Synthesis of Milk Fat from Acetic Acid ($\text{CH}_3^{14}\text{COOH}$) by the Perfused Isolated Bovine Udder

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The technique of perfusion of the isolated udder is likely to give valuable information on the mechanisms of milk formation, provided it can be shown that most of the milk collected is actually a product of the secretory activity of the gland during the experiment. This point is not easy to demonstrate by ordinary chemical methods, because present-day techniques only permit of a rather limited perfusion period, and also because in the relatively large quantity of tissue involved the various precursors, intermediates and products are distributed in an unknown manner. Moreover, although some of the milk held in the alveoli and finer branches of the duct system can be collected via the teat after administration of oxytocin, the difficulty remains that the proportion of preformed to newly formed constituents in any milk sample must remain uncertain.

Two modern methods for perfusing the isolated bovine udder have so far been described (Petersen, Shaw & Visscher, 1939; Peeters & Massart, 1947), and a number of studies, mainly on the physiology of milk ejection, have been carried out by means of these techniques. The demonstration that the udder of the intact goat synthesizes milk fatty acids from ^{14}C -labelled acetate (Popják, French & Folley, 1950, 1951) suggested the possibility of a direct test of the ability of the isolated preparation to synthesize milk constituents in the normal manner.

The results presented in this paper show that the perfused, isolated udder can use $\text{CH}_3^{14}\text{COONa}$ added to the perfusion fluid for synthesis of milk constituents, in particular fatty acids. They show further, however, that the conditions of perfusion need to be most carefully controlled if comparisons are to be made with processes occurring in the living animal. For this reason the interpretation of the results, in terms of the synthetic mechanisms occurring in the isolated organ, must be regarded as tentative at present.

EXPERIMENTAL

The udder of a lactating cow (East Flemish breed), yielding about 12 kg. milk daily, was perfused for 2 hr. by the method of Peeters & Massart (1947). An important feature of this technique is that it allows the simultaneous perfusion of two separated udder halves, each consisting of two mammary glands, with no mixing of the blood in the two circulations; one udder half can thus serve as control for the other. In this experiment the left udder half was perfused with blood containing $\text{CH}_3^{14}\text{COONa}$ while $\text{NaH}^{14}\text{CO}_3$ and heavy water (D_2O) were added to the blood perfusing the right half. Since it was expected that radioactive CO_2 would be given off continuously to the atmosphere in the artificial lungs, the apparatus was set up in the open air, and the experimenters wore service respirators fitted with soda-lime canisters.

Care was taken to choose a cow with a normal udder, showing on clinical examination no evidence of mastitis. Immediately before slaughter the cow was milked out as completely as possible with the aid of intravenously injected oxytocin. The udder, removed as soon as the cow was shot, weighed approximately 20 kg. It was perfused with saline to remove blood, completely bisected along the median septum and the halves connected to the perfusion apparatus. The teats were cut off at the tip and cannulae inserted, residual milk being allowed to drain from the cisterns; this milk was discarded.

Approximately 3 l. heparinized fresh cow blood was initially used for the perfusion of each udder half, but owing to the unusually large size of the udder it was necessary to add a further 2 l. blood to each half in the course of the first 30 min. The following additions to the perfusion bloods were made (at the outset unless otherwise stated): left ('acetate') side: 1.8 g. glucose, 600 mg. sodium acetate containing 1 mc. $\text{CH}_3^{14}\text{COONa}$, 80 ml. physiological saline, 25 mg. NaHCO_3 in 200 ml. physiological saline added by means of a constant-drip device to the arterial (i.e. oxygenated) blood continuously throughout the perfusion; right ('bicarbonate') side: 1.8 g. glucose, 600 mg. sodium acetate, 80 ml. D_2O (99.5%) containing NaCl to 0.9%, 1 mc. $\text{NaH}^{14}\text{CO}_3$ (25 mg.) in 200 ml. physiological saline added continuously to the arterial blood as for the left side. It is thus seen that the chemical composition of the bloods used for the perfusion of both udder halves was quantitatively identical. The addition of the bicarbonate

was spread over the whole perfusion period because if it were all added at the outset most of the labelled CO_2 would soon be lost in the artificial lung. The purpose of the addition of glucose and carrier acetate to the blood was to ensure a supply of milk precursors and energy-yielding substrates.

