TABLE II. Unidirectional Calcium Fluxes Across the Isolated Shell Gland and Their Relation To Egg Location in Oviduct at Time of Sacrifice (Mean ± SEM).

Egg position	No. of observations	Calcium flux (μmole/cm^{2}hr) Influx	Efflux	Net influx	Flux ratio (Ca influx/ Ca efflux)
Not in shell gland*	5	.032 ± .007	.031 ± .005	.001 ± .004	1.0 ± .1
In shell gland	6	.051 ± .004	.021 ± .001	.030 ± .004	2.4 ± .2

* In 3 cases the oviduct did not contain an egg; in one case an egg was in the magnum and in another it was in the isthmus.

absence of concentration gradients and isolation from $CaCO_3$ deposition *in vitro*, the net Ca movement seems to parallel the time course of shell deposition *in vivo*. In the live animal, the rate of deposition is initially low, followed by an increase to a maximal and constant value toward the end of the plumping period (4).

The results indicate that only when an egg was in the shell gland at time of sacrifice was a net flux of Ca observed *in vitro*. Furthermore, net movement of Ca occurred in the absence of an eggshell and isolation from the physiological variables present in the live animal. The data suggest the existence of a physiological control mechanism which, at the time the egg enters the shell gland or sometime thereafter, initiates net movement of Ca in the mucosa.

Summary. The experiments indicate that calcium movement across the avian shell gland *in vitro* is in part dependent on metabolic energy derived from oxidative metabolism, requires the generation of phosphate-bond energy, and is predetermined by the physiological state of the shell gland at time of sacrifice.

1. Schraer, R., Schraer, H., Proc. Soc. Exp. Biol. & Med., 1965, v119, 937.

2. Hohman, W., Schraer, H., J. Cell Biol., 1966, v30, 317.

3. Misra, M. S., Kemeny, A., Acta Vet. Hung., 1964, v14, 443.

4. Sturkie, P. D., Avian Physiology, Comstock Publishing Associates, Cornell Univ. Press, Ithaca, 1965.

5. Welcher, F. J., The Analytical Uses of Ethylenediamine Tetraacetic Acid, D. Van Nostrand Co., Inc., Princeton, 1958.

6. Aldred, P., J. Exp. Biol., 1940, v17, 223.

7. Ussing, H. H., Zerahn, K., Acta Physiol. Scand., 1951, v110, 23.

8. Schultz, S. G., Zalusky, R., J. Gen. Physiol., 1964, v47, 567.

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Relationship Between Circulating Red Cell Volume and Endogenous Thyroxine in Sheep.* (32456)

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Relationships of the thyroidal system to the red cell-hemoglobin axis was recently discussed by Muldowney *et al*(1) who observed red cell mass to be reduced in hypothyroid human subjects. In newborn rats, Adam and Doljansky(2) noted the development of a progressive hyperchromic-microcytic anemia following thyroidectomy. Falkheden *et al*(3) reported red cell volume and total hemoglobin to be reduced after hypophysectomy in 14 human subjects affected by mammary carcinoma, acromegaly or diabetes. These data, however, suggested that thyroid deficiency alone did not explain the observed results. In a later report by Meineke and Crafts(4), an erythropoietic effect of thyroxine was demonstrated in rats that was indepen-

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dent from the effect upon increased oxygen consumption. Conversely, Goswami(5) found daily doses of 100 mg thyroxine to actually decrease red cell count in the buffalo.

This study was initiated to investigate by radiochemical procedures the relationships of circulating thyroid hormones and red cell volume in the intact sheep, as measured under physiological conditions.

Materials and methods. The 31 crossbred castrated male sheep used in this study ranged in age from 4 months to more than 6 years, and weights ranged from 19 to 72 kg.

During the preparation and test periods, all animals were maintained in a well-ventilated shelter and were fed a nutritionally balanced 12% protein corn, soybean-oil-meal, cottonseed-hull ration at a calculated maintenance level. At periodic intervals animals were placed in individual metabolism units (6) and administered intravenously a single tracer dose of I^{131} labeled thyroxine for routine balance and blood studies. Radiochemical procedures were employed to measure degradation rates of injected I^{131} labeled thyroxine (RT-4), as described for the mathematical model of Vohnout, Hansard and Morton(7), Employing plasma thyroxine disappearance rates, the data were fitted to a curvilinear equation by a method of nonlinear squares(8), and mean PBI values were used to convert data to thyroxine units. Calculations were made using an IBM 1620 computer programmed procedure(7).

Red cell volume (RCV) was determined by the Cr-51 labeled red cell dilution procedure, and plasma volume was calculated using the dilution of dosed radio-thyroxine within the blood compartment(7). Total blood volume was calculated from the summation of plasma and red cell volume.

Results and discussion. Mean values for measured parameters in the intact sheep are shown in Table I. Accuracy of the procedure for measuring thyroxine degradation rate (RT-4), using a mathematical model for describing the system(7), and the chloric acid digestion for PBI analysis has been previously discussed(9).

Multiple relationships using RCV as the dependent variable are shown in Table II.

TABLE I. Average Values for Red Cell Plasma and Total Blood Volume, Urinary Creatinine Extrathyroidal Thyroxine (ETT_4), and Thyroxine Degradation Rate (RT_4) in 20 Wethers.

