Light and Electron Microscope Study of Chlorobiphenyl Poisoning

In Mouse and Monkey Liver

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The effects on mouse and monkey liver of long-term oral administration of chlorobiphenyls (CBP), 1.5 mg/day or more, have been investigated at selected intervals by light and electron microscopy. Hepatocytes of mice contained large amounts of acidophilic materials in the cytoplasm, and fatty vacuoles were observed later. Fine structural changes in the hepatocytes consisted of a marked increase of smooth endoplasmic reticulum, a reduction of rough endoplasmic reticulum, "myelin figure" formation in the cytoplasm, and increase of microbodies and lysosomes. A marked increase in lipid droplets was observed later. Results of electron microscopy in monkey liver showed an increase of smooth endoplasmic reticulum in the hepatocytes and swelling of Kupffer's cells, with an increased number of lysosomes and vacuoles. Judging from the findings by electron microscopy, characteristic lesions of liver cells were produced by administration of CBP.

INGESTION of rice bran oil contaminated with chlorobiphenyls compound (Kanechlor 400) produced poisoning among the general population in western Japan from summer to fall of 1968,[1] with a total of 600 patients to date. This event seems to be unique in the field of food poisoning.

It was found that contamination of the oil occurred by leaking of chlorobiphenyls compound into the rice bran oil through pinholes in a pipe used for heat exchange in the manufacturing process. Now, one year after the incident, many patients are anxious about the possible presence of latent lesions in internal organs, as well as persistent dermatological signs.

Various reports of poisoning by chlorobiphenyls (CBP)[2-4] since the first outbreak of acne-like lesions, due to high-boiling chlorinated compound in industry, was noted by Schwartz in 1936.[5] But all these cases were due to exposure to the fumes or dust of this compound.

Several animal experiments have been carried out to examine the toxic effects of CBP by the oral route[6,7] and have revealed lesions of skin and liver by light microscopy. However, the limited resolving power of light microscopy left unsolved problems of interpretation. Moreover, these earlier reports were concerned primarily with the effects of high levels of exposure for a short period. This paper extends these observations to the levels of resolution provided by electron microscopy and more thoroughly explores the effects of relatively low levels of oral exposure for a long period.

Materials and Methods

Experiment With Mice.—The ddN strain mice used were originally obtained from the National Institute of Genetics, Mishima, Japan, and raised in the mouse colony of this university for 12 years. Sixty ddN female mice ten weeks old were separated into three groups: (1) mice receiving olive oil or nontoxic rice bran oil as a control group, (2) mice receiving 0.5% v/v CBP in olive oil, and (3) mice receiving toxic rice bran oil containing CBP at a concentration of 1,600 ppm. The CBP used was a mixture of chlorinated biphenyls containing 48% chlorine (equivalent to three to four atoms of chlorine per molecule), with a trace (0.01%) of naphthalenes. Each mouse was given 0.2 ml of its respective oil by stomach tube every day. All animals were kept in metal cages, in groups of two to three per cage, and were given the commercial standard diet and water ad libitum. They were observed for toxic signs and weighed twice a week.

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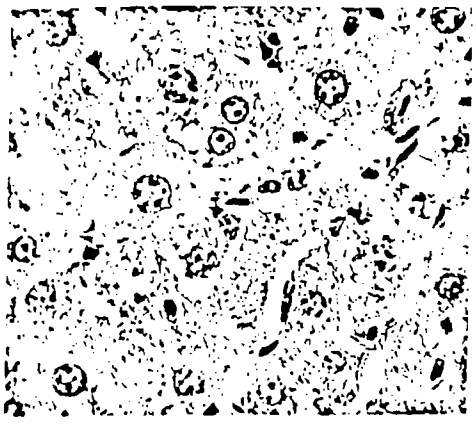


Fig 1.—Hepatocytes of control mouse that received olive oil for 26 weeks. Small vacuoles are seen in cytoplasm; 10% formaldehyde solution fixation (hematoxylin-eosin, slightly reduced from × 560).

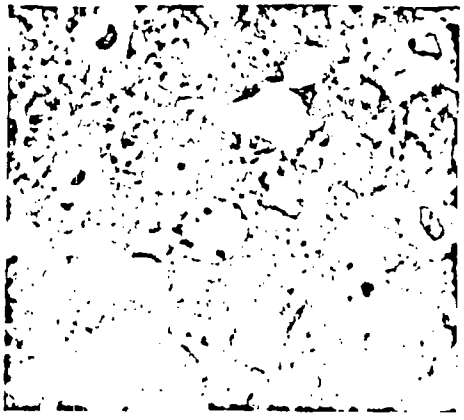


Fig 2.—Hepatocytes from control mouse that received nontoxic rice bran oil for 26 weeks. No remarkable abnormalities are present. Densely stained masses in cells represent associated mitochondria and ER; 4% glutaraldehyde fixation (toluidine blue, slightly reduced from × 800).

