THE CHRONIC TOXICITY OF *p*-NITROPHENYL DIETHYL THIOPHOSPHATE (E. 605)

A LONG-TERM FEEDING EXPERIMENT WITH RATS

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(With Plate 15)

INTRODUCTION

This paper describes a study of the chronic toxicity of the insecticide *p*-nitrophenyl diethyl thiophosphate, also known by the symbol E.605 and a legion of trade names including 'Parathion'. The insecticide is widely used in this country and abroad. It is highly toxic, and casualties have been reported amongst those making the compound and formulating it into dusts and concentrates and amongst those applying these mixtures in the field (Grob, Garlick & Harvey, 1950). This investigation is more concerned with the hazard to the consumer of fresh fruit and vegetables that have been sprayed shortly before harvesting. E.605 has been detected by chemical analysis in peas, apples, pears, strawberries and other comestibles, and levels of 0·01–0·20 parts per million have been reported (Barnes, Carman, Ewart & Gunther, 1950; Westlake & Fahey, 1950; Walker, 1950; Carman, Ewart, Barnes & Gunther, 1950). The present work is an attempt to find whether such levels of E.605 are dangerous or innocuous.

The acute and subacute toxic effects of E.605 on laboratory animals was investigated by DuBois, Doull, Salerno & Coon (1949), and it is now clear that E.605 behaves like a cholinergic drug by inhibiting the enzyme cholinesterase. Poisoning by E.605 leads to the symptoms of lachrymation, salivation, intestinal colic, muscular twitchings and respiratory embarrassment. There have been a number of scattered references to the tissue changes produced in acute poisoning by E.605 and other anticholinesterase drugs in experimental animals, and lesions have been described in almost every tissue of the body (Forssling, 1948; Jones & Landing 1948; Sallé, 1950; Valade, 1950). Most of these reports have not been confirmed in this laboratory (Denz, 1951), but instead, lesions have been found in the salivary and lachrymal glands and the acinar portion of the pancreas. Other changes were seen in the thymus and spleen. These histological changes are incidental rather than lethal, and death is probably due to failure of transmission of nervous impulses at cholinergic synapses including the myoneural junctions.

A passing reference to the chronic toxicity of E.605 has been made by Lehman (1949), who states that, when rats were fed a diet containing 25 p.p.m. of E.605, toxic symptoms were observed. Hazleton & Holland (1950) have reported experiments continued for 2 years in which E.605 was added at the rate of 10, 25, 50 and 100 p.p.m. to the diet of rats. Groups of ten rats were used and at 100 p.p.m. they occasionally showed peripheral tremors and irritability during the first few

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weeks, but were later normal. The females were more seriously affected than the males. At 50 p.p.m. the rats were normal throughout the experiment. The results recorded below fall between those in the two American reports and are offered for comparison and in amplification.

METHODS AND MATERIALS

E. 605

The material used* was a sample of a typical commercial preparation of *p*-nitrophenyl diethyl phosphate prepared by reacting thiophosphoryl chloride with sodium ethylate. An analysis of the sample was as follows:

	%
p-Nitrophenyl diethyl thiophosphate	76·8
Triethyl thiophosphate	9·2
Nitrophenol	4·9
Remainder, including traces of the di-*p*-nitrophenyl ester and sodium salts of mono- or diethyl-phosphoric acid	9·1

Rats

These were weanling albino rats, about 6 weeks old at the start of the experiment. The average weights of the rats in each group are given in Tables 3 and 4. The rats were bred at the Animal Farm, Chemical Defence Experimental Establishment, Porton.

Experimental groups

E. 605 was added to the food at five different levels. These levels and the number of rats in each group are shown in Table 1. Six rats were housed in each cage.

Table 1. *Experimental groups*

Group	Amount of E. 605 in diet (p.p.m.)	No. of rats	
		Female	Male
A	100	36	36
B	75	36	36
C	50	36	36
C1	50	36	—
D	20	36	36
E	10	36	36
F	Controls	30	30

Preparation of food

Dilutions of the original E. 605 were made in alcohol to give a stable preparation of E. 605 of appropriate concentration for each group, so that the addition of 0·1 ml. of this solution to 50 g. of dry food powder† would give a final concentration of the

* This was supplied by Messrs Albright and Wilson Ltd., Oldbury, and we are indebted to Mr B. Topley for the information about its composition and mode of preparation.

