

out leaving a residue of charcoal or coke, while in nature, according to Dr. Engler, we have no connection between deposits of coal and the occurrence of petroleum. Another theory, defended by Whitney, Hunt, Höfer and others, ascribes the origin of petroleum to animal remains. To test this latter theory, Dr. Engler has conducted a series of experiments so successful as to demonstrate clearly its possibility, at least, if not its probability, from a chemical point of view. First, some thousands of salt-water fishes were distilled under strong pressure, with the production of a liquid containing nitrogenous bases such as pyridin, but having no similarity to petroleum. Recalling experiments of Wetherill and Gregory as to the nature of so-called "adipocere," the idea was conceived that possibly in nature the nitrogenated animal substances were destroyed and the fatty residue converted into petroleum. Animal fat (train oil) was submitted to distillation under a pressure of 25 atmospheres at a moderate heat of 300°–400°, and it was found that 70 per cent (or 90 per cent of the theoretical) of the train oil was transformed into petroleum. The same results were obtained from the other fats like butter, hog fat, artificial fats, the free, fatty acids, etc. Not only illuminating oils were obtained, but also the lighter hydro-carbons, gasoline, ligwin, benzine, etc., and in those parts of the crude oil which show a high boiling point were found and separated paraffin wax and lubricating oils. "As a matter of fact," says Dr. Engler, "I have found in the distillate obtained by decomposition of train oil nearly all of the constituents which have been separated from the natural crude petroleum, and even the gases, which, like natural gas, consist essentially of marsh gas." For the chemism of the formation of the hydro-carbons, Dr. Engler refers to a recent paper in the *Berichte der Deutschen Chemischer Gesellschaft*.

RETICULATION OF SPINDLE-CELLED SARCOMA.

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No subject lends itself more freely to errors of interpretation than the description of the microscopical appearances presented by histological and pathological preparations.

Even the delineation, both manual and photographic, of the structure of the Diatomaceæ bear some semblance of uniformity in the descriptions of different observers. Still, to quote Dallinger, "In the present state of the theory and practice of microscopy, it would be extremely unwise to give absolute adhesion to what is now held, by some students of diatom structure of no mean repute and of unrivalled manipulative skill, to be the absolute structure of some of the larger forms."

The same observation applies with still more force to the former investigations, as it is impossible to compare and correlate either the methods of preparation, observation or interpretation of different observers. They all differ, as a rule, in some detail, and in addition there is not only a marked tendency on the part of histologists and pathologists to copy the methods, drawings and results of others, but also a great liability to subjective imitation through suggestion.

Before confining myself to the evidences of reticulation in sarcomal structures, I may mention that the appearance in *Polymyxa*, so interpreted, is perfectly evident in some individuals and absolutely imperceptible in others. When seen, it is extremely evanescent, and, therefore, can scarcely be looked upon as evidence of the existence of formed material, but rather as the effect of some temporary chemical or physical change in or upon the external surface of the protoplasmic mass. The same or very similar appearances may be observed in *Volvox*, which are equally erratic, but as they are unquestionably received as the

evidence of formed material, the foregoing statement is put forward as only a conditional hypothesis.

The portion of the tumor from which the accompanying sketch is taken was placed in Muller's fluid twelve hours before the sections were cut. These sections were taken from the central portion, where the fluid had evidently no time to act, then slightly stained with carmine, mounted in balsam and in the usual way. On being examined the same evening with a one-sixteenth water immersion and No. 12 compensating eyepiece, it was found that the markings forming portions of the reticulations took a definite direction, that is, obliquely lateral to the long diameter of the cell. This lateral obliquity did not change on revolution of the stage, and therefore cannot be interpreted as the result of oblique illumination. In many of the cells a granular nebular nucleus was observed, connected by slender and almost phantom branches with the oblique lateral markings. At the junction of these branches with the nucleus their point of



insertion or outgrowth, as the case may be, seemed to be placed in the hyaline substance surrounding the granules, and unconnected with the granules themselves. This latter observation is not laid down as an established fact, but simply as something more than ordinary conjecture. At the points of junction with the lateral markings there seemed to be definite nodal enlargements increasing in frequency towards the edges of the cell, and the whole section had a peculiar watered-silk appearance, which it was found impossible to represent on paper.

On examination of teased preparations, it was evident that the sections were cut obliquely, as the cells appeared very much elongated; at the same time they showed no reticulation.

Sections from the same portion of the growth were treated with osmic acid and several aniline dyes without effect. I am, however, by no means skeptical as to the results which ought to be obtained in perfectly fresh specimens with chloride of gold. Its manipulation is difficult, owing to the nature of the tissue, changes in temperature, light and color definition, therefore annoyingly variable in its results.

I cannot endorse Chatin's statement, as quoted by Dr. Stokes on p. 374, No. 517 of this journal, that reticulated structure in amœboides and in the blood corpuscles of

invertebrates is constantly and easily demonstrable. Chatin, in the previous paragraph, referred to osmic acid; it is natural to suppose that the organisms and globules were submitted to that treatment, a method which, at least in my hands, has proved extremely uncertain in its results.