Seven or eight mice in each group were killed after four weeks, and two mice in each group at a time were killed at 13, 17, 22, and 26 weeks. All of them were investigated microscopically for the presence of toxic alterations. They were killed by a sharp blow on the head, followed by decapitation, between 9 AM and 10 AM in nonfasted condition. Subsequent to exsanguination, the livers were immediately excised and weighed. Samples from a constant site on the right lateral lobe were processed for light and electron microscopy. Tissues for conventional light microscopy were fixed in neutral 10% formaldehyde solution, and paraffin-embedded tissue was stained with hematoxylin-eosin. Tissues for electron microscopy were immediately placed in a drop of 4% glutaraldehyde in *s*-collidine buffer (pH 7.4) and cut into cubes of approximately 1 mm with a razor blade. Small blocks of tissue were fixed for two hours at room temperature in buffered 4% glutaraldehyde and postfixed for one hour at 0 C in collidine-buffered 2% osmium tetroxide. Some of these tissues were also cut and fixed in 2% osmium tetroxide in *s*-collidine[8] or phosphate buffer[9] at pH 7.4 for 1½ to 2 hours. The fixed and washed tissue slices were then dehydrated in a graded series of ethanol and in propylene oxide prior to embedding in epoxy resin (Epon).[10] Thin and thick sections of the epoxy-embedded tissues were cut with glass knives on an ultramicrotome. Thick (0.5μ to 1.0μ) sections were prepared and stained with toluidine blue[11] for orientation. Thin sections of approximately 60 mμ were stained with uranyl acetate[12] and lead nitrate or Millonig's lead[13] alone. Specimens were examined with an electron microscope operating at 50 kv.

Experiment With Monkeys.—Five cynomolgus monkeys, weighing about 1 kg (2.2 lb) each, and three squirrel monkeys, weighing about 500 gm (11.0 lb) each, were used for this experiment. The CBP was administered with fresh natural diet (potatoes, cabbages, carrots, bananas, oranges, apples, and a little quantity of of small, dried fishes). The total amount of CBP administered to a cynomolgus monkey ranged from 641 mg in 40 days (16 mg/day) to 348 mg in 239 days (1.4 mg/day). That administered to a squirrel monkey ranged from 320 mg in 46 days (7 mg/day) to 67 mg in 48 days (1.4 mg/day). They were observed for general skin alterations and behavior and weighed weekly. Histological and electron microscopical examination were carried out in the same way as in mice.

Results

General Observations.—*Mice.*—The three groups of mice were normal in appearance and behavior for the first three months except for slight weight loss in two experimental groups. After three or four months, the group of mice receiving CBP in olive oil and that receiving toxic rice bran oil revealed slight decrease in activity, and gross changes of the skin began to appear. The macroscopical changes seen of the skin during the

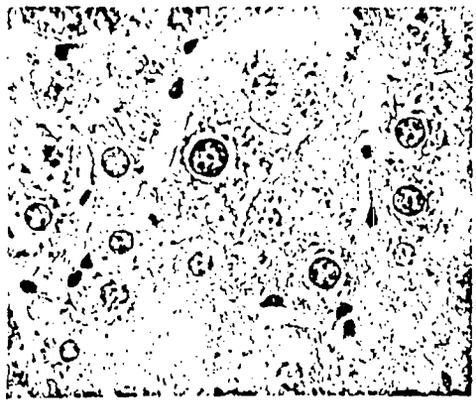


Fig 3.—Hepatocytes of mouse given CBP in olive oil for four weeks. Some of hepatocytes increase in size and contain small granular materials in cytoplasm. Nuclei of hepatocytes vary in size; 10% formaldehyde solution fixation (hematoxylin-eosin, slightly reduced from × 470).

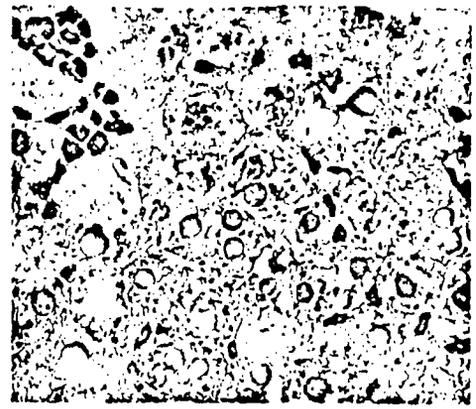


Fig 4.—Liver section from mouse receiving CBP in olive oil for 13 weeks. Hyaline granules and small vacuoles are seen in cytoplasm. Infiltration of small round cells is noted at upper left; 10% formaldehyde solution fixation (hematoxylin-eosin, slightly reduced from × 470).

Fig 5.—Tissue from liver of mouse given CBP for 22 weeks. There is marked variation in nuclear size and many small vacuoles seen in cytoplasm. Note lack of cell necrosis or alteration of other tissue components; 4% glutaraldehyde fixation (toluidine blue, slightly reduced from × 470).

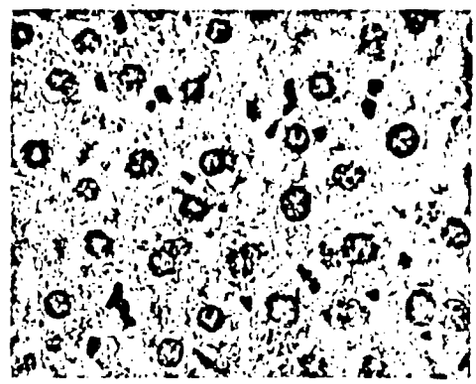


Fig 6.—Hepatocytes of mouse receiving toxic rice bran oil for 13 weeks. Some cells contain small vacuoles in cytoplasm and reveal granular structure; 10% formaldehyde solution fixation (hematoxylin-eosin, slightly reduced from × 470).

experimental period were as follows: (1) eczematous changes of the skin around the eyelids, (2) erosion, ulceration, and perforation of earlaps, and (3) loss of hair, erosion, and ulceration at the skin around the neck, forelegs, and sides of the chest.

At the end of the experiment, the changes similar to those in experimental mice were seen in some control mice. In the former group, however, the changes were much more marked.

