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Effects of Reticuloendothelial Blockade on Acute Dimethylnitrosamine Poisoning in Mice (38324)

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It has long been established that reticuloendothelial cells play an important role in the defense mechanism against bacterial invasion (1). For the explanation of such important phenomenon, the concept of microenvironmental control mechanisms have recently been proposed, based on the findings that carbon-induced blockade of reticuloendothelial system (RES) provides a favorable environment condition for recovery of irradiated haematopoietic stem cells (2–4). It has been also suggested that reticuloendothelial (RE) cells release "proliferation stimulating factors" (5–7) and interferon(s) (8). Another important role of the RE cells possibly is to provide the parenchymal cells with supporting networks through which various substances can pass.

The present investigation deals with the possibility that these networks not only support the parenchymal cells but also actively trap them under a control mechanism so that the physical rigidness or the distribution of parenchymal cells has in itself been constructed so as to provide protective mechanisms against given toxic substances.

Although the parenchymal cells of the liver play an important role in the metabolism of injected drugs and also of toxic products absorbed through the intestine, the possible interaction between endothelial cells (Kuppfer's and other phagocytic cells) and parenchyma in these processes still remains to be investigated.

In the present study, dimethylnitrosamine, which is reported to produce severe liver necrosis (9), was employed and attempt was made to protect the mice from liver damage by blockading RES prior to the administration of the drug with carbon particles. The purpose of such treatment was to prove that the rigidness of RES framework possibly contributes to maintain the parenchymal cells in a normally functioning state and to protect them from direct attack of toxic substance.

Materials and Methods. Male mice of DDD strain, at 10 weeks of age, were used. Dimethylnitrosamine (DMN) was appropriately diluted and injected ip into mice. Carbon suspension was prepared by diluting Pelikan India ink (C11/1431 a, Guenther-Wagner, Germany) with physiological saline, and 10 mg of carbon particles were injected iv into mice 24 hr before or 1 hr after the administration of DMN. Saccharated ferric oxide (SFO) was also used to blockade RES. Three or 4 mg of SFO was injected into mice 24 hr or 6 hr before the administration of DMN.

For histological study, animals were sacrificed 1, 2, 3 and 7 days after the injection of DMN, and tissues were fixed in twofold diluted Bouin's fluid. Paraffin sections of 8 μm were stained with haematoxylin and eosin.

Results. Protection from lethal effects of DMN by carbon treatment. Seven-day-survival of mice on DMN are shown in Table I. A dose of 20 mg/kg killed all mice in control group within 2 days. Those given 15 or 18 mg/kg either died within 2 days or survived at least 7 days. All surviving mice were then sacrificed for histological examination. The gross features of the surviving animals were quite similar to those reported by Barnes and Magee (9). In short, they appeared ill with apparent abdominal swelling. A large amount of chocolate-colored blood-stained fluid was seen in the peritoneal cavity. The liver was swollen, dark and mottled.

Pretreatment of mice with carbon particles decreased the susceptibility to DMN. Eighty percent of mice survived the dose of 40 mg/kg DMN. Survival time was somewhat prolonged even in mice on 45 mg DMN. Surviving mice exhibited no observable signs of illness. As carbon treatment stained the liver and the spleen black, precise observation was difficult to carry

TABLE I. Effect of Pre- and Posttreatment of Mice with Carbon Particles on Dimethylnitrosamine Poisoning.

Treatment	No. survivals/No. treated		
	at day 2	at day 7	at day 14
Control			
10 mg DMN alone		5/5 (100%)	
15 mg	5/5	4/5 (80%)	
18 mg	2/5	2/5 (40%)	1/5 (20%)
20 mg	0/5	(0%)	
Pretreatment			
10 mg C[a] + 20 mg DMN		5/5 (100%)	
+ 30 mg DMN	5/5	4/5 (80%)	4/5 (80%)
+ 35 mg DMN	5/5	4/5 (80%)	2/5 (40%)
+ 40 mg DMN	4/5	4/5 (80%)	2/5 (40%)
+ 45 mg DMN	3/4	0/4 (0%)	
Posttreatment			
10 mg DMN + 10 mg C[b]	4/5	4/5 (80%)	
15 mg DMN +	0/5	(0%)	

[a] Carbon particles given iv 24 hr before DMN.
[b] Carbon particles given 1 hr after DMN.

out, however, livers were not swollen and peritoneal exudate was absent. Conversely, the mice given carbon particles after the DMN administration died following an injection of less than 15 mg/kg DMN. Thus, the posttreatment with carbon particles was deleterious.

