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Predictability of the Changes in Hematocrit which Follow Repeated Withdrawal of Blood.* (26903)

TAKANORI ARAMAKI AND HAROLD S. WEISS (Introduced by Paul Griminger)
Department of Poultry Science, Rutgers, The State University, New Brunswick, N. J.

A formula was derived for determining hematocrits (Hcts) of successive blood samples on the basis that sampling losses were being exactly replaced by extravascular fluid. When applied to experimental data, good agreement between measured and theoretical Hcts were encountered in several series of tests, as described herein.

Procedure. With the assumption that total blood volume remains constant through replacement of lost cells and plasma by extravascular fluid, it can be shown that the hematocrit of any particular blood sample (Hct_n) in a consecutive series of samples of constant size, is given by the exponential equation:

$$Hct_n = Hct_o \left(1 - \frac{s}{BV} \right)^{n-1}$$

Where:

Hct_o = hematocrit of the initial sample in a series
s = sample size (constant over the series)
BV = total blood volume
n = number of blood samples taken

Hematocrits so calculated were compared to Hcts measured during experiments which required repeated handling and multiple blood samplings in the chicken.

All studies were conducted on unanesthetized adult male chickens of the White Leghorn breed, 12-24 months of age. Hematocrits were measured on heparinized blood samples centrifuged in graduated tubes for 20 minutes at approximately 1500 × g. As the blood volume (BV) required by the formula had not been determined on the birds for which Hcts were available, and as literature values for BV of adult male chickens varies from 9-10%(1) to 5.6 ml/100 g body weight(2), it was necessary to measure this parameter in a group of comparable animals. The method used was basically the T-1824 dilution technic(3), except that the calculations were made on a single sample of wing vein blood taken 2 min. after injection of dye.

Results and discussion. Blood volume. Total BV of 8 chickens weighing 2491 ± 48 g (SE) was determined to be 6.6 ± 0.25 ml (SE) per 100 g body weight (BW), and

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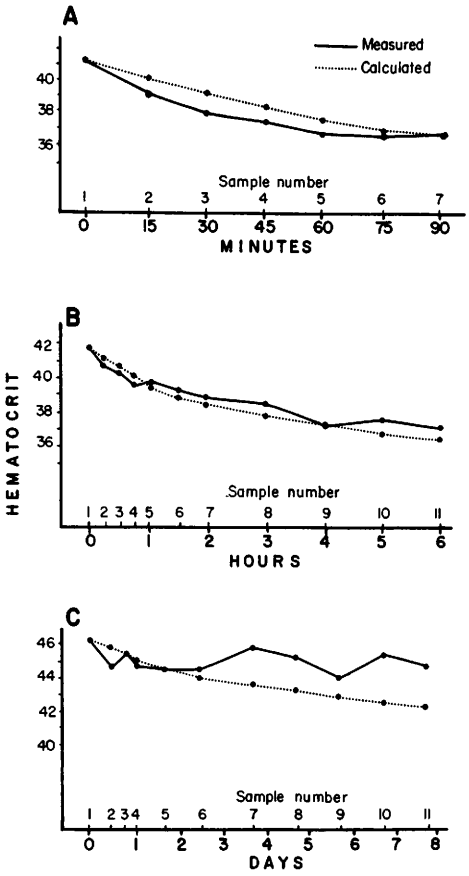

FIG. 1. Measured and calculated hematocrits following repetitive blood sampling in adult, male, White Leghorn chickens. Sample size: A—3½ ml, B—2½ ml, C—1½ ml.

this value was used to estimate the BV required in the formula. While this value for BV is close to the 5.6 ml/100 g BW found by Bond and Gilbert for White Leghorns, it should be noted that it is considerably lower than the 9-10% of BW suggested by Newell and Shaffner(1) for New Hampshire males of similar BW. Part of this discrepancy may well be due to breed differences, but it is also likely that the single 10-min. post-injection sample used by Newell and Shaffner (1) resulted in an overestimate of BV because of the rapid disappearance rate of T-1824 in the chicken(4).

Short term studies. Fig. 1-A shows the calculated and measured Hcts in a trial in which 3½ ml of blood was removed at 15-min. intervals within a 2-hr period. The 9 birds used averaged 2345 ± 88 g in BW and Hct_0 averaged 41.2 ± 1.2 (SE). At the end of the 7th sample the animals had lost approximately 16% of their BV. Measured and calculated Hcts decreased in a parallel manner in this series, although the measured values are generally lower. However, with an average difference at each sampling of 0.7 ± 0.7 Hct unit, the two sets are within experimental error, and the calculated Hcts estimated measured Hcts with an average discrepancy of approximately 2%. It is conceivable that had BV been measured separately on each of these birds, both the bias and the error could have been reduced.