Pathological examination at four weeks showed a marked difference in the size of the livers between the groups of mice receiving CBP in oil and the group receiving no CBP in oil ($P < 0.01$). The mice receiving CBP in olive oil and those receiving toxic rice bran oil had a mean absolute liver weight of 2.9 ± 0.4 gm and 2.1 ± 0.3 gm, respectively, while the control mice had a value of 1.7 ± 0.2 gm. Mean relative liver weights were 11.5 ± 1.0 gm, 8.3 ± 0.8 gm, and 6.8 ± 0.9 gm/100 gm of body weight, respectively. No gross tissue abnormalities were noted other than marked liver enlargement.

Monkeys.—All monkeys fed CBP gradual-

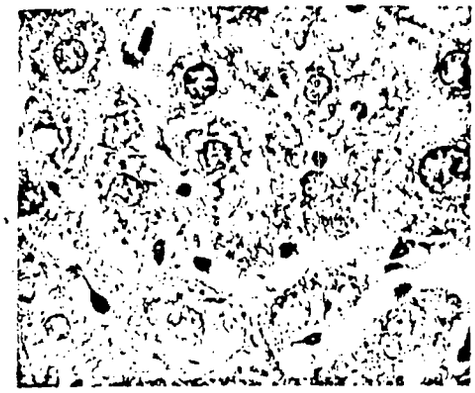


Fig 7.—Liver section from mouse given toxic rice bran oil for 26 weeks. Hepatocytes and Kupffer's cells are enlarged, and cytoplasm of some hepatocytes reveals reticular architecture containing brown pigment. Increased cell size is due to both nuclear and cytoplasmic enlargement. Not all liver cells are equally altered; 10% formaldehyde solution fixation (hematoxylin-eosin, slightly reduced from × 470).

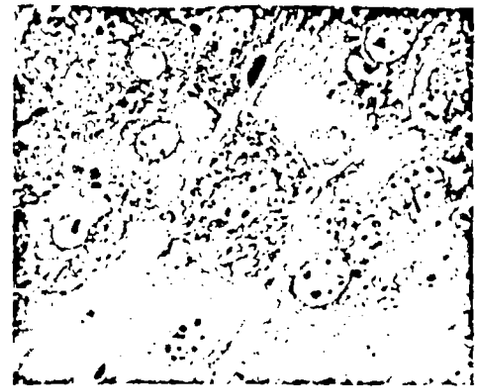
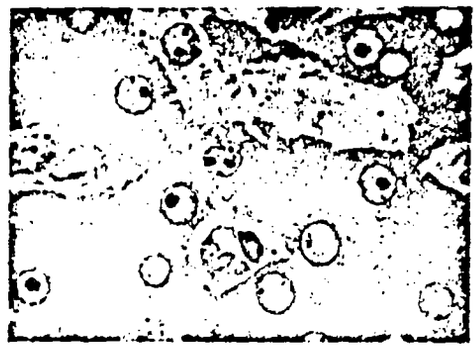
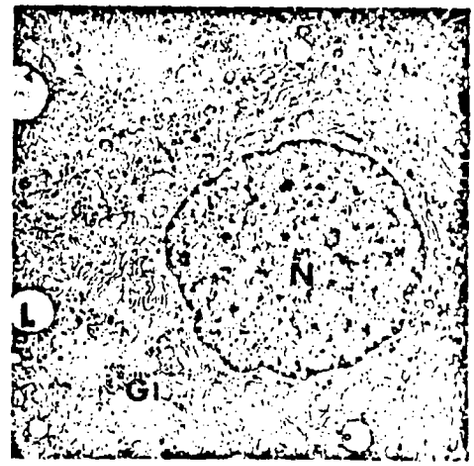


Fig 8.—Hepatocytes of mouse given toxic rice bran oil for 26 weeks, illustrating presence of light and dark cells. Phenomenon of light and dark cells was encountered more frequently in experimental specimens; 4% glutaraldehyde fixation (toluidine blue, slightly reduced from × 470).

Fig 10.—Hepatocyte from control mouse given oil without CBP for four weeks. Various intracellular organelles appeared but no increase in lipid droplets. Glycogen area (Gl), lipid droplets (L); nucleus (N); glutaraldehyde osmium sulfate fixation (lead, reduced from × 7,360).

Fig 9.—Tissue from liver of cynomolgus monkey given total of 348 mg of CBP in 229 days. Small fatty vacuoles are noted in cytoplasms of hepatocytes, and Kupffer's cells are enlarged; 4% glutaraldehyde fixation (toluidine blue, slightly reduced from × 800).





ly lost weight. One of the squirrel monkeys who received a total of 20 mg of CBP in six days revealed palpebral edema two days before death. Increased discharge from the eye with blepharitis and loss of appetite were noted in a cynomolgus monkey who received 795 mg of CBP in seven weeks and were observed for three weeks after CBP was stopped. Although two cynomolgus monkeys who received 122 mg of CBP in six weeks revealed loss of appetite and weight loss during the six weeks, they gained weight after CBP was stopped. Pathological examination just before or after death demonstrated enlargement of the liver, but it was found that pneumonia or diarrhea was the main cause of death for all monkeys.

