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3. B. O. Phinney *et al.*, *Proc. Natl. Acad. Sci. U.S.* 43, 398 (1957).
4. Gibrel is the name applied to the potassium salt of gibberellic acid by Merck & Co., Inc. The gibberellic acid used in these studies was prepared by the Chemical Division, Merck & Co., Inc., Rahway, N.J.
5. The tissues examined included skin, skeletal muscle, gonads and accessory sex organs, stomach, intestine, salivary glands, thymus, pancreas, adrenal, thyroid, parathyroid, lymph nodes, spleen, liver, kidney, urinary bladder, aorta, heart, lung, bone marrow, and usually the pituitary, brain, and spinal cord.

5 August 1957

Preparation of Cell-free Yeast Homogenate That Converts Acetate to Sterols

Cell-free yeast preparations have been applied recently to the study of sterol biogenesis (*1*). However, all these preparations require complicated apparatus for the mechanical disruption of yeast cells. A search for an easier method has been conducted in this laboratory over the last 2 years, and a method employing only the simplest equipment is described in this report (*2*).

Twenty grams of dry baker's yeast (Fleischmann) is suspended in 80 ml of 5 percent aqueous glycerol solution and stirred vigorously for 2 hours at room temperature with a Herschberg wire stirrer (Nichrome wire loops on a glass rod). The brei is then centrifuged at 1000 *g* for 30 minutes in the cold, and the supernatant is dialyzed against four changes of distilled water over a 24-hour period at 7°C to remove the glycerol. The homogenate (approximately 60 ml) contains particulate material but no whole cells or cell-wall debris. It is then diluted to 80 ml and divided into 20 Erlenmeyer flasks, each containing 1.0 mg of adenosine-5′-triphosphate (ATP), 1.3 mg of diphosphopyridine nucleotide (DPN), 1.6 mg of coenzyme A (CoA), 5 mg of methionine, 4 mg of $MgSO_4$, 8 mg of $NaNO_3$, 4 mg of K_2HPO_4, 2 mg of KCl, 0.04 mg of $FeCl_2$, and 20 mg of "tris" buffer (*p*H 7). Five microcuries of sodium acetate-1-C^{14} (0.41 mg) is added to each flask.

The incubation is carried out at room temperature in cotton-plugged flasks mounted on a rotary shaking table. After 48 hours, 4 ml of methanol and 0.8 g of potassium hydroxide pellets are added to each flask, and the mixture is hydrolyzed on a steam bath for 16 hours. The hydrolyzate is extracted with pentane, and the pentane phase is washed thoroughly with alkali. The sterols are isolated by precipitation with digitonin, followed by cleavage with pyridine and recrystallation. Radioactivity is measured in a gas-flow counter in which 1 μc is equivalent to 3×10^5 count/min.

Proper stirring of the yeast suspension in the glycerol solution is quite important. Gentle shaking on a rotary table produces weak homogenates. Suspensions digested with diammonium phosphate or treated in a Waring Blendor, in a Potter-Elvehjem or Virtis homogenizer or in a Hughes press also gave only weakly active extracts. Table 1 demonstrates the role of various cofactors in the system. The methionine requirement has been explored in experiments with methionine-methyl-C^{14}, which was found to yield ergosterol-28-C^{14} (*3*). Aeration during incubation is essential. Homogenates incubated under nitrogen gave only 17 percent of the yield in a comparable aerobic incubation. Potassium cyanide ($1 \times 10^{-3}M$), α,α-dipyridyl ($1 \times 10^{-3}M$), and digitonin ($1 \times 10^{-4}M$) strongly inhibit synthesis of sterols. In one experiment, varying quantities of sodium acetate were used to determine the capacity of the system to convert acetate into sterols, and it was found that 2 mg of acetate was the maximum that could be efficiently utilized by 4 ml of homogenate in 48 hours.

The duration of incubation determines the extent of incorporation of C^{14} into sterols. After 48 hours, one-third of the C^{14} in the nonsaponifiable fraction has been incorporated into sterols.

The homogenate remains active for a long time. In several experiments a small increase in incorporation of C^{14} into sterols was found even after a 96-hour incubation. Since the homogenate is apparently not a very good growth-supporting medium, proper care during its preparation is sufficient to prevent contamination in 90 percent of the cases. Passing it through a Seitz filter into sterile flasks prior to incubation eliminates the remaining contaminants. This has been verified by microscopic examination both before and after incubation. Contaminated flasks usually show a lower yield of sterols, probably because whole cells divert acetate to other uses.

Table 1. Cofactor requirements. Yeast homogenate was incubated for 48 hours at room temperature.

Cofactor	Total C^{14} in sterols (10^3 count/min)	Decrease in yield (%)
None	5.1	
Yeast hydrolyzate (30 mg)*	22.4	
Complete system	43.3	0
ATP omitted	24.5	43.5
DPN omitted	37.3	13.9
CoA omitted	39.7	8.2
Methionine omitted	22.4	48.4
$MgSO_4$ omitted	24.5	43.4

* Nutritional Biochemicals Corp., Cleveland, Ohio. It lacks any inherent enzymatic activity in this system.

Standing at 7°C for 24 hours prior to incubation does not materially affect the activity of the preparation, but standing in 5 percent glycerol solution decreases the efficiency of ergosterol synthesis.

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References and Notes

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24 July 1957

Differentiation of Species by Paper Electrophoresis of Serum Proteins of Pseudemys Turtles

Serum proteins of a number of vertebrates, including the turtle, have been studied by paper electrophoresis, and differences between major groups have been noted (*1*). Several workers have used protein composition in taxonomic studies, employing precipitin or electrophoretic methods (*2, 3*). The present work (*4*) was undertaken to compare the serum proteins of closely related turtle species.

Striking differences were observed when 22 individuals representing three different species of the turtle genus *Pseudemys* were analyzed by paper electrophoresis. Included were three races of *P. scripta* (*scripta, elegans,* and *gagei*) from four widely separated localities (Florida, Kansas, Louisiana, and Mexico), *P. nelsoni* from Florida, and three races of the *P. floridana* complex (*floridana, suwanniensis,* and *mobilensis*) from two localities (Florida and Louisiana). While the representatives of the *P. floridana* complex are currently considered to be subspecies of a single species (*5*), the *floridana* and *suwanniensis* examples from Florida exhibit biological relationships characteristic of distinct species—namely, reproductive isolation in microgeographic sympatry (*6*). Further evidence presented in this report indicates a difference between the serum proteins of these two forms which substantiates a species level relationship (*7*).

Our paper electrophoresis techniques were the same as those described by Durrum (*8*); we used Spinco model R, series B apparatus; barbital buffer (ionic strength 0.05; *p*H 8.6); Heath Kit constant-voltage power supply (300 v d-c);