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The effect of vitamin C supplementation on maximum physical activity toward leucocytes total

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Abstract

The purpose of study was to determine the effect of vitamin C supplementation on maximum physical activity on the total of leukocytes. Research method used a quasi-field experiment with pre-test design and post-test control group design. The study subjects consisted of four male non-athletes, aged 20-22 years. The number of leukocytes was measured before and after vitamin C supplementation with a dose of 100 mg and 500 mg in maximum activity, namely running to fatigue. Data normality was tested by Kolmogorov Smirnov and paired sample t-test was used to test for differences in the total of leukocytes before and after vitamin C supplementation. The results showed that the average number of respondents' leukocytes increased after maximal physical activity (Δ = 6,775) and after vitamin C supplementation 100 mg (Δ = 1300) and 500 mg (Δ = 550). Paired t-test showed a significant difference (p = 0.019) between the total of leukocytes before and after maximum physical activity. There was no significant difference between the supplementation of vitamin C at a dose of 100 mg (p = 0.09) and a dose of 500 mg (p = 0.95) on the total of leukocytes before and after maximum physical activity. So, it is concluded that maximum physical activity increases the number of leukocytes. However, there was no difference in the number of leukocytes before and after maximum physical activity in the supplementation of 100 mg and 500 mg of vitamin C

Keywords: vitamin C, leucocytes, physical activity

INTRODUCTION

Everyone has different physical activity needs. They require high and low activity (Adhitya, 2016). It means that everyone must pay attention to the needs of physical activity in order to maintain body resistance. They have different levels of physical activity requirements in maintaining endurance (Ariestika & Agung Nanda, 2020). Thus, the need for physical

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activity adapted to everyone's goal in keeping the immune system. They usually have a target of doing physical activity in order to achieve achievements in the sports they are engaged in. Meanwhile, ordinary people or non-athletes do physical activity to maintain health (Supriyoko & Mahardika, 2018). From the previous study, it can be seen that one has different needs for physical activity. Some aim to maintain health or achievements in the sports field. Everyone must maintain a good immune system or health, so they must maintain physical activity that suits their body's needs.

Moreover the current condition, the world has been had by the spread of the covid-19 virus which attacks the human immune system, then everyone must continue to do physical activity and consume vitamin C so that the immune system remains balanced and healthy. On 12 March 2020, WHO declared COVID-19 a pandemic. This condition has caused many deaths throughout Indonesia (Susilo et al., 2020). This is because until now, there is no vaccine to treat this disease. The Ministry of Home Affairs through the General Guidelines for Facing the Covid Pandemic 19 suggests several things that can be done by each individual to avoid Covid 19, including eating regularly and nutritionally and diligently exercising. Therefore, the immune system in the body must be strong in order to be able to fight corona virus infection as well as a functioning respiratory tract condition. A body that is experiencing a lack of oxygen supply will trigger the formation of free radicals. So, everyone must improve endurance in various ways, such as taking supplements such as vitamin C.

Vitamin C is an essential water-soluble vitamin. This vitamin functions for the formation of collagen, carnitine and neurotransmitters. Animals and plants can synthesize vitamin C in their own bodies, but this vitamin cannot be synthesized by the human body because it does not have the enzyme L-gulonolactone oxidase. It is often consumed by the public. Until now, the function of vitamin C which is known to the public is as a booster for the immune system, forming collagen, preventing aging and as a cold medicine (Setiawan, Pangemanan, & Polii, 2016). Vitamin C also plays a

role in accelerating the recovery process after physical activity. (Jannah, 2019). It can be understood that the presence of vitamin C for the body is very important because consumption of vitamin C can increase lymphocyte production and can work as an antibiotic to destroy viruses. Therefore, vitamin C has an influence on everyone's activity, because it can have an impact on the formation of immunity, both innate and adaptive. (Carr & Maggini, 2017). Vitamin C not only provides immunity to everyone, but also provides a sense of security and effectiveness against pain (Carr & McCall, 2017).

