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THE TOXICITY OF 2,2-*bis* (*p*-CHLORPHENYL) 1,1,1-TRICHLORETHANE (D.D.T.)

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The introduction of the new synthetic insecticide 2,2-*bis* (*p*-chlorphenyl) 1,1,1-trichlorethane (D.D.T.) demands that possible hazards to man be determined and potential dangers safeguarded against. We describe in this paper investigations on the toxicology of D.D.T. which we carried out during the period April, 1943, to March, 1945. These have been the subject of several reports to the Ministries of Production and of Supply, at whose request we have prepared the following account.

Methods

Our material embraces a variety of laboratory animals as well as human volunteers. Toxic effects and fatal doses (L.D.50) were determined in rats, guinea-pigs, and rabbits, D.D.T. being administered by various routes, in single or repeated doses, and dissolved in a number of solvents. Both crude and pure samples of D.D.T. were employed; the former is said to contain 5% sulphuric acid, but appears to behave similarly to pure D.D.T. Some experiments on the action of D.D.T. powder on healing of experimental wounds were also done. Human experiments were chiefly confined to the wearing of garments and underclothes impregnated with D.D.T. In all cases a close watch was kept for the development of toxic effects, and exposed animals were the subject of careful pathological examination at death or when the observational period had elapsed. Various investigations were made on selected subjects, including blood and urine examination and body-weight determination throughout the course of exposure. These will be described in the appropriate sections. Necropsies on animals were performed immediately after death or, frequently, when the animals were moribund. Microscopical examination of all the important organs and tissues, including the central nervous system in representative cases, followed upon preparation of paraffin and frozen sections. Tissues were fixed in 10% formol-saline and 10% formol-alcohol, embedded after passage through the alcohol series, and many sections stained with Ehrlich's acid haematoxylin and eosin and Weigert's iron haematoxylin and van Gieson as a routine procedure, and often with Heidenhain's azan method, v. Kossa's method for calcification, the prussian-blue method for iron, Weigert's elastica method, and Turnbull's Jenner method for bone marrow. The Scharlach-R fat stain was employed with frozen sections after 10% formalin fixation. The central nervous system was dissected out with very great care so as to avoid disturbance of relationships and trauma from handling. After prolonged fixation in 10% formol-saline, transverse wedges of tissue, a few millimetres thick, were removed at the level of the anterior third of the frontal lobes, at the level of the hypophysis, through the middle of the cerebellum and pons, and at the commencement of the medulla. Such wedges included the whole of the cerebral tissues in a cranio-caudal direction. Paraffin sections were stained with the usual haematoxylin and eosin and van Gieson methods, with toluidine blue, and with the Marchi method. Sections from the main levels of the spinal cord were also prepared from a limited number of animals.

ANIMAL EXPERIMENTS

Single Doses.—Single doses of D.D.T. were administered to groups of at least 5 animals by skin application—the skin area being shaved 24 hours previously—by stomach tube, and by subcutaneous and intramuscular injection. For skin application we used solutions of D.D.T. in ether, kerosene, dimethyl phthalate, and dibutyl phthalate. The two latter solvents have their uses in insect control. For gastric and subcutaneous administration we dissolved D.D.T. in medicinal liquid paraffin and a small amount of tragacanth. About 400 animals were used in these experiments. For skin application of single doses the L.D.50 for our rabbits was 300 mg./kg., for guinea-pigs 1,000 mg./kg., and for rats 3,000 mg./kg. Results were in close agreement when ether and kerosene were used as solvents. For subcutaneous injection the L.D.50 for rabbits was 250 mg./kg., for guinea-pigs 900 mg./kg., and for rats 1,500 mg./kg. Liquid paraffin was the solvent. For gastric administration the L.D.50 for rabbits was 300 mg./kg., for guinea-pigs 400 mg./kg., and for rats 800 mg./kg. Control animals given the largest amount of solvents used in the above tests, but without D.D.T., were unaffected. Results of tests in which dimethyl phthalate and dibutyl phthalate were employed as solvents were substantially in agreement with the above, though we did not carry out subcutaneous tests. Although the L.D.50 levels were definite enough in most cases, a certain number of animals died after doses lower than these levels. Generally speaking, the lowest dose which produced some casualties was about half to two-thirds that of the L.D.50. Even so, it is apparent that the toxicity of D.D.T. when given in solution by various routes as single doses is not high.

Repeated Administration of D.D.T.—Repeated skin application of D.D.T. has been carried out with various solutions and emulsions of D.D.T., with the dry powder, alone or mixed with pyrophyllite, and through the agency of impregnated cloth. The effect of repeated gastric administration has also been investigated, and some experiments in which animals were frequently exposed to heavy mists of D.D.T. in chambers were performed. Precautions against licking contaminated skin were always taken.

1. Solutions or Emulsions

(i) 10% Kerosene Solution.—Five rabbits were given daily skin applications of 100 mg./kg. D.D.T. as a 10% solution in kerosene; 5 guinea-pigs and 5 rats received 200 mg./kg. All of the rabbits were dead after 6, the guinea-pigs after 12, and the rats after 14 applications. All showed clinical features of D.D.T. poisoning and severe inflammation of the skin.

(ii) 1% Kerosene Solution.—Five rabbits, 5 guinea-pigs, and 5 rats were given daily applications, each 2 hours in duration, of 50 mg./kg. D.D.T. as a 1% kerosene solution. No rabbit survived more than 16 applications, no guinea-pig more than 18, but only one rat died during a series of 20 applications. Nervous symptoms were uncommon, but fatal cases showed severe liver damage. Skin irritation occurred in all. Similar groups of rabbits, guinea-pigs, and rats received daily applications of 10 mg./kg. D.D.T. Rabbits survived 20 such exposures without clinical signs of intoxication, and no pathological changes were found when they were killed off. Skin irritation, however, was present. Two guinea-pigs died after 11 applications, both showing moderate liver damage. The other three received 14 applications without ill effects. None of the rats were affected by 14 applications. Control experiments with kerosene alone showed that skin irritation developed and sometimes animals died from this. It is obvious that kerosene may have accounted for some of the deaths in the above experiments. This was more especially the case with rabbits.

(iii) 1% Ethyl Alcohol, Acetone, and Ether Solutions.—Groups of 5 rabbits, 5 guinea-pigs, and 5 rats received daily applications, each lasting 2 hours, of 10 mg./kg. D.D.T. as 1% ethyl alcohol, acetone, or ether solution. With ethyl alcohol as solvent, 2 of 5 rabbits died after 9 and 12 applications respectively; with acetone as solvent, 1 of 5 rabbits and 2 of 5 guinea-pigs died after 7, 7, and 9 applications respectively; with ether as solvent, 1 of 5 rabbits and 2 of 5 guinea-pigs died after 13, 7, and 8 applications respectively. No rats died during the experimental period of 14 applications. All fatal cases showed moderate or severe liver damage but no nervous symptoms.

