is supported by autoradiographs of the kidney slices which show general distribution of ^{31}Si throughout the organ but with a higher concentration in the medullary portion where there are tubules only.

The variation of the silica concentration in the kidney both with time and with the amount injected was studied, using the liver tissues as representative of the concentration in other organs of the body.

The time-concentration curve for the kidney after the injection of 8 ml. of a solution containing 1 mg. SiO_2/ml. into rats showed a sharp rise to the 3-hr. value followed by a gradual decline. The liver merely showed a steady drop in concentration down to a value comparable with that found in normal tissues.

Injection of varying amounts of silicate into rats gave an initial sharp rise in kidney silica with increasing dose followed by a rapid fall even to a value below that for the liver.

It would appear that at low concentrations the silicate is concentrated by the kidney and is rapidly excreted. When the silicate is injected in higher concentration the body appears to be unable to deal with it, probably because the silicate is highly polymerized.

REFERENCE

King, E. J., Stantial, H. & Dolan, M. (1933). *Biochem. J.* 27, 1007.

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Massive Talc Aspiration

Successful Treatment With Dexamethasone

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FATALITIES HAVE been reported following the aspiration of talcum powder in infants.[1-3] The incidence of nonfatal illness from the inhalation of this dusting powder is unknown. Because of the ready availability of this substance and since the clinical manifestations from aspiration mimic those of acute infectious bronchiolitis, it is likely this etiology may sometimes be overlooked in milder cases.

Talc is hydrous magnesium silicate, and commercially, the mineral talc is usually mixed with other silicates such as serpentine, tremolite, anthophyllite, and other ingredients, predominantly carbonates. Talc is insoluble, chemically inert, and, in a fine state of subdivision, it is used topically as a "mechanical protective." It acts mainly by preventing friction and by absorbing moisture, but otherwise has no therapeutic effect.

Inhalation of talc dust may result in acute or chronic lung disease. Sudden massive inhalation produces acute respiratory distress of the bronchiolitis fibrosa obliterans type[4] which is often fatal. Sustained inhalation of significant amounts of talc can produce pneumoconiosis (talcosis). The case herein presented is of the former type in which an infant accidently inhaled a large amount of talc dusting powder and suffered acute respiratory distress. The course of this infant's illness, similar to the reported fatal cases, seemed to be favorably influenced by treatment with dexamethasone (Decadron).

Report of a Case

A healthy 14-month-old white male infant was playing with a can of talcum powder on Sept 24, 1965. At 9 AM the mother was attracted to the infant because of coughing and gasping. The face was covered by and the mouth was "caked" with the powder. He vomited a small amount and by 10 AM appeared to be in slight respiratory distress. After receiving an emetic in a community hospital, he was returned home. By afternoon the infant was having more difficulty in breathing and was taken to the family physician who administered an antibiotic to prevent infection. The dyspnea did not abate and the patient was admitted to Louisville General Hospital at midnight.

On examination the infant was in moderate respiratory distress, very restless, and irritable. The rectal temperature was 101 F (38.3 C) pulse, 160/min; blood pressure, 120 (systolic) by palpation method; and the rate of respiration was 44/min. The mouth and pharynx were normal. There was moderate intercostal and subcostal retraction. Air exchange was fairly good but expiration was prolonged. No rales were detected. The abdomen was not distended, and the liver and spleen were not enlarged. There was no cyanosis but the baby coughed frequently. A roentgenogram of the chest at the time of admission revealed no abnormalities other than signs of moderate hyperinflation. The hemoglobin was 8.6 gm/100 cc; and the white blood cell count was 17,000/cu mm, with 51% neutrophils, 37% lymphocytes, and 12% monocytes. The red blood cells were hypochromic and the hematocrit was 29% and reticulocyte count, 1.4%. Dietary history substantiated the impression of iron-deficiency anemia.

Upon admission the patient was placed in a tent with oxygen mist. There was no evident response to 0.15 ml aqueous epinephrine injected subcutaneously. A dose of 300,000 units of procaine penicillin G were administered twice daily. Isoproterenol (Isuprel), 2.5 mg, was given orally every six hours.

Twenty-four hours after the aspiration, the infant was in severe respiratory distress with marked retractions of the chest. He was slightly cyanotic and the respiratory rate was between 80 and 90 per minute. With the hope of enhancing bronchodilation and reducing inflammation, 2.0 mg of dexamethasone was administered intramuscularly and continued every 12 hours for seven doses.

Within 6 to 12 hours after initiation of steroid therapy, improvement was definite and the respiratory rate was between 40 and 60 per minute. Continued improvement came rapidly and dexamethasone was discontinued on Sept 29, 1965. On this date and afterward the infant was asymptomatic and without respiratory distress. Examination on October 7 revealed no symptoms nor physical abnormalities; the chest roentgenogram was normal, and the hemoglobin had increased to 12.0 gm/100 cc with oral iron therapy.

Comment

The case presented here followed a course similar to all the reported cases of fatal talc aspiration up to the time when therapy with dexamethasone was initiated. Improvement

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appeared to follow administration of this drug. The most recently reported case by Jenkins[3] in 1963 was that of a 14-month-old infant, who had coughing and some respiratory distress immediately after aspiration of talcum powder, and was hospitalized five hours later with grunting respiration with a rate of 48/min and intercostal retraction. Respiration became increasingly more labored and finally ceased ten hours after the aspiration. Molnar and coauthors' case was a 22-month-old Negro boy whose respiratory rate reached 90/min, seven hours after admission, and death occurred 20 hours after aspiration of the talcum powder. Cless and Anger's[1] case followed an identical course. Antibiotics, oxygen mist, digitalis, morphine, and epinepherine had no demonstrable effect on these cases.

Pathologically, lesions of bronchitis and acute bronchiolitis have been demonstrated in the fatal cases, with pulmonary edema, focal atelectasis, and compensatory emphysema. The bronchial and bronchiolar lumens and alveoli may be filled with a homogenous basophilic matrix embedded with leukocytes, epithelial fragments, and refractile particles. Complete desquamation of the epithelium in many bronchi is seen with various gradations of edema, hyperplasia, and squamous metaplasia. The submucosa is swollen and often infiltrated with polymorphonuclear leukocytes and refractile particles of talc.[2] The pathological changes seen in talc pneumoconiosis have been described as both cellular and fibrotic reactions affecting primarily the alveoli, smaller bronchioles, and arterioles.[5]

Umbilical granuloma has been described secondary to the free use of talcum powder over the trunk and diaper areas of newborn infants.[6] Surgeons are familiar with granulomatous reactions in the peritoneum from glove talc. Also, skin granulomas may result from talc deposited in an incision site.

Reasoning that led to the use of dexamethasone in this case was as follows:

1. Talc pneumoconiosis resulting from chronic exposure to talc in industry requires at least ten years before pulmonary fibrosis of clinical significance appears. It would seen that if, in an acute aspiration, the infant could survive two or three days, the respiratory tract would clear itself of most of the foreign material and further damage would be unlikely.

2. Adrenocortical steroids produce considerable temporary improvement in berylliosis,[7] similar in some respects to talcosis.

3. An inflammatory reaction occurs in bronchi and bronchioles which might be reduced by dexamethasone.

If the favorable response to treatment was due to use of the steroid, it was probably due to the anti-inflammatory effect of the drug. Failure of response to epinepherine used prior to dexamethasone would suggest simple bronchodilation was not responsible for the improvement.

In conclusion, it is strongly urged that talcum powder be removed from the environment of children and the traditional association of talcum powder and babies be abandoned. It has no medicinal value; wherever placed it serves as a foreign body; and at least three deaths and an unknown morbidity have resulted from this silicate powder.

Summary

A case is presented of a 14-month-old infant who aspirated talcum powder, became severely dyspneic, and was successfully treated with dexamethasone. The clinical course is compared to the three fatal cases reported in the literature. There is no justification for the use of talcum powder in infant care.

Generic and Trade Names of Drugs

Dexamethasone—*Decadron, Deronil, Dexameth, Gammacorten, Hexadrol.*
Isoproterenol—*Aludrine, Isuprel.*
Procaine penicillin G—*Depo-Penicillin, Lentopen, Wycillin.*

REFERENCES

1. Cless, D., and Anger, R.: Erstickungstod Durch Aspiration von Kinderpuder, *Kinderaerztl Prax* 22: 506-508 (March) 1954.

2. Molnar, J.J.; Nathenson, G.; and Edberg, S.: Fatal Aspiration of Talcum Powder By a Child, *New Eng J Med* 266:36-37 (Jan 4) 1962.

3. Jenkins, M.Q.: Dusting Powder Inhalation, *J S Carolina Med Assoc* 59:62 (Feb) 1963.

4. La Due, J.S.: Bronchiolitis Fibrosa Obliterans. *Arch Intern Med* 68:663-673 (Oct) 1941.

5. Kleinfeld, M., et al: Talc Pneumonconiosis, *Arch Environ Health* 7:101-115 (July) 1963.

6. Creery, R.D.G.; McClure, D.M.; and Rutherford, R.J.: Talc Granuloma of the Umbilicus, *Lancet* 1:667-668 (March 30) 1957.

7. Kennedy, B.J., et al: Effect of Adrenocorticotropic Hormone (ACTH) on Beryllium Granulomatosis Silicosis, *Amer J Med* 10:134-155 (Feb) 1951.

Bull. Soc. Chim. Biol.
18:1769-1782, 1936

The nature of silica in living organisms

Constitutional silica and interposed silica

by

Ernest Kahane and Georges Antoine

Meeting of June 18, 1935.

Silica particles and formations can be isolated from vegetable and animal tissues. Based on the study of the chemical and physical properties of these particles, they may be divided into two categories of different origins: "constitutional silica" and "interposed silica".

Introduction

Most of the basic constituents of living beings appear in biologic substances only in diffusible or dissolved form, or else in very complex combinations.

Since, with the exception of very special cases, it is difficult to discover the proportion included in one or the other of these combinations, we are generally satisfied in biological chemistry with obtaining and describing the total content of various minerals in the organism or portions of organism (1).

This is how the problem of silica has generally been handled (2), even though it has long been known that this substance exists in some organisms in the form of distinctly isolated and characteristic particles (3). Thus silica sometimes forms fairly

complex structures of remarkable regularity, whose morphology has been carefully studied and contributes to the definition of the identifying characteristics of the different types. In other living organisms there are also silica particles, but these do not assume a characteristic appearance or show any regularity.

Aside from this formed silica, whose existence is far from general, there is no doubt that in many species, and possibly in all living organisms, there is dissolved silica, capable of circulating and diffusing. The existence of this form of silica proves that there is silica metabolism. Some silica formations in living organisms are the result and proof of this silica metabolism. Others develop without the participation of this metabolism.

In fact it seems to us that the word metabolism cannot be used when a silica particle absorbed by the living organism has been purely and simply transported, without undergoing any changes of a chemical nature, even if it played a role, such as that of support or protection, in the tissue in which it was deposited. The difference established by Kahane between constitutional silica and interposed silica (4) appears to us justified because of this fundamental difference.

At present there is no general method which reveals whether a particle from a given substance is of internal or external origin, whether it was formed by the living organism itself or whether it was mechanically introduced into the tissue in which

it is found. In the case of many chemical substances it might be thought that this is quite impossible and that the distinction made for silica does not apply to them.

But in the case of silica it seems that in the absence of certainty, some very cogent arguments can be found as to the endogenous or exogenous nature of the silica formations observed. The chemistry of silica is marked by the existence of numerous species that are individually better characterized mineralogically than chemically. These individual types have been formed under conditions of time of development, temperature and pressure different from those that may be found in living organisms. Consequently when these types are encountered in vegetable or animal tissues, it can be claimed with a high degree of probability that they are of external origin.

To isolate silica particles from the tissues studied, we have utilized the observation of Kahane in the study of nitro-perchloric destruction of biologic substances (5): the insoluble deposit may include gelatinous silica and compact particles or formations. It has been found that the procedure allows the isolation, when they are differentiated, of certain silica particles that may exist in tissues, while leaving their morphological characteristics and mineralogic identity intact, and that the compact particles collected pre-existed in this form in the analyzed tissue.

Some of the particles collected are crystalline and it is thus established that they pre-existed in this state. When changes in silicates are produced under the influence of acid

reagents, these are always in the form of an increased symmetry, that is to say by degradation, transforming the silica present in the form of complex crystalline silicate into pure gelatinous silica. The reverse mechanism has never been observed. It is highly improbable and the observation of complex crystalline silicates following nitroperchloric destruction may be considered as adequate proof of their existence in this form in the tissue studied.

We have studied Tabasheers (internodal bamboo concretions), the shell of Diatoma, sponge spikelets, the silica particles in Silicoderma and dust from human and animal lungs.

In the special cases of interest to us here, the gelatinous silica observed is quantitatively negligible and because of its lightness, it can, if necessary, be eliminated by simple decantation.

The isolated silica formations are resistant to strong acids, as demonstrated by their process of extraction. Their other characteristics divide them into two categories. Their silica content, determined by treatment with hydrofluoric and sulfuric acid, shows whether or not they are composed of pure silica. When treated with potassium hydroxide solution (one quarter sodium hydroxide) some are attacked (amorphous silica and silicates), while other are not (crystalline silica and many crystalline silicates). Viewed in the polarizing microscope they may or may not show double refraction. When pulverized and used for an x-ray diffraction diagram[1)], some behave like amorphous substances

1) We cordially thank Marcel Mathieu for kindly obtaining these diagrams for us.

and others like crystalline substances.

According to their behavior towards these chemical and physical tests, we have been able to divide the silica particles encountered in living organisms into two well defined categories, corresponding to constitutional silica and interposed silica. Constitutional silica is characterized at a first approximation by a certain morphological regularity, an amorphous state, solubility in alkaline solutions and by the nature of the bonds between the chemically combined substances, which are broken in the course of the attack by perchloric acid. Similarly, interposed ilica or silicates are characterized at a first approximation by the irregularity of their shape, their crystalline nature, their resistance to acid, their variable and irregular composition, in a word by their analogy with mineralogically defined crystalline substances.

Tabasheers

Tabasheers are rounded siliceous concretions, hard and translucent, sometimes found in the internodes of some varieties of bamboo. They have been known for a very long time and been the subject of several serious studies[1]. We have had occasion

1) A good bibliography is furnished by Czapek and by Wehmer in their classical works (6,7). See in particular the report by Guibourt (8).

to study a few samples that were kindy provided by Messrs. Rondeau du Noyer and Orcel and were part of the Zoologic collection of the Paris Pharmacy Faculty and of the mineralogic collection of the Museum of Natural History. We wish to express our deep appreciation to Messrs. Rondeau due Noyer and Orcel.

Table I.

Analysis of Tabasheers.

	Poids après calcination mg. 1	Poids après FH + SO^4H^2 mg. 2	SiO^2 p. 100 de subst. sèche 3
4 Tabaschir n° 1	93,56	1,05	98,9
— n° 2	58,68	1,01	98,3
— n° 3	22,98	0,35	98,4
5 *Après attaque perchlorique*			
Tabaschir n° 1	81,40	0,31	99,6
	70,92	0,23	99,6
— n° 2	90,30	0,22	99,7
— n° 3	88,60	0,46	99,5

Key: 1. Weight after calcination in mg. 2. Weight after $FH+H_2SO_4$ in mg. 3. SiO_3, % dry substance. 4. Tabasheer. 5. After attack by perchloric acid.

The Tabasheers studied by us were in the form of small, rounded, chalky or transparent masses, the size of a pea and generally weighing 50-100 mg. These particles are made up of almost pure silica, as indicated by the anlysis in Table I, and undergo no appreciable modification upon treatment with acid (perchloric). It is interesting to note, however, that the small quantity of non-siliceous mineral substance in Tabasheers is almost entirely solubilized in the course of perchloric digestion. The percentage of silica changes from 98.3-98.9 to 99.5-99.7.

When viewed between crossed Nicol prisms the Tabasheers revealed no double refraction. The x-ray diagram obtained from pulverized Tabasheers is identical to that obtained with amorphous silica and shows not the slightest trace of the rays characteristic of the crystalline state (see Table V). Powdered Tabasheer readily dissolves in potassium hydroxide.

The formation of these Tabasheers, which was long considered a mystery, can be very simply explained. It is known that bamboo sap is extremely rich in silica. It is enough for an acidification of the fluid to take place when a portion of it is enclosed in the hollow stem closed off by the internode, which plays the role of a semi-permeable wall, so that the passage of actual crystaloids takes place without hindrance, while that of colloid silica is obstructed. Under the influence of rain water a perfect washing may take place of the silica gel enclosed in the internodes, then, under the influence of drought, there is dessication with retraction and formation of the small pebble of amorphous silica which constitutes the Tabasheer. This is analogous to silica gel particles obtained today in the laboratory and in industry.

According to all its physical and chemical characteristics and according to the probable course of its production, the Tabasheer silica is a constitutional silica. It has taken part in the metabolism of bamboo, since it went through the form of circulating silica, and when it ceased to play this role, owing to the death of the plant or for some other reason, it escaped from this metabolism and was deposited in the form known to us.

Shells of Diatoma

The silica of shells or crusts of Diatoma has the same characteristics as the silica of sponge spikelets.

Analysis is more difficult because of the small size of Diatoma and the difficulty of isolating them in the pure state without including sand and mineral particles.

The destruction of the organic matter of Diamtoma by the nitroperchloric method was recommended by Perruche (9) for the morphological study of the shells. These are extremely highly differentiated, a feature which is used for the identification and classification of Diatoma. Their structure, like that of the sponge spikelets, is no doubt due to the mechanical peculiarities of their formation, concerning which no information is available.

The shell of Diatoma is made up of silica chemically combined with organic and mineral substances (Al, Fe, Ca, Mg, K, Na, H_3PO_4)(Brun, 10), or it is stuck to a membrane (Mangin, 11). When treated with nitric or perchloric acid, it loses minerals that were combined with it and leaves a residue of pure, amorphous (Table V), isotropic silica soluble in potassium hydroxide (12).

Regardless of the manner in which the silica is extracted from the nutritive medium and regardless of the structure of the Diatoma, there is no denying that, as in the case of Tabasheers, the silica went through the state of circulating silica before becoming a constitutent, and that the silica which has participated in the metabolism of the living organism is therefore constitutional silica.

Sponge spikelets

The silica spikelets of sponges, which often form the skeleton of these animals, assume the most varied and complex forms. These forms, which are extremely regular, are the same for sponges belonging to the same species and serve to identify them (13). We have studied the spikelets of Ephydatia fluviatilis, isolated by nitroperchloric treatment, and those of Hyalonema Thomsoni, whose skeleton was preserved in the zoological collection of the Pharmacy Faculty.

Sponge spikelets are built up around a nucleus or an organic or organo-mineral filament which is only an infinitessimal part of their substance. They do not suffer any appreciable loss of weight upon perchloric treatment, which leaves their shape intact. The residue is almost pure silica (98%)(Table II), which is readily soluble in potassium hydroxide. Viewed in the polarizing microscope it reveals no double refraction. The x-ray diagram made on powdered spikelets is identical to that of amorphous silica precipitated and dried at a low temperature (Table V).

Table II

Analysis of sponge spikelets treated with perchloric acid

4	1 Poids en mg.	2 Résidu après $FH+SO^4H^2$ mg.	3 SiO^2 p. 100 de substance sèche
Ephydatia fluviatilis, Echantillon n° 1...	11,81	0,21	98,3
Hyalonema Thomsoni, — 2...	131,26	3,20	97,6
Ephydatia fluviatilis, — 3...	32,79	0,62	98,2

Key: 1. Weight in mg. 2. Residue after $FA+H_2SO_4$ in mg. 3. SiO_2 % dry substance. 4. Sample

The chemical process by which silica is precipitated in the sponges to form the spikelets is not known, nor is the mechanical process of the build-up of the complicated structures. There is no doubt, however, as in the case of bamboos and Diatoma, that these formations are the result of the physicochemical activity of the living organism, that they are metabolic products, thus that their silica is constitutional silica.

Silica of Silicodermata

Silicodermata were discovered as siliceous animals by Alphonse Labbé (14). The chemical study of their silica has been the subject of a special publication (15) which we will summarize and discuss.

The Silicodermata which were kindly furnished by Mr. Alphone Labbé were subjected to nitroperchloric action. In certain cases and to a partial degree, this operation gave rise to the appearance of gelatinous silica, which may be the result of the attack on the circulating silica, or on organic siliceous formations, or it may be produced in the tissues of the animal, but it might also be the result of the degradation, under the influence of acid reagents, of certain siliceous particles receptive to attack that might be contained in the sample studied. In addition to this gelatinous silica, concerning whose origin no definite statements can be made at this stage, we have always observed compact, massive particles of varying size, the crystalline nature of which has been demonstrated by double refraction and by the x-ray diagram. The figures in Table V are not good, because the amount

of substance collected is extremely small, its pulverization is very laborious and it is difficult to take it far enough to obtain a good x-ray diagram. However, despite the added imperfections due to reproduction, one can see well localized dots and rays produced by the x-ray diffraction on the reticular planes of the crystals of the preparation.

Analysis of these particles has yielded the results shown in Table III.

Table III

Siliceous particles from Silicodermata

1 Echantillons	2 Particules siliceuses en mg.	3 Silice en mg. (1)	4 Silice p. 100
1	2,45	2,08	84,7
2	2,13	1,39	64,5
3	6,63	3,92	59,0
4	36,93	20,43	55,1
5	3,09	1,87	60,5
6	3,58	2,33	65,1
7	3,71	2,61	70,3
8	1,53	1,09	71,2
9	3,27	2,31	70,5
10	1,72	1,27	73,5
11	2,94	2,36	80,0

Key: 1. Samples. 2. Siliceous particles in mg. 3. Silica in mg[1]. 4. Silica %.

1) The weight of the silica is fairly arbitrarily taken as the difference between the primary weight and the weight of the residue after treatment with $FH+H_2SO_4$. A systematic error results from the presence of certain sulfates in the oxide residue, but this can be avoided by use of the $NaPO_3$ method of Galfajan and Tarajan (16). This error does not affect the order of magnitude of the results, which is all that concerns us here.

Even though they are very rich in silica, it can be seen that the siliceous particles of Silicodermata are far from possessing the 97.5 to 99.5% silica content invariably observed in the case of the Tabasheer, sponge spikelet and Diatoma shell silica. Elements combined with the silica are not removed from it by perchloric acid treatment. Potassium, calcium, magnesium and aluminum have been detected, precisely the elements most often encountered in natural silicates.

Since Mr. Alphone Labbé was kind enough to provide us with animals deprived of their digestive tube, we believe that the siliceous particles observed are exogenous elements which were purely and simply transported inside the animal tissue. These particles, which in no way take part in the chemical exchanges of the organism, meet the definition of interposed silica.

Lung particles

Particles isolated from the human lung by a nitro-sulfo-perchloric method (17) have been studied from the chemical standpoint by one of us (Antoine, 18). The whole of the particle extracted from the lungs is never made up of pure silica[1]. Its silica content is around 90% (Table IV), the rest being made up of potassium oxides, magnesium, calcium and aluminum. Viewed under the microscope, this dust has the irregular appearance of crystal

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1) These investigations have since been confirmed by those of our associate, Peyssonneau (19), who has studied siliceous particles extracted from human lungs and found that they had a similar silica content.

fragments with an average size of 3 to 4 μ. Most of the particles are birefringent. The powder yields a remarkably pure x-ray diagram, identical to that of quartz (Table V). The process of extraction shows that these particles cannot be attacked by concentrated acid. Nor can they be attacked by diluted potassium hydroxide, under the influence of which they give up virtually nothing.

Analogous observations have been made on the lungs of various animals: monkey, horse, gazelle, ostrich, etc. (see Tables IV and V).

Even though the hypothesis of the endogenous nature of lung silica has been advanced repeatedly, no one any longer disputes its respiratory origin. Siliceous dusts are mechanically introduced into the organism. Some have probably been digested, which would explain the relative abundance of quartz in pulmonary deposits, but the fixed particles undergo at the most changes of a mechanical nature, migrations grouping them in special histologic formations (20).

Not having taken part in metabolism, these siliceous particles represent the typical example of interposed silica and we propose that all siliceous particles encountered in live organisms presenting the same characteristics be considered as interposed silica.[1a)]

1a) Particles showing similarities with those in the lungs have been observed by Lematte and Kahane (21) in the blood of higher animals.

Table IV

Lung dust

1 Echantillons	2 Poids en mg	3 Résidu après FH + SO^4H^2	4 SiO^2 p. 100
5 Poumon humain n° 1.....	270,0	24,41	91,0
— — n° 2.....	222,0	29,73	86,6
— — n° 3.....	115,4	9,41	91,8
— — n° 4.....	383,8	25,34	93,4
— — n° 5..	85,8	8,56	90,0
— — n° 6.....	57,1	6,05	89,4
— 6 d'Autruche (1).....	11,59	0,60	95,0
— 7 de Gazelle.....	2,84	0,27	90,5
— 8 d'Hamadryas.....	45,60	3,39	92,5

Key: 1. Samples. 2. Weight in mg. 3. Residue after $FH+H_2SO_4$. 4. SiO_2%. 5. Human lung. 6. Ostrich lung[1]. 7. Gazelle lung. 8. Hamadryas lung.

Table V. Top to bottom: I. Shells of Diatoma. II. Sponge spikelets. III. Silica from Oncidium durum Labbé (Silicoderma). IV. Dust from human lungs. Sample 1. V. Dust from human lungs. Sample 2. VI. Dust from horse lungs. VII. Amorphous silica. VIII. Quartz.

Conclusions

Treatment of vegetable and animal tissues with mixtures of oxidizing acids results in the entire solubilization of their normal organic and mineral constituents, with the exception of

1) We thank Messrs Anthony and Clavelin, Professor and Chief Curator of the Museum, who allowed us to remove the organs required by us from the cadavers intended for their service.

silica and certain siliceous substances.

Siliceous substances that are attacked by acids, such as dissolved or colloid silicates, the organic components of silicon and certain degraded minerals such as kaolin, give up an insoluble residue of gelatinous silica (pure silica).

Siliceous substances that cannot be attacked by acid, such as amorphous or crystalline silica, glass and most natural crystalline silicates, are not changed and preserve their crystallographic and mineralogic individuality.

The insoluble residue collected after nitroperchloric acid destruction of biologic substances may thus contain gelatinous silica, compact amorphous silica and crystalline silica or silicates.

The origin of gelatinous silica can only definitely be determined by the study of intact tissue. In general this is silica belonging to the cellular stroma or the intracellular fluid, in other words taking part in the metabolism of the living organism. This is constitutional silica. It is encountered in the vegetable and animal kingdom in very variable quantities. A sufficiently sensitive method of determination might possibly reveal the general nature of its presence.

Compact amorphous silica is invariably found in certain Diatoma and sponges, and occasionally in bamboos. Whether they are normal formations, such as Diatoma shells and sponge spikelets, or accidental formations, such as bamboo Tabasheers, they certainly originate from the dissolved and circulating silica of the animal

or vegetable organism. The workings of the physicochemical mechanisms common to the species condition their build-up, and despite the apparently irreversible nature of their deposits, it would still seem to be justified to consider this amorphous silica as constitutional silica.

Crystalline silica or silicate, as encountered in Silicodermata, the human and animal lung and in numerous animal tissues (Antoine, 22) have a mineralogic individuality which does not appear to be due to the normal workings of biochemical mechanisms. Their formation appears to be subject to conditions of time of development, temperature and pressure that are foreign to the living organisms, whose siliceous products are always amorphous. Nor do they result from the acid treatment applied during extraction, which is always only manifested by increased symmetry (change of some crystalline silicates into a state of colloid silica). In view of the fact that these particles are of external origin, that they play the role of foreign bodies, sometimes migrating in the organism, but not taking part in its chemical processes, we consider them to be interposed silica.

From: Toxicology Laboratory, Faculty of Pharmacy, Prof. R. Fabre.

Bibliography

See pp. 1781-1782.

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TRAVAUX ORIGINAUX.

Sur la nature de la silice chez les êtres vivants. Silice de constitution et silice d'interposition,

par MM. Ernest KAHANE et Georges ANTOINE.

(*Mémoire présenté à la séance du 18 juin 1935*).

Des particules et des formations siliceuses peuvent être isolées des tissus végétaux et animaux. L'étude des propriétés chimiques et physiques de ces particules permet de les répartir en deux catégories d'origine distincte : la « silice de constitution » et la « silice d'interposition ».

Introduction.

La plupart des constituants fondamentaux des êtres vivants ne figurent dans les substances biologiques que sous une forme diffusible ou dissoute, ou engagés dans des combinaisons très complexes.

Comme il est difficile, sauf dans des cas très particuliers, de discerner la portion qui est engagée dans les unes ou les autres de ces combinaisons, on se contente habituellement, en chimie biologique, d'obtenir et de décrire la teneur totale des organismes ou des portions d'organismes en tel ou tel élément minéral [1].

C'est ainsi qu'a été traité le plus souvent le problème de la silice [2], bien que l'on sache depuis longtemps que cette substance existe dans quelques organismes sous la forme figurée, à l'état de particules ou de structures nettement isolées et caractérisées [3]. La silice forme parfois ainsi des édifices assez complexes et d'une remarquable régularité, dont la morphologie a été soigneusement étudiée, et contribue à définir les caractères d'identité des différentes espèces. Chez d'autres êtres vivants il existe

encore des particules siliceuses, mais qui n'affectent aucun aspect caractéristique ni aucune régularité.

Outre cette silice figurée, dont l'existence est loin d'être générale, il est hors de doute qu'il existe chez de nombreuses espèces, et peut-être chez tous les êtres vivants, de la silice dissoute, capable de circuler et de diffuser. L'existence de cette forme de silice démontre la réalité d'un métabolisme de la silice. Certaines formations siliceuses des êtres vivants sont le résultat et le témoin de ce métabolisme de la silice. D'autres ont été constituées sans intervention de ce métabolisme.

Il ne nous semble pas, en effet, que le mot de métabolisme puisse être employé dans le cas où une particule siliceuse absorbée par l'être vivant a été purement et simplement véhiculée sans subir de modifications d'ordre chimique, même si elle a joué un rôle tel qu'un rôle de soutien ou de protection dans le tissu où elle a été déposée. La différence établie par E. KAHANE entre la silice de constitution et la silice d'interposition [4] nous paraît justifiée par cette distinction fondamentale.

Il n'existe pas, pour l'instant, de méthode générale qui permette de reconnaître si une particule d'une substance quelconque est d'origine interne ou externe, si elle a été formée par l'être vivant lui-même ou si elle a été introduite mécaniquement dans le tissu où elle est rencontrée. Pour de nombreuses substances chimiques, on peut même considérer qu'il y a là une véritable impossibilité et que la distinction établie pour la silice ne peut pas leur être appliquée.

Mais dans ce dernier cas, il semble qu'à défaut de certitude on puisse trouver des arguments très probants quant au caractère endogène ou exogène des formations siliceuses observées. La chimie de la silice se distingue en effet par l'existence de nombreuses espèces, dont les individus sont caractérisés minéralogiquement plus encore que chimiquement. Ces individus ont été formés dans des conditions de durée, de température ou de pression, différentes de celles qui peuvent se trouver réunies chez les êtres vivants. Par suite, lorsque de tels individus sont rencontrés dans les tissus végétaux ou animaux, l'on peut affirmer avec une haute probabilité qu'ils sont d'origine externe.

Pour isoler les particules siliceuses des tissus étudiés nous avons mis à profit la remarque faite par E. KAHANE dans l'étude de la destruction nitro-perchlorique des substances biologiques [5] : le dépôt insoluble peut comprendre de la silice gélatineuse et des particules ou des formations compactes. Nous admettons que le procédé utilisé permet d'isoler, lorsqu'elles sont diffé-

renciées, certaines des particules siliceuses pouvant exister dans un tissu, en respectant leurs caractères morphologiques et leur identité minéralogique et que les particules compactes recueillies préexistaient sous cette forme dans le tissu analysé.

Certaines des particules recueillies sont cristallines, nous admettons donc qu'elles préexistaient à cet état. En effet, lorsque des modifications de silicates se produisent sous l'influence des réactifs acides, elles se font toujours dans le sens de l'augmentation de symétrie, c'est-à-dire par dégradation, en faisant passer de la silice engagée sous forme de silicates complexes cristallins, à l'état de silice gélatineuse pure. Le mécanisme inverse n'a jamais été observé, il est hautement improbable, et on peut considérer l'observation après destruction nitro-perchlorique de silicates complexes cristallisés comme étant une preuve satisfaisante de leur existence sous cette forme dans le tissu étudié.

Nous avons étudié les Tabaschirs (concrétions internodales des Bambous), les carapaces de Diatomées, les spicules d'Eponges, les particules siliceuses des Silicodermés et les poussières des poumons humains et animaux.

Dans les cas particuliers qui nous intéressent ici, la silice gélatineuse observée est quantitativement négligeable, et grâce à sa légèreté, elle peut, le cas échéant, être éliminée par simple décantation.

Les formations siliceuses isolées résistent aux acides forts, comme le montre leur procédé d'extraction. Leurs autres caractères les divisent en deux catégories. Leur teneur en silice déterminée par traitement aux acides fluorhydrique et sulfurique, montre si elles sont constituées par de la silice pure ou non. Traitées par une lessive alcaline (soude caustique au quart), les unes sont attaquées (silice et silicates amorphes), les autres ne le sont pas (silice et nombreux silicates cristallisés). Examinées au microscope polarisant, elles présentent ou non la biréfringence. Pulvérisées et utilisées pour la production d'un diagramme de diffractions de rayons X (1), les unes se comportent comme des substances amorphes, les autres comme des substances cristallines.

D'après leur comportement vis-à-vis de ces tests chimiques et physiques, il nous a été possible de diviser les particules siliceuses rencontrées chez les êtres vivants en deux catégories bien tranchées, correspondant à la silice de constitution et à la silice

(1) Nous remercions bien cordialement M. Marcel MATHIEU, qui a bien voulu obtenir pour nous ces diagrammes.

d'interposition. La silice de constitution est caractérisée en première approximation par une certaine régularité morphologique, par l'état amorphe, par la solubilité dans les liqueurs alcalines et par la nature des liaisons des substances chimiquement associées, qui sont rompues au cours de l'attaque perchlorique. De même, la silice ou les silicates d'interposition se caractérisent en première approximation par l'irrégularité de leur forme, leur nature cristalline, leur résistance à l'attaque acide, leur composition variable et irrégulière, en un mot, par leur analogie avec les individus cristallins minéralogiquement définis.

Tabaschirs.

Les Tabaschirs sont des concrétions siliceuses arrondies, dures et translucides, que l'on trouve parfois dans les entre-nœuds de certaines variétés de Bambous. Ils sont connus depuis fort longtemps et ont été l'objet de plusieurs études sérieuses (1). Nous avons eu l'occasion d'étudier quelques échantillons qu'ont bien voulu nous remettre M. Rondeau du Noyer et M. Orcel, et qui faisaient respectivement partie de la collection de Zoologie de la Faculté de Pharmacie de Paris et de la collection de Minéralogie du Muséum d'Histoire Naturelle. Nous exprimons à MM. Rondeau du Noyer et Orcel, l'expression de nos remerciements empressés.

TABLEAU I.

Analyse des Tabaschirs.

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— n° 2	90,30	0,22	99,7
— n° 3	88,60	0,46	99,5

(1) Une bonne bibliographie est donnée par Czapek et par Wehmer dans leurs ouvrages classiques [6 et 7]. Consulter, en particulier, le mémoire de Guibourt [8].

Les Tabaschirs que nous avons étudiés se présentent sous la forme de petites masses arrondies, crayeuses ou transparentes, de la grosseur d'un pois et pesant en général de 50 à 100 mg. Ces particules sont constituées par de la silice à peu près pure, ainsi qu'en témoignent les analyses suivantes (Tableau I), et ne subissent aucune modification importante par traitement aux acides (attaque perchlorique). Il n'est pas dépourvu d'intérêt, cependant, de faire remarquer que la faible quantité de substance minérale non siliceuse des Tabaschirs est presque intégralement solubilisée au cours de la digestion perchlorique. La silice p. 100 passe de 98,3-98,9 à 99,5-99,7.

Examinés entre nicols croisés, les tabaschirs ne présentent aucune biréfringence. Le diagramme de rayons X obtenu sur les tabaschirs pulvérisés est identique à celui que l'on obtient avec la silice amorphe et ne présente pas la moindre trace des raies caractéristiques de l'état cristallin (voir tableau V). La poudre de tabaschir se dissout aisément dans les lessives alcalines.

La formation de ces tabaschirs, qui a été longtemps considérée comme mystérieuse, peut s'expliquer de la façon la plus simple. On sait que le suc du Bambou est d'une extrême richesse en silice. Il suffit qu'une acidification du liquide se produise lorsqu'une portion en est enfermée dans la tige creuse fermée par les entre-nœuds, qui peuvent jouer le rôle de paroi semi-perméable, pour que le passage des cristalloïdes proprement dits s'effectue aisément et que celui de la silice colloïdale se trouve entravé. Sous l'influence des eaux de pluie, il peut se produire alors un lavage parfait du gel de silice enfermé dans les entre-nœuds, puis sous celle de la sécheresse, une dessication avec rétraction et formation du petit caillou de silice amorphe qui constitue le tabaschir. Celui-ci est analogue aux particules de gel de silice couramment obtenues aujourd'hui au laboratoire et dans l'industrie.

Par tous ses caractères physiques et chimiques, comme par le schéma vraisemblable de sa production, la silice du tabaschir est une *silice de constitution*. Elle a participé au métabolisme du Bambou puisqu'elle a passé par la forme de silice circulante, et c'est lorsqu'elle a cessé de jouer ce rôle, par la mort de la plante ou pour toute autre raison, qu'elle a échappé à ce métabolisme et qu'elle s'est déposée sous la forme que nous lui connaissons.

Carapaces des Diatomées.

La silice des carapaces des Diatomées ou frustules, présente les mêmes caractères que la silice des spicules des Eponges.

L'analyse est plus délicate en raison de la faible taille des Diatomées et de la difficulté de leur isolement à l'état pur, sans entraînement de sable et de particules minérales.

La destruction de la matière organique des Diatomées au moyen de la méthode nitro-perchlorique a été préconisée par PERRUCHE [9] pour l'étude morphologique des frustules. Ceux-ci possèdent une différenciation extrêmement poussée, utilisée pour l'identification et la classification des Diatomées. Leur structure tient sans doute, comme celle des spicules des Eponges, aux particularités mécaniques de leur formation, sur lesquelles on ne possède aucun renseignement.

La carapace des Diatomées est constituée par de la silice associée chimiquement à des substances organiques et minérales (Al, Fe, Ca, Mg, K, Na, PO^4H^3) (BRUN [10]), ou accolée à une membrane (MANGIN [11]). Attaquée par les acides nitrique et perchlorique, elle perd les minéraux qui lui étaient associés, et laisse un résidu de silice pure amorphe, (tableau V), isotrope, soluble dans les lessives alcalines [12].

Quelle que soit la façon dont la silice a été extraite du milieu nutritif et dont les carapaces des Diatomées ont été édifiées, il est indéniable, pour ces formations comme pour les Tabaschirs, que la silice a passé par l'état de silice circulante avant de venir les constituer, que c'est de la silice ayant participé au métabolisme de l'être vivant, et par conséquent, que c'est de la *silice de constitution.*

Spicules d'Eponges.

Les spicules siliceux des Eponges qui forment souvent le squelette de ces animaux, affectent les formes les plus variées et les plus complexes. Ces formes, extrêmement régulières, sont constantes pour les individus appartenant à la même espèce et servent à caractériser celle-ci [13]. Nous avons étudié les spicules de *Ephydatia fluviatilis* isolées par attaque nitro-perchlorique et ceux de *Hyalonema Thomsoni*, dont le squelette se trouvait dans la collection de Zoologie de la Faculté de Pharmacie.

Les spicules d'éponges sont constitués autour d'un noyau ou d'un filament organique, ou organo-minéral, qui ne forme qu'une

partie infime de leur substance. Ils ne subissent pas de perte appréciable de poids par l'attaque perchlorique, qui laisse intacte leur forme. Le résidu est de la silice sensiblement pure (98 p. 100), (tableau II), aisément soluble dans les lessives alcalines. Examinés au microscope polarisant, ils ne montrent aucune biréfringence. Le diagramme de rayons X effectué sur leur poudre est identique à celui de la silice amorphe précipitée et séchée à basse température (tableau V).

TABLEAU II.

Analyse de spicules d'Eponges traités par l'acide perchlorique.

	Poids en mg.	Résidu après $FH+SO^4H^2$ mg.	SiO^2 p. 100 de substance sèche
Ephydatia fluviatilis, Echantillon n° 1...	11,81	0,21	98,3
Hyalonema Thomsoni, — 2...	131,26	3,20	97,6
Ephydatia fluviatilis, — 3...	32,79	0,62	98,2

On ne connaît pas le processus chimique par lequel de la silice se sépare dans les Eponges pour former leurs spicules, pas plus qu'on ne connaît le processus mécanique de l'édification de ces structures compliquées. Il est cependant hors de doute, comme pour les Bambous et les Diatomées, que ces formations résultent de l'activité physico-chimique de l'être vivant, qu'elles sont un produit du métabolisme et, par suite, que leur silice est de la *silice de constitution.*

Silice des Silicodermés.

Les Silicodermés ont été découverts, en tant qu'animaux à silice, et étudiés par Alphonse LABBÉ [14]. L'étude chimique de leur silice a fait l'objet d'une publication spéciale [15], que nous résumons et discutons.

Les Silicodermés, qui nous ont été aimablement fournis par M. Alphonse LABBÉ, ont été soumis à la destruction nitro-perchlorique. Cette opération a donné lieu, dans certains cas et d'une façon seulement partielle, à l'apparition de silice gélatineuse, qui peut résulter de l'attaque de silice circulante ou de formations siliceuses organiques ou organisées dans les tissus de

l'animal, mais qui peut également résulter de la dégradation, sous l'influence des réactifs acides, de certaines particules siliceuses attaquables, que renferme peut-être l'échantillon étudié. A côté de cette silice gélatineuse sur l'origine de laquelle il ne nous est pas possible de nous prononcer avec certitude pour l'instant, nous avons toujours observé des particules compactes, massives, plus ou moins volumineuses, dont le caractère cristallin était démontré par la biréfringence et par le diagramme de rayons X. Les figures du tableau V ne sont pas belles parce que la quantité de substance recueillie est extrêmement faible, que sa pulvérisation est très laborieuse et qu'il est difficile de la mener assez loin pour obtenir un bon diagramme de rayons X. On peut cependant voir, malgré les imperfections supplémentaires de la reproduction, des points bien localisés et des raies produites par la diffraction des rayons X sur les plans réticulaires des cristaux de la préparation.

L'analyse de ces particules a donné les résultats résumés dans le tableau III.

TABLEAU III.

Particules siliceuses des Silicodermés.

Echantillons	Particules siliceuses en mg.	Silice en mg. (1)	Silice p. 100
1	2,45	2,08	84,7
2	2,13	1,39	64,5
3	6,63	3,92	59,0
4	36,93	20,43	55,1
5	3,09	1,87	60,5
6	3,58	2,33	65,1
7	3,71	2,61	70,3
8	1,53	1,09	71,2
9	3,27	2,31	70,5
10	1,72	1,27	73,5
11	2,94	2,36	80,0

(1) D'une façon assez arbitraire, le poids de la silice est considéré comme étant la différence entre le poids primitif et le poids du résidu après traitement par $FH + SO^4H^2$. Une erreur systématique, qui peut être évitée par l'emploi de la technique à PO^3Na de GALFAJAN et TARAJAN [16] résulte de la présence de certains sulfates dans le résidu d'oxydes. Cette erreur n'influence pas l'ordre de grandeur des résultats, qui seul nous intéresse ici.

Bien qu'elles soient très riches en silice, on voit que les particules siliceuses des Silicodermés sont loin de posséder la teneur de 97,5 à 99,5 p. 100 qui est régulièrement observée avec la silice des Tabaschirs, des spicules d'Eponges et des carapaces de Diatomées. Les éléments associés à la silice ne lui sont pas arrachés par attaque à l'acide perchlorique. Le potassium, le calcium, le magnésium et l'aluminium ont pu être caractérisés : ce sont précisément les éléments qui se rencontrent le plus fréquemment chez les silicates naturels.

Comme M. Alphonse LABBÉ avait bien voulu nous fournir des animaux débarrassés de leur tube digestif, nous estimons que les particules siliceuses observées sont des éléments exogènes, qui ont été purement et simplement véhiculés jusque dans l'intimité des tissus de l'animal. Ces particules, qui ne participent en aucune façon aux échanges chimiques de l'organisme, répondent à la définition de la *silice d'interposition*.

Particules du poumon.

Les particules isolées du poumon humain par une technique nitro-sulfo-perchlorique, [17], ont été étudiées au point de vue chimique par l'un de nous (ANTOINE [18]). L'ensemble des particules extraites du poumon n'est jamais constitué par de la silice pure (1). Leur teneur en silice est de l'ordre de 90 p. 100 (tableau IV), le reste étant constitué par des oxydes de potassium, de magnésium, de calcium et d'aluminium. Examinée au microscope, cette poussière présente l'aspect irrégulier de débris de cristaux d'une dimension moyenne de 3 à 4 μ. La plupart des particules sont biréfringentes. La poudre donne un diagramme de rayons X d'une remarquable pureté, identique à celui du quartz. (tableau V). Le procédé d'extraction montre que ces particules sont inattaquables par les acides concentrés. Elles sont également inattaquables par les lessives alcalines diluées auxquelles elles ne cèdent presque rien.

Des observations analogues ont été faites sur les poumons de divers animaux : Singe, Cheval, Gazelle, Autruche, etc. (Voir tableaux IV et V).

Bien que l'hypothèse ait été formulée à différentes reprises du caractère endogène de la silice du poumon, personne ne conteste plus à présent son origine respiratoire. Les poussières siliceuses

(1) Ces recherches sont d'ores et déjà confirmées par celles de notre collaborateur PEYSSONNEAU [19], qui a étudié les particules siliceuses extraites de poumons humains et leur a trouvé une teneur en silice du même ordre.

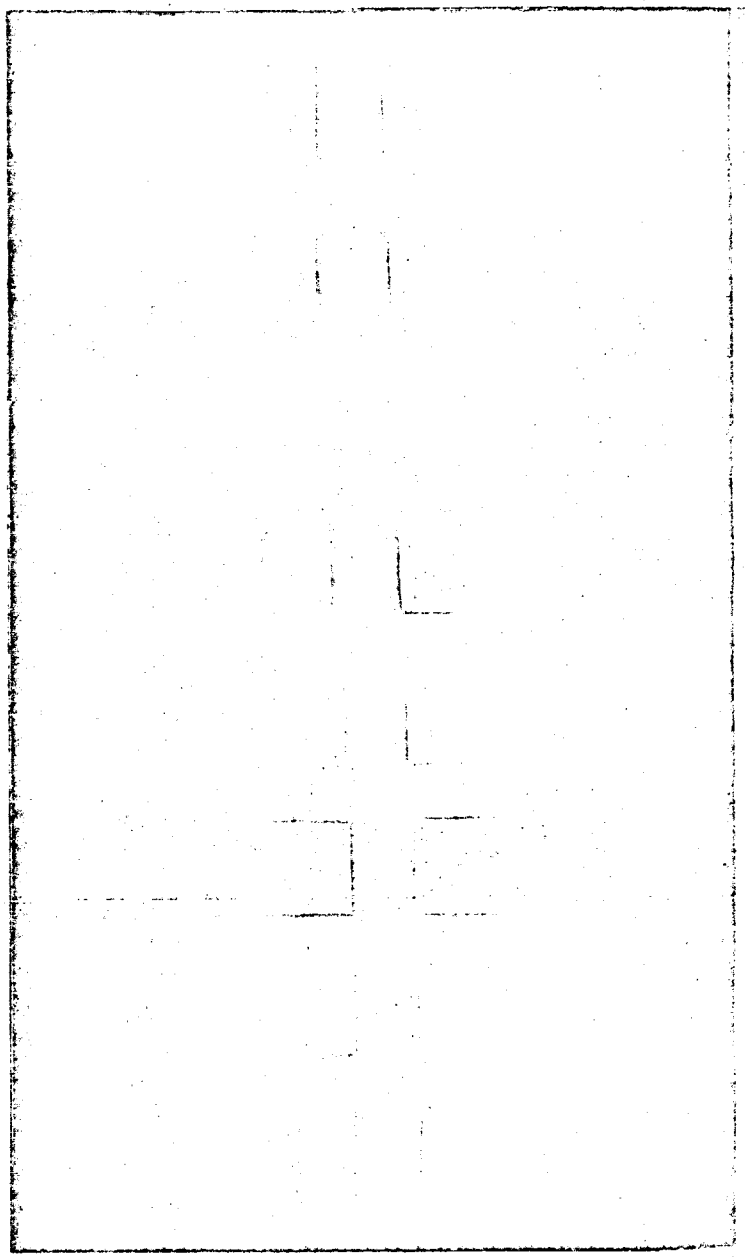

TABLEAU V (de haut en bas).

I. Tests de Diatomées ; II. Spicules d'Eponges ; III. Silice de l'*Oncidium durum* Labbé (Silicodermé) ; IV. Poussière des poumons humains, 1er échantillon ; V. Poussière des poumons humains, 2e échantillon ; VI. Poussière des poumons de Cheval ; VII. Silice amorphe ; VIII. Quartz

ont été mécaniquement entraînées dans l'organisme. Certaines ont vraisemblablement été digérées, ce qui expliquerait l'abondance relative du quartz dans le dépôt pulmonaire, mais les particules fixées n'ont plus subi que, tout au plus, des modifications d'ordre mécanique, des transports qui les ont groupées dans des éléments histologiques spéciaux [20].

TABLEAU IV.

Poussières du Poumon.

Echantillons	Poids en mg	Résidu après FH + SO^4H^2	SiO^2 p. 100
Poumon humain n° 1.....	270,0	24,41	91,0
— — n° 2.....	222,0	29,73	86,6
— — n° 3.....	115,4	9,41	91,8
— — n° 4.....	383,8	25,34	93,4
— — n° 5.....	85,8	8,56	90,0
— — n° 6.....	57,1	6,05	89,4
— d'Autruche (1).....	11,99	0,60	95,0
— de Gazelle.....	2,84	0,27	90,5
— d'Hamadryas.....	45,60	3,39	92,5

N'ayant pas participé au métabolisme, ces particules siliceuses constituent l'exemple-type de la *silice d'interposition* et nous proposons de considérer comme *silice d'interposition* toutes les particules siliceuses rencontrées dans un être vivant et qui présenteront les mêmes caractères (2).

Conclusions.

L'attaque des tissus végétaux et animaux par les mélanges acides oxydants aboutit à la solubilisation intégrale de leurs constituants organiques et minéraux normaux, à l'exception de la silice ou de certaines substances siliceuses.

Les substances siliceuses attaquables par les acides comme les silicates dissous ou colloïdaux, les composés organiques du

(1) Nous remercions MM. ANTHONY et CLAVELIN, Professeur et Chef de Travaux au Muséum, qui nous ont permis de prélever sur les cadavres réservés à leur service, les organes qui nous intéressaient.

(2) Des particules présentant des analogies avec celles du poumon ont été observées par LEMATTE et KAHANE [21] dans le sang des animaux supérieurs.

silicium, et certains minéraux dégradés comme le kaolin, abandonnent un résidu insoluble de silice gélatineuse (silice pure).

Les substances siliceuses inattaquables par les acides, comme la silice amorphe ou cristallisée, les verres et la plupart des silicates naturels cristallisés, ne sont pas modifiées, et conservent, s'il y a lieu, leur individualité cristallographique et minéralogique.

L'insoluble recueilli après destruction nitro-perchlorique des substances biologiques peut donc contenir de la silice gélatineuse, de la silice amorphe compacte, de la silice ou des silicates cristallisés.

L'origine de la silice gélatineuse ne peut être déterminée avec certitude que par l'étude du tissu non attaqué. Il s'agit en général de silice appartenant au stroma cellulaire ou au liquide intracellulaire, c'est-à-dire participant au métabolisme de l'être vivant. C'est de la *silice de constitution*. Elle se rencontre dans le règne végétal et le règne animal, en quantité très variable. Une méthode de dosage suffisamment sensible indiquerait peut-être le caractère général de sa présence.

La silice amorphe compacte se rencontre régulièrement chez certaines Diatomées et Eponges, et, irrégulièrement, chez les Bambous. Qu'il s'agisse de formations normales, comme les tests de Diatomées et les spicules d'Eponge, ou de formations accidentelles comme les tabaschirs des Bambous, elles ont pour origine certaine la silice dissoute et circulante de l'organisme animal ou végétal. C'est le jeu des mécanismes physico-chimiques habituels de l'espèce qui a conditionné leur édification et, malgré le caractère apparemment irréversible de leur dépôt, il semble encore justifié de considérer cette silice amorphe compacte comme de la *silice de constitution*.

La silice ou les silicates cristallisés, tels qu'on les rencontre chez les Silicodermés, dans le poumon humain et animal et dans de nombreux tissus animaux (ANTOINE [22]), ont une individualité minéralogique qui ne paraît pas pouvoir provenir du jeu normal des mécanismes biochimiques. Leur formation paraît être soumise à des conditions de durée, de température et de pression étrangères aux êtres vivants, dont les productions siliceuses certaines sont toujours amorphes. Elles ne résultent pas davantage du traitement acide subi au cours de l'extraction, qui ne se traduit jamais que par une augmentation de symétrie (passage de certains silicates cristallins à l'état de silice colloïdale). Considérant que ces particules ont une origine externe, qu'elles

jouent le rôle de corps étrangers, soumis parfois à des cheminements dans l'organisme, mais ne participant pas à ses échanges chimiques, nous les considérons comme de la *silice d'interposition*.

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BIBLIOGRAPHIE.

1. BERTRAND (G.). — Cours de Chimie biologique, professé à la Faculté des Sciences de Paris.
2. KÜHN (A.). — Die Kieselsaüre ; F. Enke, éd. Stuttgart,, ,1926.
3. KOHL (F.-G.). — Anatomisch. physiologische Untersuchung der Kalksalze und Kieselsaüre in der Pflanze, N. C. Elwertsche édit. Marburg, 1889.
4. KAHANE (E.). — Recherches sur le dosage de la silice et sur l'état de la silice dans les substances biologiques. (*Bull. Soc. Philomath.*, 1933, **116**, 27.
5. KAHANE (E.). — L'action de l'acide perchlorique sur les matières organiques et ses applications analytiques. (Thèse doctorat ès-sciences, Hermann, édit. Paris, 1934).
6. CZAPEK (F.). — Biochemie der Pflanzen. 2e éd. Iéna, 1920, **2**, 412.
7. WEHMER (C.). — Die Pflanzenstoffe, 2e éd., Iéna, 1931, 104 et 1277.
8. GUIBOURT (M.). — Mémoire sur le Tabaschir. (*Journ. Pharm. Chim.*, 1855, **27**, 81-95, 161-172, 252-266).
9. PERRUCHE. — Notes de technique : une nouvelle méthode de nettoyage des Diatomées. (*Bull. Soc. franç. Microscopie*, 1935, **4**, 22-24).
10. BRUN (J.). — Diatomées des Alpes et du Jura, Genève, 1880.
11. MANGIN (L.). — Sur la constitution de la membrane chez les Diatomées (C. R. Ac. Sc., 1908, 146, 770).
12. LAUBY (A.). — Nouvelle méthode technique pour l'étude paléophytologique des formations sédimentaires anciennes. (*Bull. Soc. Biol. Fr.*, 1909, **56**, Mém. n° 15, p. 32).
13. CLAUS (G.). — Traité de Zoologie, 2e éd. franç., Paris 1884, p. 249-269.
14. LABBÉ (A.). — Les Silicodermés du Muséum National d'Histoire naturelle. (*Ann. Inst. Océan.*, 1936, **16**, 101-221).
15. KAHANE (E.). — Sur la silice des Silicodermés. (*Bull. Soc. Chim. Biol.*, 1935, **17**, 1554-1558).
16. GALFAJAN (C. T.) et TARAJAN (W. M.). — Le dosage de la silice dans les silicates par différence. (*Z. anal. Chem.*, 1933, **92**, 417-420).

17. Kahane (E.). — Recherches sur le sort des poussières dans l'organisme. II. Technique de dosage des poussières charbonneuses et siliceuses dans les poumons. (*Journ. Pharm. Chim.*, 1935, **22**, 204-211).

18. Antoine (G.). — Recherches sur le sort des poussières dans l'organisme. III. Répartition des poussières siliceuses dans les poumons. (*Journ. Pharm. Chim.*, 1935, **22**, 412-430).

19. Peyssonneau (J.). — Thèse doct. Pharm. (sous presse).

20. Policard (A.). — Sur la fixation de poussières minérales dans le poumon humain. (*Bull. Hist. appl.*, 1930, **7**, 337-352).

21. Lematte (L.) et Kahane (E.). — La silice dans l'organisme et les particules siliceuses du sang. (*C. R. Ac. Sc.*, 1933, **196**, 575).

22. Antoine (G.). — Sur la présence de particules siliceuses dans les tissus animaux. (*C. R. Ac. Sc.*, 1935, **200**, 980).

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Hepatic Catalase Changes and Leucine Incorporation in Talc-injected Mice[1]

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Summary

The s.c. injection of talc, $MgSiO_3$, into mice results in a decrease of hepatic catalase activity. A transient, but marked rise in catalase activity was observed on the 4th day following talc injection. This rise in activity was manifest primarily in the mitochondrial fraction. A concomitant increased incorporation of radioactive leucine occurred in the microsomal fraction. The relationship between the increased catalase activity and increased incorporation of leucine is discussed with respect to enzyme biosynthesis.

Introduction

The depression of liver catalase during tumor growth was long considered to be specifically a manifestation of malignancy (17). This view has become less tenable as a number of diverse organic compounds have been shown to depress hepatic catalase to varying degrees (discussed in Refs. 8, 19, 20). Recently, Nishimura and co-workers have shown that liver catalase could be markedly and reproducibly depressed when mice were injected with an aqueous suspension of talc, $MgSiO_3$ (20). Maximum depression of hepatic catalase occurred at 48 hr. No inhibition of the enzyme was obtained when catalase and talc were incubated together *in vitro*.

The present study describes the changes in catalase activity in subcellular fractions of mouse liver on successive days following talc injection. Concomitantly, in a preliminary attempt to determine the site of catalase biosynthesis, the extent of incorporation of leucine ^{14}C in the cell fractions was also measured.

Materials and Methods

Animals

Inbred male Strong A mice were used. The animals, 4–6 months of age, were housed in airconditioned quarters and had free access to Rockland mouse diet and water.

Talc Suspension

The preparation and s.c. injection of talc was similar to that described previously (20), except that each animal received 1.0 ml of a 0.67% suspension of talc. The smaller quantity of talc was easier to inject and produced an effect equivalent to the larger dose used previously.

Enzyme Preparations

The following cell fractionation procedure was used except where noted otherwise. A 1% total homogenate of mouse liver was prepared by homogenizing 80-100 mg of tissue with 2 ml of 1% Triton[3] (prepared in 0.1 M phosphate buffer, pH 6.8) in a cold all-glass Potter-Elvejhem homogenizer for 1 min. The homogenate was diluted to volume with 0.1 M phosphate buffer (pH 6.8) and centrifuged at 800 × *g* for 15 min at 5°C to remove cellular debris not solublized by Triton. The supernatant was diluted 1:1 with phosphate buffer immediately before catalase was to be assayed.

Pellet and high-speed supernatant fractions were prepared from the liver in a manner similar to the above procedure, except that the tissue was homogenized in 2 ml of phosphate buffer instead of Triton. After low speed centrifugation, a 5-ml aliquot of the supernatant was again centrifuged at 18,400 × *g* for 20 min at 5°C. The supernatant was decanted, and the pellet was dissolved and made to 5 ml with 0.25% Triton (in phosphate buffer). Both supernatant and pellet were used without further dilution. Enzyme preparations were kept in ice water. This type of simple fractionation was used in the early experiments when the aim was only to observe whether or not modification in enzyme activity occurred in the particulate (presumably mainly mitochondrial and lysosomal rich particles (9, 11)) or in the supernatant fraction (which would include most of the microsomal particles).

Catalase Assay

The permanganate titration method (5, 25) was modified with respect to small reaction volume (3 ml), short reaction time (30 sec), and a single titration. The procedure and kinetic data of reliability are described below.

To a 25-ml Erlenmeyer flask was added 1.9 ml of phosphate buffer (0.1 M, pH 6.8) and 0.1 ml of enzyme preparation. The

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[3] Triton is a nonionic detergent compound (a brand of alkyl amyl polyester alcohol) distributed by Foley and Company, Foley, Alabama.

mixture was stirred with a magnetic mixer at a slow to moderate speed. The size of stirring bar and speed of mixing were not critical, but excessive agitation was avoided. At zero time, 1 ml of hydrogen peroxide (0.1 M) was blown into the reaction mixture. The tip of a 1-ml volumetric pipet was cut off to facilitate rapid delivery (2). A stop watch was started when the peroxide was in the last 1–2 inches of the pipet. At 30 sec, 1 ml of 2 N sulfuric acid was added to stop the reaction. The hydrogen peroxide not decomposed by catalase was titrated with 0.05 N potassium permanganate using a micro buret. Blank determinations were made on samples in which sulfuric acid was added prior to the hydrogen peroxide.

Highly reproducible results were obtained when the enzyme concentration was adjusted so that approximately ½ of the substrate was decomposed within 30 sec. Catalase activity is expressed in terms of the 1st order velocity constant (k) and is calculated:

$$k = \frac{2.3}{t} \log \frac{x}{a}$$

where x is the hydrogen peroxide present at zero time, a is the hydrogen peroxide remaining at time t, and t is in sec. Data presented as k/N and k/p refer to k divided by mg of nitrogen or protein, respectively, of the enzyme preparation. Nitrogen was determined by a micro-Kjeldahl method (14) and protein by the Lowry procedure (15).

CATALASE AND SUBSTRATE CONCENTRATION. Establishment of quantitative relationship between catalase activity (break-down of hydrogen peroxide) and catalase concentration is based upon 2 criteria (4): catalase concentration must be linearly proportional to the 1st order velocity constant (k), and secondly, the 1st order velocity constant must be independent of substrate concentration and of time. The validity of the 1st order equation with respect to catalase activity has been shown by Chance and Herbert (7) and by others (3, 23) in detailed kinetic studies.

Linear proportionality between the 1st order rate constant and enzyme concentration was obtained, except at very low enzyme concentration (1:30 dilution of a 1% homogenate). This deviation from linearity is probably due to inactivation of enzyme due to excessive dilution (5).

Substrate concentration between 0.008 and 0.033 M did not affect the value of k under the conditions of this procedure. A slight decrease in k values was obtained between 0.033 and 0.067 M hydrogen peroxide. The independence of k through a greater range of substrate concentration has been obtained by others (3) using the spectrographic procedure. The results of the present experiments show that k and, therefore, the [illegible] of hydrogen peroxide, was independent of the hydrogen peroxide originally present and the amount of hydrogen peroxide destroyed was proportional to the original concentration of substrate.

EFFECT OF TIME. Although the 1st order velocity constant should be independent of the time of the reaction, this condition is difficult to achieve with simple titration procedures. The k value usually decreases with increasing reaction time. Extrapolating the k values to zero time may not necessarily improve accuracy, since a straight line relationship between k and reaction time is not always attainable (5).

The calculation of k at the 30-sec reaction time interval was found to be the most expedient and simple measure of catalase activity for routine determinations under the conditions described. The 30-sec time interval was based also on the results obtained when $\log a/a - x$, from the integrated form of the 1st order

$$\left(k/2.3 = \frac{\log a/a - x}{t}\right)$$

equation was plotted against time (t). The slope of the resulting straight line should equal $k/2.3$, and is a test for the 1st order equation (18). The 30-sec $k/2.3$ values obtained with a total liver homogenate, supernatant, and pellet fraction were 0.0128, 0.0056, and 0.0179 for slopes of 0.0110, 0.0049, and 0.0157, respectively.

EFFECT OF TEMPERATURE. No appreciable change in k was observed in a temperature range of 2°–45°C. The energy of activation obtained from an Arrhenius plot yielded a value of 1.5 kcal for liver pellet fraction, and 1.2 kcal for a highly purified (19) mouse liver catalase preparation. These results are in general agreement with those obtained with other short reaction time methods: horse blood catalase, 1.7 kcal (5), beef liver catalase, 5.1 kcal (23), and bacterial catalase, 1.4 kcal (7).

Results

Charts 1 and 2 compare and show the similarity between hepatic catalase activity on successive days following implantation of Ehrlich ascites tumor and following s.c. talc injection. The catalase activity remained low throughout the period of tumor growth. Of particular interest is the transient rise of catalase activity of the pellet fraction at the 4th day of growth. No significant change in red cell catalase or kidney catalase occurred; although in some animals a small (10–15%) decrease in kidney catalase was noted after about 12 days of tumor growth. The over-all pattern of hepatic catalase activity following talc injection is similar to the tumor experiments, even to the transient rise of catalase activity on the 4th day. The general pattern

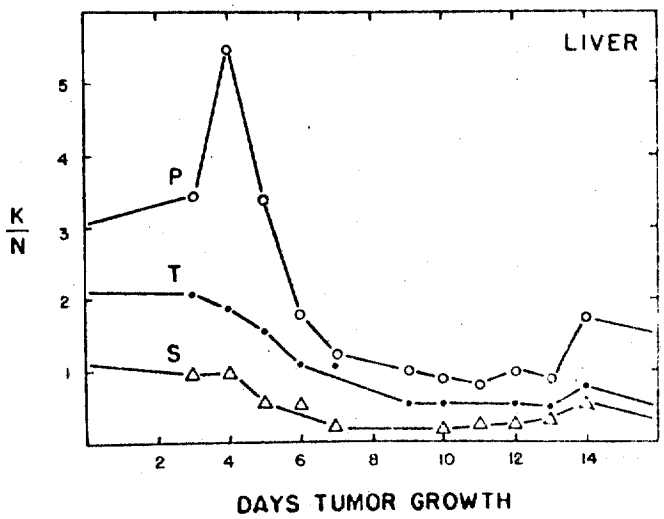

CHART 1. Hepatic catalase activity in total homogenate (*T*), pellet (*P*), and supernatant (*S*) fractions during growth of Ehrlich ascites tumor. The results are the average of 3 experiments. In each experiment, 2–4 mice were used for each point.

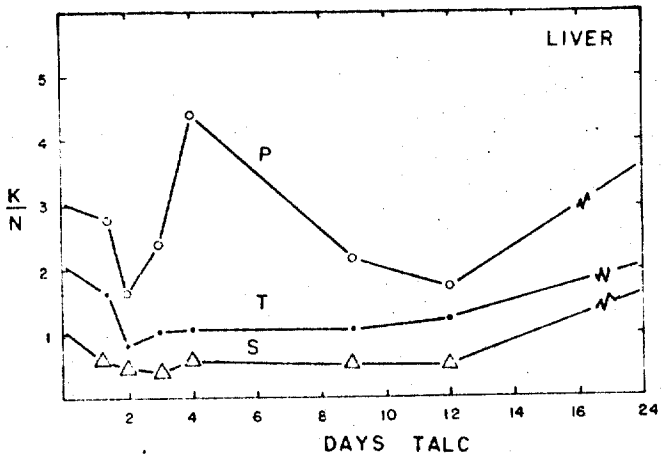

CHART 2. Hepatic catalase activity following talc injection. The results are representative of 2 experiments. Each point is an average of 3 mice.

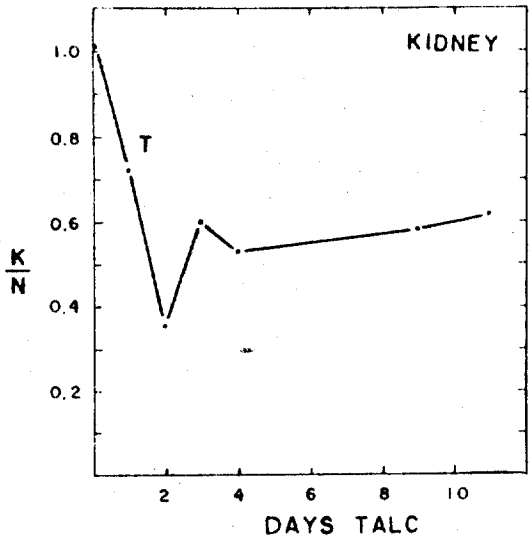

CHART 3. Kidney catalase activity in total homogenate (*T*). Each point is an average of 3 mice.

differs from the tumor experiments in that there is a gradual return to normal levels after the 12th day. No significant changes were noted in red cell catalase. However, kidney catalase showed a marked but transient depression of activity on the 2nd day (Chart 3).

A previous report from our laboratories indicated, by immunochemical evidence, that the decrease in liver catalase was due to repressed catalase synthesis (19). Thus, the rise in catalase activity on the 4th day following talc injection may be related, in part, to increased protein synthesis. Accordingly, experiments were done in which the livers were fractionated by the method of Hogeboom (13) so that catalase activity in the mitochondrial and microsomal fractions could be delineated. Also, the mice were injected with leucine-^{14}C and the cell fractions assayed for incorporation into acid-insoluble material. Chart 4 shows the catalase activity in the mitochondrial, microsomal,

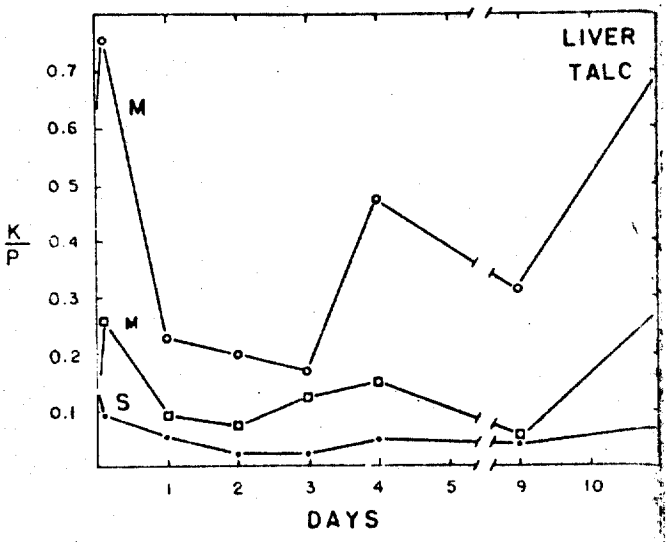

CHART 4. Hepatic catalase activity in mitochondrial (*M*), microsomal (*M*) and supernatant (*S*) fractions following talc injection. The results are representative of 2 experiments. Each value is an average of 2 mice.

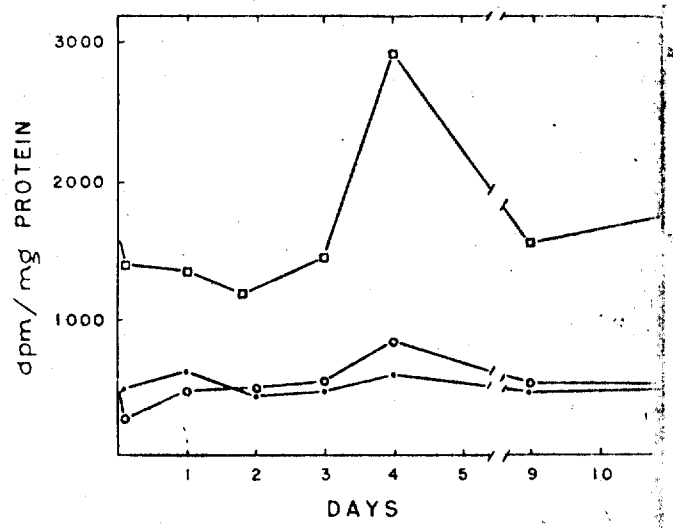

CHART 5. Leucine-^{14}C incorporation following talc injection. Same animals as in Chart 4. The labeled leucine, 3.3 μc, was injected i.p. 45 min before livers were removed. The *ordinate* represents disintegrations/min/mg protein. (dpm/mg protein). Trichloroacetic acid-insoluble material of the various fractions was solubilized with Hyamine (Packard Instrument Co.) and counted in a Packard Tri-Carb liquid scintillation spectrometer. Each sample was recounted with an internal standard to obtain disintegrations/min.

and supernatant fractions of mouse liver following talc injection. The marked rise in enzyme activity on the 4th day after the initial fall was manifested primarily in the mitochondrial fraction. The return to normal levels was not always rapid, but normal levels were reached between the 15th and 25th day.

Chart 5 shows the results of the incorporation experiments. It is of primary interest that at the time catalase activity was

increasing in the mitochondrial fraction (Chart 4) there was a concomitant increase of leucine-^{14}C incorporation in the microsomal fraction.

Discussion

The results of this investigation show the similarity in pattern between hepatic catalase depression in tumor-bearing and talc-injected mice. The pattern does differ in the talc-injected animals in that there is a gradual return to normal values, whereas tumor-bearing animals continue to maintain low levels of catalase. Greenstein *et al.* (10) were the 1st to experimentally demonstrate that rat liver catalase is depressed as long as tumor growth is maintained. This difference could be expected if one considers the tumor to be a chronic progressive process and the talc granuloma a self-limited reparative process.

The unusual observation in these experiments was the fact that on the 3rd day following tumor growth or talc injection there was a transient increase of hepatic catalase activity, exceeding normal values, and this rise was manifested primarily in the pellet fraction (Charts 1, 2). The total homogenate did not reflect this increased activity on the 4th day. This was apparently due to the formation of the inhibitory catalase-H_2O_2 complex (7). Increased catalase activity in unpurified tissue preparations has been obtained by incubating with ethanol before assay (2, 11). Catalase activity of mitochondrial or microsomal preparations does not seem to be effected in this manner (11). Recent tests on tissue preparations similar to those described under "Enzyme Preparations" concur with these observations.

The results obtained in the pellet fraction suggested the series of experiments in which the liver was fractionated into mitochondrial, microsomal, and high-speed supernatant fractions. The fractionation studies showed that most of the catalase activity as well as the transient rise of catalase activity was localized in the mitochondrial fraction (Chart 4). Very little of the particulate enzyme activity was present in the microsomal fraction, as has been shown previously for normal liver by others (9, 11, 16). The high initial proportion of intracellular hepatic catalase activity in the mitochondrial fraction and the rapid rise in activity under the conditions described is evidence for the possibility that the mitochondrion is the site of catalase formation (1). Moreover, results obtained previously, using immunochemical technics, indicated that the lowering of hepatic catalase activity represented a repression of catalase biosynthesis (19). That the depression of hepatic catalase is not due merely to an inhibitor of catalase has been indicated by others using different technics (6, 21, 22, 24). Higashi and Peters (12) have indicated that mitochondrial catalase may be formed by the ribosomes of the rough-surfaced reticulum and then transported to the mitochondria. The rapid uptake of labeled leucine into the microsomal fraction (Chart 5) at the same time that mitochondrial catalase activity was increasing is consistent with this hypothesis. The small peak of radioactivity in the mitochondrial fraction may represent mitochondrial incorporation of labeled leucine into protein or may be a result of rapid transfer (12) of labeled protein synthesized in microsomes. Contamination of the mitochondria fraction could also explain this peak but is partially ruled out by the difference in the shapes of the curve.

Additional work is necessary to determine whether the sudden rise in mitochondrial catalase represents synthesis or assembly of preformed parts of the molecule.

The immunoelectrophoretic technic was in an exploratory phase at the time these experiments were done, and no conclusive data were obtained at the time. The application of this technic to this type of study may indicate qualitative differences in the various components of the cell fractions. Immunochemical procedures will be required to quantitate the changes of the catalase molecule during the course of injury. Such technics may also aid in explaining whether or not the rapid changes in catalase activity could be due to the rapid breakdown of particles containing catalase or due to an altered rate of enzyme destruction.

Acknowledgments

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References

1. Adams, D. H. The Mechanism of the Action of Androgenic Hormones on Mouse-Liver Catalase. Biochem. J., *74:* 141–48, 1960.
2. Alexander, N. M. Catalase Inhibition by Normal and Neoplastic Tissue Extracts. J. Biol. Chem., *227:* 975–85, 1957.
3. Beers, R. F., Jr., and Sizer, I. W. A Spectrophotometric Method for Measuring the Breakdown of Hydrogen Peroxide by Catalase. *Ibid.*, *195:* 133–40, 1952.
4. ———. Catalase Assay with Special Reference to Manometric Methods. Science, *117:* 710–12, 1953.
5. Bonnichsen, R. K., Chance, B., and Theorell, H. Catalase Activity. Acta Chem. Scand., *1:* 685–709, 1947.
6. Ceriotti, G., Spandrio, L., and Agardi, A. A Study of the Synthesis of Catalase in Liver of Tumor-bearing mice by means of Radioactive Iron. Biochim. Biophys. Acta, *27:* 432–33, 1958.
7. Chance, B., and Herbert, D. Catalases and Peroxides. XIV. Enzyme-Substrate Compounds of Bacterial Catalase and Peroxides. Biochem. J., *46:* 402–14, 1950.
8. Costa, G. Cachexia, the Metabolic Component of Neoplastic Diseases. Progr. Exptl. Tumor Res. *3:* 338–46, 1962.
9. DeDuve, C., Beaufay, H., and Baudhuin, P. Intracellular Localization of Catalase in Rat Liver. Acta Chem. Scand., *17:* 210–15, 1963.
10. Greenstein, J. P., Jenrette, W. V., and White, J. The liver Catalase Activity of Tumor-bearing Rats and the Effect of Extirpation of the Tumors. J. Biol. Chem. *141:* 327–28, 1941.
11. Higashi, T., and Peters, T., Jr. Studies on Rat Liver Catalase. I. Combined Immunochemical and Enzymatic Determination of Catalase in Liver Cell Fractions. *Ibid.*, *238:* 3945–51, 1963.
12. ———. Studies on Rat Liver Catalase. II. Incorporation of C^{14} Leucine into Catalase of Liver Cell Fractions *in vivo*. *Ibid.*, *238:* 3952–54, 1963.
13. Hogeboom, G. H. Fractionation of Cell Components of Animal Tissues. *In:* S. P. Colowick and N. O. Kaplan (eds.), Methods in Enzymology, Vol. 1, p. 16. New York: Academic Press, Inc., 1955.
14. Jenden, D. J., and Taylor, D. B. Micro Kjeldahl Distillation Apparatus. Anal. Chem., *25:* 685–86, 1953.
15. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall,

R. J. Protein Measurement with the Folin Phenol Reagent. J. Biol. Chem., *193:* 265-75, 1951.

16. Miller, L. A. Studies on the Localization of Intracellular Catalase of Mouse Liver. Exptl. Cell Res., *34:* 283-92, 1964.
17. Nakahara, W., and Fukoka, F. Chemistry of Cancer Toxin Toxohormone. Springfield, Ill.: Charles C Thomas, 1964.
18. Neilands, J. B., and Stumpf, P. K. Outlines of Enzyme Chemistry, p. 92. New York: John Wiley & Sons, Inc., 1958.
19. Nishimura, E. T., Kobara, T. Y., Kaltenbach, J. P., and Wartman, W. B. Immunological Evidence of Repressed Catalase Synthesis in Livers of Tumor-Bearing Mice. Arch. Biochem. Biophys., *97:* 589-95, 1962.
20. Nishimura, E. T., Rosenheim, S., and Klein, L. Depression of Hepatic Catalase in Mice after Subcutaneous Injury. Lab. Invest., *12:* 115-21, 1963.
21. Price, V. E., and Greenfield, R. E. Liver Catalase. II. Catalase Fractions from Normal and Tumor-bearing Rats. J. Biol. Chem., *209:* 363-76, 1954.
22. Price, V. E., Stirling, W. R., Tarantola, V. A., Hartley, R. W., Jr., and Recheigl, M., Jr. The Kinetics of Catalase Synthesis and Destruction *in vivo*. *Ibid.*, *237:* 3468-75, 1962.
23. Strother, G. K., and Ackerman, E. Physical Factors Influencing Catalase Rate constants. Biochim. Biophys. Acta, *47:* 317-26, 1961.
24. Theorell, H., Beznak, K., Bonnichsen, R., Paul, K. G., and Akeson, A. Distribution of Injected Radioactive Iron in Guinea Pigs and its Rate of Appearance in some Hemoproteins and Ferritins. Acta Chem. Scand., *5:* 445-75, 1951.
25. Von Euler, M., and Josephson, K. Catalase I. Ann. Chem. Liebigs, *452:* 158-81, 1927.

COMPARATIVE EFFECT OF SODIUM BICARBONATE AND MAGNESIUM TRISILICATE ON GASTRIC SECRETION

I. V. Kas'yanenko

It is often difficult to decide whether patients should be given alkalis, even though their mechanism of action has been fairly well studied experimentally (I. P. Pavlov, A. P. Sokolov, P. P. Pimenov, P. P. Pimenov, P. P. Khizhin, and others). The stomachs of healthy persons frequently contain alkaline secretions at basal metabolism. According to V. N. Ivanov who used a thin stomach tube to examine healthy persons, about 50 ml of contents was extracted at one time from all those examined, and the contents proved to be acid in 43%.

In cases of stomach pathology, the amount of acid secretion at basal metabolism may increase substantially, a fact that should be borne in mind when prescribing alkalis for such patients.

While most investigators insist that alkalis should be taken shortly before eating in order to decrease gastric secretion, others believe that alkalis and alkaline mineral water taken at this time intensify gastric secretion.

Many investigators maintain that an alkali or mineral water introduced into the stomach with food stimulates gastric secretion. Some data are available on the efficacy of alkalis taken at the height of gastric secretion. G. F. Shattuk, Ye. L. Rozenberg, and L. Ye. Bukher and Bergman et al. showed that ingestion of sodium carbonate or magnesium oxide reduces the acidity of the stomach contents initially but increases it later on.

We investigated the effect of alkalis on gastric secretion when they were introduced into the stomach simultaneously with a standard stimulant of gastric secretion and without the latter - at the height of secretion. We used sodium bicarbonate and magnesium trisilicate, a little studied alkali introduced fairly recently into clinical practice. We chose it because while sodium bicarbonate is known to form products with succagogue action when reacting with acid stomach contents, magnesium trisilicate does not do so.

The synthetic preparation, magnesium trisilicate, was first introduced into medical practice by Much as an antacid, an adsorbing and coating substance. According to Armstrong et al., magnesium trisilicate becomes jelly-like after entering the stomach. Hydrochloric acid is neutralized without gas formation and without the appearance of increased quantities of hydrochloric acid. The great adsorption capacity of magnesium trisilicate is due to the formation of colloidal silicic acid, which likewise protects the gastric mucosa against pepsin and hydrochloric acid.

We analyzed a total of 113 portions of gastric secretion from 32 persons in 7 of whom it was normal and in 25 increased. In some of those examined, the effect of 300 ml of a 5% alcohol solution on gastric secretion when introduced into the stomach was compared with that of a 5% alcohol solution to which 1 g of sodium bicarbonate or magnesium trisilicate had been added. The method employed has already been described.*

*Fiziol. zhurn. (Journal of Physiology), 1962, Vol. 8, No. 5, p. 650.

Another group of patients was examined with a slightly modified form of N. I. Leporskiy's method. The stomach contents were first extracted in toto at 10-minute intervals and then the succagogue was introduced - 300 ml of a 5% alcohol solution. Two 10-ml portions were extracted at 10-minute intervals after which the entire contents were extracted a third time. 1 ml of the alkali diluted in 50 ml of distilled water was then introduced into the stomach through a tube.. The contents were withdrawn from the stomach at 10-minute intervals after the alkali was introduced. 50 ml of distilled water without the addition of the alkali was introduced during the control examinations. This approach made it possible to study the effect of fairly concentrated solutions of the alkali at the height of gastric secretion. Moreover, by extracting all the contents during the successive secretions we were able to investigate pure gastric juice and determine the absolute amounts of the hydrochloric acid present in it.

Of those examined, hypersecretion was noted in 17; it was constant in 11. A substantial amount of acid contents was found in the stomachs of these patients at basal metabolism. The following case is illustrative.

Patient B., age 35, with constant hypersecretion. Three studies were made. During a study run at basal metabolism 120 ml of the stomach contents was extracted; free hydrochloric acid amounted to 50 units and total acidity 64. After the introduction of a 5% alcohol solution with nothing added, free hydrochloric acid amounted to 82 units and total acidity 92. During the subsequent gastric secretion, 48 ml of the contents was extracted. During the next study run at basal metabolism, 145 ml of the contents was obtained, with acidity

the same as during the preceding study. After a 5% alcohol solution containing 1 g of sodium bicarbonate was introduced into the stomach, free hydrochloric acid was not found in the first 3 portions of the stomach contents; the level then gradually rose to 48 units and total acidity 54 units. The amount of stomach contents in the next phase was 72 ml. During the third study at basal metabolism, 170 ml of contents was obtained, with free hydrochloric acid amounting to 66 units and total acidity 76 units. After the 5% alcohol solution with 1 g of magnesium trisilicate added was introduced into the stomach, free hydrochloric acid was absent only in the first portion, after which the curve rose quite rapidly to 92 and total acidity to 102. The quantity of juice in the following secretion was 102 ml. Evacuation of the 5% alcohol solution from the stomach ended within 50 minutes and that of the stimulant admixed with the alkali after 60 minutes.

It is reasonable to conclude from the above that the addition of 1 g of sodium bicarbonate to a 5% alcohol solution resulted initially in neutralization of the hydrochloric acid followed by a rise to a lower level than after introducing the standard stimulant (without soda) and admixed with 1 g of magnesium trisilicate. In calculating the absolute quantity of free hydrochloric acid, we found that the total amount secreted did not decrease after the addition of sodium bicarbonate, that it even increased slightly compared with the examination during which the 5% alcohol solution not containing the alkali was introduced into the stomach. The absolute quantity of free hydrochloric acid in the subsequent secretion was 117 mg during the first study, 150 mg and 310 mg during the second and third studies, respectively.

The difference between the values of acidity and absolute amounts of hydrochloric acid released during the various studies was apparently due, in part, to the dissimilar functional state of the gastric secretory apparatus on the different days of the examination, as can be judged from the nature of the secretion of the jejunum.

The above-described studies suggest that the addition of sodium bicarbonate to a succagogue causes a brief decrease in the acidity of the stomach contents and some decrease in acidity of the subsequent secretion. The initial decrease in acidity is less pronounced when magnesium trisilicate is added to the succagogue and the acidity of the subsequent secretion does hot decrease.

We used the same method to study 6 persons with moderate hypersecretion and found the results to be approximately the same as in those suffering from constant hypersecretion.

In all the persons with hypersecretion, a period marked by the absence of free hydrochloric acid in the stomach contents followed the introduction into the stomach of one of the two alkalis under study. Only one case in which magnesium trisilicate was added to the stimulant proved to be an exception. After the introduction of sodium bicarbonate into the stomach, free hydrochloric acid appeared in the third portion of the stomach contents in most of the patients, whereas it appeared in the second portion (i.e., sooner) in those that received magnesium trisilicate. Thus, sodium bicarbonate exerted a greater direct neutralizing effect.

Regarding the evacuation time of the stimulant from the stomach, which we judged from the decolorization of the stomach contents extracted, it is generally determined from the last colored portion. In point of

fact, the moment of complete evacuation is found in the interval between the extraction of the last colored and first uncolored portion of stomach contents.

Our findings suggest that a 5% alcohol solution containing magnesium trisilicate remains longer in the stomach (50 to 70 minutes) than the same standard stimulant with sodium bicarbonate (40 to 60 minutes).

We obtained similar results after examining 3 persons with normal gastric secretion.

Since we did not obtain conclusive data on the effect of sodium bicarbonate and magnesium trisilicate added in the amount of 1 g simultaneously with the succagogue, we ran a series of studies involving the introduction into the stomach of 50 ml of distilled water to which 1 g of the above alkalis was added at the height of secretion. For this purpose we used, as mentioned above, a slight modification of N. I. Leporskiy's method of analyzing gastric secretion. An example of this is the studies performed with a 5% alcohol solution (No. 292), with the same stimulant and introduction into the stomach of 50 ml of distilled water at the height of secretion (No. 295), 50 ml of distilled water with 1 g of sodium bicarbonate (No. 293, and 50 ml of distilled water with 1 g of magnesium trisilicate (No. 294) in patient K., age 24 (gastritis with moderate hypersecretion).

In studies Nos. 292, 293, and 294 at basal metabolism, approximately the same amount of stomach contents was extracted with equal quantities of free hydrochloric acid. In study No. 295, the amount of stomach contents and quantity of free hydrochloric acid at basal metabolism were somewhat higher than in the other studies. After a 5% alcohol solution was introduced into the stomach, the

maximum quantity of free hydrochloric acid was 68 units and total acidity 62 units. The absolute amount of free hydrochloric acid in the next gastric secretion was 100 mg. After 50 ml of distilled water was introduced into the stomach at the height of secretion, a comparatively large amount of stomach contents was obtained with about 58 units of free hydrochloric acid. The absolute amount of free hydrochloric acid ~~in this study~~ in the next secretion was 147 mg. After 1 mg of sodium bicarbonate or magnesium trisilicate in 50 ml of distilled water was introduced into the stomach, the levels of free hydrochloric acid (64 to 76) were as high as after the introduction of the standard stimulant of gastric secretion, but somewhat less gastric juice was secreted than after the standard stimulant. The absolute amount of free hydrochloric acid in the secretion following the introduction of sodium bicarbonate into the stomach was 23 mg and after the introduction of magnesium trisilicate 55 mg. Thus, in this patient 1 g of either alkali in 50 ml of distilled water introduced into the stomach at the height of secretion ~~had~~ distinctly ~~inhibited~~ reduced the amount of acid secreted by the stomach.

We ran similar studies on 12 persons. Gastric secretion increased in 8 and remained normal in 4. These studies revealed definite patterns in the action of the alkalis on acid secretion by the stomach regardless of the nature of gastric secretion. For example, after the 12 persons were given 50 ml of water and 50 ml of sodium bicarbonate solution, we observed a distinct decrease in the absolute amount of free hydrochloric acid after introduction of the soda in 7, a decrease of 15% in 2, an increase of 12 and 18% in 2, and a substantial increase in 1.

A comparison of the absolute amount of free hydrochloric acid in the control studies with that in the studies involving the introduction of 50 ml of magnesium trisilicate solution into the stomach revealed the following. The absolute amount of free hydrochloric acid distinctly decreased in 6, increased slightly (12 to 17%) in 2 and more significantly in 2.

Thus, the secretion of hydrochloric acid decreased about as often after the introduction of sodium bicarbonate as after the introduction of sodium bicarbonate.

СРАВНИТЕЛЬНОЕ ДЕЙСТВИЕ ДВУУГЛЕКИСЛОЙ СОДЫ И ТРИСИЛИКАТА МАГНИЯ НА ЖЕЛУДОЧНУЮ СЕКРЕЦИЮ

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Решение вопросов, связанных с применением щелочей в клинике, несмотря на то, что механизм их действия достаточно изучен в эксперименте (И. П. Павлов, А. П. Соколов, П. П. Пименов, П. П. Хижин и др.), встречает нередко значительные затруднения. В желудке здоровых людей нередко натощак содержится щелочной секрет. По данным В. Н. Иванова, исследовавшего тонким зондом желудок у здоровых лиц, при однократном извлечении в желудке находилось до 50 мл содержимого у всех обследуемых, а у 43% содержимое было кислым.

При патологических состояниях желудка количество кислого секрета натощак может значительно увеличиваться, что также необходимо учитывать при назначении таким больным щелочей.

Если большинство исследователей с целью снижения желудочной секреции настаивает на целесообразности применения щелочей за некоторое время до приема пищи, то существует также мнение, что щелочи и щелочные минеральные воды, принятые в это время, усиливают желудочную секрецию.

Многие исследователи полагают, что щелочь или щелочная минеральная вода, введенная в желудок вместе с пищей, возбуждают желудочную секрецию.

Имеются данные относительно эффективности применения щелочей в период разгара желудочной секреции («на высоте секреции»).

Г. Ф. Шаттук, Е. Л. Розенберг и Л. Е. Бухер, Бергман и др. показали, что двууглекислая сода и окись магния, применяемые больными внутрь, вначале вызывают снижение кислотности желудочного содержимого и повышают ее в более поздней стадии.

Мы изучали действие щелочей на желудочную секрецию при введении их в желудок одновременно со стандартным возбудителем желудочной секреции и без него — на высоте секреции. Среди применяемых щелочей мы остановились на двууглекислой соде и на сравнительно недавно вошедшей в клиническую практику и мало изученной щелочи — трисиликате магния. Выбор его определялся еще и тем, что если относительно двууглекислой соды известно, что при взаимодействии ее с кислым желудочным содержимым могут образовываться продукты, обладающие сокогонным действием, то трисиликат магния лишен этих свойств.

Синтетический препарат — трисиликат магния впервые введен в медицинскую практику Мутчем, как противокислотное, адсорбирующее и обволакивающее средство. По мнению Армстронга и др., трисиликат магния при поступлении в желудок переходит в желеобразное состояние. Нейтрализация соляной кислоты происходит без образования газов и без появления повышенных количеств соляной кислоты. Большая адсорбционная способность трисиликата магния объясняется образованием коллоидной кремниевой кислоты, которая также защищает слизистую оболочку желудка от действия пепсина и соляной кислоты.

В общей сложности нами проведено 113 фракционных исследований желудочной секреции у 32 лиц, из них у семи была нормальная и у 25 — повышенная секреторная функция желудка.

У части исследуемых влияние 5% раствора алкоголя на желудочную секрецию, введенного в желудок в количестве 300 мл, сравнивалось с действием 5% раствора алкоголя с добавлением к нему 1 г

двууглекислой соды или 1 г трисиликата магния. Методика исследований подробно описана ранее [1].

У другой группы больных исследования проводились по несколько видоизмененной методике Н. И. Лепорского. Вначале желудочное содержимое трижды извлекалось все полностью с интервалами в 10 минут, затем вводился возбудитель секреции — 300 мл 5% раствора алкоголя, после чего с десятиминутными интервалами извлекались две порции по 10 мл, в третий раз извлекалось все содержимое, после чего вводился в желудок через зонд 1 г щелочи, растворенной в 50 мл дистиллированной воды. После введения щелочи продолжалось извлечение содержимого из желудка с интервалами в 10 минут. Во время контрольных исследований вводилось 50 мл дистиллированной воды без добавления щелочи. Такая постановка исследований позволяла изучать действие достаточно концентрированных растворов щелочи в период разгара желудочной секреции. Кроме того, извлекая во время последующей секреции все содержимое, мы могли исследовать чистый желудочный сок и определять абсолютные количества содержащейся в нем соляной кислоты.

Среди подвергшихся исследованиям у 17 больных имела место гиперсекреция, причем у 11 она носила постоянный характер; натощак у этих больных в желудке обнаруживалось значительное количество кислого содержимого.

В качестве примера приводим результаты исследования одного больного.

Больной Б., 35 лет с гиперсекрецией постоянного типа. Проведены три исследования. Во время исследования натощак извлечено из желудка 120 мл содержимого, количество свободной соляной кислоты в котором достигало 50 единиц, а общая кислотность — 64. После введения 5% раствора алкоголя без каких-либо примесей цифры свободной соляной кислоты достигли 82 ед., а общая кислотность — 92 ед. В фазу последующей желудочной секреции было извлечено 48 мл содержимого. Во время следующего исследования натощак в общей сложности получено 145 мл содержимого, цифры кислотности были такими же, как и во время предыдущего исследования. После введения в желудок 5% раствора алкоголя с добавлением 1 г двууглекислой соды в первых трех порциях желудочного содержимого свободная соляная кислота не определялась, после чего цифры ее постепенно повышались и достигли 48 ед., а общей кислотности — 54 ед. Количество желудочного содержимого в последующую фазу равнялось 72 мл. Во время третьего исследования натощак было получено 170 мл содержимого, цифры свободной соляной кислоты в нем достигали 66 ед., общей кислотности — 76 ед. После введения в желудок 5% раствора алкоголя с 1 г трисиликата магния свободная соляная кислота отсутствовала лишь в первой порции, после чего ее кривая довольно быстро поднялась до цифры 92, а общей кислотности — до 102. Количество сока в фазу последующей секреции равнялось 102 мл. Эвакуация из желудка 5% раствора алкоголя закончилась в течение 50 минут, а того же возбудителя с примесью щелочей — через 60 минут.

При оценке описанных наблюдений можно прийти к выводу, что добавление к 5% раствору алкоголя 1 г двууглекислой соды привело к начальной нейтрализации соляной кислоты с последующим подъемом ее цифр до более низких показателей, чем после введения в желудок стандартного возбудителя (без соды) и с примесью 1 г трисиликата магния. При подсчете абсолютного количества свободной соляной кислоты было обнаружено, что общее количество выделенной соляной кислоты при добавлении двууглекислой соды не уменьшилось, а даже несколько увеличилось по сравнению с исследованием, при котором вводился в желудок 5% раствор алкоголя без примеси щелочи. Абсолютное количество свободной соляной кислоты в фазе последующей секреции в первом исследовании равнялось 117 мг, во II — 150 мг, а в III — 310 мг.

Разница в цифрах кислотности и абсолютных количествах соля-

[1] Фізіол. журн., 1962, т. VIII, № 5, стр. 650.

ной кислоты, выделившейся во время отдельных исследований отчасти, очевидно, зависит от неодинакового функционального состояния секреторного аппарата желудка в разные дни исследования, о чем можно судить по характеру секреции тощего желудка.

На основании описанных выше исследований можно заключить, что добавление к возбудителю двууглекислой соды приводит к непродолжительному снижению кислотности желудочного содержимого и некоторому понижению кислотности в фазу последующей секреции. При добавлении к возбудителю трисиликата магния это начальное снижение кислотности меньше выражено, а цифры кислотности в фазу последующей секреции не снижаются.

С умеренной гиперсекрецией нами обследовано шесть человек по тому же методу, что и обследование лиц с постоянной гиперсекрецией и получены примерно такие же данные.

Во всех исследованиях у лиц с гиперсекрецией после введения в желудок одной из двух исследуемых щелочей наблюдался период отсутствия свободной соляной кислоты в желудочном содержимом. Исключение представляет только одно исследование с добавлением к возбудителю трисиликата магния. После введения в желудок двууглекислой соды свободная соляная кислота появлялась у большинства больных в третьей порции желудочного содержимого, в то время, как после введения в желудок трисиликата магния — во второй порции, то есть скорее. Таким образом, большее непосредственное нейтрализующее действие оказывала двууглекислая сода.

Что касается времени эвакуации возбудителя из желудка, о котором мы судим по обесцвечиванию извлекаемого желудочного содержимого, то принято определять его по последней окрашенной порции. В действительности, момент полной эвакуации находится в интервале времени между извлечением последней окрашенной и первой из лишенных окраски порции желудочного содержимого.

Полученные нами данные разрешают считать, что сравнительно дольше в желудке задерживается 5% раствор алкоголя с добавлением трисиликата магния (от 50 до 70 минут), в то время, как тот же стандартный возбудитель с двууглекислой содой наиболее часто находился в желудке 40—60 минут.

Аналогичные данные получены при исследовании трех человек с нормальной секреторной функцией желудка.

Не получив достаточно убедительных данных о влиянии на желудочную секрецию двууглекислой соды и трисиликата магния, вводимых в количестве 1 г одновременно с возбудителем секреции, мы провели серию исследований с введением в желудок 50 мл дистиллированной воды с добавлением 1 г указанных щелочей в период разгара желудочной секреции.

Для этого, как было указано выше, нами была использована несколько видоизмененная методика исследования желудочной секреции, предложенная Н. Н. Лепорским.

Примером могут служить исследования, проведенные с 5% раствором алкоголя (№ 292), с тем же возбудителем и введением в желудок на высоте секреции 50 мл дистиллированной воды (№ 295), 50 мл дистиллированной воды с 1 г двууглекислой соды (№ 293) и 50 мл дистиллированной воды с 1 г трисиликата магния (№ 294), у больной К., 24 лет (гастрит с умеренной гиперсекрецией).

Натощак в исследованиях № 292, 293 и 294 было извлечено примерно одинаковое количество желудочного содержимого с одинаковыми цифрами свободной соляной кислоты. В исследовании № 295 количество желудочного содержимого и цифры свободной соляной кислоты натощак оказались несколько выше, чем в других исследованиях. После введения в желудок 5% раствора алкоголя максимальные цифры свободной соляной кислоты равнялись 68 ед., общей кислотности — 62 ед. Абсолютное количество свободной соляной кислоты в фазе последующей желудочной секреции

равнялось 100 мг. После введения в желудок на высоте секреции 50 мл дистиллированной воды было получено сравнительно большое количество желудочного содержимого с цифрами свободной соляной кислоты до 58 ед. Абсолютное количество свободной соляной кислоты в этом исследовании в фазе последующей секреции равнялось 147 мг. После введения в желудок 1 г двууглекислой соды в 50 мл дистиллированной воды, а также 1 г трисиликата магния цифры свободной соляной кислоты (64—76) не уступали цифрам после введения в желудок стандартного возбудителя желудочной секреции, но желудочного сока выделилось несколько меньше, чем после стандартного возбудителя. Абсолютное количество свободной соляной кислоты в фазе последующей секреции после введения в желудок двууглекислой соды равнялось 23 мг, а после введения трисиликата магния — 55 мг. Таким образом, у представленного больного ясно выражено понижающее действие на кислотовыделительную функцию желудка щелочей, введенных в желудок в количестве 1 г в 50 мл дистиллированной воды на высоте секреции.

Подобного рода исследования нами проведены у 12 человек. Из них повышенная секреторная функция желудка наблюдалась у восьми человек и нормальная — у четырех. При этих исследованиях наблюдались определенные закономерности в действии указанных щелочей на кислотовыделительную функцию желудка, независимо от характера желудочной секреции.

Так, при исследовании 12 лиц, которым вводилось 50 мл воды и 50 мл раствора двууглекислой соды, мы наблюдали, что у семи после введения раствора соды абсолютное количество свободной соляной кислоты отчетливо уменьшилось, у двух это же количество понизилось на 15%, у двух — увеличилось на 12 и 18%, а у одного больного значительно возросло.

При сопоставлении абсолютного количества свободной соляной кислоты во время контрольных исследований и при исследованиях с введением в желудок 50 мл раствора трисиликата магния было установлено следующее: у шести после введения раствора трисиликата магния абсолютное количество свободной соляной кислоты отчетливо уменьшилось, у двух увеличилось незначительно (на 12—17%) и у двух — более значительно.

Таким образом, примерно одинаково часто как при введении двууглекислой соды, так и при введении трисиликата магния наблюдалось понижение секреции соляной кислоты.

SUMMARY

COMPARATIVE EFFECT OF SODIUM BICARBONATE AND MAGNESIUM TRISILICATE ON GASTRIC SECRETION

I. V. Kasianenko (Kiev)

In persons with a normal and increased secretory function of the stomach the difference of effect of the two agents (sodium bicarbonate and magnesium trisilicate administered per 1 g with 300 ml of 5% alcohol solution) was insignificant.

