S **89**. *The Problem of Radiation Damage when Radioactive Phosphorus is used in Fertiliser Experiments.**

By R. Scott Russell.

The results of fertiliser experiments in which ^{32}P has been used are of doubtful value if radiation from the tracer has caused marked abnormalities in the plants under investigation. There is evidence that this question has hitherto received insufficient attention. In water culture it has been shown that radiation damage may reduce phosphorus absorption significantly and also depress the rate of growth when very low levels of tracer (10 micro-curies per litre of culture solution) are employed (Russell and Martin, *Nature*, 1949, **63**, 71). These effects are due to the accumulation of phosphorus in root tips where active cell division occurs. Concentrations of more than 1000 times that of the initial culture solution have, under certain conditions, been found after treatment for 24 hours with labelled phosphate. The rate of nutrient absorption is affected by radiation more seriously than the rate of growth. Moreover, the weight of shoots may remain unaffected while that of roots is reduced by more than 40%.

So far as is known, comparable experiments on plants grown in soil have not yet been carried out. Work on this subject is in progress at the Department of Agriculture, Oxford. In a preliminary experiment winter wheat plants grown in pots filled with soil were treated with a constant level of phosphorus labelled with different levels of ^{32}P, the highest being comparable to that used by Spinks and Dion (this vol., p. S 410). The rate of phosphorus absorption was significantly affected by radiation, the effect being so great that no conclusion on the normal rate of fertiliser uptake was possible. No change in shoot growth could, however, be detected. The pattern of radiation damage appeared to be more complicated than in water culture owing to the fact that the greater part of the fertiliser phosphate is retained in the upper layers of the soil. Different horizons of roots are consequently affected to varying degrees. Further work is in progress and the results will be published in due course. It would be unwarranted on the basis of these findings to suggest that radiation damage did in fact occur in Spinks's experiments, since the conditions under which his plants were grown, and their rate of phosphorus absorption, were different. It is, however, apparent that the results of field experiments in which radioactive phosphorus has been used should be regarded as of doubtful validity unless it is established that radiation effects have not occurred. Moreover, it has been shown both in water culture and in the soil that the absence of changes in shoot weight does not justify the assumption made by Spinks, and also by Hendricks and Dean (*Soil Sci. Amer. Proc.*, 1948, **12**, 98), that radiation damage has not occurred.

Sufficient data are not available for the extent of injurious radiation effects to be determined by measurement of the amount of radiation to which root tips are exposed. It is necessary to treat replicated batches of plants with a constant level of total phosphorus labelled with different quantities of tracer and to determine rate of growth and phosphorus absorption on both roots and shoots. The assumption that radiation has caused no effect is justified only if no significant differences are found.

It has been suggested that radiation damage is less likely in the field (where Spinks's later experiments were carried out) than in the pot culture. There seems to be little justification for this contention since it is known that the greater part of the phosphatic fertiliser applied to soil is retained in the upper two or three inches. Radiation damage should therefore be equally likely in pot culture and in the soil if the amount of tracer added per unit of superficial area is the same.

In view of the wide interest which investigations of this type is now arousing, it appears desirable to stress these considerations despite the paucity of data on radiation effects in plants grown in soil.—Department of Agriculture, University of Oxford. [*Read, April 1st*, 1949.]

S **90**. *Some Chemical Problems in the Use, as a Fumigant, of Methyl Bromide labelled with* ^{82}Br.

By F. P. W. Winteringham.

^{82}Br has been used to label $Me^{82}Br$ in studies of its behaviour as a fumigant. Methods of preparation and assay are indicated. Some data are presented to illustrate how isotope exchange between different compounds may vitiate the results of isotope dilution procedure.

