still be capable of substrate binding (cf. hypoxanthine binding by cyanide-treated xanthine oxidase, Fridovich & Handler, 1958*a*). However, in this case inhibition would be due to substrate-inhibitor rather than enzyme-inhibitor combination; this type of inhibition is important only when a sufficient number of inhibitor molecules are present to bind an appreciable proportion of the substrate molecules (Friedenwald & Maengwyn-Davies, 1954), and as the total xanthine oxidase concentration calculated from the E_{450} value did not exceed $1 \mu\text{M}$ in any of the assays, this type of inhibition may clearly be neglected in this work.

SUMMARY

1. The stabilities of two xanthine oxidase preparations have been compared under a variety of conditions; one of these preparations was found to be consistently more stable than the other.

2. Kinetic experiments indicated that the more stable sample contained a xanthine oxidase inhibitor; the presence of this could account for the observed stability differences.

3. Evidence bearing on the occurrence, formation and nature of the inhibitor are discussed briefly, and it is suggested that variations in its concentration may be responsible for certain discrepancies and anomalies in the properties of xanthine oxidase recorded in the literature.

The author thanks Professor F. Bergel, F.R.S., for his interest in this work and for many helpful discussions. The xanthine oxidase samples were prepared by Mr P. G. Avis

and Mr D. A. Gilbert, and Miss A. M. Scott rendered valuable technical assistance. The work has been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

REFERENCES

- Avis, P. G., Bergel, F. & Bray, R. C. (1955). *J. chem. Soc.* p. 1100.
 Avis, P. G., Bergel, F. & Bray, R. C. (1956). *J. chem. Soc.* p. 1219.
 Bergel, F. & Bray, R. C. (1956). *Nature, Lond.*, **178**, 88.
 Bergel, F. & Bray, R. C. (1959). *Biochem. J.* **73**, 182.
 Booth, V. H. (1938). *Biochem. J.* **32**, 503.
 Bray, R. C., Malmström, B. G. & Vänngård, T. (1959). *Biochem. J.* **73**, 193.
 Dixon, M. & Thurlow, S. (1924). *Biochem. J.* **18**, 976.
 Eden, A. & Green, H. H. (1940). *Biochem. J.* **34**, 1202.
 Fridovich, I. & Handler, P. (1958*a*). *J. biol. Chem.* **231**, 899.
 Fridovich, I. & Handler, P. (1958*b*). *J. biol. Chem.* **233**, 1578.
 Friedenwald, J. S. & Maengwyn-Davies, G. D. (1954). In *Mechanism of Enzyme Action*, p. 154. Ed. by McElroy, W. D. & Glass, B. Baltimore: Johns Hopkins University Press.
 Haddow, A., de Lamirande, G., Bergel, F., Bray, R. C. & Gilbert, D. A. (1958). *Nature, Lond.*, **182**, 1144.
 Hofstee, B. H. J. (1955). *J. biol. Chem.* **216**, 235.
 Laskowski, M. & Laskowski, M. (1954). *Advanc. Protein Chem.* **9**, 203.
 Lowry, O. H., Bessey, O. A. & Crawford, E. J. (1949). *J. biol. Chem.* **180**, 399.

Arteriovenous $^{14}\text{CO}_2$ Differences and the Pentose Cycle in the Cow's Udder

By H. G. WOOD, R. GILLESPIE, R. G. HANSEN,* W. A. WOOD* AND H. J. HARDENBROOK
Department of Biochemistry, School of Medicine, Western Reserve University, Cleveland, Ohio,
and the Department of Dairy Science and College of Veterinary Medicine,
University of Illinois, Urbana, Illinois, U.S.A.

(Received 23 March 1959)

There is a large body of evidence that the pentose cycle occurs in the mammary gland. This evidence is based: (1) on studies of the conversion of different types of labelled glucose into CO_2 (Abraham, Hirsch & Chaikoff, 1954; Glock, McLean & Whitehead, 1956; Duncombe & Glascock, 1956; Abraham, Cady & Chaikoff, 1957; Black, Kleiber, Butterworth, Brubacher & Kaneko, 1957); (2) on measurement of glucose 6-phosphate and 6-phosphogluconate dehydrogenases (Glock & McLean,

* Present address: Department of Agricultural Chemistry, Michigan State University, East Lansing, Michigan.

1953, 1954); (3) on the detection of some of the intermediate compounds of the pentose cycle in mammary tissues (Peeters & Debackere, 1956); and (4) on the determination of the distribution of ^{14}C in the 6 carbons of the glucose and galactose moieties of lactose (Schambye, Wood & Kleiber, 1957; Wood, Gillespie, Joffe, Hansen & Hardenbrook, 1958*a*; Wood, Joffe, Gillespie, Hansen & Hardenbrook, 1958*b*). However, Duncombe & Glascock (1956) have indicated that the direct oxidative pathway may not be as important in the udder of ruminants (sheep) as in the rat mammary

gland. Also Glock *et al.* (1956) state that in the mammary gland of sheep there was not a large increase in glucose 6-phosphate and 6-phosphogluconate dehydrogenase during lactation such as was observed in rats.

