after the intra-articular administration of this steroid in all samples analyzed. The mobility of betamethasone acetate standard is shown in the strip to the extreme right. No betamethasone alcohol is visible in the chromatograms. This either suggests no appreciable conversion of betamethasone acetate to the free alcohol in synovial fluid or very rapid elimination of any free alcohol formed. The additional spots visible in both figures could not be identified, but are probably due to blood contamination of synovial fluid.

To evaluate the relative amounts of steroid present in the samples, experiments were conducted to determine the minimum sensitivity of the analytical method. The described procedure clearly distinguishes 20 mcg. of betamethasone acetate and 50 mcg. of betamethasone phosphate (converted to the free alcohol) from background. A direct relationship between the intensity of the corresponding chromatographic spot and the concentration of the steroid (20–300 mcg.) was also observed. In another experiment, the effect of the steroid vehicle on analysis of betamethasone phosphate was tested. The vehicle was injected intra-articularly, and the total content of synovial fluid was withdrawn after 2 hr. Betamethasone phosphate (500 mcg.) was added to this as well as to a control sample of synovial fluid. Both samples were then treated as described for the betamethasone phosphate determination. The intensity of the chromatographic spots so obtained appeared similar, indicating that this amount of the vehicle did not interfere with the analysis of betamethasone phosphate.

The *in vivo* results thus demonstrate that the concentration of both betamethasone acetate and phosphate in synovial fluid is very high 5 min. after intra-articular administration. Betamethasone phosphate, however, very rapidly disappears, evidenced by the small amount of this drug present after 2 hr. This is particularly clear if one considers that the 5-min. sample represents the drug content of 0.5 ml. of synovial fluid while the 2- and 24-hr. samples represent the drug content of the total synovial fluid available from the joint (approximately 5 ml.).

Betamethasone acetate persists in the knee joint much longer than the phosphate ester as demonstrated by the appreciable levels of the steroid acetate in the joint even after 24 hr. This observation is consistent with the *in vitro* data and can probably be similarly rationalized on the basis of the slow dissolution rate of the drug.

The results obtained in this investigation could conceivably explain the clinical data (4) obtained with a formulation containing betamethasone acetate and phosphate (formulation *B*). The onset of activity with this formulation was found to be rapid and to have a prolonged duration (5).

The rapid onset of anti-inflammatory action is most logically explained as an effect of the readily available betamethasone phosphate. The persistance of betamethasone acetate in the joint is probably responsible for the prolonged activity of the formulation. This is consistent with the suggestion of Will and Murdoch (6) that longer persistence of a steroid in a joint cavity may account, at least in part, for its prolonged duration.

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Relationship Between Hematocrit and Solubility of ^{133}Xe in Blood

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The relationship between the hematocrit and the solubility of ^{133}Xe in blood at 37° was measured in 64 samples, and an excellent linear correlation was found. The partition coefficients for erythrocytes/water and plasma/water were 2.14 and 1.02, respectively.

MEASUREMENTS of regional blood flow from the wash-out curves of the radioactive inert gas, ^{133}Xe, from a tissue has become a valuable method in clinical studies (1, 2). To convert the clearance values of the gas to quantitative values for blood flow, the relative xenon solubility in tissue and blood must be known. The present work concerns the relationship between hematocrit and solubility of ^{133}Xe in blood.

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EXPERIMENTAL

Materials.—^{133}Xe was obtained from The Radiochemical Centre, Amersham, Buckinghamshire, England. According to the specification, the maximal impurities of the gas with other radioactive isotopes, mainly ^{133}Xe m, were below 2%; only traces of ^{131}Xe m and ^{85}Kr were present. These data were confirmed by spectral analysis, where activity above the mentioned levels from elements other than the 0.081 Mev. γ emission of ^{133}Xe was not found. Nor by decay studies was contaminating radioactivity recognized.

Heparinized or citrated blood samples were obtained from voluntary donors.

The water used was redistilled.