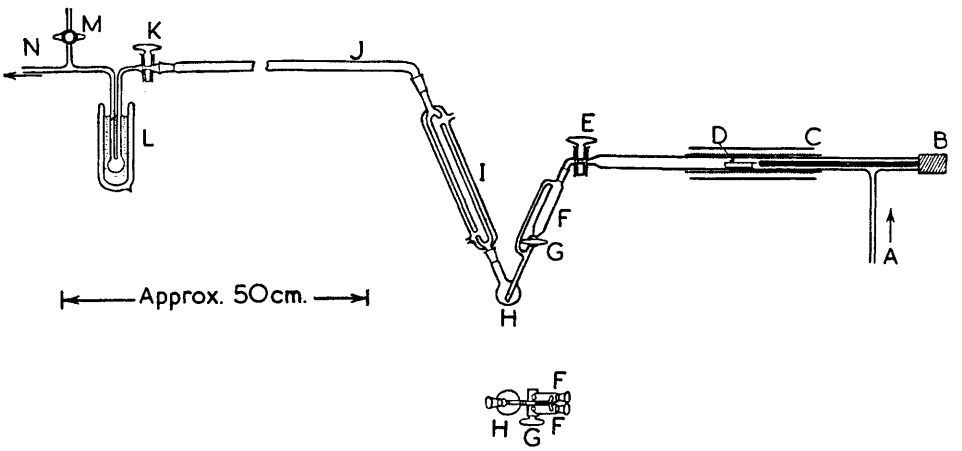
During the insecticidal treatment of insect-infested foods some insecticide (or fumigant, *e.g.*, methyl bromide) is sorbed and traces are retained by the product which may eventually be consumed as food by man or animal. It is therefore important to investigate the physical and chemical fate of these " residues." Similar studies on the fate of the insecticide in the poisoned insect may yield valuable information on the mechanism of insecticidal action. These studies sooner or later necessitate the determination or detection of traces of insecticidal substances in the presence of complex biological material and the isotope tracer technique has provided a sensitive method of doing this. This contribution briefly describes some aspects of the use of ^{82}Br as a tracer.

Preparation of ^{82}Br*-labelled Methyl Bromide.*—Hydrogen bromide is a useful starting material in the preparation of ^{82}Br-labelled compounds and it is used in the preparation of methyl bromide,

* Contribution to the Discussion of paper No. S 88 entitled " Study of Fertiliser Uptake using Radio-phosphorus," by J. W. T. Spinks and H. G. Dion.

a widely used fumigant. The apparatus used is illustrated in Fig. 1. 100 Mg. or more of AgBr* are precipitated from an aqueous solution of Pile-irradiated potassium bromide, and dried. The AgBr* in *D* is reduced in a current of hydrogen at about 700° (Mellor, " Comprehensive Treatise on Inorganic Chemistry," London, 1922, Vol. 2, p. 169) and the HBr* collected at −180°. The HBr* is condensed in a 20-c.c. reaction flask *H*, to which is attached a twin by-pass funnel *F* which enables two different reagents to be added independently during a preparation and without having to disconnect any part of the apparatus. Aqueous methanol is run from the first funnel on top of the frozen HBr* which dissolves instantaneously with negligible loss. Concentrated sulphuric acid is gradually added from the second funnel, and the reaction allowed to proceed at about 125°. The relative weights of reagents used are based on the optimum values determined by Bygden (*J. pr. Chem.*, 1922, **104**, 285). Methyl bromide passes through a condenser *I* and purifying train and the pure ester collects at −180°. The purifying train consists of a long tube *J* packed successively with cotton-wool saturated with distilled water, soda-lime, and anhydrous calcium chloride. By isolating and evacuating the cooled trap *L* the b. p. of the ester is checked by determining the temperature at which its vapour pressure reaches 76 cm. of mercury as read on an attached manometer. This generally agrees with the correct value of +3·6° to within 0·1—0·2°. The bromine content has also been checked to within 1% by decomposing a sample of the prepared ester in monoethanolamine followed by potentiometric determination of the bromide formed. The principal feature of the apparatus is that, having introduced the reagents, all manipulation can be performed from behind the necessary lead screening. The progress of the preparation is followed by means of a portable γ-monitor.

FIG. 1.



Preparation of ^{82}Br-*labelled methyl bromide.*

The methyl bromide is finally distilled into the reservoir of a high-vacuum assembly whereby micro- or milli-mole quantities of condensable gases and vapours can be quantitatively handled.

