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HEMATOLOGICAL STUDIES IN HEALTHY ONE-YEAR-OLD PUREBRED ARABIAN HORSES

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INTRODUCTION

When an outbreak of *anaemia infectiosa equorum* (swamp fever) occurred among purebred Arabian horses at the Sultansuyu Government Breeding Station in Turkey, a research committee investigated the disease. As a member of this committee, the author was responsible for hematological studies of the affected animals. At the same time the hematological studies were made in ten normal one-year-old purebred Arabians, of which the results are being reported in this paper.

It is the custom of the author - when studying the hematology of any disease - to obtain data from normal animals of comparable ages and breeding which are kept under compareble conditions in the same area in order to have data for comparison with the findings in the diseased animals. Jennings (11) stated that the results of his work on the peripheral blood picture in some diseases of animals indicated that a series of examinations of diseased animals together with representative samples from the herd would be necessary before clear-cut opinions could be expressed.

Since data on normal blood pictures of yearling purebred Arabian

horses are not available, records of our findings are being reported in this paper.

MATERIALS AND METHODS

All the ten apparently healthy one-year-old Arabians used in this study were kept under the same conditions of care and feed, and were under the permanent control of veterinarians. None of them had had any disease in the past according to the records of Breeding Station.

Blood samples were collected between 9.00 and 10.00 o'clock a.m. in the stable of the animals, care being taken not to excite them. The top of the ear of each animal to be studied was cut just enough to allow blood to ooze out drop by drop. The diluting pipettes for erythrocte and leucocyte counts were filled directly from this capillary blood. It was mixed immediately with diluting fluids. Hayem's solution was usd as the diluting fluid for erythrocyte counts and Türk's solution for leucocyte counts. The amount of hemoglobin was determined by use of Sahli's hemometer. The blood for this purpose was drawn directly into a hemoglobin pipette from blood as it dropped from the ear. Differential leucocyte counts were made from Giemsa-stained films of fresh blood on glass slides. Differential counts were based upon a count of 200 cells from each slide.

Blood samples for the determination of erythrocyte sedimentation rates were obtained by drawing 1.6 cm. blood from the jugular vein directly into a 2 cm. syringe containing 0.4 cm. 3.8 per cent sodium citrate solution. Erythrocyte sedimentation rates were determined by the method of Westergren, using pipettes with 3 mm. inside diameters. The tests were performed at room temperature.

Although it is customary to read the sedimentation rate after one hour and two hours, and express it as SR/hours, we recorded the sedimentation rate at the 5, 10, 15, 30, 60, 120 minutes intervals and at the end of 24 hours. Katz' formula, modified by Van Zijl (15)

$$SR/10\ min = \frac{\frac{a}{3} + \frac{b}{6}}{2}$$

was applied to calculate the mean sedimentation rate per 10 minutes. Here (a) represents the reading after 30 minutes, and (b) after 60 minutes.

The volume of packed erythrocytes was determined by reading the volume of packed cells in the sedimentation pipettes at the end

of 24 hours and correcting the results for the undiluted blood. As it is known, the blood sample in the Westergreen sedimentatoin tubes contains 80 per cent blood and 20 per cent citrate solution. Horse blood settles completely and reaches its constant value in 24 hours. We do not use hematocrit tubes for this purpose, because most of the time we investigate the diseases in the villages and it is not always possible to use a centrifuge because of the lack of electric power. Therefore, we had to develope a simple method for the determination of packed cell volume and use it in all our hemotological studies.

Statistical computations were made to obtain arithmetic averages, standard deviations, and the standard errors.

RESULTS

The results are given in TABLE 1 and 2. A comparison of mean values with their standard errors suggests that the mean values are reliable. The closeness of ranges to the mean values shows that atmost care was taken to avoid technical errors in individual tests. The different phases of erythrocyte sedimentation in the purebred Arabians are shown in FIG. 1. The first phase (autoaglutination) is the part of the curve between o and 15 minutes. The second phase (increased sinking velocity) is represented by the part of the curve between 15 and 60 minutes, and the third phase (red cell packing) between 60 minutes and 24 hours.

