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Liver Function Tests among Michigan and Wisconsin Dairy Farmers

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Serum activity of SGOT, SGPT, LDH, and alkaline phosphatase was measured in 614 Michigan adults exposed to PBB and 141 Wisconsin adults not so exposed. The Michigan group had higher prevalence of abnormal SGOT ($p < 0.005$) and SGPT ($p < 0.005$). A clear sex difference was observed. Michigan men had a higher prevalence of abnormal SGPT ($p < 0.005$) and LDH ($p < 0.005$) than Michigan women, and a higher prevalence than Wisconsin men of abnormal SGOT ($p < 0.005$) and SGPT ($p < 0.01$). These differences could not be ascribed to differing patterns of alcohol consumption, laboratory error, or choice of criteria for normality/abnormality. Seven Michigan subgroups were defined on the basis of the criteria by which they had been selected to participate. The two subgroups who were essentially self-invited did not differ from the remaining five randomly selected subgroups combined in prevalence of these abnormal liver function tests. Based on 364 serum PBB analyses thus far analyzed of the 614 Michigan participants, no obvious relationship between serum PBB values and liver function tests was observed. However, this is a tentative conclusion that will be further evaluated when remaining serum PBB analyses are completed.

The greater prevalence of abnormal SGPT and SGOT among Michigan dairy farm residents compared to the Wisconsin dairy farm residents is tentatively ascribed to the former group's exposure to PBB.

Introduction

Abnormalities of liver structure and function have been demonstrated in animals exposed to polybrominated biphenyls (PBB). In rats, PBB has caused hepatic hyperplasia with increase in liver size and weight (*1*, *2*), cytoplasmic inclusions (*3*), swelling and vacuolization of hepatocytes (*2*), and induction of cytochrome P-450 and P-448 microsomal enzymes (*4-6*). Hepatic enlargement has also been shown in rabbits (*7*) and guinea pigs (*2*) given PBB. Intrahepatic bile duct proliferation and other changes have been seen in bovine liver after PBB feeding (*8*, *9*).

Since many residents of Michigan ingested PBB in contaminated beef, dairy and poultry products since 1973, the question has arisen whether abnormalities of liver function tests are associated with such exposures to PBB in humans. We present the results of liver function tests among 614 adult residents of Michigan, most, if not all, of whom had ingested PBB, and compare the findings to those among 141 adult residents of Wisconsin who had not been exposed to PBB.

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Materials and Methods

A detailed description of the methods of this study appear elsewhere (*10*). A more general description is presented here.

Population Studied

The Environmental Sciences Laboratory of the Mount Sinai School of Medicine in November, 1976 conducted an extensive investigation of the health status of a group of Michigan dairy farmers, their families and consumers who purchased from these farms. A similar investigation of Wisconsin dairy farmers and their families was conducted in March 1977. These groups did not differ substantially with respect to age or sex.

Within the Michigan group, seven subgroups were defined as in Anderson et al. (*10*): Random quarantined ($n = 214$) consisted of a random sample taken from a list supplied by the Michigan Department of Agriculture of those dairy farms whose

produce had been quarantined because of high levels of PBB in the livestock and/or produce. All people living on the selected farms were invited to participate. Random nonquarantined (n = 65) consisted of a random sample of farms which had not been quarantined. All people living on the selected farms were invited to participate. Special quarantined (n = 112) consisted of people residing on quarantined farms who learned of the planned study and requested to be included. In some cases, these individuals were referred by their physicians. Special nonquarantined (n = 109) consisted of individuals living on nonquarantined farms who learned of the proposed study and requested to be included. Some of these individuals were physician-referred. Consumers of quarantined (n = 65) consisted of a random sample of nonfarm residents who were identified by the random quarantined individuals as people who had purchased produce or meat directly from their farms. Consumers of nonquarantined (n = 33) consisted of a random sample of nonfarm residents who were identified by the random nonquarantined individuals as people who had purchased produce or meat directly from their farms. Others (n = 16) consisted of a miscellaneous group of people who did not fulfill any of the above criteria.

It should be emphasized that the dispersion of PBB in the food chain in Michigan has been such that nearly all Michigan participants, regardless of subgroup, had ingested PBB in some amount.

