TABLE 1

Plant	Spray conc (%)
Tomato	0.05
Johnson grass seedlings	0.5
Established Johnson grass	3.0
Bermuda grass	1.0
Quack grass	1.0
Nut grass	3.0

The initial effect generally is leaftip dieback, beginning on the older leaves. This is followed by progressive chlorosis and retardation of growth, ending in the death of the plant. Preliminary observations in field experiments suggest strongly that 3-(*p*-chlorophenyl)-1,1-dimethylurea acts readily through the root system and is translocated upward to the leaves.

3-(*p*-Chlorophenyl)-1,1-dimethylurea was synthesized by reaction of *p*-chlorophenyl isocyanate with dimethylamine. The product melts at 169.8°–170.4° C. After crystallization from methanol, it is obtained as thin rectangular prisms which melt at 170.5°–171.5° C. It is an essentially neutral, stable substance, insoluble in water, and only slightly or moderately soluble in most organic solvents. Its solubility in acetone is sufficient for greenhouse tests such as those described here.

Preliminary toxicity tests conducted by the Haskell Laboratory of Industrial Toxicology of this company indicate that the LD_{50} of 3-(*p*-chlorophenyl)-1,1-dimethylurea by oral administration to male rats is approximately 3,500 mg/kg of body weight.

Various concentrations of 3-(*p*-chlorophenyl)-1,1-dimethylurea have been sprayed on test plants in the greenhouse, as shown in Table 1.

In these experiments, all plants used for the work were grown in 4-in. clay pots. Tomato plants, grown from seed, and Johnson grass seedlings were sprayed when 6–7 weeks old. Bermuda grass, quack grass, and Johnson grass were established from root stocks. Nut grass was established from tubers. All these established grasses were sprayed approximately 3 months after planting.

In the case of tomato plants, first symptoms appeared 3 days after spraying. The plants were dead in 7–14 days. With Johnson grass seedlings, first reaction was noted in 5 days, and the plants were dead 14–28 days after spraying. In the case of established perennial grasses, the initial response was noted in 7–10 days. Both tops and roots were killed in 2–3 months.

TABLE 2

	Age of plant at time of treatment (days)	Percentage kill at conc of	
		0.1%	0.25%
Meadow fescue	20	0	100
Wheat	12	100	100
Timothy	20	99	100
Sudan grass	20	75	99
Rye grass	29	100	100
Harting grass	29	65	100
Orchard grass	25	100	100
Prairie brome	25	65	100
Meadow foxtail	29	100	100

In another series of greenhouse experiments, a wider range of grass seedlings was sprayed with two concentrations of 3-(*p*-chlorophenyl)-1,1-dimethylurea, as shown in Table 2. The percentage kill at these concentrations is also given.

The seedlings used in this experiment were grown in flats in the greenhouse, and ranged from 2 to 6 in. in height at the time they were sprayed. In the case of most of these seedling grasses, the first symptoms appeared in 2–3 days. The percentage kill shown in Table 2 was reached 2–3 weeks after treatment.

More detailed field investigations are still in progress to determine the effectiveness of 3-(*p*-chlorophenyl)-1,1-dimethylurea against a wide variety of annual and perennial weeds. Tests are also being conducted to determine the persistence of the compound when applied in various types of soils.

Tritium in Radioautography[1]

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One of the advantages of the radioautographic technique to the biologist is its ability to localize a radioactive element or compound to a particular organ, histologic unit, or to a distinct cell group in an organism. Many labeled compounds and elements have been traced to specific zones of concentration in animal and human organs. The ultimate goal of radioautography is to identify the site of emission of the radioactive element or compound in terms of intracellular structure such as nucleus, nucleolus, cytoplasm, or mitochondria. Or, if the concentration were intercellular, the identification of areas of radioactivity as those of collagen, interstitial fluid, reticulum, or similar structures would be of considerable importance. A resolution of a few microns or less would be required for such demonstration.

