Table 1. Radioactive mustard associated with whole cells, nuclei, and DNA after reacting S- and G2-phase cells with mustard (final concentration 0.6 μg/ml) for 1 hour at 37°C. The radioactivity of all the samples, except the DNA preparations in experiment III, was measured by the liquid scintillation technique (the samples having been collected on Millipore filters). The filters were placed in toluene-phosphor solution and counted. The DNA samples in experiment III were dissolved in toluene-phosphor solution and counted. All values are the average of two estimations.

Experiment	Specific activity (count min^{-1} μg^{-1})		
	Whole cells*	Nuclei*	DNA†
	S phase		
I	0.555	0.511	
II	.541	.533	
III			12.0
IV			8.47
	G2 phase		
I	0.523	0.452	
II	.519	.531	
III			11.4
IV			8.70

* Per microgram of protein. † Per microgram of DNA phosphorus.

tivity values for whole cell and nucleus are expressed per unit of protein and those for DNA are expressed per unit of DNA (Table 1) there is no difference between the two populations.

It seems likely that the DNA in S-phase cells and the DNA in G2-phase cells have different molecular configurations and perhaps different degrees of association with histones and other nuclear proteins. Therefore it is noteworthy that the extent of mustard reaction with DNA is the same in S and G2 cells.

According to target theory, if the number of targets in the unit to be destroyed is increased, the targets behave independently with respect to the inactivating agent (*11*). The G2 cells contain almost twice as much DNA as do the S cells under the present experimental conditions. If DNA is the target component of the cells, and G2-cell and S-cell DNA do not react differently from each other with mustard one would expect the following: the linear portion of the G2-cell survival curve would be parallel to that of the S cells but would be displaced above it by a factor of almost 2. That is, the extrapolation number of the G2 curve would be twice that of the S curve. In fact the curves are displaced by a factor of about 10. This could be taken to mean that another target is involved. It could also mean that some kind of repair mechanism is operative and that G2 cells can take advantage of this mechanism.

In order to obtain some idea of the quantitative nature of the lethal effect of sulfur mustard on L-cells, the following calculations were made. From the data of experiment III (Table 1) where a reliable estimate of the counting efficiency was made it was calculated that there was one alkylation per 48,000 nucleotides in DNA. From Fig. 1, the D_0 value (*12*) or dose which on the average will inactivate a cell is 0.06 μg/ml. Since alkylation of the cellular DNA is directly proportional to the dose of mustard (*13*), the D_0 would yield therefore one alkylation per 480,000 nucleotides. If the average gene contains about 3000 nucleotides (*14*) then about one gene in every 160 (480,000/3000) could be alkylated. Some genes undoubtedly would escape alkylation, while others would suffer several alkylations. If gene inactivation is the critical action of mustard in producing cell death, then the foregoing calculations suggest the feasibility of this process. At the same time the calculations indicate the small extent of gene damage that will produce cell death. Such a consideration is even more marked if only about one in five alkylations or perhaps fewer yield cross-links in the DNA and it appears that the latter is the inactivating reaction (Brookes and Lawley, *13*).

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References and Notes

1. I. G. Walker and C. W. Helleiner, *Cancer Res.* **23**, 734 (1963).
2. Although these cells are not synthesizing DNA, they are all able to synthesize DNA immediately upon the addition of thymidine as shown by J. E. Till, G. F. Whitmore, S. Guylas, *Biochem. Biophys. Acta* **72**, 277 (1963). The response of these cells to mustard is the same as cells that are actively synthesizing DNA (*1*), and for convenience they are referred to as S-phase cells.
3. The period of DNA synthesis that follows the addition of thymidine to FUDR-blocked cells is considerably shorter than that in asynchronously dividing cells. Four and one half hours after thymidine has been added, only about 15 percent of the cells are synthesizing DNA [Till *et al.* (*2*)].
4. T. T. Puck and P. T. Marcus, *Proc. Natl. Acad. Sci. U.S.* **41**, 432 (1955); A. G. Levis and A. de Nadai, *Exptl. Cell Res.* **33**, 207 (1964).
5. R. C. Parker, *Methods of Tissue Culture* (Hoeber, New York, 1961), p. 58.
6. D. Baltimore and R. M. Franklin, *Proc. Natl. Acad. Sci. U.S.* **48**, 1383 (1962).
7. B. Djordjevic and W. Szybalski, *J. Exptl. Med.* **112**, 509 (1960).
8. V. I. Oyama and H. Eagle, *Proc. Soc. Exptl. Biol. Med.* **91**, 305 (1956).
9 K. Burton, *Biochem. J.* **62**, 315 (1956).
10. The difference between the two slopes was compared for significance by the "t" test in which "t" is given by the difference between the slopes divided by the standard error of difference.
11. This point is discussed by M. M. Elkind and H. Sutton, *Radiation Res.* **13**, 556 (1960), and they provide experimental data for the survival of mammalian cell colonies that contained one and more cells at the time of irradiation.
12. D_0 equals the dose of mustard required to reduce the surviving fraction of the population to 0.37, measured in the linear portion of the curve.
13. I. G. Walker, unpublished results; P. D. Lawley and P. Brookes, *Nature* **206**, 480 (1965).
14. For 3000 nucleotides, a codon of 3 and only one strand of the DNA being read, a protein containing 500 amino acids would be produced.
15. I thank Mrs. Patricia Whiteside, H. Chew, and Mrs. Jean Smith for technical assistance. Supported by a grant from the National Cancer Institute of Canada.

