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PATHOLOGIC CHANGES IN ANIMALS EXPOSED TO A COMMERCIAL CHLORINATED DIPHENYL [1]

By J. W. MILLER, *Surgeon (R), United States Public Health Service*

The demands of industry as a result of the war have greatly increased the use of chlorinated naphthalenes and chlorinated diphenyls. In the past few years the hazards associated with their application have attracted much interest and a number of reports regarding the systemic and dermatologic effects of exposure, including fatal cases, have been made.

Only the pathologic changes in animals exposed to a commercial chlorinated diphenyl are given here.

The chlorinated diphenyl used was viscous, almost water white, and clear at room temperature. It consisted of a mixture of isomers of diphenyl chlorinated in different positions and extent, with an approximate chlorine content of 42 percent and an approximate empirical formula of $C_{12}H_7Cl_3$. It was insoluble in water but soluble in mineral and vegetable oils, other chlorinated hydrocarbons, and fat solvents. Its specific gravity was 1.374 to 1.393.

Guinea pigs, rats, and rabbits were exposed to the above compound by subcutaneous injections, feeding, and applications to the skin and cornea. The survival times given here refer only to animals subjected to pathologic examination in each exposed group. Certain additional results reported are from groups of animals exposed solely to obtain pathologic data.

I. SUBCUTANEOUS INJECTIONS

(A) GUINEA PIGS

A series of 31 guinea pigs was injected subcutaneously with a single dose of 0.05 cc. (69 mg.) of the chlorinated diphenyl. Some of these animals were killed and examined at 2-day intervals up to 38 days after injection. Sections were made of the liver, lungs, spleen, kidneys, adrenals, pancreas, heart, and skin, and were stained with hematoxylin and eosin, eosin-polychrome methylene blue (*1*), and frozen sections with hematoxylin and Sudan IV (*2*).

Skin.—In 2 days the skin lesion at the site of subcutaneous injection consisted of a central, faintly basophilic necrotic mass containing much nuclear debris, many polymorphonuclears, and lymphocytes. Much of the nuclear material was present at the periphery. On the

[1] From the Industrial Hygiene Research Laboratory, National Institute of Health. This material is based on experiments conducted by Surgeon Benjamin F. Jones and Physiologist D. D. Donahue.

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periphery were, first a zone of fibrin, then one of monocytes, leucocytes, lymphocytes, and, externally, a few fibroblasts which extended into the adjacent muscle and subcutaneous tissue. In 4 days a poorly-defined zone of fibroblasts accompanied by numerous capillaries was noted in the periphery. In 6 days the necrotic material had become eosinophilic. There was a central portion of finely granular and fibrinoid material surrounded by a zone of nuclear fragments, and a fairly well defined capsule of connective tissue and fibroblasts which extended into the adjacent fat and muscle. If, however, the injected material had been placed in the superficial portion of the derma, the inner portion of the capsule was partially lined by stratified squamous epithelium extending down from the epidermis. The layers of cells adjacent to the necrotic debris were strongly oxyphil, flattened, and lacked nuclei, thus presenting the appearance of early keratinization. These changes are essentially those of chloracne (*3, 4, 5*), though the latter are thought to be due to mechanical occlusion of the ducts resulting in accumulation of secretion within the glands.

Encapsulation progressed from the eighth to the thirty-eighth day. When the lesion was superficial, the epithelium continued to develop until it lined the cyst wall, and the portion adjacent to the necrotic material was cornified. The outer layers of fibrous tissue became increasingly collagenous. The necrotic material remained eosinophilic in most cases and nuclear remains became less abundant. When the lesion was deep, the capsule consisted of adult connective tissue; otherwise the appearance was the same.

Liver.—Eight to 10 days after injection, fat droplets were noted in the liver cells. At early examinations, these were very few in numbers, but after 16 days they were present in moderate or very large numbers. Small fat droplets were generally scattered throughout the lobule, although in some of the sections they were present only about the portal canals. Central atrophy, usually slight or moderate in degree, occurred in practically all of the animals with hepatic fat, and made its appearance at about the same time as the fat. Congestion of the sinusoids was absent in all but 2 of the animals. At irregular intervals and with no particular regard to interval after injection, a slight to moderate amount of perinuclear, basophilic granulation was noted in the liver cells in sections stained with eosin-methylene blue. Focal necrosis occurred in only 4 of 31 animals. It was found in various portions of the lobules and may not be significant.

Spleen.—The spleen showed no particular lesions.

Kidneys.—The kidneys were essentially normal in most of the series. A slight to moderate congestion of the interstitial capillaries was noted. Fat was present in the cells of the convoluted tubules in only two animals.

