BBA 75431

INFLUENCE OF BLOOD SUPPLY ON LIPID UPTAKE FROM MICELLAR SOLUTIONS BY THE RAT SMALL INTESTINE

CHRISTER SYLVÉN

Division of Physiological Chemistry, Chemical Center, University of Lund, Lund (Sweden)

(Received January 16th, 1970)

SUMMARY

1. A micellar solution containing trace amounts of different radioactive species of lipid was injected into a rat jejunal segment, tied off at both ends, with or without blood supply. Incubation was performed for different times after which the jejunal tissue was isolated and the radioactivity uptake determined. In one experiment jejunal slices were incubated for different times in a micellar solution.

2. With intact blood supply to the intestinal segment the uptake of the different species of lipid varied between 8 and 30 % 0.5 h after administration. Octadecane had the most rapid rate of uptake followed by oleic acid, monoolein and cholesterol, respectively. The rate of sitosterol uptake was approximately one third that of cholesterol.

3. When the blood supply to the segment was interrupted, the uptake decreased for all species of lipid to 1.5–4.5 %. After 5 min there was no net uptake with time. The cholesterol uptake decreased more than the sitosterol uptake, the latter being about 0.7 of that of cholesterol.

4. When a micellar solution containing KCN was injected into a jejunal segment with intact blood supply, there was a decrease in lipid uptake similar to that produced by exclusion of the blood supply.

5. In contrast to the low uptake by segments with interrupted blood supply, the uptake of radioactive sterols by preparations *in vitro* increased markedly with time. Sitosterol was taken up to the same extent as cholesterol.

6. The results suggest that intestinal lipid absorption cannot be investigated by conventional methods *in vitro*. The penetration of lipid from micelle to mucosa epithelial cell is thought to be an active process the arrangement of which is hypothetically described in a four-dimensional membrane.

INTRODUCTION

The intestinal absorption of different species of lipid is considered to involve a transfer from an oil phase through a micellar phase to the mucosa epithelial cell[1,2]. Digestion of triglyceride by pancreatic lipase results in the production of

Definition: In this investigation, octadecane, oleic acid (9-octadecenoic acid) and monoolein (glycerol-1-monooctadecenoate) are as a group referred to as octadecanoic compounds.

monoglyceride and fatty acid. These compounds form mixed micelles together with components of the bile[3]. Transfer from the micellar phase to the epithelial cell is thought to be a passive diffusion process[1].

The micellar solubilization and hence the luminal transfer of sitosterol and cholesterol to the epithelial cell are dependent on the presence of the products of glyceride hydrolysis in the micellar phase[4]. Sitosterol and cholesterol seem to be transferred from oil phase to micellar phase to the same degree[5,6]. *In vivo* the uptake of cholesterol by the epithelial cell[7] and the transport of absorbed cholesterol in the thoracic duct lymph[8] are about 5 times greater than for sitosterol. *In vitro, i.e.* when everted sacs have been incubated, sitosterol and cholesterol are taken up to the same extent[6]. The specificity mechanism in sterol absorption, which exists *in vivo*, has been localized to an outer layer of the mucosa epithelial cell[7,8].

In this investigation lipid absorption and the specificity mechanism in sterol absorption have been studied in preparations that can be pictured as representing a gradual transition from conditions *in vitro* to *in vivo*. Different micellar solutions containing sterols, octadecane or oleic acid and monoolein have been used to study uptake into small intestinal slices *in vitro*. In addition, these solutions have been injected *in situ* into rat jejunal segments which had been tied off at either end. Conditions of both intact and interrupted blood supply were studied.

MATERIALS

[4-^{14}C]- and [7-^{3}H]cholesterol (the Radiochemical Centre, Amersham, Bucks, Great Britain) were checked by thin-layer chromatography and found to have a radiopurity of more than 98 %. [22,23-^{3}H] Sitosterol was prepared from stigmasterol by hydrogenation as previously described[5] and found by thin-layer chromatography to have a radiopurity of more than 97 %. [1-^{14}C] Oleic acid, [9,10-^{3}H] oleic acid and [1-^{14}C] octadecane (the Radiochemical Centre, Amersham, Bucks, Great Britain) were found by thin-layer chromatography to have a radiopurity of more than 98 %.