Light Microscopic Findings.—*Mice.*—In the control mice receiving nontoxic rice bran oil, livers showed nearly normal histological appearance throughout the experiment except for slight appearance of small cyto-

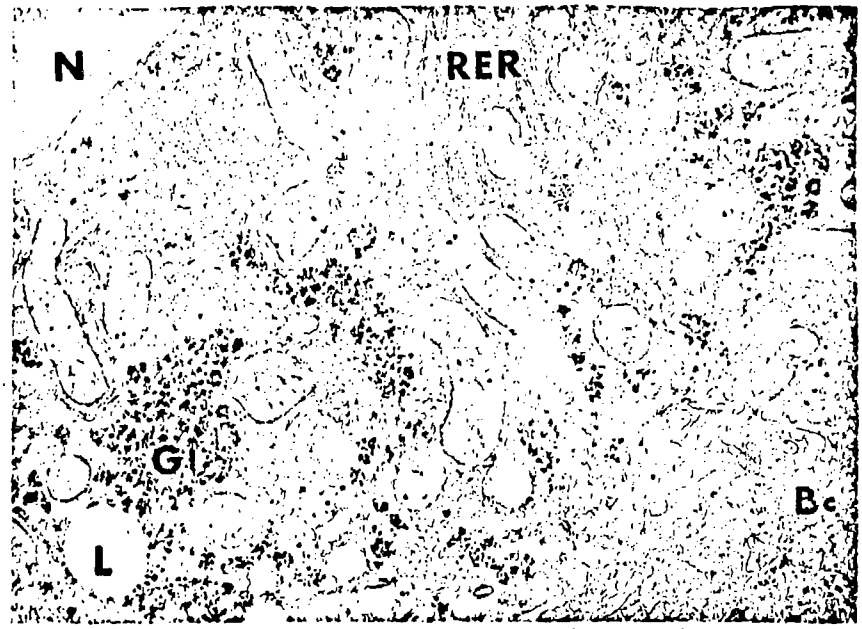
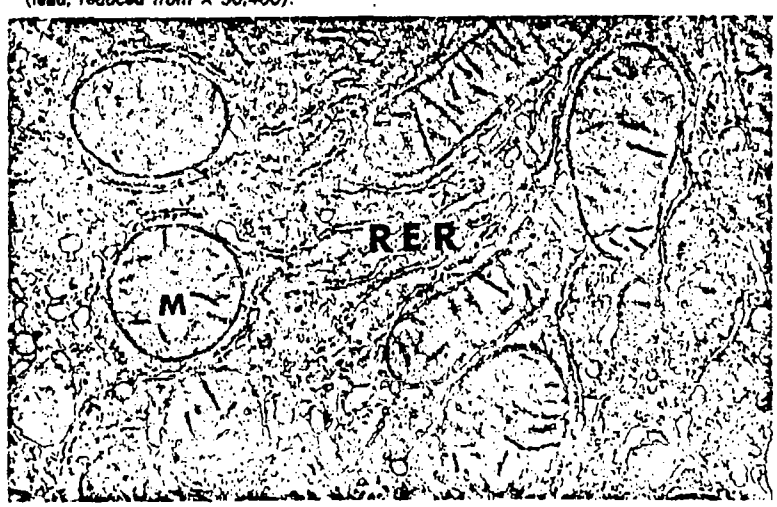


Fig 11.—Hepatocyte from control mouse given nontoxic rice bran oil for four weeks; RER are abundant. Lipid droplets (L), glycogen area (Gl), nucleus (N), bile canaliculi (Bc); osmium fixation (lead, reduced from × 37,600).

Fig 12.—Part of an hepatocyte of control mouse at 17 weeks of experimental period, showing presence of RER and abundant mitochondria (M); osmium fixation (lead, reduced from × 30,400).



plasmic vacuoles in cells of the centrilobular regions after 22 weeks or more (Fig 1 and 2).

The hepatocytes of mice receiving CBP in olive oil for four weeks increased in size and contained small acidophilic granular materials in the cytoplasm without changes of lobular structure (Fig 3). This suggested that the liver enlargement was due to cytoplasmic hypertrophy since mitotic figures were not

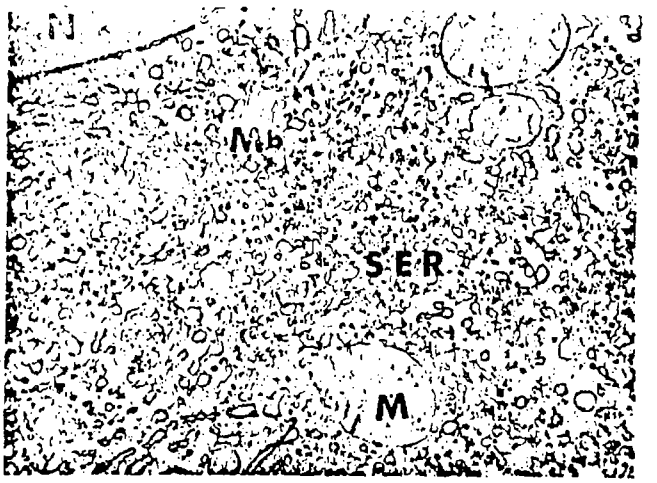


Fig 13.—Portion of an hepatocyte from mouse given CBP in olive oil for four weeks, showing an increased amount of SER and lack of RER. Glycogen granules are present in dispersion. Nucleus (N), mitochondria (M), microbodies (Mb); osmium fixation (lead, reduced from × 24,300).

