Dunphy, J. V., Littman, A., Hammond, J. B., Forstner, G., Dahlqvist, A. & Crane, R. K. (1965). *Gastroenterology* **49**, 12.

Gryboski, J. D., Thayer, W. R., Gryboski, W. A., Gabrielson, I. W. & Spiro, H. M. (1963). *New Engl. J. Med.* **268**, 78.

Haemmerli, U. P., Kistler, H. J., Ammann, R., Marthaler, T., Semenza, G., Auricchio, S. & Prader, A. (1965). *Am. J. Med.* **38**, 7.

Littman, A. & Hammond, J. B. (1965). *Gastroenterology* **48**, 237.

McMichael, H. B., Webb, J. & Dawson, A. M. (1965). *Lancet* i, 717.

Mansford, K. R. L. (1965). *J. Physiol., Lond.* **180**, 722.

Mansford, K. R. L. & Opie, R. K. (1963). *Analyst, Lond.* **88**, 646.

Miller, D. & Crane, R. K. (1961*a*). *Biochim. biophys. Acta* **52**, 281.

Miller, D. & Crane, R. K. (1961*b*). *Biochim. biophys. Acta* **52**, 293.

Newey, H., Sanford, P. A. & Smyth, D. H. (1963). *J. Physiol., Lond.* **168**, 423.

Paulley, J. W. (1963). In Symposium at 2*nd World Congress on Gastroenterology* in Munich. [H. Schon, editor.] p. 107. Basel: S. Karger.

Reid, E. W. (1901). *J. Physiol., Lond.* **26**, 427.

Rubino, A., Zimbalatti, F. & Auricchio, S. (1964). *Biochim. biophys. Acta* **92**, 305.

Schultz, S. G. & Zalusky, R. (1964). *J. gen. Physiol.* **47**, 1043.

Semenza, G. & Auricchio, A. (1962). *Biochim. biophys. Acta* **65**, 172.

Semenza, G., Auricchio, S. & Rubino, A. (1965). *Biochim. biophys. Acta* **96**, 487.

Semenza, G., Auricchio, S., Rubino, A., Prader, A. & Welsh, J. D. (1965). *Biochim. biophys. Acta* **105**, 386.

Starling, E. H. (1906). *Mercer's Company Lectures on Recent Advances in the Physiology of Digestion*, p. 127. London: Constable.

Walker, D. M. (1959). *J. agric. Sci., Camb.* **52**, 357.

Weijers, H. A., van de Kamer, J. H., Dicke, W. K. & Ijsseling, J. (1961). *Acta paediat.* **50**, 55.

Absorption of fats

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The general principles of the digestion and absorption of triglyceride fat have been outlined during recent years and are summarized in Fig. 1. Intestinal digestion of fat involves a physico-chemical transformation of the emulsified non-polar triglyceride fat catalysed by pancreatic lipase in the presence of bile, to a mixed bile salt micellar solution including mainly the polar end-products of lipolysis, monoglycerides and fatty acids. The mixed micelles of bile salts, monoglycerides and fatty acids have a diameter of 40–80 Å, assuming that they are spherical, and they appear to be highly hydrated. Recent work seems to indicate that they are spherical with the bile salt attached to the periphery, its hydroxy groups bound to the hydroxy groups of the monoglycerides, the latter forming the central core of the micelle. The micellar form seems to be the physical form preferred for absorption of lipids even if the mucosa cell can accept lipids in other forms. For detailed discussions of the various phases of triglyceride digestion the reader is referred to original articles (Borgström, 1964, 1965; Laurent & Persson, 1966; Hofmann, 1963) and I will change over to another related subject, which has interested us during the last year, the mechanism of specificity in absorption of non-glyceride fat. It has long been known that other fats, usually present dissolved in the dietary fat, are absorbed to a varying degree. It is thus known that cholesterol is absorbed only to a limited extent and that plant sterols, which chemically are very similar to cholesterol, are almost completely non-absorbable.
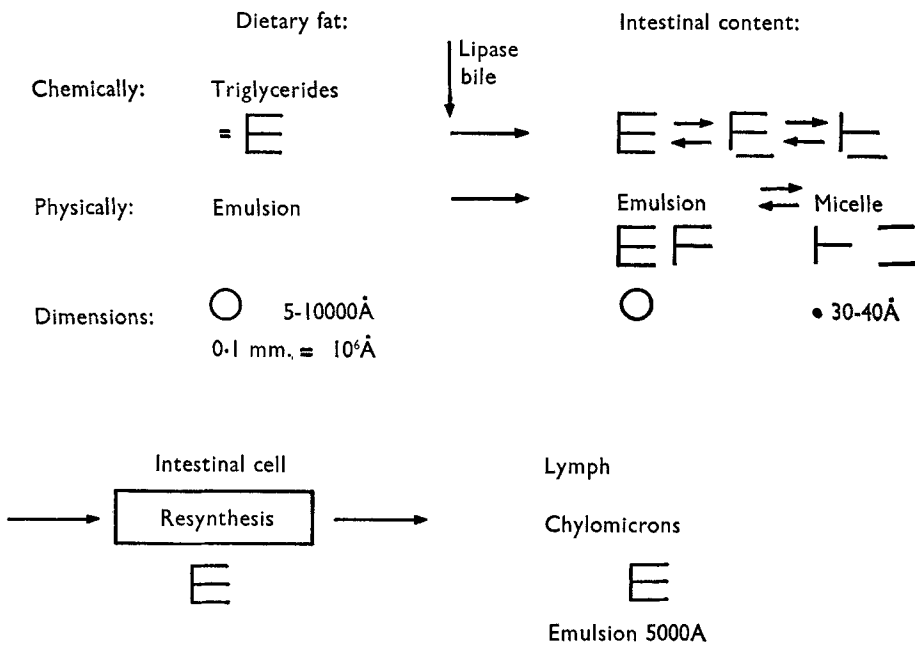
Fig. 1. Summary of the digestion and absorption process of glyceride fat.

