Experimental

Synthesis of N-Substituted Aniline MSD——Synthesis was followed to the method of Neelakantan, *et al.*[8]

Kinetic Techniques——Kinetic procedures for the determinations of formation and hydrolysis rate constants were the same as employed in the previous study.[6] Reaction temperature was maintained at 37°.

pK_a Measurement——The pK_a of N-*n*-pentylaniline was determined by spectrophotometrical method at 25°.[6] Values pK_a of other aniline derivatives were cited from literature.[7,9]

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9) A. Albert and E.P. Serjeant, "Ionization Constants of Acids and Bases," John Willy & Sons Inc., New York, N. Y., 1962, p. 148.

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Effect of Zymosan on Hepatic Drug Metabolism in Mice. II.[1] Identification of Active Component of Yeast Cell Walls

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Three glucan and a mannan preparations from baker's yeast (*Saccharomyces cerevisiae*) were examined for their effects on aminopyrine N-demethylase activity and cytochrome P-450 content of liver, and phagocytic activity in mice. Dextran and pustulan were also examined.

Among the polysaccharides tested, insoluble glucan mostly decreased cytochrome P-450 content and aminopyrine N-demethylase activity, and stimulated phagocytic activity. Alkaline soluble glucan, mannan, and pustulan decreased a little of cytochrome P-450 at high dose, whereas water-soluble glucan residue produced by acetolysis affected neither cytochrome P-450 content nor phagocytosis.

It has been reported that some modifiers of the reticuloendotherial (RE) system prolong the barbiturate-sleeping time and depress the metabolism of drugs.[3] Wooles, *et al.* had shown that prolonged intravenous administration of zymosan produced a marked prolongation of the barbiturate-sleeping time in mice,[3c] but decreased only 11% the metabolism of pentobarbital in liver slices.[3e] In previous work[1] we investigated the mechanism of zymosan-induced depression of the drug metabolism in mouse liver and found that the activities of aminopyrine N-demethylase, *p*-nitroanisole O-demethylase, and aniline aromatic hydroxylase were all markedly depressed and concomitantly cytochrome P-450 content was decreased.

Since zymosan is an insoluble cell wall complex of yeast consisting of polysaccharides as the major component, proteins, lipids, and inorganic elements,[4] it became necessary to clarify what component of zymosan would affect the drug-metabolizing enzyme system.

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2) Location: *a*) *Aobayama, Sendai*; *b*) *1–18–1, Kamiyoga, Setagaya-ku, Tokyo.*
3) *a*) S.C. Samaras and N. Diets, Jr., *Federation Proc.*, **12**, 122 (1953); *b*) W.R. Wooles and J.F. Borzelleca, *J. Reticuloendothel. Soc.*, **1**, 354 (1964); *c*) W.R. Wooles and J.F. Borzelleca, *ibid.*, **3**, 41 (1966); *d*) F.J. DiCarlo, L.J. Haynes, C.B. Countinho, and G.F. Phillips, *ibid.*, **2**, 360 (1965); *e*) D.W. Barnes and W.R. Wooles, *ibid.*, **7**, 684 (1969); *f*) W.R. Wooles and A.E. Munson, *ibid.*, **9**, 108 (1971); *g*) D. Gaillard, B. Pipy, and R. Derache, *Biochem. Pharmacol.*, **23**, 1245 (1974).
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The present study was undertaken to determined whether polysaccharides isolated from various sources decreased aminopyrine N-demethylase activity and cytochrome P-450 content in mouse liver and whether the decrease was an acompanying event to an acceleration of phagocytosis.

Materials and Methods

Polysaccharides——Glucans and mannan were isolated from baker's yeast (*Saccharomyces cerevisiae*) purchased from Oriental Yeast Co., Ltd. Insoluble glucan (glucan 1) was prepared by the method of Peat, *et al.*[5]

Alkaline soluble glucan (glucan 2) and water soluble glucan (glucan 3), water-eluted fractions on a column of diethylaminoethyl-cellulose (borate type) of the acetolysis products of alkaline soluble glucan, were prepared according to the method of Sakaguchi, *et al.*[6] The molecular weight of this water soluble glucan was estimated to be *ca.* 17000 by the method of gel filtration.

Mannan was prepared by the method of Suzuki, *et al.*[7] Pustulan was prepared from *Gyrophora esclenta* Miyoshi by the method of Shibata, *et al.*[8] These two polysaccharides were the gifts of Prof. S. Suzuki and Prof. M. Suzuki, Tohoku College of Pharmacy.

Nitrogen was not detected in all the polysaccharides isolated except glucan 1 containing 1.1% of nitrogen.

Dextran (mol. wt. 60000—90000) was purchased from Wako Pure Chemical Co., Ltd., Osaka.

Treatments——Male ddY mice maintained on commercial mouse food (Oriental Yeast Co. Ltd., Tokyo) and weighing 21—26 g were intraperitoneally (*i.p.*) injected with a saline solution or suspension of polysaccharides twice at 5 hr interval. Aminopyrine N-demethylase activity, cytochrome P-450 content, and phagocytic activity were all assayed 24 hr after the first injection when the drug-metabolizing enzyme activities had been effectively depressed by zymosan.[1] Throughout the experimental period all mice were starved.