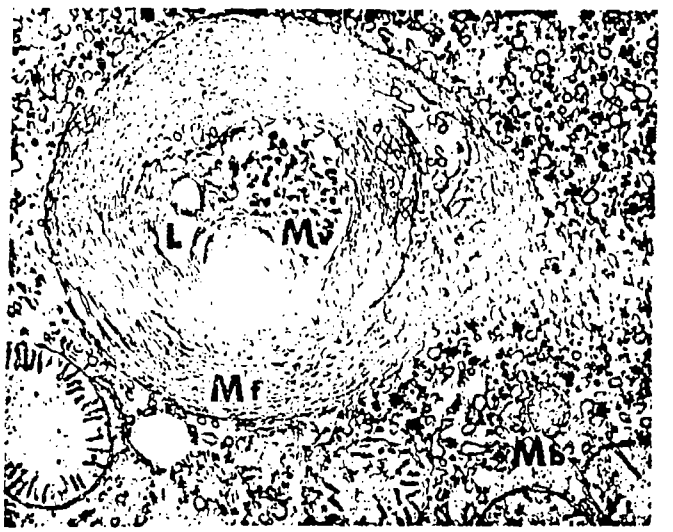


Fig 14.—Portion of an hepatocyte from mouse prepared as in Fig 13. There is "myelin figure" (Mf) in which multivesicular body (Mv) is incorporated with lipid body (L). Synthesizing SER containing electron-opaque material are noted in area surrounding "myelin figure." Microbodies (Mb); osmium fixation (lead, reduced from × 30,400).

Fig 15.—Part of an hepatocyte of a mouse given CBP for 13 weeks, showing another "myelin figure" (Mf); SER is increased markedly; glutaraldehyde OsO_4 fixation (uranyl acetate and lead, reduced from × 16,000).

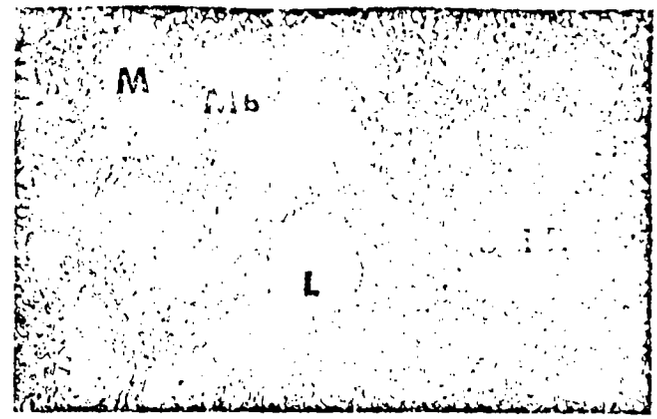


Fig 16.—Area of an hepatocyte prepared as in preceding figures, characterized by unusually abundant microbodies and proliferation of vesicular SER. Microbodies (Mb), mitochondria (M), lipid droplets (L). Osmium fixation lead, reduced from × 29,600).

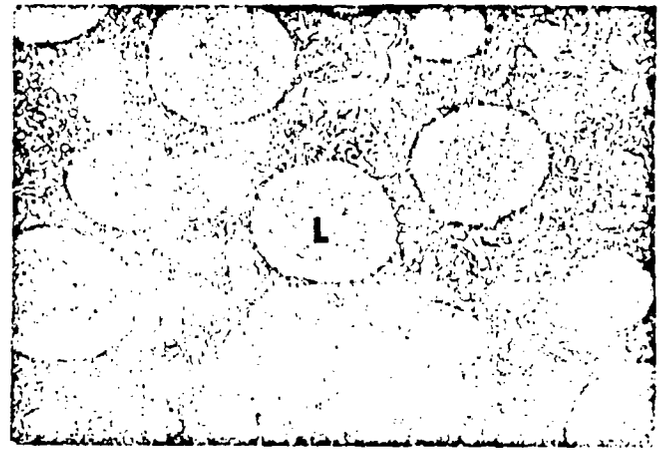


Fig 17.—Part of an hepatocyte from mouse given CBP for 22 weeks. Increases in lipid droplets (L) and microbodies (Mb) are marked. Glutaraldehyde Oso, fixation (uranyl acetate and lead, reduced from × 37,600).

seen frequently. At 13 weeks after commencement of this experiment, more eosin-stained hyaline granules and small vacuoles in the cytoplasm began to be observed (Fig 4). After 17 weeks, the nuclei of the hepatocytes revealed variation in size, and outlines were not clear in some cells. Stainability of cytoplasm generally decreased in sections stained with hematoxylin-eosin, and the cytoplasm was granular. Although lobular architecture was well preserved and no necrosis was seen, there was remarkable variation in size of hepatocytes and their nuclei at 26 weeks. Some Kupffer's cells slightly increased in size (Fig 5).

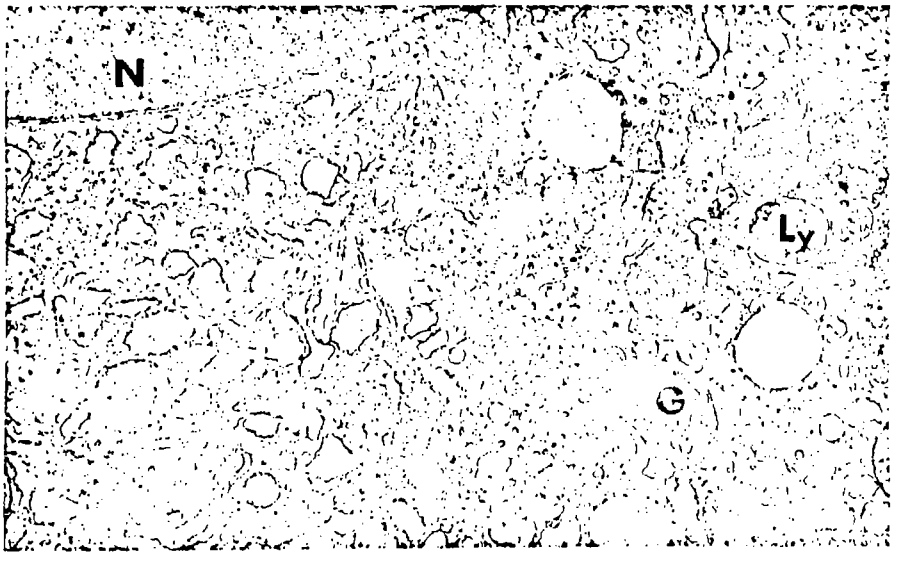
The livers of mice receiving toxic rice bran oil showed the typical lobular structure, with hepatocytes arranged in cords radiating from the central vein. However, hepatocytes in centrilobular areas contained small vacuoles in the cytoplasm at 13 weeks (Fig 6). At 22 weeks, there was a marked increase in the number of small vacuoles, and most of the nuclei increased in size ununiformly. After 26 weeks, sinusoidal endothelial cells, Kupffer's cells, and some hepatocytes around the central vein contained brown pigment in their enlarged cytoplasm. Hepatocytes and Kupffer's cells increased in size and had a reticulated architecture in their cytoplasm (Fig 7 and 8).

