the interests of speed, a short period (20 minutes) of electrical destaining followed by washing of the gel in 5% acetic acid overnight gave satisfactory results.

*Summary.* Acrylamide gel electrophoresis in a vertical slab has been adapted to the routine analysis of the Gc protein. The band patterns do not differ in principle from those obtained by starch gel electrophoresis using a discontinuous buffer system; however, the Gc bands are very much sharper and the fast Gc components are well separated from the trailing edge of the albumin. The method has good reproducibility and may be capable of still further refinement. At present it provides a simple and convenient way of confirming the results obtained from immunoelectrophoresis and is particularly advantageous in investigation of rapidly migrating Gc variants which are only with considerable difficulty distinguished by starch gel electrophoresis.

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## Plasma Trapping in Hematocrit Determination. Differences Among Animal Species.* (30402)

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When a blood sample is centrifuged for the determination of hematocrit, there is some plasma trapped in the packed cell column, resulting in a higher hematocrit reading than the actual cell percentage. The plasma trapping correction factor (cell percentage/hematocrit reading) for a given blood sample depends upon the force and duration of centrifugation. When normal human blood or dog blood is centrifuged for 30 minutes at 1500 $\times$ *g*, the plasma trapping correction factor averages 0.96(1,2,3). In the course of investigating the influence of hematocrit on the viscosity of blood in several animal species (4), it was discovered that the plasma trapping correction factor shows a considerable species difference.

*Methods.* Blood samples were drawn from elephant (ear vein), man (antecubital vein), dog (femoral artery or vein), sheep (external jugular vein) and goat (external jugular vein) into heparinized containers. The plasma trapping correction factor ($F_t$) was determined on heparinized whole blood, and, for all species except the elephant, also on suspensions of cells in Ringer's solution. To prepare the cell suspensions, plasma was removed from heparinized blood after centrifugation, and the cells were washed 3 times with a modified Ringer's solution (NaCl 9 g/l, KCl 0.42 g/l, $CaCl_2$ 0.24 g/l, $NaHCO_3$ 0.2 g/l and heparin 10,000 units/l).

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TABLE I. Hematological Findings on 5 Animal Species.

| | Red cell, % | RBC, $10^6/mm^3$ | MCV, $\mu^3$ | Hb, g/dl blood | MCHC, g/dl cells | $\rho$ cells, g/ml | $\rho$ plasma, g/ml |
|---|---|---|---|---|---|---|---|
| Elephant | 34.8 | 3.11 | 111.9 | 12.4 | 35.7 | 1.102 | 1.024 |
| Man | 44.5 | 4.92 | 90.6 | 15.1 | 33.8 | 1.077 | 1.022 |
| Dog | 46.1 | 6.38 | 72.3 | 15.9 | 34.5 | 1.116 | 1.020 |
| Sheep | 41.1 | 11.01 | 37.4 | 14.2 | 34.7 | 1.096 | 1.020 |
| Goat | 28.5 | 16.04 | 17.6 | 10.3 | 36.7 | 1.104 | 1.020 |

Determinations were made in duplicate or triplicate with the use of $I^{131}$ labelled human serum albumin (Abott Laboratories, Oak Ridge, Tenn.). In a 4-ml Wintrobe hematocrit tube, exactly 3 ml of whole blood or cell suspension was mixed with 100 $\mu$l of albumin-$I^{131}$ solution containing approximately 1 $\mu$c albumin-$I^{131}$ and 1 mg albumin, and the mixture was used for the following measurements. Micro-hematocrit capillary tubes were filled with a small volume of the mixture and centrifuged at 15,000 $\times$ $g$ for 5 minutes in a micro-centrifuge (Clay-Adams, New York City) for determination of micro-hematocrit. The Wintrobe hematocrit tubes were then centrifuged at 1500 $\times$ $g$ for 30 minutes and the macro-hematocrit read. For the purpose of calculating the $F_t$, the top of the buffy coat was read in both the micro- and the macro-methods. Following the macro-hematocrit reading, 1 ml of plasma or supernatant Ringer's solution was pipetted into a test tube for the determination of $I^{131}$-activity ($C_p$) in a gamma-ray spectrometer (Baird-Atomic, Inc., Cambridge, Mass.). For each experiment, the $I^{131}$-activity was also determined for standards ($C_s$) prepared by mixing 100 $\mu$l of the original albumin-$I^{131}$ solution with 2 ml of a detergent solution (0.5% Alconox). From these results, it is possible to calculate, for the sample of whole blood or cell suspensions, in sequence, the volume of supernatant solution contained ($V_p$), the true cell percentage (H) and the plasma trapping correction factor ($F_t$):

