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Influence of the month of the year in the hematological profile in carthusian broodmares

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Abstract

Background: This study provides data regarding seasonal changes in the hematology of 44 adult Carthusian broodmares (4-17 years old), sampled during 12 months.

Methods: On each animal 24 blood samples were taken every 14 days. A total of 1056 blood samples were analyzed. We measured red blood cells (RBC), hemoglobin (HB), hematocrit (HcT), volumetric indices (MCV, MCH and MCHC), white blood cells (WBC) and subsets, total serum proteins (TSP) and platelets (PLT).

Results: RBC and HcT reached higher values from May to August and PLT, in July and August. By contrast, HB achived the lowest concentrations in September. MCV was lower in May and July, MCH from May to August and MCHM in July and September. The highest TSP concentrations were found in January. WBC, lymphocytes, neutrophils and eosinophils had significantly higher values during the coldest months of the year, from January or February to May, even though the neutrophil/lymphocyte ratio was statistically similar during the whole study.

Conclusions: According to these results, it appears that the hematological profile of the pregnant Carthusian broodmares is subjected to seasonal variations, so it would be a factor to consider in interpreting the haematological profile in the mare.

Keywords: Horse, hematology, seasonal variations, carthusian broodmares, hemoglobin

Introduction

Normal cell physiological processes, body composition, water and food intake, organ function, and diseases are subjected to periodic variations related to biological rhythms in a number of species, including human beings and horses [1-4]. Oscillations sustained with a period of about one day are called circadian rhythms, which are superimposed upon rhythms with lower frequencies, called infradian rhythms. Those include rhythms with a period of about 1 week (circaseptan), 2 weeks (circadiseptan), 3 weeks (circavigintan), about 30 days (circatrigintan) and with a period of about 1 year (circannual and/or seasonal variations) [5-8].

Hematological determinations are commonly performed in equine medicine for a variety of reasons, as screening tests on healthy animals, to examine geriatric patients, to identify conditions that might make an animal a poor candidate for anesthesia or surgery, to diagnose a disease, to determine its severity and its consequences, to formulate a prognosis, to monitor the response to therapy and in sport medicine, to assess exercise response, fitness and training levels. Clinical pathology parameters, and particularly hematological variables, are subjected to biological rhythms [9]. Therefore, the knowledge of the rhythmic variations of the most used laboratory tests has important diagnostic, therapeutic, research and epidemiological implications that guarantee a deep investigation.

Most studies about the variations due to biological rhythms in the hematological parameters have been carried out in humans. Kristal-Boneh et al., [10] stated that hemoglobin concentration (HB) and hematocrit (HcT) were significantly lower in summer than during the rest of the year, due to a decrease in the mean corpuscular volume (MCV). These results were justified in relation to heat acclimatization with plasma and red cell volume expansion. Cheung et al., [11] found that HcT varied with the seasons, although in this case, peak values were attained in July. They also demonstrated that hemoconcentration was insufficient to explain the rise in HcT. Also in human beings, Peng et al., [12] confirmed that platelet (PLT) counts in healthy subjects are greatly influenced by seasonal variations. Similarly, circannual and circadian variations in total white cells (WBC) count and WBC subsets have been described [13].

Circadian variations in HB, red blood cell count (RBC) and mean corpuscular hemoglobin (MCH) have been analyzed in foals and adult horses, as well as in mares in different reproductive situations [14]. Other parameters analyzed

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Table 1. Enviromental conditions during the period of study.

	Relative humidity (%)	Mean temperature (ºC)	Hours of light (h:min:s)
January	76	9.9	06:15:02
February	68	13.50	07:10:07
March	59	15.20	08:16:12
April	69	19.70	07:25:00
May	65	24.40	10:31:36
June	57	24.0	11:33:24
July	57	26.40	11:32:24
August	58	26.20	11:01:36
September	64	22.70	08:10:24
October	76	20.40	06:01:48
November	69	13.00	07:38:48
December	78	12.00	05:38:00

were aminostranferases (AST and ALT), acid and alkaline phosphatases, aldolase, cortisol, thyroid hormones, glucose, lactate, pyruvate, pH, pO2, pCO2 and electrolytes [15,16]. However, the influence of month on these parameters has not been as extensively reviewed as diurnal changes. To the author´s knowledge, there is only one article of the circannual rhythm in the hematological profile of horses, but it focused on growing Thorough bred foals [5]. Furthermore, it seems that the interaction between pregnancy and biological rhythms markedly influences the hematological profile. Flisinka-Bojanowska et al., [16] concluded that gestation in mares modifies diurnal rhythms and seasonal cycles in secretion and metabolism of cortisol and thyroid hormones. In addition, these authors stated that pregnancy abolishes cyclicity in plasma lactate levels and modifies seasonal behavior of pyruvate and pCO2.

