unnecessary step in the preparation of this medium, it is recommended that the YES medium be adopted routinely as a recovery medium in thermal resistance studies on the spores of P.A. 3679. In addition to giving thermal resistance values comparable to those obtained in Yesair's pork infusion, it has the advantages of ease of production, reduced cost, and relative reproducibility.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Dr. E. Staten Wynne for making available the composition of the YESB medium prior to its publication.

SUMMARY

Data are presented which show that the constitution of the recovery medium exerts an appreciable effect on the apparent thermal resistance of spores of Putrefpork infusion, it has the advantages of ease of production, reduced cost, and a standardized method of preparation.

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Microbiological Deterioration of Vulcanized Rubber

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The present state of our knowledge of the microbiological deterioration of vulcanized rubber materials must be deemed unsatisfactory, an opinion which is also expressed by Greathouse, Wessel, and Shirk (1951) in their review on microbiological deterioration of manufactured materials. On the one hand, there is a tendency to extend the observations on microbial attack of natural, nonvulcanized rubber also to rubber objects which have been subjected to a vulcanization process. But this does not seem permissible, because in this process the long hydrocarbon chains have been linked together by sulfur bridges, and it seems quite possible that this alteration materially changes the susceptibility of the hydrocarbon to microbial attack. On the other hand, several reports which deal with the problem merely from the standpoint of testing materials leave no doubt that the deterioration is often accompanied by the development of microorganisms. However, in these cases, usually no proof is given that a certain type of organism is responsible for the attack, and it is not established with certainty that the breakdown is indeed due to a consumption of the rubber hydrocarbon in the vulcanized state. In this connection, it should be realized that all vulcanized rubber objects contain several compounds besides the hydrocarbon. This also makes it clear that vulcanized rubber may be covered by profuse growth of an organism fully unable to attack the rubber itself.

Before reporting on the experimental work performed, a survey of the main results obtained in investigations both on nonvulcanized and vulcanized rubber cannot be omitted.

As early as 1913, Söhngen and Fol (1914) isolated pure cultures of natural-rubber-consuming microorganisms from an enrichment culture in which pure rubber hydrocarbon was the main source of carbon. They prepared films of nonvulcanized rubber of high purity by dissolving pieces of sheet rubber in benzene, separating the clear upper layer of the solution, and subsequently evaporating the benzene. It should be acknowledged that the thus prepared rubber still contained 0.1 per cent nitrogen. However, it appears to be difficult to attain a lower nitrogen content without employing methods too complicated. Boggs and Blake (1936), in discussing

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the preparation of deproteinized rubber from latex, mentioned that even after repeated creaming or centrifuging of the latex the rubber still had a nitrogen content of 0.08 per cent.

Söhngen and Fol (1913) inoculated their thin rubber films, floating on an aqueous medium containing inorganic salts, with soil and observed the growth of colonies. The greater part of these colonies appeared to be *Actinomycetes*. After some time, the rubber under and around the colonies had disintegrated to such an extent that this could not be ascribed to the disappearance of impurities from the rubber.

Two of the active strains isolated in pure culture were described as *Actinomyces elastica* and *Actinomyces fuscus*.

During the next 20 years, no significant advance was made, but in 1936, Spence and van Niel introduced a new and very ingenious method.

They succeeded in preparing rather well-purified latex suspensions that could be sterilized by steam without coagulation. Then, the latex was dialyzed against repeatedly refreshed phosphate buffer of pH 6.8 to 7.2. Ammonia and water-soluble organic impurities were removed quantitatively; however, from the analytical data given in the tables it may be seen that the latex still contained about 3 per cent protein (calculated on dry weight). With this sterile latex, agar plates were prepared by pouring thin layers of a suspension of latex in hot washed agar solution on a layer of washed agar to which the necessary mineral salts had been added.

By inoculating the plates with soil particles and incubating at 25 or 30 C, colonies, chiefly of various *Actinomyces* species, appeared. These colonies produced a clear zone in the opaque medium, thus indicating that rubber globules disappeared as a result of microbial action. Experiments in which pure cultures prepared from *Actinomyces* colonies were inoculated into synthetic media, containing known quantities of latex rubber, demonstrated that the isolated strains were able readily to decompose the rubber hydrocarbon.