Samples of venous blood from each circulation were taken at the beginning of the perfusion, at 15 min. and at subsequent 30 min. intervals, for determination of CO_2 content and specific activity, and for determination of the D content of the blood water. These samples were at once sealed into ampoules and kept frozen solid until analysis. A measured volume, 0.25–0.50 ml., of thawed blood was quickly pipetted into a bulb connected with a high-vacuum train, the CO_2 liberated with lactic acid and its volume measured, after suitable purification, in a McLeod gauge. It was then introduced into an internal gas counter for radioactivity measurement. No special precautions were taken to prevent loss of gas during sampling or pipetting so that the blood CO_2 values can only be regarded as approximate.

The milk secreted by each half during the perfusion was collected, the udder halves being emptied by oxytocin at the end of the perfusion; adequate samples were frozen solid and kept in the frozen state until preparation of fatty acid fractions could be begun. Samples of udder tissue were taken from each half at the end of the perfusion, frozen and kept for preparation and fractionation of tissue fat.

Unfortunately, for a reason unknown, oxygenation and CO_2 clearance was poor on the left ('acetate') side. This was reflected in high blood CO_2 concentrations as compared with blood from the right side (see Table 1) and was probably responsible for the fact that only 140 ml. milk were secreted during the 2 hr. perfusion by the left udder half as compared with 1420 ml. secreted by the right half. The fat content of milk samples from both udder halves was practically the same, 4.7 and 4.75%.

Crude glyceride fatty acid fractions (water-soluble and insoluble, steam-volatile; non-volatile solid; non-volatile liquid acids) and some chromatographically pure fatty acids and also cholesterol were prepared from the samples of milk and udder tissue as described previously (Popják, French & Folley, 1951; Popják, French, Hunter & Martin, 1951). The distributions of the fractions obtained from each of the four samples are given in Table 2. The specific activities of the fatty acid fractions from the udder half perfused with $\text{CH}_3^{14}\text{COONa}$ were determined as before on solid preparations of salts using an end-window counter; the much less active fractions from the right side were combusted in O_2 , the CO_2 purified and counted in a gas counter. The water resulting from the combustion was purified and its D content determined by the falling-drop method (see Popják, 1950).

RESULTS

Blood CO_2 . The CO_2 content of blood samples taken at various times during the perfusion and the specific activities of the CO_2 are given in Table 1. During the first 15 min., the CO_2 content of blood from the left ('acetate') side was within normal limits for venous blood of the lactating ruminant (cf. figures for the goat given by Graham, Houchin, Peterson & Turner, 1938), but thereafter rose progressively to a high maximum value at 90 min. However, the CO_2 content of the sample taken at the

end of the perfusion (120 min.) was back within the normal range. These results illustrate the fact, already referred to, that the artificial lung on this side of the apparatus was not working efficiently during the perfusion. It was also observed that the blood in this half of the apparatus was haemolysed to some extent. Whether this was a result or a cause of the poor oxygenation and consequent retention of CO_2 is not known.

Table 1. Amount and specific activity of CO_2 in blood samples obtained during perfusion of lactating bovine udder

Sample no.	Time (min.)	Vol. % CO_2 at N.T.P.	Specific activity of CO_2 ($1 \times 10^{-3} \mu\text{c./mg. C}$)
I. Left side: 1 mc. $\text{CH}_3^{14}\text{COONa}$			
1	0	44.4	147.1
5	15	39.4	39.0
7	30	109.0	30.9
9	60	111.6	31.4
11	90	210.0	21.0
14	120	54.5	43.7
II. Right side: 1 mc. $\text{NaH}^{14}\text{CO}_3$			
3	0	48.0	0.0
6	15	87.2	3.5
8	30	94.4	9.1
10	60	63.4	11.7
12	90	55.6	29.0
16	120	42.2	121.5

The CO_2 content of the blood samples from the right ('bicarbonate') side showed some tendency to increase during the early stages of the perfusion, but the maximum value reached was not excessive and the values declined to normal in the later stages. Evidently oxygenation and CO_2 clearance were satisfactory on this side. The blood passing through this artificial lung underwent no haemolysis.