[^{14}C] Monoolein was synthesized from isopropylideneglycerol and [1-^{14}C]-oleic acid[9]. It was further run on thin-layer chromatography to a radiopurity of more than 96 %. Sodium taurodeoxycholate was synthesized according to HOFMANN[10]. Monoolein was a product of Distillation Products Industries (Rochester, N.Y.). It was purified on an alumina column. 2-(4'-*tert*-Butylphenyl)-5-(4-biphenyl)-1,3,4-oxadiazole was obtained from Ciba Ltd., Basle, Switzerland.

METHODS

White male rats (250 g) were fasted overnight prior to experimentation.

Anaesthesia was induced by administration of Nembutal intraperitoneally. The abdominal wall was opened in a 37° room. 0.30-g wet wt. jejunal segments of approx. 3 cm were ligated with intact blood supply. The first of these segments was located just distal to the ligament of Treitz. Before the segment was completely closed, 0.4 ml of a micellar solution was injected through one end. In some cases the blood supply was excluded by tying off the whole mesentery of the segment under study. The segment was resected 5, 15 or 30 min after injection. Where the blood supply had been left undisturbed for the study ligation was carried out just prior to resection.

In a few experiments KCN was included in the micellar test solution which was then injected into the proximal jejunal segment. In the adjacent segment the same test solution without KCN was administered.

In one experiment jejunal slices of approx. 2 cm (0.20 g wet wt.) were carefully resected, opened along the anti-mesenteric border and immediately transferred to 2.0 ml of the micellar solution. Using segments of this size, the tissue remained extended so that the mucosal surface was exposed to the micellar solution during the whole incubation. Gentle agitation was employed.

At the end of the experiment the mucosal surface of the segment was exposed to 5 ml 0.15 M NaCl containing 2.4 μmoles sodium taurodeoxycholate per ml. The segment was then washed 3 times in 10 ml of the same solution (5 min in each) with gentle agitation. It was thereafter dried with filter paper and weighed.

Homogenization was performed in chloroform–methanol (2:1, v/v). The lipids were extracted according to the Folch procedure. An aliquot was evaporated to dryness in a counting vial. 15 ml of toluene–dioxane–2-(4'-*tert.*-butylphenyl)-5-(4-biphenyl)-1,3,4-oxadiazole scintillation solution were added. Counting was performed to at least 95 % confidence in a Packard liquid-scintillation spectrophotometer of the 4000 series. Quench correction with internal standard was performed.

The micellar solutions were generally prepared according to HOFMANN[11]. They consisted of 10 mM monoolein, 10 mM sodium taurodeoxycholate and different radioactive tracers in phosphate buffer (pH 6.3) which was 0.15 M with respect to Na^+. The administered cholesterol tracer represented 0.0006 μmole, the sitosterol tracer 0.01 μmole, the oleic acid tracer $<$ 0.02 μmole and the octadecane tracer 0.2 μmole. When radioactive monoolein was administered the solution was, as in the other experiments, 10 mM with respect to this substance. Monoolein and tracers were evaporated to dryness and buffered sodium taurodeoxycholate solution was then added. The solution was shaken until water-clear.

Where KCN was included in the micellar test solution the composition was as follows: 10 mM monoolein, 10 mM sodium taurodeoxycholate and the radioactive tracers in glycine–NaOH buffer (pH 11.4) which was 0.15 M with respect to Na^+.

Each experiment consisted of four observations made in different rats during the same day.

RESULTS

Fig. 1 illustrates the uptake of trace amounts of radioactive cholesterol and sitosterol by six adjacent segments of rat jejunum with intact blood supply after 30 min. The uptake of cholesterol was around 22 % in the proximal two segments and 13 % in the distal four. The sitosterol uptake was 5 % in all segments. The ratio $^3H/^{14}C$ in the radioactivity uptake was 0.20–0.40 in all the segments (Fig. 1).

In order to determine whether the label left the intestinal segment during the incubation, a micellar solution was incubated in segments for different times. The radioactivity of the segment and of its intestinal content was then measured. Fig. 2 shows that there was no change in the radioactivity recovered with time. Because of a systematical error of the method, the amount recovered was about 8 % more than the amount of radioactivity injected into the segment.

