in small intestine, 2% each in spleen and lung, and 1% in kidney. When the liver has been depleted to zero xanthine oxidase activity by a purified low protein diet, the entire rat has lost about 75% of its original enzyme activity (assuming the skin is relatively unchanged).

Summary. The average xanthine oxidase activities in $Cmm O_2$/g dry weight/hour for adult rat tissues were: Liver 1862, small intestine 628, spleen 534, lung 479, kidney 135, stomach 42, skin 100-200, brain 0, muscle 0, testes 0. Two-thirds of the total activity in the entire rat was in the liver.

Methylene blue added to the Warburg flask in the aerobic determination increased the xanthine oxidase activity in liver, kidney, stomach and small intestine, but not in lung or spleen. Added xanthine oxidase was recovered quantitatively in the presence of lung, spleen and kidney; greater than added amounts of activity were recovered from liver and decreased amounts from small intestine.

As the liver xanthine oxidase was depleted by a purified low protein diet, the small intestine lost about 2/3 of its activity, and lung lost about ½. Losses from the spleen and kidney were small. The entire rat lost ¾ or more of its xanthine oxidase activity.

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17126. Relationship Between Protein Osmotic Pressure and Density in Plasma from Cats, Dogs, and Humans.

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During the course of experiments on the effective osmotic pressure of the plasma proteins in the capillary circulation[1] we have had occasion to compare protein osmotic pressure with density in a large number of samples of plasma from cats, dogs, and humans. We were surprised to find that for any given density the protein osmotic pressure of human plasma is higher than that of plasma from cats or dogs. The data given below may prove useful to other investigators who wish to use plasma density as a measure of the osmotic activity of the plasma proteins in these species.

Methods. Blood was drawn from the carotid arteries of cats and dogs anesthetized with Nembutal. Human blood was obtained by venipuncture. Heparin was used to prevent coagulation. Low protein concentrations were obtained by diluting the plasma with Ringer's solution (0.9% NaCl, 0.042% KCl, 0.024% $CaCl_2$, 0.020% $NaHCO_3$) of density 1.0068 relative to water at the same temperature. High protein concentrations were obtained by evaporating the plasma in cellophane bags placed in front of a fan. The bags were then sealed close to the liquid level and the concentrated plasma dialyzed against cold Ringer's solution until ionic equilibrium was established as indicated by the electrical conductivity.

Densities were determined by the Falling Drop Method of Barbour and Hamilton.[2] The standard deviation from the means of 68 duplicate determinations performed with this method was ± 0.00013 g/cc. Protein osmotic pressure measurements were made across collodion membranes using a Hepp osmometer.[3] All the results were corrected to 37°C by multiplying the observed values by the factor $273 + 37 \div 373 + t$, in which t = room

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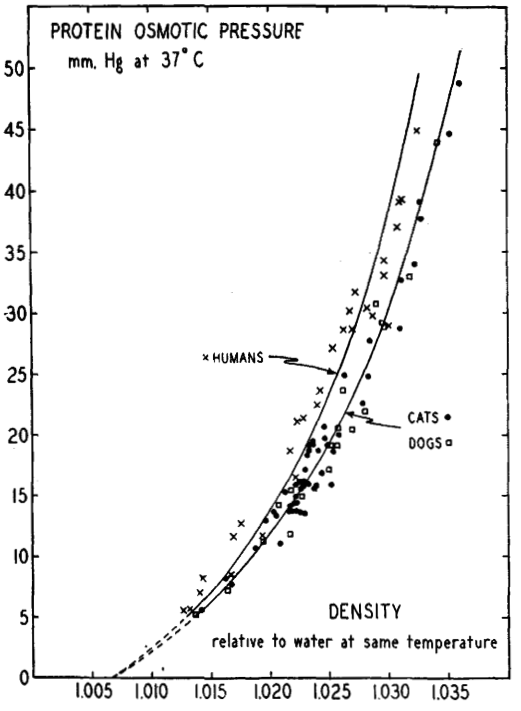

FIG. 1.

temperature °C. The standard deviation from the means of 68 duplicate measurements by this method was ± 0.4 mm Hg. The pH of the plasma samples ranged from 7.2 - 7.5 (glass electrode). For purposes of calculation, however, a value of 7.4 was assumed since a variation of ± 0.3 pH unit produces a change of protein osmotic pressure too small to be detected with our apparatus (cf. also Equation 1)

Results. Fig. 1 shows the relations between density and protein osmotic pressure in the three species. It is seen that at any given density human plasma has a higher protein osmotic pressure than that of cat or dog plasma. There is no significant difference in this respect between cat plasma and dog plasma.

