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INFLUENCE OF CADMIUM INTOXICATION ON THROMBORESISTANCE OF VASCULAR ENDOTHELIUM IN RABBITS

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Here, using our original technique for measuring of thrombogenesis on the surface of rabbit aorta endothelium superfused with whole blood, we demonstrate that the thrombogenic property of endothelium is potentiated in the course of 3 months cadmium intoxication. The loss of endothelial thromboresistance is accompanied by suppressed generation of endogenous prostacyclin, leukopenia, increased platelet aggregability and by the presence of quasi-atherosclerotic, focal proliferative, glassy-protein lesions formed in aortic endothelium. We hypothesize that the final loss of vessel thromboresistance with all sequence of events that accompany cadmium intoxication, may result from the cadmium-induced inhibition of the generation of endothelial prostacyclin. However, the exact mechanism by which cadmium intoxication may affect the generation of prostacyclin and, then, functioning of blood platelets and vascular endothelium requires further investigations.

Key words: *Vascular thromboresistance, cadmium intoxication, platelet aggregation, vascular endothelium, prostacyclin*

INTRODUCTION

Hypertensive activity of cadmium has been widely referred to in literature (1—4), although one can encounter reports about the lack of increase in blood pressure under the influence of cadmium (1). Pathomechanism of hypertension in cadmosis still remains unexplained. However, several hypotheses have been adopted in an attempt to clarify hypertension mechanisms occurring in chronic cadmosis (1, 4, 5). For example, atherogenic activity of cadmium is suggested (4). Although in the cadmium exposure conditions, an increase in total cholesterol concentration was found, along with increase of the phospholipid

concentration in aortas from rabbit, these findings did not resolve the problem in question (2). The investigators also point out to interdependence between cadmium and prostaglandins. After exposure to a small cadmium dose, an increase of thromboxane B_2 in rabbit plasma was found along with parallel reduction of prostacyclin secretion through vascular endothelium (5).

For that reason, it seemed purposeful to follow the influence of cadmium on the interaction between blood platelets and vascular endothelium during the intoxication process.

MATERIAL AND METHODS

Experimental animals and protocol

Experiments were carried-out on 36 male rabbits of 3 kg body weight kept in the same conditions and fed in the same manner. 24 rabbits were divided into three experimental groups. The animals were administered with an aqueous solution of cadmium chloride in the same dose of 0.4 mg/kg of body weight *per os* three times a week. Particular groups were different with respect to the exposure periods as follows: Group I — one month, Group II — two months, and Group III — three months. The remaining 12 rabbits were divided into three groups of control animals. Each of these groups was handled in parallel with the respective group of cadmium intoxicated rabbits.

Hematological tests

Each time, a day before the end of experimental period, i.e. after one, two, or three months, respectively, blood was colleced from the marginal auricular vein and the number of leukocytes and erythrocytes were counted by the chamber method. The hematocrit value was established by the microhematocrit method, while the hemoglobin concentration by the Drabkin cyanmethemoglobin method. The following day, animals were anaesthetized with pentobarbitone (50 mg/kg i.v.) and blood was collected from the animals by heart puncture into 3.8% sodium citrate, and separately into heparin (1000 U/ml). Then animals were killed by exsanguination. Citrated blood was used for platelet aggregation tests, for determination of thromboresistance of rabbit aortas and for radioimmunological measurements of the levels of 6-keto-prostaglandin $F_1\alpha$. Cadmium concentration was determined in heparinised blood.

Platelet aggregation

Platelet-rich plasma (PRP) was prepared from fresh citrated rabbit blood by centrifugation at $280 \times g$ for 20 min. The platelet aggregation was measured in a Born aggregometer (Upchurch Co., U.K.) using collagen and ADP at threshold proaggregatory concentrations. The principle on which this method is based concerns monitoring the light permeability through platelet-rich-plasma (PRP) after administering collagen and ADP aggregating agents.

Plasma levels of 6-keto-prostaglandin $F_1\alpha$

The concentration of 6-keto-prostaglandin $F_1\alpha$ was measured in plasma samples using radioimmunoassay kits (Biotrac, I^{125}-labeled) according to the manual.