A further contribution worth mentioning was made by Kalinenko (1938), who applied the technique of Spence and van Niel for the isolation of several rubber-decomposing organisms.

Among these organisms there were, besides *Actinomyces* species, also such molds as *Aspergillus oryzae* and a *Penicillium* species. Kalinenko claimed that all these cultures were able to consume large quantities of rubber in diluted latex. Moreover, he found that growth of one of the *Actinomyces* species on a thin film of purified natural rubber led to a perforation of this film.

The foregoing may suffice as a documentation for the suitability of the rubber hydrocarbon as a substrate for some microorganisms, among which *Actinomycetes* apparently prevail.

With respect to available evidence for the susceptibility to microbial attack of the hydrocarbon after it has been subjected to vulcanization, it should be realized that the conversion of natural rubber into a normal rubber commodity is not at all restricted to a heating of the rubber with elementary sulfur. The rubber is compounded with quite an arsenal of chemicals serving specific purposes. Examples are accelerators, antioxidants, fillers, pigments and stains, mineral oils, antiabrasives, and wear-resistors. It is clear at once that growth of a microorganism on vulcanized rubber does not necessarily imply that this organism is able to consume the rubber hydrocarbon itself.

As far as it is known, ZoBell and Grant (1942) were the first investigators who gave special attention to the question of microbial attack of vulcanized rubber. They observed that the use of rubber stoppers in experiments for determining the B.O.D. of water samples led to increased values. In special experiments it was shown that by adding vulcanized rubber cut into small pieces to inoculated water the B.O.D. increased from 0.5 to 6.0 ppm; a more or less similar effect was observed when the bottles in which the B.O.D. test was performed were coated with a thin film of purified, nonvulcanized rubber. In a second publication of ZoBell and Beckwith (1944), these observations were extended, and the most remarkable point is undoubtedly that they observed the effect after the water had been inoculated with quite divergent microorganisms. They mentioned molds, actinomycetes, and bacteria, representatives of the genera: *Aspergillus*, *Penicillium*, *Actinomyces*, *Proactinomyces*, *Micromonospora*, *Mycobacterium*, *Pseudomonas*, *Bacillus*, and so on. Some other bacterial strains, however, yielded negative results. In the light of the foregoing discussion it is clear that these experiments do not offer proof that the rubber hydrocarbon in the vulcanized state is liable to microbial attack. ZoBell and Beckwith themselves rightly remark that "the mere utilization of oxygen in the rubber products does not necessarily prove that rubber itself is oxidized." The same can be said for such other criteria used by ZoBell and co-workers as increased carbon-dioxide production and multiplication of the organisms.

However, somewhat further on, they referred vaguely to unspecified data regarding the loss in weight of rubber products when exposed to microbiological action over a long period of time, and in their summary the microbial deterioration of vulcanized rubber was put forward as a fact. As such, it has usually been quoted in later publications.

More direct evidence for a microbial attack of vulcanized rubber hydrocarbon was given by Blake and Kitchin (1949) and Blake, Kitchin, and Pratt (1950, 1953, and 1955), who studied the deterioration of the rubber insulation of electric cables. They found that when the insulation had been buried for some period of

time in soil it lost its insulating properties, whereas in parallel tests this material, exposed to sterilized soil, remained practically unaltered. Synthetic rubber proved to be more resistant in the soil burial test.

With natural rubber compounds, loss of electrical resistance of the insulation was accompanied by visible pitting, thus leaving no doubt that part of the vulcanized rubber hydrocarbon had also been consumed. In addition, micropores could be detected, but only after they had been made visible with the aid of an elegant method in which copper was electrolytically deposited in the pores.

Blake *et al.* (1949) assumed visible pitting to be caused by microorganisms having eaten away the bulk of the rubber compound, hydrocarbon included, while the micropores might have originated in imperfections in the mixing of the compound, thus resulting in minute streaks of nonrubber components susceptible to microbiological attack. In natural rubber compounds, both pitting and micropores were observed; in synthetic rubber compounds, only the latter occurred.

