J. Physiol. (1972), **222**, *pp.* 679–690
With 1 *text-figure*
Printed in Great Britain

THE EFFECTS OF BILE SALT AND RAW BILE ON THE INTESTINAL ABSORPTION OF MICELLAR FATTY ACID IN THE RAT *IN VITRO*

By ALFRED J. RAMPONE
From the Department of Physiology, University of Oregon Medical School, Portland, Oregon 97201, U.S.A.

(*Received 13 December 1971*)

SUMMARY

1. The uptake, esterification and transport of [^{14}C]oleic acid were studied using sacs of rat everted small intestine incubated in 25 ml. of a buffered mixture of sodium taurocholate, glyceryl mono-oleate and ^{14}C-labelled oleic acid in micellar form.

2. Intestine obtained from bile fistula rats (bile duct cannulated 48 hr previously) showed elevated rates of ^{14}C uptake into the tissue total lipid compared with sham-operated controls.

3. Nearly all of the excess ^{14}C uptake in the bile fistula group was in the form of free fatty acid. Both groups showed similar rates of [^{14}C]oleic acid incorporation into tissue triglyceride and also similar, though small, amounts transported into the serosal fluid.

4. In further experiments using intestine from bile fistula rats the addition of 1 ml. of fresh rat bile to the incubation mixture reduced the ^{14}C uptake to approximately control levels. The addition of 2–3 ml. of fresh bile similarly reduced the uptake and increased ^{14}C incorporation into the triglycerides of mucosal tissue and serosal fluid.

5. These responses were not entirely the result of the bile salts contained in fresh bile since increasing the taurocholate concentration *per se* caused uptake, esterification and transport all to increase. In the presence of the higher taurocholate concentration the addition of fresh bile still caused a decrease in ^{14}C uptake.

6. There was no significant effect of either fresh bile or taurocholate on the transport of the 3-*O*-methyl analogue of D-glucose under comparable conditions.

7. It is concluded that raw bile contains one or more components other than bile salts which may be important in determining fatty acid absorption.

INTRODUCTION

The major functional constituents of bile are probably the bile salts, amphipathic molecules which serve to disperse the bulk of the triglycerides of the diet into a crude emulsion form, thereby facilitating the break-down of the triglyceride by pancreatic lipase (Hofmann & Borgström, 1962). An important development was the concept that the resulting monoglycerides and fatty acids then become further dispersed into micellar form again with the aid of the bile salts (Hofmann & Borgström, 1962, 1964; Hofmann, 1966). It is likely that because of their small size (Borgström, 1965) mixed micelles of bile salts, fatty acids and monoglycerides constitute one of the important vehicles for transporting lipid into the intestinal epithelium. The micelles form spontaneously provided bile salt is present above a certain critical concentration relative to fatty acid and monoglyceride. They have been used successfully as substrates for lipid transport studies both *in vivo* (Redgrave, 1967; Webb, Hamilton & Dawson, 1969; Rampone, 1970) and *in vitro* (Johnston & Borgström, 1963; Mishkin, Yalovsky & Kessler, 1970).

In the present study we used micellar lipid in order to measure fatty acid uptake, esterification and transport by the rat small intestine *in vitro*. Since most attention in the past has centred on the role of the bile salts in bile, it was of particular interest to compare the effects of bile salt and raw bile on these parameters.

