Caltech researchers are finding out how guayule, a native wild shrub, manufactures excellent natural rubber. Will they be able to duplicate the process in the laboratory?

New source of natural rubber

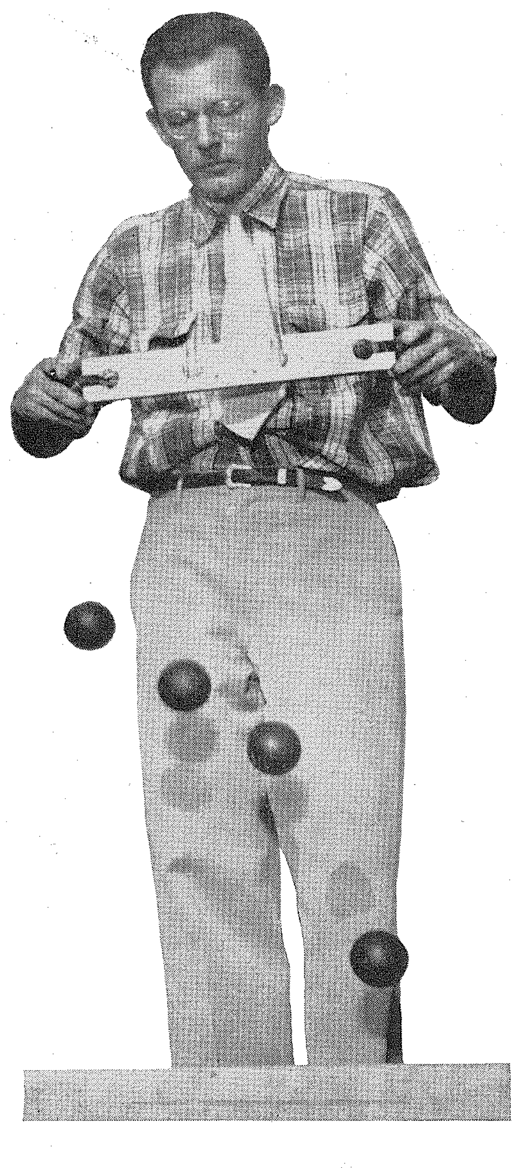
THE gentleman bouncing the rubber balls below is Dr. James Bonner of Caltech's Biology Division, and he is illustrating a point that to most is no news: For many purposes natural rubber still has it all over the synthetic product.

The four balls shown here are of identical size, and they were compounded in the same way. From left to right, the first two are natural rubber, the third is GR-s or Buna rubber, and the fourth is butyl. Dr. Bonner dropped them all at once from the same height, and the photographer caught them at the top of their bounce.

Caltech's interest in rubber centers mainly around natural rubber from guayule—one of 2,000 known rubber producing plants, and one of 600 which have been tried, at one time or another, commercially. Of the 600, guayule appears to be one of the most energetic. About 20 percent of its dry weight may be rubber. Yields of rubber from guayule vary greatly depending on the climate, but mature guayule plants have been known to yield as much as 2,700 pounds of rubber per acre. Furthermore, getting rubber from guayule plant to finished product involves about 1/20th as many man hours as is the case with rubber from the rubber tree.

Guayule is native to the Chihauhuan desert of northern Mexico and the Big Bend region of Texas, but it has been grown in parts of California, Arizona, New Mexico, and in several foreign countries—including sections of South America, Europe and Russia. The first commercial shipment of rubber from wild guayule shrubs reached this country in 1904 from Mexico, and by 1940 Mexican shipments were amounting to about 10,000 tons of rubber a year.

Production from the wild shrub still continues. While our rubber consumption is now so great that production from guayule has become less and less significant, guayule's potentialities were considered of such importance during World War II, that 32,000 acres of government-owned guayule were put under cultivation in this country. During the war a good deal of research was done on these plants— research on how to increase the plant's rubber production and on how to improve the processing of the rubber itself.