The highest specific activity of CO_2 obtained from blood taken from the left ('acetate') side was found in the sample taken at zero time. This sample was taken from venous blood immediately after the circulation through the udder half had been established, and the fact that the specific activity of its CO_2 was the greatest observed in the series illustrates the rapidity of the uptake and oxidation of acetate by the lactating mammary gland under the conditions of this experiment. In the intact animal the excretion is slower, the maximum specific activity of the respiratory CO_2 following the injection of $\text{CH}_3^{14}\text{COONa}$ occurring at 12–15 min. in the goat (Popják, French & Folley, 1950, 1951) and at 10 min. in the cow (Kleiber, Smith & Tolbert, 1950). As might be expected from the manner in which the labelled bicarbonate was added to the perfusion blood, the specific activity of blood CO_2 from the right side was low at the outset and rose slowly during the earlier stages, but more rapidly

later on, and attained its highest value at the end of the perfusion period.

Fatty acids. The distribution of glyceride fatty acids in the milk and udder tissue (Table 2) showed that the composition of the fats obtained from both halves of the perfusion was normal. The specific activities of the four crude fatty acid fractions prepared from milk fat secreted by the left udder half,

Table 2. *Distribution of glyceride fatty acids in samples of milk and udder fat obtained from perfusion experiment*

Fraction	Milk fat		Udder fat	
	Amount* isolated (g.)	% of total	Amount* isolated (g.)	% of total
I. Left side: 1 mc. $\text{CH}_3^{14}\text{COONa}$				
Volatile:				
Water-soluble	0.457	7.7	0.347	3.0
Water-insoluble	0.139	2.3	0.159	1.4
Non-volatile	5.36	89.9	11.12	95.6
II. Right side: 1 mc. $\text{NaH}^{14}\text{CO}_3$				
Volatile:				
Water-soluble	1.08	6.5	0.263	4.5
Water-insoluble	0.209	1.3	0.155	2.6
Non-volatile	15.3	92.1	5.46	92.8

* The amounts isolated were samples and do not represent total yields. The milk obtained from the left side (140 ml.) contained 4.70% and that from the right side (1420 ml.) 4.75% fat. The udder tissues from each half, weighing 10 kg. each, contained 5.0% glyceride fat.

Table 3. *Specific activities of glyceride fatty acids, cholesterol and lactose from milk and udder tissue after perfusion of left half of udder with $\text{CH}_3^{14}\text{COONa}$ (1 mc.) and of right half with $\text{NaH}^{14}\text{CO}_3$ (1 mc.)*

(Specific activities are expressed as $1 \times 10^{-3} \mu\text{c./mg. C.}$)

Substance	Left side		Right side
	Milk	Udder	Udder
Steam-volatile fatty acids:			
Water-soluble	3.020	31.450	0.053
Water-insoluble	1.590	14.890	0.006
Non-volatile fatty acids:			
Solid	0.334	2.390	0.001
Liquid	0.239	1.510	0.000
Cholesterol	0.028	0.126	—
Lactose	0.178	—	0.003*

* Isolated from the milk.

and from udder fat from both sides of the udder, are given in Table 3. On the left side there was definite incorporation of labelled C into fatty acids belonging to all four fractions, the mean specific activities of the fractions falling progressively with increase in the mean chain length. The specific activities of similar fractions isolated from fat obtained from the tissue of this udder half were in all four cases some 7–10 times greater than those of the corresponding

fractions from the milk. This may be ascribed to dilution of the milk fat with the fat of preformed milk existing in the alveoli at the beginning of the perfusion and which it was not possible completely to remove from the udder tissue even by intravenous injection of oxytocin followed by the most thorough milking-out. A rapid turnover of fatty acids in the epithelial cells themselves would also contribute to the observed difference between the specific activities of the fatty acid fractions from milk and udder tissue respectively.