METHODS

Unfasted male Sprague–Dawley rats weighing 250–300 g were used. Under sodium pentobarbitone anaesthesia the entire small bowel below the ligament of Treitz was flushed out with saline at room temperature and then removed and everted over a glass rod (Wilson & Wiseman, 1954). It was then extended by means of ligatures to a length of 90 cm in a trough filled with saline. Two 20 cm segments located 10–30 and 30–50 cm from the ligament of Treitz were removed for study. The segments, tied at both ends, were filled with 2 ml. of Krebs phosphate buffer (without calcium and magnesium) which had been gassed for 1 hr with 100 % oxygen. The filled sacs were then placed in incubation flasks containing 25 ml. of the same buffer plus bile salt (sodium taurocholate, 99 %+, K & K labs. Hollywood, Calif.), monoglyceride (glyceryl mono-oleate, 90 %, Calbiochem, San Diego, Calif.) and fatty acid (oleic acid, 99 %+, Nutritional Biochemicals, Cleveland, Ohio) in micellar form with glucose added to support metabolism. In some cases, fresh bile was also added. Except when indicated, the final concentrations in the incubation flasks were as follows: sodium taurocholate 2·4 mM, glyceryl mono-oleate 0·3 mM, oleic acid 0·6 mM and glucose 28 mM (Johnston & Borgström, 1963). The oleic acid was labelled with a trace of [1-^{14}C]oleic acid (New England Nuclear, Boston, Mass.). The solution had a pH of 6·9 and became optically clear after a few minutes of shaking. The segments were incubated for 1 hr at 38° C with shaking at 80 oscillations per minute in an atmosphere of oxygen.

After incubation, the sacs were removed from their flasks, washed thoroughly in

cold saline, emptied into tared centrifuge tubes, and the serosal fluid weighed. The mucosal tissue was then scraped off with a spatula, transferred to a separate centrifuge tube and weighed. The serosal fluid and the mucosal tissue were extracted with chloroform-methanol (2:1, v:v) and, after evaporating the extracts to dryness, the lipid content was obtained by weighing (Folch, Lees & Sloane Stanley, 1957). The lipid was then dissolved in a known volume of chloroform-methanol and aliquots (10 μl.) were spotted on 20×20 cm thin layer plates (silica gel on polyethylene terephthalate (Eastman Chromagram, Eastman Kodak Co., Rochester, N.Y.)). Similar aliquots of the initial incubation fluid extracts were also spotted. The plates were developed using a mixture of *n*-hexane, diethyl ether, acetic acid and methanol (90:20:2:3, v:v) as described by Brown & Johnston (1962). The spots were detected with iodine vapour and identified by comparison with a standard mixture of highly purified glyceryl trioleate, glyceryl dioleate, glyceryl mono-oleate and oleic acid (99 %+, Sigma Chemical Co., St. Louis, Mo.). The spots were cut out, transferred to scintillation vials and extracted with 3 ml. of chloroform-methanol (2:1, v:v). After drying the lipid in the vials, 10 ml. of standard scintillation solution (PPO-POPOP in toluene) were added and counts were taken in the Packard Tricarb model 314 EX 2 liquid scintillation system. All of the counts were converted to n-mole equivalents of oleic acid incorporated into the various lipid fractions.

In order to be certain that all traces of natural bile were absent at the time the sacs were prepared, most of the experiments were done using intestinal segments obtained from bile fistula rats. Thus, 48 hr before the sacs were made, the animals were anaesthetized with sodium pentobarbitone and the bile duct was cannulated above the entrance of the pancreatic ducts using polyethylene tubing (Clay Adams PE 50). The animals were then housed in restraining cages and allowed food and water *ad libitum*. During the last 24 hr of this period, the bile was collected in glass tubes in ice and stored in a nitrogen atmosphere for later use. The fatty acid uptake and esterification of these bile-free segments were compared with those of sham-operated controls.

In one set of experiments [^{14}C]3-*O*-methyl-D-glucose (a non-metabolized actively transported analogue of D-glucose (Csáky & Glenn, 1957; Wilson & Landau, 1960)) was used in place of [^{14}C]oleic acid in order to test the effects of raw bile and bile salts on the uptake and transport of a non-lipid solute. The experimental procedures were the same as those already described except that both the incubation fluid and the serosal fluid contained ^{14}C-labelled 3-*O*-methyl-D-glucose initially in a concentration of 5 mM and the [^{14}C]oleic acid was omitted. Uptake and transport were gauged by a direct measurement of ^{14}C activity in the serosal and mucosal fluid compartments after 1 hr of incubation. This was done by preparing a filtrate of the fluids using a solution of equal parts of 2 % $ZnSO_4$ and 1·8 % $Ba(OH)_2$ and adding an aliquot of the filtrate to 10 ml. of Bray scintillation solution for counting (Bray, 1960).