FIGURE 2 was designed to show how the distribution of sedimentation rate in different normal subjects were spread out in the phase of maximal sinking velocity, that is after 30 and 60 minutes, and why we should read the sedimentation in horses after 10 minutes fall or calculate mean SR/10 minutes.

This is the first report in Turkey in which the sedimentation rate was expressed as «mean SR/10 minutes» by application of modified Katz formula. Therefore, it was thought that the SR data, not applied to this formula, would be usefull for the others, who are not accustomed to use Katz formula, and given in TABLE 3.

DISCUSSION

There are several government breeding stations in Turkey on which purebred Arabian and English horses are raised. There are no data on the blood pictures of these animals in Turkey. There are studies on the blood pictures of native horses and mixed breds in Turkey (2), but they should not be used in evaluating similar blood stu-

TABLE 1. THE RESULTS OF HEMATOLOGICAL EXAMINATIONS OF TEN NORMAL ONE-YEAR-OLD PUREBRED ARABIAN HORSES.

Measure	Erythrocytes 10^6/cmm.	Leucocytes 10^3/cmm.	Hb. Gm/100 m'.	Color index	Sedimentation, m m. Hours 0.5	1	24	SR/10 min.	Packed cell volume %
Range	9.20—11.80	9.52—13.77	11.00—12.50	0.90—1.20	23—38	41—77	124—142	7.26—12.40	36.2—47.5
Arithmetic mean and St. error	10.61 ± 0.232	11.67 ± 0.357	11.45 ± 0.157	1.00 ± 0.026	— —	— —	— —	10.23 ± 0.608	44.15 ± 1.020
St. deviation	0.736	1.126	0.497	0.083	—	—	—	1.921	3.228

TABLE 2. THE RESULTS OF DIFFERENTIAL COUNTS OF TEN NORMAL ONE-YEAR-OLD PUREBRED ARABIAN HORSES.

Measure	Neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %
Range	31—53	1.0—4.0	0.0—0.5	40—63	1—7
Arithmetic mean and st. error	42.80 ± 2.442	2.00 ± 0.332	0.10 ± 0.076	51.50 ± 2.763	3.60 ± 0.567
St. deviation	7.728	1.077	0.241	8.658	1.794

TABLE 3. SEDIMENTATION RATES IN ON-YEAR-OLD PUREBRED ARABIAN HORSES.

Numbre of animals	Sedimentation Rates, in mm.						
	5 Min.	10 Min.	15 Min.	30 Min.	60 Min.	120 Min.	24 Hours
5/51	1.5	5	8	34	75	125	132
6/51	1	3	6	28	54	89	128
24/51	1	3	5	23	57	103	129
62/51	1.5	4	8	38	73	114	129
38/51	0.5	1	4	23	41	73	128
29/51	1.5	6	9	36	77	136	142
14/51	1	4	8	34	75	113	124
4/51	1	3	6	30	74	113	125
18/51	0.5	1	4	23	53	101	125
52/51	0.5	1	5	25	62	105	131
Mean	1.0	3.1	6.3	29.4	64.1	107	129