Table 4. *Specific activities of pure fatty acids isolated from udder tissue perfused with blood containing 1 mc. of $\text{CH}_3^{14}\text{COONa}$*

Fatty acid	Specific activity ($1 \times 10^{-3} \mu\text{c./mg. C.}$)
Butyric	43.50
Caproic (hexanoic)	27.00
Caprylic (octanoic)	24.20
Capric (decanoic)	21.90
Lauric (dodecanoic)	19.03
Myristic (tetradecanoic)	12.48
Palmitic (hexadecanoic)	3.69
Stearic (octadecanoic)	0.045
Oleic (octadec-9-enoic)	0.030

The specific activities of purified fatty acids isolated from the tissue fat of the udder half perfused with $\text{CH}_3^{14}\text{COONa}$ are given in Table 4. It will be seen that the specific activities decrease as the chain length increases from butyric acid upwards.

Table 5. *Deuterium content of milk lactose and of fatty acid fractions isolated from udder tissue perfused with blood containing D_2O*

Fraction	D (atoms % excess)
Steam volatile fatty acids:	
Water-soluble	0.02
Water-insoluble	—
Non-volatile fatty acids:	
Solid	<0.01
Liquid	<0.01
Lactose	<0.02
Blood water	0.77
Milk water	0.75

By contrast with the above results, the specific activities of fatty-acid fractions obtained from fat from the right ('bicarbonate') udder half were in all cases very low (in fact only measurable by gas-counting technique), showing that incorporation of $^{14}\text{CO}_2$ into fatty acids in the mammary gland under these conditions is negligible. Thus, in the case of the water-soluble steam-volatile fatty acids, the specific activity of the udder fat from the right side was only about 1/1000 of that from the left side, the contrast being pointed by the fact that milk was secreted about ten times faster by the right udder half than by the left udder half.

The results of deuterium determinations on the fatty acid fractions isolated from the 'bicarbonate' udder half are given in Table 5. The incorporation of deuterium was in all cases very low.

Cholesterol. Cholesterol isolated both from milk and udder tissue from the left ('acetate') udder half was radioactive, the specific activity of the cholesterol from the udder being about four times that of the milk cholesterol. The results, in confirmation of previous findings on the rabbit and goat *in vivo* (Popják & Beeckmans, 1950; Popják, French & Folley, 1950, 1951), indicate synthesis of cholesterol in the isolated udder tissue.

DISCUSSION

In planning the experiment it was assumed that each perfused udder half would secrete milk at the rate of about 100 ml./hr. and that this milk would be formed from material taken up at a fairly steady rate from the blood, the chemical composition of the alveolar tissue of the udder itself remaining constant. Such a process would presumably approximate to normal secretion. The results of the experiment were, however, quite different. The right ('bicarbonate') half of the udder produced 1420 ml. of milk containing 4.75 % fat, i.e. a total of 67.45 g. of fat. The amount of glucose and total fatty acids in the 5 l. of perfusion blood was certainly less than a third of this quantity. Most of this milk fat must therefore have arisen from other sources, presumably from the udder tissue itself. As regards the left ('acetate') side also there is evidence that the metabolites supplied were insufficient for the needs of the tissue. The specific activity of the blood CO_2 reached a peak immediately after the perfusion began, after which it dropped sharply and thereafter remained practically constant until the end of the experiment. It seems probable that the gland cells had taken up most of the acetate from the blood in a very short time, other metabolites probably being removed equally rapidly. It is calculated from the yields of udder and milk fatty acid fractions and their specific activities that a large fraction of the ^{14}C administered as acetate, nearly 400 $\mu\text{c.}$, i.e. 40 %, was present in the fatty acids at the end of the experiment. The most likely interpretation of the time course of the blood CO_2 specific activity would therefore seem to be that the initial high value was due to oxidation of free acetate, whereas the later steady excretion of $^{14}\text{CO}_2$ was due to oxidation of reserve materials in the tissue, including the labelled fatty acids formed from acetate. The fact that after the first 15 min. the specific activity of the blood CO_2 on the left ('acetate') side (Table 1) was of the same order as that of the volatile fatty acid fractions isolated from the udder (Table 3) is in keeping with this suggestion. It is also probable that the initial perfusion with saline had the effect of

