Machlin, U.S. Department of Agriculture, for their disclosure of unpublished data as well as for advice and suggestions. Machlin has recently confirmed, and in some instances extended the findings reported here.

7. Salts "A" contained, in grams per 60 g, the following compounds: $CaCO_3$, 15; K_2HPO_4, 9; Na_2HPO_4, 7.3; $Ca_3(PO_4)_2$, 14; NaCl, 8.8; $MgSO_4 \cdot 7H_2O$, 5; ferric citrate, 0.4; $MnSO_4 \cdot 4H_2O$, 0.42; KI, 0.04; $ZnCO_3$, 0.02; $CuSO_4 \cdot 5H_2O$, 0.02.
8. Choline chloride was added as 0.2 percent of the final ration and the following were added as indicated (in milligrams per kilogram): thiamine HCl, 8; riboflavin, 8; calcium pantothenate, 20; nicotinic acid, 100; pyridoxine HCl, 8; D-biotin, 0.3; folic acid, 3; vitamin B_{12}, 0.02; menadione, 1; vitamin-A acetate, 3; alpha-tocopherol, 10; vitamin D_2, 0.02.

25 July 1955

Synthesis of Rubber by Fungi

Rubber, as *cis*-polyisoprene, was isolated and identified from benzene extracts of sporophores of species of the genera *Lactarius* and *Peziza*. This is believed to be the first evidence of rubber synthesis by microorganisms.

Species of the latex-bearing genus *Lactarius* were collected throughout the growing season in the Brecksville, Ohio, area. Since some species were not abundant, the sporophores of the various species were combined and preserved in ethanol. All species of this group had white latices that did not discolor in air. Sporophores of *L. deceptiva* appeared in large numbers. These were preserved separately. Ascocarps of several saprophytic species of *Peziza* were observed to be rubbery. These were collected and preserved in ethanol.

The carpophores were separated from the ethanol and ground in a meat grinder. The coarsely-ground material was placed in a stainless steel sleeve of fine mesh and extracted for 24 hours with acetone in a large Soxhlet-type extractor. Both alcohol and acetone extracts were evaporated to dryness and the total solids were determined.

The acetone-extracted mycelia were then extracted for 24 hours with redistilled benzene that contained 0.1 percent N,N-diphenyl-p-phenylene-diamine as antioxidant. The benzene extracts, blanketed with nitrogen, were reduced to known volume and aliquots were removed for characterization, for intrinsic viscosity measurements, for total solids, and for cure.

A highly purified sample of *Hevea* rubber was prepared for use as the reference standard for infrared in the following manner. Natural rubber crepe (120 g) was placed in a 6-lit erlenmeyer flask and extracted twice with 2-lit portions of boiling acetone. The acetone-extracted crepe was then placed in 6 lit of redistilled benzene and kept 4 days at room temperature. The benzene-soluble rubber was separated from the gel rubber, which retained most of the protein, by filtration through a fine mesh stainless steel screen. The clear, colorless rubber solution, about 3 lit, was added to an equal volume of acetone. The precipitated rubber was separated, redissolved in benzene, and again precipitated with acetone. The precipitated rubber was dissolved in 4 lit of benzene and filtered through a coarse filter paper. The filtrate of about 25 g of rubber in benzene was placed in a bottle and blanketed with nitrogen. Phenyl-β-naphthylamine, 0.1 percent on the rubber was added as antioxidant. All work was done under nitrogen.

An aliquot of the reference sample was taken and prepared for analysis. The benzene was removed with nitrogen. The resulting film, after 24-hour storage under vacuum over potassium hydroxide, was submitted for analysis with some of the original crepe. Nitrogen content of the reference sample was 0.03 percent, whereas that of the original crepe was 0.45 percent. The carbon and hydrogen values of the purified rubber were 87.90 percent and 11.69 percent, respectively. The theoretical values are 88.15 percent and 11.85 percent.

Infrared spectra of films deposited from benzene extracts of the fungi and from the reference samples were obtained with the B. F. Goodrich infrared spectrophotometer. The films were prepared by evaporating the benzene extracts on sodium chloride disks with nitrogen.

The limiting intrinsic viscosity $[\eta]_0$ of the polymer extracted by benzene from the mixed species of *Lactarius* was determined. Viscosity measurements were made at 25° ± 0.01°C with Cannon-Fenske viscosimeters. The data obtained at varied dilutions are shown in Table 1.

Table 1. Intrinsic viscosity measurements on polymer from *Lactarius* sp; η_r is the relative viscosity. A plot of ln η_r/concentration versus concentration gave a limiting intrinsic viscosity $[\eta]_0$ of 0.29. This yielded an estimated viscosity average molecular weight of 13,900 using Eq. 1.