Less diluted sodium bicarbonate and magnesium trisilicate (1 g in 50 ml of distilled water) introduced at the height of gastric secretion effected in the majority of cases a decrease of the gastric acid-secreting function.

Persons with increased gastric sectretion may be advised to take sodium bicarbonate and magnesium trisilicate (small doses) at the height of gastric secretion.

ВЛИЯНИЕ БЕНЗОГЕКСОНИЯ НА ЖЕЛУДОЧНУЮ СЕКРЕЦИЮ

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Одним из наиболее эффективных способов фармакологического воздействия на функции важнейших физиологических систем организма является направленное вмешательство в процессы нервной регуля-

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THE SILICA CONTENT OF TISSUES, WITH AND WITHOUT SILICOTIC LESIONS

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(PLATES XXXVII AND XXXVIII)

WHEN we speak of silicotic lesions we have in mind the silicotic nodule. For all ordinary purposes we may regard it as the only specific lesion in silicosis. Wherever there is a silicotic nodule there is almost certainly some excess of silica.* But how far does the converse hold true? Does silica, when suitably implanted in the tissues, necessarily give rise to a specific lesion? In what follows we shall present certain evidence to the contrary.

This is essentially a quantitative study of the silica content of lungs and root glands of human subjects, both silicotic and non-silicotic. By comparing one group with another we shall see that the content of silica over and above a certain minimum bears no apparent relationship to the severity of the specific lesion. It may be argued that this finding alone can point to no valid conclusions unless we know the quality as well as the quantity of the silica and the length of time it has been present in the tissues. Though we lack exact information as to these factors we do know something of the quality of the silica in certain cases at least. We know for example that some of the non-silicotics were exposed to the same dusts as certain of the silicotics and for comparable periods, from which it is clear that a "dangerous" dust may produce the specific lesion in one individual and not in another. Whether or not these findings throw any light on the aetiology of silicosis is beside the point. Our main purpose is to show that the presence of abnormal quantities of silica in human tissues as determined by chemical analysis or micro-incineration does not necessarily point to a diagnosis of silicosis.

The material is divided into two parts: (1) the lungs of 35 persons with long occupational exposure to siliceous dusts; (2) the peribronchial lymph glands of 130 individuals without such exposure.

* The term silica is here used to signify the total concentration of silicon expressed as the oxide, SiO_2.

The silica content of the lungs

These cases were either silicotic or suspected of being silicotic and the specimens were sent to us for confirmation or exclusion of this diagnosis. We have divided them into several groups according to the final diagnosis in each case (table I). Those in the first three

TABLE I

Silica content of lungs (percentage of dry weight)

Diagnosis of pulmonary lesion	Occupation	Age	Duration of exposure (years)	Silica content of dry lung	
				Range	Average
Simple silicosis	Coal miner	47	15	3·70	1·67*
	Abrasive soap worker	24	3	0·29	
	Gold miner (S. Australia)	55	Many	1·62	
	Coal miner (hard header)	53	,,	1·09	
Anthracosilicosis	Coal miner	...	...	1·37	1·41
	,, ,,	...	...	1·91	
	Fireman (coal mine)	49	...	1·53	
	Anthracite collier	39	...	1·40	
	Fireman (coal mine)	54	...	1·66	
	Coal screener	...	...	0·95	
	Collier (house coal)	48	33	1·22	
	Fireman (coal mine)	40	25	1·28	
	Anthracite collier	53	35	1·35	
Silicosis and tuberculosis	Coal miner	28	10	2·91	1·36
	Emery worker	64	25	1·09	
	Coal trimmer	...	...	0·37	
	Coal miner	20	3	0·53	
	,, ,,	...	...	0·48	
	Granite mason	...	...	1·05	
	Granite worker	...	...	3·09	
Slight silicosis	(Unrecorded)	...	...	0·82	1·19
	Roadway worker (coal mine)	65	50	2·53	
	Anthracite collier	60	44	1·06	
	,, ,,	60	42	0·94	
	Hard header (coal mine)	69	44	0·98	
	Coal miner	58	40	0·85	
Anthracosis	Anthracite collier	...	...	1·38	1·09
	Coal screener	...	...	1·30	
	Anthracite collier	...	...	0·90	
	Roadway worker (coal mine)	...	52	1·42	
	Anthracite collier †	...	58	1·18	
	Coal miner	...	...	0·39	
Tuberculosis	Sandblaster	38	2	0·27	0·50
	Silica brick grinder	38	2	0·92	
	Abrasive soap worker	23	3½	0·31	

* The silica content of normal lungs ranges up to 0·20 per cent.
† See fig. 1.

groups were eligible for compensation under the Silicosis Scheme. Of the others, 6 had slight (non-compensatable) silicosis, 6 had only anthracosis and 3 were simply tuberculous. Twenty-five of the

cases were drawn from the South Wales coal mines, the other 10 from various dusty occupations.

In table I are given the results for the different groups. The percentages of silica range from 0·27 to 3·7 (table I). The upper limit of normal is 0·20 per cent. among persons with no exposure to industrial dust. High silica percentages are seen in both the silicosis and non-silicosis groups, and almost as low values are encountered among the silicotics as among the non-silicotics. In the "slight silicosis" group the values are of the same order as those in the silicosis and anthracosilicosis groups.

Two conclusions emerge from this study. The presence of excess silica in the lungs does not necessarily imply the presence of silicotic nodules (fig. 1), and the severity of a silicotic condition bears no relation to the amount of silica present.

In a previous communication (Belt, Irwin and King, 1936) we put forward the view that a high concentration of siliceous matter in a tissue does not of necessity mean that silicotic lesions will develop. Similar conclusions have been reached by others, *e.g.* Fowweather (1934, 1939). But the view still prevails in many quarters that excess of silica in the tissues means silicosis. This belief cannot be accepted. The presence of high concentrations of silica in the tissues is compatible with a complete absence of the silicotic lesion. Only in about half the lungs of the present series has an excess of siliceous dust led to the formation of silicotic nodules.

Whether or not a siliceous dust will produce silicosis may depend, for one thing, on the nature of the siliceous particles. Silica occurs in nature in a great many mineral forms. Some of these minerals are thought, according to current belief, to produce silicosis, others are believed not to do so. The inhaled dust of all these minerals, however, will be reported simply as "silica" when chemical analyses are made of the lungs.

In our former communication we pointed out that, in the silicotic lungs we had examined, the extent of nodule formation did not appear to be conditioned by the concentration of silica. This fact is again evident in the present series. Sladden (1933) concluded, from a study of the silica content of coal miners' lungs, that there was a direct relation between the concentration of silica and the degree of fibrosis. It might be argued that the discrepancy between Sladden's results and ours could be due to his study having been confined to a single industry where there was no qualitative difference in the dust breathed by different men, whereas our cases are from different industries carrying qualitatively different dust hazards. But cases from the same occupations, with, presumably, exposure to similar dusts, are represented both in our silicosis groups and in the slight silicosis and anthracosis groups. Thus amongst the coal miners there are both colliers and hard headers,

some hom have little or no silicotic nodulation, while in others the lesions are severe; yet the amounts of silica in their lungs are very similar. In our cases, therefore, the degree of silicosis appears to be independent of the concentration of silica.

The silica content of the peribronchial lymph glands

These were taken at random from ordinary hospital cases which came to autopsy during 1935-38. The only factor which governed the selection was the age of the individual: we sought to have equal representation from each decade of life. We chose the root glands of the lung for investigation because, as reported previously, we had found these structures to represent natural reservoirs of silica in ordinary individuals.

Embryonic tissues contain only 0·01-0·02 per cent. of silica and the content of the lymph glands from still-born babies was not in excess of these very small concentrations. But even during the first decade of life there seems to be a considerable amount of silica breathed in and this appears to be transported promptly to the lymph nodes. The amounts of silica in these glands appear small, however, when contrasted with those for the glands of older people.

In table II the results for the 132 cases investigated are presented as averages of the figures obtained in the analyses of the glands from cases in each decade of life. A marked increase with age in

TABLE II

Silica content of peribronchial lymph nodes from 132 persons without history of exposure to siliceous dust

Age group	No. of cases in group	Percentage of SiO_2 in dry tissue (average)
stillborn	4	0·02
0-9	13	0·10
10-19	4	0·25
20-29	14	0·32
30-39	10	0·63
40-49	22	0·56
50-59	17	0·82
60-69	23	0·80
70-79	21	1·09
80-89	4	1·08

the silica content of the lymph glands is seen in those people who have breathed nothing but the ordinary air of house and street. There are large variations within each group (*cf.* table III), but on the whole there is evidence of accumulation of siliceous material in the root glands in the course of years. The lungs of these cases contained only normal amounts of silica: there is no parallelism between the concentrations of silica in lungs and lymph nodes

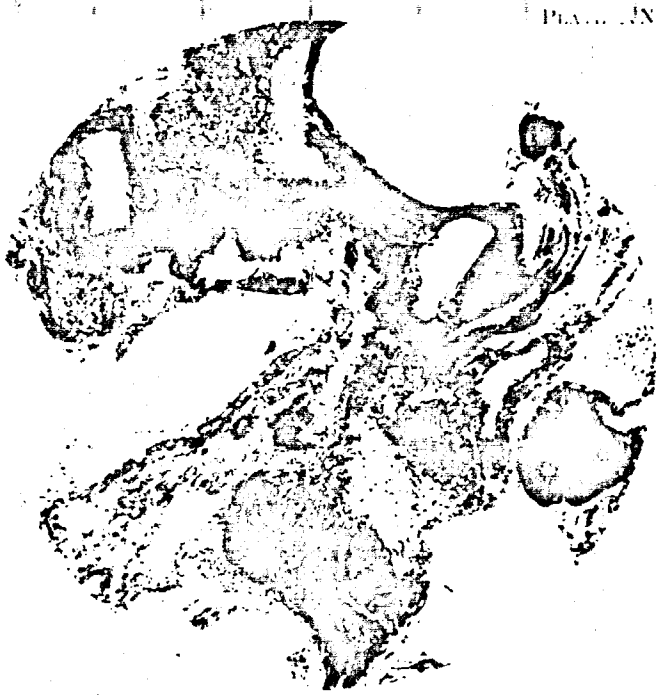

FIG. 1A

FIG. 1B

FIG. 1.—Dust-laden reticular tissue, chiefly around blood vessels, in the anthracotic lung of a Welsh anthracite miner showing heavy silica ash but no silicotic nodules. 1·19 per cent. of SiO_2 in dried lung.

A. Hæmatoxylin and eosin. B. HCl-insoluble ash (dark field). 25.

TABLE III

Silica content of peribronchial lymph nodes of normal persons aged 60-70 years

Occupation	Cause of death	Percentage of SiO_2 in dry tissue
General labourer	Pulmonary embolism	0·32
Clerk	Uræmia	2·20
Seaman	Myocardial degeneration	0·68
Housewife	Ovarian cyst	1·76
,,	Cerebral hæmorrhage	1·21
,,	Bronchopneumonia	0·23
Clerk : O.A.P.	Syphilitic aortitis	0·51
Taxi driver	Calculus, anuria	0·93
Housewife	Pulmonary embolism	0·98
Builder	Carcinoma of pancreas	0·62
Housewife	Carcinoma of colon	0·57
,,	Chronic bronchitis	0·20
Motor driver	Accident	0·62
Clerk	Angina pectoris	1·01
Housewife	Nephritis	0·53
,,	Carcinoma of peritoneum	0·83
Laundress	Cirrhosis of liver	1·28
Dry cleaner	Carcinoma of lung	1·39
Housewife	Cerebral hæmorrhage	0·69
Hairdresser	Abscess of liver	0·53
Labourer	Peptic ulcer	0·90
,,	Chronic bronchitis	0·54
Housewife	Lobar pneumonia	1·25
O.A.P.	Bronchiectasis and emphysema	0·68

(table IV). There is, of course, an increase in the silica content of the lungs during life ; but with people who have had no prolonged exposure to siliceous dust the final concentrations reached are never very high, even in cases where marked accumulations of silica are found in the lymph glands.

TABLE IV

Silica content of peribronchial lymph nodes arranged in order of silica content of lungs

Age	Occupation	Percentage of SiO_2 in	
		lungs	peribronchial lymph nodes
17	Boy	0·07	0·36
18	,,	0·08	0·25
47	Scene-shifter	0·09	0·23
47	Laundress	0·09	0·22
65	Housewife	0·10	1·29
72	Labourer	0·12	1·26
70	Housewife	0·14	1·76
79	,,	0·15	2·82
65	Labourer	0·16	0·32
45	Housewife	0·17	1·15
48	Laundress	0·18	0·31
70	Seaman	0·19	0·68
69	Clerk	0·20	1·15

The large amounts of siliceous material in these glands have not produced silicotic lesions, and that despite the fact that in many of the cases there was just as high a concentration of silica as in lungs which had well developed nodules. Fig. 2 shows sections of a non-silicotic lymph gland with a heavy siliceous deposit from a patient age 75. We may assume that much of this silica had been present for many years. Despite the very high concentration of silica (3·4 per cent.) there is no silicotic nodulation. The small amount of fibrous tissue which has developed is only sufficient to hold the dust in a loose enmeshment.

It has been shown elsewhere that the solubility of the different forms of silica in body fluids differs greatly (King and McGeorge, 1938). The siliceous deposits in the lymph nodes of normal persons may represent the accumulation of the most refractory—*i.e.* the most insoluble and hence, perhaps, the least harmful—of the siliceous particles inhaled. There seems to be no question but that the lung can handle small amounts of siliceous dust without damage. Some of the particles may be dissolved, completely or in part, and excreted. Others, too large or too refractory to be dissolved, are carried by phagocytes to the lymph nodes. There they are left, as it were in the deltas of the lymph channels, to constitute the " sedimentary deposits " of a lifetime's inhalation.

Further knowledge is needed regarding the silica deposits found in lungs and lymph nodes. It is necessary to know the nature of the siliceous matter as well as its total concentration. The determination of what siliceous minerals are present may furnish a basis for establishing a relationship between the production of fibrosis and the presence of mineral matter. Such an investigation is now in progress. With the help of mineralogical and X-ray diffraction pattern data it may be possible to determine which siliceous minerals have been relatively innocuous and which productive of silicotic nodules—further, whether or not the latter always produce the silicotic lesion.

Summary

Analyses for silica have been made in the lungs of 35 persons employed in dusty occupations and in the peribronchial lymph glands of 132 cases having no history of exposure to industrial dust.

In some cases the concentration of silica was as high in lungs showing no silicotic nodulation as in the silicotic group. No evidence was found of a direct association between the concentration of silica and the degree of fibrosis, though in all silicotic cases there was silica in excess of 0·2 per cent. of the dry weight.

There is an accumulation with age of silica in the root glands of normal persons. The heavy deposits of siliceous particles found in the glands of old people have not produced significant silicotic lesions.

SILICA CONTENT OF TISSUES

FIG. 2A

FIG. 2B

FIG. 2.—Heavy silica ash in peribronchial lymph gland without silicotic nodules.
A. Hæmatoxylin and eosin. B. HCl-insoluble ash (dark field). 15.

We wish to thank Dr C. V. Harrison for his kind assistance in collecting the lymphatic glands and Dr P. D'Arcy Hart for his helpful co-operation in securing for us several of the lung specimens. We are indebted to the Medical Research Council for a grant covering part of the expenses of this investigation. The research was also aided by a grant from the research fund of the University of London for the purchase of apparatus and materials.

REFERENCES

BELT, T. H., IRWIN, D. A., AND KING, E. J. 1936. *Canadian Med. Assoc. J.*, xxxiv, 125.
FOWWEATHER, F. S. . . . 1934. *Chem. and Ind.*, xii, 713.
" " . . . 1939. *Analyst*, lxiv, 779.
KING, E. J., AND MCGEORGE, M. 1938. *Biochem. J.*, xxxii, 417.
SLADDEN, A. F. . . . . 1933. *Lancet*, ii, 123.

a given specification was being further examined by The British Drug Houses Ltd. In reply to Mr. Spald-'s query about fines, Mr. Lord stated that the removal of fines (by which was meant material passing a o. 200, and preferably a No. 170, B.S. sieve) was essential to the proper working of the method.

Experience so far had shown that the chances of a determination being uncertain or invalid through e use of unsatisfactory bone meal were less than they were through the use of improperly activated umina or unstable alkaline alumina, which had been found to be unreliable after 3 or 4 days, even in aled ampoules. This experience was consistent with Mr. Taylor's remarks concerning the method of ecking the preparation of the aluminas. Despite rigidly defined conditions of preparation and activation, seemed that the final test of their behaviour was a purely empirical one.

In regard to Mr. Taylor's third point, Mr. Lord remarked that at the present time both methods tended give high results, and, although the need for a correction was indeed unfortunate, the choice lay between e modified method, which appeared to be amenable to such a correction, and the Official method, which d not. Both methods had been applied to many samples representative of six different brands of argarine and, from the results given, they could be seen to be in close agreement.

The Colorimetric Determination of Silicon in the Micro-analysis of Biological Material and Mineral Dusts

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and

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The basic ferric acetate precipitation procedure used for removing phosphate from solutions in which silica is to be determined has been critically investigated. It has been found that unless strict control of the pH is observed the method may give inaccurate results, owing to (*a*) incomplete removal of phosphate or (*b*) removal of silica with phosphate.

Previous work has been confirmed that both phosphate and silica couple with molybdate in weak acid, but that only the silicomolybdate complex is reduced in strong acid. On this principle a new procedure for determining silica in biological material has been developed, in which phosphate is left in solution.

Iron, in amounts greater than those occurring in tissue, has been found not to interfere.

In urines with high phosphate concentrations two methods have been evolved to overcome interference by precipitation of ammonium phosphomolybdate. In one, phosphate is partly or completely removed by precipitation with calcium hydroxide; in the other, ammonia and urea are removed with nitrous acid and sodium molybdate is used to couple with silica.

The new colorimetric procedure gives good agreement with gravimetric analyses, and good recoveries of added silica have been obtained with tissue, blood and urine. The procedure likewise gives results in good agreement with gravimetric methods, when applied to small samples of mineral dusts.

Silicon may be determined chemically by gravimetric, titrimetric and colorimetric procedures. All methods depend on the decomposition of siliceous material to liberate silicic acid. In the gravimetric method silicic acid is subsequently dehydrated to the oxide of silicon, and the silica, SiO_2, is isolated and weighed. The silica so isolated often contains small amounts of metallic salts, and for this reason it is usual to treat the impure silica with hydrofluoric acid, which completely volatilises the silicon as silicon tetrafluoride. The gravimetric procedure is described in most textbooks of quantitative analysis, *e.g.*, Hillebrand,[1] Treadwell and Hall[2] and Scott.[3]

Gravimetric procedures as ordinarily performed are not sufficiently sensitive for the accurate determination of less than 5 mg of silica. Micro-gravimetric procedures based on similar chemical principles to the above, but with the apparatus and techniques of Emich[4] and Pregl,[5] have been described by Morgan and King,[6] Gerstel[7] and Holt.[8]

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When soluble silica (silicic acid) and ammonium molybdate are present together in acid solution, silicomolybdic acid, which contains 1 molecule of silica to 12 molecules of MoO_3 (Parmentier[9]; Asch[10]; Copaux[11]; Pechard[12]; Miolati[13]), is formed. This acid forms insoluble compounds with organic bases such as brucine, coniine, pyridine, pyramidon, quinine, quinoline and strychnine. In the case of pyramidon and silicomolybdate 3 molecules of pyramidon are combined with 1 of silicomolybdic acid. King and Watson[14] described a method in which a weighable precipitate could be obtained in terms of this heavy salt of pyramidon silicomolybdate when the amounts of silicon were too small to determine in the micro-gravimetric method. Silicomolybdate can also be determined gravimetrically (or volumetrically) as an oxine - silicomolybdic acid complex (Volinetz[15]; Merz[16]), a hexamethylenetetramine complex (Duval[17]) and a 2:4-dimethylquinoline complex (Miller and Chalmers).[18]

A titrimetric method for determining silicon depends on the reaction (Tananaev and Babko[19]) —

$$SiO_2 + 6NaF + 4HCl \rightarrow Na_2SiF_6 + 4NaCl + 2H_2O.$$

The method is rapid and has been extensively used, *e.g.*, by Korol and Koluzhskaya[20] and by Kern and Jones[21]; although it is accurate with fairly large amounts of silica, it is not in our experience trustworthy as a micro method. Another procedure involves the alkalimetric finish in which a quinoline - silicomolybdate complex is used (Wilson[22]).

Colorimetric methods form the basis of all micro-analytical procedures for silicon, when it is present in quantities of less than 1 mg. These procedures depend on the formation of silicomolybdic acid, $SiO_2.12MoO_3$, a method first advocated by Jolles and Neuratl,[23] and later by Schreiner[24] and Lincoln and Barker.[25] This yellow compound, which was originally given the formula $H_8Si(MoO_7)_6.H_2O$ and later assigned the formula $H_4Si(Mo_3O_{10})_4$ by Souchay,[26] can be estimated colorimetrically. The well-known Dienert and Wandenbulcke[27] procedure is based on the formation of this highly coloured yellow silicomolybdic acid (see also Winkler[28]; Thresh[29]; Atkins[30]; Steffens[31]; Thayer[32]; Thompson and Houlton[33]; Liebknecht, Gerb and Bauer,[34] who investigated natural waters; and King,[35] who used it for determining the silica content of tissues).

A blue-colour method can be based on the reduction of the yellow silicomolybdic acid complex, and this has been made the basis of other colorimetric procedures. Procedures were described by Isaacs,[36] who used sodium sulphite and heat to effect the reduction; by Oberhauser and Schormüller[37] and Woods and Mellon,[38] who used stannous chloride; by Nemec, Lanik and Koppova,[39] who used sodium thiosulphate; by King,[40] Roman,[41] Bodnár and Török[42] and Gettler and Umberger,[43] who used quinol; by Berg and Teitelbaum,[44] who used pyrrole; by Parri and Scotti[45] and Rodillon,[46] who used hydroxylamine; by King and Stantial,[47] King,[48] Straub and Grabowski,[49] Olsen, Gee, McLendon and Blue[50] and Buntings[51] who used aminonaphtholsulphonic acid; and by Gentry and Sherrington,[52] who used ferrous sulphate. The blue colour developed from a given amount of silica is more intense than the corresponding yellow colour (Roman[41]; De Eds and Eddy[53]).

The silicomolybdic acid reaction, however, is not a simple one (Armand and Berthouy[54]). The intensity of both the silicomolybdate yellow colour and the molybdenum blue reduced silicomolybdate colour depend on the acid as well as the molybdate concentration. Optimum conditions for colour development have been extensively studied by several workers, e.g. King and Stantial.[47] Two forms of silicomolybdic acid (α and β) were recognised by Strickland,[55] both having the same empirical formula but different light-absorption properties. The form produced depends mainly on the degree of acidification of the molybdate. For analytical purposes it is desirable to ensure that only the β-complex is formed; this requires between 3 and 5 equivalents of acid per gram-ion of molybdate.

Silica, moreover, must be brought into a reproducible molecular state before the silicomolybdic acid reaction can be applied. Dienert and Wandenbulcke[27] distinguished between soluble silica, which reacts, and colloidal silica, which does not react with molybdate. Weitz, Francke and Schuchard[56] demonstrated that the degree of polymerisation of the silicic acid affects the rate of its reaction with molybdate; monomers react completely in 75 seconds, dimers in about 10 minutes and higher polymers take much longer. The study was extended by Alexander.[57] Alkali treatment has a depolymerising action, but it is not always reproducible (Adams[58]). Fusion with sodium carbonate is more reliable.

The reaction is also complicated by various interfering substances, of which phosphate and iron are most important. Interference can be avoided by first isolating the silica which

is rendered insoluble by evaporation and dehydration, as in the gravimetric procedure, when it may then be fused with sodium carbonate and re-dissolved (compare King and Stantial[47]; Holzapfel[59,60]; Ohlmeyer and Olpp[61]).

Interference by phosphate can be avoided or minimised by carrying out the reaction in acetic acid (Isaacs[58]; Brabson, Harvey, Maxwell and Schaeffer[62]), when phosphomolybdic acid formation is inhibited; or the phosphomolybdic acid may be destroyed by other organic acids, *e.g.*, tartaric (Bunting[51]) and oxalic (see Schwartz[63] for a summary of these procedures; and Straub and Grabowski[49]), or by mineral acid (Kahler[64]; Harrison and Storr[65]; Milton[66,67]).

Interference by iron, which produces a green tint in the molybdenum-blue procedures, was compensated for by Isaacs,[58] but the results are not easily reproducible (King[35]). Thayer[72] found the influence of iron and of phosphate to be complicated and difficult to eliminate. Various complexes are produced (Arnfeld[68]; Wu[69]).

Atkins and Wilson,[70] King[35,68] and King and Stantial[47] thought it advisable to remove both phosphate and iron before applying the molybdate reaction. But this is not so easily done as might at first appear. Phosphate may be precipitated by magnesium, calcium and iron, in the presence of ammonia, but in the presence of iron variable amounts of ferric silicate may be precipitated with the phosphate (Thayer[32]; King[71]). A two-stage precipitation of iron by added phosphate at an acid pH, and of phosphate by calcium chloride with ammonia, was used by King and Stantial,[47] and King[48] used the basic ferric acetate procedure of Jacobs[72] for the simultaneous elimination of phosphate and iron, phosphate being removed as ferric phosphate and excess of iron as basic ferric acetate, together with any colloidal material, such as protein, fat, pigment and debris (*e.g.*, in urine). The remaining chloride has an almost negligible effect on the colour intensity (Robinson and Spoor[73]).

Although Strickland[55] has shown that the silicomolybdic acid complex can exist in two spectrographically dissimilar forms, nevertheless it is easy to obtain reproducible results with simple silicic acid solutions under standardised conditions. It is in the elimination of the interference by phosphate and iron that uncertainty may enter. Since certain materials to be analysed (*e.g.*, water; mineral dust) may contain silicon and neither phosphate nor iron, others phosphate but no iron (*e.g.*, urine) and others (*e.g.*, blood) much iron but less phosphate, it is apparent that any procedure must be varied to suit the material being analysed. In using the ferric acetate precipitation method with animal tissues to rid them of phosphate and iron, before applying the colorimetric method for silica, we have experienced occasional uncertainty as to whether we were eliminating all the phosphate or iron or both and not losing silica, and have at times encountered inaccuracies. Attention was therefore directed not to the stage of colour development in the phosphate-free solution but rather to a re-investigation of the precipitation procedure, to determine whether errors could be introduced by the incomplete removal of phosphate from the solution or by the co-precipitation of silica with the ferric phosphate and ferric hydroxide. There is no colorimetric method alternative to the molybdate procedure for the determination of very small amounts of silica. In this procedure, phosphate and silicate give similar colours, and it is possible for losses of silicate to be obscured by the retention of phosphate in the filtrate. To examine this point some of our experiments were carried out with labelled silicon and some with labelled phosphate.

As a result of this re-investigation, we have concluded that phosphate and iron can be successfully eliminated and silica completely recovered with the basic ferric acetate method; but that this can only be achieved by very careful attention to the details of the method and particularly to concentrations of reagents and pH. By taking advantage of an observation by Milton[66,67] that both silicate and phosphate couple with molybdate in weak acid solution, but that only silicomolybdate is reduced to the blue complex if the solution is first made strongly acid, we have been able to simplify the procedure greatly and to get rid of the troublesome precipitation of phosphate and iron. Likewise, amounts of iron likely to be present in animal tissues do not interfere. This paper presents the results of our critical study of the ferric acetate precipitation method, and of the development and testing of the new modified colorimetric procedure.

Experimental

Previous blue colour method—

An amount of the filtrate that may be expected to contain from 0·02 to 0·12 mg of silica is transferred to a 25-ml calibrated flask. Then 2, 5 and 10-ml portions of the weak standard

(equivalent to 0·02, 0·05 and 0·1 mg of silica) are put by pipette into similar flasks. Water is added to about 20 ml, and then 2·0 ml of acid ammonium molybdate and 0·5 ml of reducing agent. The flasks are shaken, and kept for 10 minutes for the colours to develop. Then 1 ml of 10 *N* sulphuric acid is added to each flask to stabilise the blue colours, the volumes are adjusted to 25 ml and the contents of the flasks thoroughly mixed. Comparison of the test is then made against the appropriate standard.

INVESTIGATION OF THE BASIC FERRIC ACETATE PROCEDURE FOR REMOVING PHOSPHATE AND IRON—

Adsorption of silica on the ferric hydroxide and phosphate precipitate—To 5 ml of standard silicate solution (0·142 mg of SiO_2 per ml) were added 20 ml of the ferric chloride reagent and 20 ml of sodium acetate reagent. The pH was adjusted to one of the several values given in Fig. 1 by the addition of sodium hydroxide to the acetate reagent. The solution was diluted to 100 ml, boiled, filtered and cooled. Part of the filtrate (5 ml) was transferred to a calibrated flask and the blue colour was developed by the above method. The optical densities of the solutions were determined photo-electrically.

In Fig. 1, curve A, the amount of silica lost with the precipitate is plotted against the

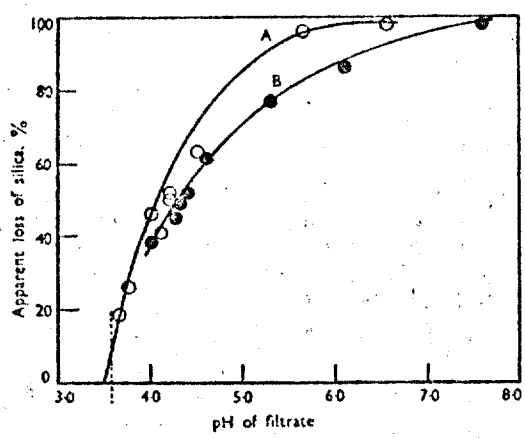

Fig. 1. Loss of silica by co-precipitation at different pH values: curve A, colorimetric measurements; curve B, radiometric measurements

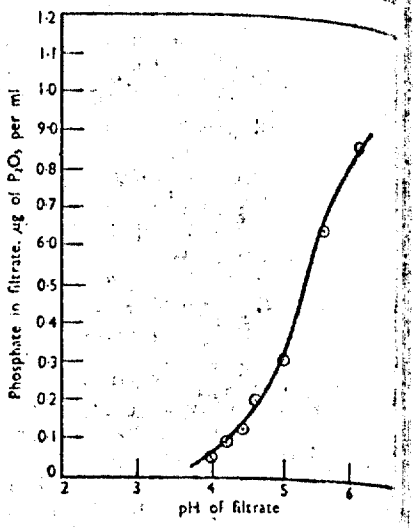

Fig. 2. Retention of phosphate by the filtrate at different pH values

pH of the solution. At pH 3·5, apparently, no silica is lost from the filtrate, but there is loss with increasing pH and at pH 5·5 practically all of the silica is retained by the precipitate. In fact, at pH 3·5 the recovery value is apparently slightly greater than the amount of silica added.

The experiment was repeated with ^{31}Si silicate. In this case the ferric phosphate ferric hydroxide precipitate was rapidly washed to remove contaminating solution, dissolved in dilute hydrochloric acid and assayed with a Geiger counter.

The curve showing the silica loss at different pH values (Fig. 1, curve B) follows that obtained by the colorimetric method. But the values are slightly lower. Having regard to an apparent small gain in silica at low pH values shown colorimetrically, it appears that some other substance present in the reagent may give a slight blue colour with the molybdate reagents.

Efficiency of the procedure for phosphate precipitation—To each of several 10-ml samples of sodium silicate solution (1 mg of SiO_2) were added 10 ml of a solution of $NH_4H_2PO_4$ (24 mg of $^{32}P_2O_5$; specific activity 2·5 microcuries per ml). In each case the pH of the mixed solution was brought to one of the values shown in Fig. 2 by adding sodium hydroxide to the acetate reagent. The ferric chloride reagent (20 ml) and sodium acetate reagent (20 ml)

re added. The solutions were diluted to 100 ml, boiled, cooled and filtered. The filtrates re assayed with a Geiger counter.

The phosphate concentrations of the filtrates decreased with decreasing pH down to 6 μg of P_2O_5 per ml in a filtrate of pH 3·9 (Fig. 2). At a lower pH it is impossible to cipitate the ferric hydroxide completely.

Effect of phosphate concentration on the pH of the filtrate—To each of several 5-ml portions silicate solution (0·5 mg of SiO_2) was added one of the volumes of phosphate shown in le I and the solutions were neutralised to Congo red with dilute sulphuric acid. The ml portions of the ferric chloride and sodium acetate reagents were added to each. The ture was boiled, cooled and filtered. The pH of each filtrate was measured.

The pH of the several filtrates is shown in Table I. Although the pH is but little affected small quantities of phosphate, larger amounts have an appreciable effect; 30 mg of osphate may alter the pH by 0·3 units.

Buffering power of the acetate reagent—The acetate reagent was made up with a number different concentrations of sodium hydroxide. The pH of each solution was determined using a glass electrode.

At the concentration used, the acetate is operative as a buffer between pH 3·5 and 5·5 g. 3), but even in this range small changes in the amount of alkali added produce appreciable nges in pH.

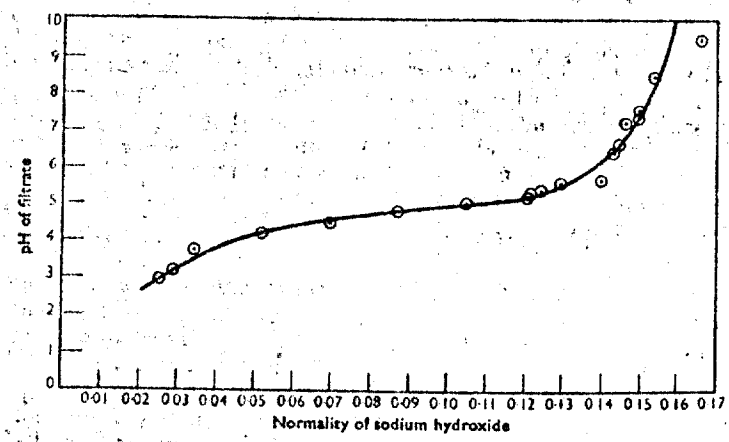

Fig. 3. Efficiency of the acetate buffer reagent

Discussion—The method of King[48] for the determination of silica in biological materials es on the removal of phosphate, which interferes with the colorimetric determination of ica, as insoluble ferric phosphate in the so-called "basic ferric acetate precipitation." cess of ferric chloride reagent, which also has a slight effect on the reduced molybdate our, is hydrolysed to insoluble ferric hydroxide. The investigation described in this per shows that phosphate is almost completely removed from the solution by this procedure the pH is less than 3·9, and that silicate remains mainly in solution. It is equally apparent at, if the pH is even slightly higher than 3·9, phosphate appears in the filtrate and, at the me time, silica is co-precipitated with the ferric phosphate and hydroxide.

The efficiency of this method of analysis depends, therefore, on careful control of the H. If substances that affect the pH of the solution are absent, the buffer used in the rocedure will give a pH of 3·6 (indicated by the dotted line in Fig. 1). At this pH the loss silica is small, but some iron remains in the filtrate as a ferric hydroxide sol. In the sence of other substances that affect the pH, it appears that the procedure as described ll give satisfactory silica recoveries.

The sodium acetate used to buffer the solution may allow some variation in pH when mall quantities of acid or alkali are added as may be seen from Fig. 3, in which is shown e effect on the pH of the filtrate of changing the sodium hydroxide concentration of the dium acetate reagent, and in most biological materials substances that act as buffers are resent. Because of this, it is possible for the buffering action of sodium acetate to be adequate for accurate pH control. Even the precipitation of phosphate may have some

effect on the pH. Whilst small amounts of phosphate have little effect, 30 mg of phosphate, a quantity that may be present in 10 ml of urine, may alter the pH by 0·3 units. This pH shift is sufficient to cause a considerable error in the silica determination. The molybdate reagent is sensitive to many anions and this fact rules out the use of most of the usual buffer solutions. It is apparent that without careful adjustment of the pH to within 0·1 unit, which is difficult and probably impracticable with large numbers of samples, erroneous results may occur.

NEW METHOD—

In an investigation of the reduced molybdenum-blue method for silica, Milton[44] studied the formation of silicomolybdate and phosphomolybdate, and their reduction. Several workers (*e.g.*, Dienert and Wandenbulcke[27]; Armand and Berthoux[54]) showed that silicomolybdic acid will form only in weakly acid solution and that it is quite stable in strong acid. Phosphomolybdic acid, on the other hand, will form over a wide range of acidity and is stable in strongly acid solution. But although phosphomolybdic acid forms over a wide range of acidity, it will not reduce to phosphomolybdenum blue in strong acid. This is in contrast to silicomolybdic acid, which can be reduced even at high acidities. Milton found that silicomolybdate forms in 0·1 *N* sulphuric acid, and that if the acidity is then raised to 2 *N* in sulphuric acid it will reduce to silicomolybdenum blue without any phosphomolybdenum blue being formed, even when considerable amounts of phosphate are present. There is no reduction of phosphomolybdate, and therefore no increased colour due to the presence of phosphate, if the solution is more than 1·5 *N* in sulphuric acid.

We have confirmed that silicate and phosphate both couple with molybdate in weak acid, but that only silicomolybdate is reduced if the solution is first made strongly acid. And we have likewise shown that small amounts of iron also do not interfere. This makes possible to determine silica in the presence of phosphate and iron, with greater accuracy and without the chance of loss or interference, which exists when it is necessary to remove these substances by precipitation procedures.

Development of blue colour at various acidities—

(*a*) *Silicate*—Five-millilitre portions of dilute silica standard (0·05 mg of silica) were diluted to 15 ml with water in 25-ml flasks. Then 2·0 ml of 5 per cent. ammonium molybdate in *N* sulphuric acid were added, mixed, and left for 10 minutes. Various amounts of 10 *N* sulphuric acid were added, mixed, and 0·5 ml of aminonaphtholsulphonic acid reducing agent. The solutions were made to volume, mixed, and the intensities of the colours measured in a Spekker absorptiometer after 10 minutes. Fig. 4 illustrates the results. The final acidity in the absence of any additional sulphuric acid is 0·08 *N* (2 ml of *N* acid diluted to 25 ml), and this mixture had slightly more colour than was obtained when extra sulphuric acid was added. But between 1 and 10 ml of added 10 *N* sulphuric acid, the intensity of colour was constant, showing that silicomolybdate blue is unaffected by acidity within the limits 0·5 to 4 *N*.

(*b*) *Phosphate*—One-millilitre portions of standard potassium dihydrogen phosphate solution (1·0 mg of phosphorus) were similarly treated. This amount of phosphate gave a very intense blue colour when the final acidity was kept at or below *N*. (The acidity usually maintained in phosphorus methods is 0·5 *N* to *N*.) Above this the phosphomolybdate blue colour decreased sharply, until at 1·5 *N* there was almost no colour, and at 2 *N* (5 ml of 10 *N* sulphuric acid) only the faintest tinge of blue, possibly due to a trace of silica, could be detected (Fig. 4). In another experiment larger amounts of phosphate, up to 5 mg of phosphorus, failed to give any appreciable colour.

(*c*) *Silicate and phosphate*—Five millilitres of silica standard (0·05 mg of silica) and 1 ml of phosphate (1·0 mg of phosphorus) were diluted to 15 ml, and similarly treated. With increasing acidity up to 1·5 *N*, the colour of the mixed solution was dominated by that contributed by the phosphate. Above 1·5 *N* it gave colours similar to the silicate solution with no phosphate. At 2 *N* sulphuric acid (5 ml extra of 10 *N*) and above, phosphate appeared to contribute no colour and does not interfere with the silicomolybdate blue (Fig. 4).

Under the conditions adopted for the test (volume of 25 ml, 2·0 ml of 5 per cent. ammonium molybdate and 5 ml of 10 *N* sulphuric acid), however, there is a limit to the amount of phosphate that can be tolerated. If phosphorus is present in an amount greater

than 1·2 mg of phosphorus with ammonium molybdate, or 1·3 mg with sodium molybdate, interference will occur (see Fig. 5). The intensity of the silicomolybdate blue colour decreases with increasing phosphorus concentration above this level, presumably owing to phosphate monopolising more and more molybdate, leaving insufficient for the formation of silicomolybdate. An attempt to counteract this was made by doubling the amount of molybdate.

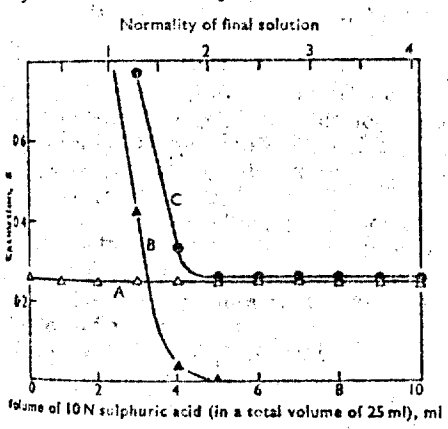

Fig. 4. Effect of acidity on the molybdenum blue colour formed by silica and phosphate: curve A, 0·05 mg of silica alone; curve B, 1·0 mg of phosphorus alone; curve C, 0·05 mg of silica plus 1·0 mg of phosphorus

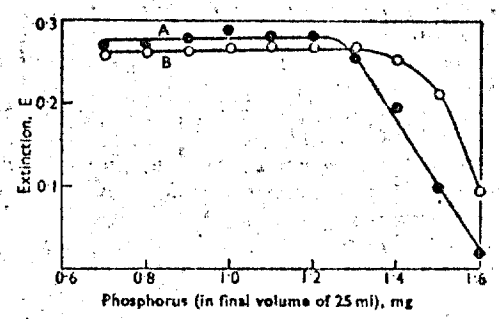

Fig. 5. Effect of phosphate on the molybdenum blue colour formed by silica: curve A, with 5 per cent. ammonium molybdate; curve B, with 7 per cent. sodium molybdate

although higher levels of phosphate could then be tolerated, a marked colour progression was produced and there was a pronounced deviation from Beer's law.

In the molybdic acid complexes ratios of 12 molybdate to 1 phosphate or 1 silica have been found, *i.e.*—

$$\frac{MoO_3}{PO_4} = \frac{12}{1} \text{ (Schwartz[74])} \text{ and } \frac{MoO_3}{SiO_2} = \frac{12}{1} \text{ (Snell and Snell[75])},$$

and it has been claimed that only a slight excess of molybdate is necessary for complete reaction. This does not appear to conform with the fact that 1·5 mg of phosphorus (≡4·5 mg of phosphate) interferes with the silicomolybdate formation even though excess (84 mg) of molybdate (MoO_3) is present. This may be due however to a change in the nature of the phosphomolybdate complex at these relatively high levels of phosphorus, which could alter the MoO_3 to PO_4''' ratio. Certainly the complex shows marked visible change with increasing phosphorus concentrations, changing from intense yellow at 1·2 mg to colourless at 2·0 mg of phosphorus.

Effect of iron—Small amounts of iron (up to 1 mg) (Fig. 6) have no effect on the silicomolybdate colour, either in the absence or presence of phosphate. This is more iron than is present in any sample of animal tissue that is likely to be taken for analysis, *i.e.*, it is more than is contained in 1 ml of blood, the highest iron-containing tissue. Phosphate is always in excess in animal tissue, *e.g.*, there are 40 to 50 mg of phosphorus (atomic weight 31) and 40 to 55 mg of iron (atomic weight 56) in blood (per 100 ml), and iron is unlikely to cause interference when such material is being analysed.

METHOD

REAGENTS—

All water used must be distilled and silica-free.

Silica standard: sodium silicofluoride—This salt is a light anhydrous non-hygroscopic powder that is hydrolysed in solution to give silicic acid. Hopkin and Williams' sodium metasilicate (pure) and Schering - Kahlbaum's natrium silicofluoride are satisfactory. Other commercial preparations may be only partly soluble in water. A solution of 314 mg of the salt in 1 litre of water gives a concentration equivalent to 1 mg of silica in 10 ml (strong

standard). A weak standard (0·1 mg of silica per 10 ml) is made by diluting the strong standard 1 in 10. It has been found advisable to make fresh solutions each week.

Silicate solutions—Standard solutions were prepared by fusing pure silica, sometimes after irradiation in the Harwell pile (BEPO), with sodium carbonate (Holt and Yates[7]). Solutions are preserved in wax or polythene bottles.

Sulphuric acid, 10 N—278 ml of concentrated acid diluted to 1 litre with water.

Sulphuric acid, N—A 1 in 10 dilution of the 10 *N* acid.

Sodium hydroxide, N—The sodium hydroxide, to be silica-free, must be prepared from the metal. Hence 2·3 g of metallic sodium are dissolved in 100 ml of water in a nickel crucible, and the solution is preserved in a wax or polythene bottle. Sodium hydroxide, pure (from sodium) as supplied by the British Drug Houses Ltd. is satisfactory. Glass pipettes should be brought into the sodium hydroxide solution only for the briefest possible times.

Phenolphthalein indicator—A 0·5 per cent. solution in ethanol.

Congo red indicator—A 0·1 per cent. solution in ethanol.

Sodium carbonate—AnalaR, and silica-free.

Acid ammonium molybdate—A 5 per cent. solution of ammonium molybdate in *N* sulphuric acid.

Acid sodium molybdate—A 7 per cent. solution of sodium molybdate in *N* sulphuric acid.

Reducing agent—A 0·2 per cent. solution of 1:2:4-aminonaphtholsulphonic acid in a solution of 2·4 per cent. of sodium sulphite, $Na_2SO_3.7H_2O$, and 12 per cent. of sodium metabisulphite in water.

Ammonium phosphate—A 2 per cent. solution of ammonium dihydrogen phosphate.

Standard phosphate solutions—Standard solutions of inactive phosphate were made from ammonium dihydrogen phosphate. These solutions were labelled by adding small quantities of $H_3{}^{32}PO_4$ (specific activity 2·0 millicuries per ml) to give a specific activity of about 2 microcuries per ml.

Potassium phosphate solution, 1 mg of phosphorus per ml—0·4388 g of AnalaR silica-free potassium dihydrogen phosphate dissolved in 100 ml of water.

Calcium chloride—A 0·5 per cent. solution of AnalaR calcium chloride, $CaCl_2.6H_2O$.

Calcium hydroxide—A saturated solution of the AnalaR solid, preserved in a wax or polythene bottle.

Sodium nitrite—A 5 per cent. solution of AnalaR sodium nitrite in water.

Trichloroacetic acid—A 20 per cent. solution in water.

Active carbon—Kahlbaum's Carbo Activ, or Neo Spectro M II, Columbian Carbon Co., Brooklyn 32, N.Y.

Ferric chloride in hydrochloric acid—A 1 per cent. solution of ferric chloride, $FeCl_3.6H_2O$, in 0·02 *N* hydrochloric acid.

Sodium acetate in sodium hydroxide—A 1·5 per cent. solution of sodium acetate, $C_2H_3O_2Na.3H_2O$, in 0·028 *N* sodium hydroxide.

Acetic acid—Glacial and 10 per cent.

TISSUE ANALYSIS—

Depending on the silica content, mix 0·05 to 0·5 g of dried powdered tissue with six times its weight of sodium carbonate in a 25 to 50-ml platinum crucible. Carefully ignite to remove organic matter and fuse to a clear melt (1 to 2 hours). Dissolve the melt in water by warming, cool, neutralise to Congo red with 10 *N* sulphuric acid and add a 0·2-ml excess of acid. Transfer to a calibrated flask and dilute to 250 ml (or to 100 ml if the tissue silica be very low). Transfer an amount of the solution containing 0·02 to 0·01 mg of silica to a 25-ml calibrated flask. To similar flasks add 2, 5 and 10-ml amounts (corresponding to 0·02, 0·05 and 0·10 mg of silica) of the weak standard and prepare a blank of water. Add water to about 15 ml and then 2·0 ml of 5 per cent. ammonium molybdate in *N* sulphuric acid. After 10 minutes add 5 ml of 10 *N* sulphuric acid and then 0·5 ml of aminonaphtholsulphonic acid reducing agent. Make the solutions to volume, mix, and measure the intensity of the colours after 10 minutes.

METHODS FOR URINE—

Some urines may not be amenable to the colorimetric silica determination by the alkali silicomolybdate method. With rat urine, precipitation may occur following the addition

of the acid molybdate reagent. To obviate this difficulty modifications of the method have been investigated.

In determining silica directly by the addition of acid molybdate to diluted urine, various factors may interfere. Phosphorus forms phosphomolybdate complexes, and ammonia in the presence of phosphorus may form a precipitate of ammonium phosphomolybdate, an interference that can be overcome by the initial removal of ammonia and urea with nitrous acid (sodium molybdate being used subsequently to develop the colour), or by removal of all or part of the phosphate. Proteins and organic bases may form precipitates or turbid solutions. This can be effectively prevented by treating the urine with trichloroacetic acid and finely divided carbon.

(*a*) *By removal of phosphate*—In a Lusteroid or plastic test tube treat 0·3 ml of rat (or other herbivore) urine high in silica, or 3 ml of human (or carnivore) urine low in silica with 10 ml of 0·5 per cent. calcium chloride solution and saturated calcium hydroxide solution until pink to phenolphthalein and dilute with water to 15 ml. Add about 50 mg of carbon (Neo Spectro M II or Kahlbaum's Carbo Activ), shake the mixture, warm at 70° C for 15 minutes, cool and filter through a Whatman No. 42 filter-paper.

In 25-ml calibrated flasks place 10-ml portions of the filtrate (≡0·2 or 2 ml of urine), and 0·02, 0·05 and 0·10 mg of silica (2, 5 and 10 ml of weak standard). Add water to about 15 ml, and then 2·0 ml of 5 per cent. ammonium molybdate in *N* sulphuric acid. Mix, and after 10 minutes add 5 ml of 10 *N* sulphuric acid. After mixing, add 0·5 ml of aminonaphthol-sulphonic acid reducing agent. Make the solutions to volume, mix, and measure the intensities of the colours after 10 minutes.

(*b*) *By removal of ammonia (and urea)*—Shake 2 ml of urine with 1 ml of 5 per cent. sodium nitrite solution, 2 ml of 20 per cent. trichloroacetic acid, 50 mg of carbon and 1 drop of caprylic alcohol for 5 minutes and filter. Transfer, for rat urine, 0·5 ml of filtrate (≡0·2 ml of urine), and for human urine 3 or 4 ml of filtrate (≡1·2 or 1·6 ml of urine), to a 25-ml calibrated flask and prepare standards as above. Add water to about 15 ml and 2·0 ml of 7 per cent. sodium molybdate in *N* sulphuric acid. After 10 minutes add 5 ml of 10 *N* sulphuric acid, mix and then add 0·5 ml of reducing agent. Make the solutions to volume and measure the intensities of the colours as before.

Blood analysis: soluble silica—

After deproteinising whole blood, serum or plasma with trichloroacetic acid, silica can be determined in the filtrate by the silicomolybdate method. The sample of the filtrate is heated in a boiling-water bath, before treatment with ammonium molybdate, to destroy most of the trichloroacetic acid, and so allow the silicomolybdate complex to form at the optimum pH.

Procedure—Mix 2 ml of whole blood, serum or plasma with 20 ml of 5 per cent. trichloroacetic acid and filter after 5 minutes. Transfer 15 ml of the filtrate to a 25-ml calibrated flask and heat for 15 minutes in a boiling-water bath. Cautiously shake the contents of the flask while hot to expel carbon dioxide. After cooling, add ammonium molybdate, 10 *N* sulphuric acid and aminonaphtholsulphonic acid as described above. Prepare a 0·02-mg silica standard.

Determination of silica in mineral dusts—

The total silica content of mineral dusts may be rapidly determined by the colorimetric silicomolybdate procedure. The method, although certainly not as accurate as the gravimetric, has been found to give acceptable results when samples of dust containing about 5 mg of silica are fused with 1 g of sodium carbonate. The fused melt is taken up in water, added rapidly to a known excess of acid, and shaken vigorously to expel carbon dioxide and dissolve any remaining particles of the melt. This rapid neutralisation, by preventing polymerisation of silicic acid (Holt and Osborne[77]), ensures complete reaction on subsequent treatment with ammonium molybdate. The tendency of iron to form insoluble fusion products can usually be overcome by initially roasting the sample in the platinum crucible and then carrying out the sodium carbonate fusion in an oxidising atmosphere (Hillebrand[1]).

Procedure—In a platinum crucible fuse with sodium carbonate (1 g) for 1 hour an amount of the dust containing about 5 mg of silica. Add water (20 ml) and warm the crucible for 30 minutes on a boiling-water bath, disintegrating the melt during this time with a plastic

stirring rod. Rapidly wash the contents of the crucible with water into a 250-ml calibrated flask containing 19 ml of *N* sulphuric acid (roughly 1 milli-equivalent of acid in excess of that required for the neutralisation of the sodium carbonate). Vigorously shake the flask until solution of the last particles is complete. Add water to the mark and mix the solution.

Transfer a sample of the solution containing about 0·05 mg of silica to a 25-ml calibrated flask and add water to 15 ml. Add 2·0 ml of acid ammonium molybdate, 5 ml of 10 *N* sulphuric acid and 0·5 ml of aminonaphtholsulphonic acid as described in the method for tissue analysis. In a photo-electric absorptiometer compare the intensity of the colour with that obtained from a 0·05-mg silica standard.

Results

Tissue—

Agreement between this colorimetric modification and gravimetric results on normal and pneumoconiotic lungs has been good. Mean results and range for 22 samples by gravimetric and colorimetric methods were 1·37 and 1·29 per cent. (4·2 to 0·1 per cent. and 4·6 to 0·1 per cent.), respectively, of silica. Analysis of variance showed no significant difference; residual standard deviation was 0·24 per cent.

Recoveries of pure powdered quartz added to dried, ground, foetal, human lung were 5·0 to 5·3 mg of silica when 5 mg were added to 100 mg of lung, and 9·8 to 10·0 mg of silica when 10 mg were added.

Urine—

Good recoveries (90 to 100 per cent.) have been obtained with the two modified colorimetric methods (Table II).

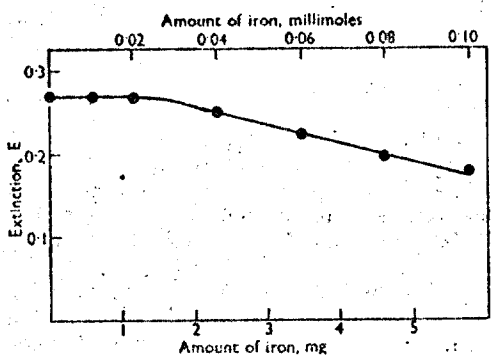

Fig. 6. Effect of iron on the molybdenum blue colour formed by silica

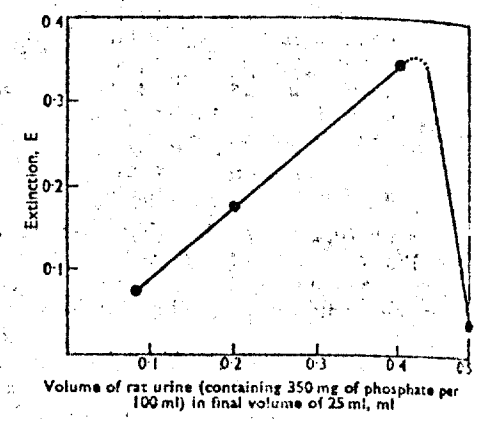

Fig. 7. Effect of increasing volume of rat urine on silica blue colour

In the sodium nitrite method not more than 1·4 mg of phosphorus should be present in the final volume (25 ml) in which the colours are developed. This method is therefore limited with human urines (with 1·4-ml samples) to those containing less than 100 mg of phosphorus per 100 ml of urine. At the risk of obtaining low absorptiometer readings, the method may be extended by using smaller samples. With rat urine, 0·2-ml samples being used in the final volume, the method should tolerate concentrations up to 700 mg of phosphorus per 100 ml of urine. This was tested by noting the effect on the silica determination of increasing the volume of rat urine. As shown in Fig. 7, there was a marked interference in colour formation when 0·5 ml of urine was used, suggesting that 0·5 ml contained an amount of phosphorus greater than 1·4 mg, *i.e.*, that the amount of phosphorus in the urine was greater than 280 mg per 100 ml. On determination it was found that the concentration of phosphorus was 350 mg per 100 ml of urine.

Blood analysis: soluble silica—

Recoveries of added silica and the silica concentration in several samples of human blood are shown in Table III.

MINERAL DUSTS—

The results (Table IV) obtained on analysing mineral dusts by the colorimetric procedure compare favourably with those determined by the standard gravimetric procedure.

TABLE I

pH OF FILTRATE AFTER THE PRECIPITATION OF PHOSPHATE

Weight of phosphate, mg of P_2O_5	0	0·5	2·0	5·0	10·0	20·0	30·0
pH of filtrate	3·77	3·74	3·76	3·78	3·85	3·98	4·09

TABLE II

COLORIMETRIC ANALYSIS OF URINE: RECOVERY OF ADDED SILICA AND COMPARISON OF SILICA ANALYSES BY DIFFERENT METHODS

	Calcium chloride precipitation method				Nitrous acid method				
Urine	Silica in urine, mg per 100 ml	Silica added, mg per 100 ml	Total silica found, mg per 100 ml	Silica recovered, mg	Urine used for analysis, ml	Silica in urine, mg per 100 ml	Silica added, mg per 100 ml	Total silica found, mg per 100 ml	Silica recovered, mg
Human 1	1·4	1·7	2·9	1·5	1·0	1·5	5·0	6·2	4·7
	1·3	1·7	2·8	1·5	1·0	1·5	5·0	6·2	4·7
					2·0	1·4	2·5	3·9	2·5
Human 2	1·2	1·7	2·9	1·7	1·0	1·1	2·5	3·4	2·3
	1·2	1·7	2·7	1·5					
Human 3	0·72				1·0	0·85	5·0	5·5	4·7
	0·74				1·0	0·95	5·0	5·5	4·6
Human 4					1·0	2·3	5·0	7·1	4·8
					2·0	2·2	1·5	3·6	1·4
Rat 1					0·2	9·2	25·0	35·0	26·0
Rat 2	10·0	10·0	20·0	10·0	0·2	10·0	25·0	35·0	25·0
					0·2	9·0	25·0	35·0	26·0

TABLE III

COLORIMETRIC ANALYSES OF BLOOD, AND RECOVERY OF ADDED SILICA

	Silica in blood, mg per 100 ml	Silica added, mg per 100 ml	Total silica found, mg per 100 ml	Silica recovered, mg
Defibrinated horse blood	0·78	1·00	1·73	0·95
	0·78	2·00	2·72	1·94
	0·78	4·00	4·67	3·89
	0·78	5·00	5·97	5·19
Mixed heparinised blood	0·33	1·00	1·23	0·90
	0·33	2·00	2·32	1·99
	0·33	4·00	4·45	4·12
	0·33	5·00	5·50	5·17
Mixed human serum	0·30	1·00	1·27	0·97
	0·30	2·00	2·40	2·10
	0·30	4·00	4·23	3·93
	0·30	5·00	5·36	5·06
Individual, heparinised human bloods .. .. .. (1)	0·37			
(2)	0·19			
(3)	0·24			
(4)	0·27			
(5)	0·31			

TABLE IV

ANALYSIS OF MINERAL DUSTS

	Silica found by colorimetric analysis, %	Silica found by gravimetric analysis, %
Quartz	99·4	99·7
Kaolin	47·1	45·6
Mica	43·8	43·0
Quartz, kaolin and mica: mixture 1	45·1	44·0
Quartz, kaolin and mica: mixture 2	51·8	52·0
Quartz, kaolin and mica: mixture 3	69·9	70·5
Coal-mine dust (Ammanford B)	61·0	61·3
Coal-mine dust (Ammanford C)	53·7	54·2
*Standard granite G–1	71·5	72·6
*Diabase W–1	50·8	52·7

* U.S. Geological Survey samples obtained from American Bureau of Standards.

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REFERENCES

1. Hillebrand, W. F., "The Analysis of Silicate and Carbonate Rocks," *U.S. Geol. Surv. Bull.*, No. **700**, 95.
2. Treadwell, F. P., and Hall, W. T., "Analytical Chemistry," Ninth Edition, Chapman & Hall Ltd., London, 1942.
3. Scott, W. W., and Furman, N. H., "Standard Methods of Chemical Analysis," Fifth Edition, D. van Nostrand Co. Inc., New York, and The Technical Press Ltd., London, 1939.
4. Emich, F., "Microchemical Laboratory Manual," translated from Second German Edition, John Wiley & Sons, New York, 1932.
5. Pregl, F., "Quantitative Organic Analysis," J. & A. Churchill Ltd., London, 1945.
6. Morgan, J. C., and King, E. J., *J. Biol. Chem.*, 1932, **95**, 613.
7. Gerstel, G., *Arch. Gewerbepath. Gewerbehyg.*, 1934, **5**, 249.
8. Holt, P. F., Thesis for Ph.D. degree, University of London, 1939.
9. Parmentier, M., *Compt. Rend.*, 1893, **97**, 788.
10. Asch, W., *Z. anorg. Chem.*, 1901, **28**, 273.
11. Copaux, M. H., *Ann. Chim. (Phys.)*, 1906, [8] **7**, 118.
12. Pechard, M., *Compt. Rend.*, 1893, **97**, 788.
13. Miolati, A., *J. prakt. Chem.*, 1908, **77**, 450.
14. King, E. J., and Watson, J. L., *Mikrochemie*, 1936, **20**, 49.
15. Volinetz, M. I., *J. Chim. Ukr.*, 1936, **11**, 18.
16. Merz, J. A., *Svensk Kem. Tidskr.*, 1941, **53**, 374.
17. Duval, C., *Anal. Chim. Acta*, 1947, **1**, 33.
18. Miller, C. C., and Chalmers, R. A., *Analyst*, 1953, **78**, 24.
19. Tananaev, N. A., and Babko, A. K., *Z. anal. Chem.*, 1930, **82**, 145.
20. Korol, S. S., and Koluzhskaya, V. M., *Zavod. Lab.*, 1934, **3**, 308.
21. Kern, E. F., and Jones, T. R., *Trans. Amer. Electrochem. Soc.*, 1930, **57**, 273.
22. Wilson, H. N., *Analyst*, 1949, **74**, 243.
23. Jolles, A., and Neurath, F., *Z. angew. Chem.*, 1898, **11**, 315.
24. Schreiner, O., *J. Amer. Chem. Soc.*, 1903, **25**, 1056.
25. Lincoln, A. T., and Barker, P., *Ibid.*, 1904, **26**, 975.
26. Souchay, P., *Ann. Chim.*, 1946, **1**, 249.
27. Dienert, F., and Wandenbulcke, F., *Bull. Soc. Chim. France*, 1923, **33**, 1131.
28. Winkler, L. W., *Z. angew. Chem.*, 1914, **27**, 511.
29. Thresh, J. C., *Analyst*, 1922, **47**, 459.
30. Atkins, W. R. G., *J. Mar. Biol. Ass. U.K.*, 1928, **15**, 191.
31. Steffens, W., *Chemikeztg*, 1930, **54**, 96.
32. Thayer, L. A., *Ind. Eng. Chem., Anal. Ed.*, 1930, **2**, 276.
33. Thompson, T. G., and Houlton, H. G., *Ibid.*, 1933, **5**, 417.
34. Liebknecht, O., Gerb, L., and Bauer, E., *Z. angew. Chem.*, 1931, **44**, 860.
35. King, E. J., *J. Biol. Chem.*, 1928, **18**, 25.
36. Isaacs, L., *Bull. Soc. Chim. Biol.*, 1924, **6**, 157.
37. Oberhauser, F., and Schormüller, J., *Z. anorg. Chem.*, 1929, **178**, 381.
38. Woods, J. T., and Mellon, M. G., *Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 760.
39. Nemec, A., Lanik, J., and Koppova, A., *Z. anal. Chem.*, 1931, **83**, 428.
40. King, E. J., *Bull. Soc. Chim. Biol.*, 1930, **12**, 903.
41. Roman, W., *Biochem. Z.*, 1931, **233**, 344.
42. Boduar, J., and Török, T., *Hoppe-Seyl. Z.*, 1939, **261**, 257.

43. Gettler, A. O., and Umberger, C. J., *Amer. J. Clin. Path. Tech. Suppl.*, **1941**, **9**, 1.
44. Berg, R., and Teitelbaum, M., *Mikrochemie, Emich Festschr.*, 1930, 23.
45. Parri, W., and Scotti, G., *J. Pharm. Chim. Paris*, 1933, **18**, 513.
46. Rodillon, C. *Bull. Biol. Pharm.*, 1934, **1**, 33.
47. King, E. J., and Stantial, H., *Biochem. J.*, 1933, **27**, 990.
48. King, E. J., *Ibid.*, 1939, 33, 944.
49. Straub, F. G., and Grabowski, J. A., *Ind. Eng. Chem., Anal. Ed.*, **1944**, **16**, 574.
50. Olsen, A. L., Gee, E. A., McLendon, V., and Blue, D. D., *Ibid.*, **1944**, **16**, 462.
51. Bunting, W. E., *Ibid.*, 1944, **16**, 612.
52. Gentry, C. H. R., and Sherrington, L. G., *J. Soc. Chem. Ind.*, **1946**, **65**, 90.
53. De Eds, F., and Eddy, C. W., *J. Biol. Chem.*, 1936, **114**, 637.
54. Armand, M., and Berthoux, J., *Anal. Chim. Acta*, 1951, **5**, 380.
55. Strickland, J. D. H., *J. Amer. Chem. Soc.*, 1952, **74**, 862.
56. Weitz, E., Francke, H., and Schuchard, M., *Chem. Z.*, 1950, **74**, 256.
57. Alexander, G. B., *J. Amer. Chem. Soc.*, 1953, **75**, 5655.
58. Adams, M. F., *Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 542.
59. Holzapfel, L., *Z. Ver. dtsch. Ing.*, 1943, **87**, 606.
60. ——, *Naturwiss.*, 1943, **31**, 189.
61. Ohlmeyer, P., and Olpp, U., *Hoppe-Seyl. Z.*, **1944**, **281**, 203.
62. Brabson, J. A., Harvey, O. W., Maxwell, G. E., and Schaeffer, O. A., *Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 705.
63. Schwartz, M. C., *Louisiana State Univ. Bull.*, 1938, **30**, No. 14.
64. Kahler, H. L., *Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 760.
65. Harrison, T. S., and Storr, H., *J. Soc. Chem. Ind.*, **1944**, **63**, 154.
66. Milton, R. F., *Analyst*, 1951, **76**, 131.
67. ——, *J. Appl. Chem.*, 1951, **1**, Suppl. Issue No. 2, 126.
68. Arnfeld, A., Dissertation, University of Berlin, 1899, pp. 1 to 60.
69. Wu, H., *J. Biol. Chem.*, 1920, **43**, 189.
70. Atkins, W. R. G., and Wilson, E. G., *Biochem. J.*, **1926**, **20**, 1223.
71. King, E. J., *Ind. Eng. Chem., Anal. Ed.*, 1931, **3**, 117.
72. Jacobs, H. R. S., private communication, 1931.
73. Robinson, R. J., and Spoor, H. J., *Ind. Eng. Chem., Anal. Ed.*, **1936**, **8**, 455.
74. Schwartz, M. C., *Ibid.*, 1942, **14**, 893.
75. Snell, F. D., and Snell, C. T., "Colorimetric Methods of Analysis," Third Edition, D. Van Nostrand Co. Inc., New York, Volume II, p. 653.
76. Holt, P. F., and Yates, D. M., *Biochem. J.*, 1953, **54**, 300.
77. Holt, P. F., and Osborne, S. G., *Brit. J. Ind. Med.*, **1953**, **10**, 153.

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CXXX. THE BIOCHEMISTRY OF SILICIC ACID.

II. THE PRESENCE OF SILICA IN TISSUES.

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(*Received May 30th, 1933.*)

The presence of silica in animal and vegetable tissues has attracted interest for a considerable time. As early as 1857 Witting recorded 0·5 % of SiO_2 in the ash of a sample of human blood. Schulz [1901] made the first thorough investigation of the presence of silica in biological materials, and Gonnerman [1917; 1918] extended the analyses and collected the results of much previous work. Vegetable matter was shown to contain silica in amounts varying from 1·2 % of the ash for yeast up to 68 % for wheat straw and 80 % for horse-tail (*Equisetum arvense*). Feathers of grain-feeding birds had a higher silica content than did those of flesh- and fish-eaters. The hair of men and of animals invariably showed the presence of silica, even in the case of the new-born, but there appeared to be no definite relation between the silica content and either the species of animal or colour of the hair. Of the many samples of animal and human tissue, organs and fluids examined, all contained silica, though in very different and variable amounts. This earlier work has recently been summarised by Kühn [1926], in whose monograph are collected the results of a large number of investigators.

In the present investigation analyses of several tissues for their content of silica have been made following the colorimetric method previously described [King and Stantial, 1933]. The results have been checked repeatedly by gravimetric controls, and are believed substantially to represent the actual silica contents of the different samples. In most cases the figures are in accord with those of previous workers, but some discrepancies have been noted and are discussed in connection with the individual tissues.

Urine. In general it may be said that the carnivorous and omnivorous animals have a much lower urinary excretion of silicic acid than do the herbivora. This point is well illustrated in Table I.

Herbivora, and particularly those feeding on whole grains (including the hulls), straw and hay, ingest large amounts of silicon. While it is improbable that a very large percentage of this silicon is absorbed, a considerable amount must pass into the blood-stream to give the high concentration which is found in the urine. The values for herbivorous urine show very marked variations, but this is to be expected because of the very marked differences in the silica contents of different vegetable foods.

That the silica content of the urine may be influenced by the diet is also well shown in Table II. A group of four rabbits, which had been kept on whole oats and carrots and which showed high urinary silica values, was placed for

Table I. *Silica content of urine.*

		mg. SiO_2 per 100 cc.		No.	mg. SiO_2 per 100 cc.
Normal human:					
Average of 10 morning samples		1·2	Cat	1	0·5
		(high 2·1)	"	2	0·3
		(low 0·8)	"	3	0·5
Average of 17 afternoon samples		1·0	"	4	0·8
		(high 2·2)	"	5	0·7
		(low 0·7)	"	6	0·4
Silicotic human:			Rabbit	1	11·1
W. L. C. (gold miner 6 years)		1·4	"	2	27·2
J. L. (gold miner 7 years)		6·6	"	3	22·7
J. L. (gold miner 7 years)		1·9	"	4	21·3
		(1 week later)	"	5	7·2
J. D. (gold miner 14 years)		1·0	"	6	14·0
J. M. (gold miner 9 years)		0·6	"	7	15·6
	No.		Guinea-pig	1	14·0
Dog	1	0·9	"	2	22·4
"	2	1·3	"	3	28·6
"	3	1·8	"	4	16·6
"	4	0·7	"	5	14·6
"	5	1·3	"	6	8·2
"	6	2·7	Sheep	1	17·2
Rat (lab. diet)	1	3·0	"	2	12·8
"	2	5·7	"	3	11·9

Table II. *Effect of diet on silica content of rabbit urine.*

Rabbit No.		232	233	234	235
Diet	Days on diet	mg. SiO_2 per 100 cc. urine			
Oats + carrots	20	8·5	12·8	10·3	31·3
White bread	7	3·6	2·5	2·1	4·0
	10	4·4	3·9	5·6	4·6
	15	0·9	2·8	1·1	2·2
	17	0·7	2·5	2·9	2·9
	22	4·1	2·6	—	3·1
	26	—	2·7	0·9	3·6
	32	5·5	2·2	1·5	0·7
Oats + wheat straw	1	15·1	27·2	25·7	19·3
	4	27·0	24·5	12·0	21·7

a month on a diet of white bread supplemented with a little canned tomato. The amount of silica excreted dropped almost immediately and remained at a lower level during the course of the white bread feeding. On placing the rabbits again on a diet of whole oats, carrots and wheat straw, the amounts of silica in the urines returned to much higher levels. This agrees with the observation of Riessner and Kindt [1928] in human beings.

Blood. The silica content of the blood of individuals in this country appears to be very different from that reported by several German investigators. Kraut [1931] and Bohme and Kraut [1932] have recently reported an average of about 15 mg. of SiO_2 per 100 cc. of blood for normal men and women. Miners with and without silicosis had considerably more. Reckoned on an ash basis, Kraut's figures agree closely with that originally recorded by Wasing [1857]. If these figures, obtained by satisfactory methods, are correct, and if our own results also substantially represent the silica content of the bloods analysed, then it appears that only about one-tenth the amount of silica found by Kraut circulates in the blood of normal human beings in this country.

Only a limited number of blood samples from cases of silicosis have been examined. These were sanitorium patients, former gold mine workers, who showed a history of silicosis, but who had been some time removed from their work. The average figure found was 0·9 mg. SiO_2 per 100 cc. of whole blood, with high and low values of 1·8 and 0·6 respectively.

It is apparent, however, that despite the low level of blood-silica, considerable amounts are passing through the body by way of the blood-stream to the kidneys at all times. Normal human beings on refined diets excrete 15 or 20 mg., and an herbivorous animal of approximately the same size would appear to be putting out 10 to 20 times this amount daily in the urine.

Foetal tissue. All the tissues and biological fluids so far examined have contained at least traces of silicon. The almost universal distribution of silicates, be it in sea-water or in the blood of mammals, makes it probable that if silicon is not an essential ingredient it is at least a likely contaminant of the developing egg. All the marine forms examined have contained silica, and added sodium silicate has been found greatly to enhance the growth of cultures of phytoplankton [King and Davidson, 1933]. Both the white and the yolk of the eggs of birds appear to contain small amounts of silicon, and several embryonic mammals have contained appreciable quantities, both in the whole foetus and in the individual organs. Table III contains the results for several such determinations.

Table III. *Silica content of foetal tissue.*

Tissue	mg. SiO_2 per 100 g. dry tissue
Mouse (whole foetus) (1)	10·7
(2)	10·5
Calf A: lung	4·4, 4·8
Calf B_1: kidney	8·2, 9·4
liver	4·4, 6·4
muscle	36·8
Calf B_2: lung	3·8, 4·2
kidney	9·6, 7·8
liver	10·0, 8·8
spleen	11·2
muscle	21·2
Calf B_3: lung	8·0, 10·4
Human: lung	9·8, 8·4
liver	3·8, 5·6
kidney	13·3
spleen	20·3
heart	20·4
brain	22·0

Adult tissues. The two paths of entry for silica into the body are the lungs and the digestive tract. The greater part of that entering by the latter route is doubtless eliminated in the fæces. A considerable amount probably passes into the blood-stream, however, and it appears that this is excreted in the urine without much retention by any of the organs of the body. The liver, spleen and kidneys of animals, even those ingesting large amounts of silicates in their food, contain only relatively small amounts of silica, and whatever silicate enters the blood by way of the intestine appears to pass quickly into the urine.

In the case of silica entering by the lung—and a considerable amount must enter as finely particulate dust even in the case of most individuals who are seldom exposed to a dusty atmosphere—there is no ready means of exit once

the particles have lodged in the parenchyma. Certainly the majority of particles breathed in are wafted up by the cilia of the bronchial passages and are either swallowed or expectorated, but the smallest particles probably lodge in the lung spaces and are then only capable of being eliminated by phagocytosis or through dissolution by the mildly alkaline body fluid which is constantly bathing them. Some particles which are ingested by cells which have passed into the air space are probably expectorated with their enveloping cell. Other particles may be ingested and drawn into the lung parenchyma, and the migration of the phagocytes may carry them appreciable distances in the lymph channels; some particles may even lodge in the bone-marrow [Chapman, 1933]. How much silica may be carried away by dissolution is entirely a matter of speculation, but it is certain that silica particles in the lung tend to become hydrated and invisible to the polarising microscope, and weak sodium bicarbonate solutions tend to dissolve surprising amounts of silicate from colloidal hydrated silica.

As is to be expected, lung tissue varies very markedly in its silica content, from what would be a normal value for any other tissue of the body up to values

Table IV. *Silica content of tissues.*

Tissue	mg. SiO_2 per 100 g. dry tissue
Human:	
Lung* (normal adult)	140
(pneumonia)	93
(silicotic)	3640
,,	1700
,,	880
,,	1630
,,	810
Liver* (stone grinder)	196
Spleen*	
(normal)	15·1
(septicaemia ♂ 30)	16·0
(bronchopneumonia ♀ 75)	43·0
(rheumatic heart ♀ 46)	14·2
(bronchopneumonia ♀ 36)	23·0
(pulmonary embolism ♀ 67)	30·0
(carcinoma ♂ 25)	45
(silicosis, fibrosis of spleen ♂)	345
(silicosis, stone grinder)	436
Mesenteric lymph gland*	
(stone grinder)	1080
Bone-marrow	26·0
(lumbar vertebra)	25·2
Ox:	
Lung	103, 109
	104, 120
	87·2, 81·2
Liver	21·6, 18·0
	12·0, 15·0
Dog:	
Lung	115
	270
Kidneys	28·7
	31·8

Tissue	No.	mg. SiO_2 per 100 g. dry tissue
Rabbit:		
Lung	430	148, 161
	369	235, 229
(young)	378	18·4, 14·2
(animal exposed to SiO_2 dust)	651	6755, 7115
,,	693	895, 785
,,	687	1002
Liver	687	12·4
	648	30·6
	378	21·4
	651	20·8, 14·3
	360	20·0
Kidney	373	16·4
	380	22·2, 17·8
	647	14·4
(animal exposed to SiO_2 dust)	651	50·0, 47·6
Lymph glands		80
Spleen	657	16·0
(animals exposed to SiO_2 dust)	360	22·5
,,	369	76·0
Hair (grey)		16·5
(white)		15·6
Guinea-pig:		
Lungs		78·0
		48·0
Hair		19·4
		20·7
Chicken feathers		15·1
		12·9

* These analyses were performed on petrol- and ether-extracted tissues obtained from the wax blocks in which they had been imbedded after fixation for microscopic section and do not represent the silica content of the tissue as a whole. We are indebted to the Department of Pathology for this material.

which show about 50 % of the mineral material present to be SiO_2. The lungs of gold miners, sand blasters, quarrymen, *etc.*, invariably contain extraordinary, though variable, amounts of silica. The presence of these large amounts in particulate form in the lungs appears to lead to the development of the fibrotic nodules typical of silicosis, which gradually extend, as snowflake-like flecks on the X-ray plate, from the mid-line to the periphery as the disease progresses. It is probable that the amount of effective alveolar tissue in some of these men is very greatly reduced. They become, moreover, very susceptible to tuberculosis, of which many of them die.

A considerable variety of animal material has been analysed with a view to gaining an insight into the possible significance of the presence of silicon in the different tissues. The results, which are illustrated in Table IV, have appeared to indicate a disposal within the body of inhaled and ingested silica along the lines suggested above. No evidence has so far been obtained that silicon is an essential element of animal protoplasm, or that it is a limiting factor in growth, as may be the case in certain microscopic marine plants. Further work will be presented in another paper [King, Stantial and Dolan, 1933], to show that whether or not silicates are an essential in the animal economy, the body possesses a very efficient mechanism for their disposal.

Summary.

Silica occurs in small but significant amounts in foetal tissue.

A considerable excretion of silicate occurs through the kidneys. The silicate level of the urine of herbivora is much higher than that of carnivora. The amount of silica appearing in the urine is markedly influenced by diet.

Circulating blood contains only very small amounts of silica.

A variety of adult tissues shows marked differences in silica content, lung having the largest amount.

References.

Bohme and Kraut (1932). *Arbeitsphysiol.* **5**, 269.
Chapman (1933). *Amer. J. Med. Sci.* **185**, 171.
Gonnerman (1917). *Z. physiol. Chem.* **99**, 257.
—— (1918). *Biochem. Z.* **86**, 401.
King and Davidson (1933). *Biochem. J.* **27**, 1015.
—— and Stantial (1933). *Biochem. J.* **27**, 990.
—— —— and Dolan (1933). *Biochem. J.* **27**, 1607.
Kraut (1931). *Z. physiol. Chem.* **199**, 81.
Kühn (1926). Die Kieselsäure. (Ferdinand Enke, Stuttgart.)
Riessner and Kindr (1928). *Z. physiol. Chem.* **174**, 40.
Schulz (1901). *Pflüger's Arch.* **84**, 67.
Witting (1857). *Arch. exp. Path. Pharm.* **140**, 155.

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DEVELOPMENT OF SILICA CALCULI AFTER ORAL ADMINISTRATION OF MAGNESIUM TRISILICATE

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Synthetic magnesium trisilicate is widely used in the treatment of hyperacidity in gastritis and gastric and duodenal ulcers. It was introduced in 1936 by Mutch,[1-3] who considered it to be superior to other antacids and absorbents by virtue of its strong neutralizing action. It has been stated that neither magnesium trisilicate nor the hydrated silica that is formed on the breakdown of the trisilicate in contact with hydrochloric acid is soluble, and therefore cannot be reabsorbed from the digestive tract.[2, 4] In this respect these substances differ from, for instance, sodium bicarbonate, which can give rise to alkalosis through disturbance of the acid base equilibrium.

The mechanism of breakdown and excretion of magnesium trisilicate has been studied by, among others, Page and associates.[5] When a silicate is acted on by an acid, part of the silica so formed is precipitated as a gel and part remains in solution as a colloid. It is probable that as a silicate passes through the digestive tract other breakdown products than silica are formed. In theory, these may include ortho-silicic acid (H_4SiO_4), which is soluble, meta-silicic acid (H_2SiO_3) and tri-silicic acid ($H_4Si_3O_8$), which are partially soluble, and di-silicic acid ($H_2Si_2O_5$), which is practically insoluble. Since the reaction between magnesium trisilicate and an acid is more complex than has previously been thought, Page and associates[5] considered it possible that part of the silica can be reabsorbed and excreted in the urine. They accordingly measured the excretion of silica in the urine of five healthy subjects before and after supply of magnesium trisilicate. It was found that the amount of silica excreted increased after intake, though not, it seemed, to a harmful extent.

There appears to have been no mention in the literature of urinary calculus composed of silica until Hammarsten and associates[6] reported in 1953 a case of recurrent renal calculus, whose chemical analysis of a stone showed it to be composed chiefly of silica. The patient had been taking tablets containing trisilicate regularly for several years for gastric ulcer. Hammarsten has since analyzed a further silica stone from a patient with a similar history.[7] Studying x-ray absorption in a plane ground rock crystal (SiO_2), she found that it closely resembles calcium oxalate and ammonium magnesium phosphate in this respect. The silica stones, however, had a fairly low radiopacity and this is ascribed to the calculus in the renal pelvis being in the gel state and to its being mixed with much fibrin.[6] One similar case with calculus caused by antacid therapy has later been reported.[8] In contradistinction to human being silica calculi are common in some animals and a serious problem among cattle in many areas.[9, 10]

The formation of calculus in connection with magnesium trisilicate therapy is presumably more common that would appear from the literature, and for this reason it has been considered of in-

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[1] Mutch, N.: Silicates of magnesium. Brit. M. J., 1: 143-148, 1936.

[2] Mutch, N.: Synthetic magnesium trisilicate. Its action in the alimentary tract. Brit. M. J., 1: 205-208, 1936.

[3] Mutch, N.: Hydrated magnesium trisilicate in peptic ulceration. Brit. M. J., 1: 254-257, 1936.

[4] Kraemer, M. and Aaron, B.: Use of hydrated trisilicate of magnesium for peptic ulcer. Am. J. Digest Dis., 7: 57-60, 1940.

[5] Page, R. C., Heffner, R. R. and Frey, A.: Urinary excretion of silica in humans following oral administration of magnesium trisilicate. Am. J. Digest Dis., 8: 13-15, 1941.

[6] Hammarsten, G., Helldorff, J., Magnusson, W. and Rilton, T.: Dubbelsidiga njurstenar av kiselsyra efter bruk av silikathaltigt antacidum. Svensk Lakartidning, 50: 1242-1246, 1953.

[7] Hammarsten, G.: Terapi-inducerade urinvägskonkrement. Opuscula Medica, 3: 19-22, 1958.

[8] Herman, J. R. and Goldberg, A. S.: New type of urinary calculus caused by antacid therapy. JAMA 174: 1206-1207, 1960.

[9] Whiting, F., Conell, R. and Forman, S. A.: Silica urolithiasis in beef cattle. Can. J. Comp. Med. 22: 332-337, 1958.

[10] Forman, S. A., Whiting, F. and Conell, R.: Silica urolithiasis in beef cattle. 3. Chemical and physical composition of the uroliths. Can. J. Comp. Med. 23: 157-162, 1959.

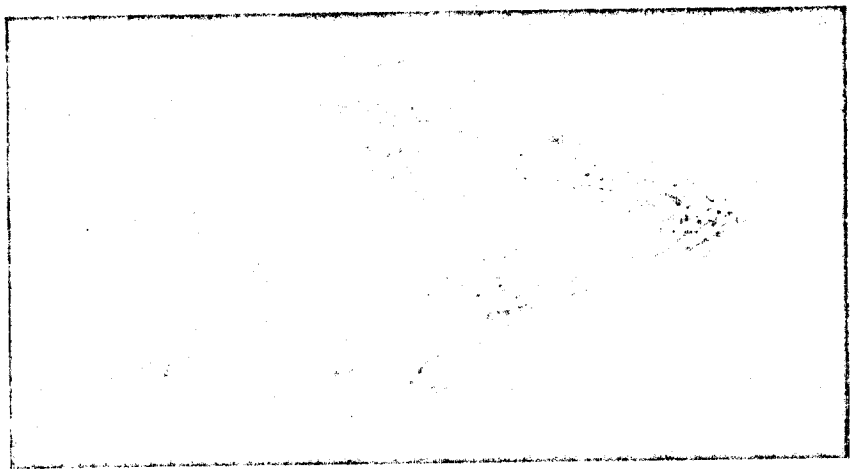

FIG. 1. Microradiogram of 100 microns thick section of silica stone. Central area is irregular structure and contains organic substance and silica. Alternating rings indicate silica and hydroxyapatite. 15×.

st to report 5 cases of silica stones where re had been regular medication with the tri-ate over a number of years.

At the Department of Radiologic Diagnosis of hospital x-ray crystallographic analysis is formed on urinary tract calculus from some 0 patients annually. During 1960 and 1961 there e been altogether 5 cases of silica stones. In e of them there were also small quantities of ium hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$; in one, all quantities of calcium oxalate mono- and hydrate, while the fifth consisted only of a. In all cases the silica was present in a fine stalline form, and as a consequence the pri-y diffraction pattern in 3 cases consisted only unterpretable diffraction rings. The two her samples showed weak but readily identifi-le powder diagrams. After the samples had been ated, clear diagrams that were characteristic of ica were obtained.

In order to study the structure of the calculi, 3 cases one half of the calculus was imbedded methyl methacrylate and cut into thin slices. ese were ground to 100 microns, and micro-ographed.[11] In all cases the part of the stone sisting of silica gave similar pictures. As is n from figure 1, where the central region of the stone consists of silica, the structure is irregular and mossy, with parts of low x-ray absorption, containing organic substance, alternating with areas of more radiopaque silica. Within the surface layer there are concentric alternate rings of silica and hydroxyapatite with a still higher x-ray absorption. In the 3 cases where the patients underwent radiologic examination prior to the passage of the stone the calculus was described as being of fairly low radiopacity, which property was clearly due to its structure. The structural picture and the finely crystalline form of the silica support Hammarsten's supposition that during the formation of the calculus the silica is precipitated in the gel form.

All 5 patients had been taking tablets containing magnesium trisilicate for gastric ulcer and gastritis more or less regularly; in 2 cases daily, for 2–5 years. There was no evidence that any of them had exceeded the doses specified by the respective manufacturers. Two of the patients had previously had attacks of the renal calculus type during trisilicate therapy but no stone has been discovered.

Since silica stones in the urinary tract do not occur unless trisilicate has been taken over a long period as an antacid, the occurrence of these stones may be confidently ascribed to this substance. There is reason to doubt the wisdom of prolonged use of such preparations. In those cases where they have been taken over a long

[11] Lagergren, C.: Biophysical investigations of nary calculi. X-ray crystallographic and roradiographic study. Acta radiol. suppl., 3: pp. 1–71, 1956.

period an examination of the renal status should be made.

SUMMARY

X-ray crystallographic analyses of calculi of the urinary tract from 800 patients disclosed 5 cases where the calculi consisted mainly of silica, which was present in a finely crystalline form. All 5 patients had been taking magnesium trisilicate in tablet form regularly over several years as an antacid for gastritis or ulcer. The dose has apparently not been remarkably large.

Since oral administration of magnesium trisilicate evidently can give rise to the formation of silica stones, the renal status of patients on this therapy should be checked. As equally effective antacids are available they are to be preferred.

Z. Ernährungswiss.
8:27-32, June 1967.

The effect of polymeric silicic acid on renal SiO_2 excretion in man

by

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From: Physiological-Chemical Institute of the University of Mainz

Sent in: Oct. 5, 1966.

There are a number of substances of the most diverse chemical origin that can be used to improve the technological qualities of certain foodstuffs. Among these are hydrophile polymeric silicic acids with a large surface, that can be used, for instance, to stabilize emulsions, as an anti-sedimentation agent for cocoa in beverage mixes, to maintain dispersability in powdered substances and as a clarifying agent for beer.

Our purpose was to investigate whether the oral administration of such preparations influences the renal SiO_2 excretion in man. Aerosil and FK 700 of the Degussa Company were available as test substances.

Aerosil is a very pure, coagulated and radiologically amorphous SiO_2 aerosol with a particle size between 10 and 40 nm and a surface of 175 m^2g. The SiO_2 content of the water-free substance is more than 99.8%. The surface is free of pores and in addition to siloxan groups, also contains silanol

groups (5-6 µMol OH-groups/m^2) and is thus capable of forming hydrogen bonds. One g aerosol is capable of taking up ca. 400 mg water by multi-layer adsorption, without losing the quality of a dry powder. Ca. 9 mg is fixed to the surface by the formation of hydrogen bonds. (For details see Wagner and Brünner). The beer clarifying agent FK 700 is a pure, hydrated silicic acid, which in addition to traces of other metal oxides contains 86.65% SiO_2. Upon drying (105°C) it loses 7.34% and upon heating (1000°C) 13.00% of its weight. The water-soluble component (membrane filtration) amounts to 0.65% SiO_2.

Method

The experimental subjects were 10 healthy men and 2 women aged between 22 and 28 years. The preparations were administered suspended in commercial apple juice (concentration 5 g/l) on the 4th day of a 7 day period of experimentation, in a quantity of 250 ml in the morning and at midday containing a total of 2.5 g of one of the two preparations. On each of the 7 days, starting with the morning urine of the first day, the experimental subjects collected all their urine in polyethylene bottles and handed it in to the laboratory during the forenoon. Otherwise they went about their usual occupations and were free to select their own diet.

After thorough mixing, a sample of 100 ml was taken from each days' urine and frozen until analyzed for its SiO_2 content. The SiO_2 was determined according to the direct method of

Baumann (1) with which the molybdate-active, or monomeric SiO_2 can be detected. The search for polymeric SiO_2 (SiO_2 determination after alkaline hydrolysis) remained fruitless, in that while as a rule somewhat higher values were found, the differences fell within the limit of error of the method, which was appreciable in itself and was further enlarged by the additional manipulation (rise of blank value, see also Baumann, 1). To check that the daily urine delivered was complete, the creatinine excretion was determined with the picrate method (standard methods of clinical chemistry, see Hinsberg-Lang). There was no indication of any grossly incomplete delivery.

Results and Discussion

The results are summarized in Tables 1 and 2. During the preliminary period the SiO_2 excretion already reveals pronounced individual variations, as well as considerable variations in one and the same subject from one day to the next, in some cases by more than 50% compared to the previous day's values, or in absolute figures, up to 64 mg (subject 2, second and third day). The highest SiO_2 excretion was 119.8 mg (subject 2, second day), the lowest 14.5 mg (subject 7, third day). The highest SiO_2 concentration in one daily urine appeared to be 86 µg/ml in subject 3 on the second day. The averaged SiO_2 concentrations (last but one line of the tables) were between 11 and 70 µg/ml, the averaged daily excretion between 15.1 and 86.9 mg SiO_2.

After the administration of 2.5 g Aerosil the SiO_2 excretion did not change unequivocally. If we compare excretion on the third and fourth day (Table 1), subjects 1, 11 and 12 revealed a more or less marked reduction, while the rest showed an increase. The average daily SiO_2 excretion in the subsequent period (see last line of the table) is practically unchanged compared to the preliminary period for subjects 1 and 3, in the case of subject 2 it was distinctly reduced and in the rest just as distinctly increased, although not to a striking degree compared to the preliminary period, either with regard to concentration or the daily SiO_2 excretion.

The situation is somewhat different after the administration of 2.5 g FK 700 (= 2.15 g SiO_2). Here the SiO_2 excretion increased in all subjects on the 4th day, with the exception of subject 6, who revealed a 26 mg decrease. The increases ranged between +7 and +23 mg. In subject 5 the mean daily SiO_2 excretion was also increased compared to the preliminary period by between +4 and +20 mg, and only in subject 9 was it slightly reduced. The highest SiO_2 excretion observed (subject 6, 142,3 mg on the 5th day) and the highest SiO_2 concentration observed (also subject 6, 6.91 µg/ml on the 6th day) occurred during the second period of the experiment. It should, however, be noted that subject 6 (and also subject 3) had participated in a summer festival on the evening of the 4th day at which they partook of considerable quantities of beer.

This also explains the striking volume of urine of 2.58 l on the 5th day. The SiO_2 concentration in beer, of 131 µg/ml, is at the saturation limit (Baumann, 2). In this group the SiO_2 concentration and daily excretion was also not markedly changed compared to the preliminary period. In both groups, not only the increased excretion in the given cases, but the total SiO_2 excretion during the four days of the second period of the experiment was in no way related to the quantities of 2.5 or 2.15 g SiO_2 administered.

It has been shown by Baumann (2,3) that orally administered monomeric silicic acid is rapidly absorbed and maximum renal excretion occurs as early as after 1-2 hours. Monomeric silicic acid is dissolved in water in stable form up to a concentration of 120 µg/ml, while at higher concentrations polymerization begins (Thomas). Baumann (2,3) only found polymeric silicic acid in the urine with very high concentrations of monomeric SiO_2 (600 µg/ml or more) and when present for a sufficiently prolonged period (urine samples every 2 hours). The development of polymeric SiO_2 harmful to the urinary tract is thus dependent upon concentration and time. The SiO_2 concentrations found in the present experiments are still far removed from the dangerous range.

If the SiO_2 preparations administered in the experiment described here had been absorbed to a greater degree - which would postulate prior depolymerization during passage through

the gastrointestinal tract - then, according to Baumann's findings, there should have been a distinct increase in the renal SiO_2 excretion. The changes observed, however, were completely within the range marked out for the preliminary period.

In experiments on cats King and McGeorge have studied the influence of various polymeric silicic acids and natural silicates on renal SiO_2 excretion after oral administration. The different preparations gave rise to a slight but variable increase in SiO_2 excretion. The most pronounced effect was that of amorphous calcium silicate, which, after the administration of 5 g, led to an excess excretion of 30 mg SiO_2 in 120 hours. In the case of the other preparations excess excretion was less than 10 mg during the same period. The cats fed with a standard diet excreted between 1.6 and 1.8 mg SiO_2 per day, and 8.6 mg in 5 days. It is apparent from the experiments that small quantities of some polymeric silicates - in the case of calcium silicate not quite 1% - are depolymerized in the intestinal juices and go into solution.

In view of the considerable spontaneous variations in SiO_2 excretion, Aerosil had no definite effect and FK 700 only a very doubtful effect on the renal excretion of SiO_2 in terms of an increase. Thus Aerosil is not absorbed at all, and FK 700 only partially.

Summary

Our purpose was to investigate whether the oral

administration of polymeric SiO_2 preparations alters the renal excretion of SiO_2 in humans. The preparations Aerosil and FK 700 were administered to 12 healthy experimental subjects as a 0.5% suspension in 500 ml apple juice. After Aerosil there was no clearcut change and after FK 700 a doubtful increase in renal SiO_2 excretion in view of the spontaneous variations in SiO_2 excretion.

Tables

1. Aerosil preparations. Key: 1. Day 2. Experimental subject number 3. Mean value 4. Column 1: urine volume, liter; column 2: daily SiO_2 excretion in mg; column 3: SiO_2 concentration in μg/ml

2. FK 700 preparation. Key as in Table 1.

Bibliography

See p. 32.

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Tab. 1. Präparat Aerosil®

1. Tag / 2. Vpn.-Nr.	1			2			3			10			11			12		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	0,80	26,2	33	1,78	85,1	48	0,93	64,7	67	2,01	51,6	26	1,05	14,0	13	1,39	34,8	25
2	0,77	28,1	36	1,84	119,8	65	1,07	92,0	86	1,62	23,6	15	0,76	24,6	32	0,75	32,5	43
3	0,98	49,3	50	1,20	55,7	46	1,28	31,7	25	1,13	45,9	41	0,75	37,3	50	1,08	41,2	38
4	0,73	30,0	41	1,91	75,2	39	0,81	61,9	76	1,69	83,9	49	0,89	35,7	40	1,05	39,9	38
5	0,69	50,0	72	1,50	56,0	37	2,40	101,5	42	1,65	66,1	40	1,21	49,1	41	1,63	65,3	40
6	0,63	23,6	37	1,15	47,7	41	0,97	34,6	36	0,82	35,1	43	0,67	57,5	86	0,83	41,5	50
7	0,54	25,7	48	2,27	78,9	35	0,72	47,0	65	1,57	58,1	37	1,00	35,5	36	1,14	64,8	57
1.–3.	2,55	103,6		4,82	260,6		3,28	188,4		4,76	121,1		2,56	75,9		3,22	108,5	
4.–7.	2,59	129,3		6,83	257,8		4,90	245,0		5,73	243,2		3,77	177,8		4,65	211,5	
Mittelwert 1.–3.	0,85	34,5	41	1,61	86,9	54	1,09	62,8	57	1,59	40,4	25	0,85	25,3	29	1,07	36,2	34
Mittelwert 4.–7.	0,65	32,3	49	1,71	64,5	37	1,23	61,3	50	1,43	60,8	42	0,94	44,5	47	1,16	52,9	45

Spalte 1: Harnvolumen, Liter; Spalte 2: tägliche SiO_2-Ausscheidung in mg.
Spalte 3: SiO_2-Konzentration in $\mu g/ml$.

Tab. 2. Präparat FK 700

Tag / Vpn.-Nr.	4			5			6			7			8			9		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	0,94	46,4	49	0,95	34,3	36	1,01	66,6	66	1,43	17,6	12	1,07	33,0	31	0,75	33,7	45
2	0,76	50,8	67	0,72	33,9	47	0,94	73,1	78	1,28	16,2	13	1,32	49,1	37	0,85	27,4	32
3	0,74	30,0	40	1,33	42,2	32	1,10	74,6	68	1,46	14,5	10	1,63	37,7	23	0,55	21,0	38
4	0,85	53,3	63	1,81	55,8	31	1,20	48,9	41	1,77	24,6	14	1,45	44,7	31	0,96	28,8	30
5	0,90	62,7	70	1,77	61,8	35	2,58	142,3	55	0,87	19,5	22	0,81	45,6	56	0,68	26,6	39
6	0,72	50,7	70	0,90	75,2	84	0,83	75,7	91	1,20	20,6	17	0,93	44,0	47	0,60	11,7	19
7	0,98	42,0	43	1,08	35,1	33	0,86	58,2	68	1,46	17,0	12	1,55	98,8	64	0,53	19,9	38
1.–3.	2,44	127,2		3,00	110,4		3,05	214,3		4,17	48,3		4,02	119,8		2,15	82,1	
4.–7.	3,45	208,7		5,56	227,9		5,47	325,1		5,30	81,7		4,74	233,1		2,77	87,0	
Mittelwert 1.–3.	0,81	42,4	52	1,00	36,8	37	1,02	71,4	70	1,39	16,1	11	1,34	39,9	30	0,72	27,4	38
Mittelwert 4.–7.	0,86	52,2	60	1,39	57,0	41	1,37	81,3	59	1,33	20,4	15	1,19	58,3	49	0,69	21,8	31

Spalte 1: Harnvolumen, Liter; Spalte 2: tägliche SiO_2-Ausscheidung in mg.
Spalte 3: SiO_2-Konzentration in $\mu g/ml$.

12) On peut attribuer au caecum une fonction de réglage car il retient la nourriture jusqu'à 30 heures et davantage.

Literatur

1. BARNES, R., G. FIALA et al., J. Nutr. **63**, 489 (1957). — 2. BARNES, R. and G. FIALA, J. Nutr. **65**, 103 (1958). — 3. BARNES, R., E. KWONG and G. FIALA, J. Nutr. **65**, 251 (1958). — 4. BARNES, R., E. KWONG and G. FIALA, J. Nutr. **67**, 599 (1958). — 5. BARNES, R., S. TUTHILL et al., J. Nutr. **68**, 121 (1959). — 6. BARNES, R. and G. FIALA, J. Nutr. **68**, 603 (1959). — 7. BARNES, R., G. FIALA and E. KWONG, Fed. Proc. **22**, 125 (1963). — 8. BERGE, E. and M. WESTHUS, Tierärztliche Operationslehre (Berlin u. Hamburg 1961). — 9. FITZGERALD, R. et al., J. Nutr. **84**, 155 (1964). — 10. FRANK, I. et al., Pflügers Archiv **253**, 173 (1951). — 11. HARDER, W., Verh. dtsch. zool. Ges. **43**, 95 (1943). — 12. KELLNER, W., Z. Morph. u. Oekol. Tiere **44**, 518 (1956). — 13. KUNZ, E. und M. REIFF, Rev. Suisse Zool. **71**, 603 (1964). — 14. REIFF, M., Acta Trop. **13**, 289 (1956). — 15. REIFF, M., Rev. Suisse Zool. **72**, 666 (1965).

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Der Einfluß polymerer Kieselsäuren auf die renale SiO_2-Ausscheidung beim Menschen

Von H. LANGENDORF und K. LANG

Mit 2 Tabellen

(Eingegangen am 5. Oktober 1966)

Eine Reihe von Substanzen verschiedenster chemischer Provenienz ist geeignet, die technologischen Eigenschaften mancher Lebensmittel zu verbessern. Zu ihnen gehören auch hydrophile polymere Kieselsäuren mit großer Oberfläche, die etwa zur Stabilisierung von Emulsionen, als Anti-Absetzmittel für Kakao in Mischgetränken, zur Streufähighaltung von Pulversubstanzen und schließlich als Bierklärmittel Verwendung finden können.

Es war zu prüfen, ob die orale Verabreichung solcher Präparate die renale SiO_2-Ausscheidung beim Menschen beeinflußt. Als Testsubstanzen standen die Präparate Aerosil® und FK700 der Degussa zur Verfügung.