RESULTS

Bile fistula vs. control rats

In the initial phase of this study, all of the lipid fractions isolated by thin layer chromatography were counted to determine the distribution of [^{14}C]oleic acid in the lipid of mucosal tissue and serosal fluid after the 1 hr of incubation. The lipid fractions were: a non-migrating fraction (NM) of unidentified composition, monoglyceride (MG), diglyceride (DG), free fatty acid (FA) and triglyceride (TG) in order of increasing R_F values. The

recovery of ^{14}C in each of these fractions is given in Table 1 expressed as n-mole equivalents of oleic acid. The last column in the table (T) is the amount measured in an aliquot of the total lipid extract before chromatography and represents the theoretical maximum recoverable.

It can be seen in Table 1 that in the sacs obtained from the bile fistula rats there was a significantly higher free fatty acid uptake than in control sacs. This was evidenced by a greatly increased labelled free fatty acid content in the mucosal tissue plus a slight increase in the serosal fluid. The incorporation of label into the total lipid of the bile fistula group was also increased in both compartments chiefly as a consequence of the increase in the free fatty acid fraction, although in the case of serosal fluid it was not statistically significant. The amount of [^{14}C]oleic acid incorporated into triglyceride was similar in both groups of animals and there were no significant differences between any of the other lipid fractions.

TABLE 1. [^{14}C]oleic acid recoveries in lipid fractions isolated from mucosal tissue and serosal fluid after 1 hr incubation in a micellar solution of [$1-{}^{14}C$]oleic acid (0·6 mM), glyceryl mono-oleate (0·3 mM) and sodium taurocholate (2·4 mM) in phosphate buffer. Values are means ± S.E. of means, expressed as n-mole equivalents of oleic acid

	n	NM	MG	DG	FA	TG	T
		Mucosal tissue (n-mole/g tissue)					
Bile fistula	18	40 ± 5	17 ± 3	48 ± 3	566 ± 47 $P < 0.01$	265 ± 16	1053 ± 49 $P < 0.01$
Controls	16	27 ± 5	19 ± 7	34 ± 3	275 ± 28	276 ± 16	649 ± 34
		Serosal fluid (n-mole/sac)					
Bile fistula	7	4 ± 1	2 ± 0·4	11 ± 5	29 ± 4	64 ± 10	112 ± 17
Controls	6	2 ± 0·5	1 ± 0·3	4 ± 0·3	17 ± 2	52 ± 8	78 ± 10

NM, non-migrating fraction; MG, monoglyceride; DG, diglyceride; FA, fatty acid; TG, triglyceride; T, total in lipid extract before chromatography.

A consistent finding was that nearly 90 % of the recovered label was found in the two fractions, free fatty acid and triglyceride, and only approximately 10 % in the remaining three fractions. Hence, only the free fatty acid and triglyceride fractions were counted in the remainder of the study.

Effects of adding raw bile vs. bile salt

In the next set of experiments, the effects of adding a small amount of fresh rat bile to the incubation fluid was studied. The bile was added in a volume of 1, 2 or 3 ml. to the 25 ml. of incubation fluid which had the same initial composition as that used in the preceding experiments. The final bile salt concentration was therefore slightly in excess of 2·4 mM because of the bile salt contained in fresh bile, but the concentration was not measured.

All of the experiments reported in this series utilized segments of small intestine taken from bile fistula rats.