Our problem has been to find out if the mechanism of glyceride digestion and absorption as outlined above can explain the various features of specificity of non-glyceride absorption.

It was of primary interest in the elucidation of the mechanism of the absorption of non-glyceride fat to find out the relationship between the dose given and amount absorbed. Such experiments were performed with cholesterol both in man and in
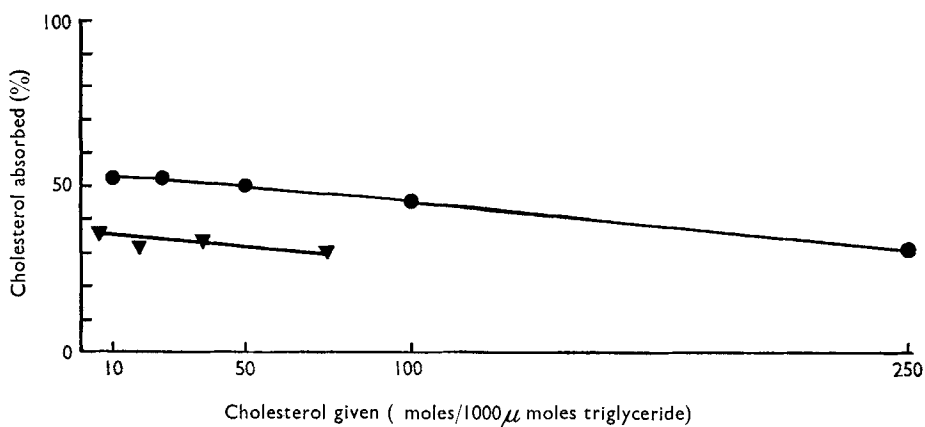

Fig. 2. Absorption curve for cholesterol in man (●) (butter) and in the rat (▲) (triolein) as obtained from single-dose isotope feeding experiments. Varying amounts of cholesterol were given in a constant amount of triglyceride fat.
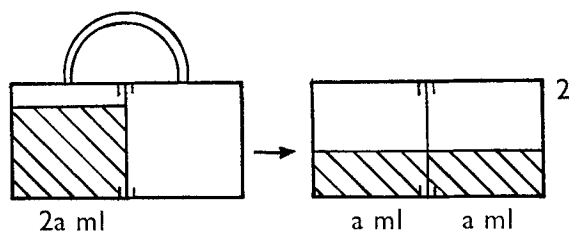
Millipore membrane

Fig. 3. Design of the experiments used for determining the partition coefficients micellar to emulsion phase = $K_{M/E}$.

the rat. Various amounts of labelled cholesterol were given in a constant amount of triglyceride fat in single-dose experiments. Absorption was calculated from the faecal excretion of sterols over a period of 5 days, and the absorption curves obtained are seen in Fig. 2. It appears that cholesterol absorption is incomplete and that approximately the same proportion of the dose is absorbed, not depending (inside certain limits) on the size of the dose. This type of absorption curve is not unique for cholesterol, it has also been obtained for cholesterol methyl ether and might be a general type of curve for non-polar lipids that are incompletely absorbed. When trying to understand the mechanism behind this type of absorption curve it appeared to us that a partition between two phases might be involved. It had already been demonstrated that, during fat absorption, intestinal contents contain two lipid phases: an emulsion phase and a micellar phase (Hofmann & Borgström, 1964). To be able to determine the partition of lipids between the two phases a method had to be used that separated at least one of the two phases.

