A very useful list of the algae of typical associations is given for various habitats such as ponds and ditches, rainpools, lakes, mountain tarns, flowing waters, bogs, salt marshes, wet rocks, damp ground, subterranean soil and tree trunks.

The greater part of the book is concerned with a systematic discussion of the eleven classes of freshwater Algae as follows: Isokontae, Heterokontae, Chrysophyceae, Bacillariales (Diatomales,) Cryptophyceae, Dinophyceae (Peridinieae,) Chloromonadales, Euglenineae, Rhodophyceae, Myxophyceae (Cyanophyceae.)

The author accepts the view of Luther, Borzi, Bohlin and others that the various classes of algae have had their origin from unicellular flagellates with similar pigments and storage products. He accordingly includes such flagellates in these respective algal classes. Zoologists will be interested in the bearings of these segregations on current. classifications by protozoologists. The desirability of some sort of protistological organization of this common ground of the two biological disciplines and jurisdictions is rapidly becoming more evident.

The author uses the term Dinophyceae in lieu of Dinoflagellata. This is to be regretted, since the latter has been so widely used for many years, and the scope of the former strictly speaking can not be legitimately extended to include the Gymnodiniaceae and Peridiniaceae. The citation of Woloszynska's observations of polygonal plates on *Gymnodinium* without criticism might lead the reader to accept the idea that the structures thus detected really belonged to a true *Gymnodinium* instead of to a young or recently exuviated member of some armored genus such as *Peridinium*.

The illustrations are mainly old, but well selected and well executed. One might wish there were more of them, especially in a systematic treatise of this sort, but the author had to choose between the Scylla of condensation and the Charybdis of expense. A brief list of important works and a full index add to the usefulness of this excellent revision of a highly valued handbook.

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Gewebezüchtung. Handbuch der Biologie der Gewebezellen in Vitro. 2 vermehrete Auflage. By AL-BERT FISCHER. München: Rudolf Müller und Steinicke, 1927. 508 pages, and 151 illustrations.

THIS volume is a German edition of Albert Fischer's "Tissue Culture," which was published in 1925 by Levin and Munksgaard, Copenhagen. Fischer may well be regarded as the most successful research worker in all Europe in this particular field. The excellent introduction was written by Dr. Alexis Carrel, the eminent authority on the subject of tissue cultivation. The book is really an entirely new edition of the first, fully revised and enlarged. A thorough description of the technique of tissue culture renders the book especially valuable for investigators in this field, which is rapidly becoming more and more important. In addition, the value of his work is greatly enhanced by the comprehensive and critical presentation of the very significant results and numerous new formulations of biological questions brought out by this method.

An historical review is followed by chapters on: (1) Media employed in the cultivation of tissues in vitro; (2) technique covering the preparation of culture media and the various substances now used; cover-glass preparations; measurement of the rate of growth; flask culture method; application of the microdissection method; photography and microscopic examination of cultures; (3) pure strains of cells; (4) tissue culture as a physiological method; (5) morphology; (6) tissue culture as a pathological method, and lastly, a most important chapter on the behavior of tumor cells *in vitro*. An excellent bibliography is appended.

The author's success in the cultivation of tumor cells is outstanding. He devised a technique whereby strains of malignant cells are made to grow permanently outside of the organism. The tumor cells do not lose their malignancy during their life *in vitro*. Even after many passages, a culture when transplanted to an animal gives rise to a tumor with metastases. This is true not only of Rous sarcoma, but also of different epithelial tumors which have been cultivated; for example, the carcinomata of mice.

The results obtained by the method of tissue culture have widened our scientific outlook and have correspondingly increased our knowledge of cell physiology, and Albert Fischer is to be highly congratulated upon his book on this subject.

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## SCIENTIFIC APPARATUS AND LABORATORY METHODS

## A SIMPLE VISUAL METHOD FOR DEMON-STRATING THE DIFFUSION OF OXYGEN THROUGH RUBBER AND VARIOUS OTHER SUBSTANCES

In experimental work in which small quantities of oxygen are to be measured, rubber connections must be avoided because of the error introduced by the diffusion of oxygen through rubber. Coating such connections with paraffine, as is sometimes done, is of negligible value, as is shown by the following experiments. A simple visual demonstration of the diffusion of oxygen may be made by using luminous bacteria as an indicator. It has been shown by Harvey and Morrison  $(1923)^1$  that these bacteria will give a just perceptible glow when the partial pressure of oxygen is only .0053 mm Hg.