depleting the reserves of metabolites in the cells, so that the available material in the blood was rapidly taken up. Beyond this point the two halves behaved differently: on the right side, where oxygenation and CO_2 removal were adequate, a large amount of milk was secreted, whereas on the left side only small amounts of the labelled fatty acids formed in the udder found their way into the milk.

The general conclusion to be drawn from these considerations is that the tissue was active in the turnover and secretion of fat, but it is very doubtful whether net fat synthesis occurred at all on the left side, and it probably occurred only to a limited extent on the right side. On the whole it is probable that breakdown of tissue fat by oxidation was the predominant process. The R.Q. of the udder can be taken as a measure of net fat synthesis or breakdown (see Folley, 1949). It is of interest that although the R.Q. of the lactating bovine udder *in vivo* has been generally found to be greater than unity, Shaw (1939) reported a mean value of only 0.8 for the perfused, isolated udder. It is known that the R.Q. of tissue slices of lactating mammary gland is very sensitive to the supply of suitable substrates in the medium (Folley & French, 1949, 1950). Further work with the perfused preparation should aim at the maintenance of continuous net synthesis of milk constituents, which might be possible by maintenance of constancy of composition in the perfusion medium.

In spite of these differences from physiological conditions, some qualitative conclusions can be drawn from the results. The secretion of radioactive milk fatty acids by the udder perfused with $\text{CH}_3^{14}\text{COOH}$ in general confirms previous results on the intact lactating goat (Popják, French & Folley, 1950, 1951) which led to the conclusion that the lactating ruminant udder brings about the synthesis of milk fat by condensation of acetate or some C_2 unit derived therefrom. The specific activities of all four crude fractions of milk fatty acids in this experiment were considerably lower than the maximum values found in the experiment on the live goat. The fatty acids isolated from the udder tissue, however, had an activity comparable to that found in goat milk several hours after the administration of labelled acetate. It is probable that the fatty acids synthesized in the cells are the direct precursors of the milk fatty acids, and the high activity of this fat, which includes 'structural' fat of the cells, illustrates the previous conclusion (Popják, French & Folley, 1951) that milk-fat synthesis is the end result of the dynamic turnover of the udder cell constituents.

The decrease of specific activity of the milk and udder fatty acids with increasing chain length indicates, in agreement with the results obtained on the intact lactating goat, that the short-chain acids could not have arisen to any considerable extent by the direct degradation of long-chain acids, mainly

oleic, as required by the theory of Hilditch (see Achaya & Hilditch, 1950). There appears to be a significant difference between the order of the specific activities of the pure fatty acids found in this experiment compared with those in the intact animal. It has been reported (French, Hunter, Martin & Popják, 1951; Popják, French, Hunter & Martin, 1951) that in the lactating goat during the first 12 hr. after the injection of labelled acetate, the specific activities of the milk fatty acids increased with chain length from butyric up to capric (decanoic). These results, together with those obtained by chemical degradation of some of the acids, could be explained only by the assumption that butyric acid synthesized in the gland from the labelled acetate was continuously being diluted with a non-isotopic C_4 compound. It was suggested that this C_4 precursor was obtained from the blood and that it might be β -hydroxybutyric acid. In the absence of this unlabelled precursor it would be expected that in the early part of the experiment the butyric acid would be the most active product of the condensation of C_2 units. This expectation appears to apply in the present experiment, in which the synthetic period was short, and the supply of β -hydroxybutyric acid in the perfusion blood was probably very small.