Gel filtration on Sephadex columns had been shown to separate the emulsion phase from a micellar phase by exclusion, but the complexity of the process did not allow partition coefficients to be calculated (Feldman & Borgström, 1966a). We then turned to the use of equilibration filtration with Millipore filters. A glyceride–fatty acid mixture containing a dissolved sterol was emulsified in a bile salt solution well above its critical micellar concentration, the emulsion–micellar solution obtained was transferred into the one side of a dialysis chamber schematically shown in Fig. 3. The solution was allowed to filter and equilibrate until the volume was the same on both sides. The filtrate was then sampled and partition coefficients were calculated, the assumption being that only one of the phases had passed through the filter. By using filters of different pore size it was found that the 100 and 500 Å diameter filters gave an optically clear filtrate that by other means could be shown to be a pure micellar solution. Increase in filter pore size gave filtrates with increasing turbidity indicating that larger particles were passing. By using different pore size filters a particle size distribution could be obtained and an example is shown in Fig. 4. In this experiment equimolar amounts of tri-, di- and mono-olein with oleic acid corresponding to the free hydroxy groups of the glyceride mixture and containing cholesterol was used. It is seen that different
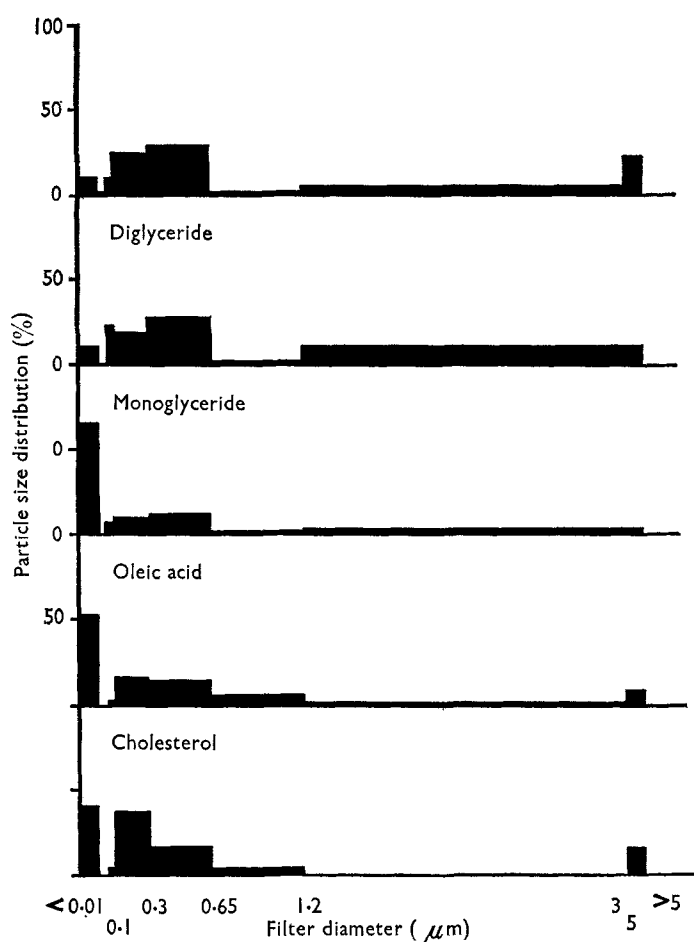
Fig. 4.   Particle size distribution of a glyceride–fatty acid emulsion in bile salt solution as measured by Millipore filters.

The emulsion contained per ml: 2·5 μmoles of triolein, of diolein and of mono-olein, 7·5 μmoles oleic acid, 0·1 μmole cholesterol and 6 μmoles sodium taourodeoxicholate (NaTDC). pH 6·3. Sodium ion concentration 0·15 M. The emulsion was produced with a Branson sonifier.

families of particles exist, a micellar fraction filtering with filters of a pore size less than 1000 Å and a fraction containing most of its particles of a size that passed the 12 000 Å filter. Only a small fraction was present that did not pass the 50 000 Å filter. The emulsion used in these epxeriments was prepared by sonication with a Branson sonifier. It was, however, then found that shaking this kind of surface-active lipid mixture in bile salt solution by hand for less than 1 min produced a rather stable emulsion with the same families of particles, the main difference being that more lipid was present in a form that did not pass the 50 000 Å filter. Continued shaking resulted in a transfer of this fraction to the 1000–12 000 Å fraction. It therefore seemed reasonable to assume that a certain particle size distribution is
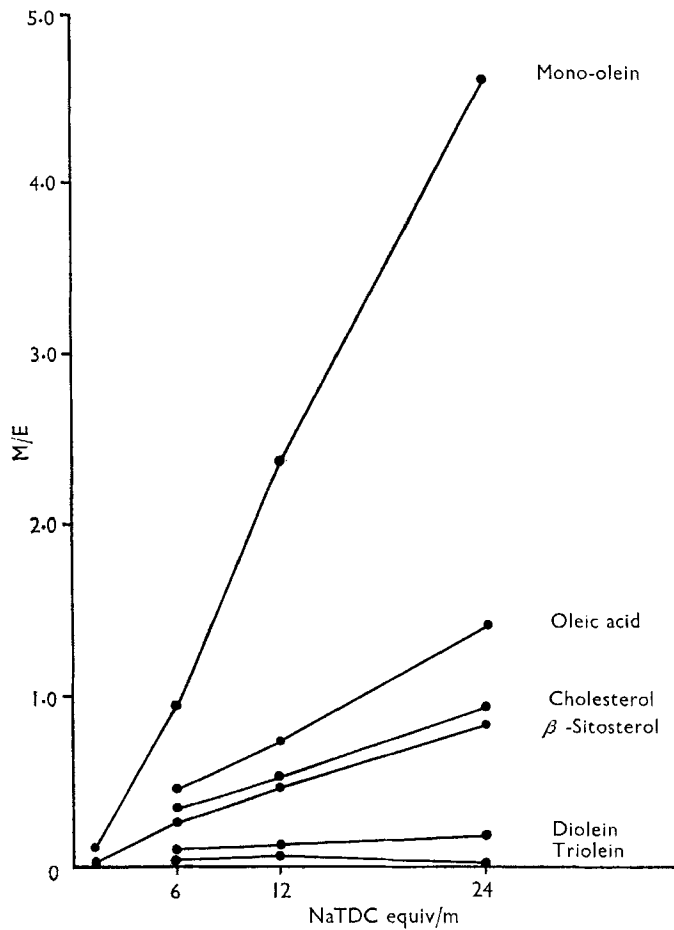
Fig. 5. Partition coefficients (M/E) for different lipids over bile salt concentration. Other conditions as in Fig. 4.

preferred from a thermodynamic point of view and is reached if sufficient energy is put into the system. In the following experiments shaking by hand for 1 min was used for the production of the emulsions. No significant differences in partition coefficients were found in sonicated or hand-shaken emulsions. In a series of experiments the partition coefficients for the micellar to emulsion phase were determined for different lipids known to be present in intestinal contents during fat digestion. In the calculations of partition coefficients no consideration has been taken of the actual volumes of the oil and micellar phases. The calculations have been based on the same total volume of the solution for both phases.