The hematological characteristics of the Carthusian broodmares, and the changes linked to pregnancy and age have been published [9,17]. This strain of the Andalusian breed constitutes a small population, located mainly in the south of Spain, and it is economically and genetically of high value. According to Haus et al., [18], some physiological rhythms are presumably genetically determined. In the present research, we want to test two hypotheses. Firstly, whether hematological profile changes in a 12-month period possibly in association to external or environmental conditions; secondly, whether influences of the month of the year are similar in Carthusian broodmares raised in the south of Spain, than those described for other equine breeds in different geographic locations.

Material and methods

Mares and general management

Forty-four healthy pregnant en el mes de marzo, correspondiente al primer mes de gestación Carthusian broodmares of the same stud-farm and aged between 4 and 17 years (7.12±2.66) were studied. All the mares were kept in pasture most of the time, being placed in the stall when the environmental temperatures were high (>35ºC) or low (<10ºC). Values of relative humidity, temperature and number of sunlight hours are shown by months in **Table 1**. The type of diet supplied to the mares was as follows: the daily diet was of a combination of fiber and concentrates. The daily amounts of concentrate was: 250 g of dried beet pulp, 1500 g of barley, 500 g of beans, 200 g of oats and 3000 g of soybean. The daily amount of fiber was provided in 5000 g alfalfa hay and 5000 g of oat hay. Water intake was ad libitum. Blood samples were withdrawn before the morning feed.

Blood sampling schedule

A total of 1056 blood samples were extracted during the research. In each mare, the first sample was obtained on day 16 of pregnancy and the last sample the corresponding day of the week of delivery. Pregnancy was detected by sequential ultrasonic scanning (Pie Medical 480®) starting on day 14 after mating. The mares were sampled periodically along the pregnancy, every 14 days, with an overall study period of 12 months. Blood extractions were always performed between 9:00 and 12:00 a.m. When the times of sampling and feeding coincided, the former preceded the latter.

Blood samples management and laboratory procedures

Immediately after venipuncture of the external jugular vein, and directly from the syringe, without anticoagulant, a blood smear was performed. The smears were air-dried. The rest of the blood was poured into EDTA-3K tubes for hematology and into glass tubes without anticoagulant to measure total proteins. Samples were kept refrigerated during the transport to the lab and the analysis was performed within the first 12 hours after extraction.

Hematological profile included the following parameters: RBC, HB, HcT, MCV, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), WBC and PLT. These determinations were made with a semiautomatic counter (System F820®). The differential WBC count was carried out in the blood smear, which was fixed with ethanol and stained with the May-Grünwald-Giemsa technique. The absolute number of the WBC populations was quantified by microscopic observation (Olympus CX 21®): lymphocytes (LYMP), band neutrophils (BNTP), neutrophils with less than three nuclear lobulations (NTP<3), neutrophils with more than three nuclear lobulations (NTP>3), segmented neutrophils (SNTP), total neutrophils (TNTP), eosinophils (EOS), monocytes (MON), basophils (BAS) and neutrophil/lymphocyte ratio (N/L). The concentration of total serum proteins (TSP) was assessed by immersion refractometry (Atago).

Statistics

Results were expressed as mean±SD. A one-way repeated measures analysis of variance (ANOVA) was used to determine significant differences in blood parameters at monthly intervals. $P<0.05$ was considered to be statistically significant.

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Table 2. RBC parameters, platelets and total serum proteins in Carthusian broodmares in the different months during the period of study (mean, standard deviation between bracketts; n=1056) (Different superscript indicates significant differences) p<0.05.