Manipulation.—Blood samples with hematocrit values over a wide range were prepared by removal of plasma or erythrocytes from whole blood after centrifuging. The hematocrits of the samples were

obtained after centrifuging at 3000 r.p.m. in 30 min. and corrected for trapped plasma. These samples were placed in small test tubes together with a sample of water in an airtight chamber containing about 100 ml. of a mixture of atmospheric air and ^{133}Xe. Equilibration took place for 24 hr. at 37°. The samples were stirred constantly with a magnetic stirring rod. After equilibration, the samples were anaerobically transferred into syringes. From the syringe about 0.1 ml. of each sample was transferred into a 2-ml. rubber-capped vial and counted in a scintillation counter to a statistical error below 1%.

Weighing of the rubber-capped vials before and after introduction of the sample gave the weight of blood and water in each vial. For every blood sample the coefficient of radioactivity per Gm. of blood to radioactivity per Gm. of water from the same experiment was calculated.

RESULTS AND DISCUSSION

A total of 64 determinations of the partition coefficient blood/water were carried out. The hematocrit (hct) varied from 0 to 88%. The results are shown in Fig. 1. An excellent linear correlation between the partition coefficients (in Gm./Gm.) against the hematocrit was found. The slope of the correlation line was 0.0112 (standard deviation, 0.0004), and the intercept (for hct = 0) was 1.02 (standard deviation, 0.02). The standard deviation of the estimate was 0.08. These values indicate a partition coefficient plasma/water of 1.02 (in Gm./Gm.) and erythrocytes/water of 2.14 (in Gm./Gm.). From known values (3) of the specific weight of plasma and erythrocytes, the following formula for the partition coefficient of tissue/blood in Gm./ml. (λ) at changing hematocrits was calculated:

$$\lambda hct_x = \lambda hct_{50} \frac{1.69}{1.05 + 0.013\ hct_x}$$

where λhct_{50} is the partition coefficient of the tissue at hematocrit 50%, and λhct_x is the partition coefficient of the tissue at hematocrit x %.

The higher solubility of ^{133}Xe in plasma and blood rather than in water is explained easily by the high affinity of ^{133}Xe to lipids (4) and hemoglobin (5).

The solubility of ^{133}Xe in plasma and erythrocytes has been investigated *in vitro* by Conn (5). He found partition coefficients (in Gm./Gm.) of erythrocytes and plasma to water of 3.75 and 1.45, respectively. However, his measurements were carried out at a temperature of 21°, and his figures are therefore not directly comparable with ours. For whole blood (hemoglobin content 15 Gm./L.), Conn found a partition coefficient of blood/water *in vivo* at 2.10, a figure not confirmed by our findings. His measurements were carried out at 25 mm. Hg partial pressure of xenon. Recently, Isbister *et al.* (6) measured the solubility of ^{133}Xe in blood and plasma with a tracer dose of xenon. His results agree exactly with ours.

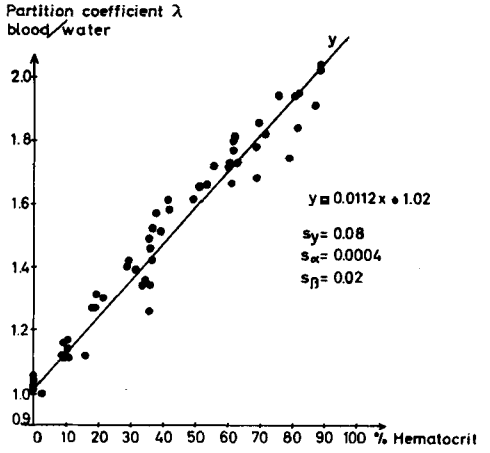

Fig. 1.—Relationship between hematocrit and solubility of ^{133}Xe in blood.

The results reported here show that estimations of the blood flow from clearance values of ^{133}Xe without correction of the partition coefficient of tissue to blood for the hematocrit may induce a random error of about 10%.

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