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Abolition of natural tolerance and the influence of the chemical allergen beryllium on autoimmune processes *

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Pulmonary berylliosis, a chronic allergic pneumosclerosis resulting from inhalation of beryllium compounds, may occur in two clinical forms: interstitial, which is mild, slowly progressive, and presents moderate skin reactions of delayed-type hypersensitivity to haptens; and granulomatous, which is rapidly progressive after a short latent period and presents systemic lesions and significant allergic skin reactions. The investigation of experimental berylliosis in rats has revealed some factors that could lead to the abolition of natural tolerance. Six new beryllium-containing autoantigens, two of which accumulated in different tissues, were identified in the lung nucleoproteins accompanying a partial loss of normal tissue and serum antigens. Antibodies to the new antigens and to normal lung tissue (in smaller amounts) were found in the sera of rats with experimental berylliosis, as well as in patients. The patients with granulomatous berylliosis also had antibodies to DNA, RNA, and extracts of normal homologous heart, spleen, liver, and thyroid in quantities that correlated with the clinical picture and with the effectiveness of glucocorticoid therapy. The fact that natural tolerance mechanisms were interrupted in all cases and autoimmune granulomatous berylliosis developed only in some patients led to the assumption that the mechanism was effective under the influence of additional endogenous factors; diseases producing an accumulation of autoantibodies could provide the stimulus for the appearance of these factors.

The interruption of natural tolerance, which leads to autoimmune syndromes and diseases, has been explained by different alternative hypotheses (1), the appearance of autoimmune processes in allergies to simple chemicals appearing to be no exception (2). Although it is known that antigens of the "hapten+protein" type produce antibodies not only to the hapten but also to the protein carrier (3–5), including homologous proteins (6, 7), the abolition of natural tolerance cannot be explained by the production of antibodies to the protein only. Because allergies to simple chemicals are seldom accompanied by specific autoimmune processes, the induction of autoallergy has been attributed to certain endogenous factors.

This point of view is supported by the results of immunological studies of chronic pulmonary berylliosis, an allergic pneumonitis caused by beryllium compounds of low solubility with the property of haptens (9–15); positive skin and cell reactions of delayed-type hypersensitivity are characteristic of this disease (16–18). In this paper are described the results of experimental and clinical investigations, which showed that the progress of berylliosis and the appearance of systemic lesions were conditioned by an autoimmune process, the development of which was probably connected with the influence of an endogenous factor.

MATERIALS AND METHODS

Patients. The diagnosis of pulmonary berylliosis in all our patients (40% interstitial and 60% granulomatous) was based on clinical and X-ray examinations, and on positive skin reactions of the delayed-type to beryllium chloride.

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Animals. A granulomatous berylliosis was induced in rats (August strain), weighing 100–120 g, by the intratracheal injection of 2.5 mg of a fine powder of beryllium oxide in 1 ml of saline solution. Morphologically typical lesions appeared in the lung and a positive scarification test of the delayed-type with beryllium chloride was obtained a month later.

Tissue antigens. The control and experimental rats (1–2 months after intratracheal injection of beryllium oxide) were killed by decapitation and the organs were immediately removed. Human tissues from men who died of trauma or berylliosis were also taken for study. The organs were washed with ice-cold saline solution, homogenized or ground up with quartz sand, and extracted with saline solution (pH 8.5) in a ratio of 1 g tissue in 10 ml saline for 18–24 h at 4°C. The extract was centrifuged at 0°C and 2 400 *g* for 30 min and the supernatant was used as the antigen. Nucleoproteins of lung (RLN) were obtained from the supernatant by Belosersky's method as modified by Silber (19). In some experiments, they were separated in two fractions—with high (B1, N1) and low solubilities (B2, N2) in neutral saline solution—by centrifuging at 0°C and 11 000 *g* for 40 min. The concentration of proteins was determined by the method of Lowry et al. (20) and the beryllium content was 0.032 mg Be per 1 mg of RLN protein (data obtained by P. A. Rosenberg). Beryllium was not analysed in our saline extracts, but there is evidence that it penetrated the liver, kidneys, and spleen after intratracheal injection (16).