Assay of endothelial thromboresistance

In vitro interaction between blood and vascular endothelium was studied under flow conditions (6, 7). Briefly, an inverted inside out tubular segment of rabbit aorta, taken from killed animal, was attached to the auxotonic lever of the Harward transducer and superfused (2 ml/min) with citrated (3,8%; 1:9 v/v) blood of rabbit (37°C) that was gassed with 95% O_2+5% CO_2. Blood for experiments was obtained from previously anaesthetized animals by heart puncture. A pre-scaled Watanabe recorder was used to monitor the formation of thrombi by registering changes in weight of superfused aorta. In this system a maximal gain in weight (expressed in mg of formed thrombi) at the steady state is inversely proportional to endothelial thromboresistance against adhesion and aggregation of morphological elements of non-coagulating blood. As checked by electron microscopy the main component of thrombi were platelets whereas fibrin, leukocytes and erythrocytes constituted its minor component.

Determination of the cadmium concentration

The remaining part of the aorta was cleaned, dried in ambient temperature, and stored in exsiccator. Samples were mineralized with addition of spectrally pure concentrated nitric acid (Merck Suprapur) in a microwave oven. The cadmium content was determined in the mineralized tissues and blood by the atomic absorption spectrophotometry (AAS) method.

Measurements of blood pressure

Arterial blood pressure was measured by the direct method in rabbits subjected to three-month cadmium intoxication and the related control animals.

Histochemical tests of the aorta arch

Three samples were taken from the aorta arches of each rabbit. The samples were preserved in formaline and stained with the following methods: hematoxiline/eosine, Van Gison, and oil red, respectively.

Statistical analysis

The results obtained from controlled and cadmium-intoxicated rats were presented as mean values $\pm$SD for n number of experiments. The statistical analysis of test results was conducted on the basis of the Student's t-test. The probability coefficient of 0.05 was accepted as significance limit. In the study of platelet aggregation effective doses of aggregating agents inducing 30% of maximal proaggregatory responses to aggregating agents were calculated by plotting the dose-response regression curve (ED_{30}).

RESULTS

The levels of cadmium

The levels of cadmium in the control-rabbit blood and aortas were presented as average values obtained from animals representing all the control groups. The level was 0.74 ng/ml$\pm$0.21 (n = 8). In the course of intoxication,

the cadmium concentration was gradually increased in blood and amounted to 2.03 ng/ml $\pm$ 0.49 (n = 5) after one month, 2.50 ng/ml $\pm$ 0.56 (n = 8) after two months, and 2.95 ng/ml $\pm$ 1.08 (n = 7) after three months. All results were characterized by a high statistical significance ($p < 0.001$) in comparison to the controls (*Fig. 1*). The cadmium content in the control aorta was 273.09 ng/1 g of the dry tissue mass $\pm$ 107.4 (n = 11). In the rabbits intoxicated during one month, the cadmium level amounted to 140.71 $\pm$ 54.6 (n = 7) and statistical significance was found in comparison to the control group ($p < 0.01$). After two-month intoxication, the cadmium concentration amounted to 348.14 $\pm$ 128.7 (n = 7) and to 284.71 $\pm$ 102.6 (n = 7) after three months. These results were not statistically significant in comparison to the control group.

