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Effect of Cocoon Magnetization on the Glycogen Content in the Tissues of Multivoltine Mulberry Silkworm *Bombyx mori* (Lepidoptera) Larvae

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Abstract: The application of magnetic field on *Bombyx mori* cocoon has been proved to be of biotechnological significance in the sericulture industry. Variation in the static magnetic strength significantly influenced the glycogen content in the silk gland, fat body and haemolymph of *B. mori* larvae. The experiments were conducted in the year 2007. The cocoons were magnetized for 24, 48, 72 and 96 h separately with the magnet of each strength just after 3rd day of spinning. The bio-magnetic interactions, if applied tactfully, may be useful for boosting up the sericulture industry as well as the economy of silkworm rearing. The glycogen content increased with the increasing exposure duration of cocoon from 24 to 96 h in 0.1, 0.2 and 0.3 tesla magnetic field. The maximum level of glycogen content in the silk gland (1.296±0.022 µg/mg), fat body (32.628±0.321 µg/mg) and haemolymph (16.324±0.013 µg/mg) was noticed in case of 0.3 tesla – 72 h exposed cocoons. The minimum glycogen content in the silk gland (0.825±0.002 µg/mg), fat body (24.008±0.007 µg/mg) and haemolymph (10.253±0.003 µg/mg) was recorded in case of 0.4 tesla – 96 h exposed cocoons.

Key words: Magnetic strength · Glycogen content · Exposure duration · *Bombyx mori*

INTRODUCTION

Nistari is a resistant variety of multivoltine mulberry silkworm *Bombyx mori* Linn. which contributes up to a great extent in the commercial production of cocoon in India. The glycogen content is the most important factor that influences the production of silk on commercial scale. Efforts are being made to evolve new technologies that are effective, labour saving and eco-friendly in order to increase, the production of silk. Attempts have been made to study the effect of ecological factors [1], relative humidity [2], refrigeration of eggs [3], refrigeration of cocoons [4], magnetization of eggs [5] and magnetization of cocoons [6, 7] etc on the performance of silkworm. The effect of temperature on amino acids content [8], nucleic acid content [9] and magnetic field also influences the protein content [10-12], amino acid content [13, 14], glucose content [15, 16] in the tissues of silkworm larvae and pupae. Nowadays, biotechnology has become leading field of scientific researches which are directly concerned with the life quality of human beings. It is hypothesized that if the cocoons of *B. mori* are exposed to different magnetic strength, there may be some beneficial effects on the life pattern of silkworm and the productivity of silk. An attempt has been made to investigate the bio-magnetic effect of cocoon magnetization on the glycogen content in the tissues of silkworm *B. mori* larvae. This study may be helpful in devising the biotechnological application of magnetic field for the heavy production of silkworm cocoon as well as new magnification in the field of biophysics.

MATERIALS AND METHODS

The seed cocoons of multivoltine mulberry silkworm (*B. mori* Nistari) were obtained from the silkworm grainage Beharich, Directorate of sericulture Uttar Pradesh and were maintained in the plywood trays (23x20x5 cm) under the ideal rearing conditions [17] in the silkworm laboratory. The temperature and relative humidity were maintained in the Biological Oxygen Demand (BOD) incubator at 26±1°C and 80±5% RH until the emergence of moths from the seed cocoons. The newly emerged moths were quickly picked up and kept sex-wise in separate trays

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to avoid copulation. The whole grainage operation was performed as per description given by Krishnaswamy *et al*., [17] and eggs were obtained.

After hatching, the larvae were reared on the mulberry leaves given as food in the trays. After completion of 5th instar, the ripe worms ceased feeding and were ready for spinning. Small mountages were provided to the ripe worms and thus, sufficient numbers of cocoon were obtained from the silkworm larvae reared in the laboratory. Further, the cocoons were taken for magnetic exposure.