Most past studies have failed to show such fine delineation. Emulsions of the required resolving power were not available. Recently, however, with the use of nuclear track plates, radioactive lines of 2.5 μ width can be distinguished, and this brings the emulsions to the intracellular level (*1*). The other factor has been the range of the emitted radiation. Even weak β-emitters such as C^{14} and S^{35} have 90- and 100-μ maximum paths, respectively, in the nuclear track emulsions. If all activity were recorded in the autograph it is obvious that the isotope would give too diffuse an image to localize the site of activity in one cell. The disadvantages of a long average path are lessened by a shorter

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exposure time and longer emulsion development, so that individual β-tracks are recognizable. By this method, C^{14} in liver and blood cells (*2, 3*), P^{32} in *Colpidium* (*4*), S^{35} in cartilage, and Fe^{59} in rat placenta have been identified (*5*). A disadvantage is that β-particles having a high energy give rather wide grain spacing at the start of their track, and intracellular localization seems unobtainable with many isotopes. Campbell estimated that tracks from S^{32} and Fe^{59} could be correlated with morphologic structures of the order of 10 μ (*5*). Very thin emulsion layers increase considerably the resolving power for some isotopes. α-Particle emitters give a distinct, recognizable path in emulsions that allows one to trace the particle to its origin (*6*), but these elements are foreign to biological material, and many problems arise in their use as a label.

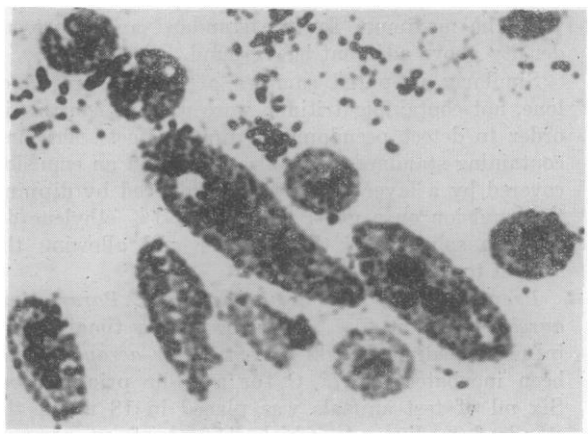

FIG. 1. Radioautograph (#51-24-K). One-micron section of many paramecia. Diffuse activity with endoplasmic and ectoplasmic concentrations. Some areas of activity between organisms (radioactive organisms or debris). Exposure 2 weeks, E. K. NTB, 10 μ, emulsion; unstained; original magnification, × 460.

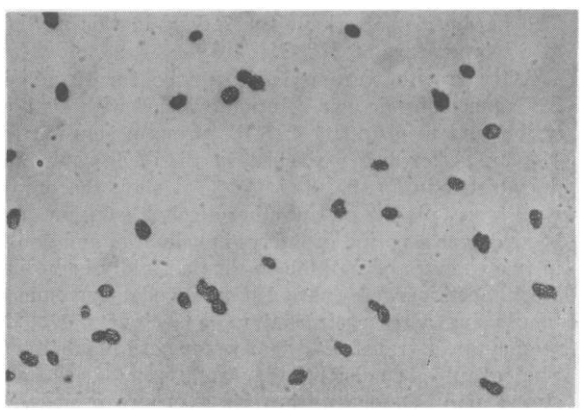

FIG. 2. Radioautograph (#51-35) of yeast cells obtained by placing Kodak Limited NTB stripping film over smear of dried organisms. Three weeks' exposure; unstained; original magnification, × 492.

Of great advantage would be a radioactive isotope of an element commonly occurring in biological material which has a very short path in emulsion. Tritium,

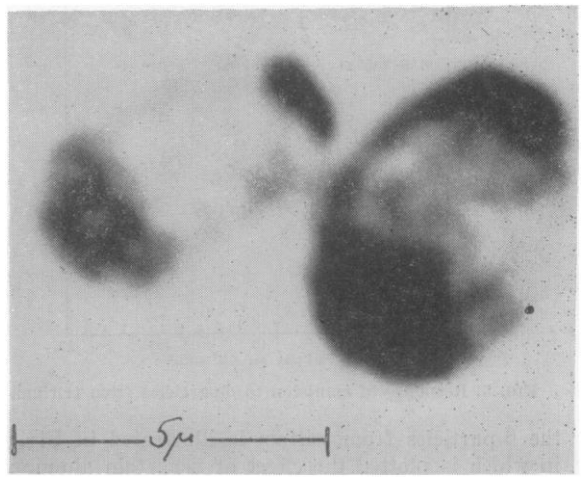

FIG. 3. Radioautograph (#51-19) of yeast cells. NTB_3 25 μ, emulsion covered with a thin Formvar film and an aqueous suspension of organisms smeared on the film. Organisms were washed off during removal of Formvar. Extranuclear polar and peripheral concentrations of the isotope. Formvar coat caused slight diffuseness of image; 8 days' exposure, 2 min development, D19; original magnification, × 1800.

