SUMMARY

1. An explicit mathematical statement for the effects of irradiation on the kinetic parameters K (Michaelis constant) and V (maximum velocity) of enzymes is provided.

2. Irradiation of glutamic acid dehydrogenase, in solution, increases K and decreases V for the substrates glutamate and α -oxoglutarate. V is decreased and K is unchanged for the coenzymes diphosphopyridine nucleotide and reduced diphosphopyridine nucleotide (DPNH).

3. The increase in K for glutamate is not associated with a change in the relative inhibitory power of dicarboxylic analogues of glutamate.

4. Irradiation reduces the main protein peak seen in the ultracentrifuge and produces slower sedimenting material. This is consistent with a splitting of the large kinetic molecular unit into sub-units. At very high doses rapidly sedimenting material, presumed to be aggregates, is produced. The decrease in the main component is coincident with a decrease in enzymic activity.

5. Irradiation of the enzyme reduces its ability to enhance the fluorescence of DPNH. This is observed whether wavelength of the exciting light is 365 m μ or 280 m μ . The change observed parallels a decrease in the maximum velocity, V .

6. The increase in K for glutamate is not accompanied by a decrease in the augmented DPNH fluorescence observed on the addition of glutamate to enzyme-DPNH mixtures. The decrease in maximum enzyme velocity is not paralleled by a marked fall in the intrinsic protein fluorescence.

7. It is suggested that the primary action of the aqueous radicals formed on irradiation leads to modification in the tertiary structure of the enzyme with resulting loss in maximum velocity.

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The Metabolism of Specifically Labelled Glucose in Bovine Ketosis

By E. G. TOMBROPOULOS* AND M. KLEIBER

Department of Animal Husbandry, University of California, Davis, California, U.S.A.

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Bovine ketosis in dairy cows is accompanied by hypoglycaemia in degrees varying from slight to severe. Shaw (1943, 1956) demonstrated that glucose tolerance and uptake were normal in the mammary gland of ketotic cows. He concluded

* Present address: Department of Physiology, University of California, Berkeley.

that the overall rate of glucose utilization, especially by the mammary gland, is not impaired in ketosis. Studies with labelled glucose have also suggested that the overall rate of glucose metabolism is not decreased during ketosis (Kronfeld, Tombropoulos & Kleiber, 1959b). Nevertheless, the pathway of glucose catabolism may be abnormal.

Black, Kleiber, Butterworth, Brubacher & Kaneko (1957) compared the relative contribution in normal cows of [$1\text{-}^{14}\text{C}$]glucose and [$6\text{-}^{14}\text{C}$]glucose to ^{14}C in respired carbon dioxide, glycerol of butterfat, and alanine and serine of milk casein. In addition, serine isolated from casein was degraded. These authors concluded that normal cows catabolize glucose mainly by two pathways: the classical Embden-Meyerhof glycolytic scheme and the hexose monophosphate oxidative pathway. The latter pathway accounted for from 37 to 80 % of the glucose catabolized, depending on the metabolic end-product chosen as indicator. The relative participation of that pathway in glucose catabolism may be altered in bovine ketosis.

The operation of the hexose monophosphate oxidative pathway may be related to lipogenesis. Experiments with rat tissues as well as with the intact rat show that starvation and diabetes reduce lipogenesis (Stetten & Boxer, 1944; Chaikoff, 1952; Brady & Gurin, 1950; Van Bruggen, Yamada, Hutchens & West, 1954) and glucose catabolism via the hexose monophosphate oxidative pathway (Agranoff, Brady & Colodzin, 1954; Bloom, 1955; Felts, Doell & Chaikoff, 1956). Langdon (1957) demonstrated that the conversion of acetate into fatty acids requires reduced triphosphopyridine nucleotide, which is the specific hydrogen donor for the conversion of crotonyl-coenzyme A into butyryl-coenzyme A. These findings have led various investigators (Milstein, 1956; Siperstein & Fagan, 1957, 1958*a, b*) to postulate that the oxidation of glucose via the hexose monophosphate oxidative pathway may lead to increased lipogenesis by providing TPNH. It is not yet established whether increased oxidation of glucose 6-phosphate and 6-phosphogluconate is the cause of increased lipogenesis or a result of accelerated regeneration of TPN $^{+}$ during increased lipogenesis (Winegrad & Renold, 1958; Cahill, Hastings, Ashmore & Zottu, 1958; Brin & Yonemoto, 1958; McLean, 1959).