Selection of participants was random within each subgroup except special quarantined and special nonquarantined. These two groups were generally composed of self-referred individuals, and therefore did not reflect a random selection process.

Examination Protocols

Examinations included complete medical history, occupational history, dietary history, history of chemicals used on the related farm, general physical examination, dermatological examination, ophthalmologic examination, pulmonary function tests, chest x-ray for those over 40 years of age, sensory-motor testing, and adipose tissue biopsies. In addition, numerous biochemical analyses were performed on serum from most adult participants. These included activity of serum alkaline phosphatase, SGOT, SGPT, and LDH, elevations of which are often associated with liver damage and/or induction of hepatic enzymes, although disorder of other organs may cause elevations as well. Isoenzymes of alkaline phosphatase and LDH were not studied.

Handling of Specimens

Blood was drawn at the time of examination and the serum immediately separated and frozen. Samples were transported to the Environmental Sciences Laboratory, refrigerated in dry ice. Michigan and Wisconsin serum was analyzed by the same laboratory (Mount Sinai Medical Center, Department of Chemistry), with samples identified only by coded numbers.

Fasting blood specimens were desired, but it was not always possible to achieve this. Results should not be assumed to be from fasting specimens.

The ranges of normal values used by the laboratory for routine clinical purposes in adults age 18 and over are as follows: alkaline phosphatase, ≤95; SGOT, ≤41; SGPT, ≤45; LDH, ≤225.

Analysis of Data

Because of the significance of liver function test results is different for children than for adults, we report results only for those age 18 and over. The χ^2 test was used to evaluate differences in prevalence of abnormal liver function test results among groups or subgroups. Student's t values were used to test differences of means of liver function test results. Univariate regression analyses were used to examine the relationship between PBB levels and values of liver function tests.

Results for the subgroups, special quarantined and special nonquarantined, were examined separately, because these subgroups were not randomly selected. Table 2 shows that these subgroups did not differ in prevalence of abnormal liver function tests from the rest of the Michigan participants. Therefore, their data were combined with that from the other subgroups for all comparisons with the Wisconsin group.

Results

Distribution of Liver Function Test Results

Six hundred and twenty-six adults (age 18 and over) participated in the Michigan survey; serum analyses for all four liver function tests were completed for 614. Of 143 adult Wisconsin participants, complete liver function test results were obtained in 141.

The distributions of values of SGPT and SGOT appear in Figures 1 and 2, respectively. The inset bar graphs demonstrate a significantly greater prevalence of abnormal SGPT and SGOT values in the Michigan group than in the Wisconsin group. How-

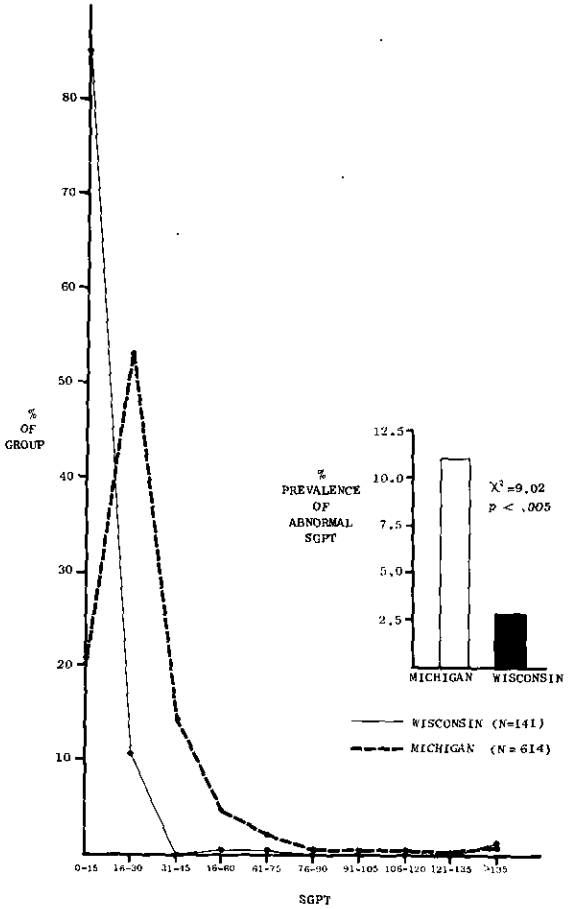

FIGURE 1. Distribution of SGPT values among adult Michigan and Wisconsin participants. Inset: Prevalence of abnormal SGPT in Michigan and Wisconsin groups.