In the preparation of labelled compounds incorporating radioactive isotopes, particularly those of short half-life, it is useful to express yields as the product of the percentage chemical yield and the ratio of the specific activity of pure product obtained to that which would be obtained were there no loss due to isotope dilution or exchange, radioactive decay, etc. This takes into account factors such as time which, though possibly irrelevant in ordinary chemical synthesis, become important in tracer work. On this basis the overall activity-yield of the methyl bromide prepared above was about 85%.

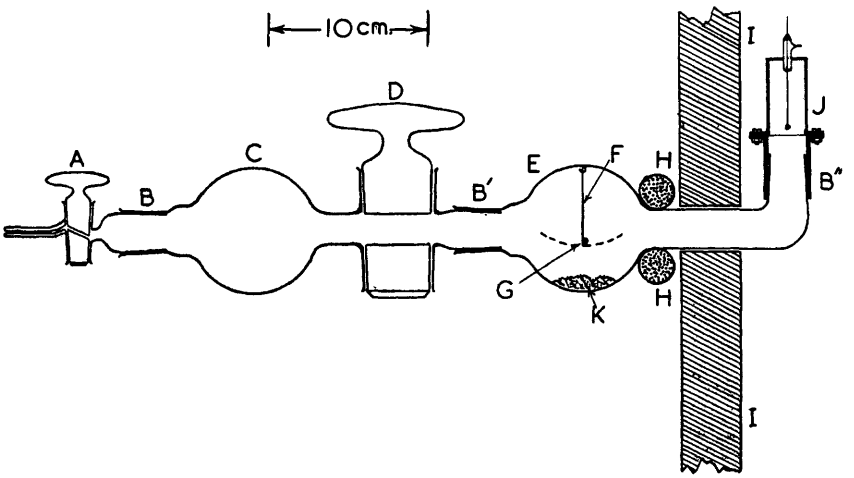
^{82}Br-Labelled methyl bromide has also been prepared by isotope exchange in solutions of active bromides and initially inactive methyl bromide. This is simple but suffers from the unavoidable loss of specific activity by isotope dilution. Nevertheless, the method has been found useful for the preparation of bromobenzene, for example, active aluminium tribromide being used (see Fairbrother *J.*, 1941, 293).

Radioactive Assay of ^{82}Br-*labelled Compounds.*—Active bromides have been assayed in solutions in Geiger–Müller counters of the type described by Veall (*Brit. J. Radiol.*, 1948, **21**,

347), and as prepared precipitates of silver bromide under thin end-window counters which enabled a much larger fraction of the β-radiation to be counted. The use of liquid samples is convenient but that of prepared precipitates is some 15 times more efficient, the higher efficiency being a decisive factor with samples of low specific activity.

Precise assay of moderate or weak β-emission from solids necessitates a method of preparing samples of good geometrical uniformity and reproducibility. This was achieved for silver bromide precipitates in the following way. The labelled bromide (together with added carrier when necessary) is precipitated as silver bromide and collected by filtration under standardised geometrical conditions on a 1·5-cm. Whatman No. 50 filter circle. The precipitate is then hot pressed at 140° against a numbered and tared lead disc faced with " Alkathene " thermoplastic, after which the filter-paper can be cleanly peeled away, leaving the precipitate as a hard flat disc mounted on the lead base. Lead is used deliberately to enhance back-scattering and so increase the number of recorded counts for a given specific activity. Silver iodide and chloride behave similarly. In a series of determinations made over about three half-lives, the overall coefficient of variation was ±1·8% on total corrected counts of 10,000; the precipitate thicknesses varied from 6·2 to 43·7 mg./cm.^{2}.

FIG. 2.



Apparatus for the continuous measurement of the sorption of radioactive fumigants by food constituents.

