a higher concentration of DDT in butterfat at feeding levels of 1.0 to 5.0 p.p.m. of added DDT. Total amount of DDT excreted in the milk of each pair of animals seemed to have no correlation to total fat production. Therefore, these data suggest that one might predict DDT excretion patterns of different animals on the same feed by comparing the fat percentage of their milk rather than their total fat production. Percentage of DDT excreted in the milk based on total daily intake, ranged between only 0.3 and 0.8% at the 1- and 2-p.p.m. DDT feeding levels, and between 1.0 and 2.2% at the 3- and 5-p.p.m. levels.

Data from Table II were plotted to calculate probable safe levels of DDT in feeds that would result in undetectable levels of DDT in milk (Figure 2). The logarithm of the highest DDT excretion level for each animal was plotted against the logarithm of the corresponding DDT concentration added to feed. The data fell along a straight line that, on extrapolation to a residue of 0.01 p.p.m. of DDT in milk, corresponded to a feeding level of 0.8 of p.p.m. DDT in feed. This value of 0.8 p.p.m. of DDT in feed is in good agreement with the observation that 0.5 p.p.m. of added DDT resulted in an undetectable residue (Table II). Extrapolating this curve to 10 p.p.m. of added DDT, the value obtained is 0.85 p.p.m. of DDT in milk, which is in good agreement with Gannon's data (*3*).

Work is in progress in our laboratories on the excretion pattern of a number of other chlorinated pesticides fed at low levels to dairy cows.

Acknowledgment

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INSECTICIDE EFFECTS ON ANIMALS

Response of Experimental Animals to Phosdrin Insecticide In Their Daily Diets

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The level of Phosdrin insecticide in the diet that induced fatal intoxication in rats was 400 p.p.m. over a period of 13 weeks; in dogs, 200 p.p.m. over a period of 14 weeks. Nonspecific toxic degeneration and necrosis of the liver and renal tubular epithelium were noted. Characteristic alterations in exocrine glands of the animals were observed and could be correlated with the quantity of the insecticide in the diet. At dietary levels below 25 p.p.m., there was no gross effect on rats or dogs. At levels of 2 to 5 p.p.m. in the diets of rats, there was moderate depression of the cholinesterase in the erythrocytes, slight depression of the cholinesterase in the plasma, and no effect on the cholinesterase of the brain. Slight inhibition of the cholinesterase activity of erythrocytes and plasma of dogs was noted at dietary levels of 2.5 and 5 p.p.m.

THE development of Phosdrin insecticide, *O,O*-dimethyl 1-carbomethoxy-1-propen-2-yl phosphate, as an effective new insecticide having worldwide application in the control of insects harmful to agricultural crops, indicated the need to determine the response of animals to the compound and to investigate its effects on the physiology of animals. Such information would make possible the formulation of procedures for the safe handling and use of the material.

Phosdrin is soluble in water and fat and can be absorbed through the skin, the lungs, or the gastroenteric tract. It is highly toxic to warm-blooded animals—for example, the LD_{50} for rats is approximately 6 mg. per kg. (*10*). The toxicity and physiological action of Phosdrin were reported to be similar to those of other well known organophosphorus insecticides, such as tetraethyl pyrophosphate (*6*).

Experimental animals were exposed to lethal and sublethal quantities to determine the type of injury to the internal organs associated with acute intoxication and to measure the effects on growth. The second phase of the experimental program was to study the inhibition of the cholinesterase of the brain and of erythrocytes and plasma in the peripheral blood.

The toxicity of parathion, diethyl *p*-nitrophenyl thiophosphate, has been studied extensively and is of the same order of magnitude as Phosdrin (*8*). For this reason parathion was used as a positive control in studies of Phosdrin.