Fig. 5 gives the partition coefficients for the micellar to oil phase for lipids at different bile salt concentrations at pH 6·3. It appears that the $K_{M/E}$ (the partition coefficient of a particular compound between the micellar and emulsion phases) is very low for tri- and di-olein and is high for mono-olein. Intermediate values for
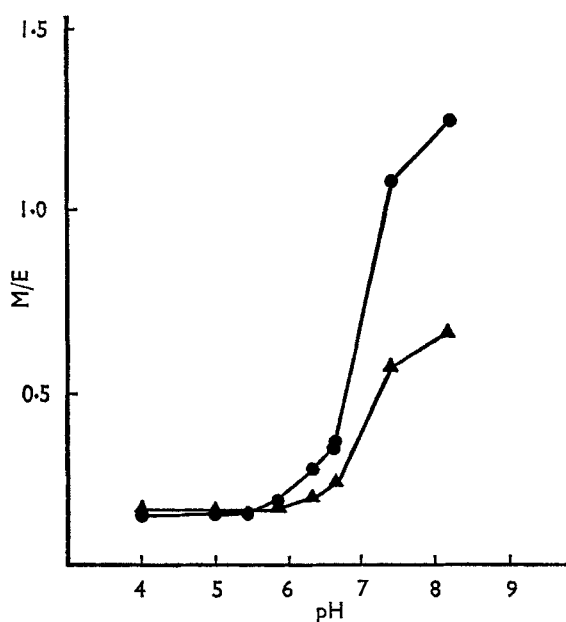
Fig. 6. Effect of pH on the partition coefficient (M/E) of oleic acid and cholesterol. Other conditions as in Fig. 4. ●, oleic acid; ▲, cholesterol.

$K_{M/E}$ were found for oleic acid, cholesterol and β-sitosterol. The latter substance has a slightly lower K value, which is in line with its lower polarity due to the presence of an additional ethyl group in the side-chain.

It was also of interest to study the effect of pH on the partition of oleic acid. Fig. 6 gives a plot of $K_{M/E}$ for oleic acid and cholesterol over pH. A sharp increase is found between pH 6 and 8. The curve can best be interpreted as representing the dissociation curve for oleic acid in bile salt solution, the pK_α being around 7. This value for the pK_α of long-chain fatty acids is in good agreement with that obtained from other types of experiment (Hofmann & Borgström, 1963; Borgström, 1964). The partition of cholesterol is also affected by the pH, indicating that inclusion of oleate in the mixed bile salt micelles favours the incorporation of cholesterol.

Also the effect of fatty acid chain length on the partition is of interest. Decrease in chain length sharply increases the $K_{M/E}$ (see Fig. 7). Also included in this effect is the partition to the water phase of the short-chain fatty acids, an effect that has not yet been studied in detail.