Item	Total	Per kg body wt*
RCV, cc	796	18.2
Plasma	1808	41.4
Blood, total cc	2604	59.6
Creatinine, mg	1289	29.5
ETT_4 μg	180	4.1
RT_4	98	2.7
Weight, kg	44	—

* Body wt empty GI tract, calculated from the formula $Y = 19.56 + 1.173X - 20.909 \log X$ where Y is empty body wt and X is measured body wt [Reid *et al*(19)].

TABLE II. Prediction of Red Cell Volume from Body Weight and Thyroxine Degradation Rate.

Equation	R square
$Y = 124 + 11.898X + .8059X_2$	.802
$\log Y = 1.9032 + .5772 \log X_1 + .000525X_2$	.823

Y = cc total red cell vol.
X_1 = Kg body wt.
X_2 = μg thyroxine degraded daily.

Logarithmic expressions improved the degree of this relationship; however, prediction of RCV from RT-4 and body weight accounts for only 82.3% (R^2) of the variation in red cell volume. Table III shows the partial correlation coefficients between the measured parameters. It was difficult to speculate the nature of the relationship between RCV and RT-4. The fact that the greatest degree of relationship was obtained when the logarithmic expression was employed for describing the dependent variable, RCV, does not necessarily imply that RCV and RT-4 are related exponentially, as the equation $Y = Ae^{bX}$. A correlation of 0.48, although significant ($P<0.01$), leaves 77% ($1 - r^2$) of the variation in one variable unexplained. The significant relationship between thyroxine degradation rate and RCV observed in these in-

TABLE III. Partial Correlation Coefficients.

Item	RCV	Log RCV
RT-4	.41*	.48†
Body wt	.84†	—
Log body wt	—	.90†

* Significant $P<.05$
† Significant $P<.01$

tact animals confirms previous results using other procedures(4,7). However, some of these results were procured under pathological conditions(1,2), or by the substitution therapy(4).

The direct mechanism responsible for this observed relationship remains speculative. Thyroid hormones have been found to stimulate amino acid incorporation into proteins under *in vitro* physiologic concentrations of the hormones(10-13), and to depress it under thyroxine intoxication(14). It has also been shown that thyroid hormones have a selective action upon body tissues. Scow(15) reported no effect of thyroxine upon muscle collagen or bone protein in rats, but myosin did respond to thyroxine stimulation. It should be emphasized that myosin is also an enzyme that can catalyze the removal of a phosphate group from ATP(16). In addition, thyroid hormones have been observed to stimulate scavenger enzymes in amphibians(17,18). Relationships between RT-4 and RCV would also suggest an indirect dependence of RCV on environmental factors, since the thyroid gland is a major part of the mechanism of body homeostasis(15). This dependence through thyroid hormones could well be a remainder of the adaptive mechanism of amphibian metamorphosis. Frieden(17) observed tadpoles and frogs to have different hemoglobins, different in composition and different in relative efficiency. Hemoglobin of tadpoles was reported to be more efficient in storing oxygen, while frogs were found to be faster in rate of oxygen delivery.

The present study has demonstrated the existence of a definite thyroidal effect upon circulating red cell volume in sheep under physiological conditions, and the procedure offers possibilities for measuring thyroid status without substitution therapy or evoked physiological changes that may mask actual mechanisms.

Summary. Radiochemical procedures with 31 castrated male sheep employed to investigate relationships between thyroxine degradation rate and total red cell volume (RCV) permitted procurement of accurate and dependable results without use of substitution therapy. The significant relationship ($P < 0.01$) between degradation and RCV in these intact animals, under physiological conditions and independent of body weight, would suggest environmental factors to affect red cells through the thyroid axis. The nature and mechanisms of these relationships, though considered speculative, were discussed.

1. Muldowney, F. P., Crooks, J., Wayne, E. J., Clin. Sci., 1957, v16, 309.

2. Adam, D. D., Doljanski, F., Endocrinology, 1958, v62, 129.

3. Falkheden, T., Sjorgren, B., Westling, H., Acta Endocrinol., 1963, v42, 552.

4. Meineke, H. A., Crafts, R. C., Proc. Soc. Exp. Biol. & Med., 1964, v117, 820.

5. Goswami, S. B., Indian Vet. J., 1965, v42, 670.

6. Hansard, S. L., Nucleonics, 1951, v9, 13.

7. Vohnout, K., Hansard, S. L., Morton, E. L., Am. J. Vet. Res., 1967, in press.

8. Vogel, R., A least Squares Algorithm for nonlinear curve fitting, 1966, IBM Computer program.

9. Vohnout, K., Hansard, S. L., Am. J. Vet. Clin. Path., 1967, v1, 89.

10. Necheles, T. F., Fed. Proc., 1961, v20, 67.

11. Sokoloff, F., Kaufman, S., J. Biol. Chem., 1964, v236, 795.

12. Tata, J. R., Ernster, L., Lindberg, C., Arrenius, E., Pedersen, S., Hedman, R., Biochem. J., 1963, v88, 408.

13. Campbell, P. L., Deibler, G. E., Gelber, S., Sokoloff, L., Endocrinology, 1964, v75, 304.

14. Hoch, F. L., Physiol. Revs., 1962, v42, 605.

15. Scow, R. W., Am. J. Physiol., 1959, v196, 859.

16. Huxley, H. F., Scient. American, 1958, Reprint 19.

17. Frieden, E., ibid., 1963, Reprint 170.

18. Usuku, G., Gross, J., Develop. Biology, 1965, v11, 352.

19. Reid, J. T., Bensadoun, A., Paladines, G. L., Van Niekerk, B. D. H., Ann. N. Y. Acad. Sci., 1963, v110, 327.

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