

pletely precipitated by $(\text{NH}_4)_2\text{SO}_4$, 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 $(\text{NH}_4)_2\text{SO}_4$. 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 $(\text{NH}_4)_2\text{SO}_4$ solution of appropriate concentration and redissolved in water, give the biuret, Milon'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.

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PRELIMINARY NOTES ON THE STRUCTURE OF PLANT PROTOPLASM

IN a recent paper¹ 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 Nasonov 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 Nasonov 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 Nasonov.

(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 Nasonov'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 spheromes occur in large numbers throughout the cytoplasm, though never intruding on the vacuolar spaces. They

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are particularly large and well developed in the root-cap cells and in developing cells of the vascular areas. They show no special features of distribution in mitosis. Each spherosome is disc-like, not spherical in shape. The body of the disc is yellowed somewhat but not blackened by osmic treatment. The rim of the disc is blackened intensely. Thus, when seen in plane view, the spherosomes appear essentially as black rings, while in profile view they appear as rods. It is these "rods" which apparently constitute the "inactive chondriome" of Guillaumond, the real morphology of which is here described for the first time.

(4) *Vacuome*—very finely demonstrated and particularly studied thus far only in *Vicia faba*. The general results are in remarkable agreement with the accounts heretofore given by the Dangeards using vital dyes. In my preparations, however, the vacuome is for the first time demonstrated in the finest detail and in a form adapted for permanent preservation and study. In the earliest meristem cells the vacuome consists of many roughly spherical masses scattered throughout the cytoplasm and blackened in a more or less complete way. Every step in the fusion of these vacuoles to form the large "cell-sap" areas of the plerome cells has been followed in detail. In the periblem cells, the history of the vacuoles is somewhat more complicated and is still being studied. In the periblem the networks early formed by fusion of the vacuoles often undergo a characteristic fragmentation during mitosis, reminiscent of the behavior of the Golgi apparatus in many animal cells.

The successful application of the methods here employed is as yet a matter largely of chance. The plastidome frequently blackens, but first-class results are only occasional. The spherome is almost always sharply impregnated, while the vacuome reacts very capriciously (frequently not at all). Furthermore, these elements may be impregnated singly or in any combination in a given cell. In general, an extensive series of serial preparations must be made in order to yield a few with really excellent fixation and impregnation.

The rather astonishing result has thus come out that methods of unusual selectivity for the Golgi material in animal cells, in plant cells will give positive results on a range of structural elements, including all known categories of cytoplasmic components (perhaps excepting central bodies). Thus all possible conclusions based on mere behavior toward osmic acid are seriously compromised from the very beginning.

The most pressing matter still remains of establishing the homologies presumably existent between the known series of cytoplasmic elements in plant and animal cells. My results incline me at present to accept the usual interpretation of the plastidome as equivalent to chondriosomes. I have followed all stages in the accumulation of starch (by the Benda

and other methods) in the plastidome of root-cap, periblem and (less completely) plerome cells, and there can be no doubt as to the facts themselves. But as to interpretation, it appears that a definite conclusion must await some light on the actual functional significance of the chondriome in animal cells. The rôle of the presumed chondriome of plant cells as a center for carbohydrate synthesis suggests that in animal cells the chondriosomes may play a related part. Thus it becomes conceivable that the function of the chondriome may be found in carbohydrate metabolism, a hypothesis which might possibly be directly tested by a careful investigation of striated muscle fibers or liver cells.

With respect to the homologue of the vacuome in animal cells, it must be confessed that the pictures in periblem cells of *Vicia* are often astonishingly suggestive of the animal Golgi apparatus. Certain features of its behavior to technical treatment do not, however, tend to bear out the comparison. At present my results afford no critical demonstration one way or the other. But the capacity of the plant vacuome for staining with neutral red perhaps suggests some possible relation to structures similarly stainable in animal cells, and for which as yet no very satisfactory accounting has proved possible.

The spherome resembles in many ways the scattered Golgi bodies in certain animal cells. Further, the reaction to the osmic Golgi methods is usually positive, tends indeed to be fairly constant. Whether we can find in the spherome the homologue of the Golgi apparatus, as I suggested in my paper already referred to, must, however, remain uncertain pending the attainment of more critical criteria of judgment—possibilities of which I now have under investigation.

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THE USE OF SUBSCRIPT AND SUPERScript EXPONENTS IN MATHEMATICS AND IN CHEMISTRY¹

SYSTEMS of notation are the tools of thought. A good workman is known by his tools, and to a greater extent than often is realized a good workman is such because of his tools. The extensive use of the abacus in countries, as ancient Rome or present-day China, whose people think in terms of symbols less convenient than the Hindu-Arabic notation, and the relatively great amount of time devoted to spelling in the schools of peoples whose writing is not phonetic,

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