Table 1. Effect of iproniazid on metabolism of brain serotonin and norepinephrine after release by reserpine. The rabbits received 100 mg/kg of iproniazid intravenously. After 6 hours some animals received 5 mg/kg of reserpine intravenously. One hour later the animals were killed and their brain stems were analyzed. Values for amines in animals given iproniazid are somewhat higher than normal because the inhibition of monoamine oxidase causes levels to rise.

Injection	Serotonin level (μg/g)	Norepinephrine level (μg/g)
Iproniazid	1.00, 1.46	0.69, 0.61
Iproniazid + Reserpine	1.15, 1.08	0.64, 0.67, 0.57

both amines. Since iproniazid blocks the deamination of brain norepinephrine without disclosing another metabolic pathway, it seems unlikely that appreciable amounts of the "hallucinogenic" adrenochrome type of compound are formed in normal brain.

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References and Notes

1. M. Vogt. *J. Physiol.* (*London*) **123**, 451 (1954); B. B. Brodie and P. A. Shore, *Ann. N.Y. Acad. Sci.* **66**, 631 (1957).
2. H. Blaschko, *Pharmacol. Revs.* **4**, 415 (1952); K. Kazuya, G. B. Koelle, H. G. Wagner, *J. Pharmacol. Exptl. Therap.* **117**, 213 (1956).
3. A. Sjoerdsma *et al.*, *Proc. Soc. Exptl. Biol. Med.* **89**, 36 (1955).
4. K. Hellman, *J. Physiol.* (*London*) **129**, 454 (1955).
5. D. Bogdanski *et al.*, *J. Pharmacol. Exptl. Therap.* **117**, 82 (1956); P. A. Shore and J. S. Olin, *ibid.*, in press.
6. The same relative preference for serotonin is shown by a purified liver monoamine oxidase preparation (H. Weissbach, personal communication).
7. M. Holzbauer and M. Vogt, *J. Neurochem.* **1**, 8 (1956); B. B. Brodie *et al.*, *Science* **125**, 1293 (1957); A. Carlsson *et al.*, Abstr. Intern. Symposium on Psychotropic Drugs, Milan, Italy (1957), p. 51.
8. P. A. Shore and B. B. Brodie, *Proc. Soc. Exptl. Biol. Med.* **94**, 433 (1957).
9. Unpublished results.
10. A. Carlsson, P. A. Shore, B. B. Brodie, *J. Pharmacol. Exptl. Therap.*, in press.
11. E. A. Zeller *et al.*, *Experientia* **8**, 349 (1952).

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Toxicologic Evaluation of Gibberellic Acid

Gibberellic acid, a metabolic product of the fungus *Gibberella fujikuroi*, produces a diversity of responses in plants, perhaps the most striking of which is rapid elongation of shoots or acceleration of the rate of organ growth. Horticulturists and agronomists are currently making application of this agent to many economic and ornamental plants with the expectation of usefully modifying the normal growth habit (*1*). The agent is produced in practical quantities from filtrates of deep cultures of the fungus (*2*). Phinney (*3*) has reported that "Gibberellin-like" materials are present also in extracts of flowering plants, thereby indicating that these agents are natural constituents.

Plants are treated with the potassium salt of gibberellic acid (*4*), by spraying (concentrations of 1 μg to 1.0 mg/ml), by painting with a paste (0.5 to 1.0 percent), or by dipping seeds in solutions of 1.0 percent, or lower, concentrations. Thus concentrations of the agent which may be accidentally inhaled or ingested, or which may come into contact with the eye or skin, vary greatly. The residue remaining on treated plants at the time of harvesting appears to be negligible. Presently available analytical methods (sensitive to less than 1 μg/ml) are unable to detect the agent in harvested plants. Toxicologic studies of gibberellic acid, designed to expose any inherent toxicity for the human being or animal, are presented in this report.

Gibberellic acid was prepared either as a 30.0 percent aqueous solution by converting the acid to the sodium salt with sodium hydroxide or as a 50.0 percent concentration in carboxymethylcellulose suspension. For determinations of the acute intravenous toxicity, the appropriate concentrations were administered into the tail veins of Carworth female white mice in volumes of 0.5 ml or less at the rate of 1.0 ml/min. For determinations of acute oral toxicity, the appropriate concentrations were administered by stomach tube. The mice were observed frequently for several hours and then were held for 7 days, when some of the surviving mice were sacrificed; various tissues (*5*) were then examined grossly and prepared for histomorphologic studies.