(iv) Emulsions.—(*a*) Emulsion I (Geigy), composed of 15 parts D.D.T., 30 parts toluene, 15 parts hexamethylenetetramine, 36 parts sulphonated castor oil, and 4 parts ammonia, was diluted 1:100 with water. Five rabbits, 5 guinea-pigs, and 5 rats received 14 daily applications each of 5 c.cm./kg. diluted emulsion (about 7.5 mg./kg. D.D.T.). Two guinea-pigs died from intercurrent infection after 14 applications. No clinical features of D.D.T. intoxication were seen in any of the animals, and the skin at the site of application was not inflamed. At the termination of the experiment all animals were killed and their main organs examined microscopically, but no pathological changes were found.

(*b*) Pymulso concentrate Mark X, containing 1% w./v. D.D.T. and about 0.2% pyrethrins, diluted 1:10 with water, was applied on 12 occasions in daily doses of 5 c.cm./kg. to the skin of 2 rabbits, 4 guinea-pigs, and 6 rats without giving rise to any evidence of skin irritation, intoxication, or pathological change in their organs.

(*c*) Pymulso concentrate Mark XI, containing 2% w./v. D.D.T. and 0.4% pyrethrins, diluted 1:20 with water, was applied in daily doses of 5 c.cm./kg. on 12 occasions to 2 rabbits, 4 guinea-pigs, and 6 rats. No ill effects were observed in the skin or organs when the animals were killed at the end of the experiment.

(*d*) Pymulso special, containing 0.5% w./v. D.D.T. and 0.02% w./v. pyrethrins, was applied on 5 occasions in doses of 5 c.cm./kg. (25 mg./kg. D.D.T.) to the skin of 5 rabbits and 5 rats. Although no symptoms of D.D.T. intoxication developed, the skin became severely irritated. Experiments were discontinued at this stage. Microscopical examination of the main organs disclosed no pathological change attributable to D.D.T.

(*e*) Special pymulso concentrate, containing 10% w./v. D.D.T. and 0.4% w./v. pyrethrins, diluted 1:20 with water, was applied on 15 occasions in doses of 5 c.cm./kg. to the skin of 5 rabbits and 5 rats. Each application, equivalent to 25 mg./kg. D.D.T., lasted 2 hours, after which the emulsion was removed with water. No inflammation of the skin developed, and the animals appeared normal throughout the experimental period. They were killed and their organs examined microscopically, but no pathological changes attributable to D.D.T. were found.

It is apparent from these experiments that the manner of preparing the D.D.T. suspensions is important for toxic effects and that some of the pymulso suspensions are more suitable than others.

2. D.D.T. Powder

Groups of rabbits, 4 animals in each group, were given 16 daily applications of 50 mg./kg. and 10 mg./kg. D.D.T. powder on the shaved skin of the back. Each application lasted two hours. During the experimental period no symptoms developed, there was no loss of weight, and the organs and skin showed no pathological changes when examined microscopically at the end of the experiment. Three rabbits, 3 guinea-pigs, and 3 rats received 9 daily applications, each of 2 hours' duration, of dry 5% D.D.T. in pyrophyllite, in doses of 200 mg./kg. (i.e., 10 mg./kg. D.D.T.) for rabbits and guinea-pigs, and 500 mg./kg. (i.e., 25 mg./kg. D.D.T.) for rats. No skin irritation developed. The animals appeared normal throughout the experimental period, and when killed showed no pathological changes in their organs. Two rabbits, 2 guinea-pigs, and 2 rats received similar treatment, the powder being wetted on application to the skin. No ill effects developed.

3. Impregnated Cloth

Lint was impregnated with 0.5% D.D.T. in benzene and allowed to dry. Portions of this lint containing known amounts of D.D.T. were placed in contact with the shaved skin of rabbits and kept there for 7 days by means of strapping. Five rabbits were so exposed to a dose of 100 mg./kg. D.D.T., five to 50 mg./kg., five to 25 mg./kg., and five to 10 mg./kg. The skin at the site of application of impregnated lint remained healthy. No symptoms of D.D.T. intoxication developed and no pathological changes were found in the organs when the animals were killed at the end of the 7 days.

Repeated Oral Administration of D.D.T.

Ten rabbits and 10 rats were given daily doses of 50 mg./kg. D.D.T. by stomach tube in the form of a 5% suspension in liquid paraffin and tragacanth. Symptoms of D.D.T. intoxication appeared in 8 rabbits, one showing marked tremors after 4 doses (total of 200 mg./kg. D.D.T.), one after 5 doses (total of 250 mg./kg.), two after 9 doses (total of 450 mg./kg.), one after 13 doses (total of 650 mg./kg.), and three after 16 doses (total of 800 mg./kg.). Four animals died after 8, 10, 11, and 20 doses respectively. The remaining 6 rabbits were killed when they had each received 20 doses—i.e., a total of 1 g./kg. D.D.T. Microscopical examination of the organs from all of the rabbits showed slight to moderate liver necrosis in 8, most marked in the 4 animals that died. In all cases necrotic areas presented signs of resolution and liver-cell regeneration without any fibrosis. Slight tubular degeneration occurred in the kidneys of these 8 animals, with calcification in one only. Other organs were unaffected. Rats proved much more resistant, for none of the group of 10 died during a period of 30 doses—i.e., they survived a total dosage of 1.5 g./kg. D.D.T. Moderately severe intoxication appeared in 4 rats after the 28th dose (total of 1.4 g./kg. D.D.T.). All were examined microscopically. No pathological changes were found in any of the organs; the animals showing signs of D.D.T. intoxication presented no abnormality, strange to say.

Repeated Exposure to D.D.T. "Mist"

Thick "mists" of D.D.T. were put up in a cubic-metre chamber by spraying, with an air-driven mechanical sprayer, 15 g. of D.D.T. in about 30 c.cm. of acetone. This gave a nominal concentration of about 1:1000 D.D.T. at the start of the experiment. Four rabbits, 5 guinea-pigs, and 10 rats were exposed to this mist for 2 hours on 11 occasions during 14 days. Two rabbits died after 8 and 9 exposures, one guinea-pig after 9 exposures and four after 10 exposures, one rat after 4 exposures, three after 5, two after 6, one after 8, two after 9, and one after 10. All showed clinical evidence of D.D.T. poisoning. Evidently rats and guinea-pigs are more sensitive than rabbits to this form of intoxication. But such exposures are severe and probably well out of proportion to practical affairs. A further experiment was carried out in which 5 rabbits, 5 guinea-pigs, and 5 rats were exposed under similar conditions for 2 hours daily on 6 separate occasions to a mist produced by spraying 5 g. of D.D.T. in 20 c.cm. of acetone. This corresponded to an initial concentration of about 1:3000 D.D.T. No symptoms of intoxication developed and no pathological changes were found in the organs when the animals were killed at the end of the experiment.