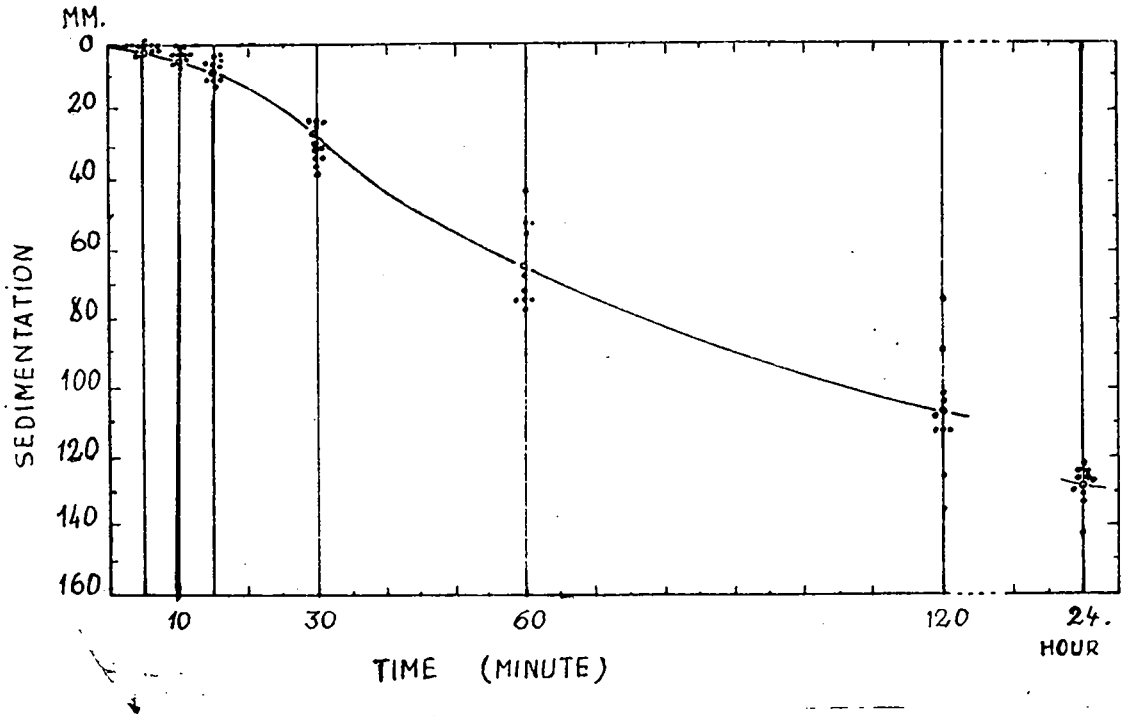

FİGURE 1. ERYTHROCYTES SEDİMENTATION CURVE OF NORMAL, ONE-YEAR-OLD PUREBRED ARABİANS (WESTERGREN).