	January	February	March	April	May	June	July	August	September	October	November	December
RBC (10^6/µl)	8.227 ^{A} (1.23)	8.795 ^{A} (1.47)	8.527 ^{A} (1.23)	8.566 ^{A} (1.45)	9.488 ^{B} (1.82)	9.147 ^{B} (1.97)	10.12 ^{B} (2.00)	9.285 ^{B} (1.71)	8.625 ^{A} (1.36)	8.831 ^{A} (1.29)	8.622 ^{A} (1.28)	8.155 ^{A} (0.85)
HB (g/dl)	12.28 ^{A} (1.34)	12.98 ^{A} (1.55)	12.75 ^{A} (1.27)	12.53 ^{A} (1.20)	12.56 ^{A} (1.42)	12.44 ^{A} (1.89)	12.40 ^{A} (1.78)	12.36 ^{A} (1.74)	11.67 ^{B} (1.31)	12.15 ^{A} (1.97)	12.06 ^{A} (1.25)	12.14 ^{A} (1.39)
HcT (%)	40.29 ^{A} (4.39)	42.44 ^{A} (4.84)	41.95 ^{A} (5.02)	42.46 ^{A} (5.40)	44.23 ^{B} (4.90)	45.53 ^{B} (5.54)	46.36 ^{B} (4.15)	45.07 ^{B} (4.04)	43.11 ^{A} (4.32)	44.33 ^{B} (4.29)	43.87 ^{A} (5.05)	43.49 ^{A} (2.42)
MCV (fl)	49.30 ^{A} (3.35)	48.75 ^{A} (4.09)	49.45 ^{A} (3.22)	50.01 ^{A} (3.91)	47.51 ^{B} (5.75)	48.64 ^{A} (5.95)	47.03 ^{B} (6.92)	49.64 ^{A} (7.05)	51.45 ^{A} (5.60)	50.64 ^{A} (4.05)	51.23 ^{A} (4.01)	53.41 ^{A} (2.42)
MCH (g/dl)	15.04 ^{A} (1.18)	14.93 ^{A} (1.46)	15.15 ^{A} (2.00)	14.89 ^{A} (1.85)	13.57 ^{B} (2.08)	13.92 ^{B} (2.08)	12.67 ^{B} (2.03)	13.54 ^{B} (1.84)	13.71 ^{A} (1.69)	13.87 ^{A} (1.97)	14.21 ^{A} (2.01)	14.92 ^{A} (1.15)
MCHC (%)	30.58 ^{A} (2.36)	30.69 ^{A} (2.43)	30.64 ^{A} (3.30)	29.65 ^{A} (2.80)	28.56 ^{A} (2.81)	28.75 ^{A} (3.77)	26.93 ^{B} (3.80)	27.55 ^{A} (3.89)	26.75 ^{B} (2.76)	27.51 ^{A} (4.10)	27.76 ^{A} (3.57)	27.95 ^{A} (2.149
PLT (10^3/µl)	147.9 ^{A} (37.0)	156.7 ^{A} (48.0)	152.9 ^{A} (40.0)	182.8 ^{A} (92.7)	239.2 ^{A} (127)	210.8 ^{A} (110)	285.9 ^{B} (125)	262.0 ^{B} (153)	220.4 ^{A} (109)	171.2 ^{A} (109)	172.1 ^{A} (59.61)	180.4 ^{A} (67.84)
TSP (g/dl)	7.572 ^{A} (0.33)	6.864 ^{B} (0.50)	6.903 ^{B} (0.45)	6.998 ^{B} (0.48)	6.891 ^{B} (0.40)	6.714 ^{B} (0.46)	6.975 ^{B} (0.43)	6.836 ^{B} (0.51)	6.862 ^{B} (0.45)	6.967 ^{B} (0.45)	7.010 ^{B} (0.46)	6.773 ^{B} (0.44)

Table 3. Total WBC and subsets in Carthusian broodmares in the different months during the period of study (mean, standard deviation between bracketts; n=1056) (Different superscript indicates significant differences) p<0.05.