With this general background of the partition of different lipids derived from triglyceride I will now discuss in more detail the behaviour of the sterols in this respect. The sterol(s) was dissolved in the glyceride oil before emulsification in the bile salt solution. Increases in bile salt concentration resulted in a linear increase in the $K_{M/E}$ for cholesterol. Increase in cholesterol in the glyceride oil had no significant effect on the partition coefficient until high levels of cholesterol were reached. In this latter instance the coefficient dropped but the solubility of cholesterol in the oil
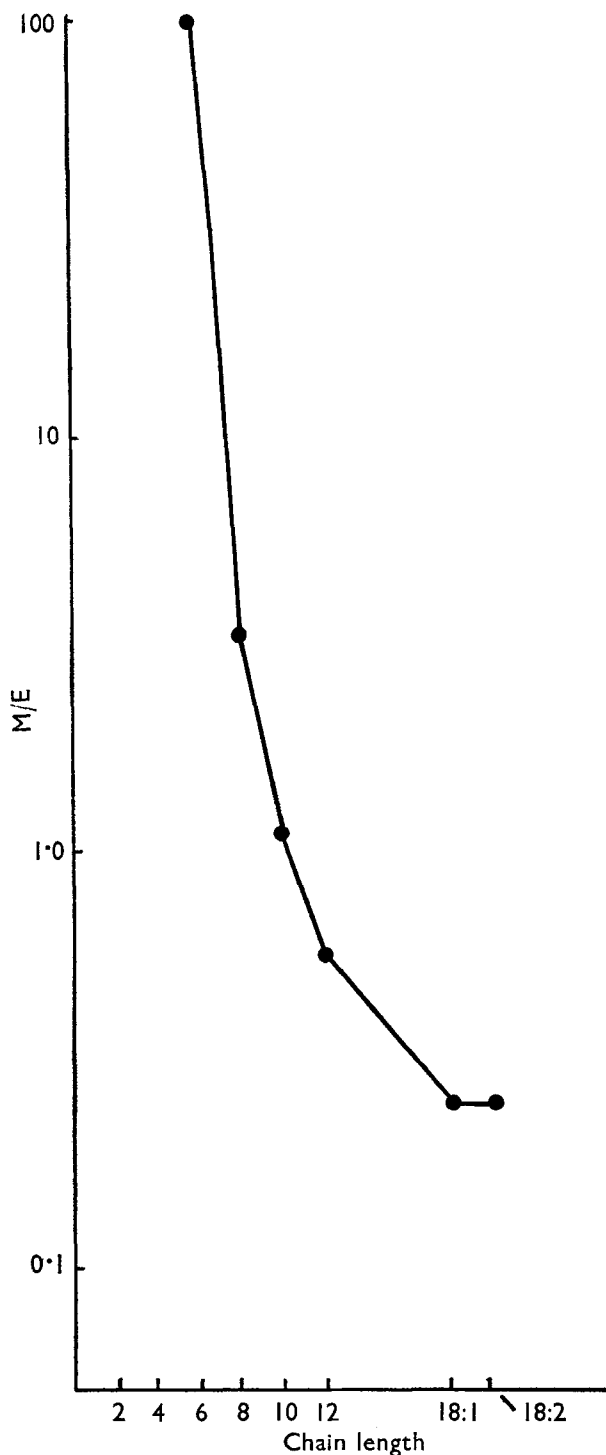
Fig. 7. Effect of chain length of the fatty acid on the partition (M/E) between 'micellar' and oil phase at pH 6·3. Conditions as in Fig. 4.
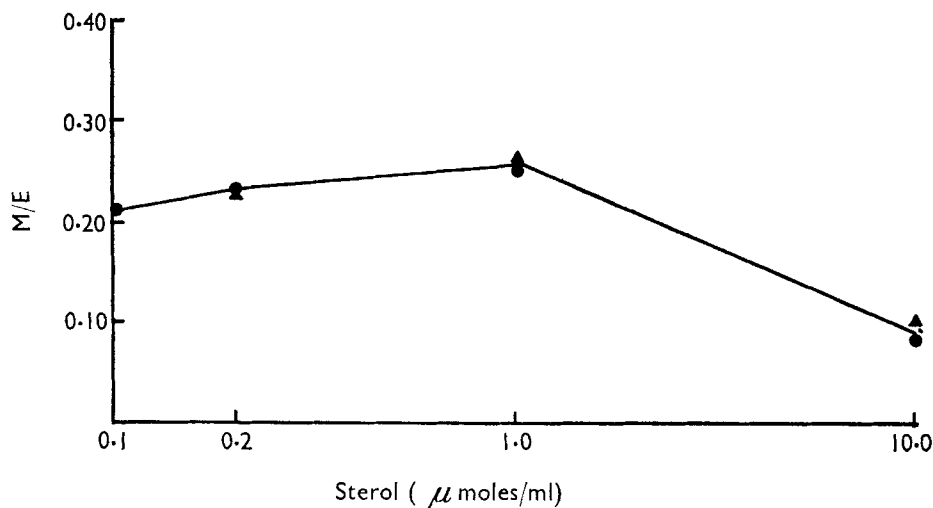
Fig. 8. Partition coefficients (M/E) micellar to emulsion phase of cholesterol at different levels of cholesterol and the effect of exchanging cholesterol for sitosterol. Except for sterols, the conditions were as in Fig. 4. ●, cholesterol; ▲, cholesterol+sitosterol.

phase was exceeded as shown by the behaviour of the emulsion. Sitosterol behaved as cholesterol and exchange of sitosterol for cholesterol had no effect on the partition of a small amount of labelled cholesterol (see Fig. 8). The results obtained thus indicated that emulsions of glyceride–fatty acids made in bile salt solution behaved as would be expected from a two-phase partition system.

Fig. 9 shows the plan of experiments subsequently used to study cholesterol transport in a kinetic system. Triolein containing labelled cholesterol was introduced into one of the compartments of the filtration cells described above and a head of bile salt solution connected. The Millipore filter was used for filtration of the micellar phase which was collected in a fraction collector. After some time, pancreatic lipase
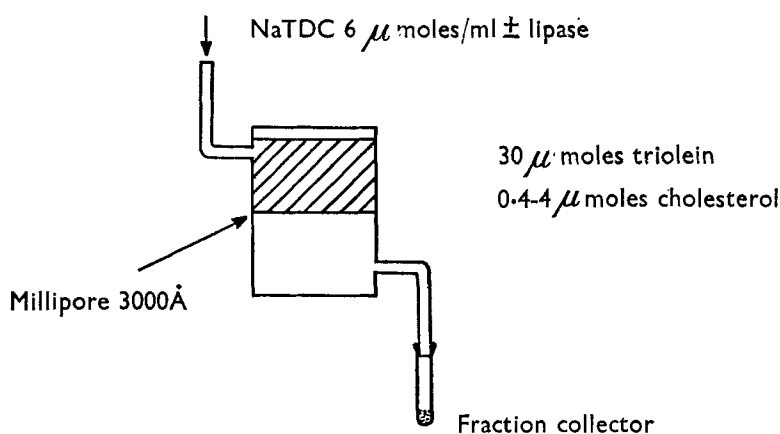