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Pesticide Residues in Total-Diet Samples

Abstract. *Small amounts of pesticide residues were found in food samples from 18 markets consisting of 82 foods collected from three different geographical areas. The samples were separated into twelve similar classes of foods, made ready to eat, and analyzed by methods capable of detecting small quantities of 50 common pesticide chemicals.*

Residues of pesticides in crops that are shipped are subject to government limitations, but obviously the real significance of the residues lies in the amount actually ingested by animals and humans. Data on the actual amounts of the residues in food as shipped do not give a clue to the amount that will be ingested. There is, therefore, a continuing need for this information specifically.

Limitations in the form of legal and safe tolerances have been established by the Food and Drug Administration for raw agricultural products as shipped in interstate commerce. Some states have established similar tolerances for intrastate control. The 1954 statement of the National Academy of Sciences with its subsequent revisions (*1*) is used as a basic guide. The report issued by the President's Science Advisory Committee discusses in some detail the mechanism and criteria used in setting tolerances (*2*).

We planned our investigation to provide additional information and expand the data furnished by experiments on specific food items and on specific types of food processing.

Laug *et al.*, in their studies of strontium-90 and cesium-137 residues in foods, used the "total diet" approach to obtain data on the amount of these residues on ready-to-eat foods. (*3*). This theoretical "total diet" represents all the food consumed over a period of 2 weeks by a male 18 to 19 years old. They discussed the advantages and disadvantages of this approach, as well as the factors of sample collection, weighting of individual food items, and sample preparation.

Available information suggests that the amount of pesticide residue is measurably reduced during preparation of food for consumption. Studies, using portions of Laug's samples, have shown that when the foods were ready to eat they contained very small amounts of pesticide residues (*4*). The importance of this type of data was recognized in the President's Science Advisory Committee report (*2*).

Accordingly, this was a study specifically of pesticide residues in food ready for consumption. We were particularly investigating the amounts and kinds of residues that different types of food contribute to the diet. We collected the food samples in the same way a customer selects his food in retail stores.

Techniques of food distribution are so highly developed that several lots of one kind of food each produced in a different region may be available for consumption at the one location. For example, a store may offer Texas-grown spinach for sale one day and California-grown spinach the following day, depending on market conditions. No uniformity can be expected for a single food item because of local variations in growing, harvesting, and processing procedures. There are no single foods or regions sufficiently representative to be used alone for this study. Therefore the samples used were not limited to a single area. They were collected bimonthly from June 1964 through April 1965 from retail food stores in Boston, Massachusetts; Kansas City, Missouri; and Los Angeles, California.

Foods that must be processed before they are eaten, such as raw meats and fresh vegetables, were prepared by professional food handlers. They were separated for analysis into 12 classes such as dairy products, leafy vegetables, or potatoes. For each class of food from one market, all samples were composited into a slurry. The composites included both uncooked and cooked portions of foods commonly eaten in both forms, and the total of composite samples was 216.

The relative amounts and specific kinds of foods included in the samples for this study are similar to those in the basic 2-week diet constructed for the earlier studies (*3, 4*).

Each composite was tested for chlorinated organic compounds (*5, 6*) at a sensitivity of 0.003 ppm; for organic phosphorus compounds (*5, 7*) at a sensitivity of 0.05 ppm; for chlorophenoxy acids and esters (*8*) at a sensitivity of 0.02 ppm; for amitrole (*9*) at a sensitivity of 0.05 ppm; for dithiocarbamates, calculated as zineb (*10*), at a sensitivity of 0.2 ppm; for carbaryl (*11*) at a sensitivity of 0.2 ppm; for bromides (*12*) at a sensitivity of 0.5 ppm; and for arsenic (*13*) as As_2O_3 at a sensitivity of 0.1 ppm.

