Technical Report No. 10

METABOLIC COMPONENTS OF CATTLE: WATER-SOLUBLE TRACERS FOR DETERMINING WATER TURNOVER AND PARTITIONING BY CATTLE

PAWNEE SITE

GRASSLANDS BIOME

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ABSTRACT

The objective of the project on water-soluble tracers for determining water turnover and partitioning by cattle is to determine the energy and nutrient components associated with the water components consumed and excreted by cattle. For example, if fecal water output can be determined, the dry matter and energy concentrations can be added to arrive at total daily amounts.

^{14}C labeled polyethylene glycol (PEG) was used to estimate fecal output, lithium (Li) was used to estimate urine output, and tritiated water ($^{3}H_2O$) was used to estimate total body water and total water turnover. Problems associated with sample collection and tracer extraction had high priority in the initial experiments.

PEG consumed in drinking water is excreted entirely in feces. However, adsorption to organic matter prevents complete recovery, and high concentrations are required for adequate sampling precision. A new extraction procedure that attains 100% recovery has been developed, and labelling with ^{14}C permits the use of very low concentrations. Since we prefer non-radioactive tracers for field studies, three non-radioactive compounds were tested as tracers of fecal output. These compounds were not satisfactory because of metabolic conversions and adsorption losses.

Li is excreted almost entirely in urine and can be used as a tracer of urine output. However, techniques for recovering Li from both urine and feces still require improvement.

Tritiated water is excreted in vapor as well as liquid phases of water, and has been used widely to determine total body water and total water turnover. Since better sampling procedures are needed, we developed equipment for collecting respired water, which requires no purification, and compared respired water

with that obtained from saliva, blood, and urine. Tritium concentrations were the same in all water sources. Therefore, field sampling can be designed to accommodate the equipment available and the objectives of a study.

In future work, we will estimate urine and fecal outputs of cattle on pastures stocked heavily (23E) and lightly (23W), as well as to continue studies on the development of water-soluble tracers. For example, fecal output of fistulated steers was measured by total collection (Tech. Rep. 12). Fecal output by the heifers used to apply the grazing routines was not measured. Urine output was not measured for any animal. Therefore, using water soluble tracers to estimate urine and fecal outputs can make the work easier and the overall determination of bioenergetics more complete. The work in 1970 will determine sampling precision and accuracy, if the radioactive tracers are given safety clearance.

INTRODUCTION

Four experiments have been conducted toward successful completion of technique development, as follows:

1. *Gastro-intestinal tract water tracers.* Three non-radioactive compounds were tested as possible water-tracers for determining gut water. A successful gut-water tracer (a) must not be absorbed or metabolized in the intestinal tract, (b) must be water soluble and pass through the animal at a comparable rate with gut water, and (c) must be chemically recoverable. The three dye compounds were tested as to their stability and recoverability. Although they show possibility of being useful, recoveries were not satisfactory and no further work was attempted.

2. *Polyethylene glycol - ^{14}C study.* Experiments were conducted to test polyethylene glycol - ^{14}C as a gut-water tracer. An initial trial using a single steer indicated that ^{14}C-PEG might be a useful gut-water tracer, but recovery problems limited the success of the study. Extraction procedures that attain 100% recovery from feces were developed. Then a second trial with sheep was conducted to determine the usefulness of ^{14}C-PEG. The samples from the second trial still are being analyzed.

3. *Methods of sample collection for determining total hydration in live animals.* Tritium labeled water has been used successfully to determine total body water. Tracer dilution has several advantages. It is sensitive, relatively inexpensive, and applicable to live animals. But, the technical problems of adapting such procedures to field situations are serious. To overcome sampling difficulties, equipment for trapping respired water was developed. Respired water, which requires no purification, was compared with water from saliva, blood, and urine for the purpose of following the course of tritium

dilution. The results of this work have been put into manuscript form and will be published.

4. *Water partitioning and flux in sheep.* An experiment was conducted using four sheep under controlled housing and feeding, to test three tracers. The tracers used were tritiated water, Li, and ^{14}C-PEG. Animals consumed these materials in their drinking water over a period of approximately 30 days. Samples for analyses were collected once or twice daily. These samples are only partially analyzed and complete data cannot be reported.

EXPERIMENT NO. 1

GASTRO-INTESTINAL TRACT WATER-TRACER STUDY

Materials Tested

F D & C Violet No. 1, F D & C Red No. 3, and phloxine B.

Purpose

To determine the feasibility of the use of these materials as water soluble tracers by the following criteria.

1. Stability of the dyes under storage and extraction conditions.
2. Recovery of the dyes after being mixed with fresh cattle feces.
3. Stability of the dyes when exposed to the action of rumen bacteria.
4. Recovery of the dyes after being placed directly into the rumen.

Procedures

1. *Stability of the dyes.* The dye materials tend to adhere to organic matter and do not equilibrate well with a liquid phase, which is mainly water. Fecal samples from which the dyes were to be extracted were dried in a forced air oven at 60°C, finely ground, and then dried in a vacuum oven at 60°C and 25 lb. vacuum. One-half gram of the dried sample was weighed into a fritted

glass crucible (medium porosity). The crucible was mounted in a Soxhlet apparatus so high that the solvent would flow continuously through the crucible. The solvent was methanol: 2.8% NH_4OH (80:20). It was observed later that the presence of the NH_4OH reduced the stability of the F D & C Violet No. 1. Therefore, the solvent used in some of the later trials consisted of only 80% methanol. The absence of NH_4OH did not seem to reduce the efficiency of extraction. Approximately 150 ml of the solvent was placed in the flask below the Soxhlet apparatus. The flask was heated by an electric jacket. Extraction times ranged from 2 to 24 hours.

In samples where a considerable amount of the dye material was present, the solvent containing the dye was diluted to 100 ml or more and the absorbence read on the spectrophotometer. In the experiments where only trace amounts of the dye were present, it was necessary to evaporate off the solvent, wash the residue with ether, and dissolve the dye in a small amount of the solvent (25 ml).

The absorbence maximums for the methanolic solution of the dyes were as follows: F D & C Violet No. 1, 585 mμ; F D & C Red No. 3, 530 mμ; and phloxine B, 540 mμ.

With samples which had to be concentrated after extraction, it was necessary to run fecal samples containing no dye for background subtraction. However, with the others the background coloring was diluted to a point at which it did not contribute measurably to the absorbence.

2. *Recovery of the dyes from fresh cattle feces.* Weighed samples of the dyes were added in solution to fresh cattle feces. The fecal samples were thoroughly mixed and then dried. In the first series of tests the amounts of

dye used were: Phloxine B, 10.45 mg/g; F D & C Violet No. 1, 10.5 mg/g; and F D & C Red, 9.29 mg/g of dry matter. The solvent used to extract these samples consisted of methanol and 2.8% NH_4OH (80:20). In the second series, smaller amounts of each dye were used; these were approximately: 0.25, 0.5, 0.7, and 1.0 mg/g of dry matter. The solvents used to extract these samples were 80% methanol.

3. *Incubation of dyes with rumen fluid.* The incubation was carried out in 15 ml culture tubes in a water bath at 39-40°C. Each tube contained 10 mg of dye, 6 ml of rumen fluid, and 4 ml of 0.25 M phosphate buffer (pH 6.7). At the end of the incubation period, which ranged from 0 to 10 days, the contents of the tubes were removed and dried prior to extraction. The methanol and 2.8% NH_4OH (80:20) solvent was used for these samples.

