decrease in hypothermia. That the decreased corticoid output in hypothermia was not due to a decreased release of pituitary ACTH was shown by the consistent corticoid fall despite the administration of an ACTH infusion. Diminished adrenal corticoid secretion in hypothermic dogs appears to be caused by direct adrenal suppression by cold, as shown by the decrease in minute corticoid output observed when local cold was applied to the adrenal gland and systemic normothermia was maintained. Cold exposure of the intact, normothermic dog at −10°C for periods up to 34 hr, in contrast to hypothermia, does not alter the normal adrenal response to ACTH.

Adrenal cortical secretion in response to exogenously administered ACTH or to the endogenous ACTH release that accompanies surgical trauma is markedly reduced in the hypothermic animal.


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## Absorption of Antibiotics by Plant Cells


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Present information indicates that animal cells are impermeable to streptomycin (*1*), but are permeable to penicillin (*2*). Penicillin has been shown to be bound and concentrated by bacteria (*3*), but not by yeast (*4*). Although the ability of antibiotics to penetrate plant cells other than microorganisms has been frequently discussed (*5*), it has not been subjected to direct investigation. In this paper (*6*), preliminary observations on the absorption of chloramphenicol, penicillin, and streptomycin by cells of the alga *Nitella clavata* (*7*) are described.

Growth of the fresh water characean *Nitella* is differentiated into nodes and internodes with whorled laterals arising at the nodes. The elongate internodes are single cells. These plant cells are ideal for permeability studies since they are large enough for the sap of an individual cell to be removed and analyzed. The cells studied are cylindrical, 0.5 to 1.0 mm in diameter and up to 10 cm in length. They are multinucleate and contain a large central vacuole surrounded by a delicate layer of protoplasm. The protoplasm is in a constant state of cyclosis.

Each of the three antibiotics that were investigated represents a different ionic species. Chloramphenicol is a neutral compound. Penicillin ($p$Ká = 2.7) is acidic (*8*) and more than 95-percent ionized in the physiologically important $p$H range of 4 to 8. Three basic groups are present in the streptomycin molecule. During titration only the weakly basic methylamino group is in evidence ($p$Ká = 7.7). The two guanido groups remain completely ionized (*9*)

Experiments were performed with freshly cut cells under fluorescent light in a constant-temperature room at 28°C. Antibiotic solutions were prepared with *M*/45 phosphate buffer. An appropriate number of cells contained in a large Petri dish (15 cm) were simultaneously suspended in a shallow layer of antibiotic solution. At various times after treatment, cells were withdrawn, washed by rapid immersion in four changes of distilled water, and placed in a buffer. The washed cells were dried against filter paper. When one end of a cell was cut off and pressure applied to the other, a droplet of sap flowed out. Sufficient sap (0.01 ml) was collected with a calibrated micropipette to uniformly wet a paper disk (7 mm in diameter). The sap of two to four cells was required to provide the necessary 0.01 ml. The concentration of the antibiotic in cell sap was determined by bioassay, using *Bacillus subtilis* as the test organism and nutrient agar as the assay medium. Standards were prepared by applying 0.01 ml of solutions of known antibiotic concentration to paper disks. The assay procedure has a lower limit of approximately 20 to 25μg/ml for chloramphenicol and streptomycin and of 8μg/ml for penicillin. All determinations were replicated at least three times. The assay measures the concentration of active antibiotic in cellular fluid. It will not detect any antibiotic that has been absorbed and inactivated. The absorption of streptomycin, chloramphenicol, and penicillin by living cells of *N. clavata* is shown in Fig. 1.

Streptomycin was rapidly absorbed and accumulated by the cells. The concentration of antibiotic in cells after 12 min of treatment was equal to that in solution. After 18.5 hr, the cells had accumulated streptomycin to more than 7 times the concentration in solution. The accumulation of streptomycin was

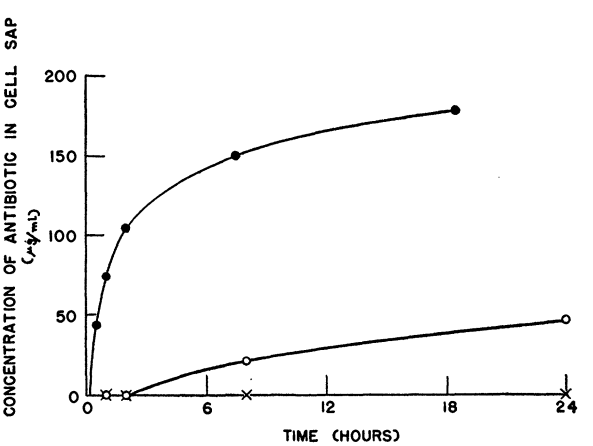


Fig. 1. The absorption of antibiotics by cells of *N. clavata*. Cells were suspended in the following antibiotic solutions prepared with *M*/45 phosphate buffer of pH 6.1: ●, 25 μg/ml of streptomycin sulfate; ○, 136 μg/ml of chloramphenicol; and ×, 128 μg/ml of potassium penicillin G.