Effect of the administration of the mixture of carbon particles and DMN. In order to determine whether or not carbon particles adsorb DMN, a mixture containing 10 mg carbon particles and 20 mg DMN was incubated for 10 min, and then injected ip into mice.

As shown in Table II, mice given the mixture of carbon and 20 mg/kg DMN died within 2 days. Thus, there was no protection when carbon was given with DMN, excluding the aforementioned possibility.

Effect of saccharated ferric oxide on DMN poisoning. Saccharated ferric oxide was also employed to blockade RES (10). The lethal dose in our mice was about 6 mg per animal. Results are summarized in Table III.

The lethal dose of SFO was so small that sufficient blockade of RES was impossible with a single injection, and a weak protection was observed by pretreatment of mice with only 3 or 4 mg. The survival rate was 3 out of 5, in mice given 20 mg DMN for 2 days after injection and among the survivors two survived for at least 7 days on 20 and 25 mg DMN each.

Histological observation. Liver. At 24 hr after the injection of 15 mg DMN, substantial damage was seen in parenchymal cells of the centrilobular regions of the liver. The cytoplasm was amorphous and eosinophilic and frequently vacuolated. The sinusoids were dilated. At 48 hr this necrotic area revealed marked haemorrhage. There were wide regions of degeneration and vacuolation, and invasions of the polymorphonuclear cells were present (Fig. 1). The RE cells were swollen and deeply stained, and occasionally lesions in the lining cells were observed. At 72 hr, most lobules consisted of amorphous vacuolated cells and reticular patterns were almost completely distorted. The liver of mice sacrificed 7 days after DMN still showed severe damage. Although the area of necrosis was li-

TABLE II. Effect of the Mixture of Carbon Particles and Dimethylnitrosamine on Mice.

Treatment	No. survivals/No. treated	
	at day 2	at day 7
Control		
15 mg DMN alone	3/5	3/5 (60%)
20 mg	0/4	(0%)
Mixture		
10 mg C + 15 mg DMN[a]	4/7	4/7 (57%)
+ 20 mg DMN	1/9	0/9 (0%)
+ 25 mg DMN	0/8	(0%)

[a] DMN was incubated for 10 min with 10 mg carbon particles, then injected ip into mice.

TABLE III. Effect of Pretreatment with Saccharated Ferric Oxide on Dimethylnitrosamine Poisoning.

Treatment	No. survivals/No. treated at day 2	at day 7
Control		
15 mg DMN alone	3/5	2/5 (40%)
20 mg	0/5	(0%)
4 mg SFO + 20 mg DMN[a]	3/5	2/5 (40%)
+ 25 mg DMN	2/5	2/5 (40%)
3 mg SFO + 15 mg DMN[b]	5/5	5/5 (100%)
+ 20 mg DMN	2/5	2/5 (40%)
+ 25 mg DMN	1/5	1/5 (20%)

[a] DMN injected ip 24 hr after SFO.
[b] DMN injected 6 hr after SFO.

mited, there were still swollen sinusoids and degenerated parenchymal cells. Proliferation of fibroblasts and young fibrous connective tissue was often observed in the necrotic area (Fig. 2). Thus, the liver of the surviving mice showed a mosaic pattern of somewhat recovered lobules among fibrillar and necrotic bands. Similar lesions were observed in the mice treated with 10 mg DMN, but the damage was less extensive.

Pretreatment of mice with carbon particles markedly reduced the liver damage. The parenchymal cells were apparently healthy at 24 hr after the DMN administration, and the RE cells appeared to be unaffected. At 48 hr, granular and vacuolar degeneration of the liver cells was occasionally present but the damage was much less than that in the control mice (Fig. 3). The recovery started earlier and at 72 hr after the DMN administration, the general feature of the liver seemed almost completely recovered, with cells containing carbon particles displaced and restricted to perilobular region (Fig. 4). Mice on 10 mg DMN after carbon treatment showed no significant damage. With administration of 20 mg DMN, significant disintegration of the liver was present. However, recovery began early and by 72 hr the necrotic areas were restricted to only small bands of perilobular regions. Even those mice which were on 30 mg DMN showed a well-recovered feature of the liver cells with slightly irregular reticular structure.

