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THE CHRONIC ORAL TOXICITY OF DDT (2,2-BIS (p-CHLOROPHENYL-1,1,1-TRICHLOROETHANE)[1]

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Recent studies on the pharmacology of DDT have treated with short-term toxicity experiments on mammals (1–6), with its storage in animal tissues (7, 8, 9), and with its excretion (8, 10, 11). No study of the lifetime effects of DDT on laboratory animals has been reported. Since long-term feeding experiments with other substances in this laboratory have revealed deleterious effects which would not have been seen in experiments conducted for shorter periods of time, it seemed advisable to feed DDT for the lifetime of the rat.

PART I. TWO-YEAR EXPERIMENTS. *Method.* Two experiments were conducted in which groups of weanling rats (21 days) from our colony of Osborne-Mendel strain were started on diets containing a commercial preparation of DDT composed of 81.8% p,p isomer and 18.2% o,p isomer. In the first experiment, started early in 1943 when our supply of DDT was small, 5 groups of 12 male rats were fed on diets containing respectively 0, 100, 200, 400 and 800 p.p.m. DDT incorporated in a 10% corn oil solution. In a second experiment, started about a year later, 7 groups of 24 rats, equally divided between the sexes, were fed on diets containing respectively 0, 200, 400, 600 and 800 p.p.m. DDT incorporated in a 10% corn oil solution, and 600 and 800 p.p.m. dry DDT for comparison with the oil solutions. Ground commercial rat biscuits with 1% added cod liver oil served as the basic diet. Litter mates were selected and assigned to the various groups in both experiments according to a randomized design of experiment (balanced incomplete blocks (12)). All animals were kept in individual cages in a room with controlled temperature and humidity and were given free access to their respective diets and water. Body weights and food consumption were determined at weekly intervals.

RESULTS. Since the second experiment involved a much larger number of animals than the first, the following discussion of results will be confined to the former except that mention will be made to any differences which occurred in the two experiments.

The production of tremors. The first noticeable effect of DDT was a hyperirritability as shown by a sensitivity of the rats to stimuli and the appearance of fine tremors, especially in those animals on 600 and 800 p.p.m. DDT in the diet. These tremors developed earlier in the female rats than they did in the male rats. The rats with severe tremors became progressively worse to the point of convulsions and death. The rats on the dosage levels of 600 and 800 p.p.m. DDT that survived for the first year exhibited only moderate tremors notably during the early part of the exposure and later appeared to recover. In the group

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on 400 p.p.m. DDT, 10 out of 24 animals showed extreme tremors before death; 8 of these 10 were female rats. Severe tremors were noted in 2 female rats in the group on 200 p.p.m. of DDT.

Since a large amount of DDT may be stored in the body tissues (7), a study was made to show the effect of starvation on the metabolism of the stored DDT. At each dosage level the DDT-containing diet was withdrawn from 3 rats which had been on the experiment for 18 months and had exhibited no severe nervous symptoms up to that time. Within 24 hours after withdrawal of all food the rats formerly on 600 and 800 p.p.m. DDT showed marked tremors. Those formerly on 200 and 400 p.p.m. DDT showed increased irritability. In a similar study to show the effect of partial starvation the DDT-containing diet was exchanged for the control diet reduced to a fourth of the daily food requirement. The partial starvation did not produce characteristic DDT tremors in rats from any group.

The effect on food consumption and growth. It was shown in a paired feeding experiment (7) that 800 p.p.m. DDT produces a significant retardation in growth without a corresponding decrease in food intake. Likewise in this experiment there was no significant ($p = .05$, or less, is significant) difference between the food intake of any group of animals and that of the controls.

Since many animals on the 800 p.p.m. DDT died early, the interval of the first 12 weeks on the experimental diet was selected as the first period of study for a comparison of growth data from all groups. During this period the retarding effect of DDT on growth was significant for the female rats at concentrations of 400 p.p.m., or more, and for the male rats only at the 800 p.p.m. DDT in the diet. There was no increase in the effect on growth of the female rats as the concentration of DDT increased from 400 to 800 p.p.m.

