years later the present writer sent a detailed description of the markings he had seen and examined to Dr. Ulke, who again asserted his belief that the structures should be referred to ancient algae. He emphasized the following points, which favor algal origin:

(1) Where best developed the markings almost always lie flat with the bedding planes, *i.e.*, they lie parallel with the bedding planes.

(2) The material inside the rods and beads is essentially like the surrounding granular limestone.

(3) Rods and beads alike are separated from the surrounding rock by a tiny groove, suggesting that there was once some sort of "skin" or shell around them.

(4) In some instances the rods bend back at an acute angle.

(5) Some of the rods branch and some possess tiny structures resembling rootlets.

(6) Small lobate markings associated with the "rod-and-bead" structures suggest the fronds of algae.

(7) Rarely the beads decrease in size in one direction, as in a budding algal branch.

(8) Transverse partition walls are indicated in some instances.

The present writer has checked most of the observations just listed and believes that the suggested origin warrants serious consideration. Interpreting these "rod-and-bead" markings as ancient algae would also be in accord with the environmental conditions which are thought to have prevailed during the deposition of the Salem limestone.[4] It has been suggested that this limestone, at least that portion which is a coquina of macerated shell matter, was deposited as a clean calcareous sand in shallow water, having been previous to its deposition a dune or beach sand. In such an environment of deposition algae, such as those which are thought to have formed the "rod-and-bead" structures, may well have found conditions favorable for the extensive growth suggested by the abundance of preserved fossils.

ROBERT R. SHROCK

DEPARTMENT OF GEOLOGY,
MASSACHUSETTS INSTITUTE OF TECHNOLOGY

THE TREATMENT OF "SNIFFLES" IN THE RAT WITH SULFANILAMIDE

"SNIFFLES" or a form of pneumonia in the rat is responsible for a high mortality in all laboratory rat colonies. Recovery is rare, although a rat may linger on for months after the first symptoms appear. In other cases the disease progresses rapidly and causes death within a week. Because no effective treatment is known and because the disease is contagious, it has been a source of considerable loss, particularly in experimental studies in which the rat is tested over a long period of time.

Since sulfanilamide has been successfully used in severe infections in humans and in experimentally induced infections in animals, we decided to test its effectiveness in the treatment of this disease characteristic to the rat.

An experimental and a control group were used, the former being given 50 mg daily (varying slightly with the animal's weight) mixed in the food and the latter given the same care except for the omission of the sulfanilamide. Both groups contained cases in which the disease exhibited varying degrees of severity.

Of the fourteen rats in the experimental group, twelve permanently recovered, whereas two died. All the seven animals in the control group died after varying lengths of time. Prompt treatment with the drug resulted in the more rapid recovery.

No detrimental effects of the drug have appeared despite the fact that some animals received it daily for nearly two months. Detailed case studies will be reported later.

NORMAN R. F. MAIER

UNIVERSITY OF MICHIGAN

SPECIAL ARTICLES

EFFECT OF OXYGEN LIGHT AND LACTOFLAVIN ON THE OXIDATION OF VITAMIN C IN MILK

THE oxidation of ascorbic acid and of fat in milk is sensitive to variations in dissolved oxygen as well as to dissolved copper and exposure to light. Lactoflavin is the sole agent in milk responsible for the sensitivity of ascorbic acid to light.

A correlation has been found between the rate of oxidation of ascorbic acid in the dark and the production of a common flavor defect of milk resulting from the oxidation of the fat. The addition of 0.005 to 0.01 per cent. of ascorbic acid delays the development of this oxidized flavor.[1] It is possible that a competition for dissolved oxygen between two distinct processes is involved.[1,2]

Mattick and Kon,[3] Kon and Watson[4] and Kon[5] have found that sunlight, more specifically the short waves of visible light, accelerates the oxidation of re-

[4] E. R. Cumings, *et al.*, 30th Ind. Rept., 1906, p. 1199; J. W. Beede, *et al.*, 39th Ind. Rept., 1915, pp. 204–206; E. R. Cumings, Handbook of Indiana Geology, Pt. IV, 1922, p. 504.

[1] P. F. Sharp, G. M. Trout and E. S. Guthrie, Tenth Ann. Rpt. N. Y. State Assoc. Dairy and Milk Inspectors, p. 153, 1936.

[2] L. Buruiana, *Biochem. Jour.*, 31: 1452, 1937.

[3] A. T. R. Mattick and S. K. Kon, *Nature*, 132: 446, 1933.

[4] S. K. Kon and M. B. Watson, *Biochem. Jour.*, 30: 2273, 1936.

[5] S. K. Kon, SCIENCE, 85: 119, 1937.

duced ascorbic acid when milk is exposed in glass bottles. They have found that the action of light on ascorbic acid does not take place in the absence of dissolved oxygen. In view of the note by Kon[5] it should be recorded that in the experiments previously reported by us,[1,6] special precautions were taken to prevent undue exposure of the milk to light. The experimental samples were collected in brown glass bottles, the bottles were never exposed to direct sunlight, and they were stored in the dark. The extent to which the commercial samples were exposed to light before shipment to the laboratory was not known.