Clinical Features and Pathological Changes in D.D.T. Poisoning

With large single doses of D.D.T. administered by any route the first signs of intoxication appear in 12 to 24 hours. The animal is cold to the touch, its fur is ruffled, and diarrhoea may be present. It seems to be nervous and very sensitive to stimuli. Muscular weakness sets in about this time, starting in the muscles of the back and soon involving the hind limbs. Fine and then coarse tremors develop in these regions, the animal shaking violently for hours on end. Movement becomes restricted, staggering, often spastic. The forelimbs are seldom affected, so that the animal can partly support itself or even drag its immobile hind quarters about. Anorexia leads to a rapid loss of body weight. Death may occur in 24 to 48 hours or be delayed for several days. Respiration fails, but the heart continues to beat until the end and sometimes for a few minutes after breathing ceases. Convulsions are rare. In animals that recover, nervous and muscular signs may develop and last for some days, eventually disappearing without apparent after-effects. No evidence of permanent damage has been seen in such cases.

Pathological examination of acute fatalities reveals little change in the organs. Pulmonary oedema is frequent, and a terminal manifestation. Mild or moderate damage of the liver and kidneys, similar to that found after repeated administration of D.D.T., may be present, but is not constant. Occasionally

the heart muscle contains more fat in its fibres than is normally the case, and the adrenals may be haemorrhagic. A careful examination of the central nervous system has failed to convince us of specific disturbances, even in the most severely affected animals. Degeneration (chromatolysis, vacuolation, pyknosis) and sometimes destruction of a few anterior horn cells in the thoracic and lumbar regions of the spinal cord have been noted, but are not pronounced. The axons of the cord appear unaffected and the higher levels of the brain and brain stem show no changes. Prolonged search of many sections stained by special histological methods has proved of no assistance in locating the seat of nervous disturbance.

With repeated administration of D.D.T. there are loss of body weight, anorexia, and sometimes diarrhoea. When doses are small nervous symptoms do not develop, but with moderate doses tremors and weakness may come and go. Hypersensitiveness to sounds and other stimuli may be striking. Some animals apparently recover from these effects only to die at a later stage; others continue to shake until the end. Death in most instances is quiet, and apparently the result of exhaustion. A different pathological picture is seen, the liver being severely damaged. Numerous areas of focal necrosis or large areas of centrolobular necrosis are uniformly distributed throughout the organ (Fig. 1).

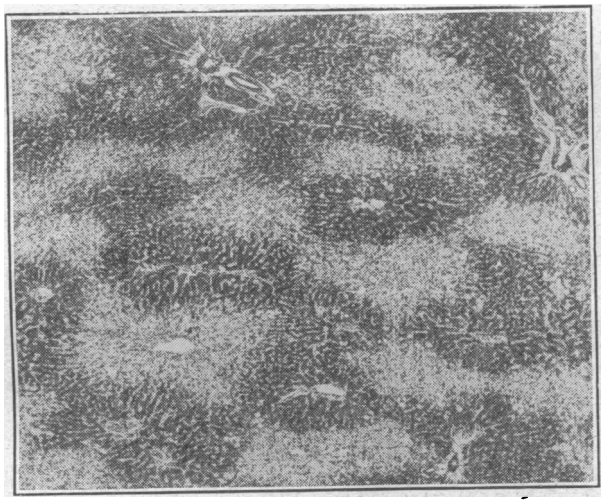

FIG. 1.—Liver of guinea-pig which received daily skin applications of D.D.T.—50 mg./kg. in kerosene—over 9 days. The dark areas are normal liver cells, the pale areas centrolobular necrosis. H. and E. (×30.)

There is much fatty degeneration, with cloudy swelling of liver cells surrounding the necrotic areas. Bile ducts are not affected. Polymorphonuclear leucocytes infiltrate the dead tissue in the early stages; later, mononuclear cells may be numerous. The degree of liver injury is sufficient to account for death. In less severe cases, and especially if D.D.T. be discontinued, necrotic material is removed in the course of a week or two by autolysis and solution as well as by phagocytic activity. Repair is complete, no fibrosis developing even when exposure to D.D.T. is prolonged. Calcification is sometimes seen in some of the necrotic areas. The kidneys, too, are affected, but renal damage is slight compared with that in the liver. Tubule cells show degenerative changes, including fatty deposition and calcification (Fig. 2); sometimes portions of the tubules are necrotic, but glomeruli escape, being congested at the most. The guinea-pig is especially susceptible to calcification. Casts appear in the collecting ducts and Henle loops, but are not numerous. The heart often undergoes mild or moderate fatty degeneration of its muscle fibres, and small focal areas of necrosis have been seen, sometimes infiltrated with polymorphonuclear leucocytes and occasionally undergoing calcification (Fig. 3). Similar necrotic and infiltrated areas have been rarely met with in voluntary muscles. Apart from the more or less rare occurrence of adrenal cortical haemorrhage, the other organs are not affected. A close study of the thyroid gland has failed to convince us of any significant alteration in its structure, but hydropic degeneration and destruction of some of the "water-clear" cells of the parathyroid have been detected during the early stages.

Repeated skin application of D.D.T. in kerosene or in some emulsions may lead to acute inflammation with destruction of the epidermis in places, much oedema of the subcutaneous tissues, and sometimes haemorrhage. Repair follows rapidly when D.D.T. is discontinued. Changes in the blood picture are induced by a large skin application of D.D.T., but are slight with repeated small doses. The haemoglobin percentage tends to decrease, but the red cell count remains unchanged when rabbits are given a large skin exposure. These findings indicate a mild secondary anaemia toxic in origin. Abnormal red cells are not found in the blood. Leucocytosis is also pronounced, starting on the second or third day after exposure usually, neutrophilic cells being mainly concerned. We have noted that animals showing such a response are those which give clinical features of D.D.T. intoxication. In other words, leucocytosis is an intimation that the toxic level is being reached. For that reason it is a useful warning of approaching danger. No characteristic features have been found in the bone marrow of such animals beyond signs of increased white cell production.

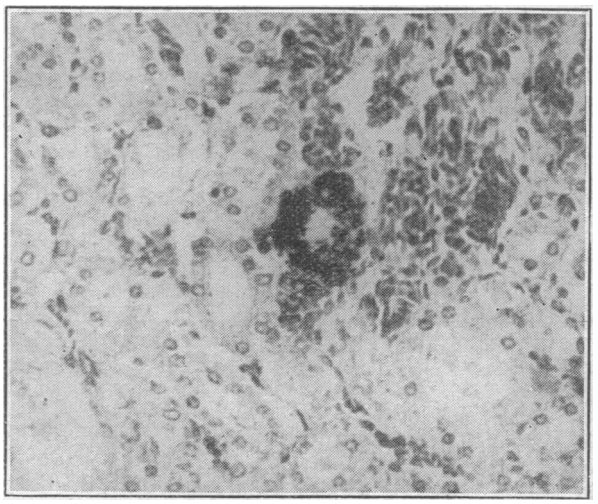

FIG. 2.—Kidney of guinea-pig treated as Fig. 1. The dark annular mass at the centre of the field is a necrotic tubule which has calcified. Near by are necrotic tubules infiltrated with leucocytes and mononuclear cells. v. Kossa and neutral red. (× 350.)