Experimental Design: To observe the influence of magnetic field on the glycogen content in the silk gland, fat body and haemolymph of *B. mori* larvae the cocoons, thus obtained were kept in the static magnetic field. The magnets of 0.1, 0.2, 0.3 and 0.4 tesla were used separately for the bio-magnetization of silkworm cocoons. The cocoons were magnetized for 24, 48, 72 and 96 h separately with the magnet of each strength. The cocoons were kept for magnetization just after the 3rd day from spinning. The control set of experiment i.e no magnetization of cocoons was also arranged. For the purpose of magnetization, initially 360 cocoons were kept within the magnetic field range of 0.1 tesla of which 90 cocoon was released after 24 h of magnetic exposure. Further, groups of 90 magnetized cocoons were released each after 48, 72 and 96 h of exposure to the static magnetic field of 0.1 tesla. These four groups of magnetized cocoons were separately transferred to the BOD incubator continuously, maintained at 26±1°C, 80±5% RH and 12±1 h photoperiod a day. Further, the incubation of exposed cocoons and the rearing of the different stages of silkworm were performed in the same BOD incubator. All the parameters of observations in the present study were determined from the respective stages obtained from the magnetized cocoons.

Glycogen Content: The glycogen content was estimated according to Van Der Vies [18] method. For determining the glycogen content took 31 mg of haemolymph, 25 mg of fat body and 30 mg of silk gland separately and homogenized in 5 ml of 5% T.C.A. (10 mg/ ml W/v) for 5 minutes and then filtered it. Added 1.0 ml of 10 N KOH solution in 1.0 ml of filtrate. The mixture was then boiled for 60 minutes in water bath. The excess alkali was neutralized by adding 0.5ml of glacial acetic acid and increased the volume up to 10 ml by adding distilled water. In 1.0 ml of above solution added 2.0ml of freshly prepared Anthrone reagent, shaked laterally and boiled in water bath for 10 minutes. Green brown colour developed. The O.D. was measured at 650 nm against blank. The blank was prepared simultaneously by using 1.0 ml of 5% T.C.A. instead of tissue filterate. The O.D. was compared with a set of glucose standard of different concentration. The result obtained was expressed as µg/mg.

The experiment was repeated in different tissues for different developmental stages. Six replicates of each experiment were made and the data obtained were analyzed statistically by two-way ANOVA and Post-hoc test.

The similar set of experiments were conducted with 0.2, 0.3 and 0.4 tesla magnetic exposure for 24, 48, 72 and 96 h. The control set of experiment was also conducted.

RESULTS

Glycogen Content in the Silk Gland of Larvae: The data given in Table 1a indicates that variation in the magnetic strength and exposure duration of cocoons influenced the glycogen content in the silk gland of 5th instar *B. mori* larvae. In 0.1, 0.2 and 0.3 tesla magnetized cocoons the glycogen content increased with the increasing duration of exposure up to 96 h while in case of 0.4 tesla magnetized cocoons, the glycogen content showed slight increase in case of 24 h exposure but further increase in the exposure duration caused decrease in the glycogen content. In 0.1 tesla magnetized cocoons, the glycogen content increased slowly which reached to the maximum level of 1.082±0.012 µg/mg in 96 h magnetized cocoons. In 0.2 and 0.3 tesla magnetized cocoons, the trend of increase in the glycogen content with the increasing exposure duration was almost of similar fashion. In case of 24 h exposed cocoons, the increase in glycogen content was of very low level while 48, 72 and 96 h exposed cocoons, the change in glycogen content was of higher level and reached to maximum of 1.296±0.022 µg/mg (43.20% increase as compared to control) in 72 h exposed 0.3 tesla magnetized cocoons. In 0.4 tesla magnetized cocoons, the glycogen content slightly increased with the increasing duration upto 24 h while further increase in the exposure duration of cocoons caused steady decline in glycogen content which reached to the minimum level of 0.825±0.002 µg/mg (8.84% decrease as compared to control) in case of 96 h exposure of cocoons.

Two-way ANOVA shows that variation in the strength of static magnetic field significantly ($P_1 < 0.01$) influenced the glycogen content in the silk gland of 5th instar *B. mori* larvae while the level of variation in the

Table 1a: Effect of cocoon magnetization on the glycogen content (µg/mg) in the silk gland of 5th instar *Bombyx mori* larvae