An association between impaired lipogenesis and increased ketogenesis in the diabetic rat was postulated by Weinhouse (1952), and a similar relationship was suggested during bovine ketosis (Kronfeld & Kleiber, 1959; Kronfeld, Kleiber & Lucas, 1959*a*). It is possible therefore that there is a decrease in the participation of the hexose monophosphate oxidative pathway during bovine ketosis with a decrease of lipogenesis.

The present investigation with ketotic cows was undertaken to study the degree of participation of the hexose monophosphate oxidative pathway in glucose catabolism as well as the utilization of glucose for the production of milk constituents.

EXPERIMENTAL

Conduct of trial. The trials were conducted in the Orland Veterinary Hospital, Orland, Calif., U.S.A. In this area bovine ketosis is prevalent. The diagnoses were performed by Dr C. Hymas, Dr C. Crenshaw and Dr E. Smith. In order to decrease the variability of the results, we selected only ketotic cows whose clinical symptoms were similar and which had been ketotic for 24–40 hr. before the experiment began. All cows used in this study were Guernseys. Table 1 describes these cows and specifies the amount of material injected.

Materials. [$1\text{-}^{14}\text{C}$]Glucose was prepared as described by Isbell *et al.* (1952) by the CaCl_2 method for the production of barium gluconate and mannono- γ -lactone. Instead of isolating the two epimeric acids at the stage of their corresponding salts, the mixture of epimeric hexonic acids resulting from the cyanohydrin synthesis was converted into a mixture of the corresponding lactones by slow concentration in methyl Cellosolve under a stream of air (Hers, Edelman & Ginsburg, 1954). The mixture of lactones in the resulting thick syrup was reduced by sodium amalgam in oxalate buffer. The Isbell procedure was followed until the salt-free sugar solution was collected from the mixed ion-exchange column. This solution was concentrated and the glucose was separated from mannose by paper chromatography on Whatman 3MM filter paper with butanol-acetic acid-water as solvent (Partridge, 1948). After elution from the paper the glucose solution was tested for purity by two-dimensional paper chromatography.

Table 1. *Description of ketotic cows used in the experiments*

Trial no.	[$1\text{-}^{14}\text{C}$]Glucose		[$6\text{-}^{14}\text{C}$]Glucose	
	XIV	XV	IX	X
Age (years)	4	6	2.5	2.5
Weight (kg.)	383	362	347	467
Week of lactation	5	2	5	8
Milk yield during experiment (kg./day)	10.9	15.1	13.9	9.2
Butterfat during experiment (g./100 ml.)	7.71	5.23	5.40	7.36
Production of CO_2 during first 3 hr. after injection of labelled glucose (l./hr.)	69.48	92.16	59.30	76.78
Blood sugar (mg./100 ml.)	38.2	32.3	38.3	36
Blood ketone bodies (total) as acetone (mg./100 ml.)	50.4	44.4	39.4	23.78
Blood acetone and acetoacetic acid (mg./100 ml.)	34.4	35.1	13.5	16.00
Injected dose of ^{14}C (mc)	1.387	0.7295	2.34	2.315

Phenol-water and butanol-acetic acid-water (Partridge, 1948) were used as solvents. Exposure of the developed paper chromatogram to X-ray film showed that the isolated [^{14}C]glucose was uncontaminated with any other [^{14}C]-labelled compound.

[6- ^{14}C]Glucose was prepared by the method of Schaffer & Isbell (1956). The glucose isolated by crystallization contained some [^{14}C]-labelled impurities. Therefore paper chromatography was used as described above as a final purification step.

Injection. The procedure used for intravenous injection of the labelled compound was described by Ralston, Kleiber & Smith (1949).