There is no doubt that the soil burial test is most useful to the rubber technologist in search for rubber compounds resistant against the destructive agents in soil. However, the need for an identification of these agents and their mode of attack remains. In their later publications Blake *et al.* (1950, 1953, 1955) made an attempt to elucidate these points. Insulations from which the copper wire had been removed were refilled with nutrient agar and sterilized. The cables thus prepared were then exposed to active soil for some period of time.

The underlying idea was that deteriorating microorganisms penetrate into the insulation and subsequently can be found as colonies on the agar medium inside the insulation wall. Indeed, Blake *et al.* found on the surface of the expelled agar-fillings growth of fungi, some of which could be identified as *Spicaria violacea*, *Metarrhizium anisopliae*, *Fusarium* species, and *Stemphyliopsis* species. In contrast to this, the agar expelled from insulations buried in sterile soil did not show any contamination. The results of parallel electrical tests on identical cables with the copper wire still inside correlated well with the appearance of colonies on the agar, insofar as electrical failure occurred after the same period of soil exposure.

The interpretation of these results, more particularly the question of whether the isolated organisms are to be considered as the causative agents of the deterioration, demands great caution. It should be realized that cracks and bursts in the rubber compound may be due to such secondary actions of the bacterial environment as a change in pH level, production of corrosive agents, like H_2S, as well as to extraction or consumption of any of the nonrubber hydrocarbon materials in microimperfections. The mere fact that fungi have forced filaments through the insulating rubber wall does not necessarily mean that the fungus itself has consumed the rubber compound; other organisms may have been the active agents and prepared the passage. These organisms may not have developed on the plain nutrient agar with which the rubber tubes had been filled. It is true that Blake *et al.* reported that they also used Sabouraud agar in some of their experiments. However, this medium is rather acid so that hardly any microorganisms not belonging to the fungi or yeasts would have had a chance to grow.

It therefore remains open to doubt whether the organisms isolated by Blake *et al.* are able to bring about a deterioration of the rubber compound.

In order to establish definite proof that a certain microbe can attack a vulcanized rubber product, it should be tested in pure culture. If in such an experiment a visible disintegration or pitting of the rubber compound occurs, the conclusion seems warranted that the organism is able to consume rubber hydrocarbon also in the vulcanized state.

Materials and Methods

From a waterwork in the western part of Holland, some specimens of rubber rings were received which had been used in connecting asbestos cement pipes in water distribution pipelines and which after several years of service showed obvious signs of corrosion.

Figure 1 shows how the ring is situated in the pipe connection; figure 2 shows a ring with inside corroded surface.

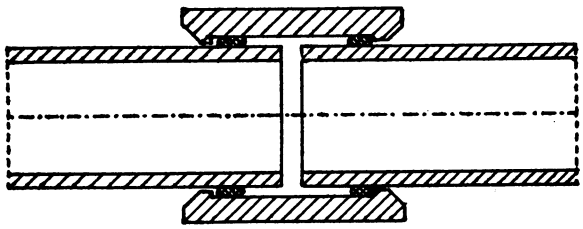

Fig. 1. Section of connection of asbestos cement pipes. Rubber rings cross-hatched.

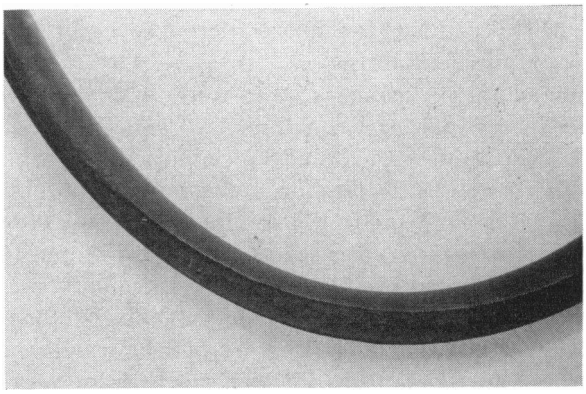

Fig. 2. A rubber ring with inside corroded surface

It was remarkable that only the side of the ring in contact with the water showed deterioration, and not the other side which was exposed to soil. There is, of course, a marked difference in the environment: the inner side is in contact with well-aerated tapwater, while the opposite side is exposed to the polder soil which, at a certain depth, rapidly becomes anaerobic.