Discussion. The following analysis of the data leads to useful formulae relating protein osmotic pressure to density and provides a basis for interpretation of the results.

As shown by Scatchard *et al.* [4,5] protein osmotic pressure is related to protein concentration by an equation of the form:

$$\pi = \frac{\pi_o\ (1 - .649)\ C}{1 - (.004 + .009\text{pH})C} \qquad (1)$$

where π = protein osmotic pressure mm Hg, π_o = protein osmotic pressure per gram protein at infinite dilution, g = ratio of globulin to total protein and C = total protein concentration in g/100 cc. For human plasma at pH 7.4 the constants are such that

$$\pi = \frac{1.97C}{1 - 0.071C} \qquad \text{.....(2)}$$

in which C is assumed to be 6.25 times the protein nitrogen. Protein concentration is related to density by an equation of the form:

$$C = K\ (\rho - \rho_o) \qquad \text{..... (3)}$$

in which ρ_o is the density at zero protein concentration. In the present experiments in which plasma was diluted with Ringer's solution ρ_o = 1.0068

Substituting Equation 3 into Equation 2 we have

$$\pi = \frac{1.97\ K\ (\rho - 1.0068)}{1 - 0.071K\ (\rho - 1.0068)} \qquad \text{....(4)}$$

The curve conforming to Equation 4 which best fits our experimental data for human plasma (method of least squares) is drawn in Fig. 1. It leads to a value for K = 353 which may be compared with values ranging from 343-360 found by previous investigators who compared protein concentration (actually protein nitrogen) with density.[6-9] For the practical purpose of predicting protein osmo-

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tic pressure from density in human plasma Equation 4 becomes

$$\pi = \frac{695\ (\rho - 1.0068)}{1 - 25\ (\rho - 1.0068)} \pm 2.4 \text{ mm Hg} \quad (5)$$

An examination of the partial specific volumes of the purified protein components of plasma by Oncley, Scatchard and Brown[10] has shown that the average specific volume of the proteins is not less than 0.74 cc/g. Since K is related to specific volume ($V\sigma$) by the relation

$$K = \frac{1}{1 - \rho_0 V\sigma} \quad \text{.....(6)}$$

we may predict that the minimum value of K for whole plasma is 389 and not 350 ± 10, as found in this and previous investigations cited above. An explanation of this discrepancy may be found in the recent studies of Armstrong, Budka, Morrison and Hasson[11] who report that protein in plasma is associated with (nitrogen-free) lipids so that the factor relating protein concentration to protein nitrogen in human plasma is closer to 6.8 than 6.25. Correction of K by the factor 6.8/6.25 yields a value of $K = 6.8/6.25 \times 353 = 385$ which is in close agreement with the value 389 predicted from the partial specific volumes of the individual components.

A similar analysis for dog and cat plasma cannot at present be made because the constants essential to Equation 2 have not been evaluated and because the effects of lipids on the factor relating protein nitrogen to protein concentration has not been determined for these species. However, for the practical purpose of calculating the osmotic pressure from density it is reasonable to suppose that the equation is of the same form as Equation 5. The curve defined by an equation of this form which best fits our experimental data for cat and dog plasma is drawn in Fig. 1. For these species

$$\pi = \frac{625\ (\rho - 1.0068)}{1 - 22.5\ (\rho - 1.0068)} \pm 1.7 \text{ mm Hg} \quad \text{....(7)}$$

The observed differences between human plasma and cat and dog plasma shown in Fig. 1 and in Equations 5 and 7 are in the direction to be expected if cat and dog plasma contain more lipid associated with plasma protein than does human plasma. Until further data are available, however, it is not possible to state that this is the sole factor involved.

Summary. 1. Data are given showing the relationship between protein osmotic pressure and density in plasma from normal humans, cats, and dogs. Formulae are derived which allow the prediction of protein osmotic pressure from density in each of the three species (Equations 5 and 7). 2. For any given density human plasma has a higher protein osmotic pressure than does plasma from cats or dogs. Possible reasons for this species difference are discussed.

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