It would appear that both phaeomelanin and eumelanin synthesis are equally sensitive to biotin deficiency. In both yellow mice and black mice deficient in biotin a marked reduction in pigmentation is found in the hairs of the second hair generation. At present, with the role of biotin in the pigmentary process undetermined, no statement can be made as to the nature of this inhibition.

Summary. Both yellow (A^y) and black (C57BL) mice produce grey hairs when weaned to a diet known to cause biotin deficiency. Histologically, the greyed hair follicles of biotin deficient mice bear a striking resemblance to the hair follicles of albino mice. The depigmented melanocytes of biotin deficient mice are similar in morphology to the follicular clear cells of the albino. Unlike clear cells, however, depigmented melanocytes react positively to tests for the presence of dopa oxidase.

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Measurement of Total Body Water in Sheep Using I^{131} Labeled 4-Iodo-Antipyrine. (22727)

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Antipyrine has been employed extensively as an indicator for the *in vivo* estimation of total body water in man(1,2) and animals (3-6). The method is based upon the dilution principle and is an adaptable and economical procedure for studies with the intact animals. Despite certain theoretical shortcomings(7,8) this method has been found to compare favorably with parallel procedures for body water measurement employing whole body analysis(3-5), specific gravity(6,9,10, 11) or the isotope dilution technic using deuterium(7), or tritium oxide(12). However, these methods require tedious procedures of sample preparation and time consuming analyses that discourage their use in routine experimentation. The recent introduction of I^{131}-labeled 4-iodo-antipyrine as a simple and rapid method for estimating total body water in man(13) made feasible the adaptation of this procedure for use with farm animals(14). This study is concerned with comparative behavior and volume of distribution of antipyrine and I^{131} labeled 4-iodoantipyrine for total body water estimation in sheep.

Materials and methods. The animals used in these studies were normal, healthy individual wether lambs and mature sheep maintained under typical summer and fall farm management conditions. The procedures employed for handling animals and performing quantitative injections of test substances have been described(15). Animals were taken off

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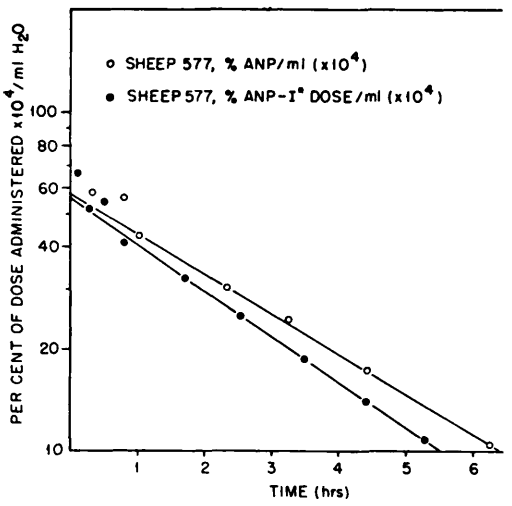

FIG. 1. Log concentration of antipyrine and I^{131} 4-iodo-antipyrine in blood of an avg sheep (577 regressed on time in hr after inj. by method of least squares to derive the theoretical concentration of total body water.

feed and water 12 to 15 hours prior to dosing intravenously with approximately 2 grams antipyrine and or 50 microcuries I^{131} labeled 4-iodo-antipyrine 100 lb body weight. Venous blood and urine samples were taken periodically during the first 5 hours and at 24 hours after injection. The blood was centrifuged and plasma removed. Radioactivity was measured in four ml of plasma using a well-type sodium iodide scintillation detector attached to a conventional type scaler unit. Radioactivity in an aliquot of standard solution was measured in the same way for calculation of relative iodine content. Total antipyrine concentration was determined by precipitation procedure(16). Radioactivity in both plasma and zinc filtrate fraction was measured for comparative purposes, and for estimation of plasma protein binding. The volume of distribution for each test substance was then calculated by plotting concentration against time after injection on semi-logarithmic paper and exterpolating back to zero time by the method of least squares. (Fig. 1). The zero value was corrected for plasma water by dividing by 0.93, and the test substance injected divided by the concentration in plasma water to give total volume of body water. Body water in liters divided by body weight (kg) X 100 gave the % body water. Thyroid uptake of I^{131} was checked periodically and at 24 hours after administration of labeled 4-iodo-antipyrine using an adapted scintillation probe attached to a count-rate meter.