Aerosil ist ein sehr reines, koaguliertes und röntgenamorphes SiO_2-Aerosol mit einer Teilchengröße zwischen 10 und 40 nm und einer Oberfläche von 175 m^2/g. Der SiO_2-Gehalt der wasserfreien Substanz liegt über 99,8%. Die Oberfläche ist porenfrei und enthält neben Siloxan- noch Silanol-Gruppen (5–6 μMol OH-Gruppen/m^2) und ist damit zur Ausbildung von Wasserstoffbrückenbindungen befähigt. 1 g Aerosil vermag rund 400 mg Wasser durch Mehrschicht-Adsorption aufzunehmen, ohne die Eigenschaft eines trockenen Pulvers zu verlieren. Davon sind etwa 9 mg durch Wasserstoffbrückenbindung an der Oberfläche fixiert. (Näheres s. bei WAGNER und BRÜNNER.) Das Bierklärmittel FK 700 ist eine reine, hydratisierte Kieselsäure und enthält neben Spuren anderer Metalloxyde 86,65% SiO_2. Es verliert beim Trocknen (105 °C) 7,34% und beim Glühen (1000 °C) 13,00% seines Gewichtes. Der wasserlösliche Anteil (Membran-Filtration) beträgt 0,65% SiO_2.

Methodik

Versuchspersonen waren 10 gesunde Männer und 2 Frauen im Alter zwischen 22 und 28 Jahren. Die Präparate wurden ihnen in handelsüblichem Apfelsaft suspendiert (Konzentration 5 g/l) am 4. Tag einer 7tägigen Versuchsperiode verabfolgt, und zwar morgens und mittags je 250 ml mit insgesamt 2,5 g eines der beiden Präparate. An allen 7 Tagen, beginnend mit dem Morgenharn des 1. Tages, sammelten die Vpn. ihren gesamten Harn in Polyäthylenflaschen und lieferten ihn vormittags im Laboratorium ab. Im übrigen gingen sie ihrer gewohnten Beschäftigung nach, und sie hatten auch freie Nahrungswahl.

Von jedem Tagesharn wurde nach gründlicher Durchmischung eine Probe von 100 ml einbehalten und bis zur Analyse auf den SiO_2-Gehalt eingefroren. Die SiO_2-Bestimmung erfolgte nach der von BAUMANN (1) angegebenen direkten Methode, mit der das molybdataktive, also monomere SiO_2 erfaßt wird. Die Fahndung nach dem Vorhandensein polymerer SiO_2 (SiO_2-Bestimmung nach alkalischer Hydrolyse) blieb insofern ohne Ergebnis, als in der Regel zwar etwas höhere Werte gefunden wurden, die Differenzen jedoch in die an sich schon nicht geringe und durch die zusätzliche Manipulation noch vergrößerte Fehlerbreite der Methode fielen [Ansteigen des Blindwertes, s. dazu auch BAUMANN (1)]. Zur Kontrolle der Vollständigkeit der abgelieferten Tagesharne wurde ferner die Kreatinin-Ausscheidung mit der Pikrat-Methode (Standard methods of clinical chemistry, s. HINSBERG-LANG) bestimmt. Es ergaben sich keine Anhaltspunkte für eine grob unvollständige Ablieferung.

Ergebnisse und Diskussion

Die Ergebnisse sind in den Tab. 1 und 2 zusammengefaßt. Bereits in der Vorperiode weist die SiO_2-Ausscheidung starke interindividuelle Unterschiede auf, aber auch beträchtliche Schwankungen bei den einzelnen Vpn. von Tag zu Tag, in einigen Fällen von über 50% des vorangegangenen Tageswertes oder, in absoluten Zahlen, bis zu 64 mg (Vpn 2, 2. und 3. Tag). Die höchste SiO_2-Ausscheidung beträgt 119.8 mg (Vpn 2, 2. Tag), die niedrigste 14.5 mg (Vpn 7, 3. Tag). Als höchste SiO_2-Konzentration in einem Tagesharn imponieren die 86 μg/ml bei Vpn 3 am 2. Tag. Die gemittelten SiO_2-Konzentrationen (vorletzte Reihe der Tabellen) liegen zwischen 11 und 70 μg/ml, die gemittelten Tagesausscheidungen zwischen 15,1 und 86,9 mg SiO_2. Nach Verabreichung von 2,5 g Aerosil verändert sich die SiO_2-Ausscheidung nicht eindeutig. Vergleicht man die Ausscheidungen am 3. und 4. Tag (Tab. 1), so ist bei den Vpn. 1, 11 und 12 eine mehr oder minder deutliche Abnahme zu bemerken, bei den übrigen eine Zunahme. Die durchschnittliche tägliche SiO_2-Ausscheidung in der Nachperiode (s. letzte Reihe der Tabelle) ist im Vergleich zur Vorperiode bei den Vpn. 1 und 3 praktisch unverändert, bei Vpn. 2 deutlich geringer und bei den anderen ebenso deutlich erhöht, allerdings weder in bezug auf die Konzentrationen noch auf die SiO_2-Tagesausscheidungen in einem gegenüber der Vorperiode auffälligen Maße.

Etwas anders liegen die Verhältnisse nach Verabreichung von 2,5 g FK 700 (= 2,15 g SiO_2). Hier nimmt die SiO_2-Ausscheidung bei allen Vpn. am 4. Tag zu, mit Ausnahme von Vpn. 6, bei der eine Abnahme um 26 mg zu verzeichnen ist. Die Zunahmen bewegen sich zwischen +7 und +23 mg. Auch die mittlere tägliche SiO_2-Ausscheidung ist bei 5 Vpn. im Vergleich zur Vorperiode zwischen +4 und +20 mg erhöht, nur bei Vpn. 9 geringfügig vermindert. In die Nachperiode dieses Versuches fallen die höchste beobachtete SiO_2-Ausscheidung (Vpn. 6, 142.3 mg am 5. Tag) und die höchste beobachtete SiO_2-Konzentration (ebenfalls Vpn. 6, 91 μg/ml am 6. Tag). Hier ist allerdings anzumerken, daß die

Tab. 1. Präparat Aerosil®

Tag / Vpn.-Nr.	1			2			3			10			11			12		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	0,80	26,2	33	1,78	85,1	48	0,93	64,7	67	2,01	51,6	26	1,05	14,0	13	1,39	34,8	25
2	0,77	28,1	36	1,84	119,8	65	1,07	92,0	86	1,62	23,6	15	0,76	24,6	32	0,75	32,5	43
3	0,98	49,3	50	1,20	55,7	46	1,28	31,7	25	1,13	45,9	41	0,75	37,3	50	1,08	41,2	38
4	0,73	30,0	41	1,91	75,2	39	0,81	61,9	76	1,69	83,9	49	0,89	35,7	40	1,05	39,9	38
5	0,69	50,0	72	1,50	56,0	37	2,40	101,5	42	1,65	66,1	40	1,21	49,1	41	1,63	65,3	40
6	0,63	23,6	37	1,15	47,7	41	0,97	34,6	36	0,82	35,1	43	0,67	57,5	86	0,83	41,5	50
7	0,54	25,7	48	2,27	78,9	35	0,72	47,0	65	1,57	58,1	37	1,00	35,5	36	1,14	64,8	57
1.–3.	2,55	103,6		4,82	260,6		3,28	188,4		4,76	121,1		2,56	75,9		3,22	108,5	
4.–7.	2,59	129,3		6,83	257,8		4,90	245,0		5,73	243,2		3,77	177,8		4,65	211,5	
Mittelwert 1.–3.	0,85	34,5	41	1,61	86,9	54	1,09	62,8	57	1,59	40,4	25	0,85	25,3	29	1,07	36,2	34
Mittelwert 4.–7.	0,65	32,3	49	1,71	64,5	37	1,23	61,3	50	1,43	60,8	42	0,94	44,5	47	1,16	52,9	45

Spalte 1: Harnvolumen, Liter; Spalte 2: tägliche SiO_2-Ausscheidung in mg.
Spalte 3: SiO_2-Konzentration in μg/ml.

Tab. 2. Präparat FK 700

Tag / Vpn.-Nr.	4			5			6			7			8			9		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	0,94	46,4	49	0,95	34,3	36	1,01	66,6	66	1,43	17,6	12	1,07	33,0	31	0,75	33,7	45
2	0,76	50,8	67	0,72	33,9	47	0,94	73,1	78	1,28	16,2	13	1,32	49,1	37	0,85	27,4	32
3	0,74	30,0	40	1,33	42,2	32	1,10	74,6	68	1,46	14,5	10	1,63	37,7	23	0,55	21,0	38
4	0,85	53,3	63	1,81	55,8	31	1,20	48,9	41	1,77	24,6	14	1,45	44,7	31	0,96	28,8	30
5	0,90	62,7	70	1,77	61,8	35	2,58	142,3	55	0,87	19,5	22	0,81	45,6	56	0,68	26,6	39
6	0,72	50,7	70	0,90	75,2	84	0,83	75,7	91	1,20	20,6	17	0,93	44,0	47	0,60	11,7	19
7	0,98	42,0	43	1,08	35,1	33	0,86	58,2	68	1,46	17,0	12	1,55	98,8	64	0,53	19,9	38
1.–3.	2,44	127,2		3,00	110,4		3,05	214,3		4,17	48,3		4,02	119,8		2,15	82,1	
4.–7.	3,45	208,7		5,56	227,9		5,47	325,1		5,30	81,7		4,74	233,1		2,77	87,0	
Mittelwert 1.–3.	0,81	42,4	52	1,00	36,8	37	1,02	71,4	70	1,39	16,1	11	1,34	39,9	30	0,72	27,4	38
Mittelwert 4.–7.	0,86	52,2	60	1,39	57,0	41	1,37	81,3	59	1,33	20,4	15	1,19	58,3	49	0,69	21,8	31

Spalte 1: Harnvolumen, Liter; Spalte 2: tägliche SiO_2-Ausscheidung in mg.
Spalte 3: SiO_2-Konzentration in μg/ml.

Vpn. 6 (ebenfalls Vpn. 3) am Abend des 4. Tages ein Sommerfest besucht und dort dem Biere kräftig zugesprochen hat. Damit ist auch das auffallende Harnvolumen von 2,58 l am 5. Tag ausreichend erklärt. Im Bier liegt die SiO_2-Konzentration mit 131 μg/ml an der Sättigungsgrenze (BAUMANN (2)). Im übrigen sind auch in dieser Gruppe die SiO_2-Konzentrationen und -Tagesausscheidungen nicht bemerkenswert gegenüber der Vorperiode verändert. Für beide Gruppen gilt, daß nicht nur die Mehrausscheidung in den entsprechenden Fällen, sondern die gesamte SiO_2-Ausscheidung in den vier Tagen der Nachperiode in gar keinem Verhältnis steht zu den verabreichten Mengen von 2,5 bzw. 2,15 g SiO_2.

Von BAUMANN (2, 3) wurde gezeigt, daß oral verabreichte monomere Kieselsäure recht schnell resorbiert wird und daß bereits nach 1–2 Stunden das Maximum der renalen SiO_2-Ausscheidung eintritt. Monomere Kieselsäure ist bis zu einer Konzentration von 120 μg/ml in Wasser stabil gelöst, bei höheren Konzentrationen beginnt die Polymerisation (THOMAS). BAUMANN (2, 3) fand polymere Kieselsäure im Harn erst bei sehr hohen Konzentrationen an monomerer SiO_2 (600 μg/ml und mehr) und bei ausreichend langer Verweildauer (Harnentnahme alle 2 Stunden). Die Ausbildung polymerer und die Harnwege schädigender SiO_2 ist somit konzentrations- und zeitabhängig. Die in diesen Versuchen gefundenen SiO_2-Konzentrationen sind noch weit von dem gefährlichen Bereich entfernt.

Wenn die in dem hier mitgeteilten Versuch verabreichten SiO_2-Präparate in stärkerem Maße resorbiert worden wären – was die vorherige Depolymerisation während der Magen-Darm-Passage voraussetzen würde – dann hätte bereits am 4. Tage, den Befunden BAUMANNs zufolge, eine deutliche Zunahme der renalen SiO_2-Ausscheidung eintreten müssen. Die beobachteten Veränderungen lagen aber durchaus in dem in der Vorperiode abgesteckten Rahmen.

In Versuchen an Katzen haben KING und McGEORGE den Einfluß verschiedener polymerer Kieselsäuren und natürlicher Silikate auf die renale SiO_2-Ausscheidung nach oraler Applikation untersucht. Die einzelnen Präparate bewirkten eine geringe, aber unterschiedliche Erhöhung der SiO_2-Ausscheidung. Den stärksten Effekt hatte amorphes Kalziumsilikat, das, nach Applikation von 5 g, in 120 Stunden zu einer Mehrausscheidung von 30 mg SiO_2 führte. Bei den übrigen Präparaten lagen die Mehrausscheidungen in der gleichen Zeitspanne unter 10 mg. Die mit der Standard-Diät gefütterten Katzen schieden pro Tag zwischen 1,6 und 1,8 mg SiO_2 aus, in 5 Tagen 8,6 mg. Aus den Versuchen geht hervor, daß geringe Mengen einiger polymerer Silikate – im Falle des Kalziumsilikates nicht ganz 1% – in den Darmsäften depolymerisiert werden und in Lösung gehen.

In Anbetracht der starken spontanen Schwankungen der SiO_2-Ausscheidung hatte Aerosil keinen eindeutigen und FK 700 einen recht zweifelhaften Effekt auf die renale SiO_2-Ausscheidung im Sinne einer Erhöhung. Demnach wird Aerosil überhaupt nicht und FK 700 nur zu einem Bruchteil resorbiert.

Zusammenfassung

Es war die Frage zu prüfen, ob die orale Zufuhr polymerer SiO_2-Präparate die renale SiO_2-Ausscheidung beim Menschen verändert. Die Präparate Aerosil® und FK 700 wurden als 0,5%ige Suspension in 500 ml Apfelsaft an 12 gesunde Versuchspersonen verabreicht. Nach Aerosil trat keine eindeutige Veränderung und nach FK 700 eine an-

gesichts der spontanen Schwankungen der SiO_2-Ausscheidung zweifelhafte Zunahme der renalen SiO_2-Ausscheidung auf.

Literatur

1. BAUMANN, H., Hoppe-Seylers Z. physiol. Chemie **319**, 38 (1960). — 2. BAUMANN, H., Hoppe-Seylers Z. physiol. Chemie **320**, 11 (1960). — 3. BAUMANN, H., Beitr. Silikose-Forsch. S-Bd Grundfragen Silikoseforschg. **4**, 19 (1960). — 4. HINSBERG, K. und K. LANG, Medizinische Chemie. 3. Aufl., S. 662 (München 1957). — 5. KING, E. J. and M. McGEORGE, Biochem. J. **32**, 426 (1938). — 6. THOMAS, K., Dtsch. Z. Verdauungs- u. Stoffwechselkrankheiten **25**, 260 (1965). — 7. WAGNER, E. und H. BRÜNNER, Angew. Chemie **72**, 744 (1960).

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Die Wirkung einiger Nahrungsfaktoren auf den Fettstoffwechsel *)

Von R. TARJÁN, MAGDALENE KRÁMER, KATALIN SZŐKE-SZOTYORI und A. A. HAFIEZ

Mit 8 Abbildungen

(Eingegangen am 1. November 1966)

In der Entstehung der Atherosklerose wird von den Nahrungsfaktoren von einigen Forschern dem Fett, von anderen den einfachen Kohlenhydraten eine krankheitserregende Rolle zugesprochen. Die Wirkung dieser Nährstoffe, in erster Linie auf den Serumcholesterinspiegel, wurde von zahlreichen Verfassern untersucht. Über Untersuchungen hinsichtlich der Wirkung der Nahrungsfette gibt C. CARROL eine umfassende Zusammenfassung (1) und die neuesten Forschungsergebnisse bezüglich der Rolle der Kohlenhydrate im Lipidstoffwechsel werden von HODGES und KREHL (2) beschrieben.

Auf Grund dieser Literaturhinweise schien es notwendig, die Wirkung verschiedener in Ungarn verwendeter Nahrungsfette auf die Fettsäurezusammensetzung des Serums und gewisser Gewebe zu untersuchen. Es war auch angezeigt den Einfluß einiger mit der Nahrung zugeführter Kohlenhydrate auf die Fettsynthese, bzw. auf die Fettsäurezusammensetzung des synthetisierten Fettes, ferner auf die Verwertung der Fettsäuren des Nahrungsfettes zu bestimmen.

Methodik

Die Wirkung der verschiedenen Nahrungsfette wurde an abgesetzten männlichen Ratten untersucht. Das Futter enthielt 60% Stärke, 20% Kasein und 20% Fett. In Gruppe I wurde das Fett in Form von Schweineschmalz, in Gruppe II in Form von

*) Vortrag gehalten auf dem VIII. Kongreß der Internationalen Gesellschaft für Fettwissenschaft, Budapest 10.–16. Oktober 1966.

C.R. Soc. Biol.
161(3):589-91, 1967

Effect of silicon compounds on experimental atheroma

by

Jacques Loeper and Jacqueline Loeper

It was shown in previous studies that in human atherosclerosis and senescence (1) the silica content of the arterial wall was considerably reduced and this was confirmed in experimental atheroma of the rabbit (2). In the present work we investigated whether the administration of silicon compounds had a preventive effect on experimental atheroma of the rabbit.

We used sodium silicate, lysine silicate and organic silicon compounds: silanol salicylate and silanol citrate.

One lot of control rabbits weighing 2 kg was kept for 2 months on an atherogenic diet that included 0.75 g/day cholesterol dissolved in oil. Another lot was kept on the same diet and during the same period also received one of the silicon compounds referred to above: a) one group received sodium silicate intravenously in a dose of 0.01 g on alternate days, b) another group received lysine silicate per os (0.01 g/day), c) a further group received silanol salicylate (monomethyltrisilanol salicylate) intravenously (0.0075 g on alternate days) and d) one group received silanol citrate (monomethyltrisilanol citrate) intravenously (0.02 g) on alternate days.

[1] J. Loeper and J. Loeper, C.R. Soc. Biol., 1961, v. 155, p. 468

[2] J. Loeper, J. Loeper and A. Lemaire, Presse Médicale, 1966, v.74, p. 865

We searched for atherosclerotic lesions 1) by macroscopic examination of entire aorta, noting atheromatous plaques; 2) by histologic examination, staining lipids with Sudan, elastic fibers with picric-indigo-carmine orcein, the basic substance by seeking metachromasia with toluidine blue in aqueous and alcoholic solution. The results are summarized in the following tables.

Diet	Rabbits	Atheromatic Rabbits
Cholesterol	8	8
Cholesterol and sodium silicate	8	3
Cholesterol and lysine silicate	5	2

Table I. First Experiment

Diet	Rabbits	Atheromatic Rabbits
Cholesterol	8	5
Cholesterol and silanol salicylate	5	0
Cholesterol and silanol citrate	4	1

Table II. Second Experiment

As can be seen, these different silicon compounds appreciably reduce the macroscopic atheromatous lesions; the frequency of plaques amounts to a total of 80% in the controls and 28% in the treated animals; the most active substances seemed to be the organic silicon compounds, which were very well tolerated when administered intravenously.

With regard to histology, the following was noted in addition to atheromatous plaques: 1) in the controls, the fine, fragmented elastic fibers were frequently altered (in 15 out of 22 rabbits); there was increased metachromasia of the basic substance in the subintimal region and the media; 2) in the treated animals alteration of the elastic fibers was rare (in 6 out of 23 rabbits), but the interesting feature is that the elastic fibers were not only intact, but often more numerous and thicker in the three aortic layers, without collagen proliferation. This reaction is particularly marked with organic silicon compounds. Metachromasia of the basic substance was increased, as in the controls.

While the atheromatous plaques were much less numerous than in the controls, in many cases there was nevertheless a slight excess of lipids in the intima and media. The chemical determination of the lipids of the arterial wall yielded comparable figures in the control and treated animals: treated animals, lipids: 61±27 mg per g aorta; control animals, lipids: 68±13 mg per g aorta.

Discussion

It was thought initially that the antiatheromatous action of silicon was due to increased impermeability of the arterial wall, possibly caused by polymerization of the mucopolysaccharides of the basic substance. Silicon does reduce the permeability of connective tissue (Holt and Osborn) and we have shown that it reduces the intradermal diffusion of stains.

Without denying these effects, we believe that the anti-atheromatous power of silicon is due to the fact that it preserves the integrity of the elastic fibers and even enhances their development, the excess lipid then being tolerated without the production of atheromatous plaques, by a biochemical or enzymatic process which is as yet unknown.

Normally, elastic fibers have a silicon content which is very much proportional to their integrity; in a normal human artery it is 500 µg per g elastin. This figure is higher than in aortic collagen, but lower than in aortic mucopolysaccharides.

It is thus possible that silicon, an element which is capable of enhancing polymerization, also gives rise to fibrillogenesis in the elastic tissue.

This activation of fibrillogenesis has been confirmed with soluble collagen. The presence of small quantities of sodium silicate accelerates the formation of collagen fibrils in vitro. In view of this fact, it might be feared that silicon would enhance the collagen reaction in the arterial wall; in the doses of these silicon compounds used by us, the effect on the development of elastic fibers is distinct, without any appreciable reaction of collagen. The most effective dose seems to us to be the lowest, i.e. with silanol salicylate we used a dose of 0.007 g on alternate days. This organic silicon compound is the most active, both with regard to its antiatheromatous action and its effect on the preservation and development of elastic fibers.

At present according to the works of Leger, Babin, Beauvieux and Coustou, only citroflavonoids are capable of enhancing the growth of elastic fibers and of protecting them against the destructive effect of an excess of lipids and even of elastase.

In conclusion, the different silicic compounds used by us, and particularly the organic silicon derivatives, have an anti-atheromatous effect; this effect seems to us more linked to the protection and the development of the elastic fibrils of the aortic wall than to an increased impermeability to lipids.

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Résumé. — Un phosphagène, la phosphoarginine, a été isolé et identifié dans *Escherichia coli* (forme ciliée), où il est accompagné d'une ATP:argininephosphotransférase assurant sa biosynthèse. La répartition naturelle de l'un et de l'autre ne se limite donc ni aux Métazoaires, ni au tissu musculaire de ceux-ci, ce qui permet d'envisager un rôle plus général des phosphagènes qu'il n'est actuellement admis.

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Médecine expérimentale.

Etude de l'action de composés siliciques sur l'athérome expérimental.

par Jacques Loeper et Jacqueline Loeper.

Dans des recherches antérieures nous avons montré qu'au cours de l'athérosclérose humaine et de la sénescence (1*) la teneur en silicium de la paroi artérielle s'abaissait considérablement et nous avons confirmé ce fait dans l'athérome expérimental du Lapin (2*). Dans le travail actuel nous avons recherché si l'administration de composés siliciques avait un rôle préventif sur l'athérome expérimental du Lapin.

Nous avons utilisé le silicate de soude, le silicate de lysine et des dérivés organosiliciques : salicylate de silanol et citrate de silanol.

Un lot de lapins témoins de 2 kg était soumis pendant 2 mois à un régime athérogène comportant 0,75 g par jour de cholestérol dissous dans l'huile. Un autre lot était mis au même régime et recevait en plus pendant le même temps un des composés siliciques précités : a) un groupe recevait du silicate de soude par voie veineuse (à la dose de 0,01 g tous les 2 jours) ; b) un groupe du silicate de lysine par voie buccale (0,01 g par jour) ; c) un groupe du salicylate de silanol (salicylate de monométhyltrisilanol) par voie veineuse (0,0075 g tous les 2 jours) , d) un groupe du citrate de silanol (citrate de monométhyltrisilanol par voie veineuse (0,02 g tous les 2 jours).

Nous avons recherché les lésions athéroscléreuses : 1) par l'examen macroscopique de la totalité de l'aorte en notant les plaques athéromateuses ; 2) par l'examen histologique : coloration des graisses par le Soudan, des fibres élastiques par l'orcéine picro-indigo-carmin, de la substance fondamentale avec recherche de la métachromasie par le

(1*) J. Loeper et J. Loeper, *C. R. Soc. Biol.*, 1961, t. *155*, p. 468.
(2*) J. Loeper, J. Loeper et A. Lemaire, *Presse Médicale*, 1966, t. *74*, p. 865.

bleu de toluidine en solution aqueuse et alcoolique. Les résultats obtenus sont résumés dans les tableaux suivants :

Régime	Lapins	Lapins athéromateux
Cholestérol	8	8
Cholestérol + silicate de soude	8	3
Cholestérol + silicate de lysine	5	2

Tableau I. — 1re expérience.

Régime	Lapins	Lapins athéromateux
Cholestérol	8	5
Cholestérol + salicylate de silanol	5	0
Cholestérol + citrate de silanol	4	1

Tableau II. — 2e expérience.

Résultats. — Comme on peut le voir ces différents composés siliciques diminuent notablement les lésions athéromateuses macroscopiques ; la fréquence des plaques est globalement chez les témoins de 80 % et chez les animaux traités de 28 % ; les corps les plus actifs nous paraissent être les composés silico-organiques qui par ailleurs sont fort bien tolérés par la paroi veineuse.

Sur le plan histologique outre les plaques athéromateuses nous avons noté : 1) chez les témoins : une fréquente altération des fibres élastiques (chez 15 lapins sur 22) qui sont fines et fragmentées, une augmentation de la métachromasie de la substance fondamentale dans la région sous-intimale et la média ; 2) chez les animaux traités : une altération rare des fibres élastiques (chez 6 lapins sur 23) ; mais le fait intéressant est que les fibres élastiques sont non seulement intactes mais souvent plus nombreuses et plus épaisses au niveau des 3 tuniques aortiques sans prolifération du collagène. Cette réaction est particulièrement nette avec les composés organosiliciques. Par ailleurs la métachromasie de la substance fondamentale est augmentée comme chez les témoins.

Enfin si les plaques athéromateuses sont beaucoup moins fréquentes que chez les témoins il existe malgré tout souvent une discrète surcharge lipidique de l'intima et de la média. Du reste le dosage chimique des lipides de la paroi artérielle donne des chiffres comparables chez les animaux témoins et traités : animaux traités, lipides : 61 ± 37 mg par gramme d'aorte ; animaux témoins, lipides : 68 ± 13 mg par gramme d'aorte.

Discussion. — Nous pensions au départ que l'action antiathéromateuse du silicium était due à une augmentation de l'imperméabilité de la paroi artérielle, peut-être par polymérisation des mucopolysaccharides de la substance fondamentale. En effet le silicium diminue la perméabilité du tissu conjonctif (Holt et Osborn) et nous avons montré qu'il diminuait la diffusion intradermique d'un colorant.

En réalité sans nier cet effet nous croyons que le pouvoir antiathéromateux du silicium tient à ce qu'il maintient l'intégrité des fibres élastiques et en favorise même le développement, la surcharge lipidique étant alors tolérée sans production de plaque athéromateuse par un processus biochimique ou enzymatique qui nous échappe actuellement.

Les fibres élastiques contiennent normalement une teneur en silicium très proportionnelle à leur intégrité ; elle est de 500 µg par gramme d'élastine sur une artère humaine normale. Ce taux est plus élevé que dans le collagène aortique tout en étant inférieur à celui des mucopolysaccharides de l'aorte.

Il est donc possible que le silicium, élément capable de favoriser la polymérisation, excite par là même la fibrillogénèse au niveau du tissu élastique.

Cette activation de la fibrillogénèse, nous l'avons vérifiée avec le collagène soluble : la présence de silicate de soude en faible quantité accélère *in vitro* la formation de fibrilles collagènes. Etant donné ce fait, l'on pourrait craindre que le silicium ne favorise la réaction collagène au niveau de la paroi artérielle ; aux doses ou nous avons utilisé ces composés siliciques, l'action sur le développement des fibres élastiques est franche sans réaction appréciable du collagène. La dose la plus efficace nous paraît être la plus faible c'est-à-dire celle que nous avons employée pour le salicylate de silanol soit 0,007 g tous les 2 jours. Ce composé organosilicique est celui qui est le plus actif tant par son action antiathéromateuse que par son effet sur la préservation et le développement des fibres élastiques.

Actuellement seuls les citroflavonoïdes sont capables d'après les travaux de Leger, Babin, Beauvieux et Coustou, de favoriser la croissance des fibres élastiques et de les préserver contre l'action destructrice de la surcharge lipidique et même de l'élastase.

En conclusion, les différents composés siliciques que nous avons employés et en particulier les dérivés silico-organiques possèdent un pouvoir antiathéromateux ; cette action nous paraît liée davantage à la protection et au développement des fibrilles élastiques de la paroi qu'à une augmentation de son imperméabilité aux lipides.

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Acta Medica Scandinavica. Vol. CXLV, fasc. II, 1953.

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Experimental Investigations on the Significance of the Size of the Particles in the Reaction of the Peritoneum to Amorphous Silica.

By

KARL DAVID LUNDGREN and ÅKE SWENSSON.

(Submitted for publication August 25, 1952.)

In the discussion of the pathogenesis of silicosis and the risks of contracting the condition the magnitude of the quartz particles has attracted great attention. It is generally considered that particles with a magnitude of 0.5—1—3 microns are of the greatest practical importance. They may have the strongest fibrogenous effect, easily make their way down into the alveoli of the lungs and are for the most part retained there (Gessner, Rüttner and Bühler (1949), Brown, Cook, Ney and Hatch (1950)).

Particles larger than 12—15 microns are to a large extent retained by the filtering apparatuses of the upper respiratory passages, and do not so frequently make their way down into the alveoli of the lungs. Their fibrogenous effect, moreover, seems to be less pronounced (Gardner 1938).

Tebbens, Schulz and Drinker (1945) have tried to show experimentally that the fibrogenous effect of quartz with a size of particle ranging between 0.5 and 3 microns is greatest for particles between 0.5 and 1 micron. Dart's (1948) statement that »particles of less than 0.6 micron may be of greatest importance in this respect» (causing silicosis) is not substantiated, and it is therefore not possible to express an opinion concerning its accuracy. Gessner, Rüttner and Bühler (1949) showed that the particles in lungs affected by silicosis were most commonly of the size of 0.9—1 micron. This latter finding may of course to a certain extent be due to the fact that smaller particles are not retained in the lung, but are exhaled with the expiratory air (Davis (1949), Brown, Cook, Ney and Hatch (1950)).

The view that the mechanical irritation of the tissues by the particles is the cause of the fibrosis is now being abandoned. Modern investigations have instead increasingly tended to favour the view that the chemical effect of dissolved quartz is of dominant importance. The size of the particles has thus once more become a matter of utmost concern, as solubility is dependent upon the increased surface which results from diminishing size of particle.

Gye and Purdy (1924) injected colloidal silica intravenously into animals. It proved to be very toxic, and caused necroses in, among other organs, the liver. Repeated injections of small amounts gave rise to fibrosis in the spleen. King (1950) gave a beautiful demonstration of the way in which the solubility of silica increases with diminishing size of particle. He did not, however, study finer particles than those of 1 micron.

Gardner (1938) reported studies of a very finely divided vitreous silica. With indirect methods the magnitude of the particles was found to be 0.0002 micron.[1] He injected a newly prepared 1 % suspension intravenously into a rabbit, according to a technique previously worked out by himself. With an observation-period of up to 1 year he was only able to observe a slight silicotic reaction in the liver. On the injection of a 10 % suspension intraperitoneally silicotic granulations appeared in the omentum in a number of animals. »Apparently the particles were so extremely small that most of them passed through the various capillary beds and were excreted in the urine.» King (1950) also showed an increase in the silica content in the urine after the administration of silica. Gardner summarizes thus: »... the experiments have demonstrated conclusively that the intensity of tissue reaction to silica is inversely proportional to the size of the particles.» Even if the figure given for the size of the particles implies a considerable error owing to the unreliability of the method of determination, these are doubtless the smallest particles that have so far been made the object of a systematic investigation, and the result is highly remarkable and significant.

The technical advances which have been made in the production of these very fine silica powders have made it possible to begin to reckon with their practical application in different industries. To judge from the preliminary investigations, they will be rather widely used, and they will therefore become very important from the point of view of occupational hygiene. For this reason we have undertaken a rather comprehensive investigation of the relationship between size of particle and the fibrotic tissue reaction following on the administration of amorphous silica.

Investigations of different forms of amorphous silica have given varying and often contradictory results. Gardner (1938) summarizes as follows: »The results obtained with these different forms of amorphous silica are perplexing and difficult to explain on any basis yet proposed. Perhaps no attempt should be made to explain them until more observations have been made.» This judgement still holds without reservation.

[1] Probably a printing error for 0.002. This quartz is generally referred to in the literature as »20 Angstrom silica».

Authors' own Investigations.

The silica powders that have been studied have been placed at our disposal by the Department for Technical Inorganic Chemistry of the Royal Institute of Technology, Stockholm.

For a general elucidation of the qualitative and quantitative tissue-reaction to quartz with particles of different magnitude we have chosen to use the technique with a single intraperitoneal injection of the substances in sterile suspension in physiological saline solution, introduced by Miller and Sayers (1934). The method is simple and easy to carry out; and it has been shown by different authors that the tissue-reaction to silica is general and uniform, with no essential difference in reaction in the various organs. One is probably justified in taking a positive result as the basis for conclusions concerning the substance tested from the point of view of the fibrogenic effect. In the case of a negative result, the possibility that the substance tested may give rise to fibrosis under other experimental conditions, *e. g.* on repeated exposure over a very long period, cannot be excluded.

Guinea-pigs weighing about 300 G at the beginning of the experiment were used for the tests. The animals were killed after an observation-period varying from 1 to 7 months. At postmortem examination the appearance of the peritoneum on the anterior abdominal wall was noted, as was also the appearance of the omentum and the abdominal organs. Different parts were taken for histological sectioning, the sections being stained with Hematoxylin-eosin, Gömöri's reticulin staining method, and a collagen staining method.

Group A. Crystalline Silica.

In order to get a starting point for the assessment of the reaction of the tissues to the finely divided amorphous silica powders used in this investigation, we tried the effect of ordinary ground, crystalline silica in a series of animals. The size and form of particle of the powder used may be seen from fig. 1. Doses of 25—100 mg/kg were injected, and the animals were then kept under observation for 1—3 months. We did not use longer periods of observation, as we were interested in the initial reaction in particular. During the entire observation-period the animals were lively and appeared in good health. Post-mortem examination showed in all of them rather large, firm, yellowish-red to greyish-red proliferations in the peritoneum of the anterior abdominal wall. In most cases the omentum was thickened and showed a diffuse scattering of larger and smaller whitish, firm granulations. It also showed frequent adhesions between different parts and with the intestine. Microscopically a very heavy, massive granulation-tissue with fibroblasts and an abundance of giant cells was observed. After a couple of months disintegrating giant cells were seen. Certain regions showed an extensive lymphocytic infiltration. The proliferations increased in size during the entire observation-period, and had an indistinctly defined boundary with the adjacent tissue. With reticulin staining one could see the formation of fibrils increasing during the period of observation. This reaction is the typical reaction to crystalline quartz described in the literature. Cf. figs. 2—5.

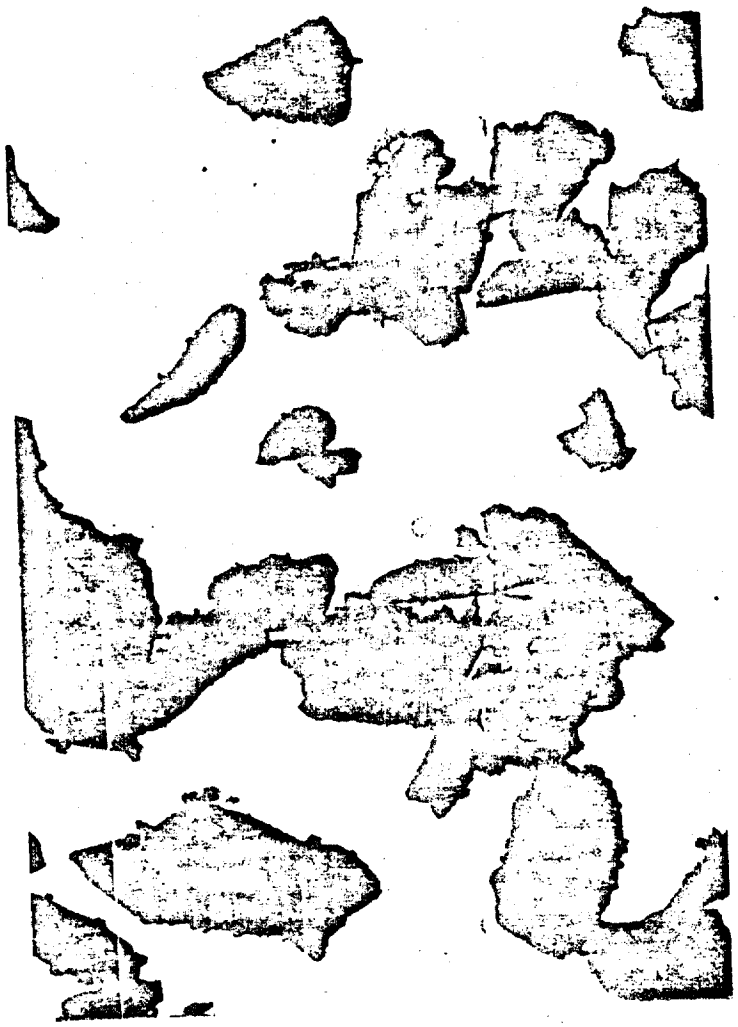

Fig. 1. Electron microphotograph of the particles of the used sample of crystalline silica (5,000).

Fig. 2.

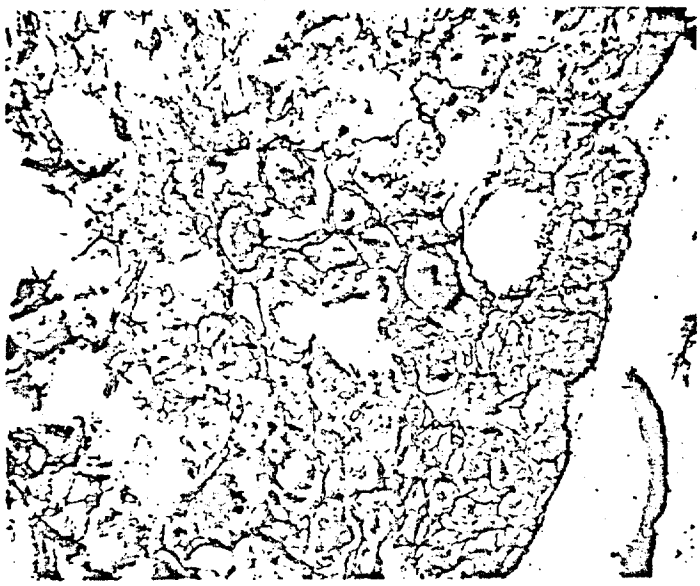

Fig. 3.

Fig. 2 and 3. Sections from the omentum of a guinea pig, injected intraperitoneally one month previously with 20 mg crystalline silica. Staining Hematoxylin-eosin resp. Gömöri (140).

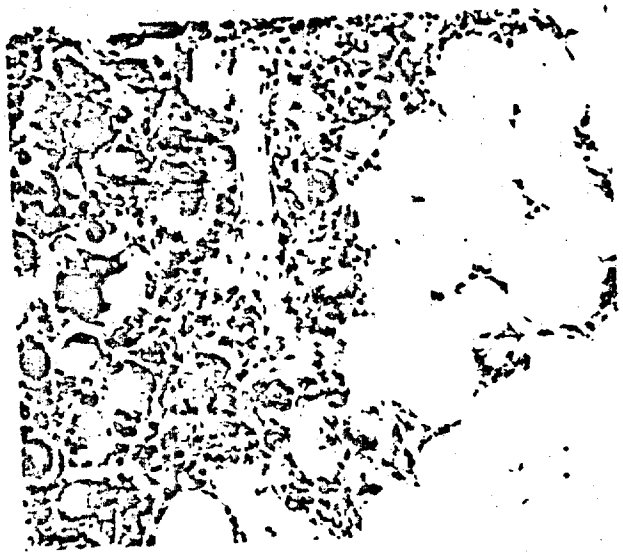

Fig. 4.

Fig. 5

Fig. 4 and 5. Section from the omentum of a guinea pig injected intraperitoneally 3 months previously with 20 mg crystalline silica. Staining: Hematoxylin-eosin, resp. Gomöri (. 140).

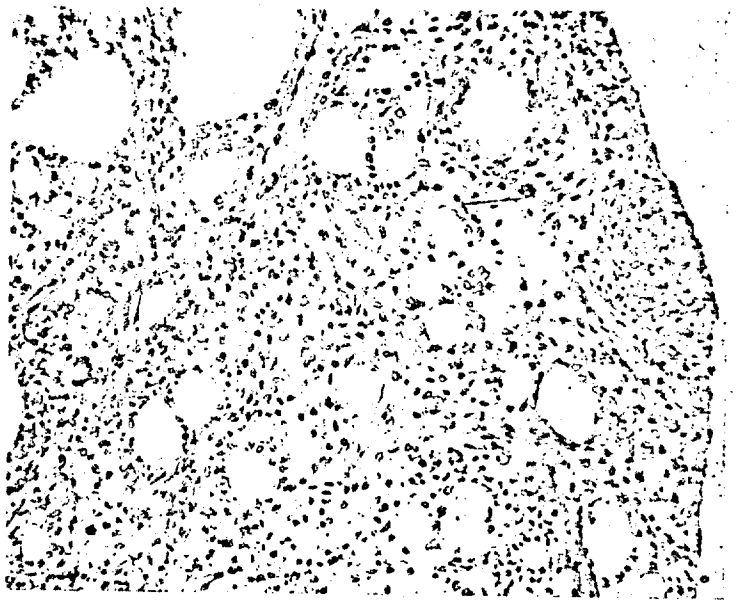

Fig 6. Section from the omentum of a guinea pig, injected intraperitoneally one month previously with 30 mg amorphous silica. Staining: Hematoxylin-eosin resp. Gömöri.

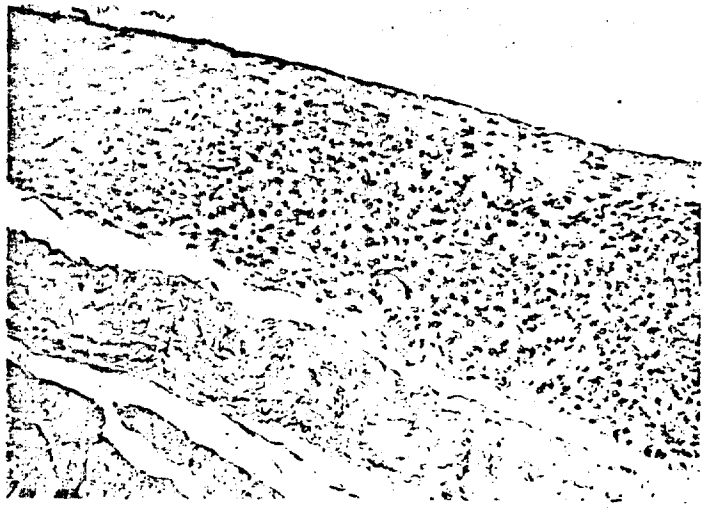

Fig. 7.

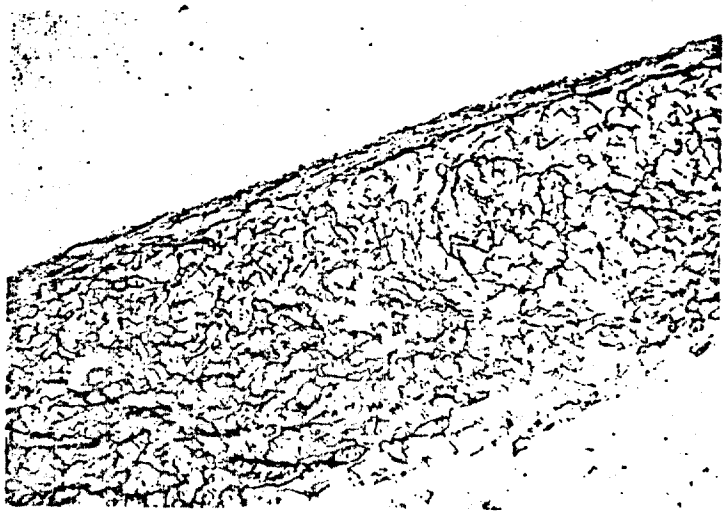

Fig. 8.

Fig. 7 and 8. Section from the peritoneum of the anterior abdominal wall of a guinea pig, injected intraperitoneally 3 months before with 16 mg amorphous silica. Staining: Hematoxylin-eosin resp. Gömori (× 140).

LUNDGREN AND SWENSSON: The Reaction of the Peritoneum to Amorphous Silica.

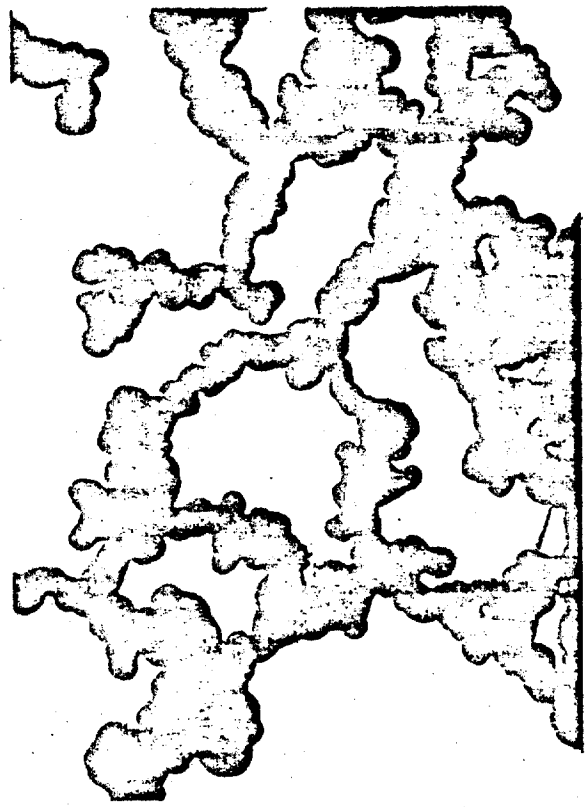

Fig. 9. Electron microphotograph of the particles of the sample of amorphous silica I (× 30,000)

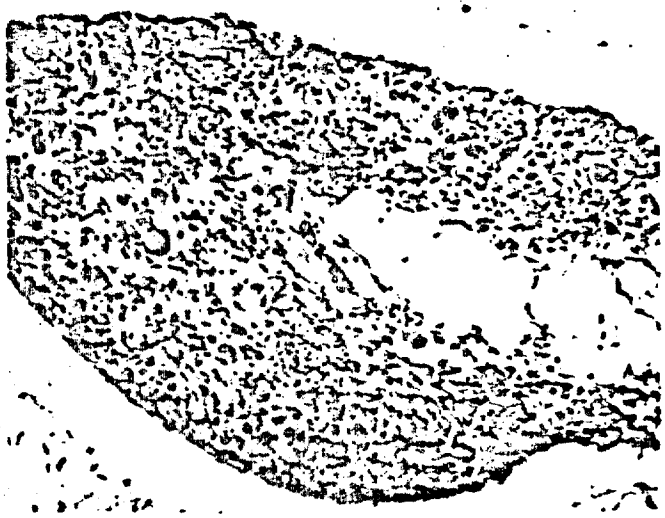

Fig. 10.

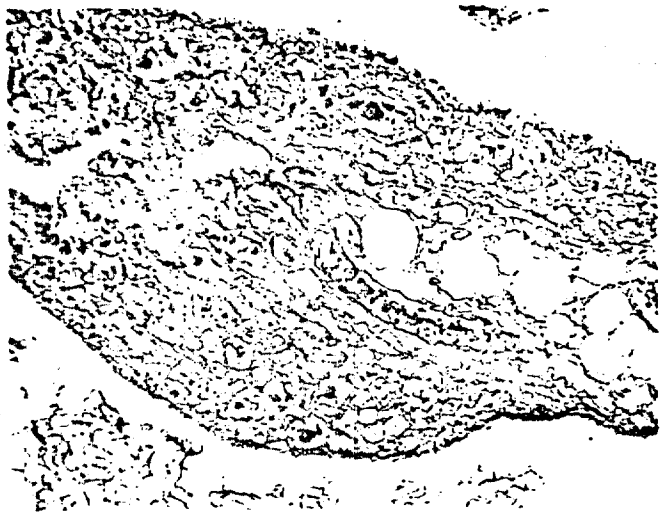

Fig. 11.

Fig. 10 and 11. Section from the omentum of a guinea pig, injected intraperitoneally one month previously with 5 mg amorphous silica I. Staining: Hematoxylin-eosin resp. Gomori (× 140).

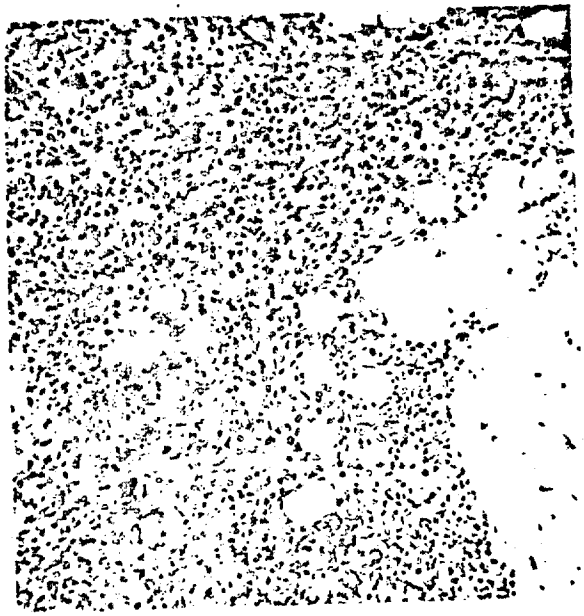

Fig. 12.

Fig. 13.

Fig. 12 and 13. Section from the omentum of a guinea pig injected intraperitoneally 2½ months earlier with 10 mg amorphous silica. Staining: Hematoxylin-eosin resp. Gömöri (× 140).

Fig. 14.

Fig. 15.

Fig. 14 and 15. Section from the omentum of the same animal as fig. 11 and 12. Staining: Hematoxylin-eosin resp. Gömori (. 140).

Fig. 16.

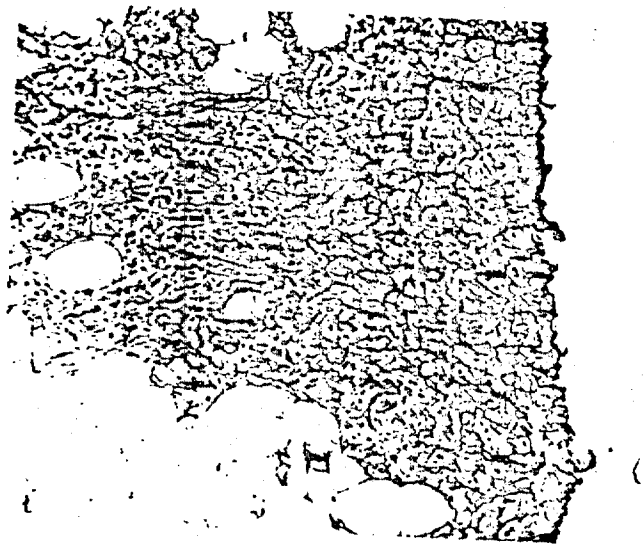

Fig. 17.

Fig. 16 and 17. Section from the omentum of a guinea pig injected intraperitoneally 7 months previously with 10 mg amorphous silica. Staining: Hematoxylin-eosin resp. Gömöri (× 14 .

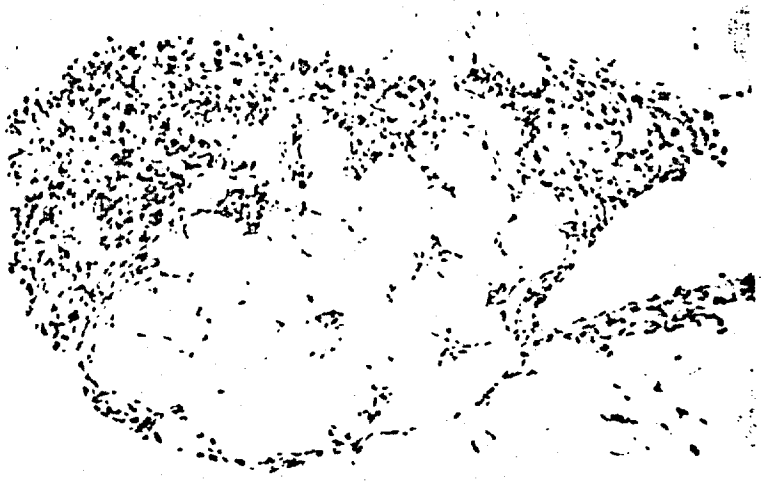

Fig. 18.

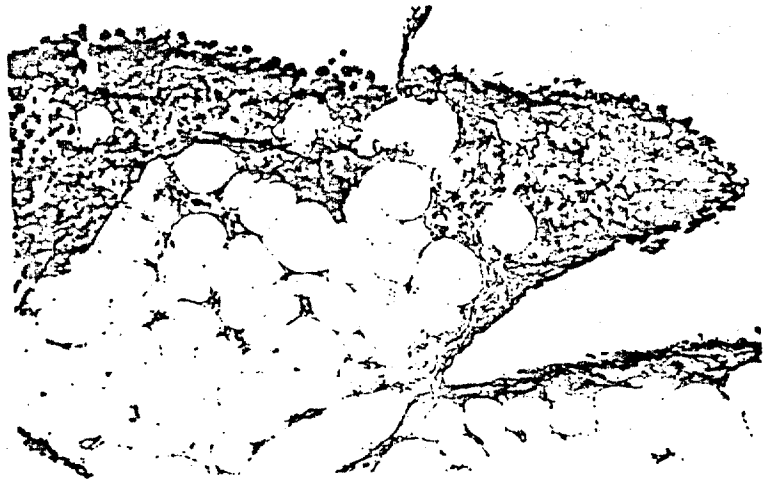

Fig. 19.

Fig. 18 and 19. Section from the omentum of a guinea pig injected intraperitoneally 7 months previously with 10 mg amorphous silica. Staining: Hemotoxylin-eosin resp. Gomori (V. 140).

LUNDGREN AND SWENSSON: The Reaction of the Peritoneum to Amorphous Silica.

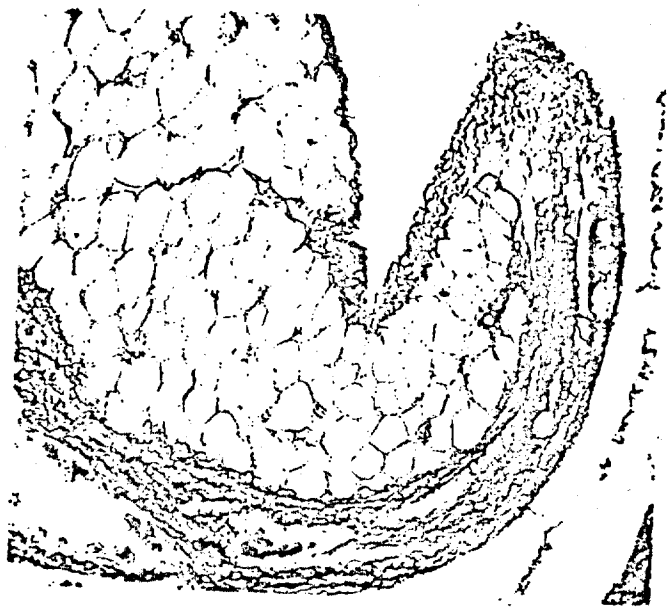

Fig. 20.

Fig. 21.

Fig. 20 and 21. Section from the omentum of a guinea pig, injected intraperitoneally 1 month previously with 20 mg amorphous silica III. Staining: Hematoxylin-eosin resp. Gömöri (× 146).

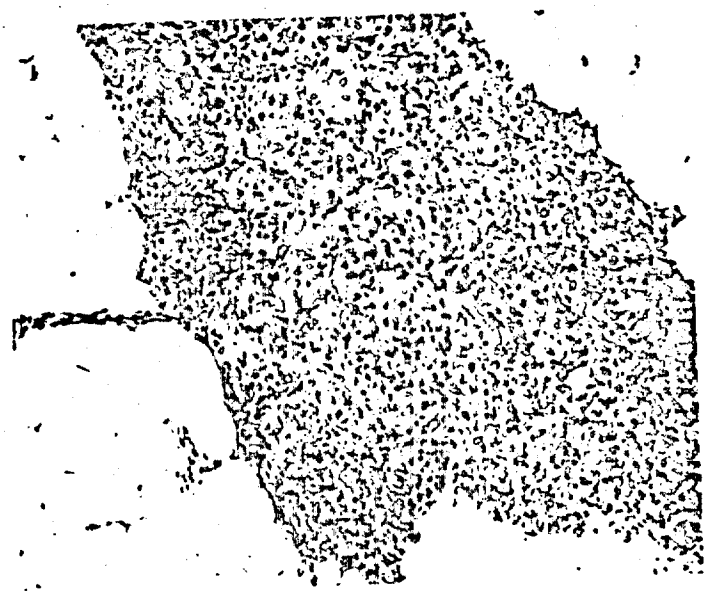

Fig. 22.

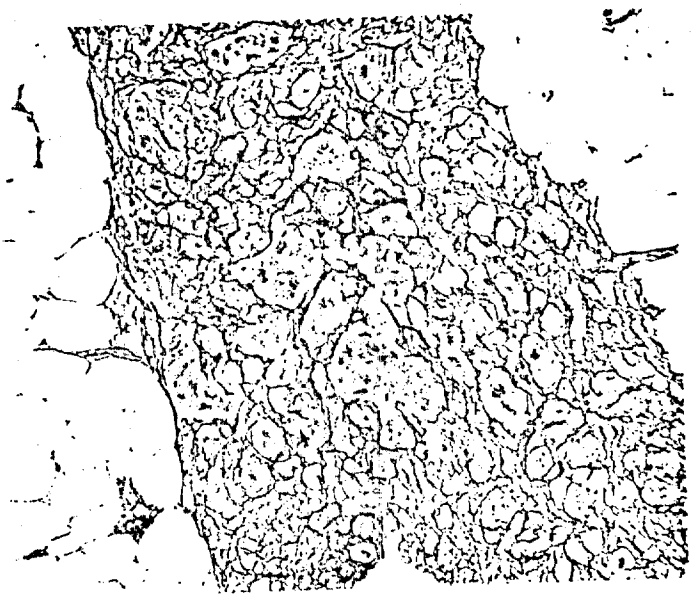

Fig. 23.

Fig. 22 and 23. Section from the omentum of a guinea pig injected intraperitoneally 2½ months previously with 10 mg amorphous silica III. Staining: Hematoxylin-eosin resp. Gömöri (× 140).

LUNDGREN AND SWENSSON: The Reaction of the Peritoneum to Amorphous Silica.

Fig. 24.

Fig. 25.

Fig. 24 and 25. Section from the omentum of a guinea pig injected intraperitoneally 7 months previously with 20 mg amorphous silica III. Staining: Hematoxylin-eosin resp. Gömöri (× 140)

Fig. 26. Electron microphotograph of the particles of the sample of amorphous silica V. [illegible] 30,000×

Fig. 27.

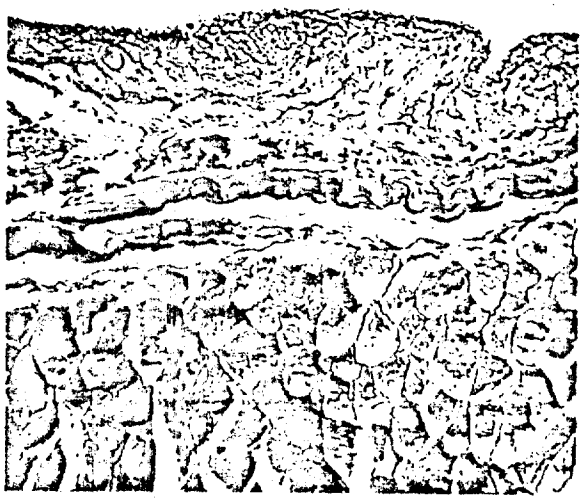

Fig. 28.

Fig. 27 and 28. Section from the peritoneum of the anterior abdominal wall of a guinea pig injected intraperitoneally 1 month previously with 20 mg amorphous silica V. Staining: Hematoxylin-eosin resp. Gomori (× 110).

Fig. 29.

Fig. 30.

Fig. 29 and 30. Section from the omentum of a guinea pig injected intraperitoneally 1 month previously with 20 mg amorphous silica V. Staining: Hematoxylin-eosin resp. Gomöri. (× 140).

Fig. 31. Section from the omentum of a guinea pig injected intraperitoneally 3 months previously with 10 mg amorphous silica V. Staining: Hematoxylin-eosin (× 140).

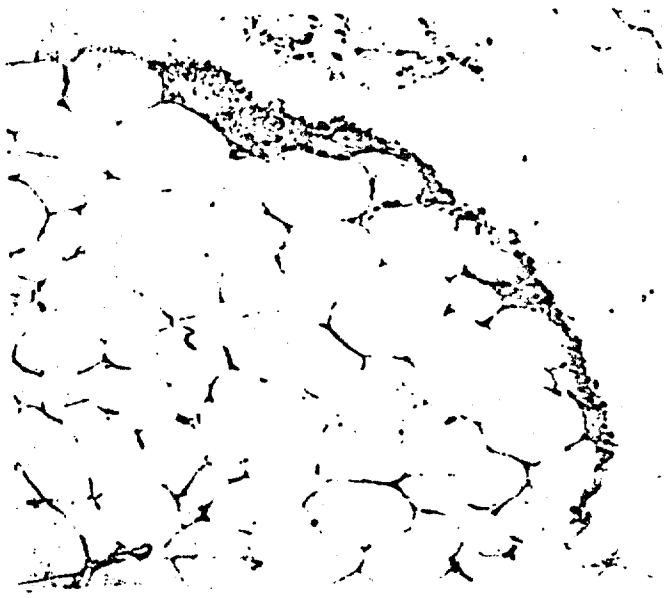

Fig. 32.

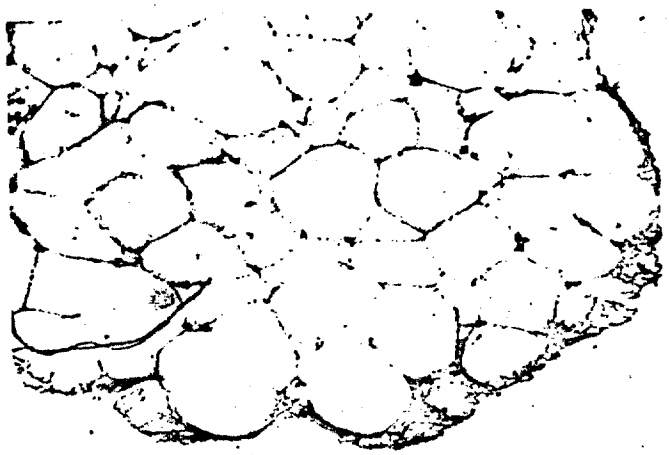

Fig. 33.

Fig. 32 and 33. Section from the omentum of a guinea pig, injected intraperitoneally 7 months previously with 10 mg amorphous silica V. Staining: Hematoxylin-eosin (× 140).

[illegible]DGREN AND SWENSSON: The Reaction of the Peritoneum to Amorphous Silica.

Group B. Amorphous Silica; Coarse Particles.

This powder was the first we received for investigation. Unfortunately, owing to a misunderstanding, no determination of size of the particles was carried out. The supplier states, however, that the particles were larger than those of the samples mentioned below.

Of this powder 25—400 mg/kg was injected. During the observation-period (1—3 months) the animals were throughout lively and unaffected. On post-mortem examination after one month it was possible to observe small, yellowish, semi-transparent granulations on the peritoneum of the anterior abdominal wall. After 2—3 months these were less apparent, resembling more patches or very flat papules. They had a quite different appearance from the granulations appearing in group A after the injection of the ground crystalline silica. In the animals in group B there was also a scattering of small whitish granulations in the omentum, though these were less extensive and smaller in size than those in group A.

Microscopically, the picture was strongly reminiscent of the one seen in group A with the crystalline silica, but nevertheless there were distinct differences between the two. The granulation-tissue was very rich in cells and compact. The polymorphic nature of the cells was less marked. There were numerous giant cells, but these were in general smaller than those in group A. The proliferations seemed more sharply defined in relation to the surrounding tissue than those in group A. Reticulin fibrils occurred to about the same extent as in group A. Cf. figs. 6—8.

Group C. Amorphous Silica I.

These silica particles were round in form and had a diameter of about 61 millimicrons. The difference in size was not particularly great. There was some tendency to agglomeration in the sample. Fig. 9.

The guinea-pigs were given a single intraperitoneal injection of 60 mg/kg. They were killed after 1—7 months. During the entire period of observation they appeared to be totally unaffected and showed normal weight curves.

Post-mortem examination revealed small proliferations of a size up to that of a pin head in the peritoneum of the anterior abdominal wall after an observation period of 1 month. Using smaller doses it was not possible to observe such proliferations at the post-mortem examination after $2^1/_2$ and 7 months. With the larger doses the picture at these later dates was unchanged as compared with the post mortem result after 1 month.

One month after the injection it was possible to find in the omentum proliferations with a rather characteristic appearance: Cellular polymorphism and numerous but rather small giant cells. In places a fairly extensive infiltration of round cells was observed. The reticulin fibrils were fine and sparsely distributed. Cf. figs. 10—11.

After $2^1/_2$ months much the same type of granulation-tissue but with a richer production of fibrils was found, cf. figs. 12—13. Of more common occurrence, however, was an other type of granulation; cf. figs. 14—15. The tendency to pro-

liferation was here less marked, and the cell-polymorphism much less pronounced. The reticulin was also sparser.

Even after an observation-period of 7 months two different types of reaction were found. The one was more compact, more proliferative and with a more pronounced cell-polymorphism and marked reticulin formation; cf. figs. 16—17. The other was less proliferative and showed a rather uniform cell-picture and only slight reticulin formation; cf. figs. 18—19.

With this long observation period the reticulin formation was more distinct than in the two previous experimental groups. In fig. 17, for example, it is very apparent. What this difference in the appearance of the types of granulation may be due to we cannot at present decide. It may possibly be connected with the quantitative distribution of the silica in different parts of the omentum. On the other hand, one always gets a typical granulation when coarser particles are supplied, if any reaction at all is forthcoming. It is also possible that the difference is due to qualitative differences in distribution over the omentum, so that particles with different degrees of agglomeration are deposited in different places. It should be possible to decide this by later determinations of the size of the particles in granulations of different type.

Group D. Amorphous Silica III.

The size of these particles is about 14 millimicrons, and they are round in form. In this preparation, too, there is some tendency to agglomeration.

After a single intraperitoneal injection of 6—60 mg/kg the guinea-pigs were allowed to live for 1—7 months. Throughout the entire observation period they showed normal weight curves and appeared quite healthy.

Post-mortem examination showed very small granulations in the peritoneum of the anterior abdominal wall in some of the animals for all the doses after an observation period of one month. With longer periods of observation the parietal peritoneum was in practically all the cases normal. The only sign of a change in tissue having taken place was the frequently occurring adhesions between different parts of the omentum and between the omentum and the intestine. Further, minimal granulations were observable in the omentum of a number of the animals.

One month after the injection, microscopic examination revealed, here and there in the omentum, small compact proliferations clearly demarcated from the adjacent tissue and with a very homogeneous cell content. None or few giant cells were seen. The reticulin formation was rather sparse; cf. figs. 20—21. After 2½ months the picture was in general unchanged. Occasionally one might find a more marked proliferation, though this was still clearly demarcated from the surrounding tissue; figs. 22—23. Here, too, the cell-polymorphism was more pronounced, and the reticulin formation more extensive. After 7 months all the sparsely occurring small proliferative areas in the omentum were of the type shown in figs. 24 and 25. The proliferation was stationary. The cells were of rather mature fibrocyte type, the reticulin fibrils fine and sparsely occurring.

Group E. Amorphous Silica V.

The size and form of these particles may be seen in fig. 26. They are round and have a diameter of about 10 millimicrons. A certain tendency to agglomeration is observable.

After a single intraperitoneal injection of 6—60 mg/kg the animals were observed for 1—7 months. During the whole of this time they appeared quite healthy. Normal weight curves.

On post-mortem examination after one month it was possible to observe in one or two of the animals an occasional very small proliferative area in the parietal peritoneum of the anterior abdominal wall. In a few of the animals the omentum was adhering to the intestine or the anterior abdominal wall. In other respects there was nothing pathological to be seen in the omentum.

With longer observation periods no proliferation was seen in any of the cases in the peritoneum of the anterior abdominal wall. Nor were any proliferative areas visible in the omentum of these animals.

Microscopically, slight cell reactions with very homogeneous cell-content were found in the omentum after one month. No giant cells. No tendency to proliferation. After $2^1/_2$ months proliferation was still less, and at 7 months very slight. Reticulin formation was almost non-existent. Cf. figs. 27—33.

Survey of the Findings.

In this experimental series one finds a continuous change in the picture of the reaction to the ordinary, coarse-grained ground silica, to that seen in the amorphous silica samples with diminishing size of particle up to the amorphous silica V, having the smallest size of particle. In the macroscopic picture it can be seen that the proliferations diminish in size and that their appearance also changes. In group A (relatively large particles of crystalline silica), large, coarse, firm, opaque proliferative areas are found in the peritoneum of the abdominal wall and in the omentum. In group B, with smaller particles of amorphous silica, though still with a fair number of relatively coarse particles, large proliferations are found in the peritoneum of the abdominal wall. These, however, have a different appearance: they are softer, semi-gelatinous and yellowish red in colour. As the size of the particles diminishes, the proliferations in the remaining experimental groups become increasingly small. It should be noted that in the groups A and B even 25 mg silica gave rise to extensive proliferation, whereas 20 mg only caused a slight reaction and no reaction at all in the group D and E. In the later series, furthermore, it is seen that the reaction is most marked with the shortest observation periods, and becomes increasingly less marked the longer the period of observation. This holds for both the abdominal-wall granulations and granulations in the omentum.

It is conceivable that the difference in the reaction on the anterior abdominal wall is connected with differences in the weight of the particles, the coarse particles sedimenting more easily to the abdominal wall and there giving rise to a reaction,

while the finer and lighter particles in the later groups sediment more slowly and may conceivably be distributed more evenly in the abdominal cavity. In this case one would get a progressively slighter reaction in the peritoneum of the anterior abdominal wall with diminishing size of particle. This assumption, however, can scarcely explain why at the same time the reaction in the omentum becomes less with diminishing size of particle, nor why the reaction to the finest silica powders is most pronounced with the shortest observation period (1 month), diminishing thereafter with increasingly prolonged period of observation. Nor can it explain why the granulations which actually appear have a totally different appearance in group E than in groups A and B.

As has already been pointed out, one finds above all in groups C and D two different types of granulation. This may possibly be explained by the tendency to agglomeration of the particles observable in these powders. The occurrence of particles of different size acts in the same way. The sedimentation might conceivably be different for the agglomerates and the rather isolated fine particles. This problem is at present the subject of further investigation.

In our opinion the differences in tissue-reaction that we have observed can only be explained as a consequence of the diminishing size of particle for the silica powders used in the different experimental groups. Whether the finest samples may possibly be dissolved, and eliminated in this way, or whether they are absorbed and carried away by macrophages is a question that we are at present not able to decide. One or the other of these possible alternatives might of course explain the fact that the reaction is less pronounced with the finer particles, and also the fact that it is in this case less pronounced with the longer periods of observation. This problem is being considered in connection with current experiments.

References.

Brown, J. H., K. M. Cook, F. G. Ney and T. Hatch: Am. J. Publ. Health *40:* 450—459 (1950). — Dart, E. E.: Patty's Industrial Hygiene and Toxicology, Vol. 1: 486. New-York, London 1948. — Davis, C. N.: Brit. J. Industr. Med. *6:* 245—253 (1949). — Gardner, L. U.: Lanza's Silicosis and Asbestosis. Oxf. Univ. Press (1938). — Gessner, H., J. R. Rüttner and H. Bühler: Schweiz. med. Wochenschr. *79:* 1241—1245, 1258—1262 (1949). — Gye, W. E. and W. J. Purdy: Brit. J. Exper. Pathol. *3:* 75—85 (1922). — King, E. J.: Die Staublungenkrankheiten. Wissenschaftl. Forschungsber. *60:* 212—230 (1950). — Miller, J. W. and R. R. Sayers: Publ. Health Rep. *49:* 77 (1934). — Tebbens, B. D., R. Z. Schulz and Ph. Drinker: J. Industr. Hyg. *27:* 199—200 (1945).

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522-523

Haemolytic Activity of Asbestos and other Mineral Dusts

SILICA in the particulate form has been shown to have a lytic action on intracellular structures[1,2] and on erythrocytes[3,4]. The present investigation has shown that chrysotile asbestos is as potent a haemolytic agent as silica powder. Other forms of asbestos lysed erythrocytes only after prolonged incubation with them, and a wide range of mineral dusts was inactive. The haemolytic action of chrysotile was completely prevented by disodium versenate and simple phosphates but only partially by polyvinylpyridine-*N*-oxide and aluminium, both of which protect cells against lysis by silica[4].

The following finely ground materials were used: crystalline and amorphous silica, silicic acid powder, silica gel, chrysotile, amosite, anthophyllite and crocidolite asbestos, serpentine, aluminium oxide, 'Sephadex G-25', high and low rank coal dust, mine dust, colloidal carbon, talc, kaolin, glass powder, carborundum and diamond dust.

For the majority of tests a standardized 2 per cent suspension of washed sheep erythrocytes in a veronal buffered isotonic saline buffer (*p*H 7·4) was used. Four millilitres of this suspension were completely lysed by 50 mg of chrysotile when this was incubated at 37° C with a fixed volume (4 ml.) of suspension for a period of 50 min. This was taken as the standard time for all subsequent quantitative tests. The effects of various agents in preventing haemolysis by silica and chrysotile were investigated after the minerals had been coated with suitable concentrations of the protective materials, followed by repeated washing with saline.

Significant haemolytic activity was found for chrysotile, serpentine and all forms of silica tested. (The particle size ranges of the active dusts were the following: chrysotile, 11 per cent $<0{\cdot}5\mu$; 37 per cent $0{\cdot}5$–2μ; 33 per cent 2–5μ; 15 per cent 5–10μ; 4 per cent 10–50μ length; serpentine, very fibrous, no size distribution obtained; crystalline silica, 15 per cent $<2{\cdot}5\mu$; 49 per cent $2{\cdot}5$–5μ; 30 per cent 5–$7{\cdot}5\mu$; 5 per cent $7{\cdot}5$–10μ; amorphous silica, 250–400 Å; silicic acid powder, not determined; silica gel, 10–40μ.) The remaining powders were either completely inactive or only weakly lytic. Quantitative values for the powders found to be lytic are shown in Table 1.

Table 1. HAEMOLYTIC ACTIVITY OF SALINE SUSPENSIONS OF CHRYSOTILE ASBESTOS AND SILICA DUSTS

Mineral	Per cent haemoglobin in supernatant
Fully haemolysed erythrocyte suspension	100
Aerosil (colloidal silica)	100
Crystalline silica	88†
Crystalline silica (acid-washed)*	89†
Silicic acid powder	68‡
Silica gel	66‡
Chrysotile asbestos	78‡
Serpentine	65‡

* Silica repeatedly washed with boiling hydrochloric acid to remove surface metals.
† No adsorption of haemoglobin – some erythrocytes left intact.
‡ Lower values caused by adsorption of haemoglobin on these dusts – erythrocytes completely haemolysed.

The rate of haemolysis by chrysotile was greater than that shown by silica, the apparent lower percentage haemolysis by the asbestos (Table 1) being the result of the adsorption of released haemoglobin during the early stages of lysis. Haemolysis by chrysotile took place rapidly, with the immediate adsorption of haemoglobin on the asbestos fibres. Because of this, the degree of haemolysis in the supernatant (as measured colorimetrically) never reached 100 per cent. This was also found in the case of the adsorbents, silicic acid and silica gel.

Several samples of amosite, anthophyllite and crocidolite asbestos gave consistently negative results when incubated for 50 min at 37° C. In the case of the first two, haemolysis started only after 5 h at 37° C and reached its maximum after 24 h. Crocidolite had a very mild haemolytic effect after 24 h.

Haemolysis by chrysotile was inhibited by serum and by low concentrations of disodium ethylenediamine tetraacetic acid (EDTA) and phosphate ions (35 mg PO_4^{3-}) in the standard test medium. In the case of EDTA it was found that 20 μmoles of chelating agent in 4 ml. of erythrocyte suspension at 37° C completely prevented the haemo-

lytic action of 50 mg chrysotile (Fig. 1). On the other hand, 2 per cent aluminium chloride, 1·25 per cent gelatine and 0·9 per cent polyvinylpyridine-*N*-oxide (all effective protective agents against haemolysis by silica) retarded lysis by chrysotile for short periods but failed to prevent it completely.

The potent haemolytic action of both chrysotile and silica has been demonstrated. The influence of any mechanical action of these materials on the erythrocyte surface can be largely excluded because other abrasive dusts, for example, carborundum, diamond dust and glass powder, gave little or no haemolysis under the same experimental conditions. Recent work[4] on silica suspensions and inert dusts confirms this observation.

The results of our investigations with protective agents suggest that silica and chrysotile differ in the nature of their haemolytic action. There is evidence that silicic acid interacts effectively with lipoprotein monolayers[5,7] and that hydrogen bonds are formed between keto-imino-acids and the hydroxyl groups of the silicic acid[6,7]. Recent investigations of the damage by silica to the phagosomal membrane of macrophages[1-3] have indicated that silica exerts its lytic effect by forming hydrogen bonds with the biological membrane of the phagosome. The preventive effect of polyvinylpyridine-*N*-oxide at the subcellular level was ascribed after experimental evidence[3] to the preferential formation of hydrogen bonds between the silica surface and the polymer. That the latter interacts more extensively with the silica surface than with the erythrocyte membrane is shown by the low degree of protection obtained when blood cells are preincubated with the polymer only[3].

The marked depression of silica solubility by aluminium (and, presumably, the protective effect of this ion against phagosomal membrane lysis by silica)[1] is caused by the formation of aluminium hydroxide by aluminium in the ionic form reacting with the hydroxyl groups of the silica surface.

In the case of chrysotile, a fibrous silicate containing 40 per cent magnesium (as oxide), the partial protection given by aluminium and polyvinylpyridine-*N*-oxide indicates that if hydrogen bond formation takes place, this must be relatively slight and may be accounted for by the molecular configuration of chrysotile which would offer not only hydroxyl groups but cations for surface interaction. Polyvinylpyridine-*N*-oxide is a neutral co-ordinating agent and would be expected to interact with cations such as magnesium (II) ions only when suitable anions are available to maintain electrical neutrality (T. Nash, personal communication).

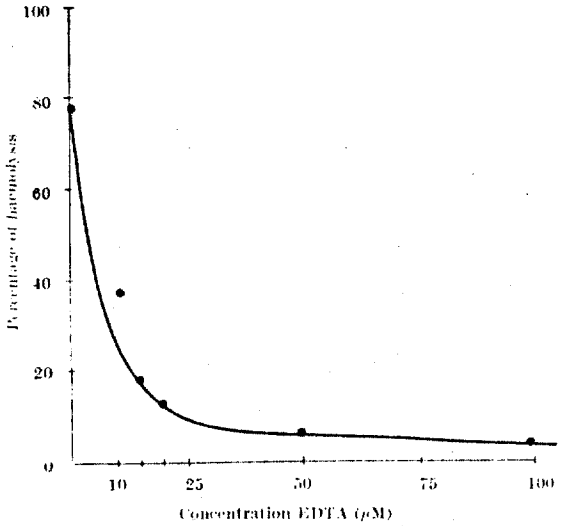

Fig. 1. Inhibitory effect of varying concentrations of EDTA on haemolysis by chrysotile (50 mg chrysotile, 2 ml. EDTA solution, 2 ml. erythrocyte suspension, 50 min incubation at 37° C; the highest value on the curve (78 per cent) represents the effect of untreated chrysotile on erythrocytes).

That magnesium in the ionic form is the principal cationic reactant on the surface of chrysotile in haemolysis seems to be indicated by the effective inhibitory action of EDTA, a material which also prevents lysis by macromolecules containing ionized iron[8]. This belief is supported by the inhibitory action of simple phosphates in the medium. Nash has suggested (personal communication) that non-ionic compounds (for example, polyvinylpyridine-*N*-oxide) or betaine-like compounds would be expected to protect cells against haemolysis by silica because of the nonionic character of the latter. If magnesium ions are the lytic agents, however, as appears to be the case with chrysotile, polyacids would seem to be suitable inhibitors. Good co-ordinators like phosphate and polyphosphate should also be effective. The iron in chrysotile (up to 2 per cent) appears to play no part in lysis by this form of asbestos, because no inhibitory effect was obtained in the presence of α,α′-dipyridyl in the medium. On the other hand, the presence of magnesium and calcium ions had no enhancing influence on the weakly lytic effect of crocidolite and amosite.

The haemolytic effects of chrysotile are of interest in view of the relative inactivity of the amphibole asbestos forms, crocidolite and amosite, and the pronounced activity of certain polymerized silicates which, unlike monomeric forms, bind firmly to the erythrocyte membrane[9]. The active fibrogenic properties common to the types of asbestos investigated here cannot yet be associated with their haemolytic characteristics, a relationship suggested for different forms of silica[1]. The inactivity of crocidolite, amosite and anthophyllite after incubation for 50 min suggested that if haemolytic activity is to be related to fibrogenicity, contact of these forms of asbestos with erythrocytes would have to be prolonged; the weak haemolytic activity observed by us for crocidolite, amosite and anthophyllite after extended incubation with erythrocytes supports this possibility.

Finally, it also seems clear that the highly active lytic property of chrysotile is related to the adsorptive capacity of this form of asbestos. The present investigation has shown that under the same conditions, chrysotile adsorbs five times more protein from serum than silica. More protein is required to "cover" the haemolytic sites of chrysotile than of silica; this is not surprising in view of the chemical structure and morphology of the two minerals. Chrysotile also has the highest Mg/Si ratio of the three forms of asbestos tested and differs also in that iron is not formally included in the lattice. It has a very high zero point of charge (*p*H 10–12), that is, over most of the *p*H range it will carry a net positive charge[10].

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[1] Harington, J. S., and Allison, A. C., *Med. Lavoro*, **56**, 474 (1965).
[2] Allison, A. C., Harington, J. S., and Birbeck, M., *J. Exp. Med.*, **124**, 141 (1966).
[3] Nash, T., Allison, A. C., and Harington, J. S., *Nature*, **210**, 259 (1966).
[4] Stalder, K., and Stober, W., *Nature*, **207**, 874 (1965).
[5] Schulman, J. H., and Rideal, E. K., *Proc. Roy. Soc.*, B 122, 46 (1937).
[6] Clark, S. G., and Holt, P. F., *Trans. Faraday Soc.*, **53**, 1509 (1957).
[7] Clark, S. G., Holt, P. F., and Wood, C. W., *Trans. Faraday Soc.*, **53**, 1500 (1957).
[8] Pickling, J., and Smith, G. M., *J. Chem. Path.*, **16**, 12 (1963).
[9] Chatterjee, P., Macleod, C. M., and White, P., *J. Gen. Physiol.*, **45**, 1417 (1962).
[10] Naumann, A. W., and Dresher, W. H., *J. Phys. Chem.*, **70**, 288 (1966).

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THE EFFECT OF COLLOIDAL SILICA ON BLOOD COAGULATION

by J. MARGOLIS

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SUMMARY.

Colloidal silica accelerates blood coagulation by adsorption and partial denaturation of a specific plasma protein, the Hageman factor. Quantitative measurements show that the activity of the silica depends on the particle diameter. It is suggested that geometrical relations between the silica and the protein molecules account for these differences in activity. The implications of certain anomalies of this reaction are considered.

INTRODUCTION.

The surface of silica is very active in the initiation of blood coagulation and of certain other related changes (Margolis, 1957, 1958a, 1959a). Although we have now a fairly coherent picture of these reactions in biological terms (Hardisty and Margolis, 1959; Margolis, 1959a, 1960a) very little is known about their physical aspects. In the course of previous work many different forms of silica were used. Quartz, tridymite, cristobalite, vitreous silica and kaolin were all excellent activating materials. They were used as granules, powders or suspensions and it was consistently found that the activity increased with the state of division of the solid, obviously owing to the increase of the specific surface area (cm.^{2}/g.). The most finely divided and therefore the most active preparations tested at the time were commercial silica aerogels and colloidal dispersions of 10-20 mμ particle diameter. But this trend did not continue with further diminution of the particle size. Silica sols made by neutralising dilute sodium silicate with HCl were almost totally inert in spite of their even smaller particle size (about 2 mμ) and hence proportionately greater surface area. Therefore somewhere between 10 and 2 mμ there is a critical minimum particle size, below which silica ceases to activate plasma (Margolis, 1959b).

Application of adequate methods for the preparation of well-specified silica dispersions and of measuring their clot-promoting ability made it possible to study quantitatively these relations. This investigation, a preliminary account of which was presented elsewhere (Margolis, 1960b), was undertaken in the hope that the results may shed some light on the physical basis of the plasma/surface reactions.

METHODS.

Vitreous silica.

Vitreous silica powders were prepared from ground silica tubing. The coarser particles were graded by passing through wire mesh sieves, retaining the 120-200 mesh fraction. They were then washed with three changes of deionised water and dried at 100° C. In order to exclude surface contamination by traces of adsorbed organic impurities, some samples were heated to 600° C. This treatment did not enhance their activity. Finer powders were graded by fractional sedimentation in deionised H_2O. The specific surface areas were estimated by adsorption of methylene blue (cf. Nevo *et al.*, 1955). A standard sample of glass microspherules was used as reference allowing for the difference in specific gravity between glass (2·8 in this case) and silica (2·2).

Colloidal silica.

The smallest particle sols were made by rapidly passing cooled solutions of 1-5 p.c. Na_2SiO_3 through a column of the cation exchange resin Zeo-Karb 225. The effluent (pH 3-4) was then stabilised at pH 8·5 by adding 2 p.c. ammonia. The colloidal particles thus prepared (2-3 mμ diam.) remain relatively stable for some days when kept at 4° C. Intermediate size particles (4-6 mμ) were prepared from the above by heating at 100° C. or autoclaving at 15 lb./inch pressure for 15-60 min. The initial pH was adjusted to various levels (5·5-8·5) and the resulting particle diameter was found to depend on pH, more alkaline suspensions yielding smaller particles in this range. Commercial preparations were used as a source of still larger particles (7-30 mμ). These were diluted with H_2O and also passed through the resin columns and adjusted to pH 8·5. The mean particle diameter was estimated by light scattering (courtesy of Mr. E. Elbing, Department of Physical Chemistry, University of Sydney), and in two cases (15 and 30 mμ) confirmed by electron microscopy (courtesy of Dr. H. Hoffman, C.S.I.R.O.). Since all these suspensions consist of fairly uniform spherical particles, the approximate surface area of each could be calculated from the particle diameter. As a corollary, the diameter of the irregular, ground particles of vitreous silica is therefore best expressed in terms of "equivalent spherical diameter" calculated from the known specific surface area. Immediately before use all

TABLE 1

Specifications of silica preparations

	Material	Surface area (m^2/g)	Equivalent spherical particle diameter*	Description
1	Vitreous silica	0·02	135 μ	Powder
2	Vitreous silica	0·07	39 μ	Powder
3	Vitreous silica	0·24	11·2 μ	Suspension
4	Vitreous silica	5·10	0·53 μ	Suspension
	Colloidal silica			
5	"Syton W20" (Monsanto)	90	30 mμ	Colloidal
6	"Ludox HS" (DuPont)	180	15 mμ	Colloidal
7	"Ludox SM" (Du Pont)	386	7 mμ	Colloidal
8	Heated silica sols (see text)	450-675	4-6 mμ	Colloidal
9	Unheated silica sols (see text)	900-1,350	2-3 mμ	Colloidal
10	Dried gels from 5-9	0·04 (external)	135 μ	Powder

* Diameter of spherical particles of equivalent surface area/g.

suspensions were adjusted to pH 7·5. The relevant physical specifications are set out in Table 1. Although the accuracy of these data is not of high order, the probable error being in the range of ± 20 p.c., this error is not significant when compared with the magnitude of the differences in biological activity of these preparations.

Dry gels.

Dry gels were obtained from the above colloidal dispersions by pouring them on to stainless steel trays and drying in a current of warm air. They were then crushed and treated as vitreous silica, retaining the 120-200 mesh fraction. The specific gravity of these gels was approximately one-half that of vitreous silica. For more detailed description of the properties of silica sols and gels the reader is referred to the monograph by Iller (1955).

Clot-promoting activity.

Clot-promoting activity was measured as follows. All surfaces with which blood or plasma came in contact were treated with silicone (M.S.200) diluted fifty times in petroleum ether. Fifty volumes of venous blood were mixed with one volume of 20 p.c. trisodium citrate and centrifuged at low speed to obtain platelet-rich plasma, and then at 2000g to obtain platelet-poor plasma. One part of platelet-rich plasma was frozen at − 20° C. for one hour, thawed and added to six parts of platelet-poor plasma. The mixture was used as the substrate in all the experiments. Silica suspensions (0·05 ml.) were delivered into a row of siliconed containers and mixed with 0·2 ml. of the substrate plasma. The mixtures were gently agitated for 2 min. at 37° C. and recalcified with 0·05 ml. of 0·1M $CaCl_2$ using an automatic delivery syringe. Conventional glass clotting tubes were found to be inade-

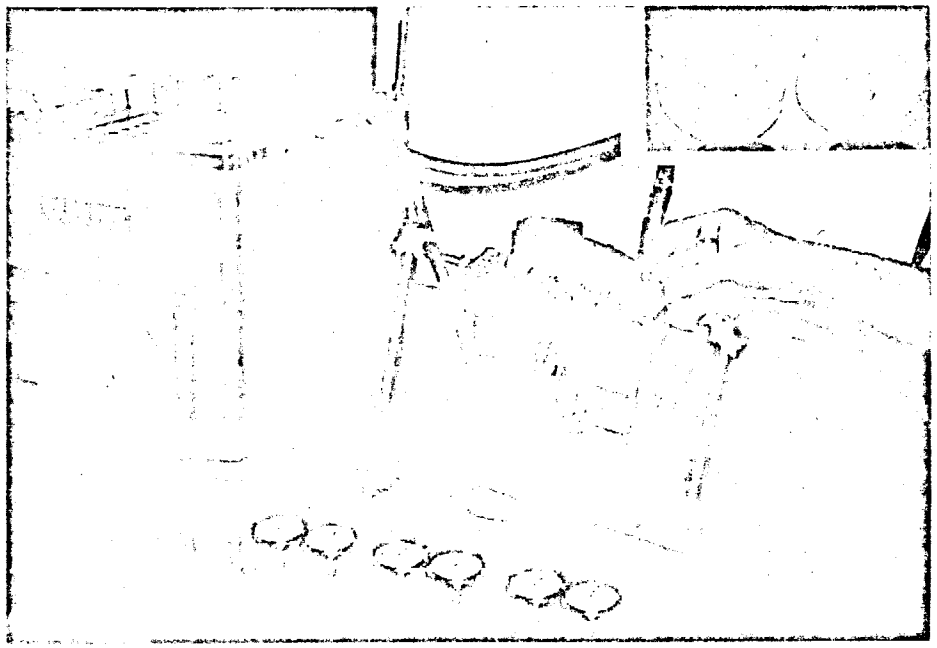


Fig. 1. Apparatus for accurate determination of clotting time. Inset shows crescents of plasma in the metal caps. See text.

quate for rapid and accurate work. The tests were therefore performed in disposable siliconed tin-plate caps ¾ in. × ½ in. which were fitted into specially cast sockets in a metal warm stage heated by circulating water at 37° C. The whole stage was mechanically rocked 90 times/min. It was also inclined about 15° to horizontal, which produced a smooth to and fro flow of the contents in a crescentic fashion along one edge of each tray (Fig. 1). The clotting times were measured using a separate stop watch for each parallel sample. Up to ten tests could thus be performed simultaneously and an extensive series (50-70 tests) completed before any significant ageing of plasma took place.

Tests for plasma kinin.

Tests for plasma kinin were performed on isolated rat uterus (Margolis, 1958a). The procedure was as described for coagulation experiments except that the substrate plasma was not enriched with platelets, activation was carried out at room temperature and instead of adding $CaCl_2$, 0·1 ml. of the plasma + silica mixture was applied for one minute to a horn of rat uterus in oxygenated Ringer's solution in 10 ml. siliconed organ bath at 30° C.

RESULTS.

Activity of suspensions.

The clotting time of recalcified plasma is quantitatively related to the amount of activating surface. When the clotting time is plotted against the concentration of powdered silica on a log/log scale a linear relation is obtained within a wide range of values (Margolis, 1957). This may be used as a basis for quantitative comparisons.

With the method described, the clotting times are reproducible well within 5 p.c. on the same sample of plasma (Margolis, 1958b). The reproducibility from day to day is somewhat less, mainly owing to small technical variations in the collection of blood. The usual clotting time with the optimal amount of silica is 45-50 sec., while that of the blank (i.e. with 0·05 ml. of H_2O instead of the suspensions) about 300 sec. Even with unsatisfactory plasma samples (due to traumatic blood collection) the internal relations are well maintained within each experiment, although all the clotting times are proportionately shortened.

Fig. 2 shows the results of a typical experiment. The clotting times were plotted as a function of the weight (2A) or surface area (2B) of the added silica. As may be expected, weight for weight the smaller particles of powdered vitreous silica are more active than the larger ones (1A). In fact, the activity is almost a direct measure of the specific surface area which is inversely proportional to the particle diameter (2B). With colloidal particle dimensions this relation no longer holds. Below 30 mμ the smaller particles are less active even on a weight basis, but since the activity is a function of the surface area the data in Fig. 2B are more informative in this respect.

The smallest particles simply fail to activate the plasma. They do not inhibit the action of larger particles. When mixtures of the two are added to plasma together the only effect is one of dilution of the active by the inactive suspensions (see Fig. 6).

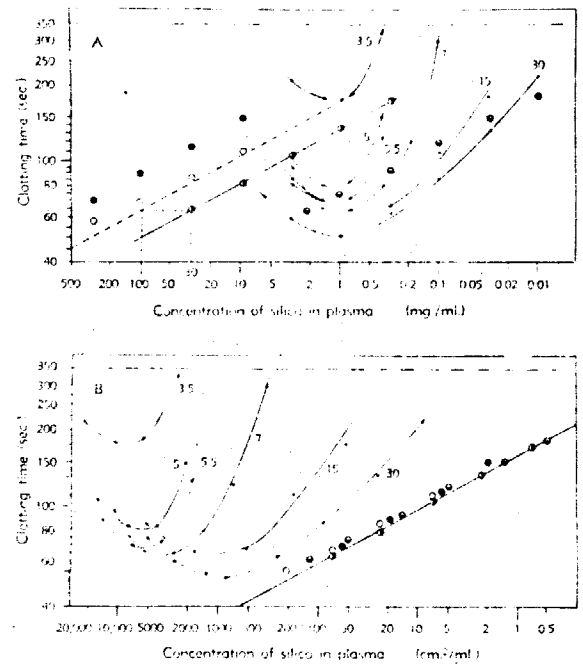


Fig. 2. Clotting time of recalcified plasma (see Methods) as a function of the concentration of silica in the plasma expressed as mg./ml. in (A) and as cm.²/ml. in (B). Equivalent spherical particle diameter of vitreous silica: (●) 135 μ; (○) 39 μ; (◑) 11·2 μ (reference standard); (◒) 0·53 μ. Colloidal silica (.): the figures beside the curves indicate the particle diameter in mμ. The method of calculating relative activity is illustrated by the interrupted lines in (A).

Two further features of the relations in Fig. 2 must be considered. Firstly, beyond a certain limit, a further increase of activating surface not only fails to shorten the clotting time, but has the opposite effect. This negative phase occurs also with powdered silica and kaolin (Margolis, 1960b) although it cannot be easily demonstrated with the larger particles because of the prohibitively large amounts which would be necessary (e.g. 5-10 gm. of the 120-200 mesh powder/ml. of plasma would be required). It is probable that the inhibition of clotting by excess surface is due to non-specific removal of one or more of the limiting factors in the chain of coagulation reactions.

The second and more interesting anomaly is the loss of linear relation between log concentration and log clotting time as the particle size decreases. Below a certain critical concentration of silica there is a rapid fading of activity with further dilution of the colloid. This anomaly will be discussed later, but in the meantime it introduces a complication in the measurements of activity which does not arise with the larger particles, when the relative activity (i.e.

activity per unit weight or surface area) remains constant throughout the relevant part of the curve. In the colloidal range, this value varies from point to point. It is therefore necessary to define some other constant as a basis for comparison. The absolute minimum clotting time could be used as such, but it is rather ill-defined and besides it cannot be measured with the coarser powders. Instead, the point of maximal relative activity was therefore chosen. It is located by drawing tangents to the curves in the direction parallel to the linear vitreous silica curves. For instance, the activity of the 3·5 mμ sample as compared with the standard powder of 11·2 μ equivalent spherical diameter is 30 p.c. on a weight basis (interrupted lines in Fig. 2A) or $\frac{30 \times 3{\cdot}5}{11{\cdot}2 \times 1000} = 0{\cdot}0094$ p.c. on a surface basis.

Using the same powder as a standard, repeated tests carried out on different days on the same samples of colloidal silica gave fairly reproducible values (co-efficient of variation ± 20 p.c.) considering the low slope and wide range of the curves.

The mean results of three separate estimations on those samples of silica which were best specified by physical measurements are shown in Fig. 3. Curve 1 represents the variation of activity/gm with the particle diameter. It may be seen that in the range 2-7 mμ small increases in the particle size produce very large increases in activity (e.g. hundredfold increase between 2·5 and 3·0 mμ). In fact, this relationship is probably a much more sensitive index of the particle size than are physical measurements. However, the limiting factor in the accuracy is not so much the assay of activity as the uniformity of the particle size and the intrinsic difficulty in physical measurements of small particles (cf. Iller, 1955). We must therefore allow for a possible error of some 0·5 mμ in the 2-5 mμ range. The activity per unit weight reaches its peak at about 40 mμ and then falls as the surface area decreases.

Curve 2 is derived from (1) and expresses the activity per unit surface area. Here there is no real maximum and the particles in the range 0·1-135 μ are approximately equally active.

Activity of dried gels.

Silica aerogels (Table 1), prepared as described, are composed of branching aggregates of the colloidal particles which still retain their individuality. The specific gravity of the gels was 1·0-1·2, i.e. about 50 p.c. that of the ultimate particles. This means that approximately half of the volume is occupied by pores. The pores are too small to allow any significant interchange of protein coagulation factors between the interior and exterior of the granules and hence only the external surface need be considered in the measurement of activity. Vitreous silica of the same particle size (120-200 mesh) was used as a standard.

The results in curve 3 (interrupted) in Fig. 3 are shown in terms of activity per unit area. As with the suspensions, the activity of the gels falls as the

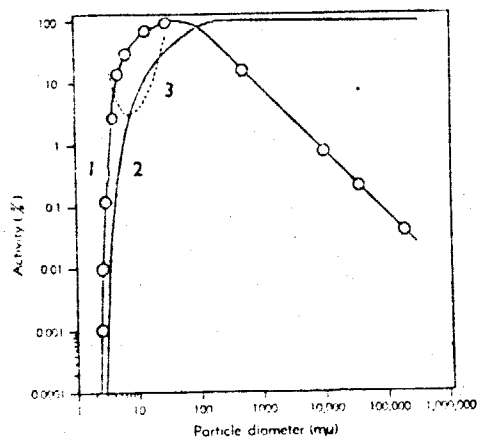


Fig. 3. Relative activity of silica as a function of particle diameter. 1, activity/gm; 2, activity/cm.²; 3 (interrupted curve), activity/cm.² of dried gel.

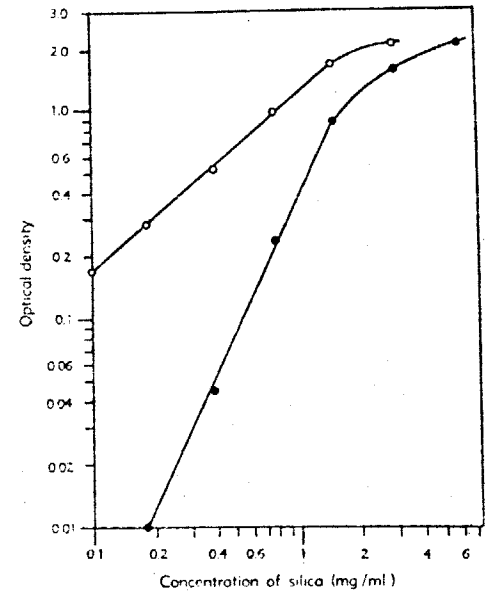


Fig. 4. Turbidity patterns of mixtures of colloidal silica (7 mμ) with citrated plasma (●) or 4 p.c. gamma globulin (○). Optical density measured at 660 mμ after 30 min. incubation.

ultimate particle size decreases from 30 to 7 mμ. Below 7 mμ, however, the activity rises again. The possible reasons for this will be discussed below.

Fading of activity.

The sudden fading of activity below a certain concentration of the smaller particle colloid (see Fig. 2) was mentioned in connection with the quantitative measurements. The simplest explanation of this phenomenon would be the dissolution of the particles in the medium. It is, however, known that this process is very slow (Iller, 1955). In a model experiment in which the haemolysis of washed red cells was used as an indicator (Harley and Margolis, 1961), 7 mμ particles were incubated for many hours, even in much greater dilutions than those used for coagulation, yet there was no evidence of any decrease in the particle size. Addition of citrate and calcium to the system had no significant effect.

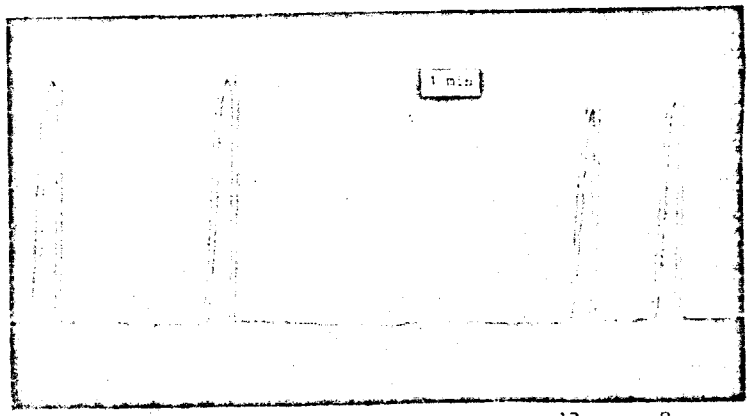
A physical counterpart of the fading of activity was found in the changes in turbidity. The plasma was incubated with serial dilutions of the 7 mμ particles and the optical density read after 30 min. In parallel, a similar series of tubes with 4 p.c. solution of human gamma globulin was used as a control. It will be seen in Fig. 4 that the optical density of the gamma globulin decreases

in proportion to the concentration of silica, whereas with plasma the fall is much steeper below 1 mg./ml. silica concentration.

It seems probable that the fading of activity is not a simple physical artefact but is due to some plasma component which can somehow "neutralise" a certain amount of colloidal silica.

Release of plasma kinin.