	Magnetic power (tesla)					
Exposure duration (h)	Control (X_1)	0.1 (X_2)	0.2 (X_3)	0.3 (X_4)	0.4 (X_5)	F_1-ratio n_1= 4
24	0.905±0.001 (100)	0.942±0.002 (104.08)	0.995±0.011 (109.94)	1.075±0.001 (118.78)	1.092±0.020 (120.66)	
48	0.905±0.001 (100	0.971±0.005 (107.29)	1.092±0.013 (120.66)	1.215±0.002 (134.25)	1.002±0.004 (110.71)	7.857*
72	0.905±0.001 (100)	1.031±0.032 (113.92)	1.296±0.022 (122.54)	1.296±0.022 (143.2)	0.892±0.003 (98.56)	
96	0.905±0.001 (100)	1.082±0.012 (119.55)	1.145±0.125 (126.51)	1.225±0.315 (135.35)	0.825±0.002 (91.16)	

F_2 –ratio = 0.286** n_2 = 3

*$P_1 < 0.01$

Each value represents mean±S.E. of six replicates

X_1, X_2, X_3, X_4 and X_5 are the mean values of glycogen content in control, 0.1, 0.2, 0.3 and 0.4 tesla magnetic strength

Figures in parentheses indicate per cent value when control was taken as 100%

Table 1b: Post-hoc Test showing effect of cocoon magnetization on the glycogen content (µg/mg) in the silk gland of 5th instar *Bombyx mori* larvae.

	Exposure duration (hours)			
Mean difference in between groups	24	48	72	96
$X_1 \sim X_2$	0.037	0.066	0.126	0.177
$X_1 \sim X_3$	0.090	0.187	0.204	*0.240
$X_1 \sim X_4$	0.170	*0.310	*0.391	*0.320
$X_1 \sim X_5$	0.187	0.097	0.013	0.080
$X_2 \sim X_3$	0.053	0.121	0.078	0.063
$X_2 \sim X_4$	0.133	*0.244	*0.265	0.143
$X_2 \sim X_5$	0.150	0.031	0.139	*0.257
$X_3 \sim X_4$	0.080	0.123	0.187	*0.920
$X_3 \sim X_5$	0.097	0.090	*0.217	*0.320
$X_4 \sim X_5$	0.017	*0.213	*0.404	*0.400

Honesty Significant difference (HSD)

$$= q\sqrt{\frac{\text{MS within}}{n}}$$

$$= 6.10\sqrt{\frac{0.0072}{6}}$$

$$= 0.211$$

MS = Mean square value of ANOVA Table

q = Studentized range static

n = No. of replicates

* = Shows significant group difference

X_1, X_2, X_3, X_4 and X_5 are mean values of glycogen content in control, 0.1, 0.2, 0.3 and 0.4 tesla magnetic strength respectively.

glycogen content due to change in the exposure duration was not of significant level. The Post-hoc test (Table-1b) shows significant group difference in glycogen content in between control and 0.3 tesla, 0.1 and 0.3 tesla and 0.3 and 0.4 tesla magnetic strength in case of 48 h exposed cocoons. In 72 h exposed cocoons, the significant group difference was observed in between control and 0.3 tesla, 0.1 and 0.3 tesla, 0.2 and 0.4 tesla and 0.3 and 0.4 tesla magnetic strength. Highly significant group difference was observed in between control and 0.2 tesla, control and 0.3 tesla, 0.1 and 0.4 tesla, 0.2 and 0.3 tesla, 0.2 and 0.4 tesla and 0.3 and 0.4 tesla, magnetic strength in case of 96 h exposed cocoons while 24 h exposed cocoons of each magnetic strength did not cause significant group difference.

Glycogen Content in the Fat Body of Larvae: It is clear from Table 2a that variation in the magnetic strength and exposure duration of cocoons, both have notable influence on the glycogen content in the fat body of 5th instar *B. mori* larvae. With the increasing duration of cocoons in the magnetic strength of 0.1, 0.2 and 0.3 tesla

Table 2a: Effect of cocoon magnetization on the glycogen content (µg/mg) in the fat body of 5th instar *Bombyx mori* larvae.

