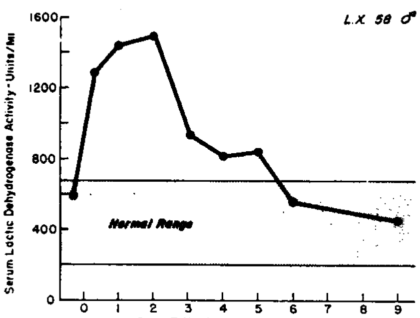

Fig. 2. Serum lactic dehydrogenase activity at the onset of chest pain and during a 10-day period following acute myocardial infarction in patient S.X.

sumed to be the result of the release of the enzyme from the infarcted heart disease. The limited number of cases presented does not permit final evaluation of these observations with regard to either their diagnostic or prognostic significance.

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Crosslinking of Latex Rubber

Most of the properties of rubber are consistent with those expected of a high-molecular-weight linear polymer of isoprene. However, there are some features of its behavior that are inexplicable on this basis. They must be the result of the presence of small amounts of structures other than the regular head-to-tail chain of isoprene units.

Bloomfield, in the first fundamental study (*1*) of the properties of rubber from freshly tapped latex, found that the tree does not continue to build indefinitely a linear polymer, but that branching reactions occur in a rested tree. These reactions eventually convert individual latex particles into substantially single molecules of enormous molecular weight. Bloomfield also observed that small amounts of oxygen are intimately associated with the hydrocarbon, even when it is isolated directly from the tree with careful exclusion of atmospheric oxygen. Craig, Juve, and Davidson (*2*) have found less certain indications of the presence of carboxyl groups, which, if they are present in the rubber in concentrations even approaching the amount indicated by their results, must be on side chains.

Watson (*3*) discovered evidence for unique links in the hydrocarbon chain at intervals of about 700,000 in molecular weight; these links dissociate at a measurable rate in solution. He has suggested that these links may be responsible for the slow "gelation" of massive rubber. Messenger (*4*) showed that this reaction is inhibited by water: samples stored in the presence of desiccants progressively increased in molecular weight (actually in solution viscosity) and finally became increasingly insoluble in the usual rubber solvents. This has recently been substantiated by further work by Wood (*5*) in connection with the "Technically Classified" rubber program (*6*).

A less well known phenomenon, although it is familiar to users of commercial latex, is the rapid development of high viscosity and relative insolubility in rubber that is obtained from preserved latex. Such rubber may become as high as 80-percent insoluble in benzene and have "Mooney" viscosities (*7*) of more than 100.

During the period 1948–50, the Plantations Division of the United States Rubber Company imported a number of samples of latex that had been preserved in a variety of ways, in the course of an intensive study of preservation methods. As a result, it was possible to obtain (*8*) data which indicate that the preservative used has a specific effect on the viscosity of the rubber (Table 1). Among other tests on the latexes, the solubility in benzene and the Mooney viscosity (ML4-212) of the rubber obtained by drying samples of the latex at room temperature were measured. Solubility was determined by the conventional procedure (developed for GR-S) in which 0.5 g of rubber supported on steel screens is left in contact with 75 ml of solvent for 24 hours in the dark, after which the concentration of an aliquot of the solution is determined.

There were not available enough high *p*H latexes containing no ammonia to eliminate entirely *p*H as a factor, but only those latexes containing free ammonia have the combination of Mooney viscosity greater than 100 and percentage gel greater than 30, indicating strongly that ammonia has a specific effect in increasing the extent of crosslinking in the latex rubber.

This is a positive effect—that is, it does not result from inhibition of degradation by oxygen during storage and handling of the latex. The rate of reaction of oxygen with latex is highest in the range of *p*H of commercial latex, and it falls sharply when the *p*H is less than 9 (*9*). The rubber from fresh latex from trees tapped regularly is usually soluble, and its Mooney viscosity is in the range of 60 to 80. Further, the viscosity increase can be induced in latexes that have been preserved without ammonia.

Table 1. Effect of preservative on gel and viscosity.

Latex No.	*p*H	Ammonia*	Other amine†	Gel (%)	ML4-212
63	11.3	–	–	0	99
47	10.4	+	–	59	118
48	10.4	+	–	38	111
66	10.4	+	–	60	> 110
68	10.4	–	+	30	98
72	10.3	+	–	65	> 110
73	10.3	+	–	49	103
57	10.3	+	–	52	108
35	10.3	+	–	58	111
40	10.2	–	+	16	87
74	9.8	+	–	39	> 110
157	9.7	–	+	12	67
58	9.6	–	+	12	64
37	9.4	–	+	18	71
38	9.4	–	+	7	64
64	9.4	–	+	12	63
62	9.3	–	+	12	72
67	9.2	–	+	9	79
60	9.1	–	+	13	84
75	8.9	–	+	9	76
27	8.7	–	+	29	70
23	8.3	–	+	8	65
61	8.3	–	+	12	76
24	7.2	–	+	17	
39	6.9	–	+	15	77
12	6.6	–	+	15	84
1	5.8	–	–	18	75

* –, preservative absent; +, present.

† Amines other than ammonia were all low-molecular-weight aliphatic amines. Dimethylamine was the sole preservative in latex 68.

Small amounts of several of the low *p*H latexes listed in Table 1 were treated with ammonia (2 percent) and left standing for 6 months. The rubber in all these samples increased markedly in viscosity as compared with controls (Table 2).

It appears quite possible that the same functional groups are responsible for the crosslinking induced by ammonia and that which occurs during storage of dry rubber. During the development of the USF rubber process (*10*) C. E. Linscott observed that brief treatment with ammonia of the latex or of freshly precipi-

Table 2. Effect of ammonia on low *p*H latexes.

