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FACTORS INFLUENCING THE PACKED CELL VOLUME OF FROG BLOOD

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At least three measurements are necessary to determine the condition of the red blood cells in health and disease. These are the red cell count, the hemoglobin concentration, and the red (packed) cell volume.

Information concerning the packed cell volume (PCV) of frog blood is available in the literature (1, 2,), but only a numerical normal value is given, and nothing is said about the factors which may influence the stated figures. In order to use the packed cell volume as a valid criterion in the study of the blood, an evaluation of the factors which may significantly influence the measurements is essential. The present study analyzes the effect of factors usually encountered in the laboratory upon the experimentally determined value of the PCV in frog blood.

Materials and Methods

Male and female frogs of the species *Rana pipiens* were used in most cases. In some trials *Rana clamitans* was employed. No differences in PCV were observed between the species.

Blood was collected only from the heart because of the reported difference in some animals between cardiac and peripheral blood (3). Care was exercised to avoid admixture of the blood sample with tissue fluid; this fluid tended to be amassed, particularly in the female during the egg-laying period, and was often evidenced by adhesions between the pericardial sac and the body wall. The fluid may inordinately reduce values and should be guarded against.

The 10 mm. Wintrobe hematocrit tube was used. The anticoagulant, 3 percent sodium citrate, was first added to the 1 mm. graduation. As much blood as possible was collected. Whereas in the early experiments only enough blood was obtained to fill approximately one-half the height of the tube (an average of 4.5 mm. in the first 126 healthy frogs), proficiency in technic later permitted filling to an average of approximately two-thirds of the tube. The resulting error (discussed later in experimental data) is very small. The centrifuge was run at 2600 r.p.m. for at least thirty minutes. The PCV was determined in percent from the proportion: packed cell level/total level = packed cell volume/100.

The factors considered included sex, red leg disease, gravidity, environmental temperature, volume of blood used, duration of centrifugation, and anesthesia. The presence of real differences from the normal was tested by statistical analysis of the data.

Frogs were considered to have red leg disease when they showed gross signs and bodily symptoms of a hemorrhagic septicemia, and when the blood smear revealed the presence of

the causative organism (4). In addition to frogs with naturally contracted red leg, frogs which contracted the disease by bacterial injection were used. Cultures of *Pseudomonas hydrophila,* from the American Type Culture Collection, serial number 9071, were inoculated daily on fresh slants of Difco brain-heart agar and incubated at 37° C. for 24 hours. The slants were washed with 5 ml. of sterile physiological saline solution and a 1:1,000 dilution made, one ml. of which was injected. Blood smears treated with Wright's stain showed the presence of bacterial invasion of the red blood cells of diseased frogs.

In the usual procedure, after the frogs had been removed from storage in the refrigerator and kept at room temperature for 30 minutes, they were rendered unconscious by a sharp blow on the head, and the blood sample was then taken. To study the influence of temperature upon PCV, blood samples were collected from frogs immediately upon their removal from the refrigerator, where they had been kept at 6° C. To study the influence of anesthesia, blood samples were taken from frogs which had been anesthetized with ether.

Experimental Data

The possibility of a sex difference in PCV was checked by a comparison of 304 healthy males with 233 healthy females. A highly significant sex difference was apparent.

The packed cell volume was significantly altered from the normal value in frogs with red leg disease.

Table 1.—Factors Influencing the Packed Cell Volume of Frog Blood

Factor studied	Number of animals	Mean PCV (in percent)	Standard deviation of mean	t value
Sex differences in normal frogs	304 male 233 female	30.04 24.77	7.69 7.42	8.0
PCV for healthy non-gravid females vs. PCV for healthy gravid females	76 non-gravid 102 gravid	23.52 25.52	7.39 7.49	1.75
Influence of disease on PCV Normal vs. red leg (Mixture of sexes)	585 normal 185 diseased	27.87 25.35	7.74 9.56	3.64
PCV for naturally acquired red leg vs. PCV for injected red leg (Mixture of sexes)	128 natural 57 injected	25.98 23.95	9.73 8.29	1.37
Frogs at room temperature vs. frogs adapted to 6°C.	419 at room temp. 48 at 6°C.	27.08 29.18	7.77 4.90	1.84
PCV in normal males vs. PCV in anesthetized males	304 normal 100 etherized	30.04 30.40	7.69 8.46	0.39

A total of 585 normal frogs were compared with 185 diseased frogs. There was no difference between frogs which had contracted red leg disease naturally and those which had contracted the disease artificially by injection of *P. hydrophila.*

There was no real difference in PCV between gravid and nongravid females. No special precaution in this regard need be taken in collecting blood samples from females.

The temperature of the cold environment (about 6° C.) in which the frogs were stored did not appreciably influence the value of the PCV. Frogs kept one-half hour to several hours at room temperature showed no real differences in PCV.

Ether anesthesia in 100 etherized frogs gave values for the PCV which were not essentially different from those obtained in 304 unanesthetized frogs.

All the above data are summarized in table 1.

In addition to the above constitutional and environmental factors which are commonly encountered in laboratory practice, errors known in other animals to be due to experimental procedure were reexamined in the frog.

Figure 1 shows that PCV depends upon duration of centrifugation and upon the volume of the blood sample. With the speed held constant at 2600 r.p.m., there was no change in PCV after centrifuging for 20 minutes.

A larger volume of blood yielded greater PCV values throughout the range tested. At the end of 45 minutes, a single 0.857 cc. sample of blood gave a PCV of 37.51 percent; another sample of 0.325 cc., from the same frog, gave a PCV of 34.46 percent.