Artificially prepared beryllium-containing antigens. For their preparation, 100 mg of beryllium oxide or chloride was added to 1 g of protein (guinea pig sera or normal nucleoprotein from rat's lung) and incubated at 4°C for a week; the mixture was stirred with a magnetic stirrer for 6 h each day.

Structure of rat lung nucleoprotein antigens. The structure of rat lung nucleoprotein antigens was studied by several methods: by Silber's method of active anaphylaxis with desensitization (19); by detecting the level of Hoigné's specific antibodies (21) in the sera of rats sensitized with the isologous nucleoproteins of a berylliosis-affected lung (BRLN); by gel precipitation and immunoelectrophoresis with rabbit antinucleoprotein serum after depletion of lyophilized normal tissue proteins; and by gel chromatography through Sephadex G–75 with saline solution at pH 6.3.

Spread of the new antigens in the body. Antigens 4 and 6 were detected in extracts of lung, liver, kidney, heart, spleen, stomach, and skin from rats with berylliosis and from normal rats by means of quantitative gel precipitation with rabbit monospecific antiserum.

Detection of antibodies. Antibodies were detected in human and rat sera by the passive haemagglutination test of Boyden (22) and, in some cases, by Hoigné's method (21).

RESULTS

Study of the new tissue antigens

A preliminary study of normal rat lung nucleoproteins (NRLN) by gel precipitation and immunoelectrophoresis revealed that they contained some serum and not less than 9 tissue antigens: 2 were organ-specific and 7 were common to different organs. In our first experiments on berylliosis (23), the disappearance of some normal lung antigens and the appearance of new antigens specific and common to both that disease and silicosis were noted. A further immunochemical study (24) showed that a part of the antigens consisted of serum, that the interorgan-specific antigens disappeared, and that the 6 new antigens had different electrophoretic mobilities (Table 1, Fig. 1).

Moreover, antigens 4 and 6 were found not only in the lung but also in the liver, kidney, heart, spleen, stomach, and skin of rats from 3 to 180 days after intratracheal treatment with beryllium oxide. In normal rats they were found in smaller quantities: antigen 4 in the lung, liver, kidney, and stomach, and antigen 6 in the kidney only. There are two possible explanations for the presence of these antigens in the normal rat: either normal tissues contain a minute quantity of the antigens that accumulate in berylliosis, or in the absence of antigens 4 and 6 from the normal body, a positive reaction may be caused by a cross-reaction with the normal protein carrier of the complex antigen.

The presence of complex beryllium-containing antigens was confirmed by an increase in skin reactions of the delayed-type to beryllium chloride in guinea pigs that were sensitized simultaneously with beryllium compounds and BRLN (Fig. 2) (25). More convincing results were obtained in a comparative study of the antigenic properties of the B1 and B2 fractions of RLN and of the artificially prepared beryllium-containing antigens in guinea pigs by the production of anaphylactic cross-reactions (Table 2). Anaphylactic shock in the animals should only just

Table 1. The relative electrophoretic mobilities of nucleoproteins from a berylliosis-affected lung (BRLN)

Experiment No.	Antigen No.					
	1	2	3	4	5	6
1	1.06	0.77	0.56	—[a]	—	—
2	1.06	—	—	—	0.43	—
3	—[a]	—	—	0.50	—	—
4	—	—	—	0.52	—	—
5	—	—	—	0.50	—	0.1
6	—	—	—	—	—	0.5–0.1
7	—	—	0.57	0.49	—	—
8	—	—	0.57	—	—	—
9	—	—	0.59	—	0.43	—
10	—	—	0.59	—	—	—
11	—	—	—	—	0.43	—
mean	1.06	0.77	0.57	0.50	0.43	0.5–0.1
zone	albumin	α_1-globulin	α_2-globulin	β_1-globulin	β_2-globulin	γ-globulin

[a] A dash means that no antigen was found in that experiment.

