ethane (DDT) based upon the formation of a red color by heating DDT with anhydrous pyridine solution containing xanthydrol and solid KOH. The reaction is sensitive to as little as 10 micrograms and is quantitative within the range of 10–240 micrograms of DDT. No mention was made of the specificity of this reaction.

In view of the studies on the metabolism of DDT and its analogues which are being carried out in this laboratory it is of interest to examine the behavior of the various analogues and derivatives of DDT towards Stiff and Castillo's new reaction. The results of the test applied to eighteen compounds are shown in Table 1.

TABLE 1

THE REACTION OF DDT, ITS DERIVATIVES AND ANALOGUES WITH XANTHYDROL-PYRIDINE; THE ABSORPTION MAXIMA AND EXTINCTION COEFFICIENTS OF THE POSITIVE REACTIONS

	Compound	Reaction	Absorption max. mμ	Extinction coefficient
(1)	$(p\text{-}ClC_6H_4)_2CHCCl_3$	+	500	10.4×10^3
(2)	$(p\text{-}ClC_6H_4)(C_6H_5)CHCCl_3$	+	500	9.2×10^3
(3)	$(p\text{-}ClC_6H_4)_2CHCBr_3$	+	506	2.9×10^3
(4)	$(p\text{-}BrC_6H_4)_2CHCCl_3$	+	520	10.8×10^3
(5)	$(C_6H_5)_2CHCCl_3$	+	495	3.1×10^3
(6)	$(p\text{-}CH_3C_6H_4)_2CHCCl_3$	+	488	2.5×10^3
(7)	$(p\text{-}CH_3OC_6H_4)_2CHCCl_3$	+	485	2.1×10^3
(8)	$(p\text{-}C_2H_5OC_6H_4)_2CHCCl_3$	+	485	1.4×10^3
(9)	$(p\text{-}C_3H_7OC_6H_4)_2CHCCl_3$	+	480	2.0×10^3
(10)	$(p\text{-}C_4H_9OC_6H_4)_2CHCCl_3$	+	482	2.0×10^3
(11)	$(p\text{-}ClC_6H_4)_2C=CCl_2$	+	500	6.6×10^3
(12)	$(C_6H_5)_2C=CCl_2$	+	490	1.9×10^3
(13)	$(p\text{-}BrC_6H_4)_2C=CCl_2$	+	482	1.5×10^3
*(14)	$(p\text{-}HOC_6H_4)_2CHCCl_3$	+	–	–
†(15)	$(p\text{-}CH_3COOC_6H_4)_2CHCCl_3$	+	–	–
(16)	$(p\text{-}ClC_6H_4)_2CHCOOH$	–	–	–
(17)	$(p\text{-}ClC_6H_4)_2CH_2$	–	–	–
(18)	$(p\text{-}ClC_6H_4)_2C=O$	–	–	–

* Compound No. 14 gave a dirty reddish violet color with bluish fluorescence.
† Compound No. 15 gave a reddish violet color and then a precipitate of brownish red.

EXPERIMENTAL

The compounds[2] used in Table 1 were weighed on the basis of 5.65×10^{-7} moles per 5 cc of anhydrous ether. Five cc of each solution were pipetted into individual test tubes 16 × 150 mm, and the colorimetric reaction as described by Stiff and Castillo was carried out on each tube in duplicate after evaporation of the ether. As pointed out by these authors, the presence of water and moisture has an inhibiting effect on color formation. We found this to be true, especially on hot humid days. Consistent results were obtained, however, by the introduction of a pellet of KOH to the tube containing 2 cc of the reagent and 4 cc of pyridine during the heating and development of color. The visible absorption curves of those compounds giving a positive reaction were obtained in a Coleman Spectrophotometer Model 11 with an effective width of 35 mμ. The extinction coefficient at 500 mμ of each of the various compounds were calculated from absorption measurements made in the same instrument in 1 cm thick absorption cells. The concentration of each compound in the reaction was such that light transmitted under this condition was approximately 50 per cent. of the incident light.

DISCUSSION

From the data in Table 1 it is seen that all compounds giving a positive reaction contain aliphatic halogen. Compounds having the structure $> CHCX_3$ or $> C=CX_2$ (X = Cl or Br) in all likelihood would give a positive reaction. On a molar concentration the strongest colors in descending order are Compounds (4), (1), (2) and (11). With the exception of compounds (4) substitution of the Cl in the para position in DDT by other groups decreases the color intensity. Likewise the substitution of Br in the side chain decreases the color intensity. The complete removal of the halogen from the aliphatic part of the molecule renders the compound negative in the reaction.

In view of these observations made on eighteen compounds the xanthydrol-pyridine reaction has no specificity for DDT.

