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A comparative histochemical study of lipid absorption in a few freshwater teleosts

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Abstract

Lipid absorption, experimentally studied in three species of fishes, namely, *Esomus danricus*, *Clarias batrachus* and *Channa striatus* is described. After starving the fishes for 72 hr, they were fed on castor oil (10 ml/kg body weight) and the absorption of neutral lipids was studied histochemically at regular intervals using Sudan black B method. The anterior portions of the intestine of all the three species of fishes depicted maximum absorption, 8 hr after feeding, and later on at 16 and 24 hr, showed progressive decline. Middle intestine of these fishes exhibited moderate absorption at different time intervals whereas the posterior part of the intestine showed comparatively less absorption at all the time intervals. The pyloric caeca of *C. striatus* showed moderate lipid absorption, 8 hr after feeding ; and after 16 hr, there was uniform increase till maximum absorption was attained at 24 hr.

Key words : Neutral lipid, absorption, intestine, *Channa striatus*, *Clarias batrachus*, *Esomus danricus*

1. Introduction

The study of lipid absorption experimentally at different time intervals, especially in fishes, has not been attempted except for the work of Sastry and Garg[1]. Moreover, this work does not give a comparative account of lipid absorption in fishes with different feeding habits. The present study is an attempt to study histochemically the absorption of neutral lipids in the intestines of *E. danricus*, *C. batrachus* and *C. striatus* with respect to their feeding habits, being herbivorous, omnivorous and carnivorous respectively[2].

2. Material and method

Control and experimental fishes were starved in the laboratory for 72 hr. Three specimens of each species for each time interval were fed orally with castor oil (10 ml/kg body weight) ; control specimens, however, were not fed with castor oil. After 8, 16 and 24 hr, the control and experimental fishes were dissected and the anterior, middle and posterior regions of intestines, and the pyloric caeca of *C. striatus* were fixed in cobalt calcium formol for 24 hr at room temperature[3]. The fixed materials were dehydrated in different grades of ethanol, cleared in xylene and embedded in paraffin wax (M.P. 56–58° C). Serial sections, 6–8μ thick, were cut and stained with Sudan black B method[3], which was preferred because this method '...is specific for neutral lipids and can be conveniently applied at room temperature.[4]'

3. Results

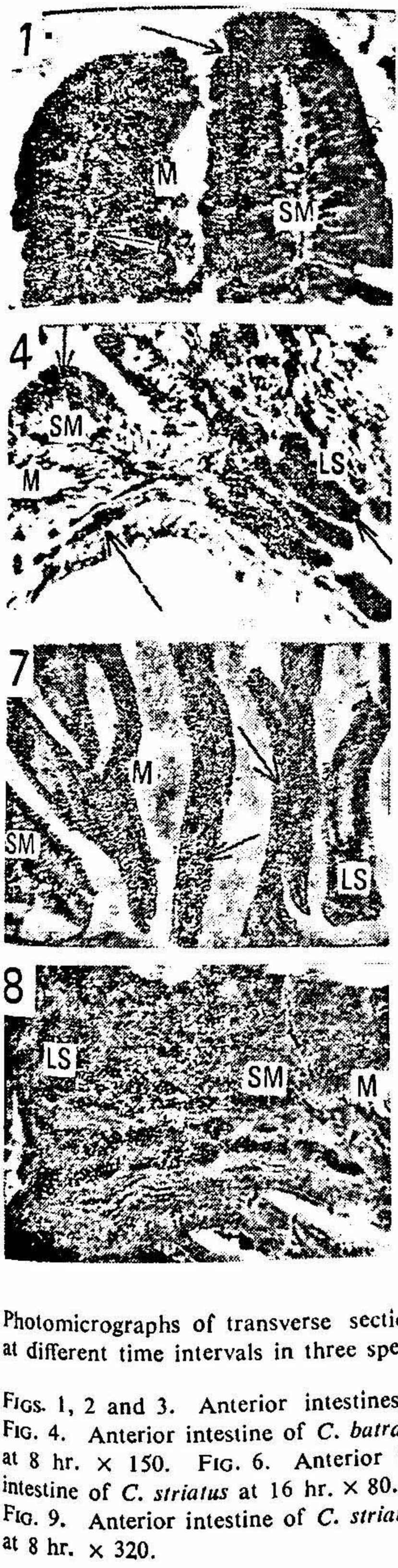
The absorption of neutral lipids as observed histochemically is described for the different regions of the intestines as under :