The results of the present experiment reinforce the previous conclusions drawn from the *in vivo* work, in that they exclude possible complications due to the incorporation in the liver or other organs of ^{14}C into carbohydrate or fat which could be transported to the udder, and there used as substrates for milk formation. Moreover, since the incorporation of $^{14}CO_2$ into milk fat in the mammary tissue has been found to be negligible, another factor which might complicate the interpretation of the previous tracer results can be disregarded. Kleiber *et al.* (1950) also found a low incorporation of $^{14}CO_2$ into milk fat in the intact cow.

The low values observed for incorporation of D into milk and udder fatty acids is not surprising when the turnover of the large quantities of fat present in the udder is taken into account. A significant proportion of D in the milk fat could only be expected if this fat arose directly from small molecules without dilution by preformed udder tissue fat. In order to study the uptake of D from body water, higher D_2O concentrations or longer experiments would be necessary.

The observed specific activities of milk and udder cholesterol agree with the conception of the turnover of udder constituents. Those constituents which are present in high concentration in the milk compared with the udder (the volatile fatty acids) are synthesized with high specific activity immediately following the administration of a single dose of precursor, whereas cholesterol, which has a higher con-

centration in the udder than in the milk, has a much lower activity, indicating a prolonged turnover time. It remains to be shown whether the low activity of stearic and oleic acids, found in this experiment as well as in the intact goat, can be entirely explained by this mechanism.

The lactose isolated from the milk on the left ('acetate') side had a significant radioactivity. Since extremely little activity was found in the milk lactose from the right ('bicarbonate') side, it appears that some pathway other than CO_2 fixation is responsible for the incorporation of acetate carbon into carbohydrate in the bovine udder. On the other hand, Kleiber *et al.* (1950) found that, following the injection of $Na_2^{14}CO_3$ into a lactating cow, lactose had the highest specific activity of the major milk constituents. In view of our results with the perfused, isolated udder, it seems probable that this was due to incorporation of $^{14}CO_2$ into glycogen in the liver followed by the transport of ^{14}C -glucose to the udder and its utilization for lactose formation.

SUMMARY

1. Two separated halves of a lactating cow udder were respectively perfused with blood containing carboxyl-labelled acetate (1 mc. $CH_3^{14}COONa$; 'acetate' half) and labelled bicarbonate (1 mc. $NaH^{14}CO_3$; 'bicarbonate' half) by a technique which permits of separate perfusion with no mixing of blood in the two circulations. Heavy water (D_2O) was added to the blood containing $NaH^{14}CO_3$.

2. The specific activity of the blood carbon dioxide in the 'acetate' udder half was maximal at the outset and thereafter fell to a fairly constant level throughout the rest of the experiment. Because of this and the fact that about 40% of the added ^{14}C was found in the udder fat at the end of the experiment, it would seem that the initial high specific activity of the blood carbon dioxide was due to the oxidation of free acetate, which was probably taken up rapidly by the udder tissue, the subsequent lower values corresponding to the oxidation of udder constituents, mainly fatty acids, into which the $CH_3^{14}COONa$ had been incorporated.

3. ^{14}C was incorporated into fatty acid fractions isolated from the milk and udder glycerides of the 'acetate' half, the mean specific activity falling with increasing mean chain length. The conclusion from previous work on a living lactating goat, that the udder utilizes acetate as a substrate for the synthesis of milk fatty acids, is therefore confirmed. The specific activities of the udder fat fractions were higher than those of corresponding fractions from the milk. This could be explained by dilution of newly synthesized fat by fat already existing in the udder tissue and by a rapid turnover of fat in the epithelial cells. The specific activities of pure fatty acids

isolated from the udder fat fell progressively with increasing chain length up to stearic and oleic acids. The short-chain acids could therefore not have been formed directly by degradation of long-chain acids.

