two types, $(n + 1)$ and n; therefore the embryo sac will have two types of nuclei. The four nuclei will probably divide again to form eight (if they divide a second time to form 16 it will not change the result as long as the nuclei are oriented with the same polarity as that of the original four megaspores). At maturation the endosperm is formed from two unlike polar nuclei which are fertilized by a single pollen nucleus to produce a $(3n + 1)$ endosperm with both genetic makers, while the embryo is produced from a single nucleus which may be either $(n + 1)$ or n, but genetically of any one of five different types $\alpha\ a^m$, $\alpha\ \alpha$, $a^m\ a^m$, α, or a^m. These events provide the type of apparent crossover endosperms observed and account for the types of corresponding and noncorresponding embryos that were associated with them.

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Water Turnover in Cattle

Abstract. *The half-life of the body water pool in cattle is unusually short, relative to their body size. The short half-life is not due to milk formation, since the same result is observed in nonlactating cattle as in cattle producing 23 kg (50 lb) of milk per day.*

A difference between cattle and other mammals became apparent during metabolic studies on rate of utilization of tritium-labeled compounds in cows (*1*). Richmond *et al.* (*2*) have established a correlation between water turnover and body weight with six mammalian species in which the half-life for body water ranged from 1 to 9 days. From this relationship one would predict a half-life of about 9 days for body water in the cow.

In contrast to this expectation we find that the half-life of water in cattle weighing 150 to 750 kg is only 3.5 ± 0.21 days, a value that is not significantly different from that obtained with 300-g rats (*2*).

Cattle were injected with tritium-labeled water or organic compounds through a plastic catheter inserted into their jugular vein. The amount of tritium injected in each trial and other characteristics of the experiments are listed in Table 1. Small samples of blood or milk or both were drawn at frequent intervals for several hours after the tritium-labeled compounds were injected and less frequently during a period lasting several days. Results reported here are for those samples collected after the tritium had equilibrated throughout the body water pool —later than 8 hours after injecting tritiated water and later than 24 hours after injecting tritiated organic compounds.

The blood was allowed to clot and, after syneresis, the serum was decanted off for lyophilization and collection of water. Three to four milliliters of water were collected in a cold trap from each blood serum or milk sample lyophilized. All samples were lyophilized to complete dryness to avoid any change in specific activity that would result from differences in rate of evaporation of tritiated versus unlabeled water molecules. A 0.1-ml aliquot of each water sample was transfered to 15 ml of liquid scintillation solution (containing dioxane for aqueous solutions) and counted for 10-minute intervals in the Packard Tricarb. The efficiency of the system (about 20 percent) was determined with an aliquot of a diluted standard prepared from the injected tracer. The efficiency factor was used to convert net counts per minute to millimicrocuries of tritium per liter of body water.

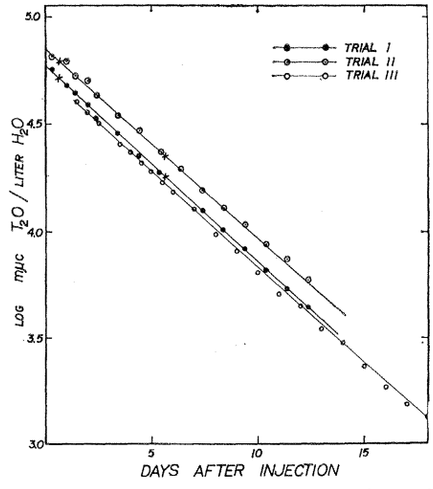

Fig. 1. Regression for concentration of tritium in body water of lactating cows after injecting tritiated water (trials 1 and 2) or tritiated acetate (trial 3). Experimental values are shown by circles; calculated regression points through which lines were drawn are indicated by X's.

When tritiated fatty acids were injected into cows, the blood or milk was made strongly alkaline by addition of dry powdered Na_2CO_3 prior to lyophilization. This treatment ensured that any tritiated acetate or propionate would remain in the residue and not influence the tritium level in the water collected.