Materials

Phosdrin. The formula for Phosdrin,

$$(CH_3O)_2POC(CH_3) = CHCOCH_3,$$

shows the possibility of cis-trans isom-

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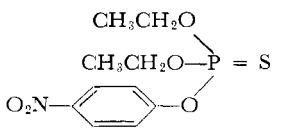
erism about the carbon-carbon double bonds. One of the two geometric isomers, designated as the α-isomer, has been found to be much more biologically active to insects and laboratory animals than the other, designated as the β (*1*). The sample employed in our experiments contained 60.1% as the α-isomer.

Phosdrin insecticide is a yellow to orange liquid, having the physical properties shown in Table I (*13*).

The vapor pressure of Phosdrin is higher than that of most chlorinated and organophosphorus insecticides and is undoubtedly one of the important factors in the rapid dissipation of its residues from crops. In general, residues of Phosdrin insecticide are well under 1 p.p.m. within 24 hours after application.

The insecticide is hydrolyzed in aqueous solutions. The rate of hydrolysis is increased in alkaline solution and is dependent not only upon the pH but also on the ionic concentration of the solution, the temperature, and other factors (*1*).

Parathion. Parathion is a dark brown liquid and has the formula



The sample used in this investigation was reported to have a purity greater than 95%.

The various concentrations of the insecticides, referred to in this report, are expressed in terms of the materials received.

Biological Methods

To ascertain the clinical signs and symptoms of intoxication associated with ingestion of Phosdrin, Carworth rats and beagle dogs (equal numbers of males and females) were fed various dietary levels ranging from 0.32 to 400 p.p.m. for periods ranging from 13 to 18 weeks. Diets containing parathion were fed also as a positive control. Weekly observations of weight gain, food consumption, and growth were made. At postmortem examination, the total body and the individual organs were weighed. The ratios of organ weight to body weight for each dog and for groups of rats were calculated.

Preparation of Diets. Since Phosdrin insecticide is volatile, the diet for each experiment was prepared daily except Sunday. A concentrated stock mixture of casein and cornstarch containing the insecticide was made weekly and refrigerated in glass-stoppered bottles except when the daily diets were prepared. For diets containing 25 to 100 p.p.m. of the insecticide, the concentrated stock was prepared once each week in a Kitchen Aid mixer by blending the appropriate amount of insecticide contained in 20 ml. of absolute alcohol with 496 grams of a mixture of equal parts by weight of casein and cornstarch. Seventy grams of the appropriate concentrate were mixed daily (except Saturday, when twice the quantity was used) in a 4-quart P-K Laboratory Twin-Shell blender for 15 minutes. Food for the

Table I. Physical Properties of Phosdrin Insecticide (*13*)

Property	Description
Odor	Mild to none
Specific gravity, 60/60° F.	1.24
Vapor pressure, mm. Hg at 70° F.	0.0029
Flash point, tag open cup, ° F.	175
Boiling point, ° F.	
At 0.03 mm. Hg	210 to 218
At 760 mm. Hg	617 (estimated)
Pour point, ° F.	−69
Refractive index, n_D^{25}	1.4493
Coefficient of expansion, per ° F.	0.00046
Viscosity, centistokes at 77° F.	6.678
Solubility	Miscible with water, acetone, carbon tetrachloride, chloroform, ethyl alcohol, isopropyl alcohol, methanol, benzene, toluene, and xylene. Slightly soluble in carbon disulfide and kerosine. Insoluble in hexane

Table II. Effects of Diets Containing Phosdrin Insecticide and Parathion Fed to Rats

Insecticide	Concn., P.P.M.	Mortality (13 Weeks) M	Mortality (13 Weeks) F	Av. Increase in Weight, Grams M	Av. Increase in Weight, Grams F	Clinical Signs of Intoxication	Pathologic Lesions: Exocrine glands	Pathologic Lesions: Viscera
Control	0	1/24	0/24	302	155	0	0	0
Phosdrin	25	0/12	0/12	305	174	+	±	0
	50	0/12	0/12	309	163	+	+	0
	100	1/12	0/12	238	126	++	++	0
	200	2/12	3/12	172	70	+++	+++	+
	150–400[a]	3/11[b]	7/12	64	44	++++	++++	+++
Parathion	100	1/12	4/12	287	94	+	++	0

[a] 150 p.p.m. for 5 weeks, 300 p.p.m. for next 2 weeks, and 400 p.p.m. for 6 weeks.
[b] Accidental death of one animal excluded.

