fected bovine tongue tissue. The Roux flask cultures are used for the production of FMV on a relatively large scale. Each flask yields 75.0 ml of fluid containing approximately $10^{7.0}$ tissue culture ID_{50} per milliliter.

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References and Notes

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Ability of Sodium Sulfate to Stimulate Growth of the Chicken

Reports demonstrating that only a trace of $Na_2S^{35}O_4$ is incorporated into sulfur amino acids (*1, 2*), that larger fractions are incorporated into taurine (*1, 3–5*), but that the largest uptake of radiosulfate occurs in the chondroitin sulfate matrix of cartilage (*2, 3*) led us to study the nutritional significance of these findings (*6*).

Chickens were fed rations containing casein, 15 percent; gelatin, 10 percent; corn oil, 4 percent; salts "A" (*7*), 6 percent; all essential vitamins in excess of their requirements (*8*); and glucose to make 100 percent. The protein furnished about 0.08 percent cystine and 0.51 percent methionine.

Sulfur or Na_2SO_4 added to this diet did not improve growth; however, when the sulfates of magnesium, manganese, and copper present in salts "A" were replaced with equimolar levels of the corresponding oxides or chlorides (new basal diet: LC2MS), it was found that sodium sulfate improved growth and feed efficiency. Typical data are given in Table 1. Dietary sulfate, in addition, appears to be capable of stimulating normal feather development, even though the sulfur amino acid content was too low to support optimal growth.

Feathers from birds that received $Na_2S^{35}O_4$ for 10 to 14 days in amounts sufficient to maintain blood levels of 0.01 μc/ml of plasma were quite radioactive (0.5 to 1.0 percent of the total isotope dose), but feather cystine and methionine accounted for less than 5 percent of this activity. Sulfate isolated after feather hydrolysis accounted for 60 percent, and about 30 percent was contributed by a nonsulfur amino acid, nonsulfate fraction that is still unidentified. This unidentified fraction also accounted for about 30 percent of the total feather activity when methionine-S^{35} was fed in a similar fashion even though the sulfate fraction then accounted for less than 5 to 10 percent.

Sulfur amino acids isolated from hydrolyzates of other tissues from birds that had received $Na_2S^{35}O_4$ for the period of 10 to 14 days incorporated only small amounts of S^{35}. We feel that the low levels of activity found in tissue sulfur amino acids merely reflect bacterial synthesis in the alimentary tract.

Taurine accounted for approximately 15 to 25 percent of liver activity; sulfates (after acid treatment) accounted for about 60 percent. Machlin (*5*) reported that about 20 percent of the sulfur from $Na_2S^{35}O_4$ that is retained by chickens is incorporated into body taurine. We found that, regardless of the ration, chickens retained initially 50 to 75 percent of a given oral dose of $Na_2S^{35}O_4$, and by 10 to 14 days, 15 to 30 percent had not been excreted. We also confirmed in a preliminary way the reported (*2, 3*) rapid uptake of radio sulfate into the mucopolysaccharide-mucoprotein "organic" sulfate fractions isolated from connective tissues.

Our work indicates that the chicken can satisfy part of its total sulfur requirement with inorganic sulfate. The sulfur amino acids meet much larger fractions of this sulfur requirement. However, large quantities of either methionine or methionine hydroxy analog did not appear completely able to satisfy the total sulfur requirement when they were administered to rapidly growing chickens that were fed a cystine-low, inorganic-sulfur-free diet. Cystine, added to such diets (provided that methionine was not limiting), did satisfy the requirement under these conditions when it was added at sufficiently high levels. Inorganic sulfate cannot replace dietary cystine or methionine for protein synthesis, and studies with $Na_2S^{35}O_4$ appear to confirm these results, although sulfate apparently will "spare" dietary sulfur amino acids for protein synthesis. The effect of elemental sulfur and inorganic compounds of sulfur, the metabolism of S^{35} and $Na_2S^{35}O_4$, the effect of sulfate on methionine-deficient diets, as well as a more detailed account of the work reported here, is in preparation.

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6. We wish to express our appreciation to G. M. Briggs, National Institutes of Health, and L. J.