4. *Addition of dyes to the rumen.* In all cases the animal used was a 43 kg Columbia ram which previously had been fitted with a rumen fistula. The animal was kept in a metabolism stall and given feed and water *ad libitum*. The dye material was dissolved in 100 ml of water and placed directly into the rumen. The flasks then were rinsed with an additional 100 ml of water. In the first trial, 20 g of phloxine B was used. Fecal collections were analyzed separately in order to show the rate of passage of the dye material. Feces were collected for seven days following the addition of phloxine B. Thirty days later, in three separate trials, 0.6 g of each dye was added to the rumen. A composite fecal sample was collected for a period of eight days following the addition of each dye. Two days were allowed between each of the last three trials. Methanol with 2.8% NH_4OH (80:20) was used as the extracting solvent in the first trial. For the last three trials, 80% methanol was used.

Results

1. *Stability of the dyes.* Both phloxine B and F D & C Red No. 3 appeared to be quite stable during extraction and under storage conditions (at room temperature in extraction solvent). However, F D & C Violet No. 1 was affected by heat and the presence of NH_4OH. Dilutions of F D & C Violet in water ranging from $1:10^3$ to $1:10^6$ were stored for more than 30 days at 5, 20 and 60°C without showing any change in absorbence. With 80% methanol used as the extracting solvent, the violet dye appeared to be stable when stored at room temperature. The heat necessary to reflux the methanol solution used in the extraction process was found to be sufficient to cause decreased stability of the F D & C Violet No. 1 dye. After about four hours of extraction, the amount of the violet dye being extracted from a sample had declined to the point where it was equal to or less than the amount being destroyed. Therefore, continued extraction resulted in less apparent dye being recovered.

2. *Recovery of dyes from fresh cattle feces.* Dye recoveries after mixing large amounts with wet bovine feces are given in Table 1. The recovery of both phloxine B and F D & C Red No. 3 was fairly good. The effect of prolonged heating on the violet dye also may be seen in Table 1. The solvent used in these extractions contained NH_4OH, which probably resulted in less of the violet dye being recovered.

The results obtained when smaller amounts of the dyes were used are shown in Table 2. The feces with which the dyes were mixed contained approximately 80% moisture. Therefore, the levels of 5, 10, 15 and 20 mg per 100 g of wet feces correspond to concentrations of about 63, 125, 188 and 250 ppm of dye material in the liquid phase of the feces. Again, the recovery of phloxine B and F D & C Red No. 3 was fairly good. The recovery of F D & C Violet No. 1

was rather poor, although the extraction time was kept to a minimum (four hours) and no NH_4OH was used in the solvent. It did appear that the recovery of the violet dye was better at high concentrations.

3. *Incubation of dyes with rumen contents.* Table 3 shows the recovery of the three dyes following incubation with rumen contents. Although the recovery of the red and violet dyes was not as high as expected, it appears doubtful that the results could be attributed to the action of rumen microbes on dyes. In light of the other result, it seems likely that the poor recovery, especially of the violet dye, might be due to its instability during the extraction process.

4. *Addition of the dyes to the rumen.* The results of teh addition of 20 g of phloxine B to the rumen of a sheep are shown in Fig. 1 and 2. Fig. 1, which is based on the analysis of individual samples, shows that after seven days, 17.6 g or 88.5% of the dye had passed into the feces. However, probably a more reliable measure was obtained from a composite of the samples, which showed that 18.2 g or 92.5% of the dye was recovered after seven days. The peak excretion occurred 70 to 80 hours after the dye had been placed in the rumen. None of the dye appeared in the urine. This large amount of dye did cause irritation of the intestinal tract. During the peak excretion period, the feces appeared quite mucous.

Results of the three experiments in which 0.6 g of dye was placed in the rumen are shown in Table 4. With each dye, the recovery was less than expected. Based on the amount of dye added and the amount of feces collected, the calculated concentrations were 0.4824, 0.4732, and 0.3299 mg/g of dry feces for phloxine B, F D & C Red No. 1, and F D & C Violet No. 3., respectively. These concentrations fall in the range similar to that obtained when 5 to 10 mg of dye was mixed to

100 g of fresh cattle feces; however, it was not possible to achieve similar recoveries. The average recoveries were 74% for phloxine B, 80% for F D & C Red No. 1, and 63% for F D & C Violet No. 3.

Summary

Although some of the data appeared encouraging, the dye recoveries in feces after addition to the rumen were discouraging. Seventy to 80% recovery would not permit the use of these dyes as gut-water tracers. The exact reason for the low recoveries is not known, but rumen microbial metabolism cannot be overlooked.

It was concluded that the further study of these dyes would not be pursued at this time and that other gut-water tracers would be tested.

EXPERIMENT NO. 2

POLYETHYLENE GLYCOL - ^{14}C STUDY

Purpose

Polyethylene glycol (PEG) is not absorbed from the gut and has been used to derermine rumen volume (Pfander et al. 1953) and intestinal tract volume (Smith 1959). To insure analytical accuracy, 10-15% concentrations, which are impractical for long-term studies, are necessary. However, tracer quantities of ^{14}C-PEG can be determined accurately. Therefore, dilution techniques can be employed to determine water volumes in the gut, and gut-water output.

Experimental Procedures and Results

In order to determine if ^{14}C-PEG could be recovered efficiently from fecal water, 255.2 µCi of ^{14}C-PEG were introduced via a ruminal cannulae into the rumen of a 510 kg steer. Fecal samples were collected over a 400-hour period,

and PEG was recovered by the method of Smith (1959). Radioactive PEG determinations were made by subsampling the extract prepared by Smith's method.

Fig. 3 shows the accumulative recovery of ^{14}C-PEG. About 94% of the ^{14}C was recovered in a 400-hour period. Although the recovery was not as high as desired, the data suggested that this method might be usable.

It was thought that part of the low recovery was due to extraction procedures employed. The method has undergone some modification and refinement. Table 5 indicates that the recovery has been improved, but there remains a serious statistical variation.

To test ^{14}C-PEG further as a water tracer, a more extensive study was undertaken, using four sheep. The PEG was administered via the drinking water. The design of that experiment is revealed in more detail in a subsequent report. The fecal samples still are being analyzed and no additional data are available.

EXPERIMENT NO. 3

DETERMINING TOTAL HYDRATION IN LIVE ANIMALS

Purpose

Within the past decade, several laboratories have used tritium labeled water successfully to estimate total hydration and water kinetics in sheep (Anand et al. 1966, and Macfarlane et al. 1966b), cattle (Black et al. 1964, Macfarlane et al. 1966c, and Ashbacker et al. 1965), chickens (Chapman et al. 1967), deer (Knox et al. 1969) and man (Foy et al. 1960, Macfarlane et al. 1966a, Richmond et al. 1962). Blood, and sometimes urine, are assayed in the determination of tritium concentrations. Blood is prepared by sublimation (Black et al. 1964, Macfarlane et al. 1966a, 1966b, 1966c) or treatment with

trichloracetic acid (Ashbacker et al. 1965, and Phillips et al. 1966). Urine is prepared by refluxing with activated charcoal (Ashbacker et al. 1965, and Knox et al. 1969) or by sublimation (Macfarlane et al. 1966a). Each of these procedures is time-consuming and may not remove all quenching substances. Additionally, and more critical to our objectives, they are cumbersome to work with in field situations. Several methods of collecting and preparing assay fluids were compared in Experiment 3.