4. The mean specific activities of fatty acid fractions isolated from udder fat from the 'bicarbonate' half were very low, indicating negligible incorporation of $^{14}\text{CO}_2$. The deuterium content of these fractions was also low, probably due to dilution of newly formed fat.

5. Cholesterol isolated from milk and udder fat from the 'acetate' half was radioactive, the specific activity of the udder cholesterol being higher than that from the milk. This confirms previous work on

the goat indicating that cholesterol is synthesized in the udder.

6. Lactose isolated from milk from the 'acetate' half was appreciably radioactive, while milk lactose from the 'bicarbonate' half was not. The udder tissue must therefore incorporate acetate carbon into lactose by some mechanism other than carbon dioxide fixation.

7. The application of the udder perfusion technique to biochemical studies of milk formation is discussed in the light of the results of this experiment, and it is concluded that the perfusion conditions must be carefully controlled if valid comparisons are to be made with the lactating udder *in vivo*.

REFERENCES

- Achaya, K. T. & Hilditch, T. P. (1950). *Proc. roy. Soc. B*, **137**, 187.
 Folley, S. J. (1949). *Biol. Rev.* **24**, 316.
 Folley, S. J. & French, T. H. (1949). *Biochem. J.* **45**, 117.
 Folley, S. J. & French, T. H. (1950). *Biochem. J.* **46**, 465.
 French, T. H., Hunter, G. D., Martin, A. J. P. & Popják, G. (1951). *Biochem. J.* **48**, vi.
 Graham, W. R., jun., Houchin, O. B., Peterson, V. E. & Turner, C. W. (1938). *Amer. J. Physiol.* **122**, 150.
 Kleiber, M., Smith, A. H. & Tolbert, B. (1950). *XVIIIth Int. physiol. Congr.*, Abstr., p. 304.
 Peeters, G. & Massart, L. (1947). *Arch. int. Pharmacodyn.* **74**, 83.
 Petersen, W. E., Shaw, J. C. & Visscher, M. B. (1939). *J. Dairy Sci.* **24**, 139.
 Popják, G. (1950). *Biochem. J.* **46**, 558.
 Popják, G. & Beeckmans, M.-L. (1950). *Biochem. J.* **46**, 547.
 Popják, G., French, T. H. & Folley, S. J. (1950). *Biochem. J.* **46**, xxviii.
 Popják, G., French, T. H. & Folley, S. J. (1951). *Biochem. J.* **48**, 411.
 Popják, G., French, T. H., Hunter, G. D. & Martin, A. J. P. (1951). *Biochem. J.* **48**, 612.
 Shaw, J. C. (1939). *J. Dairy Sci.* **22**, 438.

The Turnover Number of Cholinesterase

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Many problems in cholinesterase kinetics could be greatly simplified by a knowledge of the concentration of enzyme in the system being studied. It would, for instance, be of value to know the concentration of enzyme in various tissues, the blood in particular.

The concentration of enzyme in any system can be calculated from the activity if the turnover number (i.e. the number of molecules of substrate transformed in unit time/mol. of enzyme) is known. The turnover number (T) cannot be determined unless the enzyme is obtainable in a high degree of purity. Cholinesterase is not easily purified; in fact no pure preparation has been isolated from mammalian sources.

Easson & Stedman (1936), in discussing the fact

that certain drugs, such as physostigmine, inhibit cholinesterase in very minute amounts, assumed that 'one molecule of drug will inhibit the activity of one unit or active centre of enzyme', and based their calculation of active-centre concentration on this assumption, together with the demonstration that these drugs behaved non-competitively. They found that horse serum cholinesterase had a T of 1490 mol. of acetylcholine/sec./active centre.

The theory of inhibition by means of a bimolecular reaction between active centre and inhibitor was furthered by Straus & Goldstein (1943), but the validity of the turnover number found, 450 mol./sec., was later denied by Goldstein (1944), since physostigmine behaved competitively. Augustinsson & Nachmansohn (1949) confirmed Goldstein's