3.1. *Anterior intestine*

The anterior intestines of all the three species of fishes showed maximum intensity of lipid absorption, 8 hr after feeding (figs. 1, 4 and 5). The sites of maximum absorption in *E. danricus* included mainly the free borders and basal portions of mucoal epithelial cells, the intensity being uniform throughout the mucosal region of the villi (fig. 1). In *C. batrachus* (fig. 4) and *C. striatus* (fig. 5) the mucosal epithelial cells, however, depicted moderate absorption. During the same time interval, the submucosal region in *E. danricus* showed weak absorption, while in *C. batrachus* and *C. striatus*, this region showed strong intensity particularly in lymph spaces and blood vessels, the stain intensity being more pronounced along the basal portions of the villi in the former and uniform throughout the submucosa in the latter (figs. 4 and 5).

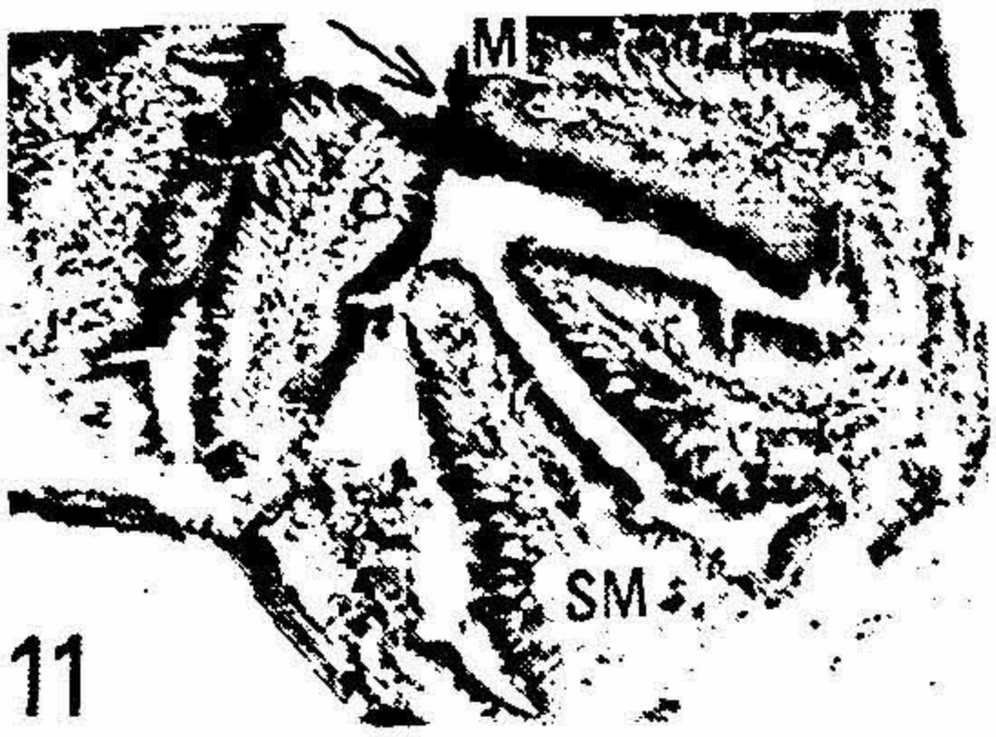
A steady decline in stain intensity for lipid absorption was recorded in all the three species of fishes 16 hr after feeding. However, moderate absorption was noted in the mucosa of *E. danricus* (fig. 2), and the submucosal lymph spaces at the bases of the villi in *C. batrachus* (fig. 6) and the lymph spaces along the apical portions of the villi in *C. striatus* (fig. 7).

In *E. danricus* and *C. striatus*, the distribution of stained lipids 24 hr after feeding was confined to the mucosal epithelial cells (figs. 3 and 9). On the contrary, the mucosa of *C. batrachus* showed negative results whereas the submucosal lymph spaces at the bases of villi showed positive results (fig. 8).



Photomicrographs of transverse sections of various regions of intestines showing lipid absorption at different time intervals in three species of fishes. Arrows indicate stained lipids.

