References and Notes

1. A. A. Nelson and G. Woodard, *Arch. Pathol.* 48, 387 (1949).
2. J. Nichols and L. I. Gardner, *J. Lab. Clin. Med.* 37, 229 (1951).
3. J. H. U. Brown, *Proc. Soc. Exptl. Biol. Med.* 83, 59 (1953).
4. J. Nichols and H. D. Green, *Am. J. Physiol.* 176, 374 (1954).
5. P. S. Larson *et al.*, *J. Pharmacol. Exptl. Therap.*, in press.


6. This investigation was supported in part by a grant from the Lasdon Foundation and in part by a grant from the Rohm and Haas Company.


7. D. H. Nelson and L. T. Samuels, *J. Clin. Endocrinol. and Metabolism* 12, 519 (1952).
8. Armour's Corticotrophin (ACTH), 25 I.U. per vial.


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## Increased Production of Carotene by Mixed + and – Cultures of Choanephora cucurbitarum

The production of carotenoid pigments by filamentous fungi is apparently quite common, but knowledge of their functions in fungus metabolism is meager. There is evidence that β-carotene is concerned with phototropic responses of certain fungi, and in many fungi, visible amounts of carotenoids are produced only in the reproductive structures. Goodwin (*1*) has reviewed the literature on this subject. *Phycomyces blakesleeanus* has been the principal test fungus, and the mycelium of either the + or the – sex (*2*) has been used. *Choanephora cucurbitarum* is not mentioned as a carotene producer.

*Choanephora cucurbitarum* is heterothallic (*3*) and zygospores have been observed and described (*4, 5*). No mention has been made of carotenoids in the mycelium, and only Wolf (*5*) reports observing "numerous yellowish oil globules" in immature zygospores.

During routine culturing of *C. cucurbitarum,* it was observed that the mycelium of combined + and – cultures in liquid medium became bright yellow within a few days, while the mycelium of either the + or – sex cultured alone was only slightly yellowish. This paper (*6*) reports results of subsequent experiments that show that the production of β-carotene by *C. cucurbitarum* is greatly increased in mixed + and – culture.

The + and – cultures of *C. cucurbitarum* used in this study were isolated from the same diseased pumpkin flower at Morgantown, W. Va., in 1954. A liquid medium (glucose, 25 g; acid-hydrolyzed casein, 2 g/lit, essential salts, and thiamine) at an initial *p*H of 6.0 was used.

The fungus was cultured at 25°C in 250-ml flasks containing 25 ml of medium without agitation and at 28°C in 9-lit bottles containing 6 lit of medium through which sterile air bubbled continuously. The experiments have been repeated a number of times with similar results, although quantitative carotene determinations were made on only some of the cultures.

Under both sets of cultural conditions, the mycelium in the mixed + and – cultures began to show yellow pigmentation after about 3 days, reaching a maximum intensity about the fifth or sixth day. The + and – mycelia produced little yellow pigment when they were grown separately.

Under all conditions of growth, the mycelium contained much oil after a few days, but only the mycelium in the mixed cultures showed conspicuous yellow pigments in the oil. Later, during sexual reproduction, these pigments usually became concentrated in the suspensor cells that subtend the immature zygospores. Some yellow pigment is evident in the oil droplets in immature zygospores.

The yellow mycelium was squeezed between layers of cloth to remove excess water, and drying was completed with absolute methanol. The pigments were extracted with petroleum ether, saponified and reextracted with petroleum ether. The β-carotene content of each extract was determined by measuring the optical density of 460 mμ in a Beckman photoelectric spectrophotometer according to the procedures of Garton *et al.* (*7*).

By using chromatographic adsorption techniques (*7*) and repeated recrystallizations from a 1-to-1 solution of ethanol and petroleum ether (bp 40° to 60°C), it was possible to isolate the primary pigment. This material was characterized by the use of chromatographic adsorption techniques (*7*), molecular extinction curves and extinction values ($E_{1\ cm}^{1\ percent} = 2400$ at 455 mμ in cyclohexane) (*8*), and identified as β-carotene. The yields of β-carotene in one typical experiment are presented in Table 1.

Under these conditions, the mycelium grown in mixed + and – cultures produced 15 to 20 times as much β-carotene per gram of mycelium as did that of either sex grown alone. The evidence suggested that the stimulatory substances responsible for the enhanced carotene production were secreted by the mycelium of the opposite sex.

When the + and – mycelia are grown on opposite sides of a cellophane membrane, both mycelia usually show increased pigment production, indicating that the stimulating substances are diffusible through cellophane and that the effect is reciprocal. The failure of these cultures to form zygospores after further incubation is evidence that the mycelium did not penetrate the cellophane.