In our opinion, these sensitivity levels represent the lowest reliable quantitative measurement that can be obtained by these methods when reasonable sample sizes are used for the prescribed extraction, isolation, and detection systems. The applicability of these procedures to the food-class composites was established by recovery studies. Recoveries of the added chemicals are comparable to those obtained when the procedures are applied to individual foods. In the development of methods of analysis for pesticide chemicals in food products, the research and regulatory laboratory staffs have collaborated extensively. Interlaboratory checks and intralaboratory control tests are used for many products and pesticide chemicals sufficiently to maintain consistent results between laboratories. Therefore, no specific intralaboratory study was deemed necessary for this investigation.

The quantitative values reported for the chlorinated organic compounds were obtained by electron-capture, gas-liquid chromatography and confirmed by thin-layer chromatography, microcoulometric gas-liquid chromatography, or both. The sensitivity level for chlorinated organic residues was based on the ability of the electron-capture, gas-liquid chromatographic system to detect heptachlor epoxide. The sensitivities of other chlorinated organic residues, such as DDT, benzene hexachloride, and toxaphene, were established by the response of the detection system relative to heptachlor epoxide.

Earlier investigators (*4*) discussed the quantitative significance of their results and pointed out that residues in amounts below 0.01 ppm had only qualitative significance. Because of refinements in methodology since these earlier studies, results above the sensitivity levels used in this study are considered to have both qualitative and quantitative significance. It must be recognized that, as in most quantitative determinations, greater deviations are observed as the lower limit of sensitivity is approached. The detectable levels are somewhat lower than the quantitative sensitivity levels and, in general, 0.001 ppm of heptachlor, heptachlor epoxide, endrin, dieldrin, and aldrin can be detected; this amount then is of qualitative significance. The method used will detect the following chlorinated organic pesticides (*14*): aldrin; benzene hexachloride (BHC); chlorbenside; chlordane; isopropyl chlorocarbanilate (CIPC); DDT (*o,p* and *p,p*); dieldrin; endrin; endosulfan; heptachlor; heptachlor expoxide; hexachlorobenzene; 1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol (Kelthane); lindane; methoxychlor; dimethyl 2,3,5,6-tetrachloroterephthalate (Dacthal) tetrachlorodiphenylethane (TDE); dichlorodiphenyl dichloroethylene (DDE); ovex; pentachloronitrobenzene (PCNB); 1,1-dichloro-2,2-bis(*p*-ethylphenyl)ethane (Perthane); ronnel; terpene polychlorinates; 1,2,4,5-tetrachloro-3-nitrobenzene (TCNB); *p*-chlorophenyl 2,4,5-trichlorophenyl sulfone (Tedion); 1,3,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanophthalan (Telodrin); and toxaphene. Reference standards of parathion, methyl parathion, malathion, Diazinon, demeton, and carbophenothion were used in the paper chromatographic analysis for organophosphorus residues.

The analytical procedure for chlorophenoxy acids and esters determines, by microcoulometric gas-liquid chromatography, 2,4-dichlorophenoxyacetic acid (2, 4-D); 4(2,4-dichlorophenoxy) butyric acid (2,4-DB); 2,4,5-trichlorophenoxyacetic acid (2,4,5-T); 2(2,4,5-trichlorophenoxy) propionic acid (2,4,5-TP); 4-chloro-2-methyl phenoxyacetic acid (MCP); pentachlorophenol (PCP), and 2,3,6-trichlorobenzoic acid (TBA). The residues reported were confirmed by paper chromatography.

Six dithiocarbamates can be detected by the method based on the evolution of carbon disulfide after decomposition of the dithiocarbamate: ferbam, maneb,

metiram, thiram, zineb, and ziram. No attempt was made to identify the specific dithiocarbamate. The residues detected were calculated as zineb.

Specific methods were used in the analysis for amitrole, arsenic, bromides, and carbaryl. No attempt was made to distinguish between organic and inorganic bromides. The values reported represent the total bromide concentration.

