Cholesterogenesis from Tritiated Water in Rats: Evidence against the Existence of Diurnal Rhythms (2. Effect of Meal-Feeding)

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In the preceding communication (Fears & Morgan, 1976), we demonstrated that the diurnal variation in cholesterogenesis observed when acetate is used as a substrate is not apparent when \(^3\text{H}_2\text{O}\) is used to measure the overall rate of the pathway.

To investigate this difference further, and more fully to control the individual response to food, we have measured the rates of lipogenesis before and after consumption of a meal of a stock laboratory diet.

Male rats of the CFY strain were trained for 3 weeks to consume their daily food intake as a meal between 10:00h and 12:00h. In each experiment, rats were maintained on a reversed light-cycle with a period of darkness from 04:00h to 16:00h.

In addition to \(^3\text{H}_2\text{O}\), sodium [l-\(^{14}\text{C}\)]octanoate was used as a substrate for lipogenesis in vivo. Use of this precursor is believed to give a more accurate measurement of lipogenesis from acetyl-CoA than is possible with acetate (Dietschy & McGarry, 1974). Measurements of lipogenesis were made as described in the preceding paper (Fears & Morgan, 1976). The ileum was used for measurements on the small intestine. The concentration of acetyl-CoA in liver was determined by the method of Williamson & Corkey (1969).

The first experiment was performed using sodium [l-\(^{14}\text{C}\)]acetate with liver slices prepared from animals killed at intervals throughout the day. The purpose of this experiment was to demonstrate the conventional diurnal variation in cholesterogenesis so that we could then select the most appropriate times for more detailed study in vivo.

Fig. 1 shows that the synthesis of both fatty acids and cholesterol increased, as anticipated, in response to the ingestion of a meal; 10:00h and 14:00h were chosen for

![Graph showing response to a meal of digitonin-precipitable sterol and fatty acid synthesis from [l-\(^{14}\text{C}\)]acetate]

Each point is the mean of five analyses. Each analysis was of tissue from a single rat. ○, Digitonin-precipitable sterol synthesis; ●, fatty acid synthesis.
Table 1. Digitonin-precipitable sterol and fatty acid synthesis in vivo before and after a meal

Each rat was given $^3$H$_2$O (1 mCi/100g) and sodium [1-$^{14}$C]octanoate (5 μCi/100g body wt.) in 0.9% NaCl by intraperitoneal injection, 1 h before it was killed. Results are the means ± S.E.M. of eight analyses; each analysis was of tissue from a single rat. Significant difference ($P < 0.05$) between pre-prandial and post-prandial is indicated *.

| Experiment 1 |  | Experiment 2 |  |
|--------------|  |--------------|  |
| Pre-prandial | Post-prandial | Pre-prandial | Post-prandial |
| Digitonin-precipitable sterol synthesis from $^3$H$_2$O (μg/h per g of liver) | 200 ± 77 | 215 ± 69 | 93 ± 15 | 95 ± 18 |
| Digitonin-precipitable sterol synthesis from [1-$^{14}$C]octanoate (d.p.m./h per g of liver) | 3240 ± 674 | 4974 ± 733 | 2929 ± 384 | 3931 ± 704 |
| Fatty acid synthesis from $^3$H$_2$O (μg/h per g of liver) | 36 ± 6 | 381 ± 41* | 22 ± 2 | 263 ± 25* |
| Fatty acid synthesis from [1-$^{14}$C]octanoate (d.p.m./h per g of liver) | 6398 ± 916 | 47846 ± 6178* | 344 ± 44 | 7964 ± 1028* |
| $^3$H(d.p.m./ml of liver water) | 0.61 ± 0.02 | 1.07 ± 0.06* | 0.74 ± 0.03 | 0.89 ± 0.06* |
| Acetyl-CoA (nmol/g of liver) | 94.7 ± 8.1 | 64.8 ± 8.8* |

subsequent experiments as times likely to give a clear difference in the rates of lipogenesis, if any difference existed, with other precursors.

An experiment was performed (twice), to measure the rates of lipogenesis \textit{in vivo}, at 10:00h and 14:00h with $^3$H$_2$O and sodium [1-$^{14}$C]octanoate (31.8 mCi/mmol) given intraperitoneally 1 h before killing. The results are shown in Table 1.