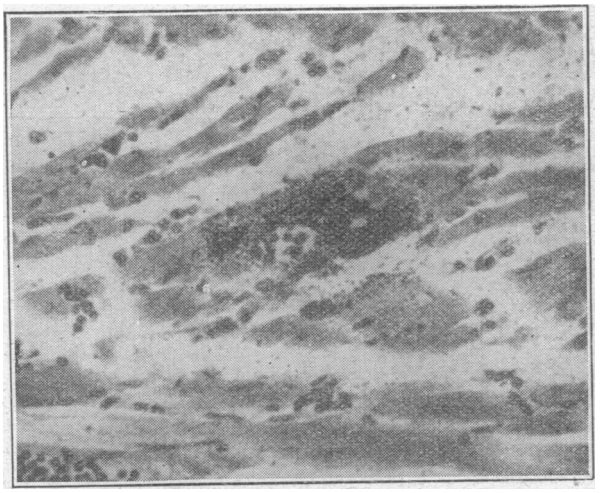

FIG. 3.—Heart muscle of guinea-pig treated as Fig. 1. The dark mass at the centre of the field is a necrotic area of muscle which has calcified. v. Kossa and neutral red. (× 350.)

In rabbits exposed to large skin doses of D.D.T. we have obtained evidence of a rise in blood calcium values. Of seven rabbits investigated for 2 to 12 days after skin application of 200 mg./kg. D.D.T. as a 10% kerosene solution, all showed increase in blood calcium during the first 2 or 3 days, followed by a transient fall below normal levels in most cases. Animals

with nervous disturbances showed the most striking changes in blood calcium, although the calcium increased in some animals which were free from nervous signs. We have also obtained similar, though not so pronounced, results in several rabbits of a group of 5 given repeated small skin applications of D.D.T. The increased blood calcium values did not persist, however.

Effect of a Single D.D.T. Contamination on the Healing of Skin Wounds

It is of interest to know whether D.D.T. contamination might influence the healing of wounds, since this possibility might arise in soldiers wearing garments impregnated with D.D.T. We have employed the method described by Young, Fisher, and Young (1941) for the study of healing of experimental wounds. Thirty unselected male rabbits, average weight 2 kg., were given standard circular skin wounds, 1 cm. diameter, on their shaved backs. Immediately after operation the animals were segregated into three groups of 10. Group 1 served as a control, the standard wounds being untreated apart from applying dry dressings. Group 2 rabbits had their wounds sprinkled with 10 mg. of D.D.T. powder immediately after operation: a sterile spatula was used to distribute the D.D.T., which covered the wound almost completely. Group 3 constituted a further control, 10 mg. kaolin being spread over the wound surface. This experiment was an attempt to control the physical property of a powder which must come into consideration when dry D.D.T. is used; it turned out to be a failure, because the kaolin apparently exerted effects on the healing of the wounds. Sepsis was not seen in any animal, and healing went on uninterruptedly. On the third day after operation tracings of the wound outlines were made on "cellophane," and the surface area was estimated in square millimetres. This was repeated at intervals until healing was complete as judged by epithelization of the wound surface and separation of any scab. The mean daily rate of healing was estimated for the period between the third day and completion of healing. Results are summarized in the table together with the statistical constants.

Table showing Mean Daily Rate of Wound Closure in Rabbits

	Number of Rabbits (n)	Mean Daily Rate of Wound Closure (sq. mm. per day)	Standard Deviation (σ)	Standard Error of Mean ($\frac{\sigma}{\sqrt{n}}$)
Control group ..	10	7·56	2·30	0·77
D.D.T. group ..	7	8·31	1·74	0·70
Kaolin group ..	9	5·02	0·66	0·23

Three of the rabbits of the D.D.T. group and one of the kaolin group died from a pasteurella infection which is not uncommon in our stock.

It can be shown that there is no significant difference between the mean daily rate of closure of wounds in the control (untreated) group and the D.D.T. group. Wounds given one application of D.D.T. powder immediately after their production heal at the same rate as uncontaminated wounds.

OBSERVATIONS ON HUMAN SUBJECTS

Observations were made on human subjects, (1) wearing undergarments impregnated with D.D.T., or (2) working in contact with D.D.T. The first group comprised 52 soldiers, the second 6 workers who were in daily contact with D.D.T. under laboratory or workshop conditions. These trials were carried out in the late autumn and early winter of 1943.

Trials with Impregnated Undergarments

(i) Twelve soldiers volunteered to wear woollen vests, long woollen drawers, and angola shirts impregnated with 1% D.D.T. (dry weight basis). Each man was examined before the trial began and at frequent intervals thereafter; examination included a careful inquiry into past and family history, the present state of health, and physical examination with full investigation of the blood picture and urine. The two latter were repeated at least once, either completely or in part. The men carried out partial military duties during the period of the trial, and on numerous occasions underwent severe exercise to induce copious sweating. The garments were worn throughout the day and night for 18 to 26 days. No symptoms of absorption of D.D.T. developed and the blood and urine remained unaltered. No loss of weight occurred. Two men showed slight dermatitis of the axillary and calf regions, but in both cases, despite the impregnated clothing being worn continuously, this disappeared within 3 days when treated with calamine cream. It does not seem likely that D.D.T. was responsible for the skin irritation, especially as one subject volunteered the information that he was susceptible to skin rashes associated with new clothing.

(ii) Forty volunteers were divided into two groups of 20 each. Group 1 wore vests, drawers, and shirts impregnated with 1% D.D.T. (dry weight basis) for 26 days without changing these garments. In no case were there features attributable to absorption of D.D.T., and physical examination at the end of the trial proved negative. One man developed a slight skin rash on the 10th and 23rd days shortly after taking a bath, but it faded rapidly and could scarcely be connected with D.D.T. Another man showed small spots on his arms on the 18th day, but these disappeared in a day or two. A third had slight acne, which was not made worse by wearing the impregnated garments. It was not possible to examine the blood or urine in this group. Group 2 had weekly changes of underclothes (prepared as in the previous groups), so that each man wore four separate lots of freshly impregnated garments, each set for a week at a time. Some of these men did not wear drawers. One man developed a small papular rash on his forearms on the 18th day, but this disappeared by the 21st day. Another man had some acne for a short period. A third showed slight dermatitis of the wrists, but this likewise cleared up rapidly after application of calamine cream. No subject experienced any ill effects suggestive of D.D.T. absorption. On the last day of the trial all seemed perfectly fit. The blood and urine were not investigated.

