experience in this field. The book has value and fills a distinct need. It deserves a wide distribution.

Tissue Culture in Relation to Growth and Differentiation. By T. S. P. STRANGEWAYS, lecturer in special pathology in the University of Cambridge. W. Heffer & Sons, Ltd., Cambridge, 1924, pp. 50.

IN this book of fifty pages is given a general survey of the results obtained by the author from certain experiments with the cultivation of tissues "in vitro," together with his interpretation of these results in relation to various biological problems, such as mitosis, differentiation, inflammation and repair, etc. A careful reading of this book has given me the impression that the material contained in it, although of value, will not have a general appeal either to workers in this field or to the average reader.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A CATHODE RAY OSCILLOGRAPH FOR SEVERAL SIMULTANEOUS WAVES WITH STABILIZED LINEAR TIME AXIS

A LINEAR time axis[1] for cathode ray oscillograph is obtained by repeatedly charging a condenser through a saturated thermionic rectifier and discharging through a neon lamp when a critical voltage is reached. The period, controlled by condenser capacity and rectifier filament current, can be made approximately synchronous with any periodic phenomenon under study, so that the oscillograph shows a nearly stationary wave or curve plotted against time in rectangular coordinates. Although, under these conditions, the wave may shift, it can be inspected and has proved highly satisfactory for certain purposes, *e.g.*, the graphical demonstration of heart sounds.[2]

For the study of physical phenomena, as alternating currents and voltages, it is desirable to have the wave or curve absolutely stationary, and, furthermore, to obtain two or more such waves simultaneously for precise comparison of phase, amplitude, frequency and wave form. In certain cases a synchronous contactor may be employed to obtain precise synchronism of the condenser discharge with the phenomenon under study, thus stabilizing the time axis and making stationary the wave. We have found this method satisfactory, but limited in its application and involving unnecessary auxiliary equipment. The desired end, however, is simply and satisfactorily obtained by tickling the neon lamp with a small alternating electromotive force[3] of the same frequency, whether it be high or low, as the periodic phenomena being studied, thus synchronizing the discharge of the condenser, without affecting its uniform rate of charge. This gives an absolutely stationary wave which may be photographed, or copied precisely on tracing cloth or by binocular vision.

A number of curves are readily obtained simultaneously by a motor-driven switch making connection to the several sources in rapid succession, the curves all appearing continuous and simultaneous due to persistence of vision. We have obtained three or four simultaneous curves (all absolutely stationary) with complete satisfaction and believe six or more could be obtained if needed. It has proved a convenient way of comparing wave forms of transformer input and output, showing the amount of distortion and amplification under different conditions. Any shifting of the curves would lead to confusion.

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SPECIAL ARTICLES

THE ISOLATION OF A CRYSTALLINE PROTEIN WITH TUBERCULIN ACTIVITY[1]

BEGINNING with Koch in 1891, many attempts have been made to separate from tuberculin the principle responsible for eliciting a skin reaction in tuberculous subjects. The exact nature of this substance has been a riddle and while most opinions proclaim it to be protein in nature, there have been many dissenting voices. A series of experiments consecutively carried out in this laboratory, and appearing in the May issue of the *American Review of Tuberculosis*,[2] seem to prove more or less definitely that the substance is of protein nature.

Briefly, these experiments show that the substance responsible for the activity of tuberculin is com-

[1] Austin Bailey, *Physical Review* (2), 25, p. 585, April, 1925.

[2] Apparatus for this purpose, developed by the Western Electric Company, was exhibited by Dr. H. Clyde Snook, 1925, at Kansas City meeting A. A. A. S. and was previously exhibited at Atlantic City meeting Am. Med. Assoc.

[3] This may be conveniently applied through a potentiometer in series with the lamp.

[1] From the Department of Pathology of the University of Chicago and the Otho S. A. Sprague Memorial Institute, aided by a grant to Dr. Esmond R. Long from the Medical Research Committee of the National Tuberculosis Association.

[2] The Chemical Composition of the Active Principle of Tuberculin. I–VII. E. R. Long and F. B. Seibert, *Amer. Rev. Tuberc.*, 1926, XIII, 393.

pletely precipitated by $(NH_4)_2SO_4$, and practically all other protein precipitants throw it out of solution to some extent. It does not dialyze, and, in fact, is always associated with protein, while it is absent when all protein is removed. Moreover, it is attacked and thereby loses its activity when treated with the enzymes, pepsin + HCl or trypsin + Na_2CO_3, which attack whole protein, while acid or alkali of corresponding strength, and erepsin or trypsin in neutral solution, do not materially reduce the activity. Chemically, it is shown that wherever the activity is lost, following enzyme treatment, there occurs also a corresponding reduction in whole protein, with an increase in proteose and residual nitrogen.