It has been shown elsewhere that the initial stages of the reactions leading to clotting and to the release of plasma kinin take place in common (cf. Margolis, 1959b). We may therefore expect that the effect of colloidal silica would be the same in both systems. The results in Fig. 5 confirm this view.



Latent period (sec.)	6		5				12	8
Silica particle diameter (mμ)	5,000	7	7	2	2	2	2 + 7	2 + 7
Concentration of silica in plasma (mg./ml.)	10	0.1	0.3	0.3	1.0	2.5	1.2+0.5	0.5+0.5

Fig. 5. Contractions of rat uterus in 10 ml. oxygenated Ringer's solution after addition of 0·1 ml. plasma activated for 2 min. with silica suspensions.

DISCUSSION.

Proteins adsorbed on to solid/liquid interfaces often undergo denaturation (Fraser, 1957). There is much evidence in favour of the view that the first stage in the activation of plasma by contact is the adsorption and partial denaturation of a specific globulin called the Hageman factor (Ratnoff and Colopy, 1955). More explicitly, this entails some degree of unfolding of the globulin. The exact dimensions of the molecules are not known, but they are probably larger than 10 mμ. In order to unfold, the protein must be attached to the surface in at least two points separated by an adequate distance. On purely geometrical grounds this is clearly impossible when the units of the adsorbing

surface are considerably smaller than the individual protein molecules, as in the case of small particle silica dispersions. In this connection it is interesting to note that weight for weight bentonite is a more powerful activator of plasma than is colloidal silica (unpublished observations). This mineral consists of thin platelets (1 mμ thick) of montmorillonite, in which the flat surfaces are almost pure silica. Thus, because of its crystal shape, bentonite combines a very large specific surface area with a sufficiently large unit of adsorbing surface.

At first sight, the behaviour of dry gels (Fig. 3, curve 3) may appear inconsistent because as the ultimate particle size decreases below 7 mμ the activity rises again. However, aggregates of the smallest silica spherules (e.g. 3 mμ) at the surface of the gel may act as a contiguous surface, whereas with 7 mμ particles the surface may be too irregular for the purpose. In gels made from 30 mμ sols the arrangement of the units does not matter because each particle itself acts as an efficient denaturation site. This hypothesis is represented schematically in Fig. 6. The geometrical interpretation is, of course, only a working hypothesis and rests on the assumption that there are no relevant differences other than size between the large and small silica particles.

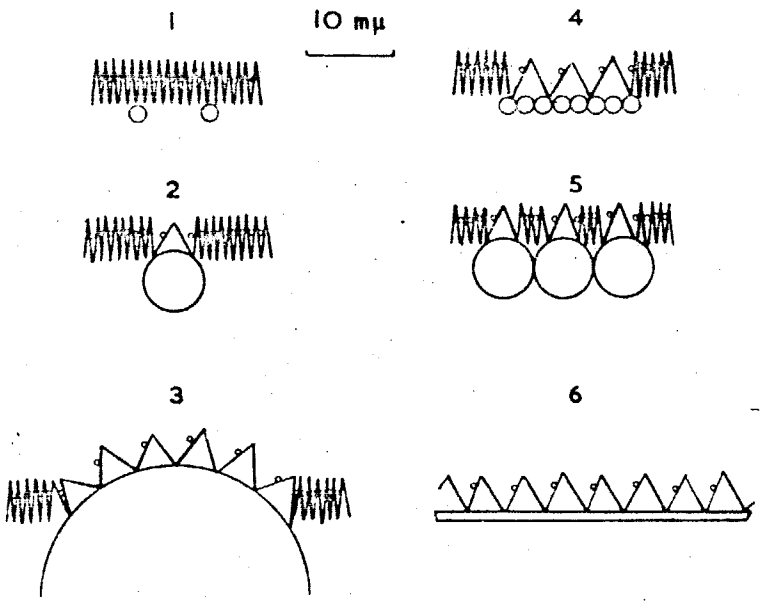


Fig. 6. Schematic representation of the hypothetical relation between the adsorbed protein (Hageman factor) and silica. The small circles between the folds represent enzymatically active sites which become exposed on unfolding of the protein molecules. (Each molecule probably has only one or two active sites. In this diagram multiple sites were drawn to represent the statistical results of activation of many molecules.) 1, 2, 3 = colloidal silica. 4, 5 = dried gel. 6 = bentonite.

The "fading" of activity has not yet been studied in detail, but the following features deserve attention. (1) It becomes pronounced only with the smaller colloidal particles (cf. Fig. 2). (2) A specific plasma component may be involved, because "fading" does not occur in model systems such as gamma globulin or red cell suspensions. The observations on turbidity changes (Fig. 4) suggest that it may be in some way connected with the problem of "colloidal stability" of plasma and not confined to coagulation alone.

The experiments with rat uterus are of little more than formal significance. Since there is not much doubt that activation of Hageman factor is the common initial step in both coagulation and the release of plasma kinin (Margolis, 1959b), it is not surprising that the surface conditions should have the same effects in both these systems.

Apart from purely academic interest, the present observations may have practical applications. (1) As was mentioned before, the coagulation methods could be applied as a very sensitive measure of particle size in the range 2-5 mμ. To do so it would first be necessary to callibrate accurately the curves in Fig. 3 with very uniform and well-specified preparations of colloidal silica. (2) If the concept illustrated in Fig. 6 is valid then the very small silica particles could be used for precipitation of certain proteins in their native state. (3) The "fading of activity" and the peculiarities in precipitation (Fig. 4) may have a bearing on the understanding of some flocculation reactions such as bentonite flocculation test (Bozicevich *et al.*, 1958).

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REFERENCES.

Bozicevich, J., Bunim, J. J., Freund, J., and Ward, S. B. (1958): Proc. Soc. exp. Biol. N.Y., 97, p. 180.
Fraser, M. J. (1957): J. Pharmacol., Lond., 9, p. 497.
Hardisty, R. M., and Margolis, J. (1959): Brit. J. Haemat., 5, p. 203.
Harley, J. D., and Margolis, J. (1961): Nature, Lond., 189, p. 1010.
Iler, R. K. (1955): "The Colloid Chemistry of Silica and Silicates". Cornell University Press, Ithaca, N.Y.
Margolis, J. (1957): J. Physiol., 137, p. 95.
Margolis, J. (1958a): *Ibid.*, 144, p. 1.
Margolis, J. (1958b): J. clin. Path., 11, p. 496.
Margolis, J. (1959a): Austral. J. exp. Biol., 37, p. 239.
Margolis, J. (1959b): "Hemophilia and other Hemorrhagic States". Ed. K. M. Brinkhous. University of N. Carolina Press, p. 208.
Margolis, J. (1960a): J. Physiol., 151, p. 238.
Margolis, J. (1960b): Proc. VIII Intern. Congress of Haematology, Tokyo (in press).
Nevo, A., de Vries, A., and Katchalsky, A. (1955): Biochim. biophys. Acta., 17, p. 536.
Ratnoff, O. D., and Colopy, J. E. (1955): J. clin. Invest., 34, p. 602.

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FURTHER STUDIES OF THE NEUTRALIZATION OF SILICA TOXICITY *IN VITRO*

BY

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A previous paper (Marks, 1957) describes work done on a number of substances to ascertain their protective effect on phagocytic cells in tissue culture against injury by silica dust. The most potent of those investigated was 48-80, a drug used in experimental pharmacology to release histamine from mast cells.

In the present paper, several drugs related to compound 48-80 have been examined for their capacity to protect phagocytic cells against the toxic effect of silica dust *in vitro*. It is shown that protection can be dissociated from histamine-releasing activity in these compounds.

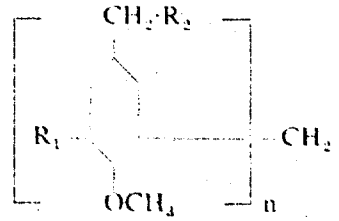
Other substances found to neutralize the toxicity of silica dust were certain phenazines, complexes of aluminium with chelating agents, and the histamine-releasing agent L 1935.

Tissue culture offers a convenient means of studying the injury of phagocytic cells by silica dust and their protection by suitable antagonists. In an earlier communication (Marks, 1957), a number of protective agents investigated in this way were described, of which the most potent was compound 48-80, a drug used in experimental pharmacology to release histamine from mast cells. Compound 48-80 was subsequently shown to protect mice against the fibrogenic action of silica dust (Marks, James, and Morris, 1958). Only small amounts of this drug are tolerated by man and therefore certain related compounds have been investigated in order to determine whether the properties of histamine-release and neutralizing action on silica are distinct. The drugs of this series were prepared in the Wellcome Research Laboratories, New York, where their effect on mast cells was assayed by Dr. Stata Norton using her published technique (Norton, 1954). In addition, the histamine-releasing agent L 1935 (Feldberg and Lecomte, 1955) was examined for protective activity.

A number of drugs unrelated to histamine release were included in the present investigations. Phenazine compounds studied in relation to experimental tuberculosis by Barry, Conalty, and Gaffney (1956) and Barry, Belton, Conalty, Dennehy, Edward, O'Sullivan, Twomey, and Winder (1957) were selected because their structure suggested the possibility of protective activity, and, in addition, three chelates of aluminium with dextran, sequestric acid and oxytetracycline respectively were prepared and tested.

Materials and Methods

The effect of silica and its antagonists on phagocytic cells was studied in tissue culture chiefly using the dehydrogenase technique described by Marks and James (1959). With this technique cells are incubated for three days with dust and their vitality then determined by their capacity to reduce tetrazolium. The dust used in the present work was a single sample of silica (tridymite) provided by Dr. G. Nagelschmidt, of the Safety in Mines Research Establishment, Sheffield. Its mean volume surface diameter was 1·3 microns and specific surface 2·5 m^2/g. The essential structure of the compounds which were related to 48-80 was as follows:—

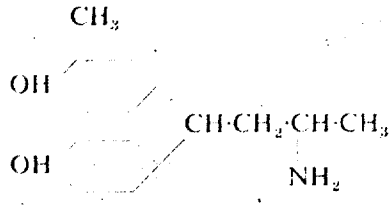


(The radicals R_1 and R_2 and values of n are listed in Table I.)

Compound 46-150 differed from the remainder in that its nitrogen was quaternary. Solutions of these drugs and also L 1935 were made aseptically in Ringer's solution freshly for each experiment.

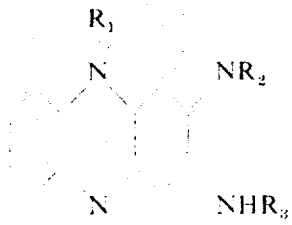
A quantity of L 1935 was obtained for examination through the courtesy of Dr. B. N. Halpern and Dr.

Dinah M. James. It is a mixture of the following compound and its dehydro form:—



The general structures of the phenazines studied were as follows:—

1



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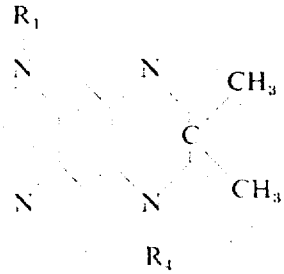

(compounds B621 and B654)

The compounds listed below were examined.

The phenazines were prepared for use by dissolving them in hot ethanol at a concentration of 0·1%. Solution was assisted in several cases by acidification with HCl, but in a number a small proportion of the drug remained insoluble. Shortly before use, 1 in 10 dilutions of the ethanolic solutions were made in 25% serum-tyrode solution.

The aluminium chelates were prepared by mixing 1 ml. volumes of 1% $AlCl_3 \cdot 6H_2O$ with 4·8 ml. of 5% clinical dextran, 1·5 ml. of 1% di-sodium diamino-ethane-tetra-acetic acid (sequestric acid), or 2·5 ml. of 0·1% oxytetracycline. Each mixture was neutralized with 0·1*N* NaOH and its volume made up to 10 ml. with isotonic saline.

Results

Compounds Related to 48-80.—Compound 46-107, which appears to be relatively harmless to mast cells, proved to be twice as effective as 48-80 in protecting phagocytic cells against the toxic effect of silica dust. Compounds 46-108 and 47-30 were approximately four times less effective than 48-80 and the quaternary compound 46-150 appeared devoid of protective action. The results recorded in Table 1 are representative of those obtained in six experiments in which the dehydrogenase method of assessing cell vitality was used. Table 1 includes details of the structure of the compounds and of their destructive effect on mast cells relative to that of 48-80 which is taken as 100. The control cell cultures had a mean dehydrogenase activity of 50 arbitrary units but cultures exposed to silica dust an activity of only 4 units. The ability of the drugs studied to protect cells against silica injury is shown in Table 1 by the degree to which dehydrogenase activity was maintained in their presence. Some of the cultures treated with 46-107 in this experiment gave high readings but the divergence from the mean control was not outside the range of variation met in control cultures nor especially characteristic of the drug. Compound 46-107 was also found to be protective in tissue culture when glucose consumption and morphological criteria of cell damage were employed (Marks, 1957). Despite its histamine-releasing activity, compound 48-80 was tolerated by cells in

Number	R_1	R_2	R_3	R_4
B283	Phenyl	H	Phenyl	—
B430	Cyclohexyl	Cyclohexyl	H	—
B595	Phenyl	Phenyl	H	—
B629	p-Chlorophenyl	p-Chlorophenyl	H	—
B663	p-Chlorophenyl	$CH(CH_3)_2$	p-Chlorophenyl	—
B673	p-Chlorophenyl	Cyclohexyl	p-Chlorophenyl	—
B740	p-Chlorophenyl	Cycloheptyl	p-Chlorophenyl	—
B778	Phenyl	C_2H_4OH	Phenyl	—
B782	p-Chlorophenyl	C_2H_4OH	p-Chlorophenyl	—
B621	Phenyl	—	—	Phenyl
B654	p-Chlorophenyl	—	—	p-Chlorophenyl

An additional compound, B749, with the following structure was also investigated.

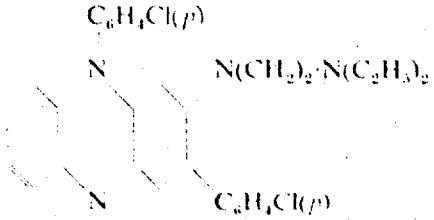

TABLE 1

PROTECTION OF PHAGOCYTIC CELLS AGAINST EFFECT OF SILICA DUST BY COMPOUNDS RELATED TO 48-80

Drug	Dehydrogenase Activity* of Cultures after Addition of 20 µg. Dust and Stated Quantity of Drug (µg.)					Polymerization and Radicals (see text)			Effect on Mast Cells
	40	20	10	5	2·5	n	R_1	R_2	
46-107	--	77	82	69	31	2	CH_3	$CH_2N(CH_3)_2$	0
48-80	55	56	51	27	--	2-4	H	CH_2NHCH_3	10
46-108	43	26	13	--	--	2	H	CH_2NHCH_3	2
47-39	38	29	14	--	--	2-3	H	NHCH	20
46-150	8	6	6	--	--	2	CH_3	CH_2NHCH_3	<5
Nil	Mean dehydrogenase activity = 4 units (untreated dust)								

*Arbitrary units.
The mean dehydrogenase activity of undusted control cultures was 50 units.

somewhat higher concentrations than compound 46-107.

For convenience, drugs were added to the cultures at the same time as the dust in most experiments but in some cells and dust were incubated together for six hours before drugs were added, a period found sufficient to permit phagocytosis. The results in the latter conditions (Table 2) were similar to those in the former, indicating in agreement with previous observations on compound 48-80 (Marks, 1957) that protection of the cells from injury by silica dust was not due to interference with phagocytosis.

TABLE 2

PROTECTION OF CELLS AGAINST EFFECT OF SILICA DUST BY ANTAGONISTS ADDED AFTER AN INTERVAL

Drug Preparation	Amount of Dust per Culture (µg.)	Dehydrogenase Activity with Stated Dose of Drug Preparation (µl.)		
		80	40	20
B283 phenazine, 0·01%	20	14	24	20
B673 phenazine, 0·01%	20	21	16	12
B749 phenazine, 0·01%	20	26	40	20
B778 phenazine, 0·01%	20	42	28	26
Oxytetracycline chelate of aluminium (0·011% aluminium)	20	42	38	36
Compound 46-107, 0·05%	20	45	39	47
Nil	20	Mean activity = 1 unit (untreated dust)		
Nil	Nil	Mean activity = 37 units (controls)		

Cells and dust were incubated together for six hours at 37°C. before antagonists were added. Readings were made after a further three days' incubation.

Compound 1, 1935.—A comparison was made between this drug and the 48-80 series in four dehydrogenase experiments. It was found to be approximately half as active as 48-80 in protecting cells against the effect of silica dust.

Phenazines. Of the phenazines examined, only B283, B673, B749, and B778 exhibited appreciable protective activity, the last two being the most effective. A comparison of their effect with that of compound 46-107 and an aluminium complex is recorded in Table 2. Protection by the phenazines appeared more obvious with the dehydrogenase technique than with the two other methods referred to above. An artefact appeared to be excluded, however, by the observation that phenazines were without effect when added at the same time as tetrazolium to cultures previously incubated with dust.

Aluminium Complexes.—The capacity of these compounds to protect cells against silica dusts was very similar to that of inorganic aluminium salts in equivalent concentration. The oxytetracycline complex was the most effective and the sequestric acid complex the least. A comparison made in a representative experiment is recorded in Table 3.

TABLE 3

PROTECTION OF CELLS AGAINST EFFECT OF SILICA DUST BY DIFFERENT ALUMINIUM COMPOUNDS

Compound Added	Amount of Dust per Culture (µg.)	Dehydrogenase Activity with Stated Dose of Aluminium (µg. metal per culture)		
		8·8	2·2	0·55
Aluminium chloride	40	27	17	10
Aluseqaestric acid complex	40	25	13	6
Aldextran complex	40	32	10	5
Al-oxytetracycline complex	40	44	26	2
Nil	40	Mean activity = 2·5 units (untreated dust)		
Nil	Nil	Mean activity = 44 units (controls)		

Discussion

Tissue culture techniques offer the advantages of convenience and speed in screening drugs for potential neutralizing action on silica dust. It is unlikely that they would fail to detect an agent of therapeutic value. However, the protective activity of a drug *in vitro* is not always reproduced *in vivo*. There are many possible reasons for such failures, for example, the agent may be destroyed, excreted, modified, or sequestrated in the intact animal before it reaches the dust-laden phagocyte in an effective form. From the results obtained with compound

48-80 (Marks *et al.*, 1958) and incomplete animal experiments on the drugs described above, it appears that some at least of the compounds selected for their activity *in vitro* offer the possibility of arresting the pathological processes of silicosis. The chief requirement now for progress in this field is a programme of synthesis of compounds suitable for study.

Experience with compound 46-107 has shown that histamine-release and neutralizing activity for silica can be dissociated. It is possible, therefore, that the 48-80 series of compounds may provide a drug of practical value. The protective activity of the phenazines is less well established since it appeared to be much greater with the dehydrogenase technique than with others. Although no artefact was detected, suspicion must remain that part at least of the apparent protection was spurious. Aluminium by inhalation has been used for a considerable period for the prevention and treatment of silicosis. The complexes of aluminium studied in the present work were prepared for possible systemic treatment with the object of reaching dust-laden cells not accessible to inhaled aluminium dust. Aluminium therapy is attractive in that its effect on silica would probably be more lasting than that of a wholly organic compound which would presumably be liable to chemical alteration. However, preliminary results suggest that the aluminium complexes are less effective than organic compounds in short-term animal experiments and are not innocuous themselves. (It is hoped to report later on the use in mice of the more important of the compounds described in the present work.)

This work was made possible by the generous provision of drugs by Dr. Stata Norton and Dr. E. de Beer of the Wellcome Research Laboratories, New York, and by Dr. V. C. Barry, Dublin. Dr. B. N. Halpern kindly provided a sample of L 1935 and Glaxo Laboratories Ltd. the therapeutic dextran. I am obliged to the Medical Research Council for a grant for apparatus and to Mr. P. J. Patterson for his technical assistance.

References

Barry, V. C., Conalty, M. L., and Gaffney, Ethna E. (1956). *J. Pharm. (Lond.)*, **8**, 1089.
— —, Belton, J. G., Conalty, M. L., Denneny, J. M., Edward, D. W., O'Sullivan, J. F., Twomey, D., and Winder, F. (1957). *Nature (Lond.)*, **179**, 1013.
Feldberg, W., and Leconte, J. (1955). *Brit. J. Pharmacol.*, **10**, 254.
Marks, J. (1957). *Brit. J. indust. Med.*, **14**, 81.
— —, and James, Diana M. (1959). *J. Path. Bact.*, **77**, 401.
— —, — —, and Morris, T. G. (1958). *Brit. J. indust. Med.*, **15**, 1.
Norton, Stata (1954). *Brit. J. Pharmacol.*, **9**, 494.

CONTRIBUTED PAPERS

RAPID DETERMINATION OF SILICA IN ACID-INSOLUBLE SILICATES

By George McClellan (Cosmetic Division, U. S. Food and Drug Administration, Baltimore, Md.)

In connection with work on the analysis of cosmetic powders, which normally contain talc or kaolin, a rapid method for the determination of silica in acid-insoluble silicates was formulated. It permits the use of strong acid for dissolving the fusion melt and dispenses with evaporation for dehydrating the silica. The single dehydration specified in the method yields a recovery of about 99.6 per cent.

The details of the method follow:

Weigh about 0.5 gram of the sample into a platinum crucible containing 6 grams of anhydrous Na_2CO_3, mix intimately, and place in a muffle furnace. Increase the temperature gradually until the contents of the crucible are in fusion, and continue to heat at 950°–1050° C. for 15 minutes.

Remove the crucible and allow it to cool. Dislodge the melt into a dry 400 ml. beaker. In a graduate mix 15 ml. of HNO_3 with 5 ml. of water, and wash the crucible with small successive portions of this mixture, adding the washings to the beaker. (The melt dissolves rapidly, with the precipitation of most (about 95%) of the silica in the sample.)

Place the beaker under a hood and add, in the order named, 5 grams of NH_4Cl, 20 ml. of $HClO_4$, and 10 ml. of H_2SO_4. (When the H_2SO_4 is added the mixture bubbles vigorously, and oxides of nitrogen are evolved.) Cover the beaker with a watch-glass, and boil over a moderate flame for 15 minutes after the evolution of oxides of nitrogen has ceased.

Cool the mixture, dilute with 150 ml. of hot water, and filter on an ashless paper. Wash paper and residue thoroughly with hot water, ignite the precipitate, and weigh as crude silica. Correct for impurities with HF in the usual manner.

DISCUSSION

The melt is dissolved in nitric acid because of its solubility even in highly concentrated (12 *N*) solutions of this acid. It is obvious that the stronger the acid used, the more silica will immediately be precipitated and the less water there will be to remove during the subsequent dehydration.

Nitric acid is an inferior dehydrating agent. Hence, when it has fulfilled its purpose as a solvent it is displaced by the less volatile sulfuric and perchloric acids.

A combination of perchloric acid and sulfuric acid is used for dehydration because it gives a reasonably pure precipitate that filters rapidly. Perchloric acid alone gives a slow-filtering, almost pure precipitate, while sulfuric acid alone gives an impure granular precipitate that filters rapidly.

The ammonium chloride has a dual and possibly a triple purpose: it ensures smooth boiling, it helps to destroy the excess of nitric acid, and it may render the precipitation of silica more complete.

RESULTS

The method outlined was used to determine silica in two samples of clay previously analyzed by the National Bureau of Standards. Sample No. 97, flint clay, was run in quadruplicate, and sample No. 98, plastic clay, was run in triplicate.

The results follow:

SAMPLE NO.	SiO_2 (AVERAGE RESULT OF NATIONAL BUREAU OF STANDARDS) *per cent*	SiO_2 (BY METHOD OUTLINED) *per cent*	RECOVERY (ON BASIS OF AVERAGE RESULT OF NATIONAL BUREAU OF STANDARDS) *per cent*
97	42.87	42.68	99.56
		42.70	99.60
		42.87	100.00
		42.71	99.63
98	59.11	58.90	99.64
		59.09	99.97
		58.87	99.59

The filtrates from the last two determinations of sample No. 97 were evaporated to fumes of perchloric acid, diluted, and filtered. The residues were ignited, weighed as crude silica, and corrected for impurities with hydrofluoric acid in the usual manner. The results follow:

SiO_2 PRESENT IN SAMPLE	RECOVERY BY FIRST DEHYDRATION		RECOVERY BY SECOND DEHYDRATION		TOTAL RECOVERY
gram	*gram*	*per cent*	*gram*	*per cent*	*per cent*
0.2178	0.2178	100.00	0.0010	0.46	100.46
0.2178	0.2170	99.63	0.0006	0.28	99.91

Two blanks were run to determine whether some of the silica recovered might come from reagents or equipment.

In blank No. 1, 6 grams of sodium carbonate was fused in a platinum crucible, dislodged into a 400 ml. beaker, and dissolved in 20 ml. of nitric acid (3+1). To the solution were added 5 grams of ammonium chloride, 20 ml. of perchloric acid, and 10 ml. of sulfuric acid. The mixture was boiled until oxides of nitrogen ceased to come off and for 15 minutes thereafter. When cool, the mixture was diluted with 150 ml. of hot water and filtered. Silica was determined in the residue.

Blank No. 2 was run in the same manner as No. 1, except that 40 ml. of perchloric acid and 20 ml. of sulfuric acid were used, and boiling was continued for 30 minutes after oxides of nitrogen had ceased to come off.

The results follow:

SiO_2 in No. 1 blank: 0.0001 gram.
SiO_2 in No. 2 blank: 0.0003 gram.

These figures indicate that recoveries by the proposed rapid method are actual and not the results of compensating errors.

SELECTED REFERENCES

Hillebrand and Lundell, Applied Inorganic Analysis, pp. 716–726.
Scott, Standard Methods of Chemical Analysis, 5th Ed., Vol. I, pp. 794–817.
Tananaeff and Buitschkoff, *Z. Anal. Chem.* 103, 349–353 (1935), through *The Analyst*, 61, 142 (1936).

A SURVEY OF METHODS FOR THE QUANTITATIVE ESTIMATION OF STARCH*

By M. P. Etheredge (Mississippi State Chemical Laboratory, State College, Miss.)

In order to make some direct starch determinations in this Laboratory it was thought that it might be well to make a survey of the literature, select the most suitable methods, and make determinations on commercial starches and also on some natural materials.

In 1898 Harvey W. Wiley and W. H. Krug (1) made a very complete report on starch methods. They were particularly interested in polarimetric methods so they tried the Guichard method, using nitric acid; the pressure method; the Baudry method; and a revision of the Reinke method, using hydrochloric acid. They believed that the Guichard method might be applicable to pure starches, but that the diastase method was the most promising. In general, they were opposed to polarimetric methods for either soluble starch or dextrose determinations. They also concluded that the sum of the several percentages obtained in the analysis of a cereal or cereal product does not approximate a hundred, and that variations are somewhat greater than they are in ordinary mineral analysis, but not so great as to warrant the assumption of the existence in cereals of a class of bodies of unknown properties and different in any marked degree from those known to exist.

Other attempts to bring starch into dispersive solution and get a quick quantitative estimation with the polarimeter include the use of hydrochloric acid alone in both concentrated and dilute solutions; hydrochloric with other acids, such as acetic; and sulfuric acid alone. According to Lintner (2) the sulfuric acid seems to give a different specific rotation with different starches.

For this investigation, it was thought best to use the current official methods. Two methods specifying hydrolysis by hydrochloric (3), and diastase-acid treatment (3), respectively, were taken from those of the Association of Official Agricultural Chemists. The Hopkins (4) revision of

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 28–30, 1940.

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TABLE 1. Porphyrin Content of Peripheral Nerves of Pigs.

	Coproporphyrin III/100 g	Protoporphyrin/100 g	Coproporphyrin/Protoporphyrin
	(μg/100 g tissue)		
	91.5	42.0	2.18
	96.0	47.3	2.03
	93.6	48.2	1.94
	99.0	44.6	2.21
	93.0	42.0	2.21
Avg	94.6	44.8	2.11

separated according to the method of Schwartz and Wikoff(6). Quantitative determinations of copro- and protoporphyrin concentrations were made fluorometrically(5).

Results. The results of 5 determinations are shown in Table 1. It is apparent from this that the amounts of porphyrin in peripheral nerves are even more abundant than in the central nervous system. The porphyrins in peripheral nerves were found to be difficult to demonstrate spectroscopically, but the emission bands of porphyrins in peripheral nerves were observed with a Gaertner quartz spectrograph.

The dominant porphyrin in peripheral nerves as in the central nervous system was coproporphyrin III. It was noted, however, that there was a constant ratio of coproporphyrin to protoporphyrin when the nerve tissue was protected from autolysis before the determination.

Summary. Both coproporphyrin III and protoporphyrin were determined in the peripheral nerves of pigs. An average of 5 determinations showed that 100 g of nerve contained 94 μg of coproporphyrin and 45 μg of protoporphyrin. The ratio of coproporphyrin to protoporphyrin was approximately 2:1 and appeared to be constant.

1. Klüver, H., *Science*, 1944, v99, 482.
2. ———, *Psychol.*, 1948, v25, 331.
3. Chu, E. J. H., Watson, C. J., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 569.
4. Blanchard, T., *ibid.*, 1953, v82, 512.
5. Solomon, H., Figge, F. H. J., *ibid.*, 1957, v94, 356.
6. Schwartz, S., Wikoff, H., *J. Biol. Chem.*, 1952, v194, 564.
7. Watson, J., Schultz, Wm., Hawkinson, V., Baker, A. B., PROC. SOC. EXP. BIOL. AND MED., 1947, v64, 73.

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Absence of Gastro-Intestinal Lesions in Rats Following Ingestion of Silica. (23732)

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The finding of solid silica in lungs and other tissues and its toxicity when inhaled or injected intravenously has led to attempts to produce lesions by ingestion. (Piezoelectric effects of crystalline silica have been blamed but no piezoelectric effect is possible unless the crystal is compressed in one direction.) Silica occurs in food and to the extent of 2% in human blood ash. We wished to determine whether 10% of the diet as silica dust would be toxic. Seventy weanling rats were divided into 7 groups of 10 rats each. One group was fed Purina dog chow and the others 90% Purina dog chow and 10% of one of the forms of silica shown in Table 1.

The rats were fed these diets 3 months and then killed and histological preparations made of the stomach, small and large intestine, liver, spleen, pancreas, adrenals, mesenteric lymph nodes and lung. No lesions were found in any of the organs studied.

It may be objected that 3 months is not sufficient time to produce lesions with silica. Clinical symptoms of silicosis have been reported in humans breathing silica dust for 6 months but most cases studied have been exposed to silica dust from 1 to 40 years. If the rat is to be used as an experimental animal it must be assumed that it lives more rapidly and that changes occur in less time than in

TABLE I.

Name	Particle size in μ
Linde silica	.02-.05
Columbia-Southern Hi-Sil 101	.03
Dow Corning silica (unpelletized)	.01-.02
Powdered silica gel	
Sodium metasilicate	
Carborundum Co. Fibrofrax (silica fibers)	

humans.

Summary. Powdered silica or Fibrofrax mixed with the rat diet to the extent of 10%, produces no tissue pathology in the gastrointestinal tract, liver, spleen, pancreas, adrenals, lung or mesenteric lymph nodes when fed continuously for 3 months.

We are indebted to Columbia-Southern Corp., Dow-Corning Corp., Carborundum Co. and Dr. Charles P. Carpenter for generous supplies of the silica.

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Cerebrospinal Fluid Enzymes in Central Nervous System Lipidoses* (with particular reference to Amaurotic Family Idiocy) (23733)

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The diagnostic usefulness of certain enzyme levels in cerebrospinal fluid (CSF) and serum derived from cases of central nervous system (CNS) disease has recently been explored (1-5). Various authors have demonstrated transient glutamic oxalacetic transaminase (GOT) elevation in CSF derived from clinically determined or experimentally induced cerebral infarction(1,2). Other investigators, in evaluating the utility of CSF GOT in a broad spectrum of neurologic disorders, were unable to report any conclusive alterations in the levels of this enzyme(4).

Significant rises in serum GOT and aldolase have recently been recorded in cases of Infantile Amaurotic Family Idiocy (IAFI) (5). These findings indicating that a number of determinable serum enzymes is increased in one of the CNS lipidoses led to an assessment of their activity within the CSF of cases in this disease category. Concurrent serum levels were also determined to clarify any possible correlation between the two biological fluids. The activity of lactic dehydrogenase (LD) was also measured in the serum and CSF of selected lipidosis and control cases.

Material and methods. a.) Clinical: Repeated serum and CSF specimens from 9 patients with IAFI (at varying stages of the illness) and 2 cases of Niemann-Pick's Disease (NPD) with CNS involvement were studied in regard to GOT, aldolase and LD levels. Similar enzyme studies were performed on 33 additional children afflicted with other neurological disorders. b.) Experimental: 10 mongrel dogs, each weighing about 13 kg, were injected intramuscularly with 1 g (GOT enzymatic activity: 80,000 u./g) of fresh heterologous canine grey matter. An additional 3 dogs were inoculated with a similar homogenate previously incubated at 100°C for 1½ hours (GOT enzymatic activity, 0.0 u./g). All dogs were then sequentially studied by daily serum GOT determinations for periods up to 8 days. The method employed for determination of serum and CSF GOT was that of Karmen, Wróblewski and LaDue(6) based on spectrophotometric measurement in the near ultraviolet with the Beckman D.U. spectrophotometer. Serum and CSF aldolase activity was estimated with the colorimetric procedure of Sibley and Lehninger(7). The LD activity of the serum and CSF samples were also determined spectrophotometrically at 340 mμ with the Beckman D.U. using the method of Wróblewski and LaDue(8).

* This project was performed under auspices of Nat. Tay-Sachs Assn.

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Electrophoretic changes in rabbits receiving intravenous injections of silica

Statistical study[1)]

by

M. Mosinger, A. Versino, J. Jouglard-Duplay and J. Fondarai.

1) Work financed by the Coal and Steel Authority of the European Community.

The variations in the blood protein fractions separated by paper electrophoresis were studied in rabbits receiving intravenous injections of silica.

We sought out normal values for these different fractions from the literature. We have noted the percentile values reported by Deutsch and Goodloe (2,3) and by Geinitz (6), and the values, also percentile, obtained by statistical studies by Scheiffarth, Kimbel and Berg (12). The figures reported by these different authors are fairly dissimilar. We thus wished first of all to determine the normal values of these different protein fractions for our own rabbits. Systematic proteinograms and glycoproteinograms were made on a number of rabbits (48) from our own stock. These were animals of the same strain (tawny Bourgogne), the same weight (average of 1500 g), the same age (ca. 3 months) and either sex. It should be noted that the term "normal" is to be taken in the broad sense. These animals had received no previous treatment of any sort, but they were not germ-free animals.

We used the usual methods of paper electrophoresis in a sodium veronal buffer of pH 8.6 and an ionic strength of 0.1, with a migration of 5.4 vV/cm and 0.31 mA/cm in 16 hours. The optimal quantity of protein deposited was 0.56 µg for the proteinograms and 2.80 µg for the glycoproteinograms. The total protein determinations were made according to the densimetric method of Phillips and Van Slyke. The proteinograms were visualized with amido black 10 B. The glycoproteinogram fractions were detected by means of the Schiff reaction, using p-rosaniline. Photometry of the bands took place with a Photovolt densimeter. Details of the method have been published elsewhere (4,5,8).

The greatest obstacle to the quantitative estimation of the different electrophoretic fractions was the fact that photometry of the paper bands does not follow a strict linear law. Thus for proteinograms stained with amido black, albumin stains more strongly than the globulins. A correction coefficient should be applied to these fractions, but this correction factor varies considerably according to different authors. Thus according to Bohle and Fischer (1) it is 1.4; according to Pugar (11) it is 1.7 and sometimes even 2.2. As to Latner (7), this author claims that albumin and the gamma globulins have the same affinity for amido black 10 B and that it is unnecessary to use a correction factor. Whether or not these coefficients are used has no effect on the statistical study. On the other hand our later studies on the behavior of these fractions after the intravenous injection of silica are strictly comparative and we used no correction

coefficient (that is to say it was taken as equal to 1). In this manner we obtained arbitrary values for our protein fractions. These values will have to be corrected by any one desiring actual values. We found it more convenient not to use the values found in the form of percentages, but to use arbitrary absolute values, obtained by expressing the fractions in g per 1000. As was stated above, we always made a total protein determination before the electrophoresis. By this means we believed that we would obtain a roughly Gaussian distribution, which would facilitate our later calculations.

Thus by calculation of Pearson's coefficient b_1 and b_2, we sought to determine whether the distribution of the values was Gaussian. These results were published elsewhere (9). It was established that the coefficients obtained were within the confidence limits of the theoretical values of these coefficients for the number of animals included in our statistics. Thus our distribution may be considered as Gaussian.

We were then able to calculate the means and standard deviations for each fraction. To avoid the risk of considering a pathologic rabbit as normal, we decided to consider all rabbits as abnormal whose values diverged from the mean by $\pm 1\sigma$, that is to say we voluntarily excluded 34% of rabbits which may have been normal, in order to minimize the risk of including a pathologic rabbit within this range. In the case of two fractions (the β-globulin fraction of the proteinograms and the α-2-globulin fraction of the glycoproteinograms) that are less regularly Gaussian than the other fractions, we imposed even

stricter rules. We considered as abnormal any value of these two fractions that diverged by $\pm$ 0.67σ from the mean, thus voluntarily excluding 50% of normal rabbits.

Tables I and II show the values of the mean m, the standard deviation σ, the limits of the selection interval chosen, the standard error of the mean sm and the confidence interval of the mean, for proteinograms, total proteins and glycoproteinograms.

Table I

Proteinograms

Albumins	m = 41.75
	σ = 5.10
	selection interval = 36.65 - 46.85
	sm = 0.73
	confidence interval of m = 40.29 - 43.21
α-globulins	m = 7.95
	σ = 2.44
	selection interval = 5.51 - 10.40
	sm = 0.35
	confidence interval of m = 7.25 - 8.65
β-globulins	m = 8.20
	σ = 2.16
	selection interval = 6.55-9.85
	sm = 0.35
	confidence interval of m = 7.19 - 8.91

γ-globulins	m = 7.60
	σ = 2.44
	selection interval = 5.16 - 10.01
	sm = 0.32
	confidence interval of m = 6.96 - 8.24
Total proteins	m = 66.13
	σ = 6.99
	selection interval = 59.14 - 73.12
	sm = 1
	confidence interval of m = 65.13 - 67.13

Table II

Glycoproteinograms

Albumin	m = 12.46
	σ = 5.51
	selection interval = 6.95 - 17.97
	sm = 0.96
	confidence interval of m = 11.5 - 13.4
α-1-globulins	m = 13.79
	σ = 4.91
	selection interval = 8.88 - 18.78
	sm = 0.85
	confidence interval of m = 12.94 - 14.64
α-2-globulins	m = 15.21
	σ = 5.67
	selection interval = 11.41 - 19
	sm = 0.99
	confidence interval of m = 14.22 - 15.20

β-globulins	m = 13.12
	σ = 5.52
	selection interval = 7.60 - 18.64
	sm = 0.96
	confidence interval of me = 13.16 - 14.08
γ-globulins	m = 10.3
	σ = 3.65
	selection interval = 6.65 - 13.95
	sm = 0.63
	confidence interval of m = 9.67 - 10.93

In the literature we found the values reported by Scheiffarth et al. (12). We changed the percentages given by this author into absolute values to be able to compare them with our own values; thus we presumed that these rabbits had average blood proteins similar to our own. It was found that the means obtained by this author after a statistical study were within the confidence interval of our means (Table III). Obviously with statistical calculations it is impossible to establish the identity of these two populations, but this excludes the conclusion that the populations are different from the usual risk coefficients.

A certain number of our "normal" animals received intravenous silica injections. Silica with a particle size of 3 μ was sorted by sedimentation (5, 8). Our animals received 100 mg silica every other month until a total dose of 500 mg was reached. A proteinogram was made on each animal before any treatment and before each new silica injection. We continued to take blood

samples for electrophoresis after the total silica dose was reached. Thus our animals were their own controls. A variance analysis in random blocks was made on our results, since the distribution of the fractions was Gaussian. The details of these calculations were published earlier (10). It was established that there was a significant increase in γ-globulins after a total dose of 200 mg, or around 43%. This increase becomes very highly significant and very great, of around 140%, after a total dose of 500 mg. The β-globulins only increased after a dose of 500 mg, by about 50% and this variation is also very highly significant. As to the albumin fraction, it shows a significant variation after 300 mg and a very significant one after 500 mg; a ca. 11% reduction is intensifed after 500 mg (19%). The α-globulin fraction shows an absolutely non-significant variation, regardless of the dose injected. If we consider the time factor, no significant variation in the protein fractions appears before the 8th month. In the 8th month there is a highly significant variation in the γ-globulins, which increase by 71%. This increase becomes more marked (100%) after 14 months of treatment. The β-globulins increase significantly from the 8th month (60%) and maintain this level of increase after 14 months (55%). The albumins show no significant variation by the 8th month, but show a significant decrease in the 14th month; this decrease is slight, of around 11%. At no time did we observe significant variations in the α- globulins. These results are shown in table IV.

Table III: Key: 1. Scheiffarth. 2. Our own results.

Table IV. Key: 1. After 200 mg. 2. After 500 mg. 3. Significance test. 4. % variation. 5. After 8 months. 6. After 14 months. 7. N.S. = not significant. 8. S= significant. 9. H.S. = highly significant. 10. T.HS. = very highly significant.

Summary and conclusions

Our statistical studies have revealed several facts:

1. The distribution of the different fractions of the proteinograms and glycoproteinograms of normal rabbits is Gaussian.

2. Having a very strict selection interval around the mean for our normal animals, an animal could thenceforth be used or rejected before any experimentation according to whether it proved to be normal or abnormal.

3. We had a rabbit population which did not differ greatly from that of another investigator. This leads us to hope that the electrophoretic fractions constitute a kind of biologic constant.

4. In our rabbits treated with repeated intravenous injections of silica, the variance analysis in random blocks showed that our animals made up a homogeneous group.

5. The same variance analysis, in which each of our rabbits was its own control, allowed a very fine analysis of the variations in the different fractions. It was found that:

a) the α-globulins showed no significant variation, either as a function of dose, or as a function of time;

b) the albumins showed a slight decrease as a function of dose and time;

c) the β-globulins showed an increase after a certain dose and time, which later remained constant;

d) the γ-globulins increased considerably and this increase was intensified with dose and time.

Bibliography

See p. 886.

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Les modifications électrophorétiques chez les lapins recevant des injections intraveineuses de silice.

(Étude statistique [1].)

Par

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(Marseille.)

Nous avons voulu étudier les variations des fractions protidiques sanguines séparées par électrophorèse sur papier chez des lapins recevant des injections intraveineuses de silice.

Nous avons cherché dans la littérature des valeurs normales de ces différentes fractions. Nous avons noté les valeurs données, en pourcentage, par Deutsch et Goodloe [2, 3] et par Geinitz [6], et les valeurs, toujours en pourcentage, obtenues par étude statistique par Scheiffarth, Kimbel et Berg [12]. Les chiffres de ces différents auteurs sont assez dissemblables. Aussi nous avons voulu, dans un premier temps, déterminer les valeurs normales de ces différentes fractions protidiques pour notre élevage personnel de lapins. Nous avons donc effectué des protidogrammes et des glycoprotidogrammes, systématiquement chez un certain nombre de lapins (48) de notre élevage. Il s'agissait d'animaux de même race (fauve de Bourgogne), de même poids (1 500 g en moyenne), de même âge (3 mois environ) et de sexe non contrôlé. Nous précisons que ce terme de normal doit être pris dans un sens élargi. Ce sont des animaux n'ayant reçu aucun traitement préalable, mais ce ne sont pas des « animaux sans germes ».

Nous avons utilisé les méthodes classiques d'électrophorèse sur papier, en tampon véronal sodique de pH 8,6 et de force ionique 0,1, avec une migration de 5,4 voV/cm et 0,31 mA/cm pendant 16 heures. La quantité optima de protéines à déposer est de 0,56 microgrammes pour les protidogrammes, et de 2,80 microgrammes pour les glycoprotidogrammes. Les déterminations des protides totaux sont faites par la méthode densimétrique de Philips et Van Slyke. La révélation des protidogrammes est effectuée à l'amidoschwarz 10 B. La mise en évidence des fractions du glycoprotidogramme se fait par la Réaction de Schiff en utilisant la p-rosaniline. La photométrie des bandes est pratiquée au densitomètre Photovolt. Nous avons publié antérieurement les détails techniques [4, 5, 8].

Le gros écueil de l'appréciation quantitative des différentes fractions électrophorétiques est le fait que la photométrie des bandes de papier ne suit pas une loi rigoureusement linéaire. Ainsi, pour les protidogrammes colorés à l'amidoschwarz, l'albu-

1. Travail financé par la Haute Autorité de la Communauté Européenne du Charbon et de l'Acier.

nous trions par sédimentation [5, 8]. Nos animaux reçoivent 100 mg de silice tous les deux mois jusqu'à une dose totale de 500 mg. Chacun de nos animaux a un protidogramme avant tout traitement et des protidogrammes avant chaque nouvelle injection de silice. Nous continuons le prélèvement de sang pour électrophorèse après que la

TABLEAU III.

	SCHEIFFARTH		RESULTATS PERSONNELS	
	p. %	g. p. 1 000	g. p. 1 000	
Albumines	61,48	40,5	40,29	43,21
Alpha-globulines	13,11	8,65	7,25	8,65
Bêta-globulines	12,08	8,00	7,49	8,91
Gamma globulines	13,07	8,55	6,96	8,21

dose totale de silice ait été atteinte. Nos animaux servent donc de témoins à eux-mêmes. Nous avons pratiqué sur nos résultats une analyse de variance en blocs casualisés, la répartition des fractions étant gaussiennes. Nous avons publié antérieurement les détails de ces calculs [10]. Nous avons constaté que les valeurs du coefficient F de Snedecor, obtenues pour les blocs et qui traduisent l'hétérogénéité du matériel expérimental, sont pour nos animaux toujours très nettement inférieures au seuil de signification. Aussi l'homogénéité de notre matériel expérimental ne peut pas être mise en cause, ce qui est satisfaisant du point de vue biologique. Nous avons étudié les variations des fractions protidiques en fonction du facteur dose totale et en fonction du facteur temps écoulé depuis la première injection de silice.

Nous avons constaté qu'il y avait une augmentation significative des gamma-globulines après une dose totale de 200 mg. Cette augmentation est de l'ordre de 43 p. 100. Cette augmentation devient très hautement significative et très importante, de l'ordre de 140 p. 100, après une dose totale de 500 mg. Les bêta-globulines n'augmentent qu'après une dose de 500 mg; leur augmentation est de l'ordre de 50 p. 100 et cette variation est aussi très hautement significative. Quant à la fraction albumine, sa variation est significative après 200 mg et très hautement significative après 500 mg et c'est une diminution de 11 p. 100 environ qui s'accentue après 500 mg (19 p. 100). La fraction alpha-globuline présente une variation absolument non significative quelle que soit la dose totale injectée. Si nous considérons le facteur temps, aucune variation significative des fractions protidiques n'apparaît avant le 8e mois. Au 8e mois, il y a une variation hautement significative des gamma-globulines, celles-ci augmentent de 71 p. 100. Cette augmentation se précise (100 p. 100) après 14 mois de traitement. Les bêta-globulines augmentent de façon significative dès le 8e mois (60 p. 100) et maintiennent ce taux d'augmentation après 14 mois (55 p. 100). Les albumines n'ont pas de variation significative au 8e mois, mais présentent une diminution significative au 14e mois, cette diminution est légère de l'ordre de 11 p. 100. A aucun moment, nous n'avons constaté de variation significative des alpha-globulines. Nous avons consigné [illegible] dans le tableau IV

TABLEAU IV.

	APRÈS 200 MG				APRÈS 500 MG			
	Alb.	Alpha glob.	Bêta glob.	Gamma glob.	Alb.	Alpha glob.	Bêta glob.	Gamma glob.
Test de signification	S.	N.S.	N.S.	S.	T.H.S.	N.S.	T.H.S.	T.H.S.
Pourcentage de variation ..	↘ 11 %	0	0	↗ 43 %	↘ 19 %	0	↗ 50 %	↗ 140 %

	APRÈS 8 MOIS				APRÈS 14 MOIS			
	Alb.	Alpha-glob.	Bêta-glob.	Gamma-glob.	Alb.	Alpha-glob.	Bêta-glob.	Gamma-glob.
Test de signification	N.S.	N.S.	H.S.	H.S.	S.	N.S.	S.	S.
Pourcentage de variation ..	0	0	↗ 60 %	↗ 71 %	↓ 11 %	0	↗ 55 %	↗ 100 %

N.S. = non significative
S. = significative
H.S. = hautement significative
T.H.S. = très hautement significative

Résumé - Conclusions.

Les études statistiques que nous avons effectuées nous ont permis de mettre en évidence plusieurs faits :

1° Les répartitions des différentes fractions des protidogrammes et des glycoprotidogrammes de lapins normaux sont gaussiennes.

2° Ayant déterminé un intervalle de sélection très sévère autour de la moyenne pour nos animaux normaux, nous pouvons dès lors, avant toute expérience, utiliser ou rejeter un animal selon qu'il se révèle être normal ou anormal.

3° Nous avons une population de lapins pas très différente de celle d'un autre chercheur. Ceci permet d'espérer que les fractions électrophorétiques constituent une sorte de constante biologique.

4° Chez nos lapins traités par des injections répétées de silice intraveineuses, l'analyse de variance en blocs casualisés nous a montré que nos animaux constituaient un lot homogène.

5° Cette même analyse de variance, où chacun de nos lapins était son propre témoin, nous a permis une analyse très fine des variations des différentes fractions. Nous avons pu constater :

a) les alpha-globulines ne présentent aucune variation significative ni en fonction de la dose, ni en fonction du temps;

mine se colore plus fortement que les globulines. Il faudrait appliquer un coefficient de correction à ces dernières fractions. Mais ce facteur de correction est fortement variable selon les auteurs. Ainsi, pour Bohle et Fischer [1], il est égal à 1,4; pour Pugar [11] à 1,7 et parfois même à 2,2. Quant à Latner [7], il affirme que l'albumine et les gamma-globulines ont la même affinité pour l'amidoschwarz 10 B, et qu'il est inutile d'employer un coefficient de correction. L'utilisation ou le non emploi de ces coefficients n'intervient pas sur la conduite de l'étude statistique. D'autre part, nos études ultérieures sur le comportement de ces fractions après injection de silice intraveineuse sont strictement comparatives. Aussi nous n'avons pas utilisé de coefficient de correction (c'est-à-dire nous l'avons posé égal à 1). Nous obtenons ainsi des valeurs arbitraires pour nos fractions protidiques. Ces valeurs devront être corrigées par un éventuel utilisateur qui désirerait avoir des chiffres réels. Nous avons trouvé plus commode de ne pas utiliser les valeurs trouvées en pourcentage, mais d'employer des valeurs absolues arbitraires, obtenues en exprimant les fractions en grammes pour 1 000. En effet, comme nous l'avons indiqué plus haut, nous effectuons toujours une détermination des protides totaux avant de pratiquer une électrophorèse. Nous pensions ainsi obtenir des répartitions sensiblement gaussiennes, ce qui devait faciliter nos calculs ultérieurs.

Nous avons donc tout d'abord cherché par le calcul de coefficient b_1 et b_2 de Pearson, si les répartitions des valeurs étaient gaussiennes. Nous avons publié ailleurs ces résultats [9]. Nous avons constaté que les coefficients que nous avons obtenus sont compris dans les limites de confiance des valeurs théoriques de ces coefficients pour le nombre d'animaux entrant dans notre statistique. Nous pouvons donc considérer nos répartitions comme gaussiennes.

Nous avons pu alors calculer les moyennes et les écarts-types pour chaque fraction. Pour ne pas risquer de considérer comme normal un lapin pathologique, nous avons décidé de considérer comme anormal tout lapin dont les chiffres s'écarteront de $\pm 1\,\sigma$ de la moyenne, c'est-à-dire que nous excluons volontairement 31 p. 100 des lapins qui sont peut-être normaux: ceci pour rendre très minime le risque de rencontrer dans cet intervalle un lapin pathologique. Pour deux fractions (la fraction bêta-globuline des protidogrammes et la fraction alpha-2-globuline des glycoprotidogrammes) qui sont moins régulièrement gaussiennes que les autres fractions, nous avons été encore plus sévères. Nous avons considéré comme anormale toute valeur de ces deux fractions s'écartant de $\pm 0{,}67\,\sigma$ de la moyenne, excluant ainsi volontairement 50 p. 100 des lapins normaux.

Dans les tableaux I et II nous donnons les valeurs de la moyenne m, de l'écart type σ, les limites de l'intervalle de sélection que nous avons choisi, l'erreur standard de la moyenne sm et l'intervalle de confiance de la moyenne. Ceci pour les protidogrammes, les protides totaux et les glycoprotidogrammes.

Tableau I.

Protidogrammes.

Albumines.

m = 41,75
σ = 5,10
intervalle de sélection = 36,65 — 46,85
S_m = 0,73
intervalle de confiance de m = 40,29 — 43,21

Alpha-globulines.

m = 7,95
σ = 2,44
intervalle de sélection = 5,51 — 10,40
S_m = 0,35
intervalle de confiance de m = 7,25 — 8,65

Bêta-globulines.

m = 8,20
σ = 2,46
intervalle de sélection = 6,55 — 9,85
S_m = 0,35
intervalle de confiance de m = 7,49 — 8,91

Gamma-globulines.

m = 7,60
σ = 2,44
intervalle de sélection = 5,16 — 10,04
S_m = 0,32
intervalle de confiance de m = 6,96 — 8,24

Protides totaux.

m = 66,13
σ = 6,99
intervalle de sélection = 59,14 — 73,12
S_m = 1
intervalle de confiance de m = 65,13 — 67,13.

Tableau II.

Glycoprotidogrammes.

Albumines.

m = 12,46
σ = 5,51
intervalle de sélection = 6,95 — 17,97
S_m = 0,96
intervalle de confiance de m = 11,5 — 13,4

Alpha-1-globulines.

m = 13,79
σ = 4,91
intervalle de sélection = 8,88 — 18,78
S_m = 0,85
intervalle de confiance de m = 12,94 — 14,64

Alpha-2-globulines.

m = 15,21
σ = 5,67
intervalle de sélection = 11,11 — 19
S_m = 0,99
intervalle de confiance de m = 14,22 — 15,20

Bêta-globulines.

m = 13,12
σ = 5,52
intervalle de sélection = 7,60 — 18,64
S_m = 0,96
intervalle de confiance de m = 13,16 — 14,08

Gamma-globulines.

m = 10,3
σ = 3,65
intervalle de sélection = 6,65 — 13,95
S_m = 0,63
intervalle de confiance de m = 9,67 — 10,93

Nous avons relevé dans la littérature, les chiffres donnés par Scheiffarth et Coll. [12]. Nous avons traduit les pourcentages de cet auteur en valeurs absolues pour pouvoir les comparer à nos propres valeurs; nous avons donc attribué à ces lapins une protidémie moyenne identique à celle de nos animaux. Nous avons constaté que les moyennes obtenues par cet auteur après étude statistique sont comprises dans l'intervalle de confiance de nos moyennes (tableau III). Evidemment, en matière de calcul statistique, on ne peut pas affirmer l'identité de ces deux populations. Mais ce fait exclut la conclusion que les populations sont différentes aux coefficients classiques de risque près.

Un certain nombre de nos animaux « normaux » ont reçu des injec[illegible] par voie intraveineuse. Nous leur injectons de la silice de dimensions [illegible] de silice [illegible] quq

nous trions par sédimentation [5, 8]. Nos animaux reçoivent 100 mg de silice tous les deux mois jusqu'à une dose totale de 500 mg. Chacun de nos animaux a un protidogramme avant tout traitement et des protidogrammes avant chaque nouvelle injection de silice. Nous continuons le prélèvement de sang pour électrophorèse après que la

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TABLEAU IV.

	APRÈS 200 MG				APRÈS 500 MG			
	Alb.	Alpha glob.	Bêta glob.	Gamma glob.	Alb.	Alpha glob.	Bêta glob.	Gamma glob.
Test de signification	S.	N.S.	N.S.	S.	T.H.S.	N.S.	T.H.S.	T.H.S.
Pourcentage de variation	↘ 11 %	0	0	↗ 43 %	↘ 19 %	0	↗ 50 %	↗ 140 %

	APRÈS 8 MOIS				APRÈS 14 MOIS			
	Alb.	Alpha-glob.	Bêta-glob.	Gamma-glob.	Alb.	Alpha-glob.	Bêta-glob.	Gamma-glob.
Test de signification	N.S.	N.S.	H.S.	H.S.	S.	N.S.	S.	S.
Pourcentage de variation	0	0	↗ 60 %	↗ 71 %	↓ 11 %	0	↗ 55 %	↗ 100 %

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5° Cette même analyse de variance, où chacun de nos lapins était son propre témoin, nous a permis une analyse très fine des variations des différentes fractions. Nous avons pu constater :

a) les alpha-globulines ne présentent aucune variation significative ni en fonction de la dose, ni en fonction du temps;

b) les albumines présentent une diminution peu importante, avec la dose et avec le temps;

c) les bêta-globulines présentent une augmentation après une certaine dose et un certain temps et cette augmentation se maintient constante ultérieurement;

d) les gamma-globulines augmentent fortement et cette augmentation s'accentue avec la dose et avec le temps.

Bibliographie.

[1] Bohle, E et Fischer, H. : *Klin. Wschr.*, 1953, **31**, 798.

[2] Deutsch, H. F. et Goodlde, M. B. : *J. Biol. Chem.*, 1945, **161**, 1.

[3] Deutsch, H. F. et Mac Chan, W. H. : *J. Biol. Chem.*, 1949, **180**, 219.

[4] Duplay, J. et Versino, A. : L'électrophorèse sur papier chez les animaux de laboratoires normaux et silicotiques. Prix P. Aymard, 1961, Marseille.

[5] Duplay, J. : *Thèse de Pharmacie*, Marseille 1962.

[6] Geinitz, W. : *Klin. Wschr.*, 1954, **32**, 1108 11.

[7] Latner, A. L. : *Biochem. J.*, 1952, **52**, xxix.

[8] Mosinger, M., Jouglard-Duplay, J. et Versino, A. : Sur la silicose expérimentale du lapin obtenue par l'administration intraveineuse de silice. *Arch. Mal. Prof.* (en cours de publication). Mars 1963.

[9] Mosinger, M., Versino, A., Jouglard-Duplay, J. et Fondarai, J. : Etude statistique de protidogrammes et de glycoprotidogrammes de lapins normaux. *C. R. Soc. Biol.*, séance du 30 avril 1963 (en cours de publication).

[10] Mosinger, M., Versino, A., Jouglard-Duplay, J. et Fondarai, J. : Etude statistique des protidogrammes de lapins silicotiques. *C. R. Soc. Biol.* (en cours de publication) séance du 21 mai 1963.

[11] Pugar, Z. : *Z. Physiol. Chem.*, 1954, **62**, 296.

[12] Scheiffarth, Kimbel et Berg : *Naturwissenschaften*, 1955, **42**, 214 5.

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Influence of Cortisone on Leptomeningeal Reaction Induced by Talc.* (20511)

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Ragan and co-workers(1) have demonstrated a marked retardation in the wound healing of rabbits treated with large doses of cortisone. This observation has been extended to the inflammatory and reparative processes of other mesodermal structures. Blunt(2) has studied delayed healing in experimental fractures of rabbits. Ducommun(3) and Scheinberg and Saltzstein(4) showed that ACTH and cortisone prevented the formation of intra-abdominal adhesions following the intraperitoneal instillation of talc, while Scheinberg, Saltzstein and Johnson(5) observed similar results in the prevention of pleural and pericardial adhesions.

Because cortisone diminished the inflammatory response of other serous surfaces, a study of its action on the meningeal reaction to the intrathecal administration of a sterile irritant was undertaken. Of several substances tried talc was the most effective in eliciting a meningeal reaction.

It was also felt that control of the meningeal reaction might be of value in the treatment of tuberculous meningitis as well as other granulomatous affections of the meninges. In such instances it seemed possible that the reduction of exudation and inflammatory response would make the offending organism more accessible to the action of the chemotherapeutic agent. Furthermore, the use of cortisone in the prevention of post-traumatic adhesions also suggested itself.

Materials and method. Eighteen white male rabbits weighing from 3.0 to 4.8 kg received a cisternal injection of 1 cc of a saline suspension of talc after a similar quantity of cerebrospinal fluid had been withdrawn. The talc suspension was sterilized by autoclaving. Ten animals were treated with cortisone, while 8 served as controls. In the cortisone-treated group 4 animals received a cisternal injection of 1 cc of a 1:9 suspension of talc, while 6

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The authors wish to acknowledge the invaluable and generous assistance of Drs. Charles Ragan, Department of Medicine, and Raffael Lattes, Department of Surgical Pathology, Columbia University College of Physicians and Surgeons.

Hydrocortisone acetate was supplied through the courtesy of Dr. Elmer Alpert of Merck and Company, Rahway, N. J.

received a 1:4 suspension. In the control series 2 animals were injected with a 1:9 suspension, whereas a 1:4 suspension was employed in the remaining 6.

The 10 treated rabbits received daily intramuscular injections of a saline suspension of cortisone acetate (Merck) for 15 days after the intrathecal administration of talc. Eight of these rabbits received cortisone for 2 days prior to the cisternal injection, while 2 received no such pretreatment. Nine rabbits received 10 mg cortisone per kilogram and one 5 mg/kg intramuscularly.

All cisternal injections were carried out under nembutal anesthesia. The talc suspension was administered slowly (15-20 seconds) to avoid respiratory arrest resulting from acute pulmonary edema (6).

All animals were sacrificed 15 days after injection. The brain and meninges were fixed in 10% formalin. Sections were stained with hematoxylin and eosin.

Results. Following cisternal injection, transient tonic and clonic convulsions were observed in several rabbits shortly after the talc administration. No permanent neurological lesions were seen. In no instance was evidence of meningeal infection found.

The average weight loss of the cortisone-treated rabbits was 625 g, whereas that of the control rabbits was 314 g. Although the difference in the mean weight loss appears significant, it must be pointed out that certain control rabbits lost more weight than some of the cortisone-treated animals. However, all the cortisone-treated animals appeared markedly lethargic and emaciated after 2 weeks.

In this series of experiments 2 animals died while receiving cortisone. One, treated with 10 mg/kg, died on the 7th day after intrathecal injection, and the other, on 5 mg/kg, died on the 11th day. The exact cause of death could not be determined in either instance.

In the control group there was diffuse thickening and opacity of the meninges, especially in the area of the brain stem, whereas in the treated animals the translucency of the normal meninges was better preserved. The differences observed grossly were not as striking as those seen histologically.

Microscopic sections from the control group revealed primarily a phagocytic, histiocytic response with some fibroblastic and minimal angioblastic proliferation. Foreign-body giant cells appeared to engulf individual talc crystals, and dense adhesions were present in certain locations between pia and arachnoid membranes. The talc particles were easily visualized under polarized light because of the doubly refractile quality of the crystals (Fig. 1).

In contrast to the controls, the meningeal response of the treated animals was consid-

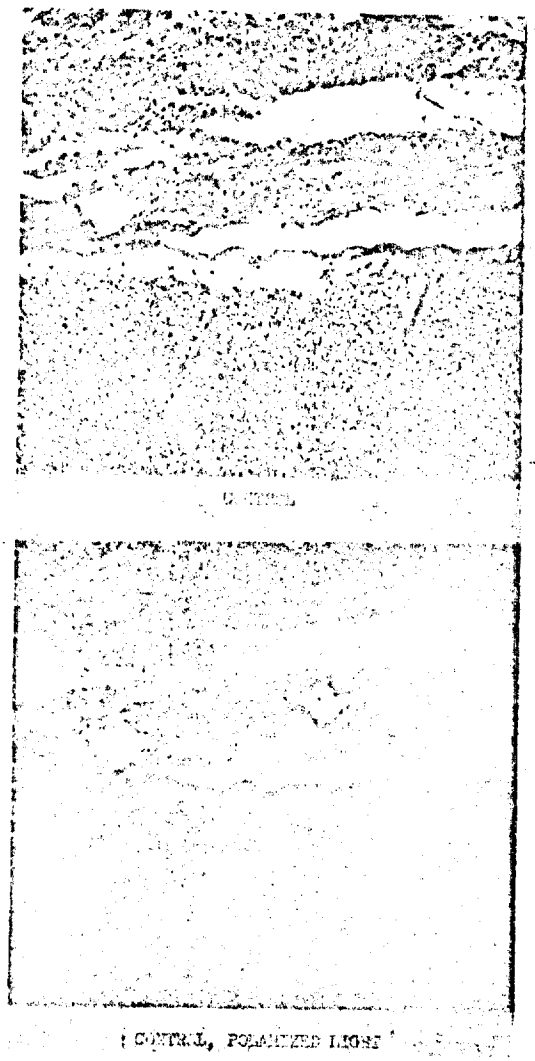

FIG. 1. Photomicrograph of leptomeningeal reaction in an untreated rabbit 15 days after cisternal injection of sterile talc. Note fibroblastic and phagocytic response. When viewed under polarized light, talc crystals may be seen. × 105.

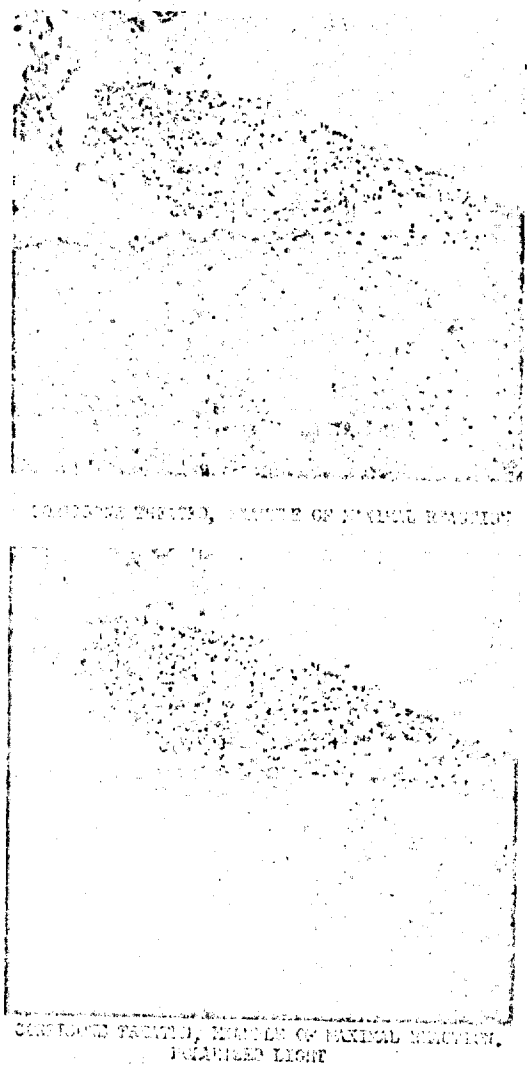

FIG. 2. Maximal reaction to talc seen in a rabbit treated 2 days before and 15 days after cisternal injection of talc with 10 mg cortisone/kg. There is minimal meningeal reaction. Talc crystals may be seen under polarized light. × 105.

crably diminished, although the degree of reaction varied from animal to animal. The extent of this variation is indicated in Fig. 2 by examples of maximal and, in Fig. 3, by the minimal meningeal response in cortisone-treated animals. Often loose collections of talc crystals could be seen lying free in the subarachnoid space without evident reaction (Fig. 3). Even when the presence of talc elicited a histiocytic response, individual crystals were generally not engulfed by phagocytes. No adhesions between pia and arachnoid were established.

Evaluation of the degree of meningeal reactivity was entirely subjective, and no attempt to quantitate the cellular response was made.

Preliminary experiments with intrathecal hydrocortisone. To avoid the systemic toxicity of parenterally administered cortisone, the use of intrathecal hydrocortisone suggested itself. Accordingly, using sterile precautions,

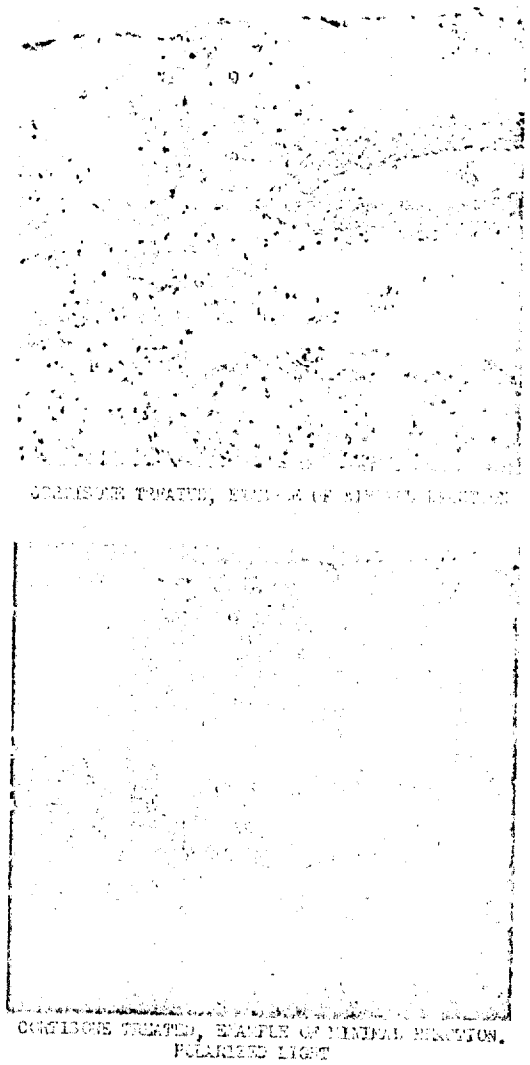

FIG. 3. Minimal reaction to talc seen in a rabbit treated similarly to that shown in Fig. 2. There is almost complete absence of meningeal reaction. Talc crystals may be seen under polarized light. × 105.

3 animals received 10 mg (0.4 cc) of a saline suspension of hydrocortisone acetate (Merck) as well as 1.0 cc of a 1:4 sterile saline suspension of talc in a single intrathecal injection. On the 7th day another dose of 10 mg hydrocortisone was administered by the same route. In order to assess the effect of the hydrocortisone suspension on the meninges, 3 animals received 10 mg of hydrocortisone plus 1.0 cc saline, followed on the 7th day by another equal dose of hydrocortisone. Following the intrathecal administration of hydrocortisone both with and without talc, the animals showed striking convulsive behavior, extensor rigidity, and marked tachypnea for approximately 1 hour following the injection. There was complete recovery in every animal, and no residual neurological abnormalities were noted. No attempt was made to determine whether the hydrocortisone or the vehicle, including 0.9% benzyl alcohol, was responsible for the immediate clinical reaction. One of the animals injected with talc and hydrocortisone died on the 12th day; the cause of death was not ascertained.

The microscopic appearance of the meninges of the 2 surviving animals injected with talc and hydrocortisone showed some diminution of the meningeal reaction, although this effect was not as striking as with cortisone. The animals, however, did not show the emaciation and lethargy characterizing those treated with cortisone, and the weight loss averaged 240 g as compared to a mean weight loss of 625 g in the cortisone group. The gross and microscopic appeance of the meninges of those animals injected only with hydrocortisone was not abnormal.

Summary. Eighteen rabbits received intrathecal injections of 1.0 cc of talc suspension and were sacrificed 15 days later. Eight rabbits were untreated, serving as controls. These developed a meningeal reaction consisting of the appearance of histiocytes, macrophages, and giant cells. Ten animals treated with daily injections of 5 and 10 mg cortisone/kg showed a marked diminution of the meningeal response. Preliminary experiments with the use of hydrocortisone, injected intrathecally (10 mg) on the 1st and 7th days, also suggest diminution of the meningeal response to talc, though not as striking as with the use of intramuscular cortisone.

1. Howes, E. L., Plotz, C. M., Blunt, J. W., and Ragan, C., *Surgery*, 1950, v28, 177.

2. Blunt, J. W., Jr., Plotz, C. M., Lattes, R., Howes, E. L., Meyer, K., and Ragan, C., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 678.

3. Ducommun, P., and Mach, R. S., *Semaine des Hopitaux*, 1951, v26, 3170.

4. Scheinberg, S. R., and Saltzstein, H. C., *Arch. Surg.*, 1951, v63, 413.

5. Scheinberg, S. R., Saltzstein, H. C., and Johnson, A. S., *Post-graduate medicine and surgery:* Surgical forum, Am. Coll. Surg., Philadelphia, Saunders, 1951, pp. 531-536.

6. Cameron, G. R., and De, S. N., *J. Path. and Bact.*, 1949, v61, 375.

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disease were belching, obstipation, and flatulence, but they were far less frequent in occurrence.

4. All of the gastro-intestinal manifestations were usually unrelenting in character and persisted until the cardiac condition was relieved.

5. Combined gastro-intestinal and heart disease may occur but it is uncommon; only three patients in this series had both conditions present at the same time.

BIBLIOGRAPHY

Brooks, H.: The Abdominal Signs and Symptoms in Cardiac Decompensation. *Ann. Clin. Med.*, 2:357, May, 1924.

Riesman, D.: Myocardial Disease and Its Gastric Masquerades. *J. A. M. A.*, 91:1521, Nov. 17, 1928.

White, P. D.: The Differential Diagnosis of Gastro-Intestinal and Cardiac Disorders. *Am. J. Dig. Dis. and Nutrit.*, 4:650, Dec., 1937.

Urinary Excretion of Silica in Humans Following Oral Administration of Magnesium Trisilicate*

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SYNTHETIC, hydrated magnesium trisilicate was introduced into therapeutics by Mutch in 1936 (1) as an antacid and adsorbent agent. Its field of greatest use appears to be as an antacid in the treatment of hyperchlorhydria as found in peptic ulcer and gastritis. Its adsorbent activity is of value in the treatment of non-specific diarrheas and in putrefactive and fermentative conditions in the intestinal tract.

It is well known that continued administration of antacid agents and in particular the soluble ones, such as sodium bicarbonate and the carbonates (even calcium carbonate in rare cases), may cause alkalosis by disturbing the acid-base equilibrium of the blood plasma. It has been stated by Mutch (2) that magnesium trisilicate cannot be absorbed and so produce direct alkali poisoning. Kraemer and Aaron (3) state that neither magnesium trisilicate nor the hydrated silica (SiO_2) formed by its interaction with hydrochloric acid are soluble, therefore neither the original compound nor this end product can be absorbed from the digestive tract to produce alkalosis.

CHEMISTRY OF MAGNESIUM TRISILICATE

Synthetic, hydrated magnesium trisilicate reacts with N/20 hydrochloric acid according to the following equation (4):

$$Mg_2Si_3O_8.nH_2O + 4HCl \rightarrow 2\ MgCl_2 + (n+2)H_2O + 3\ SiO_2$$

It is probable that this same reaction takes place in the stomach when hydrochloric acid is present. Magnesium trisilicate in the presence of hydrochloric acid assumes the state of a colloidal gel (4) and the silica evolved is in the form of an active, hydrated colloid. The total antacid effect is approximately equivalent to the magnesium content.

The hydrated, colloidal silicon dioxide (SiO_2) and the magnesium trisilicate gel formed in the stomach go into the small intestine, where they mix with the alkaline intestinal contents and it is possible that a portion of either or both are converted to Na_2SiO_3 or some other soluble form of silica. If this is true, then some of the silica will be absorbed and then excreted in the urine. It was in order to determine this action that the study outlined in this experiment was instituted.

OUTLINE OF STUDY

Five healthy, young males, members of the Resident House Staff at Grasslands Hospital, volunteered to serve as subjects in this study. In order to determine the average twenty-four-hour excretion of silica on the regular house diet, the twenty-four-hour excretion of urine was collected at several-day intervals and the silica content was quantitatively determined for each twenty-four-hour period. The results (Table I) were considered to be the mean daily excretion of silica by normal humans on regular diets.

Synthetic, hydrated magnesium trisilicate was given to each subject in the form of 0.5-gram, compressed products* which readily disintegrated in water. Five grams were given daily in five spaced doses of one gram each (9:00 a. m., 1:00 p. m., 4:30 p. m., 7:00 p. m. and 9:30 p. m.), for four consecutive days. On the second day, the collection of twenty-four-hour excretion of urine was begun, in Pyrex glass flasks so that silica from the container would not contaminate the specimen. The twenty-four-hour urine excretion was collected for five days and each specimen was analyzed for silica and the results tabulated in Table II.

RESULTS

The urine was collected for twenty-four hours and analyzed for silica content. This was done on three different days at weekly intervals and the mean twenty-four-hour excretion of all the subjects was found to be 16.2 mg. SiO_2 (Table I).

On the second, third and fourth days of the administration of magnesium trisilicate, the mean ex-

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*The preparation used in this study was 'Tabloid' Magnesium Trisilicate, gr. 7½, furnished by Burroughs Wellcome & Co., New York.

cretion rose to 172, 178 and 162 mg. SiO_2, respectively. Two days after magnesium trisilicate had been stopped, the excretion had fallen to 24.5 mg., which was within the range of the normal excretion of these subjects.

Each subject, during the course of four days, took a total amount of 20 grams of magnesium trisilicate, which contained approximately 9.2 grams of SiO_2. The increased SiO_2 excretion during the course of the experiment (not counting the first day of administration of magnesium trisilicate and the third day after its cessation) averaged 484 mg. (Table II), so that a rough approximation of the amount of SiO_2 excreted in the urine from the magnesium trisilicate taken orally is about 5.2 per cent.

TOXICOLOGY

Mutch (4) conducted an extensive toxicologic study of magnesium trisilicate. Six white rats were given daily doses of 0.6 grams for six months. They remained in perfect health. One litter was born and divided into two groups, one serving as a control and the other receiving magnesium trisilicate from the time of weaning in a dose that would correspond to a daily dose of between 3 and 4 pounds for an average man. They grew normally and had healthy litters. Tissues of the animals of the first and second generations were studied, and no evidence of pathologic change was found. He also carried out a similar study on mice and no tissue changes were found.

Sollmann (6) states that sodium silicate given by mouth acts as a mild alkali, is readily absorbed from the alimentary canal, and is excreted in the urine. There is no evidence that silicates play any physiologic role. Intravenous injection of large doses of colloidal silicic acid causes rapid death by intravascular coagulation.

DISCUSSION

Many foods contain silica, particularly vegetables, whole grains (husks), and sea food. Silica is present in the urine in amounts proportional to that in the diet.

Synthetic, hydrated magnesium trisilicate reacts in the stomach with hydrochloric acid to form magnesium chloride and silicon dioxide (SiO_2), some of which may be in the form of silica gel (silicic acid), and in the presence of hydrochloric acid, some colloidal magnesium trisilicate gel is formed. When they go into the upper intestinal tract, they enter an alkaline medium and the formation of soluble silicate may occur. Silica in some soluble form or in a colloidal form is absorbed, however it can only be excreted in solution. The excretion of silica in the urine is increased (as found by analytic methods) and reaches its peak on the second day of administration of magnesium trisilicate. It remains about level for the remaining days of administration. After the magnesium trisilicate is stopped, the silica excretion rapidly decreases, so that on the second day after its cessation the excretion is only slightly above the mean daily excretion of silica.

The total increase of silica excretion indicates that approximately 5.2 per cent of the silica in magnesium trisilicate is excreted by the kidneys, indicating either absorption of magnesium trisilicate or one of its silica compounds.

As these experiments suggest that there is absorption of more than 5 per cent of the silica in the magnesium trisilicate, it is probable that the interaction of magnesium trisilicate and hydrochloric acid produces more complex silica end products than silicon dioxide. When a silicate is decomposed by an acid, silicic acid is formed, part of which may separate as a gel and part may remain in solution as a colloid. The acids that are possible are ortho-silicic acid (H_4SiO_4), which is soluble, meta-silicic acid (H_2SiO_3), partially soluble, and tri-silicic acid ($H_4Si_3O_8$), partially soluble, and di-silicic acid ($H_2Si_2O_5$), which is nearly insoluble. When a trisilicate salt reacts with hydrochloric acid, it would be expected that the formation of a gel approximating the formula for trisilicic acid would be formed. However, other factors, such as concentration and temperature, take an active part. The loss of moisture in changing from one form to another of the silicic acids is continuous, showing that actually the acids given are hypothetical. However, the variation in the hydration of the SiO_2 molecule does give a change in the solubility, which is a point of importance in considering the possibility of a soluble silicon compound being formed in the stomach from magnesium trisilicate.

It is probable that the reaction between magnesium trisilicate and hydrochloric acid is much more complex

TABLE I

Urinary silica (SiO_2) — 24 hour totals and per 100 cc. urine

Subject	S		R		M		L		B	
Output of SiO_2 on regular diet during three different 24-hour periods	Total SiO_2 (Mg.)	Mg./100 cc.	Total SiO_2 (Mg.)	Mg./100 cc.	Total SiO_2 (Mg.)	Mg./100 cc.	Total SiO_2 (Mg.)	Mg./100 cc.	Total SiO_2 (Mg.)	Mg./100 cc.
	7.1	0.83	13.8	0.83	16.2	1.4	19.7	1.2	12.0	1.1
	13.5	1.3	17.6	1.0	11.2	1.2	10.2	1.7	11.4	0.73
	26.9	4.2	23.1	1.3	29.5	1.7	12.8	2.0	17.9	1.4
Mean daily output	16.8		18.2		19.0		14.2		13.8	
Standard deviation	± 10.11		± 4.68		± 9.38		± 4.91		± 3.59	

Mean average daily output of five normal subjects on regular diet was 16.2 mg. SiO_2.

TABLE II

Experimental study on same subjects as Table I with daily oral ingestion of 5 grams of magnesium trisilicate (Urinary silica (SiO_2) — 24 hour totals and per 100 cc. urine)

— SUBJECTS —

Day	Mg. Tri. Orally	S Total SiO_2 (Mg.)	S Mg./100 cc.	R Total SiO_2 (Mg.)	R Mg./100 cc.	M Total SiO_2 (Mg.)	M Mg./100 cc.	L Total SiO_2 (Mg.)	L Mg./100 cc.	B Total SiO_2 (Mg.)	B Mg./100 cc.	Mean Tse.
1	5 Gm.	....	...	....	...	...	...	....	...	....	...	....
2	5 Gm.	152.0	14.6	167.0	9.09	194.0	13.1	196.0	20.0	151.5	8.51	172.1
3	5 Gm.	200.0	20.0	118.0	8.69	202.0	9.09	230.0	19.0	144.0	9.01	178.8
4	5 Gm.	....	...	203.0	10.4	152.0	10.0	182.0	10.0	114.0	8.30	162.7
5	None	96.0	10.5	40.6	2.78	89.0	9.09	45.6	3.8	50.0	3.6	64.0
6	None	....	...	38.0	2.4	....	...	28.8	2.0	11.8	1.0	24.5
Total excreted from 2nd to 5th day inclusive.		448.0		528.6		637.0		653.6		459.5		545.8
Expected excretion (calculated from mean normal daily output x number of days).		*47.4		72.8		76.0		56.8		55.2		61.6
Difference =		X 400.6		455.8		561.0		596.8		404.3		483.7

*4 days. Mg. Tri. = Magnesium Trisilicate. Tse. = Total SiO_2 Excreted.

han stated in the early part of this paper, and the following reaction-steps are suggested:

$$Mg_2Si_3O_8 \cdot nH_2O + 4HCl \rightarrow$$
$$2\,MgCl_2 + H_4Si_3O_8 + nH_2O \rightarrow$$
$$2\,MgCl_2 + H_2Si_2O_5 + SiO_2 + (n+1)H_2O \rightarrow$$
$$2\,MgCl_2 + 3\,SiO_2 + (n+2)H_2O$$

Some of the tri- or di-silicic acid may react with dium compounds to form soluble sodium silicate Na_2SiO_3) in the intestine.

The highest concentration of urinary silica found uring the administration of 5 grams of magnesium isilicate daily to normal human subjects was 20 mg. SiO_2 per 100 cc. urine. Other investigators (5) report xcretions of silica in rabbits up to 27.2 mg. and 34.3 g. SiO_2 per 100 cc. urine on a diet high in silica and latively similar high figures for other herbivorous imals. Apparently such high silica excretions are t harmful, as no toxic effects are observed that can ascribed to silica.

It may be postulated that silica absorbed from foods d magnesium trisilicate by mouth is in a similar hemical and physical state. As animals excrete large mounts of silica with no apparent tissue damage, it reasonable to suspect that the moderately increased lica excretion during a course of magnesium trisilite therapy is not harmful.

CONCLUSIONS

1. Urinary silica excretion studies were carried t on five healthy, male subjects on regular diets and e mean twenty-four-hour excretion was found to be 2 mg. of silica.

2. The urinary excretion of silica varied widely and was proportional to the amount of silica in the diet.

3. Urinary silica excretion was increased in healthy subjects when magnesium trisilicate was taken by mouth.

4. These studies suggest that silica is excreted by the kidneys in direct proportion to the amount absorbed and that there is probably no definite renal threshold for silica.

5. No evidence has been found that the administration of magnesium trisilicate by mouth has any deleterious effect on the human or animal organism.

We wish to express our appreciation to Doctor Gilbert Dalldorf, Director of Laboratories, Grasslands Hospital, and to Professor William C. MacTavish, Professor of Chemistry, New York University, for advice and supervision given to us during the course of these studies.

The chemical method for determining silica in the urine will appear in the reprints.

REFERENCES

1. Mutch, N.: The Silicates of Magnesium. *Brit. M. J.*, 1:143-148, 1936.
2. Mutch, N.: Hydrated Magnesium Trisilicate in Peptic Ulceration. *Brit. M. J.*, 1:254-257, 1936.
3. Kraemer, M. and Aaron, B.: The Use of Hydrated Trisilicate of Magnesium for Peptic Ulcer. *Am. J. Dig. Dis.*, 7:57-60, 1940.
4. Mutch, N.: Synthetic Magnesium Trisilicate. *Brit. M. J.*, 1:205-208, 1936.
5. King, E. J., Stantial, H. and Dolan, M.: The Biochemistry of Silicic Acid. II. The Presence of Silica in Tissues. *Biochem. J.*, 27:1002-1006, 1933.
6. Sollmann, T.: A Manual of Pharmacology. W. B. Saunders Co., Philadelphia, p. 145, 1934. (Fourth Edition)
7. King, E. J.: Silicosis and Metabolism of Silica. *Canad. M. A. J.*, 31:21-26, 1934.
8. King, E. J. and Dolan, M.: The Biochemistry of Silicic Acid. VIII. The Determination of Silica. *Biochem. J.*, 33:944-954, 1939.

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Micro-Determination of Soluble Silica in Urine

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Silicon may be determined in solution by the spectrophotometric method of Dienert & Wandenbulche (1923), as modified by King & Stantial (1933). This involves the formation of the yellow siliconolybdate and the reduction of this complex to molybdenum blue by 1-amino-2-naphthol-4-sulphonic acid reagent. Measurement of this colour provides a means of determining silicon.

A disadvantage of this method of determining silicon is that phosphate interferes by forming phosphomolybdate, which is similarly reduced to molybdenum blue. Various methods have been used for preventing phosphate interference during silicon determination. Khaler (1941) used mineral acids to prevent the formation of phosphomolybdate. Complex-forming agents such as oxalic acid (Schwartz, 1942; De Sesa & Rogers, 1954) or citric acid (Case, 1944; De Sesa & Rogers, 1954) were used to bleach the interfering phosphomolybdate while leaving the silicomolybdate unaffected. Paul & Pover (1960*a*) have developed a method of selectively removing phosphomolybdate by ethyl acetate. In their experiments, silicon was determined in the aqueous phase after reduction of the silicomolybdate to molybdenum blue.

These methods of eliminating phosphate during the determination of silicon are successful when only small amounts of phosphate are present to interfere. However, in the presence of high concentrations of phosphate, such as are found in rat and human urines, these methods are not applicable unless the urines are diluted considerably. This is undesirable, for when silica is present in trace quantities it might escape determination.

When phosphate is present in high concentrations, it can be removed by precipitation. Thayer (1936) claimed that phosphate was completely removed by precipitation with calcium chloride and ammonia at about pH 8·0 without simultaneous removal of any silicate. King & Stantial (1933) also reported precipitation of phosphate by calcium chloride and ammonia during silica determination in urine. However, none of these workers investigated the effect of pH on silicate and phosphate precipitation. As it has been the author's experience that calcium chloride and ammonia cause precipitation of silicate at pH 8·0, a systematic study of the effect of pH on silicate and phosphate precipitation by calcium chloride and ammonia was made. From the results obtained it was possible to develop an accurate and reproducible method of determining silicon in rat and human urines.

EXPERIMENTAL

Materials

When possible, reagents of A.R. grade were used.

Silica standard. Solutions of soluble silica were prepared by fusing 10·0 mg. of finely divided quartz with 0·5 g. of powdered sucrose and 1·5 g. of sodium peroxide in a Parr bomb, and dissolving the fused material in 100 ml. of water in a polythene beaker. Further solutions of silicate were prepared by dilution and used as standards.

Phosphate standard. A stock solution of phosphate was prepared by dissolving 0·5 g. of anhydrous disodium hydrogen phosphate in water and diluting to 100 ml. Standard solutions were prepared from it by dilution.

Ammonium molybdate. The tetrahydrate (5·0 g.) was dissolved in 100 ml. of water.

1-Amino-2-naphthol-4-sulphonic acid reagent. The acid (200 mg.) was dissolved in water with 12·0 g. of sodium metabisulphite and 2·4 g. of sodium sulphite heptahydrate and diluted to 100 ml. After the solution had stood overnight it was filtered into a brown-glass bottle and kept in a cool place.

Calcium chloride. The anhydrous salt (10·0 g.) was dissolved in 100 ml. of water.

Ammonia solution. Aqueous ammonia (sp.gr. 0·880) was diluted to give a 2n-solution, which was stored in a polythene bottle. This solution contained a negligible amount of silicate.

Urine samples. Rat and human urines were collected and stored in polythene beakers containing liquid paraffin.

Trimethylhexadecylsilane. This was synthesized by the method of Paul & Pover (1959*b*). The method involved the methylation of trichlorohexadecylsilane (Found: Si 9·[illegible]; $(CH_3)_3SiC_{16}H_{33}$ requires Si, 9·4[illegible]%; the infrared spectrum of trimethylhexadecylsilane showed two bands at 8·05 and 11·8–12·02 μ).

Methods

Precipitation of phosphate in urine. Urine (2·0 ml.) was mixed with 8·0 ml. of water. A portion (4·0 ml.) of the diluted urine was acidified by adding 0·2 ml. of acetic acid

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and the mixture was shaken. Calcium chloride solution (5 ml. of 10%, w/v) was added, together with 2·0 ml. of aqueous ammonia (2 N). The mixture was then shaken and set aside for 15 min. This solution, which was at pH 7·0, was filtered and the filtrate used for the colorimetric determination. Blanks containing water in place of urine, brought to pH 7·0, were similarly prepared.

Determination of silicon. A portion (2·0 ml.) of the filtrate was pipetted into a 50 ml. separating funnel, followed by the addition, with shaking, of 2·0 ml. of N hydrochloric acid. Ammonium molybdate solution (4·0 ml. of 5%, w/v) was then added and the solution mixed by shaking for 1 min. This solution had a pH of 1·0. It was then extracted once with 20 ml. of ethyl acetate for 30 sec. The aqueous layer was separated and 4·0 ml. of 10% (w/v) citric acid solution added to it with shaking. The solution was allowed to stand for 2 min., after which 1·0 ml. of the aminonaphtholsulphonic acid reagent was added with shaking, and the resulting solution set aside for 10 min. to allow maximum colour to develop. Any precipitate formed was removed at this stage by centrifuging. The extinction of the solution, which was linear to silicon concentration in the range 5–60 μg. of SiO_2, was then measured against a similarly treated blank at 690 mμ.

Effect of pH on the precipitation of phosphate and silicate. To each of several 4 ml. portions of disodium hydrogen phosphate solution (2·93 mg./ml.) or sodium silicate (125 mg. of SiO_2/ml.) were added 4·0 ml. of 10% calcium chloride solution and 2·0 ml. of 2N-ammonia solution. The mixtures were brought to different pH values in the range 4·0–10·0, by adding acetic acid. After centrifuging, the phosphate, or silicate, was determined in the supernatant.

Determination of phosphate. The phosphate was determined by the method of King (1932).

RESULTS AND DISCUSSION

The results recorded in Table 1 demonstrate that the present method of determining silicon is accurate and reproducible. King & Stantial (1933) reported that when 1-amino-2-naphthol-4-sulphonic acid reagent was used as the reducing agent a persistent 'blank' colour was obtained. The author has found that the addition of citric acid before the reduction eliminated this 'blank' colour without affecting the reduction by the aminonaphtholsulphonic acid reagent.

In Table 2 are recorded results demonstrating the effect of pH on phosphate precipitation. These results were obtained by determining the remaining phosphate concentration in the supernatant, after precipitation and centrifuging. At pH 4·0 no precipitation of phosphate occurred; at pH 5·0, 35·9% of the phosphate was precipitated. At pH 7·2, only 5·4% of the total phosphate was present in the supernatant, 94·6% having been precipitated. Total precipitation of phosphate was obtained between pH 9·0 and 10·0.

If the results of phosphate precipitation are compared with those of Table 3 it can be seen that, unlike phosphate, silicate is not precipitated

Table 1. *Recovery of known amounts of silicon*

Samples of trimethylhexadecylsilane were fused in a Parr bomb and their silicon contents determined by the modified colorimetric method with 1-amino-2-naphthol-4-sulphonic acid reagent in the presence of citric acid, to effect reduction of silicomolybdate (for details see Experimental section).

Wt. of trimethylhexadecylsilane fused (mg.)	Silicon present (mg.)	Silicon found (mg.)	Error (%)
15·6	1·46	1·50	2·7
15·0	1·42	1·40	1·4
15·0	1·50	1·45	3·3
14·9	1·40	1·40	0·0
15·7	1·47	1·47	0·0
14·6	1·37	1·41	2·9
23·7	2·71	2·68	1·1
15·1	1·42	1·36	4·2
		Mean	1·95±0·38

Table 2. *Effect of pH on phosphate precipitation*

Solutions of sodium phosphate, each containing 11·30 mg. of anhydrous disodium hydrogen phosphate, were treated with the precipitating reagents and the resulting solutions adjusted to one of the pH values below. After centrifuging, the clear supernatant was examined for phosphate (for details see Experimental section).

pH	Amount of phosphate recovered in supernatant (mg.)	Phosphate recovered in supernatant (%)
4·0	11·30	100·0
5·0	7·29	64·5
6·0	1·16	10·3
7·2	0·605	5·4
8·2	0·467	4·1
9·0	0·275	2·4
10·0	<0·005	<0·05

Table 3. *Effect of pH on silicate precipitation*

Solutions of sodium silicate, each containing 0·500 mg. of SiO_2, were treated with the precipitating reagents and the resulting solutions adjusted to one of the pH values given below. After centrifuging, the clear supernatant was examined for silicate.

pH	Amount of silicate recovered in supernatant (mg. of SiO_2)	Silicate recovered in supernatant (%)
5·0	0·500	100·0
6·0	0·500	100·0
7·0	0·500	100·0
8·0	0·445	89·0
9·0	0·407	81·4
10·0	0·330	66·0

between pH 5·0 and 7·0. However, silicate precipitation occurred at pH 8·0, 10% of the total silicate being precipitated at this pH. Nevertheless, even at pH 10·0, at which pH all the phosphate was precipitated, only 34% of the silicate was precipitated.

In order to determine whether co-precipitation of silicate occurred during phosphate precipitation, the effect of pH on silicate precipitation in the presence of phosphate was studied. These results reported in Table 4 are in good agreement with those of Table 3, thus demonstrating that co-precipitation of silicate does not occur during phosphate precipitation.

By taking advantage of the difference at pH 7·0 between the precipitation of phosphate and silicate, a method of determining silicate in urine was developed. As only a small amount of phosphate escapes precipitation at pH 7·0, whereas silicate is not precipitated at this pH, it was possible to precipitate the bulk of the phosphate at neutral pH and then eliminate the remaining phosphate by extracting the phosphomolybdate with ethyl acetate in the manner described by Paul & Pe[illegible] (1960*a*). When this technique was applied, accurate and reproducible determinations of small amounts of silicon in rat and human urine were obtained (Table 5). As rat and human urines contain large amounts of phosphate (about 20 mg./[illegible] in rat urine), the method should be generally useful for determining trace amounts of silicon in very high concentrations of phosphate. In the experiments reported here, silica, in the presence of [illegible] about 100 times its own concentration of phosphate, was accurately determined.

Table 4. *Effect of* pH *on silicate precipitation in the presence of phosphate*

Solutions of sodium silicate, each containing 0·228 mg. of silica plus 25·0 mg. of disodium hydrogen phosphate, were treated with the precipitating reagents and the resulting solutions adjusted to one of the pH values given below. After centrifuging, the clear supernatant was examined for silicate.

pH	Amount of silicate recovered in supernatant (mg. of SiO_2)	Silicate recovered in supernatant (%)
5·0	0·228	100·0
6·0	0·228	100·0
7·0	0·228	100·0
8·0	0·210	92·1
9·0	0·189	82·9
10·0	0·156	68·4

SUMMARY

1. An accurate and reproducible method of determining small amounts of silica in rat and human urine is described. The method involves precipitating the bulk of the phosphate present in urine, by calcium chloride and ammonia at pH [illegible] and removing the remaining phosphate by selective extraction of the phosphomolybdate with ethyl acetate. Silica was then determined in the aqueous phase after reduction of the silicomolybdate by 1-amino-2-naphthol-4-sulphonic acid reagent in the presence of citric acid.

2. The method is capable of general applicability to determinations of trace amounts of silica in high concentrations of phosphate.

I wish to thank Professor A. C. Frazer for facilities to carry out this investigation, Dr W. F. R. Pover for his interest and advice, and Midland Silicones Ltd. for [illegible] trichlorohexadecylsilane. The award of an I.C.I. Research Fellowship is gratefully acknowledged.

Table 5. *Recovery of silica in rat and human urines*

Urine (2 ml.) was made up to 10 ml. with water. A portion (4 ml.) of this solution was then treated with the precipitating reagents. After centrifuging, soluble silica was determined in the supernatant. (For details see text.) To 2 ml. of the same urine, 2 ml. of silicate solution was added and the whole made up to 10 ml. After precipitation and centrifuging as described above, the total silica content of the urine was determined. All values are expressed as milligrams.

Urine	SiO_2/2 ml. of urine	SiO_2 added to 2 ml. of urine	Total SiO_2 found in 2 ml. of urine	SiO_2 recovered
Rat	0·110	0·260	0·370	0·260
	0·150	0·300	0·450	0·290
	0·110	0·400	0·510	0·390
	0·140	0·160	0·2[illegible]	0·09
	0·120	0·07	0·190	0·07
Human	0·020	0·240	0·260	0·250
	0·021	0·400	0·421	0·400
	0·030	0·300	0·330	0·300
	0·020	0·340	0·360	0·330
	0·020	0·100	0·120	0·100

REFERENCES

A. O. P. (1944). *Indust. Engng Chem. (Anal.)*, 16, 309.
M. A. & Rosen, L. G. (1954). *Analyt. Chem.* 26, 173.
F. & Wandenbulcke, F. (1923). *C.R. Acad. Sci., Paris* 176, 1478.
H. J. (1941). *Industr. Engng Chem. (Anal.)*, 13, 73.
King, E. J. (1932). *Biochem. J.* 26, 292.
King, E. J. & Stantial, H. (1933). *Biochem. J.* 27, 699.
Paul, J. & Pover, W. F. R. (1960*a*). *Anal. chim. acta*, 22, 155.
Paul, J. & Pover, W. F. R. (1960*b*). *Arch. Biochem. Biophys.* 87, 312.
Schwartz, M. C. (1942). *Industr. Engng Chem. (Anal.)*, 14, 893.
Thayer, L. A. (1950). *Industr. Engng Chem. (Anal.)*, 2, 277.

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Amino Acid-Activating Enzymes in Muscle

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The enzymic 'activation' of amino acids (Hoagland, 1955) is recognized and measured by the formation of hydroxamic acids in the presence of amino acids, adenosine triphosphate and hydroxylamine, or by the incorporation of radioactive pyrophosphate into adenosine triphosphate in the presence of amino acids. There is ample evidence (Kingdon, Webster & Davie, 1958) that activation involves the reaction of the amino acid with adenosine triphosphate to form an amino acid–adenylic acid anhydride and pyrophosphate. Magnesium appears to be essential for this reaction, which is generally considered to be the first step in the synthesis of protein from amino acids. It is likely that separate activating enzymes for the individual amino acids exist. Some have been purified, e.g. for methionine (Berg, 1956), phenylalanine (Van de Vorst, Konigsberger & Overbeek, 1957), valine (Berg & Ofengand, 1958), tryptophan (Kelley, Konigsberger & Lipmann, 1956), alanine (Hoagland & Holley, 1956) and serine (Webster & Davie, 1959).

The presence of such enzymes activating various amino acids has been demonstrated in many animal and plant tissues and in micro-organisms (Zimmermann, Bergmann & Berg, 1957; Marcus, 1959; McCorquodale & Mueller, 1958; Webster, 1958; Lipmann, 1958; Novelli, 1955; Cole, Coote & Work, 1957). In animal tissues they have been found largely in the 'soluble' fraction obtained by high-speed centrifuging of tissue homogenates. Recently (Reis, Coote & Work, 1959; Craddock & Pearson, 1960), however, their presence in mitochondria has been demonstrated.

No study of amino acid-activating enzymes in skeletal muscle has yet been reported. This paper describes such experiments, carried out with mouse and human skeletal muscle. In addition, similar measurements were made with muscle from mice with hereditary muscular dystrophy, as part of a study of muscle-protein synthesis in this disease.

EXPERIMENTAL

Mice. All the mice used were of the inbred strain 129, bred from animals obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A. This strain carries a recessive gene which produces, in the homozygote, a progressive muscular dystrophy (Michelson, Russell & Harman, 1955). The 'normal' mice of this strain may be either homozygous (double dominant) or heterozygous with respect to the gene. The animals were fed without restriction on 'breeding sow cubes' (J. N. Brown and Son, Minden Street, Newcastle upon Tyne) supplemented daily with fresh vegetables and were used when 2–3 months old. All the affected mice which were used had been frequently observed to drag their hind legs behind them.

Human muscle. Human quadriceps muscle was obtained in the course of orthopaedic operations.

Preparation of muscle 'cell-sap' fraction. The mice were killed by dislocation of the neck and the muscle from the hind legs was removed as quickly and completely as possible into a cold (0–2°) solution of 0·02M-2-amino-2-hydroxymethylpropane-1:3-diol (tris) (obtained by dilution of a 2M-solution which had been adjusted to pH 7·4 with HCl) and 0·05M-KCl. Human muscle was placed in a similar solution immediately upon removal. Visible fat was removed and the muscle chopped finely with scissors. It was homogenized in 4–5 vol. of tris–KCl in a Potter–Elvehjem homogenizer with a Perspex pestle; a loosely fitting tube was used first with subsequent treatment in a tighter tube to break up the fibres completely. The homogenate was centrifuged for 1 hr. at 70 000 *g* in a Spinco model L ultracentrifuge.