Table 2 shows the recovery of [^{14}C]oleic acid in free fatty acid, triglyceride and total lipid of the mucosal tissue and serosal fluid. The experiments with no bile added were taken from Table 1 for comparison. The addition of as little as 1 ml. of fresh bile caused a significant reduction in the labelled free fatty acid content of the tissue. Adding 2 or 3 ml. of bile

TABLE 2. [^{14}C]oleic acid recoveries in free fatty acid (FA), triglyceride (TG) and total lipid (T) of mucosal tissue and serosal fluid after 1 hr of incubation. Conditions as in Table 1 except for the addition of fresh bile as shown and the use exclusively of bile fistula rats. Values are means ± S.E. of means, expressed as n-mole equivalents of oleic acid

ml. raw bile added	*n*	FA	TG	T
		Mucosal tissue (n-mole/g tissue)		
0	18	566 ± 47	265 ± 16	1053 ± 49
1	7	235 ± 43*	294 ± 33	686 ± 81*
2	10	220 ± 23*	390 ± 34*	785 ± 65*
3	6	166 ± 28*	450 ± 64*	778 ± 49*
		Serosal fluid (n-mole/sac)		
0	7	29 ± 4	64 ± 10	112 ± 17
1	6	38 ± 6	148 ± 43	215 ± 58
2	10	34 ± 3	234 ± 24*	303 ± 25*
3	6	45 ± 8	291 ± 66*	375 ± 74*

* $P < 0{\cdot}01$, Student test comparing with zero bile added.

also reduced the labelled fatty acid content and, in addition, caused an increase in the amount of labelled oleic acid incorporated into tissue triglyceride. Furthermore, the bile appeared to stimulate triglyceride transport since there were increased amounts of labelled triglyceride recovered in the serosal fluid in the presence of fresh bile. The results suggest that some component in bile stimulates triglyceride synthesis and transport. The decrease in the tissue free fatty acid content in the presence of fresh bile may have been the result of an increased rate of fatty acid incorporation into triglyceride or a decreased rate of fatty acid uptake or both.

It has been shown by Dawson & Isselbacher (1960) that bile salts stimulate esterification of palmitic acid in the intestine *in vitro* through some cellular metabolic effect. It is possible that the effects of fresh bile seen in the present study were due solely to an increase in bile salt concentration, although an attempt was made to obtain bile with a very low

bile salt content (the bile being obtained 24 hr after the enterohepatic recirculation had been interrupted). To test for the possibility of a dependence on bile salt concentration an additional set of experiments was carried out in which the bile salt concentration of the incubation fluid was increased from 2·4 to 4·8 mM by the addition of sodium taurocholate.

TABLE 3. [^{14}C]oleic acid recoveries in free fatty acid (FA), triglyceride (TG) and total lipid (T) of mucosal tissue and serosal fluid after 1 hr of incubation. Conditions as in Table 2 except that sodium taurocholate concentration was 4·8 mM

ml. raw bile added	n	FA	TG	T
		Mucosal tissue (n-mole/g tissue)		
0	9	1070 ± 168	521 ± 57	1899 ± 161
		$P < 0{\cdot}01$		$P < 0{\cdot}01$
1	3	528 ± 142	527 ± 39	1285 ± 180
2	4	563 ± 158	428 ± 73	1197 ± 181
3	2	148 ± 8	536 ± 109	823 ± 162
		Serosal fluid (n-mole/sac)		
0	9	55 ± 8	266 ± 63	336 ± 68
1	3	34 ± 2	251 ± 61	302 ± 64
2	4	74 ± 18	272 ± 66	366 ± 66
3	2	48 ± 17	366 ± 250	412 ± 263

The results are shown in Table 3. It is noteworthy that, in the absence of fresh bile, simply doubling the taurocholate concentration caused a marked increase in free fatty acid uptake and triglyceride synthesis by the tissue as well as an increase in triglyceride transport into the serosal fluid. The values appear in most cases to be approximately double those seen at the lower bile salt concentration (Table 2).

Adding fresh bile to the incubation fluid in the presence of the doubled bile salt concentration still caused a reduction in the tissue fatty acid uptake (Table 3) as it did before. However, the fresh bile did not in this case result in additional triglyceride synthesis and transport presumably because synthesis and transport were already maximally stimulated by the higher taurocholate concentration.