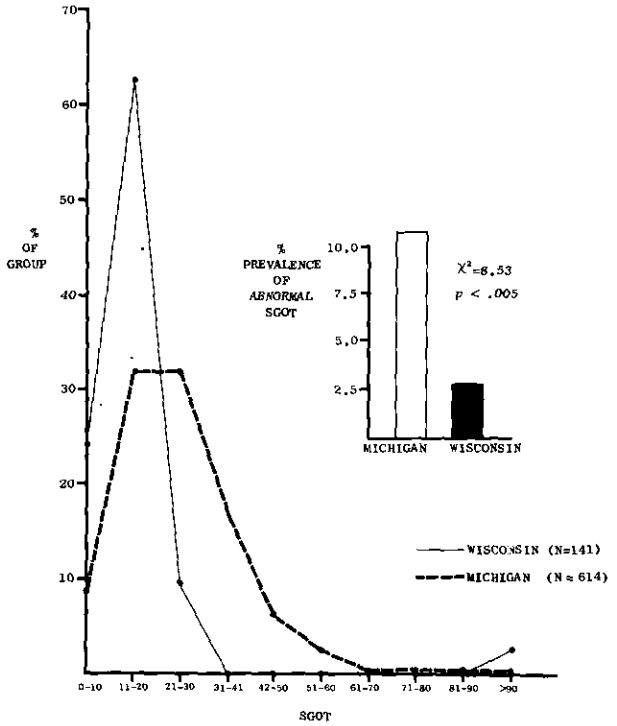

FIGURE 2. Distribution of SGOT values among adult Michigan and Wisconsin participants. Inset: Prevalence of abnormal SGOT in Michigan and Wisconsin groups.

ever, a *t*-test of the difference between the means of the distributions did not reveal statistically significant differences. This was due to the large standard deviation production by a small number of extreme values.

The distributions of values for LDH appear in Figure 3. These distributions are not significantly different, although there was a trend towards higher prevalence of abnormal LDH values among Michigan participants.

The distributions of values for alkaline phosphatase appear in Figure 4. There were no differences between the Michigan and Wisconsin groups.

Sex Differences

A clear difference between the sexes was apparent in the prevalence of abnormal liver function tests (Figs. 5 and 6). Table 1 shows that Michigan men had a significantly greater prevalence of abnormal SGPT and LDH values than did Michigan

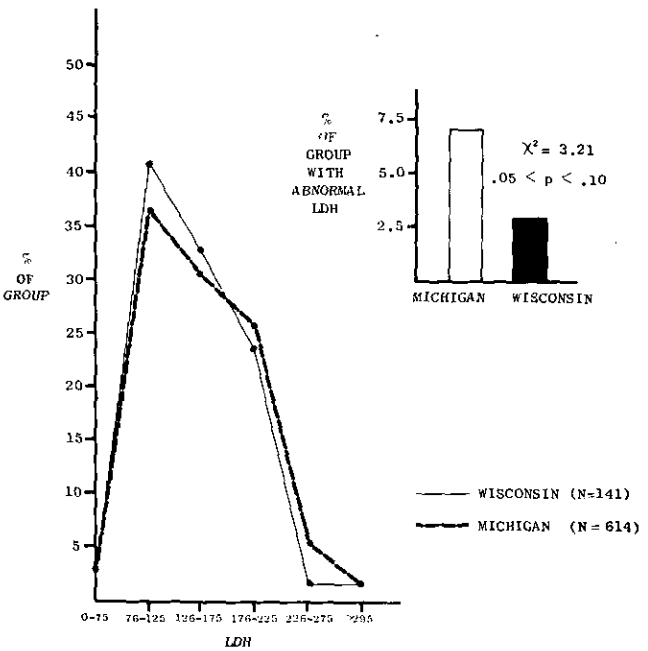

FIGURE 3. Distribution of LDH values among adult Michigan and Wisconsin participants. Inset: Prevalence of abnormal LDH in Michigan and Wisconsin groups.

