

systems. When it is permissible to neglect the increase of  $G$  along the tube, the system may be characterized by a single value; a satisfactory convention is the specification of the value of  $G$  which prevailed at the bottom of the tube, since this maximum value ( $G_{max}$ ), unlike the minimum or the average value, is independent of the height of the fluid level in the tube.

Time may be incorporated into the description by forming the product  $G_{max} \times \text{min}$  to give a single datum which is adequate for the specification of many procedures. When required, this datum should be supplemented by calculation of the force gradient along the tube and by description of special geometrical features such as the degree of angulation of the tube in relation to the axis of rotation.

The necessary calculations of relative centrifugal force,  $G$ , from the *Radius* and the *rpm* are facilitated by the accompanying nomogram (Fig. 1). A thread

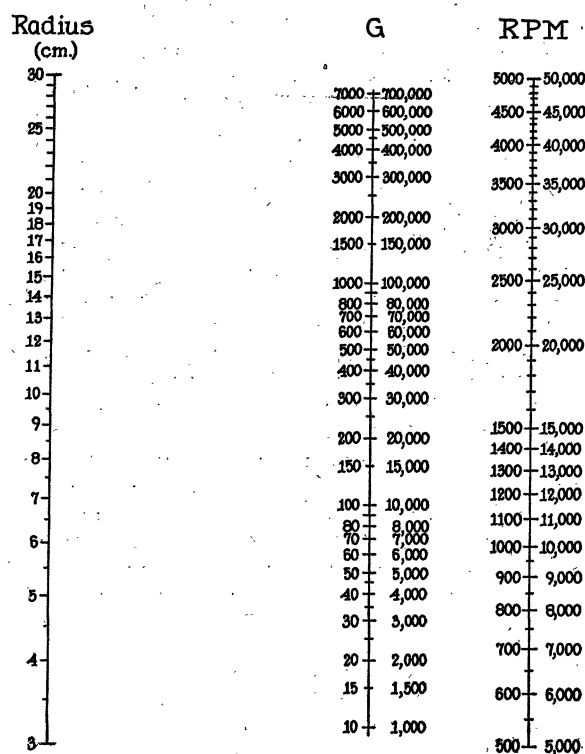


FIG. 1. Nomogram for the calculation of centrifugal force.

stretched across the chart so as to intercept the scales of any two variables at selected values will cross the third scale at a value which satisfies the equation  $G = 1.11 \times 10^{-5} (R) \times (\text{rpm})^2$ . It should be noted that the chart can be used for the calculation of values outside the indicated ranges merely by proper shifts of the decimal points since a change in *Radius* by a factor of 10 alters  $G$  to the same extent, and a change in *rpm* by a factor of 10 affects  $G$  by a factor of 100.

Use of this nomogram in our laboratory has improved the planning of centrifugal fractionations in which the proper choices of rotor head, speed, and time are required for efficient separation. With its use

there has developed the habit of thinking directly in terms of the forces at work rather than in terms of a partial datum (*rpm*) which happens to appear on a dial at the top of the instrument.

## A Laboratory Method for Evaluating the Phytotoxicity or Phytostimulation of Insecticides<sup>1</sup>

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Since the advent of numerous synthetic organic insecticides, particularly the chlorinated hydrocarbons, for the control of insects affecting plants, there has been an increasing need for a rapid laboratory technique to estimate relative phytotoxicity of these compounds. As it has been shown by Chapman and Allen (1) that DDT stimulates plant growth, the technique should also be adaptable to measuring possible plant stimulation by the insecticide. The methods currently employed for phytotoxicity studies are based largely on gross expression of plant damage when plants are grown under field or greenhouse conditions. Such methods have proved to be time-consuming and have yielded only qualitative relationships. The procedure reported here is both rapid and quantitative in nature.