† Ground rat cubes of the recipe prepared by the Rowett Research Institute and sold by the Northern Agricultural Co-operative, Aberdeen.

active ingredients in the required parts per million. Thus, in practice, 360 g. of food powder were weighed into an enamel bowl. To this 250 ml. of water containing 0·72 ml. of the appropriate alcoholic solution of E. 605 was added. The mash was thoroughly mixed and divided into twelve equal parts for the twelve cages in each group. The amount of food provided varied at different stages of growth of rats and was based on the amount consumed. The figures given above are typical but were varied according to demand.

The control group was fed rat cubes of the same composition as the powder from which the experimental diets were prepared.

Feeding regime

The rats were fed once a day and the food was freshly prepared for each feed. The E. 605 was added to the diet 6 days per week. Each Sunday all groups of rats were fed ordinary rat cubes without the addition of E. 605.

Records

A detailed diary was kept recording amounts of food provided and eaten, the occurrence of symptoms, accidents and deaths, the weights of animals and anything that might seem relevant, including the weather.

An attempt was made to find a cause of death with every animal that died. In the early stages of the experiment many animals died with obvious symptoms of E. 605 poisoning, and this was accepted as the cause of death. Three weeks from the start of the experiments the number of deaths had diminished, and thereafter all animals that died and were not completely eaten by their cage mates were examined and tissues taken for the preparation of histological sections.

Weekly weight records of the rats were kept. These were expressed as the average weight of the rats in each cage of six or less rats.

HISTOLOGICAL METHODS

The tissues taken for examination were brain (three sections), submaxillary gland, cervical lymph nodes, heart, lung, thymus, liver, kidney, adrenal, spleen, pancreas, duodenum, ovary (or testis) and bone marrow.

These were fixed in Helly's solution or Bouin. Most of the specimens of brain were fixed in Carnoy's fluid. All tissues were stained with Ehrlich's acid haematoxylin and eosin.

At the end of the experiment all the survivors of groups A and B were killed and the tissues examined histologically. In groups C, CI, D, E and F a random sample of 20 % was taken from each group for histological examination.

RESULTS

Signs of poisoning were observed in animals in the groups receiving 100, 75 or 50 p.p.m. E. 605. A general lack of liveliness associated with an increased irritability when disturbed suggested poisoning in its mildest form. A careful inspection might reveal almost continuous fine muscular fasciculations. This was considered to be pathognomonic of poisoning.

As the poisoning became more severe the fasciculations became obvious, an muscular tremors involving large muscle groups would develop. At this stag there was a very obvious general muscular weakness and the animals were in capable of any sustained activity. In the final stages the animals became more o less prostrate. Red crusts from dried lachrymal secretions appeared round th eyes and the animals had dirty wet muzzles as a result of excessive salivatior Rats might survive for several days in this condition, and if the drug was with drawn they made a rapid and apparently complete permanent recovery.

The results will be considered group by group.

Group A. 100 *p.p.m.*

Within 2 hr. of receiving the first food containing E.605 one rat was dead an many had symptoms of poisoning. On the second day six animals died. On th third day the rats refused their food and there were no deaths. Six animals wer sacrificed because of injuries. Between the fifth and sixth days the rats began t eat some food and symptoms and deaths recurred. The rats continued to eat les than 5 g. each per day as compared with a consumption of 15 g. per rat by th controls. Symptoms were continuous and deaths occurred every day.

Table 2. *Effect of dose level of E.*605 *on mortality*

Group	Duration of feeding (in days)	No. of rats in group	No. of survivors	Cause of death	
				Poisoning	Other cause
A 100 p.p.m.	19	72	7	58	7
B 75 p.p.m.	27	72	13	53	6
C 50 p.p.m.	365	72	44	26	2
C1 50 p.p.m.	365	36	28	3	5
D 20 p.p.m.	365	72	70	0	2
E 10 p.p.m.	365	72	70	0	2
F Controls	365	60	59	0	1

After 19 days there were only seven survivors (Table 2), all with symptoms an in a very poor condition. Fifty-eight rats had died with the symptoms of E.60 poisoning and seven had been destroyed owing to injuries inflicted by their cag mates. It was decided that nothing further could be learnt by continuing at thi dose level, so the rats were then put on a diet of ordinary rat cubes to see what sor of recovery they would make. The symptoms disappeared within 24 hr.