Spleen. Minor changes were present at 24 hr after DMN administration. The most obvious feature was the disintegration of sinuses. These were swollen and haemorrhage was frequently present. Large eosinophilic cells were conspicuous in the red pulp. At 48 hr, most of the germi-

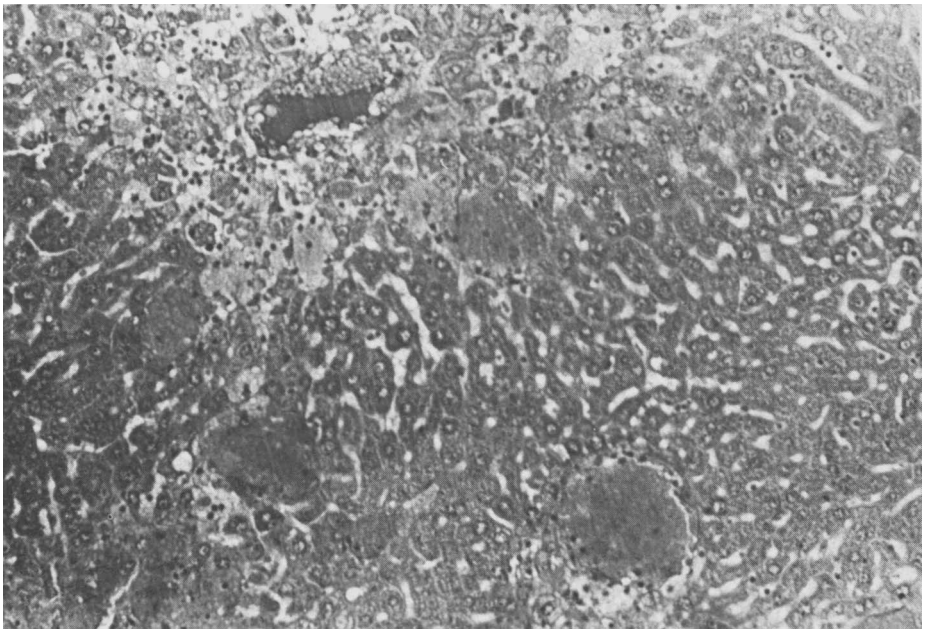

FIG. 1. Section of the liver of mouse 48 hr after the administration of 15 mg dimethylnitrosamine. Notice granular and vacuolar degeneration. 8 μm sections were stained with haematoxylin and eosin.

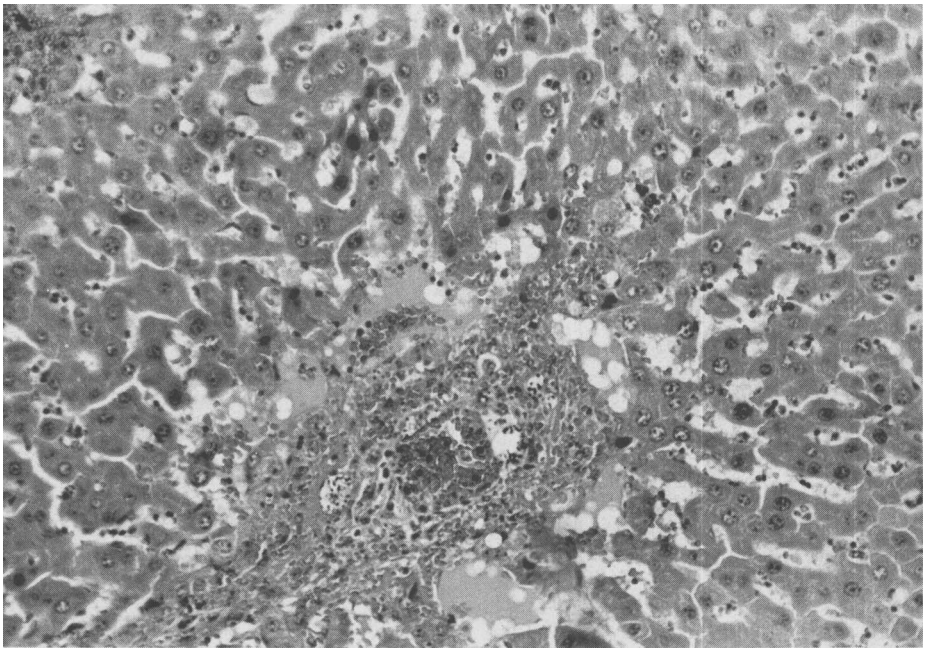

FIG. 2. Section of the liver 7 days after DMN. Notice disturbed reticular pattern and severe necrosis.

