If this procedure is to be used for research rather than for simple detection of the toxin, the following three modifications are recommended. Extract the mushroom tissue in methanol for 1 hr or longer. Evaporate the methanol extract and redissolve the residue three times to coagulate the polypeptides. Employ a 14-in. filter-paper strip and run the chromatogram for 2 hr. These modifications assist in the extraction and separation of the individual toxins.

References and Notes

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Effect of Hypothermia on 17-Hydroxycorticosteroid Secretion in Adrenal Venous Blood in the Dog

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This paper summarizes our studies on adrenal vein 17-hydroxycorticosteroid secretion in acutely traumatized dogs during the induction of and rewarming from hypothermia. Adrenal venous blood samples were obtained by a technique (*1*) that consists of placing a cannula in the lumbar portion of the lumboadrenal vein, and intermittently occluding the adrenal vein-caval junction by means of a polyethylene choker for periods of 1 min. 17-Hydroxycorticosteroids were determined by the method of Nelson and Samuels (*2*). Hypothermia was induced in 20 mongrel male dogs either by cooling an external vascular shunt or by ice water immersion. In three of these experiments the animals were given a continuous intravenous infusion of ACTH, 40 U/1000 ml of 0.99-percent NaCl at a rate of 40 drops/min.

Table 1. Experiments in which hypothermia was carried to 25 percent or less of normothermic 17-hydroxycorticosteroid minute output in adrenal venous blood.

No. of dogs	Temperature (°C) at which 25% of normothermic corticoid output was reached
6	27.0–28.0
5	26.0–26.9
4	25.0–25.9
1	23.0
1	22.0

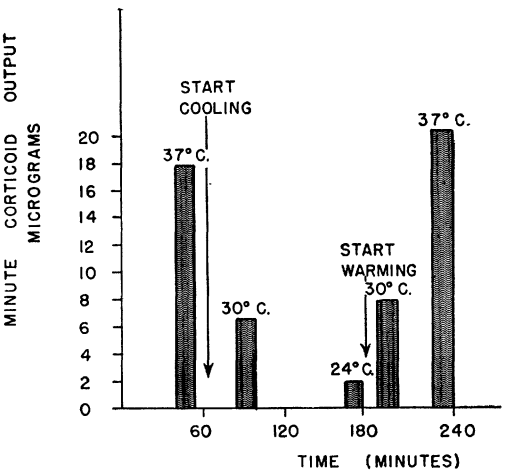

Fig. 1. Typical hypothermia experiment.

Table 1 illustrates that in most animals hypothermia to between 25° and 28°C is accompanied by a 75-percent decrease in adrenal venous blood minute corticoid output, but that an occasional animal may be cooled to lower temperatures before such a decrease is observed. Figure 1 is an example of a typical experiment demonstrating a marked fall in minute corticoid output from the adrenal gland during hypothermia, near-zero corticoid values at 24°C, and return to normal values upon rewarming. In Fig. 2, a similar sequence of events, with near-zero corticoid values at 21°C, is shown in an animal receiving an ACTH infusion during the procedure. In general, adrenal venous blood flow decreased with progressive hypothermia, as did the minute corticoid output. There were occasional exceptions, however, in which a relatively high adrenal blood flow was associated with a low minute corticoid output. Maintenance of the normal systemic blood pressure by the infusion of norepinephrine did not alter the minute corticoid output

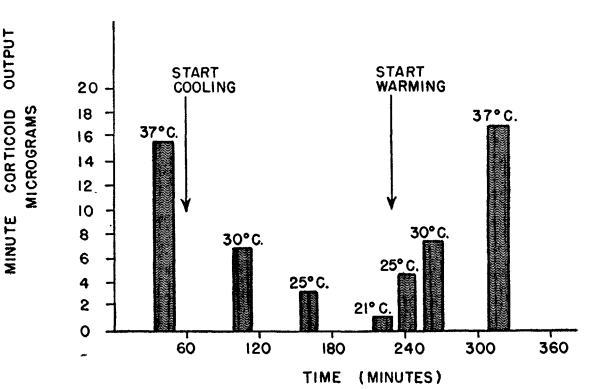

Fig. 2. Hypothermia experiment during which the animal received ACTH infusion.

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 (pKá = 2.7) is acidic (*8*) and more than 95-percent ionized in the physiologically important pH 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 (pKá = 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

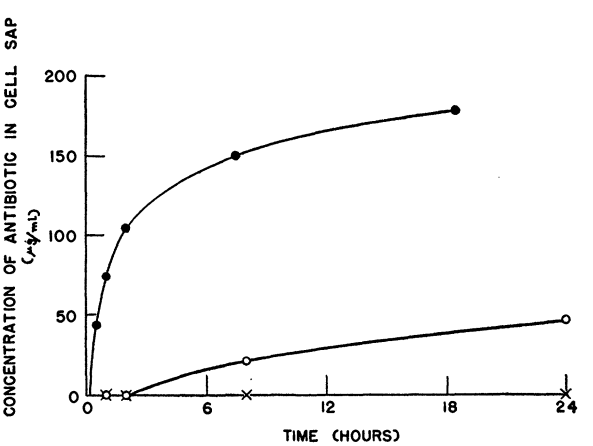

Fig. 1. The absorption of antibiotics by cells of *N. clavata*. Cells were suspended in the following antibiotic solutions prepared with *M*/45 phosphate buffer of pH 6.1: ●, 25 μg/ml of streptomycin sulfate; ○, 136 μg/ml of chloramphenicol; and ×, 128 μg/ml of potassium penicillin G.