While the rats were being fed E.605 they gained very little weight as compare with the controls (Table 3). But when removed from E.605 diet they rapidl gained weight and caught up with the control group in 3 weeks. After 9 months c normal diet their weight was very similar to that of control groups, and in thi

respect at least they seemed to suffer no ill effects of their 3 weeks' poisoning. At the end of the year the seven survivors were killed and their tissues examined histologically. Four rats had some degree of bronchiectasis, a condition relatively common in older rats of this strain. No other abnormality was found.

Table 3. *Weights of animals. Average weights (in grams) of control rats and groups A and B*

	Start of Exp.	After feeding E.605 (3 weeks)	After 9 months of normal diet	Gain in wt.
Group A: Females	78	87	225	147
Males	93	117	367	274
Group B: Females	93	96	224	131
Males	106	125	347	214
Controls: Females	100	150	258	158
Males	144	199	421	277

Group B. 75 p.p.m.

Slight symptoms were evident within 2 hr. of adding E.605 to the food and were severe at 24 hr. The first rat died on the third day. By the fifth day the rats were eating much less food and by the seventh day some rats were attacking the weaker cage mates. Six animals were sacrificed because of injuries received in this way. From the second week onwards food consumption was most irregular. Sometimes the food was taken and symptoms were severe and many deaths occurred. On other occasions the food was refused and symptoms abated somewhat.

After 27 days the thirteen survivors were put on a normal diet of rat cubes. At this time fifty-three rats had died of poisoning and six had been killed because of injuries. After 24 hr. on the normal diet all symptoms disappeared and the animals fully regained their appetites. While the E.605 was being fed the rats of group B had gained much less weight than had the controls (Table 3), but after normal feeding was resumed the survivors soon made good their arrears in weight. Nine months after the cessation of feeding E.605 the rats of group B were slightly below the control rats in weight.

Most of the rats dying in the first week were not examined histologically, since the symptoms left no doubt that death was due to E.605 poisoning. Four females and one male dying between the fourteenth and nineteenth day were examined histologically. In all five cases the vacuolation characteristic of E.605 poisoning was found in the submaxillary glands and pancreas.

These changes have been described and illustrated in a previous paper (Denz, 1951). Atrophic changes were found in the spleen and thymus, and these were more advanced than in acute poisoning where symptoms had lasted only a few hours, or at the most a few days. The spleen in poisoned rats (Pl. 15, fig. 1) is small. The lymphoid nodules of the white pulp has almost disappeared, as shown by comparison with the spleen of a normal rat (Pl. 15, fig. 2). The white pulp persists as blood vessels and connective tissue. The lymphocytes of the red pulp are also reduced. The thymus shows a considerable reduction in lymphoid tissue. (Pl. 15, fig. 3). The thymus from a normal rat (Pl. 15, fig. 4) has a well-developed cortex and

medulla. In chronic poisoning with E.605 the distinction between cortex and medulla is lost, there is an overall reduction in lymphocytes and reduction in the size of the thymus. Pl. 15, fig. 3, also shows that lymph nodes, even when in intimate contact with the atrophic thymus, do not undergo any marked retrogressive change.

A year after the start of experiment, the thirteen survivors who had then been on a normal diet for over 11 months were killed and their tissues taken for histological examination. The only abnormal findings were bronchiectasis in one rat and a few small focal lesions in the cortex of the kidneys in another.

Group C. 50 *p.p.m.*

On the fifth day of the experiment some of the rats refused to eat, and on the seventh day the first symptoms appeared and three rats died. The rats continued to eat very little food; twenty-four rats died during the second and third weeks but no deaths occurred thereafter, although symptoms persisted. The symptoms tended to disappear on Sunday when the animals were fed ordinary rat cubes without any E.605, to be slight on Monday and increase in severity during the week, until the week-end again brought some relief. The severity of the symptoms varied from cage to cage and from day to day, but during the first 2 months of the experiment symptoms could always be observed amongst some of the animals in this group. During the third month symptoms decreased in severity and frequency. After the third month the survivors were usually free from symptoms, but symptoms reappeared occasionally in all cages of this group, especially in the later stages of the experiment.