Many previous studies have examined vitamin C with physical activity performed by everyone or on the number of leukocytes. The research shows that there is an effect of physical activity, whether heavy or low, on the number of leukocytes, lymphocytes, and monocytes. However, this effect is based on statistical testing (Sandy et al., 2019). The results of the same study were shown by Resmanto (2017) that maximum physical exercise increased the number of leukocytes and administration of vitamin C did not decrease the number of leukocytes even though physical exercise was done optimally, this condition increased the number of platelets. Exercise as a form of physical activity can affect leukositoris and hemostasis in both men and women (Sand, 2013; Sellami et al., 2018; Apollo Sinaga et al., 2017) and physical activity can also protect the body from viral infectious diseases (Hemilä, 2017; da Silveira et al., 2020; Abobaker, Alzwi, & Alraied, 2020). Apart from that, vitamin C also provides excellent nutrition to the body such as skin (Pullar, Carr, & Vissers, 2017). However, other studies have shown that strenuous activity can also have an effect on lowering immunity (Nieman & Wentz, 2019). From different studies, it is stated that different intensity of physical exercise will have different effects on leukocytes (Neves et al., 2015). Vitamin C supplementation reduces the risk of upper respiratory tract infection in endurance athletes, giving Vitamin C 600 mg for 3 weeks to 90-km ultramarathon athletes has been shown to reduce the incidence of upper respiratory tract infection symptoms (Gleeson & Bishop, 2000).

From the previous research, it can be concluded that vitamin C plays an important role in physical activity and airway protection during physical activity, especially during the COVID-19 pandemic. This vitamin also provides immunity to the body so that it can protect the body from pain. However, this study has a different focus from other studies, namely testing vitamin C in physical activity on the number of leukocytes. The new thing that wants to be tested in this study is the consumption of vitamin C which is used as a supplement for physical activity. The presence of vitamin C that has been processed in the body and used in physical activity can affect the number of leukocytes. Thus, this study examines how the management of vitamin C in the body used for physical activity can increase the number of leukocytes, because leukocytes as one of the building blocks of immunity should be able to build antibodies against viruses or bacteria that enter the body. When physical activity is maximized, what is the presence of the leukocyte count from vitamin C intake. This concept is part of the assumption that vitamin C that enters the body and is used as a supplement to perform maximum physical activity can increase the number of leukocytes, so that research contributes to understand of measures of vitamin C use and the impact of vitamin C supplementation on physical activity.

Therefore, this research is important because it provides an in-depth understanding of the provision of vitamin C for the needs of the body. Especially during the Covid-19 pandemic, the need for body immunity has an effect from exposure to bacteria or viruses. In addition, vitamin C is also an antioxidant in dealing with exposure to free radicals so that physical conditions are maintained and the need for leukocyte levels is also met (Wibawaa, Arifin, & Herawatia, 2020).

From this explanation, the aim of this study was to obtain an in-depth study of the effect of vitamin C supplementation on maximum physical activity on leukocyte levels. Thus, the results of research can be useful for science related to physical activity that can be done through

exercise and also the body's need for supplements to build the immune system.

METHOD

This research used a quasi-experiment with pre-test and post-test control group design. It was carried out in July - November 2020 at the FIK Uncen Jayapura Laboratory and the Sakura Abepura Pharmacy Laboratory. The sampling technique used nonprobability sampling. It provides equal opportunities for everyone who is selected as a sample. So, the determination technique used purposive sampling. This is due to certain considerations, namely that subjects did not consume alcohol, did not smoke and did not use other supplements. The respondents involved in this study were 4 male non-athletes aged 20-22 years.

Subjects were taken from a group of youths who did physical activity running, which was carried out optimally in the Laboratory of FIK Uncen Jayapura and the Sakura Abepura Pharmacy Laboratory. The following is the profile data of the respondents involved in the study.

Table 1. Profile of Research Respondents

Number	Respondent code	Age (A)	Height (H)	Weight (W)	Body Mass Indext (BMI)
1	EP	20	160	58	21.48 (W: normal)
2	BA	21	175	80	26.1 (W : fat)
3	MM	21	165	60	22.05 (W: normal)
4	AP	22	175	70	22.9 (W: normal)

Measurement of maximum physical activity used the Multistage Fitness Test (MFT). This test is carried out in an open field with a track length of 20 meters and a track width of 1 to 1.5 meters for each test. The test used a series of rhythms to determine each shuttle (Sukadiyanto & Muluk, 2011). The measurement of maximum physical activity is determined by the standard duration of time taken by the respondent during the 20 meter run during the round trip. The standard time setting is based on the results of the running test instrument, which is from 30 seconds to 1.5 minutes (30-90 seconds). This activity is designed in the form of running back and forth on a 20 meter track until the respondent is completely exhausted.