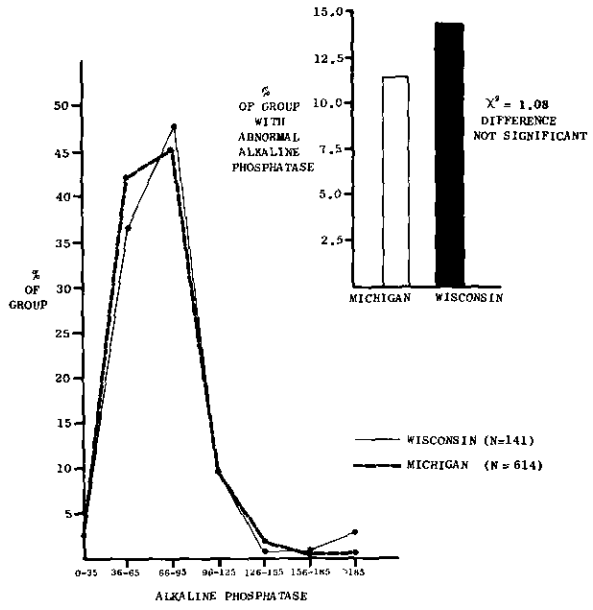

FIGURE 4. Distribution of alkaline phosphatase values among adult Michigan and Wisconsin participants. Inset: Prevalence of abnormal alkaline phosphatase in Michigan and Wisconsin groups.

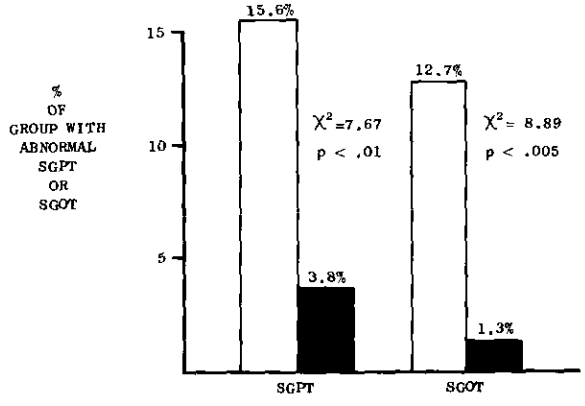

FIGURE 5. Prevalence of abnormal SGPT and SGOT among Michigan and Wisconsin male participants: (□) Michigan men; (■) Wisconsin men.

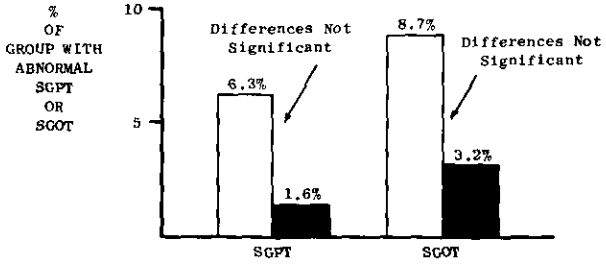

FIGURE 6. Prevalence of abnormal SGPT and SGOT among Michigan and Wisconsin female participants: (□) Michigan women; (■) Wisconsin women.

women ($p < 0.005$). In addition, Michigan men differed from Wisconsin men on SGOT ($p < 0.005$) and SGPT ($p < 0.01$), while Michigan women did not differ from Wisconsin women in prevalence of abnormality of any liver function test.

Subgroups of Michigan Participants

Table 2 shows the prevalence of abnormal liver function test results among the subgroups of Michigan participants as well as for the overall Michigan and Wisconsin group.

None of the subgroups of Michigan participants differed significantly from the remaining Michigan participants, with the exception of the consumers of quarantined subgroup. This subgroup had a significantly increased prevalence of abnormal SGOT ($p < 0.005$) and alkaline phosphatase ($p < 0.05$) compared to the other Michigan subgroups combined.

Relationship between Liver Function Test Results and Serum PBB Values

Thus far, serum PBB values are available for 364 of the 614 adult Michigan participants whose liver function tests are reported here. These 364 were selected at random from the larger group.

Table 3 shows the prevalence of abnormal liver function test values among participants at each level of serum PBB values. It can be seen that increased levels of serum PBB were not associated with increased prevalence of abnormality of any liver function test, at least among the 364 whose serum PBB values have been determined thus far. The lack of a strong relationship was further investigated by undertaking regression analyses between serum PBB levels and values of each liver function tests.