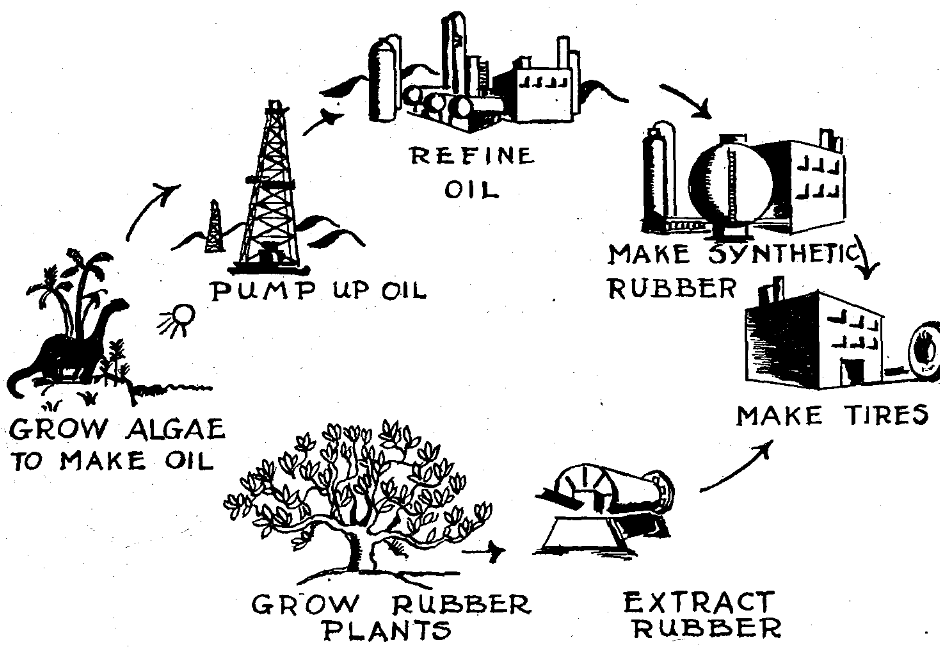
But strangely enough, in spite of all that was learned during this time about guayule nutrition, and the factors which influenced its growth—correct night temperature and light intensity, for instance—very little work was done on the actual chemical mechanism by which rubber is made in guayule.

How does the guayule do it? Plant physiologists wish to answer this question so that they can synthesize natural rubber outside the living plant. When they find out what the chemical precursors to rubber are, and what the enzymes and conditions of polymerization are in the plant, they can use this knowledge to consider the possible synthetic production of real, natural rubber—not a rubber substitute. This is the basis for continuing research on guayule at Caltech.

Chemically, natural rubber is an isoprenoid compound. But isoprene itself has never been found in plant tissues, even though it has been extensively sought there. Plant physiologists feel, therefore, that probably some other compound possessing the same carbon skeleton may be the elementary unit which is polymerized, or changed into rubber, in the plant. The search for this compound and a study of the mechanism by which it is formed—a search for the rubber precursor—is the object of a cooperative research project by the United States Department of Agriculture's Bureau of Plant Industry, Soils and Agricultural Engineering and Caltech. And, as the American Chemical Society's Symposium on Plant Synthesis heard recently in San Francisco, the project has made considerable headway.

At Caltech, research on the problem is being conducted along two parallel lines—with seedlings of guayule plants, and with cultures of the rubber-producing parts of the guayule plant, grown in isolated solutions.

Rubber is produced in the bark



A plant physiologist (understandably prejudiced) suggested this comparison of two ways of getting rubber. Starting at the left, one can either wait for algae to form oil, pump the oil, refine it and use by-products for synthetic rubber; or one can go right to the guayule and simply extract natural rubber. This reasoning has one obvious fault: production from guayule could never meet present rubber consumption. A better method would be to find out how the guayule does it, then learn how to copy its chemical process.

and stems of guayule, not in its leaves. But, as shown in the accompanying diagram, without the leaves the guayule plant cannot produce rubber at all. Obviously the leaves contain something which is essential to rubber formation in the stem tissues. As yet the nature of this compound is unknown, and researchers feel that its identification is going to be a complex job. Meanwhile, they are finding that the most fruitful approach lies in testing pure synthetic compounds for their ability to support rubber formation in isolated stem cultures.

Stems or seedlings are grown in synthetic nutrient solution, under controlled environmental conditions, so that rubber formation at the expense of rubber precursors present in the plant can be carefully controlled. To the nutrient solution, then, are added compounds which are regarded as possible rubber precursors or intermediates. If a compound causes an increase in rubber formation, it is considered a possible intermediate. Final proof is based on experiments which are being conducted with isotopically tagged molecules of the intermediate chemical compound.

When stems or seedlings are grown as described above, they produce but little rubber. But the addition of an extract of leaves from plants actively engaged in rubber formation actually increases the amount of rubber formed and accumulated in the stem tissues.

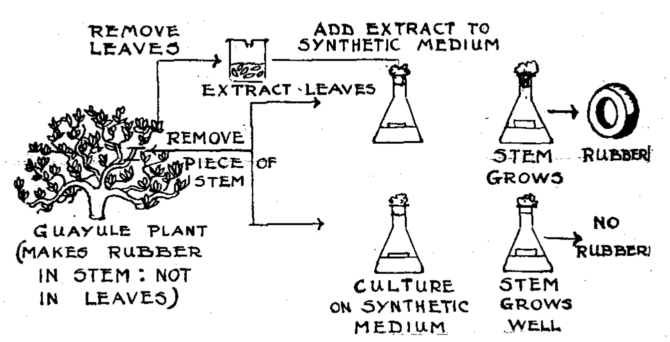
A number of compounds, such as isovaleraldehyde, tigaldehyde, tiglic acid and an amino acid, valine, which have the carbon skeleton of isoprene and which are known to appear in nature, were tried and found to be inactive in rubber production. Other compounds which have been suggested by workers in the field, such as simple terpene compounds and betamethylcrotonaldehyde, were also tried and found to be inactive.