Arzneimittel-Forschung
14:47-50, 1964.

Various forms of experimental gastric ulcers in rats and the influence of drugs thereon*

1st Report: The induction of ulcers of the antestomach in rats by administration of glucose and the influence of drugs

by

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*Work carried out with financial support from the Hans Walz Foundation.

In the discussion of the development of stomach ulcers the "peptic theory" is currently the focus of attention: the mentally induced, pathologically intensified secretion of gastric juice in the empty stomach leads to digestion of the stomach wall at certain preferred sites and therewith to a stomach ulcer (1, 14, 15).

It is therefore necessary to give a brief account of the mechanisms by which gastric secretion takes place (2, 15, 16). On the one hand the gastric mucosa is stimualted through the nerves (cerebral cortex - anterior hypothalamus - nucleus of the vagus nerve - vagus nerve - stomach mucosa), and on the other it is stimulated to secretion by tissue hormones (gastrin etc.) that are liberated by the stimulus of the digested food in the stomach or small intestine. In addition, as a result of

psychic factors during digestion, the hypothalamus causes the pituitary to secrete adenocorticotropic hormone (ACTH). ACTH causes an increased secretion of glucocorticoids by the adrenal cortex, and these intensify the acid and pepsin secretion of the gastric mucosa (3,4).

A systematic treatment of gastric ulcers must attempt to block the above-mentioned route by way of which stimulation of the gastric mucosa takes place, at one or preferably several different points. For this the following possibilities are available today (5,6,7):

1. Neutralization of gastric juice by antacids.
2. Blocking of intracellular enzymes of the gastric mucosa.
3. Blocking of the vegetative ganglia.
4. Interruption of parasympathetic postganglionic nerve fibers by drugs.
5. Influencing of humoral stimulation of the gastric mucosa.
6. Depression of the cerebral cortex and brain stem by means of sedatives.
7. Finally, attempts have been made to enhance the perfusion of the gastric mucosa by means of various drugs (for instance estrogens), so as to make it more resistant to digestive attack by gastric juices.

In the present first report the effect of various drugs was tested on experimentally induced ulcers of the antestomach

of the rat. For the influence of drugs on ulcers of the glandular stomach of the rat, see 2nd and 3rd reports.

Our experiments are based on a method developed by Grandjean (8). He has established that rats fed for a few days exclusively with glucose solution reveal numerous ulcers in the antestomach.

Experiments and calculations

Male Wistar albino rats fed on a standard diet (Latz, Euskirchen) were used as experimental animals. 468 animals were evaluated. The weight of the animals varied between 144 and 386 g, with an average of 217 g. Before beginning experimentation, the animals, kept in single cages on the above standard diet, were habituated for 3-4 days to the drinking tubes through which the glucose solution was to be administered later, but which were at first filled with water. The drinking tubes were test tubes with a 33 ml capacity with a hole blown into the bottom. Each animal received 2 tubes per day. In order to avoid coprophagy, the floor of the cages consisted of wide-mesh wire lattice. The cages were protected from direct incident light and were housed in a semi-dark room. The room temperature was kept constant at 22°C by means of a thermostat.

After the period of habituation the animals received exclusively 20% glucose solution for 8 days (Dextropur, Deutsche Maizena-Werke, Hamburg). The drugs were in part dissolved in the glucose, and in part injected subcutaneously, intramuscularly or intraperitoneally. Next the rats were killed

with chloroform and the stomach carefully removed. In order to be able to evaluate the ulcers, the stomachs were cut open along the large curvature and stretched out on a cork mat. Next the stomachs were fixed in 4% formalin. Random samples from each series were examined histologically. The ulcers were evaluated according to number and zize, the size being determined according to Grandjean (9) by comparison with small pieces of paper of known area stuck on to them. The total number of ulcers of each stomach was determined, and by addition of the individual ulcer surfaces the total ulcerated area was established.

The average number of ulcers (12.9 ulcers per rat) and the average ulcerated surface (11.5 mm^2) of 39 control rats served as basis for comparison. These rats had received exclusively glucose as diet for 8 days. The drug experimental series generally included 16 animals. If distinct differences existed between the control group and the drug series, the results were calculated statistically according to the method of t-distribution (10).

Experiments on ulcer production and morphology of the ulcers

In order to observe the daily increase in the tumor rate on a glucose diet, a total of 13 series of experiments were carried out. Isolated tumors were observed as early as after 2 days of glucose diet.

From the third to the fifth day and later ulcers appeared with great regularity (See Fig. 1 and 2). The ulcers were round to oval, with a characteristic border ridge. In some cases the ulcers were scattered over the entire antestomach, as in Fig. 3, while in others they formed small groups in the tip of the antestomach. The rat antestomach has a covering tissue consisting of several layers of hornified flattened epithelium, while the other layers correspond go the usual structure of the intestinal wall. The ulcers with border ridges did not break through the muscularis mucosae, in other words they are strictly speaking erosions. The mucosa and submucosa were loosened by edema and showed ample infiltration by leukocytes.

In certain cases starting from the 5th day, and invariably from the 8th to 9th day after the glucose diet, in addition to the ulcers with border ridges large, confluent, polygonally bordered ulcers appeared with a lardlike, shiny yellow background and the surrounding area pulled in by scars (Fig. 4). Histologic examination showed that these ulcers destroyed the wall layers of the antestomach as far as deep in the muscularis propria. The muscularis mucosae was severed and retracted from the edges of the ulcers. The background of the ulcers consisted of a fibrinoid, swollen necrosis. The stomach necroses were separated from the live tissue by a wide wall of leukocytes. Ulcer perforation was never observed.

The fundus of the stomach during the glucose experiments invariably revealed small hemorrhagic dots that were easily wiped off, beneath which there were sometimes superficial

mucosal necroses. The pylorus mucosa, which consisted of wide-lumened, very tortuous glandular ducts, revealed minute round ulcers (Fig. 3). In a random sampling the duodenum and small intestine never revealed hemorrhages or ulcers.

In summary, it may be said that with a few exception, the ulcers increased continuously both in number as well as size and depth from the 2nd to the 12th day (Fig. 1 and 2).

Experiments on ulcer healing

The animals were first fed for 8 days with glucose solution and then again received a standard diet and water for 1 day or 2-3 or 4 days (see Fig. 1 and 2). The ulcers subsided very rapidly on the normal diet, in such a way that from the edges of the ulcers wide epithelial bands advanced towards the center of the ulcers. On the second day of the healing experiments most of the former ulcers were only recognizeable from a hypertrophized scarry area. On the 4th day the ulcers were largely cicatrized with scars often running together in large, tumor-like, projecting foci.

Drug Experiments

The following drugs were tested*:

Solu-Decortin-H[1)] significantly delayed healing (see Table 1).

Belladonna-Neutralon[2)] (100 mg/kg body weight per day per os).

Enzynorm liqu.[3)] (25 ml/kg body weight per day per os.

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* For more exact data on the chemical composition and mechanism of action of the drugs used, see Röhm (11).

Vagantin[4] (500 mg/kg body weight per day per os).

Rabro[5] (300 mg/kg body weight per day per os).

Serpasil[6] (1 mg/kg body weight per day i.p.).

Robufakton[7] (100 mg/kg body weight per day per os).

Arovit[8] (15,000 I.U./kg body weight per day i.m.).

Ephynal[9] (5 mg/kg body weight per day i.m.).

Silver nitrate D_5[10] = 1:100,000 (20 ml/kg body weight per day per os).

Nux vomica Ø = tincture[11] (4ml/kg body weight per day per os.

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1) Prednisolone-21hemisuccinate-Na; E. Merck, Darmstadt.

2) Al-Na-silicate with 0.5% belladona extract; Schering, Berlin.

3) Standardized stomach extract with peptic and catheptic enzyme activity and hydrochloric acid bound by mucine; Nordmarkwerke, Hamburg.

4) Xanthene carbonic acid-diethylaminoethylester-methylbromide (Methantheline); Dr. Ch. Brunnengräber and Co., Lübeck.

5) 1 tablet = 0.33 g subnitr. bism., 0.3 g succ. liquiritiae, 0.4 magn. carbon., 0.2 sodium bicarbonate, 0.025 g cort. Fraergulae, 0.025 g Rhiz. Calami; H. Trommsdorff, Aachen.

6) Reserpine; Ciba, Wehr/Baden.

7) Extract from Brassica oleracea capitala alba with a standardized 0.1 mg S methylcysteine sulfoxide, 25 mg S-methyl-methionine-sulfonium bromide, 0.3 mg L(-)-cistine, 1.3 mg DL-methionine, 0.3 mg L-glutamine content; Robugen, Esslingen-a.N.

8) Vitamin A palmitate in oily solution; Deutsche Hoffmann-La-Roche Co., Greuzach/Baden

9) DL-α-tocopherolacetate (vitamin E): Deutsche Hoffmann-La Roche Co., Greuzach/Baden.

10) Silver nitrate has been in homeopathic use since Hahnemann. It is"one of the most valuable agents against ulcerous alterations of the stomach and duodenum"(12).

11) Nux vomica contains the alcaloid strychnine, which according to Korbsch (13) enhances gastric secretion. In homeopathy Nux vomica is regarded as a "polychrest"(=substance acting in many ways), preferably in the digestive organs (12).

It should be pointed out that the drugs were administered during the development of the ulcers. Thus it can only be said concerning the drugs tested that they inhibit, intensify or do not influence the formation of ulcers. The experiments give no information as to the effect of the drugs on the healing of pre-existing ulcers. The results of the drug experiments are shown in Table 2.

In summary, the following drugs gave rise to a statistically confirmed increase in the number of ulcers or in the ulcerated area: Belladonna-Neutralon, Enzynorm liqu., Serpasil, Robufakton, Arovit, Silver nitrate D_5 and Nux Vomia Ø. A distinct, but statistically unconfirmed reduction in the number of ulcers and in the ulcerated area was only observed with Vagantin.

Discussion

The production of ulcers of the antestomach on a glucose diet may be explained by the fact that the gastric juice secreted during drinking is not utilized for digestion at all and can thus exercise a destructive effect on the stomach wall. This is also indicated by the fact that the additional administration of gastric juice in the form of Enzynorm gives rise to a 363% increase in the ulcerated area.

A striking observation is that the ulcers were located almost exclusively in the antestomach. The rat antestomach is a sack covered by a layered, hornified, flattened epithelium with a ca. 3-4 ml capacity. It may be regarded as a storage organ

and on an exclusively liquid diet it becomes virtually inactive. Since it has no mucous glands, unlike the glandular area it lacks a protective covering of mucosa. This may be the reason that the antestomach is the primary victim of the digestive action of the gastric juice.

Since,as is known, the human stomach does not have a part corresponding to the rat antestomach, the results of our experiments can only be transferred to human pathology with great reservations. However, the results of the experiments reveal certain systematic aspects, in particular there is a close connection between the duration of the experiments and the number and area of ulcers (Fig. 1 and 2).

All our results were related to a control value, calculated from 39 animals. According to our observation the weight of the animals appears to have no effect on the development of ulcers of the antestomach. Since all the experiments were carried out in a room which was kept constantly at 22°C by a thermostat, there could be no difference in the possible effect of temperature on the development of ulcers. Despite the homogeneity of the animal material used in our experiments, the individual variations in ulcer rate were very great. This had a particularly detrimental effect on the possibility of statistical confirmation of the results. Evaluation of the results with regard to number of ulcers does not appear difficult at first sight. However, the ulcers very often run together, so that they cannot always be distinctly distinguished from one another. On the other hand the ulcerated surface is definitely

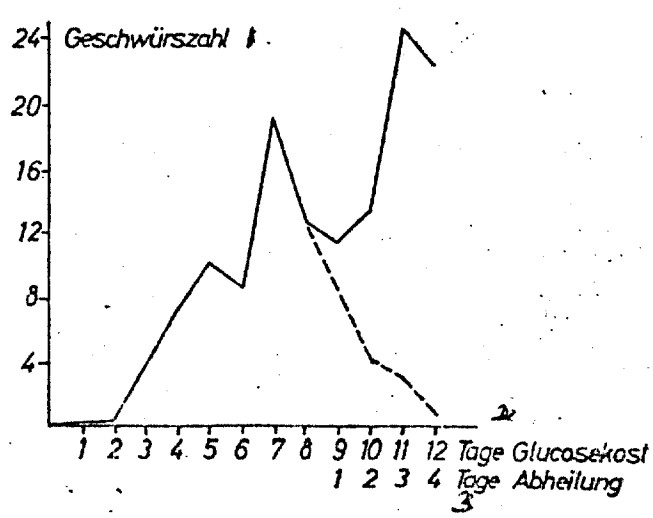
established. The surface was determined by comparison with small pieces of paper of known size, in other words by a somewhat subjective method. In order to keep this error as small as possible, all the stomachs were evaluated by the same investigator.

The ulcerated area undoubtedly is the more significant in assessing a drug. Unlike the number of ulcers, it also has some connection with the depth of the ulcers, since experience has shown that ulcers with a large surface frequently break through the muscularis mucosa. Morphologically the ulcers revealed nothing unusual under the influence of drugs. It may be said in general that substances that appreciably intensify ulcer formation elicited predominantly ulcers of the polygonal type, in other words large and deep ulcers, and conversely, under the influence of drugs that inhibit the formation of ulcers, ulcers with border ridges were induced, in other words ulcers with a small surface.

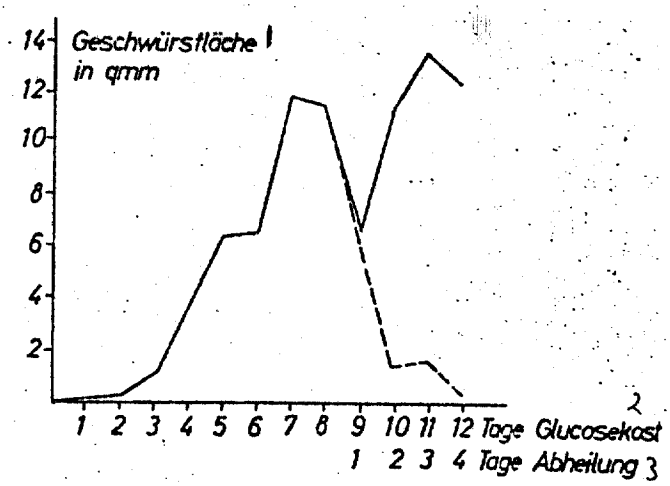
Summary

Please refer to English summary on p. 50.

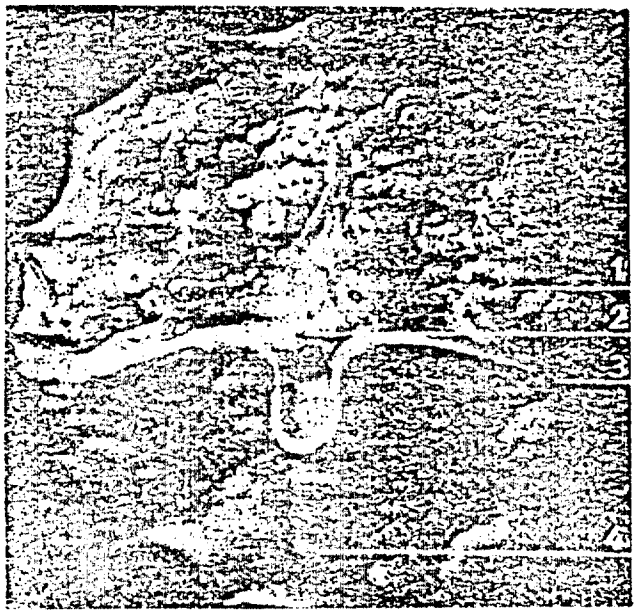
Figures



1. Increase in the number of ulcers of the antestomach with increasing duration of experimentation. Broken line means healing of the ulcers once normal food was once more administered after the 8th day. Key: 1. Number of ulcers, 2. Days of glucose diet, 3. Days of healing.



2. Increase in the surface of the ulcers of the antestomach with increasing duration of experimentation. Broken line means healing of the ulcers after resumption of normal diet after the 8th day. Key: 1. Ulcerated surface in mm^2. 2. Days of glucose diet. 3. Days of healing.



3. Ulcer of the antestomach with formation of border ridge. These ulcers already develop after 3 days of glucose diet in small numbers, but regularly. The ulcers are round to oval, not exceeding a diameter of 2-3 mm. As a rule the ulcers, as in the figure, are scattered over the entire antestomach. 1 = typical ulcer with border ridge. 2 = entry into the esophagus. 3= margo plicatus. 4 = ulcers of the pylorus.

4. Large-surfaced polygonally outlined ulcer of the antestomach (see 1). In certain cases starting from the 5th day, and invariably starting from the 8th day to the 9th day after the glucose diet, in addition to the ulcers with border ridges large, polygonally bordered ulcers developed.

Tables

	n	Z	t	F	t
1 4 Tage Abheilung unter Normalkost	16	0,8		0.13	
2 4 Tage Abheilung unter Normalkost + 5 mg/gg tgl. Prednisolon	16	3,6	2,84	2.0	2,31

1. Delay in healing of ulcers of the antestomach under the influence of Prednisolone. The figures in semi-heavy type represent statistically confirmed values (t greater than 2.01 or 2.04). n = number of animals. Z = total number of ulcers. F = total ulcerated surface. Key: 1. 4 days of healing on normal diet. 2. 4 days of healing on normal diet + 5 mg/kg Prednisolone per day.

1	n	Z	2 Änderung v. Z., gegenüber d. Kontrollwert %	t	F	3 Änderung v. F., gegenüber d. Kontrollwert %	t
Kontroll-Serie	39	12.9			11,5		
Belladonna Neutr.	16	23,4	+ 82	3,30	21,6	+ 88	3,17
Enzymorm liqu.	16	28.0	+117	4.99	54,5	+365	2,99
Vagantin	15	11,7	— 9	0,40	6,9	— 40	1,52
Babro Magentabl.	16	17,5	+ 36	1,55	10,3	— 10	0,40
Serpasil	15	22,6	+ 75	2,73	28,7	+149	4,49
Robufakton	16	25,9	+100	3,49	7,7	— 33	1,75
Arovit	15	20,6	+ 59	2,72	26,0	+126	2,94
Ephynal	16	12.0	— 7	—	12,5	+ 9	
Argent. nitr. D 5	14	14,0	+ 9	0,36	21,4	+ 86	2,46
Nux vomica ♀	16	13,2	+ 2	0,13	29,9	+160	2,91

2. Summary of results of drug experiments. Figures in semi-heavy type represent statistically confirmed values (t greater than 2.01 or 2.04). n = number of animals. Z = total number of ulcers. F = total ulcerated surface. Key: 1. Control series. 2. Change in total number of ulcers compared to control values %. 3. Change in total ulcerated surface compared to control values %.

Bibliography

See p. 50.

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Trotz der für die iv. Applikation relativ hohen Dosen von Fursemid ließ die pathologisch-histologische Untersuchung auch hier keinen direkten schädigenden Einfluß, bedingt durch das Präparat, erkennen.

Als Ergebnis der dargelegten Versuche kann somit festgestellt werden, daß Fursemid sowohl oral als auch iv. verabreicht, eine günstige salidiuretische Wirksamkeit bei Hunden aufweist: Die Natrium- und Chlorid-Ausscheidung wird aequimolar stark erhöht, während die Kalium-Ausscheidung nur geringfügig zunimmt. Für die Anwendung ist weiter von Bedeutung, daß kein Einfluß auf den Kohlenhydrat- und Harnsäurestoffwechsel beobachtet wurde. Da überdies beim oralen 28-Tage-Versuch und bei 14tägiger iv. Applikation neben guter lokaler und allgemeiner Verträglichkeit keine toxischen, biochemischen und pathologisch-histologischen Veränderungen gesehen wurden, die auf das Präparat zurückgeführt werden konnten, wurde Fursemid der klinischen Prüfung übergeben.

Zusammenfassung

In der vorliegenden Veröffentlichung werden Untersuchungsergebnisse über das Salidiureticum 4-Chlor-N-(-2-furylmethyl)-sulfamyl-anthranilsäure (Fursemid, Lasix®) erstmals, wie folgt, dargelegt:

1. Die unterste sicher wirkende Dosis für Fursemid am Hund liegt bei oraler Applikation bei 0,5 mg/kg und bei iv. Applikation bei 0,125 mg/kg. Bis zu 1 mg/kg (oral und iv.) wurde keine vermehrte Kaliumausscheidung beobachtet, obwohl bereits eine deutlich gesteigerte Natrium- und Chlorid-Ausscheidung vorlag.
2. Unter den angegebenen Versuchsbedingungen wurde die größtmögliche Salidiurese bei einer Dosierung von ca. 12,5 mg Fursemid/kg oral erreicht. Eine weitere Steigerung konnte auch mit höheren Dosen (bis zu 200 mg/kg) nicht erreicht werden. Die maximale Mehrausscheidung betrug für Wasser das 2,5fache, für Natrium und Chlorid das 7fache, für Kalium das 3,5fache gegenüber den Kontrollen.
3. Das Präparat ruft in hohen Dosen (50 mg/kg oral und 25 bzw. 50 mg/kg iv.) an der Katze eine Blutdrucksenkung um 20—50 mm Hg hervor. Die blutdrucksenkende Wirkung am Hund ist stärker.
4. Fursemid hat keinen Einfluß auf den Blutzucker (Kaninchen) und Harnsäureblutspiegel (Hund). Es wurde keine Carboanhydratasehemmung festgestellt.
5. Die akute Toxizität an Mäusen liegt oral bei 4,6 (2,0—10,1) g/kg, intravenös bei 308 (290—327) mg/kg.
6. Neurologische und pathologisch-histologische toxische Veränderungen, die im Zusammenhang mit der Substanz stehen können, sowie wesentliche Störungen im Blutserum und Fermentwerten bzw. Elektrolythaushalt wurden in einem Dosenbereich von 6 bis 50 mg/kg täglich oral über 28 Tage nicht gesehen. Intravenös wurde das Präparat bei 14tägiger täglicher Verabfolgung lokal und allgemein gut vertragen.

Summary

The Saluretic Effect of Chloro-N-(2-furylmethyl)-5-sulfamyl-anthranilic Acid

The results are reported of investigations on the saluretic agent 4-chloro-N-(2-furylmethyl)-sulfamyl-anthranilic acid Fursemid, Lasix®).

1. The minimum effective dose of Fursemid in dogs is 0,5 mg/kg after oral and 0,125 mg/kg after intravenous administration. No increase in potassium excretion was observed after doses up to 1 mg/kg (oral and intravenous) although a considerably raised sodium and chloride excretion was already present.
2. Under the test conditions described, maximum saluresis was reached by an oral dose of approximately 12,5 mg/kg Fursemid. A further increase could not be obtained even with higher doses (up to 200 mg/kg). The additional elimination of water was 2,5 times, sodium and chloride 7 times and potassium 3,5 times that of the controls after administration of the maximum effective dose.
3. High doses (50 mg/kg orally and 25 or 50 mg/kg intravenously) of the preparation had a lowering effect on blood pressure of 20—50 mm Hg in cats. This effect was more pronounced in dogs.
4. Fursemid has no influence on the blood sugar (rabbits) and on the uric acid level of the blood (dog). Inhibition of the enzyme carboanhydratase has not been found.
5. Acute toxicity in mice is 4,6 (2,0—10,1) g/kg orally, and 308 (290—327) mg/kg intravenously.
6. Oral doses ranging from 6—50 mg/kg daily during 28 days did not result in any neurologic or histopathologic changes attributable to the compound, nor were any disturbances observed in blood serum and enzyme values or in the electrolyte balance. During 2 weeks of intravenous administration of 5—50 mg daily, the compound was well tolerated locally as well as generally.

Literatur

[1] Johnston, D. H. u. Cornish, A. L., J. Amer. med. Ass. 170, 2054 (1959)
[2] Jones, M. T. u. Caldwell, F. R., New Engl. J. Med. 267, 1029 (1962)
[3] Shanklin, D. R., New Engl. J. Med. 266, 1097 (1962)
[4] Sugar, S. F., J. Amer. med. Ass. 175, 618 (1961)
[5] Langdon, R. G. u. Wolff, F. W., Brit. med. J. II, 926 (1962)
[6] Wilson, W. R. u. Okum, R., Clin. Res. 10, 184 (1962)
[7] Formanek, K., Wien. klin. Wschr. 75, 211 (1963)
[8] DBP 1 122 541 Kl. 12 q 6/04 Internationale Kl. C 07 c; d v. 28. 12. 1959.
[9] DBP 1 129 501 Kl. 12 q 6/04 Internationale Kl. C 07 c; d v. 17. 12. 1960.
[10] Council of Drugs, New Names, List No. 19, J. Amer. med. Ass. 184, 576 (1963)
[11] Fernandez, E. M., Fuertes, A. M. u. Marañon, G., El Bazo, Bd. I, Universidad de Granada 1952
[12] Philpot, F. G. u. Philpot, J. St., Biochem. J. 30, 2991 (1956)
[13] Häussler, A. u. Hajdú, P., Klin. Wschr. 38, 874 (1960)

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Die verschiedenen Formen des experimentellen Ulcus ventriculi bei der Ratte und seine medikamentöse Beeinflussung*)

1. Mitteilung: Erzeugung von Vormagengeschwüren bei der Ratte durch Glukosefütterung und ihre medikamentöse Beeinflussung

Von F. Röhm, G. Seybold und R. Pirtkien

In der Diskussion um die Entstehung der Magengeschwüre steht heute die „peptische Theorie" im Vordergrund: Die psychisch ausgelöste, krankhaft gesteigerte Leersekretion des Magensaftes führt an bestimmten Prädilektionsstellen zu einer Andauung der Magenwand und somit zum Magengeschwür [1, 14, 15].

Es ist deshalb notwendig, kurz die Mechanismen darzustellen, über die die Magensaftsekretion erfolgt [2], [15], [16]: Einerseits wird die Magenschleimhaut auf dem Nervenweg stimuliert (Großhirnrinde — vorderer Hypothalamus — Vaguskern — Vagusnerv — Magenmucosa), andererseits wird sie durch Gewebshormone (Gastrin u. a.), die durch den Reiz der angedauten Nahrung im Magen bzw. im Dünndarm freigesetzt werden, zur Sekretion angeregt. Außerdem wird durch psychische Momente während der Verdauung die Hypophyse über den Hypothalamus zur Ausschüttung von adrenocorticotropem

*) Die Arbeiten wurden aus Mitteln der Hans Walz-Stiftung unterstützt.

Hormon (ACTH) veranlaßt; ACTH seinerseits bewirkt in der Nebennierenrinde eine vermehrte Abgabe von Glucocorticoiden, die wiederum die Säure- und Pepsinsekretion der Magenschleimhaut verstärken [3, 4].

Eine zielbewußte Therapie des Magengeschwürs muß versuchen, die oben genannten Wege, auf denen die Stimulation der Magenmucosa erfolgt, an einer oder besser an mehreren verschiedenen Stellen zu durchkreuzen. Dazu stehen heute folgende Möglichkeiten zur Verfügung [5, 6, 7]:

1. Neutralisation des Magensaftes durch Antacida.
2. Blockierung der intrazellulären Enzyme der Magenschleimhaut.
3. Blockade der vegetativen Ganglien.
4. Medikamentöse Unterbrechung der parasympathischen postganglionären Nervenfasern.
5. Beeinflussung der humoralen Stimulation der Magenschleimhaut.
6. Dämpfung der Hirnrinde und des Hirnstamms durch Sedativa.
7. Schließlich wird versucht, durch verschiedene Arzneimittel (z. B. durch Oestrogene) die Durchblutung der Magenschleimhaut zu fördern, um sie so gegen eine Andauung durch den Magensaft widerstandsfähiger zu machen.

In der vorliegenden 1. Mitteilung wurde die Wirkung verschiedener Medikamente an experimentell erzeugten Vormagengeschwüren bei der Ratte ausgetestet. Die medikamentöse Beeinflussung von Geschwüren im Drüsenmagen der Ratte siehe 2. und 3. Mitteilung.

Unseren Versuchen liegt eine von Grandjean [8] ausgearbeitete Methode zugrunde; er stellte fest, daß Ratten, die einige Tage ausschließlich mit Glukoselösung gefüttert werden, zahlreiche, im Vormagen der Tiere lokalisierte Geschwüre aufweisen.

Versuchsanordnung und Berechnung

Als Versuchstiere wurden weiße, männliche Wistar-Ratten benutzt, die mit Standardkost (Latz, Euskirchen) gefüttert wurden. Zur Auswertung kamen 468 Tiere. Das Gewicht der Ratten schwankte zwischen 144 und 586 g. Durchschnittsgewicht 217 g. Vor Versuchsbeginn wurden die Tiere 3–4 Tage lang in Einzelkäfigen bei oben angegebener Standardkost an die Trinkröhrchen gewöhnt, in denen später Glukoselösung verabreicht werden sollte und die zunächst mit Wasser gefüllt waren. Bei den Trinkröhrchen handelte es sich um 35 ml fassende Reagenzgläser, in die unten ein Loch eingeblasen war. Jede Ratte erhielt täglich 2 Röhrchen. Um die Koprophagie zu vermeiden, bestand der Boden der Käfige aus weitmaschigem Drahtgitter. Die Käfige waren vor direkt einfallendem Licht geschützt und standen in einem halbdunklen Raum. Die Raumtemperatur wurde durch einen Thermostaten konstant auf 22° C gehalten.

Nach der Eingewöhnungszeit erhielten die Tiere 8 Tage lang ausschließlich 20%ige Glukoselösung (Dextropur®, Deutsche Maizena-Werke GmbH., Hamburg). Die Arzneimittel wurden teils in der Glukose gelöst, teils sc., im. oder ip. injiziert. Danach wurden die Ratten in Chloroform getötet und ihre Mägen sorgfältig herauspräpariert. Um die Geschwüre beurteilen zu können, wurden die Mägen längs der großen Kurvatur aufgeschnitten und auf einer Korkscheibe aufgespannt. Anschließend fixierten wir die Mägen in 4%igem Formalin. Einzelne Stichproben aus jeder Serie wurden histologisch untersucht. Die Auswertung der Geschwüre erfolgte nach Zahl und Größe, wobei die Größe nach Grandjean [9] durch Vergleich mit kleinen aufgeklebten Papierstückchen bekannter Fläche bestimmt wurde. Von jedem Magen wurde die Gesamtgeschwürszahl und durch Addition der einzelnen Geschwürsflächen die Gesamtgeschwürsfläche bestimmt.

Als Vergleichsbasis diente die durchschnittliche Geschwürszahl (12,9 Geschwüre pro Ratte) und die durchschnittliche Geschwürsfläche (11,5 mm²) von 39 Kontrolltieren, die acht Tage lang ausschließlich Glukosekost erhielten. Die Arzneimittelversuchsreihen umfaßten im allgemeinen 16 Tiere. Soweit deutliche Unterschiede zwischen Kontrollserie und Arzneimittelserien vorhanden waren, wurde die statistische Berechnung der Ergebnisse nach der t-Verteilung vorgenommen [10].

Versuche zur Geschwürsentstehung und Morphologie der Geschwüre

Um die tägliche Zunahme der Geschwürsrate unter Glukosekost beobachten zu können, wurden insgesamt 15 Versuchsreihen angesetzt. Bereits nach zwei Tagen Glukosekost fanden sich vereinzelte Geschwüre im Vormagen.

Vom dritten bis fünften Tag und später traten dieselben mit großer Regelmäßigkeit auf (vgl. Abb. 1 u. 2). Die Ulcera sind rund bis oval, charakteristisch ist eine deutliche Randwallbildung. Teils sind die Geschwüre wie in Abb. 3 über dem ganzen Vormagen verstreut, teils sitzen sie zu kleinen Gruppen zusammengelagert in der Spitze des Vormagens. Der Vormagen der Ratte besitzt als Deckgewebe ein mehrschichtiges, ver-

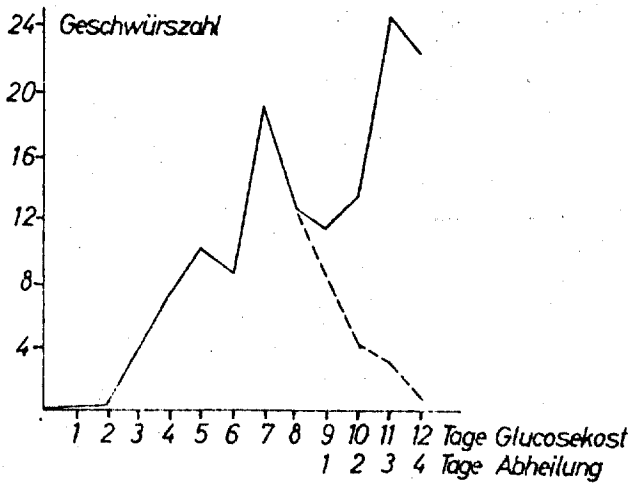

Abb. 1: Zunahme der Zahl der Vormagengeschwüre mit zunehmender Versuchsdauer. — Die gestrichelte Linie bedeutet die Abheilung der Geschwüre, wenn ab dem 8. Tag wieder Normalkost gefüttert wird.

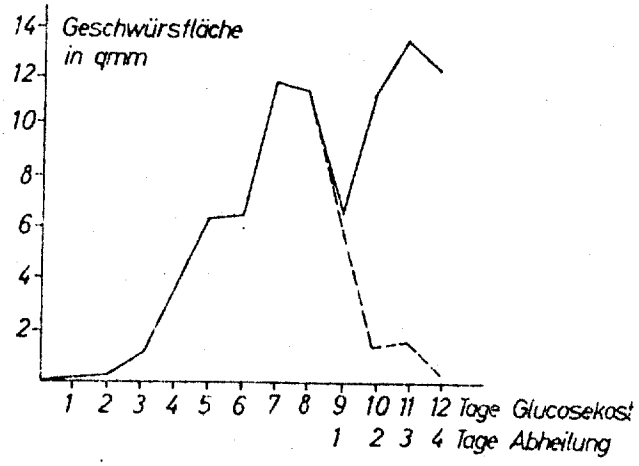

Abb. 2: Zunahme der Fläche der Vormagengeschwüre mit zunehmender Versuchsdauer. — Die gestrichelte Linie bedeutet die Abheilung der Geschwüre, wenn ab dem 8. Tag wieder Normalkost gefüttert wird.

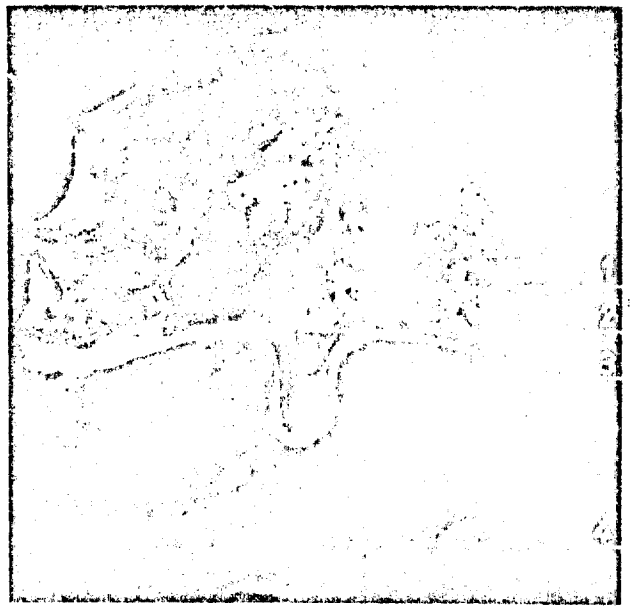

Abb. 3: Vormagengeschwüre mit Randwallbildung. Diese Geschwüre treten bereits nach 5 Tagen Glukosekost in geringer Zahl regelmäßig auf. Die Ulcera sind rund bis oval, ein Durchmesser von 2 bis 3 mm wird nicht überschritten. Meist sind die Geschwüre wie in der Abb. über den ganzen Vormagen verstreut. 1 = Typische Randwall-Geschwüre, 2 = Ösophagus-Einmündung, 3 = Margo plicatus, 4 = Pylorus-Geschwüre.

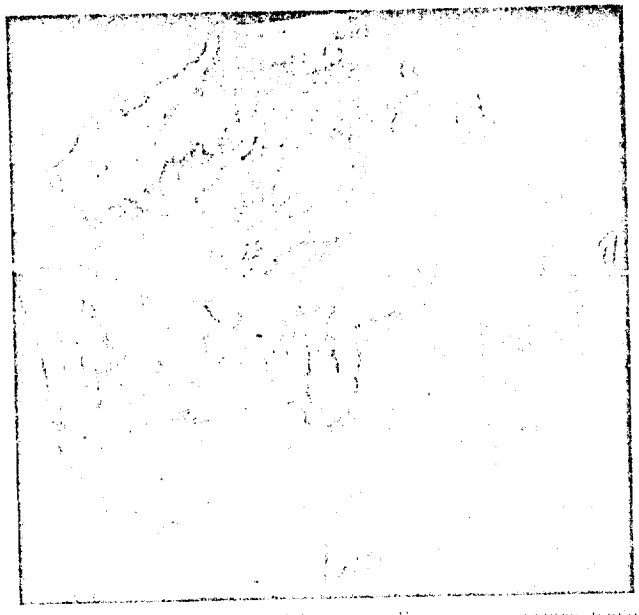

Abb. 4: Geschwüre polygonal begrenzt = Vormagengeschwüre (siehe 1). In Einzelfällen ab dem 5. Tag, regelmäßig ab dem 8. bis 9. Tag nach Glukosefütterung treten neben den Wall-Ulcera große, vielecks begrenzte Geschwüre auf.

hornendes Plattenepithel, die übrigen Schichten entsprechen dem üblichen Aufbau der Darmwand. Die Randwall-Ulcera durchbrechen die Muscularis mucosae nicht, es handelt sich also streng genommen um Erosionen. Mucosa und Submucosa sind ödematös aufgelockert und zeigen reichlich Leukozytenin filtrationen.

In Einzelfällen ab dem fünften Tag, regelmäßig ab dem achten bis neunten Tag nach Glukosefütterung treten neben den Randwall-Ulcera großflächige, konfluierende, polygonal begrenzte Geschwüre auf, mit speckig glänzenden gelben Grund und narbig verzogener Umgebung (Abb. 4). Die histologische Untersuchung zeigt, daß diese Geschwüre die Wandschichten des Vormagens bis tief in die Muscularis propria hinein zerstören. Die Muscularis mucosae ist durchtrennt und vom Geschwürsrand retrahiert. Der Geschwürsgrund besteht aus einer fibrinoiden Quellungsnekrose. Die Magenwand ist stark aufgelockert und zellig infiltriert. Die Nekrosen sind durch einen breiten Leukocytenwall vom lebenden Gewebe abgesetzt. Eine Geschwürsperforation wurde nie beobachtet.

Der Fundus des Magens zeigte während der Glukoseversuche regelmäßig kleine, leicht abwischbare Blutpunkte, denen bisweilen oberflächliche Schleimhautnekrosen zugrunde lagen. Die Pylorus-Schleimhaut, die aus weithumigen, stark geschlängelten Drüsenschläuchen besteht, wies kleinste runde Geschwüre auf (Abb. 5). Duodenum und Dünndarm zeigten bei Stichproben niemals Blutungen oder Geschwüre.

Zusammenfassend kann man sagen, daß von einigen Ausnahmen abgesehen, die Geschwüre vom 2. bis zum 12. Tag sowohl an Zahl als auch an Größe und Tiefe kontinuierlich zunahmen (Abb. 1 u. 2).

Versuche über die Abheilung der Geschwüre

Die Tiere wurden zunächst 8 Tage mit Glukoselösung gefüttert und erhielten anschließend 1 Tag bzw. 2, 3 oder 4 Tage lang wieder Standardkost und Wasser (vgl. Abb. 1 u. 2). Die Geschwüre bildeten sich unter der Normalkost sehr rasch zurück, indem von den Geschwürsrändern her breite Epithelbänder auf das Geschwürszentrum hinkriechen. Bereits am 2. Tag der Abheilungsversuche sind die meisten einzelnen Geschwüre nur noch an kleinen Delle in einem hypertrophen Narbengebiet zu erkennen. Am 4. Tag sind die Geschwüre weitgehend vernarbt, wobei die Narben oft zu großen, tumorartig vorgewölbten Herden konfluieren.

Arzneimittelversuche

Es wurden folgende Medikamente ausgetestet*):

Durch Solu-Decortin-H[*1]) ließ sich die Abheilung signifikant verzögern (s. Tab. 1).

Tab. 1: Verzögerung der Abheilung der Vormagengeschwüre durch Prednisolon. Die halbfett gesetzten Zahlen stellen die statistisch gesicherten Werte dar (t größer als 2,01 bzw. 2,04). n = Tierzahl, Z = Gesamtgeschwürszahl, F = Gesamtgeschwürsfläche.

	n	Z	t	F	t
4 Tage Abheilung unter Normalkost	16	0,8		0,15	
4 Tage Abheilung unter Normalkost + 5 mg/kg tgl. Prednisolon	16	5,6	**2,84**	2,0	**2,31**

Belladonna-Neutralon[*2]) (100 mg/kg Körper-Gew. tgl. oral).

Enzynorm liqu.[*3]) (25 ml/kg Körper-Gew. tgl. oral).

Vagantin[*4]) (500 mg/kg Körper-Gew. tgl. oral).

Rabro[*5]) (500 mg/kg Körper-Gew. tgl. oral).

Serpasil[*6]) (1 mg/kg Körper-Gew. tgl. intraperitoneal).

Robufakton[*7]) (400 mg/kg Körper-Gew. tgl. oral).

Arovit[*8]) (15 000 I.E./kg Körper-Gew. tgl. intramuskulär).

Ephynal[*9]) (5 mg/kg Körper-Gew. tgl. intramuskulär).

Argentum nitricum D_3[*10]) = 1 : 100 000 (20 ml/kg Körper-Gew. tgl. oral).

Nux vomica ♀ = Urtinktur[*11]) (4 ml/kg Körper-Gew. tgl. oral).[*12])

Es sei darauf hingewiesen, daß die Medikamente während der Geschwürsentstehung verabreicht wurden. Über die getesteten Arzneimittel läßt sich also lediglich aussagen, ob sie die Geschwürsbildung hemmen, unbeeinflußt lassen oder verstärken. Die Experimente geben:

*) Genauere Angaben über die chemische Zusammensetzung und die Wirkungsmechanismus der verwendeten Medikamente siehe bei Röhm [11].

[*1]) Prednisolon-21-hemisuccinat-Na: E. Merck AG, Darmstadt.

[*2]) Al-Na-silikat mit 0,5% *Belladonna*-Extrakt; Schering AG, Berlin.

[*3]) Standardisierter Magenextrakt mit peptischer und kathepsischer Fermentaktivität und mucingebundener Salzsäure; Nordmark-Werke GmbH., Hamburg.

[*4]) Xanthogenarbonsäure-diäthylaminoäthylester-methylbromid (Methanthelin); Dr. Ch. Brunnengräber u. Co. GmbH., Lübeck.

[*5]) 1 Tabl. = 0,35 g Bism. subnitr., 0,5 g Succ. Liquiritiae, 0,4 g Magnes. carbon., 0,2 g Natr. bicarbon., 0,025 g Cort. *Frangulae*, 0,025 g Bluz. *Calami*; H. Trommsdorff, Aachen.

[*6]) Reserpin; Ciba AG., Wehr Baden.

[*7]) Auszug aus *Brassica oleracea capitata alba* mit einem standardisierten Gehalt an 0,1 mg S-Methylcysteinsulfoxyd, 25 mg S-Methylcysteinium-sulfoniumbromid, 0,5 mg L-(+)-Cystin, 1,5 mg DL-Methionin, 0,5 mg L-Glutamin; Robugen GmbH, Plötzen a. S.

[*8]) Vitamin-A-Palmitat in öliger Lösung; Deutsche Hoffmann-La Roche AG., Grenzach Baden.

[*9]) DL-α-Tocopherolacetat (Vitamin E); Deutsche Hoffmann-La Roche.

[*10]) *Argentum nitricum* findet schon seit Hahnemann Verwendung in der Homöopathie, es ist eines der „wertvollsten Mittel gegen geschwürige Veränderungen am Magen und Duodenum" [12].

[*11]) *Nux vomica* enthält das Alkaloid Strychnin, das nach Korbsch [13] die Magensaftsekretion fördert. In der Homöopathie gilt *Nux vomica* als „Polychrest" (= vielseitig wirkende Substanz) mit Bevorzugung der Verdauungsorgane [12].

Tab. 2: Zusammenstellung der Ergebnisse der Arzneimittelversuche. Die halbfett gesetzten Zahlen stellen die statistisch gesicherten Werte dar (t größer als 2,01 bzw. 2,04). n = Tierzahl, Z = Gesamtgeschwürszahl, F = Gesamtgeschwürsfläche.

	n	Z	Änderung v. Z. gegenüber d. Kontrollwert %	t	F	Änderung v. F. gegenüber d. Kontrollwert %	t
Kontroll-Serie	30	12,9			11,5		
N. Belladonna Neutr.	16	25,4	+ 82	**3,30**	21,6	+ 88	**3,17**
Enzynorm liqu.	16	28,0	+ 117	**4,99**	31,5	+ 365	**2,99**
Vagantin	15	11,7	− 9	0,40	6,5	− 49	**1,52**
Rabro Magentabl.	16	17,5	+ 36	**1,55**	10,5	− 10	0,40
Serpasil	15	22,0	+ 75	**2,75**	28,7	+ 149	**4,49**
Robufakton	16	23,9	+ 100	**3,49**	7,7	− 33	**1,75**
Arovit	15	20,6	+ 59	**2,72**	26,0	+ 126	**2,94**
Ephynal	16	12,0	−	−	12,5	+ 9	
Argent. nitr. D 3	14	14,0	+ 9	0,36	21,4	+ 86	**2,48**
Nux vomica ♀	16	15,2	+ 2	0,13	29,9	+ 160	**2,92**

keinen Aufschluß darüber, welche Wirkung die Medikamente auf die Ausheilung bereits entstandener Geschwüre entfalten. Die Ergebnisse der Arzneimittelversuche sind in Tab. 2 dargestellt.

Zusammenfassend ergaben folgende Medikamente eine statistisch gesicherte Zunahme der Geschwürszahl bzw. der Geschwürsfläche: Belladonna-Neutralon, Enzynorm liqu., Serpasil, Robufakton, Arovit, Argentum nitricum D 5 und Nux vomica Φ. Eine deutliche, allerdings statistisch nicht gesicherte Verminderung der Geschwürszahl und Geschwürsfläche beobachteten wir lediglich bei Vagantin.

Diskussion

Die Entstehung der Vormagengeschwüre durch die Glukosekost ist wohl so zu erklären, daß der während des Trinkens abgesonderte Magensaft zur Verdauung gar nicht benötigt wird und sich deshalb zerstörend gegen die Magenwand richten kann. Dafür spricht auch die Tatsache, daß wir durch zusätzliche Zufuhr von Magensaft in Form von Enzynorm eine Zunahme der Geschwürsfläche um 365% beobachten konnten.

Auffallend ist die Tatsache, daß die Geschwüre fast ausschließlich im Vormagen lokalisiert sind. Der Vormagen der Ratte ist ein mit geschichtetem, verhornendem Plattenepithel ausgekleideter Sack von etwa 3—4 ml Inhalt. Er ist als Speicherorgan anzusehen und wird bei ausschließlich flüssiger Nahrung praktisch funktionslos. Da er keine Schleimdrüsen besitzt, fehlt ihm im Gegensatz zum Drüsenbereich eine schützende Schleimschicht; dies ist wohl der Grund, daß der Vormagen bevorzugt der verdauenden Wirkung des Magensaftes anheimfällt.

Da der menschliche Magen bekanntlich keinen dem Ratten-Vormagen entsprechenden Anteil besitzt, sind unsere Versuchsergebnisse nur mit großen Vorbehalten auf die menschliche Pathologie zu übertragen. Immerhin weisen die Ergebnisse der Versuchsanordnung gewisse Gesetzmäßigkeiten auf, insbesondere besteht eine enge Beziehung zwischen Versuchsdauer und Geschwürszahl bzw. Geschwürsfläche (Abb. 1 u. 2).

Wir bezogen unsere Ergebnisse alle auf einen Kontrollwert, der sich aus 30 Tieren errechnete. Das Gewicht der Tiere scheint nach unseren Beobachtungen ohne Bedeutung für die Entstehung der Vormagengeschwüre zu sein. Da wir alle Versuche in einem Raum anstellten, der durch einen Thermostaten ständig auf 22° C gehalten wurde, konnte sich ein eventueller Einfluß der Temperatur auf die Geschwürsbildung nicht unterschiedlich auswirken. Trotz des homogenen Tiermaterials, das wir zu unseren Versuchen verwendeten, waren die individuellen Schwankungen hinsichtlich der Geschwürsrate sehr groß. Dies wirkte sich besonders nachteilig auf die Möglichkeit der statistischen Sicherung der Ergebnisse aus. Die Auswertung der Ergebnisse hinsichtlich der Geschwürszahl erscheint zunächst unproblematisch. Sehr häufig konfluieren die Geschwüre jedoch, so daß sie nicht immer eindeutig voneinander abzugrenzen sind. Die Geschwürsfläche dagegen liegt sicher fest. Die Bestimmung der Fläche der häufig völlig unregelmäßig begrenzten Geschwüre erfolgte durch Vergleich mit Papierstückchen bekannter Größe, also mit einer etwas subjektiv gefärbten Methode. Um diesen Fehler möglichst klein zu halten, wurden alle Mägen von demselben Untersucher ausgewertet.

Der Geschwürsfläche kommt bei der Bewertung eines Medikamentes zweifellos die größere Bedeutung zu. Sie steht auch im Gegensatz zur Geschwürszahl mit der Tiefe der Geschwüre in gewissem Zusammenhang, da erfahrungsgemäß flächenmäßig große Geschwüre die Muscularis mucosa häufig durchbrechen. Morphologisch zeigten die Geschwüre unter der Arzneimitteleinwirkung keine Besonderheiten. Verallgemeinernd kann man sagen, daß solche Substanzen, die die Geschwürsbildung erheblich verstärkten, vorwiegend Geschwüre vom polygonalen Typ, also große und tiefe Geschwüre erzeugten, und daß umgekehrt unter dem Einfluß von Medikamenten, welche die Geschwürsbildung hemmen, Randwall-Ulcera, also kleine oberflächliche Geschwüre entstanden.

Zusammenfassung

Füttert man Ratten ausschließlich mit 20%iger Glukoselösung, so treten bei den Tieren regelmäßig schon nach wenigen Tagen Vormagengeschwüre auf. Die Mägen der Tiere werden anatomisch auf Geschwürszahl und Geschwürsfläche untersucht. Entstehungs- und Abheilungszeit der Geschwüre werden beobachtet, die Morphologie der Ulcerationen wird beschrieben, ihre Ätiologie wird diskutiert. Zusammen mit der Glukosekost werden 10 verschiedene, in der menschlichen Ulcustherapie gebräuchliche Arzneimittel verabreicht und ihr Einfluß auf die Geschwürentstehung untersucht.

Eine Hemmung der Geschwürsbildung scheint nur Xanthencarbonsäure-diäthylaminoäthylester-methylbromid (Metantheline, Vagatin®) zu erzielen, jedoch ließ sich das Ergebnis statistisch nicht sichern. Dagegen ergaben folgende Medikamente eine statistisch gesicherte Zunahme der Geschwürszahl bzw. der Geschwürsfläche: Al-Na-silikat mit 0,5% Belladonna-Extrakt (Belladonna-Neutralon®) standardisierter Magenextrakt (Enzynorm®), Reserpin (Serpasil®), ein Auszug aus *Brassica oleracea capitata alba* (Robufakton®), Vitamin A (Arovit®), Argentum nitricum D 5 und *Nux vomica* Φ.

Summary

Gastric Ulcers Experimentally Produced in Rats — Drug Influence Thereon

1st Report: The induction of ulcers in the gastric fundus of rats by feeding glucose and the influence of drugs thereon.

When rats are exclusively fed on 20% glucose solution, the occurence of ulcers in the upper end of the stomach can be consistently observed even after a few days. The animals' stomachs were examined anatomically for number and size of ulcers. The time elapsing until the occurrence and healing of ulcers was observed; the morphology of the ulceration is described and its etiology discussed. Together with the glucose feeds, ten drugs commonly employed in human gastric therapy were administered and their influence on ulcer formation studied.

An inhibition of ulcer formation can only be ascribed to xanthenecarboxylic acid-diethylaminoethyl ester-methylbromide (Metantheline, Vagatin®), however, this finding could not be confirmed statistically. On the other hand, the following drugs produced a statistically confirmed increase in the number or extent of ulcerations: Al-Na-silicate with 0.5% belladonna extract (Belladonna-Neutralon®), standardised gastric extract (Enzynorm®), reserpine (Serpasil®), an extract of *Brassica oleracea capitata alba* (Robufakton®), Vitamin A (Arovit®), argentum nitricum D 5 and *nux vomica* Φ.

Literatur

[1] Büchner, F., Spezielle Pathologie, Verlag Urban & Schwarzenberg, München u. Berlin 1955
[2] Rein, H. u. Schneider, M., Physiologie des Menschen, Springer-Verlag 1956
[3] Gray, S. J., Gastroenterology 37, 412 (1959)
[4] Shay, H., Amer. J. digest. Dis. 4, 846 (1959)
[5] Demling, L., Internist 1, 175 (1960)
[6] Norpoth, L., Dtsch. med. Wschr. 77, 1281 (1952)
[7] Schaper, W., Ztschr. Inn. Med. 15, 38 (1960)
[8] Grandjean, E., Helv. Physiol. Pharm. Acta 6, C 7 (1948)
[9] Grandjean, E., Rev. med. Suisse, rom. 68, 442 (1948)
[10] Koller, S., Graphische Tafeln zur Beurteilung statistischer Zahlen. Verlag Steinkopf, Dresden 1940
[11] Röhm, F., Inaug.-Diss. Tübingen (1962)
[12] Mezger, J., Gesichtete homöopathische Arzneimittellehre, Haug-Verlag, Saulgau (Württ.) 1950
[13] Korbsch, R., Münch. med. Wschr. 73, 752 (1931)
[14] Palmer, W. L., Arch. intern. Med. 106, 786 (1960)
[15] Demling, L., Dtsch. med. Wschr. 86, 1357 (1961)
[16] Gregory, R. A., Secretory mechanisms of the gastrointestinal tract, Verlag Edward Arnold, London (1962)

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TALC GRANULOMA — A SURVEY OF ITS INCIDENCE AND SIGNIFICANCE*†

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IN THE 14 YEARS since Antopol first described the entity known as talc granuloma, numerous clinical and experimental studies have been reported in the literature. However, there has been an inertia on the part of the medical profession to accept the challenge of the danger of the use of talc. In spite of what has been written practically all hospitals, save those where the problem has been made a special study, are still using talcum powder as a dusting powder for their surgical rubber equipment.

It was not until we had a death at our hospital from intestinal obstruction and fecal fistula directly attributable to talc (reported by Dr. A. J. Swingle[18]), that we became fully aware of the seriousness of the practice of using talcum powder in the operating room. This case instigated our consideration of the statistical incidence of talc granuloma as a postoperative complication. Although a review of the literature revealed many cases resembling those we subsequently found, there was no instance in which a comprehensive survey was made of the incidence of the disease in routine surgical material in a given hospital, to correlate this with total operative procedures performed. With this question in mind we reviewed the consecutive surgical pathologic material submitted to the laboratory over the past 16 months. Not only were we interested in the incidence of occurrence, but also if possible to correlate the presence of talc granuloma with actual morbidity.

HISTORY

It is not the purpose here to completely review the literature on this subject. This has previously been thoroughly done by Seelig *et al.*,[17] and others subsequent to Antopol's first paper in 1933. Knowledge of the effect of talc in tissues has been gradually developed. Erb[3] in 1935 reported six cases of dusting powder granuloma. However, he did not report lesions produced by talc crystals, his cases being due to lycopodium spores. Owen[13] in 1936 reported one case of talc granuloma which caused intestinal obstruction two years following appendectomy. In 1940 German[5] reported a case of lupoid-sarcoid reaction induced by a foreign body (silica) and in 1943 this same author[6]

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presented [illegible] series of 50 talc granulomata, 40 of which were intra-abdominal and were found in patients undergoing abdominal surgery for at least the second time. He further found that if serial sections are routinely taken through the omentum of patients previously operated upon, granulomata will almost always be found. In many of his cases the talc was merely an incidental finding and not necessarily the cause of any complaint. Ramsey and Douglass,[14] McCormick and Ramsey,[11] Byron and Welch,[2] and Ramsey[15] all have reported additional cases, and the injury now has been described in various parts of the body, including intra-peritoneal, intra-thoracic and in skin lesions, scar nodules and tumor masses; in vaginal, rectal, brain, spinal, kidney, breast, ovary, testes, penile and parts and in healing surgical wounds, *et cetera*. Lichtman *et al.*[9, 10] found granulomatous lesions in malfunctioning intestinal stomata, and demonstrated experimentally the importance of talc as the causative factor in the production of fistulas. Swingle[18] has reported a case of a patient from this hospital who had a laparotomy for removal of an abdominal tumor and then had 14 subsequent laparotomies and finally expired from intestinal obstruction and fecal fistula caused as a direct result of talc.

PATHOLOGY AND PATHOGENESIS

Talc powder is a hydrous magnesium silicate and contains 82.7 per cent talc, 8.7 per cent calcium carbonate, 7.6 per cent magnesium carbonate, and 1 per cent moisture.[7] Miller *et al.*[12] in 1934 found that quartz, which is 99 plus per cent silica, when introduced into the peritoneum produced nodules that progressively increased in size. This was apparently due to chemical irritation supplied by the solution of silicate in the tissue. Particles below 10 microns in size are particularly harmful to tissues. Talc produces a pseudo-tuberculous, non-caseating, granulomatous type of lesion which previously has been described in pathologic specimens merely as chronic granuloma or chronic foreign body reactions. Lichtman *et al.*[9] point out that "material bleached out of the talc crystals is not the same as the parent substance as the degree of hydration of this derivative substance determines its toxicity." The talc crystals are not inert particles. They immobilize the phagocytes without destroying them, and therefore are not carried into the lymph stream. Usually a circumscribed granuloma is produced at the original site of contamination. Microscopically it is a fibrous granuloma with foreign body giant cell reaction. Under polarized light the smallest talc particles can be seen to be frequently engulfed by large histiocytes, whereas around the larger particles there are typical multinucleated foreign body giant cells.

The role of talc in the actual production of adhesions is a point over which there is marked difference of opinion. From their experimental work, Lichtman *et al.*[9, 10] concluded that simple inoculation of talc with little infection and trauma produced nodules rather than dense fibrosis. This point is interesting inasmuch as German[5] states that talc alone does not cause adhesions; that trauma is necessary to produce fibrin and the talc is incorporated in the adhesions and plays a secondary role. Owen[13] also found that talc injected intraperitoneally in rabbits produced a granulomatous lesion but no adhesions. On the

other hand Lee and Lehman[illegible] state that when dry talcum powd[illegible] dusted over the serosal surfaces without trauma, massive dense uncounta[illegible] adhesions are produced. It is further interesting to note that as far back as 1934, Bethune[1] described a technic for the deliberate production of pleural adhesions as a preliminary to lobectomy by the use of pleural poudrage with talc. In retrospect it is odd that surgeons using talc therapeutically to produce adhesions did not realize that they were also powdering their gloves with the same substance for operations in which adhesions were not desirable.

Apparently there is extreme variability in the individual reaction to talc. This is seen clinically inasmuch as some wounds with talc fail to heal, or break down within a few weeks following initial healing. In other cases, a period of years may elapse between inoculation with talc and the production of symptoms. The longest time interval between talc contamination and treatment in any of our cases was nine years. Others have reported granulomata developing in scars as long as 36 years after inoculation with talc (Lichtman[9, 10]).

The presence of a talc granuloma does not necessarily presuppose a previous surgical procedure. Cases of dacrocystitis caused by talc have been reported in personnel working in surgical supply rooms where dust counts are extremely high. In one of German's cases[5] the causative agent (silica) was introduced into the tissues when the patient suffered a scalp wound while mountain climbing. Furthermore, granulomatous lesions of the rectum and vagina also may occur after the prolonged use of suppositories coated with talc.

Our own cases, however, all occurred following previous surgery, either at this hospital or elsewhere. The talc crystals may have been deposited into the wound by particles settling from the air—especially inasmuch as we do not have a separate room in which the doctors may "gown and glove"—this being done in the operating room proper. In most cases obviously the source of talc is from the surgeon's glove, both from the outside of the glove as well as following perforation. In a study by Weed and Groves[19] it was observed that in a series of 4,589 operative procedures there was a perforation of one or more gloves in 74.4 per cent of the operations and that 22.6 per cent of the gloves that were used were perforated. Also, the talc may be introduced into the wound on cotton packs and sponges as well as on rubber drains. At this hospital rubber drains were formerly liberally powdered with talc for autoclaving and it is believed that this procedure is carried out in most hospitals throughout the country. Swingle[18] states that regardless of how thoroughly the drains are washed, subsequent examination under the polarizing microscope still reveals birefringent crystals clinging to the rubber.

ANALYSIS OF MATERIAL

Talc crystals were identified by examination under a polarizing microscope. They are seen to be doubly refractile, varying in size up to 10 microns, and of irregular configuration. A positive statement cannot always be made whether or not a given granulomatous lesion is due to talc alone. Although talc can be found in many specimens, it may be merely contributory or incidental and

not necessarily the primary etiologic factor in the production of the granuloma. It was considered significant only when it produced tissue reaction characterized by phagocytosis of the talc crystals by histiocytes and giant cells and by the presence of fibrosis and tubercle formation. Other double refracting substances which must be distinguished from talc include hair, lint, suture material, cholesterol crystals, vegetable fibers, cotton fibers, *et cetera*.

With the above mentioned criteria in mind, a general review was made of 1,012 consecutive surgical specimens submitted to the pathologic laboratory. A total of 33 specimens containing talc granuloma was found. Eight of these were in recurrent pilonidal sinuses, there having been 21 recurrent pilonidal sinuses in the entire series. The remaining 25 specimens (from 21 patients) had been previously identified as nonspecific inflammatory granulomas.

CLASSIFICATION OF CASES

Talc granuloma was found in 33 specimens obtained from 29 patients (Table I). A breakdown of these cases is as follows:

I. Chronic Granulomas as Draining Sinuses: Chest Cases—9.

a. Seven of the granulomas were from five patients with pulmonary tuberculosis, the lesions being a draining sinus tract or chronic granulomatous lesion at the site of a thoracoplasty or a thoracotomy wound, and in one case at the site of a phrenicectomy wound. In none of these sections could tuberculosis be found.

b. There were two non-tuberculous chest cases:

(1) One case was a wound granuloma and sinus tract following thoracotomy for empyema from a ruptured amebic abscess of the liver. Four revisions of the wound were required before healing finally occurred.

(2) One case developed a bronchopleural fistula following a thoracotomy for post pneumonic empyema.

II. Chronic Draining Sinuses and/or Fistulae: Non-Chest Cases—9.

a. Four draining sinuses of the lumbar region all found in second or later operations following an initial operation for (1) shell fragment wound, (2) nephrectomy for tuberculous kidney, (3) lumbar sympathectomy, (4) osteomyelitis of the sacrum.

b. Two fecal fistulae following appendectomy for perforated gangrenous appendices: One of these healed on excision of the sinus tract and repair of the bowel, and the other fistula persisted for four months until the patient died.

c. One pararectal sinus, operated upon five times—(three times at this hospital) before healing finally occurred.

d. One rectal fistula which had been draining since initial surgery two years previously. This patient had pulmonary tuberculosis but no tuberculosis of the rectal lesion was demonstrated on the microscopic section.

e. One case of intestinal obstruction and fecal fistula which caused death of the patient and was directly attributable to talc.[18]

III. Inflammatory Masses:—3.

a. A tumor of the jaw which had progressively enlarged since the patient received initial treatment for gunshot wound of the mandible five years previously.

b. A tumor mass of the right spermatic cord which had developed following hemorrhaphy three months previously. (Hematoma was also a factor.)

c. Talc granuloma superimposed on an angioma which had been partially removed at the initial operation three years previously.

IV. Non-Union of Radius:—1.

V. Chronic Cholecystitis and Non-Functioning Cholecystoduodenostomy—1.

VI. Recurrent Cases of Pilonidal Sinuses:—8.

Cases described above under I b.(2), IV, and V (cases number 6, [illegible] 20 respectively in Table I), and the group of cases under VI are deserving of special mention:

Case 6.—*Bronchopleural Fistula with Talc Granuloma:*

The patient was a 52-year-old white male who entered the hospital April 29, 1947, complaining of cough and dyspnea. Symptoms had developed a few weeks following right lower lobe pneumonia. A diagnosis of empyema was made on the basis of physical and X-ray findings and on April 30, 1947, a thoracotomy and open drainage from the empyema cavity was done by Dr. Wilson Weisel, Consultant in Thoracic Surgery. The empyema was extensive with total collapse of the lung. Three large rubber drains were inserted into the thoracotomy wound for drainage. Following this the patient developed a bronchopleural fistula and a "frozen" lung. On August 12, 1947, decortication was done to permit free motion of the lung, a considerable portion of the pleura and scar and granulation tissue being removed. The involved bronchus was closed with interrupted sutures of 000 black silk and the wound was closed using a muscle flap to fill the cavity defect. The fistula remained closed and the wound healed well. The surgical specimen consisted of pleura and granulation tissue removed from the region of the involved bronchus. The removed tissue microscopically showed a marked proliferation of fibrous tissue with groups of lymphocytes, plasma cells and eosinophilic cells, with occasional foreign body giant cells which were seen engulfing small refractile crystals which were birefringent with the polarizing lens.

Case 16.—*Non-Union of Fracture of Right Radius with Talc Granuloma:*

The patient was a 23-year-old white male who sustained a simple fracture of the right radius September 28, 1946. An attempted closed reduction was unsuccessful and therefore an open reduction was performed on October 3, 1946, by Dr. P. Collopy, Consultant in Orthopedic Surgery, with insertion of a metal plate and screws. The operative wound healed well, and the patient was discharged with his arm in a plaster cast. When he returned to the Out-Patient Department nine weeks after surgery for follow-up treatment, it was found that the wound had partially broken down and there was a moderate amount of granulation tissue and a seropurulent exudate at the wound site. X-ray at that time reported "only minimal new bone formation at the fracture site." The wound failed to heal under conservative treatment which included a regime of penicillin and sulfadiazine. On March 4, 1947, five months after the initial operation, the plate, screws and fibrous tissue from the site of non-union were removed, and the fragments were immobilized by means of Kirschner wires and a plaster cast. The surgical specimen showed fragments of bone encased in fibrous tissue. In addition there was an inflammatory infiltrate and an amorphous crystalline double refractory material, identical with talc crystals, enclosed in giant cells. The wound healed by first intention and three weeks later the patient was again operated, at which time a bone graft was applied to the site of non-union. An intermedullary as well as an onlay graft was used in order to correct a one-inch defect of the radius. The wound healed postoperatively without complication.

Case 20.—*Cholecystitis, and Non-Functioning Cholecystoduodenostomy with Talc Granuloma:*

This patient was a 45-year-old white male who entered the hospital July 13, 1946, because of an incisional hernia at the site of a previous laparotomy for gallbladder disease. He gave a history of having had a cholecystoduodenostomy in 1937, and an exploratory laparotomy in 1945 because of persistent biliary tract symptoms. At the time of his second operation (done in an overseas army hospital) the surgeon noted a contracted gallbladder and a greatly distended common duct which was explored and drained. No stones were present, and there was no apparent blockage of the common duct. The reason for the original cholecystoduodenostomy is unknown. On admission to this hospital it was

TABLE I.—*Summary of Talc Granuloma Cases*

Case No.	Initial Diagnosis	Initial Operation	Complication — Reoperated For	Operation	Pathologic Lesion	No. Times Initial Lesion Re-operated	No. Specimens Sub-mitted	Results
1.	Tuberculous Empyema	Thoracotomy	Persistent drainage from operative wound.	Revision of Wound.	Talc granuloma in excised tissue.	1	1	Healed.
2.	Pulmonary Tuberculosis.	Thoracoplasty	Persistent drainage from operative wound.	Revision of Wound.	Talc granuloma in excised tissue.	2	2	Continued granulation—needs further revision.
3.	Pulmonary Tuberculosis.	Thoracotomy; Thoracoplasty	Persistent drainage from operative wound.	Revision of Wound.	Talc granuloma in excised tissue.	2	2	Healed.
4.	Pulmonary Tuberculosis.	Thoracoplasty	Persistent drainage from operative wound.	Revision of Wound.	Talc granuloma in excised tissue.	2	1	Healed
5.	Pulmonary Tuberculosis.	Phreniclasia	Granuloma of operative wound.	Excision of granuloma.	Talc granuloma in excised tissue.	1	1	Healed.
6.	Empyema (Post Pneumonic)	Thoracotomy	Broncho - pleural fistula. Persistent drainage from operative wound.	Decortication and Closure Broncho-pleural Fistula.	Talc granuloma in excised tissue.	1	1	Healed.
7.	Empyema (following ruptured Amebic Abscess)	Thoracotomy	Wound breakdown with chronic drainage from operative wound.	Revision of Wound.	Talc granuloma in excised tissue.	4	1	Healed.
8.	Rectal Fistula	Excision of Fistula.	Failure to heal and continued drainage of fistula.	Excision of Fistula.	Talc granuloma in excised fistulous tract.	1	1	Healed.
9.	Para-rectal Sinus	Incision and Drainage.	Continued drainage from sinus.	Excision of sinus.	Talc granuloma in excised sinus.	4	2	Healed.
10.	Ruptured Appendix	Appendectomy	Fecal Fistula	Biopsy of fistulous tract.	Talc granuloma in excised fistulous tract.	1	1	Fistula failed to heal. (Died)

TABLE I.—*Summary of Talc Granuloma Cases*

Case No.	Initial Diagnosis	Initial Operation	Complication — Reoperated For	Operation	Pathologic Lesion	No. Times Initial Lesion Re-operated	No. Specimens Sub-mitted	Results
11.	Ruptured Appendix	Appendectomy	Fecal Fistula	Excision of fistula and closure of bowel.	Talc granuloma in excised fistulous tract.	1	1	Healed.
12.	Shell fragment wound.	Debridement of wound and secondary closure.	Chronic draining lumbar sinus.	Excision of sinus.	Talc granuloma in excised sinus.	1	1	Healed.
13.	Tuberculosis of kidney.	Nephrectomy	Chronic draining lumbar sinus.	Excision of sinus.	Talc granuloma in excised sinus.	1	1	Healed.
14.	Peripheral Vascular Disease.	Lumbar Sympathectomy.	Chronic draining lumbar sinus.	Excision of sinus.	Talc granuloma in excised sinus.	3	2	Failed to heal after 7 months. (Died of intestinal carcinoma.)
15.	Osteomyelitis of sacrum with draining sinus of left flank.	Curettement of sacrum, sequestrectomy, and excision of sinus.	Chronic draining lumbar sinus.	Excision of sinus.	Granulation tissue in excised sinus tract.	3	1	Failed to heal.
16.	Fracture Right Radius.	Open Reduction and Plating.	Non-union right radius.	Removal of plate and curettement. Bone graft.	Fibrous talc granuloma at site of non-union.	2	1	Healed.
17.	Gunshot wound of mandible.	Surgical treatment gunshot wound.	Tumor mass of mandible.	Excision of tumor.	Fibrous talc granuloma.	1	1	Cured.
18.	Angioma, right index finger.	Partial removal of angioma.	Progressing tumor mass right index finger.	Excision of tumor mass.	Fibrous talc granuloma.	1	1	Healed well.
19.	Right Inguinal Hernia.	Herniorrhaphy	Tumor mass right groin.	Excision of tumor.	Talc granuloma and organized hematoma.	1	1	Cured.
20.	Gall Bladder Disease	Cholecystoduodenostomy.	Chronic Cholangitis.	Common duct drainage. Cholecystectomy.	Chronic talc granuloma at site of Cholecystoduodenal anastomosis.	1	1	Healed (Symptoms alleviated.)

TABLE I.—*Summary of Talc Granuloma Cases*

Case No.	Initial Diagnosis	Initial Operation	Complication—Reoperated For	Operation	Pathologic Lesion	No. Times No. Initial Lesion Re-operated	Specimens Submitted	Results
21.	Carcinoma of Bowel.	Exploratory Laparotomy and resection of tumor.	Intestinal Obstruction and Fecal Fistula.	Repeated Laparotomies for intestinal obstruction and fecal fistula.	Talc granuloma of excised gangrenous bowel.	14	1	Wound never healed. (Patient died.)
22.	Pilonidal Sinus.	Excision of sinus.	Recurrent Pilonidal Sinus.	Excision of sinus.	Talc granuloma in excised tissue.	1	1	Healed.
23.	Pilonidal Sinus.	Excision of sinus.	Recurrent Pilonidal Sinus.	Excision of sinus.	Talc granuloma in excised tissue.	1	1	Healed.
24.	Pilonidal Sinus.	Excision of sinus.	Recurrent Pilonidal Sinus.	Excision of sinus.	Talc granuloma in excised tissue.	10	1	Wound granulating and still not quite healed at discharge.
25.	Pilonidal Sinus.	Excision of sinus.	Recurrent Pilonidal Sinus.	Excision of sinus.	Talc granuloma in excised tissue.	3	1	Wound broke down and finally healed slowly by granulation.
26.	Pilonidal Sinus.	Excision of sinus.	Recurrent Pilonidal Sinus.	Excision of sinus.	Talc granuloma in excised tissue.	3	1	Healed.
27.	Pilonidal Sinus.	Excision of sinus.	Recurrent Pilonidal Sinus.	Excision of sinus.	Talc granuloma in excised tissue.	3	1	Wound granulating and still not completely healed at discharge.
28.	Pilonidal Sinus.	Excision of sinus.	Recurrent Pilonidal Sinus.	Excision of sinus.	Talc granuloma in excised tissue.	1	1	Healed.
29.	Pilonidal Sinus.	Excision of sinus.	Recurrent Pilonidal Sinus.	Excision of sinus.	Talc granuloma in excised tissue.	1	1	Healed.
29						71	33	— Total

noted that he had an icteric tinge to his skin and the icterus index was 22 preoperative diagnosis at this hospital was chronic cholangitis based on the histo ecurrent bouts of jaundice, chills and fever following cholecystoduodenostomy. Lap omy was performed on July 30, 1946, by Dr. F. Raine, Senior Consultant in Surgery. Since cholecystogram revealed patency of the common duct, the anastomosis from the duodenum was taken down and the gall bladder was removed. Postoperatively, the patient had a stormy course with exacerbations and remissions of his cholangitis, but succeeding attacks

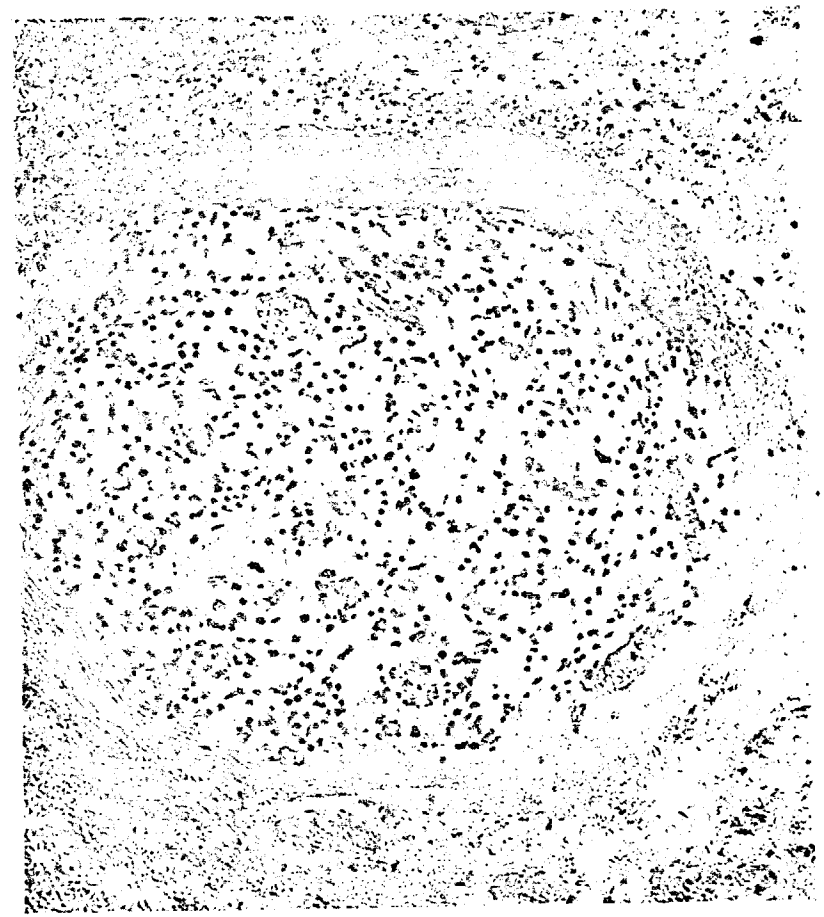

FIG. 1A.—(Case 20) Cholecystitis showing talc granuloma in wall of gallbladder at site of duodenal anastomosis (low power field).

were decreasingly severe and his icterus index gradually returned to normal. The microscopic examination of the gallbladder showed the mucosa to be normal but the wall showed round cell infiltration in the submucosal area and within the muscularis a large area was found to be surrounded by fibrous tissue, in which there were epithelioid cells and giant cells, some of which engulfed foreign particles which were identified under the polarizing microscope as talc crystals (Fig. 1a and 1b).

Talc Granuloma in Recurrent Pilonidal Sinuses. A special study was made of pilonidal sinuses. Since they are notably prone to recur, the thought was

enterta[illegible] that talc might be responsible for these recurrences. There was a total 58 pilonidal specimens, of which 37 were primary and 21 were recurrent. Talc crystals were not found in any of the primary cases. In eight (38 per cent) of the 21 recurrent cases, talc was found. However, in all recurrent cases, remnants of epithelial cyst or sinus could be demonstrated. Thus it could be established that talc was not the primary cause for recurrence of a pilonidal sinus. The possibility that the talc crystals acted as an irritant to the lesion causing symptoms necessitating another operation cannot be excluded.

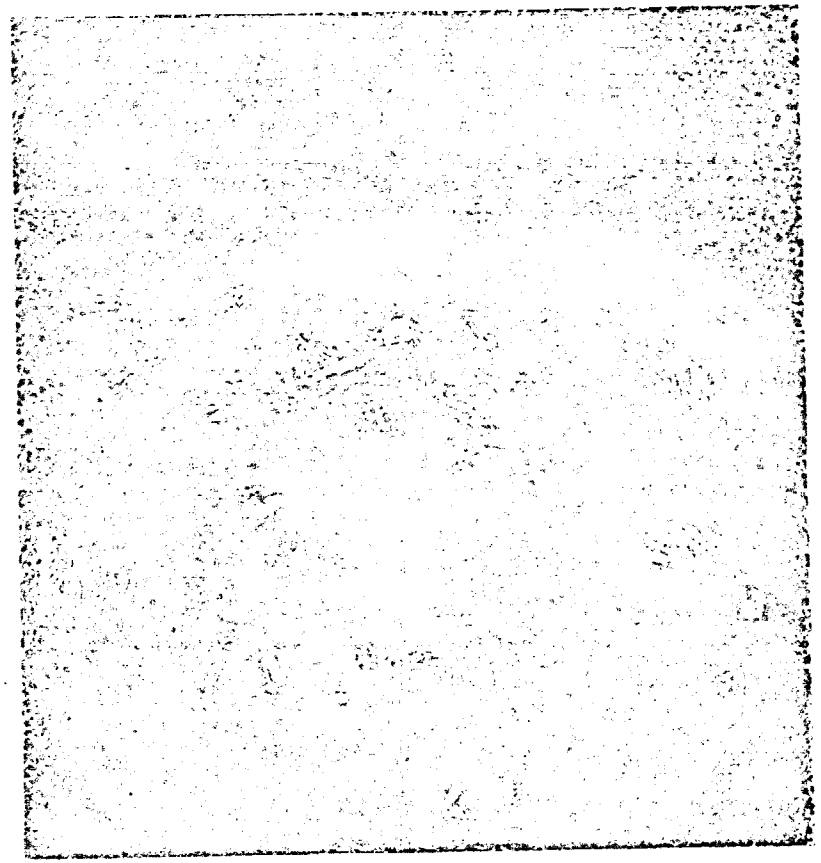

FIG. 1B.—(Case 20) Same as 1A under polarized light, showing talc crystals within macrophages and giant cells (low power field).

DISCUSSION

From our study, no support can be given for or against the previously mentioned dispute as to whether talc alone is innocuous without associated trauma. A large per cent of talc granulomata were found in thoracic (thoracoplasty and thoracotomy) cases. There were 12 specimens from thoracic cases in this series which had been diagnosed previously as non-specific inflammatory granulomas. Nine of these were found to be talc granulomas. The multiple stage procedures employed and the use of drains and frequent dressings

required in these cases afford repeated trauma as well as increa[illegible] portunity for talc implantation.

We believe that Case 6 is the first case of talc granuloma in a bronchopleural fistula to be recorded. Intraabdominal fistulae due to talcum powder have been repeatedly reported, and this has also been observed in experimental animals.[9, 10] In our case it cannot be stated how much of the responsibility talc must bear as the cause of the fistula, but at least it is a contributing factor to prevention of spontaneous closure of the sinus.

In the case of non-union of the radius (Case 16), it cannot be proved that talc alone was the cause of non-union. However, this was a clean case of elective surgery. The operative wound was healed at the time the patient was discharged. It subsequently broke down and continued to drain and would not respond to conservative therapy. This clinical picture of wound breakdown after primary healing, with the formation of a draining sinus that failed to heal on the usual regime of heat, rest, elevation, and antibiotics, is characteristic of talc granuloma. In addition, the microscopic evidence of typical talc granuloma in the fibrous tissue from the site of non-union indicates that talc in this case was the cause of non-union. The fact that primary healing rapidly occurred subsequent to the removal of the plate and fibrous tissue containing the talc, plus the fact that there is no other reasonable explanation for the failure of healing after initial plating, adds strength to the argument for talc as the etiologic agent here. We have found no previous reported case of non-union of a fracture due to interference of healing by talc.

In the case of gallbladder disease (Case 20), the introduction of talc into the wound at the time of initial surgery resulted in prolonged morbidity of the patient. The cause for the patient's original operation is not known. Nevertheless there was interference with function of the cholecystoduodenostomy as is evidenced by the observation of the surgeon at the time of the second operation for choledochotomy and drainage. He noted that the common duct was greatly distended and that the gallbladder was markedly contracted and apparently not functioning as a channel for the transport of bile to the duodenum. The talc in this case was not merely covered over with serosal cells so as to produce nodules on the peritoneal surface, as is usually the case, but was actually found in the wall of the gallbladder in the submucosal area and within the muscularis. The crystals undoubtedly were placed there when the cholecystoduodenostomy was performed. We feel justified in thus assuming that the gallbladder disease was due to talc. This was, in fact, a talc cholecystitis. We have found no similar case recorded in the literature.

There is a marked difference of opinion as to the incidence of talc granuloma. Fienberg[4] in 1937 reported two cases and stated that in a review of 30,000 specimens he had found a total of but five cases. Saxen and Tuovinen[16] in 1947 also report a low incidence, having found but six cases in 5,241 specimens. German[5] on the other hand, as mentioned above, reported 50 cases of talc granuloma, and stated that serial sections through the omentum of patients undergoing abdominal surgery for the second time would almost always contain talc. We found 33 cases of talc granuloma in a consecutive series of 1,912

surgical [illegible]ens. This would indicate that 1.7 per cent of the consecutive surgical procedures that were done were necessitated entirely or in part by talc. Actually, however, the figure is probably even higher than this, since a great number of wound granulomas and sinuses that are excised, many of which may contain talc, never reach the pathological laboratory. As an example, one patient had a chronic draining sinus excised on four different occasions and only one specimen was sent to the pathological laboratory, this specimen being a talc lesion. It is interesting to note (Table I) that of the 29 patients from whom these 33 specimens were obtained, 16 patients had one re-operation of the talc lesion concerned, whereas 13 patients (44.8 per cent) were reoperated two or more times, one patient having ten and another fourteen re-operations. Theoretically the talc could have been inserted into the wound at the time of the initial surgery or at any of the subsequent operations prior to the last one. It is further significant to note that these 29 patients underwent a total of 71 operations subsequent to their 29 initial procedures.

CONCLUSIONS

1. In a series of 1,912 routine consecutive surgical specimens, talc granuloma was found in 33.

2. Of the consecutive surgical procedures from which the above specimens were obtained, 1.7 per cent of the operations performed were necessitated in whole or in part by talc.

3. Draining sinuses following chest surgery were found to be frequently complicated by talc granuloma. In specimens from nine out of the twelve chest cases examined, talc was considered to be a major factor in the production and persistence of these sinuses.

4. To the reported cases of talc granuloma, one each of broncho-pleural fistula, failure of function of a cholecystoduodenostomy, and non-union of a fracture of the radius, is added.

5. In eight of 21 cases (38 per cent) of recurrent pilonidal sinuses, talc reaction was noted. Since in each case residuals of the sinus tract could be demonstrated, recurrence of the sinus could not be primarily attributed to talc. The talc may, however, act as an irritant necessitating revision.

6. Excision of the granuloma with the talc with resultant healing of the wound was accomplished in 21 out of the 29 cases of talc granuloma.

7. The inadvisability of the use of talc as a dusting powder is again emphasized.

Note: Photomicrographs were kindly prepared by Mr. Leo Massopust of Marquette University School of Medicine.

BIBLIOGRAPHY

1 Bethune, N.: J. Thoracic Surg., **4**: 251–261, 1935.
2 Byron, F. X., and C. S. Welch: Surgery, **10**: 766–769, 1941.
3 Erb, I. H.: Surg. Gynec. & Obst., **16**: 40–44, 1935.
4 Fienberg, Robert: Arch. Path., **24**: 36–42, 1937.
5 German, Wm. M.: Am. J. Clin. Path., **10**: 245–250, 1940.
6 *Ibid.* Surg., Gynec. & Obst., **76**: 501–507, 1943.

7 Kronenberg, Milton H.: Mod. Hosp., **49**: 84, 1937.
8 Lee, C. M. Jr., and E. P. Lehman: Surg. Gynec. & Obst., **84**: 689–695, 1947.
9 Lichtman, A. L., John R. McDonald, Claude F. Dixon and Frank C. Mann: Surg. Gynec. & Obst., **83**: 531–540, 1946.
10 Lichtman, A. L., and J. R. McDonald: Arch. Path., **42**: 69–80, 1946.
11 McCormick, E. J., and Thomas L. Ramsey: J. A. M. A., **116**: 817–821, 1941.
12 Miller, J. W., R. R. Sayers and W. P. Yant: J. A. M. A., **103**: 907–912, 1934.
13 Owen, May: Texas St. J. Med., **32**: 482–485, 1937.
14 Ramsey, Thomas L., and Fred M. Douglass: J. Internat. Coll. Surgeons, **3**: 3–10, 1940.
15 Ramsey, Thomas L.: Am. J. Clin. Path., **12**: 553–558, 1942.
16 Saxen, Arno, and P. I. Tiovinen: Acta Chir. Scandinavica, **96**: 131–151, 1947.
17 Seelig, M. G., D. J. Verda and H. F. Kidd: J. A. M. A., **123**: 950–954, 1943.
18 Swingle, Alvin J.: Ann. Surg., **128**: 144–152, 1948.
19 Weed, Lyle A., and Jessie L. Groves: Surg. Gynec. & Obst., **75**: 661–664, 1942.

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Fast-neutron Activation Analysis of Silicon in Sputum

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A method for a relatively rapid and accurate determination of silicon in sputum by activation analysis is described. The method has been developed to identify and separate the cases of silicosis from those of pulmonary tuberculosis.

The sputum is digested by trypsin, then spun in a centrifuge three times at room temperature, and once after heating with 5 per cent. trichloroacetic acid to remove phosphorus which interferes in the activity measurement. For irradiation, a neutron generator with a fast-neutron yield of 2×10^9 n per cm^2 per second is used. After exposing for 2·3 minutes and then waiting for 1 minute the activity measurements are made by using a multi-channel analyser for 4·6 minutes. The silicon content is evaluated from the aluminium-28 activity produced by the reaction ^{28}Si (n,p) ^{28}Al with a photopeak at 1·78 MeV.

The results show that the silicon content in the sputum of patients suffering from silicosis is, on the average, four times that of the samples used for reference.

It is a current problem in tuberculosis dispensaries to distinguish cases of silicosis from those of pulmonary tuberculosis, as the X-ray photographs in the two cases are often quite similar. Considering its nature, it seems reasonable to assume that the sputum of patients suffering from silicosis contains much more silicon than that of patients with tuberculosis. The determination of silicon with conventional techniques being lengthy, laborious and inaccurate, investigations have been made to develop a method for the neutron-activation analysis of silicon in sputum.

Neutron-activation analysis has been already carried out for several elements present in biological substances.[1] Silicon has not been included in these experiments mainly because it is not an essential biological constituent. Another reason for this is that silicon-31, produced by the reaction ^{30}Si (n,γ) ^{31}Si in silicon exposed to thermal neutrons in a reactor, being a β-emitter of 2·6-hours half-life, cannot be identified by gamma spectroscopy, so that complicated and lengthy chemical procedures have to be used for the separation of silicon.

The recent availability[2] of neutron generators producing fast neutrons with energies of 14 MeV offers the possibility of making use of the reaction ^{28}Si (n,p) ^{28}Al, the cross-section of which is 220 mb. Considering the 92·17 per cent. abundancy of silicon-28, the 2·3-minute half-life and the 1·78-MeV photopeak of aluminium-28 activity for the transition $^{28}\text{Al} \rightarrow {}^{28}\text{Si}^* \xrightarrow{E = 1{\cdot}78\text{MeV}} {}^{28}\text{Si}$ that one 1·78-MeV gamma photon is emitted per β-particle,[3 to 9] the product of this reaction lends itself well to gamma spectroscopic measurement after simple separation from interfering activities.

Fast-neutron activation analysis of silicon in inorganic substances has already been extensively dealt with in the literature.[10,11,12,13,14] The method is rapid, selective and of high sensitivity, *e.g.*, 0·4 μg per 100 d.p.m. for a fast-neutron flux of 10^9 n per cm^2 per second. An accuracy of 1 per cent. has been achieved by Martin, Mathur and Morgan[12] for a 2-g sample with a 1 per cent. silicon concentration.

Although fast-neutron activation analysis by the use of neutron generators has not yet been applied to biological substances, considering the above, the method also seems promising for their analysis.

Experimental

Preparation of Samples—

The sputum of various patients was collected during 3 to 6 days, depending on the individual rate of secretion.

REAGENT—

Tris(hydroxymethyl)aminomethane - formic acid buffer solution—This consists of a mixture of 50 ml of 0·2 M tris(hydroxymethyl)aminomethane (24·2 g dissolved in 1 litre of distilled water) and 26·8 ml of 0·2 M formic acid made up to 100 ml with distilled water.

DIGESTION—

Trypsin was dissolved in 0·1 M tris(hydroxymethyl)aminomethane - formic acid buffer to give a 1 mg per ml concentration and the solution added to the sputum collected over 24 hours in amounts equal to that of the measured sputum. The well mixed solutions were digested for 24 hours at 37° C, during which period the viscous sputum became fully digested.

REMOVAL OF PHOSPHORUS—

To eliminate any interference from aluminium-28 produced in phosphorus by the reaction $^{31}P\ (n,\alpha)\ ^{28}Al$, with a cross section of 130 mb, this element had to be removed from the sample before activation. This is achieved by spinning the solutions three times in a centrifuge after acidifying them to 5 per cent. with 100 per cent. trichloroacetic acid. The opalescent supernatant liquid is discarded each time after spinning for 30 minutes and the precipitate suspended in 10 to 15 ml of 5 per cent. trichloroacetic acid. To remove the inorganic phosphorus bonded to the macromolecule, the precipitate obtained in the third spin is hydrolysed with 5 per cent. trichloroacetic acid for 30 minutes at 80° C before spinning it a fourth time, the precipitate being suspended each time in 20 ml of ethanol, which removes not only the phosphoric acid released by the hydrolysis from the macromolecule, but also the phosphorus bonded to the lipoids of the cellular fragments, as well as the trichloroacetic acid. The final precipitate is evaporated to dryness. In the samples prepared by this method no phosphorus could be determined by the Fiske - Subba Row test.[15] The detection limit for this test is 0·5 μg of phosphorus. In the samples prepared without the addition of trichloroacetic acid the phosphorus concentration, checked by the Fiske - Subba Row method, was found to vary from 10 to 80 μg per 100 mg of dry substance. By "spiking" the sample with 1 mg of silicon dioxide we determined that after the phosphate separation the recovery of silicon was 95 per cent.

IRRADIATION—

The dried samples are put into 2 × 2-cm polythene bags and transferred in a cylindrical polythene holder to the neutron generator by means of a vacuum facility. Their arrival at the place of irradiation is checked by photo diodes, and causes the automatic start of the assembly that measures the neutron yield, and of the timer that times the operation of the relay and valve by which the sample is returned to the place of measurement after the pre-set time for irradiation.

The irradiations were carried out in the KFKI NA-1-type neutron generator producing 14-MeV neutrons at a maximum rate of 10^{10} n per second.[16] The neutron flux was measured by Gamma Production, Emmerich-type plastic phosphor fast-neutron detector and 2 to 3 minutes' irradiation times were applied with a neutron flux of about 2×10^9 n per cm per second.

ACTIVITY MEASUREMENT—

Two different assemblies were used for the activity measurements. Assembly A consisted of a 3·5 by 2·75-inch sodium iodide - thallium crystal detector in a lead chamber constructed from 10-cm thick lead bricks and a KFKI-type 128 channel analyser. The irradiated samples could be measured immediately after irradiation. The samples were left to cool for 1 minute to remove the 7·3-second half-life, 6 to 7-MeV nitrogen-16 activity produced by the $^{16}O\ (n,p)\ ^{16}N$ reaction.

Assembly B consisted of a 6 by 4-inch sodium iodide - thallium crystal detector in an iron chamber of 15-cm wall thickness and Raduga-type 100 channel analyser. This assembly was located farther from the neutron generator, so that the activity measurement could not be started until 5 to 6 minutes after irradiation. It proved to have a lower background and a higher counting efficiency than assembly A. The measuring times were 4·6 minutes.

RESULTS AND DISCUSSIONS

The silicon concentration was evaluated from the 1·78-MeV peak activity measured in the repeatedly calibrated energy-channel width of 1·78 ± 0·11 MeV. As the measurements

TABLE I

MEASURED SILICON ACTIVITIES AND CONCENTRATIONS IN FAST-NEUTRON ACTIVATION ANALYSIS OF SPUTUM FOR SILICOTIC AND NON-SILICOTIC PATIENTS

The values are normalised for 1·78 ± 0·11 MeV to 100 mg of dry material, 2·3 minutes' activation, 1 minute's decay, 4·6 minutes' measuring times and given neutron flux

		A_{st}, counts per minute		Concentration of silicon in sputum, p.p.m.	
		Assembly A	Assembly B	Assembly A	Assembly B
Background		91 ± 10·5	89 ± 9·3	—	—
Silicosis Samples	No. 1	68 ± 13·4	253 ± 34·5	17·6 ± 2·1	18·7 ± 4·1
	2	82 ± 20·2	222 ± 29·2	18·2 ± 2·8	15·6 ± 1·8
	3	99 ± 18·3	190 ± 25·3	14·7 ± 3·2	17·2 ± 3·2
	4	80 ± 12·5	210 ± 37·2	20·1 ± 2·3	21·3 ± 1·9
Control	5	27 ± 8·6	49 ± 14·0	3·9 ± 1·5	4·2 ± 1·7
	6	15 ± 7·0	36 ± 9·8	2·8 ± 0·9	1·8 ± 0·8
	7	25 ± 6·7	92 ± 15·2	5·1 ± 1·7	3·7 ± 0·43

were carried out under somewhat different conditions, the measured activities, A_m, were normalised to 100-mg samples, 2·3 minutes' activation, 1 minute's waiting time and 4·6 minutes' measuring time with a half-life of 2·3 minutes for the given reading on the meter measuring the neutron yield. The normalised activity values were taken as standard and have been calculated from the formula—

$$A_{st} = 100\, A_a/\pi\eta.f.w$$

where A_a is the measured activity A_m *minus* background counts; $\pi\eta$ is the total time coefficient and equals $\eta_a.\eta_w.\eta_m$, the subscripts a, w and m denoting the time coefficients of the activation, waiting and measuring times, respectively; f is the reading of the neutron yield, meter counts; and w is the weight of the sample in milligrams.

The average values of the standard activity obtained by using either of the assemblies are seen to be much higher for silicosis than for the samples used for the check (Table I): for assembly A, 83·3 counts as compared with 22·2 and for assembly B, 226 as compared with 59. Even the minimum values measured for silicosis are found to be more than twice the maximum value found for the control samples. The results thus seem definitely to support the diagnostic value of the silicon analysis in sputum.

Samples from which the phosphorus had not been removed were also measured. The results obtained spectacularly confirmed the need for the separation from phosphorus.

In view of the limited number of cases investigated to date, no definite conclusions can be drawn, but the results are promising enough for the experiments to be continued on a more extensive scale and under increasingly uniform conditions.

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REFERENCES

1. Berton, M., *CEA*, 1964, **50**, 82.
2. Meinke, W. W., and Shideler, R. W., *Nucleonics*, 1962, **20**, (3), 60.
3. Lbov, A. A., and Naumova, I. I., *Atomn. Energ.*, 1959, **6**, 468.
4. Coleman, R. F., *Analyst*, 1961, **86**, 39.
5. Gillespie, A. S., and Hill, W. W., *Nucleonics*, 1961, **19**, (11), 140.
6. Gunn, P., *Ibid.*, 1961, **19**, (8), 81.
7. Maddock, R. S., Meinke, W. W., *T.I.D. Prog. Rep.* 10, TID-14310, November, 1961, p. 61.
8. Steele, E. L., "Proceedings of the International Conference on Modern Trends in Activation Analysis," Texas, 1961, 161.
9. Kusaka, Y., Tsuji, H., Fujii, J., Muto, H., and Miyoshi, K., *Bull. Chem. Soc. Japan*, 1965, **38**, (4), 2.
10. Turner, S. E., *Analyt. Chem.*, 1956, **28**, 1457.
11. Caldwell, R. L., and Mills, U. R., jun., *Nucl. Instrum. Meth.*, 1950, **5**, 312.
12. Martin, T. C., Mathur, S. C., and Morgan, J. L., *Int. J. Appl. Radiat. Isotopes*, 1964, **15**, 331.
13. Wing, J., *Analyt. Chem.*, 1964, **36**, 559.
14. Vogt, J. R., and Ehmann, W. D., *Geochim. Cosmochim. Acta*, 1965, **29**, 373.
15. Colowick, S. P., and Kaplan, N. O., "Methods in Enzymology," Academic Press Inc., New York, 1957, p. 843.
16. Pásztor, E., and Klopfer, E., *Közp. Fiz. Kut. Int. Közle.*, 1964, **12**, 143.

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SILICA METABOLISM IN GUINEA PIGS[1]

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Abstract

The excretion of silica (SiO_2) in urine and feces was determined following the oral and intraperitoneal administration of silica to guinea pigs. The urinary excretion of silica after oral administration generally reached a maximum value that was not exceeded by increasing the dose. The urinary excretion after intraperitoneal administration was considerably greater than after oral administration. The results of these experiments suggested that orally and intraperitoneally administered silica is depolymerized to the soluble form prior to excretion in the urine.

Introduction

The economic losses resulting from silica urolithiasis in Western range steers have been recognized for some time (1). A recent report by Connell *et al.* (2) described the incidence and geographical distribution of this condition in Canada. X-ray diffraction patterns and chemical analyses carried out in this division (3) and elsewhere (1) have shown that the calculi obtained from these animals were composed primarily of amorphous silica and organic matter.

The effects of various diets on silica excretion in urine have been reported by King *et al.* (4, 5), who noted that urinary silica excretion was much greater in herbivore than in carnivore. An increased urinary silica excretion was observed following the oral administration of powdered quartz or freshly precipitated silicic acid to dogs (6). Although the amount of silica excreted in the urine was readily influenced by diet these changes were never reflected in the blood levels and it was suggested that animals possess a very low renal threshold for silica.

King (7) studied the urinary excretion of silica following the intraperitoneal injection of either quartz dust or amorphous silica to cats and noted that the excretion of extra silica was transitory and in no instance lasted more than 3 or 4 days. Only a small portion of the administered silica was recovered. Holt (8), using mice, studied the urinary excretion of silica following the intraperitoneal injection of cement, quartz, carborundum, and molding sand. The administration of cement resulted in a definite increase in silica excretion that lasted for 3 or 4 days. Holt (9, 10) studied the distribution of labelled silicon in rat tissues following the intraperitoneal injection of radioactive silica sols. It was noted that alkaline sols were rapidly absorbed from the peritoneal cavity and deposited in the liver and kidneys. Acid sols, however, were poorly absorbed, presumably because of rapid polymerization of silica in the pH range of 5.5 to 6.0.

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Scheel *et al.* (11) studied the urinary silica excretion following the intraperitoneal injection of silica gel and sodium silicate into rats. Following the intraperitoneal injection of 100 mg of silica gel, the urine silica excretion increased from 0.2 mg per day to 0.9 mg per day and was still elevated 30 days later. It was noted that the silica content of urine could exceed 0.016% without forming a colloidal suspension.

The determination of silica levels in biological material has been greatly facilitated through the use of an improved silicomolybdate colorimetric technique (12). The interference of phosphorus is minimized in this method by reducing the silicomolybdate complex in 2 *N* sulphuric acid. A useful aspect of the silicomolybdate reaction is that it permits differentiation between 'soluble' silica and 'insoluble' or colloidal silica (13). This was also reported by Weitz *et al.* (14), who noted that molybdate reacted with monosilicic acid at 20° C. in 75 seconds, disilicic acid in 10 minutes, higher oligo acids in 1 hour, and the remainder not at all. Alexander (15) extended this study to include reaction rates.

In the present work a study of silica metabolism in guinea pigs has been undertaken as part of a general project related to silica urolithiasis in steers.

Materials and Methods

The following silicates were used:

1. Sodium metasilicate ($Na_2SiO_3.5H_2O$) obtained from Fisher Scientific Company Limited, Montreal.

2. Precipitated silicic acid ($H_2SiO_3.nH_2O$) obtained from Fisher Scientific Company Limited, Montreal.

3. A 30% silica sol with average particle size of 250 Å supplied by Monsanto (Canada) Limited, Montreal.

4. Tetraethyl orthosilicate obtained from Matheson, Coleman & Bell, Division of The Matheson Company Inc., East Rutherford, New Jersey.