In conclusion, allow me to request some of your very numerous correspondents to inform us if the spectroscope would give any material assistance in the solution of the true nature of these markings. (I, of course, mean the diffraction spectrum), my acquaintance with the instrument being limited to test fluids.

Since writing the above my colleague, W. F. Pentland, has persuaded me not to be too dogmatic with regard to the reticulation of the invertebrate corpuscles and individual (especially conjunctival) cells of invertebrates till after next spring, as in the meantime he intends working up the subject.

THE BACTERIOLOGICAL ANALYSIS OF WATER.

BY J. H. STÖILER, UNION COLLEGE, SCHENECTADY, N. Y.

WHEN, in 1881, Koch announced the gelatine culture method for bacteria devised by him, it was believed that one of its most important applications would be in the examination of waters with reference to their potable use. This method, as is now well known, renders possible an exact determination of the abundance of bacteria in water. But it was soon discovered that the mere demonstration of the presence of bacteria was of little value in estimating the qualities of waters, inasmuch as waters of unquestionable suitability for potable use often contained bacteria in considerable abundance. However, the general result was established that the numbers of bacteria are in relation to the amount of putrescible organic matter in the water.

The ideal value of the gelatine culture method not having been realized, it is probable that its true usefulness in water analysis has not been estimated as highly as it deserves. An experimenter who has familiarized himself with the distribution of bacterial life in waters will be able to form definite and reliable conclusions upon the basis of numbers of bacteria. This is especially true in the case of river water subject to pollution by sewage from towns. Numerical determinations of abundance of bacteria having been made of samples taken at various points from the same river, a fair judgment may be formed of the amount of sewage pollution at any required point. The first step requisite to be taken is to determine, for use as a standard, the numbers of bacteria in unpolluted water *in the stream under investigation*. Comparisons made with this standard give reliable quantitative indications of pollution. Any access of sewage raises the number of bacteria above the normal for that stream and the excess is a definite indication of the extent to which the water has suffered pollution. The standard is obtained by testing the water, both at such points and at such times as give the condition approaching nearest to purity for that stream. In general, samples taken from the head waters of the river, above the first town from which sewage pollution is received, and at a time of continued fair weather when the water is free from rain-wash, are best suited for the control tests. In regard to the effects of surface washings from the land by rains, as indicated by turbidity of the water, it is necessary to eliminate them from all tests by taking samples only when the water is clear. This rule being observed, comparisons of results give indications of the extent of contamination due to sewage.

It should be added that there are other conditions which enter in a minor degree as factors in the results of numerical determinations of bacteria. These are temperature of water, depth at which the sample is taken, point at which the sample is taken with reference to rifts and pools in the stream, free exposure to air and light (prevented in winter by ice), etc. Consideration should always be given to these conditions and as far as possible samples should be taken under similar conditions throughout in order to render the results comparable.

The writer, working in association with Prof. C. C. Brown, consulting engineer for the New York State Board of Health, in furtherance of his work in investigating rivers as sources of water supply, has made numerical determinations of bacteria for some six hundred samples of water from the Hudson and Mohawk rivers. A statement of the results of this work is given in the annual reports of the State Board of Health of New York for the years 1891 and 1892.

It naturally occurred to us, early in the work here alluded to, that a method of differentiating sewage bacteria from ordinary water bacteria would be of great value as affording a more exact means of ascertaining the degree of sewage pollution than is possible by the method outlined above. Dr. Theobald Smith, of Washington, D. C., was then consulting bacteriologist for the New York State Board of Health and upon submitting the idea to him he informed us of a method of differentiating gas-producing bacteria from others which he had devised and published some time previously (*Centralblatt für Bakteriologie*, Vol. VII, p. 302 and Vol. XII, p. 367) and which he believed was applicable to the end sought by us.

The method thus placed at our disposal consists in the use of a culture fluid of which sugar (glucose) is a component and which is placed for inoculation in tubes similar in principle to the ureometer employed by chemists. Bacteria capable of causing sugar-fermentation when introduced into such culture-tubes give rise to a gas the quantity and composition of which can be ascertained. In the application of this method to the bacteriological analysis of water its value rests upon the fact that the most common species of bacteria present in feces are gas generators. As is well known the most constantly occurring species of bacteria in feces is *Bacillus coli commune*; and for some time our experiments related to the determination of the abundance of this species in the waters under investigation by means of the characteristic quantity and composition of the gases which it generates in the fermentation-tubes. Later others of the more common fecal bacteria were isolated and studied with reference to their gas-generating character. In this way a method was elaborated by which, it is believed, there can be determined with approximate exactness the numbers of prevailing species of fecal bacteria in a unit quantity of water. This determination is taken as a definite indication of the amount of sewage pollution.

In the practical use of this method the procedure is as follows: The saccharine culture fluid contained in a set, say eight, of fermentation-tubes is inoculated with a measured quantity of water from the source of supply under investigation. The tubes are immediately placed in an incubator and kept at a temperature of thirty-eight degrees centigrade for forty-eight hours or somewhat longer. (This is favorable to the development of fecal bacteria and probable destruction of the greater number of ordinary water bacteria.) Those tubes in which gas has been developed are then examined with reference to the amount and composition of the gases present and note is taken of those which agree in these respects with the effects produced by known fecal bacteria. Finally