The investigations of the pentose cycle in mammary gland by means of labelled glucose have for the most part been done *in vitro* with slices. The investigation of Black *et al.* (1957) is an exception. These authors injected [$1\text{-}^{14}\text{C}$]- and [$6\text{-}^{14}\text{C}$]-glucose intravenously into lactating cows and found 1.6–3.6 times as much ^{14}C in the respiratory CO_2 with [$1\text{-}^{14}\text{C}$]- as with [$6\text{-}^{14}\text{C}$]-glucose. They also found more ^{14}C from [$6\text{-}^{14}\text{C}$]- than from [$1\text{-}^{14}\text{C}$]-glucose in the alanine and serine of casein and in the glycerol of fat. Both results indicate a pentose cycle and they conclude that the cycle plays a major role in glucose metabolism in the lactating cow.

Previous studies have shown that it is possible to investigate the metabolism of the udder in the intact cow (Wood, Siu & Schambye, 1957; Wood *et al.* 1958*a, b*) by injection of ^{14}C compounds directly into a pudic artery which supplies blood to only one side of the udder. A high specific activity of ^{14}C was observed in the milk from the injected side, which reflects the metabolism of the compound directly in the udder, and a lower activity was observed in the milk from the uninjected side which reflects the metabolism of the compound in the whole animal. In the present study the metabolism of the udder has been investigated by injecting labelled glucose into the arterial supply and then determining the arteriovenous difference in specific activity of CO_2 of the blood supply of the udder. The C-1 of glucose is oxidized in the udder to CO_2 far more rapidly than is C-6. [$1\text{-}^{13}\text{C}$]- or [$1\text{-}^{14}\text{C}$]-acetate was also included in the study and it is oxidized to CO_2 as rapidly as is the C-1 of glucose.

METHODS

The arterial blood supply of the cow's udder flows via the posterior aorta to the right and left external iliac arteries and then to the corresponding right and left pudic arteries. The major return of blood from the udder is through the right and left subcutaneous abdominal veins and the right and left pudic veins (Espe, 1948). There are collateral connexions in the venous circulation of the two sides of the udder, but relatively few in the arterial circulation (Turner, 1952). Arterial blood usually was obtained from either the posterior aorta or pudic artery, but in one experiment the blood of the jugular vein was taken as representative of arterial blood. The ^{14}C of the CO_2 leaving the udder was determined in blood from the subcutaneous abdominal vein. The arteriovenous difference in the specific activity of the CO_2 of the blood of the udder is considered to be proportional to the rate of oxidation of the labelled carbon of the compound tested.

A Holstein cow weighing about 750 kg. and producing about 22 l. of milk per day was used in the experiments of

Tables 1–3. She had been used previously in experiments by Wood *et al.* (1958*a, b*). For the experiment of Table 4 a Holstein cow was used which weighed about 500 kg. and produced about 18 l. of milk/day. Local anaesthesia of the left flank of the cow was produced by injecting procaine into the left epidural space of the lumbar spinal column (St Clair & Hardenbrook, 1956; Wood *et al.* 1958*b*). With the cow in a normal standing position an incision was made through the abdominal wall, and the posterior aorta and left pudic artery were located by palpation. The labelled compound was infused into the pudic artery or posterior aorta by using a constant-speed mechanical syringe attached with Tygon tubing (Arthur H. Thomas, Philadelphia) to a hypodermic needle. Blood was drawn at as nearly the same rate as possible from the selected vessels. It required about 30 sec. to draw the blood and the time given is the mid-point of the collection.

The CO_2 was liberated from the blood samples in an all-glass apparatus with ground-glass joints consisting of a 500 ml. flask fitted with a funnel and connected by a large-bore tube to a 50 ml. flask. The 500 ml. flask contained 100 ml. of H_2O , 5 ml. of 5*N*- H_2SO_4 and 1 ml. of octyl alcohol. A volume (3 ml.) of 2.5*N*-NaOH was in the 50 ml. flask and 2 ml. of mineral oil and 3 ml. of 3.3% sodium citrate were in the funnel. The assembled equipment was evacuated before the experiment. The blood was introduced in the funnel under the mineral oil and then was rapidly admitted to the 500 ml. flask. The 500 ml. flask then was shaken in a hot-water bath for 5 min. while the 50 ml. flask was cooled in ice. The ^{14}C and ^{13}C of the CO_2 were determined with a gas-phase proportional counter (Bernstein & Ballentine, 1950) and a double-beam mass spectrometer (Nier, 1947) respectively.

In some of the experiments the acetate or glucose, or both, were isolated from the blood. The acidified blood from the 500 ml. flask was neutralized to pH 7 with NaOH solution and then deproteinized with 40 ml. of 5% ZnSO_4 and 40 ml. of 0.3*N*-Ba(OH) $_2$. The filtrate from this mixture was made just alkaline to phenolphthalein and was then freeze-dried. The dry powder was dissolved in water, acidified to a Congo red end-point and extracted continuously with ether into water. The combined ether and water of the extract was steam-distilled, the volatile acids were titrated and the solution was evaporated to dryness. The volatile acids were then chromatographed on a Celite column (Swim & Krampitz, 1954) to obtain the acetic acid, which was titrated, diluted with carrier acetate, and then oxidized to CO_2 (Van Slyke & Folley, 1950). The ^{13}C content of the CO_2 was determined and the total excess of ^{13}C was calculated for the carboxyl carbon of the acetate.