Fig. 9. Experimental design used as an 'artificial intestine'. For details see above.
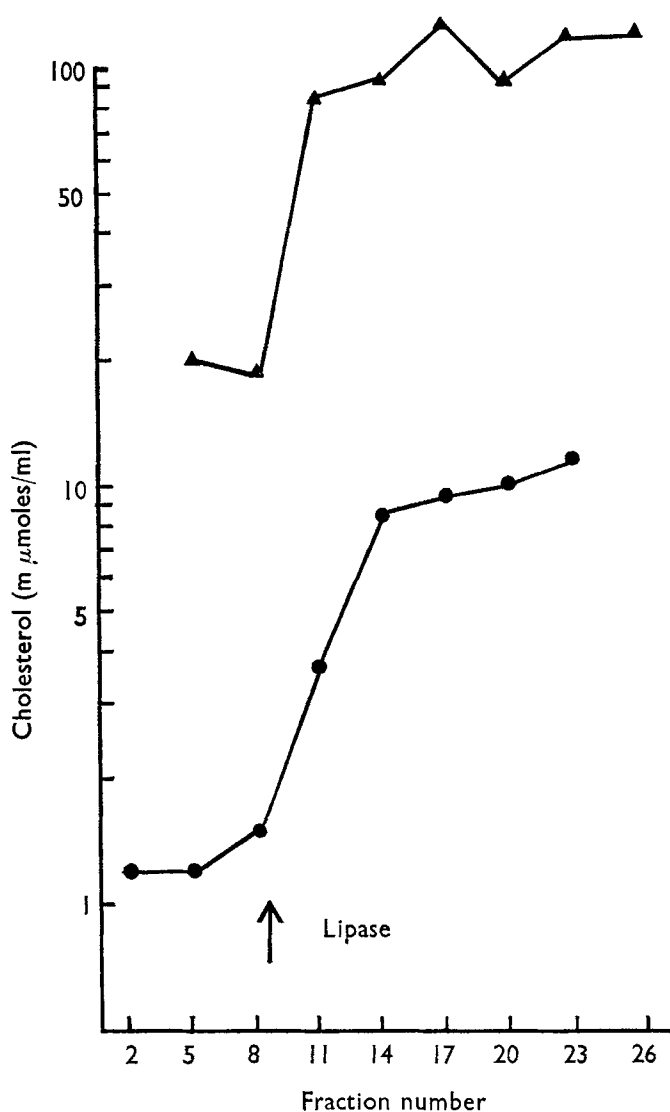
Fig. 10. Results of experiments obtained with the design illustrated in Fig. 9. The two curves show the cholesterol concentration in the filtrates at two different levels of cholesterol in the oil phase. ●, 0·4 μmoles cholesterol, 30 μmoles triolein; ▲, 4·0 μmoles cholesterol, 30 μmoles triolein.

was added to the bile salt solution entering the upper compartment. The results of two experiments, identical except for the amount of cholesterol in the triolein, are seen in Fig. 10. It can be seen that the concentration of cholesterol in the filtrate was dependent and proportional to the concentration of cholesterol in the oil phase. It can also be seen that the addition of lipase sharply increased the concentration of cholesterol in the filtrate and also that the level reached was dependent on the concentration of cholesterol in the oil phase. The lipase catalyses the formation of the

more polar monoglycerides and fatty acids which form mixed micelles with the bile salt solution favouring the partition of cholesterol into the micellar phase. This 'artificial intestine' thus produces a micellar phase in which the concentration of cholesterol is proportional to its concentration in the fed triglyceride and it is obvious that the same mechanism can be expected to operate in the intestinal content, being the basis of the absorption curves found for cholesterol in vivo.

Table 1. *Comparison of the $K_{M/E}$ values for certain sterols and cholesterol ethers and the rates of absorption of these compounds from the intestine of the rat*

Compound	Partition micellar phase/ oil phase	Uptake intestinal slices	Absorption in whole animal (%)
Cholesterol	0·25	1·0	50
Cholesterol methyl ether	0·068	2·5	41
Cholesterol propyl ether	0·047	2·0	11
Cholesterol decyl ether	0·021	2·4	7
Sitosterol	0·23	1·1	5

The $K_{M/E}$ values for some sterols and a homologous series of cholesterol ethers are shown in Table 1, from which it may be seen that the $K_{M/E}$ values decreased with increasing chain length of the aliphatic alcohol, an effect that parallels rather well their absorption as measured by their appearance in the thoracic duct lymph of rats (Table 1). It seems reasonable, therefore, to predict that the partitioning between the oil phase and a micellar phase can constitute one level of specificity in the absorption of fats.

Sitosterol does not, however, fit into this scheme. It has a partition coefficient very close to that of cholesterol but is only absorbed to a very minor extent compared to cholesterol. Therefore another level of specificity must operate to effect this difference.

To explore another level of the absorption process, experiments were performed using intestinal rings from rat or hamster in vitro (Feldman & Borgström, 1966b). In these experiments the intestinal preparation was incubated with an emulsion of glycerides and fatty acids containing different labelled non-polar lipids prepared in bile salt solution. The results indicated that the uptake of lipids by the intestinal tissue increased as the polarity of the lipid solute decreased. Thus the non-polar cholesterol decyl ether showed considerable uptake (see Table 1). Again there was found no difference in the behaviour of cholesterol and sitosterol. In this type of experiment an equilibrium is measured between the three phases present: emulsion, micellar solution and the membrane of the cell, that obviously does not bear any direct relationship to their overall absorption process in vivo.