Assays of Aminopyrine N-Demethylase Activity and the Contents of Cytochrome P-450 and Protein——$9000 \times g$ supernatant and microsomes of livers were prepared as reported previously.[1] Aminopyrine N-demethylase activity was assayed by the method of Cochin, *et al.*[9] Cytochrome P-450 content was determined by the method of Omura and Sato.[10] Protein content was assayed by the method of Lowry, *et al.*[11]

Assay of Phagocytic Activity——Phagocytic activity was evaluated by measuring the clearance of carbon particles from the blood as described previously[1] except that the withdrawals of blood samples were done at 0 and 10 min after the administration of carbon particles.

Results and Discussion

Table I shows a comparison of the effects of glucan 1 isolated in present study with those of zymosan reported in previous work on aminopyrine N-demethylase and phagocytosis. Phagocytosis was enhanced at the dose of 10 mg/kg and 40 mg/kg of glucan 1, but at the dose of 80 mg/kg the enhanced phagocytosis was not observed. These results supported the work by Riggi and DiLuzio[12] that insoluble glucan (β-1,3-glucan) was one of the active components of zymosan concerning phagocytosis. On the other hand, aminopyrine N-demethylase activity was significantly depressed in the liver of glucan 1-treated mice and the ratio of depression was proportional to the dose administered.

Glucan 2 showed a weak potency on the RE system and a smaller and water-soluble glucan residue (glucan 3) produced by mild acetolysis had no measurable potency (Table II). Mannan and pustulan (β-1,6-glucan) did not enhance phagocytosis although these polysaccharides

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Table I. Effects of Glucan 1 and Zymosan on Aminopyrine N-Demethylase and Phagocytosis

Treatment (mg/kg)		Phagocytosis[a] ($K \times 10^2$)	Aminopyrine N-demethylase[b] (μmoles HCHO/g liver/hr)
Control		2.55±0.12	2.25±0.10
Glucan 1	1	2.68±0.55	
	10	4.28±0.30[c]	1.99±0.08
	40	3.51±0.16[c]	1.69±0.30[d]
	80	2.62±0.46	1.26±0.21[d]
Control		2.53±0.46	2.92±0.06
Zymosan[e]	1	2.52±0.44	2.80±0.06
	10	3·93±0.73[c]	2.27±0.03[c]
	40	4.10±0.64[c]	1.78±0.02[c]
	80	3.19±0.74	1.69±0.04[c]

a) expressed as the mean±S.D. of the values obtained with 6 mice
b) expressed as the mean±S.D. of the values obtained with 6 mice, the activity being determined on 3 paired liver preparations
c) significantly different from control, $p < 0.01$
d) significantly different from control, $p < 0.05$
e) the data in previous study

Table II. Effect of Various Polysaccharides on Phagocytosis

Treatment (mg/kg)		Phagocytosis[a] ($K \times 10^2$)	Treatment (mg/kg)		Phagocytosis[a] ($K \times 10^2$)
Control		2.60±0.31	Control		2.55±0.14
Glucan 1	40	3.83±0.64[b]	Glucan 3	40	2.65±0.41
Glucan 2	40	2.94±0.41	Pustulan	40	2.69±0.28
Mannan	10	2.44±0.33	Dextran	40	2.54±0.10
	40	2.72±0.39			

a) expressed as the mean±S.D.of the values obtained with 4—6 mice;
b) significantly different from control, $p < 0.05$

Table III. Content of Cytochrome P-450 in Liver Microsomes of Polysaccharides-Treated Mice

Treatment (mg/kg)		Cytochrome P-450[a] (nmoles/mg protein)	% control
Control		1.10±0.03	100
Glucan 1	10	1.04±0.07	94.5
	40	0.91±0.05[b]	82.7
	80	0.81±0.05[b]	73.6
Glucan 2	40	1.02±0.07	92.7
Mannan	40	1.04±0.05	94.5
Control	10	1.07±0.04	100
Glucan 1	120	0.71±0.04[b]	66.4
Glucan 2	120	0.88±0.01[b]	82.2
Glucan 3	120	1.09±0.09	101.9
Mannan	120	0.84±0.03[b]	78.5
Pustulan	120	0.92±0.08[c]	86.0
Control		1.10±0.06	100
Dextran	120	1.08±0.04	98.2

a) expressed as the mean±S.D of the values obtained with 12 mice, the specific content being determined on 4 paired liver preparations
b) significantly different from control, $p < 0.01$
c) significantly different from control, $p < 0.05$

had the same host-mediated antitumor activity[13] that is considered to correlate with the function of the RE system as glucan[14] and zymosan.[15]

Table III shows the cytochrome P-450 content in the liver microsomes of polysaccharide-treated mice. The specific content of cytochrome P-450 was effectively reduced by glucan 1 among the polysaccharides tested. Glucan 2, mannan, and pustulan induced mild decrease in the cytochrome P-450 content at high dose. Glucan 3 and dextran had no effect.

The present results indicated that the polysaccharides that were active on the RE system such as glucan 1 reduced the cytochrome P-450 content although there was no correlation between the functional state of the RE system produced and the reduction of cytochrome P-450 content.

It remains still obscure why do the agents modifying the RE system affect on the cytochrome P-450 located in the microsomes of parenchymal cells. Agarwal and Berry[16] presented the similar phenomena that RE-modifiers depressed tryptophan pyrrolase activity located in the cell sap of parenchymal cells.

It has been suggested that the enhanced phagocytosis by the RE-modifiers may be due to an influx of phagocytic cells into liver, an increase of function of existing cells, or new cell formation within the liver. However, since the number of phagocytic cells in the liver did not increase one day after zymosan administration,[17] there was no possibility that the decrease of cytochrome P-450 content observed above was due to the predominance of the phagocytic cells in cell populations in the liver.

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