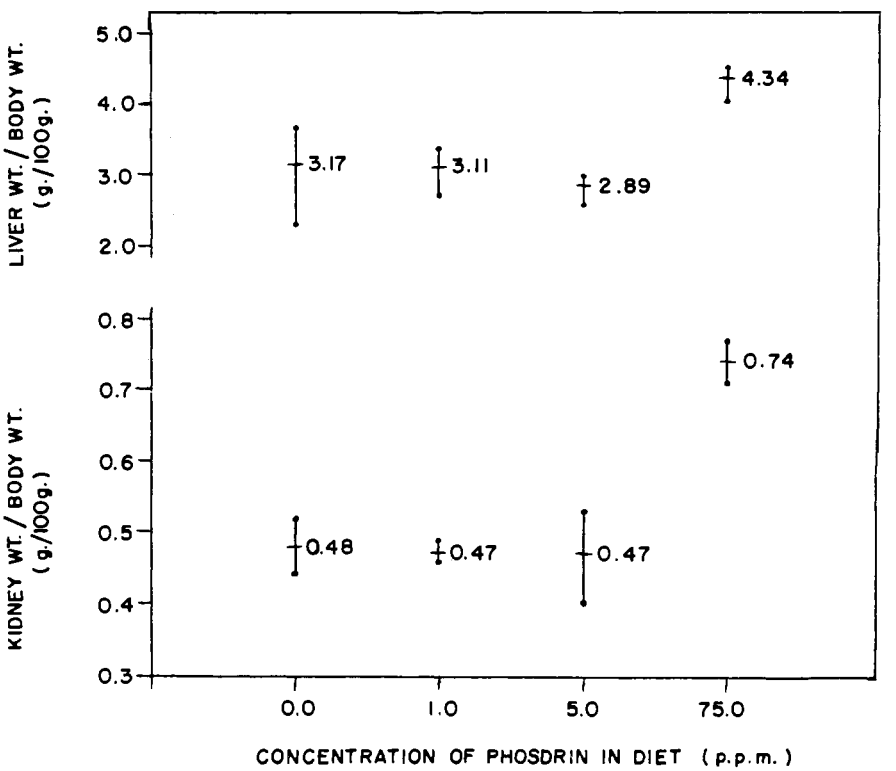


Figure 1. Ratio of weights of liver and kidneys to body weight of male and female dogs fed Phosdrin insecticide for 14 weeks

Table III. Extent of Degenerative Lesions of Exocrine Glands of Rat

Insecticide	Concn., P. P. M.	Submaxillary M	Submaxillary F	Sublingual M	Sublingual F	Parotid M	Parotid F	Harderian M	Harderian F	Lacrimal M	Lacrimal F	Thymus M	Thymus F	Pancreas M	Pancreas F
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phosdrin	25	+	±	±	0	0	0	+	±	+	±	0	0	0	0
	50	++	+	+	0	0	0	++	+	++	+	±	±	±	±
	100	+++	++	+	0	0	0	+++	++	+++	++	+	+	+	+
	200	+++	++	++	+	++	+	+++	++	+++	++	+	0	+	+
	150–400	++++	+++	++	++	+++	+	++++	+++	++++	+++	+	±	++	+
Parathion	100	++	++	+	+	++	++	++	++	++	++	+	+	+	+

Table IV. Response of Dogs to Diets Containing Phosdrin

(14 weeks)