The results were: PBB vs. SGPT, $r = 0.0104, p = 0.422$; PBB vs. SGOT, $r = -0.0073, p = 0.445$; PBB vs. LDH, $r = -0.0359, p = 0.247$; PBB vs. alkaline phosphatase, $r = 0.0141, p = 0.394$. The regression analyses confirm the absence of an obvious association between increasing serum PBB values and results of liver function tests.

Relationship between Liver Function Test Results and Reported Symptoms

The χ^2 statistic was used to test the association between abnormality of each liver function test and each of six commonly reported symptoms among Michigan participants. The six symptoms were: anorexia, loss of 10 lb or more present at the time of examination, constant fatigue, joint pains, abdomi-

Table 1. Prevalence of abnormal liver function tests by sex.

	Number examined	SGOT >41		SGPT >45		LDH >225		Alkaline phosphatase >95	
		No.	%	No.	%	No.	%	No.	%
Michigan									
Men	314	40[a]	12.7	49[b,c]	15.6	32[d]	10.2	39	12.4
Women	300	26	8.7	19[b]	6.3	10[d]	3.3	29	9.7
Total	614	66[e]	10.7	68[f]	11.1	42	6.8	68	11.1
Wisconsin									
Men	79	2[a]	2.5	3[c]	3.8	4	5.1	15	19.0
Women	62	2	3.2	1	1.6	0	0.0	5	8.1
Total	141	4[e]	2.8	4[f]	2.8	4	2.8	20	14.2

[a,b,d,e,f] Paired superscripts denote significant difference, $p < 0.005$.
[c] Significant difference, $p < 0.01$.

Table 2. Prevalence of abnormal liver function tests among Michigan and Wisconsin study participants.

	Number examined	SGOT > 41		SGPT > 45		LDH > 225		Alkaline phosphatase > 95	
		No.	%	No.	%	No.	%	No.	%
Michigan									
Random quarantined	214	20	9.3	18	8.4	16	7.5	20	9.3
Random nonquarantined	65	8	12.3	6	9.2	6	9.2	6	9.2
Special quarantined	112	7	6.3	13	11.6	8	7.1	10	8.9
Special nonquarantined	109	15	13.8	15	13.8	5	4.6	15	13.8
Consumers of quarantined	65	13	20.0	10	15.4	4	6.2	12	18.5
Consumers of nonquarantined	33	3	9.1	4	12.1	2	6.1	4	12.1
Others	16	0	0	2	12.5	1	6.3	1	6.3
Michigan total	*614*	*66*	*10.7*	*68*	*11.1*	*42*	*6.8*	*68*	*11.1*
Wisconsin	141	4	2.8	4	2.8	4	2.8	20	14.2

Table 3. Prevalence of abnormal liver function tests by level of serum PBB among Michigan participants.

		SGOT > 41		SGPT > 45		LDH > 225		Alkaline phosphatase > 95	
Serum PBB, ppb	Number	No.	%	No.	%	No.	%	No.	%
Nondetectable–0.2	16	2	12.5	3	18.8	1	6.3	4	25.0
0.21–1.0	69	9	13.0	5	7.2	3	4.3	9	13.0
1.1–5.0	169	17	10.1	22	13.0	15	8.9	17	10.1
5.1–10.0	52	5	9.6	7	13.5	4	7.7	5	9.6
>10.0	58	5	8.6	11	19.0	4	6.9	9	15.5
Total	364	38	10.4	48	13.2	27	7.4	44	12.1

nal pain, and nausea. The only associations that reached statistical significance were those between abnormal SGPT and constant fatigue ($p < 0.05$); and abnormal SGPT and joint pain ($p < 0.05$). When analyzed by sex, the association with constant fatigue was significant for men ($p < .05$) but not for women. For joint pain, a trend was apparent among men ($p < 0.10$), but not among women.

Discussion

The results presented above demonstrate a greater prevalence of abnormal SGOT and SGPT among Michigan dairy farm residents and consumers of products from these farms, compared to Wisconsin dairy farm residents.