the hydrogen isotope of mass 3, emits β-particles having a maximum energy of only 17.9 kev (*7*) and a maximum range of about 6 μ in a medium of unit density. Since the average density of nuclear plates is between 3 and 4,[2] the longest tracks would be about

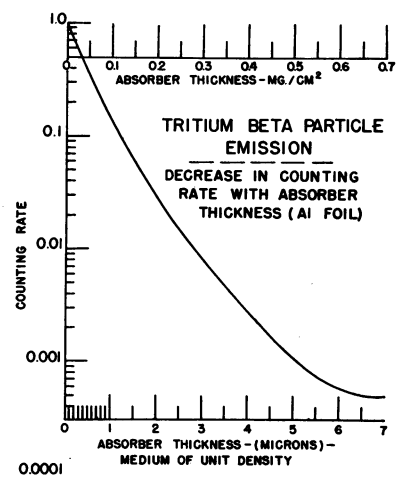

FIG. 4. Counting rate of a tritium standard as a function of absorber thickness.

2 μ, although most tracks would be shorter. This small path would be compatible with intracellular resolution. It is shown in this report that tritium can be used for radioautography and that it gives resolution of a few microns in individual yeast and paramecium cells (Figs. 1–3). Actually, by short development of emulsion, localization of activity to individual grains can be achieved.

Effect of absorbers on tritium. The short range of

[2] A nuclear track emulsion consisting of equal volume percentages of silver halide and gelatin with densities of 6.5 and 1, respectively, has an average density of 3.75. The grain diam are 0.2–0.4 μ (*8*). There is, therefore, the strong possibility that only one grain will be reduced by most β-particles.

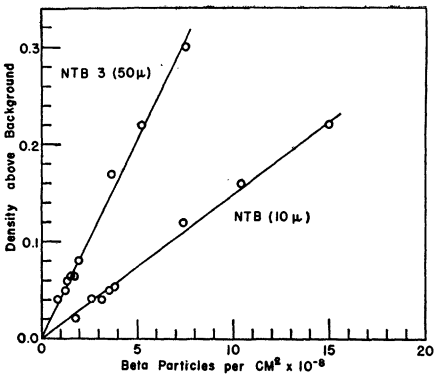

FIG. 5. Response of emulsion to β-particles from tritium.

the β-particles from tritium is illustrated in Fig. 4, in which is plotted the effect of very thin aluminum foil absorbers on the counting rate of a solid sample containing tritium.[3] The counting rate relative to the absorber-free sample is plotted as ordinate. As abscissas are indicated absorber thicknesses in units of mg/cm² and microns in a medium of unit density. The flux of β-particles is reduced to 0.1, 0.01, and 0.001 of the surface value by absorber thicknesses (of unit density) of 1.2, 2.8, and 5.1 μ. For emulsion densities averaging 3.5, these thicknesses correspond to 0.34, 0.80, and 1.5 μ, respectively.

Tritium-containing standards. Tritium-containing samples having an area of 1 cm², a thickness of approximately 1 mg/cm², and counting rates ranging from 4×10^3 to 4×10^4 cpm were used. The active compound was 11-keto-pregnanolone containing randomly labeled tritium.[4] Samples were prepared by adding methanol solutions of the compound to a flat aluminum disk and allowing the solvent to evaporate while being stirred.

The standards were counted as solid samples, using a windowless flow gas counter, following the method described by Eidinoff and Knoll (*10*). The β-particle emission per cm², plotted as abscissa in Fig. 5, was obtained by multiplying the observed counting rate by the exposure time. The efficiency of counting in the gas volume directly over the sample was taken to be unity. This assumption is supported by the observation that measured counting rates are equal when using the upper end of the proportional region for a gas mixture at close to 4,000-v operating voltage or the Geiger-Müller region for a gas mixture at 1,400 v. Displacement of the sample by 1.5 mm toward the anode wire along the axis of the cylindrical tube did not change significantly the observed counting rate. The total area of the sample pans was 10 cm², and only the central 1-cm² area was used for counting.