Experimental Procedures

To test the reliability of several methods, two trials were conducted. The first trial was conducted to check the feasibility of several procedures and the second to compare further two methods. In Trial 1, a Holstein steer weighing 510 kg was given a single injection containing 4.28 mCi of tritium labeled water (^3H_2O) via jugular catheter. The following samples were taken daily: respired air water, jugular blood, saliva, urine (24-hour composite) and urine by palpation.

Body water samples were obtained from respired air by trapping in an apparatus shown in Fig. 4. The animal breathes into a face mask fitted with two one-way valves and a rubber diaphragm containing one hole, through which the animal's nose is inserted. The face mask is connected to a drier column filled with 8 mesh anhydrous $CaSO_4$ containing an indicator. Thus the air inhaled by the animal is water-free. Exhaled air is exhausted through a removable tygon tube (1/4" I.D.), which is coiled in a dewar flask containing dry ice and alcohol. The one-way valves insure that exhaled or inhaled air enters or leaves the appropriate valve. The face mask is attached to the animal for three minutes to empty the lungs of residual ambient air and possible contamination fr-

water in the residual air. The collection tube is then connected to the face mask for one to six minutes to collect enough respired water (about 0.5 ml) for assay.

Jugular blood (2 ml) was collected daily by vein puncture. The proteins were precipitated with 200 mg of anhydrous trichloracetic acid. Then the sample was centrifuged and the supernatant frozen until assay.

Saliva was collected as accumulated in the face mask when respired water was being obtained. This fluid was assayed directly.

Urine samples were collected on a 24-hour basis, using a collection harness of our design, and once daily by palpation. The urine samples were prepared by refluxing with activated charcoal and filtering.

All samples were assayed for tritium by pipetting 0.05 ml of prepared animal fluid in 10 ml of scintillation fluid containing 5 gm 2, 5-diphenyl-oxazole, 0.1 gm 1,4-bis-(2, 5-phenyloxazole)-benzene, 380 ml dioxane, 50 gm naphthalene, 380 ml of toluene and 240 ml absolute ethanol per liter. The assay efficiency of each sample was determined by external standardization.

The data points were plotted on semilogarithmic coordinates and the best fitting straight lines were calculated by the method of least squares.

A second experiment was conducted to further test the reliability of the respired-air-water collection procedure. Twelve wether lambs were given a pulse dose of approximately 784 μCi $^{3}H_2O$ via jugular catheter. At daily intervals, jugular blood was drawn by vein puncture and processed as previously described. Simultaneously, respired H_2O was collected and assayed for tritium.

Results and Discussion

The calculated regression equations for the dilution of tritium in the various fluids are shown in Table 6. Analysis of covariance (Li 1964) indicates no statistical difference at the 5% confidence level among any of the fluids assayed. Therefore, one can use, with equal reliability, any of the techniques to determine tritium dilution.

There are several technical and theoretical problems associated with each of the procedures used. Respired air water offers the most desirable sample to assy in that it requires no processing, and the collection apparatus is simple and easy to use in laboratory or field sitatuions. However, if all residual lung air is not free of ambient H_2O, this method leads to a lowered specific activity of water.

Assay of urine water is cumbersome, in that it requires animal collection equipment that restricts animal movement and does not always function properly. Urine formation and evacuation times must be estimated. Urine collection by palpation is subject to the same kind of time error as that of total urine collection. Furthermore, urine constituents containing tritium that are not removed by activated charcoal are assayed as ^3H_2O because refluxing does not remove all light-absorbing components.

Precipitation of blood proteins with TCA causes a variable amount of quench (Phillips et al. 1966) and may be a source of error unless corrected for. Additionally, non-TCA precipitable compounds containing tritium can result in error.

Saliva may offer the possibility of easier sample collection. However, it does contain mucoproteins, inorganic salts, and other non-water substances containing hydrogen.

Since the data in Trial 1 were collected from only one animal and the respired water was theoretically the best fluid to assay, a second trial was conducted to test further the reliability of the respired-water method.

The results of the blook water specific activity and that of respired water are shown in Table 7. A paired "t" test (Li 1964) indicated no statistical difference between the results using the two sampling methods. The data indicate that respired H_2O collected in the manner described is reliable for assay of tritiated water in animals and for the study of water partitioning and kinetics.

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EXPERIMENT NO. 4

WATER PARTITIONING AND FLUX IN SHEEP

Previous work indicated that total body water can be determined by isotope dilution, fecal water by ^{14}C-PEG dilution, and urine water by Lithium dilution. In order to test the utility of all three tracers, four sheep were given water and feed *ad libitum* in a controlled environment. Each animal was maintained in a metabolism stall and all feces and urine collected. The water consumed by the sheep contained $^{3}H_2O$, Lithium Chloride and ^{14}C-PEG. Table 8 shows a flow chart of analyses conducted.

A total of 4160 analyses were to be performed, not including any rechecks. This experiment was terminated during mid-August and the samples currently are being analyzed. Much of the raw data from respired-air water is complete and diagrammatically shown in Fig. 6-9. Preliminary calculations suggest that

total body water, feed water, water flux, metabolic water and respired water can be ascertained from these data. Fecal and urine water determinations are not complete. Until all samples are analyzed, evaluation and modelling will not be attempted.

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Table 1. Recovery of dyes added to fresh feces.

Dye	Hours Extracted	Hours with ether	Added (mg/g DM)	Found (mg/g DM)	Recovery (%)
Phloxine B	4	0	10.45	10.09	96.6
	4	0	10.45	10.09	96.6
	4	16	10.45	9.47	90.6
Violet	2	48	10.15	5.28	52.0
	2	0	10.15	9.38	92.4
	3	0	10.15	9.33	91.9
	5	0	10.15	9.23	90.9
	5	0	10.15	8.32	82.0
	16	4	10.15	7.95	78.3
	16	4	10.15	7.38	72.7
Red	4	0	9.29	9.19	98.9
	4	0	9.29	8.64	93.0
	4	0	9.29	8.54	91.9
	4	0	9.29	8.98	96.7

Table 2. Recovery of graded amounts of dyes when added to fresh feces.

Dye	Wet feces (mg/100g)	Added (mg/g DM)	Found (mg/g DM)	Recovery (%)
Phloxine B	5	0.2528	0.241	95.3
		0.2528	0.272	107.6
	10	0.5200	0.471	90.6
		0.5200	0.492	94.6
	15	0.7696	0.729	94.7
		0.7696	0.757	98.4
	20	1.0331	1.006	97.4
		1.0331	1.010	97.8
Violet	5	0.2617	0.206	78.7
		0.2617	0.209	80.0
	10	0.5221	0.467	89.4
		0.5221	0.436	83.6
	15	0.7696	0.671	87.2
		0.7696	0.662	86.0
	20	1.0325	0.919	89.0
		1.0325	o.941	91.1
Red	5	0.2626	0.251	95.3
		0.2626	0.216	82.1
	10	0.5241	0.479	91.4
		0.5241	0.457	87.1
	15	0.7886	0.722	91.5
		0.7886	0.804	101.9
	20	1.0346	0.985	95.2
		1.0346	1.040	100.4

Table 3. Recovery of dyes from rumen contents.