In the process of digestion and absorption in vivo a lipid such as cholesterol is transported from the oil phase via the micellar phase to the cell membrane. The concentration of the solute in the micellar phase is dependent on the partition coefficient for the substance between the oil phase and the micellar phase. The concentration in the micellar phase in a kinetic system must be a determinant for the rate of transfer from the oil phase to the cell membrane. As the partition oil to

micellar phase is dependent on the presence of the products of lipolysis of the tri-glyceride, monoglyceride and fatty acid (and soaps), it is sharply decreased when all triglyceride has been absorbed. Non-polar lipids not absorbed together with the products of triglyceride hydrolysis therefore remain mainly dispersed in bile salt solution and are largely unavailable for absorption.

The difference in absorption of cholesterol and sitosterol, however, still remained unexplained. A series of experiments was therefore performed on the whole rat. Rats were given a dose of triolein containing labelled sterol and the distribution was determined in different parts of the gastro-intestinal tract at various times after dosing.

When labelled triolein was given digestion and absorption took place rapidly and efficiently and only minor amounts of labelled fat were found at any time in any of the different parts of the gastro-intestinal tract (except the stomach). If cholesterol was included in the triglyceride oil, part of it was rapidly taken up by the intestinal mucosa at the same time as the triglyceride split products; what was absorbed was present in the intestinal content and was rather rapidly transported to the large intestine where it is no longer available for absorption. (See Figs. 11 and 12.) In
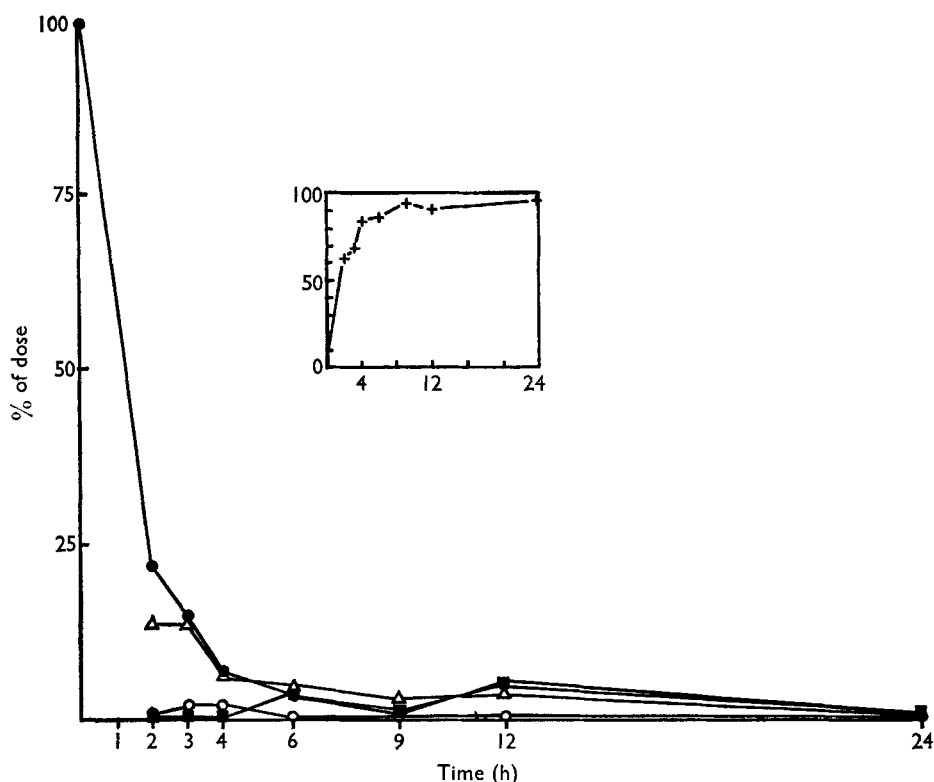

Fig. 11.   Time curves for the distribution of labelled triolein in the different parts of the gastro-intestinal tract of the rat after a dose of 200 μmoles triolein. ●, stomach; ○, intestinal contents; △, intestinal wall; ■, large intestine; +, absorption.
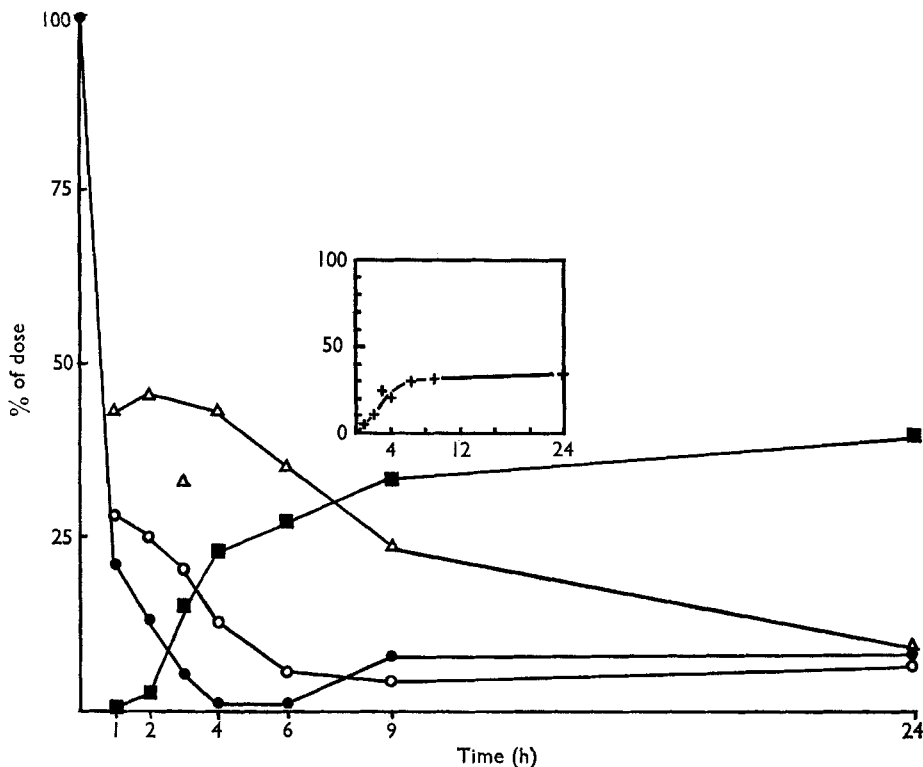
Fig. 12. Time curve for the distribution of labelled cholesterol over the gastro-intestinal tract of the rat after a dose of 200 μmoles triolein with 5 μmoles cholesterol. ●, stomach; ○, intestinal contents; △, intestinal wall; ■, large intestine; +, absorption.