The tritium-containing standards were sandwiched between a glass slide with emulsion and a plain glass slide. The flat surface of the compound was in contact with the emulsion and the specimen was held in place by steel clips. Nuclear track types of emulsion (Eastman Kodak, NTB, NTB_2, NTB_3, NTA), No-Screen X-ray Emulsion, and Medium Contrast Lantern slide emulsions were used.

Standards were exposed for periods varying from 1 to 4 weeks. All exposures were in black, light-tight, plastic boxes kept in a refrigerator at about 5° C. Development was in D19 solution for 4 min at 20° C, except for a few slides developed for 2 min to show differential concentration, and some for 20 min for possible tracks. An acid stop bath of 1% acetic acid was used, and fixation was accomplished with 28% sodium thiosulfate.

The density of the radioautographic image produced by the standards was measured by a Sweet-Ansco densitometer, which was calibrated by an optical film wedge. The aperture was 2 mm. All densities recorded were the maximum for the standard and represent density above adjacent background.

Similarly prepared samples of 11-keto-pregnanolone, not containing tritium, were used as controls in order to detect pseudophotographic effects. Tritium-containing standards were also placed on an emulsion covered by a layer of Formvar prepared by dipping the emulsion on a glass slide into a 1% ethylene dichloride solution of the polymer and allowing the solvent to evaporate.

Preparation of biological specimens. Paramecium aurelia,[5] variant 51.7, was grown in a timothy hay infusion medium in which *Aerobacter aerogenes* had been incubated at 27° C for a 48-hr prior period. Six ml of test animals was placed in 18 ml of the standard medium, to which 0.5 ml of an aqueous solution of sodium acetate containing tritium was added. The activity of the water was 2×10^7 disintegrations/min/mg, and the sodium acetate, labeled in the methyl groups with tritium, had an activity of 4×10^7 disintegrations/min/mg of the anhydrous salt. The paramecia were kept for 7 days in this medium at a temperature of 27° C.

After growth, paramecia were fixed for 10 min in 2% aqueous osmic acid solution, washed with distilled water, and fixed in 2% U.S.P. formalin solution for 2½ hr. After being washed in distilled water and dehydrated in 70, 95, and 100% alcohol, the organisms were placed in an ether-alcohol solution, embedded over a period of 2 days in celloidin, and finally in two changes of paraffin to give a rigidly embedded block. Sections were cut at 1 μ on a wedge-type microtome using a reservoir containing acetone (*11*). The sections were transferred to a water bath, floated onto photographic emulsions, deparaffinized, dried, and stored at 5° C for appropriate exposure periods.

Whole organisms were put through the same initial procedures but after being fixed, were washed, centrifuged, and kept in distilled water. A control group of organisms was processed simultaneously under sim-

[3] The measurements were made using a windowless flow counter; details will be published elsewhere (*9*).

[4] This compound was prepared in a collaborative study with T. F. Gallagher and D. Fukashima, of the Division of Steroid Biochemistry.

[5] The paramecia were prepared and kindly made available by Leonard Hamilton, of the Division of Experimental Chemotherapy.

ilar circumstances, with the exception that the medium contained no radioactivity.

Torula utilis, a saprophytic yeast, was grown in a salt solution (*12*) in which the only source of nitrogen was ammonium ion and the only source of carbon was 2% sodium acetate containing tritium.[6] The test organisms were grown in 2 ml of medium in a shaken culture for 4 days at 25° C. The activities of the water and sodium acetate in this medium were 2×10^6 and 4×10^7 disintegrations/min/mg, respectively. The yeast were washed with distilled water, centrifuged 6 times, and kept in distilled water. Control organisms were simultaneously processed, with the exception that the medium contained no radioactivity.