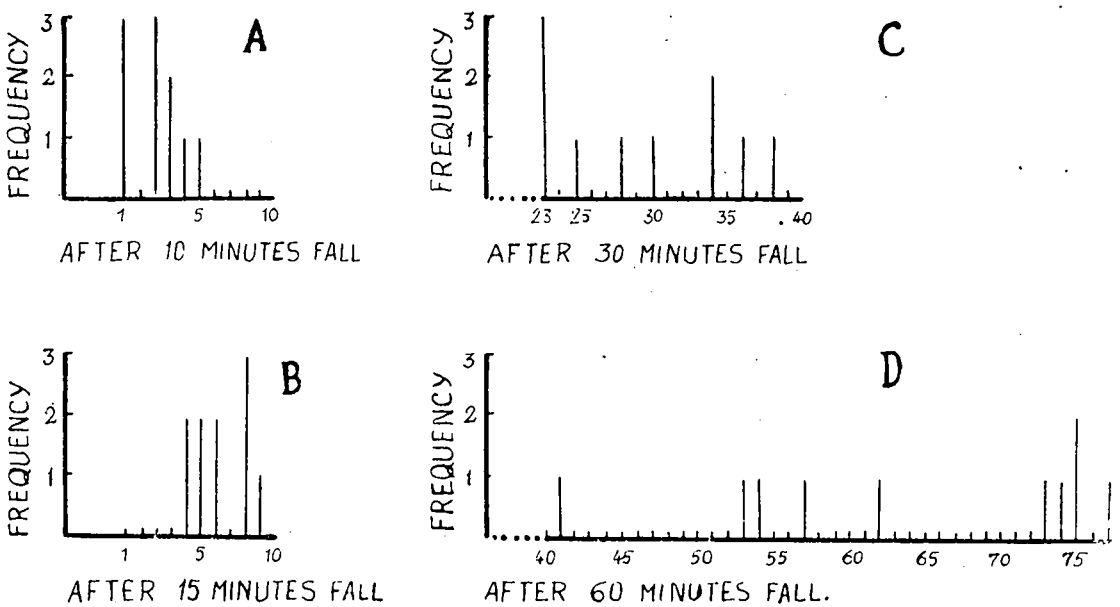

FİGURE 2. — GRAPHICAL REPRESENTATİONS OF DISTRİBUTİONS OF ERYTHROCYTE SEDİMENTATİON RATES İN NORMAL, ONE-YEAR-OLD, PUREBRED ARABİANS (ALL IN MİLLİMETERS).

dies in the purebred Arabians or Thoroughbreds. There may even b differences in blood pictures between the same breeds raised in di ferent countries. Brenon's (1) statement is a good example for th necessity of determining the normal in different localities. He stated «The variables in the hemoglobin percentages in other parts of th nation should also be kept in mind. This report is based on 15.6 Gm as being 100 per cent while Illinois used 16.0 Gm. as 100 per cent, and Wintrobe reports two figures of 14.5 and 15.0 Gm., respectively.»

There is no record in the literature on the hematology of one year-old purebred Arabians. However, there are hematological stu dies on the Thoroughbred yearlings and weanlings.

The average erythrocyte count in our cases is 10,600,000, which is some what less than the values reported in the literature for Ara bians and Thoroughbreds. The results of blood picture studies o purebred Arabians and Thoroughbreds may adequatly be compared Mac Leod *et al.*, (12) reported that there was no significant difference in the count of the Thoroughbred and that of the purebred Arabians Fundamentally all Thoroughbreds descend three Arabian stallions bred to native English mares (12).

Taking the age into consideration the blood values of weanlings and yearlings might be compared with that of one-year-old Arabians. TABLE 4 includes the mean values obtained by different authors and present author.

TABLE 4. COMPARISON OF MEAN VALUES OBTAINED BY DIFFERENT AUTHORS

Author	Typ of animals	Number of animals	Erythrocytes 10^6/cmm.	Leucocytes 10^3/cmm.	Hb. Gm./100 ml.
Mac Leod et al., (12)	Thoroughbred Weanlings	22	11.90	11.60	13.30
Mac Leod et al., (12)	Thoroughbred Yearlings	16	11.60	13.40	13.60
Hansen et al., (7)	Thoroughbred Weanlings	70	11.24	13.64	11.97
Mac Leod et al., (12)	Arabians (different age)	4	11.50	11.00	13.00
Present author	Arabians one-year-old	10	10.61	11.67	11.45

As is seen in TABLE 4 the present author fonud lower erythrocyte and leucocyte counts and less hemoglobin per 100 ml blood than other authors found in both Thoroughbred weanlings and yearlings, and Arabians. We assume that this difference may be due to the methods used. Mac Leod *et al.*, (12) used heparinized blood drown from jugular vein and Hansen, *et al.*, (7) oxalated blood also drown from jugular vein. We used, however, capillary blood from ear drown directly into the counting and hemoglobin pipettes, without using any anticoagulant.

If an exact comparison is to be made the same procedures and conditions must be followed implicitly. The time of securing blood samples, degree of exitation of animals and other small procedural differences can cause variation in results.