The MFT was carried out at the FIK Uncen Jayapura Laboratory, while the leukocyte examination was carried out at the Sakura Abepura Pharmacy Laboratory which is about 1 km away. Examination of leukocytes at Apotik Sakura was carried out as soon as possible after the respondent had done a maximum run. The implementation of MFT is based on patented audio, so the researcher only records the results shown by the respondents

Research procedure vitamin C tablets with a dose of 100 mg and 500 mg were given to 2 groups of respondents, namely 1 group given vitamin C 100 mg and 1 group given vitamin C 500 mg. Vitamin C supplementation was given for 7 days. Physical activity referred to in this study is maximum physical activity. The test implementation procedures are;

1. Respondents are given brief instructions regarding the implementation of the test on counting backwards for five seconds before the start of the test played from the patented audio.
2. The test start mark is carried out according to the instructions played via the MFT audio
3. Respondents who have reached the one-minute interval are called level one which consists of 7 returns, so that to complete the next level, the respondent must run faster. Respondents who complete the 20-meter run are marked with one foot stepping on the 20 meter mark.
4. The researcher wrote the respondent's run activity.
5. Respondents must be able to survive as long as possible according to the predetermined speed and time. However, if the respondent is unable to run again or is tired, it can be stopped on the condition that the participant fails if he does not reach the 20 meter limit (Rachman & Nasution, 2017).

Leukocytes referred to in this study are leucocytes obtained from the results of the respondent's blood tests that have been carried out in the laboratory and refer to the standard of body leukocyte requirements for people who have good immune system. Leukocyte data collection was

carried out 4 times, 2 times, namely before and after maximum physical activity before giving vitamin C supplements of 100 mg and 2 times before and after maximum physical activity after giving vitamin C supplements of 500 mg. The time lag between giving vitamin C 100 mg and 500 mg was 7 days.

Data analysis technique the direct counting method was used to determine the number of leukocytes (antal leukocytes) before and after maximum activity and vitamin C supplementation with doses of 100 mg and 500 mg. The leukocyte count begins with a volume dilution of the blood with a Turkic solution containing 2% acetic acid and 1% gentian violet. The leukocyte count is counted under a microscope. Furthermore, multiplying the calculation factor, then the number of leukocytes is obtained in units of blood volume.

The data were processed with statistics at a significance level of 5%, using SPSS version 22 program. Descriptive statistical tests were used to see the average number of leukocytes and their increase. The normality of the data used Kolmogorov Smirnov and testing the difference in the number of leukocytes before and after vitamin C supplementation using the Paired Sample t-test. The results of the data normality test show that the four variables have a sig> α (0.05), so it can be concluded that the data for the four variables are normally distributed and meet the requirements to proceed to the paired t-test. The following are the results of the Kolmogorov Smirnov test of the respondent's leukocyte count before and after maximum physical activity after giving the supplement of 100 mg and 500 mg of vitamin C.

Table 2. Kolmogorov Smirnov Test Results for Number of Respondents' Leukocytes Before and After Maximum Physical Activity After Giving Vitamin C Doses of 100 mg and 500 mg

Respondent		α	Sig
Vitamin C dosage 100 mg	Before Training	0,05	0,99
	After Training	0,05	0,99
Vitamin C dosage 500 mg	Before Training	0,05	0,99
	After Training	0,05	0,99

Source Primary Data Processing, 2020

RESULT

1. Descriptive Data Analysis Results of Leukocyte Levels Before and After Physical Activity Maximum Before Giving Vitamin C

The following is the number of respondents' leukocytes before and after maximum physical activity before giving vitamin C.

Table 3. Number of Leukocyte Before and After Physical Activity Maximum Before Giving Vitamin C

Respondent	Number of Leukocyte (**thousand/mm^3**)	
	Pre	***Post***
EP	9.100	14.600
BA	6.600	17.200
MM	10.000	17.200
AP	6.400	10.200
Average	8.025	14.800

Source Primary Data Processing, 2020

Table 3 shows the results of the average number of leukocytes respondents before the maximum physical activity and after maximum physical activity before giving vitamin C respectively 8025 and 14,800 (Δ = 6775).

