A second trial was conducted with birds that had received some flight training, as outlined in Table 1.

TABLE 1

SCHEDULE OF TRAINING FOLLOWED FOR BIRDS USED IN SECOND TRIAL

Flight number	Date	Approximate distance from home loft	Direction from home loft
		(miles)	
1	April 17	2	North
2	April 18	2	North
3	April 19	5	West
4	April 20	8	North
5	May 8	11	Northeast
6	May 9	22	North-northwest
7	May 12	16	North-northeast
8	May 19	40	North-northeast
9 (test flight)	June 1	80	North-northeast

The ten birds used were trained as a group, all birds being taken for each of the training flights. They were released individually at 5-minute intervals in New Brunswick on June 1. Previously they had returned home as a group from a point half way between the home loft and New Brunswick, a distance of approximately 40 miles. The weather was warm and partly cloudy with no perceptible wind. Of the ten birds released, two found their way back to the home loft, one 30 days later, and the other 48 days later. As a check group, eight untrained birds were released the same day. None of these ever returned to the home loft. The trained birds were 4½ months old, and the untrained birds 3½ months old.

In comparison with the first trial and the control group, even the training of the birds over 40 miles in the same direction as the 80-mile test flight failed to assist them materially in finding their way home when flown individually.

A third trial was conducted with birds flown over a longer period of time and as a group over the territory to be covered by the individual test flight, namely, from New Brunswick to Millville. The flying experience of the birds used is shown in Table 2, together with the results obtained. Of the nine birds flown, three never were heard from. Each bird carried a message holder with instructions enclosed to notify the author if the bird was picked up at any point, but no word ever was received regarding the three birds that did not return home. The six birds that returned home found their way back on the day of their release, with one exception, and that particular bird—which previously had not flown the course—came in two days later. Previous experience in flying over a particular territory certainly appeared to be highly desirable for birds when flown individually.

TABLE 2

PREVIOUS FLYING EXPERIENCE AND RESULTS OF HOMING PIGEONS FLOWN INDIVIDUALLY IN THIRD TRIAL

Bird No.	Age of bird (months)	No. of group flights in training	No. of group flights over test course (80 miles)	Time required to reach home
6906	9	5	2	7 hrs., 2 min.
6911	8	9	2	4 hrs., 50 min.
6919	8	8	2	Lost
6930	8	6	1	Lost
6931	8	18	1	6 hrs., 40 min.
6947	7	15	0	2 days
6954	7	16	1	4 hrs., 37 min.
6966	6	2	2	4 hrs., 28 min.
6972	6	1	1	Lost

A point of interest in connection with the third trial was the fact that when flown as individuals the birds required from 4½ to 7 hours to find their way home, whereas on a previous flight as a group the birds covered the same distance in 2½ hours. Even on an additional flight in a fourth test, the birds as individuals required over 4 hours to come home. Weather conditions on all occasions were favorable and could not be considered as having affected the time required by the birds to find their home loft. Just why a group would know its way any better than the same birds as individuals is a point worthy of consideration and experimentation.

In summary, homing pigeons untrained in flights did not possess an instinct that would automatically take them back home when they were released individually at a point 80 miles from home. Even training for half the distance was of no avail. After once having flown with a group over the distance indicated, the majority of the birds, when flown as individuals, were able to find their way home.

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NEW JERSEY AGRICULTURAL EXPERIMENT STATION

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A COLORIMETRIC METHOD FOR THE MICRO-DETERMINATION OF 2,2, BIS(P-CHLOROPHENYL) 1,1,1 TRICHLORETHANE (DDT)[1]

THE efficiency of the compound 2,2 bis(p-chlorophenyl) 1,1,1 trichlorethane (DDT) as an insecticide, its tendency to remain effective for a long period of time and its cumulative toxic action,[2,3,4] makes it desirable that methods for the estimation of this sub-

[1] From the Chemistry Section, Antilles Department Medical Laboratory, U. S. Army.

[2] R. D. Lillie and M. I. Smith, *Pub. Health Rep.*, 59: 979–984, July 28, 1944.

[3] A. A. Nelson, J. H. Draize, G. Woodard, O. G. Fitz-

stance be developed in order that its usage may be controlled to the best advantage.

The current method of Smith and Stohlman[4] is open to certain objections. The ideal method should be sensitive enough to work with very low concentrations, accurate enough to detect small differences in these concentrations, and simple enough to be used for routine analysis. The proposed method fulfills these requirements.

