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[GANN, 64, 105~108; February, 1973]

UDC 616-006-021.6[616.36] :547.621

NEOPLASTIC CHANGES IN THE RAT LIVER INDUCED BY POLYCHLORINATED BIPHENYL

(Plate XII)

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Polychlorinated biphenyls (PCBs) are now widely dispersed in the global ecosystem. There are many reports that PCBs and such chlorinated insecticides as BHC, Dieldrin, and DDT have extensively polluted the animals in the world, and that high concentrations of PCB have been detected in fish, shell-fish, seals, porpoises, and wildlife in general.[4,5,7,14] PCBs have also been detected in human adipose tissue and mother's milk.[1,2] Not only accumulation of PCBs in human tissue but also their hazard is evident from the unfortunate and miserable accident of "Yusho" (oil disease), a mass food poisoning involving more than 1,000 people who over a long period had ingested rice oil contaminated with PCB that had leaked from a heat exchanger in the course of manufacture, that occurred in 1968 in western Japan.[3,6]

The studies we made this time on the carcinogenic potency of PCB were started by the findings that there were specific morphological alterations in liver cell nucleoli of the animals ingesting PCB as demonstrated by electron microscopy.[16] Similar nucleolar changes caused by several agents known as potent carcinogen have been reported.[9,10,13]

Materials and Methods

Kanechlor-400, a commercial brand of PCB and the pollutant in the rice oil of the Yusho case, was employed for the present study. Six compounds were recently isolated from Kanechlor-400, and five of them were chemically identified as 2,4,3′,4′-, 2,5,3′,4′-, 2,3,4,4′-, and 3,4,3′,4′-tetrachlorobiphenyls, and 2,3,4,3′,4′-pentachlorobiphenyl.[15] Thirty rats of Donryu strain were used. They were 10 weeks of age at the start of the experiment, and 185~260 g in weight in male group (average, 207 g) and 155~225 g in female group (average, 186 g). They were randomly divided into 2 groups for experiment and control. The experimental group consisted of 10 male and 10 female rats, and the control group had 5 each. The diet for both, experiment and control, were prepared as follows: For the experimental group olive oil containing a certain concentration of Kanechlor-400 was mixed in a concentration of 0.3 ml/g with powdered diet (CE-2, Oriental Co., Tokyo). For the control group, pure olive oil was mixed in the same concentration with the powdered diet. The diet thus prepared and water were given freely. Initial concentration of Kanechlor-400 in the test diet was 38.5 ppm and given for 4 weeks, then referring to the body weight increase of the animals the concentration of Kanechlor-400 was increased by 2 times of the initial one and given for 8 weeks, 4 times for 3 weeks, 8 times for 3 weeks, and 16 times for 8 weeks. The change in body weight of the animals was individually checked every week. Since the body weight decreased remarkably when the diet containing 16 times of the initial concentration (616 ppm) of Kanechlor-400 was given for 8 weeks, its concentration was decreased to 12 times (462 ppm). This dose was maintained for 32 weeks. Total feeding period to the experimental and control animals was 400 days. During the last half of the

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Table I. Pathological Findings Induced by Long-term Oral Administration of Kanechlor-400 in Rats