influenced by temperature ($Q_{10} = 2.0$), $p$H (optimum $p$H = 5.0), and the respiratory inhibitors $NaN_3$ and 2,4-dinitrophenyl; $o$-phenanthroline and $NaAsO_2$ had no effect. These results suggest an active transport of the basic streptomycin ion across the cell membrane.

Chloramphenicol was not detected in cells treated for 2 hr, but was present after 8 hr. Following 24 hr of treatment, the concentration of antibiotic in cells was less than one-half that in solution. The relatively slow absorption of chloramphenicol appears to depend on simple diffusion.

No antibiotic activity was demonstrated in cells treated with penicillin for 25 hr. This may indicate that if the penicillin ion is able to penetrate the cells at all, it does so at a greatly reduced rate in relation to streptomycin and chloramphenicol. Alternatively, penicillin may be absorbed but inactivated by the cells and, therefore, not detected by the bio-assay procedure. This possibility is being investigated. Detailed studies on the absorption of chloramphenicol, penicillin, and streptomycin are being continued and will be published elsewhere.

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## Isolation of Organic Carbon from Bones for $C^{14}$ Dating


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A method recently used in the U.S. Geological Survey (*1*) for isolating the carbon from two fossil animal bones to determine their age by the carbon-14 technique (*2, 3*) may be of interest to investigators attempting to date old bones.

The bone samples, partly coated with clay, came from a sandstone deposit in Craven Canyon, South Dakota. Because secondary carbonates were present as white incrustations and impregnations of exposed porous bone structure, the carbon for age determinations had to be restricted to organic carbon. Nevertheless, the possibility of the presence of secondary organic matter should be taken into account.

Table 1. Sample weights and carbon yield (g).

| Samples as received | Clean samples | Char | Carbon recovered |
|---|---|---|---|
| 290 | 196 | 161 | 1.8 |
| 190 | 146 | 119 | 3.8 |

Because relatively small samples were available (196 and 146 g of clean bone), it was decided not to attempt the isolation of organic matter by selective solution of mineral matter; poor separations resulting in a low yield of organic matter would have yielded insufficient carbon for age determinations.

The method used consisted of pyrolysis of the bone in a nitrogen atmosphere, followed by acid solution of the mineral matter. The residual carbon was collected by centrifuging. The sample weights and carbon yield are shown in Table 1.

Material that was obviously extraneous (mostly clay) was removed mechanically from the bones. The cleaned bones were then broken into small pieces of about 4-mesh size. Charges of 40 to 50 g were placed in the central part of a 3-ft length of quartz tube 1 in. in diameter. The tube was heated by a 1-ft hinged-type combustion furnace that was controlled by an autotransformer. Nitrogen from a gas cylinder was passed over heated copper to remove oxygen and then through the quartz tube. The effluent gas was piped into a hood to remove malodorous vapors formed during the pyrolysis.

The quartz tube containing the sample charge was flushed with nitrogen for 10 to 15 min, and then the furnace was heated at a low-voltage setting (maximum temperature 340°C) for 60 min. The voltage was then increased and the sample was heated for an additional 90 min (maximum temperature 650°C). The bones were converted to a black char at the high temperature. A small amount of liquid condensed in the cool portion of the tube, but no attempt was made to collect the liquid. After completion of the charring, the tube was taken from the furnace and cooled to room temperature without interrupting the flow of nitrogen.

The total char from each sample was then treated in a beaker with 6*N* hydrochloric acid. After the initial effervescence subsided, the samples were digested on a steam bath for several hours. They yielded a residue consisting largely of quartz and carbon. The suspended carbon was separated from most of the quartz by decantation. The carbon and remaining insoluble matter were then separated from the solution by centrifuging. The residue was washed three times with 3*N* hydrochloric acid, and the insoluble matter was separated after each washing by centrifuging.

The remaining mineral matter was dissolved by transferring the residue to a platinum dish and digesting it overnight with hydrofluoric acid on a steam