Bromide residues in excess of the quantitative sensitivity limits established for the investigation were found in 178 of the composite samples. The existence of residues of chlorinated organic pesticides was confirmed in 129 composite samples. Some of the confirmed residues were reported in amounts below the quantitative sensitivity limits that we prescribed. Residues of chlorophenoxy compounds and dithiocarbamates were less common; 14 and 5 composite samples contained these classes of agricultural chemicals, respectively. Carbaryl was found in 15 composite samples. No residues of organophosphorus chemicals or amitrole at or above the quantitative sensitivity limits were found in the samples.

Twenty-five different residues were found in the samples. The frequency with which we found these residues and ranges in their amounts are shown in Table 1. The most common residues, maximum amounts of those residues, and residues reported less frequently are described below for each food class.

The values for residues reported in dairy products, meat, fish, and poultry, and oils, fats, and shortening represent fat-soluble residues found in the fat portion. No precise quantitative determination of the fat in these composites was made; however, the dairy products composites contain approximately 8 to 13 percent fat, the meat, fish and poultry composites contain approximately 17 to 23 percent fat, and the oils, fats, and shortening composites contain approximately 83 to 88 percent fat.

Dairy products (fresh, canned, and dry milk; ice cream; cottage cheese; natural and processed cheese; butter and margarine): (i) Small residues of chlorinated organic pesticides were present in varying combinations in 15 of 18 composite samples. The most common were DDT, DDE, and TDE, with maximum values of 0.15 ppm, 0.22 ppm, and 0.053 ppm, respectively. (ii) Also present were dieldrin, heptachlor epoxide, lindane, and BHC. (iii) Bromides were present (1.1 ppm to 31.7 ppm) in 17 of 18 composite samples.

Meat, fish and poultry (including beef, pork, lamb, chicken, luncheon meat, frankfurters): (i) Small residues of chlorinated organic pesticides were present in varying combinations in all composite samples. The most common were DDT, DDE, and TDE, with maximum values of 0.86 ppm, 0.92 ppm, and 0.25 ppm, resectively. (ii) Also present were dieldrin, heptachlor epoxide, BHC, lindane, aldrin, endrin, heptachlor, and PCP. (iii) Bromides were present (2.3 ppm to 35.5 ppm) in 16 of 18 composite samples. (iv) Arsenic was present (0.12 ppm) in 1 of 18 composite samples.

Grain and cereal products (including general purpose and self-rising flour; corn flakes; oatmeal; macaroni; raw, canned, and frozen corn): (i) Small residues of chlorinated organic pesticides were present in varying combinations in 17 of 18 composite samples. The most common were DDT and lindane, with maximum values of 0.026 ppm and 0.032 ppm, respectively. (ii) Also present were TDE, DDE, heptachlor epoxide, dieldrin, aldrin, endrin, PCP, MCP, carbaryl, and dithiocarbamate. (iii) Bromides were present (4.4 ppm to 111 ppm) in 17 of 18 composite samples. (iv) Arsenic was present (0.10 ppm) in 1 of 18 composite samples.

Potatoes (white—baked, boiled, and fried; potato chips; sweet potatoes and yams, both fresh and canned): (i) Small residues of chlorinated organic pesticides were present in varying combinations in 9 of 18 composite samples. The most common were heptachlor epoxide, DDE, and endrin, with maximum values of 0.020 ppm, $>$ 0.003 ppm, and $>$ 0.003 ppm, respectively. (ii) Also present were dieldrin, DDT, lindane, TDE, TCNB, and carbaryl. (iii) Bromides were present (1.5 ppm to 38 ppm) in 15 of 18 composite samples. (iv) Arsenic was present (4.7 ppm) in 1 of 18 composite samples.

Leafy vegetables (including raw and canned beet tops; raw, canned, and frozen spinach; fresh and frozen broccoli): (i) Small residues of chlorinated organic pesticides were present in varying combinations in 12 of 18 composite samples. The most common were DDT, DDE, and TDE, with maximum values of 0.047 ppm, 0.096 ppm, and 0.29 ppm, respectively. (ii) Also present were chlorbenside, heptachlor epoxide, BHC, lindane, endrin, carbaryl, dithiocarbamate, and 2,4-D. (iii) Bromides were present (1.1 ppm to 16.3 ppm) in 16 of 18 composite samples.

Legume vegetables (including raw, canned, and frozen peas; raw, canned, and frozen green beans; raw, canned, and frozen lima beans): (i) Small residues of chlorinated organic pesticides were present in varying combinations in 5 of 18 composite samples. The most common was DDT, with a maximum value of 0.13 ppm. (ii) Also present were DDE, TDE, and dieldrin. (iii) Bromides were present (0.9 ppm to 17.9 ppm) in 14 of 18 composite samples. (iv) Arsenic was present (0.11 ppm) in 1 of 18 composite samples.