Results. The adaptation of a new test substance requires that certain physiological studies be made for comparison with proven and acceptable methods of analysis. Since the antipyrine procedure has been used generally for estimation of body water volume in man and animals and has compared favorably with various other procedures(4,5,11, 13), it was of interest to consider results from iodinated antipyrine with data obtained concurrently with stable antipyrine. The use of the labeled compound further permitted a simple investigation of rate and behavior patterns of test substances.

Partition in the plasma. The uniform distribution of both antipyrine and the I^{131} labeled 4-iodo-antipyrine in sheep occurs in about one hour (Fig. 1). The initial rate of disappearance for the 2 test substances was not much different, averaging approximately 30% per hour for animals in the 2 age groups. If it is assumed that the labeled and carrier antipyrine are similarly metabolized by sheep, then the degree of antipyrine binding by plasma proteins may be determined simply by measuring plasma radioactivity before and after deproteinization with zinc hydroxide (1). Furthermore, if plasma could be used directly without deproteinization to reflect the water content from radiochemical analysis, the procedure would be simplified. Therefore, all blood samples were centrifuged and aliquots measured for radioactivity before and after deproteinization and the % reversibly bound to plasma(1) proteins calculated

$$\text{as } 100 \times \frac{\%\text{ dose plasma } I^{131}/\text{cc minus } \%\text{ dose filtrate } I^{131}/\text{cc}}{\%\text{ plasma } I^{131}/\text{cc}}$$

Results from analysis of plasma samples taken periodically from sixty-two sheep indicated that 10 to 20% of plasma iodine was bound to proteins during the critical 5 hour test period and increased to 60% of that remaining in the blood at 24 hours. In quantitative studies where *absolute* volume data are

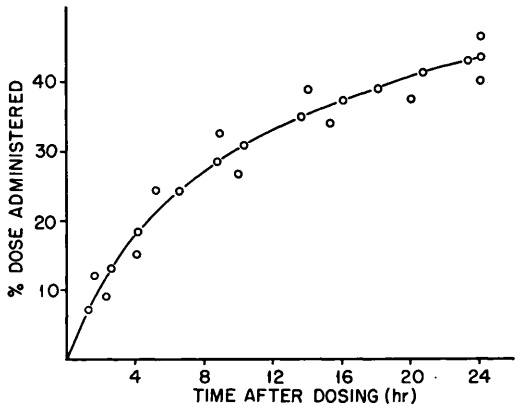

FIG. 2. Accumulative percent excretion of I^{131} in urine of sheep dosed intrav. with I^{131} labeled 4-iodo-antipyrine.

required corrections, therefore, would be necessary. However, in those studies where relative body water volume values are desired, the simplicity of the plasma analysis procedure would be justified.

Thyroid uptake. Measurements of thyroid uptake of I^{131} from labeled antipyrine were made periodically throughout the test period by external counting over the thyroid area with an adapted probe type scintillation counter. The fact that only 3 to 5% of the dose administered was found in this area during the 5 hour test period would indicate the degree of firmness of I^{131} binding to the antipyrine. Six wethers were sacrificed after 24 hours and the thyroids analyzed radiochemically were found to contain 35 $\pm$ 8 percent of the I^{131} administered.

Excretion of metabolized antipyrine. Urinary and fecal excretion values and radioanalysis of gastrointestinal tract contents from 8 sheep sacrificed 24 hours after administration of test substances indicated that insignificant amounts of I^{131} were excreted in feces. The accumulative average % of administered dose excreted in urine of 12 sheep is shown in Fig. 2. Of 44% voided during the first day, approximately 20% was excreted during the critical 5 hour test period of the study.