Table 1. Effect of Na_2SO_4 and graded levels of sulfur amino acids and analogs on growth of chickens.

Ration and supplements	Gain (g)	Standard error*	Feed efficiency†	Feather score‡
Five weeks				
Basal (LC2MS)§	371.5	24.5	2.84	1.2
Basal + 0.5% Na_2SO_4	488.1	24.6	2.70	3.6
Basal + 0.22% DL-methionine	516.7	23.8	2.43	1.8
Basal + 0.22% methionine hydroxy analog‖	521.0	19.7	2.41	2.1
Basal + 0.22% analog + 0.5% Na_2SO_4	617.0	16.4	2.38	3.8
Three weeks				
Basal (LC2MS)#	119		4.01	
Basal + 0.5% Na_2SO_4	214		2.54	
Basal + 0.22% DL-methionine	163		2.26	
Basal + 0.22% DL-methionine + 0.5% Na_2SO_4	301		2.15	
Basal + 0.44% DL-methionine	259		2.46	
Basal + 0.44% methionine hydroxy analog	263		2.36	
Basal + 0.49% analog	274		2.14	
Basal + 0.44% analog + 0.5% Na_2SO_4	308		1.71	
Basal + 0.4% L-cystine	311		1.75	

* Standard error for one group of 10 to 12 New Hampshire cockerels raised in starting batteries of conventional type with raised wire screen floors.
† Feed consumed divided by weight gained. Under these conditions, an improvement (less feed consumed per unit gain) in excess of 0.10 was found to be significant.
‡ Average of scoring by two persons of each group; a difference in excess of 0.5 was found to be significant on a scale of 0 to 4.0.
§ 4.1 g $MgCl_2$, 0.37 g $MnCl_2$, and 0.03 g $CuCl_2$ used instead of corresponding sulfates in salts "A."
‖ Calcium DL-2-hydroxy, 4-methylthiobutyrate.
1.1 g MgO, 0.32 g MnO_2, and 0.03 g $CuCl_2$ used instead of corresponding sulfates in salts "A."

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.

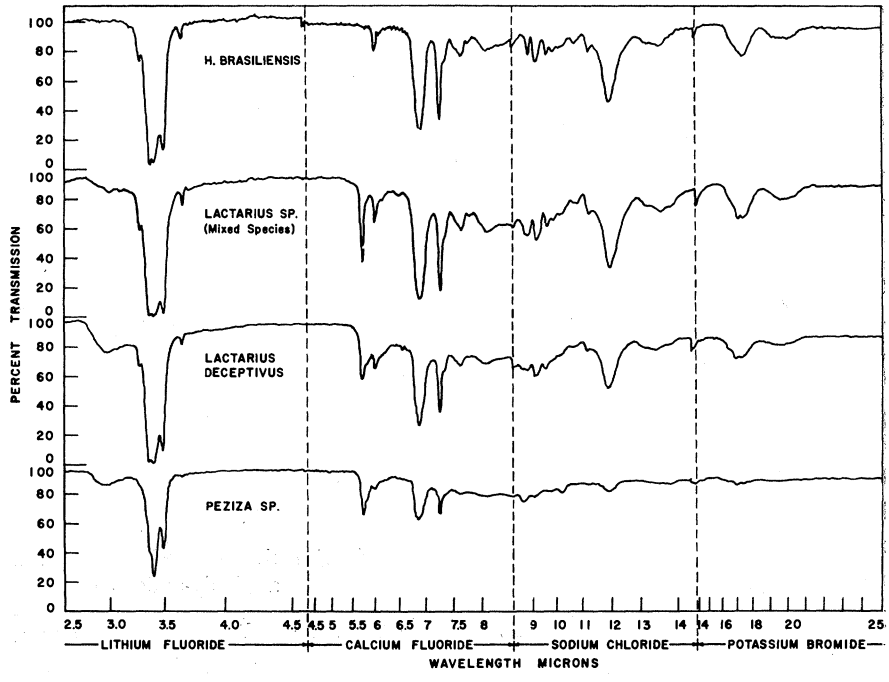

Fig. 1. Comparison of the infra-red absorption spectra of benzene extracts of various fungi with the spectrum of purified rubber from *H. brasiliensis*.