$$V_p = 2.1\ C_s/C_p \text{ ml},$$
$$H = 100(3.1 - V_p)/3.1\%,$$
and $$F_t = H/Hct$$

where Hct is the hematocrit reading obtained with the macro- or micro-method. The macro-$F_t$ and micro-$F_t$ were separately calculated from the respective Hct readings. It is to be noted that a sample with complete cell packing and no plasma trapping would have a $F_t$ of 1, whereas decreases in cell packing and increases in plasma trapping would result in $F_t$ values progressively lower than 1.

Other hematological correlates of the blood samples were measured with the following methods. The number of red cells per unit volume of blood (RBC counts) was determined in an electronic particle counter (Coulter Electronics, Hialeah, Fla.) and the mean corpuscular volume (MCV) calculated as the ratio of red cell percentage (excluding the buffy coat) to RBC counts. The hemoglobin concentration (Hb) was determined with the cyanomethemoglobin method(5) and the mean corpuscular hemoglobin concentration (MCHC) calculated as the ratio of Hb to red cell percentage. The density of blood ($\rho_b$) and the density of plasma ($\rho_p$) were determined at room temperature (22-25°C) by weighing a known volume of the sample, and the density of cells ($\rho_c$) was calculated from the following formula:

$$\rho_c = [100\rho_b - (100 - H)\rho_p]/H.$$

*Results.* The hematological data on the blood obtained from the 5 animal species are listed in Table I. The most striking species difference is found in the MCV which ranges from 18 (goat) to 112 (elephant) $\mu^3$. As a result, although the cell percentage is not much different between goat blood and elephant blood, goat blood contains more than 5 times as many red blood cells per unit volume as elephant blood.

The results on $F_t$ for whole blood are listed in Table II. With the macro-method (30 minutes of centrifugation at 1,500 $\times$ $g$), the mean $F_t$ values for elephant blood, human blood and dog blood are all between 0.96 and 0.94, *i.e.*, 4 to 6% of the packed cell column is occupied by plasma, whereas the mean $F_t$ values for sheep blood and goat blood are significantly lower. Goat blood has

TABLE II. Plasma Trapping Correction Factor in Whole Blood.

| | Macro-method Mean ± S.D. | (n) | Micro-method Mean ± S.D. | (n) |
|---|---|---|---|---|
| Elephant | .94 ± .017 | (4) | .96 | (2) |
| Man | .96 ± .031 | (6) | .99 ± .026 | (6) |
| Dog | .95 ± .023 | (5) | .97 ± .020 | (5) |
| Sheep | .91 ± .034 | (5) | .96 ± .031 | (5) |
| Goat | .82 ± .016 | (9) | .91 ± .022 | (7) |

the lowest $F_t$ of 0.82, indicating that the packed cell column contains the largest volume % of plasma (averaging 18% of the packed cell column).

With the micro-method (5 minutes of centrifugation at 15,000 × *g*), the whole blood obtained from each species shows a $F_t$ higher than that determined with the macro-method. The elevation of $F_t$ toward unity is especially pronounced in sheep blood and goat blood. Therefore with the micro-method, $F_t$ of sheep blood becomes the same as that of elephant blood; and $F_t$ of the goat blood, though still being the lowest, also approaches the values found for the blood of the other 4 species.

Table III shows the $F_t$ values for Ringer suspensions of cells (man, dog, sheep, and goat) determined with the macro- and the micro-methods. The general trend of species difference found in whole blood is also found in the Ringer suspensions. For each species, $F_t$ of Ringer suspension determined with the micro-method is higher than the corresponding value determined with the macro-method. For goat, macro-$F_t$ of Ringer suspension is significantly higher (Student t test $p<0.05$) than that of whole blood.