	Magnetic power (tesla)					
Exposure duration (h)	Control (X_1)	0.1 (X_2)	0.2 (X_3)	0.3 (X_4)	0.4 (X_5)	F_1-ratio n_1= 4
24	28.532±0.292 (100)	28.929±0.127 (101.39)	29.826±0.003 (104.53)	30.124±0.035 (105.57)	30.526±0.315 (106.98)	
48	28.532±0.292 (100)	29.608±0.003 (103.77)	30.623±0.120 (107.32)	30.728±0.031 (107.69)	28.026±0.032 (98.22)	4.417*
72	28.532±0.292 (100)	30.325±0.153 (106.28)	31.352±0.152 (109.88)	32.628±0.321 (119.35)	27.531±0.005 (96.49)	
96	28.532±0.292 (100)	30.525±0.123 (106.98)	31.928±0.032 (111.90)	32.025±0.003 (112.24)	24.008±0.007 (84.14)	

F_2 –ratio = 0.187** $n_2 = 3$

*$P_1 < 0.025$

Each value represents mean±S.E. of six replicates

X_1, X_2, X_3, X_4 and X_5 are the mean values of glycogen content in control, 0.1, 0.2, 0.3 and 0.4 tesla magnetic strength

Figures in parentheses indicate per cent value when control was taken as 100%

Table 2b: Post-hoc Test showing effect of cocoon magnetization on the glycogen content (µg/mg) in the fat body of 5th instar *Bombyx mori* larvae.

	Exposure duration (hours)			
Mean difference in between groups	24	48	72	96
$X_1 \sim X_2$	0.397	1.076	1.793	1.993
$X_1 \sim X_3$	1.294	2.091	2.820	3.396
$X_1 \sim X_4$	1.592	2.196	*4.096	3.493
$X_1 \sim X_5$	1.994	0.506	1.001	*4.524
$X_2 \sim X_3$	0.897	1.015	1.027	1.403
$X_2 \sim X_4$	1.195	1.120	2.303	1.500
$X_2 \sim X_5$	1.597	1.582	2.794	*6.517
$X_3 \sim X_4$	0.298	0.105	1.276	0.097
$X_3 \sim X_5$	0.700	2.597	3.821	*7.920
$X_4 \sim X_5$	0.402	2.702	*5.097	*8.017

Honesty Significant difference (HSD)

$$= q\sqrt{\frac{\text{MS within}}{n}}$$

$$= 6.10\sqrt{\frac{2.363}{6}}$$

$$= 3.825$$

MS = Mean square value of ANOVA Table

q = Studentized range static

n = No. of replicates

* = Shows significant group difference

X_1, X_2, X_3, X_4 and X_5 are mean values of glycogen content in control, 0.1, 0.2, 0.3 and 0.4 tesla magnetic strength respectively.

the glycogen content increased while in 0.4 tesla, the glycogen content decreased with the increasing exposure duration of cocoons. In the case of 0.1 tesla magnetized cocoons, the increase in the glycogen content with the increasing exposure duration is very slow which reached to the level 30.525±0.123 µg/mg in case of 96 h exposed cocoons. In case of 0.2 and 0.3 tesla magnetized cocoons the increase in the glycogen content was considerable with the increasing exposure duration and reached to the maximum level of 32.628±0.321 µg/mg (14.35% increase as compared to control) in case of 0.3 tesla- 72 h exposed cocoons. In 0.4 tesla magnetized cocoons, the glycogen content showed a slow increase in case of 24 h exposure of cocoons which further declined deeply to the level of 24.008±0.007 µg/mg (15.86% decrease as compared to control) in case of 96 h exposed cocoons.

Two-way ANOVA indicates that variation in the static magnetic field significantly (P_1<0.025) influenced the glycogen content in the fat body of *B. mori* larvae while exposure duration did not cause significant effect. The post-hoc test (Table-2b) shows significant group difference in the glycogen content in between control

Table 3a: Effect of cocoon magnetization on the glycogen content (µg/mg) in the haemolymph of 5th instar *Bombyx mori* larvae

	Magnetic power (tesla)					
Exposure duration (h)	Control (X_1)	0.1 (X_2)	0.2 (X_3)	0.3 (X_4)	0.4 (X_5)	F_1-ratio n_1= 4
24	12.872±0.032 (100)	13.190±0.003 (102.47)	13.325±0.012 (103.51)	13.829±0.039 (107.43)	13.995±0.004 (108.72)	
48	12.872±0.032 (100)	13.425±0.032 (104.29)	13.829±0.018 (107.43)	14.829±0.052 (107.69)	12.315±0.031 (95.67)	
72	12.872±0.032 (100)	13.924±0.231 (108.17)	14.237±0.035 (110.6)	16.324±0.013 (126.81)	11.137±0.005 (86.52)	5.450*
96	12.872±0.032 (100)	14.339±0.001 (111.39)	14.556±0.025 (113.08)	15.225±0.042 (118.27)	10.253±0.003 (79.65)	