Forty-four rats were alive at the end of a year's feeding with E.605. Of the twenty eight fatalities, twenty six were the result of poisoning, the diagnosis being reached in the case of fourteen rats on the basis of symptoms observed immediately before death. In fourteen animals the diagnosis of E.605 poisoning was based on histological findings. If the histological changes regarded as characteristic of E.605 poisoning were found in any two of the four tissues, submaxillary gland, pancreas, thymus and spleen, this was considered to be diagnostic. Of the two animals dying of causes other than poisoning, one was destroyed because it was suffering from middle-ear disease, and the other died of bronchiectasis and associated renal abscesses. At the conclusion of the experiment after a year of feeding E.605 nine animals were killed and examined histologically. One animal had bronchiectasis, but all the others were normal. The submaxillary glands, pancreas, thymus and spleen were examined particularly and did not show any acute or chronic lesions. Any changes that may have occurred at times when the animals were showing symptoms must have returned to normal before the animals were killed.

The group C rats failed to gain as much weight as did the controls or groups D and E (Table 4). This applied to both males and females, and could not be attributed to decreased intake of food. After the first 3 weeks, during which the group C rats consumed 5 g. less food per day per rat than the controls, the appetite of the group C rats improved and thereafter they ate as much as the control rats. The discrepancy in weight between group C rats and control rats was continuous and

steadily progressive over the year; and was not due to any single period in the experiment.

As the 50 p.p.m. dose seemed to be the borderline between toxic reactions and normality a second group of thirty six female rats (group C1) were fed at this level to provide a breeding stock of chronically poisoned rats. Some symptoms were recorded on the second day, but were not common or severe until the tenth day. One rat died during the fourth week and another in the fifth week. Symptoms occurred until the seventh or eighth week and then decreased in incidence and severity. The animals were sometimes ruffled and apprehensive and an occasional rat had tremors.

Table 4. *Average weights (in grams) of control rats and of groups C, C1, D and E*

		Start exp.	After 12 months	Gain
Group C:	Females	105	214	109
	Males	118	322	204
Group C1:	Females	98	212	114
Group D:	Females	101	253	152
	Males	111	381	270
Group E:	Females	103	271	168
	Males	107	416	309
Controls:	Females	100	253	153
	Males	144	406	262

The group C1 rats also failed to gain as much weight as the controls (Table 4). This failure was not associated with decreased food intake and was not related to any skeletal or other deformities. At the end of the experiment a random sample of eight of the animals were killed and examined histologically. Three had bronchiectasis but no other abnormalities were found.

Group D. 20 *p.p.m.*

These rats were fed for a year without showing any symptoms or other untoward effect. One rat died in the second week of the experiment of bronchiectasis and bronchopneumonia. Another rat died in the fifth month, but its corpse was eaten by its cage mates and no cause of death could be established.

The rats gained weight satisfactorily, and at the end of the year they compared favourably with the control rats. At the end of the experiment fourteen rats were killed for histological examination. One rat was found to have bronchiectasis and another a spindle cell sarcoma of the mediastinum. A third rat had a cholesteatoma of the lung and a fourth showed a moderate degree of fatty infiltration of the liver.

Group E. 10 *p.p.m.*

These rats did not show any symptoms at any time. One female rat in the eighth month had a sudden vaginal haemorrhage and died during the night. It was eaten by its cage mates and so could not be examined histologically. Another rat suffered a shoulder laceration which did not respond to treatment and the rat was killed in the ninth month. Two rats developed the symptoms of acute middle-ear disease but were successfully treated with penicillin and survived to the end of the experiment.

The rats gained weight satisfactorily throughout the experiment. The males gained appreciably more than the control males (Table 4). It should be noted that the control males were rather large at the start of the experiment and required less gain in weight to reach maturity.

At the end of the experiment fourteen rats were examined histologically. Two were found to have bronchiectasis, but there was no other abnormality.