nal centers were rather atrophic. Peripheral and medullary sinuses appeared swollen and extensive haemorrhage was present. Similar features were still present at 72 hr. At 7 days after DMN administration, the spleen again showed severe lesions. Peripheral and medullar sinuses were destroyed and haemorrhagic or vacuolated regions were dispersed all over the organ (Fig. 5).

In this case again, carbon treatment reduced the damage markedly. Germinal centers appeared normal and sinuses were not swollen. Carbon particles were trapped in the cells surrounding the germinal centers (Fig. 6). These patterns did not change greatly during the period of the present experiment. The patterns were similar in mice on 20 mg DMN.

Discussion. The most distinct observation of the present experiment was that previous treatment of mice with carbon particles reduced the lethal effect of dimethylnitrosamine whereas posttreatment appeared to magnify the damage. It seems that once DMN had entered into the liver cells, RES-blockading could not change the effect or was rather deleterious. However, the possibility of the adsorption of DMN by injected carbon particles was excluded, since the injection of the mixture of carbon and DMN had the same effect as DMN alone.

It was also shown that a certain duration of time was required after injection of carbon particles before the appearance of a protective effect. Since carbon particles are taken into sinusoidal endothelial cells and macrophages and not into parenchyma (5), it is likely that such duration is attributable to the time required for the "reinforcement" of RES, which as a result leads to the modification of the parenchymal disintegration by some mechanism interacting between the parenchymal cells and the fine network of RES. The fact that saccharated ferric oxide had a similar effect suggests that the protection was not specifically attributable to carbon particles and that any other particulate material capable of blockading RES may indeed have a similar effect.

Studying saponin-induced disintegration of RES in the bone marrow and the liver of rabbit, Osogoe and his colleagues found that the disintegration was almost completely prevented by previously blockading RES, however they did not study the effect of the treatment on the damage of parenchymal cells (10, 11). By using DMN, which acts primarily as a liver poison and produced severe liver necrosis (9), it was possible to present evidence for the possibility that the RES-blockade would result in a decreased degeneration of parenchymal cells as well as RES.

We have suggested in a previous paper that

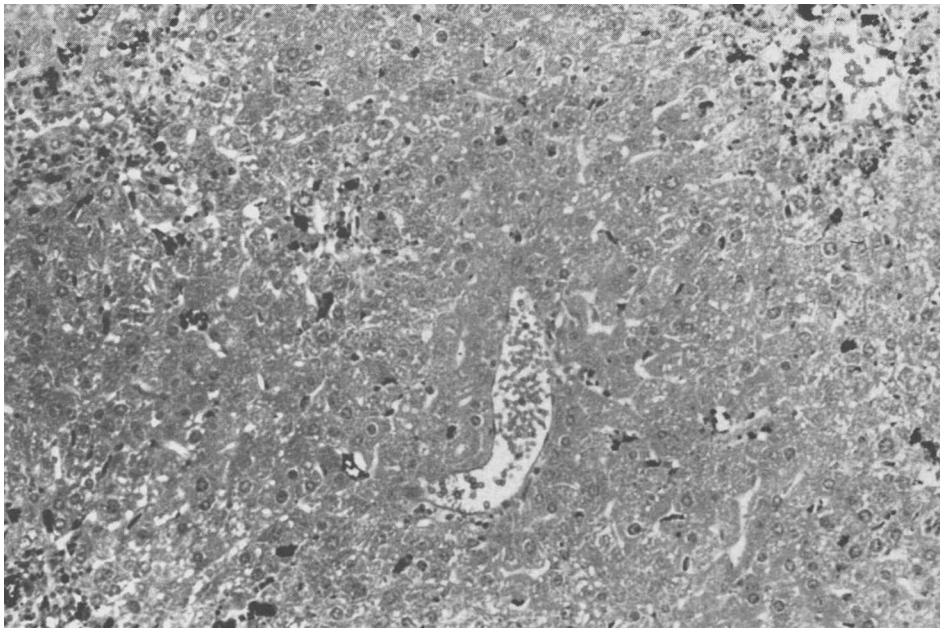

FIG. 3. Liver of the carbon treated mouse 48 hr after 15 mg DMN. Moderate disturbance of the liver feature is seen.