Effects of raw bile and bile salt on 3-O-methyl-D-glucose transport

The inhibitory effect of fresh bile on fatty acid uptake appears not to be due to bile salt, but may possibly be due to some other component of bile or to a non-specific effect. It is possible that some of the bile salts in the bile had become deconjugated or that some other process of deterioration had taken place during collection and storage even though care was taken to keep the bile at approximately 0° C and in an atmosphere of nitrogen

before use. If the observed effects were non-specific or 'toxic' in nature causing altered membrane permeability or changes in cell metabolism, one might expect the uptake or transport of non-lipid materials to be similarly affected. Some evidence against this idea was provided in a series of experiments utilizing [^{14}C]3-*O*-methyl-D-glucose in place of [^{14}C]oleic acid. The transport of this non-metabolized actively transported analogue of glucose was studied in the presence and absence of sodium taurocholate at

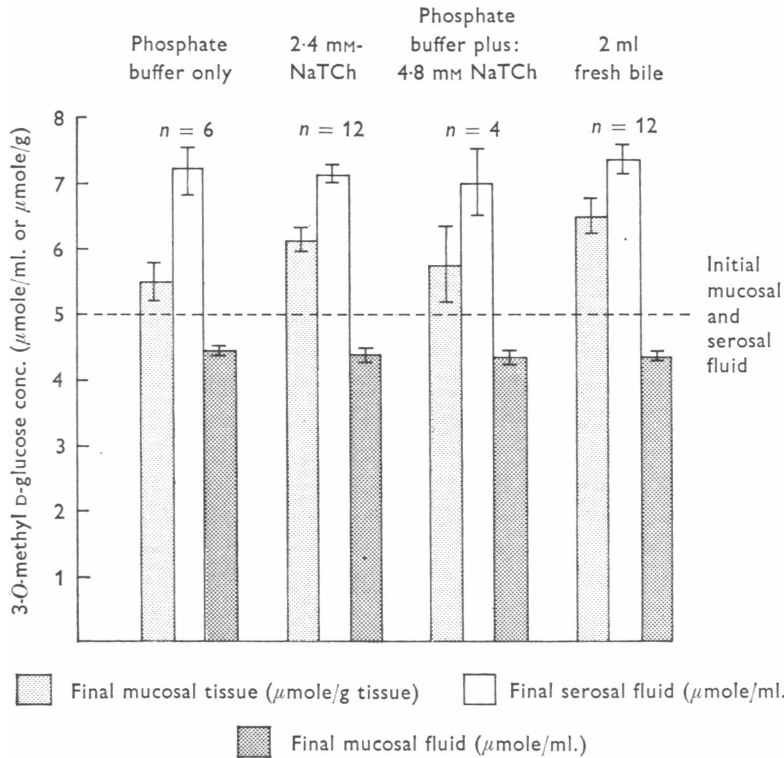

Fig. 1. Comparison of the effects of sodium taurocholate (NaTch) and fresh bile on the uptake and transport of 3-*O*-methyl-D-glucose by everted sacs. NaTch was used in two concentrations as shown. Fresh bile was added in the presence and absence of the same NaTch concentrations and the data pooled.

2·4 and 4·8 mM concentrations and in the presence of 2 ml. of bile under conditions similar to those of the preceding experiments. Initially, 3-*O*-methyl-D-glucose was present in both the incubation fluid (mucosal) and the serosal fluid at 5 mM concentration and glucose was omitted to avoid competition for the sugar carrier. Active transport was denoted by a net transfer of the analogue from mucosal to serosal fluid. Fig. 1 shows the

results. In all conditions, the final serol 3-*O*-methyl-D-glucose concentration exceeded the initial concentration. This was accompanied by a slight fall in the final mucosal fluid concentration, despite the large volume of fluid used (25 ml.). Hence, there was evidence for transport against a concentration gradient, and in no case did the addition of either bile salt or fresh bile significantly alter the transport capabilities of the tissue for this compound. This is in clear contrast to the effects on fatty acid uptake described in the preceding experiments. A further contrast with the fatty acid experiments can be seen in regard to the amount of 3-*O*-methyl-D-glucose taken up by the tissue which either did not change or was increased in the presence of both fresh bile and bile salt.