Radioautographic technique. Only the nuclear track emulsions were used with the biological specimens. Eastman Kodak NTA, NTB, NTB_2, and NTB_3 emulsions and Kodak Limited Autoradiographic Stripping Plate (*13*) were used. (The use of the Autoradiographic Stripping Plate greatly increases resolution for most isotopes because only a few grains are present to record a portion of an otherwise long track and the diffusion of the image is less [*13*]. With tritium, since only one grain is ordinarily made developable, thickness of emulsion is not significant and the Stripping Plate is used because of its low background.) A drop of distilled water containing the radioactive organisms was placed on these emulsions. Drops of the organisms in suspension were placed on other glass slides. After drying, the slides were apposed to emulsion surfaces. Sections of the paraffin-celloidin–embedded paramecia were transferred to a water bath, floated onto photographic emulsions, deparaffinized, dried, and exposed. Other deparaffinized paramecium sections on a glass slide were covered with the Kodak Limited stripping film.

To rule out pseudophotographic effects, two types of control experiments were performed. Yeast and paramecia grown in a nonradioactive medium, but otherwise identical with the respective radioactive organisms, were processed simultaneously, placed on similar emulsion, and exposed for equal time periods. In a second group, one half of the emulsion on a glass slide was coated with Formvar by dipping the slide in a 1% ethylene dichloride solution of the polymer and allowing the solvent to evaporate. A drop of suspension containing nonradioactive organisms was placed on uncoated emulsion, and a drop of the radioactive suspension was placed on the Formvar-coated surface of the same emulsion. All biological specimens were exposed on emulsion from 1 to 4 weeks and processed as described above for the tritium standards.

Results. The photographic density of the standards was proportional to the intensity up to densities of at least 0.3 and 0.2 in the case of NTB_3, 50 μ (Lot #458, 371-47), and NTB, 10 μ (Lot #458, 380-47), respectively (Fig. 5). Exposure was expressed as total disintegrations/cm² of surface and was calculated from the counting rate as described above. Beischer observed a similar proportionality in the case of C^{14} up to an optical density of 0.4, using EK No-Screen X-ray Film (*14*). In the case of P^{32}, proportionality was observed by Kaplan (*15*) up to a density of 1.1 for No-Screen X-ray film and 0.1 for EK Nuclear-Track, NTA, emulsions. The particular emulsion lot of NTB_3 mentioned above was found to be more sensitive than NTB, NTB_2, Medium Lantern Slide, and No-Screen X-ray emulsions when measured densitometrically, using the same standards for each. As was expected, the sensitivity of factory-coated NTB_3 was slightly less than that of the uncoated emulsion. Application of a thin layer of 1% Formvar decreased sensitivity slightly. Fairly good linear response in density against intensity was also noted with the other films. Control nonradioactive standards of 11-keto-pregnanolone gave no detectable darkening of emulsion.

Yeast grown in the medium containing tritium gave evidence of radioactivity after a week's exposure. Clusters of yeast showed an image in the radioautograph that corresponded identically with their pattern of arrangement on the slide. Usually a complete silhouette of each yeast cell was given in the radioautograph when organisms were distinct. Individual budding yeast cells are shown (Fig. 2). In some preparations with short development periods variations in the concentration of radioactivity could be observed. Extranuclear concentration was greater than nuclear. Often a differential concentration at the poles or along the periphery of the organism was apparent (Fig. 3). Discrete areas of concentration as small as 0.5 μ were resolved and measured with a filar ocular micrometer calibrated by a standard stage micrometer. Peripheral outlining of the yeast cells 1 grain in width was seen in some specimens purposely underexposed. Control yeasts did not cause reduction when placed directly on emulsions, when placed on a Formvar coat overlying emulsions, or when apposed to emulsion.

Paramecia grown in the medium containing tritium gave well-defined radioautographic images after a week's exposure. Whole organisms on the emulsion were sufficiently opaque to hide the underlying radioautographic image. When the organism was removed, a sharply defined radioautographic image was seen. Emulsions apposed to organism on a glass slide also gave a sharp image.