SUMMARY

The Stiff and Castillo colorimetric test for DDT was extended to eighteen analogues and derivatives of DDT. The absorption maxima and extinction coefficients of the colored reactions were also obtained. The test is not specific for DDT. Of the compounds tested, the reaction was given by those having the structure $> CHCX_3$ or $> C=CX_2$.

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[1] H. A. Stiff and J. C. Castillo, SCIENCE, 101: 440, 1945.

[2] Compounds Nos. 1, 2, 3 and 13 were kindly furnished by Dr. H. L. J. Haller, of the Department of Agriculture. Compound No. 18 was obtained from Eastman Kodak Company. Compounds Nos. 16 and 17 were supplied by Dr. T. R. Sweeney of this laboratory. The rest of the compounds were synthesized by one of us (N.E.S.) following published material.

PHYSIOLOGICAL EVIDENCE OF A SITE OF ACTION OF DDT IN AN INSECT

WHEN DDT (1-trichloro-2,2-bis (*p*-chlorophenyl) ethane) solution[1] is injected into a roach, *Periplaneta*

[1] The following definitions apply to this paper: DDT solution, a high concentration (*ca.* 10 per cent.) of DDT in corn oil. Nicotine solution, a high concentration (*ca.*

americana (L.), the ensuing symptoms of toxicity involve increased activity, the eventual appearance and persistence of contractions and tremors in the appendages and body, erratic behavior, and loss of equilibrium. The typical DDT contractions and tremors[1] have been used as a sign of a toxic but not necessarily a lethal action of the poison. Qualitative experiments of the following kinds were performed, with the results indicated. In each experiment controls were treated with corn oil alone and yielded negative results. For brevity, individual experiments are not described, and results and conclusions are much condensed.

Experiments and results.—(1) DDT solution injected into an isolated leg, *a,* taken from a normal roach, an attached leg, *b,* of a cauterized roach,[1] or the leg, *c,* of a leg-ganglion preparation[1] caused symptoms in, and only in, each injected leg. Severing legs *b* and *c* at the point of their attachment to the body did not stop the symptoms. The DDT symptoms in leg *a* were made to vanish at once by sectioning the leg just distad to the region injected. Conclusion 1: DDT can[2] provoke symptoms[1] in a leg without having to act at any site not located within that leg.

(2) Local injection of DDT solution into the region of a thoracic ganglion, *d,* of a cauterized roach, or local application to the ganglionic region of a leg-ganglion preparation, I, caused symptoms in, and only in, the two legs innervated by *d* and in the leg of I. Severing an affected leg at its point of attachment to the body stopped the symptoms, which continued when such legs were not severed. Conclusion 2: DDT can cause symptoms in a leg without having to act at any site located within that leg.

From conclusions 1 and 2 it follows that the DDT can provoke these symptoms by acting at a site (or sites) common to leg and body, without having to act at other sites. A site, common to leg and body, that readily accounts for these results is that part of a nerve between its ganglionic origin in the central nervous system and the terminations of its fibers in the leg.

(3) Nicotine solution was injected into an isolated leg, *e,* from a normal roach, an attached leg, *f,* of a cauterized roach, and the region of a thoracic ganglion, *g,* of a cauterized roach, II. It was put on the ganglionic region, *h,* of a leg-ganglion preparation, and was also injected into a normal roach, III. Legs *e* and *f* showed no reaction to the nicotine. Roaches II and III at once exhibit violent tremors involving all the legs, but severing a tremoring leg caused the symptoms in that leg to vanish. Similarly violent tremors appeared in the leg innervated from *h,* but vanished when the tremoring leg was severed at its attachment to the body segment. Conclusion 3: Nicotine can produce violent tremors in all legs of a roach by excitatory action at a single thoracic ganglion, and seems not to excite the motor fibers of the nerves.

(4) Repetition of the experiments under (3), using DDT solution instead of nicotine solution, gave the following results: Legs *e* and *f,* the two legs innervated by *g,* the leg innervated from *h,* and the legs and body of roach III soon exhibited DDT symptoms. The symptoms in leg *f* and in a leg of roach III continued after the legs were severed, but severing a leg innervated by *g* or from *h* caused the symptoms to vanish from the severed leg. The DDT symptoms in leg *f* were the only ones exhibited by that insect, and the symptoms of roach II occurred only in the two legs innervated by *g,* whereas the symptoms in roach III involved the entire body. Conclusion 4: DDT did not cause ganglionic excitation, as did nicotine, but apparently did excite the motor fibers of the nerves.

(5) Normal roaches were injected with DDT solution. Typical DDT symptoms appeared. Then the insects were injected with nicotine solution. Violent nicotine tremors, followed by paralysis, of all the appendages ensued, and masked the DDT symptoms. Severing a violently tremoring leg caused the nicotine tremors to vanish, after which the DDT symptoms reappeared and continued. Severing a paralyzed leg also was followed by the reappearance of the DDT symptoms. Similar results were obtained by applying nicotine solution to DDT-affected leg-ganglion preparations. Conclusion 5: Nicotine symptoms may mask DDT symptoms, but nicotine action does not necessarily stop DDT action, nor does prior DDT action necessarily prevent the action of nicotine.