Concn., P. P. M.	Mortality	Clinical Signs of Intoxicotion	Pathologic Lesions: Exocrine glands	Pathologic Lesions: Viscera
0	0/4	0	0	0
0.3	0/4	0	0	0
1.0	0/4	0	0	0
2.5	0/4	0	0	0
5.0	0/4	0	0	0
75	0/4	+	±	0
200	4/4	+++	++	++++

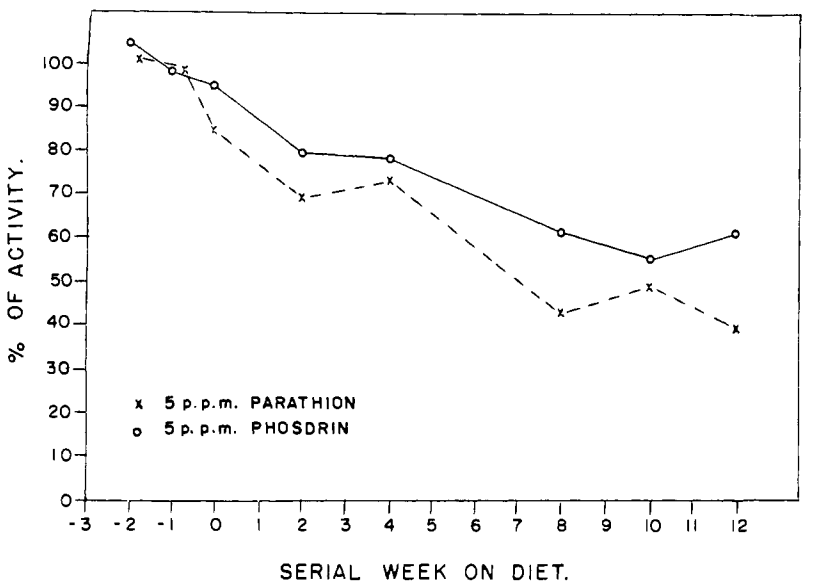


Figure 2. Inhibition of cholinesterase in erythrocytes of male rats

Average of 5 determinations. Expressed as percentage of control value

control animals was prepared in the same manner with uncontaminated ethyl alcohol. The diets were put in the hoppers within the cage every day (Monday through Saturday) after the food from the previous day had been removed. The high-grade cornstarch employed was obtained from the Janszen Co. and the pure grade of casein was obtained from the Matheson Co.

The diets which contained the insecticide at levels of 10, 5, or 0.5 p.p.m. were prepared daily by dilution of the next more concentrated dietary mixture with the appropriate quantity of uncontaminated ground Purina Laboratory Chow. For example, the diet at 10 p.p.m. was prepared by blending 140 grams of a freshly prepared diet at 25 p.p.m. with 210 grams of uncontaminated ground Purina Laboratory Chow. As it was not known whether Phosdrin insecticide would be stable for a week as a prepared mixture with the Purina Chow, it was necessary to prepare this mixture daily. After the insecticide mixed with cornstarch and casein and refrigerated was found to be stable for at least 4 months, this mixture was used as the starting point in the preparation of the daily diet.

To obtain accurate data in the conduct of the experiments, the stability of the insecticide in the final diet was determined. Accordingly, the insecticide was blended into a mixture of equal parts of cornstarch and casein, to yield concentrations of 10, 50, and 1000 p.p.m. Portions of these blends then were mixed into ground Purina Laboratory Chow in a ratio of 1 part of the starch casein concentrate to 9 parts of chow, so that the dietary concentrations were 1, 5, and 100 p.p.m. Analogous procedures were used in preparing the diets for the dogs.

Samples of these mixtures were analyzed by the following procedure: The Phosdrin insecticide was extracted from the food with hexane in a Soxhlet extraction flask and then transferred to water by evaporation of the hexane phase from a two-phase system of hexane and water. The quantity of insecticide was determined by a spectrophotometric procedure whereby the extent of the inhibition of cholinesterase activity was measured. The data showed that approximately 90% of the insecticide was recovered after it had been stored at room temperature in open wide-mouthed glass jars for one day. These data, confirmed by bioassay, reflected the maximum loss that might be expected if the chow was allowed to stand in hoppers for 24 hours during the course of the feeding to animals. In a similar test in which comparable diets were stored in closed glass jars, no appreciable loss was shown when the material was examined by bioassay.