The residue of the ether extraction was deionized with Amberlite resin, the reducing sugar was determined (Somogyi, 1945; Nelson, 1944), carrier glucose was added and the phenylosazone was prepared and recrystallized (Baker, Shreeve, Shipley, Incefy & Miller, 1954). The osazone was oxidized to CO_2 (Van Slyke & Folley, 1940), its ^{14}C content was determined and the specific activity of the osazone was calculated.

RESULTS AND DISCUSSION

Comparison of oxidation of [$1\text{-}^{14}\text{C}$]- and [$6\text{-}^{14}\text{C}$]-glucose to CO_2 . In the first experiment 100 μC of labelled glucose was injected into the left pudic

artery over a period of 15 sec. and then blood was collected at intervals from the left subcutaneous abdominal and jugular veins. The metabolism of [1-¹⁴C]- and [6-¹⁴C]-glucose was tested alternately (Table 1). The CO₂ of the blood of the jugular vein was assumed for purposes of this experiment to be comparable with that of the arterial blood supplying the udder. This assumption was valid, as illustrated in the experiment of Table 3, in which blood from both the posterior aorta and jugular vein was collected.

There was an immediate rise in the specific activity of the CO₂ of the subcutaneous abdominal vein above that of the jugular vein when [1-¹⁴C]-glucose was injected (tests *A* and *C*). The highest difference was observed at the time of the first collection, 2 min. after the injection. The values for the two tests were not the same, however, test *A* giving an arteriovenous difference of 12.0 counts/min./μmole of CO₂, and test *C* giving 3.8. It seems likely that the principal cause of disagreement was the timing of the injection and of the collection of the blood. Apparently the specific activity of the CO₂ rose and fell rapidly and was falling rapidly at 2 min. With [6-¹⁴C]glucose, test *B*, there was only a small arteriovenous difference in the specific activity of the CO₂ of the blood and in test *D* the arteriovenous difference was actually reversed, i.e. it was slightly higher in the jugular vein. The results clearly show that [1-¹⁴C]glucose gives rise to CO₂ more rapidly than does [6-¹⁴C]glucose and thus indicates that the pentose cycle occurs in the udder. These experiments, therefore, supplement those of Black *et al.* (1957), which were based on the composite metabolism of the whole cow.

The changes in the specific activity of the CO₂ of the blood in the subcutaneous abdominal vein were very different from those found in the respiratory CO₂ by Black *et al.* (1957). They found that the maximum specific activity was reached in about 30 min. and then the ¹⁴C activity fell slowly. This delayed maximum and prolonged high activity apparently occurred because the labelled glucose mixed with the large body pool of glucose and after the initial equilibration the specific activity of the glucose did not change rapidly (Black *et al.* 1957; Kleiber *et al.* 1955). In addition, the bicarbonate and CO₂ pools of the body are large and consequently considerable time is required for these pools to reach their maximum specific activity. In contrast, injection via the pudic artery rapidly raises the specific activity of the glucose in the udder, but the specific activity of the glucose in the whole body pools remains relatively low. However, as soon as the injection is stopped the ¹⁴C activity of the glucose in the udder drops because glucose with low activity now enters the udder. Consequently there was a rapid rise and fall in the specific activity of the blood CO₂ in the subcutaneous abdominal vein. However, the changes in specific activity of the CO₂ of the blood from the jugular vein were similar to those observed by Black *et al.* (1957) in the respiratory CO₂, i.e. reaching a maximum at about 26 min. and then remaining constant. This similarity is to be expected, since both the blood CO₂ of the jugular vein and respiratory CO₂ reflect the changes in the pools of the entire body.

Equilibration between blood and tissue bicarbonate in the udder. The fact that the specific activity of

Table 1. *Arteriovenous difference in specific activity of CO₂ of the blood supply of the udder after injecting [1-¹⁴C]- or [6-¹⁴C]-glucose in the left pudic artery of a cow*

Aq. 0.09% NaCl containing 100 μc of labelled glucose (National Bureau of Standards) (5 ml.) was injected into the left pudic artery in approximately 15 sec. Blood (20 ml.) was withdrawn at the indicated times simultaneously from the left subcutaneous abdominal vein and the jugular vein, and the CO₂ was collected from the blood. The injections of labelled sugars were at 3.12, 3.58, 4.49 and 5.40 p.m. After completion of test *B*, 50 units of oxytocin were injected in the jugular vein and the cow was milked; 12 l. was obtained.

Values are specific activities expressed as counts/min./μmole of CO₂. The time is that after completion of the injection of the labelled glucose.