FIGS. 1, 2 and 3. Anterior intestines of *E. dantricus* at 8, 16 and 24 hr respectively. × 320. FIG. 4. Anterior intestine of *C. batrachus* at 8 hr. × 150. FIG. 5. Anterior intestine of *C. striatus* at 8 hr. × 150. FIG. 6. Anterior intestine of *C. batrachus* at 16 hr. × 320. FIG. 7. Anterior intestine of *C. striatus* at 16 hr. × 80. FIG. 8. Anterior intestine of *C. batrachus* at 24 hr. × 320. FIG. 9. Anterior intestine of *C. striatus* at 24 hr. × 80. FIG. 10. Middle intestine of *E. dantricus* at 8 hr. × 320.



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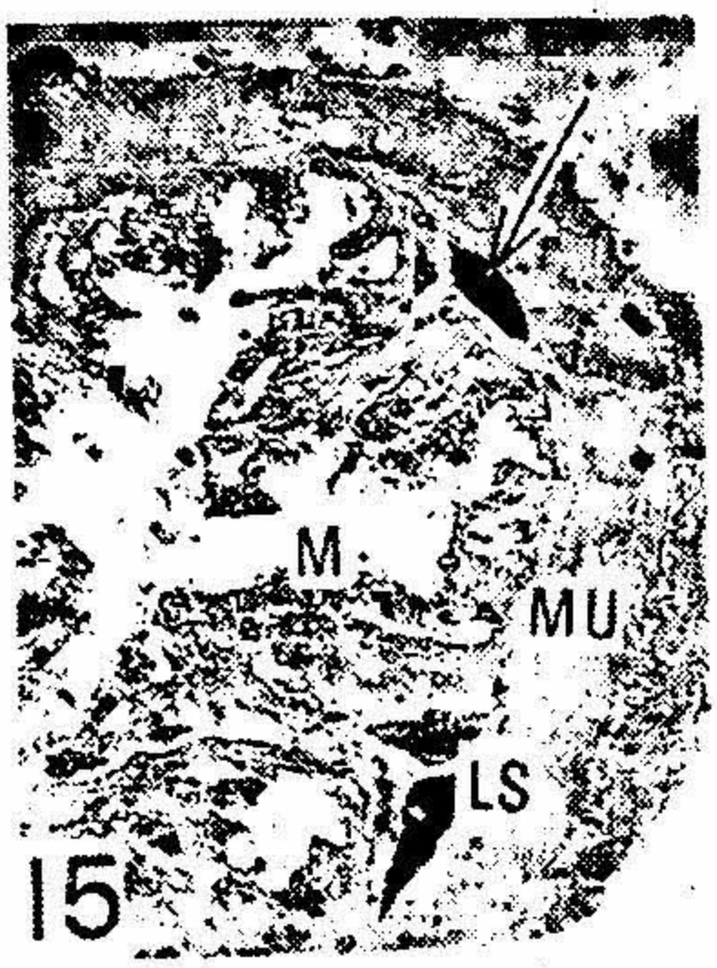

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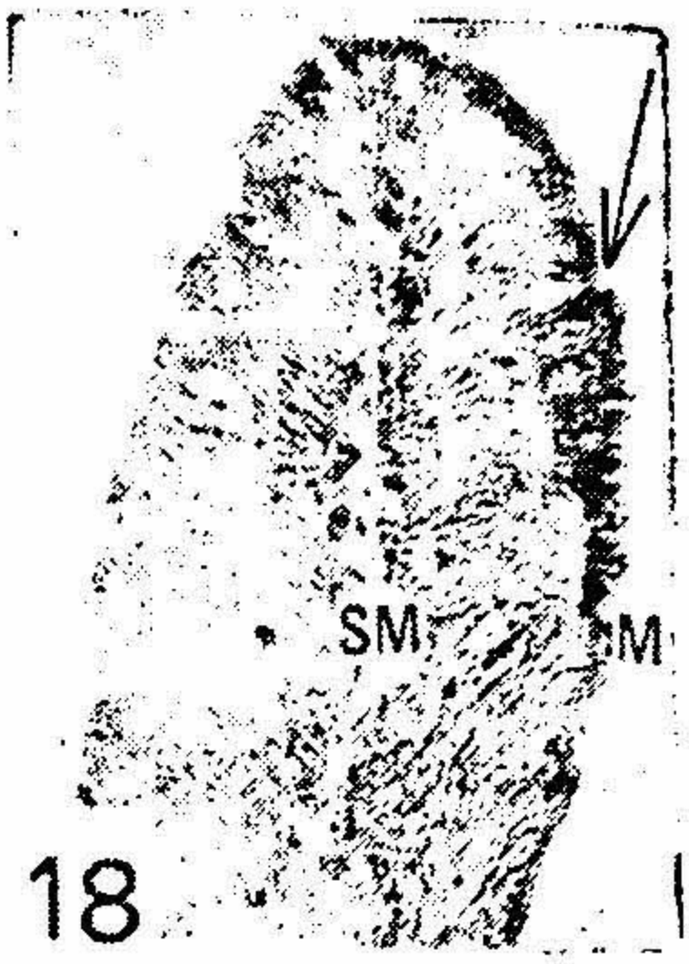