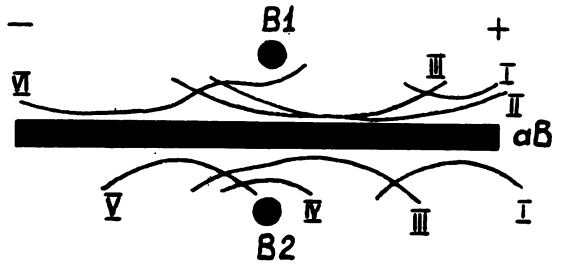

Fig. 1. Dislocations of the 6 new antigens (I-VI) in the immunoelectrophoretic plate; B1 and B2 are the fractions of BRLN; AB is rabbit antiserum to BRLN.

be induced by antibodies that contained the beryllium determinant, because for sensitization and intracardiac tests heterologous protein carriers were used as in experiments 3–5; antibodies to the homologous carriers were depleted preliminarily in experiments 6–8.

Another experiment (experiment 9) showed that the molecules of some antigens specific to berylliosis did not contain any beryllium (Table 2). The appearance of these natural autoantigens could be connected with a disturbance in protein synthesis in berylliosis, which was shown by an abnormal subfraction of proteins of low molecular weight (<70 000) and of peptides in the B1 fraction by gel chromatography through Sephadex G–75 with saline (26).

Search for specific humoral antibodies

Although it appeared that the new antigens must force the production of antibodies, all our attempts to find the specific humoral antibodies in experimental animals were unsuccessful for a long time (27). These antibodies were found in patients only when BRLN antigens were used, but the specificity of the antibodies was only relative (28). This could be explained by the fact that the substance used as the antigen also contained many normal antigens and probably inflammatory or necrotic antigens as well. We therefore now use more specific fractions of lung nucleoproteins as the antigen. Owing to the spread of antigens 4 and 6 into several organs and the frequent development of pathologic processes in the liver, heart, and spleen of patients, we also used as antigen a set of extracts of different normal human and rat tissues, including the normal lung–heart antigen made from an isogenous heart by preparatory immunoelectrophoresis. The antibodies

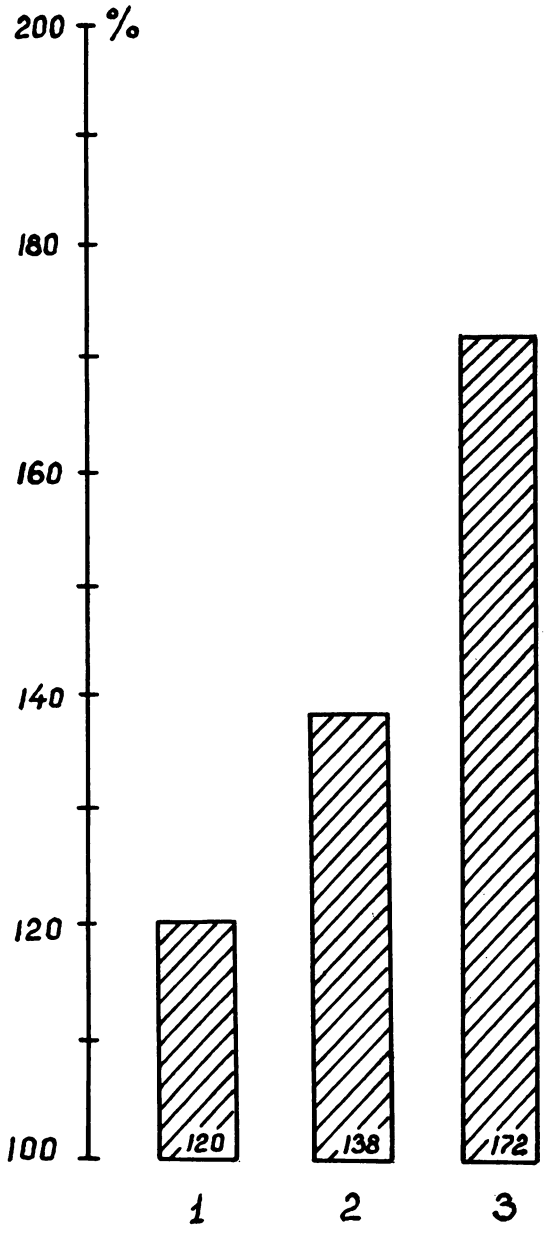

Fig. 2. Mean levels of delayed-type skin reactions to the intradermal injection of 2.5 mg of beryllium chloride in sensitized guinea pigs expressed as a percentage of the mean level of reactions in normal control animals; column 1 is after sensitization with 100 mg BeO, 2 with 10 mg BRLN, and 3 with 3 mg $BeCl_2$ + 10 mg BRLN.

to all the antigens used were found more often in rats with experimental berylliosis than in control animals (Table 3). It should be noted that the mean titres of the antibodies to the extracts of normal heart, liver, and spleen were higher than those of antibodies to the corresponding antigens made from rats with berylliosis. These results are probably due to a simplification of the normal antigenic structure of tissues in berylliosis.