Special Histologic Techniques. Complete post-mortem examinations were performed on all animals, including microscopic examination of brain, heart, lungs, liver, kidneys, spleen, and stomach. The exocrine glands, submaxillary, major sublingual, parotid, Harderian, exorbital lacrimal, and pancreas and thymus, were examined using Bensley's technique (*11*) for demonstrating mitochondria and zymogen granules. All organs were examined using the hematoxylin and eosin staining technique.

Cholinesterase Activity. The cholinesterase activity of erythrocytes, plasma, and brain of rats was assayed. Similar assays were made on the erythrocytes and plasma of dogs fed diets containing 5 p.p.m. or less of the insecticides The method of Michel (*12*) as modified by Hamblin (*7*) and Frawley (*6*) was used. The cholinesterase activity of erythrocytes and plasma was determined at weekly intervals during the experimental program and the activity of the brains was determined at the termination of the experiment.

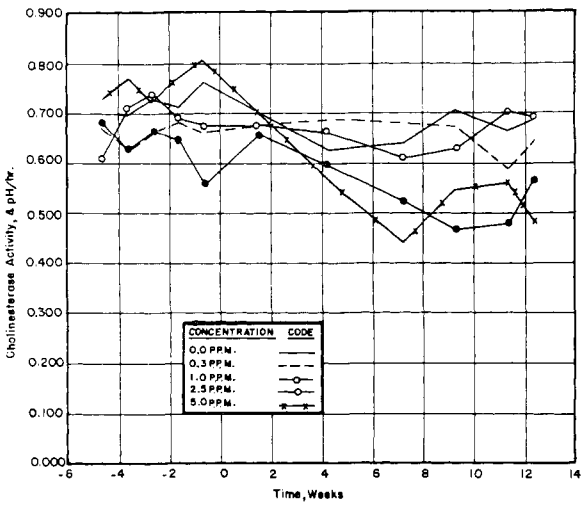


Figure 3. Effect of Phosdrin on cholinesterase activity of erythrocytes of male dogs

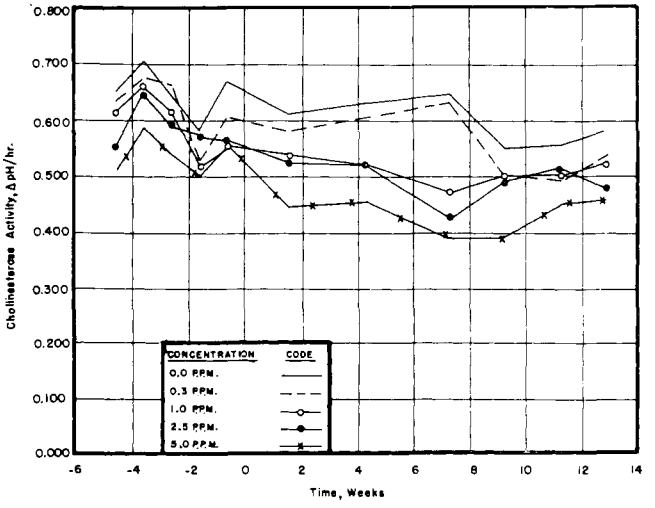


Figure 4. Effect of Phosdrin on cholinesterase activity in plasma of male dogs

Results

Rats. In Table II are shown the results of feeding rats diets containing Phosdrin or parathion at levels ranging from 25 to 400 p.p.m.