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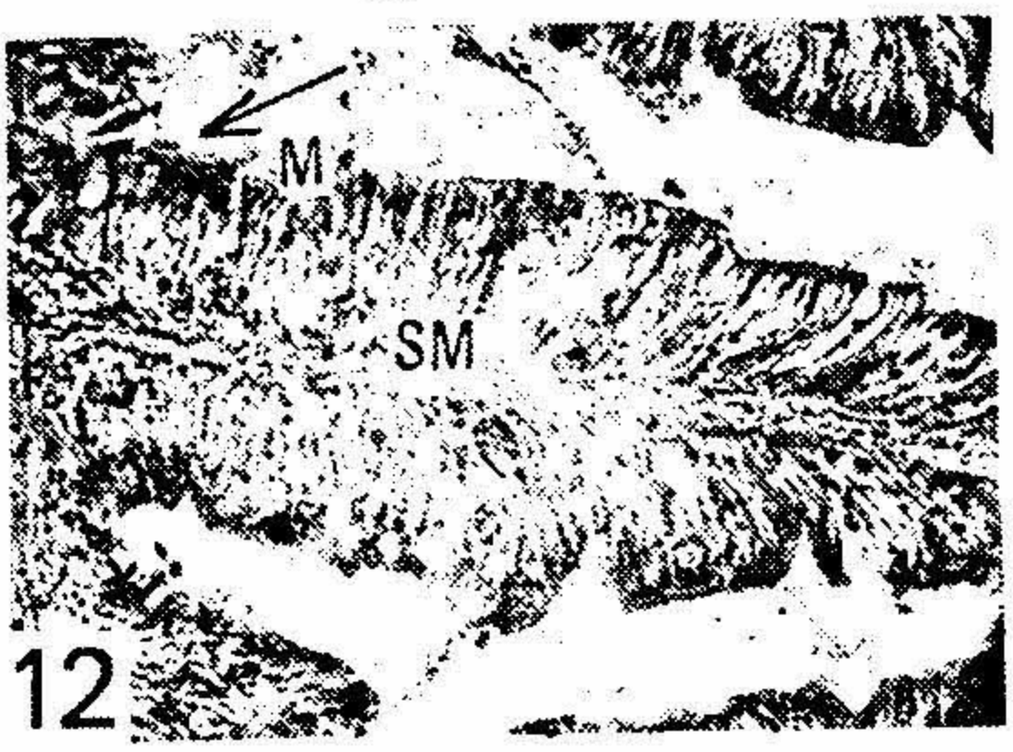