Methods initially tested for possible adaptability in measuring phytotoxicities were based on the techniques that have been used in the detection of plant auxins. The *Avena* coleoptile test (2) and the slit pea stem test (2) proved inadequate. A modification of the slit pea stem test, using the pole bean or cucumber, proved usable but did not allow a distinction between phytotoxicity and growth stimulation. Growth of cucumber roots was found very sensitive and was used to a large extent; the principal difficulty, however, was in the interpretation of growth inhibition, since stimulatory (auxins) as well as inhibitory substances to aerial parts of the plant cause root inhibition. Respiration and permeability tests were discarded because the various compounds to be tested varied in their mode of action. Of the different procedures tested, it was found that readily interpretable and consistent results could be obtained by the use of the straight growth of stem sections of the Kentucky Wonder pole bean grown under standardized conditions in a nutrient solution.

Pole bean seeds from a uniform standard lot were immersed in water for 1 hr and planted in moist Vermiculite<sup>2</sup> in darkness and held at a temperature of 23°–26° C. At 7 days the plants have developed a long hypocotyl and the epicotyl is just ready to emerge. Plants selected for testing are taken from those in which the epicotyl has not yet emerged and

<sup>1</sup> Approved for publication by the director of the Wisconsin Agricultural Experiment Station.

<sup>2</sup> Expanded mica used commercially for insulation.

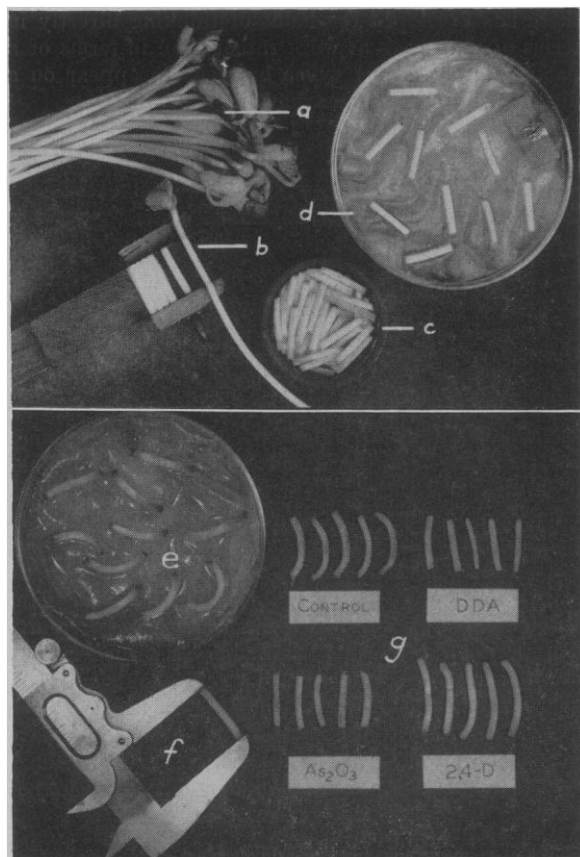


FIG. 1. Procedure for testing straight growth of bean stem sections. A uniform lot of bean seedlings (a) is cut (b) until sufficient sections are collected (c) to place in Petri dish (d) with toxicant. After 48 hr the sections (e) are measured (f), and the differences compared with the control and standard  $\text{As}_2\text{O}_3$  and 2,4-D (g). (DDA at  $1 \times 10^{-3}M$ ;  $\text{As}_2\text{O}_3$  at  $1 \times 10^{-3}M$ ; 2,4-D at  $1 \times 10^{-4}M$ .)

which range in height from 10–20 cm. Particularly slender or robust seedlings are discarded. A 20.0-mm section is removed from the hypocotyl of each plant beginning at a point 1 cm below the node at which the cotyledons are attached. A cutter consisting of 2 razor blades permanently mounted 20.0 mm apart in a wooden handle is convenient for removing the sections.

Serial dilutions of the substance to be tested are prepared in a nutrient solution of 0.5 mg/l IAA (indole-3-acetic acid) and 1% sucrose, a volume of 40 ml being convenient. The pH is critical, and in those cases where any constituent might alter it, the solution should be adjusted to pH 6.0 and buffered with pH 6.0 M/40 phosphate buffer.