Studies of the acute intravenous toxicity of gibberellic acid gave an LD_0 of 4.2 g/kg, and LD_{50} of 6.3 g/kg, and an LD_{100} of 8.7 g/kg. The signs of toxicity were nonspecific. No deaths and only minimal signs of toxicity were observed after the oral administration of 25.0 g/kg. Gross and histomorphologic studies did not reveal lesions or tissue changes that could be attributed to an effect of administration of gibberellic acid.

Twenty-seven male and 27 female Holtzman white rats were fed a diet containing 5.0 percent gibberellic acid. One-third of these animals were sacrificed after 5 weeks' feeding on the diet, and one-third after 8 weeks' feeding. The remaining rats are being continued on the diet. Three groups of control rats were fed the basal diet, and one group was sacrificed with each experimental group.

Body weights, food consumption, and hematologic values were normal for all groups of rats. Gross and histomorphologic studies of the various tissues (*5*) did not reveal lesions or alterations that could be attributed to the administration of gibberellic acid. Weights of organs were within normal limits.

Two groups of male and female Holtzman rats were exposed to an aerosol produced by spraying a solution containing 200 or 400 parts per million of gibberellic acid. The aerosol was produced continually for 10 minutes in a closed 88-1 chamber containing one group of rats. The rats then were held in the chamber for an additional 50 minutes. This procedure was repeated twice a day for 3 weeks. One-half of the rats were sacrificed at the termination of the study, one-fourth of the original group were autopsied after 1 month, and the remaining rats 2 months after the exposure. Control rats were exposed to an aerosol of the vehicle and sacrificed in the same temporal sequence. Gross and microscopic examinations of the various tissues (*5*) did not reveal abnormalities.

A single application of a 1.0 percent aqueous suspension of gibberellic acid to the eye of the rabbit did not produce immediate or delayed signs of irritation.

A concentration of 100 μg/ml in cultures of Rhesus testicular cells, Hela cells, or stable human amnion cells did not result in toxic reactions or in stimulation of cell growth. Repeated subcultures through several passages in the presence of the agent did not reveal cytotoxic activity. The yield and morphology of the cells were not influenced.

The order of acute and subacute toxicity of gibberellic acid is such that it is relatively harmless when administered orally, parenterally, by inhalation, or by topical application. This is the more remarkable since the agent is so potent that it may be employed effectively in amounts that leave no detectable residue on plants. The present evidence indicates that the agent presents no apparent hazard either to the individual who uses the material for agricultural purposes or to the individual who consumes products on which gibberellic acid or salts have been used.

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References and Notes

1. A. Kahn, J. A. Goss, D. E. Smith, *Science* **125**, 645 (1957); A. Lang, *Naturwissenschaften* **43**, 257, 284 (1956); C. Leben and L. V. Barton, *Science* **125**, 494 (1957); D. G. Morgen and G. C. Mees, *Nature* **178**, 1356 (1956); W. G. Whaley and J. Kephart, *Science* **125**, 234 (1957).
2. F. H. Stodola *et al.*, *Arch. Biochem. Biophys.*

54, 240 (1955); F. H. Stodola, G. E. N. Nelson, D. J. Spence, *ibid.* 66, 438 (1957).
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.

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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

1. P. M. Nossal, *Australian J. Exptl. Biol. Med. Sci.* 31, 583 (1953); H. P. Klein and Z. K. Booher, *Proc. Soc. Exptl. Biol. Med.* 89, 43 (1955); L. M. Corwin, L. J. Schroeder, W. G. McCullough, *J. Am. Chem. Soc.* 78, 1372 (1956).
2. This work was supported by the U.S. Public Health Service (grant No. C321), by the Jane Coffin Childs Fund, and by an institutional grant from the American Cancer Society.
3. G. J. Alexander, A. M. Gold, E. Schwenk, *J. Am. Chem. Soc.* 79, 2967 (1957).

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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);