The logarithm of tritium concentration in body water (millimicrocuries per liter) is plotted as a time series in Fig. 1, for three trials. The concentration decreased exponentially with time, the individual values fitting very closely the linear regression calculated by the method of least squares. The results for trials 1 and 2 show that the body water pool in cattle behaves kinetically as a single pool undergoing simple dilution at times greater than 8 hours after injecting tritiated water and during an interval extending almost 2 weeks.

The slope of the regression line, (k), multiplied by 2.3 (to convert to natural logarithms) represents the turnover rate of the body water pool, and from the relationship

$$0.693/k = T_{½}$$

the half-life $(T_{½})$ of body water was calculated.

Table 2 lists the half-life for body water found in several experiments with cattle. In dairy cows, trials 1 to 8, the half-life ranged from 3.0 to 3.9 days, with an average value of 3.54 ± 0.10 days. The Jersey cow used in trial 8 was not lactating at the time of the trial but had been through several lactation periods, the last one terminating only a few weeks prior to the experiment. This animal was included with the dairy cows, group I, instead of group II, which consisted only of steers, and a heifer.

The half-life for body water in nonlactating cattle, group II, in Table 1, ranged from 2.8 to 4.1 days with an average value of 3.4 ± 0.18. This average is not significantly different from that found for the dairy cows.

The average half-life calculated for all animals in groups I and II was 3.5 ± 0.21 days, a value not significantly different from 3.5 ± 0.12 days (*2*), measured in rats which were 1000 times as small.

A bull calf was injected with tritiated water first when he was 7 days old (trial 16) and again when he was 6 months old (trial 17). The shorter half-life, 2.8 days, occurred at the younger age but the result obtained at each age fell within the range observed for the older animals; thus, it is not possible to decide from our limited data whether the younger calf has a greater turnover rate than mature cattle.

In trial 4, tritium-labeled acetate was injected into a cow suffering from bovine ketosis, a metabolic disease associated with reduced intake of food and water and characteristically resulting in marked dehydration of the animal. During a period of 3 weeks this animal lost 112 kg from an original weight of 518 kg. Although the cow was under treatment during the time water samples were collected, she had not recovered from the ketosis as shown by her inability to maintain blood sugar levels above 40 mg/100 ml and ketone body levels below 10 mg/100 ml unless glucogenic substances were administered. Several weeks after recovering from ketosis, she was again injected with tritium-labeled acetate (trial 5). The half-life measured under these two conditions was 3.8 days, during the period of ketosis, and 3.7 days, after recovery. This small difference indicates that the ketosis with its associated dehydration and marked change of body size (22 percent of body weight) had no appreciable effect on the half-life of her body water pool.

In some of the trials, tritium was administered in the form of T-acetate (trials 3 to 6), T-propionate (trial 7), or glucose-1-T (trial 8). Samples of body water collected 24 hours or later after injecting these compounds decreased in specific activity with first-order kinetics. The results for trial 3, shown on Fig. 1, are typical of all results with tritiated fatty acids or glucose, for water samples collected between 1 and 18 days after injecting the tritiated compounds. The rate of change of specific activity is indistinguishable from that observed after injecting cows with tritiated water, which indicates that the tritium released during metabolism of the acetate, propionate, and glucose must appear in the body water largely during the first 24 hours and that any released subsequently had no detectable influence on the tritium turnover in the body water. Thus, to evaluate the metabolism of organic compounds in cattle, with tritium used as a tracer, one must measure the appearance of tritium in body water within the first 24 hours.

Table 1. Conditions for trials and experimental animals.