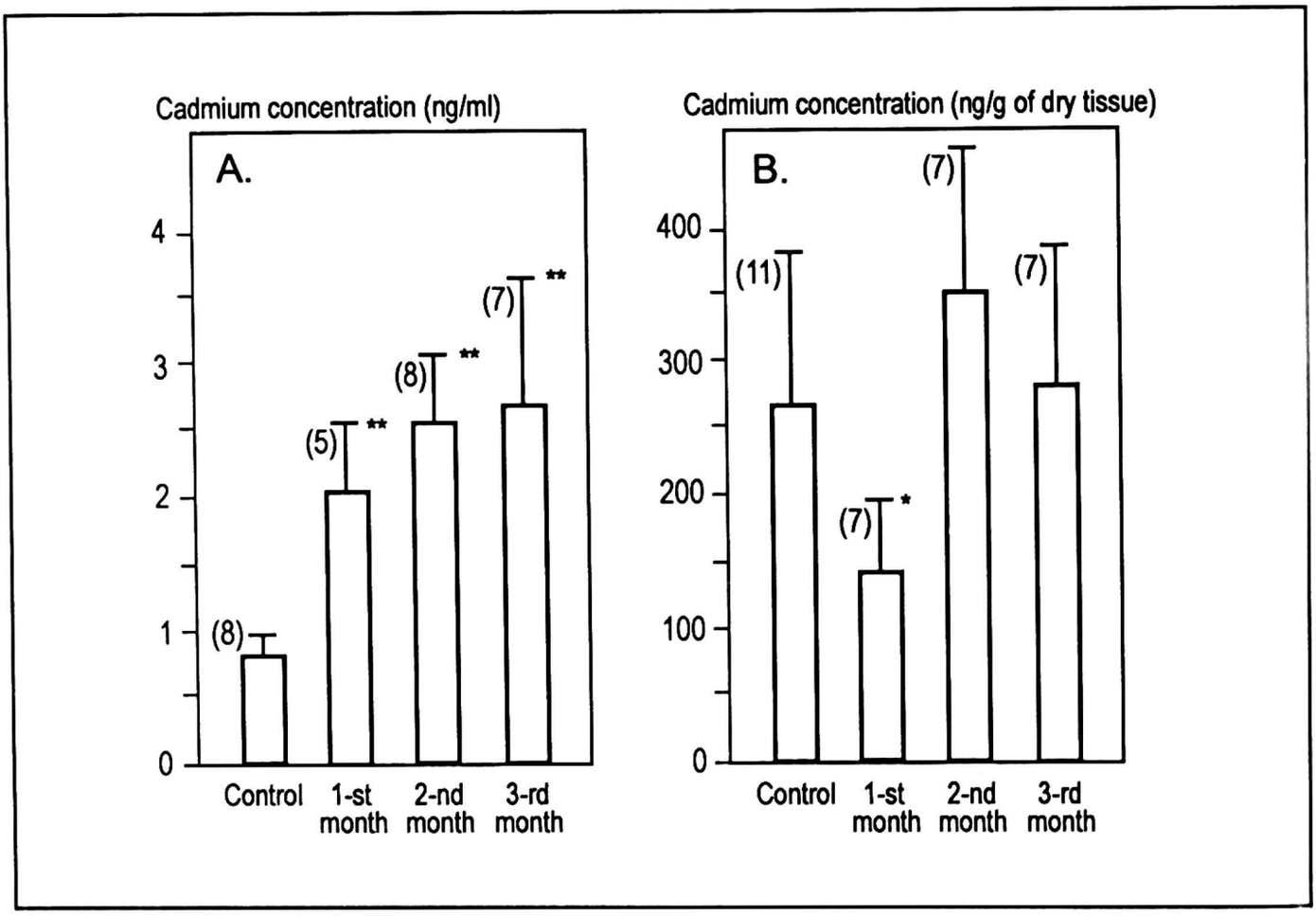

Fig. 1. The levels of cadmium concentration in rabbit blood (A) and in the mineralized segments of rabbit aorta (B) during the course of cadmium intoxication. Results are expressed as a mean $\pm$SD for (n) number of experiments (*$p < 0.01$; **$p < 0.001$)

Endothelial thromboresistance

After one month of cadmium intoxication, the thromboresistance of rabbit aorta endothelium amounted to 91.5 mg $\pm$ 40 (n = 8) as compared to 97.0 mg $\pm$ 27.6 (n = 4) in the control animals. Administering of cadmium during two months caused an essential decrease of thromboresistance ($p < 0.05$) against

formation of thrombi which amounted to 152.9 mg $\pm$ 37.0 (n = 8) in these animals and 88.5 mg $\pm$ 18 (n = 4) in the respective control group. Inhibition of thromboresistance of vascular endothelium after three-months cadmium intoxication was statistically highly significant ($p < 0.001$) as compared to related control (*Fig. 2*). Nevertheless, endothelial thromboresistance after three-months diet, in comparison to two-months cadmium diet, was slightly increased both in experimental (138.4 mg $\pm$ 14; n = 7) and in the control group (70.2 mg $\pm$ 21; n = 7).