There were 2- to 3-mm-deep cavities in the rings on the water side. Experiments carried out in the Rubber Research Institute T.N.O. at Delft made it probable that the corrosion was due to local oxidation. This conclusion was based on the differences in the rate of oxygen uptake at 70 C of slices of the attacked surface, of the normal surface, and of the interior part of a ring. The attacked surface was distinctly more liable to oxidation than the other parts.

Microscopical preparations from the corroded surface of rings, freshly removed from the water pipes, showed an amorphous material in which now and then some bacteria could also be discerned. After streaking some of this material on various media, a variety of bacterial colonies developed. It was attempted to cultivate rubber-consuming organisms directly, by inoculating a slice cut from the deteriorated surface into a sterilized tapwater medium containing a weighed piece of a normal rubber ring. After some weeks' incubation at 25 C, the pieces of rubber showed a cloud of fungus growth enveloping the rubber. A *Fusarium* species could be readily isolated. However, even after two months it was impossible to detect any weighable loss of rubber. The observed mold growth must, therefore, have been due to extractable organic materials present in the rubber compound. This was checked by making cold- and hot-water rubber extracts; both proved to be excellent nutritional media for the isolated *Fusarium* strain, as well as for several other fungi and bacteria.

In a later phase of the investigation, the presence of rubber-decomposing organisms on the surface of a deteriorated rubber ring was tested with the aid of latex-agar plates prepared according to Spence and van Niel (1936).

The latex used was purified by repeated centrifuging in distilled water, to which a small amount of aerosol M.A. (dimethylamyl ester of sulfonated succinic acid) had been added. The addition of this dispersing agent had a stabilizing effect on the latex, thus permitting a more thorough elimination of the nonrubber substances naturally occurring in the latex. In this way, the latex was washed six times. After this, the latex was diluted to 20 per cent and dialyzed against 0.1 N phosphate buffer of pH 6.9. The buffer solution was renewed three times.

The latex thus prepared could stand sterilization in the autoclave at 115 C. A sufficient amount of the diluted latex was added to a solution containing 2 per cent washed agar, 0.1 per cent potassium dihydrogen phosphate, 0.1 per cent ammonium sulfate, and 0.05 per cent magnesium sulfate to make a medium containing about 1 per cent of purified rubber. The pH level was adjusted to 7.0. Small pieces cut from the damaged surfaces of a rubber ring were put upon the latex-agar plates. On other plates, slices of the damaged surface were streaked in order to obtain isolated colonies of rubber-consuming organisms. Then the plates were incubated, partly at 25 C, partly at 30 C.

Since, as mentioned above, the purified latex is never free from all nitrogenous matter, it is not surprising that colonies of nonrubber-decomposing organisms developed on the plates. Various *Pseudomonas* species, among which *Pseudomonas fluorescens* prevailed, grew profusely on the plates. These colonies were never surrounded by clear zones. After 10 days, however, several other colonies were surrounded by distinctly transparent zones in the opaque latex-agar. On the plates seeded with the rubber fragments, local clear zones had also appeared. Around the rare and poorly developed colonies of fungi no transparency was ever observed.

The colonies that had produced clear zones were easily recognized as *Actinomycetes*. A few were pink-rose without spore formation; in others, spores were responsible for a black center in the colony. A latex-agar plate streaked with the latter organism is shown in figure 3. Some of these organisms, obviously belonging to the genus *Streptomyces*, were brought into pure culture by subcultivating on latex-agar.

Two of these strains were then tested for their ability to attack vulcanized rubber. For this purpose, the manufacturer of the rubber rings kindly placed at our disposal thin strips of the same composition as the rings.