Results of the experiments conducted at Caltech did indicate, however, that a reaction in which acetic acid and acetone combine to produce a compound known as betamethylcrotonic acid, is of importance in rubber formation. When acetate was added to the nutrient solution in which either stem tissues or seedlings were grown, a considerable increase in rubber formation took place. And acetone, like acetate, was found to affect rubber formation by seedlings. Another substance, glycerol, was found to influence rubber accumulation too. But glycerol is known to be rapidly converted to acetate and to acetone by various microbial systems, and it was thought to be entirely possible that the activity of glycerol in rubber formation in plant tissues was caused by some such conversion.

How do acetate and acetone bring about rubber formation in guayule? The Caltech plant physiologists decided to find out whether or not betamethylcrotonic acid might be a condensation product of the two substances. When tested for its ability to support rubber formation, this substance turned out to be as active as acetate and acetone, both with the seedlings and with the isolated stem tissue cultures.

The next step was to find out how acetone might arise in the stem tissue. And at this point it was found

THE SECRET OF GUAYULE'S RUBBER MAY LIE IN ITS LEAVES.



Caltech researchers have devised a striking experiment, left, to show how an unidentified chemical, produced in leaves, is vital for manufacture of rubber in stems. Stem sections alone can be made to grow in synthetic medium, but produce no rubber. When leaf extract is added to medium, stems produce rubber as usual.

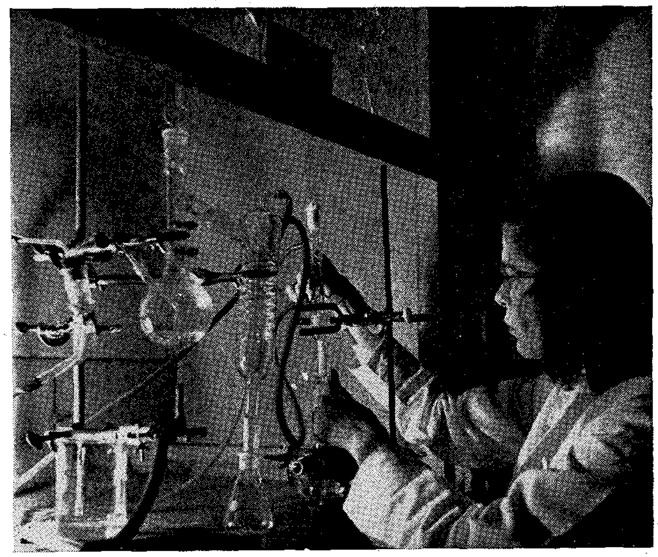
that acetoacetic acid, which is known to be an intermediate in the forming of acetone from acetate by various species of bacteria, is active in replacing acetone in rubber formation in guayule tissue. It seemed possible, therefore, that acetone arises in guayule, too, through acetoacetic acid.

These investigations were then extended to a study of the metabolism of radioactive acetate—acetate tagged with $Carbon_{14}$. When the tagged acetate was fed to seedlings or to mature plants, it turned up within three days in the plant's rubber. In one experiment 75 per-cent of the acetate metabolized by the plant appeared in the form of rubber within three days, and the rest of the radioactive acetate metabolized appeared as resins which are also isoprenoid compounds. When radioactive acetone was supplied to the plants the same thing happened. Radioactivity was recovered in the rubber the plant made.

As for betamethylcrotonic acid—it has actually been found in plants, but efforts to isolate this substance from guayule have been unsuccessful. But a striking experiment recently performed at Caltech indicates that it is indeed formed when acetate is metabolized by guayule.

In this experiment, guayule plants were fed with radioactive acetate. After three days the plants were harvested, and the organic fraction which *should* contain betamethylcrotonic acid was isolated. To this fraction researchers added a large amount of inactive betamethylcrotonic acid, recrystallized the material and found that it contained radioactivity. The radioactivity could only have come from radioactive betamethylcrotonic acid in the plant extract, and is strong evidence that this acid is a by-product of acetate.

In establishing this relationship between acetate, acetoacetate, acetone and betamethylcrotonic acid, the Caltech researchers have made a big step forward in an understanding of the biosynthesis of natural rubber. The next step is to find out how betamethylcrotonic acid turns into rubber itself. The compound possesses the same arrangement of carbon atoms as the isoprene of which rubber is composed. If betamethylcrotonic acid molecules are combined to form rubber, the process must necessarily involve reduction and elimination of oxygen, since rubber contains only carbon and hydrogen. This kind of a condensation and reduction would not be unprecedented in nature, however, since biochemists have shown that the long hydrocarbon chains of fatty acids are formed by such a condensation and reduction of acetate. In this further work, scientists will be on the final lap: reproducing the plant's rubber producing process in the laboratory, and perhaps on a commercial scale.



Research Assistant Betty Jean Wood traces acetate metabolism in guayule with radioactive C14. Research Fellow on project is Dr. Barbarin Arreguin, Dep't. Agriculture.