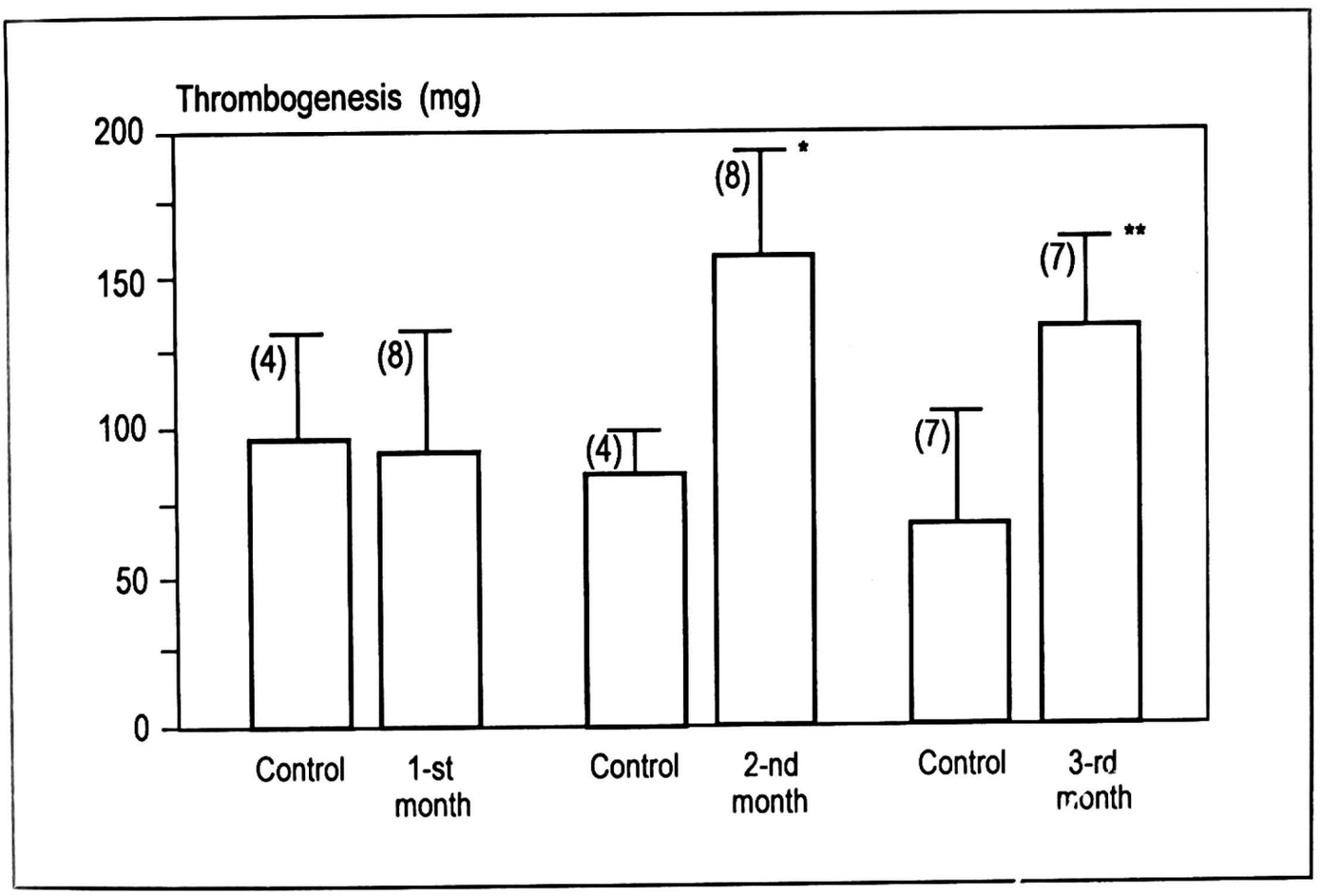

Fig. 2. Exacerbation of thromboresistance of rabbit aorta endothelium during the course of cadmium intoxication as compared to respective controls. Results are expressed as a mean $\pm$ SD for (n) number of experiments (*$p < 0.05$; **$p < 0.001$)