Total body water in sheep. The results of total body water determinations using both antipyrine and I^{131} labeled 4-iodo-antipyrine procedures with 62 young and mature sheep are shown in Table I. These values for young lambs are slightly higher than the 59 $\pm$ 5.1 and 61 $\pm$ 3.4% reported earlier(14) using antipyrine and I^{131} labeled 4-iodo-antipyrine concurrently in 36 trials with 12 growing lambs. This is probably due to the greater number of younger lambs used in the current study since the young animals have invariably demonstrated a higher body water volume. Mature wethers showed a lower body water volume and checked closely with reports for beef cattle(3), averaging 55.3 $\pm$ 6.3 and 56.2 $\pm$ 6.6% body water respectively by the antipyrine and labeled antipyrine procedure. The range shown for the two methods was not much different, and reflected the rate of disappearance from plasma (Fig. 1), indicating results from the isotope procedure to compare favorably with that for the stable antipyrine method. The advantage of the labeled antipyrine method lies in simplicity and possible rapidity. It is necessary only to inject intravenously and quantitatively the activity in a minimum volume and to measure the radioactivity in the plasma of blood samples drawn periodically over a 1 to 5 hour period (13). The fact that the labeled test substance appeared to give a slightly higher body water volume may well be accredited to inherent errors involved in quantitative admin-

TABLE I. Percent of Body Water in Young and Mature Sheep as Measured by Antipyrine and I^{131} Labeled 4-Iodo-Antipyrine.

Age	Wt, lb	No. of animals	Method*	Mean†	Stand. dev.	Range
Young	65 $\pm$ 10	34	A.N.P.	61.8	6.5	45-72
	62 $\pm$ 12	44	I^{131} A.N.P.	62.3	6.8	48-74
Mature	135 $\pm$ 49	16	A.N.P.	55.3	6.3	37-63
	138 $\pm$ 51	18	I^{131} A.N.P.	56.2	6.6	35-65

* Refers to antipyrine (A.N.P.) and I^{131} labeled 4-iodo-antipyrine (I^{131} A.N.P.).

† Percent body water calculated as $100 \times \frac{\text{Body water (l)}}{\text{Body wt (kg)}}$.

istration of very small volumes of solution. The activity usually required was contained in 0.25 to 1.0 ml. It should be noted, however, that for dilution methods in general the experimental errors almost invariably lead to high values for body water. Some of the more important sources of error include (1) nonquantitative injection into the blood stream, (2) excess water in the gastrointestinal tract and, (3) rapid metabolism of the test substance or its removal from the circulating blood following administration. In this study every precaution was taken to eliminate or to correct for these errors, and by management practices to avoid physiological changes that are known to affect the normal individual.

Discussion. The ultimate objective of establishing a predictable relationship between body water and weight in farm animals is obviously that of using such data to evaluate gain or loss of body energy in nutritional investigations or physiological studies without the need for sacrificing the animal. Several investigators have demonstrated the relative merits of estimating body fat from body water measurements(3,4.5.11.14) and mathematical expressions have been proposed that would permit estimations for other body constituents(17). It is evidenced from the data presented that, although the I^{131} labeled 4-iodo-antipyrine procedure does not rule out all of the recognized shortcomings of the antipyrine method, it offers a simple and rapid means of estimating *relative* body water in mature and in growing farm animals, and is well adapted for routine application to experiments requiring a measurement of this vital metabolic pool. These values employed, for the calculation of total body fat and their extension to prediction of chemical composition and energy values(17) would thereby provide additional criteria for the response measurement of animals to various experimental treatments.

Summary. A simple and rapid procedure is presented for the *in vivo* measurement of total body water in sheep using I^{131} 4-iodo-antipyrine as the test substance. Body water values for 62 young and mature sheep averaged 61.3 ± 6.5 and 55.3 ± 6.3, respectively, by the antipyrine method, and 62.3 ± 6.8 and 56.2 ± 6.6%, respectively by the labeled antipyrine procedure. Thyroid uptake of I^{131} from labeled test substance amounted to 3 to 5% and urinary excretion was less than 20% of administered dose during the critical 5-hour test period. Protein binding was evidenced, indicating that in sheep both test substances were reversibly bound to plasma protein. Consideration is given to the use of these body water volume values in establishing a predictable relationship to body constituents and to provide additional criteria for estimating animal response to experimental treatments.

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