	January	February	March	April	May	June	July	August	September	October	November	December
WBC (10^3/µl)	10.20 ^{A} (1.94)	11.06 ^{A} (2.58)	11.42 ^{A} (2.37)	11.33 ^{A} (2.57)	11.20 ^{A} (2.23)	10.13 ^{A} (1.98)	9.754 ^{A} (2.46)	8.803 ^{B} (2.00)	8.973 ^{B} (1.99)	8.472 ^{B} (2.39)	8.592 ^{B} (1.71)	8.965 ^{B} (1.73)
LYMP (10^3/µl)	3.931 ^{A} (0.95)	4.489 ^{A} (1.43)	4.818 ^{B} (1.34)	4.751 ^{B} (1.32)	5.059 ^{B} (1.37)	4.378 ^{A} (1.22)	4.271 ^{A} (1.21)	3.845 ^{A} (1.13)	3.978 ^{A} (1.06)	3.833 ^{A} (1.14)	3.795 ^{A} (0.98)	4.131 ^{A} (0.93)
BNTP (10^3/µl)	0.233 ^{A} (0.11)	0.260 ^{B} (0.12)	0.246 ^{A} (0.10)	0.228 ^{A} (0.12)	0.221 ^{A} (0.09)	0.216 ^{A} (0.09)	0.110 ^{C} (0.55)	0.171 ^{C} (0.05)	0.181 ^{C} (0.08)	0.065 ^{D} (0.08)	0.153 ^{C} (0.10)	0.088 ^{D} (0.09)
NTP<3 (10^3/µl)	1.295 ^{A} (0.66)	1.327 ^{A} (0.62)	1.218 ^{A} (0.72)	1.605 ^{B} (1.18)	1.724 ^{B} (1.09)	1.105 ^{A} (0.59)	1.097 ^{A} (0.55)	1.060 ^{A} (0.52)	1.061 ^{A} (0.73)	2.586 ^{C} (1.64)	1.517 ^{B} (1.15)	2.578 ^{C} (1.28)
NTP>3 (10^3/µl)	3.776 ^{A} (1.15)	4.138 ^{B} (1.42)	4.200 ^{B} (1.24)	3.811 ^{A} (1.73)	3.315 ^{A} (1.13)	3.619 ^{A} (1.16)	3.449 ^{A} (1.19)	3.103 ^{A} (0.88)	3.043 ^{A} (1.08)	1.319 ^{C} (1.35)	2.524 ^{D} (1.18)	1.340 ^{C} (1.25)
NTP (10^3/µl)	5.242 ^{A} (1.49)	5.731 ^{A} (1.66)	5.635 ^{A} (1.51)	5.639 ^{A} (1.67)	5.260 ^{A} (1.16)	4.955 ^{A} (1.37)	4.745 ^{A} (1.45)	4.328 ^{B} (1.08)	4.266 ^{B} (1.11)	3.947 ^{B} (1.22)	4.207 ^{B} (1.06)	4.040 ^{B} (1.04)
EOS (10^3/µl)	0.784 ^{A} (0.46)	0.662 ^{A} (0.42)	0.737 ^{A} (0.50)	0.671 ^{A} (0.41)	0.677 ^{A} (0.43)	0.559 ^{B} (0.38)	0.549 ^{B} (0.36)	0.459 ^{B} (0.25)	0.525 ^{B} (0.35)	0.564 ^{B} (0.26)	0.452 ^{B} (0.30)	0.504 ^{B} (0.36)
MON (10^3/µl)	0.246 ^{A} (0.10)	0.263 ^{A} (0.14)	0.226 ^{A} (0.08)	0.270 ^{A} (0.16)	0.235 ^{A} (0.10)	0.211 ^{A} (0.08)	0.189 ^{B} (0.08)	0.172 ^{B} (0.05)	0.203 ^{A} (0.09)	0.280 ^{A} (0.177)	0.211 ^{A} (0.13)	0.277 ^{A} (0.18)
N/L ratio	1.367 ^{A} (0.48)	1.424 ^{A} (0.72)	1.259 ^{A} (0.53)	1.253 ^{A} (0.45)	1.087 ^{A} (0.37)	1.228 ^{A} (0.56)-	1.166 ^{A} (0.40)	1.183 ^{A} (0.36)	1.130 ^{A} (0.35)	1.023 ^{A} (0.28)	1.176 ^{A} (0.50)	1.022 ^{A} (0.34)

Results

Results for the months of the year are shown in (**Tables 2** and **3**), for RBC, PLT and TSP, and WBC parameters, respectively. Analysis of variance showed that RBC and HcT (from May to August), and PLT (July and August), were significantly higher than the other months of the year. HB was significantly lower in September than the rest of the months (**Table 2**). MCV was significantly lower in May and July, MCH was lower from May to August whereas MCHC was lower in July and September and TSP was higher in January (**Table 2**).