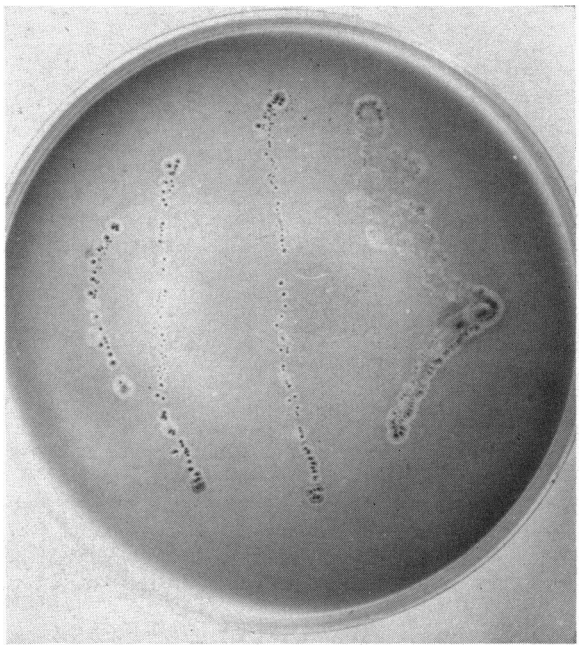

FIG. 3. Colonies of a *Streptomyces* species on a latex-agar plate. Clear zones around the colonies indicate rubber attack.

These circular strips were made by the immersion vulcanization process and were about 0.2 mm thick. The strips were mounted on glass spanners, the strips being stretched to give a circumference elongation of approximately 10 per cent. The mounted rings were put in round flasks so as to be partly immersed in a solution of 0.1 per cent K_2HPO_4, 0.05 per cent $(NH_4)_2SO_4$, and 0.02 per cent $MgSO_4$ in distilled water. The assembly was closed with a cotton plug and sterilized 15 minutes at 115 C. A photograph of such a flask is shown in figure 4. Two flasks were inoculated, each with one of the isolated *Streptomyces* strains. A third flask served as an uninoculated control.

After they had been kept at room temperature for 8 months, small holes became visible to the naked eye in one of the inoculated strips. After 12 months, the holes had reached a diameter of 1.5 mm. The rubber strip inoculated with the second *Streptomyces* did not show distinct holes, but it had become wrinkled and limp. That these effects both must have originated in microbial action is testified to by the appearance of the control. The control strip still had the same appearance as at the beginning of the experiment. The strips were then taken out for inspection. A picture of the perforated strip is shown in figure 5. By microscopical examination of the edges of the holes, filaments of *Streptomyces* could easily be detected. Figure 6 is a photomicrograph of one of the holes in the perforated strip; a dense growth of *Streptomyces* filaments within the area of destruction can be clearly discerned. Tensile strength measurements, kindly carried out by the Rubber Institute T.N.O. at Delft, showed the average values.

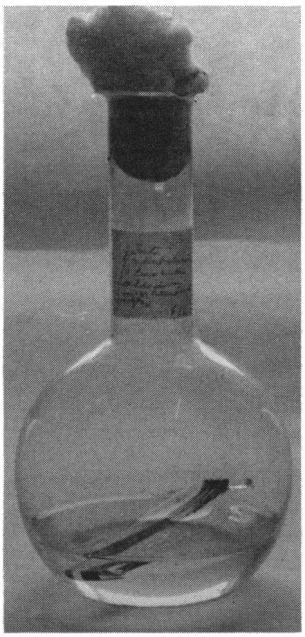

FIG. 4. Device for the study of the attack of vulcanized rubber strips by pure cultures.

This test leaves no doubt regarding the destructive action of the two *Streptomyces* strains examined. The conclusion therefore seems warranted that these two strains, which had been isolated from corroded rubber rings, are able to attack vulcanized rubber.

The significance of this result for the problem of the deterioration of the rubber rings in the pipelines of the water-distribution net again demands a cautious interpretation. It is certainly highly significant that from a corroded part of such a ring a microorganism has been isolated which in pure culture is able to attack a vulcanized rubber compound having the same composition as the ring in question. However, it should not be lost sight of that the conditions prevailing at the surface of the corroded ring are distinctly different from those which are realized in the pure culture experiment. One needs only point out the low concentration of mineral nutrients in the tap water. It seems possible that the continuous supply offered by the running water can make up for the difference in this respect. However, if the deterioration of the ring would be solely due to the action of some *Streptomyces* species, one should have expected that a microscopical examination of the material adhering to the corroded rubber should have revealed the presence of easily recognizable *Streptomyces* filaments. In reality, such filaments were only scantily observed.