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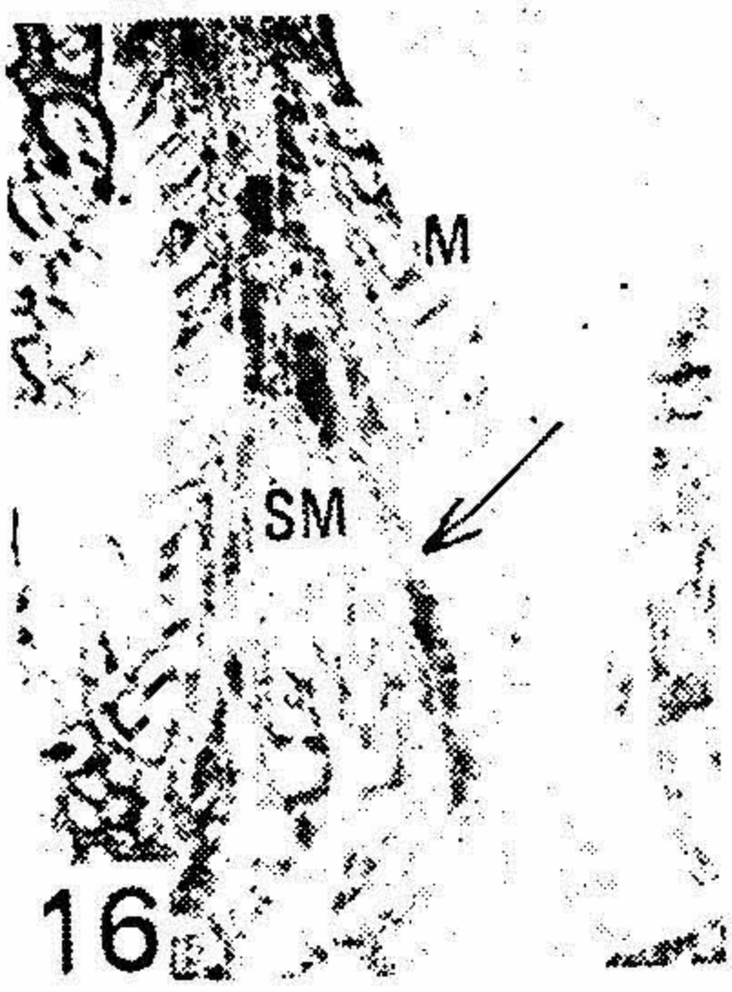

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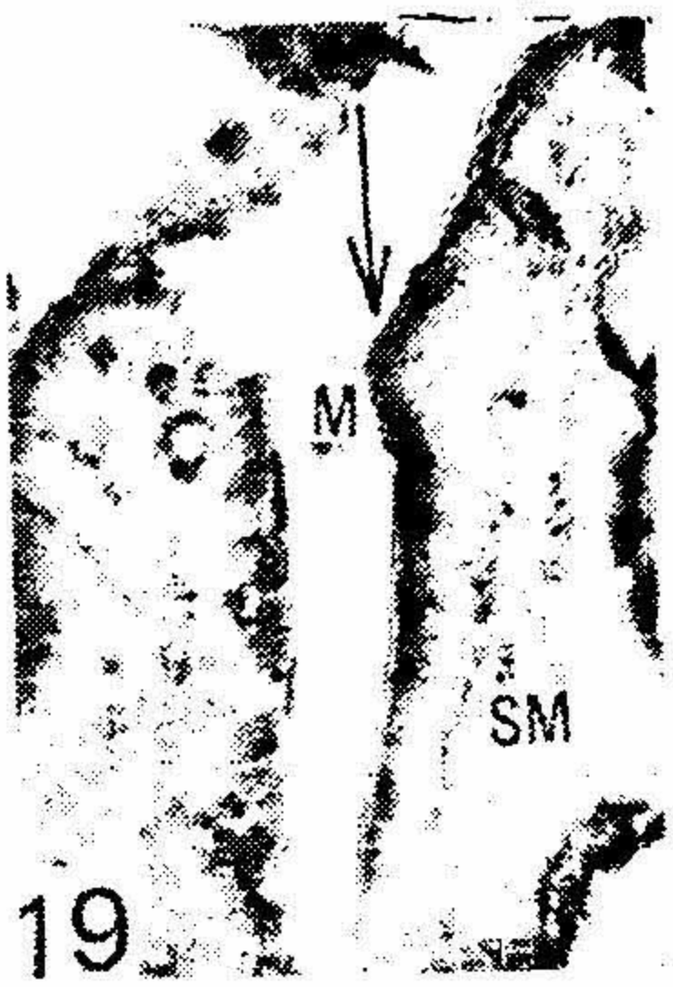