(6) A small amount of DDT solution was injected through a pinhole made in the eye of a cauterized roach. Later DDT symptoms appeared in one or both antennae (different experiments). The DDT symptoms did not appear posterior to the head. Other roaches were treated similarly, except that they were injected with nicotine solution instead of DDT solution. Violent tremors appeared, involving the

10 per cent.) of nicotine in corn oil. Nicotine symptoms, only the violent tremoring and paralysis caused by nicotine. DDT symptoms, only the typical contractions and tremors caused by DDT; these consist usually of slower contractions upon which are superimposed at times tremors or rapid twitches. Cauterized roach (a roach with its entire heart region cauterized to prevent cardiac circulation of the hymolymph. Leg-ganglion preparation, a dissected preparation consisting of the ventral part of a thoracic body segment with its ganglion and one attached leg, and with the nerve connections between ganglion and leg intact.

[2] "Can" is used in this paper to denote that the conditions referred to are sufficient to produce the specified results without implying whether or not these are the only conditions that may produce them.

whole body. Decapitation of a roach showing violent nicotine tremors caused the tremoring posterior to the neck to stop at once. Conclusion 6: DDT applied in this way does not cause general excitation, as does nicotine.

General discussion.—These results indicate that the action of DDT was different from that of nicotine, the latter affecting the ganglia and DDT affecting the nerves somewhere along their length. The results suggest that the DDT can act more readily on the motor than on the sensory fibers and, further, that the DDT can bring about repetitive discharges of nerve impulses somewhere along the motor fibers.

General conclusion regarding a mode of toxic action of DDT in the roach.—Certain symptoms of toxicity, referred to as typical DDT contractions and tremors of a leg, can result from the action of DDT at a site (or sites) common to leg and body. It is strongly indicated that the site (or sites) referred to consist of that region of a nerve lying between the origin of its fibers in the ventral nerve cord and the terminations of its fibers in the leg exclusive of the origin and the endings, that is, the myo-neural junctions, of the fibers. It may be said also that all these results are consistent with the idea that DDT can provoke contractions and tremors in other appendages, or in the body, by acting at a similar site on other nerves.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

AN ADJUSTABLE RESISTANCE WITH LINEAR RESPONSE TO AIR FLOW FOR RESPIRATION EXPERIMENTS

IT is often necessary in respiration experiments and clinical work to apply resistance to inspiration or expiration. It is desirable to have this resistance increase linearly with increasing air flow as Davies, Haldane and Priestley[1] have pointed out. The value of such a characteristic is that it represents the change in resistance produced in humans in diseases such as asthma and bronchitis. It is also the type of resistance applied to man by protective respiratory devices such as gas masks and respirators. Davies *et al.* have used canisters filled with cotton wool to give linear response to inspiratory resistances. Obviously, such resistances are not suitable for expiratory resistances because moisture will wet the wool and alter the resistance. Killick[2] has used a pair of flanged inverted funnels with filter papers clamped between them for inspiratory resistances and these also yield a linear response. We[3] have used the same type of funnels but with a glass filter cloth placed between the funnels. By heating the funnels with an electric heating element they may be used for inspiratory and expiratory resistances without interference of moisture absorption or condensation.

Several investigators, principally Hill,[4] Matthes[5] and Barach,[6] have used orifices of varying sizes for introducing resistance to respiration. The resistance to air flow of an orifice varies nearly as the square of the flow or parabolically, hence a doubling of flow increases the resistance fourfold. The use of varying sizes of orifices for adjustable resistance is not entirely satisfactory because of this parabolic flow-resistance relationship. For our experiments a linear response was desired and changes were to be made during the experiment by gradual increase (occasionally decrease) of resistance while the subject was sedentary or exercising. It was not practicable to add layers of glass filter cloth to funnel devices in place or change to different funnel devices because of the time and manipulation necessary.

Flow-measuring instruments[7] were placed in the inspiratory and expiratory tubes thus making it impractical to remove and insert different resistances without the subject's knowledge while an experiment was in progress.

The apparatus described here gives linear resistance response with air flow and is adjustable in resistance from 0.1 to 1.0 mm of water per liter of air flow per minute.

DESCRIPTION OF APPARATUS

A diagrammatic sketch of the apparatus is shown in Fig. 1. It consists of two concentric plastic tubes (lucite) with an annular space between them. The central tube is sectioned so that four supporting strips approximately 3 mm wide remain. A piece of glass filter cloth (WB–0048, Filter Media Corporation, New York) is wound cylindrically around this section and the edges are cemented to the lucite tube by means of

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