Control group

With two exceptions these sixty rats were normal during the year of the experiment..

One rat died, after 3 weeks, of bronchopneumonia superimposed on bronchiectasis. At 8 months one rat developed symptoms of middle-ear disease but was effectively treated with penicillin. Twelve rats were examined at the end of the year. Four had bronchiectasis and one had an adenoma of the lung.

BREEDING EXPERIMENTS

These were carried out during the course of the feeding experiment.

The two females surviving in group A were mated with a male from the same group, 66 days after the cessation of E.605 feeding. These produced eleven and nine young respectively and raised them successfully.

The seven surviving females in group B were mated with two group B males, 57 days after the cessation of E.605 feeding. These females had litters of 11, 9, 8, 8, 6, 4 and 2 and all the young survived.

Five females of group C were mated with one group C male after 355 days of feeding, and feeding was continued during the period of gestation and after parturition. Throughout this time the animals were all showing slight symptoms of E.605 poisoning. Four of the five females littered but all the young died within 2 days (Table 5).

Table 5. *Effect of E.605 feeding on fertility and on viability of the young*

	No. of days from start of exp. until mating					
Group	Days E.605 fed	Days E.605 not fed	No. of females	No. of females littering	Average no. in litter	% surviving
A	19	66	2	2	10	100
B	27	67	7	7	7	100
C	355	—	5	4	4·5	0
C1	289	—	10	4	7	0
D	353	—	6	3	7	43
E	93	—	6	5	8	100
E1						
2nd generation	178	—	6	5	7	19
Control		135	6	3	8	100
		379	5	2	5	70

Similar results were obtained with group C1. Ten females were mated with two males at the 289th day of the feeding experiment. At this time and throughout gestation the females had symptoms of E.605 poisoning. Four females produced litters but all the young died within a few days. One female died during par-

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turition, and other females showed severe symptoms of E.605 poisoning during parturition.

In group D, six females were mated with one male at the 353rd day of the experiment. The feeding of E.605 was continued until the surviving young were weaned. Three females produced litters, but more than half the young died within a few days of birth. During the period of mating, gestation and lactation the group D rats did not show any symptoms of poisoning, and except for the foetal deaths were quite normal.

With group E, six females were mated with one male early in the experiment after 93 days. Five females conceived and produced a total of forty two young, all of which survived. When these young were weaned they were placed on the same dietary regime of 10 p.p.m. of E.605. During the first month on this diet three died and were consumed by their cage mates, so the cause of death is unknown. During this time the young (group E 1) did not show any symptoms or abnormalities and grew at the normal rate. After 178 days on this diet six females and one male

Table 6. *Cross-breeding experiments. Group E* 1 *(second generation fed* 10 *p.p.m.) and normal animals*

Females	Males	No. of litters	Average young per litter	% survivors
6 group E 1	2 group E 1	2	7	27
5 normal	1 group E 1	5	7	93
5 normal	1 normal	5	8	100
6 group E 1	1 normal	2	7	38

of group E 1 were mated. Five littered producing a total of thirty seven young, but thirty of these died within a few days. This question was further investigated by repeating the matings and also cross-mating normal females with group E 1 males and group E 1 females with normal males. The results are shown in Table 6. Mating group E 1 females with normal males did not improve the survival rate of the young. Conversely, normal females did not have unduly high rates of fatalities with their litters when mated with group E 1 males.

The control animals were also mated during the course of the experiment. At 135 days six females were mated with one male, but only three litters were obtained (Table 5). Later in the experiment at 379 days five females mated with one male. Only two litters were produced, and in one of these, three of six young died.

DISCUSSION

Dietary levels of 10 and 20 p.p.m. of E.605 when fed to rats 6 days a week for a year did not lead to any symptoms of poisoning. The rats gained weight in the normal way, had no higher incidence of intercurrent disease than control rats, and when examined at the end of the year did not have any pathological lesions that could be attributed to the action of E.605.

The level of 50 p.p.m. was definitely toxic. The symptoms were more common and severe at the start of the experiment when the rats were small. This was probably due to the relatively high food intake of young rats, so that when they were young they were consuming a larger amount of E.605 relative to their body

weight than later when they were more mature. During the experiment symptoms constantly recurred and were recorded for different cages at least once a week throughout the year.