Days	% Recovered		
	Violet	Phloxine B	Red
0	84.0	97.3	88.0
1	67.5	93.3	93.3
2	58.3	93.3	93.5
3	80.3	93.5	93.5
4	65.8	95.8	86.1
5	74.5	93.8	86.8
6	62.3	94.0	61.9
7	61.0	94.5	80.4
8	lost	92.7	85.8
9	64.5	91.5	82.1
10	61.5	90.6	84.2

Table 4. Recovery of dye administered intrarauminally and collected in the feces.

Dye	Hours extracted	Recovery (%)
Phloxine B	4	70
	15	78
	20	77
Red	15	73
	16	70
	24	88
Violet	3	61
	4	52
	48[1/]	69

[1/] No heat applied.

Table 5. Recovery of added PEG from sheep feces.

No. of determinations	Average recovery (%)	Standard error of mean
8	100.0	±2.53

Table 6. Linear regression equations[1/] for tritium concentrations in body water.

Sample assayed	Intercept $_{-4}$ uCi/ml X 10	Slope
Jugular blood	138.7 ± 2.8[2/]	-0.00319
Urine, grab sample	142.2 ± 3.7	-0.00320
Urine, 24 hr. composite	139.5 ± 3.2	-0.00316
Respired air water	138.0 ± 3.3	-0.00349
Saliva	138.4 ± 2.4	-0.00338

[1/] Linear regression equation; Y = a + bX, when Y = specific activity (uCi ^3H_2O/ml of H_2O) at any time X, a = intercept at time zero, b = slope of line, X = time.

[2/] Standard deviation of intercept (7).

Table 7. Comparison of body water percentage and turnover rate calculated from either respired water of jugular blood[1/].

Sample source	No. of animals	Body water (%)	Turnover rate half time (days)
Respired water	12	67.6 ± 2.4[2/]	4.48 ± 0.16
Jugular blood	12	66.5 ± 3.1	4.45 ± 0.16

[1/] Paired t test showed no difference at the 10% level of significance between parameters determined from blood or respired water.

[2/] Standard error of mean.

Table 8. Schematic of samples taken for analyses.

	Consumption			Analytical determinations made				
	Feed	Water						
		Feed	Drinking	$^{3}H_2O$	Amt.	DM	Li	^{14}C-PEG
Feed	X	X			X	X		
Water			X	X				
Respired Air				X				
Urine				X	X	X	X	
Feces				X	X	X	X	X

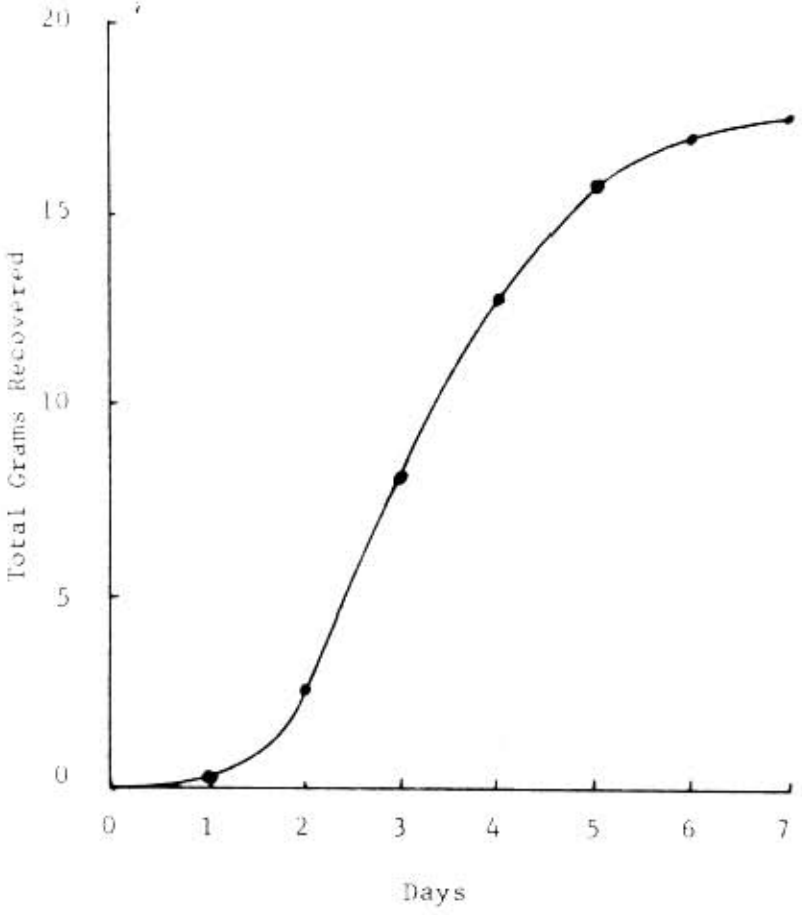


Figure 1. Accumulative recovery of phloxine B.

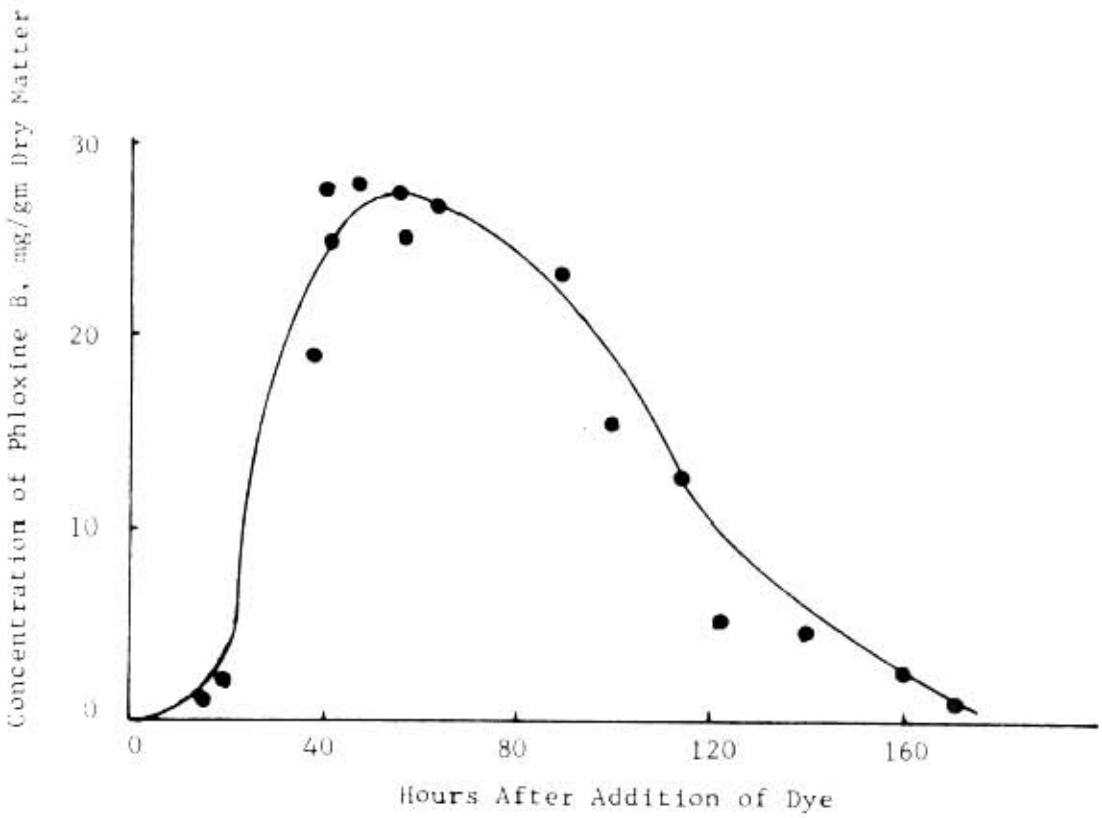


Figure 2. Concentration of phloxine B in feces at various times after initial dose.