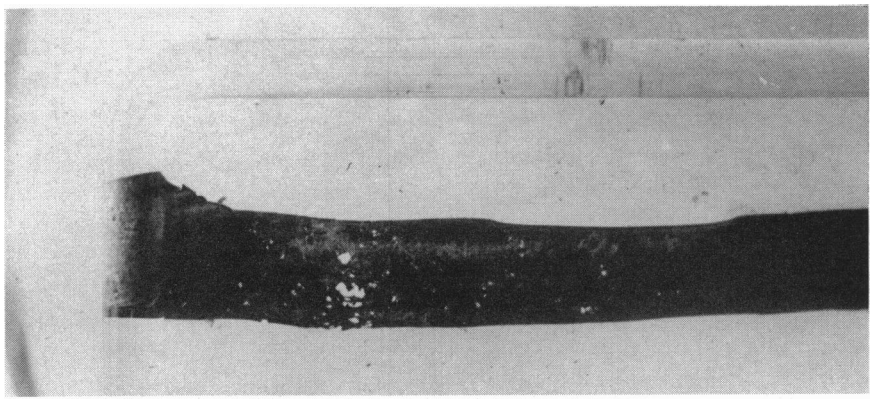

FIG. 5. Vulcanized rubber strip perforated by a *Streptomyces* species.

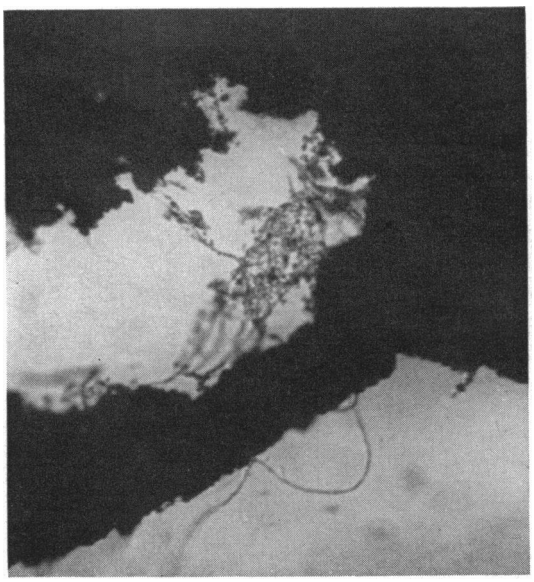

FIG. 6. Photomicrograph of cavity in rubber strip showing *Streptomyces* filaments.

TABLE 1. *Tensile strength measurements of vulcanized rubber strips inoculated with Streptomyces*

Vulcanized Rubber Strips	Tensile Strength	Elongation
	kg/cm²	%
Uninoculated control	122	480
Perforated strip (inoculated with a *Streptomyces* sp.)	80	390
Wrinkled strip (inoculated with a *Streptomyces* sp.)	70	300

In summarizing, we can only conclude that further investigations are indispensable for a final decision in this matter.

The Effect of the Addition of Some Fungicides

In following this line of research, it is, of course, tempting to search for fungicides or other germicides that render rubber compounds unassailable to microbial activities.

Soon after ZoBell and Grant in 1942 had drawn attention to a possible microbial attack of vulcanized rubber, such a suggestion was made by Dimond and Horsfall (1943). These investigators proposed the use of the fungicidal properties of the well known vulcanization accelerators mercaptobenzothiazole (Captax)[2] and tetramethylthiuram disulfide (Tuads).[2] They therefore examined the germicidal effect when the said compounds were mixed with zinc oxide, as normally would happen in compounding rubber products. In spore germination tests, the Captax in mixtures with zinc oxide lost its germicidal properties, while Tuads was not inactivated. The authors accept the theory that the thiuram compound will remain present as such in rubber after vulcanization. Nevertheless, it is clear that such experiments cannot decide on the ability of the said fungicides to protect vulcanizates against microbial attack.

[2] R. T. Vanderbilt Co., New York.