Later publications will cover the application of this method to the determination of DDT in water, body fluids, tissues and foods.

Principle of the Test

It was found that when DDT is heated in an anhydrous pyridine solution containing xanthydrol (9-hydroxyxanthene) and solid potassium hydroxide, a red color develops which under proper conditions is proportional to the amount of DDT present. The reaction will detect as little as 10 gammas of DDT.

Reagents and Special Apparatus

(a) DDT Powder, dissolving, commercially pure 100 per cent. (Merck).

(b) Pyridine (Baker's Analyzed). It should be clear and colorless.

(c) Xanthydrol (Eastman Kodak).

(d) Potassium Hydroxide Pellets (Merck) U.S.P., average weight 125 mg per pellet. They should not have undergone deliquescence.

(e) Reflux Condenser Assembly. A 500 ml round bottom flask fitted with a ground-in all glass reflux condenser. In the absence of an all glass assembly, tinfoil covered cork connections may be employed. Rubber stoppers can not be used, as pyridine dissolves rubber.

(f) Oil Bath. A 500 ml beaker containing 200 to 250 ml of heavy liquid petrolatum, heated with a flame regulated so as to keep the temperature at 120° C ± 2°.

(g) Photoelectric Colorimeter. A Hellige-Diller Model No. 400 was used throughout. The colorimeter tubes were Hellige No. 452-D and the green filter used was No. 520.

Procedure

Preparation of the Xanthydrol-KOH-Pyridine Reagent: The reagent is prepared by placing in a dry 500 ml round bottom flask of an all-glass reflux-condenser assembly 50 ml of a 0.2 per cent. solution of xanthydrol in clear colorless pyridine. The assembly is set up in such a way that the contents of the flask may be mixed by swirling during the preparation of the reagent. The solution is brought to boiling by heating with a moderate flame through an asbestos-center wire gauze. As soon as boiling starts, 25 pellets of potassium hydroxide are added through the condenser. In order to prevent absorption of moisture by the potassium hydroxide, the pellets should be added directly from the bottle by using a small test-tube as a spatula. The boiling is continued and the contents of the flask mixed by swirling at 15 to 20 second intervals until the supernatant becomes dark green in color. The flame is then removed and when the boiling subsides the condenser is disconnected and the supernatant decanted, while hot, into a clean dry pyrex flask, being careful to leave the undissolved potassium hydroxide behind. Upon being transferred to the cold flask the supernatant immediately loses its green color. The reagent is now ready for use. It should be mixed well just before using, and it should be prepared fresh each day.

Reaction of the Reagent with DDT: A solution of DDT in ether containing 0.1 mg per cc is pipetted into a series of dry test-tubes (16 mm × 150 mm) so that the tubes contain 0, 10, 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 gammas of DDT. The tubes are then placed in the oil bath at 120° C for a few minutes to evaporate the ether. When the evaporation is complete the tubes are removed from the bath, 2 cc of Xanthydrol-KOH-Pyridine reagent added to each, and then replaced in the bath for 8 minutes. At the end of this time the tubes are immersed in cold water for 1 minute, removed and wiped free of oil and water and placed in a rack. (During the heating period the contents of the tubes undergo various color changes involving green, red and brown, depending upon the concentration of DDT. However, once the tubes are cooled by immersing in cold water, only the red or the initial yellow color of the reagent remains.) To each tube is now added 4 cc of pyridine. The contents are then mixed by inversion and transferred to dry photoelectric colorimeter tubes. Using a green filter (No. 520) the blank is set at zero on the Direct Reading (Concentration) scale. Readings are taken on all tubes within ten minutes after removal from the oil bath.

Discussion

Graph I is a curve plotted from data obtained by the above procedure. The reaction follows Beer's law to the extent that it can be used for the quantitative colorimetric determination of the substance. The sensitivity is such that small differences in concentration of DDT can be readily detected.

Different batches of reagent prepared on different days give constant results with known amounts of

hugh, R. Blackwell and H. O. Calvery, *Pub. Health Rep.*, 59: 1009–1020, August 4, 1944.

[4] M. I. Smith and E. F. Stohlman, *Pub. Health Rep.*, 59: 984–993, July 28, 1944.

