thromboplastic activity is related to the degree of polymerization of the hyaluronate, which is readily altered, resulting in loss of viscosity, the activity of low-viscosity preparations is best demonstrated in the two-stage purified system. The addition of 1% sodium chloride, a slight change of pH in either direction, successive freezing and thawing, or even standing for any length of time at room temperature are among the factors that cause both a loss in viscosity and in thromboplastic activity.

Thus it can be seen that sulfate-free hyaluronate, as it occurs naturally in the body, perhaps in combination with a protein, is an active thromboplastic agent. Its activity in coagulating blood is related to its viscosity. Like thromboplastin of tissue origin, it is dependent upon calcium, is affected by accelerator globulin, and has similar kinetics. Unlike tissue extracts, however, its activity is destroyed by hyaluronidase or by any physical procedure resulting in depolymerization. It is noteworthy that the simple sulfonation of hyaluronic acid produces a strong heparinlike anticoagulant (*10*). The widespread distribution of hyaluronic acid in tissues, and the accumulation of it or a similar substance in areas of arteriosclerosis, suggest that it may play an important role in the coagulation of blood *in vivo*.

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Tobacco Mosaic Virus Mutation

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All plant viruses that have been sufficiently studied reveal the existence of one or more strains which have evidently arisen by mutation. This subject has been frequently reviewed, most recently by Bawden (*1*). The number of strains reported for a single virus is often large, but the frequency of virus mutations has not been clearly established.

Much of the claim for high rates of virus mutation rests on the earlier studies of the yellow tobacco mosaic virus. McKinney (*2*) concluded from a study of more than 5,000 plants that the occasional yellow spots resulted not from viruses introduced from the outside, but from strains that originated as mutants in the tissues involved in the spots. Kunkel (*3*) reported that 232 plants, inoculated with 4 green strains, yielded over 5,000 yellow spots, or an average of about 6.6 spots/plant, and 0.5–3.0 spots/leaf. Jensen (*4*) and others have come to similar conclusions. Hence, the common occurrence of yellow spots on otherwise green mosaic plants has been attributed to mutations of the green virus, rather than to yellow strain contaminants in the green types.

Efforts have been made in this laboratory to determine the number of strains of the tobacco mosaic virus that are definitely determinable as such, by the available techniques. The number of these strains is found to be relatively low (*5*). If the numerous yellow variants reported to occur should be considered as mutations they would necessarily seem to be identical or recurring mutations.

To secure further information on this matter it would appear to be necessary to eliminate all possibility of contamination of green strains with yellow strains. This has been possible with improved technique as far as differentiation between green and yellow strains is concerned. The local-lesion technique in serial transfers was used to eliminate the yellow strain contamination. When green strains yield only green isolates they may yet only be assumed to be free of yellow, since more than one strain may exist in a green strain obtained from a single lesion.

Nicotiana sylvestris is well known as a test plant for yellow strains, but it unfortunately also gives local lesions with some green strains (*6*). Nevertheless, if local lesions are not obtained on *N. sylvestris* from green isolates, it appears to be satisfactory evidence that a yellow strain is not present in a totally green plant. However, local lesions on *N. sylvestris* must be verified by yellow symptoms on Havana Seed test plants to establish that a yellow strain is present if prior symptoms do not confirm this point. Tests on *N. sylvestris* often reveal the probable presence of a yellow strain long before yellow symptoms have appeared. In some instances where a single yellow spot has developed on an otherwise green mosaic plant the presence of a yellow strain has been demonstrated in areas considerably distant from the tissue involved in the spot. At least 8 strains of green mosaic have been secured that are free of yellow contamination. Many of these plants have been grown in the greenhouse, under optimum conditions for yellow strains to develop, for a period of 3 months, and to the 15–20 leaf stage without the appearance of any yellow mutations.

The reasons for easy misinterpretation of results with yellow spots are due to several circumstances, such as interference phenomena and environmental conditions. Altogether, these factors may yield an infinite variety of symptoms within fairly restricted boundaries, beyond which, however, further variation is not to be expected.

Our experimental results appear to have established the following: (1) Green and yellow strains may be readily separated where they exist in mixtures. (2) Green strains, if completely free of yellow strains, were not found to yield yellow spots. (3) Yellow areas very similar to those reported as "mutations" may be "synthesized" by proper mixtures of known yellow and green strains under controlled environmental conditions. (4) The known yellow strains, of which only 5 have been encountered in our studies, show that new isolates are similar to older known strains. (5) No evidence was secured that some strains mutate and others do not.