Root vegetables (including raw and canned carrots; raw and canned beets without tops; raw turnips without tops; raw rutabagas): (i) Small residues of chlorinated organic pesticides were present in varying combinations in 9 of 18 composite samples. The most common

Table 1. Numbers of composites where pesticide residues were found and ranges in the amounts.

Pesticide	No. composites with residue	No. with residues below sensitivity level	Range at and above sensitivity level (ppm)
Bromides	178		0.7–261.6†
DDT	85	4	0.003–0.862
DDE	82	24	0.003–0.915
TDE	48	6	0.003–0.291
Dieldrin	46	7	0.003–0.142
Lindane	37	13	0.003–0.21
Heptachlor epoxide	33	9	0.003–0.082
Carbaryl	15	2	0.2–0.5
BHC	14		0.004–0.141
Aldrin	13	5	0.003–0.014
Endrin	11	6	0.004–0.017
2,4-D	10	1	0.02–0.158
Arsenic (As_2O_3)	6		0.10–4.7
Dithiocarbamates*	5		0.4–0.8
Kelthane	4		0.108–0.166
Chlorbenside	4	1	0.010–0.038
PCP	3	1	0.02–0.03
TCNB	2		0.011–0.216
Heptachlor	2	1	0.008
Tedion	2		0.006–0.044
TBA	2	1	0.02
Perthane	1		0.016
PCNB	1		0.003
MCP	1	1	
Chlordane	1		0.033

* Calculated as zineb. † Twenty residues above 25 ppm.

were DDT, DDE, and dieldrin, with maximum values of 0.071 ppm, 0.051 ppm, and 0.018 ppm, respectively. (ii) Also present were TDE, TCNB, endrin, chlorbenside, and carbaryl. (iii) Bromides were present (2.6 ppm to 22.1 ppm) in 14 of 18 composite samples. (iv) Arsenic was present (0.10 ppm) in 1 of 18 composite samples.

Garden fruits (raw peppers, fresh and canned tomatoes, raw cucumbers, catsup, raw eggplant, and raw and frozen summer squash): (i) Small residues of chlorinated organic pesticides were present in varying combinations in 16 of 18 composite samples. The most common were DDT, DDE, TDE, lindane, and dieldrin, with maximum values of 0.15 ppm, 0.009 ppm, 0.049 ppm, 0.025 ppm and 0.012 ppm respectively. (ii) Also present were heptachlor epoxide, chlordane, BHC, endrin, and carbaryl. (iii) Bromides were present (1.7 ppm to 18.9 ppm) in 15 of 18 composite samples.

Fruits (including fruit filling from pies, fresh oranges, bananas, raisins, raw and canned apricots, and raw and canned pears): (i) Small residues of chlorinated organic pesticides were present in varying combinations in 16 of 18 composite samples. The most common were DDT, DDE, and aldrin, with maximum values of 0.027 ppm, 0.005 ppm, and 0.020 ppm, respectively. (ii) Also present were lindane, Kelthane, dieldrin, TDE, PCNB, Tedion, Perthane, and carbaryl. (iii) Bromides were present (0.7 ppm to 31.4 ppm) in 12 of 18 composite samples. (iv) Arsenic was present (0.18 ppm) in 1 of 18 composite samples.

Oils, fats, and shortening (French dressing, mayonnaise, salad dressing, shortening, and peanut butter): (i) Small residues of chlorinated organic pesticides were present in varying combinations in 7 of 18 composite samples. The most common were heptachlor epoxide, DDT, DDE, and TDE, with maximum values of 0.004 ppm, 0.049 ppm, 0.014 ppm, and 0.037 ppm, respectively. (ii) Also present were dieldrin, lindane, endrin, heptachlor, BHC, aldrin, 2,4-D, and TBA. (iii) Bromides were present (1.1 ppm to 261 ppm) in 16 of 18 composite samples.

Sugar and adjuncts (including white sugar, jam or jelly, pudding mix, blended syrup, molasses, candy bars, baking powder, and salt): (i) Small residues of chlorinated organic pesticides were present in varying combinations in 9 of 18 composite samples. The most common were 2,4-D and DDT, with maximum values of 0.16 ppm and 0.085 ppm, respectively. (ii) Also present were aldrin, BHC, lindane, TDE, DDE, heptachlor epoxide, dieldrin, and carbaryl. (iii) Bromides were present (0.7 ppm to 55.1 ppm) in all composites.