Concentration (g/100 ml of benzene)	ln η_r/concentration
1.017	0.281
0.508	0.285
0.254	0.298
0.127	0.296
0.127	0.281

The sporophores of the mixed *Lactarius* species contained, on a dry-weight basis, 1.7 percent of a rubbery polymer that was soluble in benzene. The infrared absorption curve (Fig. 1) shows that the rubber is *cis*-polyisoprene. Its curve is identical with that of *Hevea* rubber except for the carbonyl peak at 5.8 μ. This may be ascribed either to an impurity or to oxidative degradation of the rubber during extraction.

The $[\eta]_0$ values of the polymer in benzene at 25.00°C was 0.29. In order to estimate the molecular weight M, we employed the equation

$$[\eta]_0 = 5.02 \times 10^{-4} M^{0.667}, \qquad (1)$$

which indicated a viscosity average molecular weight of 13,900 for the polymer.

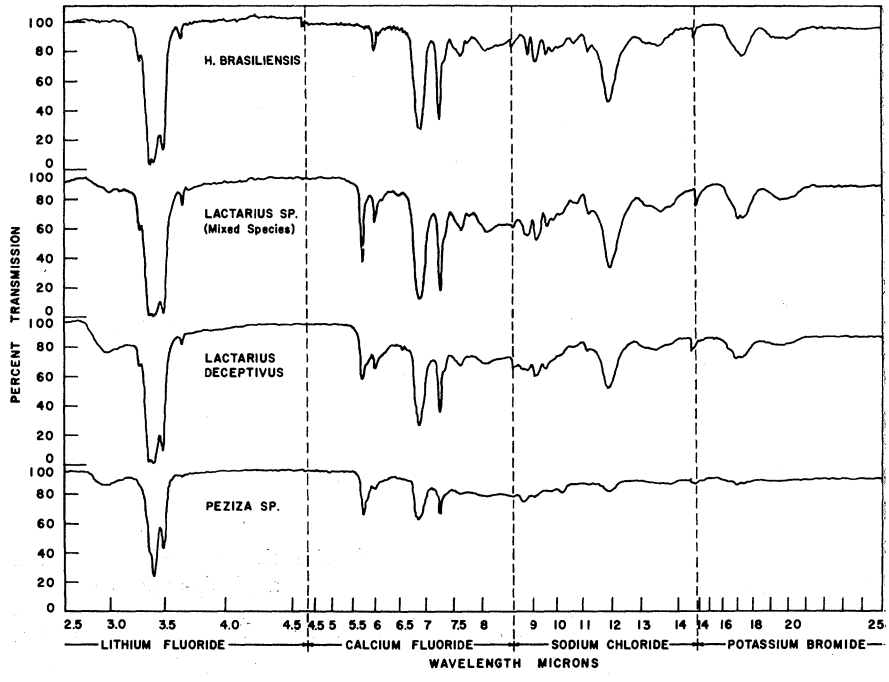

Fig. 1. Comparison of the infra-red absorption spectra of benzene extracts of various fungi with the spectrum of purified rubber from *H. brasiliensis*.

This equation was derived by W. Carter, R. Scott, and M. Magat for natural rubber in toluene (*1*). Since toluene and benzene have similar μ values for natural rubber, the equation was employed for the purpose of estimating molecular weight of the polymer in benzene. The molecular weight of the rubber is low; however, benzene would not extract polymer of high molecular weight—that is, gel rubber—even if it were present. A better method of extraction is needed. Since this is a mixed collection, some species are probably contributing more than the average content of 1.7 percent rubber.

An attempt was made to cure the soft, tacky, low-molecular-weight rubber using the "Peachy" cure and sulfur chloride. The cured products were of low moduli, and elongations did not exceed 200 percent. There was insufficient material for milling to incorporate curing agents.

Rubber extracted from sporophores of *L. deceptiva* was similar to that obtained from the mixed species. It too was *cis*-polyisoprene (Fig. 1). The rubber content was low—0.16 percent on a dry weight basis. It was not characterized further.

The rubber extracted from ascocarps of *Peziza* was much tougher than that from *Lactarius*. Solubility in benzene was very low and deposition of polymer on the salt cakes was not uniform. Satisfactory films were not obtained, but the infrared spectrum (Fig. 1) suggests that this rubber is also *cis*-polyisoprene.

Fungi belonging to two of the major classes of fungi, Ascomycetes and Basidiomycetes, are able to synthesize rubber as *cis*-polyisoprene. Since fungi, phylogenetically, were derived from algae—that is, they are degenerate forms with loss of chlorophyll—the probability of rubber synthesis by species of this phylum is implied.

