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

separate, drain off and discard the lower aqueous layer. Drain the toluene layer into a 50-ml. beaker. Determine the absorbance of this solution in a 10-cm. cell (Pyrocell Mfg. Co., Cell S25-220, 10 mm. in O.D., capacity about 4.5 ml.) at 420 mμ using a Beckman DU spectrophotometer with toluene in the reference cell.

Prepare the calibration curve (0 to 6 μg. of selenium) as follows:

Pipet 0, 1, 2, 4, and 6 ml. of a standard sodium selenite solution (1 μg. of selenium per ml.) into a series of 200-ml. beakers and make up with water to a total volume of 150 ml. Proceed as in the analysis of samples beginning with the addition of hydroxylamine solution. The calibration curve follows Beer's law from 0 to 6 μg. of selenium; the 6 μg. standard corresponds to a reading of about 25% transmission.

Results and Discussion

The method was used to recover selenium added as sodium selenite from oats. The recoveries are shown below:

Added, P.P.M.	Recovery, %
0.5	95.0
0.5	122.0
0.5	108.0
2.0	87.5
2.0	80.5
2.0	89.0

In recovery studies, the oat pellet is fortified after it has been placed in the platinum holder. The 2.0-p.p.m. level is attained by pipetting 0.1 ml. of a sodium selenite solution (20 μg. of selenium per ml.) onto a 1-gram pellet of ground oats. This volume of liquid does not prevent combustion and the sample may be burned immediately without drying. The 0.5-p.p.m. level is attained by pipetting 0.1 ml. of a 5 μg. per ml. selenium solution onto each of two 1-gram pellets which are burned successively in the flask; the same 100 ml. of absorbing solution is used for each burning.

The method was used to determine the selenium content of three oat samples from South Dakota in which selenium had been determined by the method of Klein (*4*). The results are given below:

	Selenium, P.P.M.	
Code No.	Klein method	Flask combustion
1	0.9	0.86
2	2.1	2.06
3	4.7	4.23

The check value for six analyses averaged 0.10 p.p.m. of selenium. The method will detect 0.25 p.p.m. of selenium in a 2-gram oat sample. The colorimetric procedure is an adaptation of that of Cheng (*1*). The selenium stock solution (50 μg. of selenium per ml.) is stable for about 1 week. After this, precipitation takes place and the solution must be freshly prepared. The aqueous solution of 3,3′-diaminobenzidine darkens rapidly during storage. The rate of darkening can be greatly reduced by purging the headspace of the container in which the reagent is stored with nitrogen before letting it stand for prolonged periods. Store the reagent in a refrigerator. When measuring absorbance, rinse the cuvet with about 1 to 2 ml. of the solution whose density is to be determined.

This method may be applicable to the determination of selenium in other biological material, since the flask has been used to burn cabbage, cherries, onions, and potatoes prior to determination of arsenic, bromine, chlorine, and manganese (*3*), and to burn organic soils for determination of organic carbon content.

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SOIL EFFECTS ON PESTICIDES

Determination of Carbon in Organic Soils by Oxygen Flask Combustion

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A method is described for determining organic carbon in organic soils by match-produced combustion of a pelleted sample of dry soil in an oxygen-filled flask. Excellent agreement is obtained by comparing carbon analysis by this method and the electric furnace combustion procedures. Soils containing 14% of organic carbon burn spontaneously. The method is simple, inexpensive, and requires about 30 minutes per sample.

Dry combustion is considered the most accurate method for determination of organic carbon in soils. The most common of these methods employs an electric furnace in which the soil is burned in a stream of oxygen in the presence of one or more catalysts. Carbon dioxide may be absorbed with Ascarite and weighed, collected for Orsat analysis, or measured by other means. The equipment, however, is expensive: difficulties arise from leaks in the system and plugging of absorbers.

In the course of a study of the disappearance of pesticides in soils as related to their organic matter content, a much simpler method was devised. In this method, the dry soil sample is pelleted and burned in an oxygen-filled flask. Evolved carbon dioxide is absorbed in sodium hydroxide, magnetically stirred, and contained in the flask. Carbon is determined as bicarbonate by titration of the base with standard acid between the phenolphthalein and methyl orange end points.

Procedure

Weigh approximately 0.5 gram of well mixed, dry soil accurately and place in a Paar pellet press having a 0.5-inch diameter bore. Pelletize and transfer the resulting disk of soil gently into the platinum holder of the 5-liter combustion flask (*1*) using forceps. Place a fuse (1 mm. wide and 8 cm. long) cut from Whatman No. 42 filter paper in the basket touching the sample. Pipet exactly 100 ml. of approximately 2*N* sodium hydroxide in the flask for absorption of carbon dioxide. Place the magnetic stirring bar in the flask and purge the flask with oxygen. Ignite the fuse and gently insert the platinum holder in the flask. After combustion, allow the magnetic stirrer to mix and splash the solution vigorously inside the flask for 10 minutes.

Transfer exactly 4 ml. of the absorbing solution to a 125-ml. Erlenmeyer flask. Add about 5 ml. of water and a few drops of 0.1% phenolphthalein in methanol. Titrate the solution with approximately 0.25*N* hydrochloric acid