As indicated previously, the intestinal segment was washed 3 times. The

efficacy of the washing procedure was studied by measuring the timed release of radioactivity from the segment into each of the three wash solutions (Fig. 3). It was found that 3 min was the optimal time period, since no further radioactivity was recovered after this interval.

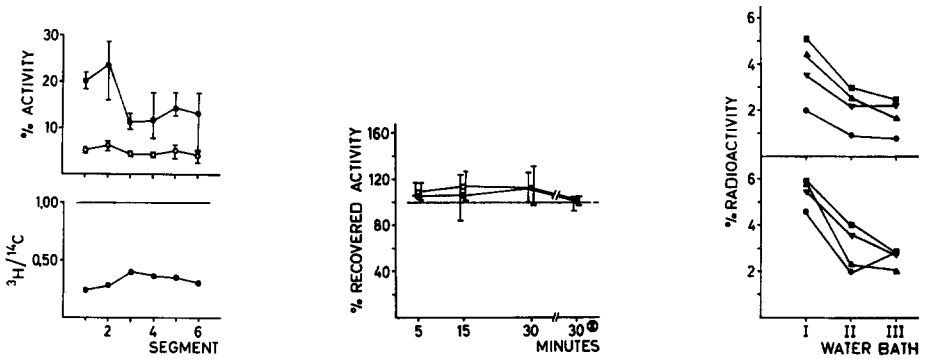


Fig. 1. Above: Uptake of [22,23-^{3}H] sitosterol ± range (○—○) and [4-^{14}C] cholesterol ± range (●—●) by six adjacent jejunal segments. Below: Ratio of uptake $^3H/^{14}C$. The ratio was taken as 1.0 in the micellar test solution. 0.4 ml of this containing tracers of [22,23-^{3}H] sitosterol and [4-^{14}C] cholesterol was injected 30 min before isolation.

Fig. 2. Percent radioactivity recovered ± range of [^{14}C] monoolein (○—○) and [^{3}H] cholesterol (●—●) in intestinal segments and their intestinal content. The segment was isolated at different times after the injection of 0.4 ml of a micellar test solution containing the radioactivities. ⊗ refers to one segment without blood supply.

Fig. 3. Percent radioactivity released from an intestinal segment into washing Solutions I, II and III containing 5 ml 2.4 μmoles sodium taurodeoxycholate per ml in 0.15 M NaCl. Above: [^{14}C]-Monoolein. Below: [^{3}H] Cholesterol. The segment was washed in each solution for 1 min (●—●), 3 min (▼—▼), 5 (▲—▲) or 10 min (■—■). 0.4 ml of a micellar solution with the two radioactivities was injected 30 min before isolation of the segment. Each point represents eight observations. After isolation the segment was opened and rinsed with 5 ml sodium taurodeoxycholate solution. Then it was transferred to the wash solutions. 'Percent radioactivity' denotes the fraction found in the wash solution divided by the radioactivity recovered from the segment and the wash solutions.

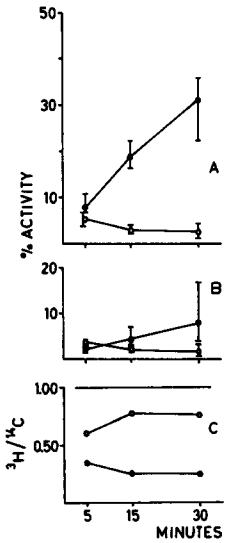
Studies on lipid uptake

It is assumed that the percentage of isotope taken up by the tissue equals mass uptake, *i.e.* that no exchange of labeled molecules for unlabeled molecules took place.

The cholesterol and sitosterol uptake both increased with time (Figs. 4A and 4B). However, the cholesterol uptake was 4 times as large as that of sitosterol. When the blood supply to the jejunal segment was excluded the cholesterol uptake, 5 min after administration, was 5 %. After 15 or 30 min there was rather a decrease to 3 %. A parallel effect was observed on the sitosterol uptake so that 15 or 30 min after administration 2 % was recovered in the jejunal segment. Fig. 4C shows that the ratio $^3H/^{14}C$ of the radioactivity uptake by segments with blood supply was 0.25–0.35. With interruption of blood supply there was an increase to 0.60–0.80.