DISCUSSION

In this study everted intestinal sacs of the upper half of the small intestine of the rat were used to evaluate certain aspects of micellar fatty acid uptake, esterification and transport. When the sacs were incubated in the presence of sodium taurocholate (2·4 mM), glyceryl mono-oleate (0·3 mM) and ^{14}C-labelled oleic acid (0·6 mM) in micellar form at pH 6·9 there was considerable uptake of the labelled fatty acid by the tissue and a measurable incorporation of the fatty acid into tissue glyceride. At the end of 1 hr of incubation nearly 90 % of the label recovered in the tissue was recovered in the sum of the free fatty acid and triglyceride fractions with the proportion varying under different experimental conditions.

Bile fistula vs. control rats

A surprising finding was a marked discrepancy between bile fistula and control animals. Sacs obtained from bile fistula rats took up 60–70 % more of the ^{14}C label than did control sacs and most, if not all, of the excess label in the tissue was in the free fatty acid fraction (Table 1). Therefore, diverting the bile from the intestine (in this case for 48 hr) resulted either in an increased rate of fatty acid uptake or a decreased rate of fatty acid mobilization through the tissue. There was evidence against a decrease in the rate of fatty acid mobilization: no statistically significant differences between the two groups were found in terms of the amount of label incorporated into tissue glycerides or the amount transported into serosal fluid. However, the amounts transported into serosal fluid were small in both groups of animals, perhaps reflecting a limited capacity of the intestine for transporting lipids *in vitro*. In a previous *in vivo* study in rats we were able to show that bile fistula rats demonstrated a less than normal capacity to transport lipids into lymph when the lipids were perfused through the intestine in micellar form (Rampone, 1970). In that same study, as in

the present one, the bile fistula rats showed a tendency to accumulate the absorbed lipid in the wall of the gut.

Effects of adding raw bile or additional bile salt in bile-free intestinal segments

The greater tissue uptake of fatty acid in the bile fistula group suggested that there may be some component in natural bile which was effective in inhibiting fatty acid uptake and which was absent in the bile fistula animals. However, since all of the sacs were washed in saline during preparation, this hypothetical component must have been effective in only trace amounts, or it was firmly bound to the tissue. A further possibility is that biliary drainage induced changes of a non-specific nature in the bile fistula group.

Some support for the idea that there may be components in bile in addition to bile salts which affect fatty acid uptake was obtained in the experiments in which small amounts of fresh bile were added to the incubation fluid. When this was done, the sacs showed a marked reduction in fatty acid uptake and an increased incorporation of fatty acid into triglyceride. The taurocholate concentration in the first such series of experiments (Table 2) was 2·4 mM before the bile was added. The bile salt concentration would have been increased somewhat by the addition of fresh bile even though the amounts added were small (1–3 ml.) and greatly diluted in the large volume of incubation fluid (25 ml.). Some idea of the bile salt concentration of rat bile can be obtained from the study of Klaassen (1971), who found the taurocholate concentration in rat bile to be in the range 10–15 μ-equiv/ml. and the taurochenodeoxycholate concentration to be of the order of 4–5 μ-equiv/ml. Therefore, assuming a total bile salt concentration of 20 μ-equiv/ml. (as a maximum) in the fresh bile used in the present study, adding 1–3 ml. to the incubation fluid would raise the bile salt concentration by 0·8–2·4 mM, a substantial increase which could possibly have accounted for the observed effects of the fresh bile. This idea was tested by determining the effects of doubling the initial taurocholate concentration from 2·4 to 4·8 mM and then noting any additional effects of adding fresh bile as before. It was found that doubling the bile salt concentration alone caused significantly more fatty acid to be taken up and was accompanied by a correspondingly large increase in the amount of fatty acid incorporated into triglyceride by the tissue as well as an increase in the amount of both fatty acid and triglyceride transported into the serosal fluid. We had assumed that the 2·4 mM concentration used in the preceding experiments was above the critical micellar concentration since the solutions were water clear. However, no measurements of actual light scattering or micellular particle sizes were made. In view of the extra