Trial	Animal No. and breed	Sex	Age (yr)	Wt (kg)	Milk (kg/day)†	Diurnal temp. (°C) Min.	Diurnal temp. (°C) Max.	Tritium injected (mc)	Compound injected
				Group I					
1	1186-Holstein	F	6	763	22.7	42	67	32.5	T-H_2O
2	8-Ayrshire	F	8	588	17.7	42	67	32.5	T-H_2O
3	1186-Holstein	F	5	783	20.5	47	78	62	T-acetate
4	H-28-Jersey	F	8	450	9.1	54	91	10.2	T-acetate
5	H-28-Jersey	F	8.5	407	9.6	39	55	16.8	T-acetate
6	8-Ayrshire	F	8	598	24.1	50	85	12.5	T-acetate
7	1186-Holstein	F	5.5	738	12.8	39	62	55.9	T-propionate
8	253-Jersey	F		437	0			14.7	Glucose-1-T
				Group II					
9	868-Holstein	M	1	309	0	49	74	30‡	T-H_2O
10	1120-Holstein	M	1	237	0	49	74	30‡	T-H_2O
11	1048-Holstein	M	1	300	0	49	74	30‡	T-H_2O
12	1125-Holstein	F	1	177	0	49	74	30‡	T-H_2O
13	1069-Hereford	M	1.5	207	0	53	84	30‡	T-H_2O
14	1357-Hereford	M	1.5	218	0	53	84	30‡	T-H_2O
15	1189-Hereford	M	1.5	252	0	53	84	30‡	T-H_2O
				Group III					
16	H-28A-Jersey	M	7‡	29	0	54	91	10	T-H_2O
17	H-28A-Jersey	M	6§	130	0	39	54	10	T-H_2O

* One kg = 2.2 lb. † Approximately 30 mc of T-H_2O was injected into each of these animals. Unfortunately, a mixup in records leaves an uncertainty about how much each received; this information is not needed to calculate half-life but is necessary to calculate pool size. ‡ Days. § Months.

Factors which influence the turnover rate of body water are: the size of the body water pool; the amount of water gained per unit time by drinking, eating, or by metabolism of food; and the amount of water lost per unit time by breathing, sweating, and excretion (urine, feces, and in the case of lactating cows, milk).

The size of the body water pool of the cows used in trials 1 and 2 was 73 to 74 percent of the body weight (Table 2). This is larger than the values that have been reported for other mammalian species (*2*) and at the high end of the range of values reported for dairy cows (*3*). Richmond *et al.* (*2*) report body water, in percentage of body weight, to be 60 (rat), 55 (man), and 66 (horse). Hansard (*3*) has compiled data from several sources for mature animals, which averaged about 48 percent (swine), 56 percent (sheep), 56 to 60 percent (beef cattle), and 65 percent (dairy cattle). The range of values for dairy cattle was given as 49 to 77 percent.

For a given water flux, a larger body pool will have a greater half-life than a small pool. Thus, the relatively large pool size in cattle would not account for their unusually short half-life (relative to body size); on the contrary, the larger pool would make the half-life longer than it would be if the same amount of water per unit time passed through a smaller pool. Neither can lactation, per se, be responsible for the abnormally fast turnover rate in the bovine, since the average half-life was as great in cows giving 9 to 23 kg (20 to 50 lb) of milk per day as it was, on the average, in nonlactating cows, heifers, and in steers (Table 2). Close inspection of the data suggests that the half-life may be somewhat shorter for cows with higher levels of lactation than for those giving little or no milk, but our data are too limited to calculate a correlation.

Other factors that would be expected to influence the water turnover are sweating and respiration, both of which would result in greater losses during the hot, dry summer months than during the colder, damper winter months. Again the data collected are inadequate to critically assess this influence but it appears that, as with lactation level, the effect of seasonal change is small, since the half-lives determined during May and June (trials 9 to 15) or July (trial 4) overlap those measured during October, November, or December (trials 3, 1 and 2, and 5, respectively).

It would not be justified to state that the level of lactation or environmental

Table 2. Kinetic parameters for water pool in cattle. For the cow used in trial 1, the body water pool was 73.4 percent of body weight; for that used in trial 2, it was 72.4 percent.

Trial	Half-life (days)	Water turnover (pool fraction/day)
	Group I	
1	3.3	0.21
2	3.4	0.204
3	3.5	0.198
4	3.8	0.082
5	3.7	0.187
6	3.0	0.231
7	3.9	0.178
8	3.7	0.187
	Mean 3.54 ± 0.105	
	Group II	
9	3.4	0.204
10	4.1	0.169
11	2.8	0.247
12	3.8	0.182
13	3.6	0.192
14	2.9	0.239
15	3.2	0.217
	Mean 3.4 ± 0.179	
	Group III	
16	2.8	0.247
17	3.5	0.198

conditions do not effect the half-life of body water. Rather, it appears that their effect was very small and, with our limited data, cannot be determined. More extensive investigations are in progress to evaluate the individual effects of these factors on water turnover.