With the blood supply excluded, the uptake of sitosterol and cholesterol by a jejunal segment was not further changed when the connections with the peritoneum and the rest of the jejunum were excised. After 5 min incubation there was no net uptake. The uptake of sitosterol and cholesterol in jejunal slices incubated *in vitro* increased with time (Fig. 5).

In both experiments the ratio $^{3}H/^{14}C$ (Fig. 5) increased with time, *i.e.* there was an increased uptake of sitosterol relative to cholesterol with time. In the experiment *in vitro* the ratio $^{3}H/^{14}C$ was 0.74–1.04. Thus, after 30 min the ratio was 1.04, indicating that sitosterol was taken up to the same extent as cholesterol.



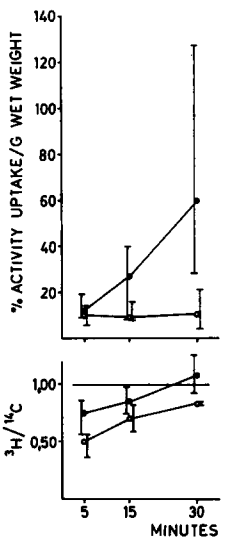


Fig. 4. Uptake of [4-^{14}C] cholesterol ± range (A) and [22,23-^{3}H] sitosterol ± range (B) by a jejunal segment the blood supply of which was intact (●—●) or ligated (○—○).
Ratio of uptake $^{3}H/^{14}C$ in relation to a ratio of 1.0 in the micellar test solution (C). 0.4 ml of this containing tracers of [22,23-^{3}H] sitosterol and [4-^{14}C] cholesterol was injected. Six tests were carried out in each animal, every second one with ligated blood supply.

Fig. 5. Above: Percent uptake per g wet wt. of [4-^{14}C] cholesterol ± range by isolated jejunal segments *in situ* (○—○) and by jejunal tissue *in vitro* (●—●). Below: Ratio of uptake $^{3}H/^{14}C$ ± range. The ratio was taken as 1.0 in the micellar test solution. In the experiment *in situ* 0.4 ml of the micellar test solution containing [22,23-^{3}H] sitosterol and [4-^{14}C] cholesterol was injected, and in the experiment *in vitro* the tissue was incubated in 2.0 ml of the same solution.

In the following three experiments a micellar solution with the same concentrations of monoolein, sodium taurodeoxycholate and Na^{+} in phosphate buffer (pH 6.3) was administered but with other pairs of radioactive lipids, *i.e.* monoolein and cholesterol (Fig. 6A), oleic acid and monoolein (Fig. 6B) and octadecane and cholesterol (Fig. 6C). The uptake of all the tracers by jejunal segments with intact blood supply increased with time.

Fig. 7 shows the uptake of the different species of lipid in these experiments relative to that of octadecane, taken as unity. In order of decreasing uptake octadecane is followed by oleic acid, monoolein, cholesterol and sitosterol. The relative uptake of the different species of lipid changed little with time.

With excluded blood supply the uptake of all lipid species investigated was identical. Up to 5 min after injection of the test solution there was a net uptake. After 15 min there was a net decrease, which was unchanged after 30 min.

