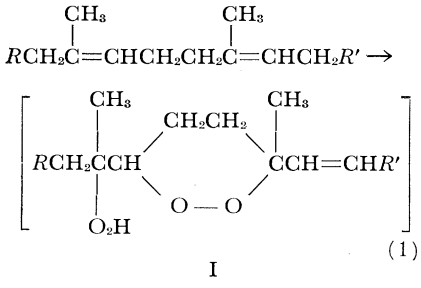
Reports

End Groups of Oxidized Rubber

Chain scission during the oxidation of polymeric 1,5-dimethyl-1,5-dienes occurs nearly quantitatively through the scission of an unusual peroxidic intermediate (*1*), followed ultimately by evolution of a group of fragments of low molecular weight at each scission (*1–4*). The composition of this group depends on the reaction conditions. The first known stable intermediates to be formed subsequent to scission are levulinaldehyde and formaldehyde (*4*):



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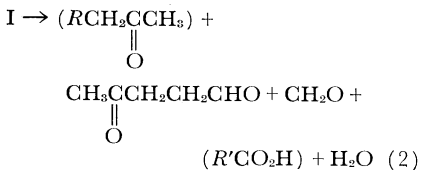

(2)

Bolland's kinetic (*5*) and analytic (*6*) studies combined with the requirements of the observed stoichiometry of scission form the basis on which it is possible to write a detailed mechanism (*1*) for the transformation represented by Eq. 1. Nothing is known in detail of the sequence of reactions resulting in Eq. 2. It has been suggested (*7*) that scission is associated with propagation rather than initiation—that is, with decomposition of a peroxy radical rather than of a hydroperoxide as is indicated in this scheme. This is still a moot point. It is now known, for example, that over-all scission efficiency is strongly determined by temperature (*1, 3, 8*) and that benzothiazolethione (*9*) and *o,o'*-dibenzamidodiphenyldisulfide (*10*) both accelerate oxygen consumption as well as scission, in contrast to earlier belief.

Assignment of the end groups on the newly formed chain ends (in parentheses in the equations) in the original outline of this mechanism (*1, 2*) was based on Naylor's (*11*) analyses of samples of a commercial oxidized rubber. It is assumed that the initial scission of a carbon-to-carbon bond occurs between the two adjacent atoms each attached to oxygen, with the formation of a methyl ketone as one new chain end. This is followed by opening of the six-membered heterocycle and subsequent evolution of the two aldehydes. The chain end remaining has been presumed to go rapidly through the stages RCHO → RCO$_2$H. The high oxygen content of Naylor's samples indicates that they were oxidized at relatively low temperature (*3, 8*), where over-all scission efficiency is low. Although "depolymerized" rubber of both low molecular weight and low oxygen content is available commercially, it seemed desirable to attempt to confirm directly the formation of the postulated end groups on samples oxidized under controlled conditions and not so severely "degraded."

The results of estimates of acetyl and carboxyl end groups on a group of similar samples adjusted in molecular weight in the range 10^5 to 10^6 by oxidation at high temperature are presented in Figs. 1 and 2 (*12*).

Analyses were made on samples of a relatively pure rubber obtained from a commercial latex concentrated by creaming. The only further treatment consisted of leaching the samples with hot water (95° to 99°C) after they had been coagulated on forms. The samples were treated with oxygen at 140°C in an apparatus which has been described (*4*). In this apparatus, the rubber is exposed to a flowing stream of gas so that volatile products are swept away. Under the conditions of this experiment, the measured scission efficiency (moles of chain cuts per mole of oxygen absorbed) is close to 0.1.

An unsuccessful effort was made to estimate total carbonyl content of the oxidized rubber by adaptation of a published method claimed to give high sensitivity and precision (*13*). The iodoform reaction (*14*), which should be specific for one end group, proved to be unexpectedly easy to use. By making use of an observation by Fuson (*15*) the reaction was carried out on a solution of rubber in a mixture of chloroform and dioxane. Rubber was precipitated by the addition of methanol, and iodoform was determined in the supernatant by comparison of the absorption at 347 mμ with a solvent blank. In Fig. 1 the results are plotted as methyl ketone end groups versus oxygen absorbed. The solid line is that expected if one CH_3CO group is formed per scission.

Carboxyl groups were estimated by titration of 0.5- to 1.0-g samples of rubber with potassium methoxide in benzene solution, using phenolphthalein as indicator. The results for one series of samples are plotted in Fig. 2.