A test tube is completely filled with a dilute suspension of luminous bacteria and closed with a rubber dam, care being taken to see that no air bubbles are included. The suspension will glow brightly until practically all the oxygen has been consumed. The light then disappears except in contact with the membrane and for a short distance from it. If a suspension of the proper concentration has been chosen a gradient of brightness is obtained, most brilliant in contact with the membrane and fading out toward the other end of the tube. The critical worker who desires a control may use for the purpose a glass-stoppered tube or graduate of the same diameter as the experimental tube. This becomes completely dark.

If it is desired to form a rough estimate of the relative amounts of oxygen diffusing through various substances, this may be done by comparing the length

<sup>1</sup> Harvey, E. Newton, and Morrison, Thos. F., "The Minimum Concentration of Oxygen for Luminescence by Luminous Bacteria," J. G. P., 6, 13, 1923. of the luminescent columns, provided that all the tubes are filled with a suspension of the same concentration and that convection currents are reduced to a minimum. In a recent experiment, the results shown in the table were obtained. The thermostat was kept at  $24.6 \pm .3^{\circ}$  C., and top and bottom temperatures of the double walled box in which the apparatus was set up were recorded by Beckmann thermometers. The maximum difference between top and bottom was  $.054^{\circ}$  C., and the average difference for the time of the experiment was  $.024^{\circ}$  C. It is not believed that this difference was sufficient to set up disturbing convection currents.

It will be apparent from a glance at the table that for detecting the presence of minute quantities of oxygen very dilute suspensions are best, while for estimating relative quantities, suspensions somewhat more concentrated must be used. There is then established a mobile equilibrium, oxygen diffusing in at a definite rate, and being consumed at a definite rate, the ratio of the two rates determining the length of the luminescent column.

The long-continued luminescence under kerosene and xylol is surprising, as both of these are cytolyzing agents. On pouring off the oils at the end of the sixth hour, those under these two substances were found to be somewhat injured, as they did not exhibit full brilliance. Small quantities of all the substances used were shaken with bacterial suspensions, and all

	Suspension 1				Suspension 2	
· ·	2	hours	6	hour	rs	3 hours
Open control	10	cm.	10	cm.		10 cm.
Glass stoppered control	(Dark in 45 min.)					(Dark in 3 hours)
Wet collodion	4	cm.	4	cm.		10 cm.
Kerosene	4	cm.	3	cm.	(dim)	10 cm. (dim)
Paraffined cork	3	cm.	3	cm.		10 cm.
Paraffine oil	3	cm.	3	cm.		10 cm.
Paraffine (52°)	3	cm.	3	cm.		10 cm.
White vaseline	3	cm.	3	cm.		10 cm.
Amber vaseline	2	cm.	<b>2</b>	cm.		10 cm.
Wet parchment	<b>2</b>	cm.	<b>2</b>	cm.		10 cm.
Paraffined parchment	<b>2</b>	cm.	<b>2</b>	cm.		10 cm.
Olive oil	1.5	cm.	1.5	cm.	(dim)	10 cm. (dim)
Thin lubricating oil	1.5	cm.	1.5	cm.		10 cm.
Xylol	1.5	cm.	1.5	cm.	(dim)	10 cm. (dim)
Rubber dam	.5	cm.	.5	cm.		4 cm.
Paraffined rubber stopper	$\mathbf{Tr}$	ace	0			trace
Medium motor oil	0		0			.5 cm.
DeKhotinsky on rubber dam	0		0			0
DeKhotinsky on cork	0		0			0
Dried collodion on linen	0		0			trace

Length of luminescent columns of bacteria under various oils, etc. The tubes used contained 10 cm. of the bacterial suspension.

were found harmless except olive oil, kerosene and xylol, each of which caused a considerable diminution of brilliance. Evidently the slight solubility and slow diffusion downward of these substances saved the bacteria from cytolyzing effects.