TABLE 2. *Composition of mixtures in parts by weight vulcanized into thin films*

Compounds	Number of Compound Mixtures					
	1	2	3	4	5	6
Smoked sheet	100.0	100.0	100.0	100.0	100.0	100.0
Zinc oxide	—	10.0	10.0	10.0	10.0	10.0
Stearic acid	—	—	2.0	2.0	2.0	2.0
Lubricating oil	—	—	—	2.0	2.0	2.0
Cannel black	—	—	—	20.0	20.0	20.0
Sulfur	8.0	3.0	—	3.0	3.0	3.0
Phenyl-β-naphthylamine	—	1.0	—	1.0	1.0	1.0
Diphenylguanidine	—	1.0	—	0.5	—	—
Mercaptobenzothiazole	—	—	—	0.8	—	—
Benzothiazyl disulfide	—	—	—	—	0.5	0.5
Tetramethylthiuram disulfide	—	—	3.0	—	0.2	—
Zinc dimethyldithiocarbamate	—	—	—	—	—	0.2
Vulcanization time in minutes	135	30	45	15	10	10

It seemed desirable to investigate this point more directly. For this purpose, special mixtures were made and vulcanized in thin films of about 0.2-mm thickness.

Six different mixtures were prepared; their composition is indicated in table 2.

Vulcanization took place at 142 C, the vulcanization time was chosen in connection with the vulcanization rate of each mixture. Mixture no. 3 contained 3 parts by weight of the tetremethylthiuram disulfide compound per 100 parts of rubber, which is far more than normally applied in rubber processing. Mixture no. 5 is based on a normal technical formula and contains the usual quantity of tetramethylthiuram disulfide. Mixture no. 6 is compounded with the zinc derivative of the latter. The mixtures 3, 4, 5, and 6 thus contained the fungicides to be tested.

Strips of about 12 cm in length were cut and made into circular strips by sticking both ends together. In mounting them on glass U-rods, the strips were slightly stretched, the elongation being restricted to 5 per cent.

Inasmuch as a rapid test was wanted, the soil burial test was applied. Strips of each sample were put into wide glass tubes. Then the tubes were filled with loose garden soil, half way up the rubber strips, as is shown in figure 7. One series of tubes was filled with soil sterilized at 120 C. Both series were kept at 30 C in an incubator room with moisture control in order to avoid drying.

After a period of five months the tubes were opened and the rubber strips were examined. The strips from the tubes with sterilized soil did not show any sign of deterioration; only samples no. 1 and no. 3 (without

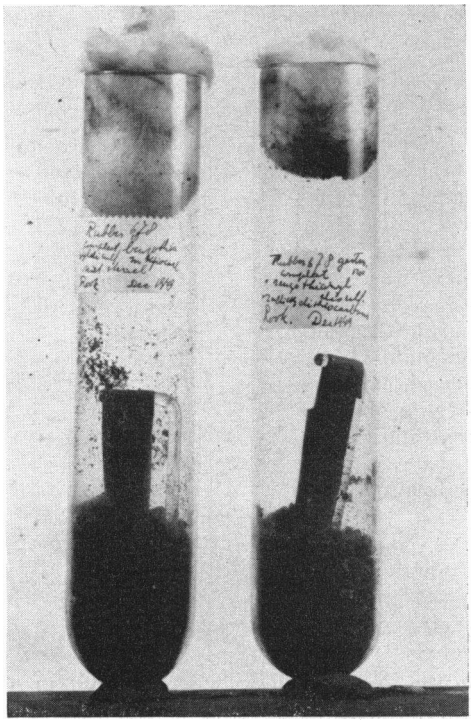

FIG. 7. Soil burial test of rubber strips in glass tubes

antioxidant) had lost some elasticity; the others were quite normal.

In the nonsterile series, sample no. 1 had become slimy and sticky, whereas the samples no. 4, 5, and 6, which corresponded to normal technical rubbers, showed several distinct holes. It is remarkable that sample no. 2 showed only superficial black spots, a result for which no explanation can be offered. Sample no. 3 had become only as limp as the corresponding control.

From the behavior of samples no. 4 and 5 it can be concluded that neither the presence of mercaptobenzothiazole, nor that of tetramethylthiuram disulfide in the normally applied amount does prevent microbial deterioration. Only a large quantity of the latter seems to be adequate in this respect, but such an addition may well influence the general qualities of the final rubber product in an unfavorable way.

In order to determine whether the holes in the samples 4, 5 and 6 had their origin in fungal or bacterial action, the edges of the holes were examined under the microscope. Filaments of *Actinomycetes* were abundantly found. There was a striking similarity of the image to that observed in the experiment with the pure culture of *Streptomyces*.

It is remarkable that the samples having a composition as technically applied (nos. 4, 5, and 6) showed the clearest signs of attack. One might be inclined to connect this with the high content of carbon black in these samples, although this product itself is certainly not attacked by microorganisms.