In order to study the effect of DDT on growth for a longer period, a second interval of the first year on the experimental diet was selected. At the end of the year only 1 female rat was living from the two groups on 800 p.p.m. DDT. An analysis (table 1) shows that there was a retardation of growth in all groups of female rats; however, the value for the group on 200 p.p.m. remained nonsignificant. Slight retardation of growth occurred in the male rats on 600 and 800 p.p.m. DDT. The difference between the group on 800 p.p.m. dry DDT and the controls was significant at 3 months. Because of the small number of rats surviving for a year, this difference became not significant.

As shown in table 1 the dissolving of DDT in corn oil did not change the toxic effect of DDT on growth at the concentrations used in this experiment.

The effect on mortality. The mortality rate of the rats fed diets containing DDT was related to the dosage of DDT and to the sex of the rats (table 2). DDT at 800 p.p.m. in the diet produced severe nervous symptoms which were followed by death in many animals within the first few months. Severe nervous involvement occurred earlier in the female rats than in the male rats and death occurred earlier, therefore, in them. At the 800 p.p.m. level only 1 female rat lived for as long as a year. There was an increase in mortality rate for the female rats over that of the controls on all concentrations of DDT. The per cent difference in

TABLE 1

Mean gain in weight of rats fed diets containing DDT (second experiment)

TIME	DOSAGE OF DDT	SEX	NO. OF ANIMALS	MEAN GAIN IN WEIGHT
months	*p.p.m.*			
	0	M	11	310.2 ±13.3
		F	12	205.3 ± 6.8
	200	M	12	300.8 ± 9.5
		F	12	203.7 ± 6.8
	400	M	12	316.6 ± 5.3
		F	12	177.3 ± 6.4†
3	600	M	12	279.9 ±11.4
		F	12	178.8 ±60.0†
	600 Dry	M	12	280.0 ± 9.9
		F	10	176.7 ± 4.1†
	800	M	12	273.7 ±14.8
		F	12	172.8 ± 7.7†
	800 Dry	M	10	255.7 ± 8.5†
		F	9	177.2 ± 7.6†
	0	M	10	486.6 ±18.9
		F	11	293.5 ±10.7
	200	M	9	488.1 ±26.5
		F	10	282.0 ±10.9
	400	M	10	537.8 ±24.2
		F	9	253.7 ±10.8*
12	600	M	11	481.4 ±23.2
		F	5	240.4 ±14.9*
	600 Dry	M	11	463.6 ± 8.7
		F	3	238.7 ± 4.4†
	800	M	10	473.7 ±21.0
		F	1	
	800 Dry	M	6	459.8 ±12.9
		F	0	

* $p < .05 - > .01$.
† $p < .01$.

the mortality rate between the experimental groups of females and those of the control at a year on the experimental diets ranged from 8.3% for those on 200 p.p.m. DDT to 91.7% for those on 800 p.p.m. At the end of the experimental

period the differences appeared to have a definite break at 400 p.p.m.; however, because of the small number of rats the difference at 600 p.p.m. was not significant. When all groups of female rats on 400 to 800 p.p.m. DDT were compared jointly with those on the 200 p.p.m. DDT and the control group the difference in mortality rate was found to be highly significant.

Fewer male rats were living at the end of the experimental period in the groups

TABLE 2

Per cent mortality of rats fed diets containing DDT

DOSAGE OF DDT	SEX	12 MOS.	18 MOS.	24 MOS.
First experiment				
p.p.m.				
0	M	25	58.3	66.7
100	M	33.3	50	58.3
200	M	33.3	41.7	66.7
400	M	25	58.3	83.4
800	M	75	83.4	91.7
Second Experiment				
0	M	16.6	50	75
	F	8.3	25	58.3
200	M	25	33.3	50
	F	16.6	25	83.4
400	M	16.6	75	91.7
	F	25	50	100
600	M	8.3	33.3	83.4
	F	41.7	66.7	83.4
600 Dry	M	8.3	41.7	83.4
	F	58.3	66.7	91.7
800	M	16.6	41.7	83.4
	F	91.7	91.7	100
800 Dry	M	50	66.7	83.4
	F	100	100	100

on 400 p.p.m. or more of DDT than in the control group, but the difference between groups was not significant. There was no definite relation of the mortality rate to the dosage of DDT as mentioned above for the female rats. In the first experiment the death of 5 male rats on 800 p.p.m. DDT within the first two weeks accounts for the difference in mortality in the 2 experiments. No explanation can be advanced for the death of these rats early in the experimental

period. The paired feeding experiment referred to previously (7) gave results similar to the second experiment.