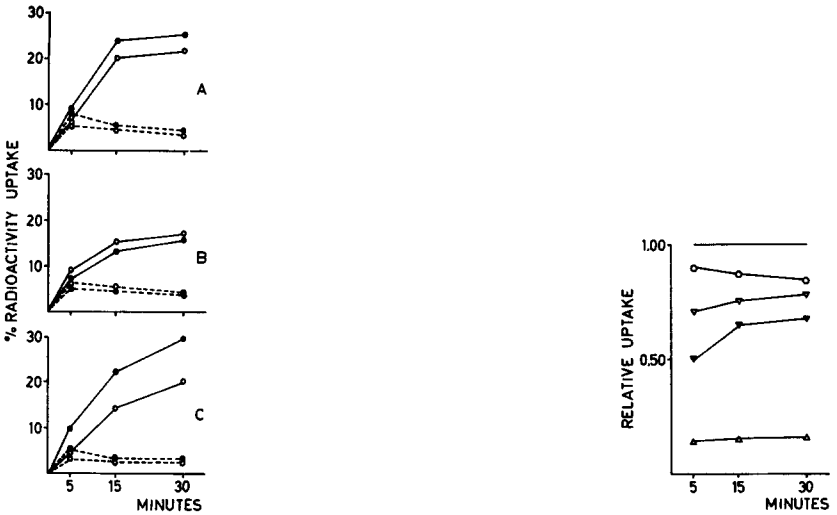


Fig. 6. Uptake of (A) (O—O) a [^{3}H] cholesterol tracer and [^{14}C] monoolein (●—●), (B) an [^{3}H] oleic acid tracer (O—O) and [^{14}C] monoolein (●—●), (C) a [^{3}H] cholesterol (O—O) and an [^{14}C] octadecane tracer (●—●) by a jejunal segment with intact blood supply. ●- - - -● and O- - - -O, uptake of the same tracers by segments with ligated blood supply. The pairs of radioactive substances were injected, dissolved in 0.4 ml of a micellar test solution. Six tests were carried out in each animal, every second one with ligated blood supply.

Fig. 7. Relative uptake of tracers of different radioactive species of lipid by a jejunal segment into which 0.4 ml of a micellar test solution was injected. The percent uptake of the different lipids was divided by that of octadecane (octadecane equal to unity). O—O, oleic acid; ▽—▽, monoolein; ▼—▼, cholesterol; △—△, sitosterol.

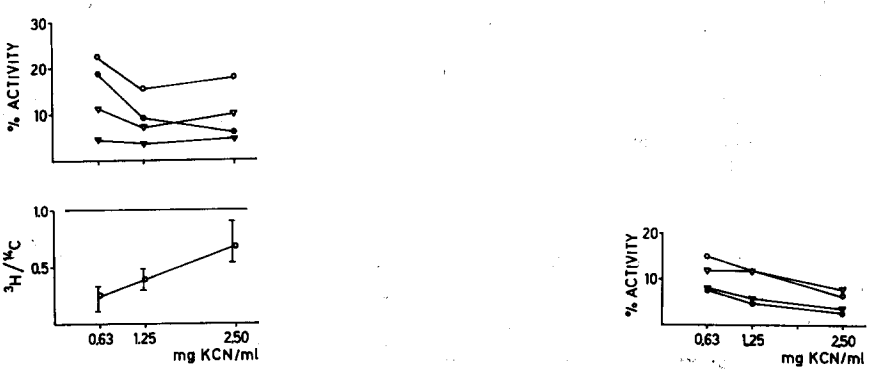


Fig. 8. Above: Uptake of [22,23-^{3}H]sitosterol and [4-^{14}C]cholesterol after 30 min by a jejunal segment when tracers of sterols were administered in 0.4 ml of a micellar solution with or without varying concentrations of KCN. O—O, [4-^{14}C]cholesterol without KCN; ●—●, [4-^{14}C]-cholesterol with KCN; ▽—▽ [22,23-^{3}H]sitosterol without KCN; ▼—▼, [22,23-^{3}H]sitosterol with KCN. Two incubations, the first with and the second without KCN, were carried out in each animal. Below: Ratio of uptake ^{3}H/^{14}C ± range as before.

Fig. 9. Uptake of a [^{3}H] cholesterol tracer and [^{14}C] monoolein after 30 min by a jejunal segment when the lipids were injected in micellar form with or without varying concentrations of KCN. O—O, [^{3}H] cholesterol without KCN; ●—●, [^{3}H] cholesterol with KCN; ▽—▽, [^{14}C]-monoolein without KCN; ▼—▼, [^{14}C] monoolein with KCN. Two incubations, the first with and the second without KCN were carried out in each animal.

Fig. 8 shows that the uptake of cholesterol decreased with increasing concentration of KCN in the micellar test solution injected in the first segment. With sitosterol the uptake was low even with the smallest concentration of KCN used. With increasing concentrations this low uptake was not further decreased. The uptake of radioactivity of both sterols was always larger in the second segment where the same micellar solution without KCN was injected. The ratio $^{3}H/^{14}C$ in the first segment increased with increasing concentration of KCN (Fig. 8).