2. Descriptive Data Analysis Results of Leukocyte Levels Before and After Physical Activity Maximum After Giving Vitamin C

The results of data processing on the number of respondents' leukocytes before and after maximum physical activity after giving vitamin C describe in the following table.

Table 4. Number of Leukocyte Before and After Physical Activity Maximum After Giving Vitamin C

Respondents Dose of Vitamin C 100 mg	Number of Leukocyte (**thousand/mm^3**)		Respondents Dose of Vitamin C 500 mg	Number of Leukocyte (**thousand/mm^3**)	
	Pre	***Post***		***Pre***	***Post***
EP	5.700	7.200	BA	6.500	6.500
AP	5.300	6.400	MM	5.900	7.000
Average	5.500	6.800	Average	6.200	6.750

Source Primary Data Processing, 2020

Table 4 shows the average score of the respondent's leukocyte count before and after maximum physical activity after giving the dose of vitamin C 100mg were 5,500 and 6,800 (Δ = 1300). Meanwhile, the

average number of respondents' leukocytes before and after maximum physical activity after giving the dose of vitamin C 500mg were 6,200 and 6,750 (∆ = 550).

The results of the Paired Sample t-test were used to see the difference in the number of leukocytes before and after physical activity before giving vitamin C. The significance of the test results was seen by comparing the Sig value with α= 0.05. If Sig <α = 0.05, it is stated that there is a significant difference, and if Sig> α = 0.05, it is stated that there is no significant difference.

Table 5. The Result of Paired t-Test for the Number of Leukocytes Before and After Physical Activity Before Giving Vitamin C

Respondent	Mean	n	Sig
Before Physical Activity – After Physical Activity	8.025 – 14.800	4	0,019

Source Primary Data Processing, 2020

Table 5 shows the sig value <α = (0.05), so it can be concluded that there is a significant difference between the number of leukocytes before and after maximum physical activity. While the results of the Paired Sample t-test before and after maximum physical activity after the study subjects were given a dose of vitamin C 100 mg and a dose of 500 mg as shown in the following table.

Table 6. The Result of Paired t-Test for the Number of Leukocytes Before and After Physical Activity After Giving Vitamin C Doses of 100 mg and 500 mg

Respondent	Mean	n	Sig
Before Physical Activity 100 mg – Before Physical Activity 500 mg	5.500 – 6.200	2	0,09
After Physical Activity 100 mg – After Physical Activity 500 mg	6.800 – 6.750	2	0,95
Total		4	

Source Primary Data Processing, 2020

Table 6 shows the value of sig> α = 0.05, so it can be concluded that there is no significant difference between giving 100 mg of vitamin C or 500 mg of vitamin C to the number of leukocytes before and after maximum physical activity.

DISCUSSION

The results of the data findings showed that the respondents had differences in the number of leukocytes after being given 100 mg of vitamin C and 500 mg of running physical activity. In general, these results are in line with the research hypothesis. The results of this study indicate that physical activity using running increases the number of leukocytes after maximum physical activity with the consumption of vitamin C. The mechanism of the increase and decrease in the number of leukocytes after vitamin C supplementation can be understood using the theory of cell damage and free radicals during maximum physical activity and the role of vitamin C as an antioxidan.

The average number of respondents' leukocytes before maximum physical activity and after maximum physical activity before vitamin C supplementation were 8,025 and 14,800 / mm3, respectively (Δ = 6,775). The normal number of leukocytes in adults is 5,000-9,000 / mm3, which means that the leukocyte count before physical activity is within the normal range. After maximum physical activity, the leukocyte count showed leucocytosis (> 10,000 / mm3) with an increase of 6,775 / mm3. This is the body's physiological response to stimulation of the adrenal glands due to maximal physical activity. The immune system is very responsive to physical activity, the weight of activity according to its intensity and duration determines physiological stress. Heavy activity that exceeds the physiological limit causes leukocytosis (Nieman & Wentz, 2019). Leukocyte secretion is as part of the innate immune system which acts as the main defense against pathogens (Actor, 2012). The increase in leukocytes after physical activity has been proven by other studies conducted by (Said et al., 2009; Sinaga, Sinaga, & Sinaga, 2017; Marpaung, Sinaga, Rismadayanti, Ginting, & Fitri, 2018). Said, et.al. (2009) found an increase in leukocytes in 14 athletes and 7 non-athletes after running 2 tracks to fatigue.