There was no difference between the mortality rate of the rats on diets with the DDT dissolved in corn oil, and that of rats on diets with the same concentration of dry DDT.

The effect on the weights of the liver, kidneys and spleen. At autopsy it was noted that the livers, and to a lesser extent the kidneys and the spleens, of the experimental animals were larger than those of the controls. Weighing these

TABLE 3

The effect of chronic ingestion of DDT on the weight of the livers, kidneys and spleens of rats

DOSAGE OF DDT	SEX	NO. OF RATS	MEAN WEIGHT (GRAMS PER KGM. OF BODY WEIGHT)		
			Liver	Kidneys	Spleen
p.p.m.					
0	M	6	25.6 ±2.9	6.6 ±0.5	1.1 ±0.2
	F	7	32.7 ±3.5	7.4 ±0.4	1.7 ±0.3
100	M	4	32.2 ±1.6	7.4 ±0.5	1.6 ±0.1
200	M	7	33.2 ±2.6	6.3 ±0.3	1.9 ±0.2
	F	9	48.7 ±3.8†	8.5 ±0.4	2.1 ±0.4
400	M	6	39.9 ±2.9†	6.8 ±0.2	1.4 ±0.4
	F	7	42.7 ±2.3*	8.3 ±0.8	1.7 ±0.4
600	M	7	41.4 ±3.5†	8.5 ±0.5*	2.0 ±0.4
	F	4	67.3 ±3.3†	9.1 ±0.5*	2.3 ±0.7
600 Dry	M	5	44.1 ±6.1*	8.5 ±0.3*	1.3 ±0.6
	F	4	60.6 ±2.1†	9.2 ±0.7*	1.8 ±0.4
800	M	8	47.3 ±3.7†	8.3 ±0.4*	1.6 ±0.2
800 Dry	M	4	44.2 ±1.5†	8.7 ±0.5*	2.0 ±0.3

* p. <.05 — >.01.
† p. <.01.

organs of rats, which had been on diets containing DDT for 18 months, or more, confirmed this observation, the results of which are shown in table 3. The differences in weights of these organs increase with the increase in concentration of DDT in the diets. The data in table 3 show that the mean liver weights of all groups of female animals and of those of males on diets containing 400 p.p.m. DDT, or more, were significantly larger than the corresponding controls. The kidneys and spleens showed less striking effects than the livers; however, the differences for the kidneys of all groups on 600 and 800 p.p.m. DDT were significant; the differences in the mean weight of the spleens were not significant. The hypertrophic effects of DDT become more striking when it is remembered that the lighter organs came from the heavier control animals, and the heavier organs from the lighter experimental animals. We have shown in another experiment from this laboratory (7) that this hypertrophy is not a hydration but an actual increase in the amount of tissue as shown on a dry basis.

Pathology. The findings in the first ten rats dying during the course of the first experiment have already been published (13) as part of a study of the histopathological changes following administration of DDT to 9 species of animals. The 38 rats discussed in that paper had been treated for periods ranging from a few days to 32 weeks. In general, those subjects treated for the longer periods showed changes of the type about to be described, while with shorter periods of treatment the affected animals showed toxic lesions of a more acute and nonspecific nature.

Of the 60 rats started on the first experiment, 47 were sent to the pathology laboratory for examination, and of this latter number, 36 were examined microscopically as outlined in the next paragraph for the rats of the second experiment, with minor exceptions. Since the second experiment involved a much larger number of animals, and since the findings in the two series were essentially similar, the principal portion of the description will refer to the findings in the second series, with mention of any difference in the first series when such a difference was present. Of the 168 rats started on the second experiment, 145 were sent to the pathology laboratory for examination, and of this latter number 126 were examined microscopically in a uniform manner. Advanced post mortem autolysis accounted in the majority of instances for the difference between the number in each of the last two categories and the number in the preceding one.