Höhnke (10) reported that the number of erythrocytes and the amount of hemoglobin did not show noticeble daily variation in horses. However, he found that the number of leucocytes were higher in the evening hours than the morning. He also reported that Kudrjasov investigated the blood picture of the horses during one year period and found that the number of erythrocytes and the amount of hemoglobin showed maximum value from May to july, and minimum from October to December. The number of leucocytes were found higher in Summer than in Winter. Trum (14) stated that a breed and age difference in blood cell counts was indicated as well as differences during lactation. Pregnancy seems to results in a near term anemia and as postpartum polycythemia (14). Excitation of animals causes high blood values (6, 8, 9, 13).

The mean values of the percentage distribution of leucocytes reported in this paper are very similar to values obtained by other authors (see TABLE 5).

The sedimentation rate of erythrocytes was also determined in ten normal, one-year-old, purebred Arabians. We did not find any report in the literature on sedimentation rates in purebred Arabians.

The sinking velocity of erythrocytes in horse is very high. Therefore. we applied Katz formula, modified by Van Zijl (15) and the sedimentation rate is expressed as millimeters per 10 minutes (SR./10 min.) This makes the result more easily analyzed. Gilman (3) used the same formula to evaluate his findings on the sedimentation rate of horse blood. He also reported that the sedimentation rate at the end of ten minutes appeared to be the only one showing data of possible statistical significance. Gilman (3) cited from Coffin that the sedimentation rate in the horse was very fast and recommended to read

TABLE 5. COMPARISON OF MEAN VALUES OF PERCENTAGE DISTRIBUTION OF LEUCOCYTES.

Author	Type of animals	Numbre of animals	Leucocytes %				
			Neutros.	Eos.	Basos.	Lymphos.	Monos.
Mac Leod et al., ([12])	Thoroughbred Weanlings	16	37.0	4.0	—	55.0	4.00
Mac Leod et al., ([12])	Thoroughbred yearlings	22	44.0	3.0	—	49.0	4.00
Hansen et al., ([7])	Thoroughbred Weanlings	70	45.0	2.3	<0.10	49.7	2.72
Present author	Arabians one - year - old	10	42.8	2.0	0.10	51.5	3.60

the fall at the end of ten and twenty minutes. Gsell (5) read the rate at the end of fifteen minutes, and Van Zijl (15) recommended to calculate mean SR/10 minutes from two readings, first at the end of 30 minutes and then at the end of 60 minutes. On the other hand, most of the veterinarians in this country read the fall either after 30 minutes or 60 minutes. However, FIG. 2 shows definitelly that one can not make a good interpretation with a reading of SR after 30 ar 60 minutes, because the distribution spread out considerably (see FIG. 2, C and D). The least spreading of distribution occured in «after ten minutes fall» (FIG. 2, A), then in the «mean SR/10 minutes» (FIG. 2, E). The data on after 15 minutes fall seems to have possible statistical significans, too. However, when this data is obtained from the horses, which are not purebreds and have normally less erythrocytes than our subjects, distribution of the data on 15 minutes fall would also be spread considerably. Because the less the number of erythrocyte in the blood, faster it reaches the maximum sedimentation velocity. We have observed this fact epecially in the cases of infectious anemia.

From ours and also from Gilman's findings it seems that in the first order «after 10 minutes fall», and in the second order «mean SR/10 minutes» would give the most possible statistically significant resutls. However, these results were from normal animals. It must also be tried in the blood samples from diseased animals, in which the sinking velocity of erythrocytes increased or decreased. After

ese experiments, it would be proper to deside on wheather «after minutes fall» or «mean SR/10 minutes» should be used to express e sedimentation rate in the horse. The results of sedimentation and cked cell volume are shown in TABLE 1.

There are several methods for the determination of packed volne of erythrocytes. Gösling (4) applied three different methods in his tensive study to determine the packed cell volume in different anial species. He used the most frequently used hematocrit method, einbach's modification of Bleibtreusch's method, and the polarizaon method. In Steinbach's method the nitrogen content of plasma d then that of the diluted serum are determined and from these cked cell volume is calculated. In the polarization method saccharse is added to the blood and the sinistrorotatory ability of the blood reduced. From the amount of added saccharose the amount of cell lume is calculated. This method was tried for the first time by Gösng and the results were satisfactory.