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The adrenals, heart, lungs, and pancreas showed no noteworthy changes.

In another series of 23 animals receiving larger single doses of 0.1 to 0.5 cc. (138 to 690 mg.), which were killed or died from 2 to 24 days after injection, pathologic changes consistent with those found in the previous series were noted. Fatty degeneration in the liver was more marked, appearing on the second day in animals injected with 0.5 cc. (690 mg.). Approximately the same degree of central atrophy resulted from the injection of 0.5 cc. (690 mg.) as from 0.05 cc. (69 mg.); central atrophy was more frequent in animals dying or killed before the tenth day. Changes in the spleen were essentially the same except that lymphoid hyperplasia was more frequent and few to moderate numbers of hemosiderin-bearing cells were seen in 12 of 23 animals. In the kidneys congestion of capillaries was more frequent. Fat was absent. The adrenals showed cortical congestion in most animals, sometimes also medullary. Similar adrenal congestion has been observed with carbon tetrachloride (*6*). However in the latter it often goes on to hemorrhage and necrosis. The lungs in the animals which died during the test showed congestion of the alveolar capillaries and focal extravasation of red blood cells and serum into alveoli in only 4 animals. The changes in the skin were essentially the same as those noted before. However, in contrast with the findings following administration of smaller doses, with larger doses the necrotic material in the derma remained basophilic in reaction. The heart and pancreas were normal.

A third series of 10 guinea pigs received a single 0.5 cc. (345 mg. as chlorinated diphenyl) dose of the chlorinated diphenyl dissolved in an equal amount of mineral oil. These all died within 13 days. The pathologic picture was essentially the same as noted in the previous series. The lungs showed somewhat greater congestion and edema in 9 of 10 guinea pigs.

(B) RATS

A series of 20 rats received a single dose of 0.05 cc. (69 mg.) of chlorinated diphenyl subcutaneously. Animals were killed at 2 and 7 days and at 10-day intervals thereafter up to 90 days.

Liver.—Fat appeared in liver cells of rats much earlier than in guinea pigs. It was already present 2 days after injection and was fairly constant throughout the remainder of the series. Central atrophy was less marked. There was no greater periportal lymphocytic infiltration than in untreated rats. Irregular basophilic stippling of liver cell cytoplasm was noted, as much in untreated controls as in experimental animals.

Spleen.—Congestion of the cavernous veins was more frequent, while reticuloendotheliosis was only slight and often absent. Pulp

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myelosis, most about trabeculae, occurred in all but two of the animals and varied from slight to marked, as commonly seen in rats.

The lungs, kidneys, adrenals, and pancreas showed no changes. The lesions in the skin were identical with those noted in the guinea pigs in which the materials had been injected deeply. In all but one of the cases the capsule about the necrotic mass consisted of fibrous tissue and fibroblasts. In one animal killed 60 days after injection, a partial epithelial lining of the cavity was noted.

A second series of 10 rats, receiving a 0.5 cc. (690 mg.) dose, was killed at the same time intervals, the last at 40 days. In these, fatty changes in the liver were more marked. The spleen showed considerable hypertrophy in most cases. The other organs were essentially the same.

Two other groups of 4 rats each were given 10 doses (total of 690 mg.) on alternate days and 27 doses (total of 1,863 mg.) daily of 0.05 cc. (69 mg.), respectively. Half of each group was killed 60 and 90 days after the first injection. In all of the rats receiving 10 such doses peculiar round or oval intracellular bodies were observed in the livers of the animals in both 60- and 90-day groups. However a negligible number were found in only 1 rat injected 27 times.

These bodies varied considerably in size. The smaller were homogeneous and usually oxyphilic. Somewhat larger ones showed a denser periphery and paler center. As size increased the central portion became either granular, reticular, or foamy in appearance and occasionally contained a small faintly eosinophilic body about the size of a nucleolus. With the increase in size the shell became sharply demarcated. It was hyaline and deeply eosinophilic, with a scant basophilic outer margin, and sometimes presented a concentric lamination. The thickness of this hyaline shell varied. Many of the bodies were often within a single cell. They were generally present in the cells in the central one-third to four-fifths of the lobule. The changes in the other organs were consistent with the findings in the previous groups.

(C) RABBITS

Two rabbits were given 0.5 cc. (690 mg.) and another 1.0 cc. (1,380 mg.) for 10 consecutive days. They died 16, 72, and 14 days after injection. Three more received 10 similar daily doses of 345 and 690 mg. as a 50 percent solution of the material in mineral oil and died in 46, 360, and 42 days. Both groups showed essentially the same pathology.