Rat No.	Sex	Total exp. period (days)	Approx. amount of KC-400 ingested (mg)	Body weight (g)		Liver			Other pathological findings*
				Initial	Final	Ratio to body wt. (‰)	Fatty degeneration	Multiple adenomatous nodules	
1	♂	159	450	199	309	4.1	+	—	SA, Pn, LA
2	♂	256	900	192	273	5.4	+	—	
3	♂	323	1200	201	192	6.8	+	—	SA, Pn, FDAd
4	♂	339	1300	201	199	6.8	+	—	Pn, D, FDAd
5	♂	347	1300	200	328	6.2	+	—	Pn, LA
6	♂	353	1300	230	210	5.4	+	—	SA, Pn, IA
7	♂	355	1300	201	296	5.2	+	—	Pn
8	♂	357	1300	185	226	6.3	+	—	Pn, LA, FDAd
9	♂	373	1300	234	325	6.5	+	—	LA
10	♂	530	1800	228	297	5.8	+	—	LA
11	♀	244	700	158	137	5.9	+	—	LA
12	♀	357	1100	161	184	6.2	+	—	Pn, D
13	♀	366	1100	154	177	7.1	+	—	LA, D
14	♀	396	1100	165	125	7.8	+	—	Pn, LA, D
15	♀	402	1200	202	212	6.7	+	+	LA, D
16	♀	461	1500	168	148	8.4	+	+	LA, D, IA, FDAd
17	♀	477	1500	178	165	7.8	+	+	LA, D, IA, SA,
18	♀	482	1500	225	145	6.3	+	+	LA, IA, SA
19	♀	504	1500	191	167	6.5	+	+	LA, D, FN
20	♀	560	1500	209	253	6.4	+	+	D, AAd
21	♂	255		257	471	2.5	—	—	FDAd
22	♂	332		140	432	3.1	—	—	Pn, FDAd
23	♂	538		190	426	2.7	—	—	FDAd
24	♂	538		182	523	2.5	—	—	
25	♂	538		256	456	2.7	—	—	FDAd
26	♀	331		192	198	4.2	+	—	Pn
27	♀	448		192	495	2.6	+	—	Pn
28	♀	538		202	292	2.9	—	—	
29	♀	538		201	320	3.0	—	—	
30	♀	538		193	314	2.6	—	—	

* SA = spleen atrophy, Pn = pneumonia, LA = lung abscess, FDAd = fatty degeneration of the adrenal cortex, D = depilation, IA = intracranial abscess, FN = extensive fat necrosis in the abdomen, AAd = adenoma of the adrenal cortex.

Schedule of feeding:

Concn. of Kanechlor-400 (ppm)	38.5	77	154	308	616	462	——	462	——	462	
Period of feeding (days)	26	57	21	21	56	39	28	98	28	82	Total 400

A pause (— —) in PCB feeding was inserted similarly in experimental and control groups, during which and after termination of diet feeding, the rats had free access to the conventional diet (CE-2).

experimental period, a pause in the diet feeding for 4 weeks was inserted twice. Total amount of Kanechlor-400 intake in each rat, the schedule of the feeding, and the results obtained are shown in Table I.

Results and Discussion

The most remarkable changes induced were noticed in the liver. Grossly, all the rats ingesting more than 700 mg of PCB showed hypertrophy of the liver. Pinhead- to pear-sized

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round and pale brown flecks or nodules were scattered on the surface and on the cut-surface of the liver of all the female rats in the experimental group ingesting more than 1,200 mg of Kanechlor-400 (Photo 1). On the contrary, none of the male rats showed such nodular changes in the liver in spite of having ingested a corresponding or even higher amount of PCB than females.

Microscopically, very remarkable changes in the liver of experimental rats were fatty degeneration and multiple adenomatous nodules (Photo 2). The former was seen, irrespective of sex, in all the animals of the experimental group, but it was seen only in 2 females in the control group. The latter, which appeared to be a benign neoplastic lesion, was seen in all the female rats ingesting more than 1,200 mg of Kanechlor-400, as has been predicted in the gross examination. In sharp contrast, however, the liver specimens of the male rats revealed no such nodular changes even in the animals who had ingested a comparable or higher amount of Kanechlor-400 than females.

Lung abscesses, pneumonia, spleen atrophy, and intracranial abscesses were found frequently in the experimental group, and this suggested that the resistance of the rats treated with Kanechlor-400 to infection was lowered. Depilation also appeared frequently in the experimental group especially in the females when the PCB intake amounted to about 600 mg.

It should be emphasized from the present result that PCBs induced a benign neoplastic change, though it seemed to be still short of malignancy, and that the change appeared exclusively in the females. The results of this experiment may offer many problems that should be clarified in the future. Such a neoplastic change as adenomatous nodules in the liver may progress by other specific or non-specific carcinogenic stimuli if introduced into the animals simultaneously or summationally with PCB. It has been reported, as a matter of fact, that nontoxic amounts of PCB enhanced the toxicity of DDT or of some organophosphate insecticides by as much as 100% in insects.[2] Recently, Nagasaki *et al.*[11,12] reported the hepatocarcinogenicity of benzene hexachloride in dd male mice. It must be considered, however, that spontaneous hepatomas have been found frequently in male mice. As to the Yusho case (oil disease) mentioned at the beginning, it was reported that PCB residues were still detected at a high concentration in the adipose tissue of the Yusho patients even 2 years after the onset of the Yusho symptoms.[8]

It is urgent to know beforehand that a lesion would occur in the human body by taking much PCBs on the one hand, and to establish an effective means for therapy against the lesion on the other.