Paraffin-embedded sections stained with hematoxylin and eosin were routinely made of lung, heart, liver, spleen, pancreas, stomach, small intestine, colon, kidney, adrenal, testis, thyroid and (except in the 200 p.p.m. and control groups) hind leg muscles. Ovary and uterus were sectioned in about half the females, and parathyroids were encountered in about half the thyroid sections. Other structures such as lymph nodes, hing leg bones, and bone marrow, were sectioned in a moderate number of instances, about two dozen of each. Special stains for fat and for iron-containing pigment were done in a few instances.

Perhaps the one outstanding gross change in the treated animals was the increased size of the liver, as shown in table 3. In about a fourth of the animals the liver had a "nutmeg" appearance, more frequent on the higher than on the lower dosage levels, and not seen in the controls. Five rats, three in the 800 p.p.m. in oil group, had a yellowish or tan tinge to the liver. In 4 rats, scattered among the various dosage levels, the adrenals were specifically noted to be large. Eight animals, 4 receiving 800 p.p.m. in oil, had bloody material in the stomach or small intestine. The gross characteristics concerned in the slight tendency of DDT to produce liver tumors will be dealt with later in this summary. The

external appearance of the rats, and the lungs, heart, spleen, lymph nodes, pancreas, kidneys, testes, uterus, ovaries, thyroid and parathyroids, showed no gross effects from DDT, except for weight reduction in some instances.

Microscopically, the observations led to the same conclusion as was found in the first two-year experiment, namely, that the chief lesion in long-term experimental rats is a moderate degree of liver damage of a characteristic type. Also noted in both series was a minimal hepatocarcinogenic tendency, evident late in the experimental period. A finding not noted in the first series, because it contained no females, was a stromal fibrosis and cellular proliferation in the ovary; this, like the tumorigenic tendency, occurred only after about the ninetieth week of feeding. Fatty change in the liver, although not of high grade, was more evident in the second series. Also evident microscopically were a slight generalized increase in size of the adrenals, a slight generalized increase in number of interstitial cells in the testis, and a slight brown pigmentation of the epithelium of the renal convoluted tubules. The latter three changes were all of minor degree, but some of them are perhaps of physiological interest.

The characteristic microscopic change in the liver was proportional to dosage level, although the lower grades of the change were generally present even at the lowest dosage level of 200 p.p.m., and in the first series at 100 p.p.m. No difference in intensity could be observed between dry and oily dosage forms at the same dose levels, or between sexes. The lesion consisted principally in hypertropy and increased cytoplasmic oxyphilia of the centrolobular hepatic cells, plus increased basophilia and margination of the cytoplasmic granules, and a tendency to hyalinization of the remainder of the cytoplasm. This general type of change in the liver has been noted by us (13) in animals other than the rat (mice, rabbits) given DDT, and also in at least one other laboratory (14) in animals receiving DDT. In a small proportion of the rats the typical change appeared to have migrated peripherally in the lobule, or to have been overshadowed by other changes, principally necrosis. The periportal hepatic cells, only rarely showing the characteristic DDT changes, were generally somewhat atrophic. The increased weight of the livers of the treated animals could easily be explained on a basis of centrolobular cellular hypertrophy, when it is considered that doubling each dimension of the hepatic cell would increase its volume approximately eight times; an increase in the number of hepatic cells would not have to be invoked.

Centrolobular necrosis, or focal necrosis within centrolobular areas, both superimposed on the typical DDT change, occurred in many of the livers of the treated rats. The same condition was seen to a lesser extent in the first series. It was somewhat less frequent at the lower dosage levels, and was absent in the controls. It was rather distinctly more frequent in those animals found dead than in those surviving the experimental period. The necrosis generally had an acute appearance and it seems reasonable to assume that much of the necrosis was a terminal phenomenon in dying animals, occasioned by the release of DDT from storage in body fat (7) when such fat was used up during the terminal period of semi-starvation. Vacuolation of the hepatic cells in the paraffin sections,

interpreted as evidence of fatty degeneration, was noted in 13 of the treated rats and in one control. It was generally of slight degree.