Respiratory carbon dioxide. The respiratory CO_2 was collected continuously for the first 3 hr., and every hour thereafter for an additional 7 hr. The 'respiration apparatus' used was described by Kronfeld *et al.* (1959a) and the methods used for the collection and [^{14}C] assay of the respiratory CO_2 were described by Kleiber & Edick (1952).

Separation of milk constituents. The cows were milked 3, 10 and 25 hr. after the administration of the labelled compound. These samples were allowed to stand at 2° for 24 hr. and the cream was separated. The skim milk was acidified with HCl to pH 4.5-4.7 and the precipitated casein removed and purified as described by Black *et al.* (1957). The resultant supernatant was adjusted to pH 5.5 with aq. NH_3 soln. and the albumin removed by filtration, washed with water, ethanol and ether and then dried. The filtrate containing the lactose was deionized by passage through two ion-exchange columns. The resins used were Dowex 50 (H form) and Dowex 2 (formate), which were washed with ethanol and water before use. The eluent containing the lactose was concentrated to a thick syrup under reduced pressure and the disaccharide was crystallized by adding ethanol. Citric acid retained by the Dowex 2 (formate) resin was eluted and isolated as described by Lucas, Kaneko, Hirohara & Kleiber (1959).

The butterfat was isolated from the cream as described by Gottlieb (1922).

Glycerol and fatty acids were isolated from butter as follows: a sample (5 g.) of butterfat was saponified by treatment with 50 ml. of 1N-KOH solution in ethanol under reflux for 3 hr. Water (about 50 ml.) was added and the mixture was extracted three times with ether to remove the non-saponified lipids. Excess of cupric sulphate was added to the aqueous solution and the mixture was concentrated on a steam bath to a volume of about 15 ml. The cupric salts of the fatty acids were precipitated by adding acetone and water to a final concentration of acetone-water (1:1, v/v) in a final volume of 75 ml. The mixture was allowed to stand overnight at 2° and the resulting precipitate was filtered, washed with acetone-water (1:1, v/v) and dried *in vacuo* at 40° . The glycerol was isolated by concentrating the filtrate and extracting the residue with 80 ml. of 95% ethanol. The ethanol extract was evaporated and the resulting crude glycerol weighed. Glycerol tribenzoate was prepared according to the method outlined by Abraham & Hassid (1957).

Combustion and [^{14}C] assay of samples. Glycerol tribenzoate, casein and albumin were oxidized by the method of Gabourel, Baker & Kock (1955) in a Sargent microcombustion apparatus. Lactose and citrate were completely oxidized to CO_2 by the persulphate procedure of Katz, Abraham & Baker (1954). Butterfat and fatty acids were

oxidized by the method of Van Slyke & Folch (1940), in a pear-shaped flask fitted with a reflux condenser to minimize any loss of short-chain fatty acids.

The preparation of BaCO_3 planchets from each method of combustion (except of fatty acids) and the method of expressing the [^{14}C] activity of samples were the same as previously described by Black, Kleiber & Baxter (1955).

Because of the low activity of the BaCO_3 derived from the combustion of fatty acids, these samples were assayed in a liquid-scintillation chamber (Packard Tri-Cab) in *m*-hyamine [*N*-*p*-(diisobutylresoxyethoxyethyl)-*NN*-dimethyl-*N*-benzylammonium hydroxide)] solution in methanol. The method used for the regeneration and absorption of CO_2 was described by Eisenberg (1958).

Analytical determinations. Blood ketone bodies were estimated by the method of Thin & Robertson (1952). The blood-sugar content was estimated with ferricyanide (Folin & Malmros, 1929) on a ZnSO_4 -NaOH filtrate (Somogyi, 1930). The milk fat was determined by the Babcock (1890) method.

RESULTS

The results were obtained during a period of 25 hr. after the injection of isotope. The ketotic cows were then treated for ketosis. These data were compared with results obtained from normal cows in the first 25 hr. after injection. The data for 48 hr. for normal cows were reported by Black *et al.* (1957).