DISCUSSION

From the foregoing experiments it follows that results of investigations on the microbial attack of complex materials like vulcanized rubber must be interpreted with great caution. In this connection, we need only remind ourselves of the fact that a *Fusarium* strain which gave rise to a luxuriant surface growth on a piece of vulcanized rubber was found to be unable to attack the rubber hydrocarbon present in a plate containing purified latex. This same strain, however, grew profusely in an aqueous extract of the vulcanized rubber. The same observations were made for some strains of *Penicillium* and *Aspergillus*. Growth of molds on vulcanized rubber may, therefore, proceed entirely at the expense of the nonrubber constituents of the vulcanized product.

The conclusion that the ability to attack rubber hydrocarbon is never encountered among fungi would go too far. Nevertheless, we wish to state explicitly here that the stray colonies of fungi which from time to time developed on our latex plates, inoculated either with material removed from the corroded rings or with soil particles, never led to the formation of clear zones in the latex plates, as the simultaneously growing *Streptomyces* colonies did. Moreover, it also seems significant that the rubber-attacking organisms which both Söhngen and Fol (1914) and Spence and van Niel (1936) isolated from their enrichment cultures all belonged to the *Actinomycetes*. Only Kalinenko (1938) claimed to have observed consumption of nonvulcanized rubber by pure cultures of some *Aspergillus* and *Penicillium* strains. However this may be, there is no doubt that we may consider the ability to attack rubber hydrocarbon to be a property most frequently encountered among the *Actinomycetes*.

It may be stressed that a first convincing demonstration has been given of a disintegration of vulcanized rubber by a pure culture of some microorganism. The extent of the breakdown was such that the rubber hydrocarbon must also have been involved. Apparently, the presence of sulfur bridges does not offer an effective protection in this respect, although they certainly decrease the vulnerability.

A way to protect vulcanized rubber products against microbial deterioration seems to be the addition of some product toxic for *Streptomyces* species. Experiments in which the fungicides mercaptobenzothiazole and tetramethylthiuram disulfide were tested did not yield satisfactory results.

A second approach to the solution of the problem may possibly be found in a procedure which makes the rubber product less accessible to water. In this connection, it may be remarked that Boggs and Blake (1936) showed that the water absorption of rubber is largely determined by its protein content. With deproteinized

rubber, the water absorption of a vulcanized compound was reduced to one-sixth of that of a similar compound in which untreated natural rubber had been used. Accordingly, they established that by compounding deproteinized rubber cable insulation could be obtained with much greater resistance against exposure to water. It seems likely that a decrease in water absorption will also markedly reduce the liability of a vulcanizate to microbial attack.

Finally, it should not be lost sight of that the work of Blake *et al.* (1949, 1950, 1953, 1955) has shown that at least certain types of synthetic rubber compounds either are invulnerable to microbial attack or deteriorate at a much slower rate than corresponding compounds of natural rubber.

Bakanauskas and Prince (1955) emphasize that the addition of certain fungicides is harmful to the physical properties of both natural and synthetic rubber compounds. Some fungicides can, however, be used with impunity. This supplements earlier work by Stief and Boyle (1947).

Acknowledgments

The author is greatly indebted to Professor A. J. Kluyver for his helpful discussions during both the experimental work and the preparation of the manuscript, to the Rubber Research Institute T. N. O. at Delft for valuable cooperation, and especially to Mr. D. Luteyn for making the different rubber compounds and for his advice.

Summary

A brief survey of the principal publications on microbial attack of rubber is given. It is stressed that many data dealing with vulcanized rubber are vague and should be interpreted with great caution.

With the aid of purified latex plates, as devised by Spence and van Niel, several *Streptomyces* strains were isolated which attack rubber hydrocarbon. The ability of one of these strains to attack rubber in the vulcanized state was definitely proved; in thin rubber strips, distinct holes were formed.

An attempt was made to check the microbial attack by adding two well-known fungicides, mercaptobenzothiazole and tetramethylthiuram disulfide, to the rubber compound. The result of a series of soil burial tests was unsatisfactory. Other possible ways of prevention of microbial deterioration of vulcanized rubber compounds are briefly discussed.

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