There was no increase in mortality below the level of 400 p.p.m. of Phosdrin in the diet, and female rats were more susceptible than males. There was a significant increase in mortality of female rats fed parathion at 100 p.p.m. There was no interference with growth at levels of Phosdrin below 100 p.p.m. Clinical signs of intoxication were minimal at 25 p.p.m., increased progressively with increasing dietary levels, and could be correlated with morphologic lesions of the exocrine glands.

The clinical signs of intoxication included hypersensitivity to external stimuli, occasional tremors, ataxia, weakness, anorexia, pallor of the mucous membranes, and increased respiratory rate. An unusual lesion was ulceration and subsequent rupture of the cornea and loss of the aqueous humor.

Pathology. The following visceral lesions were observed in the organs of animals fed levels of Phosdrin at or above 25 p.p.m.: nonspecific diffuse, toxic degeneration of the liver and renal tubular epithelium, and degeneration of the epithelial cells lining ducts and acini of the exocrine glands.

The lesions of the salivary and lacrimal glands reflect the concentration of the insecticide in the diets and indicate that the response of the males was greater than that of the females.

Dogs. The results of feeding Phosdrin to dogs are shown in Table IV.

Clinical evidence of intoxication included anorexia, vomiting, muscular twitching, generalized tremors, prostration, and convulsions.

Pathology. The visceral lesions were similar to those observed in rats, although the lesions of the exocrine glands were less severe than those noted in rats at the same concentrations. While no microscopic evidence of intoxication in the viscera was noted, there were significant changes ($p < 0.01$) in the ratio of liver and kidney weights to the body weight (Figure 1) at 75 p.p.m.

Inhibition of Cholinesterase Activity by Phosdrin. RATS. In Figure 2 are presented representative data on serial determinations of the cholinesterase inhibition observed in the erythrocytes of male rats.

Inhibition was progressive over the 12-week period at the level of 5 p.p.m. of both Phosdrin and parathion. Phosdrin at 2 p.p.m. in the diet caused a depression to 75% of the normal values in male rats. The degree of inhibition was similar in the females. Below the level of 2 p.p.m., there was no inhibition of the cholinesterase activity of the erythrocytes. There were no significant differences in the effects of Phosdrin or parathion. The cholinesterase inhibition proved to be reversible, activity returning to normal within 2 to 4 weeks on an uncontaminated diet.

The cholinesterase of the plasma of rats fed rations containing either Phosdrin or parathion at a level of 5 p.p.m. was decreased to approximately 80% of that of the controls, whereas at 2 p.p.m. or below, Phosdrin had no effect on the cholinesterase activity. No significant inhibitory effect on the cholinesterase activity of the brain was noted at any concentration ranging from 0.32 to 5.0 p.p.m. in the diets of the rats.

DOGS. Male and female beagles fed diets containing 5 p.p.m. of Phosdrin had inhibition of cholinesterase activity of the erythrocytes to approximately 70% of that of the controls; at 2.5 p.p.m. inhibition was present at approximately 82% of that of the controls (Figures 3 and 4). Phosdrin below 2.5 p.p.m. in the diet had no effect on cholinesterase of erythrocytes. Only minimal depression of the cholinesterase activity of the plasma was noted at 5 p.p.m. of Phosdrin in the diet (90% of control values); below 5 p.p.m. plasma cholinesterase was normal. The cholinesterase activity of the brains of both males and females was normal after 12 weeks on diets containing from 0.03 to 5.0 p.p.m. of Phosdrin.

Discussion

The clinical signs of intoxication observed in this investigation were similar to those reported by Kodama and other investigators (*2, 6, 8, 10*), who noted increased swallowing and munching, twitching of ears, myosis, muscular twitching and fibrillation, chattering of teeth, coarse generalized tremors, lacrimation, salivation, urination, defecation, depression, prostration, and tonic or clonic convulsions. Differences in response according to sex have been noted and have been corroborated in the present study. Female rats generally were more susceptible than males. Recovery from the effects of intoxication after removal of the contaminated diet was evidenced by increased consumption of food, improvement in general physical appearance, rapid regression of abnormalities of the eyes, and diminution in the morphologic changes in the affected viscera.