Glass wool is added to the dishes to support the plant sections so that air as well as the solution is accessible. After the addition of the glass wool, 10 sections of plant material are placed in each Petri dish. At this point insoluble materials may be applied directly to the surface of the sections, or the sections may be variously exposed to dusts or sprays. The Petri dish is then covered and kept in darkness for

30–48 hr to allow the sections to grow. At the end of 30 hr, when plant growth is essentially completed, the lengths of the sections are either measured directly with a vernier caliper or interpreted by shadow-graphs. Curvatures developed during growth may be useful when making comparisons but do not interfere with the accuracy in measuring the length of sections. Observations may be taken as convenient up to 48 hr, but extended holding of the plants may result in interference by yeasts and molds. The authors have tested each treatment in duplicate series. The procedure is illustrated in Fig. 1.

Comparisons of different treatments are conveniently made on the basis of percentage inhibition of growth, or the “phytotoxic index.” By the term “phytotoxic index” is meant the measure of differences or percentage of inhibition of growth expressed as

$$100 \left( \frac{\text{elongation in control} - \text{elongation in treatment}}{\text{elongation in control}} \right).$$

Standards employed for comparing the degree of phytotoxicity or stimulation are arsenic trioxide and 2,4-D, both of which are available in high purities. Fig. 2 illustrates the relative action of these standards as compared with that of DDA (dichlorodiphenyl acetic acid), an analog of DDT (dichlorodiphenyl trichloroethane).

This method has been used to compare the relative phytotoxicity or phytostimulation of a large series of new organic insecticides, as well as their impurities and breakdown products. It has also been found useful in comparing the effect of different methods of insecticide application on phytotoxicity. The results of these studies are to be presented in detail in a forthcoming publication.

By this technique further information can be obtained relative to the mode of phytotoxic action of insecticides, as has been recently reported with other growth inhibitors (3, 4) by a somewhat similar method. Because of variance in susceptibility of plant species to insecticides (1), this method has been

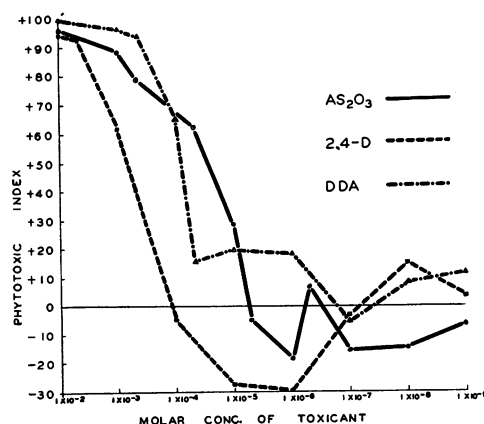


FIG. 2. Relative action of arsenic trioxide, 2,4-dichlorophenoxy-acetic acid, and dichlorodiphenyl acetic acid in inhibiting the growth of bean stem sections.

adapted for use with a series of different plants, but is only illustrated here with the bean. The results with this technique are not directly interpretable to conditions other than those actually tested. However, this procedure allows a rapid laboratory screening technique to test for both stimulatory or inhibitory effects of chemicals.

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## Excretion of Gallium by the Rat and by Man<sup>1</sup>

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Dudley (1-3), working with rats, dogs, rabbits, and goats, found the principal route of excretion of gallium to be the kidney. Confirmation of this has been noted in previous reports (4, 5). The purpose of this report is to detail the comparative excretion of gallium in the rat following intravenous administration of (1)  $\text{GaCl}_3$ , a rapidly ionizing salt, prepared by dissolving irradiated  $\text{Ga}(\text{NO}_3)_3$  in  $\text{HCl}$  and then neutralizing with  $\text{NaOH}$  to a pH of 2.2; (2) the slowly ionizing complex, gallium citrate, which was prepared in an ion exchange column and stabilized at pH 7.0 with sodium citrate (6). A second comparison is made between the excretion by the rat and by man of tracer and larger intravenous doses of gallium citrate prepared according to a method described by Dudley (7).