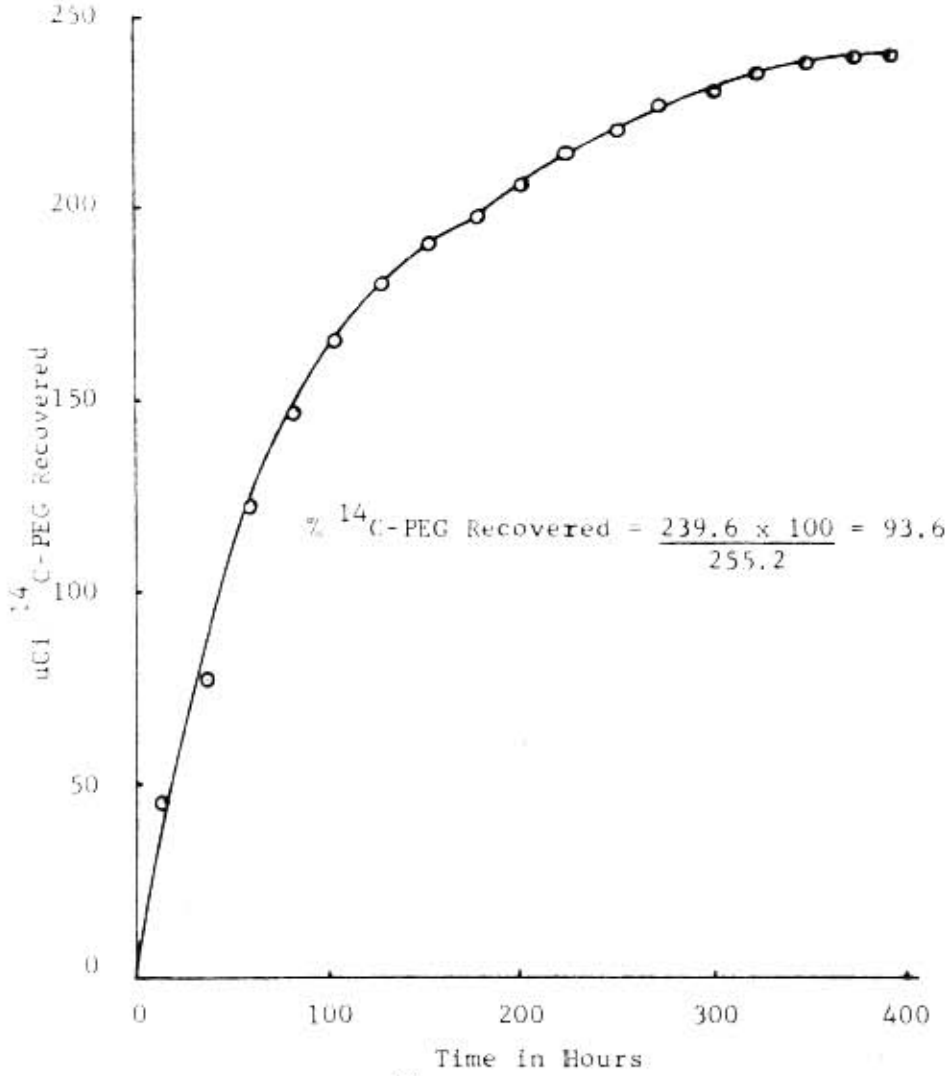
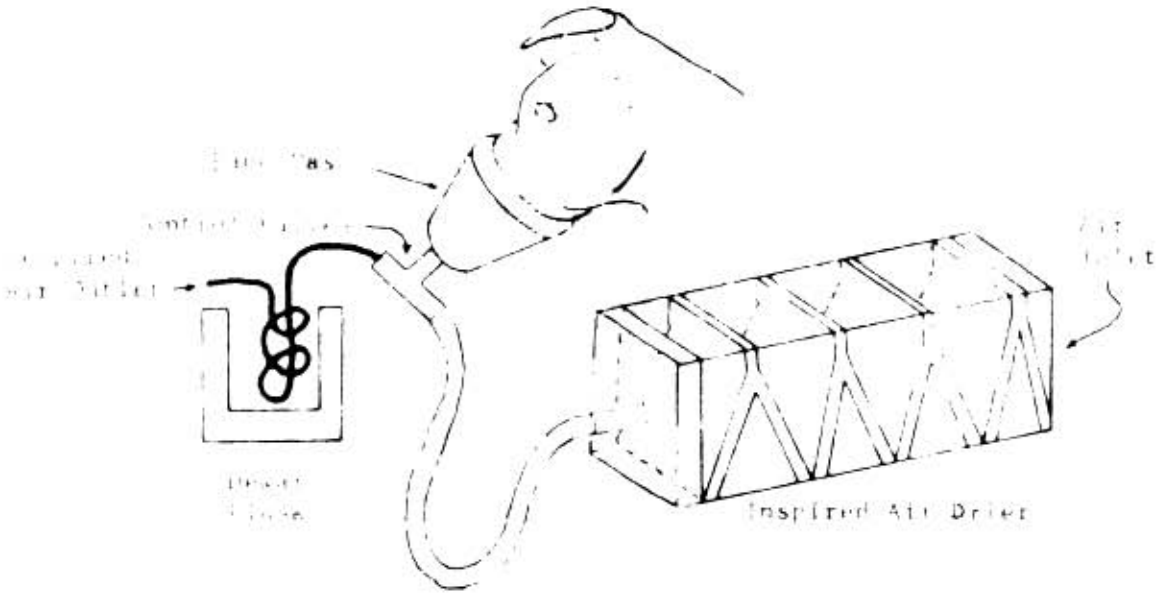


Figure 5. Recovery of ^{14}C-PEG following ruminal administration of 255.2 uCi PEG.