Total WBC and subsets showed significant differences when comparing the different months of the year, with the highest values during the coldest months of the year, mainly from January or February to May (**Table 3**). EOS count was higher from January to May (**Table 3**). N/L ratio remains unchanged during the whole period of study.

Discussion

Most of the analyzed data of our study reached statistical significance when comparing by months, suggesting that these variations were not accidental; that is, they reflected seasonal changes in the hematological profile of the Carthusian broodmares.

Red blood cell parameters

Early reports in Arabian mares and Thoroughbreds found higher

RBC, HB and HcT in autum and winter [19]. These changes were interpreted on the basis of metabolic acclimation to the environmental conditions. Lower ambient temperatures would have required a higher metabolic capacity for regulating body temperature, stimulating erythropoiesis [20]. Additionally, enhanced sympathetic activity in winter could lead to increased spleen mobilization, with the release of blood into the bloodstream [21]. These data contrast with a previous report that indicated that intense cold decreases RBC due to a reduction of its half-life [22].

In our case, however, higher RBCs were found from May to August, with the lowest values in December. Similarly, HcT was higher in the same months than RBC. There are some ideas that could justify our data, such as heat, degree of physical activity and composition of the grass. During the summer months, the higher temperatures could develop an adaptative response to heat stress [23]. The higher temperatures and the associated decrease in body fluids in association with the thermoregulatory mechanisms could have influenced RBC and HcT [23]. However, TSP did not follow the same trend as RBC and HcT, limiting the importance of hemoconcentrations because of evaporative sweating and/or breathing in our data. A second consideration could be the physical activity performed by the animals during the study period. Sexual activity in horses occurs mainly in spring and summer, decreasing in later summer and autum, and ceasing completely in winter [24]. Further, the mares could have also had greater physical activity because they stayed longer in the pasture. Likewise, one might think that the differences in the results obtained could have been due to the result of feeding. During spring, water intake with grazing might have limited the effect of hemoconcentration induced by physical activity and/or thermolysis.

It should be kept in mind that during the months of May to August, the mares were pregnant and in lactation of the foals of the previous pregnancy. In our opinion, the effect of the month of the year might have masked the effect of pregnancy and lactation on erythrocyte parameters. Thus, a previous report Satué et al., [17] show a significant effect of pregnancy on erythrocyte parameters in mares.

The increased HcT from May to August conditioned significantly lower MCV values during this period of time. These results have been also found in other animal species, such as Mountain gazelle [25]. However, work in experimental animals found that during winter, erythrocytes appear smaller in size than in spring and summer [18]. These researchers found a type of genetic intervention on changes in the size of RBC that occurs independently of heat acclimatization, that is not shown in the horse. Similarly, in human beings, Kristal-Boneh et al., [10] found a reduction in HcT in August in association with a reduction in MCV. Although it appears that the results should be determined by the heat stress and the degree and type of adaptation to this heat, interspecific differences might exist.

On the other hand, the reduction in MCH and MCHC during the hottest months of the year (i.e., May, June, July and August) in our broodmares agrees with the data provided by Kristal-Boneh et al., [10] in humans. It has been indicated that these lower levels of HCM suggest a reduced efficiency in the transport of oxygen from the erythrocyte. This fact could result in a compensatory increase in the number of red blood cells [26].

Total serum proteins

The evolution of TSP in the current research is unexpected, because in the hottest time of the year, they reached significantly lower values, mainly in comparison with January. Furthermore, these changes were not in parallel with HcT. It can be supposed that the broodmares in pasture have access to fresh grass in months with more rain. At this time of the year, the content of protein in the plants is higher and therefore, the mares should have higher TSP, as reported in Arabian mares [27]. Seasonal ciclicity has been found in TSP, as well as in protein fractions (i.e., albumin, α1, α2, β1, β2 and γ globulins). However, ciclicity depended on the reproductive state of the studied mares (pregnant, barren and with foals) [28].