Platelet aggregation

ED_{30} for ADP-induced aggregation in platelet rich plasma was the same in all the control animals and amounted to 31.0 μM $\pm$ 11.0 (mean $\pm$ SD; n = 4). In cadmium intoxicated rabbits, ED_{30} after the first month was decreased to 20.6 μM $\pm$ 8.0 (n = 5), 16.4 μM $\pm$ 4.0 (n = 5) after two months, ($p < 0.05$), and 24.0 μM $\pm$ 5.0 (n = 4) after three months.

Platelet aggregation evoked by collagen developed in a similar manner in the control and cadmium intoxicated rabbits during the whole study. The

means of ED_{30} for collagen-induced aggregation in control animals were 9 μm/ml after the first (n = 3) and the second months (n = 3) and 10 μg/ml after three months (n = 3). ED_{30} for collagen in cadmium intoxicated rabbits was 10 μg/ml both after the first (n = 4) and the third month (n = 6) of intoxication and 13 μg/ml (n = 4) after the second month. The above differences were not statistically significant.

RIA for 6-keto-prostaglandin $F_{1\alpha}$

The mean concentration of 6-keto-prostaglandin $F_{1\alpha}$ (stable metabolite of prostacyclin) in rabbit blood amounted to 350 pg/ml ± 37 (n = 2) in control animals and was decreased to 152.83 pg/ml ± 10.9 (n = 6) in cadmium-intoxicated animals after the first month of experiments. These results were statistically significant ($p < 0.05$). After the second month of the experiment, the prostaglandin concentration amounted to 335 ± 18.7 pg/ml (n = 4) in control animals and 356.7 ± 46.7 (n = 6) in cadmium-intoxicated rabbits, while in the final stage of the experiment, the prostaglandin concentration amounted to 400 pg/ml ± 60 (n = 6) in control animals and 383.3 pg/ml ± 81.5 (n = 5) in cadmium-intoxicated rabbits. However, the above-mentioned results were not significant.

Blood pressure

The mean arterial blood pressure did not change as a result of three-month cadmium intoxication (104/80 mmHg, n = 4) and was similar to that of control animals (103/74 mmHg, n = 4).

Hematology tests

During hematological examination, a statistically significant decrease in leukocytes from $8.28 \times 10^3/\mu l \pm 2.06$ (n = 9) in control rabbits to $5.50 \times 10^3/\mu l \pm 0.45$ (n = 7) in three months cadmium-intoxicated animals was observed. After a month of cadmium administration, a significant increases in erythrocytes and hematocrit value were observed in comparison to the control groups (*Table 1*). After the second and third months, a number of erythrocytes and hematocrit value were at the level obtained in the control animals. The hemoglobin concentration did not change during the whole experiment (*Table 1*).

Table 1. The number of leukocytes, erytrocytes, hematocrit value and hemoglobin concentration in rabbit blood, including control and cadmium chloride intoxicated rabbits (mean ± SD). A p-value of <0.05 was taken as significant.

Protocol	Leukocytes ($\times 10^3/\mu l$)	Erythrocytes ($\times 10^6/\mu l$)	Hematocrit Value %	Hemoglobin (g/dl)
Group I-control	8.28 ± 2.06	5.52 ± 0.41	45.00 ± 2.68	13.14 ± 1.05
Group II, cadmium chloride administered during 1 month	9.80 ± 1.68	6.82 ± 0.54	48.57 ± 2.81	14.24 ± 1.23
Group III, cadmium chloride administered during 2 months	8.38 ± 1.76	5.28 ± 0.40	46.87 ± 6.17	12.23 ± 0.78
Group IV, cadmium chloride administered during 3 months	5.50 ± 0.45	5.55 ± 0.80	45.37 ± 4.59	13.44 ± 1.27
Statistical analysis:				
Group I compared to Group II	$p < 0.05$	$p < 0.001$	$p < 0.02$	$p < 0.05$
Group I compared to Group III	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
Group I compared to Group IV	$p < 0.005$	$p < 0.05$	$p < 0.05$	$p < 0.05$

Histological assessments

As based on histological results, normal endothelium, smooth muscles, and aorta arch adventitia were found in all the control rabbits (n = 7). However, bulged endothelium with focal proliferative and glassy-protein lesions were found in all the cadmium-intoxicated rabbits (n = 7). In five cases oil-red stain showed red pollen granules in the aortic endothelium.