Sections of paramecia cut at 1 μ gave the best radioautographs (Fig. 1). The sections were thin enough to permit the passage of light through the organism and to reveal the underlying emulsion image. Radioactivity was evident throughout the organisms, both in endoplasm and ectoplasm, with a concentration of activity in the latter. Many discrete round areas of concentration of tritium were present in the endoplasm. Most corresponded to food vacuoles, but a few large areas resembled the macronucleus. The mouth was sharply outlined in some organisms. In

[6] The yeast were prepared and kindly made available by H. Christine Reilly, of the Division of Experimental Chemotherapy.

some preparations, areas of radioactivity between organisms much smaller than the endoplasmic concentrations or the yeast cells were noted. It could not be decided whether these were foci of radioactive debris or organisms.

Since about 90% of the β-particles are absorbed by a thickness of 1.2 μ in a medium of unit density or by a thickness of 0.2 μ in a silver bromide medium, very few β-particles will affect more than two grains. This factor leads to increased resolution. We were unable to observe β-particle tracks from samples containing tritium. Some of the smaller individual yeast organisms gave radioautographic images 3 μ in diameter, and in some organisms extranuclear polar or peripheral concentrations of radioactivity as small as 0.5 μ were resolved. Resolution of less than 1 μ was readily discernible (Fig. 3). This observation was made in a radioautograph which contained a thin coating of Formvar over the emulsion. The image here was not as distinct as in some preparations, where peripheral concentrations were represented by a single-grain outline of the cell wall. Ectoplasmic concentrations of radioactivity in the paramecium disclosed that distinct images of 1-μ size were clearly resolved.

The usefulness of tritium, allied with radioautography, in tracer studies depends on the specific problem under study. Tritium atoms bonded stably to carbon can be used for the labeling of carbon atoms just as deuterium has been used (*16*). The preparation of certain tracer compounds using radiocarbon is sometimes very difficult. In these cases, it may be less difficult to introduce tritium by catalytic reduction of a related compound or isotopic exchange with the inactive compound. For example, the introduction of radiocarbon into the skeletal framework of a steroid compound is considerably more difficult, in general, than the introduction of hydrogen atoms bonded to carbon. Among the general synthetic methods may be included exchange between the organic compound and water, sulfuric acid or hydrogen gas containing tritium, the hydrogenation of a double bond, and biosynthetic techniques involving the culture of organisms in media containing tritium (*17*). The lability of hydrogen linked to oxygen and nitrogen in —OH and —NH_2 and to carbon atoms at sites of enolization must be kept in mind by the investigator.

Tritium has recently been made available by the U. S. Atomic Energy Commission in 100-mc and 1-curie lots at relatively small cost. Its half-life of 12.1 ± 0.5 years (*18*) is more than adequate for radioautographic studies. Its use in human beings requires information about the biological half-life and any specific sites of localization of radioactivity.

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A Method for Initiating the Absorption of Carbon Dioxide during a Manometric Experiment

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When the respiratory quotient of tissues respiring in the presence of carbon dioxide is determined, it is necessary to absorb this gas after the period of metabolism. Many techniques for accomplishing this (*1, 2*) require special glassware, such as the flasks designed by Dickens and Simer (*3*) or by Dixon and Keilin (*4*). Other methods (*2*), adapted for the generation of alkali in the side arm of a Warburg flask, require an extended period of time for the complete absorption of carbon dioxide. The method reported here permits the addition of strong alkali to a filter paper strip in the side arm of a standard Warburg flask with a minimum of special equipment. The alkali, thus distributed throughout the area of the filter paper strip, causes a rapid absorption of the carbon dioxide in the flask.

The apparatus consists of a stopcock (ST 7/22) mounted on a ground-glass connection which fits into the opening of a Warburg flask side arm (Fig. 1). The stopcock contains a well in the form of a groove with a capacity of 0.1–0.2 ml. The well and part of the reservoir may be filled with alkali by inserting the needle of a hypodermic syringe through the reservoir. Care must be taken that no air bubbles are trapped in the alkali of the well. The reservoir is then sealed with a small cork. The stopcock remains in the position for filling until the alkali is to be added to the flask. A strip of filter paper is then fitted into the ground-glass connection so that it touches the stopcock, the remainder of the strip projecting into the side arm of the flask. The apparatus is then inserted into the side arm.

Following a period of respiration, acid is tipped

[1] American Cancer Society fellow, upon recommendation of the Committee on Growth of the National Research Council.
[2] Atomic Energy Commission predoctorate fellow in the biological sciences.