These results are in agreement with the hypothesis that the methyl ketone end groups are formed in the initial scission reaction and that the final structure of the other end group produced is determined by secondary reactions. Un-

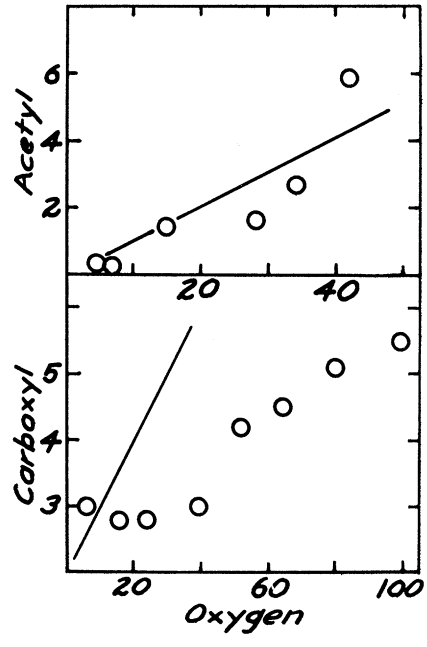

Fig. 1. (Top) Methyl ketone. Fig. 2. (Bottom) Carboxyl. Both are given as a function of oxygen consumed. Units are moles (equivalents) per 10^5 g of rubber. Solid lines show the expected relations if one such end group is formed per scission.

All technical papers and comments on them are published in this section. Manuscripts should be typed double-spaced and be submitted in duplicate. In length, they should be limited to the equivalent of 1200 words; this includes the space occupied by illustrative or tabular material, references and notes, and the author(s)' name(s) and affiliation(s). Illustrative material should be limited to one table *or* one figure. All explanatory notes, including acknowledgments and authorization for publication, and literature references are to be numbered consecutively, keyed into the text proper, and placed at the end of the article under the heading "References and Notes." For fuller details see "Suggestions to Contributors" in *Science* 125, 16 (4 Jan. 1957).

der the conditions used in the experiments reported here, just under half the product is oxidized as far as carboxylic acid (*16*).

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References and Notes

1. E. M. Bevilacqua, *Rubber Age (N.Y.)* 80, 271 (1956).
2. ———, *J. Am. Chem. Soc.* 77, 5396 (1955).
3. ———, *J. Org. Chem.* 21, 369 (1956).
4. ———, *Abstr. 130th Meeting Am. Chem. Soc.* (1946), p. 4T; *J. Am. Chem. Soc.*, in press.
5. J. L. Bolland, *Proc. Roy. Soc. (London)* 186A, 218 (1946).
6. J. L. Bolland and H. Hughes, *J. Chem. Soc.* 1949, 492 (1949).
7. L. Bateman, *Trans. Inst. Rubber Ind.* 26, 296 (1950).
8. E. M. Bevilacqua, *J. Am. Chem. Soc.* 77, 5394 (1955).
9. B. Dogadkin *et al.*, *Rubber Chem. and Technol.* 23, 563 (1950); A. S. Kuzminskii and N. N. Lezhnev, *Rubber Chem. and Technol.* 26, 352 (1953); G. J. van Amerongen, *Ind. Eng. Chem.* 47, 2565 (1955).
10. J. E. Newell and E. M. Bevilacqua, unpublished.
11. R. F. Naylor, *Trans. Inst. Rubber Ind.* 19, 45 (1944).
12. This is communication No. 164 from the General Laboratories of the United States Rubber Company.
13. G. R. Lappin and L. C. Clark, *Anal. Chem.* 23, 541 (1951); M. F. Pool and A. A. Klose, *J. Am. Oil Chemists' Soc.* 28, 515 (1951).
14. F. D. Snell and C. J. Snell, *Colorimetric Methods of Analysis* (Van Nostrand, New York, ed. 3, 1953), vol. 3.
15. R. L. Shriner and R. C. Fuson, *The Systematic Identification of Organic Compounds* (Wiley, New York, ed. 3, 1948).
16. I am indebted to C. L. Hilton and O. E. Beckvold for spectrographic analyses, and to B. E. Jacobs, A. Denice, and J. Luethy for microtitrations.

29 May 1957

Inhibition of Cell Division of Escherichia coli by Low Doses of Ultraviolet Light

Several workers have observed and worked with the division inhibition of *Escherichia coli B* by ultraviolet light (*1, 2*). However, little quantitative work has been done on the division inhibition as measured microscopically. Ultraviolet light inhibits the cellular division and causes the cells to grow into long "filaments," or "snakes," of about the same diameter as the normal cells but of many times the normal length. The filaments, as they will be called in this report, have no constrictions and appear as long, continuous structures under a phase-contrast microscope.