F_2 –ratio = 0.734** $n_2 = 3$

*$P_1 < 0.01$

Each value represents mean±S.E. of six replicates

X_1, X_2, X_3, X_4 and X_5 are the mean values of glycogen content in control, 0.1, 0.2, 0.3 and 0.4 tesla magnetic strength

Figures in parentheses indicate per cent value when control was taken as 100%

Table 3b: Post-hoc Test showing effect of cocoon magnetization on the glycogen content (µg/mg) in the haemolymph of 5th instar *Bombyx mori* larvae.

	Exposure duration (hours)			
Mean difference in between groups	24	48	72	96
$X_1 \sim X_2$	0.318	0.553	1.052	1.467
$X_1 \sim X_3$	0.453	0.957	1.365	1.684
$X_1 \sim X_4$	0.957	2.051	*3.452	2.253
$X_1 \sim X_5$	1.123	0.557	1.735	*2.619
$X_2 \sim X_3$	0.135	0.404	0.313	0.217
$X_2 \sim X_4$	0.639	1.498	2.400	0.886
$X_2 \sim X_5$	0.805	1.110	*2.735	*4.086
$X_3 \sim X_4$	0.504	1.094	2.087	0.669
$X_3 \sim X_5$	0.670	1.514	*3.100	*4.303
$X_4 \sim X_5$	0.166	*2.608	*5.187	*4.972

Honesty Significant difference (HSD)

$$= q\sqrt{\frac{\text{MS within}}{n}}$$

$$= 6.10\sqrt{\frac{1.035}{6}}$$

$$= 3.532$$

MS = Mean square value of ANOVA Table

q = Studentized range static

n = No. of replicates

* = Shows significant group difference

X_1, X_2, X_3, X_4 and X_5 are mean values of glycogen content in control, 0.1, 0.2, 0.3 and 0.4 tesla magnetic strength respectively.

and 0.3 tesla and 0.3 and 0.4 tesla magnetic strength in case of 72 h exposed cocoons. In case of 96 h exposed cocoons, the significant group difference was observed in between control and 0.4 tesla, 0.1 and 0.4 tesla, 0.2 and 0.4 tesla and 0.3 and 0.4 tesla magnetic strength in glycogen content in the 5th instar *B. mori* larvae.

Glycogen Content in the Haemolymph of Larvae: It is clear from the data given in Table 3a that the change in the static magnetic strength and exposure duration of *B. mori* cocoons, influenced the glycogen content in the haemolymph of 5th instar *B. mori* larvae. With the increasing duration upto 96 h, the glycogen content increased in the haemolymph of larvae obtained from 0.1, 0.2 and 0.3 tesla magnetized cocoons. In case of 0.1 tesla magnetized cocoons, the glycogen content steadily increased and reached to the higher level of 14.339±0.001 µg /mg at 96 h of exposure. In 0.2 and 0.3 tesla magnetized cocoons, the initial increase in the glycogen content was higher at 24 h of magnetization which further increased slowly and reached to level of 14.556±0.025 and 15.225±0.042 µg/mg respectively in case of 96 h exposed

cocoons. In 0.4 tesla magnetized cocoons, the glycogen content slightly increased in 24 h exposed cocoons but with the further increase in the exposure duration, the glycogen content decreased to the lowest level of 10.253±0.003 µg/mg in 96 h magnetized cocoons. The maximum level (16.324±0.013 µg/mg) of glycogen content (26.81% increase as compared to control) was noticed in 0.3 tesla -72 h exposed cocoons.