Starting with bromide irradiated in B.E.P.O., this technique has enabled weights of the order of 0·005 μg. of labelled bromide to be determined to within a few units % up to one week after removal from the pile. The sensitivity could, of course, be enormously increased by using carrier-free bromides prepared, *e.g.*, by Glueckauf's method. ^{82}Br-labelled methyl bromide has also been assayed directly in the gas phase in an apparatus of the type illustrated in Fig. 2. This apparatus is used for studying the rates of sorption of methyl bromide by substances such as wheat protein under atmospheric conditions, so providing data on the physical adsorption and chemical reaction kinetics in the adsorbed phase. A sample of the active gas is drawn into the evacuated chamber *C*. Tap *A* is closed after admission of air to atmospheric pressure and *D* is opened, allowing the gas to diffuse throughout the apparatus. *G* is the armature of a stirrer vane intermittently swung to and fro over the adsorbent *K* by means of the external electromagnet *H*. The concentration of unadsorbed gas is proportional to the corrected rate of count by the screened Geiger–Müller counter *J*. A control unit, charged with a known concentration of gas, operates simultaneously so that significant variations in the characteristics of the amplifying and scaling equipment can be allowed for.

Labelled bromides deposited in tissue exposed to methyl bromide can be detected qualitatively by means of the so-called autoradiographic technique. In our applications, the resulting bromides were soluble in water, so the Altmann–Gersh freeze-drying method in high vacuum is being used instead of the more usual liquid fixatives, etc. When using Pelc's stripping emulsion technique (*Nature*, 1947, **160**, 749) it is necessary to deposit a thin film of nitrocellulose over the sections before water immersion in order to avoid almost complete solution of the active bromides.

Gorbman (*Nucleonics*, 1948, **2**, 30) quotes a figure as low as 2×10^6 disintegrations per sq. cm. of tissue as being required for a satisfactory autoradiograph. Dr. Herz, of Kodak Ltd., in a private communication, has recommended a figure of 10^9 disintegrations per sq. cm. We can confirm this as a suitable figure for ^{82}Br. Autoradiographs at an estimated density of the order 10^8 disintegrations per sq. cm. have been obtained by using Ilford lantern slide plates. These figures suggest that a figure less than 10^7, however, would be rather low for the autoradiographic location of ^{82}Br.

Isotope-dilution Procedure and Exchange Reactions.—Residual bromide in wheat meal which has been aired after exposure to methyl bromide can largely be accounted for as soluble ionised bromide, but it is possible that traces of unchanged methyl bromide may be firmly retained physically, *e.g.*, by adsorption. An attempt was made to apply the isotope dilution procedure to the detection of such traces. Dry wheat meal was exposed to ^{82}Br-labelled methyl bromide for several days and then aired at room temperature. The total methyl bromide sorbed and that recovered by aeration were determined, so the total residual active bromine was known. The meal was then shaken in inactive methyl bromide vapour for several hours at room temperature, during which time it was assumed that at least part of any active methyl bromide present would have become homogeneously diluted by the added methyl bromide. A sample of the pure methyl bromide was finally isolated and the specific activity determined.

Let the initial specific activity expressed, say, as c.p.m. per mg. of bromine under some standard conditions of measurement be a_1. Let the total weight of residual bromine be w_1 mg., and suppose x mg. of this residue to remain as undecomposed methyl bromide. Let w_2 mg. be the weight of bromine added as inactive methyl bromide, and a_2 the specific activity of the final sample. Then,

$$a_2 = a_1x/(x + w_2) \quad \text{and} \quad x = a_2w_2(a_1 - a_2)$$

Now suppose that, despite any assumptions to the contrary, isotope exchange occurs between the active bromine atoms of decomposed ester and the inactive bromine atoms of the added ester, then at equilibrium it follows that $a_2 = a_1w_1/(w_1 + w_2)$ while x assumes the fictitious value w_1.

When applying isotope-dilution procedure, therefore, it is most important to ensure that no isotope exchange occurs between different compounds. This was done before proceeding with the experiment on wheat meal as follows. No significant exchange could be detected at room temperature between dry silver, barium, or potassium bromides and methyl bromide. In the wheat-meal experiment x was, in fact, found to have the value w_1, indicating that all the residual bromide was present as unchanged methyl bromide, a result hardly consistent with the known properties of the bromide in aqueous extracts. A more likely alternative was that of a rapid exchange between the active bromide resulting from exposure to methyl bromide and the bromine of the added methyl bromide. This led to one or two interesting observations.