We thank Professor Torao Yamamoto, Faculty of Medicine of Kyushu University, for generous gift of Kanechlor-400 for our study.

(Received October 2, 1972)

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Explanation of Plate XII

Photo 1. Liver of a female rat (No. 20) ingesting ca. 1,500 mg of Kanechlor-400. Note pinhead- to pea-sized multiple nodules scattering on the surface of the liver.

Photo 2. Photomicrograph of the liver of the rat No. 20. Large and small adenomatous nodules are shown. The nodules consisted of somewhat atypical parenchymal cells with clear and enlarged cytoplasm. Some nuclei are pyknotic but not much atypical. Mitosis is seen infrequently. Liver cells surrounding the nodules are atrophic and seem to be compressed by the nodules. Fatty degeneration is also seen in the parenchymal cells. ×40.

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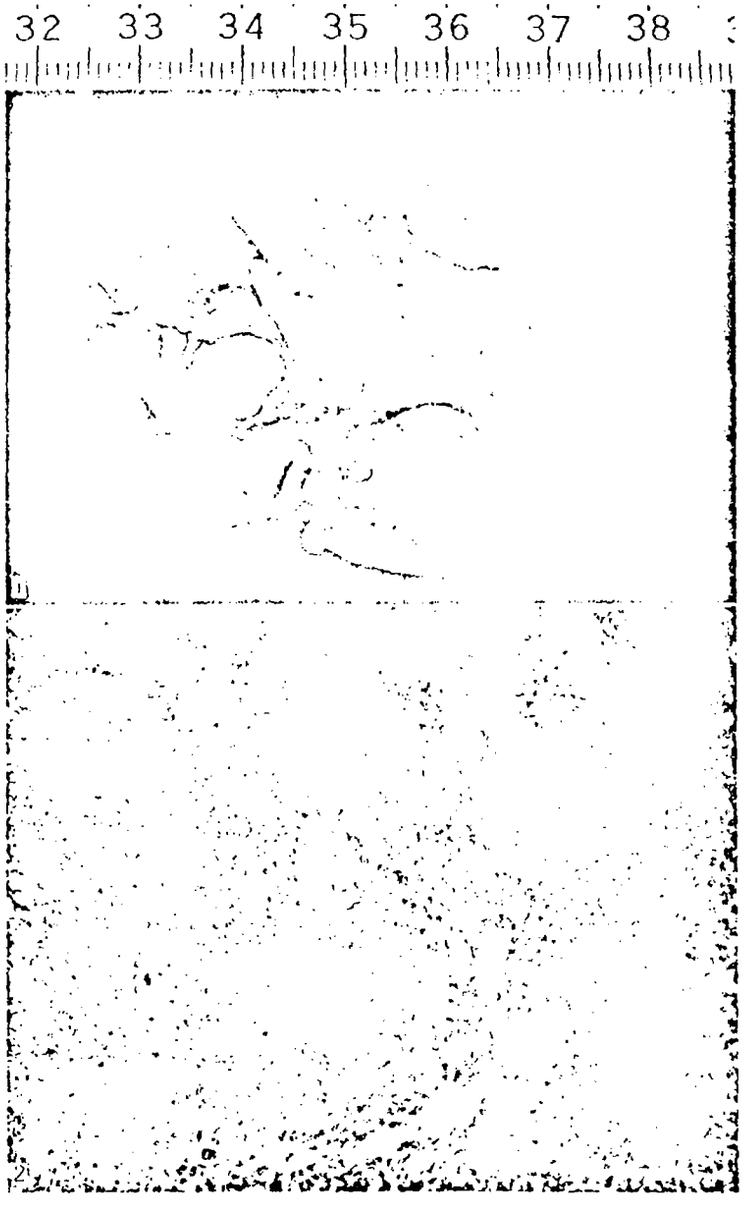

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