	Test A [1- ¹⁴ C]Glucose					Test B [6- ¹⁴ C]Glucose				
	2	6	14	26	42	2	6	14	26	42
Time (min.) ...	...	...	...	...	...	...	...	...	...	...
Subcutaneous abdominal vein	12.2	3.08	1.62	1.21	1.18	1.29	1.34	1.10	1.13	1.13
Jugular vein	0.2	0.99	1.13	1.24	1.10	1.06	1.16	1.31	1.13	1.29
Arteriovenous difference	12.0	2.09	0.49	-0.04	0.08	0.23	0.18	-0.21	0.00	-0.16

	Test C [1- ¹⁴ C]Glucose					Test D [6- ¹⁴ C]Glucose				
	2	6	14	26	42	2	6	14	26	42
Time (min.) ...	...	...	...	...	...	...	...	...	...	...
Subcutaneous abdominal vein	5.32	—	2.26	2.29	2.19	2.18	2.45	2.48	2.66	2.82
Jugular vein	1.52	1.94	2.31	2.74	2.76	2.78	2.89	2.87	2.65	3.14
Arteriovenous difference	3.80	—	-0.05	-0.45	-0.57	-0.60	-0.44	-0.39	-0.01	-0.32

the CO_2 was sometimes higher in the jugular vein than in the subcutaneous abdominal vein (Table 1) was of interest and was not expected. It seemed likely that this dilution of $^{14}\text{CO}_2$ of the blood was due in part to an equilibration with unlabelled bicarbonate of the different pools in the udder, including that of the milk. The experiment in Table 2 was done to obtain information on the rate of this equilibration. This information was essential for interpretation of data from the experiments with labelled glucose and acetate. A solution of $\text{NaH}^{14}\text{CO}_3$ was injected at constant rate into the posterior aorta and blood samples were obtained during the course of the injection from the left pudic artery and the left subcutaneous abdominal vein. The amount of CO_2 in the blood and its specific activity were determined and from these values the total ^{14}C in the blood was calculated. The results are shown in Table 2.

The specific activity of the CO_2 of the blood decreased greatly as it passed through the udder. At 8 min. the decrease was 32 %. The increase in the arteriovenous CO_2 was from 533 to 584 $\mu\text{moles}/20\text{ ml.}$ of blood and therefore was not enough to account for the dilution of $^{14}\text{CO}_2$ which occurred in the blood. Clearly this decrease in specific activity was in large part due to equilibration with unlabelled bicarbonate of the udder. This fact is apparent since the total counts/min. as CO_2 in the blood decreased as it passed through the udder, while there was no net uptake of CO_2 from the blood. The decrease in ^{14}C activity varied from 43 % at 2 min. to 26 % at 8 min. The results indicate clearly the complications in experiments involving arteriovenous differences of CO_2 formed from labelled compounds. It should theoretically be possible by use of the isotope-dilution technique

to determine the total CO_2 produced from unlabelled compounds in the metabolism of the udder or, conversely, to determine the amount of CO_2 produced from a labelled compound. However, this would be difficult because it would be necessary to know the size of the bicarbonate pool of the udder and blood and the rate of equilibration of the bicarbonate of the blood and udder. During a period of time sufficient for complete equilibration to occur, compounds other than the one injected would acquire ^{14}C activity owing to metabolism in the udder or in other organs of the body, and these compounds would in turn contribute to the $^{14}\text{CO}_2$.

Comparison of oxidation of C-1 of glucose and C-1 of acetate to carbon dioxide. Baxter, Kleiber & Black (1955) have estimated that the lactating cow derives only 10 % of its CO_2 from pathways involving oxidation of glucose and that glucose is less important in the energy metabolism of the cow than it is in the metabolism of dogs and rats. This is in accord with the fact that most of the non-nitrogenous material absorbed from the digestive tract of ruminants is in the form of volatile fatty acids (Barcroft, McAnally & Phillipson, 1944). It is possible, however, that the metabolism of glucose may play a proportionally greater part in the udder than in the whole animal, since the pentose cycle may serve as a major source of reduced triphosphopyridine nucleotide for the synthesis of fat in the mammary gland (Seubert, Greult & Lynen, 1957; Lachance, Popják & de Waard, 1958). It therefore was of interest to compare the oxidation of the C-1 of glucose with the C-1 of acetate. For this purpose [$1\text{-}^{14}\text{C}$]glucose and [$1\text{-}^{13}\text{C}$]acetate were injected into the left pudic artery, and the glucose and acetate from the blood of the left subcutaneous abdominal vein were isolated and their ^{14}C and ^{13}C contents

Table 2. *Arteriovenous difference in the specific activity of the bicarbonate of blood during its passage through the udder*

Bicarbonate solution (2.5 ml.) containing about 9.8×10^6 counts/min. was injected per min. into the posterior aorta. The collection of blood from the left pudic artery and the left subcutaneous abdominal vein was started 2 min. after the beginning of the injection. The injection of $\text{NaH}^{14}\text{CO}_3$ was continuous until all samples of blood were collected.

An average of 533 μmoles of CO_2 was found in 20 ml. of blood of the pudic artery and 584 μmoles in 20 ml. of blood of the subcutaneous abdominal vein. The total ^{14}C was calculated from these values and the corresponding specific activities of the CO_2 (e.g. $533 \times 26.5 = 14120$ or 14.12×10^3).