With the thesis that the active principle of tuberculin is protein in nature, as a basis, an attempt was made to learn if the specifically active protein could be isolated in very pure form and possibly be among the few known proteins which will crystallize. Large quantities of tuberculin prepared by Parke Davis Co. and the Mulford & Co. for the National Tuberculosis Association, and made upon the non-protein synthetic medium recommended by Dr. E. R. Long, were used, and from it all the protein was precipitated by complete saturation with $(NH_4)_2SO_4$. This precipitate proved to consist of a water soluble non-coagulable protein, a water soluble heat-coagulable protein, and a water insoluble protein, the first two of which contained most of the tuberculin activity.

The water soluble non-coagulable dialyzed fraction was treated by Hopkins' method for isolating crystalline ovalbumin, and burrs or bundles of crystalline needles appeared on three different occasions from three different lots of material. These crystals take the methylene blue stain, and when thoroughly washed with an $(NH_4)_2SO_4$ solution of appropriate concentration and redissolved in water, give the biuret, Millon's and Molisch tests. These washed needles also give a marked skin reaction in tuberculous guinea pigs and none in normals, *i.e.,* satisfy the requirements for the recognition of true tuberculin activity. The purest lot of crystals so far obtained, nevertheless, still contains a few small particles admixed with the needles, which may be amorphous, and attempts are being made to make a preparation entirely free of such particles. However, it is possible that these amorphous-appearing particles are the precursors of needles, or needles whose formation has been retarded, since, as with ovalbumin, the amorphous precipitate which appears immediately, transforms itself into needles on standing.

There has, therefore, been found in tuberculin a protein which can be crystallized. Moreover, this crystallized protein apparently is able to elicit a tuberculin skin reaction. The more detailed analyses and the quantitative potency of the protein will be reported in greater detail in the near future.

FLORENCE B. SEIBERT

PRELIMINARY NOTES ON THE STRUCTURE OF PLANT PROTOPLASM

IN a recent paper[1] on problems touching the Golgi apparatus, I was led to offer some rather rash suggestions as to the homologue of this element in plant cells, where thus far nothing certain is known concerning even the presence of Golgi material. A paper of some years ago by Nassonov had demonstrated the possibility of impregnating with osmic acid (Kolatchev's method for Golgi material) certain elements of plant cytoplasm. The question which I put to myself was therefore an apparently simple one. Were some of the materials impregnated by Nassonov equivalent to the Golgi apparatus of animal cells—thus bearing out one of my theoretical suggestions? With this as a point of departure, I have now under way an extensive study of the structural elements of plant cytoplasm as revealed particularly by the various methods of osmic acid impregnation now in common use by zoologists for demonstrating the Golgi apparatus. The results thus far achieved have been very unexpected, and since their publication will be delayed pending the possible solution of several debatable points, it seems worth while to make now a brief preliminary statement.

Thus far root-tips from Vicia, Pisum, Hyacinthus, Ricinus, kidney bean, pumpkin and barley have been examined after preparation by various modifications of the techniques of Kolatchev and Weigl. Positive results of some sort have been obtained in practically all cases, but the different forms vary considerably in the degree of success attained and exhaustive tests have been made thus far only on Vicia faba. Taken as a whole, my trials show that practically everything in the root-tip cells can be impregnated with osmic acid, save only the chromatic materials. Following, where applicable, the non-committal terminology of the Dangeards, the components demonstrated are as follows:

(1) *Spindle fibers* and cytoplasmic network—substantially as described by Nassonov.

(2) *Plastidome*—particularly in the meristem at the root-tip end but usually not in older cells where leucoplasts have become differentiated. These bodies blacken intensely in accord with Nassonov's original finding.

(3) *Spherome* (probably equivalent to the bodies so designated by the Dangeards)—in cells of all kinds and ages in all the species carefully examined. The spherosomes occur in large numbers throughout the cytoplasm, though never intruding on the vacuolar spaces. They

[1] *Anat. Rec.*, Vol. 32, 1926.