Increased carotene production by the mycelium in mixed + and – cultures of a fungus does not seem to have been previously reported. Since there is normally no anastomosis between the + and – vegetative hyphae of the Mucorales, which would result in heterocaryotic mycelium, the stimulation in *C. cucurbitarum* must originate as secretions from the mycelium of the opposite sex. These secretions are possibly of the nature of hormones. It seems probable that some relationship exists between the production of carotene and sexual reproduction in *C. cucurbitarum.*

Table 1. Weight of dry mycelium and β-carotene after 6 days in 6-lit aerated cultures when + and – sexes were grown separately and together.

| Culture | Dry mycelium (g) | β-carotene (μg) | Amt. β-carotene in dry mycelium (μg/g) |
|---|---|---|---|
| + | 25 | 1,140 | 45.6 |
| – | 22 | 1,377 | 62.6 |
| ± | 18 | 16,560 | 920.0 |

These investigations, as well as others designed to give more information on the role of carotenoids in the fungi and studies particularly concerning the relationship of carotene to sexual reproduction of *C. cucuribitarum,* are being continued.


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References and Notes

1. T. W. Goodwin, *Botan. Rev.* 18, 291 (1952); *Ann. Rev. Biochem.* 24, 497 (1955).
2. The term *sex* is used in this paper, following E. A. Bessey, *Morphology and Taxonomy of Fungi* (Blakiston, New York, 1950) and E. A. Gaumann, *The Fungi* (Hafner, New York, 1952), in preference to the less specific term *strain.*
3. A. F. Blakeslee *et al.*, *Botan. Gaz.* 99, 27 (1927).
4. J. F. Dasteur, *Ann. Botany London* 34, 399 (1920); S. Sinha, *Proc. Indian Acad. Sci.* 11, 162 (1940).
5. F. A. Wolf, *J. Agr. Research* 8, 319 (1917).


6. Published with the approval of the director of the West Virginia Agricultural Experiment Station as scientific paper No. 511.


7. G. A. Garton *et al.*, *Biochem. J. London* 48, 154 (1951).
8. W. H. Seberell, Jr., and R. S. Harris, *The Vitamins* (Academic Press, New York, 1954).


12 August 1955

## Fate of Radiostrontium Fed to Habrobracon Females

Strontium-89 has been of interest to us, not only because it is a biologically important product of nuclear fission, but more specifically because it is the only pure beta-emitting radioisotope with which we have been able to produce per-

manent sterility in *Habrobracon* feeding experiments (unpublished). This report (*1*) gives observations on the biological half-life, on the proportionate gross distribution, and on the egg radioactivity after these female braconid wasps have been fed $Sr^{89}$. The results differ, not only from findings on *Habrobracon* after $P^{32}$ feedings (*2*), but also from strontium retention studies in vertebrates (*3, 4*).

Forty virgin females from wild-type stock 33 of *Habrobracon juglandis* [*Microbracon hebetor* (Say)] were starved at least 4 days, and each was weighed on a precision balance (Roller-Smith). They were then fed $Sr^{89}$ citrate in a honey mixture at a level of 227 μc/g of mixture. With the stock solution of citrate available, this level resulted from mixing in equal proportions with honey. After feeding, each wasp was reweighed. Four that had not fed satisfactorily were discarded. Then, in order to obtain a measure of initial radioactivity for each animal, counts per second were obtained using a thin end-window Geiger tube with standard scaler. Subsequent maintenance followed standard practice in our laboratory. All wasps were provisioned with two host larvae (*Ephestia*) per day and maintained in individual Stender dishes at 30°C.

Twelve wasps were chosen at random to be followed in a study of total body radioactivity. The intact wasp (2 by 0.6 mm), periodically immobilized by brief cooling, can be subjected to Geiger-Müller counting. Thus counts per second were obtained for each animal of the sample 12 hours subsequent to feeding. The emissions were counted again at the end of 24 hours and at daily intervals for 11 days. After this time, the wasp radioactivity was barely above background. Figure 1 presents a summary of the results in net counts per second.

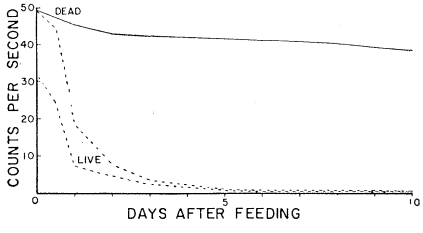


Fig. 1. The relative radioactivity of female *Habrobracon* fed $Sr^{89}$ in honey mixture. The lower broken line represents average radioactivity for nine wasps that ingested the usual quantity, 0.2 to 0.3 mg of the mixture. The upper broken line shows the same for two females that ate 0.5 mg. The solid line, which extrapolates to an adequate representation of typical $Sr^{89}$ decay, is taken to represent this phenomenon as measured in a dead wasp. The female expired shortly after its heavy feeding (0.5 mg).