The impermeability or very slight permeability of viscous motor oil to oxygen is in agreement with the finding of Kruse  $(1926)^2$  that alkaline pyrogallate solutions under medium motor oils were not perceptibly oxidized after eight weeks, while those covered with kerosene and mineral oil were oxidized 30 per cent. and less than 5 per cent., respectively. This difference Kruse ascribes to the greater viscosity of the motor oils.

It will be noted that of the liquids used the medium motor oil is by far the best for exclusion of oxygen. Heavy rubber stoppers and thick tubing, if time is allowed for diffusion out of the oxygen dissolved in the surface, will introduce only small errors, the diffusion of oxygen through rubber as thick as 1 cm. being slight.

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## SPECIAL ARTICLES

## THE PRODUCTION OF MUTATIONS AND REARRANGEMENTS OF GENES BY X-RAYS

MUTATIONS and rearrangements of genes have been produced by Muller by subjecting *Drosophila melano*gaster to the action of X-rays.<sup>1</sup> Similar experiments have since been performed by the writer; and these, while not so extensive, are in entire agreement with those of Muller. As a result of X-ray treatment, there have been obtained mutations producing visible and lethal effects, as well as genetic modifications of the frequency of crossing over, and attachments between genes of different chromosomes.

Males of *Drosophila melanogaster* were exposed to X-rays in dosages corresponding approximately to those designated by Muller as T4 and T2, the former being about twice the latter. The treated males and untreated brothers used as controls were mated to untreated females. The fertility of the irradiated

<sup>2</sup> Kruse, T. K., "The Relative Efficiency of Several Oils for the Exclusion of Oxygen," J. Phar. and Exp. Ther., 27, No. 3, April, 1926.

<sup>1</sup> Muller, H. J., "Artificial Transmutation of the Gene," SCIENCE, Vol. 66, pp. 84-87 (1927); "The Problem of Genic Modification," Proc. Fifth Int. Congress of Genetics (in press); "The Effects of X Radiation on Genes and Chromosomes," abstracts in Anat. Record, Vol. 37, p. 174, and SCIENCE, Vol. 67, p. 82.

males and of their offspring was reduced, the stronger treatment being the more effective in each case. As has been suggested by Muller, sterility might be produced in the treated males by mutations resulting in dominant lethal genes, and it might be produced in the offspring by mutations resulting in dominant genes for sterility.

The experiment was designed primarily to test the effect of the treatment on the X-chromosome. In order that the treated and control X-chromosomes might be recognized in subsequent generations, the males were mated to females that differed from them in certain sex-linked genes. Since every daughter received a treated or control X, any change in this chromosome would be inherited by half her sons and would be detected if it produced a visible or lethal effect.

Nine of the thirty-seven  $F_1$  females in the T4 series and ten of the forty-seven  $F_1$  females in the T2 series were found to have inherited altered X-chromosomes from their fathers. No changes in the X were observed in the fifty-six  $F_1$  females of the controls. (Only the fertile females are included in the reckoning.) In X's of the T4 series there were three mutant genes (one dominant, two recessive) producing visible effects, and six lethals. In X's of the T2 series there were two genes (both recessive) producing visible effects, and eight lethals. Of the non-lethal mutant genes, three recessives are allelomorphs of genes already known (deltex, furrowed, uneven); the others seem to be changes in hitherto unknown loci.

The experiment was not designed to detect mutations in the autosomes; and such changes resulting in recessive genes would, for several reasons, have escaped discovery. (One autosomal recessive was found in an  $F_2$  culture in the control series, but it must have been present in heterozygous form in either the male or the female of the  $P_1$  generation.) New autosomal dominants, however, could be recognized; and three were found, all in the T2 series. One of these (an eye color—the only dominant mutant eye color known in *D. melanogaster*) arose in a treated male; the two others (probably allelomorphs of Star and Hairless, respectively) may have originated in either the males or the females of the  $P_1$ generation.

In the offspring of the treated flies there were found six translocations in which genes of the second chromosome behave as if attached to the X; that is, they are inherited in sex-linked fashion. In at least some of these cases, genes along the entire length of the second chromosome behave in this way. The point of attachment in the X differs in different cases; whether or not the point of attachment in the second chromosome differs is not known. The results would