Cultures of *Escherichia coli B*, carried on nutrient agar slants, were inoculated into C-1 minimal medium (*3*) and grown overnight at 37°C with aeration. By morning, the cells were adapted to growth in the minimal medium and were growing exponentially at a concentration of about 10^9 cells per milliliter. These were diluted to about 10^8 cells per milliliter into fresh C-1 medium and grown to a concentration of about 2×10^8 cells per milliliter. These cultures were then transferred to quartz cuvettes, kept at 37°C, and stirred during the subsequent irradiation. Irradiation was done with a water-prism ultraviolet monochromator at the desired wavelength. Incident intensities were measured with a calibrated photocell, and the average intensity through the sample was obtained by the method of Morowitz (*4*). Irradiation times were of the order of 10 to 100 seconds, and reciprocity held over a factor of at least 25 in intensity. Following irradiation, the cultures were grown at 37°C in C-1 medium in subdued light for the desired time.

For doses up to about 100 erg/mm² at 2652 A, the rate of total mass increase of the irradiated cultures as determined by the turbidity at 650 mμ was indistinguishable from the rate of increase of unirradiated cultures. Microscopic observation and Petroff-Hauser counts of the irradiated cells during growth indicated that there were two important classes of cells: (i) those that continued to divide and grow at the usual rate, and (ii) those which increased in length but did not undergo division. During growth after irradiation, the normally dividing cells increased in number according to the division rate. The nondividing cells increased in length, so they were easily distinguishable from the normal cells. By phase microscopy, the relative number of normal cells and filaments was then determined. Knowing the factor by which the normal cells had increased since irradiation, the ratio of dividing cells to nondividing cells could be calculated for the culture as it existed immediately after irradiation.

This microscopic technique does not have the complications that arise in the determination of colony-forming ability, which is highly sensitive to the plating conditions. The cells are irradiated at 37°C during growth in the log phase with no significant interruption of growth conditions before, during, or after irradiation. The only stress being imposed on the system is the action of the ultraviolet light.

The logarithm of the survival of the ability to divide, determined as outlined, is proportional to the ultraviolet dose down to about 10-percent survival, as is shown in Fig. 1. An ultraviolet cross section for the ability to divide can thereby be found from the relation, $N/N_0 = e^{-\sigma D}$, where N/N_0 is the division survival ratio, D is the dose of ultraviolet, and σ is the sensitivity parameter called the cross section for division inhibition.

The ultraviolet doses to inhibit the division in this strain of *E. coli* relative to those needed to alter appreciably other metabolic processes in the same strain have been determined. These results, also shown in Fig. 1, are for random, nonsynchronized cultures. Irradiations were done at 2652 A on C-1 cultures at 37°C.

For determination of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), the cells were extracted with 70 percent ethanol and hot ethanol-ether to remove lipid material, cold 5 percent trichloroacetic acid to remove acid-soluble small compounds, and then hot trichloroacetic acid (5 percent) to remove the RNA and DNA. The hot trichloroacetic acid fraction was assayed for RNA by the orcinol reaction (*5*) and for DNA by the Keck modification of the Ceriotti reaction (*6*).

The doses which inhibit the microscopically observable division of almost all the cells have no detectable effect on the rate of DNA and RNA synthesis. Thus, the DNA and RNA content of the filaments is the same per unit cell mass as for the normally dividing cells. With

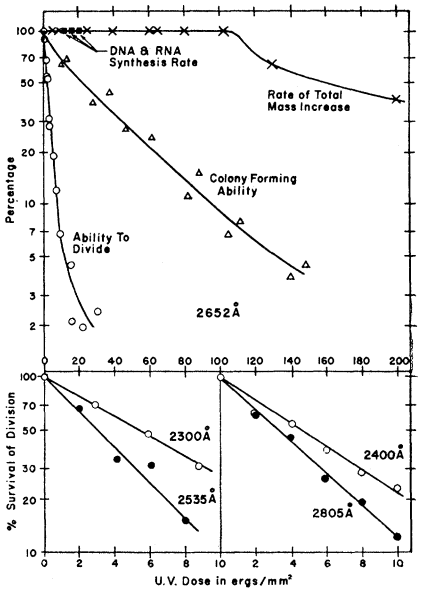

Fig. 1. (Top) Curves of log survival ratio for the given cellular property versus incident ultraviolet dose: ○, ability to divide within 3 hours in C-1 medium during continuous growth after irradiation; △, colony-forming ability on nutrient agar (plating done at about 23°C within 1 hour after irradiation; dilution series done in ice cold C-1 medium without glucose; 37°C incubation); ×, ratio of the rate of turbidity increase after irradiation to the rate for unirradiated samples; ■, ratio of the rate of RNA and DNA increase in irradiated to that in controls (this ratio is 1.00 to within experimental error of 5 to 10 percent for at least 2 hours after irradiation). (Bottom) Microscopically determined survival curves for the division versus ultraviolet dose at several wavelengths. These curves have been corrected to account for the spontaneously arising filaments in unirradiated cultures.