

influenced by temperature ($Q_{10}=2.0$), pH (optimum pH=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.

References and Notes

1. J. R. May, A. E. Voureka, and A. Fleming, *Brit. Med. J.* **1**, 627 (1947); W. W. Umbreit and N. E. Tonhazy, *J. Bacteriol.* **58**, 769 (1949); R. L. Magoffin and W. W. Spink, *J. Lab. Clin. Med.* **37**, 924 (1951).
2. H. Eagle, *J. Exptl. Med.* **100**, 117 (1954).
3. A. V. Few, P. D. Cooper, and D. Rowley, *Nature* **169**, 283 (1952); H. Eagle, *J. Exptl. Med.* **99**, 207 (1954); ———, *ibid.* **100**, 103 (1954).
4. E. A. Maass and M. J. Johnson, *J. Bacteriol.* **57**, 415 (1949).
5. A. Stokes, *Plant and Soil* **5**, 132 (1954); D. Pramer, *Ann. Appl. Biol.* **40**, 617 (1953); S. H. Crowdy and D. Pramer, *Chemistry & Industry*, No. 7, 160 (1955).
6. Journal Series Paper, New Jersey Agricultural Experiment Station, Rutgers University, State University of New Jersey, New Brunswick, N.J.
7. I am indebted to E. T. Moul for identification of the species.
8. R. B. Woodward, A. Neuberger, and N. R. Trenner, *The Chemistry of Penicillin* (Princeton Univ. Press, Princeton, N.J., 1949), p. 415.
9. J. Fried and O. Wintersteiner, *J. Am. Chem. Soc.* **69**, 79 (1947).

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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. Neverthe-

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

less, the possibility of the presence of secondary organic matter should be taken into account.

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

bath. The hydrofluoric acid was evaporated; the residue was digested and then washed five times with 3*N* hydrochloric acid. After each washing the carbon was separated by centrifuging and was finally dried in an oven at 105°C.

The digestion with hydrofluoric acid is probably not essential, but it does result in a cleaner specimen of carbon and was done with these samples to determine the yield of carbon.

References and Notes

1. Publication authorized by the director, U.S. Geological Survey.
2. W. F. Libby, *Radiocarbon Dating* (Univ. of Chicago Press, Chicago, 1953).
3. H. E. Suess, *Science* **120**, 5 (1954).

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Rearing of Honeybee Larvae on Royal Jelly in the Laboratory

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For the first 3 days of life, the female larva of the honeybee (*Apis mellifera* L.) has the potential of developing into a queen or a worker, the direction of differentiation depending on the food that the larva receives. Queen larvae are mass fed a superabundance of royal jelly, which is secreted, at least in part, by the pharyngeal glands of adult bees. Worker larvae are also given a glandular food for about the first 3 days of life; thereafter they are progressively fed on small amounts of a food that normally contains pollen.

Rhein (1) collected royal jelly from queen cells and fed it to larvae in the laboratory. Only workers were produced. The experiment has been repeated by others (2) with the same results. It has been commonly assumed that the nurse bees give some substance directly to queen larvae rather than including it in the royal jelly, but since alternate explanations are possible, it seemed worthwhile to investigate the matter further (3).

In the first series of experiments, queen larvae, about 36 hr old, were removed from their cells and replaced by larvae of about the same age from worker cells. A screen lined with dampened cotton excluded adults from further contact with the larvae. Every 2 hr the larvae were transferred to fresh queen cells. Two adults were produced by this technique. Larvae were also reared in glass queen cells in a constant-temperature cabinet held at $34^{\circ} \pm 1^{\circ}\text{C}$ and a relative humidity of more than 75 percent. Every 2 hr royal jelly was pipetted directly from queen cells into the glass cells containing larvae of about the same age. Two adults were produced. The adults that were reared by these techniques were not workerlike in any characteristic observed, and they were queenlike in appearance, abdomen size, form of metathoracic legs

and mandibles, number of barbs on the sting, tongue length, and size of mandibular glands, spermatheca, and ovaries. The number of ovarioles fell at the lower range of variation for normal queens, varying from 102 to 161 (mean 124) ovarioles per ovary, as compared with 115 to 238 (mean 169) in a typical group of normal queens. Of three pupae in which some differentiation was evident before death, one appeared to be queenlike in the few characteristics that could be observed; the others were intermediate in tongue length, and in the size of the abdomen and spermatheca, and they had workerlike metathoracic legs.

Royal jelly that had been removed from queen cells daily and had been refrigerated at about 5°C for 1 to 4 wk was fed every 2 hr to larvae in glass cells in the constant-temperature cabinet. The one adult obtained was intermediate between queen and worker. The metathoracic legs were workerlike in form; the sting was queenlike; the abdomen, mandibular glands, ovaries, and spermatheca were intermediate in size, the mandibles were intermediate in form, and there were 66 ovarioles in the ovary in which a count was possible. One pupa was also produced by this treatment. It was intermediate in tongue length and in the size of the abdomen and ovaries, and workerlike in all other characteristics that could be observed.

In an attempt to develop an easier, more efficient feeding technique, five larvae were placed in royal jelly in a Petri dish containing a ball of dampened cotton. The larvae were transferred to a different dish of royal jelly daily. Only one adult was reared on royal jelly that had been collected daily and stored in the refrigerator under carbon dioxide for about 2 wk. The metathoracic legs, mandibles, and sting were workerlike; the weight, size of the abdomen and mandibular glands, and tongue length were intermediate; the ovaries and spermatheca were queenlike; there were 135 ovarioles per ovary. Another adult was produced on the same royal jelly from which the aforementioned larvae had been removed. The jelly was slightly desiccated and had been held at 34°C for 24 hr when the larvae were placed in it. This individual was workerlike in every respect; there were four ovarioles per ovary.

Nineteen adults were produced by the same technique from royal jelly that had been stored at 5°C for about a year. Some of the jelly was left in an open container in the laboratory to allow desiccation to the consistency of a heavy paste; some was diluted 10 and 20 percent with distilled water; and some was left unaltered. All the adults, except those reared on the drier royal jelly, had larger abdomens and were heavier than normal workers. This greater size and weight appeared to have been caused by extensive fat bodies in the abdomens. The ovaries of several individuals were not found, but it is not certain that they were absent since, in the laboratory-reared queens and in the intermediates produced by other techniques, the ovaries sometimes had about the same consistency as fat bodies. All 27 of the ovaries found were well within the range of normal workers and had a mean