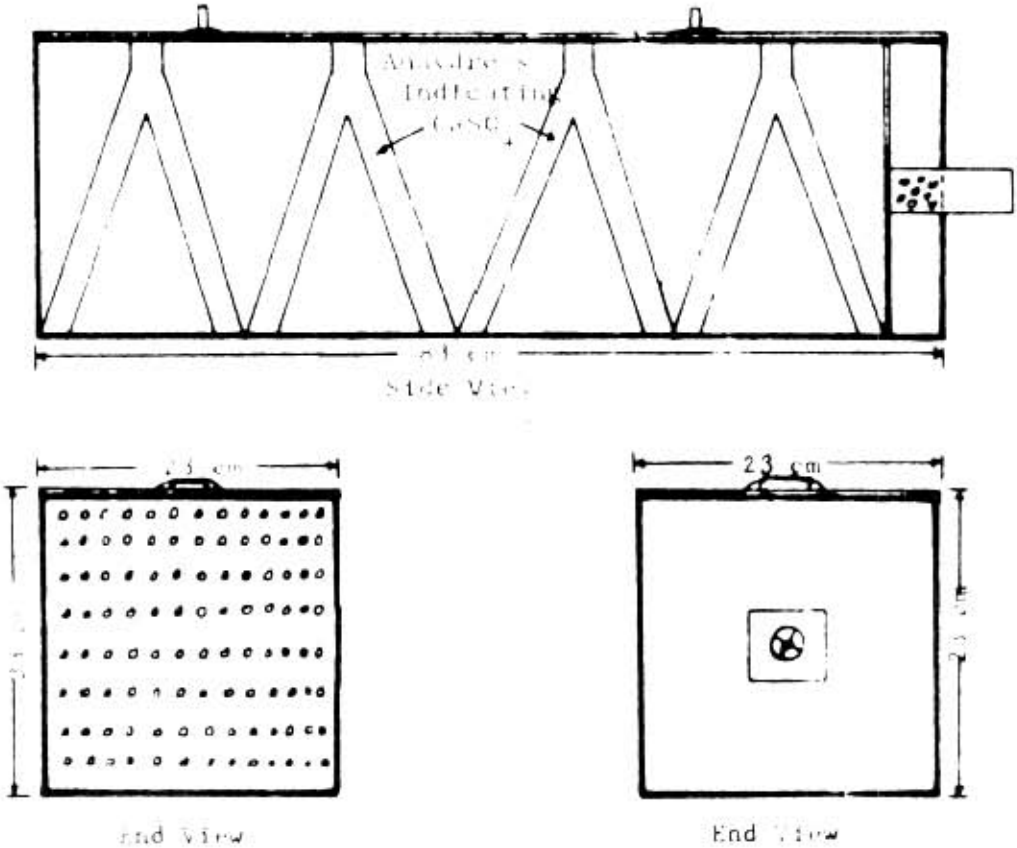


Figure 4. Schematic of apparatus for collecting respired water.

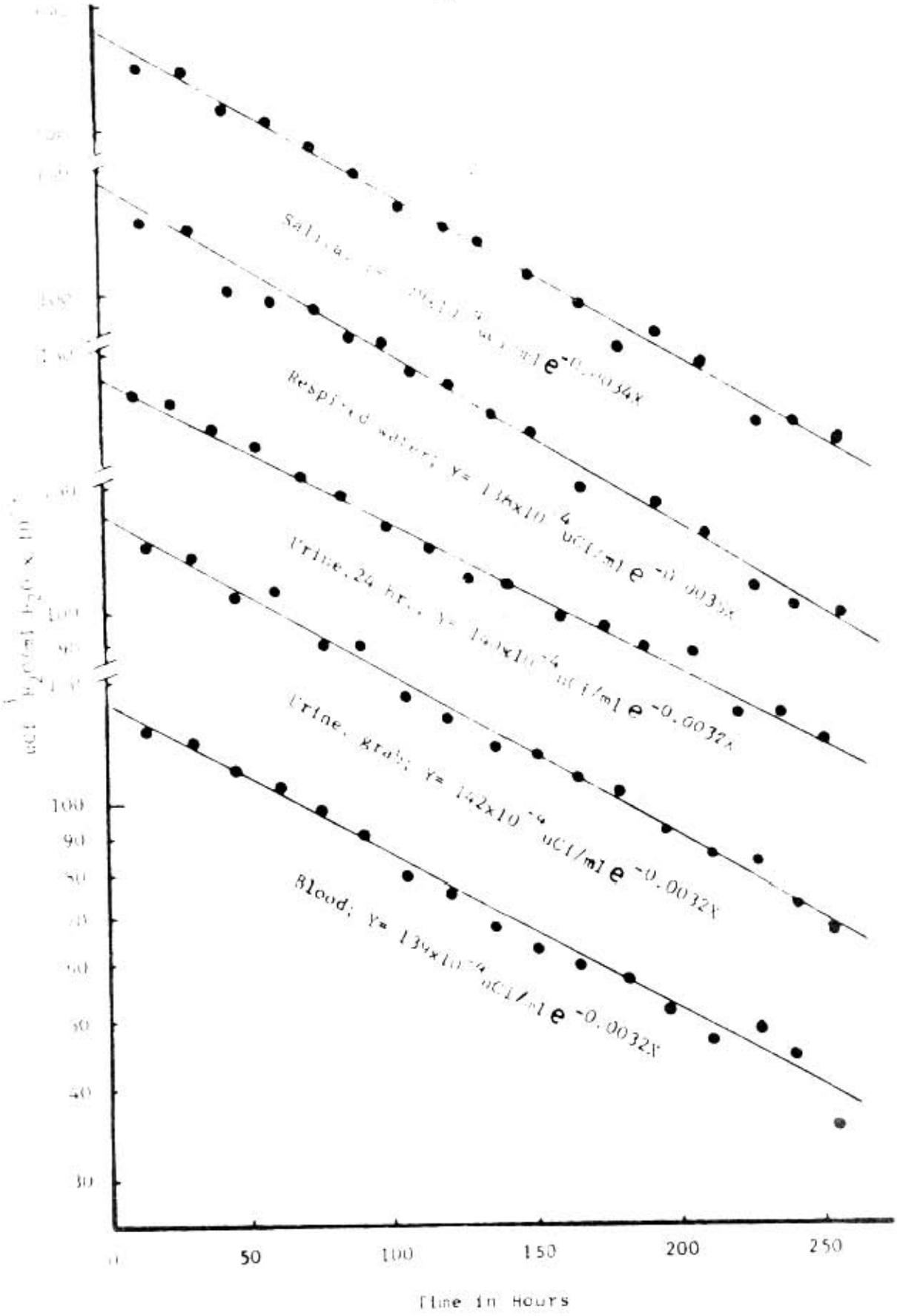


Figure 5. Regression for concentrations of tritium in body water over time lapse determined by 5 different procedures.