Beverages (tea leaves, ground coffee, cocoa, soft drinks, and drinking water): (i) One residue of a chlorinated organic pesticide was found in 1 of 18 composite samples (DDE, > 0.003 ppm). (ii) Also present was carbaryl (maximum value 0.5 ppm) in 4 of 18 composite samples. (iii) Bromides were present (0.9 ppm to 17.0 ppm) in 10 of 18 composite samples.

The analytical scheme used in this investigation will detect at least 50 of the most commonly used pesticide chemicals. The amounts of pesticide residues found in the foods ready for consumption were very small; they were substantially less than the tolerances established for specific pesticides and products in those instances where the tolerances are finite. Residues of all pesticides reported in earlier studies (*4*), except methoxychlor, were found in this series of samples. The amounts of these residues were of the same order of magnitude as those reported in the earlier studies. Aldrin, carbaryl, dithiocarbamates, TCNB, Tedion, PCP, 2,4-D, MCP and TBA residues, however, have not been reported in studies of foods ready for consumption.

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References and Notes

1. "Principles and Procedures for Evaluating the Safety of Food Additives," *Natl. Acad. Sci.–Natl. Res. Council, Publ.* 750 (1960).
2. "Use of Pesticides," *President's Science Advisory Committee Rept.*, 15 May 1963.
3. E. P. Laug *et al.*, *J. Assoc. Offic. Agr. Chemists* **46**, 749 (1963); **47**, 821 (1964).
4. P. A. Mills, *ibid.* **46**, 762 (1963); S. Williams, *ibid.* **47**, 815 (1964).
5. H. C. Barry, J. G. Hundley, L. Y. Johnson, "Pesticide Analytical Manual," *U.S. Food Drug Admin. Publ.* (1964).
6. P. A. Mills, *J. Assoc. Offic. Agr. Chemists* **44**, 171 (1951); P. A. Mills, J. Onley, R. Gaither, *ibid.* **46**, 186 (1963); L. Y. Johnson, *ibid.* **45**, 363 (1962); M. Eidelman, *ibid.* **45**, 672 (1962); *ibid.* **46**, 182 (1963).
7. M. Getz, *ibid.* **45**, 393 (1962); *ibid.* **45**, 397 (1962); R. Gajan, *ibid.* **46**, 216 (1963).
8. G. Yip, *ibid.* **47**, 343 (1964).
9. R. W. Storherr and J. Burke, *ibid.* **44**, 196 (1961).
10. T. E. Cullen, *Anal. Chem.* **36**, 221 (1964).
11. D. P. Johnson, *J. Assoc. Offic. Agr. Chemists* **47**, 283 (1964).
12. D. A. Mapes and S. A. Schrader, *ibid.* **40**, 189 (1957).
13. I. Hoffman and A. D. Gordon, *ibid.* **46**, 245 (1963); *ibid.* **46**, 142 (1963).
14. Chemical formulas of all pesticides mentioned in this report may be found in *Chem. Abstracts Subject Index* **60** (1964) and D. E. H. Frear, Ed., *Pesticide Index* (College Science Publishers, State College, Pa., 1963) except metiram, a mixture of ethylene-bis (dithiocarbamato) zinc and [dithiobis (thiocarbonyl) iminoethylene] bis (dithiocarbamato) zinc.
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Gibberellin and Growth in Isolated Wheat Embryos

Abstract. *Gibberellic acid promoted elongation growth in the coleoptiles and leaves of embryos from gamma-irradiated wheat grains whether the embryos were isolated or attached to the endosperm. Thus gibberellic acid affected this growth directly rather than indirectly through an effect upon endosperm. However, root growth was promoted by gibberellic acid only on embryos attached to endosperm, suggesting an indirect effect of gibberellic acid upon growth.*

Shoot growth in cereal seedlings, after the treatment of whole seedlings with gibberellin, has been used to investigate the basis of the effects of gibberellin upon plant development (*1*, *2*). In his recent review of the mechanisms of gibberellin action, Paleg (*3*) has stated that the interpretation of results so obtained must be queried because the effects upon growth are confounded with another effect of gibberellin upon cereal grains—the mobilization of nutrient reserves in cereal endosperm.

To decide whether gibberellin has a direct effect upon growth, or only an indirect effect through a changed nutritional status of the seedling due to greater or earlier endosperm mobilization, we have compared the effect of gibberellin upon embryos attached to endosperm and upon isolated embryos from which the endosperm, the potential source of a confounding effect upon growth, was removed. We have also taken the opportunity to make this com-