DISCUSSION

In our tests concerning the cadmium content, considerable and statistically high concentration of this element was found in blood and it increased in the course of intoxication. However, the cadmium level in aorta indicated certain fluctuations during the experiment, but it was similar to that of the control animals after three months. It should be emphasized that cadmium is not accumulated in the rabbit aorta despite the constantly increasing cadmium level in blood. The above results comply with our earlier observations since we did not find any changes of the cadmium content in the rabbit aorta under a similar experimental model (2).

The lack of cadmium concentration in the animals' vessels is probably associated with their individual susceptibility to cadmium related to species characteristics. It is known that the cadmium content is different in vessels of various animals for instance in rats, unlike in rabbits, it is concentrated mostly in aorta (1, 2).

Arterial blood pressure measured in the final stage of tests was identical both in the control and intoxicated animals. Studying of the hypertensive activity of cadmium was initiated by Schröder (8), who found the increase of the arterial blood pressure in animals receiving cadmium in drinking water. However, other authors did not always observe blood pressure fluctuations affected by cadmium (1) and this was basically what we found as well.

Application of the cadmium diet caused the decrease of the aggregation threshold of ADP in ADP-induced aggregation at all the test stages. Although statistically significant changes occurred only after two-month cadmium intoxication, it is tempting to speculate that cadmium load led to the increased platelet activation and possibly to the release of biologically active substances from platelets that could be responsible for the increase of platelet aggregability. Indeed, an increase of plasma level of thromboxane B_2 was already observed by other investigators (5) after exposure to a small cadmium dose.

In our tests, we also made an attempt at evaluation of the vascular endothelium secretory function based on 6-keto-prostaglandin $F_{1\alpha}$ level in plasma. We observed that the content of determined prostaglandin decreased by 50% after one month intoxication. This indicates inhibition of prostacyclin synthesis in the vascular endothelium by cadmium. In the successive months, the prostacyclin level was set at the level observed in control animals, but this might be connected with the occurrence of compensatory mechanisms which seemed to appear at least in the course of experimental atherosclerosis (9).

A change of the endothelium secretory function was reflected in decrease of endothelial thromboresistance. In the course of the experiment, endothelial thrombogenicity increased considerably in the aorta-blood system within the same animal. These observations make us presume the existence of a very close interaction between the vascular endothelium and blood platelets in aim to regulate any changes in endocrine function of endothelium as we suggested earlier (10). In our experiments, we were able to demonstrate for the first time the increase of endothelial thrombogenicity in cadmosis. Such a changes correlate well with the histological results since proliferation of the aorta vascular endothelium with glassy-protein lesions was found in all the rabbits. Other investigators also indicate the occurrence of quasi-atherosclerotic lesions and proliferation of aorta smooth-muscle cells stimulated by cadmium (4). It is possible that the difference in the leukocyte count in peripheral blood found by us may be associated with the above observations. Advanced leukopenia observed after three months of intoxication may be associated with the participation of the related blood cells in the aorta vascular endothelium inflammation changes, as well as infiltration of aorta by leukocytes (11).

In conclusion, the changes observed in the presented experimental model discussed above make us believe that it is possible that the very first effect of

cadmium intoxication may be an inhibitation of the generation of endothelial prostacyclin. This is followed by a sequence of events like formation of quasi-atherosclerotic lesions in the vessel endothelium and an increase of platelet aggregability that finally result in a loss of the vessel thromboresistance. However the exact mechanism by which cadmium intoxication may affect the generation of prostacyclin and, then, functioning of blood platelets and vascular endothelium requires further investigations.

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