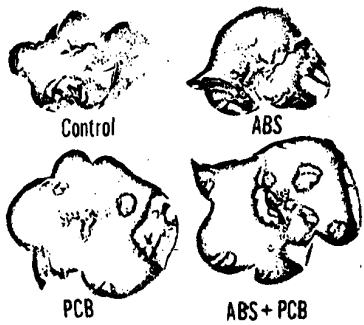
Toxicity of Polychlorinated Biphenyls Increased with Simultaneous Ingestion of Alkylbenzene Sulfonic Acid Salt

Polychlorinated biphenyls (PCBs) are chemicals used internationally for industrial application in insulations, plastics and coatings. The first report on PCBs poisoning was that by SCHWARZ[1] in 1936 and was followed by *further documentation of* the toxicological and pathological effect of PCBs in animal bodies[2,3]. In 1968 public attention was drawn to PCBs when more than 1,000 persons living in Northern Kyushu district of Japan experienced a dermatitis which was attributed to PCBs[4]. Alkylbenzene sulfonic acid salt (ABS) is an industrial neutral cleansing agent used widely in Japan, and this substance plus PCBs have gradually accumulated in the waterways. As removal of these substances is incomplete while utilizing present water purification methods, considerable amounts of these substances are being ingested into the human body via drinking water and seafood, the latter having a high consumption rate in Japan. As the combined action of PCBs and ABS on the animal body has to date not been documented, their study has now been undertaken.

Material and methods. Male Wistar rats weighing 80 to 120 g were divided into 4 groups of 8 animals. To each group the following diets were administered: Group I: ordinary diet and tap water. Group II: ordinary diet and water containing 1,000 ppm of ABS (Laurylbenzene sulfonic acid sodium salt). Group III: PCBs (Kanechlor 500) supplement diet (500 ppm) and tap water. Group IV: PCBs supplemented diet (500 ppm) plus ABS (1,000 ppm) added to tap water.

Diets and water were provided ad libitum. After 3 and 7 months on these dietary regimens, 4 rats of each group were sacrificed and the tissues dissected and weighed. Portions of liver were used for determination of total cholesterol, enzyme activities of aniline hydroxylase, Na-K-Mg-dependent ATPase and Mg-dependent ATPase. Total liver cholesterol was determined by the method of SPERRY and WEBB[5]. Aniline hydroxylase activity was assayed by the method of IMAI et al[6]. Na-K-Mg-dependent ATPase and Mg-dependent ATPase were assayed by the method of NAKAO et al[7]. Protein was determined by the method of LOWRY et al[8].

Results. Table I demonstrates liver weight expressed as a percentage of body weight for each group. In groups III and IV the liver weight showed a significant increase (Figure), total cholesterol levels increased markedly and cholesterol deposits were more marked in group IV, PCBs-ABS co-administered rats rather than in the PCBs alone administered group III (Table II). Liver cholesterol levels increased in proportion to the lenght of PCBs administration as demonstrated by a comparison between rats on the 3 and 7 month dietary regimens. Activity of aniline hydroxylase, a drug metabolizing microsomal enzyme, increased significantly in the PCBs-administered rats. This increase of activity was strengthened significantly when ABS was administered together with PCBs. On the other hand, liver Na-K-Mg-dependent ATPase decreased markedly in PCBs-administered rats and the tendency to a decrease in activity was more significant in the PCBs and ABS co-administered rats. No significant difference was observed in Mg-dependent ATPase among the groups.



Livers of groups I to IV (7 months).

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Table I. Ratio of liver weight to body weight (%)

Group	Additives		Experimental period	
	Diet	Water	3 months	7 months
I	none	none	2.85 ± 0.15^{a}	2.57 ± 0.12^{a}
II	none	ABS	3.26 ± 0.46^{a}	2.50 ± 0.30^{a}
III	PCBs	none	6.11 ± 0.43^{b}	6.24 ± 0.83^{b}
IV	PCBs	ABS	7.07 ± 1.08^{b}	7.86 ± 1.50^{b}

Values represent Mean ± SEM of 4 rats. Different letter superscripts[a,b] denote significant difference ($P < 0.05$).

Table II. Total cholesterol levels in liver

Group	Additives		Experimental period	
	Diet	Water	3 months (mg/100 g wet weight)	7 months
I	none	none	255 ± 31^{a}	257 ± 22^{a}
II	none	ABS	253 ± 25^{a}	$320 \pm 33^{a,b}$
III	PCBs	none	$439 \pm 32^{b,c}$	$548 \pm 34^{c,d}$
IV	PCBs	ABS	$611 \pm 47^{d,e}$	768 ± 67^{e}

Values represent Mean ± SEM of 4 rats. Different letter superscripts (a, b, c, d, e,) denote significant difference ($P < 0.05$).