Contact with D.D.T. in the Laboratory and Workshop

Six male workers in the Chemistry Section, Porton, were kept under observation during a period of 77 days, in which they were more or less actively engaged in work concerned with D.D.T. The work was classified as (1) laboratory scale, (2) bulk impregnation scale. The latter included such duties as making up large volumes of D.D.T. solution, impregnation of clothing, distillation of residues. Protective clothing, gloves, and gum-boots were worn. Contact through spilling occurred at times. There was also exposure to carbon tetrachloride. Two men had fairly severe exposure in the course of bulk impregnation of garments over 14 and 15 days. One, though having no abnormal symptoms and appearing fit, showed a rise in blood calcium from 11.2 mg. per 100 c.cm. before contact to 18 mg. per 100 c.cm. on the 15th day, falling to 13.4 mg. two days after he left off working with D.D.T. The other man remained perfectly well during his exposure period. The four cases with less severe exposures presented no abnormal features. In no instance was there evidence of skin irritation.

These human trials on 52 soldiers wearing undergarments impregnated with D.D.T., and the laboratory and workshop observations, suggest there is little risk of local skin irritation and general systemic effects attributable to D.D.T., even with prolonged exposure. In many of these experiments there were ideal conditions for absorption from hot sweaty skin.

Discussion

Our investigation shows that acute exposure to D.D.T., applied to the skin, injected subcutaneously, or administered by stomach tube, is tolerated in large amounts by a variety of animals. Repeated administration of much smaller doses is also possible, though there is some evidence that cumulative effects may occur. It is nevertheless true that D.D.T. can exert toxic effects. These are characterized by striking nervous signs, especially muscular weakness, incoordination, and widespread fine and coarse tremors, when the lethal level is approached; and severe damage to the liver, which presents evidence of cell necrosis and functional impairment, when much smaller amounts of D.D.T. are repeatedly absorbed into the system. For that reason it is essential to have some idea of toxic levels of the agent, and the factors which may promote its entry into the body. Although it is not known what is the fatal dose of D.D.T. for men, it can be assumed that this lies somewhere within the range of lethality for experimental animals, hence the importance of collecting such toxicological data. Already there exists much information on this subject, which we summarize below, and to which our own experiments contribute. The ultimate decision whether D.D.T. may be safely employed as an insecticide must depend on how and in what amounts it is used, the precautions taken against accidents, and the elimination of conditions which may assist in the absorption of the compound. Each of these factors is corollary to the other, and no one can be neglected.

Careful studies of D.D.T. have been made by several groups of American workers, with whose results we are substantially in

agreement. Woodard, Nelson, and Calvery (1944), using mice, rats, guinea-pigs, rabbits, and chickens, conclude that for oral administration of D.D.T. dissolved in corn oil the L.D.50s are as follows: mouse, 448 mg./kg.; rat, 180 mg./kg.; guinea-pig, > 562 mg./kg.; rabbit, > 400 mg./kg.; chicken, > 300 mg./kg. They stress the fact that its action may be irregular, owing perhaps to irregularities in absorption. Toxic doses were higher for intramuscular, intraperitoneal, and subcutaneous injection. The solvent, too, is important, for toxic doses were higher when D.D.T. was suspended in gum acacia. Toxic effects were more readily obtained with solutions than with suspensions. Diets containing D.D.T. were fed to rats, mice, guinea-pigs, and chickens for 3 days to 20 weeks. Ill effects appeared in rats and mice when the food contained D.D.T. to the extent of 500 parts per million, in guinea-pigs with 1,000 p.p.m., and in growing chicks with less than 500 p.p.m. A wide variation in individual susceptibility was noted. Clinical features and pathological changes were similar to those we have described above.

Draize, Nelson, and Calvery (1944) also studied the effect of the application of D.D.T. to the skin. Powders containing 5% D.D.T. produced no evidence of systemic disturbance or irritation of the skin in rabbits, even when a total amount of 4 g./kg. was applied. In similar acute experiments a 10% solution of D.D.T. in corn oil was applied at dosage levels of 390, 600, and 940 mg. of D.D.T./kg. Apart from slight skin erythema, which persisted about 2 days, no ill effects were experienced. In further experiments rabbit skin-doses amounted to 1.17, 1.8, and 2.82 g. of D.D.T./kg. in dimethyl phthalate and to 0.975, 1.5, and 2.35 g. D.D.T./kg. in dibutyl phthalate. No deaths occurred at any of these dosage levels, although all animals showed symptoms of intoxication, which were very severe at the highest levels. The material, it is true, was poorly absorbed, for approximately 25% of the largest doses could be accounted for on the skin dressings. Subacute experiments were also carried out; 0.5 c.cm. of a 5% suspension of D.D.T. in diethylene glycol monoethyl ether was applied daily for 3 weeks to a 2.5 sq. cm. area of the back of each of 6 albino rabbits. A mild erythema developed during the second week of application, but apparently no toxic symptoms. Ninety-day experiments on rats, guinea-pigs, rabbits, and dogs were performed, using a 30% solution of D.D.T. in dimethyl phthalate. The rabbits, rats, and guinea-pigs were inuncted at dosage levels of 150, 300, 600, and 1,200 mg./kg., the dogs at levels of 300, 600, and 1,200 mg. D.D.T./kg. None of the dogs showed any symptoms of toxicity. Careful microscopical examination of their organs disclosed little change apart from slight to moderate degenerative changes in the livers and some monocellular infiltration of the gall-bladder. We find it difficult to attach much importance to these histological findings. Rabbits, rats, and guinea-pigs appeared equally susceptible to such inunctions. Wide individual variations were encountered. The authors state that inunction of doses as low as 0.5 ml. of a 30% solution of D.D.T. per kg. per day (150 mg. per kg. per day of D.D.T.) may cause death in some cases after 30 days. Some animals survived 6 to 8 doses of 600 and 1,200 mg. D.D.T./kg. We would point out that when these doses are expressed in total amounts of D.D.T. administered the amounts tolerated are impressive, even in the experiment in which 150 mg./kg. was applied for 30 days. Obviously the solvent is highly important in such tests, for in our own tests, with different solvents, we observed deaths after the application of much smaller total amounts of D.D.T. It is a matter of great importance, therefore, to make sure that the solvent does not encourage absorption of the insecticide.