Tendency to hepatic tumor formation was, on the basis of comparison with many hundreds of rats of similar age, definite but minimal in both two-year series. Altogether, in both experiments, 4 rats each had one or more small hepatic cell tumors, from 5 to 12 mm. in diameter, paler than the surrounding liver tissue on gross examination, not sharply circumscribed microscopically, and composed of cells larger than those in the rest of the liver. Lobular architecture was almost obliterated. Mitoses were not noted. Some cells had foamy cytoplasm; some cells showed DDT changes of a degree greater than that elsewhere. Tumors of this type are not a sharply defined entity, and the question of their nomenclature cannot be treated here. They would probably be generally called ademonas because of their relative size, discrete gross appearance, and almost total loss of lobular architecture. There might be almost as much justification for considering them low grade hepatic cell carcinomas.

Eleven other rats showed varying amounts of nodular adenomatoid hyperplasia; the nodules were generally of 1 to 3 mm. diameter, and were usually noted grossly as scattered yellowish foci. Nodules smaller or less distinct microscopically were not diagnosed as adenomatoid hyperplasia. The microscopic appearance was essentially the same as in the larger tumor masses; difference in size is chiefly responsible for the difference in terminology. Nodular adenomatoid hyperplasia is almost never seen in our rat livers except after treatment with a few distinctly tumorigenic substances. About 1% of our older rats will spontaneously show distinct hepatic cell tumors, usually 1 to 2 cm. in diameter. The exact incidence has not yet been calculated, but it appears to be 1% plus or minus a few tenths of one per cent, there being about a dozen tumors in as many hundred rats over 18 months of age. By chance, then, one or at the most two tumors in the liver might be expected in the 75 or so rats fed DDT for 18 months or more. Taken together, the 15 rats having either liver tumor or nodular adenomatoid hyperplasia are numerically enough to strongly suggest a distinct although minimal tumorigenic tendency of DDT. All 15 rats had survived 84 or more weeks of the experimental period. This age distribution is essentially the same as in those rats with spontaneously occurring liver tumors, or in those developing tumors after the administration of selenium in the diet (15).

Increased size of the adrenal, and an increased number of interstitial cells in the testis, were changes which were generally minimal enough in degree so that in an individual instance a diagnosis of abnormality would not be warranted; however, they were frequent in occurrence, and in a few instances were great enough in degree to be distinctly seen. The renal tubular pigmentation, wherein clumped masses of brown non-ferrous pigment were present in generally small quantity in the epithelial cells of the convoluted tubules, was noted in 9 scattered rats among the treated groups of the second series, and not in the first series. This change is of minor significance and has been noted after treatment with several substances other than DDT.

Nearly all ovaries in rats surviving 89 or more weeks of the experimental period

showed a peculiar change, present at all dosage levels and not in the controls, in fact not noted previously in any of our rats. The change consisted in an actual as well as a relative increase in amount of stroma, with the presence within this stroma of rather numerous relatively large, generally irregular, pale cells with a moderate amount of cytoplasm. Some of the cells were arranged in glandlike formations. So little has been written on the pathology of the senile rat ovary that the significance of these changes is uncertain. Both treated and control rats showed decreased numbers of follicles, which is to be expected as a senile change.

Focal necrosis of voluntary muscle (hind leg muscles were sectioned) was generally of minimal or questionable degree, but was distinct in a few instances at 600 or 800 p.p.m. A high grade of this lesion had not been expected, but since voluntary muscle had been, next to liver, the structure most damaged by DDT in more acute experiments, its examination was desired in this one as an additional factor of evaluation between dose levels. It is very difficult to determine a minimal degree of old muscle damage (after replacement with scar tissue) with certainty; however, even a minimal amount of acute necrosis is easy to see. Some degree of change was noted in one-fourth to one-half the rats on 600 and 800 p.p.m., and curiously enough, more frequently at the lower of these levels than at the higher. No difference between dosage forms could be distinguished. At 400 p.p.m., only 2 rats in 21 showed changes and these were of ± degree. Since this is about the expected incidence in untreated rats, muscles in the 200 p.p.m. and control groups were not examined routinely.