The experiments were carried out with groups of guinea pigs raised from eighth generation inbred stock. The animals were fed a diet of soybean oil meal supplemented with vitamins and minerals (16). The silica content of the diet was 0.75 ± 0.023* mg SiO_2 per gram of feed.

Five guinea pigs were used for each experiment. The animals were kept in plastic metabolism cages and excreta collected in polyethylene containers. Forty-eight-hour urine and fecal collections were analyzed for silica.

The colorimetric technique of King *et al.* (12) was used for silica analysis in these experiments. There was no phosphate interference when phosphorus concentrations remained below 4.8 mg per 100 ml in the final dilution. The mean of eight recoveries obtained when silicates were added to urine or fecal samples was 101 ± 1.16%.* Sodium fluosilicate standards were used in this work because results were reproducible and followed Beer's law with silica

*Standard error of the mean.

concentrations of 0.04 to 0.24 mg per 100 ml. Absorbancy values were determined in a Beckman Model B spectrophotometer at a wavelength of 812 mμ.

Silica analyses on urine samples were generally done in duplicate. An aliquot was analyzed for soluble silica and hydrochloric acid (6 *N*) was added to the remainder in order to dissolve the carbonate precipitates. This acid urine was then adjusted to a known volume and an aliquot slowly dried in a platinum crucible. The residue was ashed, fused with sodium carbonate, and dissolved in distilled water. The dissolved melt was acidified to Congo red with 6 *N* HCl, diluted as required, treated with ammonium molybdate, and reduced in 2 *N* H_2SO_4 with 1,2,4-amino naphthol sulphonic acid reducing agent.

More than 100 urine samples were analyzed in duplicate using both methods and it was found that with concentrations of silica up to 0.0184% there was no significant difference between 'total and 'soluble' silica ($P > 0.05$). At concentrations above 0.0184% the difference between total and 'soluble' silica was significant ($P < 0.01$). These data indicate that although the solubility of silica in urine is greater than the reported solubility of silica in water (11, 17, 18), polymerized silica may be present when the concentration of silica in urine exceeds the solubility of silica in water.

The 48-hour fecal collections were ashed, weighed, thoroughly mixed, and a 50-mg sample weighed into a platinum crucible and fused with 3 g sodium carbonate. The silica analyses were carried out as described above.

Results

The urinary excretion of silica in five guinea pigs on the soybean oil meal diet was 2.1 ± 0.28, 2.0 ± 0.21, and 1.5 ± 0.28* mg SiO_2 respectively for three consecutive 48-hour periods.

TABLE I

Urinary SiO_2 excretion following oral administration of (A) single dose sodium silicate (80.0 mg SiO_2); (B) four doses of sodium silicate (80.0 mg SiO_2) at 48-hour intervals

Period	Total SiO_2 excretion (mg)	
	A	B
1	2.7 ± 0.11* (5)†	1.5 ± 0.16* (5)†
2	9.7 ± 0.86 (5)	10.2 ± 0.43 (5)
3	5.7 ± 0.75 (5)	10.3 ± 0.64 (5)
4	3.9 ± 0.65 (5)	10.3 ± 0.82 (5)
5	3.1 ± 0.44 (5)	10.3 ± 0.49 (5)
6		5.9 ± 0.87 (4)
7		5.0 ± 0.60 (4)
8		4.0 ± 0.60 (4)

*Standard error of the mean.
†Number of animals.

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TABLE II

Urinary silica excretion following the intraperitoneal injection of a 2% silica sol* (A) 100 mg SiO_2; (B) 60 mg SiO_2

Period	Total SiO_2 excretion (mg) A	B
1	2.2 (3)†	1.9* (2)†
2	28.0 (3)	37.1 (2)
3	17.3 (1)	3.5 (2)
4	11.0 (1)	2.5 (2)

*Prepared from a solution of sodium silicate and dilute HCl.
†Number of animals.

TABLE III

Urinary silica excretion following oral administration of (A) 1% silica sol* (93.1 mg SiO_2); (B) 5% silica sol* (567.0 mg SiO_2)

Period	Total SiO_2 excretion (mg) A	B
1	2.9 ± 0.30† (4)‡	2.0 ± 0.14† (5)‡
2	3.9 ± 0.15 (5)	5.5 ± 0.67 (5)
3	3.6 ± 0.32 (5)	4.0 ± 0.32 (5)
4	3.0 ± 0.31 (5)	3.5 ± 0.31 (5)
5		3.1 ± 0.22 (5)
6		2.9 ± 0.13 (5)
7		3.3 ± 0.22 (5)

*Prepared by diluting a commercial silica sol.
†Standard error of the mean.
‡Number of animals.

TABLE IV

Urinary silica excretion following the intraperitoneal injection of 10 ml of 4% silica sol* (438.8 mg SiO_2)

Period	Total SiO_2 excretion (mg)
1	3.6 ± 0.58† (5)‡
2	50.8 ± 4.21 (5)
3	46.6 ± 1.41 (4)
4	40.1 ± 3.41 (4)
5	33.1 ± 0.25 (3)
6	25.0 ± 3.69 (3)
7	20.3 ± 2.49 (3)
8	13.5 ± 1.96 (3)
9	9.1 ± 1.28 (3)
10	6.3 ± 0.13 (3)

*Prepared by diluting a commercial silica sol.
†Standard error of the mean.
‡Number of animals.

TABLE V

Urinary silica excretion following (A) oral and (B) intraperitoneal administration of 10 ml of a precipitated silicic acid suspension (390 mg SiO_2)

Period	Total SiO_2 excretion (mg) A	B
1	2.3 ± 0.26* (4)†	1.6 ± 0.20* (3)†
2	5.5 ± 0.50 (5)	36.2 ± 3.49 (3)
3	7.1 ± 0.69 (5)	26.6 ± 0.78 (3)
4	5.7 ± 0.43 (5)	21.2 ± 1.06 (3)
5	4.1 ± 0.19 (5)	15.4 ± 0.79 (3)
6	3.3 ± 0.36 (5)	17.0 ± 1.87 (3)
7	2.7 ± 0.27 (5)	13.4 ± 1.11 (3)
8		11.8 ± 1.85 (3)
9		9.2 ± 1.02 (3)
10		8.2 ± 1.23 (3)
11		5.1 ± 0.89 (3)
12		5.1 ± 0.89 (3)
13		4.9 ± 0.75 (3)
14		3.8 ± 0.61 (3)
15		3.3 ± 0.60 (3)

*Standard error of the mean.
†Number of animals.

TABLE VI

Urinary silica excretion following the feeding of (A) pelleted Western hay diet containing 4.65% SiO_2; (B) pelleted soybean oil meal diet containing 4.68% SiO_2

Period	Total SiO_2 excretion (mg) A	B
1	13.4 ± 1.17* (5)†	15.8 ± 1.39* (5)†
2	15.0 ± 1.28 (5)	15.4 ± 1.19 (5)
3	14.9 ± 0.82 (5)	17.8 ± 1.91 (5)
4	13.5 ± 0.87 (5)	15.7 ± 1.89 (5)
5	14.2 ± 0.93 (5)	18.8 ± 2.79 (5)
6		14.6 ± 1.74 (5)

*Standard error of the mean.
†Number of animals.

The experimental results are recorded in Tables I to VI. The term 'period' is used in this paper to indicate a 48-hour time interval.

The data listed for the first period represent the normal silica excretion of the particular groups of animals on test. All silica doses were administered at the end of the first period unless stated otherwise.

Table I, column A, shows the silica excretion following the oral administration of a dose of sodium silicate containing 80.0 mg SiO_2. The urinary silica excretion rose to a maximum of 9.7 ± 0.86* mg 48 hours after dosing

*Standard error of the mean.

and returned to the normal level after 8 days. The term 'net silica excretion' is used in this paper to indicate the difference between the silica ingested and the silica excreted in the urine and feces. This amounted to 48.3 ± 3.58* mg SiO_2 in this experiment and represented approximately 60% of the administered dose.

Table I, column B, shows the results of administering four successive doses of sodium silicate at 48-hour intervals, each containing 80.0 mg SiO_2. The urinary silica excretion reached a maximum of 10.3 mg per 48-hour period and did not increase with subsequent doses. The experiment was terminated at period 8 when the urinary silica excretion approached the normal value. The net silica excretion was 307.0 ± 7.85* mg, or approximately 96% of the administered dose.

These experiments suggested that there might be a well-defined limit to silica absorption from the intestinal tract. Therefore, in the next experiment five guinea pigs were injected intraperitoneally with a 2% silica sol of pH 5.0 prepared from sodium silicate. The animals were injected with 3- and 5-ml doses containing 60 and 100 mg of SiO_2 respectively. The results of this experiment (Table II) indicated that the 48-hour urinary silica excretion was approximately three times as great as that obtained in the previous two experiments.

Table III shows the urinary silica excretion following the feeding of a silica sol with an average particle size of 250 Å. The undiluted sol gelled rapidly at a pH below 9, but gelling did not occur at any pH if the sol were first diluted to a concentration of 5% or less.

The urinary silica excretion did not increase appreciably after the oral administration of this sol in doses equivalent to 93.1 and 567.0 mg SiO_2 respectively. The urinary silica was present entirely in the 'soluble' or 'molybdate reactive' state, indicating that silica particles of the size present in the sol were not excreted without a prior depolymerization to 'soluble' silica. With the group receiving the 5% silica sol containing 567.0 mg SiO_2, the net silica excretion was 487.2 ± 21.57* mg SiO_2, or approximately 86% of the administered dose.

A large increase in urinary silica excretion was obtained after an intraperitoneal injection of a silica sol containing 438.8 mg SiO_2. The results recorded in Table IV show that the 48-hour urinary silica excretion at period 2 reached a maximum of 50.8 ± 4.21* mg and returned approximately to the normal level at the end of period 10. The net silica excretion in this experiment was 277.5 ± 20.99* mg SiO_2. This represented 63% of the injected dose.

The results recorded in Table V were similar to those described above except that precipitated silicic acid of undetermined particle size was used instead of the silica sol. The urinary silica levels obtained following the oral administration of this precipitated silicic acid (Table V) were similar to those recorded with the silica sol (Table III). The net silica excretion in

*Standard error of the mean.

this experiment was 251.2 ± 25.80* mg at the end of period 7. This represented approximately 64% of the administered dose of 390 mg SiO_2. This relatively low recovery may have been due to silica retention in the intestinal tract.

The urinary silica excretion following the intraperitoneal injection of a 5% precipitated silicic acid suspension containing 390 mg SiO_2 is recorded in Table V. The urinary silica excretion as measured at 48-hour intervals reached a maximum of 36.2 ± 3.49* mg and subsequently dropped to normal values after 28 days. The results from two of the animals were not included in the table. Their urinary silica excretion rose to a maximum of 13.4 mg and remained high for 48 days. The net silica excretion of the entire group was 187.0 ± 15.16* mg. This accounted for approximately 48% of the injected dose. It is probable that the rest of the injected silica was retained by the tissues.

There was no well-defined increase in silica content of the fecal ash following the intraperitoneal injection of silica. The question of silica excretion into the intestinal tract following intraperitoneal injection requires further study.

Table VI shows the urinary silica levels obtained after feeding a pelleted diet of Western hay to guinea pigs. This diet, supplemented with vitamins, minerals, and casein, contained 4.65 g SiO_2 per 100 g air-dried material. Over a 10-day period silica excretions during successive 48-hour intervals ranged from 13.4 ± 1.17* mg to 15.0 ± 1.28* mg SiO_2. During the same 48-hour intervals the feces contained from 1 to 2 g SiO_2. Table VI also shows the urinary silica excretion following the feeding of a pelleted soybean oil meal diet containing silica gel. This diet contained 4.68 g SiO_2 per 100 g air-dried material. The silica excretion during 48-hour intervals over a 10-day period ranged between 14.6 ± 1.74* mg and 18.8 ± 2.79* mg.

Discussion

In this study urine samples were analyzed for total and soluble silica. The results showed that silica in guinea pig urine does not polymerize at concentrations less than 0.018%. The method for "soluble" silica, however, could not be applied when the concentration of silica exceeded 0.018% because polymerization occurred in some samples. Although these results indicated that the solubility of silica in urine approximates the reported solubility of silica in water (11, 17, 18), some of the urine samples that were analyzed contained quantities of "soluble" silica far in excess of those present in aqueous systems.

In the experiments listed in Tables I and II a solution of sodium silicate was administered orally. Although a gel was probably formed on contact with the hydrochloric acid of the stomach, higher urinary silica levels resulted from the feeding of this material than were obtained with comparable doses of other forms of inorganic silica.

*Standard error of the mean.

The results showed that silica absorption and excretion following a single oral dose of sodium silicate reached a maximum within 48 hours and gradually returned to normal values after 8 days. The experiment recorded in Table I, column B, was carried out in order to determine if the silica excretion could be increased by administering four doses of sodium silicate at 48-hour intervals. The results showed that the increase did not occur and that the excretion over a 48-hour period did not exceed 10.3 mg. The urinary excretion of silica was apparently limited by restricted absorption from the gastrointestinal tract and not by the blood transport mechanism or kidneys since in the succeeding experiment (Table II) a threefold increase in silica excretion followed the intraperitoneal injection of a comparable silica dose. Apparently the kidneys are capable of excreting much larger amounts of silica than are normally absorbed from the gastrointestinal tract.

The results in Tables III and V showed that only small increases in silica excretion followed the oral administration of the silica sol and the precipitated silicic acid. Since in these experiments all of the silica in the urine was in the 'soluble' or 'molybdate reactive' form it appears that the silica particles underwent depolymerization prior to excretion.

From these results the possibility may be considered that the absorption and urinary excretion of the ingested silica is limited by its solubility in the contents of the gastrointestinal tract. This was supported by later work which showed that when guinea pigs were dosed with 0.96 ml of tetraethyl orthosilicate in 2.0 ml of ethanol, urinary excretion values in excess of 40 mg per 48 hours were obtained.

Comparative data are given in Tables IV and V for the urinary excretion of silica after the intraperitoneal injection of a silica sol or a precipitated silicic acid. A marked increase in the excretion of urinary silica followed the use of the intraperitoneal instead of the oral route of administration. The urinary silica excretion after the intraperitoneal injection of the silica sol was increased above normal for 18 days and at that time 63% of the administered silica was accounted for. The urinary silica excretion after the intraperitoneal injection of precipitated silicic acid was increased above normal for 28 days and at that time 48% of the administered silica was recovered. Although some of the silica in the urine polymerized at the higher concentrations, the greater part was present in the soluble form indicating that the injected silica particles were depolymerized prior to excretion.

The reason for the greater silica excretion following intraperitoneal injection is not definitely known. These results indicate that conditions in the peritoneal cavity favor the depolymerization and subsequent excretion of silica.

The results in Table VI indicate the urinary silica excretion obtained when animals were fed pelleted diets of either Western hay (4.65% SiO_2) or silica-supplemented soybean oil meal (4.68% SiO_2). These animals ingested approximately 2.0 g silica during each 48-hour period. The results show that when large quantities of either silica gel or plant silica were ingested,

the amount of urinary silica excreted was comparable with that obtained with much smaller doses of sodium silicate. The results indicate that when inorganic silica is fed to guinea pigs, the silica excretion in urine, during a 48-hour period, approaches a maximum. The amount of silica required to produce this maximum depends primarily on the solubility of the administered silicate.

Acknowledgments

The authors wish to extend thanks to Mr. P. M. Richard for his assistance with the laboratory animals and to Monsanto (Canada) Ltd. for their generous supply of the silica sol used in these experiments.

References

1. SWINGLE, K. F. Am. J. Vet. Research, **14**, 493 (1953).
2. CONNELL, R., WHITING, F., and FORMAN, S. A. Can. J. Comp. Med. Vet. Sci. In press. 1958.
3. FORMAN, S. A., WHITING, F., and CONNELL, R. Can. J. Comp. Med. Vet. Sci. In press. 1958.
4. KING, E. J., STANTIAL, H., and DOLAN, M. Biochem. J. **27**, 1002 (1933).
5. KING, E. J. and BELT, T. H. Physiol. Revs. **18**, 329 (1938).
6. KING, E. J., STANTIAL, H., and DOLAN, M. Biochem. J. **27**, 1007 (1933).
7. KING, E. J. Biochem. J. **32**, 426 (1938).
8. HOLT, P. F. Brit. J. Ind. Med. **7**, 12 (1950).
9. HOLT, P. F. Brit. J. Exptl. Pathol. **35**, 52 (1954).
10. HOLT, P. F. and YATES, D. M. Biochem. J. **54**, 300 (1953).
11. SCHEEL, L. D., FLEISCHER, E., and KLEMPERER, F. W. A. M. A. Arch. Ind. Hyg. and Occupational Med. **8**, 564 (1953).
12. KING, E. J., STACY, B. D., HOLT, P. F., YATES, D. M., and PICKLES, D. Analyst, **80**, 411 (1955).
13. DIENERT, F. and WANDENBULCKE, F. Compt. rend. **176**, 1478 (1923).
14. WEITZ, E., FRANCK, H., and SCHUCHARD, M. Chemiker-Ztg. **74**, 256 (1950).
15. ALEXANDER, G. B. J. Am. Chem. Soc. **75**, 5655 (1953).
16. WESSON, L. G. Science, **75**, 339 (1932).
17. ALEXANDER, G. B., HESTON, W. M., and ILER, R. K. J. Phys. Chem. **58**, 453 (1954).
18. GOTO, K. and OKURA, T. Kagaku (Tokyo). **23**, 426 (1953).

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Scientific Notes

Demonstration of Siliceous Deposits in the Kidneys of the Guinea Pig

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Ottawa, Ontario

[illegible]E OCCURRENCE of siliceous urolithiasis in [illegible]ge steers of western Canada and the United [illegible]tes has been reported by Swingle,[6] Connell, et [illegible] and Whiting et al.[7] A description by Forman [illegible]al[4] indicates that siliceous calculi are composed [illegible]mainly of hydrated, noncrystalline silicon di[illegible]de and organic matter.

[illegible]Work in this laboratory[4] has shown that [illegible]ly administered particulate silica with an over[illegible] particle size of 250Å is not excreted in the [illegible]e as such, but is first depolymerized to the [illegible]le state. It was also noticed that soluble [illegible] in urine did not polymerize unless the con[illegible]tration exceeded 18 mg./100 ml. This indi[illegible]s that the nucleus of a siliceous calculus is [illegible]ly formed at some point in the urinary [illegible].

[illegible]his study presents evidence to show that, [illegible]ing the administration of relatively large [illegible]nts of soluble silica to guinea pigs, the for[illegible]n of siliceous deposits can occur in the [illegible] tubules.

[illegible]RIALS and METHODS

[illegible] solution of sodium metasilicate ($Na_2SiO_3 \cdot 5H_2O$) [illegible] prepared for intraperitoneal administration. [illegible]diately before use, the solution was adjusted [illegible] 5 by rapidly adding 3N hydrochloric acid. [illegible]rganic silicate, tetraethyl orthosilicate, was [illegible] for oral administration. The 15-ml. intra[illegible]neal dose contained 20 mg. of silicon dioxide [illegible]milliliter; the oral dose of 2 ml. of tetraethyl [illegible]cate solution contained 253.8 mg. of sili[illegible]xide per milliliter.

[illegible] guinea pigs, raised on a low-silica diet,[4] [illegible] killed 24 hours after the administration of [illegible]. Preliminary tests showed that, with the [illegible] of silica administered, many animals did [illegible]vive longer than 48 hours. At necropsy, the [illegible]s were pale, greatly enlarged, and had [illegible]ened surfaces; when sectioned, they were [illegible] to have tubular deposits similar to those [illegible] in the present experiment.

[illegible]al sections of kidney, 10 μ in thickness, were [illegible]. Figures 1, 2, and 3 show sections taken from an adult male guinea pig that were given silica intraperitoneally. The first section (fig. 1) was stained with hematoxylin and eosin and examined by transmitted light. The silica deposits were seen in the kidney tubules as lightly stained, homogeneous-appearing masses. An adjacent section (fig. 2) was ashed in a muffle furnace at 600 C. for one hour after removal of paraffin. The slides were always ashed in covered Petri dishes in order to avoid surface contamination. The silica deposits seen in figure 1 are shown in figure 2 as they appear after ashing when viewed by reflected dark-field illumination. An outline of the cell structure of the kidney is readily visible in this section.

In figure 3, the same section is viewed by reflected dark-field illumination after a 30-minute immersion in 50 per cent (v/v) hydrochloric acid. This treatment completely removed the tissue salts that were left after ashing and, in kidney sections from control animals, removed all visible background. The silica deposits (fig. 3) were optically isotropic when viewed by polarized light. The ashed section was then treated with 0.5 per cent hydrofluoric acid for 60 seconds, dried, and examined. The first hydrofluoric acid treatment severely attacked the particles shown; one or two additional 60-second exposures to hydrofluoric acid completely removed them.

Figures 4 and 5 are photomicrographs of kidney sections that were ashed but not treated with acid. These were taken from 2 guinea pigs that were each given silica orally. It was again noticed that the deposits were not attacked by hydrochloric acid, but were readily removed by treatment with dilute hydrofluoric acid.

RESULTS and DISCUSSION

These results indicate that, when guinea pigs are given relatively large quantities of soluble silica, siliceous deposits may form in the renal tubules.

Previous work with labeled silicon[8] has shown that, when a freshly prepared sol is orally administered to guinea pigs, the silicon is rapidly excreted in the urine. It was also noticed that, when labeled silicon was given in the form of sodium silicate

[illegible] the Animal Research Institute, Research Branch, [illegible] Department of Agriculture, Ottawa, Ont. Con[illegible]on No. 11.

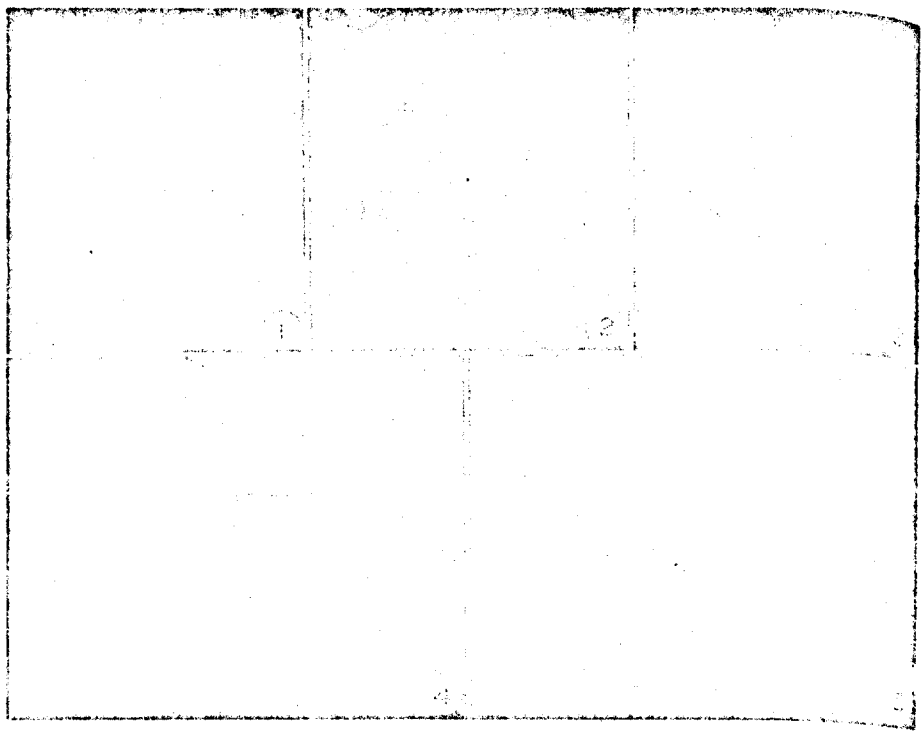

Fig. 1—Silica deposits in kidney of guinea pig given silica sol intraperitoneally. H & E stain; x 88.

Fig. 2—Section of kidney adjacent to that shown in figure 1. After ashing, same silica deposits are shown, viewed by reflected dark-field illumination. A microscopic outline of the kidney structure remains after ashing. x 88.

Fig. 3—Section shown in figure 2 following a 30-minute exposure to 50 per cent hydrochloric acid. The kidney ash has been completely removed but the silica deposits are not affected. Reflected dark field; x 88.

Fig. 4—Silica deposits in ashed sections of kidney from guinea pig given an oral dose of tetraethyl orthosilicate. Reflected dark field; x 88.

Fig. 5—Silica deposits in an ashed section of kidney from guinea pig given tetraethyl orthosilicate orally. Reflected dark field; x 88.

instead of a sol, the radioactivity in the kidney was several times greater than that present in other tissues.

It therefore appears likely that, when soluble silica is administered to guinea pigs, the silica concentration in the glomerular filtrate and urine increases until polymerization occurs and silica deposits are formed. The possibility may be considered that a similar process plays a role in the development of silica calculi.[3]

SUMMARY

Evidence showed that siliceous deposits form in the kidneys of guinea pigs given large doses of soluble silica orally or intraperitoneally. The amount of soluble silica administered was considerably greater than animals would normally ingest on a forage diet, and it cannot be concluded that silica calculi in range steers form from 'nuclei' of polymerized silica similar to those found in the guinea pig kidney. Nevertheless, this work indicates that insoluble deposits of silica may form in kidney tubules if large quantities of soluble silica are administered to an animal.

References

[1] Connell, R., Whitting, F., and Forman, S. A.: Urolithiasis in Beef Cattle. 1. Observation on its occurrence. Canad. J. Comp. Med. & Vet. Sci., 23, (1959): 41–46.

[2] Forman, S. A., Whitting, F., and Connell, R.: Urolithiasis in Beef Cattle. 3. Chemical and Physical Composition of the Uroliths. Canad. J. Comp. Med. & Vet. Sci., 23, (1959): 157–162.

[3] Forman, S. A., Sexton, F., Loughlinod, D. H., Sexton, W. M.: Volume and Acidity of Urine of Steers Fed Rich in Silica and Effect of Dietary Silica. Nature, 182, (1958): 1555.

[4] Sauer, F., Loughland, D. H., and Davidson, W. M.: Silica Metabolism in Guinea Pigs. Canad. J. Biochem. & Physiol., 37, (1959): 183-191.

[5] Sauer, F., Loughland, D. H., and Davidson, W. M.: The Silica Content of Guinea Pig Tissues as Determined by Chemical and Isotopic Techniques. Canad. J. Biochem. & Physiol., 37, (1959): 1173-1181.

[6] Swingle, K. F.: The Chemical Composition of Urinary Calculi from Range Steers. Am. J. Vet. Res., 14, (Oct., 1953): 493-498.

[7] Whiting, F., Connell, R., and Forman, S. A.: Silica Urolithiasis in Beef Cattle. The Incidence on Different Rations and on Range. Canad. J. Comp. Med. & Vet. Sci., 22, (1958): 332-337.

SUMMARIO IN INTERLINGUA

Le Demonstration de Depositos Silicee in le Renes de Porcos de India

Depositos silicee esseva demonstrate in le renes de porcos de India tractate con grande doses de silice per via oral o intraperitoneal. Le quantitates de solubile silice administrate esseva considerablemente plus grande que lo que animales ingere normalmente in un dieta de forrage, e le conclusion non es justificate que le calculos de silice incontrate in boves de pastura es formate ab nucleos de polymerisate silice del genere demonstrate in le renes de porcos de India. Nonobstante, iste investigation demonstra que le formation de insolubile depositos de silice es possibile in le tubulos renal, si satis grande quantitates de silice solubile es administrate a un animal.

Meetings • • •

• • • American Veterinary Medical Association, 97th Annual Meeting. Denver, Aug. 15-18, 1960. H. E. Kingman, Jr., 600 S. Michigan Ave., Chicago 5, Ill., executive secretary.

• • • First International Congress of Histochemistry and Cytochemistry. Paris, Aug. 28-Sept. 3, 1960. Dr. R. Wegmann, Laboratoire d'Histochimie, Faculté de Médecine, 54, rue des Saints-Peres, Paris 6e, France, secretary general.

• • • Tenth International Congress of Cell Biology. Paris, Sept. 4-9, 1960. Prof. M. Chevremont, 20, rue de Pitteurs, Liege, Belgium.

• • • European Conference on Electron Microscopy. Delft, Netherlands, Aug. 29-Sept. 3, 1960. Prof. J. B. LePoole, Mijnbouwplein 11, Delft, Netherlands.

Research Tools

Further information on these products may be obtained by writing to: Research Tools, American Veterinary Medical Association, 600 S. Michigan Ave., Chicago 5, Ill.

Radioactivity Measuring Device

A new device for measuring radioactivity (Magnescaler, manufactured by the Picker X-Ray Corp.) can be used with scintillation detectors, Geiger tubes, and proportional counters with pre-amplifiers. The unit collects more than 600,000 counts per minute with an accuracy of 99 per cent.

A built-in odometer-type timer measures up to 999.99 in steps of 0.01 minute and is accurate within 0.02 of 1 per cent. The instrument can be pre-set in 11 time positions and 11 count positions. Counts can be read directly from six electronic decades. The unit can be combined with automatic sample changers and printers to read out time and count data.

Automatic Nitrogen Analyzer

A fully automatic nitrogen analyzer, manufactured by Coleman Instruments, Inc., utilizes the micro-Dumas process for determinations.

The instrument is compact (18 in. wide by 15 in. deep), fast (4 to 5 determinations per hour), and accurate (determinations down to the 0.01% level, results within ± 0.15% of theory). Conventional stopcock and furnace manipulations are eliminated by automatic timing and performance of all sequential operations.

Each of the twin combustion chambers has a ten-stage temperature control. The entire cycle can be adjusted to optimum temperatures for complete pyrolysis. An auxiliary timer permits a pre-set extension of the final period from one to six minutes. The cycle can be interrupted and maintained at any point with a master "cycle delay" switch. Thermocouples monitor individual furnace

Ann. N.Y. Acad. Sci. 84:844-856, 1960

RECENT DEVELOPMENTS IN AUTOMATIC COLORIMETRIC CHEMICAL ANALYSIS INSTRUMENTS

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Continuous on-stream instrumentation has been receiving increasing attention and study during the last five to ten years. Previously, use of this form of instrumentation had been limited almost to measurement of physical variables or electrical characteristics of the stream. The wide interest in infrared analyzers and chromatography is indicative of this.

The control laboratory, however, has remained a part of the control loop for many variables because, until recently, all too many of the analyses of these variables did not lend themselves to measurement by any type of industrial instrumentation available. A need existed, therefore, for an instrument or a group of instruments designed as rugged industrial devices that would eliminate or minimize routine, repetitive laboratory chemical analysis. Such instrumentation would make these analyses with the greater frequency required for continuous control and would be adaptable to feedback and automatic correction loops within the system.

This paper is concerned specifically with one class of on-stream, colorimetric industrial analyzers developed for measurements in which the color is the result of a chemical reaction. Development of such instruments started 6 years ago in the German Federal Republic, where it progressed concurrently with its development in the United States during the past 3 years. This form of instrumentation is now in use in many plants in Europe. Over 500 instruments have been sold and installed during the past 3 years. In the United States, a corresponding instrument, the Quantichem† has been developed to a still higher degree of refinement before commercial marketing. It has been field-tested also, and a representative number of field installations indicate that it has reached the point of practical industrial use.

The commercial instrument has little resemblance to the original laboratory assembly of glassware. The original design objectives were:

(1) A mechanical system capable of metering with a high degree of repetitive accuracy exact volumes of both sample and reagent or reagents. The system was to be constructed of rugged materials selected to withstand corrosive effects of chemical reagents required. The instrument was designed for industrial use, not for the laboratory.

(2) An optical system that, with the proper filters and photocells, would produce results of highest accuracy and reproducibility consistent with the accuracy.

(3) An electrical system for optimum amplification of photocell signals for recording, and for feedback controls.

* Now with Milton Roy Co., St. Petersburg, Fla.
† Milton Roy Co., Philadelphia, Pa.

(4) A chemical method of analysis that, in its adaptation, would permit the preparation and storage of chemicals without deterioration for periods varying from one week to one month. The method of analysis used must combine the mechanical, optical, and electrical systems in a completely reliable industrial instrument that would require a minimum of maintenance.

The instrument could have been designed either for continuous or semicontinuous analysis, or for repetitive cyclic analysis. Since continuous analysis offered little advantage, if any, from the standpoint of time elapsing between sample procurement and read-out, it was decided to proceed with the cyclic design because of the higher accuracy possible and the flexibility as to sample spacing, which would permit more frequent sampling close to the time

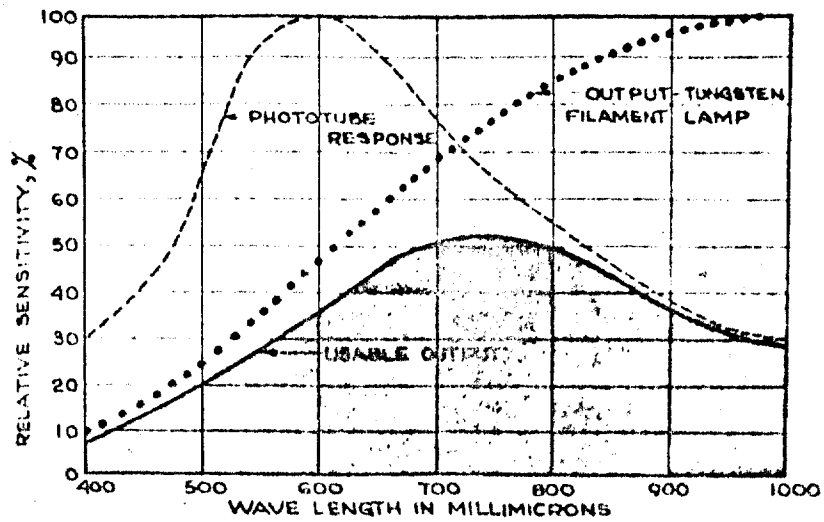

FIGURE 1. Curves of typical responses for light source and phototube.

of an anticipated process change. Furthermore, cyclic analyses obviously could be more economical of costly chemical reagents required for the tests.

FIGURE 1 illustrates a typical set of response curves for a light source (*dotted line*) and a photocell (*dashed line*). From this, it is possible to determine the useful range of the instrument (*solid line*). In this case, the normally useful range of the combination would lie between 400 and 1000 mμ. Similar curves would be drawn for the other components of the system.

In order to make use of the instrument for chemical analysis, it is necessary to be able to measure the percentage transmittance at a specific wave length. An optical filter is used for this purpose. In essence, a filter is a selective shade that ideally completely blocks out light of all wave lengths except the desired one. In practice, a curve similar to FIGURE 2 results. The minor deviations due to transmittance at wave lengths other than the desired one are usually unimportant.

Why is colorimetry preferable to other methods of analysis? Recent work, particularly in the power and nuclear fields, has required accurate determina

tion of materials in solution at concentrations of less than one part per million and, in some cases, considerably lower. At these low concentrations almost all materials follow Beer's law. In many cases, colorimetric analyses can be made accurately at one-thousandth or less the concentration that can be detected by conductivity or *p*H value. Colorimetric analysis usually can resolve ion species in the presence of other material at several thousand times the concentration of the ion being analyzed.

Instrument Development

The commercial development of a colorimetric on-stream chemical analyzer was initiated in about 1952 by Hans Fuhrmann at the firm of Bran & Lübbe*

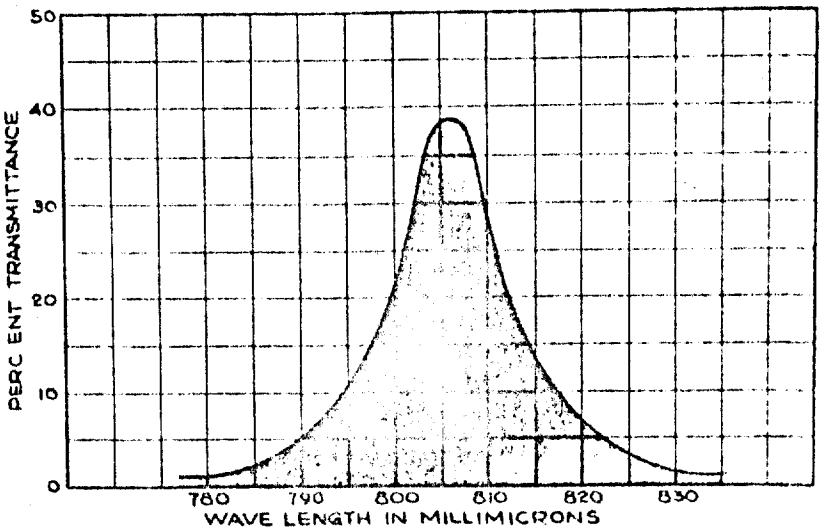

FIGURE 2. Effect of narrow band interference filter in producing monochromatic light

in the German Federal Republic. The first instruments[1-3] were designed specifically for the determination of residual hardness in water following a zeolite or an ion-exchange process. Many commercial installations of this instrument were made in power plants to monitor water softeners, to signal any breakthrough of hardness, and to initiate the regeneration process.

FIGURE 3 illustrates typical transmittance curves covering the water hardness ranges of 0 to 3.5 ppm and 0 to 7 ppm calcium carbonate. The two curves were obtained with two different monochromatic light filters. It is obvious that the read-out scale of the instrument must be designed to correspond to curve for the light filter selected. Fixed quantities of sample, indicator, buffer, and magnesium dipotassium ethylenediaminetetraacetate (MgK_2EDTA) are employed. The color developed, varying from blue to violet to red-violet to red over a range of 0 to 7 ppm total hardness, is a

* Bran & Lübbe Wasseraufbereitung Machinenzand Apparatebau, Hamburg 39, German Federal Republic.

measure of the quantity of calcium and magnesium in the sample. Eriochrome Black T, with a sufficient amount of the complexing agent MgK_2EDTA, is used as the indicator. The sole function of the MgK_2EDTA is to chelate the free calcium ions in solution and release an equivalent amount of magnesium ions, the Eriochrome Black T being sensitive only to the magnesium.

Following an initial and thorough study of the first German instruments, development of similar colorimetric analyzers was undertaken in the United States by the Milton Roy Co. Design objectives were established as already outlined. It was felt that the ultimate instrument should eliminate, as far as possible, internal laboratory-type glassware and should provide positive, accurate volumetric measurement of both sample and reagents. The present design of the Quantichem has achieved these objectives.[4]

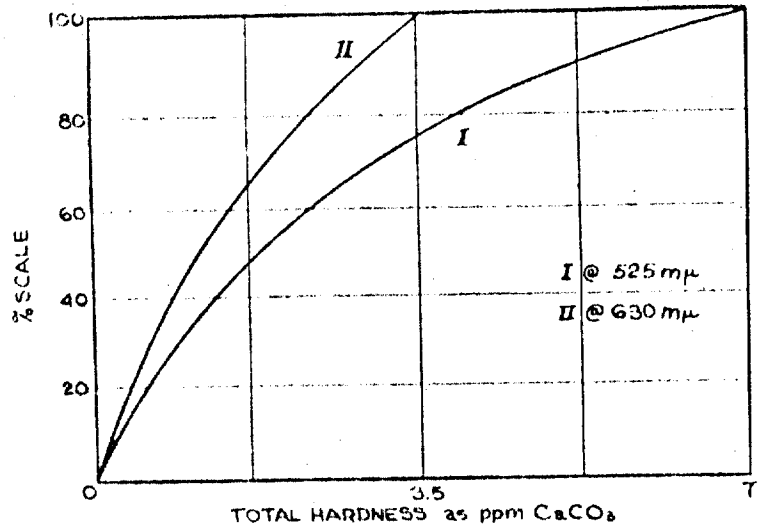

FIGURE 3. Effect of wave length on light transmission for hardness measurement.

The two units that comprise the instrument (FIGURE 4) are the analyzer and the recorder. The analyzer houses the sample- and reagent-metering pumps, the sample cells, the light source, light filters, photocells, and the program-timing system. To facilitate maintenance by the operator, three telltale lights are located prominently on the face of the analyzer unit below a master program timer. The center lamp lights when the instrument is on power. The light on the right (FIGURE 4) flashes when the cells need cleaning. The light on the left flashes if the light source in the analyzer fails.

The master program timer, whose dial is visible on the face of the instrument, controls all steps of the analysis. A manual-automatic switch placed immediately below the timer enables the operator to stop the analysis at any point in the circuit and check the functioning of the instrument by manually operating the timer.

The recorder is a standard instrument, modified to meet the requirements of this application. It contains the amplifier, bridge circuits, and the DC-AC

converter. AC amplification is used because of its inherent stability and freedom from drift. This feature permits a single calibration of the instrument, eliminating periodic standardization required by DC amplification systems.

The schematic of the Quantichem in its simplest form, as designed for water-hardness determination, is shown in FIGURE 5.

A unique pneumatic timing system that works on the principle of the player piano is employed. The air supply at 20 psi is admitted to a manifold and flows through small orifices to the individual control lines. Each line terminates in an O-ring Teflon nozzle, sealed by the solid surface of the program

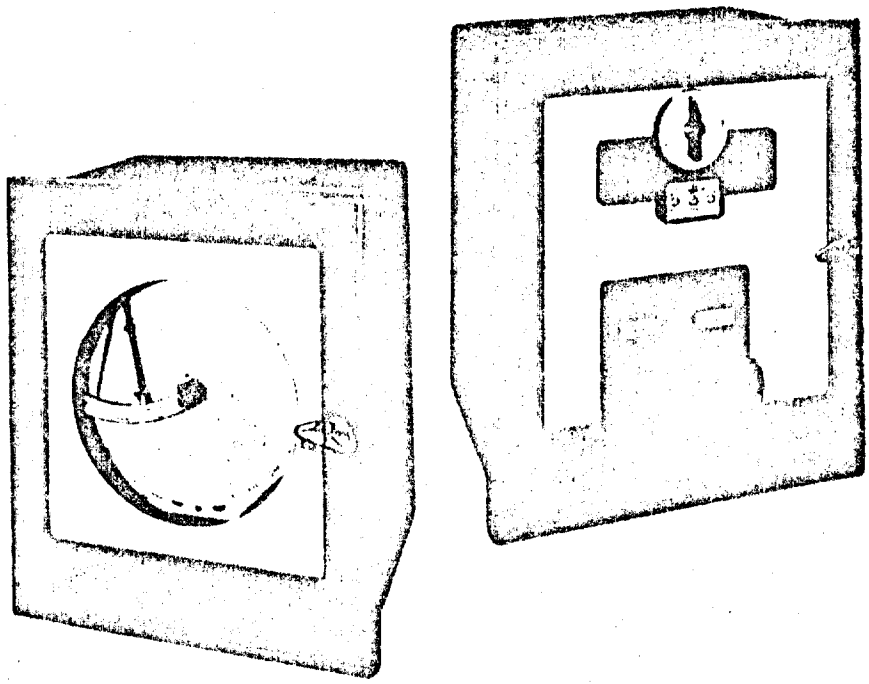

FIGURE 4. Quantichem colorimetric end-point analyzer and recorder.

ming drum, and the other end of the line terminates at either a pressure-actuated electrical switch, a pressure-actuated diaphragm valve, or a pressure-actuated reagent pump. The programming drum (FIGURE 5) makes one revolution for each cycle of analysis. At the appropriate time in the cycle, when a valve is to be opened or a pressure-actuated switch energized, air is released from the individual control line through an orifice or a slot in the programming drum so that the pressure drops in that line, the air pressure build-up at the small orifice being insufficient to compensate for the loss of pressure through the programming drum. This system of programming has simplified valve design and functioning of the instrument. Furthermore, electrical controls are minimized in what might prove to be somewhat corrosive atmospheres, facilitating maintenance.

At the start of the cycle, clean sample, filtered if necessary, flows via the pressure-regulating valve through both the sample cell and comparison cell, filling the cells to overflowing with known volumes of sample. The initial sample flow acts to flush the previous sample from the cell and to replace it with sample representative of the process stream. The sample inlet valves then are closed by the program timer.

The zeroing relay and zeroing motor now are energized to standardize the bridge circuit automatically. The purpose of standardizing this portion of the system in each cycle is to compensate for irregularities due to such factors as sample turbidity, coating of cell walls, and dust on lenses. Although these problems have not proved severe in practice, standardization nevertheless is

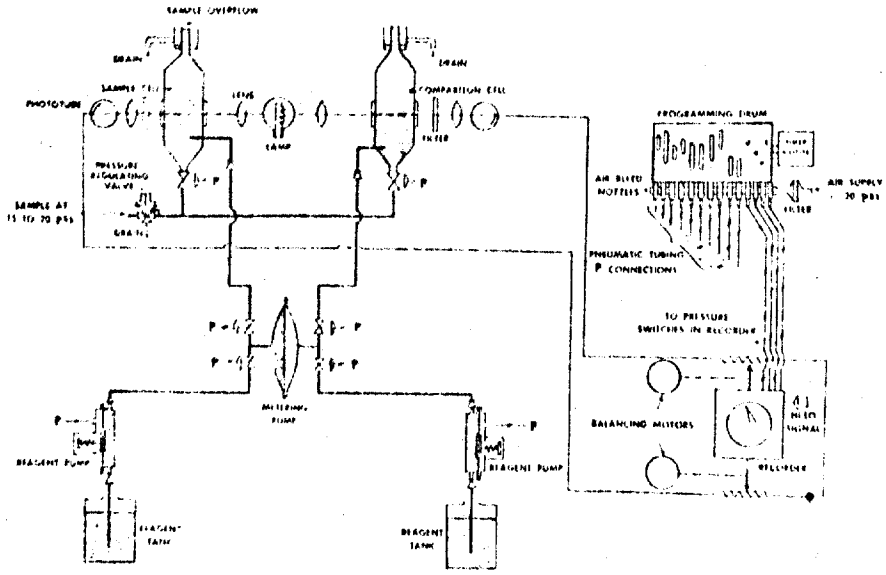

FIGURE 5. Schematic design of hardness analyzer.

incorporated in every cycle. Tests have shown little appreciable drift or change from standard zero on two-day tests. The standardization motor is connected to an adjustable bridge resistor. Since the sample cells are filled with clear water, both phototubes should be subjected to the same amount of light. If there is any variation due to dirt, turbidity, aging, or other factors, there will be a slight unbalance signal, and the amplifier will drive the standardizing motor in the proper direction to balance the bridge circuit. If the bridge cannot be balanced by moving the slide wire to its extreme position, a little cam operates a contact that lights the "clean-the-cells" alarm. The use of such a null-balance type of circuit provides maximum sensitivity in the system.

The design of the metering pump used for measuring the reagents is of interest. This pump consists of two identical segmented spheroidal cavities clamped together, with a slack diaphragm between them. In operation, reagent from the first reagent tank, under pressure from the air-actuated reagent

pump, forces reagent through the first inlet valve behind the slack diaphragm. This forces the diaphragm to the opposite side of the cavity and completely displaces the previously measured reagent from the second reagent tank to the point of application in the comparison cell. The reagent metered to the sample cell is Eriochrome Black T plus EDTA, used to give a 0 hardness reference color. By action of the other inlet and outlet valves on the metering pump, the second reagent is fed behind the diaphragm, forcing the metered quantity of the first reagent to the sample cell. The cavity is designed to meter exactly 2 ml. of reagent with one movement of the slack diaphragm within the pump cavity. This metering is exact and will deliver the desired quantity of reagent to within a fraction of 1 per cent. It has been found that with a single reagent, tangential injection into the sample cell without the use of a mixing motor assures adequate mixing and reaction.

In the programming a sufficient time interval is allowed for proper color development. During this interval, light of different intensities falls on the zero-cell and measuring cell phototubes and, the bridge is unbalanced. As in the zeroing cycle, a second balancing motor, that drives the chart pen and a second balancing potentiometer in the bridge circuit, drives in the proper direction to rebalance the bridge. The recorder then indicates and records the results of the analysis. Limit switches with alarm contacts adjustable to any desired concentration are provided; these can actuate high- or low-concentration alarms such as horns, bells, or lights. Following recording, the instrument is ready to repeat the cycle, beginning with flushing of the sample cells. A cycle for water-hardness testing can be completed as often as every 3 min. or it can be extended, depending on the requirements of the individual plant. A 6-min. cycle is normal; with this timing, 480 ml. of each reagent is required for each 24 hours of continuous operation. The standardized reagents are supplied in 5-gal. polyethylene-lined containers so that each set of containers holds reagent sufficient for over a month's operation at this cycling rate.

Determination of Dissolved Oxygen

The determination of oxygen dissolved in boiler feed water is most important and can be achieved with this type of instrument. FIGURE 6 shows the adaptation of the Quantichem to the indigo carmine method of analysis for 0 to 30 parts per billion (ppb) dissolved oxygen. In this case, the sample flows through a closed cell to avoid any contact with atmospheric oxygen and with sample and comparison cells containing fixed volumes. This is accomplished by building into the top of each cell a closure consisting of a diaphragm held in place by air pressure exerted by a piston. Following the flushing cycle and the entry of the desired amounts of sample into the two cells, the pressure is released by the piston, the diaphragm is flattened, and the cell volume expands to accommodate the introduction of reagent. In this case the reagent is indigo carmine and alkali. The indigo carmine and alkali must react in the accumulator and reaction cells before being introduced to the sample, where a specific, quantitative color reaction to the dissolved oxygen develops.

Analysis for Silica

In silica analysis,[5,6] one of the more complex applications of the instrument, four specific reagents are metered to the zero and sample cells (FIGURE 7). The first reagent is a buffer to maintain the pH as close as possible to 1.7. An-

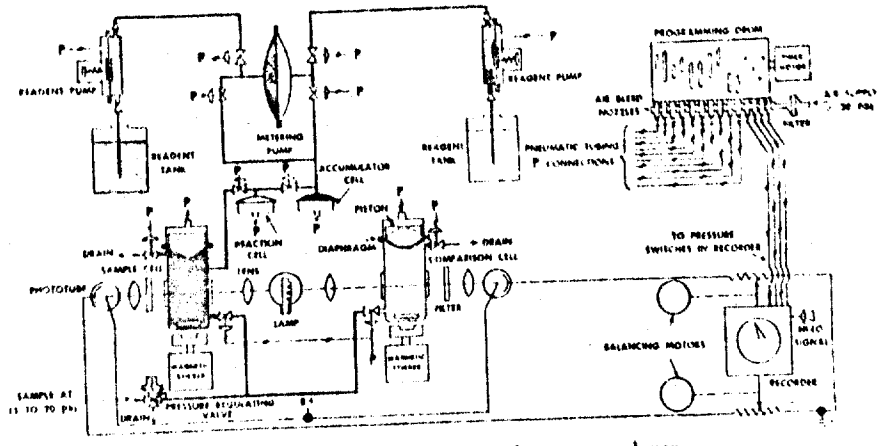

FIGURE 6. Schematic design of oxygen analyzer.

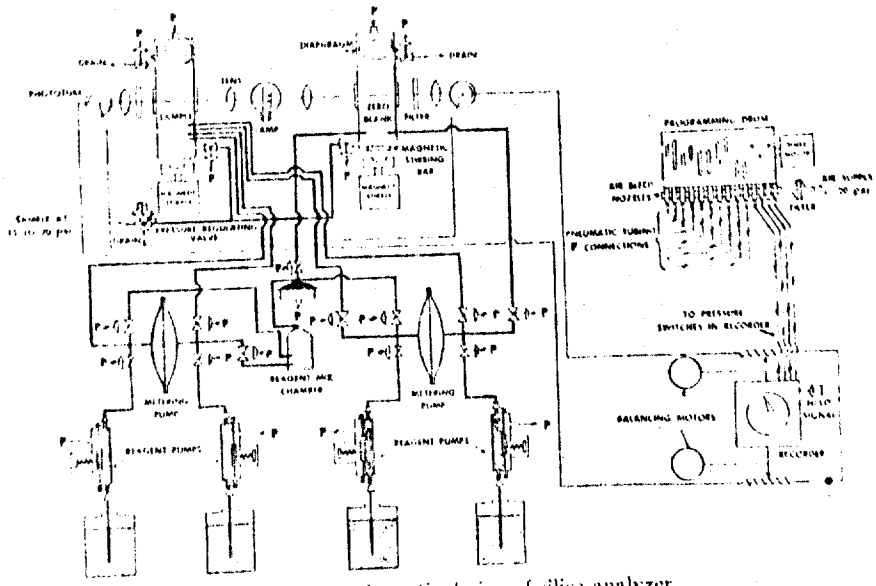

FIGURE 7. Schematic design of silica analyzer.

other reagent is ammonium molybdate. At low pH the molybdate ions polymerize to form the complex ion, $(Mo_6O_{21})_6$, which then forms heteropolymolybdic acids with the silica and phosphorus in solution.

The third reagent, a complexing agent, then is metered to both cells and

attacks the phosphomolybdic acid, destroying it completely. The reagent has no effect on the silicomolybdic acid. Finally, a reducing agent is added, resulting in the formation of colloidal molybdenum blue. This reaction always goes to completion, and therefore the color intensity is a direct measure of the soluble silica present.

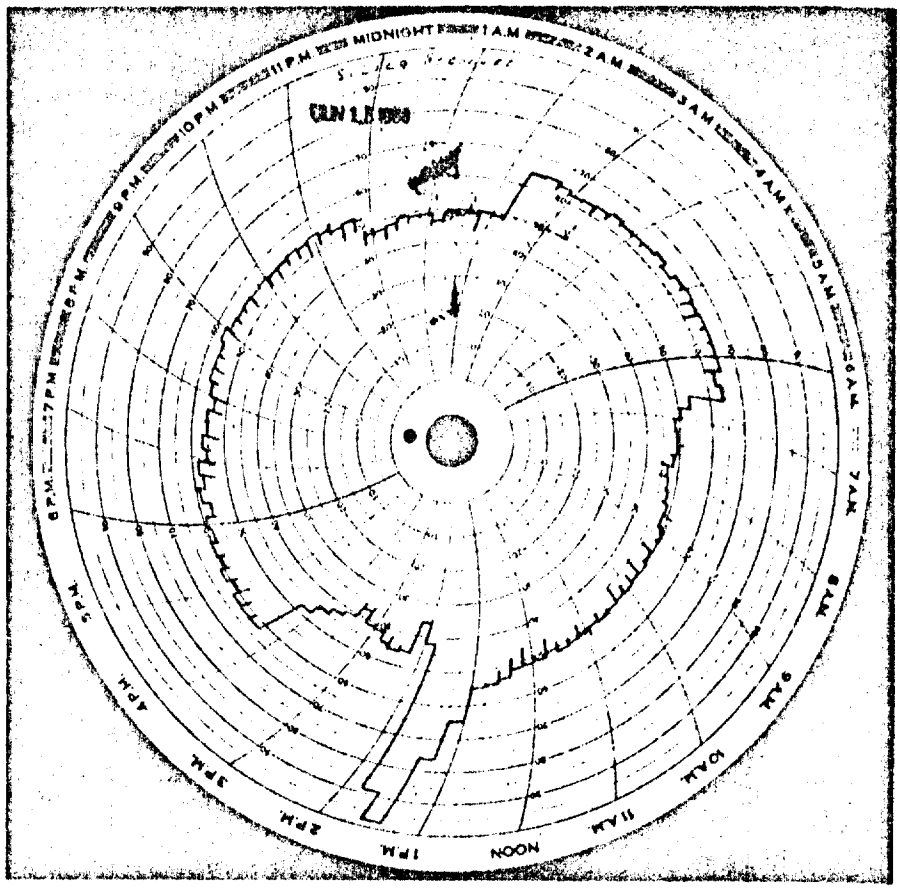

FIGURE 8. SiO_2 chart for Philadelphia Electric Company.

For the blank cell comparison, chemical reagents are fed to a cell to complete their reactions before being fed to the zero cell. Therefore they introduce to that cell only intrinsic color or color due to any trace of silica included. The color difference between the sample and the comparison then will be due only to the silica content of the sample. Each complete cycle normally lasts 12 min. The range of the instrument is normally 0 to 50 ppb, and a change of 1 ppb in the water may be detected accurately.

As the result of refinements in design, the Quantichem is capable of approximately five times the sensitivity of its German prototype. It has been under test and refinement for more than a year and is now in commercial production.

Quantichems have been installed for the determination of hardness and silica in boiler feed waters. FIGURE 8 is a typical SiO_2 chart from an analyzer installed in a large public utility power station. The variation of silica from the ion exchanger with the rate of flow through the exchanger is evident at, for

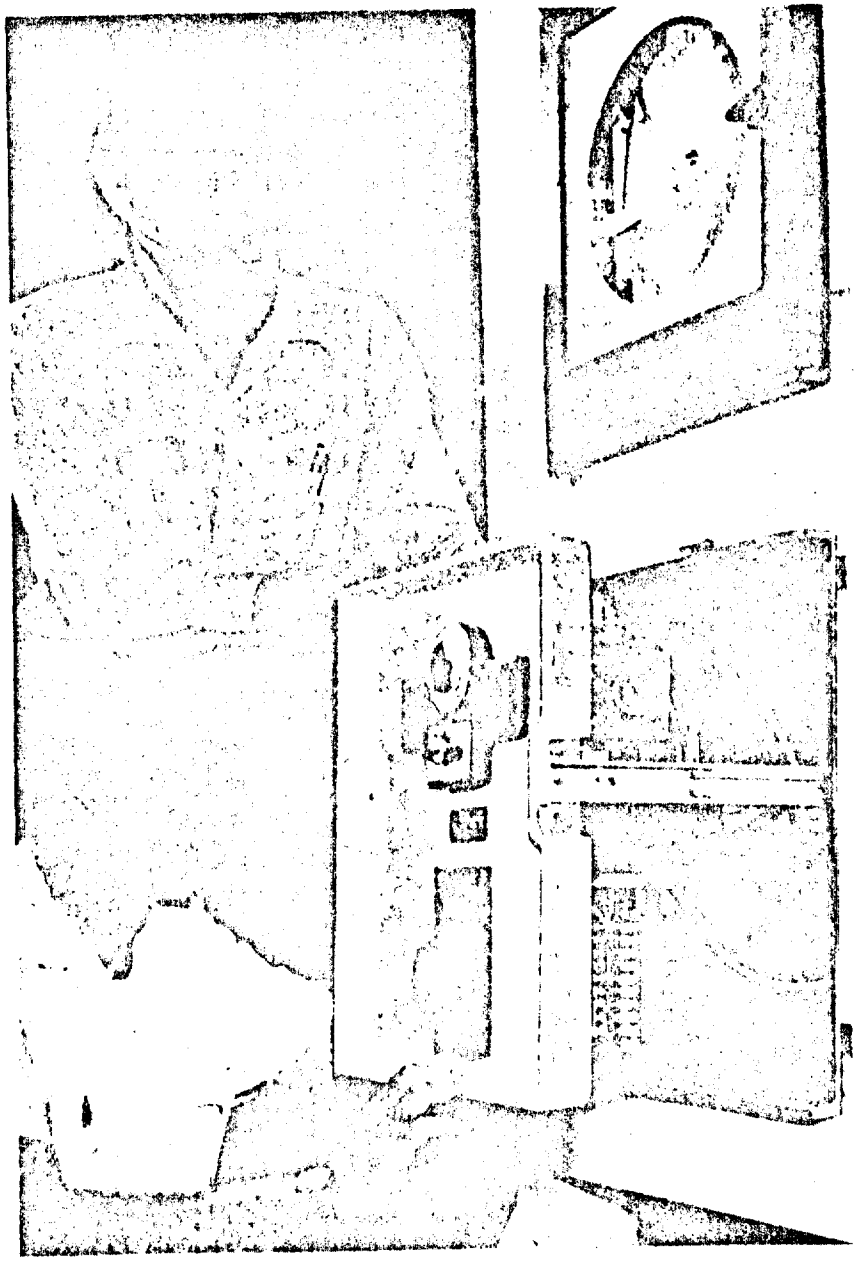

FIGURE 9. Dissolved oxygen analyzer at Florida Power Corporation.

example, 2:20 A.M. and 6:35 A.M. on the chart. The instrument recorded the first breakthrough of silica at 1:00 P.M., signalling the operator to remove that unit from the line for regeneration.

Dual-range silica units can be furnished. For example, an instrument with the ranges of 0 to 50 ppb and 0 to 500 ppb can be designed, simply by modifying the resistances within the instrument. Other silica units can be furnished with a dual range of 0 to 50 and 0 to 2500 ppb. In the latter case, the more silica-laden water (boiler water) is diluted accuratedly by a proportioning system with controlled volume pumps to 50:1 with silica-free water obtained by passing condensate through a mixed-bed cartridge at the instrument.

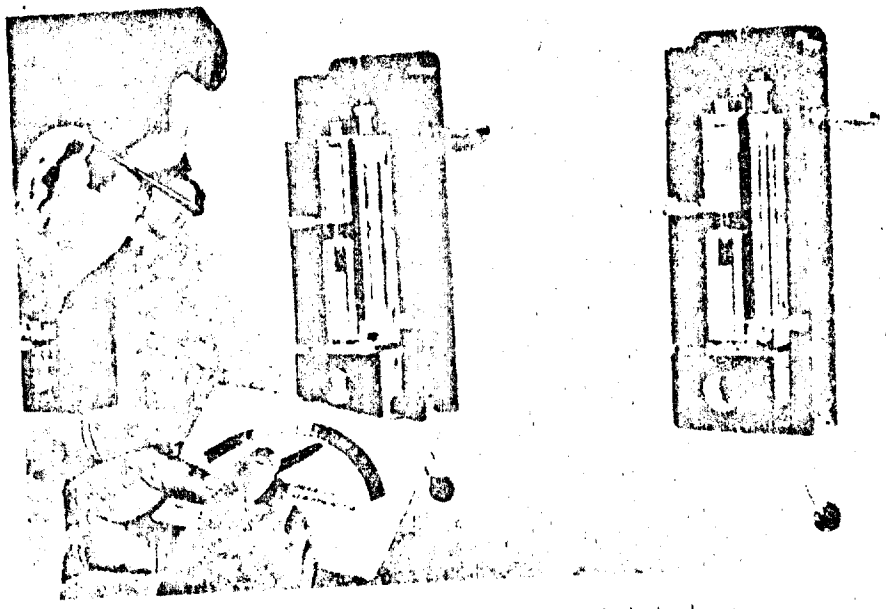

FIGURE 10. Picture of Belgian Chem O Matic Analyzer.

The instrument thus "sees" water in the same SiO_2-range as the water from the large ion exchangers.

FIGURE 9 shows an installation at a large public utility plant in Florida for determination of oxygen dissolved in boiler-feed and condenser water.

Applications

One instrument can be used for a large number of different analyses by appropriate changes in program timers and monochromatic light filters, provided the original instrument is supplied with sufficient number of metering reagent pumps. A maximum of four reagents is required, and all interfering ions in the system being analyzed can be eliminated within a four-reagent system or by an ion exchange.

Procedures are available for the use of the Quantichem in the following colorimetric determinations without pretreatment of sample and with appro-

priate instrument chemistry: Free chlorine, chlorides, chromium, copper, cyanide, fluoride, formaldehyde, hydrazine, iron, dissolved oxygen, orthophosphates, sulfites, dissolved silica, and total hardness (Ca + Mg).

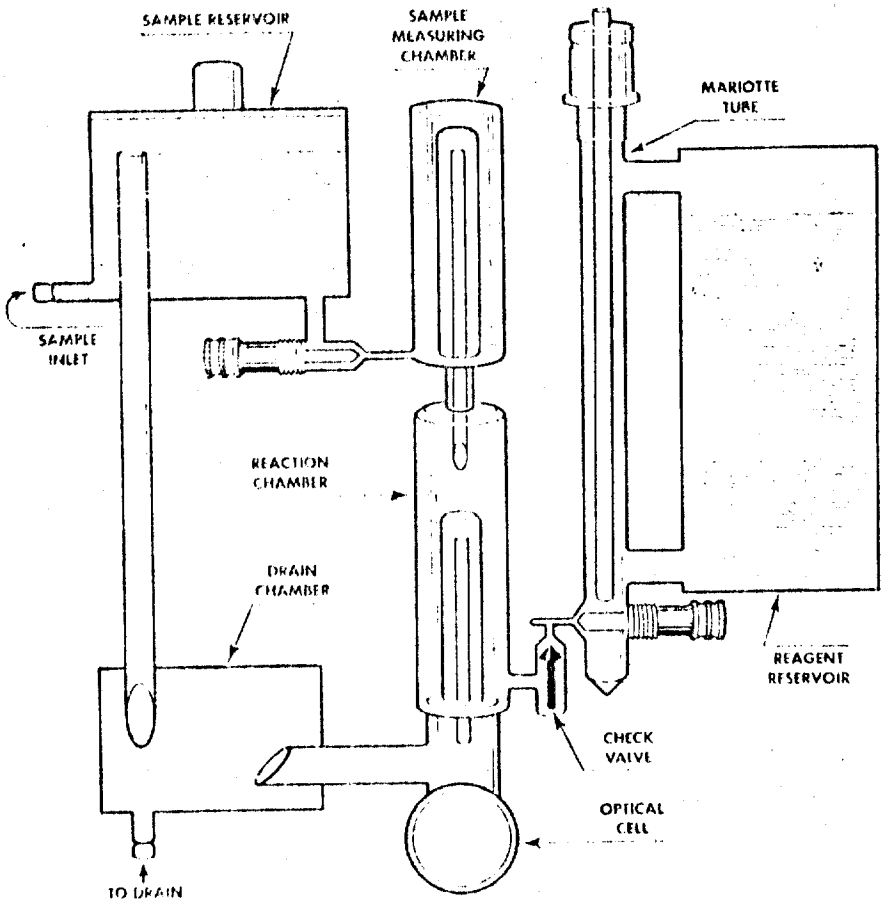

FIGURE 11. Schematic of Belgian Chem-O-Matic Analyzer.

"Go-no-go" Colorimetric Analyzers

The instruments described above are the most refined of the on-stream colorimetric chemical analyzers available at present. However, there exists also a need for simple, inexpensive instruments of a "go-no-go" design. Typical of these is a simple instrument of Belgian design, the Chem-O-Matic* (FIGURE 10), that works entirely on hydraulic principles. Sample and reagent flow is entirely by gravity.

The sample-measuring chamber (FIGURE 11) is filled by regulated flow of sample to the point of discharge. It is then siphoned into a reaction chamber,

* Interindustrie, Brussels, Belgium. Available through Milton Roy Co., St. Petersburg, Fla.

where it is mixed with a previously measured volume of colorimetric indicator. The mixture from this chamber in turn overflows via a siphon into a visual optical cell. Sample is measured as the contents of the measuring chamber, approximately 18 cc. Reagent is allowed to flow from the reagent reservoir to the reaction chamber through a small check valve until it reaches a level corresponding to the bottom of the mariotte tube. The effect of this tube is to bring air pressure to a constant level regardless of variations in the reagent reservoir pressure. The resulting color can be seen and compared with a color chart or with color standards. The instrument can respond also to simple photoelectric signals for a "go-no-go" alarm signal or chemical feed system. Such instruments can be employed for colorimetric *p*H, hardness in water, phosphate, and other simple colorimetric analyses.

Conclusion

The instruments described are new tools for on-stream chemical analysis and control based on variables hitherto controllable only by laboratory procedures. Many more applications of chemical end-point colorimetric analysis are possible than have been described. The development and field-testing of the present instruments now are so advanced that their ultimate success and commercial acceptance can be promised.

References

1. FUHRMANN, H. 1951. Chem. Ing. Tech. **26**: 401-404.
2. FUHRMANN, H. 1957. Chemiker Ztg. **81**: 678-680.
3. FUHRMANN, H. & G. W. SCHNEIDER. 1958. Brennstoff Wärme Kraft. **10**(3): 115-120.
4. SHEEN, R. T. 1959. ISA Journal. **6**(1): 41-8.
5. ROBISON, H. E., E. A. PIRSH & E. GRIMM. 1951. A.S.M.E. Paper 51-A-92.
6. ROBISON, H. E., E. GRIMM & C. BROWN. 1951. A.S.M.E. Paper 51-A-93.

Zh. Prikl. Khim. 42(10): 2363-2365
(J: Applied Chemistry - translation)

INFLUENCE OF STORAGE TIME ON THE ADSORPTION PROPERTIES OF SILICA XEROGEL

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UDC 661.183.2

We have noticed that storage of silica xerogels at room temperature leads to gradual loss of their adsorption capacity. To the best of our knowledge, only fragmentary information on this subject is to be found in the literature [1, 2]. At the same time it probably often happens that investigators find that data on the adsorptive or catalytic activity of silica gels used for a particular purpose over a prolonged period are not reproducible and do not know the cause of this effect. Therefore information on changes in the properties of silica gels during prolonged storage under normal conditions is of great interest.

In this communication we present data on changes in the adsorption properties of a number of silica gel specimens kept for a long time in loosely stoppered jars. Xerogel specimens Nos. 1 and 3 were prepared from hydrogels, and specimens Nos. 2 and 4 from the same hydrogels by displacement of inter-

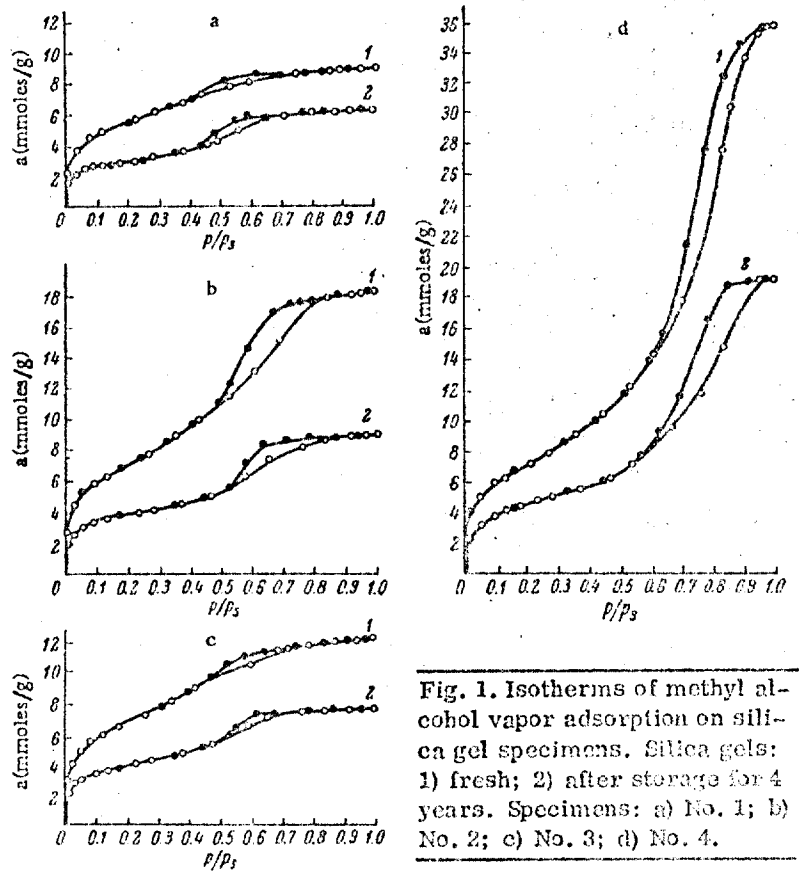

Fig. 1. Isotherms of methyl alcohol vapor adsorption on silica gel specimens. Silica gels: 1) fresh; 2) after storage for 4 years. Specimens: a) No. 1; b) No. 2; c) No. 3; d) No. 4.

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LE 1

[illegible]	Storage time (years)	Structural characteristics		
		V_p (cm³/g)	S (m²/g)	r_0 (Å)
[illegible]	0.1	0.37	6[illegible]0	11.0
	4	0.15	370	13.0
[illegible]	0.1	0.73	810	17.0
	4	0.37	490	16.0
[illegible]	0.1	0.76	850	12.0
	4	0.39	490	12.0
[illegible]	0.1	1.41	870	33.0
	4	0.77	500	28.0

[illegible]ples Nos. 1 and 3 differ in the time of [illegible] of the hydrogel.

micellar water with acetic acid by the method described earlier [3]. Silica hydrogel was prepared from water glass and sulfuric acid at pH ≈ 7, stabilized by mineral acid, and washed free from salts with acidified water at pH ≈ 3. The specimens were dried at 200°C. The adsorption and structural characteristics of the silica gels were determined from isotherms of methyl alcohol vapor adsorption at 20°. The specimens were dried at 200° before the adsorption determinations.

Isotherms of methyl alcohol vapor adsorption on fresh and aged silica gel specimens are given in Fig. 1. It is seen that, regardless of the original structure, prolonged storage of silica gels leads to decreased adsorption over the entire range of relative pressures of the adsorbate. The data in Table 1 show that the pore volume and specific surface decrease during aging of silica gels.

In our opinion, the decrease of adsorption capacity may be correlated with increasing density of the structure as the result of aging. In the case of silica gels obtained from acetic acid gels having an easily [illegible]able skeleton this effect can be attributed to the action of atmospheric moisture. This hypothesis is [illegible] agreement with the previously observed [4] contraction of the skeleton of silica gels of this type [illegible] saturation with water vapor and subsequent removal of water condensed in the capillaries by drying. [illegible]lar effect was observed [2] after water treatment and drying of a xerogel prepared from a propanol [illegible]. It is possible that in the case of xerogels prepared from hydrogels the structural changes are also [illegible] the action of atmospheric moisture. A final solution of the problem of the possible causes of the [illegible] adsorption activity of xerogels during storage is not possible at this stage in the investigations. [illegible]tional experiments are needed for explanation of our data. It is evident that the aging rate and there[illegible] the changes in the adsorption activity of silica gels must depend on the initial structure and prepara[illegible] conditions.

CONCLUSIONS

Prolonged storage of silica xerogels at room temperature increases the density of their structure, [illegible]ading to decrease of adsorption activity.

It is suggested that these changes are due to the action of atmospheric moisture on the xerogels.

LITERATURE CITED

1. S. Brunauer, Adsorption of Gases and Vapors [Russian translation], Vol. 1, Izd. Inostr. Lit., Moscow (1948), p. 563.
2. R. Yu. Sheinfain, O. P. Strast', and I. E. Neimark, Kolloidn. Zh., 27, 916 (1965).
3. I. E. Neimark and R. Yu. Sheinfain, Kolloidn. Zh., 15, 145 (1953).
4. B. M. Mitsyuk and Z. Z. Vysotskii, DAN SSSR, 152, 1116 (1963).

Beitr. Silikose-Forsch. vol. S. Grundfragen Silikoseforsch.
vol. 4 (1960), p. 121/153.

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10. ANIMAL EXPERIMENTS ON CHEMICALLY ALTERED SiO_2 SURFACES

BY F. J. STRECKER

Mr. Stöber has just recently described the chemical and physico-chemical bases of the changes in SiO_2 surfaces investigated here. I should like to report to you now on animal experiments with dusts of the same kind.

We are dealing with three different large groups of experiments:

A. Masking of SiO_2 surfaces by defined monomolecular chemical conversion of the surface SiOH groups;

B. The same monomolecular layers covering biologically inert TiO_2 dusts (as control experiments to test the covering layers for a possible auto-effect; and

C. Testing of SiO_2 surface layers with special properties. Here we are dealing with a monomolecular layer of SiO_2 on Al_2O_3.

A. Masked SiO_2 Surfaces:

In the first experimental group we are dealing with SiO_2 dusts; the surface no longer consists, however, of SiOH groups, but of a monomolecular film of non-SiO_2 molecules, in which each one of these molecules is combined in actual chemical combination with the SiO_2 particles.

I. Quick Test for Toxicity

An initial insight into the behavior of these masking monomolecular layers on SiO_2 particles in animal experiments is provided by evaluating the toxicity of the whole animal as a standard; for very finely divided SiO_2 preparations such as the aerosil are fatal even in relatively small dosage in the intraperitoneal test with mice. Thus a decrease in the death rate and a delay in the occurrence of deaths shows that the SiO_2 surfaces must be effectively masked.

Ill. 1 shows the results with various aerosil coverings (150 m^2g):

1. Untreated aerosil in 20 mg dosage is fatal to all animals in 2 days.

2. Esterification with ethandiol (the surface remaining hydrophilic) masks the aerosil effect for hours at most.

When the free SiOH groups are completely esterified with ethanol, butanol, and octanol -- that is, with hydrophobic outer members -- the ethanol esterification shows a distinct decrease and a delay in general toxicity; with increased chain length there is considerable further decrease.

A protective effect can also be seen with butanol esterification of every second surface SiOH group, and even with every fourth one; in the latter case, however, the effect is only alight.

The delayed and weakened general toxicity noted in these series of tests can easily be explained by a hydrolization of the esters and the new release of the SiOH surfaces, with all their consequences.

Conclusion: The ester compound as such (Si-O-C-) has little stability *in vivo*, but with complete covering -- that is, when combined with the monomolecular surface film -- is relatively stabilized by the hydrophobic character of the outer members of the chain and by an increasing chain length. Inversely, a thinning out of the covering decreases stability. Nevertheless, in no case does esterification produce complete suspension of general toxicity, i.e., in all preparations even greater proportions of the SiO_2 particles are again "unmasked" within the first 14 days.

3. Trimethylsilyl cover (Si-O-Si- compound): With complete as well as with half covering, general toxicity is completely suspended in practically all applicable doses. This compound might therefore be considerably more stable than the ester compound. But it is also certain that stability is basically favored here by the umbrella-like protection of the Si-O-Si- bridge.

4. The testing of general toxicity with aluminum oxydhydrate covering shows a distinct decrease, but also a considerable death rate. At first glance this seems to be in contradiction to what Mr. Stöber has reported on the stability of the Al-oxydhydrate covering *in vitro*. But in this case we are not dealing unconditionally with deaths from aerosil, that is, the effect of again released SiOH surfaces, but more probably with an auto-effect of the Al-oxydhydrate surface; for example, even the γ-Al_2O_3 under comparable conditions shows a comparable general toxicity. Thus here it is impossible to draw any conclusion from the quick test for toxicity regarding the stability or the masking effect of the Al-oxydhydrate layer on SiO_2.

II. Local Tissue Reaction

More exact knowledge regarding the behavior of the surface compounds in vivo can be obtained only from animal experiments of long duration. Here we are dealing with the reaction of the local tissue at the point where the dust is deposited.

I shall now explain briefly the course of the tissue reaction in its basic features, dealing first of all with the esterification of the aerosil and its trimethylsilyl covering. Here I beg your indulgence if I proceed somewhat schematically:

1. The first stage (in the ideal case), with effective masking of the SiOH surface of the aerosil, is a simple storing of the dust particles, while the condition of the cell remains unchanged, i. e., when the covering is intact and complete we are dealing with an innocuous "SiO_2 particle," made innocuous by the covering (Ill. 2).

2. In the further course of the tissue reaction, we encounter another apparently quite innocuous storing, here in the form of so-called "shining particles," which consist of aerosil aggregates and substances specific for the particles, and therefore represent "enveloped" particles (Ill. 3). This result is well known even with untreated aerosil.

3. Between these two "harmless" forms of storing there is, however, a phase in which the aerosil particles produce cellular lesion up to necrosis. At this stage we are clearly dealing with the phase in which the surface covering is being dissolved. Thus in the esterifications and the trimethylsilyl covering we can use the occurrence of cell lesion as a chronological indicator for the stage of dissolution.

During this dissolution the surface SiOH groups are again released. At the same time, however, the molecules of the masking layer are also released. We therefore had to test whether this release of the masking molecules in itself results in cellular lesions.

When layers of inert TiO_2 dusts of the same molecules were used as covering, however, no corresponding tissue effect could be shown (see under B). Thus cellular lesion formation is attributed to the release of the SiOH surface.

4. Tissue reaction during the dissolution of the covering molecules is determined mainly by the speed of dissolution. If this is high, i. e., if the release of large SiOH surfaces locally takes place quickly, then we find extensive, massive necroses (especially with ethanol esterification); at the other extreme, only individual cells are involved.

5. Fiber formation occurs which is obviously intimately associated with dust cell necroses: Here the "rhythm" of the dust cell necroses seems to be of significance for fiber formation. With the aerosil preparations this whole process goes on as long as free SiOH surfaces are provided, i. e., as long as the supply of masked particles lasts. The unmasked aerosil particles, however, are clearly rendered harmless quickly by transformation into so-called "shining particles." For the duration of the dissolution process, fiber formation is "progressive"; when the supply of masked aerosil particles is exhausted, the SiO_2 disappears rather quickly from the granulomas, and the origins shrink; finally all that remains is rather small "scars."

Up to now we have consciously omitted from this discussion of tissue behavior the aerosil covering of Al-oxydhydrate. Basically, however, the course here is the same, beginning with the initial storing with no cellular change, and ranging up to lesion formation and necrosis, and to fiber formation. But the Al-oxydhydrate must be discussed, in order that here too the cellular lesion formation with its consequences can be attributed to the release of the SiOH surface, that is the dissolution of the covering layer; in this connection we have the results of King and colleagues[1,2,3], that an Al-oxydhydrate in the form of boehmite produces a severe fibrosis.

Opposed to fiber formation as a result of the surface covering with our Al-oxydhydrate coverings are the following results:

1. An Al-oxydhydrate covering of inert TiO_2 causes no cellular lesion and no fibrosis (see under B).

2. During the course of the experiment the amount of dust in the abdominal cavity decreases considerably; at the same time the signs of a silicic acid secretion can clearly be seen in the kidney tubules.

3. Chemical analysis of the dust granulomas in the abdominal cavity shows a distinct shift in the proportion of Al to SiO_2, to the disadvantage of the Al. The first columns of Ill. 4 show two preparations TiO_2/with Al-oxydhydrate covering and serious dissolution after 9 months <u>in corpore</u>. Thus it is evident that the organism brings Al into solution. The remaining groups of columns in turn show decrease in Al content (after 6 and 9 months) in the tested SiO_2 dusts, in relation to the SiO_2 content; here the original Al content is in each case set at 100%. In good agreement with the

results for fine tissue, the Al content decreases with respect to the SiO_2 in the granulomas. Likewise in good agreement with the morphological results in the forms of individual origins, this takes place at varying speeds.

To summarize these results, cellular lesion and fibrosis, even with Al-oxydhydrate covering, can be related to the protective layer and the release of the SiO_2 surface, as with the other maskings.

After this general description of tissue reaction, several concrete examples will be used to illustrate the test results; here the time factor and the extent of the dust cell necroses and fiber formation are characteristic of the various surface coverings. Here only the predominant changes in each case can be referred to:

α. Chemically Altered Aerosil Preparations (150 m^2g):

1. Esterifications:

a) Ethanol esterification (6 μ mol/m^2): The masking of the surfaces is so lacking in stability that here massive cellular necrosis is still the predominant factor affecting the tissue.

b) Butanol and octanol esterification (5.2 and 6 μ mol/m^2): Dust cell necroses begin even in the first month of the experiment, but after one month simple storing conditions generally prevail. After 2 months necroses of the dust cells increase noticeably with butanol esterification, but hardly at all with octanol esterification. After 4 months, however, the dust cell origins are almost all undergoing a change involving a high rate of necrosis and vigorous fiber formation with both aerosil preparations. Only with octanol esterification are there still some mostly pure

storing origins (without "shining particles"); with butanol esterification, however, a decrease in the size of some granulomas, a "shrinking," can be seen (with decrease in the amount of dust). The intermediate stage of fiber formation in the dust granulomas at this time stage is shown in Ill. 5. After 8 months of tests, only residual scars (without dust) remain with both aerosil preparations.

c) Butanol esterification (3.6 μ mol/m^2 and 1.6 μ mol/m^2):

As might be expected, when butanol is used to cover only ½ or ¼ of the surface, the delay in cellular lesion formation is less than with complete esterification. With ½ covering the described tissue changes still last into the fourth month, with the high point after 2 months, and fiber formation is still quite considerable. With ¼ covering, individual minute origins can be found only with difficulty after 4 months; even with this preparation there is distinct fiber formation in the granulomas, which on the whole are less widespread.

2. Trimethylsilyl covering:

a) 2.9 μ mol/m^2: Even after one month the whole process of necrosis and fiber formation is in full swing. After 3-4 months a considerable amount of fiber formation is present. After 4 months, however, the granulomas begin to grow definitely smaller. After 6 months very little dust can be seen, and for the most part only dust-free residual scars of the former granulomas remain[4,5].

b) 5.2 μ mol/m^2: When the trimethylsilyl covering is practically complete, on the other hand, there is a simple storing with no

cellular change and in almost all cases only a few individual cell necroses occur, until about the sixth test month (Ill. 2 and 6). Not until after this point in time do cell necroses occur more frequently, as well as quite considerable fiber formation up to highly hyaline fiber nodules (Ill. 7 and 8).

3. Al-oxydhydrate covering (5.7 μmol/m^2):

After a predominantly unchanged storing stage of about 2 months, the dust cells show an increasing tendency toward necrosis with fiber formation (Ill. 9); here, however, dust cell areas also occur up to the ninth month (the present length of the test); in these areas the simple type of storing predominates. At the same time, dust displacements can be observed, especially in the peritoneal sac; from rather large "fiber fields" small strips and origins as it were migrate radially into the sac. After 9 months the amount of dust and the extent of the dust origins have clearly decreased. Ill. 10 shows a characteristic picture of fiber formation after 9 months, with a group of nodules ranging from those with abundant fibers to hyaline nodules.

β. Chemically Altered Surfaces of Silica Fume, Quartz Glass, and Quartz:

We have also investigated the trimethylsilyl and Al-oxydhydrate coverings of so-called silica fume and several quartz glass and quartz preparations:

1. Silica fume (surface 43 m^2/g):

With trimethylsilyl covering (5.4 μmol/m^2) and the use of aerosil preparations, the dust cell origins begin to change from a predominantly unchanged storing condition in about the sixth month;

with Al-oxydhydrate, the change occurs in about the third month. In the following test months fiber formation then reaches a stage which is not attained by untreated dust (Ill. 11).

2. Quartz glass:

We have tested the trimethylsilyl covering with a quartz glass preparation with a 6.8 m^2g surface and a cover thickness of 3.2 $\mu mol/m^2$. This cover can actually protect the dust cells from any significant lesion formation for 5-6 months. In the seventh month dust cell necroses and fiber formation increase steadily; after 10 months there is almost nothing to be seen but fiber origins and nodules with extensive hyalinization (Ill. 12).

When the same dust preparation was tested again after standing in the laboratory for several months, the change began after 4 test months, obviously because atmospheric humidity had caused partial hydrolysis of the covering (Ill. 13).

The Al-oxydhydrate covering was tested with quartz glass dust with a surface of 11.4 m^2/g and complete covering (6.9 $\mu mol/m^2$). Even after 3-4 months abundant cellular necroses and fiber formation can be seen in part of the dust cell origins (Ill. 14), but widespread areas show only the first signs of the transformation stage. Having developed gradually, after 9 months pure fiber cells practically free of cells occur with widespread hyalinization (Ill. 15). Dust cells of the simple storing type are found only in very small origins in the peritoneal sac.

3. Quartz (rock crystal):

With a complete covering of trimethylsilyl (5.7 and 5.8 $\mu mol/m^2$), quartz powder with a large surface (11.2 m^2g) after 8-10 months

showed signs of an SiO_2 effect (beginnings of dust cell necroses, augmented mitoses, increasing fiber formation; individual origins already showing considerable change); another type of quartz powder with coarser particles (4.9 m^2g), however, even after 14 months still exhibited practically the same simple dust storing conditions (Ill. 16).

With Al-oxydhydrate covering (7.4 μmol/m^2 with 9.2 m^2/g surface) cellular lesions were clearly observed after 4 months. After 6 months collagen and nodule formation occurs increasingly; but there are still many areas where almost no fiber formation is found. After 9 months the tissue reaction has already progressed to widespread occurrence of fibrous-hyaline nodules; only small cell areas and origins still exhibit generally unchanged cell storing conditions.

Thus, even for these SiO_2 dusts, the tissue reaction shows the same relationship previously described in detail for the aerosil preparations: Release of the SiOH surface, then dust cell lesion formation up to necrosis, and finally fiber formation.

The central importance of the dust cell necrosis during the transition to fibrous-hyaline nodules can be especially well seen by blocking the mitoses with colchicine so that an insight can be obtained into the new cell formation per time unit in the dust cell granuloma. Ill. 17 shows a section of a trimethylsilyl-quartz glass origin in the transformation phase with the mitoses of the last 4 hours before killing. Without the colchicine blocking, it could hardly have been foreseen that here the dust cells are converted and worn out on such a large scale and with such great rapidity.

III. Summary of Results with Fine Tissue

The results of all groups of tests show that the monomolecular layers covering the surface of SiO_2 particles investigated by us to date lead to a more or less stable but always transitory masking of the SiO_2 effect on tissue.

The speed with which the masking surface compounds dissolve in the tissue, as shown by local tissue changes in the dust granulomas, corresponds very well to behavior in vitro and to theoretical expectations (dependence on the stability of the hydrolysis of the compound with the SiOH surface groups, on the thickness of the covering, etc.); with the exception of the Al-oxydhydrate covering, it also corresponds to results of the quick toxicity test on mice. As a result of local tissue reaction, the Al-oxydhydrate covering can likewise be assigned a place according to the degree of stability in vivo; it belongs approximately between the univalent alcohols (C_4 and C_3)with longer chains and the trimethylsilyl covering.

The effect of the SiO_2 surfaces always reappears with the dissolution of the masking molecules; with quartz glass and quartz dust preparations it is essentially only delayed, but with the finely divided so-called silica fume it is not only delayed but at the same time strengthened (as compared with the untreated dust), and with the still more finely divided aerosil it is for the first time clearly evident. Thus the results of our tests show that the unique position of the untreated, very fine SiO_2 preparations with respect to the usual quartz and quartz glass dusts is based on properties connected with the extremely small size of the granules;

on the one hand, these properties produce a masking effect on the SiO_2 properties (intracellular aggregate formation and enveloping, equivalent to masking by substances specific for the particles); on the other hand, they make comparable testing for a long-lasting effect on tissue difficult or impossible (high toxicity for the whole animal; rapid dissolution of particles). These "disturbing" factors are at least partially outwitted by temporary masking, and now the SiO_2 effect also comes to the fore with very finely divided forms of SiO_2, which have those basic reactions that we recognize as typical signs of the "silicotic tissue reaction" with coarser SiO_2 particles.

B. Control Experiments with TiO_2 Dusts

In describing our tests with temporarily masked SiO_2 dusts (see under A), we have already pointed out that the tissue molecules of the masking layer, dissolved more or less slowly from the SiO_2 particles, have to be tested for a possible auto-effect. Thus, these tests are intended to make sure that the release of the SiOH surface is the factor responsible for the demonstrated cellular lesion up to necrosis and for fiber formation. Similar control experiments must guarantee, as with SiO_2 dusts, a release of the molecules in each case from a surface layer of particles in dust cells. These particles themselves, however, must not cause cellular lesion and fiber formation.

To meet these requirements, we have tested, as a control of coverings on SiO_2 dusts, the same coverings on TiO_2 dust, known to be innocuous; for this purpose we used esterification with ethanol, butanol, and octanol, and coverings of trimethylsilyl and

a layer of Al-oxydhydrate. All these coverings were tested with a very finely divided TiO_2 dust (P 25; Anatas; surface 49 m^2g) from Degussa; in addition, some were also tested with a coarser TiO_2 preparation from Titan (Kronos A; Anatas; surface 9 m^2g).

Both types of dust (P 25 and Kronos A) when untreated lead only to simple intracellular storing of dust without producing cellular necroses or fiber formation (Mohr[6]; these results also confirmed by our own "blank control" tests). But even the animal tests with covered TiO_2 dusts (experiments with trimethylsilyl covering not completed at this time) yielded no support for the formation of cellular lesions and fibers as an auto-effect of the covering molecules being dissolved in the tissue. The TiO_2 particles are also stored with the various surface layers in simple cellular form in the abdominal cavity and in the lymph nodes (Ill. 18, 19, 20). Here it can be accepted as certain that the molecules of the surface film are dissolved from the TiO_2 particles during the test period, because of their behavior *in vitro* and results *in vivo* with the SiO_2 dusts. The results of chemical analysis shown in Ill. 4 also provide direct evidence of the dissolution of the Al-oxydhydrate layer from both types of TiO_2 dust tested.

C. Monomolecular SiO_2 Layer on Al_2O_3

In this group of experiments we are dealing with an artificial monomolecular SiO_2 layer on γ-Al_2O_3 particles (16.6 μ mol SiO_2 m^2 with surface of 90 m^2g). This SiO_2 layer was distinguished *in vitro* by its "insolubility."

The following results were obtained:

1. The toxicity of γ-Al_2O_3 for the whole animal is heightened by the SiO_2 layer, but does not nearly reach the degree of a comparable SiO_2 dust (Ill. 21).

2. In the chronic tissue reaction no SiO_2 effect can be seen. The γ-Al_2O_3 with an SiO_2 layer behaves exactly like the comparable preparation without this layer. Both kinds of dust are stored in simple cellular form in the abdominal cavity, as well as in the lymph nodes (Ill. 22,23). Only in dust depots in the abdominal cavity, which were not organized with phagocytosis of the dust, is there a collagenic fiber formation between the coarse, free aggregates of dust (Ill. 24, 25). Such origins occur in both test groups; they correspond more to ordinary granulomas consisting of foreign bodies and may appear in all injection tests. No conclusions whatever as to a possible fiber forming effect for a specific type of dust, such as we have found with the SiO_2 dusts, can be drawn from them; nor can any comparable fiber formation effect of γ-Al_2O_3, present in both dusts, be diagnosed. It is true, however, that the relatively strong tendency of the γ-Al_2O_3 toward formation of aggregates results more frequently in such "unspecific" origins than with many other types of dust.

It also seems possible with this γ-Al_2O_3 dust in dust depots organized primarily on a cellular basis (especially in larger origins) to arrive later at coarse aggregate formations with results corresponding to the character of the origin; when the cells die out here, the cell content remains as a closed bundle of dust and albumen and can not be restored to phagocytosis again. Through this tendency of γ-Al_2O_3 to form aggregates, the long-lasting process characteristic

of SiO_2 tissue reaction -- ranging from lesion of the dust cells up to necrosis, then the taking up again of the released dust into new cells, and so forth -- could under certain conditions be interrupted and masked in the first cellular generation. That, however, would presuppose an effect of γ-Al_2O_3 comparable to SiO_2 cellular lesion; but thus far such an effect can not be proved from the tissue pictures. The essential result from the comparison, of primary interest in our tests, of γ-Al_2O_3 with and without an SiO_2 layer, remains the similarity of the tissue reaction on both types of dust and the absence of an "SiO_2 effect" in those origins that permit evauation.

3. Chemical analysis of the tissue shows that, contrary to expectation, in comparison with the behavior in vitro a considerable proportion of the SiO_2 layer passes in the course of time into solution, to such a degree, in fact, that in the dust granulomas of a rat 3.8 mg of SiO_2 are dissolved in 6 months (Ill. 26).

Thus from these experiments it is evident that neither this "SiO_2 surface" nor the silicic acid going from it into solution fulfills the requirements for the beginning of the "SiO_2 tissue [illegible] reaction," which previously could be proved for all compact SiO_2 particles investigated by us.

Conclusions from Animal Experiments on Chemically Altered SiO_2 Surfaces

1. None of the surface compounds of SiO_2 dusts is capable of masking over a long period the SiO_2 effect, not even an Al-oxydhydrate layer which has been discussed within the limits of the

so-called Al-therapy of silicosis. For the remainder, indisputable relationships exist between the stability of the covering *in vivo* and the chemical and physico-chemical properties. To date the most stable masking of the SiO_2 effect has been obtained by a complete covering of trimethylsilyl.

2. It is evident from all tests with SiO_2 particles that the lesion of dust cells up to necrosis occupies a central position in the SiO_2 tissue reaction and leads in turn to formation of fibers and hyalinization.

3. This effect is clearly not confined to the crystalline structure of the particles, but is likewise produced by the amorphous SiO_2 particles.

4. A monomolecular SiO_2 layer (Al_2O_3), however, does not produce this effect, although here too there is an "SiO_2 surface" and although a not inconsiderable amount of silicic acid from the dust particles goes into solution.

This means, however, that so far as we can draw conclusions from our tests, the reaction mechanism we have described is connected with the free SiOH surface of solid particles constructed from SiO_2 tetrahedrons.

(Note: For bibliographic items (1-6) referred to in text, see original, p. 144. - Transl.)

ILLUSTRATIONS (1-26)

Column 1 - Aerosil surface

Column 2 - -

Column 3 - Dose in mg

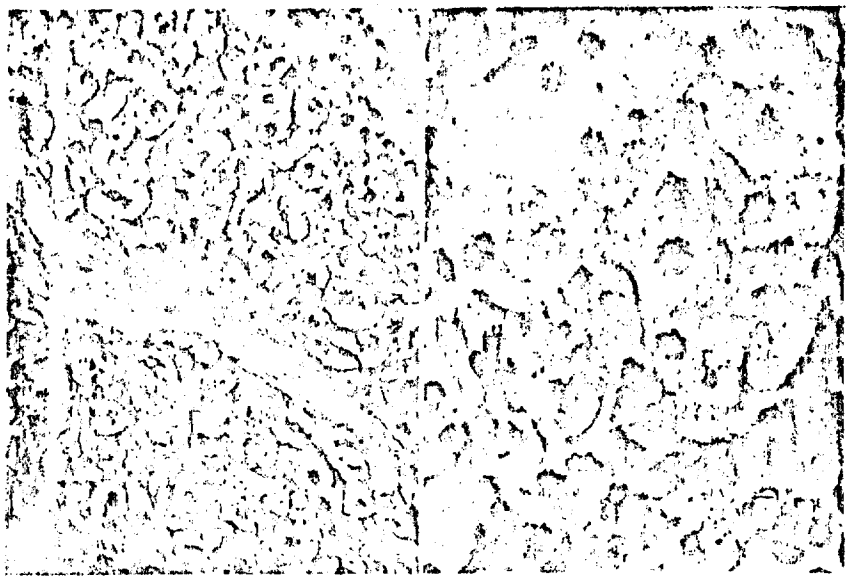
Column 4 - Death rate

Std. = Hours Tg. = Days

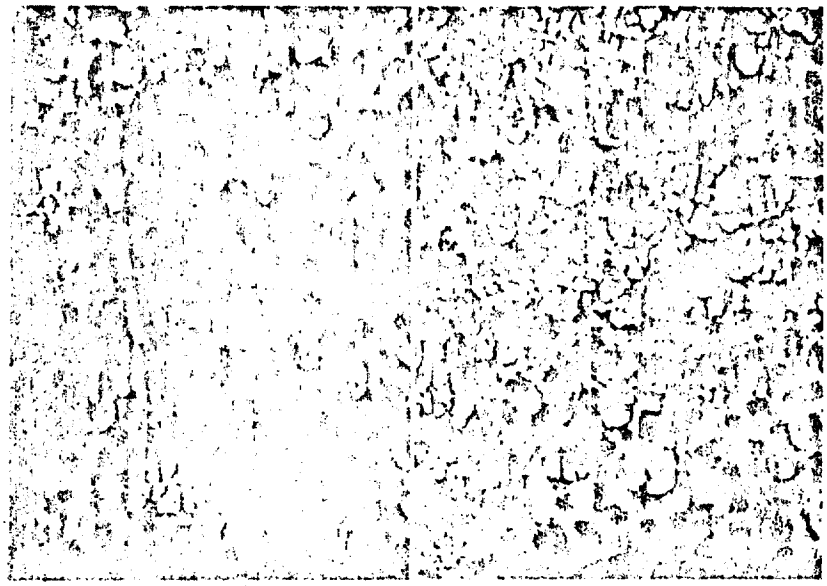
1	2	3	4
Aerosil-Oberfläche		Dosis: mg	Todesrate:
≡Si-OH		20	100% in 48 Std.
≡Si-O-C(H)(H)-C(H)(H)-OH		20	100% nach 48 Std
≡Si-O-C(H)(H)-C(H)(H)-H		30 60	90% in 10 Tg. 100% in 3 Tg.
≡Si-O-C(H)(H)-C(H)(H)-C(H)(H)-H		30 60	~50% in 14 Tg.
≡Si-O-C(H)(H)-C(H)(H)-C(H)(H)-C(H)(H)-C(H)(H)-H		30 60	~30% in 14 Tg.
≡Si-O-C(H)(H)-C(H)(H)-C(H)(H)-C(H)(H)-H		20 40	—o— 10% in 14 Tg.
		20 40	30% in 3 Tg. 50% in 3 Tg. 80% in 14 Tg.
≡Si-O-Si(CH3)(CH3)-CH3		60	—o—
		60	—o—
≡Si-O-Al(OH)(OH)		30 60	20% in 60 Std. 40% in 60 Std

Ill. 1: Toxicity of aerosil with various surface coverings.

(Fatalities in mice with intraperitoneal application.)

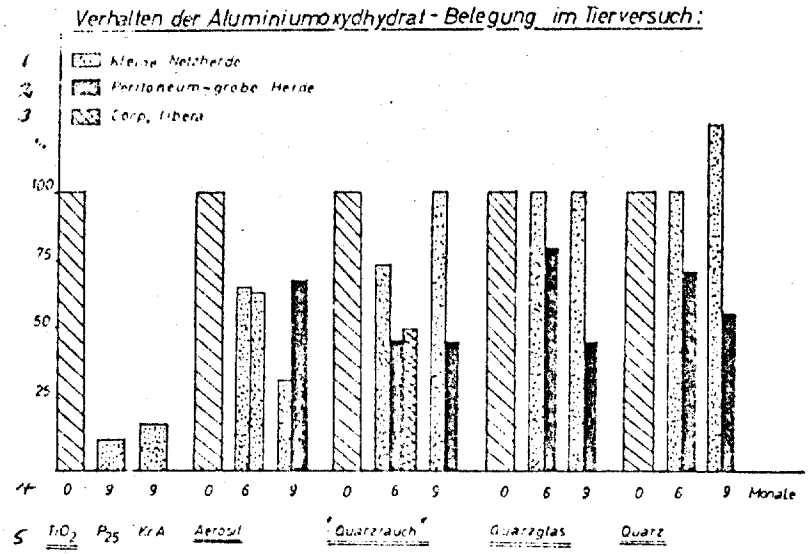


Ill. 2: Mainly simple storing of trimethylsilyl aerosil (5.2 μmol/m^2) in form of very small and uniformly fine particles; unchanged cell condition (2 months after injection of mouse: v. Gieson:positive phase contrast; left 320 X, right 1000 X; in reproduction of the large magnification, fine individual particles lying close together unfortunately appear in part as coarse aggregates).



Ill. 3: "Shining particles," with high degree of refraction, irregular in size, much coarser than the storing particles of Ill. 2; trimethylsilyl aerosil (2.9 μ mol/m^2), 2 months after injection of mouse (v. Gieson: pos. phase contrast; 320 X).