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1 August 1955

Arsenic Tribromide Immersion Liquids of High Index of Refraction

Immersion liquids of high index of refraction containing arsenic tribromide as the solvent, prepared in recent years (*1, 2*), have proved to be stable. In Table 1 are given the starting materials and their relative proportions by weight used to prepare the liquids, the indices of refraction of the liquids (n_D), the change in index of refraction with time ($\Delta n_D \times 10^{-3}$), and the temperature coefficients (dn/dT) and dispersion of some of the liquids (*3*). The liquids were prepared as described in an earlier paper (*1*). The disadvantages of the arsenic tribromide liquids have been listed in a more recent publication (*2*).

Table 1. Arsenic tribromide immersion liquids. Composition is given in relative proportions by weight. Dispersion is measured by the difference in index between the *e* line of mercury (546 mμ) and the *D* line of sodium (589 mμ).

Composition	n_D	Δn_D ($\times 10^{-3}$)	Time (yr)	dn/dT ($\times 10^{-4}$)	Dispersion ($\times 10^{-4}$)
$6AsBr_3$, 2S, $2As_2S_3$	2.00	−2	1¼	6 (*1*)	174 (*1*)
$6AsBr_3$, 2Se, $2As_2S_3$	2.11	−2	3	6	288
$12AsBr_3$, 1S, $7As_2S_3$	2.07	−1	2½		
$6AsBr_3$, 2Se, $2As_2S_3$	2.04	−2	3		
$14AsBr_3$, 3S, $3As_2S_3$, 2HgS	1.99	−1	3	6	173
$14AsBr_3$, 3S, $3As_2S_3$, 4HgS	2.01	−3	3		
$6AsBr_3$, 2S, 1Se, $2As_2S_3$	2.02	−1	3½	6	195
$6AsBr_3$, 2S, 1Se, $2As_2S_3$	2.00	−5	3		
CH_2I_2	1.74			7 (*4*)	88 (*1*)

The liquid with an index of 2.11 is very viscous and dark red, but a thin film is light yellow. It is possible that even higher indices can be obtained with these mixtures.

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22 July 1955

Mechanism of Antibody-Antigen Reaction

Antiserums to yeast crystalline alcohol dehydrogenase (*1*) were produced in rabbits by subcutaneous injections of 10 mg at weekly intervals. The antibody partially inhibited the activity of the enzyme but did not inhibit liver alcohol dehydrogenase. The enzyme (antigen) reacted with the antibody to form a precipitate of antibody-antigen complex that nevertheless possessed enzymatic activity (*2*). Kinetic studies on the inhibition of the enzyme activity with increasing increments of antiserum (*3*) led to the formulation of the following concept.

When an antigen is first introduced into the body, it stimulates the formation of specific antibodies to special sites on its surface that possess the necessary configuration. If it is still present in the body or is again introduced into the animal at a time when antibodies are already present in the circulation, it reacts rapidly with these earlier antibodies to form an antibody-antigen complex (complex I). This complex, in which the configuration of the antigen and antibody are mutually altered, behaves as a new antigen. Specific antibodies are then formed against the new sites of altered configuration. Similarly, if the antigen is still present in the body, or upon the introduction of the antigen for a third time, the earlier antibodies react with it to form complex I, which then reacts with its specific antibody to form complex II, and this in turn behaves as a new antigen with a newly altered antigenic surface. Complexes III and IV, and subsequently others, may be formed in like manner. This process presumably continues with each introduction of the antigen until a stage is reached when the final complex ceases to be antigenic or fails to differ antigenically from the immediately preceding complex.

The concept just developed represents no departure from already accepted principles of immunology and protein chemistry. There are many examples of compounds, termed *adjuvants,* that act on some substances to influence their immunological reactivity (*4*). Furthermore, a staphylococcus toxin reacts with rabbit muscle extract to render it antigenic when it is injected into rabbits (*5*).

Proteins are capable of astonishing structural transformations. They can unfold to expose new groups, hydrophilic or hydrophobic as the case may be, depending on the environment (*6*). It is therefore to be expected that the structural configuration on the surface of the antibody-antigen complex must necessarily differ at certain sites from the original configurations of the individual antigen and antibody. It has already been shown that an antibody molecule does in fact increase in volume upon reacting with the antigen (*7*). Such an increase in volume is interpreted to signify an unfolding of the molecule with a structural modification of its surface configuration. The very fact that a precipitate is formed can readily be explained by the unfolding of both antibody and antigen so as to expose a large number of hydrophobic groups.

In support of this concept, it has been possible to show the following (*8*).

1) The subcutaneous injection of a thoroughly washed antibody-antigen complex into rabbits produced antiserum that reacted more rapidly with the complex than with the enzyme antigen itself. This