Certain minor changes were best seen on the higher dosage levels. Splenic atrophy and reduced numbers of secretory granules in the acinar cells of the pancreas were accompaniments of inanition. A slight degree of testicular atrophy may also belong here, or it may be a direct toxic effect of the DDT. While some spleens were atrophic, other were slightly hyperplastic, suggestively a DDT effect; there was also a suggestion of minimal thyroid hyperplasia. Bone marrow was sectioned in 21 treated rats and, as in the thyroid, there was suggestive minimal hyperplasia at the higher dose levels. The bleeding into the gastrointestinal tract mentioned under the gross findings came from small foci of hemorrhagic necrosis in the mucosa of the glandular part of the stomach; this is not specific for DDT.

Bile duct proliferation, splenic and adrenal pigmentation, small ulcers in the forestomach (proventriculus), renal tubular atrophy, protein material in renal tubules, focal thyroid hyperplasia, islands of Langerhans, lymph nodes, heart, lung, stomach, small intestine, colon, uterus, parathyroids, and hind leg bones were the same in the DDT-treated groups as in the controls and in the general run of our comparable rats. Lymphosarcomas of the lung were less frequent in the second series than in the first, but this fact has probably no relation to DDT. Other tumors, and leukemia, showed the usual incidence.

PART II. LIVER RESTORATION IN RATS FED DIETS CONTAINING DDT FOR 12 WEEKS. A group of 16 female rats were given 1000 p.p.m. DDT in the diet for

12 weeks. At this time 4 rats were sacrificed, and the remainder was sacrificed in pairs after placing them on the same diet without DDT for additional periods of 1, 2, 4, 6, 8 and 10 weeks respectively. In the 4 rats sacrificed at the termination of feeding, the histopathological changes were typical of DDT poisoning. Changes were almost entirely confined to the liver, in which there was an enlargement of the centrolobular hepatic cells, with more oxyphilic cytoplasm and more distinct large basophilic granules than in the slightly atrophic cells of the peripheral halves of the lobules. There was a slight tendency to cytoplasmic hyalinization and peripheral segmentation of the basophilic granules in the centrolobular cells. The thyroid showed slight colloid depletion and the accumulation of debris within follicles. Leg muscle, heart, lung, spleen, pancreas, gastrointestinal tract, kidney, adrenal, bone and bone marrow were negative.

In the rats sacrificed at intervals after cessation of feeding DDT, those sacrificed at 1 and 2 weeks afterwards showed livers very similar to those described in the preceding paragraph, while at 4 weeks and 6 weeks there was little of this change, and the livers could have been passed as showing no distinct damage, had not residues of a specific nature been sought. The livers at 8 and 10 weeks had a normal appearance.

DISCUSSION. The observations of this experiment show that chronic poisoning with small amounts of DDT is characterized by degenerative changes in the liver and other organs. This toxicity places a definite and inherent danger in the consumption of small amounts of DDT for a long time. The storage and the slow excretion of DDT increase the possibility of chronic poisoning. Another characteristic is the wide variation in individual susceptibility within the same species as well as between different species of animals (3). In order to correlate the dosage levels with the body weights of animals, the intake of DDT was calculated in terms of the daily intake per kgm. of body weight (chart 1). These values were calculated from the weekly food intake of the rats from both experiments at the various monthly intervals. The DDT intake of the rats at any given dosage level decreased rapidly during the first 2 months and then became almost constant as shown by the straight line (chart 1) at about 6 months on the experimental diet. This fact is accounted for by the change in the growth rate of the rats from the fast growing period to the plateau period, while at the same time their daily food intake remained almost constant. As noted above, surviving rats on concentrations of 600 and 800 p.p.m. DDT recovered from severe tremors after they had reached the plateau period. The chart shows that the daily intake of DDT in mgm. per kgm. of body weight varied from 52.5 and 73.3 at 1 month to 27.8 and 3.82 at 6 months for the male rats on 600 and 800 p.p.m., respectively. Similarly females ingested from 57.1 and 78.9 to 33.2 and 43.1 mgm. DDT per kgm. of body weight during the same time. Therefore, this early toxicity was produced at least in part by the increased intake of DDT during the first 6 months of the experimental period. Furthermore, part, if not all, of the increased toxicity observed in the female rats was undoubtedly caused by the greater amount of DDT per kgm. of body weight consumed by them than by the male