DDT, so that a standard color curve such as that shown in Graph I may be used as a basis for the determination of unknown amounts of this substance. Table 1 gives the direct colorimeter readings obtained with five different batches of reagent, prepared on

TABLE 1

COLORIMETER READINGS OBTAINED WITH DIFFERENT BATCHES OF REAGENT WITH KNOWN AMOUNTS OF DDT

Reagent No.	Concentration of DDT in Gammas										
	0	10	20	40	60	80	100	120	140	160	180
1	0	3.0	6.0	15.5	24	37	45	51	56	62	65
2	0	2.5	6.0	15.5	25	35	45	52	58	62	65
3	0	2.5	6.5	17.0	28	35	43	52	57	63	66
4	0	3.0	7.5	16.0	26	36	43	53	56	64	68
5	0	3.0	7.0	18.0	26	36	45	52	58	64	68

different days, when the reaction is carried out on known amounts of DDT.

The stability of the red color was studied by taking direct colorimeter readings on known concentrations of DDT at 5, 10, 15, 20, 30 and 60 minute intervals after removing the tubes from the oil bath. The results are shown in Table 2. It is recommended that readings be completed within 10 minutes after removal of the tubes from the oil bath, in order to render the effect of fading negligible.

During the course of the experimental work it was found that the reaction is most sensitive in detecting small differences in the concentration of DDT when applied to quantities of this substance ranging from 10 to 200 gammas. Water and alcohol must be absent, as both of these substances inhibit the production of the red color.

It will be noted that this reaction is similar to that

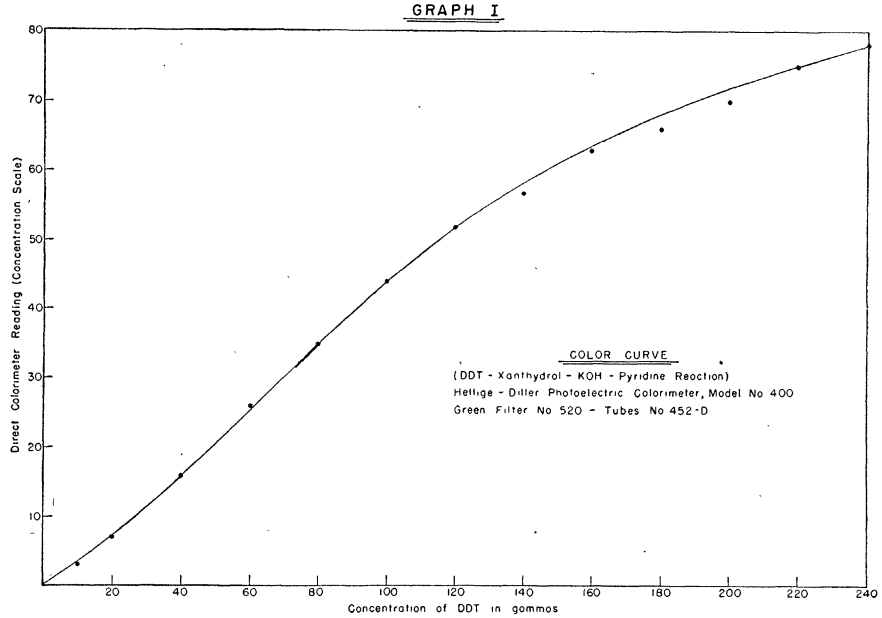
TABLE 2

COLORIMETER READINGS OBTAINED ON KNOWN CONCENTRATIONS OF DDT AT VARIOUS TIME INTERVALS AFTER HEATING

Minutes after heating period	Concentration of DDT in Gammas					
	20	60	100	140	180	220
5	6.0	28	46	58	68	75
10	5.5	27	45	57	67	74
15	5.0	24	43	55	65	72
30	4.5	21	41	51	62	72
20	3.7	17	35	47	60	65
60	3.0	13	26	39	48	56

described in the literature for chloroform and other R-C-halogen compounds with pyridine and sodium hydroxide.[5,6] However, DDT does not give the red color with pyridine and sodium or potassium hydroxide unless xanthydrol is present in the reaction. The possible interference of chloroform and allied substances is partially offset by the fact that most of these compounds will be eliminated during the process of evaporation of the ether at 120° C.

GRAPH I



We are unable at present to explain the part that xanthydrol plays in the reaction. There seem to be two possibilities. The first is that the green dye formed when xanthydrol and solid potassium hydroxide are heated together may be the reactive substance. The other possibility is that xanthydrol, at the temperature used in the reaction, may break down DDT into one or more R-C-chlorine compounds which give the red color with pyridine and alkalie.

Due to the offensive odor of pyridine, the procedure for the test has been arranged in such a way as to

[5] W. H. Cole, *Jour. Biol. Chem.*, 71: 173–179, 1926.