A further search for antibodies was carried out in the clinic on 4 groups of patients: 2 control groups of patients with silicosis or other chronic occupational lung diseases and 2 groups of patients with different forms of berylliosis, differing not only in the X-ray and pathomorphologic features but also in the rate of development and intensity of clinical signs. Interstitial berylliosis was characterized by a prolonged latent period and often developed after 8–12 years of contact with beryllium, whereas in 75% of patients with granulomatous berylliosis the clinical picture appeared after a short contact with beryllium. However, the active concentration of beryllium was not important. The positive X-ray findings in 67% of our patients with interstitial berylliosis preceded the appearance of clinical signs, which were few and only slowly progressive. On the other hand, the clinical signs of granulomatous berylliosis either preceded the X-ray findings or developed simultaneously and were characterized by a wavy course that was increasingly progressive. Some of these patients suffered from high fever, bronchitis, bronchiolitis, and a progressive disturbance of respiration which resembled the Hamman–Rich syndrome. They often had an enlarged liver and lymphopathy together with an enlarged spleen in 15% of cases, a manifest dysproteinaemia, a disturbance in oxidative processes, and sometimes primary myocarditis. They often had also different allergic symptoms, an idiosyncrasy to drugs, vivid skin reactions of the delayed-type to beryllium, increases in the number of basophilic cells (degranulate forms) and in the activity of histamine synthesis, and a decrease in histaminase. Specific granulomas were found not only in the lung but also in the liver, spleen, and myocardium after death.

The patients with granulomatous berylliosis had the highest levels of antitissue and antinucleoprotein antibodies when compared with the patients in the control groups and those with interstitial berylliosis (Table 4). It is interesting to note that the levels of antiheart and antiliver antibodies in the sera of patients with granulomatous berylliosis almost equalled

Table 2. Search for beryllium-containing antigens of BRLN in guinea pigs with anaphylaxis by Silber's method [a]

Experiment No.	No. of animals	Antigen for sensitization	Test for desensitization		Intracardiac test	
			antigen	no. with anaphylactic shock after the last injection	antigen	no. with anaphylactic shock after the first injection
1	4	not done	not done		SGP + $BeCl_2$	0
2	5	not done	not done		N2 + BeO	0
3	10	B1	not done		SGP + $BeCl_2$	6
4	7	B2	not done		SGP + $BeCl_2$	6
5	10	SGP + $BeCl_2$	not done		B2	4
6	9	N1 + BeO	N1	0	B1	8
7	8	N2 + BeO	N2	0	B2	7
8	7	B2	N2	0	N2 + BeO	6
9	8	B2	S + BeO N2 + BeO	0 0	B2	7

[a] SGP: serum of normal guinea pigs.
B1, B2: the 1 and 2 fractions of BRLN.
N1, N2: the 1 and 2 fractions of RLN from normal animals.
S: RLN from animals with experimental silicosis after the intratracheal injection of 50 mg of quartz

Table 3. Titres of antitissue antibodies in rats with berylliosis and in normal rats

Antigen separated from		Sera from					
		experimental rats (after 3 months)			normal rats		
animals	organs	no. of sera	no. positive	titres (mean ± s.d.)	no. of sera	no. positive	titres (mean ± s.d.)
with berylliosis	lung B1	25	21	1.7 ± 0.2	25	2	0.1 ± 0.05
	lung B2	25	16	1.2 ± 0.1	25	2	0.1 ± 0.08
	liver	25	19	1.5 ± 0.1	25	3	0.1 ± 0.09
	spleen	25	20	1.5 ± 0.1	25	4	0.3 ± 0.1
	heart	25	15	1.1 ± 0.1	25	1	0.4 ± 0.5
normal	lung N1	25	16	1.2 ± 0.1	25	3	0.1 ± 0.05
	lung N2	25	19	1.4 ± 0.1	25	2	0.1 ± 0.09
	liver	25	21	1.8 ± 0.1	25	2	0 1 ± 0.08
	spleen	25	22	1.9 ± 0.1	25	3	0.2 ± 0.1
	heart	25	21	1.6 ± 0.1	25	3	0.3 ± 0.09
	lung–heart antigen	10 [a]	10	2.9 ± 0.2	10	3	0.6 ± 0.3

[a] No. of sera from rats after 1 month.