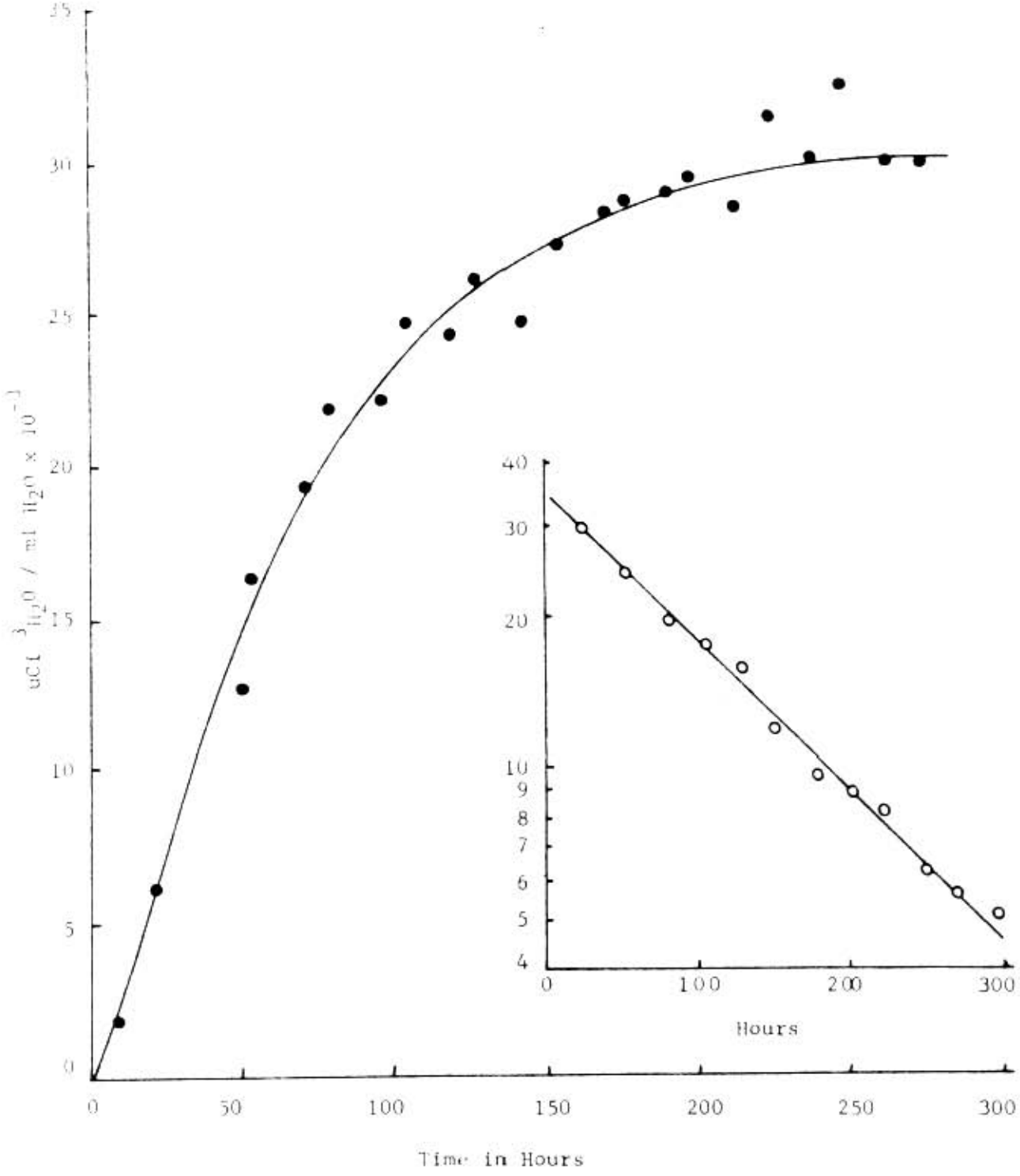


Figure 6. Specific activity of respired water during and after consumption of ^3H_2O in sheep number 1.

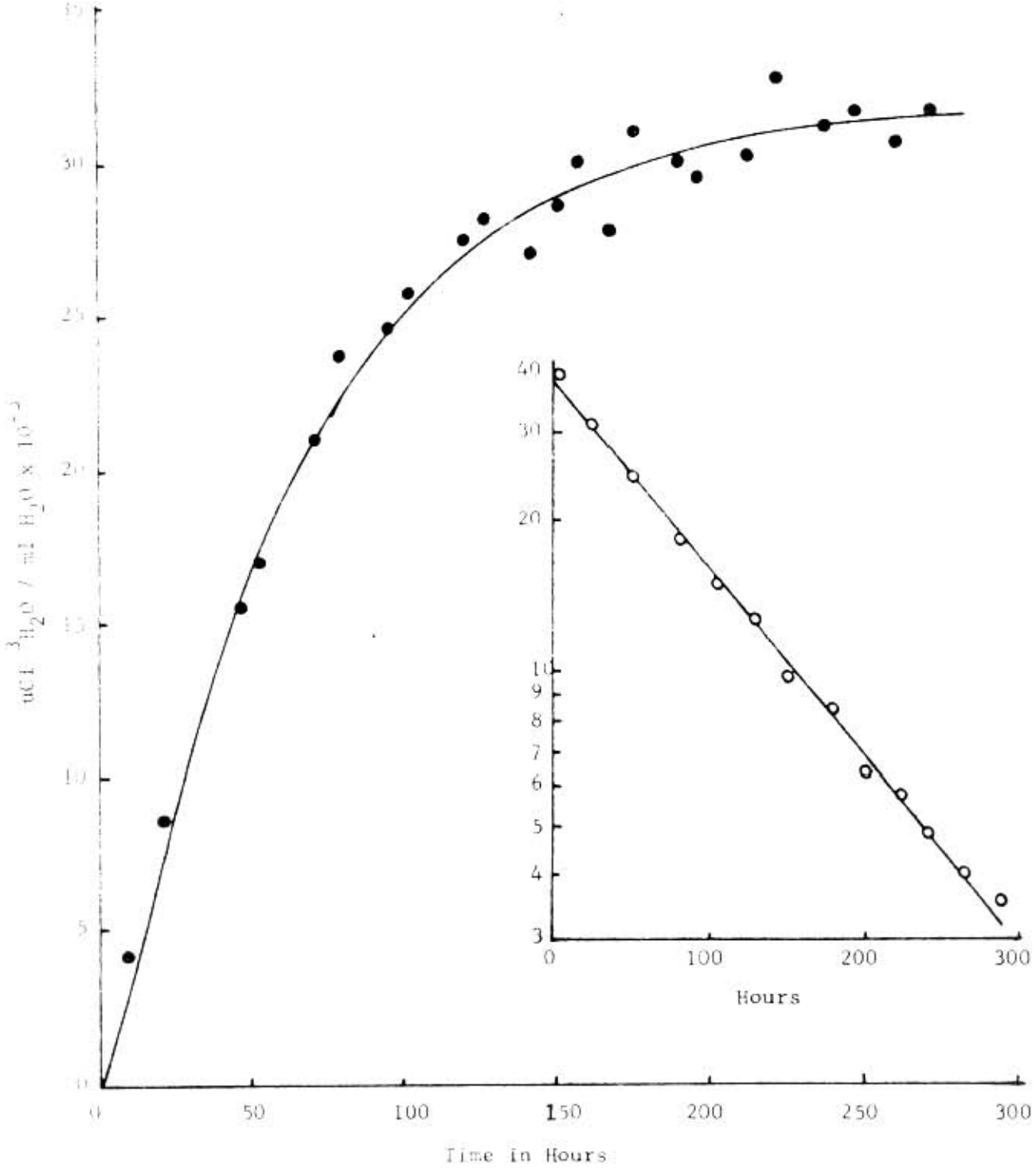


Figure 7. Specific activity of respired water during and following consumption of ^3H_2O in sheep number 2.

