

was found that the absence of cinchophen choleresis in the rabbit was not due to an acute intoxication of the liver, because after cinchophen sodium dehydrocholate caused a brisk choleresis, it is tentatively concluded that cinchophen does not cause choleresis in the rabbit because it is not excreted in the bile.

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Radioactive Ion Distribution in Protoplasmic Granules.

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The *Nitella* cell has an extensively granulated protoplasm, measurements on frozen sections giving up to 15% of the total volume of the protoplasm as being made up of nuclei, plastids, mitochondria and non-ergastic bodies. It seems important to inquire whether these granules play a significant rôle in ion transfers, and indeed, whether these bodies might not be the main seat of ion accumulation.

To determine to what extent the granuloplasm took up ions, single internodal coenocytes 5-10 cm in length of *Nitella coronata* were prepared by destroying alternate cells in the chain. Care was taken to preserve a short extension of the cellulose wall (about 5 mm long) from the adjacent cell. Surgical thread could then be tied around this cellulose projection and the cell left for 24 hours in pond water in the cold room (15°C) under conditions of intermittent light which simulated sunlight. After this treatment the cell was immersed in radioactive solutions for the desired time and then placed in a centrifuge tube, the thread tied about a cork in the tube and its length adjusted so that the cell was held clear of the sides and bottom of the tube. Centrifugal force sufficient to displace the granules in the cell was applied without any other observable damage. Control centrifuged cells kept under the previous conditions would show a gradual redistribution, reaching substantial completion in about 60 hours, of the stratified granules. These cells lived as long as untreated cells. After stratification had been obtained the experimental cells were bisected into centripetal and centrifugal halves, or fractions, and the activity of each half of the protoplasm measured under a Geiger-Muller counting tube. The only apparent error due to mixing was in the sap, which almost certainly became mixed during the sampling.

Preliminary studies showed that relatively great centrifugal forces (4,000 rpm at 15 cm) were necessary to displace the plastids in the cortical plasmagel whereas to displace the granules in the mobile plasmasol relatively small centrifugal forces (1,000 rpm at 15 cm) were required. As can be seen from the tables these cortical plastids (mostly chloroplastids) apparently were inactive as ion accumulation centers, and only served to dilute the granule fraction in general. However, when centrifugation was sufficient the cytoplasm became absolutely clear showing that all granules were sedimented.

The data obtained were quite reproducible and the maximum error did not exceed $\pm 5\%$. Tables I and II show the change K^+ or $HP^*O_4^-$ ion concentrations in the granuloplasm after various periods of immersion of the cell in 0.01M K^+Cl or $Na_2HP^*O_4^-$ in pond water at pH 8.2 temperature $15 \pm .01^\circ C$. Control cells, those which had not been centrifuged, were immersed in radioactive salts and then cut in half and each half measured for radioactivity. This showed that these halves each had the same ion radioactive concentration.

The times of sampling were chosen to correspond with various phases of the ion penetration process, namely: .25 hr corresponding to a maximum of induced accumulation (*cf.* Brooks¹); .5 hr corresponding to a minimum of ion concentration, and 4-8 hr cor-

TABLE I.
Granule Accumulation Ratios for Various Times of Immersion of Cells in Radioactive Solutions.

| Ratio: K^+ Ion Concentration in Granular Protoplasm | | | Time of immersion, hr |
|---|------------------------|--|--------------------------|
| K^+ Ion Concentration in Hyaline Protoplasm | | | |
| Low centrifugal force | High centrifugal force | | |
| 0.30 | 0.20 | | 0.25 |
| 0.64 | 0.55 | | 0.50 |
| 1.10 | 0.60 | | 1.00 |
| 2.20 | 0.75 | | 4.00 |

TABLE II.

| Ratio: $HP^*O_4^-$ Concentration in Granular Protoplasm | | Time of immersion hr |
|--|-------|-------------------------|
| $HP^*O_4^-$ Concentration in Hyaline Protoplasm (Low centrifugal force) | | |
| | 0.80 | 0.25 |
| | 1.48 | 0.50 |
| | 2.80 | 1.00 |
| | 4.44 | 2.00 |
| | 10.55 | 8.00 |

¹ Brooks, S. C., *J. Cell. Comp. Physiol.*, 1939, 14, 4.

responding to true primary accumulation of ions by the cell. It appears that as the accumulation of ions progresses, the concentration of these ions takes place in the granules of the cell. An objection to this technic may be that the whole protoplasm is centrifuged to the bottom of the cell under the conditions of the experiment. This, however, does not seem likely in view of the relatively strong turgor of the cells and the low centrifugal forces applied. A general discussion of ion permeability phenomena will be found in Mullins² so that only a brief explanation of the data will be given here.

Since a large concentration of ions distributed throughout the hyaline protoplasm might alter markedly the colloidal nature of the protoplasm by causing excessive solution of the colloid, it seems reasonable that there must be some stations in the cell where ions are accumulated and rendered ineffective. Apparently the vacuole of the *Nitella* cell functions in this manner, and the data here given would indicate that the granules also supplement the vacuole in this process.

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A Micro-Photoelectric Photometer.

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Photoelectric analytical procedures depend for accuracy upon a depth of color in solution sufficient to produce satisfactory deflection of a galvanometer or other recording device. Ideally the volumes of reagents and sample are adjusted to yield 20-80% absorption of the incident light. Since the necessary intensity of color varies with the length of the path of the light beam in the solution, the dimensions of the absorption cell are limiting factors in adapting procedures to microanalysis. Present instruments on the market do not permit an absorption path longer than 20 mm when the volume of solution is limited to 1 cc.

An instrument in use at the Babies Hospital during the past 2½ years demonstrates the feasibility of constructing apparatus in which the light beam traverses a path of 50 mm through a tube-cell of narrow bore and only 1 cc capacity. These specifications have made

² Mullins, L. J., *Ionic Equilibria in Protoplasm*, in press, 1940.