uptake, esterification and transport observed in the presence of 4·8 mM concentration (Table 3) the question of what constitutes a critical micellar bile salt concentration may perhaps need to be re-evaluated.

In the presence of a doubled bile salt concentration it is important to note that the addition of fresh bile still caused a marked reduction in tissue fatty acid uptake. One ml. was sufficient to reduce it by approximately one half. This was similar to the effects noted previously at the lower bile salt concentration. Therefore, with respect to fatty acid uptake, the effects of adding fresh bile were clearly not the same as the effects of doubling the bile salt concentration.

In the presence of the doubled bile salt concentration, but in the absence of fresh bile, as already noted, there was evidence of increased esterification as well as transport (Table 3). However, adding fresh bile to the system in this case did not bring about a significant increase in esterification and transport as it appeared to do in the previous experiments (Table 2). Therefore, it is reasonable to suggest that the stimulation of esterification and transport induced by the fresh bile in the previous experiments may have been due to the bile salts contained in the fresh bile which had been added.

Failure of raw bile or bile salt to alter transport of 3-O-methyl-D-glucose

The results lead naturally to the question of a possible non-specific or 'toxic' effect of fresh bile affecting membrane permeability or cell metabolism. This question has not been completely answered. The experiments showing that raw bile did not affect the transport of 3-*O*-methyl-D-glucose suggest that the bile did not alter the permeability of the cell membrane to this compound, nor did it affect the cellular machinery responsible for its active transport. It is worthy of note, however, that there is disagreement on the effects of bile salts and raw bile on the absorption and transport of aqueous solutes (Faust & Wu, 1965; Pope, Parkinson & Olson, 1966; Roy, Dubois & Philippon, 1970).

Other possible non-specific effects of raw bile

During the collection of bile, precautions were taken to minimize deterioration by maintaining the bile in the cold and in an atmosphere of nitrogen. Samples showing signs of pigment discoloration were discarded. There may have been some loss of CO_2 with a change of pH during storage. However, the pH change, if any, was not sufficient to cause a measurable change in pH of the final micellar solution when the bile was added to it. Finally, it would be difficult to explain the differences observed between

the bile fistula and control animals in this study on the basis of a toxic or pH effect.

There is a question as to whether or not the use of a combination of bile salts would have been a better choice than the single bile salt, taurocholate, as used in the present study. In a few experiments we used sodium taurocholate in combination with sodium taurodeoxycholate in concentrations of 1·8 and 0·6 mM respectively without any apparent differences in results. It was considered unlikely, therefore that the raw bile effects observed in the present study were due to some changes in the quality of the bile salts comprising the micellar solutions used.

Finally, the reduced fatty acid uptake in the presence of raw bile cannot be explained by a simple dilution of the micellar solution, because equal volumes of added phosphate buffer also had no measurable effect.

The data are as yet too incomplete to allow reasonable speculation as to possible mechanisms of action of the raw bile. The predominant effect seems to be an inhibitory one on fatty acid uptake by the tissue, but uptake was not measured directly inasmuch as some of the fatty acid taken up was synthesized to triglyceride and some was transported into the serosal fluid. Attempts are underway currently to measure the effects on uptake directly by lowering the incubation temperature to 0° C with the view to suppressing the esterifying enzyme systems.

Future studies should perhaps also take into account the possible effects raw bile on the charge and mobility of micellar lipid particles since these factors may influence their rate of penetration through epithelial membranes.