In contrast with the results obtained with acetate, neither $^3$H$_2$O nor [14C]octanoate showed a significantly increased incorporation into digitonin-precipitable sterols post-prandially compared with pre-prandially. In agreement with the acetate incorporation data, the synthesis of fatty acids was greatly increased. Similar results were obtained for the small intestine to those for the liver.

The importance of calculating lipogenesis in terms of the specific radioactivity of $^3$H$_2$O in the liver is shown by the fact that an equilibrium existed between the proportion of $^3$H$_2$O in the serum and liver after the meal, but not before. Thus, if the results had been expressed in terms of radioactivity as d.p.m. per unit weight of liver, then an apparent difference in cholesterogenesis would have been observed.

The total concentration of acetyl-CoA in the liver was decreased post-prandially. If a similar change occurs in the cytoplasmic fraction, then the addition of a standard amount of precursor that generates acetyl-CoA would lead to a substrate of increased specific radioactivity post-prandially. Hence there will appear to be a higher rate of throughput into lipids if measurements are expressed as d.p.m. incorporated. This probably accounts for the small change in octanoate incorporation, compared with $^3$H$_2$O, after a meal.

Fatty acid synthesis was increased after a meal, whichever way it was measured. The necessity for such an increase to store energy from a diet containing 4% fat is apparent.

With acetate, we obtain conventional variations in cholesterogenesis entirely in agreement with the results of other workers. As the rate of synthesis measured \textit{in vivo} with $^3$H$_2$O is very much higher than that found \textit{in vitro} with acetate, and as $^3$H$_2$O

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measures total flux whereas the incorporation of acetate may reflect events essentially unconnected with the rate of cholesterogenesis, we consider that the results obtained in vivo are more representative of physiological events.

The conclusion from the present experiments therefore confirms the one made in the preceding paper. That is, cholesterogenesis in the rat at the principal sites, liver and small intestine, is not subject to diurnal variation under normal conditions.


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A New Approach to Metabolite Compartmentation in Muscle

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Phosphorus n.m.r. (nuclear-magnetic-resonance) spectra of intact tissues provide information about the concentrations, co-ordination and interconversions of phosphorus-containing metabolites (Moon & Richards, 1973; Hoult et al., 1974). The shape of any particular signal can indicate the number of environments in which the corresponding compound finds itself. It is thus a monitor for compartmentation of that compound. The present communication shows that phosphorus n.m.r. studies of muscle can give information about (a) the compartmenta-

tion of $P_i$ in the tissue, and (b) variations in phosphate metabolism between different muscle types.

Fig. 1 shows phosphorus n.m.r. spectra of a rabbit intact white muscle from the hind leg, recorded 20 and 90min after excision. In each spectrum the $P_i$ signal is much broader than that of creatine phosphate. The linewidth of the phosphate resonance increases with time after excision, and after 90min the signal has two components. In addition, the phosphate signals shift to lower frequency owing to acidification of the sarcoplasm caused by lactic acid accumulation. The linewidth of the creatine phosphate signal, however, is time-independent.

To establish that the anomalously broad phosphate resonance of each spectrum is not a result of a powerful relaxation mechanism, we have measured the transverse relaxation time, $T_2$ of phosphate magnetization using a multiple-pulse sequence (Carr & Purcell, 1954; Meiboom & Gill, 1958). The value obtained for $T_2$ is about 100ms, which corresponds to a linewidth of only 3Hz. As the observed resonance has a width of approx. 50–200Hz (depending on time from excision of the tissue), we conclude that the signal from phosphate in muscle consists of many narrow and partially overlapping components. The ions must therefore be partitioned among a large number of environments. The frequency observed for phosphate ions in each environment is different. This heterogeneity may occur within the individual cell or between muscle fibres. Since the resonance position of phosphate is very sensitive to pH, it is possible that a distribution of pH within the muscle could account for the observation. The multicomponent phosphate line-shape, which is measured 90min after excision of the muscle, would be produced if phosphate experienced one set of environments with pH values ranging around 6.8 and another set with pH values around 6.4. This would be consistent with the narrowness of the creatine phosphate signal: as its $pK_a$ is 4.6, the resonance position in unaffected by small variations in pH around neutrality. Frequency shifts can be generated by effects other than pH (Dwek, 1974); a distribution of $H^+$ concentration is merely the most plausible explanation presently available.

Further evidence for the existence of multiple environments for $P_i$ has been obtained from experiments with ‘dialysed’ muscle. Muscle samples were first washed