The most simple method of determining of cell volume is the ontaneous sedimentation method. Wirth (16) said: «although this ethod is not accurate, it gives useful results for comparison and sed extensively in veterinary medicine.»

However, one can use either spontaneous sedimentation or the ematocrit method for obtaining cell volume, because the two gives ery similar results. This fact was prooved experimentally by Gsell). It is further interesting that the standard deviation of the cell olume gained by spontaneous sedimentation is much smaller (0.40) han the one gained by hematocrit method (0.86) (Gsell). Most of the me, we make our hematological studies under the field conditions nd therefore use this method in all our blood cell volume determinaons.

SUMMARY

1) A hemotologic study of ten apparantly healthy one-year-old urebred Arabians revealed the following mean values with their standard errors: erythrocytes per cubic millimeter, 10.61 ± 0.232 million; eucocytes per cubic millimeter 11.67 ± 0.357 thousand; hemoglobin er 100 ml. of blood, 11.45 ± 0.157 grams.

2) The differential leucocyte counts showed following mean perentages with their standard errors: neutrophils, 42.80 ± 2.443; eosiophils, 2.0 ± 0.332; basophils 0.10 ± 0.076; lymphocytes, 51.50 ± 2.736, nd monocytes, 3.60 ± 0.567.

3) The erythrocyte sedimentation rate, applying Katz' formula a expressing as SR per 10 minutes, was 10.23 millimeter with a sta dard error ± 0.608, and standard deviation 1.921.

4) It was shown that expressing the SR as «after 10 minutes f in millimeters» (Gilman's recommendation) or «mean SR/10 minut in millimeters» (Van Zijl's recommendation) gave the best results f a good interpretation.

5) The packed volume of erythrocytes was determined in the We tergren sedimentation pipettes leaving the blood in the pipette unt the eryhrocytes settled completelly (24 hours) and correcting the resul for the undiluted blood. The mean percentage of packed volume erythrocytes with its standard error was 44.15 ± 1.020 per cent.

ÖZET

1) Görünüşte sıhhatli bir yaşında on baş safkan Arap atlarını hematolojik muayenelerinde, standard hataları ile birlikte, aşağıdal kıymetler bulunmuştur: Alyuvar 1 mmk.'te 10 610 000 ± 0.232; aky var 1 mmk.'te 11 670 ± 0.357; hemoglobin 100 sk. kanda 11.45 ± 0.1 gram.

2) Akyuvar formülü için standard hatalariyle birlikte aşağıdal kıymetler bulundu: *Neutrophil*'ler % 42.80 ± 2.443; *eosinophil*'l % 2.0 ± 0.332; *basophil*'ler % 0.10 ± 0.076; *lymphocyte*'ler % 51.50 2.736 ve *monocyte*'ler % 3.60 ± 0.567.

3) Alyuvar sedimentasyon hızı, modifiye Katz formülü ile hesap landı ve ortalama SH/10 dakikada 10.23 ± 0.608 milimetre bulundu Ortalamanın standard dağılma ölçüsü 1.921 idi.

4) Sedimentasyon hızının «10 dakika sonraki çöküş»ten okunma sı (Gilman'ın tavsiyesi) veya «ortalama SH/10 dakika» olarak hesap lanması (Van Zijl'in tavsiyesi) ile elde edilen kıymetlerin en inanılı oldukları gösterildi.

5) Alyuvar volümü, SH tayini için kullanılan kanın Westergre pipeti içinde 24 saat bekletilmesiyle tayin edildi. Netice % 100 kana yani antikoagülant ile karışmamış kana göre düzeltildi. Bu şekilde el de edilen ortalama spontan sediment kıymeti, standard hatasiyle bir likte. % 44.15 ± 1.020 bulundu.

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