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Table III. Aniline hydroxylase, Na-K-Mg-dependent ATPase and Mg-dependent ATPase activities in liver

Group	Additives		Aniline hydroxylase		Na-K-Mg-ATPase		Mg-ATPase	
	Diet	Water	3 months (nmoles p-aminophenol/min/mg protein)	7 months	3 months nmoles Pi/min/mg protein	7 months	3 months nmoles Pi/min/mg protein)	7 months
I	none	none	1.16 ± 0.06[a]	1.48 ± 0.21[a]	140 ± 7[a]	153 ± 3[a]	401 ± 34[a]	328 ± 47[a]
II	none	ABS	1.42 ± 0.12[a]	1.22 ± 0.14[a]	122 ± 21[ab]	130 ± 7[a]	424 ± 32[a]	329 ± 36[a]
III	PCBs	none	3.94 ± 0.38[b]	4.19 ± 0.46[b]	78 ± 8[b]	76 ± 6[b]	387 ± 31[a]	342 ± 35[a]
IV	PCBs	ABS	6.72 ± 0.37[c]	6.90 ± 0.66[c]	45 ± 12[ab]	44 ± 10[c]	343 ± 29[a]	399 ± 85[a]

Values are represent Mean ± SEM of 4 rats. Different letter superscripts (a, b, c) denote significant difference ($P < 0.05$) in each enzyme.

Discussion. Reports have confirmed that PCBs increased liver cholesterol levels[9, 10] as well as microsomal drug metabolising enzyme activity[11, 12]. YAP et al.[10] have reported that PCBs inhibits Na-K-Mg-dependent ATPase activity in fish. The present study not only confirmed these results but clarified that the effect of PCBs on enzymes or cholesterol levels in the liver increased when PCBs and ABS were simultaneously administered.

Further investigation is expected to clarify the probability that ABS potentiates an increase in the toxicity of PCBs.

Zusammenfassung. Nachweis, dass in PCB vergifteten Lebern von Ratten die Quantität des Cholesterins und die Aktivität des Anilin-Hydroxylase-Enzyms zunahm, während sich die Na-K-Mg-abhängige ATPase verminderte. Gleichzeitige Verabreichung von ABS bewirkte eine signifikante Steigerung der PCB-Vergiftung.

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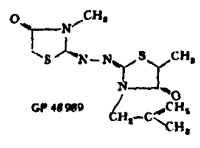
Carcinostatic Activity of a Thiazolidinonylidene-Hydrazonothiazolidinone Derivative Against DMBA-Induced Mammary Carcinomata in Female Sprague-Dawley Rats

The cytostatic agents currently in clinical use were originally selected on account of their inhibitory activity against transplantable tumours and leukaemias. With a view to finding new agents with different chemotherapeutic properties, we began to employ tests based primarily on chemically induced carcinomata. One such method which proved satisfactory in this respect was the technique of inducing mammary carcinomata in female Sprague-Dawley rats by administering DMBA (7,12-dimethyl-benz[a] anthracene), originally introduced by HUGGINS[1] for studies of hormonal dependence and sensitivity. In the present studies, some derivatives of a newly synthesised series of 2-[(4-oxo-2-thiazolidinylidene)hydrazono]-4-thiazolidinones were tested by this method. Among these, GP 48 989, 5-methyl-3-(2-methylallyl)-2-[(3-methyl-4-oxo-2-thiazolidinylidene)hydrazono]-4-thiazolidinone, showed very promising carcinostatic activity and has been subjected to extensive investigations.



GP 48 989

GP 48 989 was prepared by reacting 1-acetyl-4-methyl-3-thiosemicarbazide with chloracetic acid, to form 3-methyl-2, 4-thiazolidinodione-2-(2-acetylhydrazone). Treatment of the deacetylated hydrazone intermediate with 2-methylallyl isothiocyanate then yielded 3-methyl-2, 4-thiazolidinedione-2-[4-(2-methylallyl)-3-thiosemicarbazone], which, after being reacted with 2-bromo-propionic acid, gave GP 48 989, a white crystaline substance with an m.p. of 155–156 °C.

The carcinostatic activity of GP 48 989 was assessed in the following way. The administration of a single oral dose of 15 mg DMBA in 1 ml sesame oil by stomach tube to 50-days-old female Sprague-Dawley rats induced mammary tumours in 90% of the animals. Histologically, 85% of the tumours were carcinomata, mostly adenocarcinomata. The growth of the tumours was estimated at intervals by comparing them with plastic balls of graded sizes. Between the 7th and the 20th week after the administration of DMBA, rats with 1–2 tumours of 8–12 mm in diameter were allotted at random to several groups of at least 7 animals each.

Various doses of GP 48 989 were administered by stomach tube on 5 days a week for 3, 6 or 12 weeks. In

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