

for which were recorded relatively high frequencies of sickness or respiratory diseases. The mean proportion of short absences for all sickness varied in the 4 quarters from 52 percent in the first quarter to 62 percent in the third, with 58 percent and 60 percent representing the second and fourth quarters, respectively. The corresponding upper and lower limits for the respiratory diseases were 51 and 64 percent, with 58 and 61 percent for the second and fourth quarters, respectively. Thus the elimination of the nonrespiratory diseases effected relatively little change in the proportions for all sickness.

However, the range of the proportions corresponding to the 40 quarter-years increased from 45-68 percent to 41-74 percent when the nonrespiratory diseases were eliminated, the frequency range changing from 400-1,700 to 100-1,300. Thus, while the range of the proportions widened, the frequency range remained almost the same but was translated necessarily to a lower level, the downward movement covering approximately 300 frequency units.

The inverse association of frequency and proportion of short-term absences is of particular interest from the standpoint of seasonal variation. The effect of the winter months on absence frequency is well known; it now appears that the periodic increase in frequency is not evenly distributed among the disabilities of different durations but is less in evidence among the short-term absences. Likewise, the general decrease in frequency during the summer months is accompanied by a relatively smaller decrease in the frequency of the short-term absences.

SUMMARY

The present inquiry, the fifth of a series on the duration of disabling sickness, is concerned with the frequency of short-term absences lasting less than 4 days and its relation to the total frequency.

Based on the 10-year disability experience of a public utility company, it is shown that high total frequencies regularly occurring in the first quarter of each year are associated with relatively small proportions of short-term absences, the proportion tending to become smaller in epidemic periods; on the other hand, the low total frequencies generally appearing in the third quarter of each year are associated with relatively large proportions of such absences. A further investigation of this relationship by means of scatter diagrams for all sickness and the respiratory group of diseases revealed, in general, an inverse association between the magnitude of the total frequency and the proportion of short-term absences. Two cubic equations describing the trend of the relationship are presented for all sickness and the respiratory group of diseases.

REFERENCES

- (1) Gafafer, W. M., and Frasier, E. S.: Studies on the duration of disabling sickness. I. Duration of disability from sickness and nonindustrial injuries among the male and female memberships of 25 industrial sick benefit organizations, 1935-37, inclusive. Pub. Health Rep., 55: 1892-1903 (Oct. 18, 1940). (Reprint No. 2201.)
- (2) Gafafer, W. M., and Frasier, E. S.: Studies on the duration of disabling sickness. II. Duration of disability from sickness and nonindustrial injuries among male workers, disabilities lasting one calendar day or longer. Pub. Health Rep., 57: 1378-1384 (Sept. 11, 1942). (Reprint No. 2404.)
- (3) Gafafer, W. M., Sitgreaves, R., and Frasier, E. S.: Studies on the duration of disabling sickness. III. Duration of disability from sickness and nonindustrial injuries among the male employees of an oil refining company with particular reference to the older worker, 1933-39, inclusive. Pub. Health Rep., 57: 112-125 (Jan. 23, 1942). (Reprint No. 2350.)
- (4) Gafafer, W. M., and Sitgreaves, R.: Studies on the duration of disabling sickness. IV. Duration of disability from the nonrespiratory-nondigestive diseases among male employees with particular reference to the older worker. Pub. Health Rep., 58: 969-979 (June 25, 1943). (Reprint No. 2487.)
- (5) Gafafer, W. M.: Absenteeism. Chapter 24 in Manual of Industrial Hygiene and Medical Service in War Industries. W. B. Saunders Co., Philadelphia, 1943.
- (6) Gafafer, W. M.: Frequency and duration of disabilities causing absence from work among the employees of a public utility, 1938-42. Pub. Health Rep., 58: 1554-1560 (Oct. 15, 1943). (Reprint No. 2520.)

PATHOLOGIC CHANGES IN ANIMALS EXPOSED TO A COMMERCIAL CHLORINATED DIPHENYL¹

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

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.

DEPOSITION
EXHIBIT

Papageorge
10-1D 8-28-81

results reported are from groups of animals exposed solely to obtain pathologic data.

1. STOMACH AND PANCREAS

(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, finely basophilic necrotic mass containing much nuclear debris, many polymorphonuclears, and lymphocytes. Much of the nuclear material was present at the periphery. On the 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 cosinophilic. 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 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 chloroma (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.

Liver.—Eight to 10 days after injection, fat droplets were noted in the liver cells. At early examinations, these were very few in number, 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.

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

In another series of 23 animals receiving larger single doses of 0.1 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. Fatly 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 resisted from the injection of 0.5 cc. (690 mg.) as from 0.05 cc. (69 mg.); 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

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.

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 prickly 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.

