

"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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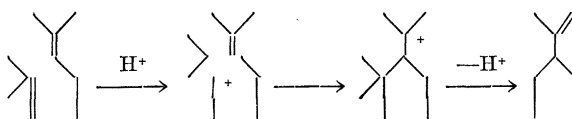
The formation of cycloparaffins, found so abundantly in petroleums, has never been plausibly explained. In a previous paper (1) evidence was presented that the chemical reactions resulting in petroleums involved typical carbonium reactions caused by acid silicate catalysts in the form of natural clays, 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).

The equilibrium between cyclohexane and methyl cyclohexane in the presence of aluminum chloride at 65° C shows 21.0% methyl cyclopentane.

These considerations have a most important bearing on the nature of the possible organic source material of petroleum and require that it be rich in olefinic material, and accordingly indicate that unsaturated fatty acids, such as are known to occur widely in marine organisms and algae, probably constitute the chief organic source of petroleum. High molecular weight polymers of the unsaturated fatty oils or acids may have been formed in the early stages, as suggested by Stadnikoff (8). The fatty acids and naphthenic acids found in petroleum, and usually considered together as "naphthenic acids," are probably vestigial remnants of the original fatty acids present in the original source material. Stevens (7) has suggested that chaulmoogric acid may be formed from linolic acid, or more probably from eleostearic acid, since in the latter the double bonds are so situated that cyclization may take place in a way analogous to the ring closing of citronellal to isopulegol. The cyclization of unsaturated fatty acids, or of olefinic material derived from them, by acid mineral catalysts affords a plausible explanation of the formation of the cycloparaffins that are so abundant in petroleum.

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### Effect of Colchicine on Regeneration in *Pelmatohydra oligactis*

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The effect of colchicine on lower animals has been little studied. Hausman and Kolmer (1) found that higher temperatures increased the toxicity of colchicine for *Paramecium*; many other observers have reached the conclusion that colchicine is more toxic in warm-blooded than in cold-blooded animals. Barros (2) reported that colchicine stimulated growth in *Paramecium*, whereas others (3,4) failed to obtain any detectable effects. Beams and Evans (5), however, reported a lowering of the viscosity or a prohibition of increased gelation, with a subsequent inhibition of cleavage, in *Arbacia* eggs.

The influence of colchicine on plants and higher animals has been well established (6), but its effects

TABLE 1

TENTACLES REGENERATED AT EACH OBSERVATION  
(Each Series, 5 Hydras)

Series	A 1		A 2		A 3		C	
	Hr	Total	Av	Total	Av	Total	Av	Total
24	0	0.00	0	0.00	9	0.60	46	3.07
36	4.5	.30	18	1.20	30	2.00	52	3.47
48	9	.60	34	2.27	46	3.07	56	3.73
60	9	.60	42	2.80	50	3.33	57	3.80
72	12	0.80	41	2.73	50	3.33	56	3.73
84	21	1.40	41	2.73	50	3.33	56	3.73
96	13	0.87	41	2.73	50	3.33	56	3.73
108	13	.87	41	2.73	48	3.20	56	3.73
120	12	0.80	38	2.53	48	3.20	56	3.73

on protozoa and lower animals have not been well delineated; it was therefore decided to test the rate of regeneration of a lower animal in a colchicine medium. It was believed that the rates from young, rapidly dividing animal cells subject to the influence of colchicine might show the typical stathmokinesis (c-mitosis).

*Pelmatohydra oligactis* males of one clone, which had been well fed with cladocera, were sectioned just below the tentacles. After 30 min, 5 hydras were placed in each of 3 stender dishes containing 30 ml of the following concentrations of colchicine in pond water: A 1, 0.0033%; A 2, 0.000033%; A 3, 0.00000033%. For the controls, the same procedure was carried out with the animals in pond water (Series C). All the dishes were covered, placed in the dark, and examined every 12 hr for evidence of regeneration.

The pond water used had previously been filtered free of organic debris. Water analysis showed it to contain 0.050 g of organic matter and 0.171 g of inorganic matter per liter, carrying the following ions: Ca<sup>++</sup>, Ba<sup>++</sup>, Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>++</sup>, Fe<sup>+++</sup>, Cl<sup>-</sup>, SO<sub>4</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>. *Vorticella*, *Halteria*, *Dileptus*, and dinoflagellates were abundant in the medium.

At the close of the experiment the specimens were examined cytologically by removing the regenerated sections, placing in 1 N HCl for 10 min, staining in acetocarmine for 2 min, and then squashing in the stain and mounting by ringing the cover slip with paraffin (salivary gland technique).

Hydra commonly regenerates 2 opposite tentacles immediately, followed shortly by the third. Then a fourth, fifth, or even more may arise by budding; nevertheless, 5 tentacles are characteristic for this species (7). The regeneration rate in a small sample is not uniform, and, as a result of individual variation in metabolic history and physiology, wide variations in numbers of tentacles at any one time level occasionally occur. This is particularly true in the case of an individual that is dying or entering a state of physiological depression. Not only are there wide variations from individual to individual, but also from trial to trial; these are due to the differences in the immediate physical and biotic environment and the