RES might be one of the suspected control mechanisms for the recovery and proliferation of haematopoietic stem cells after irradiation (4). The possibility has been also considered that reinforcement of RES plays an important role in the prevention of parenchymal damage as well as the control by excreting humoral factor(s). This was clearly demonstrated by the histological ob-

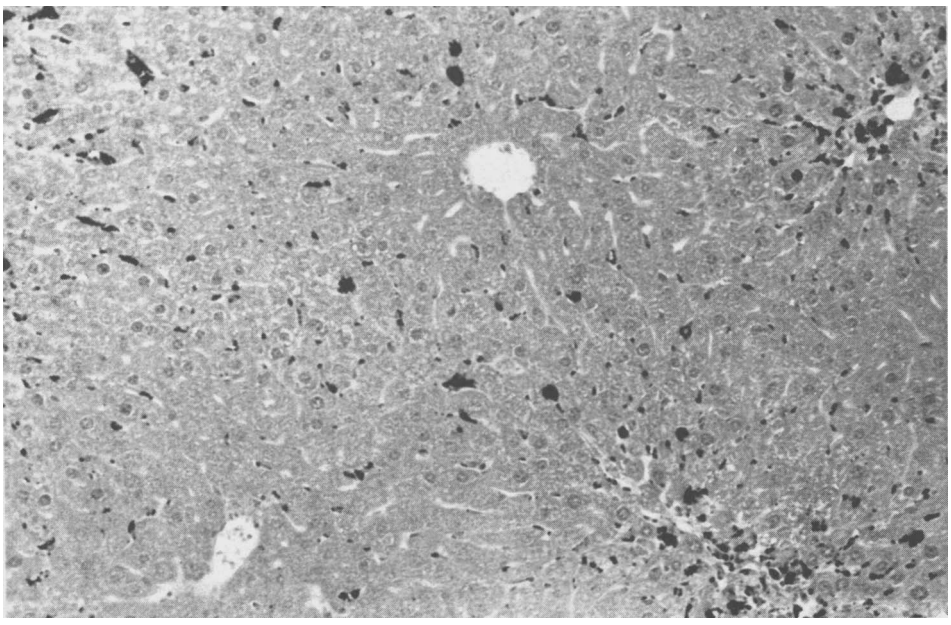

FIG. 4. Liver of the carbon treated mouse 72 hr after DMN. Recovery is almost complete.