addition to the partition effects occurring in the intestinal content, the rate of movement of the intestinal content thus is an important factor in the absorption of non-polar lipids.

The fraction of the cholesterol transferred to the intestinal wall is only slowly transported further, i.e. from the intestinal cell to the lymph. This transfer seems to involve esterification of the cholesterol. The half-life of the 'absorbed' cholesterol being around 12 h (Borgström, Lindhe & Wlodawer, 1958).

This retention in the intestinal cell might be unique for sterols and it might be that the rate of transfer from the mucosa cell determines its extent of actual absorption into the body. The other determinant is the half-life of the intestinal cell. As was discussed earlier by Dr Margot Shiner, the half-life of the intestinal cell is so short, 24–36 h, that it is obvious that it might be an important factor in regulating the absorption of substances only slowly removed from the intestinal cell into the circulation. It therefore seems plausible that sitosterol, although taken up by the intestinal mucosa at a rate similar to that of cholesterol, in the absence of an efficient mechanism for its cellular metabolism (esterification) will be largely excreted with shed intestinal cells.

This kind of mechanism thus would constitute a second level in the specificity

of absorption of non-glyceride fat. Preliminary results with labelled sitosterol, however, indicate that, although both these levels of specificity might be important for the absorption of sitosterol, other factors as yet unknown are also operating.

REFERENCES

Borgström, B. (1964). *J. Lipid Res.* **5**, 522.
Borgström, B. (1965). *Biochim. biophys. Acta* **106**, 171.
Borgström, B., Lindhe, B.-A. & Wlodawer, P. (1958). *Proc. Soc. exp. Biol. Med.* **99**, 365.
Feldman, E. B. & Borgström, B. (1966*a*). *Biochim. biophys. Acta* **125**, 136.
Feldman, E. B. & Borgström, B. (1966*b*). *Biochim. biophys. Acta* **125**, 148.
Hofmann, A. F. (1963). *Biochem. J.* **89**, 57.
Hofmann, A. F. & Borgström, B. (1963). *Biochim. biophys. Acta* **70**, 317.
Hofmann, A. F. & Borgström, B. (1964). *J. clin. Invest.* **43**, 247.
Laurent, T. C. & Persson, H. (1966). *Biochim. biophys. Acta* **106**, 616.

16 July, Second Session

Chairman : PROFESSOR D. H. SMYTH, MD, *Department of Physiology and Medical Research Council Research Group on Intestinal Absorption, University of Sheffield*

Sodium chloride absorption by the small intestine and the relationships between salt transport and the absorption of water and some organic molecules

By D. S. PARSONS, *Department of Biochemistry, University of Oxford*

Ionic composition of contents of intestinal lumen

A feature of the luminal environment of the mucosal cells of the small intestine is that it normally contains sodium in a concentration which is approximately that in the plasma. Distilled water damages the mucosa of the small intestine (Reid, 1900; Dennis, 1940), and was indeed used by Waymouth Reid to poison intestinal segments during experiments on absorption. Under physiological conditions the mucosal cells of the small intestine never come in contact with plain water, water taken by mouth being considerably mixed with gastro-intestinal secretions before absorption. For example, when meals and liquids are consumed by human subjects, the contents of the duodenum and jejunum are rapidly diluted and sodium chloride is added (Reitmeier, Code & Orvis, 1957; Borgström, Dahlqvist, Lundh & Sjövall, 1957; Fordtran, Levitan, Bikerman, Burrows & Inglefinger, 1961; Hindle & Code, 1962).

There appear to be two causes for these phenomena. Firstly, the gastro-intestinal secretions provide a daily circulation of fluid and salt which considerably exceeds the dietary intake (Table 1). The entry of these secretions into the upper intestine will continually modify the composition of the contents. The second factor in modifying the composition of the gastro-intestinal contents is the occurrence of massive bidirectional fluxes of water and of electrolytes across the intestinal cells. The existence and magnitude of these fluxes was first demonstrated for dog intestine by the classical experiments of Visscher and his associates (cf. Visscher, Varco, Carr, Dean & Erickson, 1944; Visscher, Fetcher, Carr, Gregor, Bushey & Barker, 1944).