Platelet count

It has been confirmed that seasonal variations exist in hemostatic markers, including PLT number [29]. Previous studies in human medicine corroborates our results, with higher PLT counts in the hot months of the year vs. the cold months [12]. However, other studies in dogs have shown no seasonal variations in platelet count [30]. In laboratory animals, Goryshima [31] found that the release of PLT from bone marrow to peripheral blood is limited in winter.

Total white cells and subsets

The results derived from our investigation show a marked seasonality in relation to the white series, WBC, LYMP, and the different types of NTP, N/L and EOS. Among the causes of seasonal variations in hematology, we can cite the influence of daylight, stress, climate changes (temperature and atmospheric pressure) and changes in the diet [32]. The increase in WBC, LYMP and NTP with different degrees of maturity can be due to three reasons. Firstly, some researches have shown that stress associated with cold weather in winter may suppress the immune response [33]. In fact, in human beings, Bokenes et al., [34] found a decrease in NTP in winter associated with increased activation and increased adhesion to vascular endothelium. By contrast, others showed that the highest levels of WBC are found in winter, because lymphoid organs reach their greatest size in autumn and winter [35]. These patterns could reveal the organic adaptation to harsh winter weather conditions. Secondly, increased WBC and subsets is a moderately good marker of inflammation – infection in horses, with significant correlations with acute phase proteins [36]. There is seasonal variation in the prevalence of different

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infectious diseases, mainly those affecting the respiratory tract, both in humans [37] and horses [38]. In horses, there are two peaks of incidence: during the training-competing season (spring-summer-early autumn) and during winter [38]. Thirdly, the reproductive season starts in late January or February, with more activity and cortisol release [28]. Previous investigations revealed the existence of rhythmic variations in the number of WBC in peripheral blood chronobiologically subjected to the release of endogenous corticosteroids [39]. A seasonal activity was described in circulating levels of cortisol, with a direct impact on the white blood cell count [40]. In Arabian, Thoroughbred and Standardberd mares, an increased WBC, mainly NTP in association with concentrations of cortisol have been found during the winter season [28,40]. This was caused by the action of low temperatures and atmospheric pressure. However, increased cortisol leads to increased WBC, NTP, but to a reduction in LYMP. However in our study, we found an increase in LYMP count. Therefore, in our opinion, cold temperature with humidity and the influence of possible subclinical infections could have been the main determinants of the higher WBC and subsets from February to May in our research.

The peak in EOS in the present study from January to May was an expected finding. Firstly, the mares spent more time in the pasture during this part of the year, and secondly, during spring, the parasite level in pasture is expected to rise [41].

In our opinion, the results of the present study associated with the month of the year would not have occured exclusively from the influence of environmental conditions. One should not ignore the influence of the hormones involved in pregnancy, such as estrogens and progesterone. From the literature, it appears that the role of these hormones in hematology is controversial. High doses of estradiol have been associated with increased WBC and NTP because hyperplsia of the myeloid line of the bone marrow [42]. Other studies described that estrogens exert a suppresive effect of hematopoiesis, with neutropenia, lymphopenia and eosinopenia [43]. It would be interesting in the future to analyze the relationship and the interactions between the hematological profile and the reproductive hormones in Carthusian broodmares in different periods of their reproductive season.

On the basis of the present investigation, we conclude that our results confirm that hematological indices in pregnant Carthusian broodmares suffer seasonal cyclical changes. Even though these changes reach statistical significance, they did not have clinical significance, as the values obtained were within the reference range for this breed [9,17]. It would be interesting to analyze whether these seasonal variations also exist in diseased animals and how they could influence the diagnosis of different diseases.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions

Authors' contributions	KKS	MAJ	GJ
Research concept and design	✓	--	--
Collection and/or assembly of data	✓	--	--
Data analysis and interpretation	✓	✓	--
Writing the article	--	--	--
Critical revision of the article	--	--	✓
Final approval of article	✓	✓	✓
Statistical analysis	--	✓	--

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