		Time (min.)			
		2	4	6	8
Specific activity of CO_2 of blood (counts/min./ μmole)	Pudic artery	26.5	—	30.6	29.6
	Subcutaneous abdominal vein	13.8	15.3	19.0	20.1
Arteriovenous difference		12.7	—	11.6	9.5
Decrease in specific activity (%)		48	—	38	32
$10^{-3} \times$ Total ^{14}C in CO_2 of 20 ml. of blood (counts/min.)	Pudic artery	14.12	—	16.32	15.80
	Subcutaneous abdominal vein	8.06	8.94	11.10	11.72
Decrease in total ^{14}C		6.06	—	5.22	4.08
Decrease in total ^{14}C (%)		43	—	32	26

determined. The arteriovenous ^{14}C and ^{13}C differences of the blood CO_2 were calculated. The relative rates of oxidation of the $[1\text{-}^{14}\text{C}]\text{glucose}$ and $[1\text{-}^{13}\text{C}]\text{acetate}$ to CO_2 were to be compared on the basis of the following ratios: (1) specific activity of blood CO_2 divided by specific activity of blood glucose; (2) percentage excess of ^{13}C in CO_2 divided by percentage excess of ^{13}C in the acetate. Since the $^{13}\text{CO}_2$ and $^{14}\text{CO}_2$ presumably would undergo dilution in the same pools, complete equilibration of the blood bicarbonate and tissue bicarbonate would not be necessary for purposes of this comparison.

The results are shown in Table 3. After injection of labelled glucose and acetate there was no detectable difference between the ^{14}C activity of the CO_2 of the jugular vein and that of the posterior aorta, apparently because of the low specific activity of the blood glucose in the general circulation. On the injected side of the udder a substantial arteriovenous $^{14}\text{CO}_2$ difference was observed and it increased continuously with time to 6.37 counts/min./ μmole . On the uninjected side of the udder the arteriovenous difference was much less. This was to be expected, since the specific activity of the $[1\text{-}^{14}\text{C}]\text{glucose}$ was low on this side of the udder. There was very little ^{13}C in the CO_2 , even in the blood from the injected side of the udder, and the excess of ^{13}C was too low to be detected in the remainder of the circulation. The ^{13}C content was low not because the $[1\text{-}^{13}\text{C}]\text{acetate}$ was oxidized

slowly; rather it was because of the large dilution of ^{13}C in the acetate and bicarbonate pools (see Table 4).

There was very little mixing of venous blood from the left and right sides of the udder, even though there are collateral connexions in the venous circulation of the udder (Turner, 1952). If there had been mixing of blood from the left and right sides of the udder, the blood from the two sides would have been more nearly alike and the specific activity of the CO_2 of the right subcutaneous abdominal vein would have been substantially above that of the posterior aorta.

The specific activity of the glucose of the blood from the subcutaneous abdominal vein increased with time from 2435 counts/min./ μmole at 2.7 min. to 3075 at 4.3 min. There also was an increase in the percentage excess of ^{13}C in the acetate from 0.34 to 0.84 % during this time. The $[1\text{-}^{14}\text{C}]\text{glucose}$ entering via the pudic artery has been estimated to have contained 5.0×10^3 counts/min./ μmole . Turner (1952) states that approximately 7 l. of blood pass through the udder/min. in a cow yielding 20 l. of milk/day. The cow of this experiment produced about 22 l./day, and if 4 l. of blood passed through one-half of the udder and the blood contained 49 mg. of glucose/100 ml. (Baxter *et al.* 1955), there would have been $11 \times 10^3 \mu\text{moles}$ of glucose passing/min. Approximately 55×10^6 counts/min. were injected/min. as $[1\text{-}^{14}\text{C}]\text{glucose}$ and therefore the calculated specific activity for glucose is

Table 3. *Arteriovenous difference in the specific activity of CO_2 of the blood during constant injection of $[1\text{-}^{14}\text{C}]\text{glucose}$ and $[1\text{-}^{13}\text{C}]\text{acetate}$ into the left pudic artery*

The experiment was done with the cow used in the experiment of Table 2 and there was 1.6 hr. between the two experiments. For this reason the blood contained about 1.57 counts/min./ μmole of CO_2 at the start of the experiment. 12 ml. of a solution containing $150 \mu\text{C}$ of ^{14}C in 0.33 m-mole of $[1\text{-}^{14}\text{C}]\text{glucose}$ and 3.2 m-moles of $[1\text{-}^{13}\text{C}]\text{acetate}$ with 40 % excess of ^{13}C in the carboxyl group was injected at a constant rate into the left pudic artery during 4.5 min (approximately 2.7 ml./min.). During the injection blood was removed simultaneously from the posterior aorta, the jugular vein, the left subcutaneous abdominal vein and the right subcutaneous abdominal vein.

Time (min.)	...	...	...	1.2	2.7	4.3	2.7	4.3
				Counts/min./ μmole of CO_2			Counts/min./ μmole of glucose	
Posterior aorta				1.58	1.61	—	125	310
Jugular vein				1.58	1.57	1.63	—	—
Subcutaneous abdominal vein (injected side)				2.08	4.12	8.00	2435	3075
Subcutaneous abdominal vein (non-injected side)				1.65	1.66	1.86	—	—
Arteriovenous difference (injected side of udder)				0.50	2.51	6.37*	—	—
Arteriovenous difference (non-injected side of udder)				0.07	0.05	0.23*	—	—
				% Excess of ^{13}C in CO_2			% Excess of ^{13}C in C-1 of acetate	
Subcutaneous abdominal vein (injected side)				—	0.002	0.009	0.34	0.84

* Specific activity of the CO_2 of the blood from the jugular vein was used for these calculations.