Draize *et al.* report that patch tests in human beings and the daily contact of hands of operators with 30% solution of D.D.T. in dimethyl phthalate produced no evidence of irritation. They also describe moderate leucocytosis in animals and successful attempts to sensitize guinea-pigs to D.D.T. The pathological investigations connected with these experiments are separately described by Nelson *et al.* (1944). The main macroscopic features were scaliness or hyperkeratosis of the skin, a pale liver or one showing darkened centrolobular areas, occasionally slight pitting of the kidneys, pulmonary infection in a few animals, chiefly guinea-pigs, occasionally focal haemorrhages in the gastric mucosa, jaundice in one dog, and slight or moderate atrophy of muscles and viscera due to lessened food intake. The surface of two rabbits' gall-bladders was mottled, with oedema of the wall in one. Microscopical findings fell into two groups: (1) those found regularly, and (2) inconsistent changes. Among the former are included moderately severe liver damage in all species except chicks ("little or no liver damage"); focal necrosis of small segments of voluntary muscle, especially with higher doses; hyperkeratosis of skin; and, in rabbits, slight focal epidermic necrosis. Colloid depletion and epithelial desquamation in the thyroid seemed to be common in dogs, rabbits, and guinea-pigs, but not in mice (only two examined). Inconsistent changes include an increased incidence of "spontaneous" encephalitis and focal nephritis of rabbits, gastric mucosal haemorrhages and necrosis, myocardial necroses, haemolytic pigmentation in the gastro-intestinal tract, bone-marrow hyperplasia, and inanition testicular atrophy. The central nervous system was thoroughly searched for lesions, but with negative results. Some information about the response of large animals to D.D.T. are included in this paper. Three cows, three sheep, and one horse received 100 to 200 mg./kg./day of D.D.T. for 3 weeks (one week for one cow and one sheep), either mixed with the food or in capsules when the appetite fell off, as usually happened. Two of the cows developed slow tremors or shaking, especially in the hind legs and neck; none of the other animals of this group developed tremors. None died. Slight fatty degeneration of the liver was found in one cow, and very slight focal necrosis of this organ in another cow. One sheep had slight central necrosis of the liver. Other lesions included atrophy of the spleen, probably inanitional, terminal subendocardial haemorrhages, and very slight focal necrosis of voluntary muscles. No other lesions attributable to D.D.T. were found. It will be seen that the significant changes described by these authors closely agree with those we have observed, with the exception of thyroid alteration, which did not impress us.

In a study of the pharmacological action of D.D.T., Smith and Stohlman (1944) give data for toxicity to animals. They assess the L.D.50 for rats and rabbits given D.D.T. in 1 to 5% solution in olive oil by stomach as 150 and 300 mg./kg. respectively. Two cats receiving 100 and 200 mg./kg. survived. Another cat died after 200 mg./kg. Of eight cats receiving 300 mg./kg. 62% died.

Symptoms were typical and similar to those described by other observers. Cats showed persistent extensor opisthotonos with fine and coarse muscular twitchings, especially of the muscles of the head and neck, lasting for several days, after single oral doses of 300 mg./kg. Rats fed 1,000 parts per million in a semisynthetic adequate diet containing 18% casein died in from 18 to 80 days, but survived 3 months' feeding with 500 p.p.m. D.D.T., though showing tremors and hyperexcitability. In rabbits daily oral administration of 50 mg./kg. D.D.T. in olive oil resulted in death in from 15 to 23 days after a total dose of 0.75 to 1.25 g. per kg. had been given. Parenchymatous liver degeneration with centrolobular necrosis was the most pronounced finding in these. Mild anaemia developed, but white cell counts were not abnormal. Two cats receiving 50 mg./kg. every day or every second or third day developed the characteristic features of poisoning and died—one within 12 days after a total dose of 500 mg./kg., the other within 15 days after a total dose of 300 mg./kg. A third cat, which received 4 doses of 90 mg./kg. within 10 days, died with typical tremors, ataxia, spasticity, paralysis, and terminal extensor rigidity. Skin absorption occurred when 5% solution of D.D.T. in dimethyl phthalate was applied daily over 12 to 14 days in amounts equivalent to 100 mg./kg. D.D.T. The addition of 10% cyclohexanone to such solution did not increase toxicity. Smith describes a useful method for the estimation of D.D.T. in the tissues, body fluids, and excreta, and gives valuable figures for the organ contents. D.D.T. was found in the urine, blood, liver, kidneys, and central nervous system in experimental poisoning.

Lillie and Smith (1944) report on the pathological findings in the animals studied by Smith and Stohlman. The spinal cord of one cat displayed partial tigrolysis of anterior horn cells with pericellular vacuolation; in another cat Nissl bodies were absent from these cells and pericellular and paranuclear vacuolation present. The liver of cats showed fine fatty degeneration, increasing with longer periods of ingestion of D.D.T. A single focus of coagulation necrosis was found in the liver of one cat. The spleen and kidneys showed no definite changes. Focal

haemorrhages occurred in the lungs of one cat, congestion and serous exudation in another. Rats receiving daily doses by stomach equivalent to two-thirds of the lethal dose showed some myelosis and haemosiderosis of the spleen, cloudy swelling, fine fatty degeneration and hyaline casts in some kidneys, hydropic central fatty degeneration, congestion, atrophy, and isolated necrotic cells with some fibroblastic proliferation in the liver. Partly organizing foci of coagulation necrosis were found in this organ in two animals. The brain of rats, killed 3½ to 5½ hours after large doses orally, presented vacuolation of some nerve cells, tigrolysis, and basophilic reticulation of cytoplasm of cells in tegmentum pontis only. Feeding D.D.T. produced fatty and hyaline change in the liver and inconstant fatty change in the proximal convoluted tubules of the kidneys. Rabbits killed in less than five days showed moderate to finely marked centrolobular fat in the liver, splenic haemosiderosis, and occasionally fatty change in the kidneys. Liver lesions were more severe, including centrolobular degeneration and necrosis, in rabbits repeatedly fed on olive-oil suspensions of D.D.T. (50 mg./kg./day total amounts, 0.9 to 1.3 g./kg./day D.D.T.). The brain and spinal cord showed vacuolation around large neurones. The remaining organs were not affected or showed very slight inconstant changes.