The effect of KCN on the uptake of monoolein and cholesterol is shown in Fig. 9. There is a parallel net decrease in the uptake of both lipids with increasing concentration of KCN. The uptake of both radioactivities in the second segment was always greater than in the first segment. It decreased with increasing KCN concentration in the first segment.

DISCUSSION

Reliability of the method

In each experiment the uptake was studied in a maximum of six intestinal segments. In order to test if the treatment of the jejunum during this procedure resulted in a modified uptake, the same micellar solution containing radioactive sitosterol and cholesterol was administered into six sequential segments. In the more distal segments there was a decreased uptake. The characteristics of uptake, however, expressed as the difference in uptake between sitosterol and cholesterol, were not changed. At least the proximal three fourths of the small intestine is involved in fat uptake[7]. In the present study experiments were performed in the proximal jejunum. The difference in uptake between segments therefore may not be due to biological differences between segments but rather to traumatic effects on the tissue exerted by the experimental technique.

The volume of test solution to be injected into each jejunal segment was 0.4 ml. This volume filled up the intestinal channel of the segment used. No radioactivity left the segment during the time of the experiment probably because of the fact that the lacteals in the tissue were occluded through the special handling of the segment. This may explain the difference between these results and others[12].

In preparations *in vitro* or in preparations *in situ* without blood supply the possibility exists that the bile salts used may have damaged the tissue. This possibility cannot at present be excluded. The bile salt concentration employed was in the same range as that used in previous studies *in vitro*[6,12,13].

Studies on lipid uptake

In the following the term uptake is thought to include the whole transfer of substances from the luminal micellar phase through the membrane of the epithelial cell into the cell.

The uptake of every species of lipid tested was dependent on the blood supply to the jejunal segment. By administration of KCN (a respiratory inhibitor) this dependence could be further traced to an energy supply. Based on this evidence lipid uptake seems to be an energy-dependent process. This tentative conclusion is at variance with the current view on lipid absorption[1,14] but does find support in earlier studies[12,15].

The results are similar to the findings on the absorption of glucose. This de creased, as did the ATP concentration, in a segment of the small intestinal wall when its blood supply was diminished[16].

In segments with intact blood supply different species of lipid were taken up at different rates. This has been observed before *in vivo*[4] and *in vitro*[13]. Octadecane was taken up at the fastest rate followed by oleic acid and monoolein. The polarity of the compounds seems to be associated with a decreased rate of uptake. These results favour the view that individual compounds dissolved in the micelle are released from the micelle at the membrane. The rate of release may partly be due to the fact that the polarity of the individual micellar compound influences its solubilization in the lipid part of the membrane of the epithelial cell.

The rates of uptake of cholesterol and sitosterol were lower than those of the octadecanoic compounds. *In vivo* cholesterol has been shown to be taken up about 5 times more rapidly than sitosterol[7,17]. This difference in absorption rate was also observed in the present investigation.

The limited intestinal absorption of cholesterol and sitosterol in comparison with the quantitative absorption of glyceride fat is explained as being due to two limiting steps in the transfer of sterol from the intestinal content to the mucosa epithelial cell[8,18]. These steps are the micellar solubilization of sterols and, as is also shown in this investigation, differences in rates of transfer for different species of lipid across the plasma membrane of the mucosa epithelial cell.

The mechanism responsible for the difference in absorption between sitosterol and cholesterol has earlier been localized to 'an outer layer of the mucosa epithelial cell'[8]. Evidence in the present investigation suggests that this mechanism in addition may be energy-dependent and that it might be closely related to the energy-dependent uptake of different species of lipid by the epithelial cell. Possibly, therefore, the mechanism as well as the active uptake process are located in the plasma membrane of the epithelial cell.

When the blood supply was excluded the ratio $^{3}H/^{14}C$ increased to 0.60–0.70. This indicated that cholesterol uptake decreased more than sitosterol uptake. There was no net uptake with time for any lipid species tested. However, when the jejunal tissue was further manipulated, *i.e.* when it was carefully cut into intestinal slices which were incubated *in vitro* in vessels that were not oxygenated, there was a marked uptake of radioactivity with time. In this case cholesterol and sitosterol were taken up to the same extent. This has also been observed when incubations were performed under oxygenation[6]. A high uptake *in vitro* has in addition been recorded for other species of lipid[13,14].