Monkeys.—Histological findings in livers of monkeys fed CBP generally revealed the presence of enlarged Kupffer's cells, with vacuoles in the cytoplasm of hepatocytes.

In a squirrel monkey that received a total of 51 mg of CBP per kilogram and died eight days from commencement of the experiment,

Fig 18.—Part of an hepatocyte from mouse given toxic rice bran oil for four weeks; SER and microbodies (Mb) are fairly abundant, but decrease in RER is not evident. Nucleus (N); glutaraldehyde Oso, fixation (uranyl acetate and lead, reduced from × 30,400).

Fig 19.—Portion of an hepatocyte from mouse given toxic rice bran oil for 26 weeks, illustrating appearance of lysosomes (Ly) and well-developed Golgi's complexes (G). Nucleus (N); osmium fixation (lead, reduced from × 28,400).



the liver showed slight enlargement of Kupffer's cells. The liver of a squirrel monkey that received a total of 134 mg of CBP per kilogram in six weeks also showed enlargement of Kupffer's cells containing erythrocytes and dilatation of sinusoidal spaces.

In two cynomolgus monkeys that were fed about a total of 500 to 600 mg of CBP per kilogram in four to six weeks, notable changes included the appearance of large vacuoles in the hepatocytes at the periphery of the lobules and enlargement of Kupffer's cells. Two other cynomolgus monkeys were

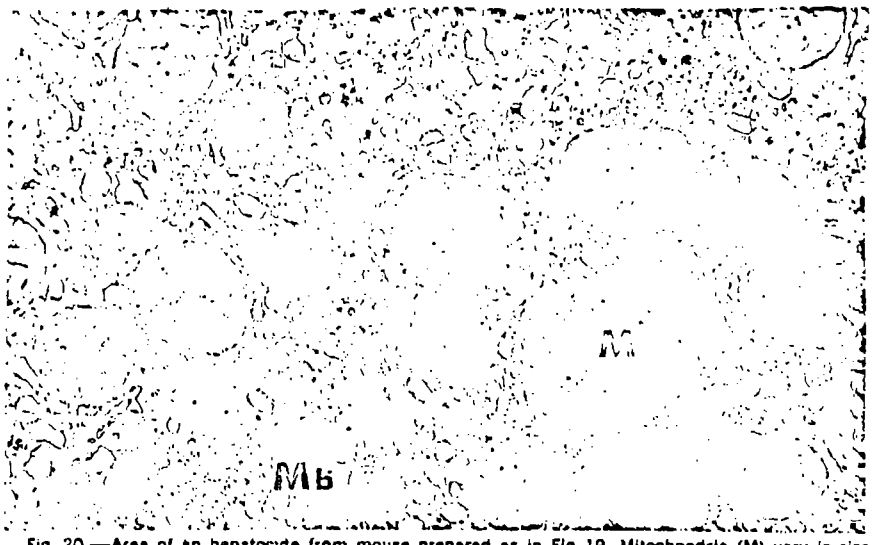


Fig 20.—Area of an hepatocyte from mouse prepared as in Fig 19. Mitochondria (M) vary in size. Microbodies (Mb) are dispersed. Osmium fixation (lead, reduced from × 29,600).

given a total of about 300 mg of CBP per kilogram in 20 to 32 weeks. In these cases, many vacuoles were seen in enlarged Kupffer's cells, and some hepatocytes contained granular materials and vacuoles (Fig 9). The hepatocytes of the monkey who died after 32 weeks revealed slight fatty degeneration in peripheral lobular areas. These microscopic alterations in the monkey livers resembled those in mice.

Electron Microscopy.—*Mice Given CBP-Free Oil.*—In electron microscopy, no obvious differences were seen between the livers of mice given olive oil and those given nontoxic rice bran oil. After four weeks, slight increase in cytoplasmic lipid droplets were noted, and the abundant mitochondria varied in size and were spherical to ellipsoidal in form. The nuclei were round in outline, and many contained nucleoli. Other cell organelles such as endoplasmic reticulum, Golgi's complexes, and microbodies were normally distributed. Stores of densely stained glycogen varied in amount and localization from cell to cell (Fig 10 and 11). After 13 weeks, the livers showed slight increase in microbodies, as well as lipid droplets. Some of the lipid droplets were partially or completely surrounded by mitochondria. In 22 to 26 weeks, moderately increased lipid droplets were noted in most of the hepatocytes. Golgi's complexes and SER (smooth-surfaced membranes of the endoplasmic reticulum) were more abundant and contained electron-opaque substances. Stacks of RER (rough-surfaced membranes of the endoplasmic reticulum) were fairly abundant for the duration of the experiment and, for the most part, were arranged in parallel (Fig 12). No novel observations were made with regard to lysosomes, peribiliary spaces, and nuclear structure.