When E. 605 was fed at higher levels, at 75 and 100 p.p.m., the rats soon showed evidence of severe poisoning and the experimental feeding could not be prolonged beyond a few weeks. From these feeding experiments a clear-cut result was obtained. The level of 50 p.p.m. was the borderline at which symptoms appeared. Above this animals could not survive, and below at 20 and 10 p.p.m. no symptoms or lesions attributable to E. 605 were detected, even when the experiment was continued for a year.

In the animals fed the higher levels of E. 605 the symptoms were always those of acute poisoning, salivation, lachrymation, intestinal hyperperistalsis and muscular tremors, occurring as recurrent episodes of acute poisoning. No new signs or symptoms made their appearance after weeks of poisoning. The pathological lesions found in the poisoned animals were the same as those found in acute E. 605 poisoning. They consisted of vacuolation and collapse of the acinar structure of the submaxillary gland and pancreas and atrophy of the thymus and spleen. The changes in the pancreas and salivary glands were associated with rapid autolytic changes, and as the animals were often picked up some hours after death the characteristic vacuolation in these organs was frequently obscured by post-mortem autolysis. There was one slight difference in the histological findings between acute and chronic poisoning. The lymphoid hypoplasia of the thymus and spleen are not common findings in acute poisoning. In rats dying of chronic poisoning, as observed in groups B and C, atrophy of the thymus and spleen was always found and was frequently extreme. The loss of lymphocytes and decrease in size of the thymus is not associated with any consistent atrophy of the lymph nodes. Indeed, normal lymph nodes and an atrophied thymus are often seen side by side in the same section. This confirms the findings in acute poisoning reported previously (Denz, 1951) and fails to confirm the report of Jones & Landing (1948) that generalized hypoplasia, including the lymph nodes, resulted from experimental poisoning with two phosphine oxide anticholinesterases.

The occurrence of incidental diseases was no higher in the experimental animals than in the control group. There was a certain amount of bronchiectasis and a few other spontaneous diseases, but no more than might be expected when several hundred rats were closely observed for a year. On the basis of Koppanyi's (1948) predictions changes in the brain were anticipated but were not found. No unexpected hazard or new pathological lesion was detected even after a year of feeding at 50 p.p.m. and at lower levels. It is interesting to note that E. 605 did not produce any lesions in the liver or kidney, a record that is almost unique with toxic substances.

As judged by the criteria of survival, of health of the animals and of normal growth, the toxic level of E. 605 can be put at 50 p.p.m., and lower levels such as 20 and 10 p.p.m. seem to be without adverse effect. This clear and satisfactory picture is somewhat obscured by one of the findings in the breeding experiments carried out during the course of the main experiment.

The group C rats on 50 p.p.m. produced normal litters but these died within a few days although the young were apparently normal at birth. This may possibly be due to an upset in lactation. The function of exocrine glands is disturbed by E.605 as shown by the symptoms of salivation, lachrymation and excessive mucous secretion in the intestines and by the occurrence of lesions in the salivary and lachrymal glands and in the acinar portion of the pancreas. If mammary function is disordered death of the young may result. Unfortunately, mammary tissue was not included in the sixteen tissues taken for the histological examination of poisoned rats, and so no evidence can be offered to support the hypothesis that death of the young was due to a disturbance of lactation.

The group D rats failed to raise all their young, but examination of Table 5 shows that these rats were old at the time of mating. The control rats, mated a second time when more than a year old, showed a similar mortality in the young. The results of the breeding experiments summarized in Table 5 show that breeding at the end of a long-term feeding experiment has resulted in low fertility in the rats of the control as well as the experimental groups. Where breeding has occurred within 6 months of the start of the experiment fertility has been reasonably high.

In group E the rats were mated when young (3 months after weaning) and they successfully raised their litters. These young, forming the second generation, were fed E.605 at 10 p.p.m., and when mated after 6 months raised only 19% of their litters. Neither group E nor group E1 showed any symptoms, and the failure of the third generation to survive is the only untoward event recorded at a level of 10 p.p.m. of E.605. This result recalls the report of Fitzhugh (1948), who found that when D.D.T. was fed to rats at a level of 600 p.p.m., the second generation produced very few viable young. The interpretation of such observations is difficult, particularly in the E.605 experiments, where the results of the breeding experiments are in conflict with the other data which suggest that 10 p.p.m. of E.605 in the diet is innocuous to rats. All that can be done is to record this unexpected and anomalous finding.