[6] A. O. Gettler and H. Blume, *Arch. Pathology*, 11: 554–560, 1931.

obtain the least degree of dissipation of the substance when heated. For example, the test-tubes used are long and narrow, thus acting as a reflux condenser when the reagent is heated in the oil bath.

If deemed advisable all unused reagent and mixture from the determination may be saved and the pyridine recovered by simple distillation.

SUMMARY

A colorimetric method for the micro-determination of 2,2 bis(p-chlorophenyl) 1,1,1 trichlorethane (DDT) is presented. The test is based on the discovery that when DDT is heated in an anhydrous pyridine solution containing xanthydrol and solid potassium hydroxide a red color develops, which under proper conditions is proportional to the amount of DDT present.

The reaction is sensitive to as little as 10 gammas of DDT. It will detect small differences in concentration within the range of 10 to 200 gammas. The test is relatively simple and can be run in a comparatively short time.

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A SIMPLE VOLUMETER

DURING the course of some recent work it became necessary to determine the volume of turtle eggs. The volumeter described here was originally constructed for measuring eggs of 4 cc to 5 cc volume, but the principle can be utilized in similar devices of other size ranges. Methods for determination of the volume of very small glands have been described recently,[1,2] but the principles involved in these procedures could not be used in our problem.

The volumeter was made from a wide-mouthed glass-stoppered bottle of 250 cc capacity. The diameter of the mouth, not the volume, is the determining factor in selection of the bottle. A hole 10 mm in diameter was drilled down through the glass stopper. A 10 cc serological pipette graduated in tenths was then cemented into the hole, mouth end down, by means of plaster of Paris. Another hole of the same diameter was drilled through the side of the bottle 15 mm from the bottom and into this hole was inserted a rubber stopper of a size suitable to insure a tight fit. The tip of a 20 cc Luer syringe was inserted firmly into a small hole bored through the rubber stopper.

To operate, the bottle is first filled with water and a few cubic centimeters are drawn back into the syringe. The glass stopper carrying the pipette is inserted in the bottle and held in place to prevent lifting out when all the water in the syringe is returned to the bottle by pushing in the plunger. A few trials will determine the amount of water that must be first placed in the bottle and withdrawn into the syringe in order to obtain an initial reading near the lower graduations of the pipette. After the initial reading has been taken, sufficient water is then drawn back into the syringe to permit the removal of the stopper and allow the object to be measured to be placed in the bottle. With the object immersed in the water, the stopper is replaced, held firmly in position and the syringe plunger again pushed in all the way. The distance the column of water rises in the pipette above the initial reading indicates the amount of water displaced by the object. The final reading is then made.

Readings are easily reproducible to 0.1 cc and readings to 0.05 cc can be made if a pipette with graduations wide enough apart is selected for use.

The construction of the stopper and the insertion of the pipette should be such that the trapping of air-bubbles is avoided. While the use of a 10 cc pipette might seem to limit the usefulness of this particular device to a range within the capacity of the pipette, the measurement of eggs up to 15 cc volume was made. Eggs close to 10 cc volume were frequently encountered and when the initial reading lay near the 1 cc mark on the pipette, the final reading would go beyond the limits of the pipette if the plunger were pushed back into the syringe all the way. In these cases it was found that with a little practice accurate final readings could be made by returning the plunger only to the 5 cc mark on the barrel of the syringe, this 5 cc being added to the final reading obtained on the pipette. By using a syringe with a precision line engraved on the end of the plunger, accuracy is increased. Similarly, still larger eggs can be measured by returning the plunger only to the 10 cc mark. Another method of arriving at the same end would be to withdraw 5 cc or 10 cc of water from the bottle by means of a pipette after making the initial reading before putting back the stopper to make the final reading. This method would necessitate replacing water after each measurement to make initial readings. It is also possible that a number of stoppers of transparent plastic material might be made and fitted to the bottle so that a series of pipettes of different sizes would be available for the same device.

In our work the placing of the eggs in the bottle was facilitated by making use of a small wire net dipper with which to lower the eggs into the bottle and to remove them. With ordinary care, loss of water can be avoided in the operations attendant upon removing the glass stopper and lifting the wire dipper. The dipper is allowed to remain in the bottle

[1] C. A. Swinyard, *Anat. Rec.*, 74: 71, 1939.
[2] H. O. Burdick, *Endocrinology*, 28: 676, 1941.