**Procedures.** The rats, young adult Wistar-strain males weighing  $275 \pm 25$  g, were injected via the tail vein. One of the human subjects had multiple myeloma, one had osteogenic sarcoma, and one had Hodgkin's disease, but all three were considered essentially normal from the standpoint of excretory mechanisms. Other details, such as care of the animals, collection techniques, tissue sampling, and counting procedures, have been described previously (5). In performing the  $\text{GaCl}_3$  studies, 5 rats were sacrificed at 6, 12, 48, and 96 hr and 4 at 24 hr following injection. For the citrate study, 4 rats were sacrificed at 12, 24, 48, 72, and 96 hr after injection. Depending upon the time of sacrifice, the rats received between 200 and 300  $\mu\text{c}$  of  $\text{Ga}^{72}$  in 1.5-2 mg of stable gallium. The patients received the mc doses noted in Table 1 of the gallium citrate preparations, which had a

specific activity of about 1 mc/5 mg of gallium. In all instances, the urine and feces were collected with negligible or no cross-contamination (8).

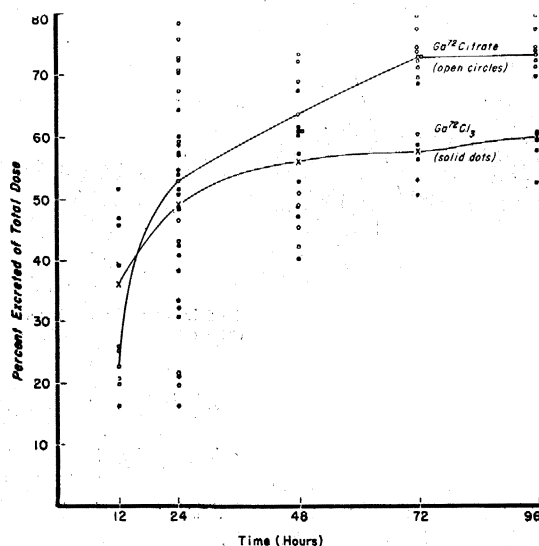


FIG. 1. Urinary excretion by the rat of intravenously injected  $\text{Ga}^{72}$ , expressed in terms of percentage of total dose excreted as a function of time in hours.

The data have been corrected for radioactive decay to the time of injection.

**Results.** Fig. 1 indicates the variation from animal to animal of the fraction of the administered dose recovered from the urine. However, the arithmetical

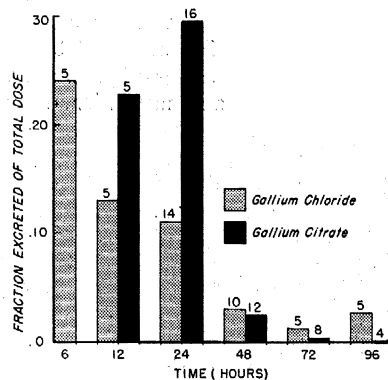


FIG. 2. Daily urinary excretion rate of gallium; average of the fractions of total recovered dose during the periods indicated. The numbers above each bar indicate the number of samples.

average and the median values check each other closely, so that the lines, as drawn, picture a reasonable central tendency. Thus, about 73% of the gallium administered as the citrate was excreted by the fourth day compared with 59% for the chloride salt. In Fig. 2 the excretion is drawn on a nonaccumulative basis to show that during the first 6 hr gallium as the chloride is excreted more rapidly, and also to demonstrate the fact that its subsequent excretion rate is lower than that of the citrate. Essentially all the gal-

<sup>1</sup> Gallium used in the study was furnished by Comdr. H. C. Dudley (MSC), USN, of NMRI, Bethesda, Md.

<sup>2</sup> Comdr. (MC) USN. The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsement of the Navy Department.