Especially during the Covid-19 pandemic, physical activity must be carried out to maintain endurance. The need for vitamin C can also have

an impact on the body's immune level. From the results of this study, it provides an understanding that consumption of vitamin C and physical activity must be done well before or after the Covid-19 pandemic. This is related to the need for the body's leukocyte count which has an impact on body health. In addition, physical activity that must be done must also be adjusted to the condition of each person's body, because everyone has different needs for physical activity, whether light or heavy physical activity.

Increasing the number of leukocytes also occurred after giving vitamin C at doses of 100 mg or 500 mg. After giving 100 mg of vitamin C, the average number of respondents' leukocytes increased by 1300 / mm3. Meanwhile, the increase in the average number of leukocytes after giving of vitamin C at a dose of 500 mg was 5500. When it compared with the increase in the number of leukocytes without vitamin C supplementation and vitamin C supplementation at 100 mg doses, the increase in leukocytes was known to be lower after vitamin C supplementation with a dose of 500 mg. It shows that as an antioxidant, vitamin C plays a role in inhibiting and neutralizing free radicals produced by the body under normal conditions and after maximum activity which in turn can cause oxidative stress. Giving vitamin C to maximum physical exercise reduces oxidative stress in mice (Khasan, 2015; Fauzi, 2018). In addition, vitamin C plays a role in helping improve immune function related to physical conditions (Ozaslan, Aytekin, & Kolić, 2004; Fauzi, 2018; Indika, P, Yuniarti, & Yosnengsih, 2019; Sadeghi, 2019).

Meanwhile, the difference in the number of leukocytes before and after giving vitamin C showed quite good results. Hypothesis testing proves that there is a significant difference between the number of leucocytes before and after maximum physical activity and before and after giving vitamin C. Although these results are different from experimental research conducted by Mushidah & Muliawati (2019) on mice with strenuous activity, these results are in line with previous research conducted by Apollo Sinaga, et.al. (2017); which has proven an

increase in the number of leukocytes in 10 respondents after doing maximum physical activity. The difference in the results of this study may be due to differences in the sample and treatment in which the study was conducted Mushidah & Muliawati (2019) at the in vitro stage with samples of mice and this study used human samples. Second, the type of activity applied is swimming and weight bearing, while the maximum activity in this study is running. Thus, the physiological response that occurs in mice may be different from the human body as well as by different types of activity. Every body's ability between humans and mice is different in managing vitamin C to form leukocytes

During physical activity, oxygen uptake was significantly increased in active muscle cells (100 times) and all parts of the body (20 times). Strenuous physical activity can cause muscle damage due to mechanical trauma called Delayed Onset Muscle Soreness (DOMS). Furthermore, DOMS causes oxidative stress. Generally, DOMS occurs in untrained people (Ulvie, Lestariana, & Sofro, 2014) because of the motor unit requirements. Damage to muscle cells triggers the secretion of neutrophils and monocytes in the damaged areas studied by Reactive Oxygen Species (ROS). ROS will be neutralized by endogenous antioxidants. Oxidative stress occurs when exogenous antioxidants are unable to neutralize the ROS produced. Oxidative stress due to excess exercise causes an increase in body temperature and stimulates the production and activation of leukocytes. Leukocytosis is significantly found in high intensity physical activity, which is mediated by lifocytosis and monocytosis followed by neutrophilia. (Neves et al., 2015). The number of leukocytes including neutrophils, lymphocytes, monocytes and eosinophils increases within the fifth minute of strenuous physical activity in untrained men and women (Sand, 2013).