Table 4. Titres of antitissue antibodies in patients

Antigen	Sera from patients with: granulomatous beryllliosis			interstitial beryllliosis			silicosis			other chronic occupational lung diseases		
	no. of sera	no. positive	titres (mean ± s.d.)	no. of sera	no. positive	titres (mean ± s.d.)	no. of sera	no. positive	titres (mean ± s.d.)	no. of sera	no. positive	titres (mean ± s.d.)
BLN [a]	34	30	1.8 ± 0.01	16	11	1.2 ± 0.01	18	8	0.6 ± 0.02	29	9	0.3 ± 0.01
NLN [a]	41	32	1.5 ± 0.01	17	11	1.0 ± 0.01	18	14	1.3 ± 0.02	29	11	0.5 ± 0.01
extracts [b] from:												
heart	40	24	1.2 ± 0.01	15	2	0.1 ± 0.01	18	9	0.8 ± 0.01	29	9	0.5 ± 0.01
liver	40	25	1.1 ± 0.01	15	2	0.1 ± 0.007	18	11	1.0 ± 0.02	29	12	0.6 ± 0.01
spleen	22	14	1.2 ± 0.02	12	2	0.3 ± 0.01	18	10	0.8 ± 0.01	26	9	0.4 ± 0.01
thyroid	40	18	0.8 ± 0.01	15	0	—	18	8	0.6 ± 0.01	29	8	0.5 ± 0.01
kidney	19	6	0.6 ± 0.01	11	3	0.3 ± 0.01	18	10	0.8 ± 0.01	24	9	0.6 ± 0.01
suprarenal	23	11	0.8 ± 0.01	10	1	0.1 ± 0.01	—	—	—	—	—	—
DNA	30	16	1.0 ± 0.01	13	6	0.6 ± 0.01	18	9	0.7 ± 0.01	25	7	0.4 ± 0.01
RNA	19	11	1.1 ± 0.02	12	3	0.2 ± 0.01	18	7	0.6 ± 0.01	24	8	0.4 ± 0.01

[a] BLN: lung nucleoproteins from a patient who died of berylliosis.
NLN: lung nucleoproteins from a patient who died of trauma.
[b] Extracts were separated from the organs of a patient who died of trauma.

the level of the antilung antibodies, whereas the level of the antibodies to the less involved kidneys was very low. Both forms of berylliosis were similar in having a higher level of antibodies to nucleoproteins of the pathologic lung than to nucleoproteins of normal lung; the control groups showed the reverse of this.

It should be noted that the combination of berylliosis with other allergic diseases promoted an increase in the titre of antitissue antibodies and their appearance in patients with interstitial berylliosis. Effective glucocorticoid therapy caused a decrease in antibody production; when such treatment was ineffective, the level of antibodies did not change or sometimes even increased.

DISCUSSION

The data given above indicate that pulmonary berylliosis is characterized not only by a pneumosclerosis but also by an allergy to beryllium and, in the case of granulomatous berylliosis, by systemic autoimmune processes. Granulomatous berylliosis resembles the collagenoses in some respects: both are characterized by hypergammaglobulinaemia, the presence of antibodies to the lung, liver, heart, DNA, and RNA, and the proliferation of plasma cells in the lymph nodes, spleen, and other organs. Antithyroid antibodies were found both in systemic lupus erythematosus and in granulomatous berylliosis. Consequently, granulomatous berylliosis could be considered to be an immunologic disease of chemical etiology (29). The immunopathologic process is a result of the abolition of natural tolerance to autoproteins. In pulmonary berylliosis, there are at least 4 factors which could lead to the abolition of natural tolerance:

(A) The formation *in vivo* of complex beryllium-containing antigens as a result of the hapten properties of beryllium (9–15); the presence of such antigens was confirmed by our experiments (Table 2);

(B) The appearance of autoantigens as a consequence of a disturbance in protein synthesis (26, 30–32);

(C) The appearance of autoantigens as a result of changes in the protein molecule which unmask certain hidden determinants; this fact was indirectly

confirmed by the protein-denaturing properties of beryllium (33);

(D) A significant decrease or the complete disappearance of certain normal tissue and serum proteins (23, 24, 34).