The liver contained numerous fine fat droplets accompanied by focal necrosis in 2 of the rabbits receiving the undiluted material. No fat or other significant changes were observed in those injected with the 50 percent solution. Occasionally slight centrolobular atrophy

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of liver cell cords was noted. The pathology of the other organs and of the skin lesions was identical with that encountered in the guinea pig and the rat.

II. SKIN APPLICATION TESTS

(A) GUINEA PIGS

One series of 11 guinea pigs received 11 daily skin applications of about 1/40 of a cc. (34.5 mg.) of the undiluted chlorinated diphenyl. These died at various intervals up to 21 days following first application. The pathologic changes in the liver, kidneys, lungs, adrenals, and heart were essentially those noted in the subcutaneous injection series. The spleen showed less pathology. The skin lesions, however, differed from those in the subcutaneous group. In another series of 2 animals receiving 11 (17 mg.) doses with an equal amount of mineral oil, similar changes were noted.

Changes in the skin were not constant in the animals which died in 12 to 21 days after the first of 11 applications of the undiluted material. The principal changes were injection of the capillaries of the derma and occasional thickening of the epidermis. The congestion was sometimes accompanied by edema of the derma adjacent to the small blood vessels. In one instance, a moderate infiltration of lymphocytes occurred.

In a group of 14 animals painted daily with 10, 20, 30, and 50 percent (3.5 to 17 mg.) chlorinated diphenyl in mineral oil, and which died or were killed 7 to 15 days after first application, the primary change was thinning and often destruction of the superficial, cornified layers of the epithelium. When destruction of the epidermis occurred, the superficial cells contained fine, granular material, and in some eosinophilic inclusions. The amount of involvement of the skin appeared to have no relationship with the dose or length of exposure. The reaction was not that of an acute inflammation.

(B) RATS

Sixteen rats received 25 daily applications of about 1/40 of a cc. (34.5 mg.) of the undiluted chlorinated diphenyl and were killed at 10-day intervals beginning at 30 and ending at 90 days after initial treatment.

Very little fat was found in the liver of 6 of the animals. Three rats showed the hyaline bodies in the liver cells at the 60- and 90-day intervals.

Slight to moderate degree of congestion of the cavernous veins of the spleen was noted. Pulp myelosis was present in a moderate degree. In 7 of the 16 animals, a slight to moderate amount of intracellular hemosiderin was noted.

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FIGURE 1.—Intracellular hyaline bodies in livers of rats exposed to a chlorinated diphenyl.

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No significant changes were found in the kidneys, heart, pancreas, and lungs.

In some of the rats the treated skin was much thickened, and the hair follicles were swollen and poorly defined.

(C) RABBITS

Eleven rabbits received cutaneous applications, at 2-day intervals, of about 86 mg. for the first 7 and 172 mg. for the last 8 applications of the undiluted chlorinated diphenyl. The animals were examined as death occurred between 17 and 98 days. The number of applications varied from 9 to 15. Seven of the rabbits received the latter number.

Fatty degeneration and central atrophy of the liver cells were more prominent than in the previous series of experiments.

The spleen showed less pathology and in two of the animals (90 and 98 days) was normal. The changes noted were slight to marked congestion of the cavernous veins and slight to moderate follicular lymphoid hyperplasia. Focal caseous necrosis occurred in four instances, but was probably due to intercurrent infections.

Changes in the heart, lungs, adrenals, and kidneys were slight and consisted primarily in congestion.

The skin showed thinning of the prickle cell layer and relative thickening of the outer cornified layers.

III. FEEDING EXPERIMENTS

(A) GUINEA PIGS

A series of 8 guinea pigs received 2 doses of 0.05 cc. (69 mg.) of the chlorinated diphenyl 1 week apart. Death occurred in 11 to 29 days after initial feeding.

The changes in the liver were more marked in this series than in the others. Fatty metamorphosis was extensive in all of the animals and was of mixed fine, medium, and large droplet variety. Central atrophy was noted in only 2 animals dying 18 and 29 days after the initial feeding.

The changes in the other organs were of the same negligible character and degree as observed in the other experiments. The gastrointestinal tract showed no abnormal histology. No changes were noted in the central nervous system.

(B) RATS

Twelve rats receiving 25 daily doses of 0.1 cc. (138 mg.) were killed and examined at 10-day intervals, beginning at 30 and ending at 90 days after initial feeding.