rats fed a similar concentration. For example, female rats on the concentration of 200 p.p.m. DDT consumed about 10.5 mgm. per kgm. of body weight per day for the greater part of the experiment, while male rats consumed 8.5 mgm. per kgm. of body weight per day for the same period.

As shown by the chart the dosage level of 100 p.p.m. DDT corresponds to about 4.5 mgm. per kgm. of body weight per day for the rat over the greater part of the 2-year experiment. The 100 p.p.m. concentration in the diet appears to be a dosage level of DDT at which only slight chronic poisoning occurs in all

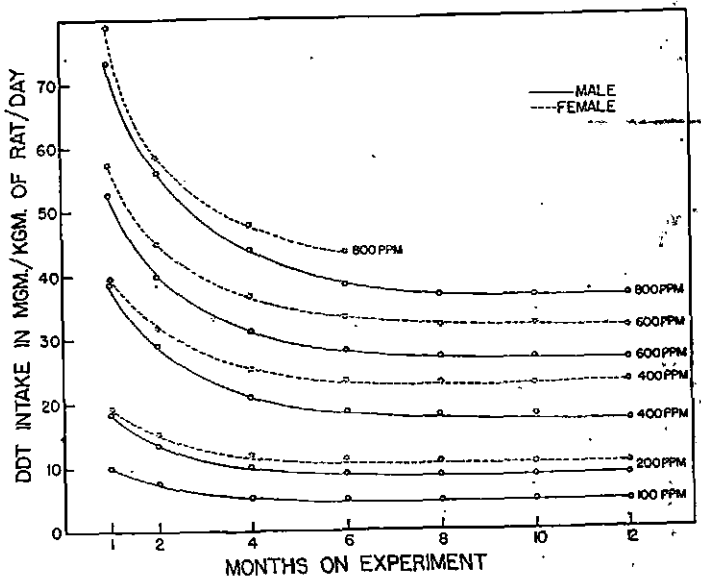

CHART 1. CALCULATED DDT INTAKE IN MGM./KGM. OF BODY WEIGHT IN RATS RECEIVING VARIOUS LEVELS OF DDT IN THE DIET

rats. Another experiment with DDT in dosage levels of less than 100 p.p.m will be reported later.

SUMMARY

In rats fed diets containing from 100 to 800 p.p.m. DDT for a period of 2 years, the following effects were noted.

1. DDT produced chronic toxicity in rats at all concentrations.

2. The outstanding and characteristic histopathological lesion caused by DDT under the conditions of this experiment was a hypertrophy of the centrolobular hepatic cells with an increased cytoplasmic oxyphilia, plus increased basophilia and margination of the cytoplasmic granules, and a tendency to hyalinization of the remainder of the cytoplasm. Frequently there was superimposed more or less of centrolobular hepatic cell necrosis, much of which had an appearance of being recent.

3. Slight focal necrosis of hind leg muscles was found frequently. This was a much less prominent lesion than liver damage.

4. DDT showed a minimal tendency to cause formation of hepatic cell tumors. This tendency did not operate until after 18 months of feeding.

5. In the later months of the experimental period the ovarian stroma underwent fibrosis and cellular proliferation.

6. The microscopic lesions observed, except for focal necrosis of the hind leg muscles, showed a fairly distinct gradation with dosage level. They varied from slight at 100 p.p.m. to marked at 800 p.p.m. DDT.

7. An increased intake of DDT-containing diet per kgm. of body weight during the fast growing period of the rat produced an increase in toxicity.