Fig. 1 shows the specific activity of CO_2 as a function of time. The specific activity of respired

Fig. 1. [^{14}C] in respiratory CO_2 after intravenous injection of [^{14}C]glucose into ketotic cows. An aqueous solution of 0.9% NaCl containing labelled glucose was injected into the jugular veins of lactating cows. Samples of CO_2 were collected continuously as indicated above and assayed for [^{14}C] activity. ●, [6- ^{14}C]Glucose (trial no. X); ×, [6- ^{14}C]glucose (trial no. IX); ○, [1- ^{14}C]glucose (trial no. XIV); △, [1- ^{14}C]glucose (trial no. XV). ρ_s , ($\mu\text{C}/\text{mole of CO}_2$) / ($\mu\text{C injected/kg. body wt.}$).

Table 2. *Conversion of injected [¹⁴C]glucose into ¹⁴CO₂ during the first 3 hr. after injection*

An aqueous solution of 0.9% NaCl containing the labelled glucose was injected into the jugular vein of lactating cows. ¹⁴CO₂ samples were collected continuously during the entire 3 hr. period after injection. The amount of CO₂ in the expired air was determined and assayed for ¹⁴C activity. The total activity for the 3 hr. period was determined by integration.

Position of label	Normal cows		Ketotic cows	
	Trial no.	Percentage of injected isotope recovered in CO ₂	Trial no.	Percentage of injected isotope recovered in CO ₂
[1- ¹⁴ C]Glucose	IV	27.8	XIV	7.0
	VI	24.5	XV	6.8
	Average	26.2		6.9
[6- ¹⁴ C]glucose	III	16.6	IX	8.3
	V	7.7	X	5.5
	Average	12.2		6.9
Average ¹⁴ C from [1- ¹⁴ C]glucose		2.15		1.00
Average ¹⁴ C from [6- ¹⁴ C]glucose				

Table 3. *Recovery of ¹⁴C in milk constituents during 25 hr. after intravenous injection of [1-¹⁴C]- or [6-¹⁴C]-glucose into cows*

An aqueous solution of 0.9% NaCl containing the labelled glucose was injected into the jugular veins of lactating cows. The cows were milked 3, 10 and 25 hr. after administration of the labelled glucose. The amount of milk was measured and then separated into the different milk constituents recorded below. Samples from each milk constituent were oxidized and assayed for ¹⁴C activity. The amounts of ¹⁴C of each milk constituent collected for the three different time periods were added, yielding values for the total 24 hr. period. Recovery of ¹⁴C is expressed as: $\frac{\text{amount (mc) of } ^{14}\text{C in product}}{\text{amount (mc) of } ^{14}\text{C injected}} \times 100$. Ratio C-1/C-6 is:

$$\frac{\text{average recovery of } ^{14}\text{C (from the two trials) in milk product after [1-}^{14}\text{C]glucose injection}}{\text{average recovery of } ^{14}\text{C (from the two trials) in milk product after [6-}^{14}\text{C]glucose injection}}$$

Trial no.	[1- ¹⁴ C]Glucose				[6- ¹⁴ C]Glucose				Ratio C-1/C-6	
	Normal cows		Ketotic cows		Normal cows		Ketotic cows		Normal cows	Ketotic cows
	IV	VI	XIV	XV	III	V	IX	X		
Milk constituent isolated										
Citrate	0.27	0.15	0.17	0.16	0.47	0.61	0.27	0.28	0.38	0.60
Lactose	40.00	40.36	61.47	67.59	37.5	45.49	40.58	35.22	0.93	1.83
Albumin	0.76	0.44	0.49	0.26	1.34	1.08	0.44	0.39	0.49	0.90
Casein	2.12	2.12	1.60	0.95	4.37	4.12	3.08	2.24	0.50	0.48
Butterfat	2.83	2.02	3.8	3.11	6.32	6.71	6.78	3.60	0.37	0.66
Glycerol	2.02	1.97	3.73	3.06	5.40	6.02	5.85	2.87	0.34	0.78

CO₂ by the ketotic cows followed a curve of similar shape and reached a maximum about the same time after the injection of either [1-¹⁴C]glucose or [6-¹⁴C]glucose. The curves resemble the corresponding curves for CO₂ activity observed after [6-¹⁴C]glucose injection in normal cows and have similar maxima. On the other hand, the ¹⁴CO₂ curves for ketotic cows differ from those obtained after [1-¹⁴C]glucose injection into normal cows (Black *et al.* 1957). The ratio of total ¹⁴C respired as CO₂ derived from [1-¹⁴C]glucose to that derived from [6-¹⁴C]glucose during the first 3 hr. was lower in ketotic than in normal cows (Table 2).