Mice Given CBP in Olive Oil.—Administration of CBP led to widespread alterations of the ER (endoplasmic reticulum), which persisted for the duration of the experiment. Appearance of cytoplasmic inclusions and increase in microbodies, lysosomes, and lipid droplets were also observed. Serial observations were as follows:

1. Four weeks after commencement of the experiment, proliferation of the SER was apparent, and individual cisternae containing electron-opaque materials were swollen and vesiculated (Fig 13). The RER were decreased with reorganization into rough-surfaced sinuous cisternae. One to four myelin figures were present in about 10% of the hepatocytes (Fig 14 and 15). Slight increase in microbodies was observed at this period, but lipid droplets were not yet increased.

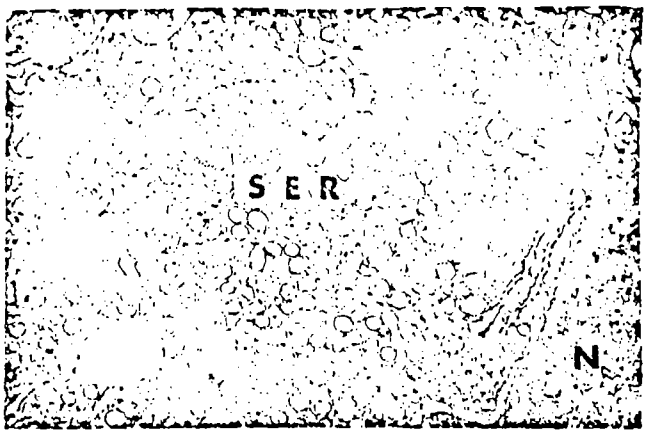


Fig 21.—Area of an hepatocyte from cynomolgus monkey fed total of 319 mg of CBP in 22 weeks, showing increased vesicular SER. Nucleus (N); glutaraldehyde Oso, fixation (lead, reduced from × 32,800).

Fig 22.—Enlarged Kupffer's cell of monkey fed total of 348 mg of CBP in 34 weeks, showing numerous vacuoles and nucleus of blood cell (Nbc) in cytoplasm. Nucleus of Kupffer's cell, (Nk) glutaraldehyde Oso, fixation (lead, reduced from × 12,000).

Glycogen was dispersed among vesiculated or tubular SER.

2. After 13 weeks, the hepatocytes contained an unusually large population of microbodies (Fig 16) and slightly increased lipid droplets. Mitochondria showed variation in size and form, and lipofuscin granules were observed in peribiliary areas. Fine glycogen granules were dispersed among vesicular SER.

3. After 17 to 22 weeks, lipid droplets were increased considerably with groups of microbodies (Fig 17). Mitochondria showed a possible increased variation in size and shape, and, occasionally, intramitochondrial inclusions were found. Golgi's region was well preserved and appeared active, as judged by accumulation of electron-dense products within its membranes.

4. After 26 weeks, marked alterations of ER were sustained. An increase in lipid droplets or fat vacuoles was noted, and many microbodies were present close to lipid droplets.

Mice Given Toxic Rice Bran Oil.—After four weeks, alterations of RER were not as remarkable as in mice given CBP in olive oil, but an increase in SER was clearly noted (Fig 18). The mitochondria varied in size. The hepatocytes contained abundant glycogen stores, and lipid droplets were slightly increased.

After 13 to 17 weeks, increase in SER and decrease in RER were remarkable. Most mitochondria were surrounded by parallel-arranged RER but were almost normal in size and shape. Glycogen deposits were preserved fairly well. At 26 weeks, increases in microbodies and appearance of a well-developed Golgi's complex were revealed in addition to the alterations of ER (Fig 19 and 20). Glycogen granules were dispersed sparsely.

In conclusion, the hepatic cell alterations induced by administration of toxic rice bran oil were essentially very similar to those caused by administration of CBP in olive oil. It was considered that the slight difference in severity of alterations was based upon the difference in the concentrations of CBP: about 1,600 ppm in the toxic rice bran oil and about 5,000 ppm in olive oil.

Monkeys Fed CBP.—The hepatocytes of monkeys fed CBP revealed the increase in SER, with dilated vesicles (Fig 21). The mitochondria were generally decreased in the density of the internal matrix and varied in size. Some mitochrondria contained intramitochondrial myelin figures. Lipid droplets and microbodies were of normal appearance, but increase in lysosomes was slightly noted.

Kupffer's cells showed moderate cytoplasmic swelling, with dilated ER. In the cytoplasm, there were an increased number of lysosomes and many vacuoles, some of which contained granular materials (Fig 22). In a few places, there were discontinuities of the cell membrane, suggesting increased fragility and subsequent rupture, possibly during processing.

Comment

The oral administration of CBP in oil resulted in enlargement of the liver and vacuolar or fatty degeneration of liver cells in light microscopy. These findings were in accordance with the observations of others.[6,7,14] Bennett et al[14] pointed out that chlorinated biphenyl resulted in liver changes markedly different from those caused by other well-known toxic agents such as carbon tetrachloride and chloroform poisoning and thought that this type of injury was persistent with slow recovery. In our study, the morphological alterations induced in hepatocytes by administration of CBP are generally slight when judged by conventional light microscopy, but several marked alterations in cellular organelles were found by electron microscopy.