In view of the physical half-life of 55 days, the biological half-life was achieved in the surprisingly short time of less than 1 day, which indicates very little retention. The dead animal carried for comparison demonstrates the loss in radioactivity owing to isotope decay only (Fig. 1). This picture is quite different from that in vertebrates, in which more than half of a dose may persist after 9 days (*4*) and in which the rate of elimination can be less than the rate of radioactive decay (*5*).

A further point brought out by this experiment is that differences in wasp radioactivity following heavy and average feedings are not significant after the biological half-life has been exceeded. Even at the end of the first day, the standard error of the mean for the animals from average feedings is 9.97 counts per second. On the second day, the range of the groups includes the mean value for the high feedings. Ultimately, as is shown by Fig. 1, the curves converge when the means become identical.

The remaining 36 animals were sacrificed in groups of four by transection at the petiole, so that the anteriors and posteriors could be counted separately (sacrifice schedule: days 2, 4, 6, 7, 8, 9, 10, 11, and 12). During the first week after feeding, it was evident that more than 90 percent of the radioactivity that was demonstrable for each female came from the abdomen. Indeed, in the majority of cases, 97 to 99 percent of the radioactivity was shown to be abdominal. In the second week, when radioactivity of the entire animal was barely above background, abdominal radioactivity fell off only slightly. On the 12th day, the abdomens held 86 to 92 percent of total radioactivity. The abdomen contains most of the digestive tract and all the organs of excretion and reproduction.

Eggs laid by the wasps of the transection experiment were collected until the day of sacrifice, and their radioactivity was determined. Eggs laid on the first 3 days were slightly radioactive, an indication that at least some $Sr^{89}$ gets into the physiological interior of fed females. Eggs laid on the third day were barely above background radioactivity. Those laid on subsequent days were not demonstrably radioactive. The average net counts per second per egg were 0.0094 on day 1, 0.0040 on day 2, and 0.0007 on day 3.

When these results were compared with the radioactivity of the ovipositing females, it was found that little more than 0.03 percent of the radioactive material lost from the animal was lost by way of the eggs. This is consistent with the indications from the biological half-life that egestion of feces and excretion plays the important role for this isotope, a finding quite different from that with $P^{32}$, where the majority of the fed isotope is incorporated into the eggs and eliminated by this route (*2*).

These discoveries concerning the fate of radiostrontium explained a perplexity that is met in autoradiographic technique. In direct contrast to the results obtained when autoradiographs are made after radiophosphorus feedings (*2*), radiostrontium shows up only in the oocytes and not even there if more than 2 days have elapsed since its ingestion. Apparently adult insects have no tissue functionally analogous to bone in a biochemical sense. In vertebrates, the presence of bone with its propensity for fixing the alkaline earth elements is responsible for the retention of strontium. On the other hand, it is well established that the radioactivity of soft tissue of vertebrates is negligible after doses of $Sr^{89}$ (*3, 4*). Our results are essentially a demonstration of this latter point for an adult insect.

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References and Notes

1. This report is based on work done for the U.S. Atomic Energy Commission under contract No. At-(40-1)-1314.
2. D. S. Grosch and R. L. Sullivan, *Biol. Bull.* 105, 296 (1953).
3. C. Pecher, *Proc. Soc. Exptl. Biol. Med.* 46, 86 (1941); J. H. Lawrence, *Am. J. Roentgenol. Radium Therapy* 48, 283 (1942).
4. B. Kidman, M. L. Tutt, J. M. Vaughan, *J. Pathol. Bacteriol.* 62, 209 (1950).
5. G. Hamilton, *Revs. Mod. Phys.* 20, 718 (1948).
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## Alkaline Phosphatase in Kidneys of Aglomerular Fish

In a recent issue of *Science*, I discussed the implications of the presence of alkaline phosphatase in the aglomerular renal tubule (*1*). This discussion was based on unpublished observations by Danielli and Lorch and on my own experience with the kidney of *Opsanus tau*. Since the publication of this note, my attention has been called to an earlier paper by Browne and associates (*2*), who reported the presence of this enzyme in the tubules of a number of teleosts, including three aglomerular species, and in particular, *Opsanus tau*. From their observations, these authors drew essentially the same conclusions about alkaline phosphatase and tubular function as I did. Priority on these points therefore rests with them.

Our conclusions with respect to the significance attached to previous reports of negative results differ. Browne and associates believed that slight variations