5.0×10^3 counts/min./ μ mole. In the 4.3 min. about 17.2 (4.3×4) l. of blood would enter the injected side of the udder, and it seems likely that this quantity of blood would displace all the pre-injection blood from the injected half of the udder. The fall in specific activity of the blood glucose as it passed through the udder was apparently due to equilibration with unlabelled glucose of the tissue of the udder. The increase with time in the specific activity of the outflowing glucose would be expected as equilibration progressed. The increase cannot be accounted for by the return of ^{14}C from the general circulation, since there was little increase in the specific activity of the glucose of the posterior aorta (125 counts/min./ μ mole at 2.7 min. and 310 at 4.3 min.).

The estimated value for the carboxyl group of acetate was 3.8 % excess of ^{13}C . McClymont (1949, 1951) states there is about 10 mg. of acetate/100 ml. in the blood of cows. Therefore 7.42 mmoles of acetate were passing/min. Since 0.72 mmole of acetate with 40 % excess of ^{13}C in C-1 was injected/min., the calculated excess of ^{13}C for the C-1 of acetate is 3.8 % ($0.72/7.42 \times 40.0$). The highest value observed in the outflowing blood of the udder was 0.84 %. It would appear from this calculation that there was a very rapid decrease in the specific activity of the blood acetate which probably was caused by equilibration with unlabelled acetate present in the udder.

Unfortunately the data of Table 3 do not permit comparison of the rates of oxidation of C-1 of glucose and C-1 of acetate, because the excess of ^{13}C in the CO_2 was too low to permit accurate determination of the ^{13}C . For this reason an experiment was done with $[1-^{14}\text{C}]\text{acetate}$, in which the acetate was injected into the posterior aorta. This permitted direct measurement of the specific activity of the $[1-^{14}\text{C}]\text{acetate}$ entering the udder via the pudic artery and thus avoided the necessity of calculating the ^{14}C activity on the basis of an

assumed blood flow and acetate content in the blood. The procedure also avoided the possibility of dilution of the $[^{14}\text{C}]\text{acetate}$ through collateral venous circulation, since both sides of the udder receive the labelled acetate from the posterior aorta.

The results are shown in Table 4 and confirm the observation that there is a decrease in the specific activity of the $[1-^{14}\text{C}]\text{acetate}$ during the passage through the udder. The $[^{14}\text{C}]\text{acetate}$ was diluted 2.5 (14 900/6080) times at 4 min. in the experiment of Table 4, whereas it was calculated to be diluted 4.5 (3.8/0.84) times at 4.3 min. in the experiment of Table 3.

Unfortunately only one set of values were obtained from the blood CO_2 (Table 4) and these were at 7 min. The specific activity of the CO_2 of the subcutaneous abdominal vein was 0.17 % [100 (15.4/8850)] of that of the $[^{14}\text{C}]\text{acetate}$ of the vein. The value was 0.21 % [100 (6.37/3075)] (Table 3) with $[1-^{14}\text{C}]\text{glucose}$ at 4.3 min. It would therefore appear that the C-1 of glucose and C-1 of acetate were oxidized to CO_2 at approximately the same rate. However, the comparison probably cannot be accepted at face value since the calculations involve a number of assumptions which may not be valid. For example, it is assumed the C-1 of glucose and C-1 of acetate undergo equal dilution of ^{14}C during their conversion into CO_2 . It is probable, however, since the pools of intermediates of the Krebs cycle are interconvertible with amino acids and fatty acids, that the C-1 of acetate undergoes a greater dilution than does the C-1 of glucose in the intermediates of the pentose cycle. Further, the glucose and acetate may attain steady-state isotope concentrations at different rates with their counterparts in the tissues of the udder and this would lead to error. Clearly the comparison of the rates of oxidation of glucose and acetate oxidation is only a rough approximation.

Oxidation of $[1-^{14}\text{C}]\text{glucose}$ to $^{14}\text{CO}_2$ by blood in vitro. As a control for the above experiments

Table 4. *Arteriovenous difference in the specific activity of the CO_2 and acetate of blood during the constant injection of $[1-^{14}\text{C}]\text{acetate}$ into the posterior aorta*

A solution of $[1-^{14}\text{C}]\text{acetate}$ (Volk) containing 25 μC of ^{14}C and 7.2 μ moles of acetate/ml. was injected at a rate of 3.2 ml./min. in the posterior aorta. Blood was collected from the left pudic artery and left subcutaneous abdominal vein. The CO_2 samples at 2 and 4 min. were lost.

		Time (min.)		
		2	4	7
Specific activity of acetate of blood (counts/min./ μ mole)	Pudic artery	10 400	14 900	10 100
	Subcutaneous abdominal vein	4 070	6 080	8 850
Arteriovenous difference of specific activity of acetate		6 330	8 820	1 250
Specific activity of CO_2 of blood (counts/min./ μ mole)	Pudic artery			16.5
	Subcutaneous abdominal vein			31.9
Arteriovenous difference of specific activity of CO_2				15.4

blood was incubated *in vitro* with [1-¹⁴C]glucose. Quite unexpectedly it was found that the initial value of the ¹⁴CO₂ was high and that there then was a slow increase in the activity of ¹⁴CO₂. Further study showed that the initial evolution of ¹⁴CO₂ was due to a slight contamination of the [1-¹⁴C]glucose with [¹⁴C]-bicarbonate or -carbonate. The slow continuous evolution apparently was due to oxidation of [1-¹⁴C]glucose by the blood cells. The results of these experiments are shown in Table 5. In experiment A at 1 min., the CO₂ contained 2.88 counts/min./μmole. There were 588 μmoles of CO₂ in the 20 ml. of blood; thus this is equivalent to 1693 counts/min. Each 20 ml. of blood contained 1 μC of [1-¹⁴C]glucose, or 1.65×10^6 counts/min. on the counting equipment used. Therefore, about 0.1 % of the counts added as glucose was CO₂.