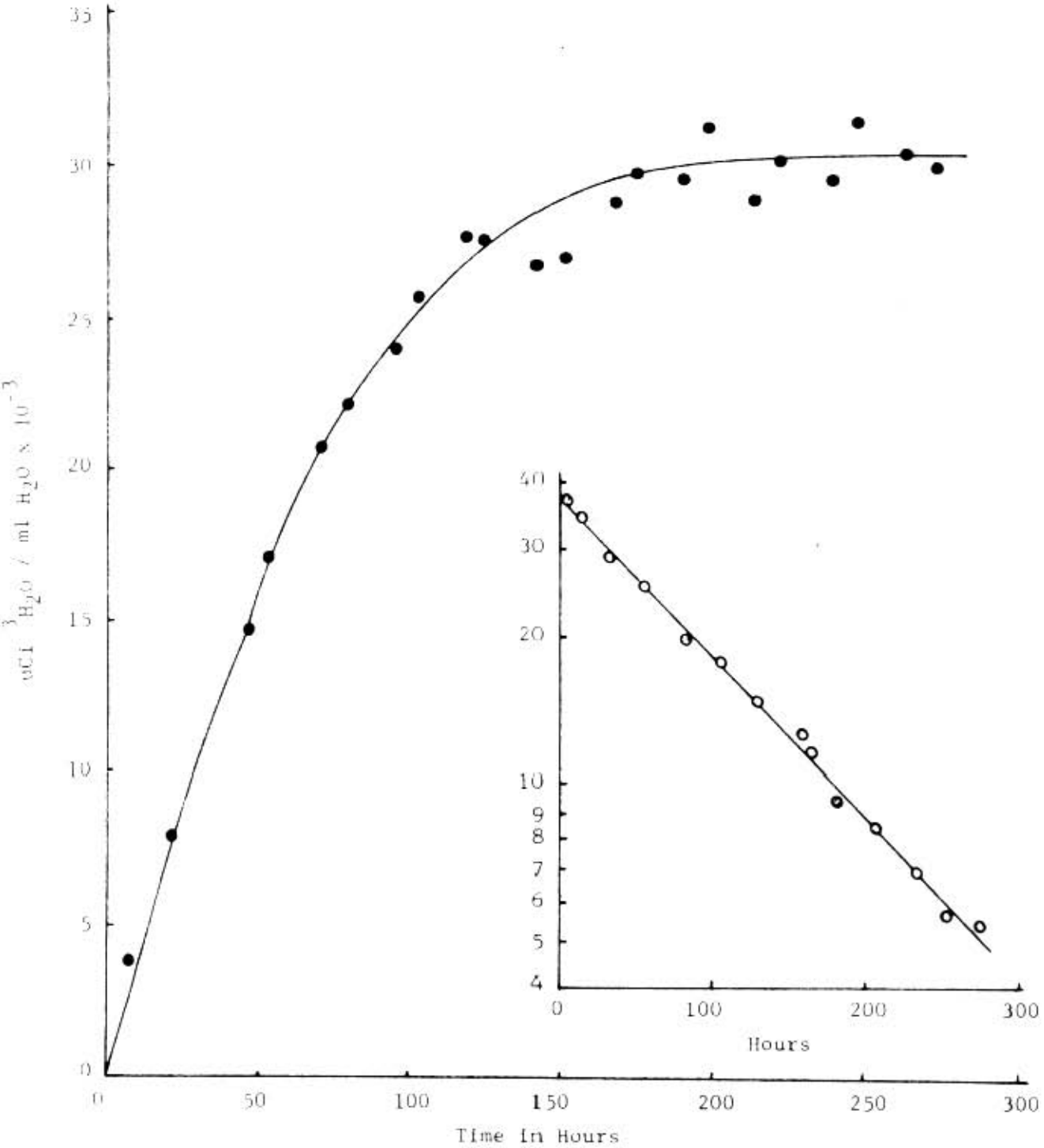


Figure 8. Specific activity of respired water during and following consumption of ^3H_2O in sheep number 3.

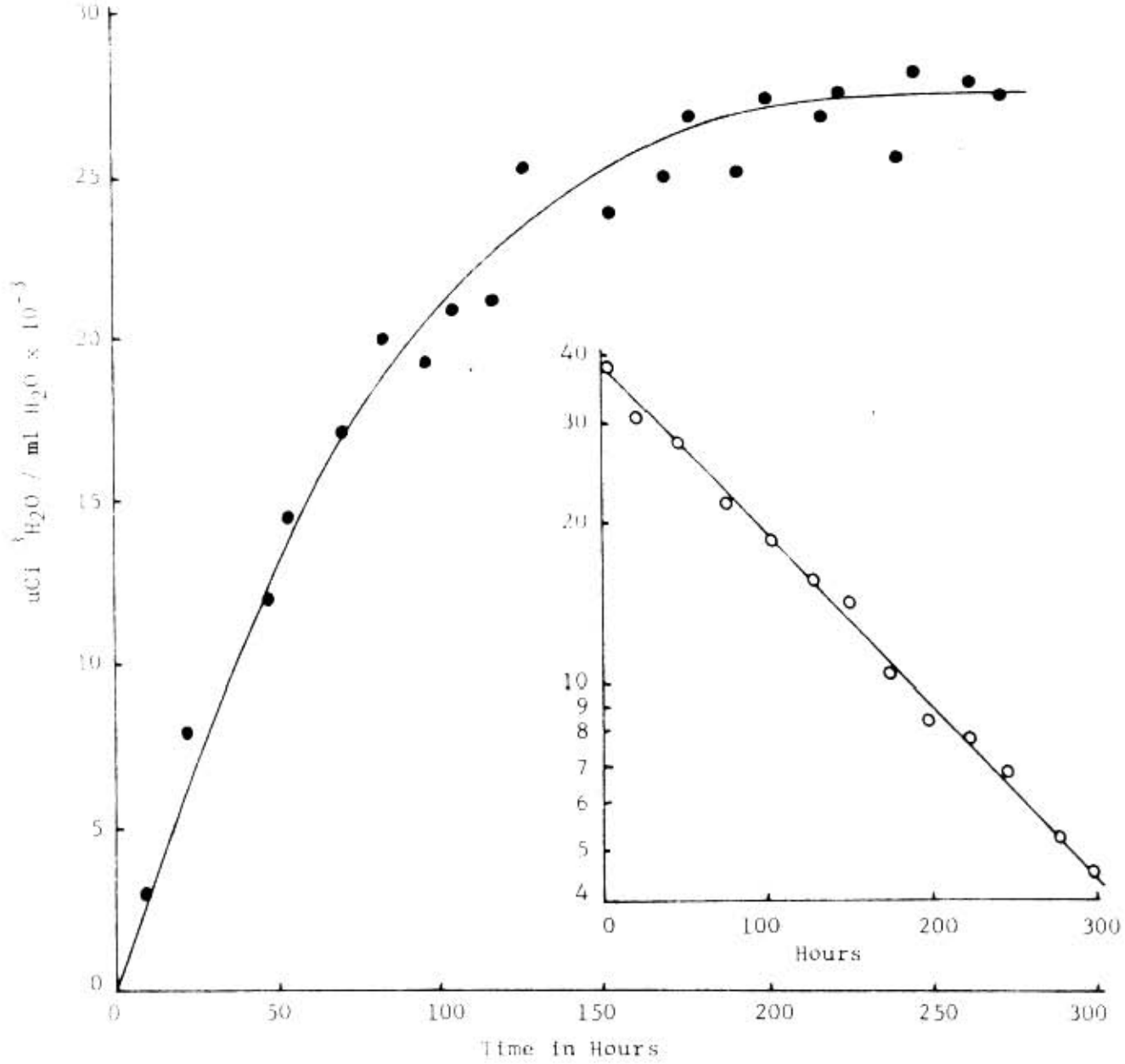


Figure 9. Specific activity of respired water during and following consumption of ^3H_2O in sheep number 4.