*Discussion.* The plasma trapping correction factor shows species differences, being high in man, dog and elephant, lower in sheep, and lowest in goat. The hematological data on these species exhibit several differences (Table I), one of which is the cell percentage of the blood. As pointed out by Chaplin and Mollison(2), the plasma trapping factor of a sample depends on the cell percentage (the height of cell column in the hematocrit tube). This dependence, however, is not great and its direction would not account for the species differences in $F_t$ observed in the present experiments. For example, goat blood has the lowest cell percentage (Table I), and this factor alone would tend to give the highest $F_t$, but the actual $F_t$ determined for goat blood is the lowest among the 5 species. The species differences in $F_t$ seem to be related to the MCV (Fig. 1), which shows the descending order:

TABLE III. Plasma Trapping Correction Factor in Ringer Suspensions of Cells.

| | Macro-method Mean ± S.D. | (n) | Micro-method Mean ± S.D. | (n) |
|---|---|---|---|---|
| Man | .97 | (2) | .99 | (2) |
| Dog | .95 ± .013 | (4) | .98 ± .024 | (4) |
| Sheep | .93 ± .021 | (4) | .96 ± .029 | (4) |
| Goat | .88 ± .015 | (5) | .93 ± .022 | (5) |

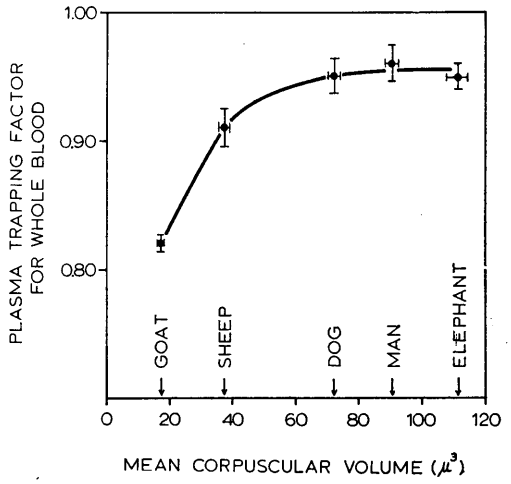


FIG. 1. Relationship between plasma trapping correction factor (macro-method) for whole blood and mean corpuscular volume in 5 species. Bars extending from each point represent standard errors of the mean.

elephant, man, dog, sheep, and goat. The goat blood has the smallest MCV and the highest $F_t$.

The Ringer suspension of goat cells shows a higher $F_t$ than goat blood, indicating that the absence of plasma proteins favors the packing of cells during centrifugation. Several mechanisms could conceivably result in such increase in $F_t$ by the removal of plasma proteins. First, in whole blood, cells form aggregates as a result of their interactions with plasma proteins and some plasma constituents can be entrapped in the aggregates. Therefore removal of proteins would reduce such trapping of fluid within the aggregates

and increase the $F_t$. Second, the removal of proteins from the suspending medium lowers the density of the medium, increasing the density gradient between the cells and the medium and thus favoring cell packing. Although this change in density gradient could play a part in bringing about the difference in plasma trapping between Ringer suspension of cells and whole blood in a given species (goat), it is not the factor causing the difference in plasma trapping of whole blood among the species studied. As shown in Table I, the densities of cells and plasma do not exhibit striking species differences, and the small differences observed cannot be correlated with variations in $F_t$. The amount of albumin in albumin-$I^{131}$ solution added to the Ringer suspensions was sufficient to avoid adsorption of albumin-$I^{131}$ onto glassware(6). If a significant degree of adsorption had occurred, the Ringer suspensions would have had lower, instead of higher, $F_t$ values than the corresponding whole blood.

For a given species, $F_t$ is greater with the micro-method than with the macro-method. Since a shorter duration of centrifugation was used in the micro-method, the lesser plasma trapping or better cell packing is undoubtedly due to the greater force of centrifugation employed.

*Summary.* With the use of albumin-$I^{131}$, the volume of plasma trapped in the packed cell column of hematocrit tubes was determined for blood samples obtained from 5 animal species. The volume of plasma trapped was least in blood samples obtained from elephant, man and dog (MCV 112 to 72 $\mu^3$), larger in sheep blood (MCV 37 $\mu^3$) and largest in goat blood (MCV 18 $\mu^3$). Increasing the force of centrifugation or removal of plasma proteins reduced the volume of fluid trapped in the packed cell column, and the reduction was most pronounced for goat blood.

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## Antigenic Relationship Between Strains of Echovirus Types 6 and 30.* (30403)

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Minor antigenic relationship between the prototype strain and one prime variant of echovirus type 6 and 2 strains of echovirus type 30 has been reported from this laboratory(1). This relationship was discussed at a recent meeting of the Panel for Picornaviruses; further study of the above problem was suggested.† This communication reports

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