After [1-¹⁴C]glucose injection the recovery of ¹⁴C in casein and albumin tended to be lower in ketotic cows than in normal cows, but the recovery

of ¹⁴C in lactose, butterfat and glycerol appeared to be higher (Table 3).

After [6-¹⁴C]glucose injection the recovery of ¹⁴C in albumin, casein, citrate, butterfat and glycerol was lower in ketotic than in normal cows (Table 3). The differences tended to be smaller or non-existent for butterfat and glycerol than for albumin, citrate and casein. The ¹⁴C recovery in lactose was about the same for normal and ketotic cows.

Since the ¹⁴C activity in the fatty acids of butterfat was very low, the correspondingly large error in determination ruled out justifiable comparisons. The activity of the butterfat was due almost entirely to the activity of the glycerol (Table 3). The ratio of the ¹⁴C-1 to ¹⁴C-6 (Table 3) in each milk product was calculated from the average

percentage recovery of the injected ^{14}C after $[1\text{-}^{14}\text{C}]$ -glucose injection and after $[6\text{-}^{14}\text{C}]$ -glucose injection. This ratio was higher in ketotic cows for all compounds except casein, which indicates that the metabolism of C-1 and C-6 of glucose was more nearly the same in ketotic cows than it was in normal cows.

DISCUSSION

The recovery of ^{14}C in respiratory carbon dioxide, albumin, casein and citrate, which, on the average, was lower in ketotic than in normal cows, suggests that ketosis is accompanied by decreased glucose catabolism. The reduction of glucose catabolism might result from the following causes.

(1) An interference with the reactions leading from glucose to the tricarboxylic acid cycle. In line with this explanation is the report by Bach & Hibbit (1959) that showed increased amounts of serum pyruvate in ketotic cows. They suggested that ketosed animals had a decreased capacity to decarboxylate pyruvate.

(2) A greater influx of non-glucose carbon atoms into the tricarboxylic acid cycle might displace glucose carbon atoms from these reactions in the absence of any disturbance at a specific enzymic step. It was shown earlier that ketotic cows had lower specific activities in butterfat than did normal cows after injection of $[^{14}\text{C}]$ acetate (McLeod, 1955; Kronfeld *et al.* 1959*a*). McLeod attributed this decrease to a greater utilization of body fat for synthesis of butterfat in the ketotic animals. In addition it was found that injection of $[^{14}\text{C}]$ acetate resulted in higher specific activity in albumin, casein and citric acid isolated from the milk of ketotic cows than in these compounds isolated from normal cows. It was concluded that ketotic cows metabolized a greater part of acetate via the tricarboxylic acid cycle than did normal cows. The greater influx of acetate carbon into the tricarboxylic acid cycle might function either directly or indirectly to reduce the metabolism of carbon from glucose in the cycle, which could account for our observed results.

(3) The possibility should be considered that reactions in which glucose carbon atoms are utilized for synthesis of glycerol and lactose might effectively compete with reactions for glucose catabolism in the presence of lower glucose concentrations in ketotic cows. Such an effect might be expected to result in a greater recovery of ^{14}C in lactose and glycerol than in other milk products (derived from intermediates of glucose catabolism) and carbon dioxide when the cow is ketotic. These relationships were observed when the glucose was labelled on C-1 (Table 3). However, when $[6\text{-}^{14}\text{C}]$ -glucose was injected the ^{14}C recovered in lactose and glycerol was no greater than it was in the normal animal.