In Table 1 values of 12.0 and 3.8 counts/min./μmole of CO₂ were obtained for the arteriovenous differences at 2 min. Since this is the time after termination of the injection of [1-¹⁴C]glucose it is probable that most of the contaminating [¹⁴C]-bicarbonate had passed out of the udder with the blood before collection of the blood sample. Therefore the results probably were not greatly affected by the presence of the ¹⁴C contaminant in the [1-¹⁴C]glucose. The slow oxidation of the [1-¹⁴C]glucose by the blood *in vitro* gave a CO₂ value of 12.6 counts/min./μmole at 40 min. in Expt. A, Table 5. A comparable value would not be expected for the experiment *in vivo* because the conditions are quite different. In Expt. A, Table 5, there were 10 μC of [1-¹⁴C]glucose in 200 ml. of blood which contained approximately 100 mg. of glucose. In the experiment *in vivo* 100 μC of [1-¹⁴C]glucose was injected and passed through the udder and was distributed in the body glucose pool. Baxter *et al.* (1955) have calculated that the body pool in a 457 kg. cow contains 62.4 g. of glucose. Thus after the [1-¹⁴C]glucose entered the general

circulation it was diluted many times more than in the experiment *in vitro*.

Expt. B was designed to serve as a control for the experiment of Table 3. The glucose contained 5000 counts/min./μmole, which was comparable with that of the glucose which flowed through the left side of the udder in the experiment of Table 3. It is seen that the CO₂ at zero time contained 0.22 count/min./μmole, and that there was very little increase in the specific activity of the CO₂ during 4.5 min. of incubation. There was some increase in 15 min. Apparently a correction value of less than 0.22 count/min./μmole should be subtracted from the specific activity of CO₂ from the left subcutaneous abdominal vein of Table 3. It would be less than 0.22 because equilibration with unlabelled bicarbonate pool would lower the specific activity of the CO₂ (Table 2). This correction would not alter significantly the value of 6.37, which was obtained for the arteriovenous difference at 4.3 min. It therefore is concluded that the [1-¹⁴C]glucose was oxidized to ¹⁴CO₂ by the udder and that the oxidation by blood and the contamination by ¹⁴CO₂ contributed very little to the activity of ¹⁴C observed in the CO₂.

SUMMARY

1. The metabolism of the lactating cow's udder was investigated by determinations of arteriovenous differences in the isotope concentrations of ¹⁴CO₂ or ¹³CO₂ of the udder during a continuous injection of [1-¹⁴C]acetate or [1-¹⁴C]glucose and [1-¹³C]acetate. The isotope concentrations of the blood glucose and acetate were also determined in the venous blood of the udder.

2. The relative rates of oxidation of the C-1 of glucose and of the C-1 of acetate to CO₂ were compared on the basis of the ratios of the isotope concentrations of the substrates and of the CO₂. By

Table 5. Oxidation of [1-¹⁴C]glucose to carbon dioxide by blood *in vitro*

Experiment A. 250 ml. of blood was drawn from the jugular vein of a cow into 500 units of heparin. 10 μC of [1-¹⁴C]glucose (9.2 mg.) in 10 ml. of aq. 0.09 % NaCl was added to 200 ml. of the blood and the mixture was incubated at 37° in a closed flask. At the indicated times 20 ml. of blood was withdrawn and the CO₂ was liberated and collected.

Experiment B. 100 ml. of blood was drawn into a syringe containing a marble for mixing of the blood and glucose. 5 ml. of a solution of [1-¹⁴C]glucose containing 0.78 mg. of glucose and 0.85 μC of ¹⁴C was drawn into the syringe. The mixed solution was incubated at 37° in the syringe and at the indicated times CO₂ was liberated from 20 ml. of blood. The zero-time CO₂ was obtained by adding 1 ml. of the [1-¹⁴C]glucose to acid solution, and then citrate and 19 ml. of blood were added to the acid solution.

Expt. A				Expt. B	
Time (min.)	CO ₂ (counts/min./ μmole)	Time (min.)	CO ₂ (counts/min./ μmole)	Time (min.)	CO ₂ (counts/min./ μmole)
1	2.88	5	3.34	0	0.22
2	2.85	10	4.18	1.8	0.23
3	2.90	20	6.97	4.5	0.25
4	3.14	40	12.60	15	0.43

this method C-1 of acetate was estimated to be oxidized to CO₂ in the udder at about the same rate as is C-1 of glucose. This estimate may be subject to considerable error because of assumptions made in the calculation.

3. [1-¹⁴C]- and [6-¹⁴C]-Glucose were alternately injected into the pudic artery of a cow and the specific activities of the CO₂ of the venous blood of the udder were determined. After injection of [1-¹⁴C]glucose the specific activity of the CO₂ was much greater than it was after [6-¹⁴C]glucose. These results indicate that the pentose cycle occurs in the udder of the lactating cow.