The pathologic findings were practically identical with those of the series of rats receiving skin applications of 25 daily doses. The intracel-

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lular hyaline bodies mentioned previously were found in the livers of 2 of the animals. No changes were noted in the gastrointestinal tract.

IV. APPLICATIONS TO CORNEA

RATS

A small amount (1 drop from a 25-gage hypodermic needle, approximately 1/80 cc. or 17 mg.) of chlorinated diphenyl was instilled into the eyes of 8 rats for 25 consecutive days. Beginning 30 days after the initial dose, 2 of the animals were killed and examined each 10 days.

The pathologic changes were very scant in degree. Slight central atrophy of the liver cords occurred in half of the rats at various irregular intervals. Slight to moderate congestion of cavernous veins of the spleen was constant. The presence of blood pigment, both free and intracellular, occurred rather frequently in the spleen.

The conjunctival tissue presented no gross changes when examined under magnification in the living animal.

SUMMARY AND DISCUSSION

Guinea pigs, rats, and rabbits were exposed to a commercial chlorinated diphenyl by subcutaneous injections and applications to the skin. The material was also administered to guinea pigs and rats by ingestion and to rats alone by corneal instillations. The doses varied from 17 to 1,380 mg. and were either single or were repeated at regular intervals.

Two conspicuous pathologic findings were observed—liver damage in all series of experiments and skin changes in the animals receiving subcutaneous injections or applications of the material to the skin.

Fatty degeneration and atrophy of the centrolobular cells were present in varying amounts and in varying numbers of animals in the different test groups. In the rat an additional finding, hyaline bodies within the liver cells, was noted in certain animals. It was possible to detect a difference in response of the three species to the material on the basis of liver damage. Most liver damage was found in the guinea pig, less in the rabbit, and least in the rat. This same species order was followed, regardless of dose, duration of test, or mode of administration.

Hepatic fat appeared in the guinea pigs receiving 0.05 cc. subcutaneously in 10 days and remained in significant amounts throughout 38 days. Rats receiving the same amount showed fat in 2 days but only small amounts were noted in some of the animals over a period of 90 days. Fat appeared earlier in both rats and guinea pigs receiving larger doses. The involvement was generally centrolobular.

Fatty degeneration, when compared with the other routes of administration, was most marked in the guinea pigs fed the chlorinated

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hydrocarbon but was absent in rats similarly treated. Central atrophy was more or less the same throughout the series of tests. Only slight central atrophy was present in about half of the rats treated by application of the material to the cornea.

Intracellular hyaline bodies were found in the liver of the rat alone. They were present, usually in large numbers, in all of the rats receiving 10 0.05-cc. doses and in some of the animals receiving 25 doses by skin and corneal applications and ingestion, but were not observed in any of the animals subjected to single doses. These bodies were noted in the animals sacrificed 50, 60, and 90 days after first exposure. None were observed in rats examined prior to 50 days on test. They occurred in from 20 to 38 percent of the animals treated in the various ways. They were somewhat less marked in degree and in number of animals when the chlorinated diphenyl was ingested. These findings agree with Bennett (7) who reported similar hyaline bodies in liver cells of white rats exposed to mixtures of chlornaphthalenes and chlorinated diphenyl, chlorinated diphenyl, and less frequently to mixtures of chlornaphthalenes. To date such bodies have only been observed in rats exposed to such chlorinated compounds.

It is interesting to note that while the bodies appeared in all of a series of 4 rats receiving 10 0.05-cc. subcutaneous doses given on alternate days, they were absent in 4 rats treated with the same amount by 27 consecutive daily injections after 60 and 90 days following first exposure. The liver cells in the latter series showed very slight deviation from normal, namely, areas of oxyphil granulation of cytoplasm and occasional loss of nucleus. It is possible that the gradual accumulation of 1,863 mg. over a period of 27 days so damaged the liver cells functionally that they were unable to form the hyaline material. The hyaline bodies are morphologically different from those produced by butter yellow in hepatic tumor cells (8). They probably represent further development of the same general type of hyaline degeneration as has been observed with certain azobenzenes (9).

The skin lesions produced by subcutaneous injection were histologically similar to those of chloracne in man. The changes produced by direct application to the skin were not constant and were essentially those of low-grade irritation. The failure of local applications to produce acne lesions may be due to relatively early deaths from systemic toxic action before pathology could be produced in the skin or the amount tolerated without causing death was too small to cause such lesions.

Attention is called to the fact that the chlorinated diphenyl used in the above experiments produces liver changes in the rat having marked differences from those resulting from other toxic substances and that such changes were not found in the guinea pig and rabbit

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