An outstanding abnormality of liver cells caused by administration of CBP to mice and monkeys is an increase in the SER and a reduction in the RER throughout the experiment. It is considered that proliferation of the SER in the hepatocytes corresponds to hyaline cytoplasmic inclusions (hyaline degeneration) recognized with the light microscope and is related to the liver cell hypertrophy. Proliferation of SER in the hepatocytes is generally seen after treatment with various toxic substances such as 3'-methyl-4-dimethylaminoazobenzene,[15] dimethylnitrosamine,[16] thioacetamide,[17] ethionine,[18] α-naphthyl isothianate,[19] phenobarbital sodium,[20] and chlorophenothane (DDT).[21] Clearly, proliferation of SER cannot be considered to be a specific response induced by CBP. Fouts[22] has pointed out that the SER of hepatocytes may be an important site of action of several drugs and poisons. In fact, it has been observed that an increase of SER in liver cells is closely associated with an increase of drug-metabolizing enzyme in the microsomal portion of liver homogenates.[22,23] A suggestion that these changes reflect adaptive phenomena for enhanced drug detoxification could also be made from CBP poisoning. Consequently, it is also fully considered that disturbances of lipid metabolism such as increase of triglyceride level in blood[24] are induced as the result of the function of proliferated SER. These changes of SER were accompanied by a disorganization of the polarized arrays of the RER and by loss of surface ribosomes from the structually intact paired membranes.

Cytoplasmic inclusions, which are composed of concentric whorls of closely packed smooth membranes, were seen in mice receiving CBP. These cytoplasmic inclusions have been generally referred to as "finger prints,"[16,19] "nebenkern,"[25] or "figures myeliniques"[26] and have been observed after treatment with various toxic chemicals.[16-21,27,28] Herdson et al[20] suggested that some enzyme functions reside in these cytoplasmic inclusions. Norback and Allen[29] considered that the membrane arrays would provide a great-

ly increased membrane surface area to ensure maximal contact of the toxic agent with the detoxifying enzymes. Various suggestions have been made as to the origins and functions of these inclusions on the basis of the morphological relationships of the formations to other organelles and the overall effects of the inducing agent on the cell. However, many details still remain for later work.

Diminution of glycogen stores is another interesting feature in the present experiment. This is partly due to the fact that the mice receiving toxic oil consumed approximately 80% of the amount of the diet consumed by the control mice. However, a hypothesis has been proposed to explain the reciprocal behavior of the SER and glycogen stores.[15] Bruni suggested that when diminution or loss of glycogen and permeation of glycogen areas by the SER occur almost simultaneously, circumscribed acidophilic areas might be seen.[30] As for our findings in light microscopy, appearance of acidophilic granular materials in the cytoplasm early and of vacuoles or hyaline cytoplasmic inclusions later might be the results of hypertrophy of SER and diminution of glycogen stores. Four months after the administration of CBP in olive oil was stopped, an increase of vesicular ER, with dilated sinuous cisternae, was still observed, but in the same period, no alterations of ER were noted in the hepatocytes of mice given the toxic rice bran oil. It was suggested that these alterations of ER are adaptive phenomena to CBP and persist in proportion to the concentrations of administered CBP.

The microbodies in experimental mouse liver slightly increased in number early, but these organelles were markedly increased after 13 weeks. It has been reported that the microbodies take part in lipid metabolism and glyconeogenesis and that increase in number accompanies changes of their structure in liver injury.[31] In this experiment, an increase in microbodies might be recognized as the morphological expression of metabolic changes induced by CBP.

In control mice, fat was abundantly observed as cytoplasmic globules from an early period of the experiment whereas in experimental mice, increase in lipid droplets was observed after 20 weeks. These findings suggest that altered metabolism induced by CBP prevents an accumulation of lipid in the early period of the experiment and results in an increase of neutral fat in the blood. In the monkey, an increase in lipid bodies was noted in Kupffer's cells of the liver injured by CBP, but hepatocellular lipid droplets were not increased. This fact does not resolve the question of whether this disparity is due to the difference in animals or other factors. For example, monkeys were fed CBP not dissolved in oils while mice received it in oils. In our laboratory, other work is going on to clarify the differences between these two cases. In the near future, more refined combinations of pathological and chemical analysis may elucidate its mechanism of action.

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ULTRASONIC COMMUNICATION

The upper frequency limit of human hearing is generally about 17-20 kHz in young people and, although for technical reasons audiometry is seldom continued above 12 kHz, it is fairly easy to show that the limit tends to decline with age as an expression of presbycusis. It has been claimed, however (Pumphrey, 1950), that the limitation is entirely due to conduction loss and that sounds presented at sufficient intensity by bone conduction are audible, without further sensation of pitch change, to more than 100 kHz. It appears that the human cochlea is capable of responding to high "ultrasonic" frequencies but does not normally experience them and is unable to perform frequency analysis upon them.

These limitations of the human ear are not shared by many smaller mammals, for there is good evidence that many species not only hear and analyze ultrasound, but use these frequencies for communication. Ralls (1967) has shown that mice and *Peromyscus* show posterior collicular responses to at least 100 kHz, with greatest sensitivity in the range 10-30 kHz. Noirot (1966) found that baby mice communicate with their mother at 60-90 kHz. Sewell (1967) has found similar behavior in ten other species of Myomorph rodents and shown that the adults of some species indicate aggressive and submissive intentions by ultrasonic signals.

The hearing of bats is especially interesting, because the Microchiroptera show a highly developed form of ultrasonic echolocation.—Pye, J.D.: "Hearing in Bats" in de Reuck, A.V.S., and Knight, J. (eds.): *Hearing Mechanisms in Vertebrates*, Boston: Little, Brown & Co., 1968, p 66.