Latex	ML4-212 after 6 months	
	Control	Ammonia added
24	90	105
39	85	114
40	87	107
60	78	108
62	78	102

tated rubber from formaldehyde-preserved latex caused an immediate rise in Mooney viscosity and accelerated the later slow rise in viscosity during storage of the dry rubber.

The fragility of the swollen gel makes it difficult to determine precisely the volume ratio of solvent to rubber. Molecular weights between crosslinks (*11*) estimated from the figures available range from $\sim 5 \times 10^5$ for the samples with higher gel contents to more than 10^6 for the very tenuous gels frequently obtained from samples with low gel. The maximum observed extent of crosslinking is thus in reasonable accord with Watson's estimate of the concentration of unique linkages in rubber.

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6 February 1956

Biological Origin of Native Sulfur in Salt Domes of Texas and Louisiana

Elemental sulfur in the cap rock of the Gulf Coast salt domes is crystalline, and it fills fractures and replaces wall rock of the porous calcite cap rock (*1*). Its origin is commonly thought to have been reduction of the sulfate, gypsum, and anhydrite of the cap rock with the formation of hydrogen sulfide, which became oxidized to elemental sulfur (*2*).

Although sulfate is reduced to sulfide by methane and other organic compounds at high temperatures (700° to 1000°C), there is evidence that the temperature in the salt domes has not exceeded 100°C. Tests were made of the chemical reduction of sulfate by organic compounds (*3*). Samples of salt-dome crude oil and methane were mixed with solutions saturated with calcium sulfate from cap-rock samples. Carbonate and sodium chloride, as well as iron sulfide, were added in some of the experiments. At temperatures between 40°C and 100°C, no hydrogen sulfide was produced in tests that lasted several months. This suggested that nonbiological reactions were not instrumental in the reduction of sulfate to sulfide in the salt domes.

Sulfate-reducing bacteria have been detected in the formation waters issuing at the ground surface and in drill cores (*4*), and it has been suggested that these bacteria converted the sulfate to sulfide, the initial transformations of the reactions that resulted in deposition of elemental sulfur. This hypothesis is supported by evidence of Thode, Kleerekoper, and McElcheran (*5*) of the fractionation of the stable sulfur isotopes S^{32} and S^{34} during bacterial reduction of sulfate to sulfide. The sulfide was richer in S^{32} than the sulfate from which it originated. The percentage fractionation F was calculated as follows:

$$F = 100 \times \frac{S^{32}/S^{34} \text{ of product} - S^{32}/^{34} \text{ of reactant}}{S^{32}/S^{34} \text{ of reactant}}$$

F was found to be 1.1 percent in experiments carried out at 25°C, where the product was sulfide and the reactant was sulfate.

Thode, Wanless, and Wallouch (*6*) found that the sulfide and elemental sulfur of Texas and Louisiana sulfur deposits have higher ratios S^{32}/S^{34} than associated sulfate, indicating enrichment in S^{32} during their formation from sulfate. Tests on ten Gulf Coast salt domes showed percentage fractionations of 4.6 percent for the sulfide sulfur and 3.9 percent for the elemental sulfur.

Laboratory studies were made of the amount of fractionation of the sulfur isotopes resulting from reduction of sulfate to sulfide by the sulfate-reducing bacterium, *Desulfovibrio desulfuricans*. The bacteria were cultivated in a 5-lit fermentor in which the medium was continuously agitated to prevent local variations in sulfate concentration. Determinations of the S^{32}/S^{34} ratios of the sulfate used to prepare the medium and of the sulfide produced from sulfate by the bacteria were made by means of a dual collector mass spectrometer. During bacterial growth the sulfide was collected from a stream of nitrogen gas that was passed through the substrate, and determinations were made on the sulfide recovered at various incubation periods.

The amount of fractionation varied from negligible values to a maximum of 2.7 percent, and it was affected by factors that influence the rate of bacterial development and by the concentration of sulfate. The slower the growth, the greater was the ratio S^{32}/S^{34} of the sulfide, indicating enrichment in S^{32} during reduction of sulfate to sulfide. Fractionation was greatest when the incubation temperature was low (10° to 20°C), when the concentration of soluble sulfate was high, and when only a small portion of the total sulfate became reduced. There should be still greater fractionation than that noted when the rate of bacterial reduction of sulfate is slower than it was under the experimental conditions used.

The source of energy for development of the bacteria in the salt domes is still uncertain. Some components of crude oil may have been utilized, for it has been reported (*7*) that the bacteria can develop on crude oil, and additional positive results have been obtained in preliminary experiments in our laboratories.

Although it is likely that the oxidation of sulfide to elemental sulfur was nonbiological, it is improbable that it was due to reaction with oxygen, for the oxygen content of salt-dome waters is negligible.

The reaction rate between hydrogen sulfide and calcium sulfate in laboratory experiments was sufficiently rapid so that it may be assumed that this sulfide-sulfate reaction produced the elemental sulfur of the salt domes. The fact that the elemental sulfur in the formations has a ratio of S^{32}/S^{34} that is intermediate between the ratios of the sulfate and hydrogen sulfide of the liquid phase supports this hypothesis.

The composition and environmental conditions of the salt-dome formations and the results of the laboratory investigations indicate that sulfate-reducing bacteria played an important role in the formation of the sulfur in the deposits. These bacteria probably reduced dissolved sulfate to hydrogen sulfide. The subsequent formation of elemental sulfur was most likely due to a nonbiological process such as a reaction between sulfide and sulfate.

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