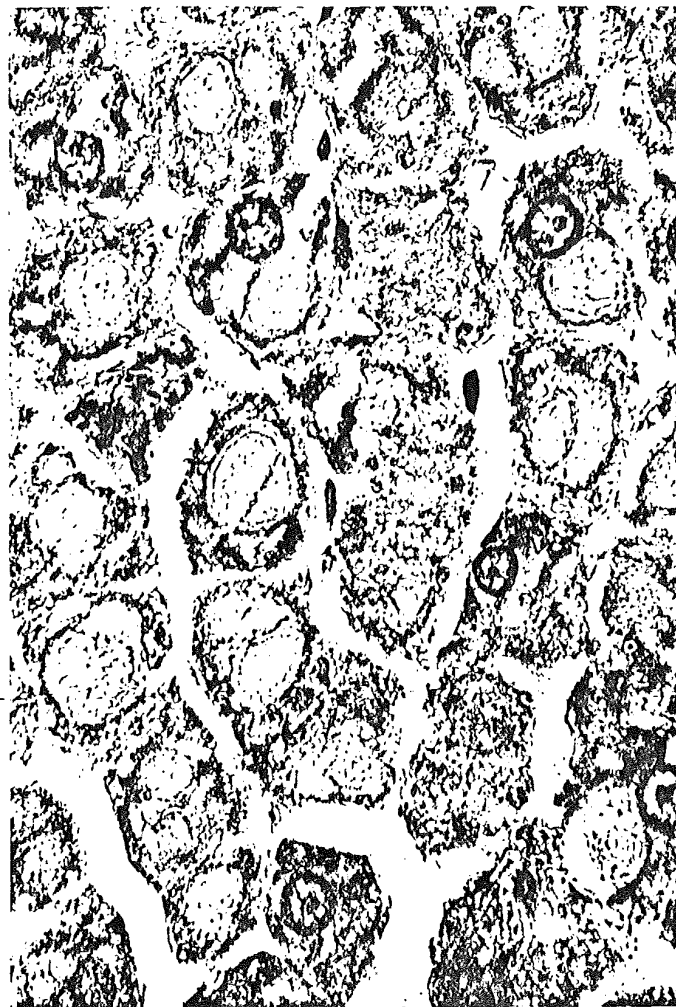


FIGURE 1. Intracellular hyaline bodies in livers of rats exposed to a chlorinated diphenyl.

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 intracellular 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 centrilobular 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 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 chlornene 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.

REFERENCES

- (1) Lillie, R. D.: Romanowsky staining with buffered solutions. III. Extension of the method to Romanowsky stains in general. *Stain Tech.*, **16**: 1-6 (1941).
- (2) Hershheimer, G.: Zur Fettfarbung. *Centralbl. f. Allg. path. u. Anat.*, **14**: 891 (1903).
- (3) Schwartz, L., and Peck, S. M.: Occupational acne. *New York State Med. J.*, **43**: 1711-1718 (Sept. 15, 1943).
- (4) Schwartz, L., and Barlow, F. A.: Chlornene from cutting oils. *Pub. Health Rep.*, **57**: 1747-1752 (Nov. 20, 1942).
- (5) Schwartz, L.: An outbreak of halowax acne ("cable rash") among electricians. *J. Am. Med. Assoc.*, **122**: 158-161 (May 15, 1943).
- (6) Lillie, R. D.: Personal communication.
- (7) Bennett, G. A., Drinker, C. K., and Warren, M. F.: Morphological changes in the livers of rats resulting from exposure to certain chlorinated hydrocarbons. *J. Indust. Hyg. and Toxicol.*, **20**: 97-123 (February 1938).
- (8) Edwards, J. E., and White, Julius: Pathologic changes with special reference to pigmentation and classification of hepatic tumors in rats fed *p*-dimethylaminoazobenzene (butter yellow). *J. Nat. Cancer Inst.*, **2**: 157-183 (October 1941).
- (9) Smith, M. I., Lillie, R. D., and Stohlman, E. F.: The toxicity and histopathology of some azo compounds as influenced by dietary protein. *Pub. Health Rep.*, **58**: 304-317 (Feb. 19, 1943).

DEATHS DURING WEEK ENDED AUGUST 5, 1944

[From the Weekly Mortality Index, issued by the Bureau of the Census, Department of Commerce]

	Week ended Aug. 5, 1944	Correspond- ing week, 1943
Data for 93 large cities of the United States:		
Total deaths.....	8,125	8,286
Average for 3 prior years.....	7,801	
Total deaths, first 31 weeks of year.....	288,008	294,030
Deaths under 1 year of age.....	654	639
Average for 3 prior years.....	600	
Deaths under 1 year of age, first 31 weeks of year.....	19,218	20,927
Data from industrial insurance companies:		
Policies in force.....	66,691,804	65,698,408
Number of death claims.....	11,548	10,889
Death claims per 1,000 policies in force, annual rate.....	9.1	8.6
Death claims per 1,000 policies, first 31 weeks of year, annual rate.....	10.3	10.1