Paired t-test showed that there was no statistically significant difference between giving of 100 mg of vitamin C and 500 mg of vitamin C on the number of leukocytes before and after maximum physical activity. However, the leukocyte count increased with the second supplementation

of vitamin C, where the increase in leukocytes at the 500 mg dose was lower than the 100 mg dose. Other studies with supplementation of one type of antioxidant have shown inconsistent results on the number of leukocytes (neutrophils). Supplementation of more than one type of antioxidant can offset the increase in the number of leukocytes due to free radicals (Mahfida, Kandarina, & Farmawati, 2015). Ulvie, et.al (2014) proved that giving vitamin C through red guava juice as an antioxidant inhibits injury and inflammation so that leukocytes do not increase sharply. Vitamin C as a non-enzymatic antioxidant combats oxidant compounds in the body, thereby suppressing tissue damage and further oxidative stress. Vitamin C scavenges free radicals and cuts the oxidation chain reaction of free radicals (Khasan, 2015). Vitamin C is a water soluble vitamin that is involved in the enzyme system and energy metabolism, which is easily digested by the body (Almatsir, 2009). Increased excretion of vitamin C can be seen in urine and sweat during strenuous physical activity and exercise. In addition, adequate consumption of vitamin C can reduce physiological stress (Dewi & Wirjatmadi, 2018).

The increase in leukocytes after vitamin C supplementation is in line with research conducted by Resmanto (2017); who has found an increase in the number of leukocytes by giving 65 mg / KgBB / day of vitamin C to 32 mice at maximum physical exercise. Similar results were also confirmed previously by Ozaslan, et.al.(2004), Vitamin C supplementation significantly increased the lymphocyte count as well as increased the swimming performance duration of mice when the vitamin C dose was increased. Vitamin C is found in many leucocytes which act as anti-infective and scavenger agents from hypochlorous-acid (HOCl) which is formed due to protein and deoxyribonucleic acid (DNA) damage. (Mahfida et al., 2015). The concentration of vitamin C in neutrophils increases after vitamin C supplementation. Neutophils are white blood cells that regulate the production of ROS during exercise. So that the regulation of neutrophil production is influenced by vitamin C supplementation. Thus, vitamin C and different types of physical activity have a considerable effect on

increasing the number of leukocytes. Moreover, if physical activity is carried out with different duration of time, the number of increased leukocytes can be known precisely according to the level of vitamin C given.

This research resulted in the conceptual understanding that increasing the number of leukocytes in the human body through the provision of vitamin C is different, because it is adjusted to the amount of vitamin C consumed. In addition, different maximal physical activity also affects the increase in the number of leukocytes in the body. This means that research provides choices for everyone to consume vitamin C. So, the different findings of this study are shown from the conclusion that each person can determine the amount of vitamin C levels that are appropriate for physical activity, so that vitamin C consumed before and after physical activity provides maximum benefit to the needs of the body. This condition occurs for young people aged between 20-22 years and who are not athletes. So, the physical activity of running that is carried out has different targets with athletes who have targets for attaining endurance.

The results of this study have limitations because the evidence of vitamin C consumption in maximal physical activity is limited to running activities, so it is not known the diversity of leukocyte counts for other physical activities. In addition, the results of this study also occurred in youths aged 20-22 years with four subjects. This limitation is in performance, so it is not known the number of leukocytes for the female sample. Therefore, this research can still be developed through sample diversity with different gender types for both athletes and non-athletes.

Overall, the results of data analysis have a big impact on increasing knowledge in consuming vitamin C for body needs. Excessive consumption of vitamins can also have a negative impact on the condition of the body. In addition, every woman and man also have different endurance in doing physical activity. Further research needs to be carried out by increasing the number of samples and dose groups, describing the types of leukocytes and controlling food intake. Research can also be

developed with more diverse types of physical activity and respondents who are divided between men and women so that levels of increased leukocytes and vitamin C can be seen to be more varied with the types of physical activity, both light and strenuous. In addition, next studies can also be linked to gender and body weight. This is because men and women have different levels of vitamin C needs.

CONCLUSION

Maximum physical activity was shown to increase the number of leukocytes in untrained subjects, but statistically there was no difference in the number of leukocytes before and after vitamin C supplementation with a dose of 100 mg or 500 mg. Thus, the existence of various types of physical activity gives a difference in the number of leukocytes both before and after vitamin C.

This research has implications for understanding the use of supplements such as vitamin C to maintain body immunity. In addition, the physical activity that everyone must do also consider the presence of a level of immunity with the right level of vitamin C supplement requirements. Therefore, everyone can use vitamin C and perform physical activity appropriately according to the body's needs to increase immunity.

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