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 Or the vacuum can be released and the greater portion of the milk withdrawn from the bottom of the evaporating tank, and run without contact with air into milk bottles by means of a filter tube which is small enough to maintain a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.

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[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.

part in the reaction showed a marked increase over that of controls. In an attempt to analyze the significance of the various factors which might play a part, the leucocytes and the streptococci were treated in various ways before setting up the tests for phagocytosis. It was found (1) that when leucocytes were well washed of plasma, (2) that when the leucocytes were treated with varying dilutions of the drug and then well washed, or (3) that when the streptococci were treated with varying concentrations of the drug and then washed, no more phagocytosis occurred than in controls. It appears that serum or a factor in it is necessary to obtain the effect of the drug.

The protocol given is typical of the results obtained when sulfanilamide or Prontosil was used. These drugs

THE EFFECT OF VARYING DILUTIONS OF SULFANILAMIDE ON PHAGOCYTOSIS OF HEMOLYTIC STREPTOCOCCI

Dilution of drug	Number of cocci phagocytosed	Percentage of leucocytes taking part
1/1000	192	20 per cent.
1/10,000	246	32 " "
1/50,000	514	60 " "
1/100,000	296	48 " "
1/250,000	82	12 " "
Control	96	16 " "

clearly enhanced the phagocytosis of hemolytic streptococci *in vitro*. Fresh serum or plasma appeared to be necessary for the completion of the reaction. Not only did these drugs increase the number of bacteria phagocytosed per leucocyte but also the number of leucocytes taking part in the reaction.

While our work was in progress, a paper by Osgood[4] appeared in which are reported results essentially the same as ours; he used bone-marrow cultures as leucocytic suspensions. He concluded that increased phagocytosis is due to a neutralization of the bacterial toxins by sulfanilamide and that the drug has no direct effect on either the leucocytes or bacteria. The increased phagocytosis in the extremely high dilution ranges that we observed compares very favorably with his findings.

Just how sulfanilamide enhances phagocytosis is not clear, but our results briefly reported here suggest that sulfanilamide or a serum-sulfanilamide complex acts as an opsonin.

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INCREASE IN VITAMIN A ACTIVITY OF CORN CAUSED BY DOUBLING THE NUMBER OF CHROMOSOMES[1]

A QUANTITATIVE gene action in corn has been demonstrated by comparing the carotinoid content of pure yellow diploid and tetraploid strains. Since tetraploid corn contains twice as many chromosomes as ordinary diploid corn, it was of interest to see what effect doubling the number of genes for yellow would have on the amount of pigment. The interesting possibility presented itself that the vitamin A activity of corn might be increased by formation of the tetraploid.

The diploid corn was found to contain 0.0267 $\pm$ 0.0004 milligrams and the tetraploid corn 0.0380 $\pm$ 0.0007 milligrams of carotinoid per gram of dry meal. These values are the means of seventeen analyses of the diploid and sixteen of the tetraploid together with their standard errors. Thus the doubling of the number of chromosomes and genes for yellow, resulted in an increase of 43 per cent. in the total carotinoid content per unit weight. There was the same percentage increase in vitamin A activity, since both the active carotinoids, beta carotin and cryptoxanthin, and the inactive zeaxanthin were increased to the same extent. Approximate measurements showed no difference in the density of diploid and tetraploid kernels. Hence the 43 per cent. increase per unit weight is also the increase in carotinoid content per unit volume.

The volume of the endosperm cells in the tetraploid yellow corn used in these analyses was approximately 3.5 times as great as the volume of the endosperm cells of the comparable diploid, as determined by direct measurements of cell dimensions in different regions of the endosperm. Because of this marked increase in cell size which resulted from chromosome doubling, the concentration of the genes per unit volume was actually less in the tetraploid than in the diploid, even though the individual cells of the former contained twice as many genes as did the cells of the latter. However, the amount of carotinoid per cell was five times as great in the tetraploid as in the diploid. In terms of gene concentration within the endosperm tissue, there was in the tetraploid 2.5 times as much carotinoid per gene as there was in the diploid. These proportional differences may be summarized as follows:

	Diploid	Tetraploid
Cell volume	1	3.5
Carotinoid per unit volume	1	1.43
Carotinoid per cell	1	5
Genes per cell	1	2
Genes per unit volume	1.75	1
Carotinoid per gene	1	2.5

The diploid and tetraploid strains of yellow corn selected for this study were produced by crossing the F_1 hybrid between an inbred line of Webbers Dent

[4] E. A. Osgood, *Jour. Amer. Med. Asn.*, 110: 349, 1938.

[1] Cooperative investigation of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Departments of Botany and Dairy Industry, New York State College of Agriculture, aided by a grant from the Committee on Radiations of the National Research Council.