Thus, in berylliosis, autoantibodies to normal tissue could appear as a consequence of either the production of antibodies to the protein carriers of complex antigens (A) or the disappearance of the antigens that support tolerance (D). Normal proteins could also react with the antibodies to produce denatured (C) or abnormal (B) proteins at the expense of partial preservation of the normal determinants in the proteins. In fact, patients with pulmonary berylliosis always had antibodies that reacted to extracts from normal tissues and to fractions of these (28, 35; Tables 3 & 4). Besides, the immune system was made more active in this disease by the adjuvant actions of beryllium itself (36) and of the products of protein disintegration (37).

All these factors require the entry of beryllium into the body and are conditioned by its allergic and toxic properties, which could equally well affect any patient. However, the characteristic clinical, X-ray and immunologic pictures allow the identification of two forms of pulmonary berylliosis. A systemic autoallergy, like that in the collagenoses, develops only in granulomatous berylliosis, whereas in the interstitial form the pathologic process is strongly local and limited to the lungs. Granulomatous berylliosis may develop as a primary process or, more rarely, may follow the interstitial form of this disease.

Although the responsible factors may not always be evident, when once they begin to act they may influence different stages of the disease. Thus, it is assumed that the factor that interrupts natural tolerance to the autoprotein requires an endogenous starting mechanism, perhaps a somatic mutation in accordance with the hypothesis of Burnet (1). Granulomatous berylliosis is often provoked by processes and diseases in which autoantibodies accumulate, e.g., pregnancy, operations, exophthalmic goitre, and different allergic and autoallergic diseases. These may precede the contact with beryllium or may occur in the latent period of berylliosis.

Thus, granulomatous pulmonary berylliosis may be considered as an example of an autoimmune process, which develops under the combined action of an endogenous factor and sensitization to the chemical allergen.

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RÉSUMÉ

SUPPRESSION DE LA TOLÉRANCE NATURELLE ET INFLUENCE DE L'ALLERGÈNE CHIMIQUE BÉRYLLIUM SUR LES PROCESSUS D'AUTO-IMMUNITÉ

La bérylliose pulmonaire est une sclérose pulmonaire chronique de nature allergique due à l'inhalation de composés de béryllium. Elle peut revêtir deux formes cliniques: une forme interstitielle, bénigne et lentement évolutive, avec apparition de réactions cutanées peu intenses du type hypersensibilité retardée aux haptènes; une forme granulomateuse, évoluant rapidement après une brève période de latence, et caractérisée par des lésions généralisées et des réactions cutanées notables. Les recherches sur la bérylliose expérimentale du rat ont mis en évidence certains facteurs qui pourraient être responsables de la suppression de la tolérance naturelle. Six nouveaux auto-antigènes renfermant du béryllium, dont deux concentrés dans différents tissus, ont été identifiés dans les nucléoprotéines pulmonaires avec disparition partielle ou complète de certaines protéines tissulaires ou sériques. Des anticorps actifs contre les nouveaux antigènes et, en plus petite quantité, contre le tissu pulmonaire normal ont été décelés dans les sérums de rats en cas de bérylliose expérimentale et chez des malades. Les sujets atteints de bérylliose granulomateuse étaient aussi porteurs d'anticorps dirigés contre l'ADN, l'ARN et des extraits de tissus normaux homologues (cœur, rate, foie, thyroïde) en quantités en accord avec le tableau clinique et l'efficacité thérapeutique des glucocorticoïdes. Le fait que les mécanismes de la tolérance naturelle ont été supprimés dans tous les cas alors que la bérylliose granulomateuse par auto-immunité n'a atteint qu'un certain nombre de sujets donne à penser que le processus ne se développe que sous l'influence d'autres facteurs endogènes. Des maladies produisant des auto-anticorps en grande quantité pourraient fournir le stimulus nécessaire à l'apparition de ces facteurs.

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