8. The greater intake of DDT per kgm. of body weight by female rats than that by male rats on similar concentrations produced an increased toxicity in the females.

9. At concentrations of 400 to 800 p.p.m. DDT, rats showed characteristic nervous symptoms of poisoning. Lower dosages produced an increased irritability. Muscle tremors were more pronounced in female rats than males.

10. Concentrations of 400 to 800 p.p.m. DDT in the diet retarded growth of female rats. In male rats only 800 p.p.m. DDT retarded growth.

11. The livers, and to a lesser extent the kidneys of experimental animals, were larger than those of the controls. These differences were more pronounced in the groups on 600 and 800 p.p.m. DDT.

12. At concentrations of 400 to 800 p.p.m. DDT in the diet, female rats showed an increased mortality rate. DDT at concentrations used in this experiment did not produce any effect on mortality rate of male rats.

13. DDT did not affect the food consumption of rats.

14. The withdrawal of all food from the chronically fed rats on 400 to 800 p.p.m. DDT produced characteristic tremors within 24 hours.

15. No difference in incidence or degree of changes occurred between animals given DDT in dry form and those given DDT in corn oil solutions.

In rats sacrificed at intervals after cessation of feeding 1000 p.p.m. DDT in the diet for 12 weeks, those sacrificed at 1 and 2 weeks afterwards showed damaged livers, while at 4 and 6 there was little change. The livers of rats sacrificed after withdrawal of DDT for 8 and 10 weeks exhibited a normal appearance.

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PHARMACODYNAMIC STUDIES OF A NEW ANTIHISTAMINIC AGENT, PYRIBENZAMINE (N,N-DIMETHYL-N'-BENZYL-N'-(α-PYRIDYL)-ETHYLENE DIAMINE HYDROCHLORIDE)

II. EFFECTS ON SMOOTH MUSCLE OF THE GUINEA PIG AND DOG LUNG

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In previous communications (1) the antihistaminic and antianaphylactic properties of N,N-dimethyl-N'-benzyl-N'-(α-pyridyl)-ethylene diamine hydrochloride (Pyribenzamine) were demonstrated. Important tests of such properties concern the drug's effects on smooth muscle, notably that of the bronchial apparatus as demonstrated by Mayer (1) in his studies on histamine-induced asthma and anaphylaxis in vivo. We chose other approaches to this problem and this report deals with such studies in the lungs of normal and sensitized guinea pigs and dogs.

A. STUDIES OF THE GUINEA PIG LUNG. *Method.* Lungs of normal and "horse-serum sensitized" guinea pigs of varied ages were prepared according to the modified method of Tainter, Pedden and James (2) in which the lung was continuously perfused with a specially prepared, aerated fluid at a temperature of 37.5°C. Perfusate was measured at regular intervals and modifications of fluid output by the lung were effected by intratracheal injections of drugs to be studied. After an interval of approximately 15 minutes for control purposes, the following drugs were instilled into the perfusion line just above the suspended trachea: Histamine phosphate, 50 micrograms; Pyribenzamine, 25 to 50 micrograms; Horse serum (Lederle), and in a few experiments, N'-phenyl-N'-benzyl-N-dimethylethylene diamine HCl, known as Antergan (3), for the purpose of comparing this antihistaminic with Pyribenzamine.

Several guinea pigs were sensitized to horse serum some 15 to 20 days previously by injections of 0.5 cc. intramuscularly. Some of these animals were given Pyribenzamine subcutaneously at varying intervals prior to lung excision.

Results. The bronchial tonus of lungs excised from 41 normal and 14 "sensitized" guinea pigs showed no significant deviation from normal even after 3 to 4 hours of non-medicated perfusion. The age of the animals made no difference in responses obtained.

The standard dose of 50 micrograms of histamine consistently produced a marked and immediate constriction of the bronchial musculature as reflected by the great diminution in perfusate per minute (fig. 1). This histaminic contraction was decidedly reduced by 25 to 50 micrograms of Pyribenzamine (fig. 1). These amounts of Pyribenzamine were without marked effect on normal bronchial tonus; however, slight evanescent constriction occasionally followed such ad-