

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⁺⁺, Ba⁺⁺, Na⁺, K⁺, Mg⁺⁺, Fe⁺⁺⁺, Cl⁻, SO₄⁻, NO₃⁻. *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

clonal metabolism. For these reasons, it was believed that the best indication of the relative rates of growth in any one concentration would be the mean number of tentacles present; these are graphed as the logarithm against the time in hours (Table 1).

The results, as illustrated by Fig. 1, indicate that

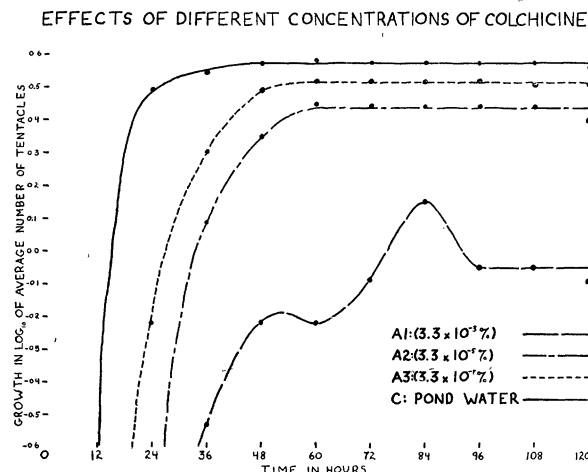


FIG. 1. Effects of different concentrations of colchicine.

the degree of inhibition of regeneration is a direct function of the concentration of colchicine. This agrees with the results of Bernhard on *Rana* (8), although he also obtained abnormal morphogenesis in regenerating tails.

The rates of regeneration as seen in Curves A 2 and A 3 appear to be depressed from the control (Curve C) by the toxicity of the alkaloid. Curve A 1, however, displays irregularities when compared to the other three. The distinct lag in absolute and relative growth rates seems to have been caused by stathmokinesis in conjunction with alkaloidal toxicity.

To test this hypothesis, cytological analyses were made. These examinations revealed, first, the fact that all the hydra (experimental, control, and nonexperimental from the same clone supply) showed polysomaty.¹ Second, an actual count was made comparing 2 regenerated sections (120-hr) from 0.0033% colchicine with the water controls, as to number of metaphases and anaphases. The following results per regenerated segment were obtained: control, 6 metaphases and 6 anaphases; colchicine-treated, 33 metaphases and 17 anaphases. In general, more mitotic figures were seen in the experimental animals, with a higher percentage of prophase being found in the control.

Therefore, the greater proportion of mitotic figures in the colchicine-treated specimen, as compared to the water control, indicated that the latter had completed the period of rapid growth in regeneration, whereas the former had not. The excess number of metaphases in the colchicine-treated specimen was visible proof of the effectiveness of the stathmokinetic properties of

¹ Discovered by G. H. Mickey, of Northwestern University.

colchicine on animal cells. These results are in harmony with the observation of stathmokinesis in *Arbacia* eggs, mentioned above (5).

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The Use of a Precision Lathe in the Preparation of Biological Thin Sections

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Through the use of a small precision lathe of the type used by jewelers and instrument-makers it is possible to prepare sections of biological materials by a technique similar to that employed in the preparation of plywood.

The basic techniques and equipment for such a method are fairly simple. Biological materials to be sectioned are embedded in the usual manner in paraffin, and the trimmed block is mounted on the face of a suitable wire or wheel chuck. In order to affix the block to the chuck the latter is heated slightly, the block is pushed onto it, and both are cooled in water.

By means of an appropriate set of pulleys the speed of the lathe is reduced to approximately that of a rapidly operated microtome. The speed can be increased as the operator's technique improves. The cutting tool, which can be a simple razor blade mounted in a holder of the type used in removing paint from glass surfaces, is held firmly against the tool rest of the lathe at an angle corresponding to that used for the knife holder of a conventional rotary microtome. It is imperative that the cutting tool be held firmly against the tool rest and the block in order to prevent vibrations that would ruin the ribbon.

This technique affords a means of preparing conventional transverse and longitudinal sections in addition to "peeled" or veneer sections. If the long axis of a tissue specimen be mounted perpendicular to the axis of rotation it is possible to remove anterior and posterior transverse sections alternately. Longitudinal sections may be obtained by mounting the specimen eccentrically in the block, but with its long axis parallel to the axis of rotation. If longitudinal sections of the entire specimen are desired the material must be so placed as to be removed completely from the rotating center of the spindle.

It should be possible to peel completely a perfectly cylindrical specimen by this technique and, in the case of forms showing concentric growth patterns, to obtain a histological "spectrum" of the tissues composing