Behavior of Aluminum Oxydhydrate Covering in Animal Experiments



Line 1 - Small origins in periton. sac

Line 2 - Peritoneum, coarse origins

Line 3 - Free bodies

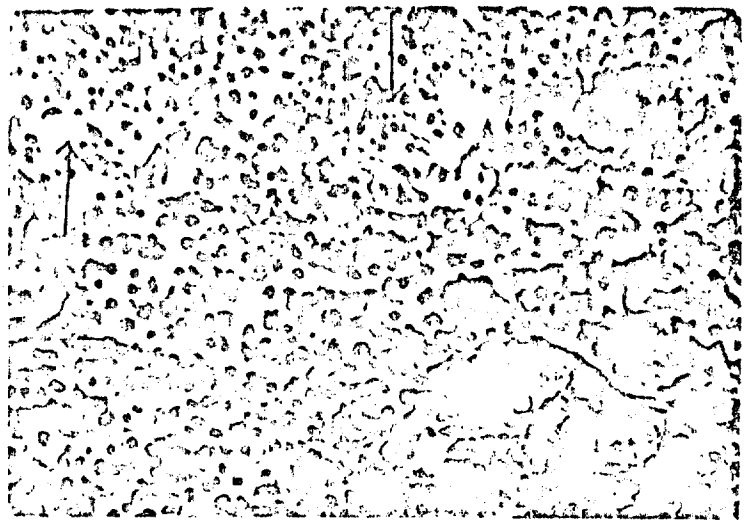
Line 4 - Monate = Months

Line 5 - Quarzrauch = Silica fume Quarzglas= Quartz glass

Quarz = Quartz

Ill. 4: Behavior of Al-oxydhydrate covering in animal experiments (Al content in relation to SiO_2 content of dust granulomas in % of Al content in dust powders before animal experiments).

Ill. 5: Intermediate stage of fiber formation with butanol aerosil ($5.2\ \mu mol/m^2$); 4 months after injection (Ag; 130 X).



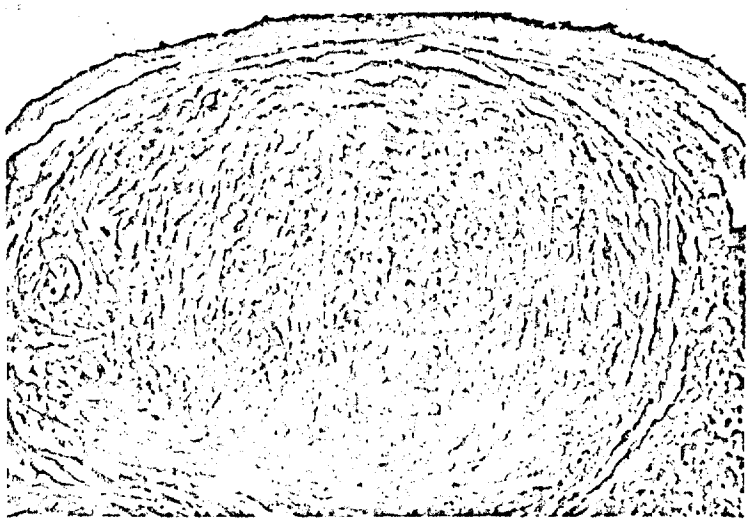
Ill. 6: Trimethylsilyl aerosil ($5.2\ \mu mol/m^2$). 6 months after injection of mouse; unchanged storing condition in lymph node sinus: (top left and top center, arrows show two small origins with beginning cellular lesion and very tender fibers) (v. Gieson; 160 X).

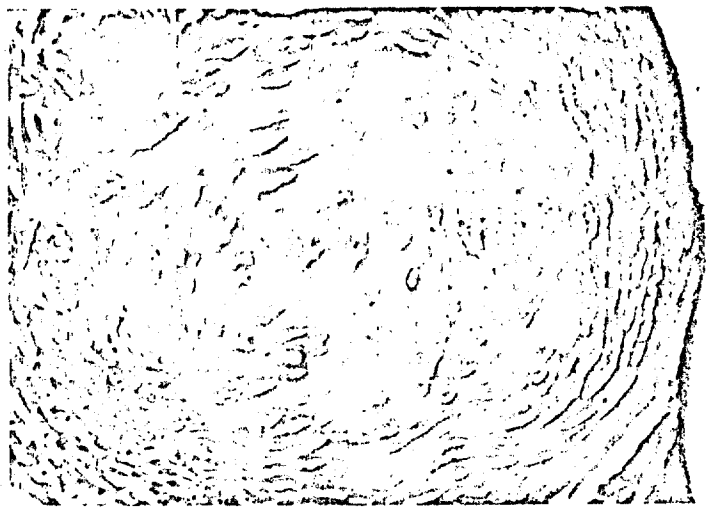
Ill. 9: Al-oxydhydrate aerosil covering (5.7 μmol/m^2), 2 months after injection of rat. Beginning of fiber formation and cellular necroses in small origin in perit. sac (v. Gieson, 240 X).

Ill. 10: Al-oxydhydrate aerosil covering (5.7 μmol/m^2), 9 months after injection of rat. Group of small fibrous nodules in perit. sac, in part with hyaline formation (v. Gieson, 140 X).

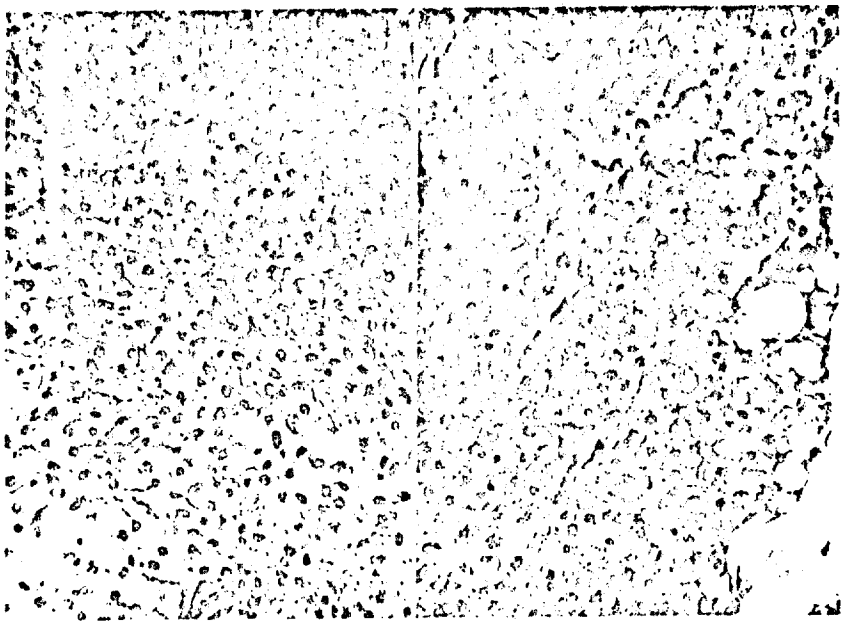
Ill. 11: Al-oxydhydrate with silica fume (5.8 μ mol/m^2), 9 months after injection of rat. Section of a hyaline fibrous nodule (v. Gieson, 130 X).

Ill. 12 and 13: Trimethylsilyl with quartz glass (3.2 μ mol/m^2); mouse: above, fibrous nodule with few cells 10 months after injection; below, hyaline fibrous origin 4 months after injection of dust "aged" in laboratory (above, v. Gieson, 130 X; below, H.-E., 150 X).

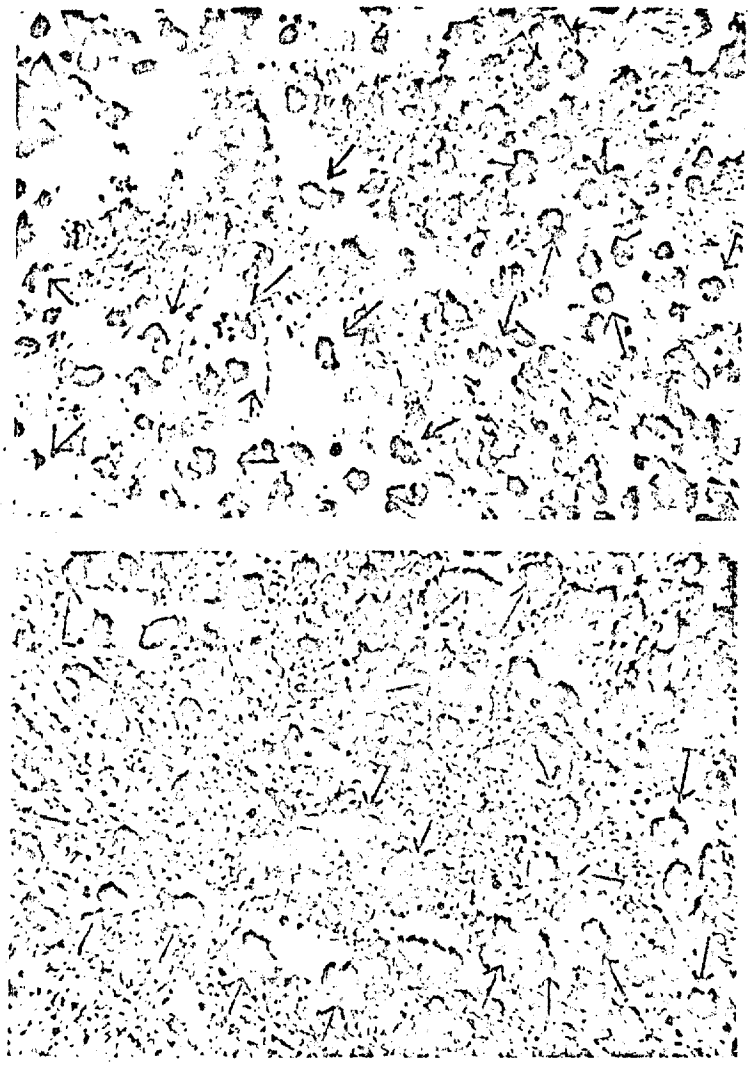




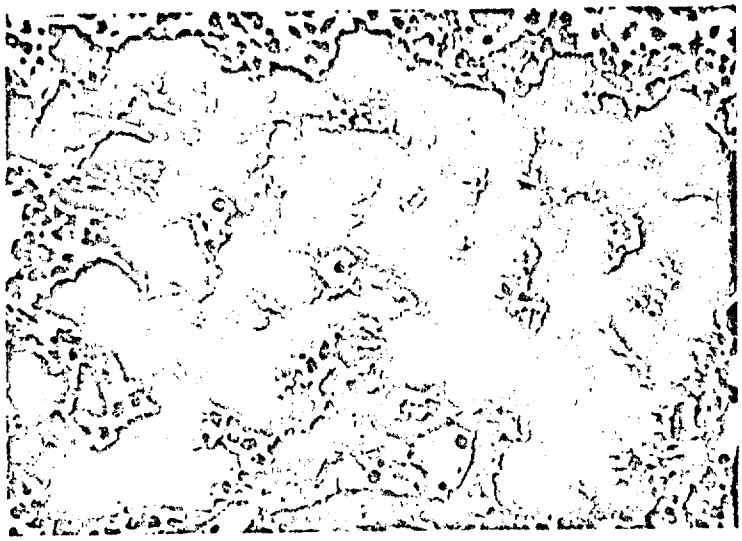
Ill. 14 and 15: Al-oxydhydrate with quartz/glass (6.9 μmol/m^2); rat: above, beginning fiber formation and dust cell necroses, 3 months after injection (v. Gieson, 100 X); below, hyaline fibrous nodule not yet free of cells, 9 months after injection (H.-E., 130 X).

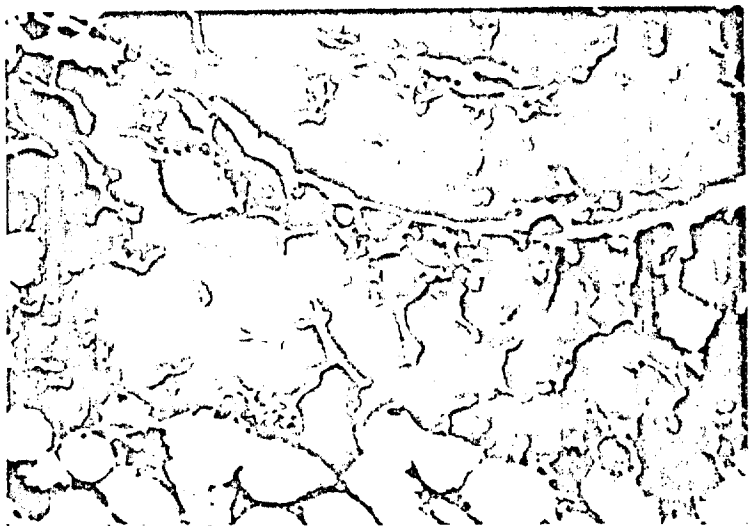


Ill. 16: Trimethylsilyl with quartz (5.3 μ mol/m^2; 4.9 m^2g). Mouse: dust storing condition mainly unchanged 12 months after injection (v. Gieson, 200 X).

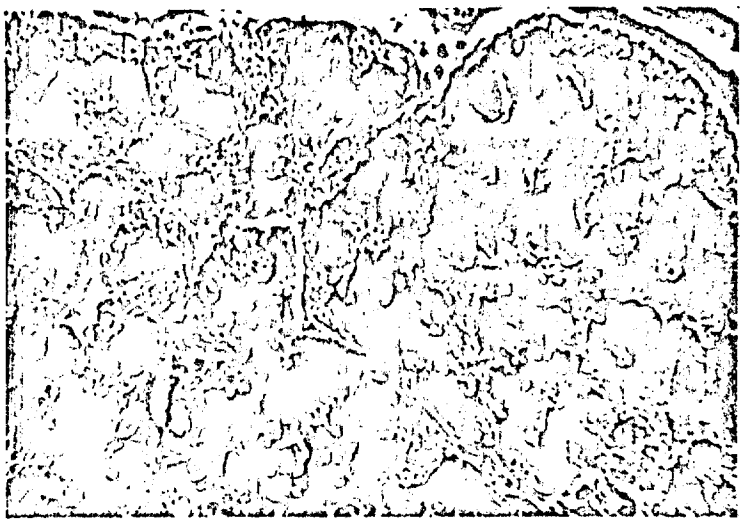


Ill. 17: Granulomas of trimethylsilyl and quartz glass with numerous mitoses (shown by arrows), 4 hours before killing with colchicine injection (v. Gieson: above 400 X; below, 600 X).





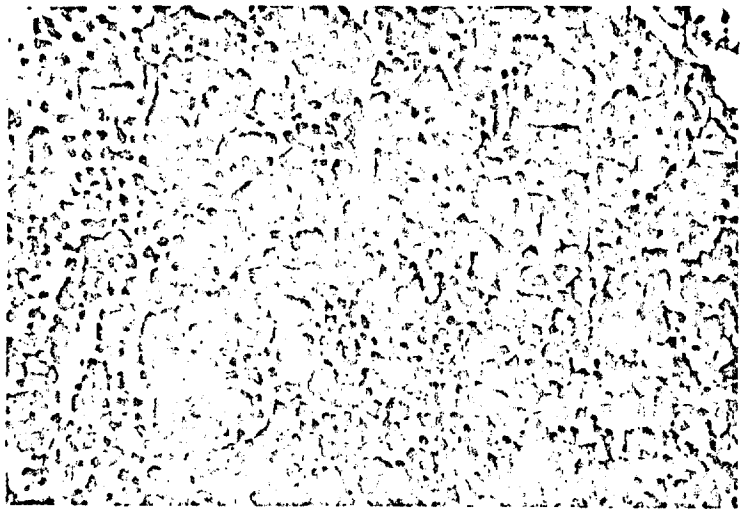
Ill. 18 and 19: TiO_2 - P 25 with Al-oxydhydrate covering. Dust storing without fiber formation in perit. sac (above, mouse, 5 months after injection, v. Gieson, 200 X); below, rat, 10 months after injection, v. Gieson, 130 X).



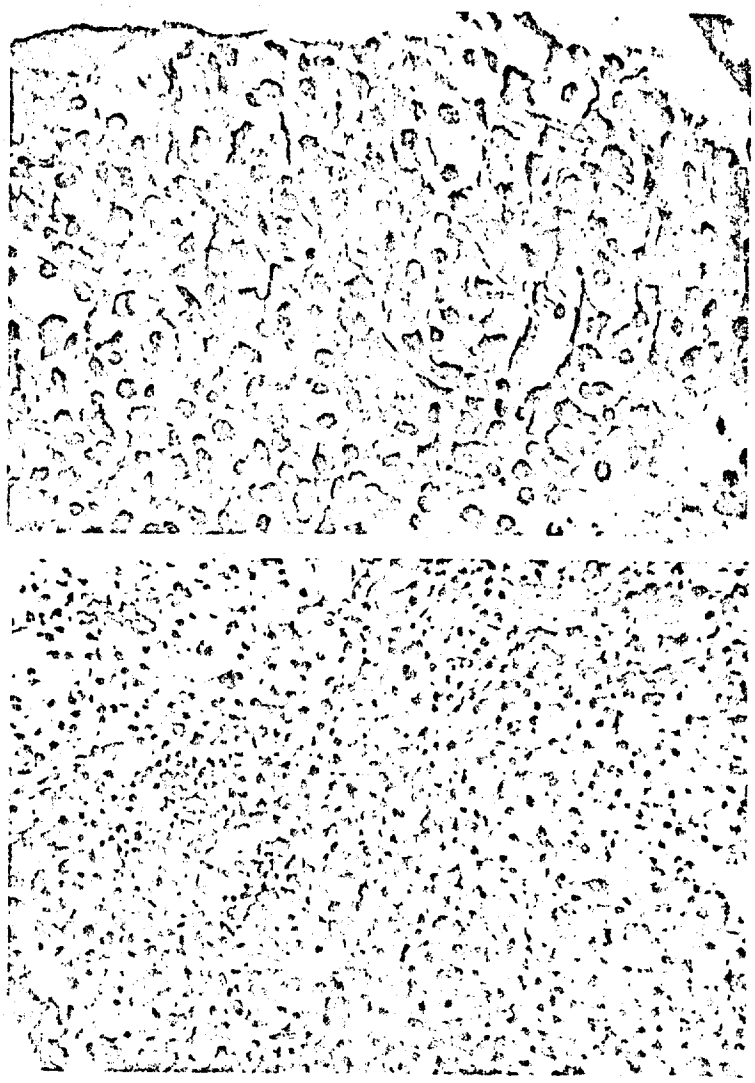
Ill. 20: As in Ill. 18 and 19. Dust storing in cells of lymph nodes without fiber formation. Rat, 10 months after injection (v. Gieson, 130 X).

Dust Preparation	Dosage in mg	Death Rate % in 48 hrs.
γ-Al_2O_3 without SiO_2 layer		
(90 m^2g)		
1. Untreated	60	40%
2. Heated at 800°C.	60	10%
γ-Al_2O_3 with SiO_2 layer		
(90 m^2g; 16.6 μmol SiO_2 m^2)		
1. Heated at 400°C.		
2. Heated at 800°C.		
SiO_2 dusts (untreated)		
1. Aerosil (150 m^2g)	20	100%
2. "Silica fume" (43 m^2g)	20	40%
	30	100%

Ill. 21: Toxicity of γ-Al_2O_3 without and with SiO_2 covering in comparison with SiO_2 dusts (intraperitoneal test with mouse).

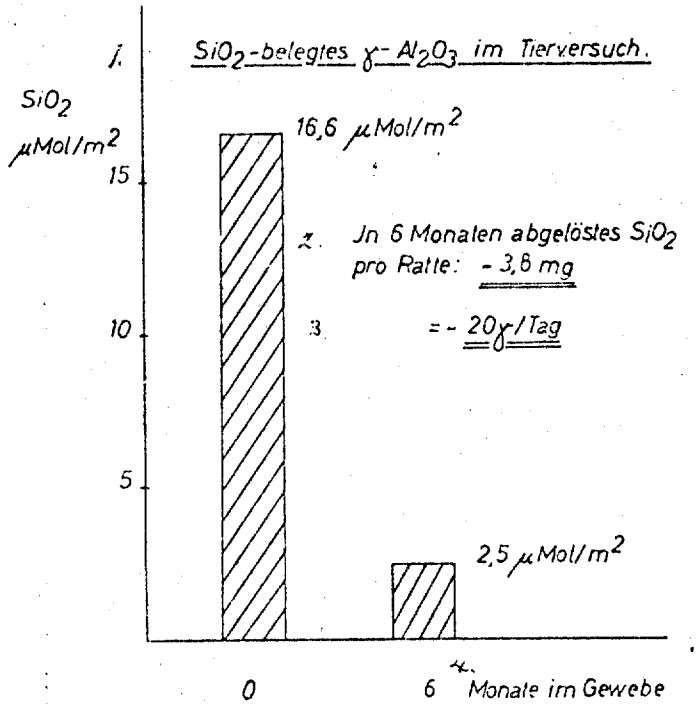


Ill. 22: γ-Al_2O_3 with SiO_2 layer. Simple dust storing in cells of lymph nodes, 6 months after injection of rats (v. Gieson, 200 X).



Ill. 23: γ-Al_2O_3 with SiO_2. Cellular storing origins without fiber formation in perit. sac, mostly with some multinuclear giant cells, as in lower picture (mouse, 6 months after injection; v. Gieson: above 320 X; below, 130 X).

Ill. 24 and 25: Fiber formation in coarser dust origins with abundant coarse aggregates of dusts; above, using γ-Al_2O_3 with SiO_2 layer (6 months after injection of mouse; v. Gieson, 100 X); below, using γ-Al_2O_3 without SiO_2 layer (3 months after injection of mouse; v. Gieson, 130 X).



Line 1 - γ-Al_2O_3 with SiO_2 Covering in Animal Tests

2 - SiO_2 dissolved in 6 months per rat: - 3.8 mg

3 - - 20γ/day

4 - Months in tissue

Ill. 26: γ-Al_2O_3 with SiO_2 covering in animal tests (SiO_2 content of dust granulomas in relation to Al_2O_3 content, given in number of μ mol/m^2 of Al_2O_3 dust surface).

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Aus der Medizinischen Forschungsanstalt der Max-Planck-Gesellschaft,
Arbeitsgemeinschaft Silikoseforschung, Göttingen
(Leiter: Professor Dr. K. Thomas)

10. Chemisch veränderte SiO_2-Oberflächen im Tierversuch

Von F. J. Strecker

Herr Stöber hat soeben die chemischen und physikalisch-chemischen Grundlagen der bei uns untersuchten Veränderungen von SiO_2-Oberflächen geschildert. Ich möchte Ihnen nun über die tierexperimentellen Untersuchungen mit derartigen Stäuben berichten.

Es handelt sich um 3 verschiedene große Versuchsgruppen:

A. Maskierung von SiO_2-Oberflächen durch definierte monomolekulare chemische Umsetzung der oberflächenständigen SiOH-Gruppen,

B. gleichartige monomolekulare Belegung biologisch inerter TiO_2-Stäube (als Kontrollversuche zur Prüfung der Belegung selbst auf eine etwaige Eigenwirkung) und

C. Prüfung von SiO_2-Oberflächenschichten mit besonderen Eigenschaften. Hier handelt es sich um eine monomolekulare SiO_2-Schicht auf Al_2O_3.

A. Maskierte SiO_2-Oberflächen:

In der ersten Versuchsgruppe haben wir es mit SiO_2-Stäuben zu tun; die Oberfläche besteht aber nicht mehr aus SiOH-Gruppen, sondern aus einem monomolekularen Film von Nicht-SiO_2-Molekülen, in dem jedes einzelne dieser Moleküle in echter chemischer Bindung mit den SiO_2-Teilchen verbunden ist.

I. Toxizitäts-Schnelltest:

Einen ersten Einblick in das Verhalten derartiger maskierender monomolekularer Schichten an SiO_2-Teilchen kann man im Tierversuch gewinnen, wenn die Toxizität für das Ganztier als Maßstab gewertet wird; denn sehr feinteilige SiO_2-Präparate wie das Aerosil sind bereits bei relativ geringer Dosierung im i-p.-Mäusetest tödlich. Eine Verringerung der Todesrate und eine Verzögerung im Auftreten der Todesfälle zeigen also bereits an, daß die SiO_2-Oberflächen wirksam maskiert sein müssen.

In der Abb. 1 sind die Befunde bei verschiedenen Belegungen von **Aerosil** (150 m^2/g) dargestellt:

1. Unbehandeltes Aerosil tötet bereits bei 20 mg alle Tiere in 2 Tagen.
2. Veresterung mit Äthandiol (die Oberfläche bleibt hydrophil) maskiert die Aerosilwirkung höchstens für Stunden.

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Oberfläche		Dosis: mg	Todesrate
≡Si-OH		20	100% in 48 Std.
≡Si-O-C-C-OH (H H / H H)		20	100% nach 48 Std.
≡Si-O-C-C-H (H H / H H)		30 60	90% in 10 Tg. 100% in 3 Tg.
≡Si-O-C-C-C-C-H (H H H H / H H H H)		30 60	~50% in 14 Tg.
≡Si-O-C-C-C-C-C-C-C-C-H (H H H H H H H H / H H H H H H H H)		30 60	~30% in 14 Tg
≡Si-O-C-C-C-C-H (H H H H / H H H H)		20 40	—o— 10% in 14 Tg.
		20 40	30% in 3 Tg. 50% in 3 Tg. 80% in 14 Tg
≡Si-O-Si-CH3 (CH3, CH3)		60	—o—
		60	—o—
≡Si-O-Al (OH, OH)		30 60	20% in 60 Std. 40% in 60 Std.

Abb. 1: Toxizität von Aerosil bei verschiedener Oberflächenbelegung. (Letalität bei intraperitonealer Applikation an der Maus)

Unter den vollständigen Veresterungen der freien SiOH-Gruppen mit Aethanol, Butanol und Oktanol — also bei hydrophoben Außengliedern — zeigt bereits die Aethanolveresterung eine deutliche Verminderung und Verzögerung der Allgemein-Toxizität: mit zunehmender Kettenlänge vermindert sich diese noch beträchtlich.

Selbst bei Butanolveresterung nur jeder zweiten und sogar noch jeder vierten SiOH-Gruppe der Oberfläche ist eine Schutzwirkung zu erkennen, im letzten Fall allerdings nur noch gering.

Die in diesen Versuchsreihen verzögert und abgeschwächt auftretende Allgemeintoxizität läßt sich zwanglos durch eine Hydrolyse der Ester und die Wieder-Freilegung der SiOH-Oberflächen mit allen ihren Folgen erklären.

Schlußfolgerung: Die Esterbindung als solche (Si-O-C-) ist in vivo wenig stabil, wird allerdings bei vollständiger Belegung — also im Verband des monomolekularen Oberflächenfilms — relativ stabilisiert durch den hydrophoben Charakter der Kettenaußenglieder und durch eine zunehmende Kettenlänge. Umgekehrt verringert eine Auflockerung der Belegung die Beständigkeit. Eine völlige Aufhebung der Allgemeintoxizität gelingt jedoch bei Veresterung in keinem Fall, d. h., in allen Präparaten werden innerhalb der ersten 14 Tage bereits größere Anteile der SiO_2-Partikel wieder „demaskiert".

3. Trimethylsilylbelegung (Si-O-Si-Bindung): Sowohl bei vollständiger als auch noch bei halber Belegung ist in allen praktisch noch applizierbaren Dosen die Allgemeintoxizität vollständig aufgehoben. Diese Bindung dürfte also wesentlich stabiler als die Esterbindung sein. Die Stabilität wird hier sicher aber auch wesentlich begünstigt durch die regenschirmartige Abdeckung der Si-O-Si-Brücke.

4. Betrachtet man nun die Allgemeintoxizität bei Aluminiumoxydhydrat-Belegung, so findet sich zwar eine deutliche Verminderung, aber doch eine beträchtliche Todesrate. Das scheint zunächst im Widerspruch zu dem zu stehen, was Herr Stöber über die Stabilität der Al-Oxydhydrat-Belegung in vitro berichtet hat. In diesem Fall handelt es sich jedoch nicht unbedingt um Aerosil-Todesfälle, also um die Wirkung wieder freigesetzter SiOH-Oberflächen, sondern mit größerer Wahrscheinlichkeit um eine Eigenwirkung der Al-Oxydhydrat-Oberfläche: z. B. zeigt auch das γ-Al_2O_3 unter vergleichbaren Versuchsbedingungen eine vergleichbare Allgemeintoxizität. Hier ist also aus dem Toxizitäts-Schnelltest noch keinerlei Aussage möglich über die Beständigkeit und die maskierende Wirkung der Al-Oxydhydrat-Schicht auf SiO_2.

II. Lokale Gewebsreaktion:

Einen genaueren Einblick in das Verhalten der Oberflächenverbindungen in vivo kann man nur aus dem langdauernden Tierversuch erhalten. Hier geht es um die lokale Gewebsreaktion am Ort der Staubablagerung.

Ich möchte Ihnen nun kurz den Ablauf der Gewebsreaktion in den Grundzügen erläutern, und zwar zunächst für die **Veresterung und die Trimethylsilylbelegung des Aerosils:** Ich muß Sie bitten, dabei etwas schematisierend vorgehen zu dürfen:

1. Zunächst kommt es (im Idealfall) bei wirksamer Maskierung der SiOH-Oberfläche des Aerosils zu einer einfachen Speicherung der Staubteilchen mit ruhigem Zellbild, d. h., bei intakter vollständiger Belegung haben wir es mit einem harmlosen, durch die Belegung harmlos gewordenen „SiO_2-Teilchen" zu tun (Abb. 2).
2. Im weiteren Ablauf der Gewebsreaktion begegnen wir dann noch einer anscheinend ebenfalls recht harmlosen Speicherung, und zwar in Form ziemlich grober sog. „Glanzkörperchen", die wohl aus Aerosil-Aggregaten und körpereigenen Substanzen bestehen, also „eingehüllte" Teilchen darstellen (Abb. 3). Dieser Befund ist auch beim unbehandelten Aerosil bekannt.
3. Zwischen diesen beiden „harmlosen" Formen der Speicherung findet sich aber eine Phase, in der die Aerosilteilchen zellschädigend bis zur Zellnekrose wirken. Bei diesem Stadium

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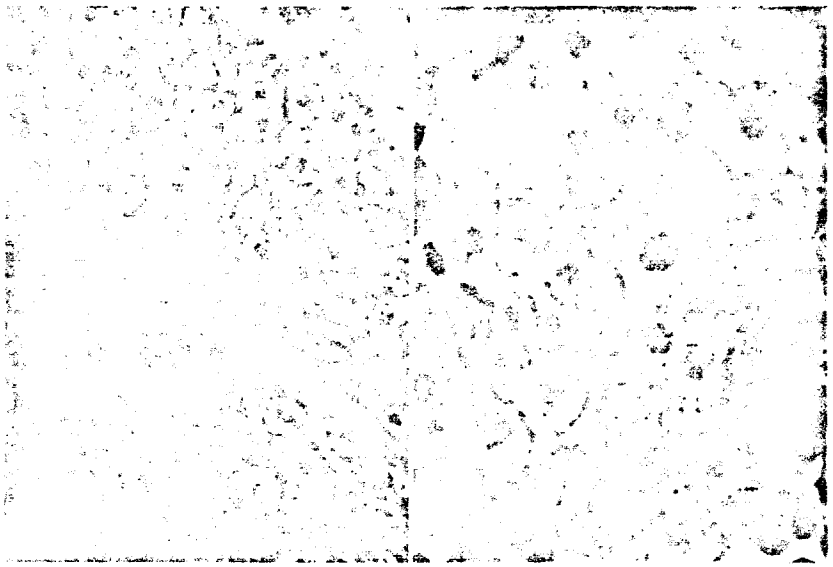

Abb. 2. Vorwiegend einfache Speicherung von Trimethylsilyl-Aerosil (5,2 μ Mol/m²) in Form kleinster und ziemlich gleichmäßig feiner Partikel; ruhiges Zellbild (2 Mon. p. inj., Maus; v. Gieson; pos. Phasenkontrast; li: 320 ×, re: 1000 ×; in der Reproduktion der starken Vergrößerung treten leider dicht beieinander liegende feine Einzelpartikel teilweise als größere Aggregate in Erscheinung).

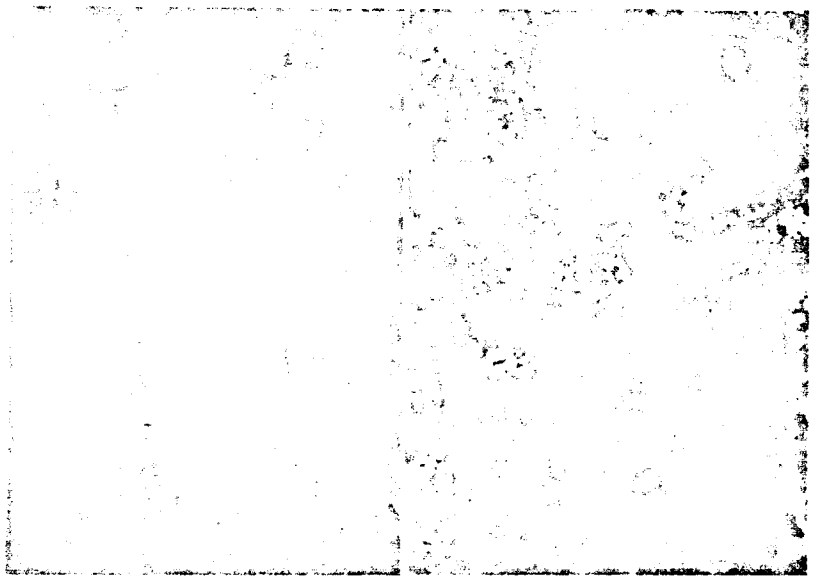

Abb. 3. „Glanzkörperchen", stark lichtbrechend, unregelmäßig groß, wesentlich gröber als die Speicherpartikel der Abb. 2; Trimethylsilyl-Aerosil (2,9 μ Mol/m²), 2 Mon. p. inj., Maus (v. Gieson; pos. Phasenkontrast; 320×).

handelt es sich offenbar um die Phase der **Ablösung der Oberflächenbelegung**. Wir können also bei den Veresterungen und der Trimethylsilylbelegung das Auftreten der Zellschädigung als Indikator für den Zeitpunkt der Ablösung benutzen.

Bei dieser Ablösung werden die SiOH-Gruppen der Oberflächen wieder freigesetzt. Gleichzeitig werden aber auch die Moleküle der Maskierungsschicht frei. Wir mußten daher prüfen, ob die Freisetzung der maskierenden Moleküle selbst zellschädigend wirkt.

Bei **gleichartiger Belegung von inerten TiO_2-Stäuben** ließ sich jedoch keine entsprechende Gewebswirkung nachweisen (siehe B). Damit ist die **Zellschädigung auf die Freisetzung der SiOH-Oberfläche zurückgeführt**.

4. Die Gewebsreaktion bei der Ablösung der maskierenden Moleküle wird wesentlich von der **Ablösungsgeschwindigkeit** bestimmt: Ist diese groß, d. h., werden schnell große SiOH-Oberflächen lokal frei, so kommt es zu ausgedehnten, massiven Nekrosen (besonders bei Aethanol-Veresterung); im anderen Extrem werden nur einzelne Zellen betroffen.
5. Offensichtlich in engstem Zusammenhang mit den Staubzellnekrosen kommt es zu einer **Faserbildung**: Dabei scheint für die Faserbildung der „Rhythmus" der Staubzellnekrosen von Bedeutung zu sein. Bei den Aerosilpräparaten läuft dieser ganze Prozeß **so lange ab, wie freie SiOH-Oberflächen nachgeliefert** werden, d. h., solange der Vorrat an maskierten Teilchen reicht. Die demaskierten Aerosil-Teilchen aber werden offenbar schnell durch Umwandlung in die sog. „Glanzkörperchen" unschädlich gemacht. Für die Dauer des Ablösungsvorganges ist die Faserbildung „progredient"; nach Verbrauch des Vorrates an maskierten Aerosilteilchen verschwindet das SiO_2 jedoch relativ rasch aus den Granulomen, die Herde schrumpfen; schließlich bleiben nur mehr oder weniger kleine „Narben".

Bisher wurde aus dieser Deutung des Gewebsgeschehens bewußt das **aluminiumoxydhydratbelegte Aerosil** erst einmal ausgeklammert. Grundsätzlich ist hier jedoch der Ablauf gleichartig, angefangen bei der initialen ruhigen Speicherung bis zu Zellschädigung und -nekrose und bis zur Faserbildung. Die Al-Oxydhydratbelegung muß aber noch besonders besprochen werden, um auch hier die Zellschädigung mit ihren Folgen auf das Freiwerden der SiOH-Oberfläche, also die Ablösung der bedeckenden Schicht zurückführen zu können; denn hier gibt es von **King** und Mitarbeitern[1, 2, 3] den Befund, daß ein Al-Oxydhydrat in Form des Boehmits eine schwere Fibrose erzeugt.

Gegen eine fibrosierende Wirkung der Oberflächenbelegung bei unseren Al-Oxydhydrat-Belegungen sprechen folgende Befunde:

1. Eine Al-Oxydhydrat-Belegung an inertem TiO_2 macht keine Zellschädigung keine Fibrose (siehe B).
2. Im Verlauf des Versuchs nimmt die Staubmenge in der Bauchhöhle beträchtlich ab; gleichzeitig sind in den Nieren-Tubuli deutlich die Kennzeichen einer Kieselsäure-Ausscheidung zu sehen.
3. Die chemische Analyse in den Staubgranulomen der Bauchhöhle zeigt eine deutliche Verschiebung im Verhältnis Al zu SiO_2 zuungunsten des Al. Die ersten Säulen der Abb. 4 zeigen 2 TiO_2-Präparate mit Al-Oxydhydratbelegung und die starke Ablösung nach 9 Monaten in corpore. Es erweist sich also, daß der Organismus Al in Lösung bringt. Die folgenden Säulengruppen zeigen jeweils die Abnahme im Al-Gehalt (nach 6 und 9 Monaten) bei den

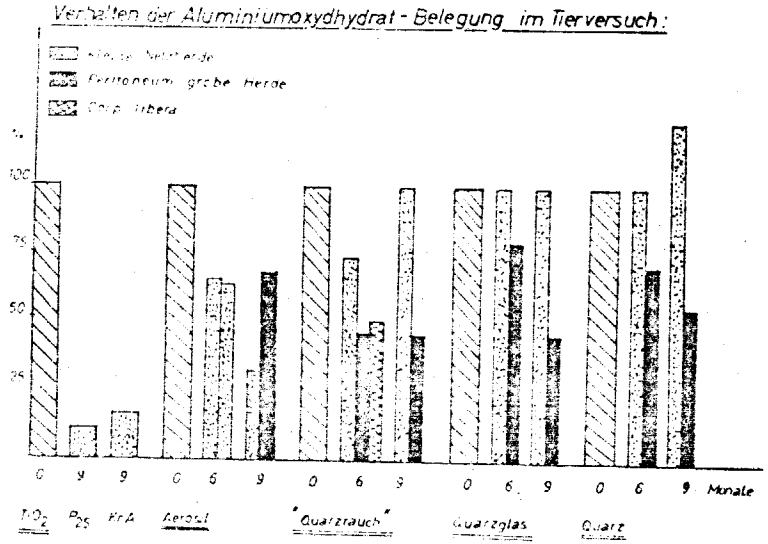

Abb. 4: Verhalten der Al-Oxydhydrat-Belegung im Tierversuch (Al-Gehalt bezogen auf den SiO_2-Gehalt der Staubgranulome in % des Al-Gehaltes der Staubpulver vor dem Tierversuch).

geprüften SiO_2-Stäuben, bezogen auf den SiO_2-Gehalt; dabei ist der ursprüngliche Al-Gehalt jeweils gleich 100 % gesetzt. In guter Übereinstimmung mit den feingeweblichen Befunden nimmt der Al-Gehalt relativ zum SiO_2 in den Granulomen ab, und zwar ebenfalls in guter Übereinstimmung mit dem jeweiligen morphologischen Befund in den einzelnen Herdformen verschieden schnell.

Faßt man diese Befunde zusammen, so darf also die Zellschädigung und die Fibrose auch bei Al-Oxydhydrat-Belegung auf die Ablösung der Schutzschicht und das Freiwerden der SiOH-Oberfläche wie bei den anderen Maskierungen bezogen werden.

Nach dieser allgemeinen Schilderung der Gewebsreaktion sollen die **Befunde an einigen konkreten Beispielen** erläutert werden, wobei Zeitpunkt und Ausdehnung der Staubzellnekrosen und der Faserbildung für die verschiedenen Oberflächenbelegungen charakteristisch sind. Dabei können hier nur die jeweils im Vordergrund stehenden Veränderungen angeführt werden:

a) **Chemisch veränderte Aerosilpräparate (150 m²/g):**

1. Veresterungen:

a) Aethanolveresterung (6 μ Mol/m²): Die Maskierung der Oberflächen ist so wenig beständig, daß hier noch ganz überwiegend die massive Zellnekrose das Gewebsbild beherrscht.

b) Butanol- und Oktanolveresterung (5,2 bzw. 6 μ Mol/m²): Staubzellnekrosen beginnen bereits im ersten Versuchsmonat, nach 1 Monat überwiegt jedoch noch das Bild der einfachen Speicherung. Nach 2 Monaten nehmen die Nekrosen der Staubzellen bei Butanolveresterung

deutlich zu, bei Oktanolveresterung praktisch noch nicht. Nach 4 Versuchsmonaten sind aber bei beiden Aerosilpräparaten die Staubzellherde ganz überwiegend in einem mit lebhaften Nekrosen und kräftiger Faserbildung einhergehenden Umbau begriffen. Nur bei Oktanolveresterung finden sich noch einige vorwiegend reine Speicherherde (ohne „Glanzkörper"), während bei Butanolveresterung teilweise bereits ein Kleinerwerden der Granulome, eine „Schrumpfung", erkennbar wird (Abnahme der Staubmenge). Die mittlere Faserbildung in den Staubgranulomen zu diesem Zeitpunkt zeigt die Abb. 5. Nach 8 Versuchsmonaten sind bei beiden Aerosilpräparaten nur noch Restnarben (ohne Staub) nachzuweisen.

Abb. 5: Mittlere Faserbildung bei Butanol-Aerosil (5,2 μ Mol/m²), 4 Mon. p. inj. (Ag; 120 ×).

c) Butanolveresterung: 3,6 μ Mol/m² und 1,6 μ Mol/m²: Bei ½- und ¼-Belegung mit Butanol ist die Verzögerung der Zellschädigung erwartungsgemäß geringer als bei vollständiger Veresterung. Bei ½-Belegung dauert der geschilderte Gewebsprozeß immerhin noch bis in den 4. Versuchsmonat mit Höhepunkt nach 2 Monaten, und die Faserbildung ist noch recht beträchtlich. Bei ¼-Belegung sind nach 4 Monaten nur mit Mühe noch einzelne kleinste Herdchen nachzuweisen; selbst bei diesem Präparat kommt es aber zuvor noch in den insgesamt weniger ausgedehnten Granulomen zu einer deutlichen Faserbildung.

2. Trimethylsilyl-Belegung:

a) 2,9 μ Mol/m²: Bereits nach 1 Monat ist das ganze Spiel mit Nekrosen und Faserbildung im Gange. Nach 3—4 Versuchsmonaten liegt eine recht beträchtliche Faserbildung vor. Nach 4 Monaten beginnen die Granulome aber auch schon deutlich kleiner zu werden. Nach 6 Monaten ist nur noch wenig Staub nachzuweisen, meist nur noch die staubfreien Restnarben der früheren Granulome[4,5].

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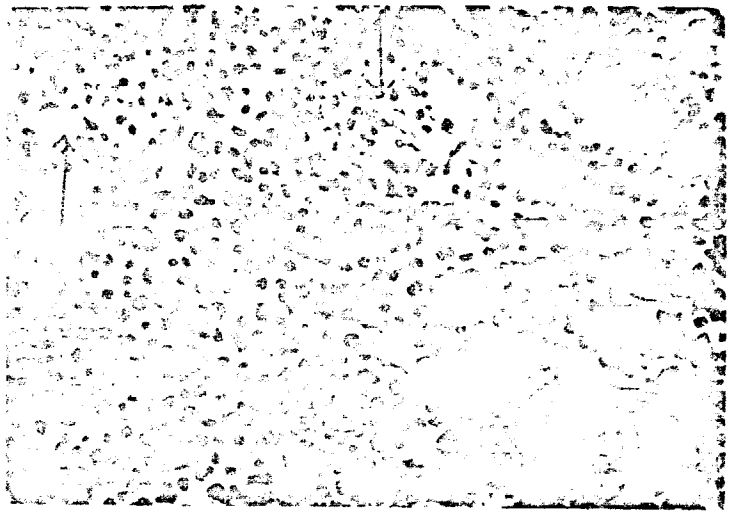

Abb. 6: Trimethylsilyl-Aerosil (5,2 μ Mol/m²), 6 Mon. p. inj., Maus: Ruhige Speicherung in Lymphknoten-Sinus; (oben li und oben Mitte (*) zwei kleine Herdchen mit beginnender Zellschädigung und ganz zarten Fasern) (v. Gieson: 160 ×).

b) 5,2 μ Mol/m²: Bei praktisch vollständiger Trimethylsilylbelegung sieht man dagegen etwa bis zum 6. Versuchsmonat ganz überwiegend eine einfache ruhige Speicherung und nur wenige Einzelzellnekrosen (Abb. 2 u. 6). Erst nach diesem Zeitpunkt kommt es zu stärkeren Staubzellnekrosen und nun teilweise ganz beträchtlicher Faserbildung bis zu stark hyalinisierten Faserknötchen (Abb. 7 und 8).

3. Al-Oxydhydrat-Belegung: 5,7 μ Mol/m²:

Nach einem überwiegend ruhigen Speicherstadium von etwa 2 Monaten findet sich eine zunehmende Nekrosetendenz der Staubzellen mit Faserbildung (Abb. 9), wobei aber bis zum 9. Monat (jetzige Versuchsdauer) auch noch Staubzellareale vorkommen, in denen das einfache Speicherbild vorherrscht. Zugleich kann man besonders im Netz Staubverschiebungen beobachten: aus größeren „Faserfeldern" wandern gleichsam kleinere Streifen und Herdchen von Staubzellen strahlig in das Netz hinein. Nach 9 Monaten haben Staubmenge und Ausdehnung der Staubherde in der Bauchhöhle deutlich abgenommen. Ein charakteristisches Bild der Faserbildung nach 9 Monaten zeigt die Abb. 10 mit einer Gruppe von faserreichen bis hyalinen Knötchen.

β) Chemisch veränderte Oberflächen von Quarzrauch, Quarzglas und Quarz:

Die Trimethylsilyl- und Al-Oxydhydrat-Belegung haben wir auch an sog. Quarzrauch und mehreren Quarzglas- und Quarzpräparaten untersucht:

1. Quarzrauch (Oberfläche 43 m²/g):

Bei Trimethylsilylbelegung (5,4 μ Mol/m²) setzt das bei den Aerosilpräparaten gekennzeichnete Umwandlungsstadium der Staubzellherde aus einem vorwiegend ruhigen Speicherzustand etwa im 6. Versuchsmonat ein, bei Al-Oxydhydrat-Belegung (5,8 μ Mol/m²) etwa im 3. Monat. Die Faserbildung erreicht in den folgenden Versuchsmonaten dann aber einen Grad, der vom unbehandelten Staub nicht erzeugt wird (Abb. 11).

Abb. 7 u. 8: Trimethylsilyl-Aerosil (5,2 μ Mol/m²) 6 bzw. 8 Mon. p. inj., Maus: Faserknötchen in Lymphknoten und Netz, teilweise mit breiten hyalinen Bändern (v. Gieson: LK = 100 ×; Netzknötchen 320 ×).

2. Quarzglas:

Die Trimethylsilylbelegung haben wir an einem Quarzglaspräparat von 6,8 m²/g Oberfläche und einer Belegungsdichte von 3,2 μ Mol/m² geprüft. 5—6 Monate vermag diese Belegung praktisch die Staubzellen vor einer wesentlichen Schädigung zu schützen. Im 7. Monat setzen zunehmend Staubzellnekrosen und Faserbildung ein; nach 10 Monaten sind fast nur noch

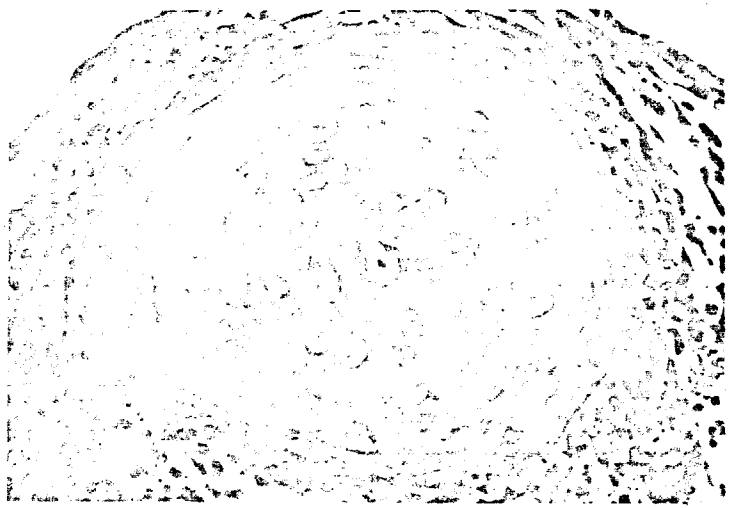

Abb. 9: Al-Oxydhydrat-belegtes Aerosil (6,7 μ Mol/m²), Ratte, 2 Mon. p. inj.: Beginnende Faserbildung und Zellnekrosen in kleinen Herdchen im Netz (v. Gieson: 280×).

Abb. 10: Al-Oxydhydrat-belegtes Aerosil (6,7 μ Mol/m²), Ratte, 9 Mon. p. inj.: Gruppe von kleinen Faserknötchen im Netz, teilweise mit Hyalinbildung (v. Gieson: 140×).

reine Faserherde und -knötchen mit ausgedehnter Hyalinisierung zu sehen (Abb. 12).

Als das gleiche Staubpräparat nach mehrmonatigem Stehen im Labor erneut geprüft wurde, trat die Umwandlung bereits nach 4monatiger Ver-

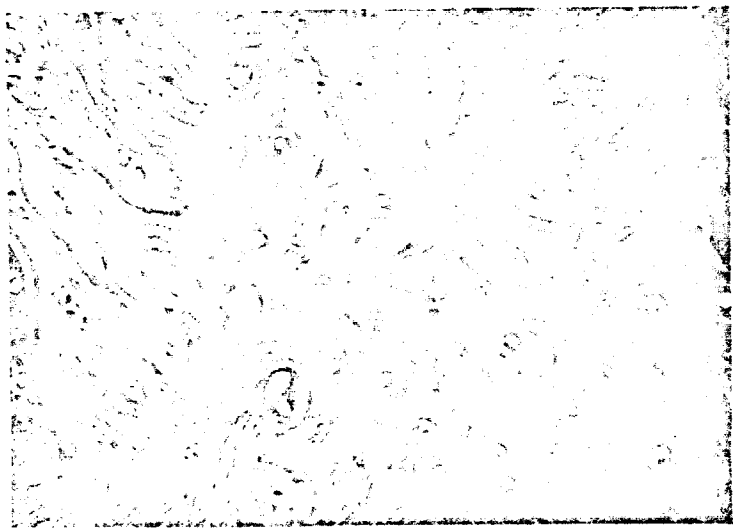

Abb. 11: Al-Oxydhydrat-Quarzstaub (5,8 μ Mol/m²), Ratte, 9 Mon. p. inj.: Ausschnitt aus einem hyalin-fibrösen Knötchen (v. Gieson: 130×).

suchsdauer ein, weil offensichtlich durch die Luftfeuchtigkeit schon eine teilweise Hydrolyse der Belegung erfolgt war (Abb. 13).

Die Al-Oxydhydrat-Belegung wurde an einem Quarzglasstaub von 11,4 m²/g Oberfläche und vollständiger Belegung (6,9 μ Mol/m²) getestet. Bereits nach 3—4 Monaten sind in einem Teil der Staubzellherde reichliche Zellnekrosen und Faserbildung zu erkennen (Abb. 14), aber ausgedehnte Bezirke stehen noch in den ersten Anfängen der Umwandlungsphase. Nach und noch stärker nach 9 Monaten liegen ganz überwiegend praktisch zellfreie reine Faserknötchen vor mit ausgedehnter Hyalinisierung (Abb. 15). Nur sehr kleine Netzherdchen zeigen noch mehr den einfachen Staubzellspeichertyp.

3. Quarz (Bergkristall):

Bei vollständiger Trimethylsilylbelegung (5,7 bzw. 5,8 μ Mol/m²) ließ ein Quarzpulver mit großer Oberfläche (11,2 m²/g) nach 8—10 Monaten die Zeichen einer SiO_2-Wirkung erkennen (beginnende Staubzellnekrosen, vermehrte Mitosen, zunehmende Faserbildung; einzelne Herde bereits in stärkerer Umwandlung); ein zweites Quarzpulver von geringerer Korngröße (4,9 m²/g) bot aber auch nach 14 Versuchsmonaten praktisch noch das einfache Staubspeicherbild (Abb. 16).

Bei Al-Oxydhydrat-Belegung (7,4 μ Mol/m² bei 9,2 m²/g Oberfläche) tritt die staubzellschädigende Wirkung nach 4 Monaten deutlich in Erscheinung. Nach 6 Versuchsmonaten findet sich zunehmend Kollagen- und Knötchenbildung; daneben bestehen allerdings noch viele Zonen mit sehr geringer oder nahezu fehlender Faserbildung. Nach 9 Monaten ist die Gewebsreaktion bereits ausgedehnt zu fibrös-hyalinen Knötchen weiter gelaufen; nur kleinere Staubzellfelder und -herde zeigen noch das Bild einer weitgehend ruhigen Staubspeicherung.

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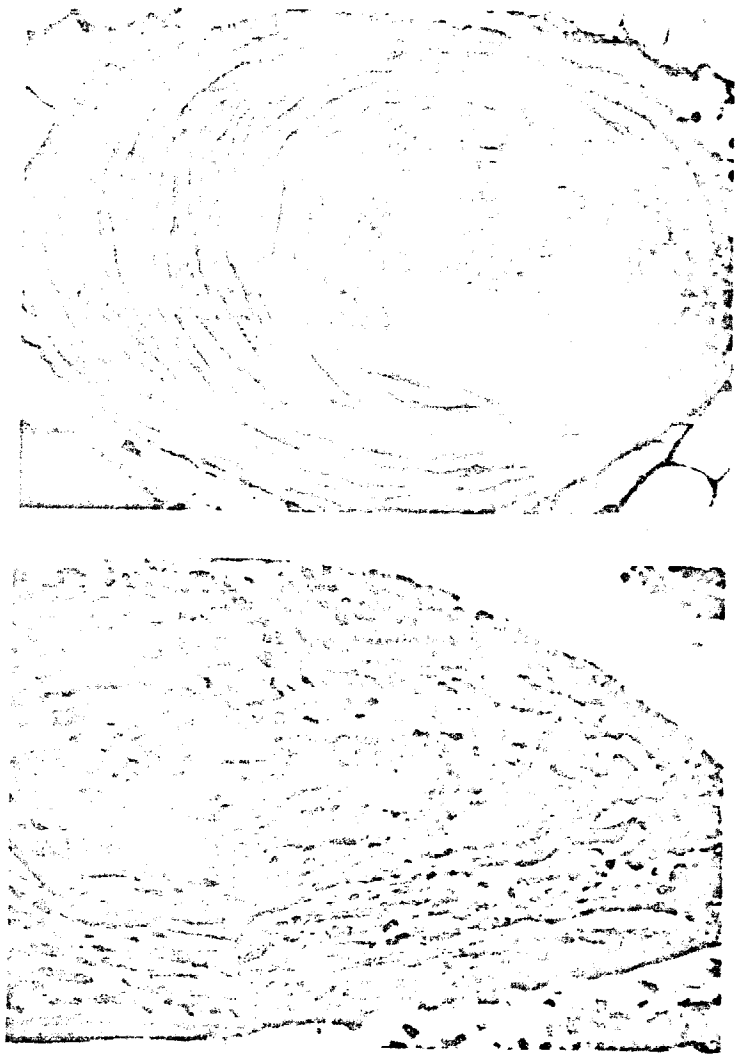

Abb. 12 u. 13: Trimethylsilyl-Quarzglas (6,2 μ Mol/m²). Maus: oben: zellarmes Faserknötchen 18 Mon. p. inj.; unten: hyalin-fibröser Herd 4 Mon. nach Inj. des im Labor „gemörserten" Staubes (oben v. Gieson, 130 ×; unten H.-E., 150 ×).

Die Gewebsreaktion zeigt also auch für diese SiO_2-Stäube die gleiche Beziehung, welche zuvor für die Aerosilpräparate ausführlich beschrieben wurde: Freisetzung der SiOH-Oberfläche → Staubzellschädigung bis zur Nekrose → Faserbildung.

Die zentrale Bedeutung der Staubzellnekrose auf dem Wege zum fibrös-hyalinen Knötchen ist besonders gut zu erkennen, wenn die

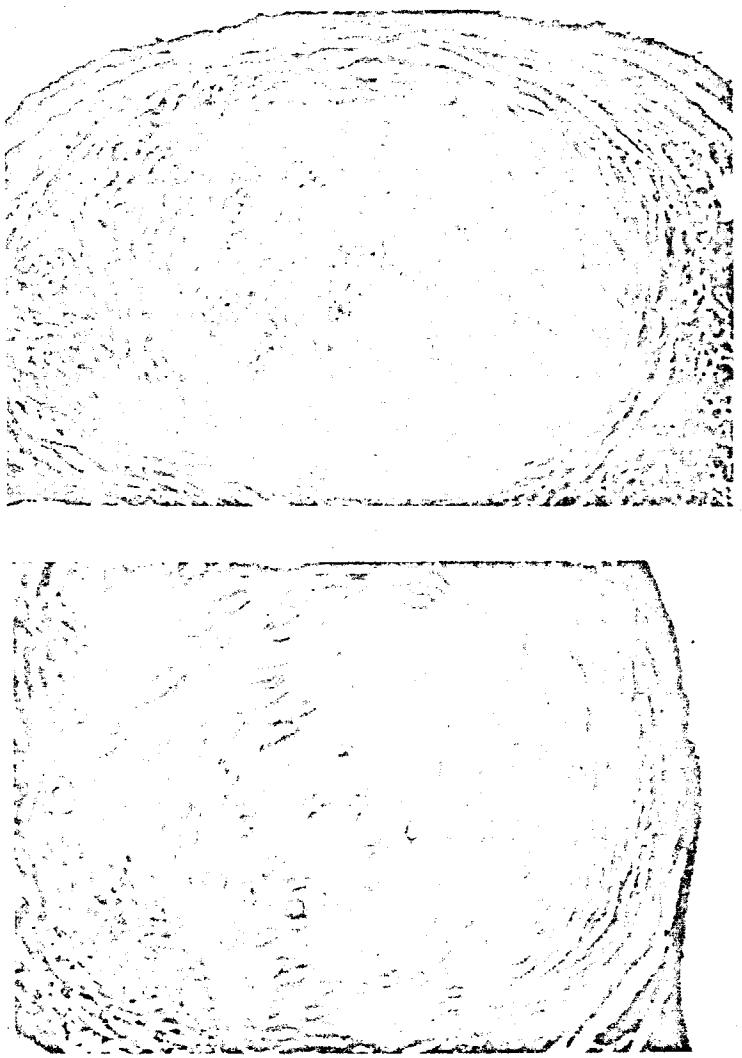

Abb. 14 u. 15: Al-Oxychlorat-Quarzglas (6,9 μ Mol/m²). Ratte: oben: 3 Mon. p. inj. mit beginnender Faserbildung und Staubzellnekrosen (v. Gieson, 160 ×); unten: hyalines, aber noch nicht zellfreies Faserknötchen 9 Mon. p. inj. (H.-E.; 130 ×).

Mitosen durch Colchicin blockiert werden und auf diese Weise ein Einblick in die Zellneubildung pro Zeiteinheit im Staubzellgranulom möglich wird. Die Abb. 17 zeigt einen Ausschnitt aus einem Trimethylsilyl-Quarzglasherd in der Umwandlungsphase mit den Mitosen der letzten 4 Stunden vor der Tötung. Aus dem feingeweblichen Bild hätte man ohne Colchicinblockierung kaum vermuten können, daß hier die Staubzellen in so großem Maße und mit solch hoher Geschwindigkeit umgesetzt und verschlissen werden.

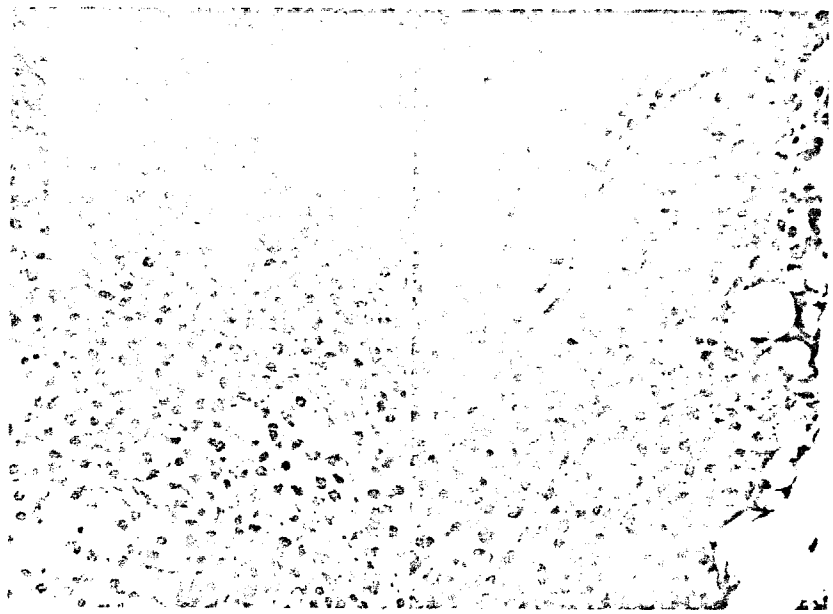

Abb. 16: Trimethylsilyl-Quarz (5,8 μ Mol m²; 4,9 m²/g). Maus: überwiegend ruhige Speicherung des Staubes 12 Mon. p. inj. (v. Gieson: 200 ×).

III. Zusammenfassung der feingeweblichen Befunde:

Aus der Gesamtheit der Versuchsgruppen ergibt sich, daß die von uns bisher untersuchten monomolekularen Molekülschichten auf SiO_2-Stäuben zu einer mehr oder weniger beständigen, aber stets nur zeitweiligen Maskierung der SiO_2-Gewebswirkung führen.

Die aus den lokalen Gewebsveränderungen in den Staubgranulomen zu erschließende Ablösungsgeschwindigkeit der maskierenden Oberflächenverbindungen im Gewebe zeigt dabei eine gute Parallele zum Verhalten in vitro und den theoretischen Erwartungen (Abhängigkeit von der Hydrolysebeständigkeit der Bindung mit den SiOH-Gruppen der Oberfläche, von der Belegungsdichte etc.) sowie mit Ausnahme der Al-Oxydhydratbelegung den Befunden im Toxizitäts-Schnelltest an der Maus. Die Al-Oxydhydratbelegung läßt sich aus dem Bild der lokalen Gewebsreaktion ebenfalls in die Beständigkeitsskala in vivo einordnen, und zwar etwa zwischen den längerkettigen einwertigen Alkoholen (C_7 und C_9) und der Trimethylsilylbelegung.

Mit der Ablösung der maskierenden Moleküle tritt stets wieder die Wirkung der SiO_2-Oberflächen hervor: bei den Quarzglas- und Quarzstaubpräparaten im wesentlichen nur verzögert, bei dem feinteiligen sog. Quarzrauch dagegen verzögert und zugleich verstärkt (im Vergleich zum unbehandelten Staub) und bei dem noch feinteiligeren Aerosil überhaupt erstmalig in kennzeichnbarer Weise. Die Sonderstellung der unbehandelten feinstteiligen SiO_2-Präparate gegenüber den üblichen Quarz- und Quarzglasstäuben beruht

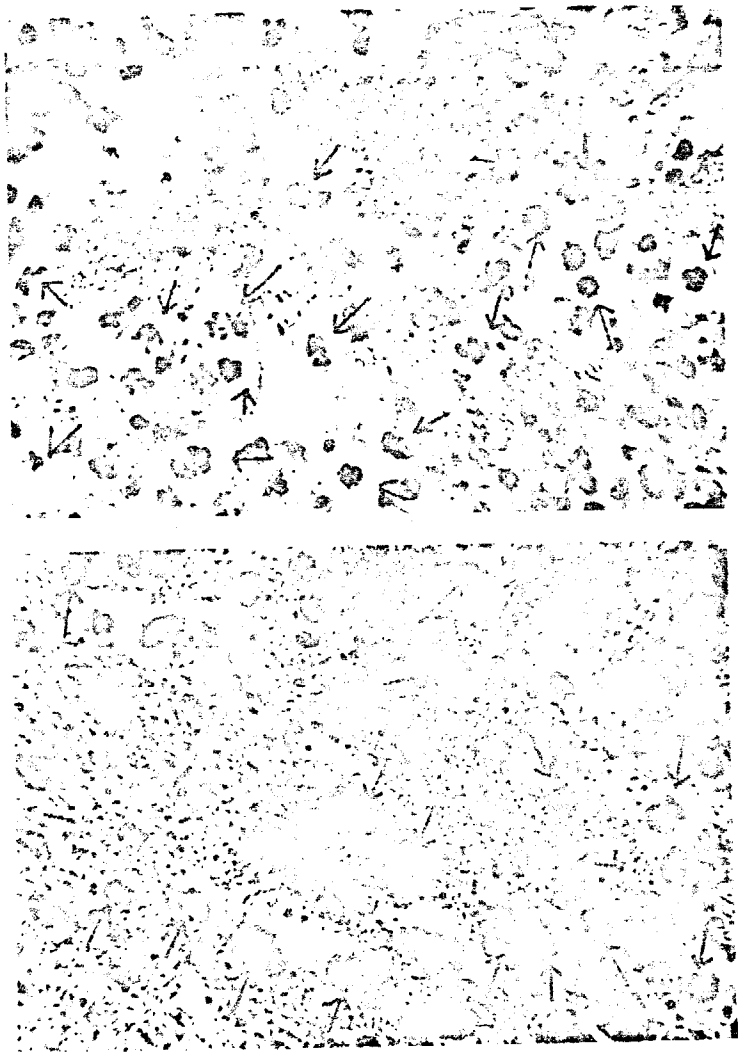

Abb. 17: Trimethylsilyl-Quarzglas-Granulome im Netz mit zahlreichen Mitosen (↑) (4 Std. vor Tötung Colchicin-Injektion); (v. Gieson, oben 400×; unten 600×).

nach unseren Befunden also auf den mit der extrem geringen Korngröße verbundenen Eigenschaften, die einerseits zu einer Verdeckung der SiO_2-Eigenschaften führen (intrazelluläre Aggregatbildung und Einhüllung = Maskierung durch körpereigene Substanzen) und zum anderen die Prüfung auf eine langdauernde Gewebswirkung überhaupt erschweren bzw. praktisch vergleichbar gar nicht gestatten (hohe Toxizität für das Ganztier; schnelle Auflösung der Teilchen). Durch die zeitweilige Maskierung werden diese „störenden" Faktoren wenigstens teilweise überlistet, und nun kommt die SiO_2-Wirkung auch bei

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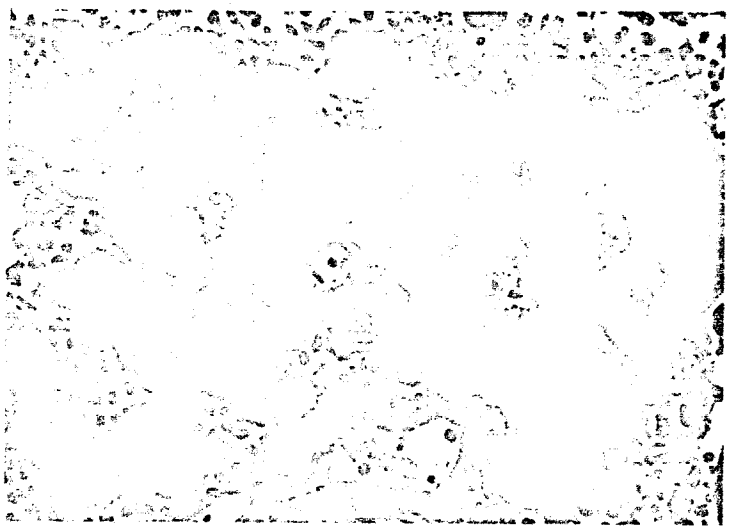

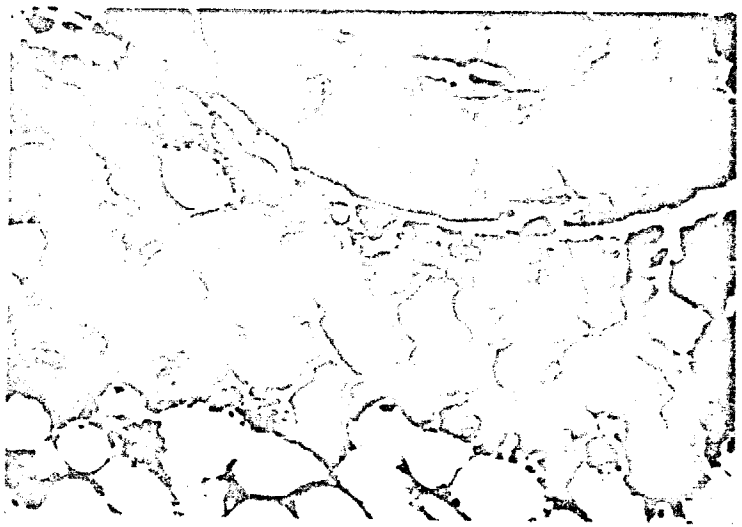

Abb. 16/19: TiO_2 — P 25 mit Al-Oxydhydrat-Belegung: Staubspeicherung ohne Faserbildung im Netz (oben: Maus, 5 Mon. p. inj., v. Gieson, 200 ×; unten: Ratte, 10 Mon. p. inj., v. Gieson, 150 ×).

diesen feinstteiligen SiO_2-Formen mit denjenigen Grundreaktionen zum Vorschein, die wir bei den gröberen SiO_2-Partikeln als die typischen Merkmale der „silikotischen Gewebsreaktion" finden.

Abb. 22: Wie Abb. 15/19: Zellige Staubspeicherung in Lymphknoten ohne Faserbildung. Ratte, 10 Mon. p. inj.; v. Gieson, 100 ×.

B. Kontrollversuche an TiO_2-Stäuben

Bei der Beschreibung unserer Versuche mit den zeitweilig maskierten SiO_2-Stäuben (A) wurde bereits diskutiert, daß die im Gewebe von den SiO_2-Teilchen mehr oder weniger langsam abgelösten Moleküle der Maskierungsschicht auf eine etwaige Eigenwirkung überprüft werden müssen. In diesen Versuchen ist also zu sichern, daß die Freisetzung der SiOH-Oberfläche derjenige Faktor ist, welcher beim Ablösungsvorgang von den SiO_2-Teilchen für die gefundene Zellschädigung bis zur Nekrose und für die Faserbildung verantwortlich ist. Derartige Kontrolluntersuchungen müssen wie bei den SiO_2-Stäuben eine Freisetzung der jeweiligen Moleküle von einer Teilchenoberfläche in Staubzellen gewährleisten. Diese Teilchen dürfen dabei aber nun nicht selbst eine zellschädigende und faserbildende Wirkung besitzen.

Um diesen Forderungen nachzukommen, haben wir zur Kontrolle der Belegungen auf SiO_2-Stäuben gleichartige Belegungen auf dem bekannt harmlosen TiO_2-Staub geprüft, und zwar die Veresterung mit Aethanol, Butanol und Oktanol, die Trimethylsilylbelegung und die Belegung mit einer Al-Oxydhydrat-Schicht. Alle genannten Belegungen wurden an einem sehr feinteiligen TiO_2 (P 25; Anatas; Oberfläche 49 m^2/g) der Degussa geprüft, ein Teil ergänzend auch an einem gröberen TiO_2-Präparat der Titangesellschaft (Kronos A; Anatas; Oberfläche 9 m^2/g).

Beide Stäube (P 25 und Kronos A) führen unbehandelt nur zu einer einfachen, intrazellulären Staubspeicherung ohne Zellnekrosen und Faserbildung (Mohr[6]: in den eigenen „Leerkontrollen" bestätigt). Aber auch die Tierversuche mit den belegten TiO_2-Stäuben (bei der Trimethylsilylbelegung Tierversuche z. Z. noch nicht abgeschlossen) ergaben keinen Anhalt für eine zellschädigende und faserbildende Eigenwirkung der sich im Gewebe ablösenden Belegungsmoleküle. Die TiO_2-Teilchen werden auch mit den verschiedenen Oberflächenschichten in der Bauchhöhle

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[illegible] den Lymphknoten nur einfach zellig gespeichert (Abb. 18, 19 und 20). [illegible] des Oberflächenüberzuges sich von den TiO_2-Teilchen während [illegible] Versuchszeit ablösen, ist dabei nach dem Verhalten in vitro und den in vivo-Befunden mit den SiO_2-Stäuben als sicher anzunehmen. Die in der Abb. 4 [illegible] chemisch-analytischen Befunde beweisen aber auch direkt die Auflösung der Al-Oxyhydratschicht von den beiden geprüften TiO_2-Stäuben.

C. Monomolekulare SiO_2-Schicht auf Al_2O_3

In dieser Versuchsgruppe handelt es sich um eine künstliche monomolekulare SiO_2-Schicht auf γ-Al_2O_3-Teilchen (16,6 α Mol SiO_2 m^2 bei einer Oberfläche von 90 m^2/g). Diese SiO_2-Schicht zeichnete sich in vitro durch ihre „Unlöslichkeit" aus.

Staubpräparat	Dosis mg	Todesrate % 48 Std.
γ-Al_2O_3 ohne SiO_2-Schicht (90 m^2/g)		
1. unbehandelt	60	40 %
2. geglüht bei 800° C	60	10 %
γ-Al_2O_3 mit SiO_2-Schicht (90 m^2/g; 16,6 α Mol SiO_2/m^2)		
1. geglüht bei 400° C		70 %
2. geglüht bei 800° C		55 %
SiO_2-Stäube (unbehandelt)		
1. Aerosil (150 m^2/g)	20	100 %
2. „Quarzrauch" (45 m^2/g)	20	40 %
	30	100 %

Abb. 21: Toxizität von γ-Al_2O_3 ohne und mit SiO_2-Belegung im Vergleich zu SiO_2-Stäuben (i. p. Test, Maus).

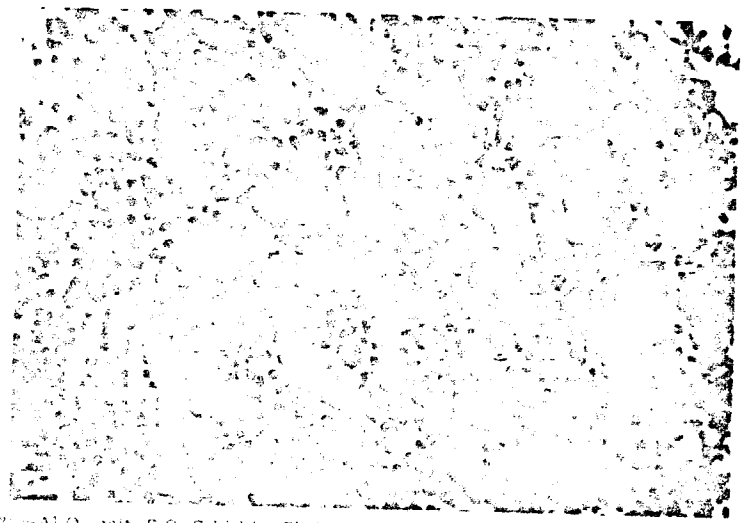

Abb. 22: γ-Al_2O_3 mit SiO_2-Schicht: Einfache zellige Staubspeicherung in Lymphknoten, 6 Mon. p. inj. (Ratte, v. Gieson, 200 ×).

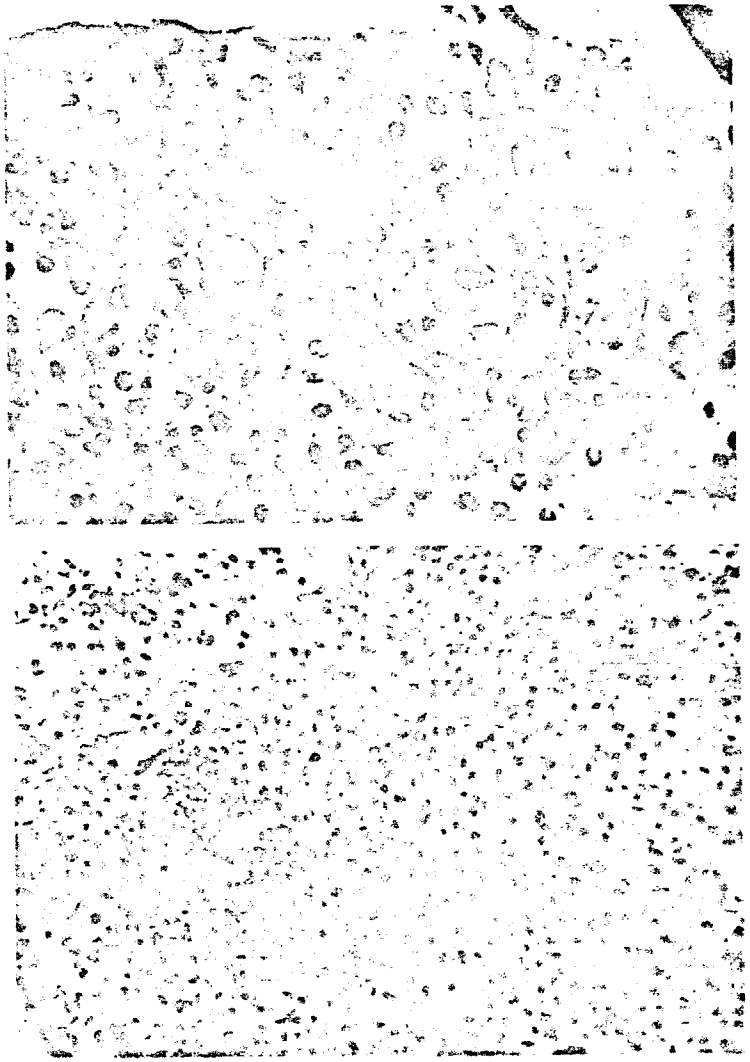

Abb. 22: γ-Al_2O_3 mit SiO_2: Zellige Speicherherde ohne Faserbildung im Netz, meist mit einigen mehrkernigen Riesenzellen wie im unteren Bild (Maus, 6 Mon. p. inj., v. Gieson: oben 320×; unten 130×).

Es ergaben sich folgende Befunde:

1. Die Toxizität des γ-Al_2O_3 für das Ganztier wird durch die SiO_2-Schicht gesteigert, erreicht jedoch bei weitem nicht den Grad eines vergleichbaren SiO_2-Staubes (Abb. 21).
2. In der chronischen Gewebsreaktion ist keine SiO_2-Wirkung zu erkennen. Das γ-Al_2O_3 mit SiO_2-Schicht verhält sich genauso wie das Vergleichspräparat ohne diese Schicht. Beide Stäube werden sowohl in der

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Abb. 24 u. 25: Faserbildung in gröberen Staubherden mit reichlich groben Staubzusammenballungen; oben bei γ-Al_2O_3 mit SiO_2-Schicht (6 Mon. p. inj., Maus, v. Gieson, 100 ×); unten bei γ-Al_2O_3 ohne SiO_2-Schicht (3 Mon. p. inj., Maus; v. Gieson, 130 ×).

Bauchhöhle als auch in den Lymphknoten einfach zellig gespeichert (Abb. 22, 23). Nur in Staubdepots der Bauchhöhle, die nicht unter Phagocytose des Staubes organisiert worden sind, kommt es zwischen den groben freien Staubzusammenballungen zu einer kollagenen Faserbildung (Abb. 24 u. 25). Derartige Herde gibt es bei beiden Versuchsgruppen; sie entsprechen mehr gewöhnlichen Fremdkörpergranulomen und können bei allen Staubinjektionsversuchen auftreten. Sie erlauben keinerlei Rückschlüsse auf eine etwaige

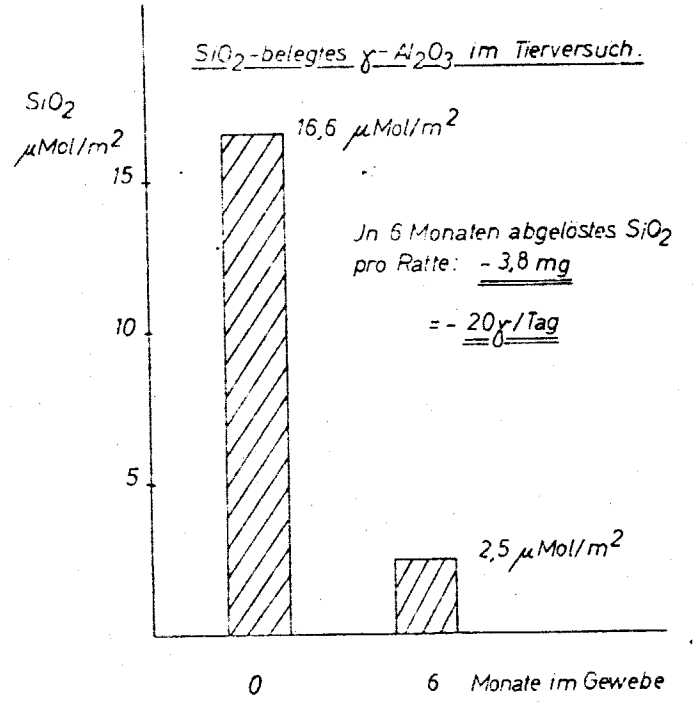

Abb. 26: SiO_2-belegtes γ-Al_2O_3 im Tierversuch (SiO_2-Gehalt der Staubgranulome bezogen auf Al_2O_3-Gehalt, angegeben in μ Mol/m² Oberfläche des Al_2O_3-Staubes).

staubeigene fibrosierende Wirkung, wie wir sie von den SiO_2-Stäuben kennen, und können daher auch nicht etwa eine vergleichbare faserbildende Wirkung des in beiden Stäuben vorliegenden γ-Al_2O_3 diagnostizieren lassen. Die relativ große Neigung des γ-Al_2O_3 zur Aggregatbildung führt allerdings häufiger zu solchen „unspezifischen" Herden als bei manchen anderen Stäuben.

Auch in primär zellig organisierten Staubdepots (speziell in größeren Herden) scheint es später noch bei diesem γ-Al_2O_3-Staub zu groben Aggregatbildungen mit entsprechenden Folgen für den Herdcharakter kommen zu können, indem der Zellinhalt bei Absterben der Zellen als geschlossener Staub- und Eiweiß-Ballen liegenbleibt und nicht wieder phagocytiert werden kann. Durch diese Ballungstendenz des γ-Al_2O_3 könnte nun etwa das die SiO_2-Gewebsreaktion kennzeichnende langdauernde Spiel der Schädigung der Staubzellen bis zur Nekrose, dann wieder Aufnahme des freigewordenen Staubes in neue Zellen und so fort — eventuell schon bei der ersten Zellgeneration unterbrochen sein und verdeckt werden. Das würde aber eine der SiO_2-Zellschädigung vergleichbare Wirkung des γ-Al_2O_3 voraussetzen, die aus den Gewebsbildern bisher nicht zu beweisen ist. Für den in unseren Versuchen primär interessierenden Vergleich des γ-Al_2O_3 mit und ohne SiO_2-Schicht bleibt das wesentliche Ergebnis die Gleichartigkeit der Gewebsreaktion auf beide Stäube und das Fehlen einer „SiO_2-Wirkung" in denjenigen Herden, die eine Auswertung zulassen.

[illegible] Gewebsanalyse zeigt, daß wider Erwarten gegen[illegible] Verhalten ein beträchtlicher Teil der SiO_2-Schicht in [illegible] Zeit in Lösung geht und zwar so viel, daß in den Staubgranu[illegible] Ratte in 6 Monaten immerhin 3,8 mg SiO_2 gelöst werden (Abb. 26).

Aus unsern Versuchen geht aber hervor, daß weder diese „SiO_2-Oberfläche" noch die von ihr in Lösung gehende Kieselsäure die Voraussetzungen für den Ablauf der „SiO_2-Gewebsreaktion" erfüllt, die vorausgehend für alle von uns untersuchten kompakten SiO_2-Körper nachgewiesen werden konnte.

Schlußfolgerungen aus den Tierversuchen mit chemisch veränderten SiO_2-Oberflächen:

1. Keine der von uns geprüften Oberflächenverbindungen an SiO_2-Stäuben vermag auf die Dauer die SiO_2-Wirkung zu maskieren, auch nicht eine Al-Oxydhydratschicht, die ja im Rahmen der sog. „Al-Therapie der Silikose" diskutiert worden ist. Im übrigen finden sich eindeutige Beziehungen zwischen Beständigkeit der Belegung in vivo und den chemischen und physikalisch-chemischen Eigenschaften. Die bisher beständigste Maskierung der SiO_2-Wirkung ist durch eine vollständige Trimethylsilylbelegung zu erreichen.

2. Aus allen Versuchen an den geprüften SiO_2-Körpern geht hervor, daß die staubzellschädigung bis zur Zellnekrose offenbar eine zentrale Stellung in der SiO_2-Gewebsreaktion einnimmt und daß es in ihrer Folge zur Faserbildung und Hyalinisierung kommt.

3. Diese Wirkung ist offensichtlich nicht an die Kristallstruktur der Teilchen gebunden, sondern wird ebenso durch die amorphen SiO_2-Körper hervorgerufen.

4. Eine monomolekulare SiO_2-Schicht auf Al_2O_3 zeigt diese Wirkung jedoch nicht, obwohl hier ja auch eine „SiO_2-Oberfläche" vorliegt und obwohl eine nicht unerhebliche Kieselsäuremenge von den Staubteilchen in Lösung geht.

Das heißt aber:

Soweit wir das aus unseren Versuchen ableiten können, ist der geschilderte Reaktionsmechanismus an die freie SiOH-Oberfläche von soliden, aus SiO_4-Tetraedern aufgebauten Teilchen gebunden.

Zusammenfassung

Es wird über Tierversuche berichtet, in denen geprüft wurden:

1. SiO_2, dessen Oberfläche mit einer monomolekularen Schicht von Alkoholen, Trimethylsilyl-Gruppen und Al-Oxydhydrat bedeckt war,
2. TiO_2, dessen Oberfläche mit gleichartigen monomolekularen Schichten überzogen war, und
3. eine monomolekulare Schicht von SiO_2 auf der Oberfläche von Al_2O_3.

In den Versuchen mit den SiO_2-Teilchen (Aerosil, Quarzrauch, Quarzglas, Quarz) waren die SiOH-Gruppen der Oberfläche mit Aethandiol, Aethanol, Butanol und Oktanol verestert (→ ≫ Si-O-C [illegible]) oder mit Trimethylchlorsilan (→ ≫ Si-O-Si(CH$_3$)$_3$) und Triisobutyl-Al (→ ≫ Si-O-Al[OH]$_2$) umgesetzt, so daß jeweils eine monomolekulare Bedeckung der SiO_2-Teilchen vorlag. Diese Oberflächenschichten führten zu einer zeitweiligen Aufhebung der SiO_2-Gewebswirkung. Die Dauer des Schutzes in vivo war verschieden und ging den unterschiedlichen chemischen und physikalisch-chemischen Eigenschaften der Oberflächenschichten parallel. Durch die vorübergehende Maskierung wurden auch bei dem sehr feinteiligen Aerosil die kennzeichnenden Merkmale der „silikotischen Gewebsreaktion" erkennbar (Staubzellschädigung bis zur Nekrose und Faserbildung).

Die gleichartigen monomolekularen Belegungen der Oberfläche von unbelegt harmlosem TiO_2 ergaben keinen Anhalt für eine staubzellschädigende und faserbildende Eigenwirkung der belegenden Molekülschichten.

Eine monomolekulare SiO_2-Schicht auf Al_2O_3 zeigte keine „silikotische Gewebsreaktion".

Résumé

Il est rapporté des recherches, dans lesquelles ont été examinées par le test péritoneal:

1. SiO_2, couvert à la surface avec une couche monomoléculaire des alcools, des groupes de triméthylsilyle- et d'Al-oxydhydrate,
2. TiO_2, couvert avec les couches monomoléculaires du type identique, et
3. une couche monomoléculaire de SiO_2 à la surface de Al_2O_3.

Dans les expériences avec les particules de SiO_2 (aérosil, silice de condensation, verre de quartz, quartz) les groupes de SiOH à la surface avaient été estérifiées avec éthandiol, éthanol, butanol et octanol (→ ≫ Si-O-C [illegible]), ou elles avaient réagies avec triméthyle-chlorosilane (→ ≫ Si-O-Si (CH$_3$)$_3$) et triisobutyl-Al (→ ≫ Si-O-Al (OH)$_2$). Par ces réactions, une couverture monomoléculaire des particules de SiO_2 avait été formée. Ces couches à la surface causaient une abolition de l'effet de SiO_2 au tissu, plus ou moins durable, mais n'ayant été que toujours transitoire. In vivo, la durée de la protection était différente et correspondait aux propriétés chimiques et physico-chimiques différentes des couches à la surface. Par la couverture temporaire, les signes caractéristiques de la „réaction silicotique du tissu" devenaient reconnaissable aussi chez l'aerosil à grains très fins (désion des phagocytes jusqu'à nécrose et formation des fibres.

Les couches monomoléculaires de mêmes molécules, couvrantes les surfaces de la poussière non réactive de TiO_2, ne conduisaient pas aux lésions des phagocytes et à une formation des fibres et, ainsi, ne donnaient pas un appui pour un auto-effet des molécules couvrantes.

La couche monomoléculaire de SiO_2 à la surface de Al_2O_3 ne démontrait pas une „réaction silicotique du tissu".

Summary

Report on animal experiments in which have been examined:

1. SiO_2, the surface of which had been covered by a monomolecular layer of alcohols, groups of trimethylsilyl- and Al-oxydhydrate,
2. TiO_2, the surface of which had been covered by the same monomolecular layers, and
3. a monomolecular layer of SiO_2 on Al_2O_3.

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In the experiments with SiO_2-particles (aerosil, silica fume, quartzglass, quartz) the SiOH-groups on the surface had been esterified with ethandiol, ethanol, butanol and octanol ($\rightarrow \geqslant$ Si-O-C...) or had been brought to reaction with trimethylchlorosilane ($\rightarrow \geqslant$ Si-O-Si[CH$_3$]$_3$) and triisobutyl-Al ($\rightarrow \geqslant$ Si-O-Al[OH]$_3$) to form a monomolecular layer on the SiO_2-particles. In vivo these surface-layers abolished the SiO_2-effect on tissue. This protecting effect of the layers lasted for different periods of time but was always only a transitory one. The duration of the protection varied due to different chemical and physico-chemical properties of the surface-layers. By temporary covering the characteristic signs of the silicotic reaction of the tissue (lesion of dust-cells up to necrosis and formation of fibers) became recognizable even with the very fine aerosil.

The monomolecular layers of the same molecules which had covered the surfaces of the innocious TiO_2 did not lead to lesions of the dust-cells and formation of fibers and therefore did not support for an auto-effect of the covering molecules.

The monomolecular layer of SiO_2 on the surface of Al_2O_3 showed no „silicotic reaction of the tissue".

Literatur

1. E. J. King, C. V. Harrison, G. P. Mohanty und G. Nagelschmidt: J. Path. Bact. 69, 81 (1955)
2. E. J. King, C. V. Harrison, G. P. Mohanty und M. Yoganathan: J. Path. Bact. 75, 429 (1958)
3. B. D. Stacy, E. J. King, C. V. Harrison, G. Nagelschmidt und S. Nelson: J. Path. Bact. 77, 417 (1959)
4. F. J. Strecker: Beitr. Silikose-Forsch., Grundfragen aus der Silikoseforschung, Sonderband II, S. 313 (1957)
5. F. J. Strecker: Die Staublungenerkrankungen, Bd. 3, S. 229 (1958)
6. H.-J. Mohr: Beitr. Silikose-Forsch., Grundfragen aus der Silikoseforschung, Sonderband II, S. 381 (1957)

Diskussion zu den Vorträgen 9 und 10

Thomas, Göttingen

Mit dem Aluminium-triisobutyl muß man unter Sauerstoff- und Wasserabschluß arbeiten, das ist nicht ganz so einfach, aber es geht, wie Sie gesehen haben. Auch wenn man einen großen Überschuß von dem Aluminiumalkylat nimmt, bekommt man trotzdem nur eine monomolekulare Schicht; erst wenn man hydrolysiert und neue OH-Gruppen entstehen, dann geht die Belegung weiter.

Zu den Strecker schen Versuchen: ein Restbestand von SiO_2 in fester Form muß da sein, damit, wenn die Zelle phagocytiert hat, dann die fibrotischen Reaktionsfolgen entstehen.

Kuchen, Aachen

Es würde mich interessieren, wie die Veresterung des Aerosils mit Alkohol präparativ durchgeführt wurde, und zweitens: wie und auf welche Weise der Veresterungsgrad, d. h. das Verhältnis Si-OR/Si-OH, ermittelt wurde?

Stöber, Göttingen

Das Verfahren ist sehr einfach. Wir haben in einen Autoklaven Aerosil eingebracht und darunter einen großen Überschuß von wasserfreiem Alkohol. Dann haben wir die Temperatur auf etwa 200 bis 250° steigen lassen, wobei sich der Dampfdruck des Alkohols auf etwa 15—25 atü einstellt. Dann haben wir das 8 Stunden reagieren und anschließend den Autoklaven abkühlen lassen, wobei sich die flüssige Phase im wesentlichen schon abtrennte. Das Aerosil lag in seinem Behälter verhältnismäßig trocken vor, es ließ sich aber in der Vakuumapparatur noch eine größere adsorbierte Menge Alkohol abpumpen. Wir gingen dabei in der Hochvakuumapparatur bis zu einem Absaugdruck von 10^{-4} Torr bei 150° C. Der Alkohol, der dann noch auf der Oberfläche war und den wir durch Abbrand der Oberfläche und Bestimmung der abgebrannten CO_2-Menge ermittelten, haben wir als irreversibel adsorbierten oder chemisorbierten Alkohol bezeichnet.

Kuchen, Aachen

Und die Feststellung der Hydroxylgruppen in Aerosil? Es ist doch nicht anzunehmen, daß eine restlose Umsetzung stattgefunden hat, besonders bei den sperrigen Alkoholen mit langen Alkylresten.

Stöber, Göttingen

Die Gesamtmenge der OH-Gruppen auf der SiO_2-Oberfläche haben wir auf verschiedene Weise festgestellt[1]: Wir haben den thermischen Wasserabbau und die Adsorption des SiO_2 gegenüber Ammoniak gemessen. Man kann dann Relationen aufstellen zwischen der Konzentration der OH-Gruppen und der adsorbierten Ammoniakmenge. Wir sind danach auf einen Wert von 6,2 μ Mol/m² Hydroxylgruppen gekommen, der sich nicht in dieser Exaktheit aufrechterhalten läßt, aber man kann sagen, im allgemeinen haben die SiO_2-Oberflächen eine Oberflächenbelegung an OH-Gruppen zwischen 5 und 6,3 μ Mol/m². Das reproduziert sich immer wieder und ist offenbar die maximal mögliche Belegung an porenfreien kompakten SiO_2-Körpern.

Seifert, Münster

Herr Strecker hat aus seinen Untersuchungen einen sehr folgenschweren Schluß gezogen, den ich zwar nach dem Vorgetragenen noch nicht zu übersehen vermag, da mir das Biologische etwas fern liegt. Er hat gesagt, die Kristallstruktur wäre nicht maßgebend für die Entstehung der Silikose, denn das Aerosil machte es ja auch. Er hat dann zum Schluß, und das ist zunächst für mich ein gewisser Widerspruch, gesagt, das SiO_4-Tetraeder sei aber notwendig. Daher zunächst zu meiner Orientierung die konkrete Frage, ob Herr Strecker meint, daß das Aerosil SiO_4-Tetraeder enthalte.

Stöber, Göttingen

Selbstverständlich besteht Aerosil aus SiO_4-Tetraedern. Im übrigen sollte mit der Formulierung von Herrn Strecker weiter nichts zum Ausdruck gebracht werden, als daß für die Entstehung der Fibrose, wie sie Herr Strecker findet, Voraussetzung ist, daß ein festes SiO_2-Teilchen, d. i. ein Teilchen aus vernetzten SiO_4-Tetraedern, das nach unseren Untersuchungen OH-Gruppen an der Oberfläche trägt, vorliegt, d. h., daß wir Fibrosen nicht erzeugen können dadurch, daß wir nur gelöste Kieselsäure in den Körper schicken; daß wir Fibrosen

[1] W. Stöber: Kolloid-Z. 145, Bd., Heft 1, S. 17 (1956)

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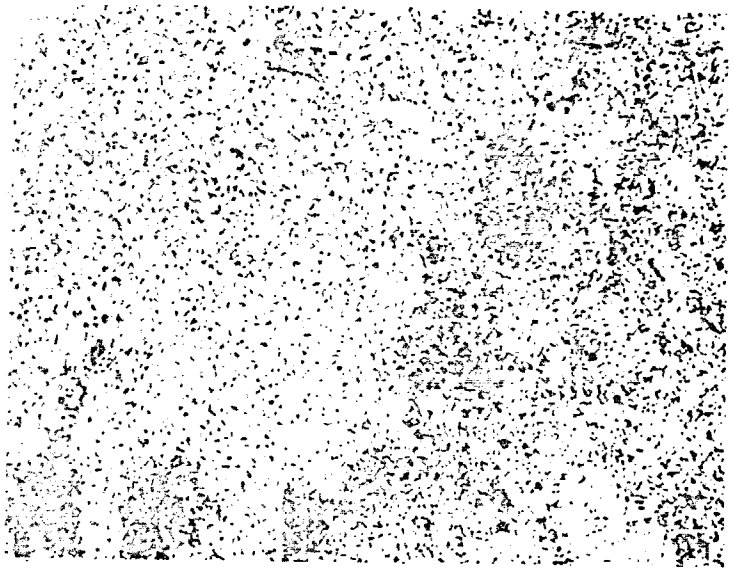

Abb. 3: Mediastinale Lymphdrüse zu Abb. 1. Weitgehende reticulohistiocytäre Umwandlung. Färbung H.E., Vergrößerung 125 ×.

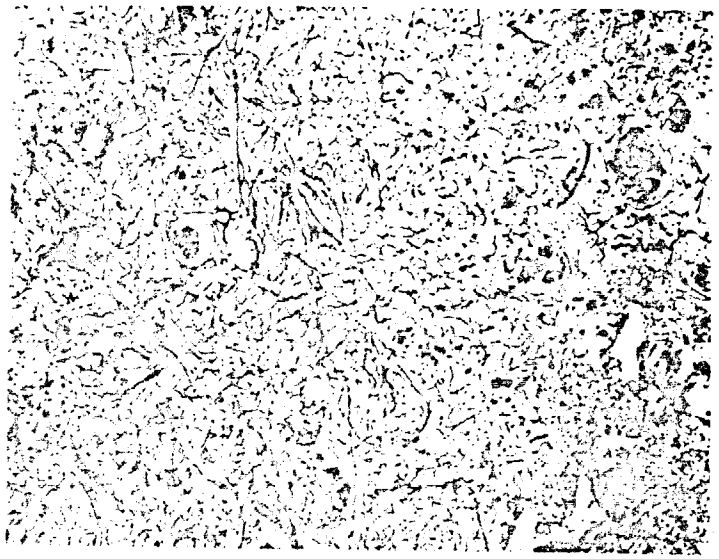

Abb. 4: Mediastinale Lymphdrüse zu Abb. 1. Färbung n. Gömöri. Vergrößerung 125 ×.

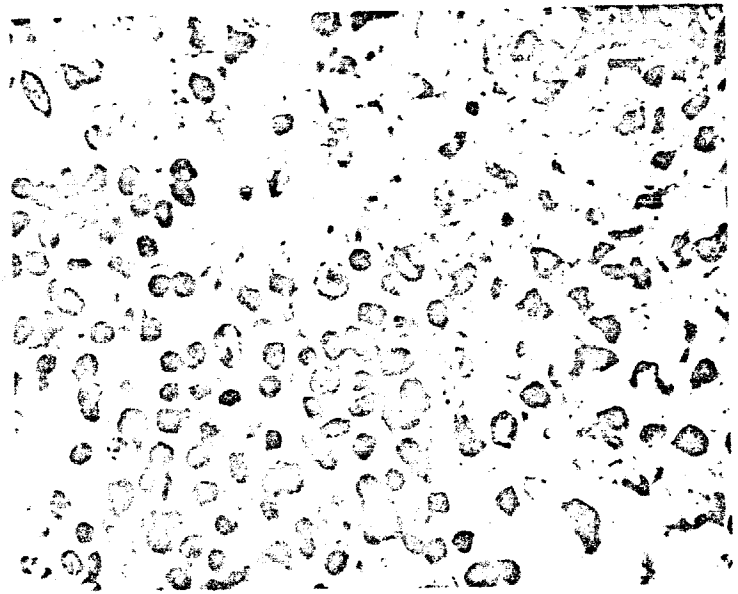

Abb. 5: Mediastinale Lymphdrüse zu Abb. 1. Färbung H.E., Vergrößerung 500 ×.

Die Gewebsreaktion auf Aerosil gleicht weitgehend derjenigen auf Quarz in frühen Stadien. Ganz besonders kann man das nach längerer Inhalation (6 Mon.) bei Ratten in den mediastinalen Lymphdrüsen sehen. Diese zeigen praktisch die gleiche reticulohistiocytäre Umwandlung mit Zelluntergang wie unter Quarzinhalation. Auch die Lungenherde gleichen frühen Quarzgranulomen. Aus der Gleichartigkeit der Veränderungen darf man wahrscheinlich auf ein gleichartiges Wirkungsprinzip schließen. Quarz wirkt allerdings als „Depotgift" unvermindert weiter, während die Aerosilwirkung nach einiger Zeit nachläßt, und zwar durch Lösung und Ausscheidung und vermutlich durch die von uns beschriebene „Einhüllung" der phagocytierten Aerosilaggregate. Zum Schluß hier noch einige Bilder aus Ratten-Inhalationsversuchen mit 150 m^2/g-Aerosil.

Harder, Münster

Ist es denn ein so wesentlicher Unterschied zwischen kristallisierter Kieselsäure und röntgenamorpher Kieselsäure? Sind es nicht nur größenordnungsmäßige Effekte, wenn nicht mehr so viel Zellen gittermäßig geordnet sind, daß wir keine Abbeugung bekommen? Ist das so wesentlich? Ich glaube doch nicht. Denn man muß ja auch mit einer Nahordnung der SiO_4-Tetraeder in der röntgenamorphen Kieselsäure rechnen, die kann ja ganz ähnlich sein wie die des Quarzes.