4. The equilibration between the glucose, acetate and bicarbonate of the blood and that of the pools of the udder was investigated. This equilibration alters the specific activity of the compounds and makes it difficult to apply tracer-dilution methods in the intact animal.

A large group of students and staff from the Department of Dairy Science and from the College of Veterinary Medicine assisted in collecting the blood samples and in liberating the CO₂. Their assistance is gratefully acknowledged. The ¹⁴C determinations were done at the University of Minnesota and the authors thank Dr Nathan O. Lifson for assistance in these measurements.

This investigation has been supported by grants from the Atomic Energy Commission under Contract No. AT-(30-1)-1320 at Western Reserve University and Contract No. AT-(11-1)-67 at the University of Illinois. The ¹⁴C was obtained on allocation from the U.S. Atomic Energy Commission.

REFERENCES

- Abraham, S., Cady, P. & Chaikoff, I. L. (1957). *J. biol. Chem.* **224**, 955.
- Abraham, S., Hirsch, P. F. & Chaikoff, I. L. (1954). *J. biol. Chem.* **211**, 31.
- Baker, N., Shreeve, W. W., Shipley, R. A., Incefy, G. E. & Miller, M. (1954). *J. biol. Chem.* **211**, 575.
- Barcroft, J., McAnally, R. A. & Phillipson, A. T. (1944). *J. exp. Biol.* **20**, 120.
- Baxter, C. F., Kleiber, M. & Black, A. L. (1955). *Biochim. biophys. Acta*, **17**, 354.
- Bernstein, W. & Ballentine, R. (1950). *Rev. sci. Instrum.* **21**, 158.
- Black, A. L., Kleiber, M., Butterworth, E. M., Brubacher, G. B. & Kaneko, J. I. (1957). *J. biol. Chem.* **227**, 537.
- Duncombe, W. G. & Glascock, R. F. (1956). *Biochem. J.* **63**, 326, 332.
- Espe, D. (1948). In *Secretion of Milk*, p. 24. Ames: Iowa State College Press.
- Glock, G. E. & McLean, P. (1953). *Biochim. biophys. Acta*, **12**, 590.
- Glock, G. E. & McLean, P. (1954). *Biochem. J.* **56**, 171.
- Glock, G. E., McLean, P. & Whitehead, J. K. (1956). *Biochim. biophys. Acta*, **19**, 546.
- Kleiber, M., Black, A. L., Brown, M. A., Baxter, C. F., Luick, J. R. & Stadtman, F. H. (1955). *Biochim. biophys. Acta*, **17**, 252.
- Lachance, J. P., Popják, G. & de Waard, A. (1958). *Biochem. J.* **68**, 7P.
- McClymont, G. L. (1949). *Biochem. J.* **45**, i.
- McClymont, G. L. (1951). *Aust. J. agric. Res.* **2**, 158.
- Nelson, N. (1944). *J. biol. Chem.* **153**, 375.
- Nier, A. O. (1947). *Rev. sci. Instrum.* **18**, 398.
- Peeters, G. & Debackere, M. (1956). *Arch. int. Physiol. Biochim.* **64**, 527.
- St. Clair, L. E. & Hardenbrook, H. J. (1956). *J. Amer. vet. med. Ass.* **129**, 405.
- Schambye, P., Wood, H. G. & Kleiber, M. (1957). *J. biol. Chem.* **226**, 1011.
- Seubert, W., Greult, G. & Lynen, F. (1957). *Angew. Chem.* **69**, 359.
- Somogyi, J. (1945). *J. biol. Chem.* **160**, 61.
- Swim, H. E. & Krampitz, L. O. (1954). *J. Bact.* **67**, 426.
- Turner, C. W. (1952). In *The Mammary Gland*, p. 56. Columbia: Lucas Bros.
- Van Slyke, D. D. & Folley, S. J. (1940). *J. biol. Chem.* **136**, 509.
- Wood, H. G., Gillespie, R., Joffe, S., Hansen, R. G. & Hardenbrook, H. (1958a). *J. biol. Chem.* **233**, 1271.
- Wood, H. G., Joffe, S., Gillespie, R., Hansen, R. G. & Hardenbrook, H. (1958b). *J. biol. Chem.* **233**, 1264.
- Wood, H. G., Siu, P. & Schambye, P. (1957). *Arch. Biochem. Biophys.* **69**, 390.

Phospholipid Metabolism in Nervous Tissue

1. A RECONSIDERATION OF BRAIN AND PERIPHERAL-NERVE PHOSPHOLIPID METABOLISM IN VIVO

By A. N. DAVISON AND J. DOBBING

Department of Pathology, Guy's Hospital Medical School, London, S.E. 1

(Received 20 April 1959)

Experiments designed to investigate the metabolic activity of the chemical components of myelin have led to a suggestion that these substances, once incorporated into the nerve sheath during development, may remain there unchanged for long periods in relation to the lifetime of the animal. Thus [4-¹⁴C]cholesterol injected into the residual yolk sac

of the developing chick (Davison, Dobbing, Morgan & Wright, 1958) or intraperitoneally into the developing rabbit (Davison, Dobbing, Morgan & Wright, 1959) is slowly and continuously incorporated into the brain during myelination, much of it remaining there for a year or more with little or no change in quantity or molecular configuration