Approximately 4 months intervened between the time the laboratory analyzed the Michigan serum samples and the Wisconsin serum samples, since it was not practical to keep the Michigan samples in prolonged storage until the completion of the Wisconsin investigation months later. Because the two groups of samples were studied at different times, a systematic deviation of results for one group or the other may have occurred due to variability in reagents or equipment operation. It is well known that even in carefully managed laboratories, measurements of hepatic enzyme activities may vary as much as ± 15% within a day.

It is unlikely that this could explain our results. The Mount Sinai Medical Center Department of Chemistry routinely performs quality control checks every 15 min to detect systematic or sustained deviations (as opposed to the inevitable random fluctuations). Moreover, samples from Michigan men were examined simultaneously with those of Michigan women and, four months later, samples from Wisconsin men were studied with those of Wisconsin men. A systematic deviation of results due to technical variations would be expected to have affected men and women equally. However, the data reveal clear sex differences.

The results could not be explained by group differences in alcohol consumption. Only five individuals in the Michigan group and two in the Wisconsin group reported that they drank as much as a quart of whiskey or 24 cans of beer per week. Of these seven individuals, none had any abnormality of liver function tests.

The definition of normal values for each liver function test was that currently used by the laboratory for routine clinical purposes in adults. The choice of an absolute cut-off between normal and abnormal is always somewhat arbitrary, and some clinical chemists might be of the opinion that the upper normal limits for SGOT and SGPT should be somewhat higher. The data of the control group (Wisconsin) do not support this suggestion, at least in this situation, since only 2.8% of the group were "abnormal" on each of these tests using the present criteria for "normal."

Nevertheless, we re-analyzed our data using the following criteria for normal: SGOT ≤49, SGPT ≤52. With these criteria, the Michigan group still showed a greater prevalence of abnormal SGPT than the Wisconsin group ($p < 0.05$); and again this held for Michigan men compared to Wisconsin men ($p < 0.05$), but not for women.

With the revised criteria, there was no significant difference between the two overall groups in prevalence of abnormal SGOT; but if men alone were compared, a trend was apparent that nearly achieved significance at the 0.05 level.

The sex differences seen in our data are of considerable interest. Results of animal studies have not emphasized this aspect of PBB toxicity. However, recent work presented at this workshop may bear on these findings. Dent stated that sex differences in enzyme induction have also been demonstrated in rats fed PBB (*11*). His written abstract did not elaborate. Kimbrough et al. (*12*) found a somewhat different histological picture in the livers of female rats administered PBB compared to those of male rats. In addition, hepatic porphyria was observed only in the females (*12*). Reported experimental studies do not yet provide an understanding of sex differences in the hepatic response to PBB. Our data would support the need for further study of this problem.

It is of interest that a higher prevalence of abnormal SGPT was associated with reports of constant fatigue and joint pain, two of the most widely reported and subjectively severe symptoms reported by the Michigan group (34% of the adults complained of constant fatigue, while 31% complained of joint pain). These complaints were significantly more prevalent in the Michigan group than among the Wisconsin controls (*10*). The association between these reports and an objective physiological measure such as SGPT lends credence to the Michigan participants' complaints.

It is intriguing to speculate on the reasons for the significantly different liver function tests among the consumers of quarantined subgroup. A possible explanation emerged in the course of dietary interviews with the examinees. Many owners of quarantined herds, who witnessed cows sicken and die, stopped consuming their own produce. Nonresident consumers of produce from these farms, however, had often purchased a long-term supply of beef. Without the opportunity to observe the deterioration of the health of the cattle, many of these consumers continued to eat the contaminated beef even after the farm from which they had purchased it had been quarantined.

No obvious relationship between serum PBB values and liver function tests was found in the Michigan group. However, this is based on only those 364 whose serum PBB values have yet been analyzed, of 614 participating adults. Moreover, no analysis has yet been performed by sex; nor have we yet studied the relationship after examining the complete medical data on the small number with extreme values of liver function tests. These few individuals may have clear non-PBB causes for their abnormal liver function tests. Even a small number

of extreme values may destroy an otherwise strong result of a regression analysis. Finally, target-organ levels of PBB, rather than serum levels, may turn out to be the important variable. Therefore, the lack of a relationship is viewed as still tentative.

In the absence of other plausible hypotheses, we tentatively ascribe the greater prevalence of abnormal SGPT and SGOT in the Michigan group to their exposure to PBB.

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