Since ascorbic acid solutions and dissolved oxygen are colorless, they do not absorb through heavy glass bottles the light which accelerates the oxidation of ascorbic acid in milk. The oxidation of pure solutions of ascorbic acid is insensitive to the accelerative effect of visible light. Therefore some secondary substance must be involved in the mechanism of the photochemical oxidation of ascorbic acid in milk. Lactoflavin had long been suspected of playing a part in the oxidative changes in milk, and the fact that it absorbs blue light led to experiments to see if lactoflavin was the intermediary in the accelerative effect of light on the oxidation of ascorbic acid. Martini[7] found that certain dyes such as methylene blue, and lactoflavin, act as photosensitizers in the oxidation of ascorbic acid.

After lactoflavin and ascorbic acid have been destroyed by prolonged exposure of milk to sunlight, light no longer causes the photo-oxidation of additional ascorbic acid. Addition of lactoflavin restores the photochemical sensitivity. Sensitivity of ascorbic acid to light can also be prevented by removal of the lactoflavin by adsorption. The ascorbic acid, which is relatively high in mares' milk, is relatively more stable towards light, because mares' milk contains little or no lactoflavin.[8]

The effect of lactoflavin on the photochemical oxidation of reduced ascorbic acid was studied, using pure compounds and artificial light. The photochemical oxidation occurred between the approximate pH limits of 5 and 9.6, and was not accelerated by the addition of copper (Table I).

TABLE 1

			Per cent. ascorbic acid oxidized in 10 minutes (*pH 5.8*)
Light	Air	Lactoflavin	48
Light	Nitrogen	Lactoflavin	2
Light	Air	.....	1
....	Air	Lactoflavin	1

[6] P. F. Sharp, SCIENCE, 84: 461, 1936.
[7] E. Martini, *Bull. Soc. Ital. Biol. Sper.*, 10: 1235, 1934.
[8] R. A. Rasmussen, R. Bogart and L. A. Maynard, Private communication.

Some investigators, who have shown marked effects of pasteurization on the destruction of vitamin C, have wrongly attributed the major influence of the variation observed to the amount of oxygen present during the heating, whereas the amount of dissolved copper,[6] amount of catalyst of enzyme-like nature,[6,9] previous exposure to light[4] and time of holding after pasteurization were perhaps of more significance. Variations in dissolved oxygen are of practical importance during subsequent holding, rather than during pasteurization. This has not been clearly recognized and controlled by previous investigators.

Our experiments have shown that milk from which the oxygen has been removed can be exposed to sunlight in clear glass bottles for considerable periods of time without any appreciable diminution in the amount of reduced ascorbic acid and without developing the true oxidized flavor. (Oxygen-free milk when exposed to sunlight does develop an off flavor of a different kind.) Furthermore, when stored in the dark for a week, even after 0.1 mg of dissolved copper per liter is added, the oxidized flavor does not develop, and there is no appreciable decrease in the amount of reduced ascorbic acid. Milk freed from oxygen was heated for 3 hours at 63° C. (145° F.) after the addition of 0.1 mg per liter of dissolved copper, with no appreciable oxidation of reduced ascorbic acid.

We have devised a simple procedure for satisfactorily freeing milk from dissolved oxygen. The milk is subjected to evacuating equipment capable of lowering the temperature of the milk about 10° C. by the water vapor removed. The water vapor sweeps out the oxygen dissolved in the milk. The reduced pressure may be applied to the milk immediately after pasteurizing, or the milk may first be cooled to about 30° C. or less, at which temperature foaming is reduced. If cooling by this method is carried too far or performed too slowly, the formation of gravity cream is not satisfactory because of the agitation after the fat globules have begun to cluster.[10] If milk has been held cold for some time it should first be warmed to 40–45° C. before cooling to 30° C. for vacuum cooling, or the vacuum should be applied to the milk at a temperature near 10° C. to avoid churning.

This procedure, which we call vacuum cooling, removes some types of off flavors from the milk,[11] and we have some evidence that it lessens the cooked flavor of pasteurized milk. Simply applying the reduced pressure without the sweeping-out effect of the removal

[9] E. Stotz, C. J. Harrer and C. G. King, *Jour. Biol. Chem.*, 119: 511, 1937.
[10] H. C. Troy and P. F. Sharp, *Jour. Dairy Sci.*, 11: 189, 1928.
[11] E. S. Guthrie and F. V. Beck, Paper delivered before Eastern Div., Amer. Dairy Sci. Assoc., September 20, 1937.

of water vapor does not reduce the oxygen in the milk sufficiently to give the best results.