If the type of calculation by Black *et al.* (1957) is applied to recovery of ^{14}C in glycerol, a result is obtained that the hexose monophosphate oxidative pathway accounts for about 24% of the glucose catabolized in ketotic cows. That is less than half of the 56% found for normal cows (Black *et al.* 1957), and indicates considerable reductions of glucose metabolism by the hexose monophosphate oxidative pathway in ketotic cows. This decrease in participation appears even more pronounced if the effect of fixation of carbon dioxide in glycerol formation on this type of calculation is considered. Fixation of carbon dioxide reduces the (C-6-C-1)/C-6 ratio products, e.g. glycerol, synthesized in normal cows, because the mean standardized specific activity of carbon dioxide was much higher after $[1\text{-}^{14}\text{C}]$ -glucose injection than after $[6\text{-}^{14}\text{C}]$ -glucose injection. In this case the effect of fixation of carbon dioxide would be to increase the role of the Embden-Meyerhof pathway.

From consideration of the known pathways of glucose metabolism in dairy cows, the deviation of C-1/C-6 from unity indicates a pathway in addition to the Embden-Meyerhof pathway. The C-1/C-6 ratios shown in Table 3 indicate in all cases, except for lactose, hexose monophosphate oxidative pathway activity. The higher ratio in the ketotic animals indicates that the hexose monophosphate oxidative pathway had a lower role in glucose metabolism in ketotic than in normal animals.

The quantitative evaluation of the hexose monophosphate oxidative pathway presents some difficulties which have been discussed by Wood (1955), Korkes (1956), Racker (1956), Wood, Gillespie, Joffe, Hansen & Hardenbrook (1958*b*) and Wood & Katz (1958). Most important is the possible indeterminable randomization of ^{14}C due to transaldolase and transketolase. If, however, these factors influence the calculations to the same extent in normal and in ketotic animals, the comparison may be valid. Therefore the evaluation of the hexose monophosphate oxidative pathway in ketotic and normal cows, though only an approximation, serves for a comparison showing reduced participation of the hexose monophosphate oxidative pathway during ketosis.

The higher recovery of ^{14}C in lactose after $[1\text{-}^{14}\text{C}]$ -glucose injection than after $[6\text{-}^{14}\text{C}]$ -glucose injection into ketotic cows could be attributed to exchange reactions catalysed by transaldolase and transketolase (Korkes, 1956; Wood *et al.* 1958*b*; Wood & Katz, 1958). These reactions might have a more obvious effect on the ^{14}C transfer to lactose during ketosis if the rate of decarboxylation of 6-phosphogluconate in ketotic cows is lowered by the decrease in degree of participation of hexose monophosphate oxidative pathway. Randomization of carbon atoms of glucose by transketolase

and transaldolase can occur very rapidly in tissue such as liver (Murphy & Muntz, 1957) and cow mammary gland (Wood *et al.* 1958*b*; Wood, Siu & Schambye, 1957; Wood, Seymour, Gillespie, Hansen & Hardenbrook, 1958*a*).

The value of the C-1/C-6 ratio, 1.83, in the lactose from the ketotic cows observed here might be explained by a combination of factors, including the redistribution of [^{14}C]glucose positions C-2 and C-3 of the hexose moieties of lactose due to the transaldolase and the transketolase activity.

SUMMARY

1. Specifically labelled [$1\text{-}^{14}\text{C}$]glucose and [$6\text{-}^{14}\text{C}$]glucose were synthesized and injected intravenously into ketotic cows. The results were compared with those obtained previously with normal cows.

2. The recovery of ^{14}C in respiratory carbon dioxide, casein, albumin and citrate after [$6\text{-}^{14}\text{C}$]glucose injection was less during ketosis than in the corresponding recovery in normal cows. This observation indicates a decrease of ^{14}C transfer from glucose to carbon dioxide, casein, albumin and citrate or dilution of ^{14}C from glucose by a relative increase in incorporation of carbon atoms from other precursors, or both of these possibilities, during ketosis. The results after [$1\text{-}^{14}\text{C}$]glucose injection support these findings.

3. The recovery of ^{14}C in lactose and glycerol was the same in ketotic and normal cows after [$6\text{-}^{14}\text{C}$]glucose injection, but higher in ketotic cows after [$1\text{-}^{14}\text{C}$]glucose injection.

4. Comparison of ^{14}C recovery in the different milk constituents after [$1\text{-}^{14}\text{C}$]glucose and [$6\text{-}^{14}\text{C}$]glucose injection shows a decrease of participation of the hexose monophosphate oxidative pathway during ketosis. The decrease seems to be correlated with impaired lipogenesis in ketotic cows.