Hazards from the use of aerosols, mists, and dusting powders containing D.D.T. have been investigated by Neal *et al.* (1944). Dogs, rats, and guinea-pigs exposed to initial concentrations of 54.4, 12.44, and 6.22 mg./litre D.D.T. in air for 45 minutes under static conditions apparently develop no toxic signs or symptoms. Mice tolerate 6.22 mg./litre if the aerosol contains only 6% sesame oil, but if the concentration of the latter be increased to 9.5% toxic features are seen. Higher concentrations than 6.22 mg./litre cause symptoms and death in a few hours, with pathological changes in liver, kidney, spleen, and spinal cord. The chronic toxicity of aerosols was also carefully investigated. Two puppies were exposed for 45 minutes to a concentration of 12.2 mg. D.D.T./litre, using 1% D.D.T., 6% sesame oil, and 93% freon, on 2 successive days in one week and on 4 successive days in the following week. They showed no signs of intoxication and gained weight. Their organs were unaffected. Ten mice, covered with gauze to prevent fur contamination and exposed in the same way, showed no toxic symptoms, but one died from an unknown cause 3 days after the last exposure. Ten unprotected mice died with typical symptoms of D.D.T. poisoning. Ten mice protected against ingestion showed slightly delayed and somewhat less severe symptoms than unprotected animals, the majority dying 5 days after exposure. Pathological changes in mice included enlargement and fatty change of liver cells with variable congestion, fatty change in proximal and distal convoluted tubules with occasional hyaline casts and congested glomeruli, haemosiderosis of spleen, chromatolysis, occasionally vacuolation, and karyolysis of many of the anterior motor neurones at various levels of the spinal cord. Daily exposure of 2 monkeys and 10 mice for 45 minutes, 5 days a week, over 5 weeks, to intermittent concentrations of 0.183 mg. D.D.T./litre in air—prepared by dispersion every 15 minutes of 1.5 g. aerosol containing 5% D.D.T., 10% cyclohexanone, and 85% freon—caused no toxic effects. If repeated 3 times daily for 4 weeks mice were affected, but monkeys showed no signs of intoxication. The organs of the monkeys were normal; the mice presented rather indefinite liver changes, some nuclei showing various stages of karyolysis. Two human subjects were kept for 1 hour daily on 6 consecutive days in a sealed chamber, 14,750 litres capacity, in which was dispersed every 15 minutes 10.40 g. aerosol containing 5% D.D.T., 10% cyclohexanone, and 85% freon. Neither showed symptoms. Exposure to 10.40 g. of a similar aerosol dispersed every 5 minutes for one hour daily on 5 consecutive days was likewise without ill effects apart from some irritation of the eyes and upper respiratory tract. Higher concentrations of D.D.T. in air cannot be built up by repeated dispersion of an aerosol, as the D.D.T. settles out so rapidly and sticks firmly to any surface. In other words, the maximal aerosol concentration is tolerated by man. Daily exposure for 3 hours, over 4 weeks, to concentrations of 12.48 mg./cubic metre of pure D.D.T. in air, using 10% D.D.T. pyrax dust, produced no toxic effects in dogs. One dog presented microscopical evidence of healing acute liver necrosis. Mice exposed in the same way to 13.9 mg./cubic metre pure D.D.T. experienced intoxication, possibly due to licking dust from their fur. Daily insufflation of 100 mg./kg. pure D.D.T. 6 days a week for 7 weeks caused toxic symptoms and damage of liver, kidneys, and nervous system in one of 3 dogs after 18 days. A fourth dog receiving 100 mg./kg. for 2 weeks and thereafter 200 mg./kg. in 8 fractions for 5 weeks did not show any definite symptoms, though it lost weight and vomited once. Daily oral administration over 7 weeks of 100 mg./kg. pure D.D.T. in 4 fractional doses caused no toxic manifestations in dogs. Rabbits exposed for 48 minutes daily over 4 weeks to a heavy mist of 1% D.D.T. deobase mixture showed some irritation of the mucous membranes of the eyes, upper respiratory tract, and skin, but no evidence of poisoning.

Neal *et al.* conclude that the use of D.D.T. in 1 to 5% solution in 10% cyclohexanone with 90 or 85% freon as aerosols should offer no serious health hazards when used under conditions required for insecticidal purposes. Powders of D.D.T. containing concentrations up to 10% also present no serious risks owing to the relative insolubility of D.D.T. and the large particulate size of the dust. Sprays of 1% D.D.T. deobase mixture should be safe. Ingestion of massive doses of D.D.T., however, is dangerous.

Conclusion

It is apparent from this summary of American investigations and the results of our own experiments that animal tests, though indicating the toxic features of D.D.T., make it clear there is a wide margin of safety in its use as an insecticide. We have always maintained that, provided a maximum concentration of 0.5% D.D.T. be insisted upon for sprays, there is no reason to anticipate any danger to man. Only gross carelessness would be likely to lead to serious features. Even with long-continued exposure to such sprays it is difficult to see how ill effects would be incurred. On the other hand, we are convinced that men handling higher concentrations should take precautions against skin contamination. Cleanliness is essential, the use of gloves and protective garments advisable. In spraying concentrates the use of respirators is advocated. We are in agreement with other workers that dry powders of D.D.T. present no danger of absorption from the skin. It is only when oily solvents are employed that such risks are likely to arise; and here again there is much variation according to the type of solvent used. Moreover, it is our experience that ample warning of the approach to toxic levels is given in the form of anorexia, muscular weakness, and fine tremors. If at this stage D.D.T. is discontinued complete recovery is the rule in animals. Even when liver damage has developed, a fatal issue is not necessarily inevitable, for the organ may still retain its capacity to regenerate if further D.D.T. absorption be prevented. Difficulty arises when liver degeneration sets in without premonitory symptoms or associated nervous signs and a fatal degree of hepatic insufficiency may be reached without warning. We have learned to attach importance to rapid decrease in body weight in such instances. Other features of value in assessing toxic absorption are the development of anaemia and leucocytosis. A rise in blood calcium may also be suggestive, though it is too inconstant to be of any great diagnostic value.

Animal investigations of a new compound have their value and must of necessity be the first approach in the assessment of toxicity risks, but they can never completely replace observations on man. Such limited data as we have been able to collect, together with the few American tests on human subjects, indicate that under practical conditions there is little reason to expect danger to health. Increasing experience of the handling of D.D.T. in factories, in the field, and in Army services seems to bear out these conclusions. Nevertheless, it must be emphasized that there are risks when carelessness leads to the contamination of body surfaces with concentrated oily solutions of D.D.T.

Summary

An experimental investigation of the toxicology of D.D.T. on a variety of animals is described, together with observations on human subjects exposed for some time to this compound.

D.D.T. is tolerated in fairly large amounts when administered as single or repeated doses. Toxic levels are not easily reached when dilute solutions suitable for insecticidal purposes are employed. Danger to health is likely to arise only from careless use of concentrates.

Prolonged contact with undergarments impregnated with D.D.T. has not produced any local or general disturbance in human subjects.

D.D.T. poisoning is characterized by nervous symptoms and the development of severe damage to the liver. Premonitory symptoms and changes in the blood picture give warning of the onset of the toxic stage.

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REFERENCES

Draize, J. H., Nelson, A. A., and Calvery, H. O. (1944). *J. Pharmacol.*, **82**, 159.
Lillie, R. D., and Smith, M. I. (1944). *Publ. Hlth. Rep. Wash.*, **59**, 979.
Neal, P. A., von Oettingen, W. F., Smith, W. W., Malmo, R. B., Dunn, R. C., Moran, H. E., Sweeney, T. R., Armstrong, D. W., and White, W. C. (1944). Ibid., Supp. No. 177.
Nelson, A. A., Draize, J. H., Woodard, G., Fitzhugh, O. Garth, Smith, R. Blackwell, jun., and Calvery, H. O. (1944). Ibid., **59**, 1009.
Smith, M. I., and Stohlman, E. F. (1944). Ibid., **59**, 984.
Woodard, G., Nelson, A. A., and Calvery, H. O. (1944). *J. Pharmacol.*, **82**, 152.
Young, J. S., Fisher, J. A., and Young, M. (1941). *J. Path. Bact.*, **52**, 225.