The data collected in trial 1 (or 2) can be used to calculate the daily water flux through the cow. For example, in trial 1, the body water pool for cow 1186 was 73.4 percent of 763 kg, or 560 kg of water, and her turnover was 0.21 of the pool per day. The water flux, based on these figures, would be 118 kg/day (560 kg × 0.21). This cow was producing 23 kg of milk per day, which would contain 19.8 kg of water, or about 17 percent of the total flux. Although water losses via feces and urine were not measured in these trials, data reported by Adolph (*4*) can be used to estimate that daily losses of 24 kg occurred (0.04 and 0.09 percent of body weight per hour for urine and feces, respectively). If this cow stopped lactating, so that her water flux decreased to 98 kg/day while her water pool remained 560 kg, her water turnover would become 0.18 of the pool per day and the half-life would increase to 4.0 days. This value for half-life is on the high side of those obtained with nonlactating cattle but still falls within the maximum observed with a steer (4.1 days). Since the average half-life for nonlactating cattle was not significantly different from that for cows with various lactation levels (9 to 24 kg of milk per day) it seems likely that other adjustments generally occur which minimize the change in half-life with changes in water flux. One possibility would be a compensatory decrease in water loss via urine and feces as the lactation level rises; however, this would be of limited magnitude, since, of the total amount of water lost in this way, most is needed for removal of undigested and nonmetabolized waste from the body. Another, and perhaps more reasonable, adjustment would be a parallel change in total pool size—the pool increasing as the water flux increases with higher levels of lactation. A parallel change of pool size and water flux would minimize any change in half-life. The greater percentage of body water in dairy cattle compared to beef cattle (*3*) would support this explanation. Also, the fact that the half-life of body water was nearly the same during ketosis (trial 4) when the cow was undergoing marked decrease in body content (22 percent weight loss in 3 weeks), as when the cow had recovered and was slowly gaining weight (trial 5), indicates that adjustments involving pool size and turnover must occur rather rapidly.

An increase in pool size associated with greater water flux could have important physiological significance. In lactating animals it would provide a greater reservoir of soluble metabolites for biosynthesis of milk. It would also provide greater water reserve for hot weather conditions when losses due to respiration and body cooling increase. The larger pools with their greater capacities would resist, for a longer time than smaller pools, the adverse physiological effects of depletion associated with high fluxes through these pools.

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"Electrical Transmission" at an Excitatory Synapse in a Vertebrate Brain

Abstract. *A type of excitatory synaptic transmission which is novel for the vertebrate brain has been found in the medulla of the goldfish. Certain synaptic terminals (the club endings of Bartelmez) appear to stimulate the Mauthner neuron (M-cell) by means of the passive spread of their action currents across the synaptic membrane. After stimulating the ipsilateral eighth cranial nerve, an excitatory postsynaptic potential (EPSP) appears in the M-cell with a latency which is very brief (about 0.1 msec) and which probably represents a negligible synaptic delay. This response is attributed to the club endings: there were steep gradients of potential along the lateral dendrite of the M-cell during activity and the early EPSP was maximal in the distal part of the dendrite where the club endings predominate. Potential changes in the M-cell spread (passively) backwards into certain eighth-nerve fibers (probably club endings) indicating the presence of special low-resistance connections between them and the M-cell.*

The Mauthner cells (M-cells) are a pair of giant neurons found in the medulla of most fish. They are large enough to permit intracellular recordings to be obtained from their axons, cell bodies, and dendrites; their rich synaptic input is morphologically specialized, certain distinctive groups of nerve endings being localized to particular regions of the cell (Fig. 1).

In previous studies of goldfish M-cells (*2*) very early intracellular changes in potentials were sometimes observed after a stimulus was applied to the ipsilateral eighth cranial nerve. The study reported herein was undertaken to examine these short-latency responses more closely.

The experimental methods were similar to those previously described (*2*), but a more radical dissection was made which exposed the utricle and parts of the eighth nerve. The fine silver wires used for stimulating were insulated except at the tips and could be manipulated independently. Intracellular recordings were made with glass micropipettes filled with either 0.6*M* K_2SO_4 or 3*M* KCl.

The responses with short latencies could be evoked in the M-cell by stimuli of relatively low intensities when the