It is first necessary to derive a working equation so that the exchange velocity constants determined in heterogeneous systems of this kind can be compared with those values determined by earlier workers using homogeneous systems, *e.g.*, Le Roux and Sugden (*J.*, 1939, 1279 and later papers) using solutions containing alkyl bromides and inorganic bromides.

Consider a bimolecular exchange of the type

$$\underset{A}{CH_3Br} + \underset{Y}{^{82}Br^-} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \underset{X}{CH_3{}^{82}Br} + \underset{B}{Br^-}$$

taking place in a heterogeneous system of net volume V containing solid or liquid sorbent of weight w, concentrations in the adsorbed or dissolved phase being given by $B/w = b$, etc. Now, over small ranges of concentration in the gas phase the concentration a' of gas sorbed may be written $A'/w = \phi(A - A')/V$, where ϕ is a constant.

If, at equilibrium, $X/A = Y/B$, and $X \ll A$ and $Y \ll B$, then $k_1 = k_2$ and it can be shown that

$$k = \frac{2{\cdot}303(V + w\phi)}{\phi t(A + B)} \log_{10}\left[\frac{1}{1 - \theta}\right]$$

where θ is the exchange after time t expressed as a fraction of the equilibrium value. When V/ϕ approaches zero, A' approaches A and the equation reduces to the simpler form applicable to single-phase systems (McKay, *Nature*, 1938, **142**, 997), *viz.*,

$$k = \frac{-2{\cdot}303}{t(a + b)} \log_{10}(1 - \theta)$$

The former equation has been applied to heterogeneous systems where adsorbed or dissolved methyl bromide, in physical equilibrium with its free vapour, was allowed to exchange with active bromide present in a liquid or solid phase. Its validity was checked by varying A, B, t, w, etc., in the experiments using potassium bromide solutions. Tentative values of k for different systems are collected in the table below. The exchange reactions were allowed to proceed for periods of 5—48 hours.

In some of the experiments with wheat protein (gluten) exchange reached the equilibrium value within the limits of experimental error.

Solid or liquid phase.	Temp. of exchange.	Gas phase.	k (mg.-mol.$^{-1}$ hr.$^{-1}$).
(1) Solid KBr (active)	20—27°	MeBr (inactive)	No exchange detected
(2) Solid AgBr (active)	20—27	,,	Slight exchange
(3) Solid $BaBr_2$ (active) (crystals possibly hydrated)	20—27	,,	No exchange detected
(4) Aqueous solutions of KBr (active) (pH 6·25—7·50)	20—27	,,	0·1—0·2
(5) Active bromide sorbed on desiccated wheat protein (gluten) resulting from exposure to CH_3Br* followed by aeration	20—37 (*sic*)	,,	2·3—25·9
(6) As in (5) but protein moistened by exposure to water vapour and redried *in vacuo* over P_2O_5	—	,,	No exchange detected
(7) As in (5) but protein moistened with EtOH and redried	—	Inactive MeBr	1·6
(8) Active bromide deposited in desiccated wheat protein by exposure to free active bromine or HBr. In some experiments the exposure was followed by ammonia treatment to neutralise free sorbed acid	—	,,	0·02—0·3
(9) As in (8) but active KBr deposited from alcoholic solution	—	,,	0·01

The disappearance of the exchange facility of the active bromide in wheat protein resulting from active CH_3Br*-exposure followed by temporary moisture increase (expt. 6) is interesting and suggests that the bromide is deposited at sites which may subsequently adsorb methyl bromide in such a way as to facilitate exchange, the bromide diffusing away from the sites in the presence of condensed moisture. Attempts to achieve the same result by exposure to bromine in other forms did not succeed (expts. 8 and 9).

In conclusion, these experiments emphasise that when exchange reactions might influence the interpretation of a tracer study, particularly when it is desired to label integral molecules or radicals, it is important to investigate the possible exchange reactions under the conditions obtaining during tracing.

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Pest Infestation Laboratory, London Road, Slough, Bucks. [*Read, April 1st*, 1949.]