Two-way ANOVA indicates that variation in the static magnetic field significantly ($P_1 < 0.01$) influenced the glycogen content in the haemolymph of *B. mori* larvae while exposure duration did not cause significant effect. The Post-hoc test (Table-3b) shows significant group difference in glycogen content in between 0.3 and 0.4 tesla magnetic strength in case of 48 h exposed cocoon. In case of 72 h exposed cocoons, the significant group difference was observed in between control and 0.3tesla, 0.1 and 0.4 tesla, 0.2 and 0.4 tesla and 0.3 and 0.4 tesla magnetic strength. In case of 96 h exposed cocoons, the significant group difference was observed in between control and 0.4 tesla, 0.1 and 0.4 tesla, 0.2 and 0.4 tesla and 0.3 and 0.4 tesla magnetic strength in glycogen content in the 5th instar *B. mori* larvae.

DISCUSSION

The level of glycogen content in the silk gland of *Bombyx mori* larvae increased considerably with the increasing exposure duration of cocoons from 24 to 96 h in 0.1 and 0.2 tesla and reached to the highest level in 0.3 tesla magnetic strength in 72 h exposed cocoons. In 0.4 tesla magnetic field, the glycogen content was noticed to be increased up to 24 h while further increase in the exposure duration caused decline in the glycogen content in the silk gland of 5th instar larvae (Table -1a). The total glycogen content in *Philosamia ricini* consistently increased reaching to the maximum level in the silk gland of 5th instar larvae accompanied by a corresponding pattern of amylolytic activity in the whole larval homogenate [19].

The glycogen level in the fat body of *B. mori* larvae was influenced due to variation in the strength of magnetic field in all the conditions of cocoons exposure. The maximum level of glycogen content was noticed in 0.3 tesla magnetic field with the exposure duration of 72 h while the minimum glycogen content was noticed in 96 h exposure of cocoons in 0.4 tesla static magnetic field. The glycogen reserve of animals has been recognized as an important polysaccharide constituents of the insect tissues particularly of the fat body [20]. The normal pattern of the changes in glycogen level in the tissues during growth and metamorphosis of insects is a substantial rise in its level during larval growth interrupted by a decrease during each moult, ultimately reaching an intensified accumulation in last larval instar [20]. The decreased level of glycogen, trehalose and glucose associated with the lowering of the super cooling points in the larvae of *Isia isabella* has been reported [21] and similar type of decrease in the glycogen content of the larvae of *Pieris brassicae* was also noticed [22]. An increase in the glycogen but decrease in glucose content was noticed from August to October when the temperature falls to 7-3°C showed the primary function of glycogen for the improvement of cold hardiness in *Gorpholitha funsbrana* due to seasonal acclimatization [23]. An inverse relationship between the glycogen and trehalose in the fat body was observed in silkworm *Bombyx mori* [24].

The moulting period is accompanied by an enhanced chitin biosynthesis and the demand for glucose may be met through trehalose of the haemolymph which is replaced by the continuous influx of trehalose from the fat body which was synthesized at the expense of glycogen of the tissue [25]. The present results are in conformity to the findings of [19] who reported an increased glycogen level in the fat body of insects. Just prior to the larval-pupal transformation, a parallel study of glycogen and trehalose metabolism was made and observed that the two sugers have inverse relationship, therefore, trehalose metabolism surpasses the glycogen metabolism in adult *Tenebrio molitor*, whereas, reverse was true in the larval stage [26]. The trehalose/glycogen ratio decreased in the late 5th instar of *Bombyx mori* due to comparative increase in the fat body glycogen and further the trehalose/glycogen ratio of the silk gland tissue was remarkably very high in comparison to that of the fat body during all developmental stages which clearly indicates that the trehalose metabolism significantly exceed the glycogen metabolism in the silk gland, while in the fat body reverse was the true condition [27]. Decrease in glycogen level during larval-pupal transformation has also been observed In *Cercopia silkmoth* [28] and *Eucilia cuprina* [29]. The total carbohydrate level in the fat body decreased during the spining process in *Rhynchosciora americana* [30]. The occurrence of similar decrease in glycogen content during the process of spinning in oak silkworm led to assume that the products of glycogen break down act as a carbon source for the essential amino acids needed for fibroin synthesis [31].

Thus the variation in the magnetic strength and exposure duration of cocoons influenced the glycogen content level in different tissues of larvae. The low strength of magnetic field may cause activation of cytochrome system which increased the metabolic rate of larvae. These changes may cause the activation of enzyme system responsible for the production of energy inside the cells in different tissues of silkworm larvae, whereas, high strength of magnetic field may cause stress response causing decline in the glycogen level in *B. mori*.

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