Evidently conditions exist in the preparations *in vitro* which allow uptake of different lipid species by passive diffusion from micelles to lipoproteins in the epithelial cell. However, these conditions do not describe the penetration *in vivo* of different lipid species from the micelle into the mucosa epithelial cell. This is the case since they leave unexplained the lack of uptake of any lipid species by jejunal segments without blood supply. In addition, in preparations *in vitro* there is no distinction between cholesterol and sitosterol. In order to fully describe the penetration it seems to be mandatory to use methods in which the metabolic integrity of the epithelial cell is not disturbed.

Recent advances[19–22] in the understanding of membrane phenomena have

led to the view that energy brought to the membrane is transformed and conserved as conformational energy, which can be used for various purposes. When this takes place, energized structures are transformed into non-energized ones which thereafter are thought to become reenergized.

It is tempting to suggest that lipid absorption in general and the specificity mechanism in sterol absorption depend on such energized, labile structures. When the energy flux is decreased, the energized structure no longer exists, and therefore no absorption of lipid can occur.

Such a hypothesis, however, describes the absorption which must be considered to be a flux of substances in structural terms only. It does not describe the flux *per se*.

In order to do this the following theoretical consideration should be made. The structural part of the membrane is described in the Cartesian coordinate system with the help of the three dimensions of space (x), (y) and (z), while the dynamic part, *i.e.* the change in the membrane, is added to the three spatial dimensions in the form of the time dimension expressed as (it). When the membrane is to be described adequately in the four-dimensional space, every dimension must be taken into consideration. The general view on the membrane created by this idea implies a highly structuralized process or state, which is continuously renewed and destroyed. This hypothesis is supported by the observation that there is a turnover of membrane components[23,24].

In the present investigation the uptake in lipid absorption might be schematically explained as in Figs. 10A and 10D. The membrane is regarded as consisting of units continuously renewed which partly consist of lipid-soluble material. These units might vibrate in an ordered fashion. Through this vibration lipid-soluble spaces are propagated from outside to inside the membrane by the membrane. Depending on the movement in the membrane, different lipid-soluble spaces will be generated, whereby a differentiation between molecules of different shape will take place, *i.e.* in the present investigation between octadecanoic compounds and cholesterol and between cholesterol and sitosterol.

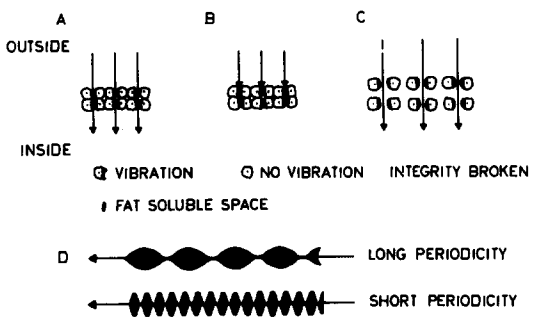


Fig. 10. Schematic representation of the plasma membrane. It consists of vibrating units with lipid-soluble parts. A. The arrows indicate flux of lipid from outside to inside the membrane by the active membrane. In B it is indicated that there is no flux of substance by the membrane without energy supply, while C indicates a flux of substance through the membrane in preparations *in vitro*. D shows the different types of lipid-soluble spaces which might be created by different frequencies in the membrane vibrations.

With the exclusion of the blood supply, the energy supply to the membrane is broken so that movement within it cannot take place. No net uptake of any lipid species will occur (Fig. 10B).

In incubations *in vitro* there is a marked uptake. In this case the integrity of the membrane seems to be broken so that the physicochemical properties of the membrane lipoproteins allow a passive uptake of radioactivity from the micelle (Fig. 10C).

In this hypothesis on membrane transport the idea of a hypothetical carrier as a well-defined spatial unit transporting substances from one side to the other of a equally hypothetical spatially well-defined membrane has been transformed into a particular process accomplished by the overall process that constitutes the membrane. Through this transformation of the idea, membrane transport-processes need no special explanation as the movement and generation of transport flows are viewed as fundamental properties of the membrane itself. Energy brought to the membrane is thought not only to be transformed into conformational energy but also into ordered movement.