Previous investigators (*3, 4, 9*) had noted the lack of specific morphologic lesions, although Denz had recorded

originally the effects on the various exocrine glands. The present study has correlated the morphologic lesions of the lacrimal and salivary glands with the level of insecticide in the diet. These glands have been shown to be the sites of high cholinesterase activity; thus harmful effects of the organophosphorus compounds may be demonstrated both by inhibition of cholinesterase and by histologic changes in the exocrine glands.

The chromodacryorrhea observed by Kodama was explained by Denz as being related to the hyperactivity of the Harderian gland. Similarly in the present study the sero-sanguineous discharge from the eyes of rats and the ulceration and subsequent rupture of the cornea may well be attributable to the injurious effects on the Harderian and lacrimal glands. Analogously it may be postulated that effects upon the digestive mechanism might result from the alterations observed in the salivary glands and pancreas.

As shown by the present and other investigations (*5, 6, 7*) the degree of cholinesterase inhibition may be used as a sensitive indicator of exposure to organophosphorus compounds. However, fatal poisoning may occur without total depression of activity and the response of the organism (man or animal) varies considerably with each specific compound, as does the recovery of the cholinesterase activity following depression (*5*). Further, the cause of death in animals or man cannot be explained on the basis of cholinesterase inhibition.

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PESTICIDE RESIDUES

Determination of Selenium in Oats by Oxygen Flask Combustion

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In this study, selenium is determined in oats by dry combustion of a pelleted sample of ground oats in an oxygen-filled, 5-liter flask. In this closed system, combustion gases are absorbed in distilled water in the flask. Selenium is determined spectrophotometrically. Flask combustion is rapid and eliminates the usual wet ashing and selenium tetrabromide distillation steps. The method is sensitive to about 0.25 p.p.m. of selenium.

DRY COMBUSTION of apple tissue in a 5-liter, oxygen-filled flask has been successfully used to oxidize fruit prior to determination of mercury (*2*). Selenium, like mercury, is difficult to retain during oxidation of biological tissue by wet ashing procedures. In the present work, the flask combustion technique is used to burn oats prior to determination of their selenium content. Combustion in the closed system eliminates volatilization losses of elements such as selenium and mercury.

Procedure

Weigh approximately 1 gram of well mixed, ground oats accurately and place in a Parr pellet press having a 0.5-inch diameter bore. Pelletize and transfer the pellet gently, using forceps, into the platinum holder of the 5-liter combustion flask (*2*). Place a fuse (2 mm. wide and 8 cm. long) cut from filter paper in the holder touching the sample. A convenient method is to fold about 1 cm. of the fuse into a right angle and place the pellet on top of this tab. Pipet exactly 100 ml. of distilled water into the flask for absorption of gases. Place the magnetic stirring bar into the flask and purge the flask with oxygen. Light the fuse and gently insert the platinum holder into the flask. After combustion, allow the magnetic stirring bar to mix and splash the solution vigorously inside the flask for 10 minutes.

Transfer the entire absorbing solution to a 200-ml. beaker. Rinse the flask and balloon twice with 25 ml. of water and combine the rinses with the absorbing solution in the beaker. Add 10 ml. of 20% hydroxylamine hydrochloride to the solution and adjust the pH of the mixture to 2.5 with 90% formic acid. Add 5 ml. of 0.5% 3,3′-diaminobenzidine hydrochloride to the solution. Mix and allow to stand for 1 hour. Then add 5 ml. of 0.2*M* disodium ethylenediaminetetraacetic acid (EDTA), mix, and adjust the pH of the solution to 7 with concentrated ammonium hydroxide. Transfer the solution to a 250-ml. separatory funnel. Add exactly 8 ml. of toluene and shake for 1 minute. Allow the mixture to stand for about 5 minutes and then shake again for 1 minute. After the layers