ACUTE POISONING DUE TO PETROL VAPOUR

BY

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On July 3, 1942, it became necessary, for security reasons, to place a charge of explosive in the vicinity of a large storage-tank used for aviation spirit. A boring had been made alongside the tank a month previously, the last 12 feet being completed on the day of the accident. Into it a workman descended, and as petrol had been leaking from the tank into the boring he wore an oxygen-breathing apparatus. This had been carefully tested beforehand and found to fit accurately. About 10 minutes after he had descended those above saw that he was behaving oddly. He was standing at the bottom of the boring, holding on to a cross-bar and reeling drunkenly. His mate, who was near by, was immediately informed, and, though unprotected from the fumes, he promptly climbed down in the pit to render assistance. Arrived at the bottom, he could see that petrol was floating on some water there, and the air was filled with the fumes, so that his eyes smarted. He called for a rope and started to heave his mate, who was heavier than himself, laboriously from one horizontal strut to another up the vertical sides. After the first minute or two he could scarcely breathe owing to a sense of constriction in his chest, and he felt sick and began to retch. Nevertheless he persisted doggedly and had heaved his mate on to the top strut, within reach of the helpers above, when he was overcome by dizziness and fell to the bottom again. The shock of the fall brought him to his senses, and he once more began to climb. He had again reached the top strut, when he completely lost consciousness. This time, fortunately, he was seized from above and pulled to safety.

Case I

When the first workman had been lifted out it was found that he had ceased to breathe, and artificial respiration had to be employed for half an hour before normal breathing could be restored. The patient then became restless and violent and had to be given half a grain of omnopon to keep him quiet during transit to hospital. On admission there, soon after, he was still semiconscious, but resisted attempts at examination. He was cyanosed and his respirations were slow and shallow. The pulse was strong—75 a minute—and there was no fever. He was given continuous oxygen with a B.L.B. mask, and in a few hours had regained consciousness. Next day he complained of discomfort in the right iliac fossa, and examination revealed tenderness in this region. He was now perfectly rational and showed no cyanosis. Examination of the cardiovascular and respiratory systems revealed no abnormal physical signs. The urine contained no albumin or sugar, nor were abnormal amounts of lead detected in urine or faeces. A blood film showed no punctate basophilia.

By the fourth day he felt well, apart from slight residual discomfort and tenderness in the right iliac fossa, which persisted till the 12th day. He was discharged from hospital on the 14th day and remained well subsequently. There had been no sequelae when he was heard of two years later.

Case II

The second workman had also stopped breathing when carried to a place of safety. Spontaneous breathing, however, returned after artificial respiration had been applied for five minutes, and he regained consciousness shortly after. He then complained of a choking sensation and a feeling as of a ball in his chest, and felt that he could not take a deep breath. In the ambulance on the way to hospital he had several attacks of abdominal pain. These had become very severe when he reached hospital and were recurring frequently, being associated with much restlessness and excitement. A third of a grain of morphine failed to give relief, so that evipan was injected slowly into a vein till a quiet sleep was procured. In a few minutes, however, he was again restless, and became so violent that it required four people to hold him down. Further evipan had to be given, and this produced an adequate depth of sleep. Next day the pain had returned, but was less severe, and was readily controlled by morphine. There was also retention of urine, requiring catheterization, and headache was still severe.

On examination he was perfectly rational. His pupils were small but equal and reacted to light. The knee- and ankle-jerks were equal and active, and the plantar responses flexor. There was marked abdominal tenderness and guarding, especially in the epigastric region. Examination of the alimentary system proved otherwise negative, and no abnormal signs were found in the cardiovascular or respiratory system. On flexion of the cervical spine there was complaint of pain in the chest. The temperature, pulse, and respiration rates were unaffected. There was no albumin or sugar in the urine, and no abnormal amounts of lead were detected in urine or faeces. A blood film showed no punctate basophilia. The cerebrospinal fluid was clear and colourless, had a pressure of 150 mm., with protein 36 mg. per 100 c.cm., and no increase of globulin or cells.

Attacks of abdominal pain requiring morphine for relief continued till the fourth day, headache being troublesome up to the third day. A urinary infection then supervened, but this responded rapidly to sulphanilamide. The patient was discharged from hospital on the 18th day, having made a complete recovery. A cough developed soon after discharge and persisted for a year. When seen two years later he was in excellent health.

Factors in Toxicity of Petrol

Originally motor spirit was derived entirely from petroleum by simple distillation. From the crude petroleum the portion distilling up to 150° C., and known as raw benzine or naphtha, was first separated, and from this, by re-distillation, petrol boiling at 50–140° C. and pure benzine boiling at 120–150° C. were obtained. Petrol so produced is known as straight-run petrol, and consists largely of pentane, hexane, and heptane. Petrol is also obtained by "cracking" or splitting up the higher paraffins in the petroleum; it then contains olefines as well. The hydrocarbons in these two types of petrol are subject, when used as a motor spirit, to "knocking," or detonative explosion with air. Petrol containing a high proportion of octane is less prone to this fault. As neither crude nor cracked petroleum yields petrols containing an appreciable amount of octane, this is made by the re-forming of either "cracker" gases or straight-run petrol into branched-chain hydrocarbons such as iso-octane (2 : 2 : 4-trimethylpentane) by processes of catalytic combination. Such processes are used particularly in the production of aero spirit. Unfortunately the toxicity of the aliphatic hydrocarbons increases in proportion to the specific gravity and boiling-point (Tschernikow *et al.*, 1935). N-octane, for example, is seven times as toxic as pentane. It is possible that the iso-octane in aero spirit may not be quite so toxic as n-octane—as dimethyl hexane, for instance, is only three times as toxic as pentane. Petrol produced by "cracking" petroleum has a toxicity similar to that of straight-run petrol (Lazarew, 1929).

Aero spirit was the toxic agent in the cases here described. It has a 10% evaporation point of 75° C., whereas in ordinary winter motor spirit the 10% evaporation point is 52° C. The final boiling-point is 180° C., as compared with 140° C. for motor spirit. Like the latter, it may contain a small percentage of benzene and other aromatic hydrocarbons, and these are of still greater toxicity. Benzene, or benzol, which is normally obtained from coal-tar distillation but is also present in certain petroleums, particularly those from Borneo, Rumania, and Galicia, should not be confused with benzine, which comes solely from petroleum, as already described. As benzine and