A more typical picture in an average healthy group of frogs was then sought. A series of trials in ten male frogs, in which the blood sample filled the Wintrobe tube to the 10 mm. mark, gave a mean PCV value of 35.9 percent, whereas in another ten frogs the experimental mean for a 4.5 mm. column of blood was 34.0 percent. In order to completely eliminate this source of error in an animal as small as the frog, the alternatives are the use of pooled bloods or substitution with a micromethod involving capillary blood. The latter technic was tried and found to be unreliable. For most of the data presented herein, the average sample height attained was approximately 7.5 mm.

Discussion

The packed cell volume appears to be a fairly stable factor which resists many internal bodily and external environmental influences.

Sex is a constitutional factor which exerts a most marked influence on the PCV of frog blood. The PCV of the males is about 6 percent higher than that of the females. This agency must be considered in presenting hematologic data for the PCV in frogs. A similar sex difference has been established by the senior author (5) for red blood cell counts and hemoglobin percentages in frogs.

The discovery of a sex difference in frog hematocrit data is novel and unexpected. Schnitter (6) claims that there are no hematocrit differences in human beings resulting directly from sex. We are now in-

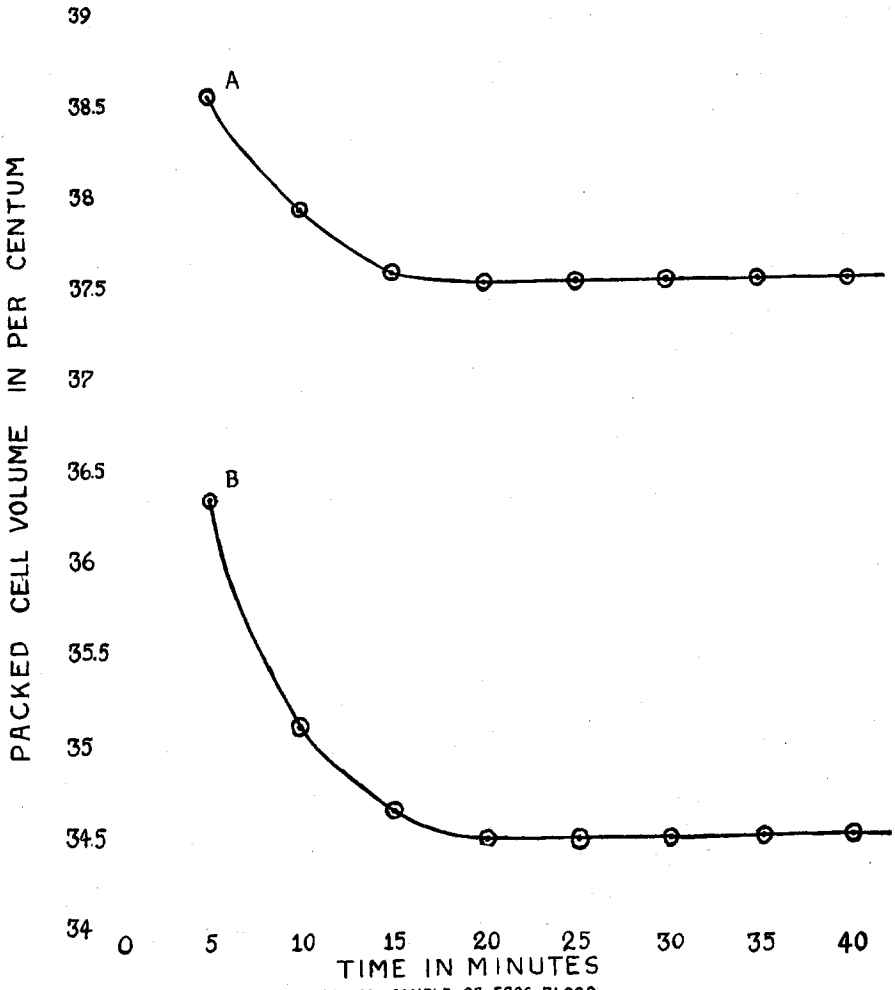

FIG. I - CURVE A IS A .857 CC. SAMPLE OF FROG BLOOD. CURVE B IS A .325 CC. SAMPLE OF FROG BLOOD FROM THE SAME SPECIMEN AS USED IN CURVE A.

vestigating this situation more fully by examining the packed cell volumes of several other vertebrates.

There is a significant difference between the PCV of healthy frogs and those with red leg disease. Efforts were made to use animals which were all in the same advanced stage of the disease, but there were probably variations in the severity of the infection. This could reduce the expression of maximal differences from the normal condition.

Etherization produces no immediate effect on the PCV.

In man, it is considered inadvisable to take blood samples for determination of the PCV for about 24 hours after etherization. Hausner et al. (7) state that in dogs, ether pro-

duces constriction of the spleen and increases the number of erythrocytes in the blood. We were not concerned in the frog about the delayed effects of ether on the blood. The blood does darken in the etherized frog, suggesting hemoconcentration. No conclusions concerning the immediate effects of anesthetics other than ether should be drawn from these data.

Summary

The manner in which the packed cell volume of frog blood may be significantly altered by various factors has been presented. Sex appeared to be the greatest factor in normal frogs. There was a significant difference between the PCV of diseased and normal frogs. Animals acquiring red leg disease in nature had essentially the same packed cell volumes as frogs inoculated with red leg organisms. Ether anesthesia in healthy animals caused no immediate changes in the PCV. Gravidity and environmental temperature did not affect the PCV to any real extent. Both the volume of the blood sample and the time of centrifugation had a marked effect upon the value of the PCV.

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