This work was supported in part by grant AM 01389 from the U.S.P.H.S.
I should like to thank Lawrence R. Long for his excellent technical assistance.

REFERENCES

BORGSTRÖM, B. (1965). The dimensions of the bile salt micelle: measurements by gel filtration. *Biochim. biophys. Acta* **106**, 171–183.

BRAY, G. A. (1960). A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Analyt. Biochem.* **1**, 279–285.

BROWN, J. L. & JOHNSTON, J. M. (1962). Radioassay of lipid components separated by thin-layer chromatography. *J. Lipid Res.* **3**, 480–481.

CSÁKY, T. Z. & GLENN, J. E. (1957). Urinary recovery of 3-methylglucose administered to rats. *Am. J. Physiol.* **188**, 159–162.

DAWSON, A. M. & ISSELBACHER, K. J. (1960). Studies on lipid metabolism in the small intestine with observations on the role of bile salts. *J. clin. Invest.* **39**, 730–740.

FAUST, R. G. & WU, S. M. L. (1965). The action of bile salts on fluid and glucose movement by rat and hamster jejunum *in vitro*. *J. cell. comp. Physiol.* **65**, 435–448.

FOLCH, J., LEES, M. & SLOANE STANLEY, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. biol. Chem.* **226**, 497–509.

HOFMANN, A. F. (1966). A physicochemical approach to the intraluminal phase of fat absorption. *Gastroenterology* **50**, 56–64.

HOFMANN, A. F. & BORGSTRÖM, B. (1962). Physico-chemical state of lipids in intestinal content during their digestion and absorption *Fedn Proc.* **21**, 43–50.

HOFMANN, A. F. & BORGSTRÖM, B. (1964). The intraluminal phase of fat digestion in man: the lipid content of the micellar and oil phases of intestinal content obtained during fat digestion and absorption. *J. clin. Invest.* **43**, 247–275.

JOHNSTON, J. M. & BORGSTRÖM, B. (1963). The intestinal absorption and metabolism of micellar solutions of lipids. *Biochim. biophys. Acta* **84**, 412–423.

KLAASSEN, C. D. (1971). Does bile acid secretion determine canalicular bile production in rats? *Am. J. Physiol.* **220**, 667–673.

MISHKIN, S., YALOVSKY, M. & KESSLER, J. I. (1970). The *in vitro* uptake and kinetics of release of palmitic acid and taurodeoxycholate from hamster small intestinal segments. *Biochim. biophys. Acta* **225**, 335–346.

POPE, J. L., PARKINSON, T. M. & OLSON, J. A. (1966). Action of bile salts on the metabolism and transport of water-soluble nutrients by perfused rat jejunum *in vitro*. *Biochim. biophys. Acta* **130**, 218–232.

RAMPONE, A. J. (1970). Intestinal absorption of micellar lipid in normal and bile-deficient rats. *Proc. Soc. exp. Biol. Med.* **135**, 666–670.

REDGRAVE, T. G. (1967). The absorption of micellar lipid into the lymph of unanaesthetized rats. *Q. Jl exp. Physiol.* **52**, 130–138.

ROY, C. C., DUBOIS, R. S. & PHILIPPON, F. (1970). Inhibition by bile salts of the jejunal transport of 3-O-methyl glucose. *Nature, Lond.* **225**, 1055–1056.

WEBB, J. P., HAMILTON, J. D. & DAWSON, A. M. (1969). A physicochemical study of fat absorption in rats: limitation of methods *in vitro*. *Biochim. biophys. Acta* **187**, 42–52.

WILSON, T. H. & LANDAU, B. R. (1960). Specificity of sugar transport by the intestine of the hamster. *Am. J. Physiol.* **198**, 99–102.

WILSON, T. H. & WISEMAN, G. (1954). The use of sacs of everted small intestine for the study of the transference of substances from the mucosal to the serosal surface. *J. Physiol.* **123**, 116–125.