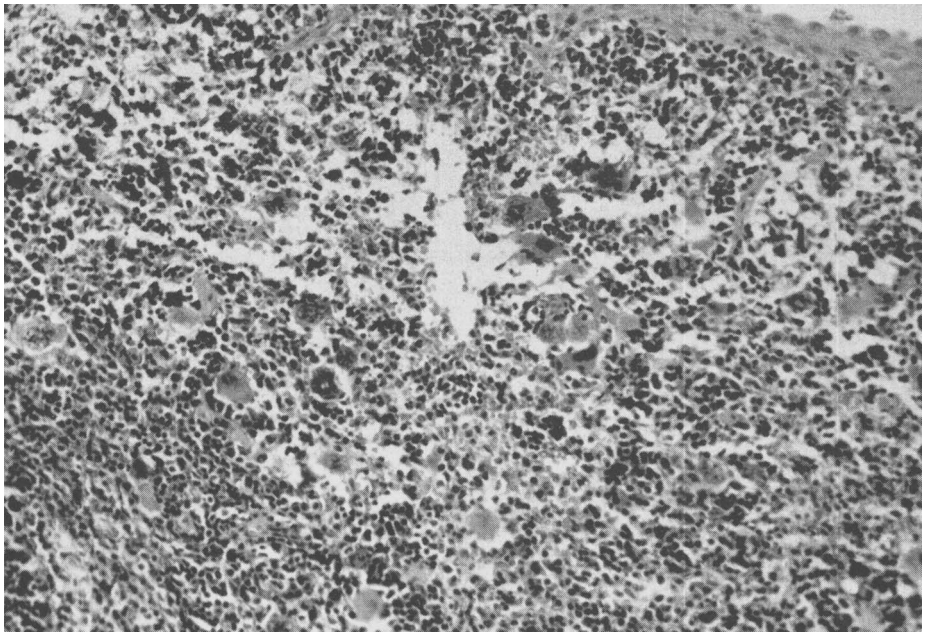

FIG. 5. Spleen section of mouse 7 days after 15 mg DMN. Sinuses are distorted and large eosin-stained cells are conspicuous.

servation in the present study. DMN produced a centrilobular necrosis followed by the haemorrhagic peritoneal exudate. Carbon treatment also prevented such a haemorrhage. The centrilobular necrosis and the following haemorrhage seems to be the result of impairment of the hepatic sinusoids and blood vessels with the carbon-induced reinforcement of the endothelium pre-

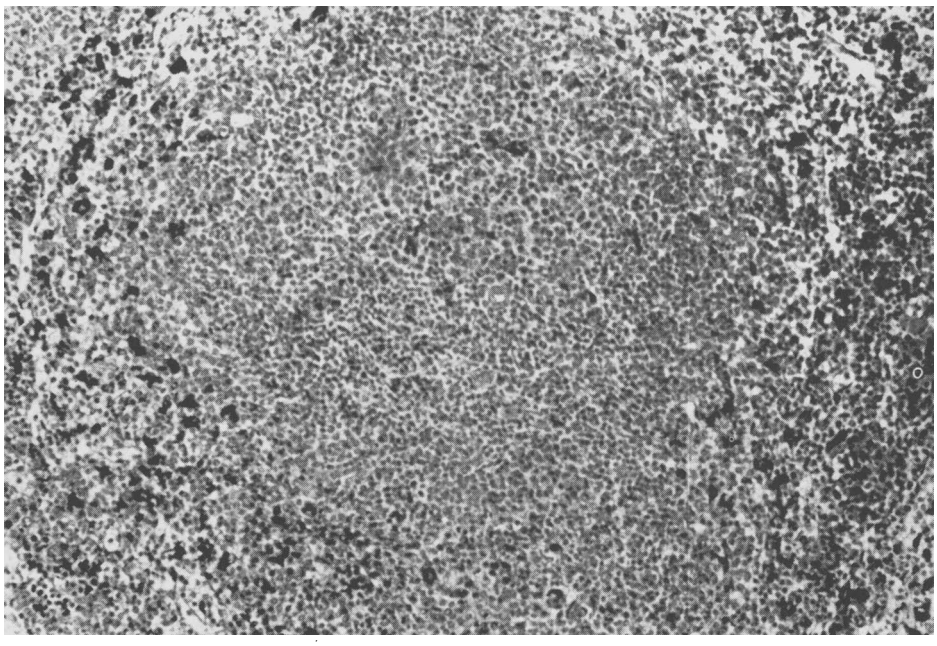

FIG. 6. Spleen section of carbon treated mouse 72 hr after DMN. No significant change is seen.

venting the damage. The mechanism by which carbon particles reinforces RES remains to be investigated.

The role of RES on the immunological or antibacterial activity of animals has been well documented (1). There are also several reports which suggest the environmental control mechanisms of RES on haematopoietic differentiation and proliferation (4–7). In addition, it is proposed that the parenchymal cells can function normally and most effectively only when they are finely trapped in the framework of RES, and therefore, that the disintegration of RES will positively result in the disturbed functioning of the parenchyma and in the unlimited degeneration.

Summary. The blockading of RES with carbon particles prior to the administration of dimethylnitrosamine prevented the damage of the liver cells caused by the drug. Histological observations revealed that both parenchymal and endothelial cells of the liver were protected. However, simultaneous treatment with carbon particles had no protective effect and the post-treatment was even deleterious. Blockading of RES with saccharated ferric oxide also protected the animals from the lethal effect of DMN to some extent.

From these results, it is suggested that the reinforcement of blood vessels and sinusoids of the liver is responsible for the protection of liver parenchyma from the toxicities.

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