Stöber, Göttingen

Im wesentlichen ist das eine Definitionsfrage. Bei früheren Diskussionen ist an dieser Stelle immer die Frage aufgetaucht, ob man SiO_2 überhaupt amorph nennen kann. Bisher haben wir uns dabei stets so geeinigt, daß wir sagten eine kompakte SiO_2-Phase ist amorph, wenn lediglich eine Nahordnung zwi

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Verfliegen der freien SiO_2-Oberfläche gebunden ist. Es zeigte sich aber auch, daß die Aerosolteilchen nach Verlust ihrer experimentell gesetzten monomolekularen Maskierungsschicht nicht nur recht schnell weggelöst werden, sondern auch im Gewebe schnell aggregieren und mit Bindegewebe umhüllt werden. Eine derartige Einhüllung, sieht man gleichfalls bei Inhalation unbehandelter Aerosilpräparate. Diese eingehüllten Teilchenaggregate sind nach dem Gewebsbefund offensichtlich unwirksam. Der Organismus ist also in der Lage, derart feine SiO_2-Teilchen durch Einhüllung wirksam zu maskieren, obwohl der Lösungsprozeß auch in der Einhüllung weitergeht. Größere SiO_2-Teilchen — kristallin oder amorph — können dagegen offenbar nicht in dieser Form vom Organismus unschädlich gemacht werden.

Aus diesen Ergebnissen glauben wir ableiten zu dürfen, daß die sehr feinteiligen SiO_2-Präparate, wie das Aerosil, unter gewöhnlichen Versuchsbedingungen nicht deshalb so wenig im Sinne einer silikotischen Gewebsreaktion wirken, weil ihnen die hochgeordnete Fernstruktur des Kristalls fehlt, sondern weil sie so sehr feinteilig sind. Die Ergebnisse mit den zeitweise maskierten Aerosilpräparaten sprechen insgesamt sogar sehr dafür, daß die Aerosolteilchen an sich durchaus alle Grundbedingungen für die Auslösung der „silikotischen Gewebsreaktion" erfüllen.

b) Monomolekulare SiO_2-Schicht auf Al_2O_3:

In diesen Versuchen zeigte sich, daß voraussichtlich nicht einfach eine beliebige SiOH-Oberfläche, sondern offenbar ein kompakter SiO_2-Körper Voraussetzung für die „silikotische Gewebsreaktion" ist. Möglicherweise könnten dabei Gruppierungen der SiO_4-Tetraeder in Nahordnungsbereichen eine Rolle spielen.

c) Aus allen Versuchen geht weiter hervor, daß einfach gelöste Kieselsäure kaum das schädigende Agens sein kann; denn es gibt keine Parallelen zwischen der Menge an lokal pro Zeiteinheit in Lösung gehender Kieselsäure und dem Ablauf der silikotischen Gewebsreaktion, wie dies ja auch sonst bekannt ist. In unseren Versuchen haben wir sogar erhebliche Anhaltspunkte dafür, daß einfach gelöste Kieselsäure auch dann, wenn sie in Staubzellen frei wird, nicht zur silikotischen Gewebsreaktion führt. (Auflösung der „Glanzkörper" bei den Aerosilpräparaten; Ablösung der SiO_2-Schicht von Al_2O_3.)

Im übrigen möchte ich noch kurz auf einen Unterschied zwischen Quarz und Quarzglas im Tierversuch hinweisen: Aus den Lösungsversuchen, die Herr Stöber demonstriert hat, geht sehr schön hervor, daß Quarzglas sich wesentlich schneller auflösen muß als Quarz. Das ist auch im Gewebe deutlich zu erkennen, wenn man gut vergleichbare Korngrößen von Quarz und Quarzglas im Tierversuch einsetzt und dann die Versuchszeit sehr lang wählt. Mit zunehmender Versuchszeit werden beim Quarzglas die Staubdepots in den Granulomen deutlich kleiner, und es ergibt sich eine eindeutige Differenz zum Quarz. Das ändert aber nichts daran, daß Quarzglas eine sehr massive „silikotische Gewebsreaktion" hervorruft, gibt jedoch eine gute Erklärung dafür, daß Quarzglas bei den intratrachealen Rattenversuchen von King und Mitarbeitern[2] nicht zum Stadium V der Kingschen Klassifikation geführt hat.

[2] E. J. King, G. P. Mohanty, C. V. Harrison u. G. Nagelschmidt: Brit. J. Industr. Med. 10, 9—17 (1953).

H. Seifert, Münster (nachgereicht)

Vom Standpunkt der Matrizentheorie möchte ich nach Überdenken der gesamten Diskussion und Kenntnisnahme einer Reihe wichtiger Änderungen und Nachschriften kurz folgendes feststellen:

Die sehr interessanten tierexperimentellen Untersuchungen von Herrn Strecker berechtigen nach unserer Auffassung nicht zu der eminent wichtigen Folgerung, daß die Kristallstruktur von Staubteilchen nichts mit der Silikose zu tun habe. Im Gegenteil berechtigen sie zu der Aussage, daß die menschliche Silikose nur durch kristalline Kieselsäure hervorgerufen wird.

In der gewählten unsicheren Formulierung der Notwendigkeit „kompakter" Partikel amorpher SiO_2 vermag ich nur ein halbes Zugeständnis an die theoretische Grundlage der Matrizentheorie zu sehen — soll sie überhaupt einen tieferen Sinn haben und, was gewiß nicht beabsichtigt ist, nur eine Verlegenheitslösung darstellen.

Die Ursache der durch amorphe SiO_2 hervorgerufenen zeitlich sehr begrenzten Gewebsveränderungen bleibt nach Strecker unbekannt. Ebensowenig vermag die von den meisten der Herren Diskutierenden geteilte Auffassung Streckers die frappanten Intensitätsunterschiede der silikotischen Schädigungen der menschlichen Lunge und tierischen Gewebe durch die verschiedenen kristallisierten Modifikationen der SiO_2 zu erklären. Das vermag nur die Matrizentheorie, wie zu zeigen sein wird.

Anm. der Redaktion: Die nachgereichte obige Diskussionsbemerkung wurde Dr. Strecker zur Kenntnis gegeben. Dr. Strecker verzichtete auf eine Antwort unter Hinweis auf seinen Vortrag und die Diskussion, insbesondere auf die Diskussionsbemerkungen von Professor Hofmann (S. 150) und Dr. Stöber (S. 149).

allen SiO_4-Tetraedern mit einer gewissen Spielbreite verschiedenartiger Anordnung besteht. Dieser Nahordnung haben wir in der Kontakttheorie nach dem bekannten principle of least motion die entscheidende Bedeutung zugesprochen, wenn z. B. die ganze SiO_2-Wirkung etwa auf einem katalytischen Effekt beruhen sollte. Als kristallin sollte dagegen erst eine Phase gelten, die zwischen sich wiederholende Lasbeziehungen, d. h. eine Fernordnung ihrer SiO_4-Tetraeder aufweist. Der bisherige Gegensatz zwischen der Kontakttheorie und der Matrixentheorie kam daher, daß nach der Matrixentheorie die Nahordnung als unzureichend angesehen wurde, und daß eine Fernordnung über ausgedehnte kristalline Flächenbereiche als notwendig erachtet wurde, um silikogene Prozesse einleiten zu können. Dieser Auffassung können wir nicht zustimmen.

Hofmann, Heidelberg

Selbstverständlich ist es sinnvoll, beim Siliciumdioxyd zwischen den kristallinen Modifikationen, z. B. dem Quarz und den amorphen Formen, wie sie z. B. im Quarzglas und im Aerosil vorliegen, zu unterscheiden. Hier ist zwar jedes Siliciumatom genauso wie im Quarz tetraedrisch von 4 Sauerstoffatomen umgeben. Aber die weitere Anordnung ist nicht mehr periodisch regelmäßig. Die verschiedenen Möglichkeiten des Verknüpfungstyps führen zu einer regellosen Folge der SiO_4-Tetraeder. Der Unterschied zum Quarz ist ganz erheblich. Die Frage ist nun, wie die Oberfläche wirkt, falls sie überhaupt wirkt. Bestehen Unterschiede zwischen der kristallin geordneten Oberfläche, bei der über große Bereiche eine periodische Regelmäßigkeit der Anordnung der SiO_4-Tetraeder wiederkehrt, und der amorphen Oberfläche, bei der statistisch alles durcheinander folgt? Und da scheinen mir die Versuche von Herrn Strecker zu zeigen, daß keine Unterschiede in der Wirkung bestehen. Es scheinen also einzelne SiOH-Gruppierungen zu wirken, denen es gleichgültig ist, ob sie an einer regelmäßig geordneten Kristalloberfläche oder an einer unregelmäßig aufgebauten Oberfläche sitzen.

Schiller, Homberg

Faserknötchen mit zentraler Verschwielung entstehen an Mäusen, denen nach einmaliger intraperitonealer Applikation von 10 mg Diamantstaub (Teilchengröße 1—2 μ) über 60 Tage täglich je 1 mg Aerosil in 0,1 ml Aqua bidestillata intraperitoneal injiziert wurde*). Aerosil allein führt bei gleicher Dosierung zu Nekrosen (Breicysten), die bindegewebig organisiert werden.

Strecker*), Göttingen

Ich habe aus unseren Befunden als Schlußfolgerung abgeleitet, daß die Staubzellschädigung bis zur Zellnekrose und in ihrer Folge die Faserbildung usw. „an die freie SiOH-Oberfläche von soliden, aus SiO_4-Tetraedern aufgebauten Teilchen gebunden" ist, jedoch „nicht an die Kristallstruktur der Teilchen", da diese Wirkung „ebenso durch die amorphen SiO_2-Körper" hervorgerufen wird. Ich habe also nicht schlechthin von amorpher Kieselsäure, sondern von soliden Siliziumdioxydpartikeln gesprochen. Daß es zwischen den amorphen und kristallinen Formen der soliden, aus SiO_4-Tetraedern bestehenden SiO_2-Partikel erhebliche Unterschiede im Ordnungszustand gibt, ist in der Diskussion wohl ganz klar zum Ausdruck gekommen. In der biologischen Wirkung gibt es jedoch keine grundsätzlichen Unterschiede zwischen diesen beiden SiO_2-Modifikationen, wie dies vor allem aus den Versuchen mit Quarzglas und Quarz (in vergleichbaren Korngrößen!) hervorgeht.

Verschiedene Aerosile und sog. Quarzrauch haben wir primär nicht etwa deshalb so eingehend untersucht, um unbedingt beweisen zu wollen, auch diese amorphen SiO_2-Präparate seien „silikogen". Wir haben speziell Aerosil immer wieder benutzt, weil dieses sehr reine und gut definierte Präparat ausgezeichnete Versuchsbedingungen sowohl für die chemische und physikalisch-chemische Untersuchung der Oberflächenreaktionen als auch für den Tierversuch geboten hat. Im Tierversuch wollten wir in erster Linie das Verhalten der Oberflächenverbindungen in vivo kennen lernen. Dabei haben wir dann aber auch einen besseren Einblick in das Verhalten sehr feiner SiO_2-Teilchen im Gewebe bekommen, die wir in kristalliner Form leider aus methodischen Gründen nicht ergänzend prüfen können.

Um Mißverständnisse auszuschließen, möchte ich die gewerbehygienischen Belange und die Folgerungen für die Theorien der SiO_2-Gewebsreaktion noch einmal gesondert zusammenfassen:

1. Gewerbehygienisch ist
   a) gewöhnliches Aerosil (unbehandelt) sicher nicht „silikogen", d. h., man hat nicht damit zu rechnen, daß es silikotische Lungenveränderungen erzeugt. Um einen Orientierungspunkt zu geben, möchte ich sagen, daß dies vorerst für diejenigen Aerosilpräparate gelten darf, die eine spezifische Oberfläche von mehr als 100 m^2/g aufweisen.
   b) Dagegen können spezielle gröbere Aerosilpräparate von 50 m^2 Oberfläche/g und weniger — das ist eine Korngröße, die etwa dem sog. Quarzrauch entspricht — nach allen bisherigen experimentellen Befunden nicht einfach als „nicht-silikogen" angesehen werden. Derartige Aerosilpräparate werden z. Z. noch bei uns untersucht.
   c) Oberflächenveränderte Aerosilpräparate mit relativ beständigen Maskierungen (Veresterungen mit Alkoholen ab C_4, Trimethylsilylbelegung, Aluminiumoxydhydratbelegung) spielen zunächst z. Z. industriell keine Rolle, haben also vorerst keine gewerbehygienische Bedeutung.

   Wenn wir hier von gewerbehygienischen Fragen sprechen, möchte ich aber doch auch daran erinnern, daß „nicht-silikogen" nicht einfach mit „unschädlich" gleichgesetzt werden darf. Betrachtet man die feinstteiligen SiO_2-Präparate unter diesem Gesichtspunkt, so ist nach unseren tierexperimentellen Erfahrungen z. B. nicht auszuschließen, daß die erhöhte Kieselsäurepassage durch die Nieren bei Belastung mit solchen sehr feinen und relativ schnell in Lösung gehenden SiO_2-Präparaten bis zur Schädigung der Nieren gehen könnte. Diese Überlegung war mit ein Grund, weshalb wir uns in Göttingen u. a. näher mit den durch Kieselsäure im Tierexperiment zu erzeugenden Nierenveränderungen befassen.

2. Nun zu den Folgerungen unserer Versuche für die Theorien der SiO_2-Gewebsreaktion:
   a) Belegte Aerosile: Durch den Trick der Oberflächenbelegung gelingt es, diese sehr feinteiligen SiO_2-Präparate notdürftig über eine längere Zeit im Gewebe zu beobachten. In diesen Versuchen zeigte sich, daß die nun auch hier über einen längeren Zeitraum hin auftretende zellschädigende und über diesen Weg faserbildende SiO_2-Wirkung an das

E. Schiller: Arch. Gewerbepath., Berlin 14, 1—9 (1955); Grundfragen aus der Silikoseforschung, Sonderband 2, 421 (1956)

*) *Anm.: Des besseren Verständnisses wegen bei der Korrektur ausführlicher, aber nicht sinnverändernd formuliert.*

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auch nicht erreichen können, wenn wir die kompakten SiO_2-Partikel durch [illegible] Substanz abdecken; denn solange sie abgedeckt waren, war im Tierversuch nichts zu finden. Erst nachdem die Partikeloberfläche freigesetzt wurde und nur wenn solide SiO_2-Partikel da waren, konnte man eine Fortentwicklung der Fibrose sehen. Daher erklärt sich heute wahrscheinlich sehr einfach, daß man früher fand, das Aerosil erzeuge keine Silikose, denn normalerweise wird es ohne Belegung so schnell weggelöst, daß kaum eine Fibrose entstehen kann, beziehungsweise der toxische Effekt alles überdeckt. Wir sehen keinen grundsätzlichen Unterschied zwischen der Reaktion des Gewebes auf die kristalline Phase und der Reaktion auf die amorphe Phase. Die Unterschiede sind bestenfalls nur quantitativ.

Seifert, Münster

Hier ist aber wörtlich gesagt worden, die Kristallstruktur ist nicht mehr von Bedeutung, sie ist nicht maßgeblich beteiligt.

Thomas, Göttingen

Grundsätzlich ist sie nicht maßgeblich. Das amorphe SiO_2 macht ja auch die Fibrosen.

Klosterkötter, Münster

Um möglichen Mißverständnissen bezüglich des „Aerosils" vorzubeugen, mit dem in Münster ja sehr viel experimentiert worden ist, möchte ich zu Herrn Streckers Vortrag einige Bemerkungen machen. Die vier Jahre dauernden Inhalationsversuche mit Kaninchen von Jötten[2] und Gärtner[3] sind unter gewerbehygienischen Gesichtspunkten durchgeführt und ausgewertet worden. Das verwendete Aerosil (Oberfläche über 100 m^2/g) erwies sich bekanntlich als schädlich für die Lungen, jedoch als „nicht silikogen". Durch die Untersuchungsreihen von Schepers[4] sind diese Ergebnisse später eindeutig bestätigt worden. Inzwischen hat Volk[5]) aus Säckingen über die 14jährige röntgenologische Überwachung von 215 Aerosil-Arbeitern berichtet. Sie fand unter diesen keinen Fall von Silikose und schließt: „Die Inhalation von weniger als 120 mg hochdisperser amorpher Kieselsäure — bekannt als Aerosil — pro Tag zeigt keinen schädigenden Effekt (such as silicosis) auf die Respirationsorgane."

Man darf also daran festhalten, daß die hochdispersen Aerosile unter gewerbehygienischen Bedingungen keine Silikose hervorrufen. Man wird das in erster Linie auf die relativ hohe Lösungsgeschwindigkeit und rasche Ausscheidung zurückführen dürfen. Abgesehen davon ist Aerosil aber eine recht toxische Substanz, die bei hochdosierter Inhalation die Atemwege und die Lungen ernsthaft schädigen kann.

Unter den Bedingungen des Tierexperiments (intraperitoneale Injektion bei Ratten, intravenöse Injektion bei Kaninchen) konnten wir mit Aerosil (150 m^2/g) sehr kollagenreiche Fibrosen erzeugen, die meist später wieder abgebaut werden. Durch den Trick der Oberflächenbelegung hat Herr Strecker diese Wirkung noch verstärkt darstellen können.

[2]) K. W. Jötten und W. Klosterkötter: Arch. Hyg. Bakt. 136, 1 (1952) 1—4

[3]) H. Gärtner: Arch. Hyg. Bakt. 136, 6 (1952) 451—467

[4]) G. W. H. Schepers: Reprint from the American Medical Assoc. Arch. Industrial Health, Baird-Ward Printing Company, Nashville 1957

[5]) H. Volk: A. M. A. Arch. Environmental Health 1 (1960), 125—128

Abb. 1: Aerosil-Granulom in Rattenlunge, Inhalationsdauer 6 Mon. Färbung H.E., Vergrößerung 125 ×.

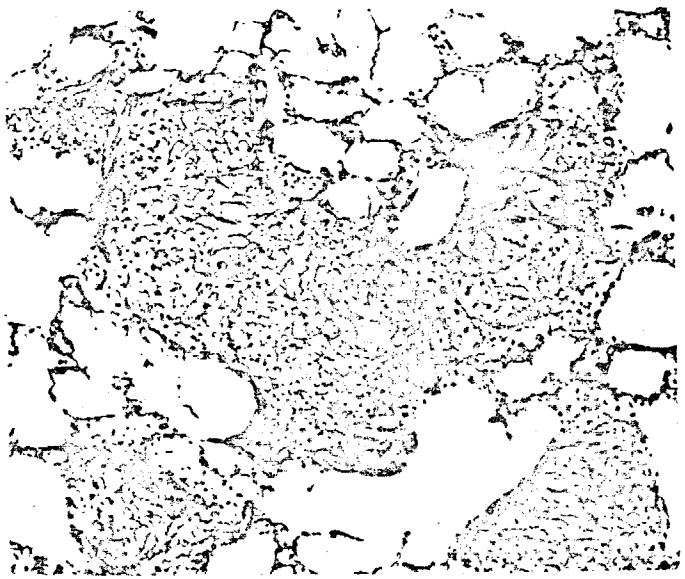

Abb. 2: Aerosil-Granulom in Rattenlunge, Inhalationsdauer 6 Mon. Färbung n. Gömöri. Vergrößerung 125 ×.

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Inhaled Particles Vapours II, Proc. Int. Symp., 2nd, Cambridge, Engl. 1965 (Publ. 1967): 111-119

SOME BIOLOGICALLY ACTIVE SUBSTANCES PRODUCED BY THE ACTION OF SILICA AND THEIR POSSIBLE SIGNIFICANCE

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Abstract — One of the striking features of an animal in a state of silica shock is the marked peripheral vasoconstriction which occurs before death. The serum of animals in a state of silica shock has been investigated and an increase of a kinin, probably bradykinin, has been demonstrated.

Premedication with substances of the anti-amine group prevents death from silica shock, and if the silica is suspended in some of these anti-amine substances, and after shaking, re-suspended in normal saline, the silica suspension is no longer toxic.

It is suggested that the release of a kinin may be a factor in the cause of death in a state of silica shock.

The urine of animals exposed to a silica dust cloud has shown an increase in 5-hydroxy-indole-acetic acid. Extractions from macrophages from animals exposed to a silica dust cloud show the presence of larger amounts of histamine than are to be found in macrophages from animals not exposed to dust.

A possible theory of the action of silica is based on the above findings.

EVER since silicosis was recognised as an industrial disease many theories have been propounded to explain the unique toxic action of silica. None of these theories have been substantiated. If it were possible to find a basic change which would account for the death of an animal after the intravenous inoculation (silica shock) and the production of silicotic fibrosis, the action of silica might well be prevented.

The state of, and death from "silica shock" has been described by many workers and was reviewed recently by HARINGTON and SUTTON (1963). From a study of the literature and from our own observations the following causes appeared to require investigation

(1) Pulmonary emboli — GYE and PURDY (1922), SIMSON and STRACHAN (1940), HARINGTON and SUTTON (1963)

(2) Anaphylactoid shock — GROSS (1955)

(3) Bronchial constriction

(4) Increased permeability of vessels — RÜTTNER *et al.* (1954)

(5) Vasoconstriction of pulmonary vessels — ANTWEILER (1953, 1954), JÖTTEN and KLOSTERKÖTTER (1952), SCHEPERS and DELAHANT (1961)

Although silica emboli can be demonstrated in the pulmonary vessels and there is evidence of a perivascular oedema in an animal dying of silica shock, these lesions do not appear to be sufficiently extensive to cause death. In none of the experimental animals were the lungs so voluminous as to suggest such marked bronchiolar vasoconstriction that there was sufficient bronchiolar obstruction to cause death by respiratory obstruction. This also cast some doubt on the cause of death being anaphylactoid shock.

One of the striking features of the state of silica shock is the well marked peripheral vasoconstriction which occurs soon after inoculation but before any alteration of the respiratory rate of the animal is evident — Figs. 1 and 2. This is followed by respiratory distress and death. During the state of silica shock it becomes increasingly difficult to withdraw blood from the ear vein of a rabbit, because of the apparent increased viscosity of the blood. In 30–40% of the animals, post-mortem examination showed the presence of thrombi in the right atrium especially in the right auricle.

EXPERIMENTAL PROCEDURES

Materials

(1) *Animals* — the rabbits and guinea pigs used in these experiments were obtained from the South African Institute for Medical Research. The rabbits weighed between 2 and 3 kg.

(2) *Silica suspension*

(a) "Aerosil" (colloidal silica supplied by Degussa, Frankfurt) containing 99·8% of silica dioxide and of particle size 100–400 Å.

(b) Ground silica (Dowson and Dobson Limited, Johannesburg). This was further ground to give a particle size of less than 1 micron.

From both of these, suspensions were made to contain 25 mg in 2 ml.

(3) *Therapeutic and prophylactic compounds*

(a) By courtesy of Sandoz Limited, Basle, Switzerland

BP 400 = 9-(*N*-methyl-piperidylidene-4′)-thioxanthene hydrogen maleate
AP 800 = [9-(*N*-methyl-piperidylidene-4′)]-1-anathioxanthene
Mellaril = 2-methylmercapto-10-[2-(*N*-methyl-2 piperidyl)ethyl]-phenothiazine
BO 329 = 3-chloro-9-(*N*-methyl-piperidylidene-4′)-thioxanthene-10-oxide
KB 95 = 1-(*N*-methyl-piperidyl-4′)-3-phenyl-4-benzyl-pyrazolone-5

(b) By courtesy of Pfizer S.A. Boehringer Ingelheim Division

AD 205 = [Dimethyl n-octyl-(Benzil acid ethyl ester)] Ammonium Bromide
AD 122 = (Atropine-*N*-n-Octylbromide)
AD 251 = (Tropinedephenyl Acetic acid Ester Bromomethylate)
NA 274 = *N*-cyclohexyl-*N*-methyl-(2-Amino-3,5-Dibromobenoxyl)-Diammonium chloride

FIG. 1.

FIG. 2.

(c) By courtesy of Petersen Limited, Johannesburg
Brontina = 3-(10,11-Dihydro-5H-Dibenzo(a,d)-cycloheptcn-5-γ-oxy) tropane citrate

(d) By courtesy of Lilly Laboratories (S.A.) (Pty.) Limited
Aerolone = Cyclopentamine hydrochloride aludrine hydrochloride Propylene glycol ascorbic acid

(e) By courtesy of Professor Schlipköter, Düsseldorf, Germany
Polyvinyl Pyridine-*N*-oxide

(f) Polybrene = Hexadimethrine Bromide

Methods

The silica suspensions were inoculated into a vein in one ear and the blood samples were taken from the other ear.

(a) Kinins—the plasma kinins were estimated using the method described by MARGOLIS (1962) and that of ARMSTRONG and MILLS (1963). In this procedure uteri from virgin rats weighing between 120 and 180 g were placed in a Burne-Dale perfusion bath and their contractions recorded on a kymograph. Rabbit blood was collected in heparinised tubes, centrifuged and acetic acid saline was added to the plasma. After boiling, the precipitate was discarded and the supernatant fluid tested for activity in the Burne-Dale perfusion bath.

(b) Histamine—the amount of histamine in plasma was measured after precipitating the protein with ammonium sulphate. The supernatant fluid was centrifuged at 15,000 revolutions per minute and a few drops of hydrochloric acid and ammonium sulphate were added. After centrifuging the fluid again the pH was adjusted to 7 and the histamine adsorbed onto charcoal. It was eluted from the charcoal by a mixture of hydrochloric acid and ethanol in the proportions 2 : 1, after which the mixture was concentrated on a water bath and tested for activity on guinea pig ileum, CODE and MCINTIRE (1956).

(c) 5-Hydroxy-indole-acetic acid—5-Hydroxy-indole-acetic acid in the urine of guinea pigs using the method described by UDENFRIEND *et al.* (1958).

(d) 5-Hydroxytryptamine—5-Hydroxytryptamine was estimated by adding 5 ml of blood to 25 ml water and 5 ml of 10% zinc sulphate. After thorough mixing 2·5 ml of normal sodium hydroxide was added. After shaking this was centrifuged at 2500 rev/min for 30 min. The supernatant was placed in a stoppered tube and the pH adjusted to 10 with sodium carbonate. Five millilitres of borate buffer (pH 10) was added and the solution saturated with sodium chloride. Fifteen millilitres of N butanol was then added and the mixture shaken. After standing the butanol layer was transferred to another stoppered tube, containing 15 ml of heptane and 2 ml of 0·1N hydrochloric acid.

After shaking well, the mixture was centrifuged and the HCl layer removed. This was then concentrated over nitrogen and evaporated to dryness and taken up in water for testing in the Burne-Dale perfusion bath, UDENFRIEND *et al.* (1958).

Chromatography

Paper chromatographic separation of plasma extracts were performed on Whatmans No. 1 paper strips, using water, acetic acid, butanol in the proportions of 125:125:100 respectively as a solvent during a descending run. Synthetic bradykinin, supplied by Sandoz Limited, was used as a marker and detected by spraying the chromatogram with 0·3% Ninhydrin in acetone. Duplicate spots of plasma extracts from blood taken before and after the inoculation of silica were examined.

Silica-Solubility Depression

This was carried out using the modified method of DALE and KING (1953).

RESULTS OF EXPERIMENTS

Kinins

There was an increase in one of the kinins in the blood of the rabbit after the inoculation of silica when the animal went into a state of silica shock. Such an increase did not occur in a rabbit not exhibiting silica shock — Fig. 3.

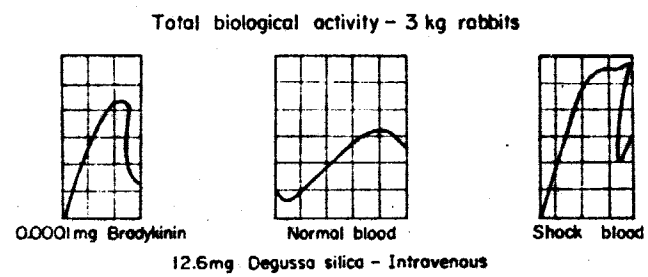

FIG. 3.

On paper chromatography this kinin has a Rf value of bradykinin and elution of the segments of these chromatograms with Ringers solution and subsequent testing for activity showed that an active substance was present. This kinin had a similar action to bradykinin in the Burne-Dale bath.

Lungs of guinea pigs not exposed to dust and exposed to a silica dust cloud were homogenised and the homogenate examined for the presence of kinins. Although the results were not always consistent there was evidence of an increased kinin content in the lungs of guinea pigs exposed to silica dust.

Serotonin

24 hour specimens of urine were examined for 5-hydroxy-indole-acetic acid both by colorimetric estimation and paper chromatography. After 5 months of exposure to a silica dust cloud there appeared to be a tendency to an increase of 5-hydroxyindole-acetic acid.

Although the daily excretion of an individual guinea pig varied from day to day the 5-HIAA value for the urine collected from guinea pigs exposed to a silica dust

cloud was 110 μg/24 hr sample as compared with 75 μg/24 hr sample from those guinea pigs not exposed to dust.

Following on the intratracheal inoculation of a silica suspension there was an increase of 5-HIAA in the urine 4–5 days after inoculation.

Silica-solubility depression

The various anti-amine preparations were tested for their effect on the amount of silicic acid released from silica in a fluid medium.

TABLE 1. ANTI-AMINES

Compounds	Silica shock prevention	Percentage silica solubility depression
Brontina	Positive	87
BP 400	Positive	81
Polyvinyl Pyridine-*N*-oxide	Negative	82
AP 800	Negative	64
NA 274	—	38
Polybrene	Positive (24 hr protection)	31
BO 329	Negative	29
Mellaril	—	26
AD 122	Negative	20
Aerolone	Negative	20
AD 251	Negative	12
AD 205	Negative	12
Sch 2544	Partial protection	0
Sch 5030	Negative	0
KB 95	Negative	—

From these results both BP 400 and Brontina could depress silica solubility almost to the same extent as aluminium oxide. These compounds have an antibradykinin and antiserotonin action pharmacologically and are used in the treatment of asthma and bronchospasm.

Rabbits were given 3 mg of BP 400 intravenously 10 min before the inoculation of 25 mg of silica, and one-third of the animals died. Others were given 4 mg of BP 400 followed immediately by the silica suspension into the opposite ear. Half of this group were given further inoculations of 1 mg of BP 400.

None of these animals showed any evidence of silica shock after inoculation with the silica suspension.

The silica powders were then suspended in BP 400 and Brontina, the suspensions shaken and centrifuged. After resuspending the silica in saline the suspensions were inoculated into rabbits. There was no evidence of silica shock even when 5 times the amount of silica was used.

Alveolar macrophages were harvested from guinea pigs and rabbits exposed to a silica dust cloud and extracts from them were compared to those from macrophages

harvested from control animals. The lipoid fraction was remov d inoculated subcutaneously into guinea pigs. No reaction was obtained.

The non-lipoid residues of both groups were then inoculated subcutaneously and the residue from the "dusted macrophages" produced a large granuloma whereas the residue from the "non-dusted macrophages" elicited a minimal response. Silica was added to the "non-dusted macrophage residue" to give the same amount as was found in the "dusted macrophage residue". The inoculation of "non-dusted macrophage residue" with the added silica failed to elicit any reaction.

TABLE 2. WEIGHT OF SUBCUTANEOUS NODULES (mg) INOCULATION OF MACROPHAGE RESIDUES

"Dusted"	"Non-dusted"	"Non-dusted" plus silica
159	16	9
198	9	10
326	16	17
520		
482		
91		
Average 294·3	13·7	12·0

This finding suggested that some factor was present in the "non-lipoid dusted macrophage residue" which was responsible for producing a granuloma when inoculated subcutaneously. The macrophage extracts were then examined and it was found that, in addition to a large increase in histamine a further substance was present in the "dusted macrophages" which was absent in the non-dusted macrophages. This substance has not yet been identified. Smears made from the suspension of alveolar macrophages did not show any mast cells or basophilic leucocytes.

TABLE 3. HISTAMINE IN ALVEOLAR MACROPHAGES

Substance tested	Average contraction	Concentration histamine (μg)	Total amount in harvesting
Histamine	8	1	
Histamine	19	2	
Histamine	29	4	
Control macrophages	15	1·531	2·143
"Dusted" macrophages	19	1·95	27·26

In view of this finding it was interesting to note the case recorded by HALONEN *et al.* (1962) from Finland. As a young woman this patient worked for a short period in a ceramic factory — a period of time which was not considered sufficient to produce severe pneumoconiosis. Radiological examinations did not reveal any abnormality until 15 years after leaving the factory. At that time she was found to be suffering from a carcinoid tumour and at autopsy this was shown to have metastasised to the liver. The marked silicosis was confirmed at post-mortem.

DISCUSSION

The possible implications of these findings are that silica either from the cells of the connective tissues or from the macrophages may release small amounts of a similar active principle as produced by the carcinoid tumour. It is known that these tumours will produce fibrotic changes in the pulmonary vessels which are not related to the associated pulmonary hypertension. It is suggested that the continual release of such an active substance may cause the perivascular fibrosis which makes up the peripheral part of the silicotic islet. Micro-incineration of these islets show that there is little dust in the centre of the islet and it is suggested that the fibrosis of the central part of the islet is caused by the organisation of a thrombus with subsequent collagenisation. Thrombus formation may be initiated by the local release of the Hageman factor — Fig. 4.

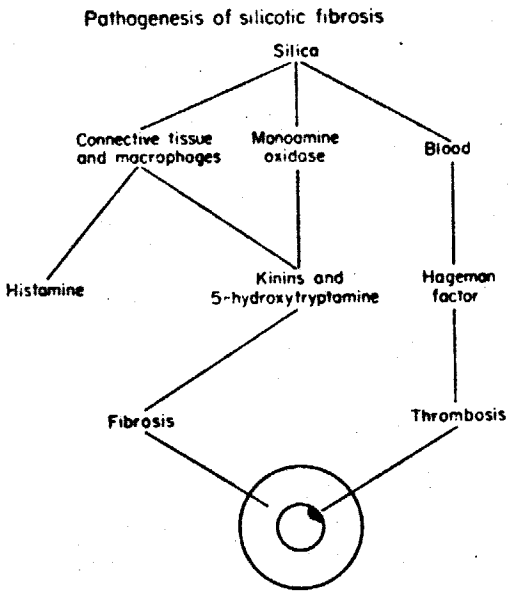

FIG. 4.

DAUTREBANDE (1962) has indicated the use of an "Aerolone" aerosol in certain mines in Katanga. Although this is used to relieve bronchospasm he suggests that if the dust load is low, Aerolone may affect the action of silica. From a comparison of the relative effects of Aerolone, BP 400 and Brontina on the surface of silica it is evident that the latter two compounds are much more effective. Unfortunately, it does appear that neither BP 400 nor Brontina remain on the silica particle for long periods. After a discussion with MARKS (1964) it was decided to determine this by estimating the amount of silicic acid in the urine after the intraperitoneal inoculation of silica and silica coated with BP 400 and Brontina. The results of this experiment are shown in Fig. 5 and indicate that BP 400 did prevent the release of silicic acid to some extent.

Further research work is necessary before the validity of this theo be assessed, but those aspects which have already been investigated tend to suggest that it may well explain the action of silica.

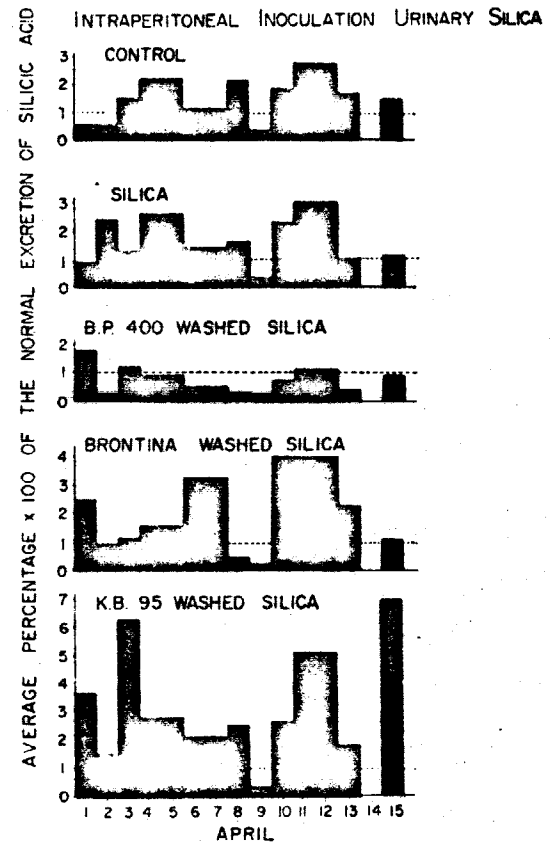

FIG. 5. Some biologically active substances produced by the action of silica and their possible significance.

Acknowledgement — We would like to express our appreciation of the co-operation and assistance which we have had from Dr. Stein of Sandoz, Dr. Durr of Pfizer and Dr. Tonkin of Scherag.

REFERENCES

ANTWEILER, H. (1953) *Beitr. Silikose-Forsch.* **29**, 19.
ANTWEILER, H. (1954) *Die Staublungenerkrankungen* (edited by JÖTTEN, J. W., KLOSTERKÖTTER, W., PFEFFERKORN, G.). Vol. 2, p. 182, Steinkopff, Darmstadt.
ARMSTRONG, D. A. J. and MILLS, G. L. (1963) *Nature (Lond.)* **197**, 490.
CODE, C. F. and MCINTIRE, F. C. (1956) *Methods of Biochemical Analysis* (edited by GLICK, D.). Vol. 3, p. 49, Interscience, New York.
DALE, J. C. and KING, E. J. (1953) *Arch. Industr. Hyg.* **7**, 484.
DAUTREBANDE, L. (1962) *Microaerosols, Physiology, Pharmacology, Therapeutics.* Academic Press, New York.
GROSS, P. (1955) *Industrial Hygiene Foundation of America Trans. Bull.* No. 28.

GYE W. E. and PURDY, W. J. (1922) Brit. J. Exp. Path. 3, 75.
HALONEN, P. I., KOSKELO, P., FRICK, M. H. and MERENMIES, L. (1962) *Acta Med. Scand.* **171**, 477.
HARINGTON, J. S. and SUTTON, D. A. (1963) *Med. d. Lavoro* **54**, 701.
JÖTTEN, W. and KLOSTERKÖTTER, W. (1952) *Arch. Hyg. (Berl.)* **136**, 1.
MAROOLIS, J. (1962) *Aust. J. Exp. Biol. Med. Sci.* **40**, 505.
MARKS, J. (1964) Personal Communication.
RÜTTNER, J. V., WILLY, W. and BAUMAN, A. (1954) *Schweiz. z. allg. Path* **17**, 352.
SCHEPERS, G. W. H. and DELAHANT, A. B. (1961) *Arch. Environm. Hlth.* **2**, 9.
SIMSON, F. W. and STRACHAN, A S. (1940) *Publ. S. Afr. Inst. med. Res.* **9**, 95.
UDENFRIEND, S., WEISSBACH, H. and BRODIE, B. B. (1958) *Methods of Biochemical Analysis* (edited by GLICK, D.). Vol. 6, p. 95, Interscience, New York.

J. Ind. Hyg. Toxicol., 19(10): 590-597, 1937

THE EXCRETION OF SILICA IN URINE AFTER ORAL ADMINISTRATION OF SILICA AND SILICATE DUSTS AND THE SOLUBILITY OF SILICA AND SILICATE DUSTS IN ACID AND ALKALINE SOLUTIONS AT 37°C*

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THE opinion now generally held regarding silicosis is that injury to the lung tissue by very sharp particles of silica is not responsible for the disease, but rather changes resulting from solution of silica, or possibly silicates, by the mildly alkaline fluid bathing the lung tissue. The solubility of air borne dusts containing silica in body fluids is, therefore, of importance, but the literature on this subject is not very extensive.

King, Stantial and Dolan (1) found that the introduction of a suspension of finely powdered quartz into the stomach of a dog caused a definite increase in the urinary output of silica, without however any corresponding increase of blood silica. Their experiments showed that dogs apparently possess a very low renal threshold for silicate. It was thought by the late Dr. J. S. Haldane that somewhat similar experiments on human beings would be useful, and this work, commenced under his direction, forms the subject of the present paper.

The experiments consisted in swallowing suspensions of fine dusts, and determining the amount of silica excreted in the urine. All the experiments were carried out by the author on himself, since the physiological effects (if any) of swallowing fairly massive doses of powdered silica are not known.

First of all it was necessary to determine the normal output of silica in urine, and a large number of samples of the author's urine were examined. The micro-colorimetric method of King (2) was adopted for the estimation of silica in the urine. It is essential in this method to employ reagents as free from silica as possible, otherwise a somewhat unsatisfactory "blank" is obtained. The distilled water employed must also be carefully checked, for in one case it was found that the "blank" was chiefly due to silica in the distilled water, the result of a badly designed still.

The silica in separate samples of urine was found to vary considerably, viz. from 0.30 to 2.0 mgm. per 100 cc., this being due partly to difference in specific gravity of the urine and partly to variation in diet. It was clear therefore that to measure any increased excretion of silica, urine collected over a period of 24 hours, or longer, would have to be used. A large number of samples of 24 hours' urine were collected during the year, and gave a fairly consistent daily

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output averaging 12.0 mgm. SiO_2 (limiting values of 7.0 to 16.0 mgm.). Certain articles of diet, in particular beer, may cause abnormally high outputs of silica, and must of course be eliminated from the diet during experiments of this kind. After drinking beer the silica content of the urine may reach as high as 9 mgm. per 100 cc. This fact is important if the urine of manual workers such as colliers, metal miners, etc. is being examined.

The dusts used in the experiments were as below:

1. Quartz: rock-crystal was ground in a steel mill and dust up to maximum particle size of 6μ separated by air elutriation.

2. Flint: ground and separated as above.

3. Scotch whinstone: ground and separated as above. A basic rock, containing no free silica, the silicates being present chiefly as labradorite, augite and serpentine (64 per cent SiO_2).

4. Precipitated silica: obtained in usual way from sodium metasilicate. Dried at 300° for 4 hours. Loss on ignition 13.3 per cent.

The dusts were taken by mouth in suspension with 200 cc. of distilled water about half an hour before a meal (mid-day). In view of the known and reputed ill effects of sand and various insoluble powders, it was deemed prudent to start with comparatively small doses of dust, and to increase the dose gradually if no ill effects were observed.

The results in table 1 show that 1 gm. of quartz (rock crystal) caused no increase in the daily output of silica in the urine, and 2 gm. of whinstone gave a similar negative result. Further doses of 3 and 6 gm. of quartz also showed no increase in urinary silica, and no other symptoms.

In order to examine how soluble silica would be eliminated from the body, a dose of 0.2 gm. of silica as silicic acid was taken by mouth. The silicic acid was prepared by neutralizing a solution of sodium metasilicate in 200 cc. water with dilute hydrochloric acid. The output of silica in the urine during the following 24 hours showed a huge increase, rising to 125 mgm., i.e. at least 110 mgm. in excess of the normal figure. From the analysis of the separate samples of urine in table 2 it will be seen that nearly 80 mgm. of SiO_2 were excreted during the first 5 hours after the dose.

The effect of precipitated silica was also investigated, the first dose consisting of 0.5 gm. being negative. A larger dose of 2.5 gm., however, raised the 24 hours' urinary silica to 36 mgm., i.e. at least two and a half times the normal output.

No significant increase in urinary silica was obtained even after a dose of 9.3 gm. of quartz, and a similar negative result occurred with 6 gm. of flint.

In the case of a dose of 6 gm. of Scotch whinstone the daily output of silica in the urine was increased to 45 mgm., i.e. at least three times the normal figure. This was an interesting result, particularly as it was somewhat unexpected. The whinstone is a basic rock containing practically no free silica, and the silicates are chiefly labradorite, augite and serpentine. As a result of experiments on the solubility of dusts in acid and alkali, referred to in the second part of the paper, it seems clear that solution of

the whinstone had been caused by the action of the acid gastric juice.

The experiments referred to above had not given any indication of solution of either quartz or flint dusts, as far as could be judged by analysis of the urine. To obtain positive results, it was evident that either the amount of the dose would have to be increased, or the range of particle size of the dust reduced. Since doses of nearly 10 gm. of dust had already been swallowed, it was considered advisable to reduce the particle size.

Flint dust of particle size up to 1 μ was, therefore, prepared by water sedimentation, and the dust suspended in water was taken by mouth as in former experiments. With a dose of 2.3 gm. of the dust, a definite increase in urinary silica was found, approximately twice the normal amount of silica being excreted in the following 24 hours. Analysis of separate sam-

TABLE 1

THE EFFECT OF ORAL ADMINISTRATION OF SILICA ON THE EXCRETION OF SILICA IN URINE

EXPERIMENT NUMBER	DOSE TAKEN BY MOUTH	DATE	VOLUME OF URINE	SPECIFIC GRAVITY OF URINE	SiO_2 IN URINE	TOTAL SiO_2 IN 24 HRS. URINE
			cc.		mgm./100 cc.	mgm.
	—	16/ 5/35	1724	1.016	0.85	14.7
	—	17/ 5/35	1718	1.015	0.73	12.5
	—	22/ 5/35	1506	1.019	0.90	13.6
I	2 gm. whinstone	23/ 5/35	1297	1.019	1.27	16.5
	—	29/ 5/35	1545	1.018	0.78	12.1
II	1 gm. quartz	30/ 5/35	1387	1.019	0.62	8.6
	—	5/ 6/35	1783	1.014	0.61	10.9
III	3 gm. quartz	6/ 6/35	1610	1.017	0.67	10.8
	—	7/ 6/35	1626	1.016	0.43	7.0
	—	17/ 6/35	925	1.023	1.28	11.8
IV	6 gm. quartz	18/ 6/35	1596	1.016	0.74	11.8
	—	27/ 6/35	1850	1.014	0.70	13.0
V	0.2 gm. silica as silicic acid	28/ 6/35	1590	1.020	7.82	124.4
	—	3/ 7/35	1345	1.018	0.78	10.5
VI	9.3 gm. quartz	4/ 7/35	1595	1.014	1.12	17.8
	—	5/ 7/35	1270	1.019	0.83	10.5
	—	1/ 8/35	1480	1.020	0.82	12.1
VII	0.5 gm. precipitated silica	2/ 8/35	2117	1.019	0.67	14.2
	—	22/ 8/35	1095	1.026	1.44	15.8
VIII	2.5 gm. precipitated silica	23/ 8/35	1485	1.020	2.40	35.7
	—	30/ 9/35	1775	1.018	0.63	11.2
IX	6 gm. flint	1/10/35	1985	1.019	0.55	11.0
	—	28/10/35	1650	1.015	0.88	14.5
X	6 gm. whinstone	29/10/35	1855	1.014	2.40	44.5
	—	1/ 4/36	1260	1.017	0.68	8.6
XI	0.22 gm. flint*	2/ 4/36	1545	1.016	1.07	16.5
	—	8/ 9/36	1720	1.013	0.76	13.1
XII	2.28 gm. flint*	9/ 9/36	2040	1.014	1.38	28.1

* Maximum particle size 1 μ.

ples of the urine (table 2) showed quite clearly that solution of the dust was almost negligible 12 hours after the dose. The rather high concentration of silica in sample (*d*) was no doubt due to the greatly increased specific gravity of the urine. The effect of variation in specific gravity of urine on its silica content has been published by Goldwater (3), who has also studied the 24 hour urinary silica excretion in persons receiving known diets.

In view of the work of King (4) on the influence of diet on the silica content of urine, the liquid and solid food consumed by the author during the necessary periods was carefully selected. Foods known to have a high silica content were avoided, and the amounts of liquids (water, tea and coffee) drunk were kept constant. With these precautions the excretion of silica in the urine could be kept within the limits stated above (7 to 16 mgm. per 24 hours). It is important to note that some supplies of drinking water may contain comparatively large amounts of silica. One supply (from a well) examined by the author was found to have 15 mgm. per L., and in this case of course the water intake has a great influence on the daily excretion of silica in the urine. The city water supply used in the author's experiments fortunately contained only 1.8 mgm. SiO_2 per L.

The above experiments on oral

TABLE 2

URINARY EXCRETION OF ADMINISTERED SILICA

EXPERIMENT NUMBER	DOSE TAKEN BY MOUTH	SAMPLE	PERIOD OF URINE COLLECTION	VOLUME OF URINE	SPECIFIC GRAVITY OF URINE	SiO_2 IN URINE	TOTAL SiO_2
			hrs. after dose	*cc.*		*mgm./100 cc.*	*mgm.*
V	0.2 gm. silica (as silicic acid)	(a)	0–5	452	1.015	17.52	79.2
		(b)	5–12	560	1.018	5.72	32.0
		(c)	12–21	490	1.023	2.28	11.2
		(d)	21–24	88	1.024	2.28	2.0
VIII	2.5 gm. precipitated silica	(a)	0–4	246	1.030	6.03	14.8
		(b)	4–10½	313	1.028	4.74	14.8
		(c)	10½–12	114	1.023	1.68	1.9
		(d)	12–22	753	1.017	0.45	3.4
		(e)	22–24	59	1.026	1.43	0.8
X	6 gm. whinstone	(a)	0–4	350	1.020	5.42	19.0
		(b)	4–8½	1080	1.012	0.85	9.2
		(c)	8½–12	227	1.023	4.02	9.1
		(d)	12–24	198	1.029	3.63	7.2
XII	2.28 gm. flint	(a)	0–1½	680	1.011	1.04	7.1
		(b)	1½–4	250	1.015	2.77	6.9
		(c)	4–8	400	1.014	1.12	4.5
		(d)	8–12	225	1.028	2.39	5.4
		(e)	12–24	485	1.018	0.86	4.2

administration of dusts are of interest in connection with the possible application of urinary silica content in diagnosis of silicosis. The excretion of silica in the urine of persons exposed to silica dust has been investigated by Bloomfield, Sayers and Goldman (5), who found close correlation between the silica dust exposure of a number of anthracite coal workers for a specific number of years and the amount of urinary silica, although it is admitted that there are insufficient data to determine the value of urinary silica examination as an aid in diagnosis. In this connection the author's experiments serve to indicate further points that must be taken into consideration, viz.

(*a*) The silica content of human urine may be greatly increased as a result of swallowing dusts containing either free silica or certain silicates.

(*b*) Dust that is initially trapped in the lungs may subsequently be conveyed into the digestive system, and as in (*a*) increase the urinary silica content.

The Solubility of Silica and Silicate Dusts in Acid and Alkaline Solutions at 37°C.

The previous experiments have shown that when fine siliceous dusts are swallowed some of the finest particles undergo fairly rapid solution; but no indication is afforded as to whether this occurred in the acid gastric juice, in the alkaline intestinal fluid, or in some combination of both. This point is of importance since the fluid bathing the lung tissue is mildly alkaline. The solubility of various dusts in solutions of known acidity and alkalinity has, therefore, been examined.

The method consisted of suspending a known weight of dust in 100 cc. of N/10 hydrochloric acid, N/10 sodium hydroxide, or 1 per cent sodium carbonate solution at 37°C., and withdrawing 10 cc. samples of liquid after intervals of 3 hours, 24 hours, and in some cases 48 hours. The samples were centrifuged for 2 hours, the clear solution siphoned off, and, after neutralization, the silica was determined colorimetrically by the method referred to previously. Nickel beakers, covers and stirrers were used with alkaline solvents, and glass bottles for acid. In the latter case the dust was kept in suspension by drawing air through the liquid, the air being passed firstly through a bottle containing N/10 hydrochloric acid in order to prevent concentration of the acid in the second bottle through loss of water. All vessels were immersed in a water bath regulated at a temperature of 37°C. Before each experiment the dust was wetted by shaking it in a bottle with the solvent.

The finest dusts (maximum particle size 1μ) were prepared by water sedimentation, and for solubility experiments the calculated amount of strong acid or alkali was added to a known volume of suspension. The other dusts were separated by air elutriation.

The flint and whinstone dusts were the same as used in the oral administration experiments, details of the other dusts being as follows:

Bull quartz: from a Canadian gold mine. Ground in steel ball mill.

Somerset greys: coal measure sandstone. Air borne dust produced by drilling, containing 49 per cent free silica.

Granite: from a Cornish tin mine. Air borne dust produced by drilling,

containing about 30 per cent free silica.

Pennant: coal measure sandstone. Air borne dust produced by drilling, containing 42 per cent free silica.

Stone dust: sample of dust used for stone-dusting underground. Fraction obtained by water sedimentation containing about 5 per cent free silica.

For three of the above samples the author is indebted to Mr. A Shaw, B.Sc., of this Laboratory, who was also responsible for the determinations of free silica. The sample of bull quartz was kindly supplied by Dr. E. J. King, British Postgraduate Medical School, and the stone dust by Dr. D. G. Skinner of this Laboratory.

The results in tables 3 and 4 show that the whinstone dust was appreciably soluble in acid, the flint dust being very slightly soluble, while the rate of solution of whinstone in sodium hydroxide was only about one-third that of flint. It seems clear, therefore, that the increase in urinary silica after swallowing whinstone dust (Part I of this paper) must have been due to the action of the acid gastric juice.

The flint and bull quartz were much

TABLE 3

RATE OF SOLUTION OF DUSTS IN N/10 HYDROCHLORIC ACID AT 37°C.

TYPE OF DUST	MAXIMUM PARTICLE SIZE	WEIGHT OF DUST	SILICA	
			3 hrs.	24 hrs.
	μ	gm.	mgm./100 cc.	mgm./100 cc.
Flint	6	1	0.4	0.8
Flint	1	0.3	—	2.7
Bull quartz	6	1	1.4	1.9
Somerset greys	6	1	3.6	5.8
Granite	5	1	5.1	8.6
Pennant	6	1	1.4	3.9
Scotch whinstone	6	1	11.3	14.4
Stone dust	1	2.3	—	9.5

TABLE 4

RATE OF SOLUTION OF DUSTS IN N/10 SODIUM HYDROXIDE AT 37°C.

TYPE OF DUST	MAXIMUM PARTICLE SIZE	WEIGHT OF DUST	SILICA	
			3 hrs.	24 hrs.
	μ	gm.	mgm./100 cc.	mgm./100 cc.
Flint	6	1	6.2	18.1
Flint	1	0.3	—	8.5
Bull quartz	6	1	2.4	7.3
Bull quartz	6	3	7.6	18.4
Bull quartz	1	0.27	2.5	7.3
Somerset greys	6	1	4.8	9.3
Granite	5	1	4.6	7.5
Pennant	6	1	2.5	5.1
Scotch whinstone	6	1	3.0	6.4
Scotch whinstone	6	3	7.5	13.2
Stone dust	1	2.3	—	4.0

more soluble in alkali than in acid, as would be expected, while the reverse of this was shown by the whinstone and stone dust. The other dusts, which included sandstones and granite, lay between these extremes, each showing fairly similar rates of solution in either acid or alkali.

If the amounts of silica from the coarser dusts dissolved by sodium hydroxide in 24 hours are compared, the figures for bull quartz, whinstone, Somerset greys and Pennant will be seen to be very similar. With the exception of the whinstone, these rocks contain considerable free silica and would be classed as dangerous with regard to silicosis. The whinstone, however, contains practically no free silica, and is regarded as harmless. Although these dusts behave so similarly in decinormal sodium hydroxide, it does not follow, however, that they would do so in a very faintly alkaline solution.

The effects on solubility of factors such as particle size and concentration of dust are quite evident in the case of bull quartz (table 4). If these factors are taken into consideration, of the dusts examined, the stone dust appears to be much the least soluble in alkali.

A few experiments on the rate of solution of some of the dusts in 1 per cent sodium carbonate solution have been carried out (table 5), and rather remarkable differences from the results with sodium hydroxide are evident. The rate of solution of flint in sodium carbonate was nearly twice as much as in the hydroxide, little difference was shown by the bull quartz, while granite was much less soluble in the carbonate than in the hydroxide.

The solubility of silica and silicates in water and blood-serum has been investigated by Titus (6), who measured the silica in true solution with the aid of an ultrafilter. No such procedure to exclude the presence of colloidal silica was employed in the author's experiments, and there would have been no object in so doing. The work of Kettle (7) has shown that colloidal silica is a cell poison, and that fibrosis will result from its action.

TABLE 5

RATE OF SOLUTION OF DUSTS IN 1 PER CENT SODIUM CARBONATE SOLUTION AT 37°C.

TYPE OF DUST	MAXIMUM PARTICLE SIZE	WEIGHT OF DUST	SILICA		
			8 hrs.	24 hrs.	48 hrs.
	μ	gm.	mgm./100 cc.	mgm./100 cc.	mgm./100 cc.
Flint	6	1	11.8	32.7	42.7
Bull quartz	6	3	—	16.6	19.2
Granite	5	3	—	4.8	5.0

The action of water and acid on different forms of silica has been studied by Lenher and Merrill (8), who suggest that the true solubility of ignited silica is the same as that of gelatinous silica, but saturation in the former case is not reached in any short period of time.

Quite recently Emmons and Wilcox (9) have given figures for the solubility of silica and silicate dusts in blood-serum. The solubility of quartz was less than either sericite or asbestos, but the results would have been more

conclusive if the preliminary treatment of the dusts with hydrochloric acid (to remove iron) had been omitted.

Measurement of the solubility of sparingly soluble substances is usually rather difficult, and this is particularly so if it is desired to obtain information as to the action of body fluids *in situ* on dusts. In the lungs, for instance, the fluid bathing the tissue is being continuously replenished. The treatment of dusts *in vitro* with a fixed volume of blood-serum for periods of about 2 months will give results that may or may not indicate the action of serum on dusts in a living organism.

Summary

1. The excretion of silica in human urine after swallowing doses of silica and/or silicate dusts, precipitated silica, and silicic acid solution has been measured. The daily excretion of silica was definitely increased with very fine flint dust, a silicate dust and precipitated silica; in the case of silicic acid solution, the increase was much greater.

2. The rate of solution of some silica and silicate dusts in acid and alkaline solutions at 37° has been determined. The amount of silica dissolved by N/10 sodium hydroxide was greatest with flint, lower values being obtained from quartz, coal-measure sandstones, granite and whinstone, while a sample of stone dust gave much the lowest figure. With N/10 hydrochloric acid as solvent, the dusts arranged in order of solubility were whinstone, granite, sandstone, quartz and flint. The amount of silica dissolved from flint by N/5 sodium carbonate was much greater than by N/10 sodium hydroxide, while the reverse of this was the case with granite. The influence on the rate of solution of such factors as particle size and ratio of dust to solvent are shown.

3. The daily excretion of silica in urine is influenced by diet, particularly if the silica ingested is in such a state that it is readily absorbed e.g. as in beer, water, etc.

4. In view of the above, it is considered that great care should be exercised in attempting to correlate urinary excretion of silica with silicosis.

BIBLIOGRAPHY

1. King, E. J., Stantial, H., and Dolan, M.: The biochemistry of silicic acid. Pt. III. The excretion of administered silica. Biochem. J., *27*, 1007 (1933).
2. King, E. J., and Stantial, H.: The biochemistry of silicic acid. Pt. I. Micro-determination of silica. Ibid., *27*, 990 (1933).
3. Goldwater, L. J.: The urinary excretion of silica in non-silicotic humans. This J., *18*, 163 (1936).
4. King, E. J., and Dolan, M.: Silicosis and the metabolism of silica. Canadian Med. Ass. J., *31*, 21 (1934).
5. Bloomfield, J. J., Sayers, R. R., and Goldman, F. H.: The urinary excretion of silica by persons exposed to silica dust. U. S. Pub. Health Repts., *50*, 421 (1935).
6. Titus, A. C.: Silica and silicate solubilities. This J., *19*, 138 (1937).
7. Kettle, E. H.: The action of harmful dusts. Trans. Inst. Min. and Met., *43*, 471 (1934).
8. Lenher, V., and Merrill, H. B.: The solubility of silica. J. Am. Chem. Soc., *39*, 2630 (1917).
9. Emmons, R. C., and Wilcox, R.: A mineralogic study of silicosis. Am. Mineralogist, *22*, 256 (1937).

Fibrogenic Activity of Different Forms of Free Silica

The Action of Fused Silica, Quartz, Cristobalite, and Tridymite on the Livers of Mice

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In a previous paper King, Mohanty, Harrison, and Nagelschmidt [1] studied different forms of free silica, i. e., fused silica, quartz, cristobalite, and tridymite, of high purity and equal particle size by injecting these substances intratracheally into the lungs of rats. In spite of almost identical silica content, size distribution, and silica solubilities, the fused silica acted less rapidly and produced less fibrosis than the crystalline forms. Of these, quartz was the next most rapidly acting and fibrogenic; cristobalite was still faster reacting and more fibrogenic; and tridymite was the most rapid in producing severe fibrosis. It appeared probable that tissue activity in the presence of the different dusts was somehow related to the crystalline structure of the particles; but the reason for this cannot yet be explained. Another possible explanation of the different pathogenicities of these several free silica dusts might be that they are removed in different amounts and at different rates from the lungs by phagocytosis and lymphatic drainage.

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From the Postgraduate Medical School of London (Dr. Zaidi, Dr. King, and Dr. Harrison) and the Safety in Mines Research Establishment (Dr. Nagelschmidt).

The present investigation was planned in order to study the differences in pathogenicity of these different forms of free silica and the intensity of tissue reaction in the livers of mice. With this organ it seemed unlikely that the results could be complicated by any variable rate of elimination of the particles, as might be the case with the lung, since it seemed that all injected particles would probably be caught in either the liver or the spleen. Moreover, we wished to determine whether the fibrogenic response to these dusts was the same in a different tissue and a different animal as that which we had found in the lungs of rats; we also wished to test whether intravenous injection of powdered minerals would result in more rapid development of fibrosis in mice livers than we had found in rats' lungs and whether such a method might be an easier and more rapid means of testing mineral dusts than the intratracheal procedure with rats and guinea pigs which we had used in the past.

The same dusts as previously used, i. e., fused silica, quartz, cristobalite, and tridymite, have accordingly been injected intravenously into mice in order to study the effect of their crystal structure (or lack of it in the case of fused silica) on the liver and spleen. The "one injection," as employed by Gardner [2] in 1938, was used; the dust was suspended in isotonic saline and injected into the tail vein of mice.

EXPERIMENTAL STUDY

Description of Samples.—Fused silica was obtained from the Thermal Syndicate and had been made from pure quartz crystals from Madagascar.

The quartz was prepared from a Belgian glass sand of high purity; the cristobalite, by heating Loch Aline quartz sand for one hour at 1620 C without flux, and the tridymite, by dissolving the glassy impurities of a silica cement, which had been for a long time in a gas retort house at approximately 1380 C. From these samples fractions below 2μ Stokes' diameter were prepared.

The quartz contained 99.2% SiO_2, and the other materials 99.5% by chemical analysis. Their silica solubilities corresponded to 13 to 14 mg. of SiO_2 per 100 ml. when a 2% suspension of them in saline buffered at pH 7.4 was agitated at 37 C for one week. Their particle sizes were all below 3.6μ, and more than 95% were below 2.6μ by microscopic measurement. Their calculated surface areas lay between 1.8 and 2.6m^2/gm. These characteristics of the samples used are set out fully in the previous paper, together with their x-ray diffraction pattern diagrams.

Preparation of Dust Suspensions.—Amounts of 200 mg. of the individual dusts were weighed and transferred into a dust grinder. Ten milliliters of isotonic saline was added, and the mixture was thoroughly ground to obtain a uniform suspension. The suspension was transferred into a screw-cap bottle and made to 20 ml. by addition of an extra 10 ml. of saline. The bottles were sterilized by autoclaving. One-half milliliter of this suspension contained 5 mg. of the dust.

Animals.—Thirty male mice were used for each of the dusts. They were Swiss strain, and their average weight was 20 gm.

Experimental Procedure.—The bottle containing the dust suspension was well shaken, and 0.5 ml. of the suspension was drawn into a 1 ml. Record syringe, to which a short No. 17 needle was fitted. The mouse was held in a short wide glass tube with its nose against a wire mesh and its tail protruding through a notch cut in a thin cork stopper which was inserted into the end of the tube following the mouse. The syringe was kept well agitated until the moment of injection, when the needle was introduced into the tail vein and the suspension injected.

Duration of Experiments.—In the quartz group a few extra animals were introduced in order to study the initial reaction to this dust; these were killed 5 minutes, 10 minutes, 15 minutes, 1 hour, 24 hour, 4 days, and 15 days after the injection.

During the course of the experiment, 9 of the fused-silica animals died, 5 of the quartz, 12 of the cristobalite, and 10 of the tridymite. Two animals from each of the groups were killed at monthly intervals up to 180 days and then at fortnightly intervals up to 273 days.

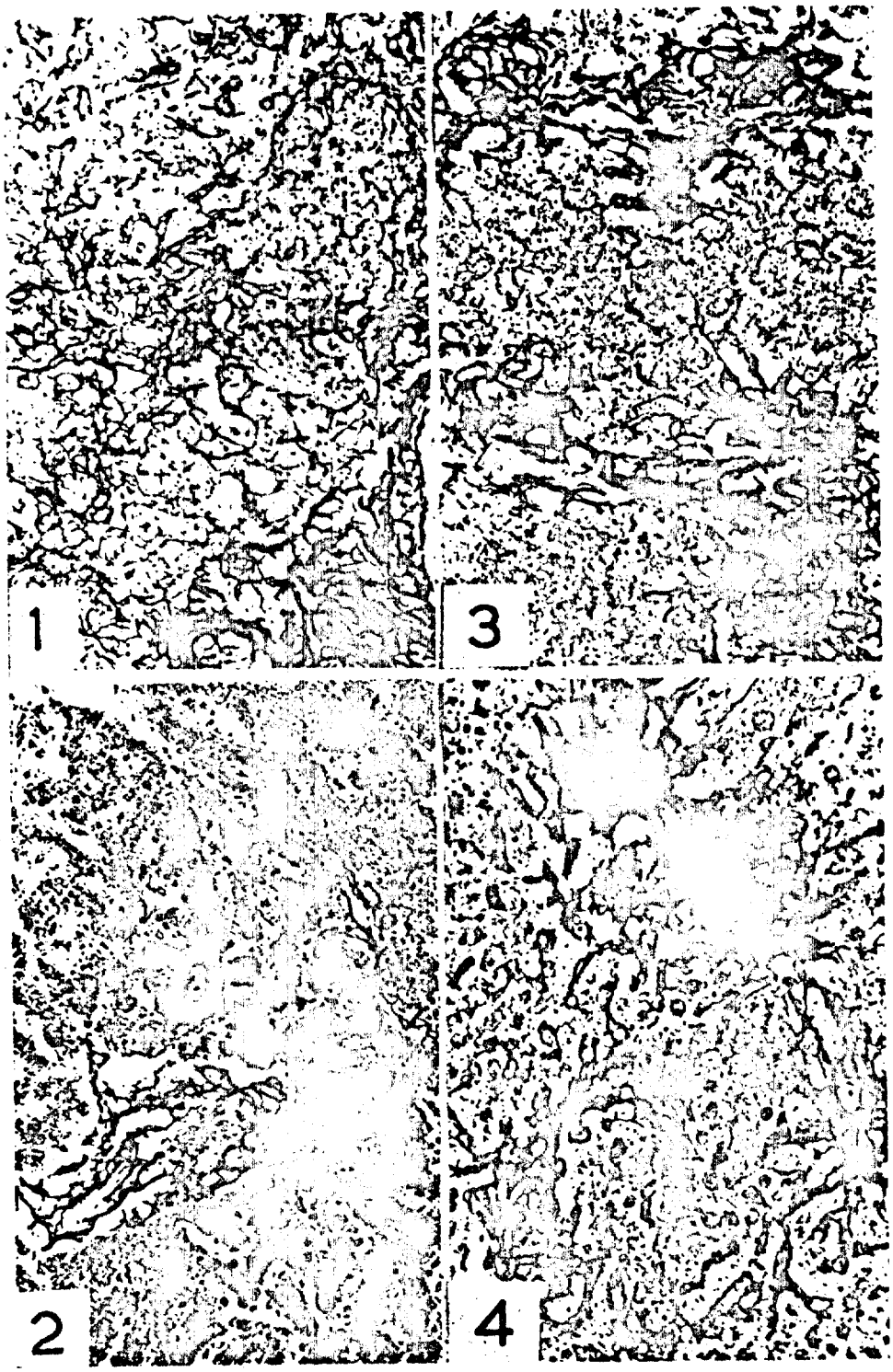
Pathological Technique.—Routine necropsies were carried out on dead and killed mice. The liver, spleen, kidneys, and lungs were fixed in 10% formol saline. In the quartz group, in addition to the above procedure, small samples of fresh tissue from the liver, spleen, lung, kidney, and bone marrow were teased out in isotonic saline and examined as wet preparations under polarized light.

After preliminary fixation, blocks were selected along the vertical axis of the liver to include the maximum representative areas of the liver for histology, and similarly from the spleen and kidneys to include their maximal diameters. The whole lungs were blocked in the earlier killings of the quartz group and the last killings of all the groups. The fixation was completed with fresh fixative, and the blocks were embedded in paraffin and sectioned at 5μ. Four serial sections were taken from each block. The first section was stained by Gordon and Sweet's[3] silver impregnation; the second was stained with hematoxylin and eosin; and the third was microincinerated. The fourth section was kept as a spare.

ASSESSMENT OF RESULTS

The liver appears to store effectively all injected silica particles, resulting in the formation of silicotic nodules (Gardner [2] and Harrison and associates [4]). These nodules resemble histologically the silicotic nodules seen in the lungs, and the classification of Belt and King [5] was easily applied in the present experiments to assess the pathological changes produced in the livers. The maturity of fibrosis with the largest number of lesions has been graded as follows: Grade 1, loose reticulin fibers with no collagen formation; Grade 2, compact reticulin fibers, with or without some collagen formation; Grade 3, mostly collagen and cellular; Grade 4, accellular collagen; Grade 5, accellular, collagenous, and confluent.

Gross Appearance of the Liver and Other Organs.—Naked-eye appearance of livers, spleens, and kidneys did not vary much. There was some enlargement of the livers and the spleens up to 90 days. The livers were pale and the cut surface showed some fine streaks, while the spleens were congested. Later in the experiment the appearance of the liver became normal, but enlargement of the spleen persisted. It was difficult to detect any naked-eye fibrosis in any of the tissues examined.



Figs. 1-4.—Sections of mouse livers 30 days after intravenous injection of 5 mg. of free silica dusts. *Fig. 1,* fused silica; nodules of very fine and loose reticulin fibers (Grade 1 fibrosis, minimal). *Fig. 2,* quartz; nodules with loose network of reticulin fibers (Grade 1 fibrosis). *Fig. 3,* cristobalite; nodules composed of compact reticulin fibers (Grade 2). *Fig. 4,* tridymite; collagenous silicotic nodule; the collagen fibers are mostly cellular (Grade 3). Silver impregnation; × 200.

HISTOLOGICAL FINDINGS

FUSED SILICA

Liver.—Fused silica produced only moderate fibrosis in the livers. At 30 days some of the dust was collected near the centrilobular veins where the particles were held together by loose reticulin fibers (Grade 1, minimal, Fig. 1); similarly at 60 days. At 90 days the small circumscribed nodules consisted of compact reticulin, but there was no evidence of collagen. By 120 days the reticulin fibers were thicker, and in some nodules collagen fibers were being laid down (Grade 2, Fig. 5). From 150 days to 225 days the lesions were mostly composed of collagen and some reticulin; but the nodules remained cellular (Figs. 9*a* and 9*b*). They did not show any tendency to spread and were small in size as compared with those produced by the other forms of free silica.

Other Organs.—The spleens, kidneys, and lungs did not show any evidence of fibrosis.

QUARTZ

Liver.—The earliest change observed was an initial concentration of particles in fixed macrophages in the liver. The concentrations of particles were focal and had a tendency to be in the region of the centrilobular veins. The livers of the mice killed at 5, 10, 15, and 60 minutes showed, on the average, 75 particles per field (× 6) lying in Kupffer cells. There were definite aggregates of quartz particles at 60 minutes. At 24 hours small aggregates of particles lying in macrophages appeared in the portal tracts. At four days more aggregates were found in the vicinity of the central vein, and fewer single particles were seen in the Kupffer cells. The aggregations near the central vein had increased in size at 15 days and were embedded in fine reticulin fibers. Particles were seen in the vicinity of the portal vein, but they were far fewer than those seen near the central vein.

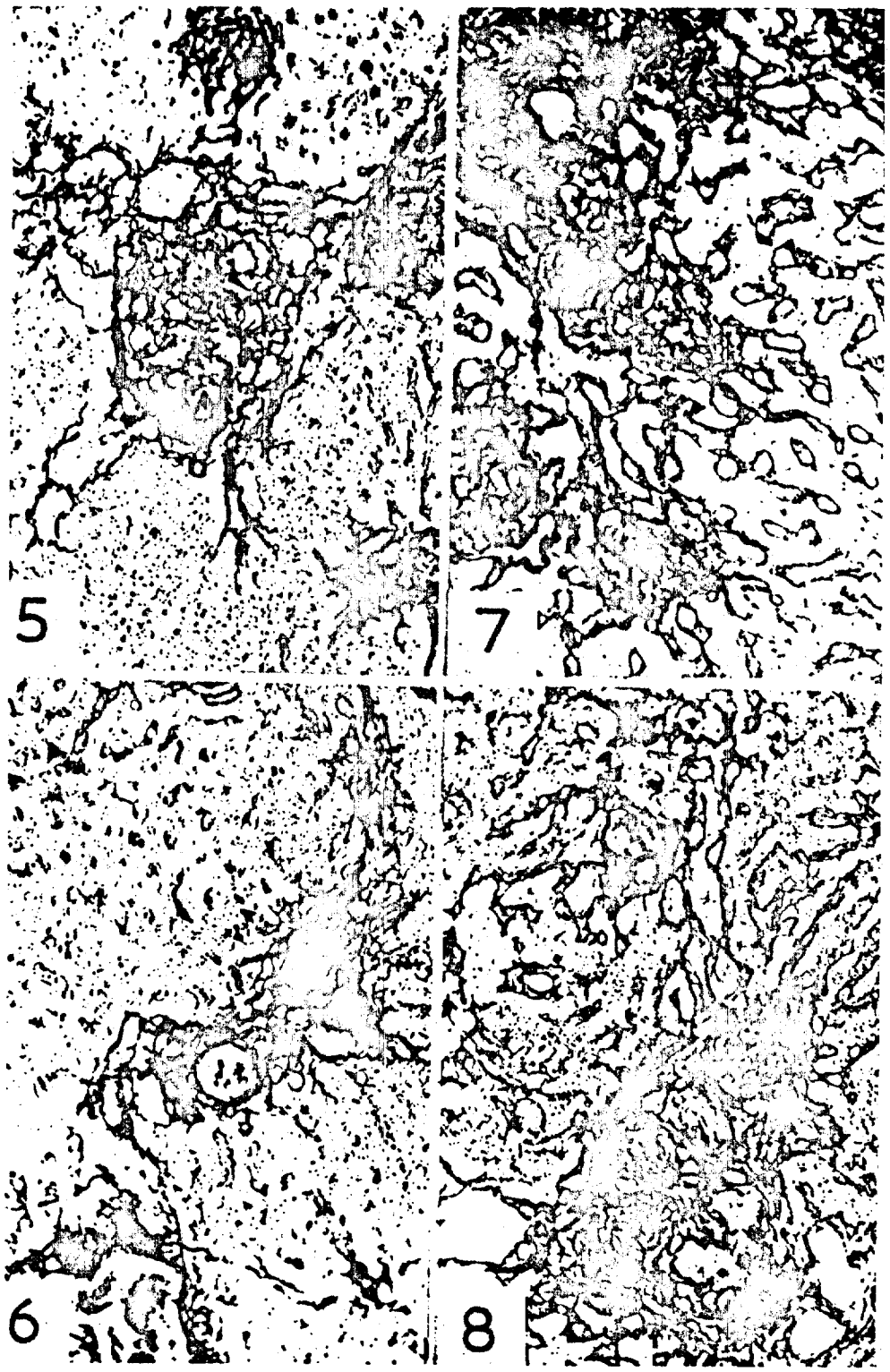
At 30 days the dust particles had collected into definite nodules in relation to the central vein and were held by reticulin fibers (Grade 1, Fig. 2). A few isolated particles were also seen in Kupffer cells. Lesions near the portal tracts were small and very cellular. In some areas there was regeneration of liver cells as shown by mitoses. Bigger lesions composed of compact reticulin fibers (Grade 1, maximal) were found at 60 days. Lesions associated with the portal tracts were more cellular than those near the central vein. More reticulin was laid down, and the nodules were denser at 90 days. At 120 days nodules near the portal tract had increased in size but were still cellular. Those near the central vein were somewhat collagenous (Grade 2, maximal, Fig. 6). At 150 days some of the nodules were largely collagenous (Grade 3). From 180 days to 225 days the livers of all the animals had nodules composed of cellular collagen fibers, but the cellularity was more marked in the lesions near the portal tract (Grade 3, Fig. 10).

Spleen.—The spleens at 5, 10, 15, and 60 minutes and at 24 hours contained a high concentration of quartz particles. Most of them lay freely in sinusoids of the spleen, but some had been taken up by macrophages. Between one and four days the activity of the macrophages increased, and big aggregates of particles were seen. At 15 days some dust particles were seen in the sinusoids; some were present in macrophages; and a few were also seen adhering to the fibrous trebeculae. A similar picture was found in the spleens up to 225 days, except that the numbers of dust particles and aggregates were considerably decreased in the later animals. There was no evidence of fibrosis in the spleen at any period.

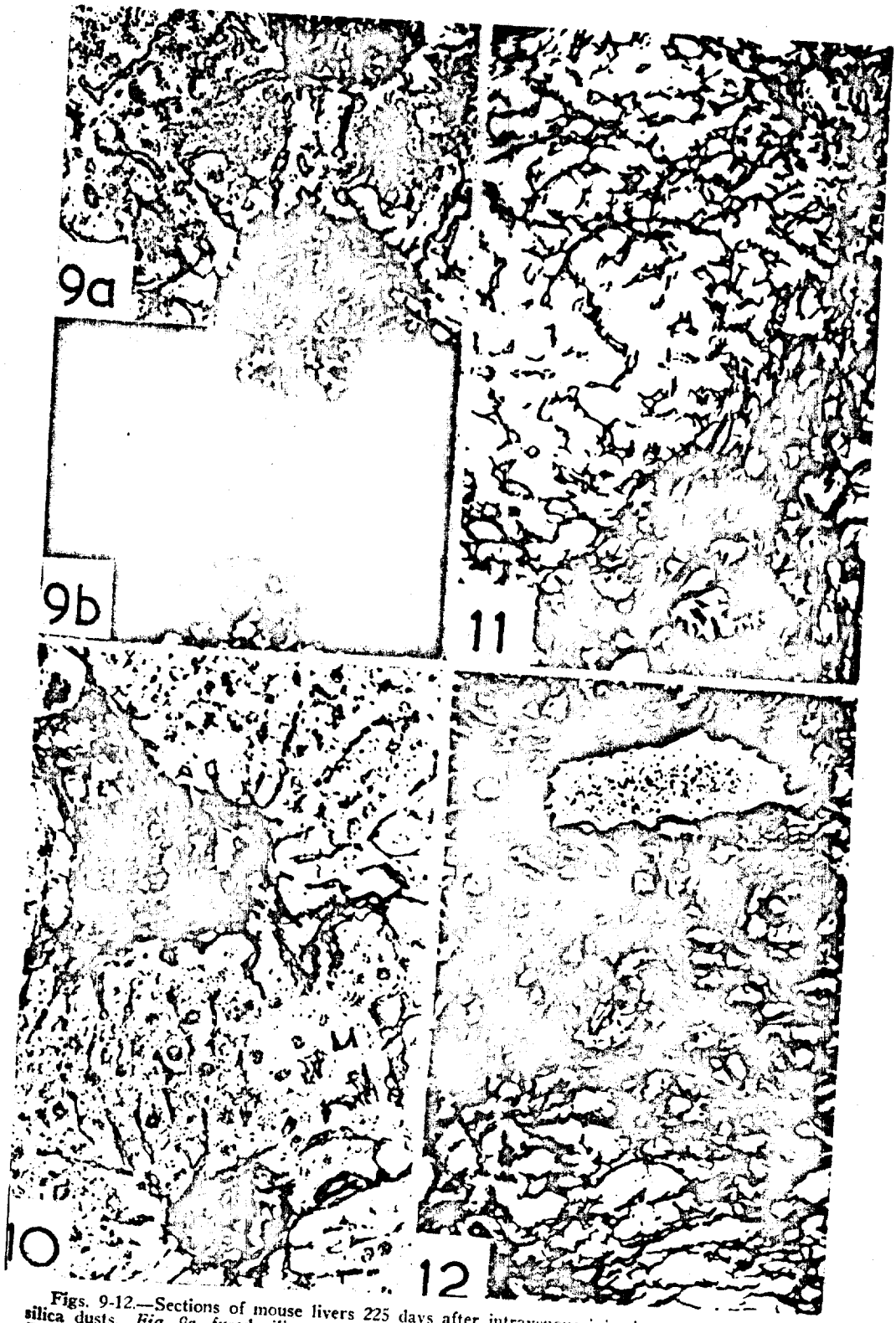
Other Organs.—Up to 24 hours after the injection a few particles could be found in the lung parenchyma, the kidneys, and the bone marrow. But after this time no particles could be detected, and there was never any evidence of fibrosis.

CRISTOBALITE

Liver.—At the 30th day there were rounded focal nodules near the central vein which were composed of compact reticulin fibers (Grade 2, Fig. 3). Lesions near the portal tracts were of loose reticulin fibers.



Figs. 5-8.—Sections of mouse liver 120 days after intravenous injection of 5 mg. of free silica dusts. *Fig. 5*, fused silica; large nodules composed of compact reticulin and a few collagen fibers (Grade 2, maximal). *Fig. 6*, quartz; nodules composed of compact reticulin and a few collagen fibers (Grade 2, maximal). *Fig. 7*, cristobalite; nodules with cellular collagen (Grade 3). *Fig. 8*, tridymite; nodules composed of cellular and acellular collagen fibers (Grade 3, maximal). Silver impregnation; × 200.



Figs. 9-12.—Sections of mouse livers 225 days after intravenous injection of 5 mg. of free silica dusts. *Fig. 9a*, fused silica; nodules composed of cellular collagen fibers (Grade 3). *Fig. 9b*, fused silica; dense aggregates of dust particles; microincinerated section (not acid washed). *Fig. 10*, quartz; nodules composed of cellular collagen fibers (Grade 3). *Fig. 11*, cristobalite; large nodule composed of cellular and acellular collagen fibers (Grade 3, maximal). *Fig. 12*, tridymite; fibrosis is fully collagenous and confluent (Grade 4, maximal). Silver impregnation; × 200.

By the 60th day most of the lesions had progressed rapidly, and they were composed of densely packed coarse reticulin fibers. Cellular collagen was being laid down at the 90th day, and at 120 days most of the nodules were composed of cellular collagen, a few being entirely acellular (Fig. 7). Maturity of fibrosis progressed from 120 to 225 days, when the nodules were nearly all acellular collagen, with only a few showing cellularity (Grade 3, maximal, Fig. 11).

Other Organs.—The lungs, kidneys, and spleens did not show any evidence of fibrosis.

Tridymite

Livers.—Two mice dead at 10 days showed central necrosis in which silica particles were seen. After as little as 30 days nodules of cellular collagen were found near the central vein (Grade 3, Fig. 4). Lesions in the portal tract were very cellular and composed of compact reticulin. The two definite types of lesions persisted; at 60 days many of the nodules near the central vein were completely collagenous and acellular; those in the portal tract were only about half acellular. Marked degeneration of liver cells was seen at 90 days, with many mitotic figures in the residual cells. Although some of the nodules were still composed of cellular collagen, more were acellular collagenous (Grade 3, maximal); similarly at 120 days, although there was now some coalescence (Fig. 8). Lesions in the portal tract were also becoming less cellular. Between 180 and 250 days there was progressive irregularity in size and shape of the nodules which now had a spreading character. By the end of the experiment the lesions were almost all acellular collagenous (Grade 4, maximal, Fig. 12).

Assessment of Fibrosis in the Livers of Mice Produced by Different Modifications of Free Silica

	Fused Silica		Quartz		Cristobalite		Tridymite	
Days of Survival	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis
0- 30	D (4)	—	D (4)	—	D (7)	—	D (7)	—
	K (1)	1 min.	K (1)	1	K (1)	2	K (1)	3
	K (1)	1 min.	K (1)	1	K (1)	2 min.	K (1)	3
31- 60	D (1)	—	D (1)	1	D (4)	—	D (2)	—
	K (1)	1	K (1)	1 max.	K (1)	2	K (1)	3 max.
	K (1)	1	K (1)	1 max.	K (1)	2	K (1)	3 max.
61- 90	K (1)	1 max.	K (1)	2 min.	K (1)	2 max.	K (1)	3 max.
	K (1)	1 max.	K (1)	2 min.	K (1)	2 max.	K (1)	3 max.
91-120	D (3)	—	K (1)	2	K (1)	3	D (1)	—
	K (1)	2 max.	K (1)	2 max.	K (1)	3	K (1)	3 max.
	K (1)	2					K (1)	3 max.
121-150	D (1)	—	K (1)	3 max.	K (1)	3 max.	K (1)	3 max.
	K (1)	3	K (1)	3	K (1)	4	K (1)	3 max.
	K (1)	3			D (1)	4		
	K (1)	3	K (1)	3	K (1)	3	K (1)	4
151-180	K (1)	3	K (1)	3	K (1)	3	K (1)	3 max.
181-195	K (1)	3	K (1)	3	K (1)	3	K (1)	3 max.
			K (1)	3	K (1)	3		
196-210	K (1)	3	K (1)	3	K (1)	3 max.	K (1)	3 max.
	K (1)	3	K (1)	3	K (1)	3 max.		
			K (1)	3				
211-225	K (1)	3	K (1)	3 min.	K (1)	3 max.	K (1)	4
	K (1)	3	K (1)	3	K (1)	3 max.	K (1)	4 max.
			K (1)	3				
226-273	K (1)	3	K (1)	3 min.			K (1)	3 max.
	K (1)	3 min.	K (1)	3 min.			K (1)	3 max.
	K (1)	3	K (1)	3			K (1)	3 max.
	K (1)	3	K (1)	3			K (1)	3 max.

K = killed; D = died; max. = maximal; min. = minimal, and — = no histology done.
Figures in parentheses indicate number of mice.

Other Organs.—No fibrosis was seen in lungs or kidneys. A few areas of thickened reticulin fibers were the only incidental findings in some of the spleens in later stages of the experiment.

ASSESSMENT OF RESULTS

A summary of the pathological changes in the livers of mice injected with the various forms of free silica is given in the Table and in Figure 13. These findings confirm the results on rats' lungs reported in our previous paper: Fused silica produces the least rapidly developing fibrosis; quartz, the second; cristobalite, the third, and tridymite, the fastest developing and severest fibrosis. Silverman and Moritz [6] studied the tissue reaction of quartz and fused silica by injecting them intraperitoneally into rats and found that quartz was more pathogenic than fused silica. Gardner [2] also found differences in the pathogenicity of the various modifications of silica in both rabbits and guinea pigs, which were similar to those reported by ourselves.

The spleen reaction to the various dusts was very slight. Particularly in the tridymite group the only evidence of any tissue reaction was the laying down of a few compact reticulin fibers. No definite silicotic nodules could be discerned in any of the spleens in any group. This is in contrast to the findings of Gardner with the spleens of guinea pigs and rabbits and of those of Tebbens.

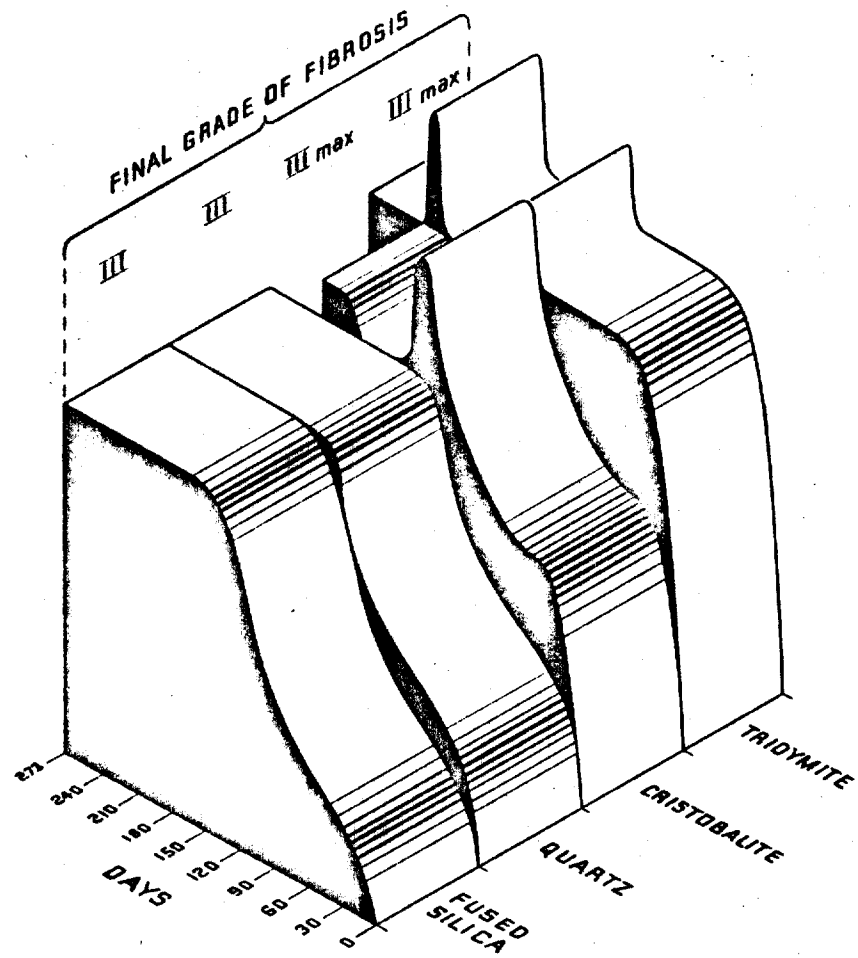


Fig. 13.—Grades of fibrosis of livers of mice after intravenous injection of free silica dusts.

Schulz, and Drinker [7] and may be due to a species difference or to differences in particle size, though it is possible that repeated injections might have produced more fibrosis of the spleen.

Aschoff [8] suggested that the cells of the reticuloendothelial system quickly phagocytose particulate material present in the blood stream. Cappell [9] showed that the carbon particles of India ink are removed from the circulating blood and are stored within the reticuloendothelial cells which line the blood channels of the liver, spleen, and bone marrow and to a slighter extent in the suprarenal and pituitary glands. We looked for silica particles in the various organs by means of polarized light and found a concentration of particles in the Kupffer cells and the sinusoids of the spleen. Apart from finding a few particles in the bone marrow, kidneys, and lungs in the initial stages, we were not able to find particles in any other tissues, and it is possible that we could not find them in these organs at a later stage because of their being thrown back into the blood circulation. Cappell [9] showed that carbon particles initially deposited on the capillary walls of the lungs appeared later to be washed away and passed on to the spleen.

The spleen in the present experiment initially picked up a high concentration of quartz particles which were seen in the macrophages and the sinusoids, but the quartz particles appeared later largely to disappear from the spleen. It may be suggested that the active phagocytes from the pulp of the spleen possibly took up these particles and were later destroyed, the particles being thrown back again into the sinusoids and from there via the splenic vein into the portal vein. This may explain why a high concentration of silica particles was found only in the liver in the later stages of the experiment, resulting in the limitation of fibrosis to this organ. And it may be the reason for the presence of lesions of two stages of maturity, the cellular nodules usually in the vicinity of the portal tract and the acellular collagen nodules near the centrilobular veins. The liver ultimately got nearly all the injected silica, both directly from the hepatic artery and from the spleen via the splenic vein.

The rate of production of fibrosis in the livers of these mice was no faster and did not progress to as completely collagenous and confluent a state as in the rat lungs of the previous experiment.

It is not possible to offer a satisfactory explanation of the differences of pathogenicity of the various forms of crystalline and fused silica. All that can be said is that there must be some factors additional to silica solubility. If test-tube experiments on the dissolution of silica particles mean anything, they will indicate that these several free silica dusts should have the same pathogenicity, since they have the same solubility. Since they have markedly different pathogenicities, there must be some other explanation, possibly related to the character of the surface of the particles, which in its turn should be related to the crystal structure or the lack of crystal structure.

SUMMARY

Different forms of free silica, i. e., fused silica, quartz, cristobalite, and tridymite, of high purity and almost identical silica content, size distribution, and silica solubility, were injected intravenously into the tail vein of mice to study the pathology produced in the livers. Tridymite was the most rapid in producing acellular collagenous silicotic nodules; cristobalite was next; quartz, third; and fused silica, the least fibrogenic. Silica particles were found only initially in the spleen, and there was very little fibrosis produced there. No fibrosis was observed in any other tissue. These observations are difficult to reconcile with the silica solubility theory of silicosis. Since these free silica dusts have the same solubilities, then either our test-tube measurements of solubility do not reflect adequately the dissolution of silica particles in the tissues, or there must be some other explanation, possibly concerned with crystal structure and surface activity.

Grants to defray the expenses of this investigation were made by the Medical Research Council and

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REFERENCES

1. King, E. J.; Mohanty, G. P.; Harrison, C. V., and Nagelschmidt, G.: Action of Different Forms of Pure Silica on the Lungs of Rats, Brit. J. Indust. Med. **10**:9, 1953.

2. Gardner, L. U.: Experimental Pathology, in Lanza, A. J., editor: Silicosis and Asbestosis, New York, Oxford University Press, 1938, pp. 257-345.

3. Gordon, H., and Sweets, H. H., Jr.: Simple Method for Silver Impregnation of Reticulum, Am. J. Path. **12**:545, 1936.

4. Harrison, C. V.; Wright, B. M., and King, E. J.: Inhibition of Quartz-Induced Fibrosis of the Liver by Aluminium, J. Path. & Bact. **62**:443, 1950.

5. Belt, T. H., and King, E. J.: Tissue Reactions Produced Experimentally by Selected Dusts from South Wales Coalmines, in Chronic Pulmonary Disease in South Wales Coalminers: III. Experimental Studies, Report 250, Medical Research Council, Special Report Series, London, H. M. Stationery Office, 1945, pp. 29-68.

6. Silverman, L., and Moritz, A. R.: Peritoneal Reaction to Injected Fused (Spherical) and Unfused (Spiculate) Quartz, Arch. Indust. Hyg. & Occup. Med. **1**:499, 1950.

7. Tebbens, B. D.; Schulz, R. Z., and Drinker, P.: Potency of Silica Particles of Different Size, J. Indust. Hyg. & Toxicol. **27**:199, 1945.

8. Aschoff, L.: Lectures on Pathology, New York, Paul B. Hoeber, 1924.

9. Cappell, D. F.: Intravitam and Supravital Staining: Principles and General Results, J. Path. & Bact. **32**:595, 1929.

Geparin (Heparin)
1969, pp. 68-69

EFFECT OF SILICA GEL ON THE HEPARIN NUMBER OF BLOOD AND HEPARINOCYTES

I. F. Zaks and A. P. Galeniyetse

Silicon actively participates in mucoid metabolism by stimulating the production of a metachromatic substance rich in mucopolysaccharides.

The purpose of this work was to study the effect of perorally administered silica gel on the heparin number of blood and heparinocytes. Experiments were performed on 11 healthy persons and 38 white rats. The former received silica gel (2.5 ml of a 20% sodium solicate solution neutralized with normal hydrochloric acid solution) for 21 days. The later received 1.3 ml of the same neutralized solution for 60 days. In the human subjects, we determined the heparin number of blood by Piptea's (?) method and the number of basophilic leukocytes in peripheral blood (calculation of basophilic leukocytes per 1000 leukocytes in a blood smear). In the experimental animals, we used a modification of Piptea's method to determine the heparin number. We determined the condition of mast cells in omentum and mesentery preparations and counted them in the mesentery but determined the rate of formation and decomposition in the omentum. The results are summarized in Tables 4 and 5.

Table 4

Effect of Silica Gel on the Heparin Number and Number of Basophils

(1) Показатели	(2) До начала наблюдений	(3) Дни после ежедневного введения силикогеля			
		7	11	14	21
(4) Гепариновое число (ед.)	9.4 ±1.0	9.6 ±0.3	10.2 ±2.8	10.1 ±0.9	11.4 ±0.9
(5) Базофильные лейкоциты (на 1000 лейкоцитов)	35.0 ±29.8	11.1 ±18.0	12.7 ±31.6	26.4 ±5.1	22.4 ±17.5

1 - Indices

2 - Prior to the start of observations

3 - Days after daily administration of silica gel

4 - Heparin number (units)

5 - Basophilic leukocytes (per 1000 leukocytes)

Table 5

Effect of Silica Gel on the Heparin Number and Number of Heparinocytes

(1) Показатели	(2) Группы	До начала эксперимента	(3) Дни после ежедневного введения силикогеля				
			3	6	10	30	60
(4) Гепариновое число (ед.)	(6) Контрольная	7.5 ± 0.9	6.0 ± 0	6.0 ± 0	6.0 ± 0	6.0 ± 0	7.0 ± 0.2
	(7) Экспериментальная	7.5 ± 0.9	6.3 ± 2.0	6.4 ± 0.1	7.0 ± 0	10.0 ± 0	4.0 ± 3.1
(5) Тучные клетки в брыжейке	(6) Контрольная	37.0 ±44.5	42.2 ±23.0	41.1 ±20.4	37.8 ±17.3	42.1 ±11.3	34.8 ± 0.5
	(7) Экспериментальная	37.0 ±44.5	36.4 ±27.8	45.4 ±13.5	44.9 ±32.2	33.5 ±14.5	42.2 ±19.6

1 - Indices

2 - Groups

3 - Days after daily administration of silica gel

4 - Heparin number (units)

5 - Mast cells in the mesentery

6 - Control

7 - Experimental

After receiving silica gel daily, the healthy human subjects exhibited an increase in the heparin number the symptoms of which appeared as early as observation day 7. A statistically significant increase in the heparin number was observed on day 21. The number of basophilic leukocytes decreased on days 7 and 11 and increased on days 14 and 21, but did not reach the baseline level.

In the experimental rats, daily peroral administration of silica gel increased the heparin number starting day 10 of the experiment. The peak was reached on day 30. It decreased sharply on day 60. There were no changes in the mesentery where the number of mast cells was counted (Table 5).

Qualitative changes in the mast cells took place in the milk spots of the omentum along with changes in the heparin number. The intensity of formation of the cells was highest on days 10 and 30. The disintegration of mast cells intensified at the same time. Fewer immature mast cells were noted in the milk spots of the omentum on day 60 and disintegrating forms predominated numerically.

The changes that we noted in the heparin number of blood were obviously related to the qualitative changes in the mast cells of the omentum because the most intensive mutually compensatory formation and disintegration of the mast cells coincided in time with an increase in the the heparin number while the start of exhaustion of their formation coincided with the lowest heparin number.

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ВЛИЯНИЕ СИЛИКОГЕЛЯ НА ГЕПАРИНОВОЕ ЧИСЛО КРОВИ И ГЕПАРИНОЦИТЫ

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Кремний принимает активное участие в мукоидном обмене, стимулируя продукцию метахроматической субстанции, богатой мукополисахаридами.

В настоящей работе мы поставили себе задачей изучить действие перорально введенного силикогеля на гепариновое число крови и гепариноциты. Наблюдения проведены на 11 здоровых людях и 38 белых крысах. Здоровые люди получали в течение 21 дня силикогель (2.5 мл 20%-го раствора силиката натрия, нейтрализованного нормальным раствором соляной кислоты). Экспериментальные животные в течение 60 дней получали по 1.3 мл такого же нейтрализованного раствора. У здоровых людей мы определяли гепариновое число крови по Пиптеа и количество базофильных лейкоцитов в периферической крови (подсчет базофильных лейкоцитов в кровяном мазке на 1000 лейкоцитов). В эксперименте мы определяли гепариновое число по модифицированной нами методике Пиптеа. В препаратах сальника и брыжейки определяли состояние тучных клеток — в брыжейке подсчитывали их количество, а в сальнике определяли интенсивность генеза и распада. Полученные результаты показаны в табл. 4 и 5.

У здоровых людей после ежедневного введения силикогеля наблюдается повышение гепаринового числа, признаки которого появляются уже на седьмой день наблюдения. Статистически достоверное повышение гепаринового числа наблюдается на 21-й день. Количество базофильных лейкоцитов крови у здоровых

Таблица 4

Влияние силикогеля на гепариновое число и количество базофилов

Показатели	До начала наблюдений	Дни после ежедневного введения силикогеля			
		7	11	14	21
Гепариновое число (ед.)	9.4 ±1.0	9.6 ±0.3	10.2 ±2.8	10.1 ±0.9	11.4 ±0.9
Базофильные лейкоциты (на 1000 лейкоцитов)	35.0 ±29.8	11.1 ±18.0	12.7 ±31.6	26.4 ±5.1	22.4 ±17.5

Таблица 5

Влияние силикогеля на гепариновое число и количество гепариноцитов

Показатели	Группы	До начала эксперимента	Дни после ежедневного введения силикогеля				
			3	6	10	30	60
Гепариновое число (ед.)	Контрольная	7.5 ± 0.9	6.0 ± 0	6.0 ± 0	6.0 ± 0	6.0 ± 0	7.0 ± 0.2
	Экспериментальная	7.5 ± 0.9	6.3 ± 2.0	6.4 ± 0.1	7.0 ± 0	10.0 ± 0	4.0 ± 3.1
Тучные клетки в брыжейке	Контрольная	37.0 ±44.5	42.2 ±23.0	41.1 ±20.4	37.8 ±17.3	42.1 ±11.3	34.8 ± 0.5
	Экспериментальная	37.0 ±44.5	36.4 ±27.8	45.4 ±13.5	44.9 ±32.2	33.5 ±14.5	42.2 ±19.6

людей под влиянием введения силикогеля снижается на седьмой и одиннадцатый дни наблюдения, далее на 14-й и 21-й день повышается, не достигая исходного уровня.

В эксперименте на белых крысах под влиянием ежедневного перорального введения силикогеля происходит увеличение гепаринового числа, начинающееся с 10-го дня эксперимента. Максимальных величин гепариновое число достигает на 30-й день наблюдения. На 60-й день гепариновое число резко снижается.

В брыжейке, где подсчитывалось количество тучных клеток, изменений не наблюдается (табл. 5).

В млечных пятнах сальника параллельно с изменениями гепаринового числа происходят качественные изменения тучных клеток — на 10-й и 30-й день наблюдается наиболее интенсивный генез их. Одновременно происходит интенсификация распада тучных клеток. На 60-й же день в млечных пятнах сальника наблюдается меньшее количество юных форм тучных клеток и количественный перевес берут дезинтегрирующие формы.

Отмеченные нами сдвиги гепаринового числа крови, очевидно, связаны с качественными изменениями тучных клеток в сальнике, так как наиболее интенсивный взаимнокомпенсирующий генез и распад тучных клеток совпадает во времени с повышением гепаринового числа, а начало истощения генеза их — с наименьшим уровнем гепаринового числа крови.