One implication of the hypothesis is that fundamental biological phenomena are to be explained not only on a molecular basis but also by properties that are original to higher forms of structure (in four dimensions).

The membrane hypothesis expressed above is in agreement with that put forward by NIMS[25] who classifies membrane properties into two classes, intensive and extensive. According to him "it is to gradients of intensive properties that one looks for "causes" of material transfer through biological barriers".

ACKNOWLEDGEMENTS

This project was supported by grants from the Swedish Nutrition Foundation (to C. S.) and by grants from The Scientific Council of the Swedish Margarine Industry and from Albert Påhlssons Stiftelse, Malmö, Sweden (to Prof. B. Borgström). Miss A. Larsson provided skilful technical assistance.

REFERENCES

1 J. M. JOHNSTON, in C. F. CODE, *Handbook of Physiology*, Section 6, The Alimentary Canal, Am. Physiol. Soc., Washington, D. C. ,1968, p. 1353.
2 A. F. HOFMANN AND B. BORGSTRÖM, *J. Clin. Invest.*, 43 (1964) 247.
3 A. F. HOFMANN AND B. BORGSTRÖM, *Federation Proc.*, 21 (1962) 43.
4 W. J. SIMMONDS, A. F. HOFMANN AND E. THEODOR, *J. Clin. Invest.*, 46 (1967) 874.
5 B. BORGSTRÖM, *J. Lipid Res.*, 8 (1967) 598.
6 E. B. FELDMAN AND B. BORGSTRÖM, *Biochim. Biophys. Acta*, 125 (1966) 148.
7 CH. SYLVÉN AND CH. NORDSTRÖM, *Scand. J. Gastroenterol.*, 5 (1970) 57.
8 CH. SYLVÉN AND B. BORGSTRÖM, *J. Lipid Res.*, 10 (1969) 179.
9 H. C. BLACK AND C. A. OVERLEY, *J. Am. Chem. Soc.*, 61 (1939) 3051.
10 A. F. HOFMANN, Doctoral thesis, University of Lund, Sweden, (1964).
11 A. F. HOFMANN, *Biochim. Biophys. Acta*, 70 (1963) 306.
12 J. P. W. WEBB, J. D. HAMILTON AND A. M. DAWSON, *Biochim. Biophys. Acta*, 187 (1969) 42.
13 A. G. THORNTON, G. V. VAHOUNY AND C. R. TREADWELL, *Proc. Soc. Exptl. Biol. Med.*, 127 (1968) 629.
14 J. M. JOHNSTON AND B. BORGSTRÖM, *Biochim. Biophys. Acta*, 84 (1964) 412.
15 R. J. C. BARRY, M. J. JACKSON AND D. H. SMYTH, *J. Physiol. London*, 182 (1966) 150.
16 V. VARRÓ, L. CSERNAY AND I. JUNG, *2nd World Congress of Gastroenterology, Munich, 1962*, Vol. 2, Karger, Basel, 1963, p. 803.

17 B. BORGSTRÖM, *J. Lipid Res.*, 9 (1968) 473.
18 CH. SYLVÉN AND B. BORGSTRÖM, *J. Lipid Res.*, 9 (1968) 596.
19 I. T. PENNISTON, R. A. HARRIS, J. ASAI AND D. E. GREEN, *Proc. Natl. Acad. Sci. U.S.*, 59 (1968) 624.
20 R. A. HARRIS, J. T. PENNISTON, J. ASAI AND D. E. GREEN, *Proc. Natl. Acad. Sci. U.S.*, 69 (1968) 830.
21 D. E. GREEN, J. ASAI, R. A. HARRIS AND J. T. PENNISTON, *Arch. Biochem. Biophys.*, 125 (1968) 684.
22 J. T. PENNISTON AND D. E. GREEN, *Arch. Biochem. Biophys.*, 128 (1968) 339.
23 J. S. HAGERMAN AND R. G. GOULD, *Proc. Soc. Exptl. Biol. Med.*, 78 (1951) 329.
24 M. E. MCLEOD AND R. BRESSLER, *Proc. Soc. Exptl. Biol. Med.*, 130 (1969) 268.
25 L. F. NIMS, *Advan. Biol. Med. Phys.*, 11 (1967) 159.