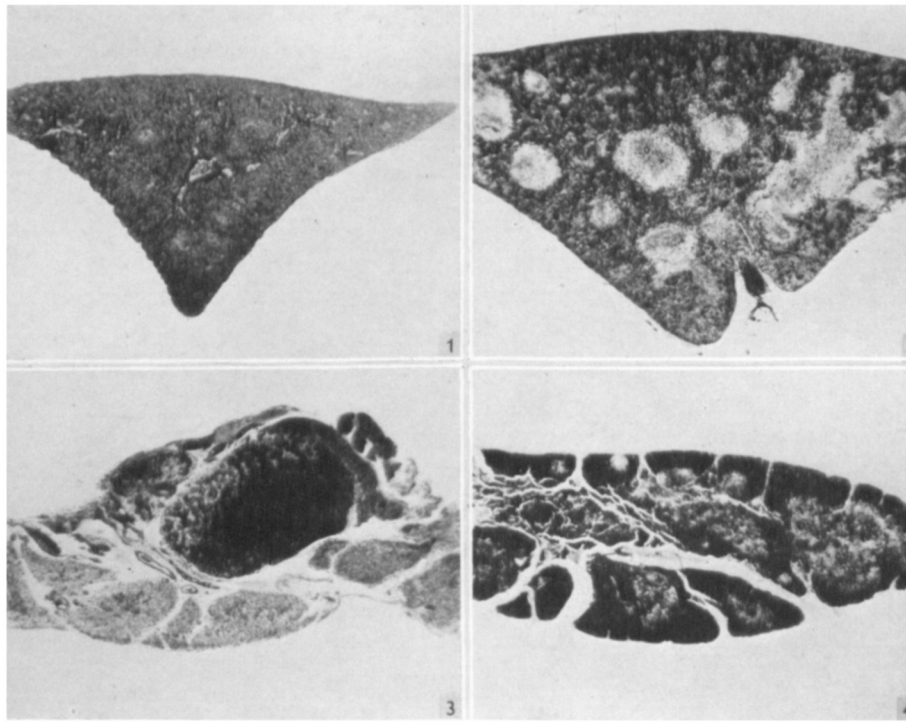
SUMMARY

1. The results of feeding albino rats on diets containing 100, 75, 50, 20 and 10 parts per million of the insecticide *p*-nitrophenyl diethyl thiophosphate (E.605, Parathion) are described. The feeding of 50, 20 and 10 p.p.m. was continued for a year.

2. At 100 and 75 p.p.m. most of the rats died in the first few weeks. At 50 p.p.m. a few deaths resulted from poisoning, and symptoms were seen on and off throughout the year. At 20 and at 10 p.p.m. no evidence of poisoning was found.

3. Histological examination of animals dying with symptoms showed the lesions of the exocrine glands and the hypoplasia of spleen and thymus that have been reported in acute poisoning by E.605. No other lesions attributable to chronic poisoning were found.

4. Breeding experiments were done with animals from all groups. The second generation of rats fed at 10 p.p.m. produced many dead in their litters. No explanation has been found for this observation.



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EXPLANATION OF PLATE 15

Fig. 1. Spleen of rat dying after 2 weeks on 75 p.p.m. of E.605 (group B). Spleen small with decrease in white pulp. Heidenhain's azan. × 24.

Fig. 2. Spleen of normal rat for comparison with Fig. 1. Normal size with usual proportion of white pulp showing as pale areas. Heidenhain's azan. × 24.

Fig. 3. Thymus of rat dying after 2 weeks on 75 p.p.m. of E.605 (group B). The shrunken thymus surrounds a normal lymph node. The thymic tissue is depleted its lymphocytes and distinction between cortex and medulla is lost. Heidenhain's azan. × 24.

Fig. 4. Thymus of normal rat for comparison with fig. 3. The cortex and medulla can easily be distinguished. Heidenhain's azan. × 24.

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