The conclusion has been reached that the yellow spots occurring only occasionally, or frequently, in green tobacco mosaic strains are usually the result of contamination with yellow strains. A more detailed account of the results is planned for a later paper.

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The Formation of Cycloparaffins in Petroleums

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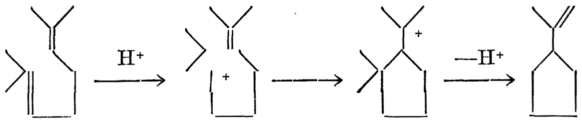
The formation of cycloparaffins, found so abundantly in petroleums, has never been plausibly explained. In a previous paper (*1*) evidence was presented that the chemical reactions resulting in petroleums involved typical carbonium reactions caused by acid silicate catalysts in the form of natural clays, and possibly other minerals, in contact with the heavy oil undergoing change. Some of the evidence was more fully given in two previous papers (*2*).

In the first paper referred to (*1*), it was pointed out that carbonium ions offer the only plausible explanation for the formation of aromatic hydrocarbons, always present and sometimes in substantial proportions in petroleums, within the low temperature limits which the chemical evidence, and particularly the geological and field evidence, impose. The same type of carbonium ion reactions explain the large number of paraffin hydrocarbons of normal and branched chain structure found in straight-run gasolines. Neither paraffins nor aromatic hydrocarbons are found in the heavy bitumens or heavy asphaltic oils containing no gasoline, which D. C. Barton (*3*), on geological evidence, regarded as the intermediate material between typical petroleums containing gasoline and the original organic source material. In a very recent study of the Wilcox, Sparta, and Cockfield formations of the Louisiana Gulf Coast area, Bornhauser (*4*) concluded that "the fact that the light oils are found in the shale facies or in the transition zone to the sand facies appears to lend considerable support" to the theory of catalytic action.

It is just as important, however, to explain not only the formation of the paraffins and aromatics, but the cycloparaffins as well. It has been previously pointed out (*5*) that the cycloparaffins, including many cyclopentanes and cyclohexanes, found in substantial proportions in petroleum, cannot be accounted for by the degradation of any known possible source material, nor by the hydrogenation of aromatic hydrocarbons. They can be accounted for, however, by cyclization of unsaturated hydrocarbon structures, for which a carbonium ion mechanism, in the presence of acid catalysts, has already been proposed, in certain cases, by Stevens and Spalding (*6*).

The instances in which cyclization of mono-olefins has been observed experimentally have been rare. Isobutene has been converted to 1.1.3-trimethyl cyclopentane in high yields by heating under pressure. However, many instances are known of the formation of cyclohexane or cyclohexene derivatives by the dimerization of dienes. As pointed out by Stevens and Spalding (*6, 7*), such cyclizations probably take place, by the action of acid catalysts, through a carbonium ion mechanism. They also showed that cyclopentanes can be formed in a similar manner, as in the case of 2.7-dimethyl octadiene-2.6:



They conclude that a cyclopentane ring can be formed by cyclization without difficulty when the acyclic diene is constituted so that formation of the cyclohexane ring is difficult because of steric factors, or because shift of the initial carbonium ion to positions favorable to cyclohexane formation involves too much energy. In view of the large number of instances in which C_6 rings are formed exclusively in such cyclizations, it seems reasonable to conclude that the cyclohexane ring is formed by preference. This suggests that the cyclopentanes in petroleum have been formed largely by isomerization of cyclohexanes. In this connection, however, it should be pointed out that the ratios of cyclohexane and methyl cyclopentane in gasolines vary widely and do not correspond to equilibrium proportions for any particular temperature.

TABLE 1

Crude source	Percentage by volume, in gasoline			
	Winkler, Texas	Conroe, Texas	Coalinga, California	Saxet, Texas
Methyl cyclopentane	1.63	2.97	10.29	5.52
Cyclohexane	0.64	4.34	7.63	15.07

The ratios of total cyclopentanes and cyclohexanes in the gasoline fractions of seven petroleums examined by the U. S. National Bureau of Standards showed variations between 8.4% cyclopentanes with 20.5% cyclohexanes (by vol), and 41.0% cyclopentanes with 27.5% cyclohexanes (Table 1).