After vacuum cooling, the milk can be drawn continuously into ordinary milk bottles by vacuum bottling technique. Or the vacuum can be released and the greater portion of the milk withdrawn from the bottom of the evaporating tank, and run without contact with air into milk bottles by means of a filter tube which is small enough to maintain a column of milk, and which reaches to the bottom of the bottle. In this way only the surface of the milk comes temporarily in contact with the air, and such bottles when completely filled and capped are practically oxygen free and remain so.

The results given in Table 2 are typical of the effect of the removal of the oxygen when bottled milk is stored at 1–3° C. The reduced ascorbic acid was determined by direct titration.[6,12] The numbers in

TABLE 2

	Mg reduced ascorbic acid per liter after holding:	
	3 days	7 days
Raw milk (fresh, 20 mg./liter)	14 (0)	2 (0)
Raw milk (vacuum treated)	19 (0)	19 (0)
Pasteurized, surface cooler	14 (3)	4 (3)
Pasteurized, vacuum cooled	20 (0)	20 (0)
Past., 0.1 mg.Cu/l.,surface cooler ..	2 (4)	0 (4)
Past., 0.1 mg.Cu/l.,vacuum cooled ..	20 (0)	20 (0)

parentheses represent the degree of intensity of the oxidized flavor, (0) represents no oxidized flavor, and (4) represents the maximum intensity.

If after a few days oxygen-free milk is shaken with air, then oxidation of the reduced ascorbic acid proceeds at the rate which is normal for the sample and its treatment if it had not been freed from oxygen.

Milk pasteurized by the holder method (63° C. for 30 minutes) is generally believed to be more susceptible to oxidative changes than is raw milk, although pasteurization exerts little accelerative effect on the oxidation of the reduced form of vitamin C.[4, 6, 13]

By utilizing water vapor generated in the milk by vacuum distillation to sweep out the oxygen, and preventing reabsorption of oxygen, flavor defects due to oxidation can be avoided and the reduced ascorbic acid naturally present in the milk or added to the milk can be preserved. Pasteurized milk subjected to this treatment is much higher in ascorbic acid after holding than is untreated raw milk held for the same length of time, and is much less subject to flavor defects.

DAVID B. HAND
E. S. GUTHRIE
PAUL F. SHARP

CORNELL UNIVERSITY

[12] P. F. Sharp, *Jour. Dairy Sci.*, 21: 85, 1938.
[13] G. Rundberg, *Acta Paediatrica*, 15: 357, 1933–34.

THE MODE OF ACTION OF SULFANILAMIDE AND PRONTOSIL[1]

THE chemotherapeutic activity of sulfanilamide and Prontosil is now well established. There is general agreement as to their effectiveness in combatting a number of infections but little agreement as to their mode of action. Most workers consider, however, that their effectiveness as chemotherapeutic agents is not paralleled by their bacteriostatic and bactericidal action *in vitro*.

It has been suggested by a number of investigators and denied by others that these compounds stimulate phagocytosis. Bliss and Long,[2] as a result of their work on *Clostridium welchii* infections in mice, have presented evidence that sulfanilamide retards the rate of bacterial multiplication and suggest that its effectiveness is due to its bacteriostatic action *in vivo*; that is, the rate of multiplication of the bacterium is retarded or stopped. The phagocytes are then able to engulf the infecting agents.

The work of Gordon and Thompson,[3] and others on artificial opsonins suggested to us the possibility that sulfanilamide and Prontosil might function in this capacity. Accordingly, the action of these drugs was determined by means of a technique for measuring their opsonizing power in leucocyte-bacteria mixtures. The technique, the details of which will appear in a later paper, consists in placing into small agglutination tubes constant amounts of ascending dilutions of the drug, suspensions of bacteria and appropriate quantities of leucocytes. Parallel control tests consisting of the diluent for the drug (buffered saline), bacteria and leucocytes were conducted in every instance.

All leucocytes used were obtained from guinea-pig blood and, unless otherwise indicated, were well mixed with fresh guinea-pig plasma at the time of use. At the end of 30 minutes incubation at 37° C. with intermittent shaking, thick smears were made from each tube and were stained by Wright's method. Although a variety of bacteria was used, only the results with hemolytic streptococci are here presented. All observations were made in the same manner: the first 50 polymorphonuclear neutrophiles seen were counted, and the presence or absence of ingested bacteria, the number of bacteria phagocytosed, and the number of leucocytes taking part in the reaction were recorded.

Upon examination of these slides it was noted that the greatest degree of phagocytosis occurred in dilutions of 1/50,000 to 1/100,000 of both sulfanilamide and Prontosil, and that the percentage of cells taking

[1] Preliminary report.
[2] E. A. Bliss and P. H. Long, *Jour. Am. Med. Asn.*, 109: 1524, 1937.
[3] J. Gordon and F. C. Thompson, *Brit. Jour. Exp. Path.*, 18: 390, 1937.