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Biochem. J. (1961) **80**, 420

Some Aspects of Amino Acid Metabolism in the Adult Housefly, *Musca domestica**

By G. M. PRICE

Agricultural Research Council, Pest Infestation Laboratory, London Road, Slough, Bucks.

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The concentration of free amino acids in insect haemolymph is in general much greater (often 10–50 times) than it is in vertebrate blood (Auclair & Dubreuil, 1953; Bishop, Briggs & Ronzoni, 1925; Buck, 1953; Duchateau & Florkin, 1955; Duval, Portier & Courtois, 1928; Florkin, 1954; Pratt, 1950). Other invertebrates, such as the lobster (*Homarus vulgaris*) and the crayfish (*Astacus fluviatilis*) possess high concentrations of amino acids in their tissues but the concentrations in their blood are of the magnitude found in vertebrate blood (Florkin, 1954). The significance of the high concentration of free amino acids in insect haemolymph has intrigued many workers and it has been wondered whether some of the roles played by amino acids in insects are particular to that class. Winteringham (1958) has suggested that in insects some of the amino acids may provide a soluble and readily available substrate reserve for the Krebs cycle. Winteringham & Harrison (1956) showed that α -alanine, aspartic acid, glutamic acid, glutamine and proline became labelled in adult houseflies (*Musca domestica*) after these insects had been injected with [2-¹⁴C]acetate as labelled precursor. Kasting & McGinnis (1958) found that certain amino acids became labelled in *Phormia regina* after it was fed with [¹⁴C]glucose. Clements (1959) found that when preparations of the fat-body of the desert locust (*Schistocerca gregaria*) were incubated with [2-¹⁴C]acetate, aspartic acid, glutamic acid and proline became labelled. In the present work the distribution in the fly and the metabolic relationships of some of the amino acids are investigated.

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METHODS

The L- or DL-forms of amino acids were purchased from L. Light and Co. Ltd, Colnbrook, Bucks. Each amino acid gave only one ninhydrin-positive spot after two-dimensional chromatography (see below) and they were therefore used without further purification. Anhydrous sodium [2-¹⁴C]acetate, with a specific activity of 5.16 $\mu\text{C}/\mu\text{mole}$, was purchased from The Radiochemical Centre, Amersham, Bucks. A pyridine-acetate buffer, pH 6.0, was prepared by mixing 10 ml. of pyridine, 1 ml. of acetic acid and 89 ml. of water. Methyl Cellosolve (ethylene glycol monomethyl ether) and lutidine were redistilled before use. Phenol was redistilled according to the method of Draper & Pollard (1949). Ninhydrin and isatin (indoline-2:3-dione) were used as 0.2% solutions in acetone.

Sarcosine-isolation medium. The final concentrations were: 0.32 M-sucrose; 0.01 M-2-amino-2-hydroxymethylpropane-1:3-diol (tris), pH 7.2; 0.01 M-ethylenediamine-tetra-acetate, pH 7.2; 1% of bovine plasma albumin.

Albumin-free isolation medium. The same as above but without albumin.

Breeding of houseflies (*Musca domestica*). Adult houseflies were kept at 27° and 60% relative humidity and were fed on glucose, milk and water. Only females, 5–6 days old, were used in the experiments.

Injection with [2-¹⁴C]acetate. Each fly was anaesthetized with cyclopropane for 1 min. and then injected from an Agla micrometer syringe with 2 $\mu\text{l.}$ of a solution containing 63.6 $\mu\text{g.}$ of anhydrous sodium acetate (4 $\mu\text{C.}$). The hypodermic needle was inserted 1–2 mm. into the dorsal posterior end of the thorax just under the left-wing base. After being injected, each fly was put into a 150 mm. $\times$ 15 mm. test tube which was sealed with a rubber bung. Soda lime was present in the base of each tube to absorb the CO₂ expired, and the fly was prevented from coming into contact with the alkali by a loosely packed plug of cotton wool placed about 30–40 mm. from the open end. The fly was left in the tube at room temperature (approx. 22°) for the required period, after which it was treated in one of three