In a second series (Fig. 1-B) 2½ ml of blood were taken first at 15 min., then at 30 min., and finally at 60 min. intervals over a 6-hour period. The BW of the 7 birds used averaged 2626 ± 104 g, and Hct_0 41.8 ± 2.4. By the 11th sample, some 16% of BV had been removed. The parallel trend of both measured and calculated Hcts toward lower values is clearly evident. No statistical significance attaches to the average difference of 0.5 ± 1.0 between the 2 sets at each sampling, and the average error in estimating the Hct here was approximately 1¼%.

Thus in trials where multiple, closely spaced blood samples are taken, and up to 16% of blood volume is removed, Hct can be predicted by the formula with an average error of 2% or less, even when using an estimated value for BV. Since the calculations are based on replacement of lost cells and plasma entirely by extravascular fluid, it appears that the chicken, like the dog(5), is capable of "rapid and delicate" readjustment and maintenance of BV. It follows further that the mechanism by which this is accomplished involves little or no addition of cells to the vascular compartment, a fact which is not in disagreement with the normal rate of replacement of RBC volume of less than 1% in 6 hours,(6), and the lack of function of the spleen as an erythrocyte reservoir in the chicken(7). Theoretically then, under these conditions significant differences between calculated and measured Hct could be interpreted to mean that the movement of extravascular fluid has not been able to main-

tain BV constant, the proportional changes in BV being estimated by the ratio: calculated Hct/measured Hct.

Long term study. By way of contrast with the previous 2- and 6-hour trials, Fig. 1-C depicts the Hcts observed in an 8-day series. The 10 birds here were on a feed deprivation test and had lost 21% of their initial 2326 ± 60 g BW before feed was restored on the 8th day. Sample size was 1½ ml, taken at 12, 18, and 24 hours, and daily thereafter. Initial Hct was 46.2 ± 1.0. Except for the period between 18 and 60 hours, there was little agreement between measured and calculated Hcts. At 12 hours, after a single 1½ ml sample, measured Hct was lower than expected, very suggestive of the hemodilution noted by Sturkie and Newman(3) under similar conditions. From the 3rd day on, measured Hcts were significantly larger than calculated values (pooled SE of differences = ± 0.8) and, in general, had returned close to the initial level. While this is highly suggestive of addition of cells to the vascular compartment, it must not be overlooked that inanition itself can raise Hct due to relative loss of plasma(8). In any event, the equation obviously cannot be used to predict Hcts under these conditions.

It should be noted that effects on Hct of such factors as size of tube, force of centrifugation, trapped plasma, and site of sampling should not alter the relationship between measured and calculated values as long as the test conditions remain uniform within any one series of measurements.

Summary. A formula has been derived for calculating the hematocrit of successive blood samples based on the assumption that blood volume is maintained constant through replacement of lost cells and plasma by extravascular fluid. The formula predicted, with an error of 2% or less, decreasing hematocrits which followed a removal of 7-11 successive blood samples of 2½-3½ ml each, within a period of 6 hours or less in the adult male chicken. The formula failed to predict the Hcts observed in an 8-day test on fasting chickens in which 1½ ml blood samples were obtained at 12-24-hour intervals. In the course of this study, the blood volume of the 2.5 kg White Leghorn male was determined to be 6.6 ± 0.25 ml/100 g body weight.

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Adaptation of Columbia SK Virus to Sarcoma 180 Ascites Tumor Cells *in vivo* and Passage in the Cells *in vitro.* (26904)

Eiichi Furusawa and Windsor Cutting
Department of Medical Microbiology, Stanford University, Calif.

Viruses growing in tissue culture provide a desirable means for testing the effects of antiviral compounds. Unfortunately, the Columbia SK virus has not in the past been successfully cultured serially in established cell cultures(1), although we have reported (2,3) that the virus could be adapted to Ehrlich ascites tumor cells *in vivo.* We now show that Columbia SK virus adapted to Ehrlich cells *in vivo* can also be adapted to Sarcoma 180 ascites tumor cells *in vivo* after serial passage in mixtures of the 2 cells, and that the new strain can propagate efficiently even in *in vitro* cultures of Sarcoma 180 as-