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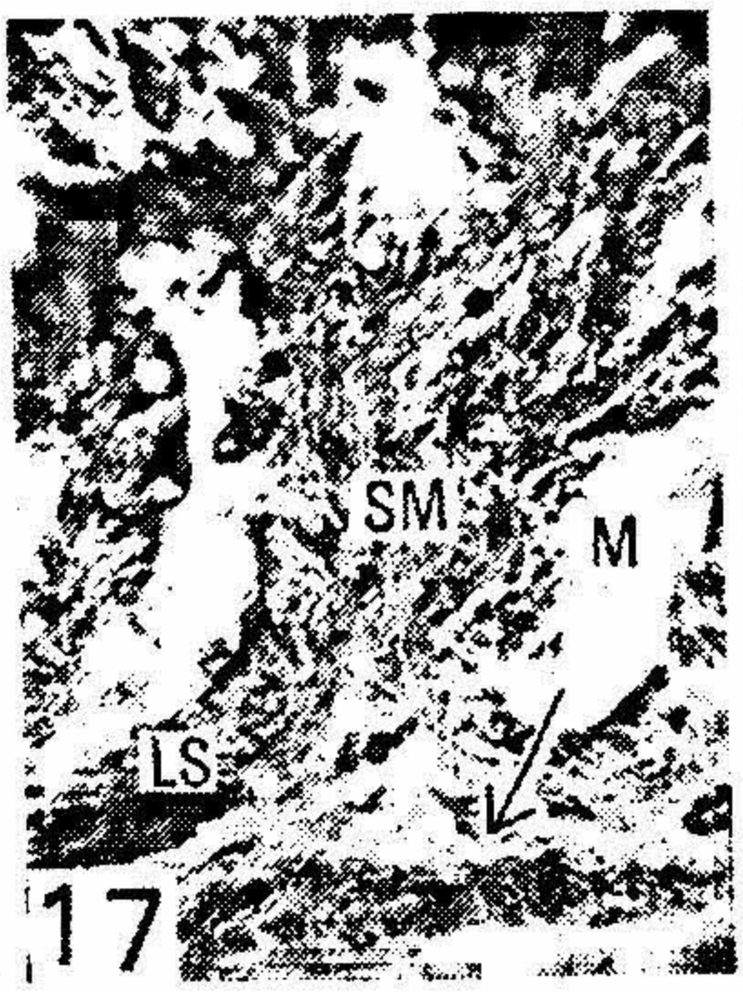

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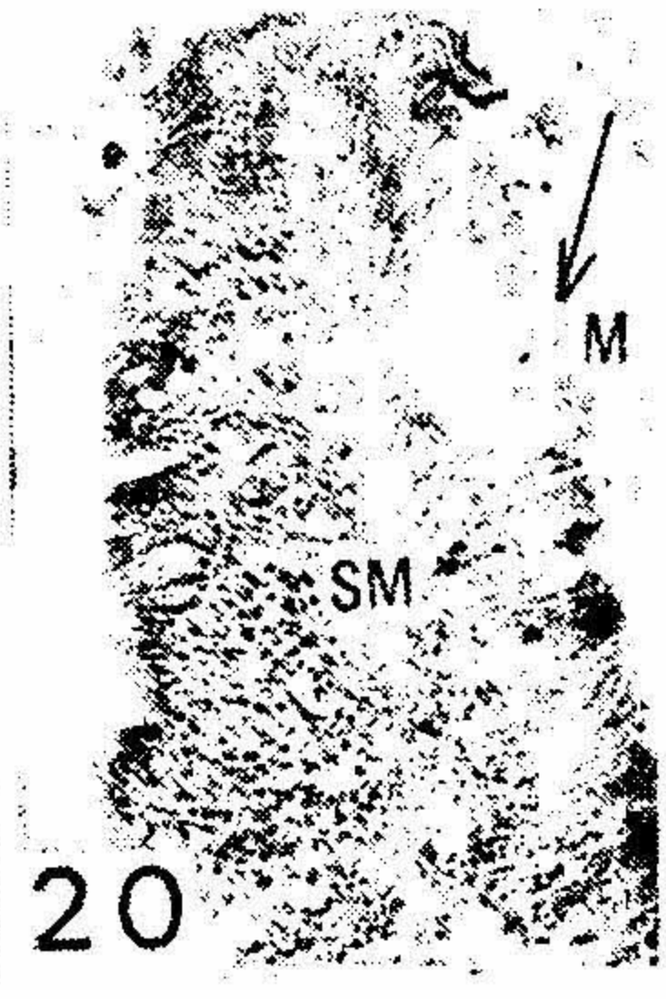

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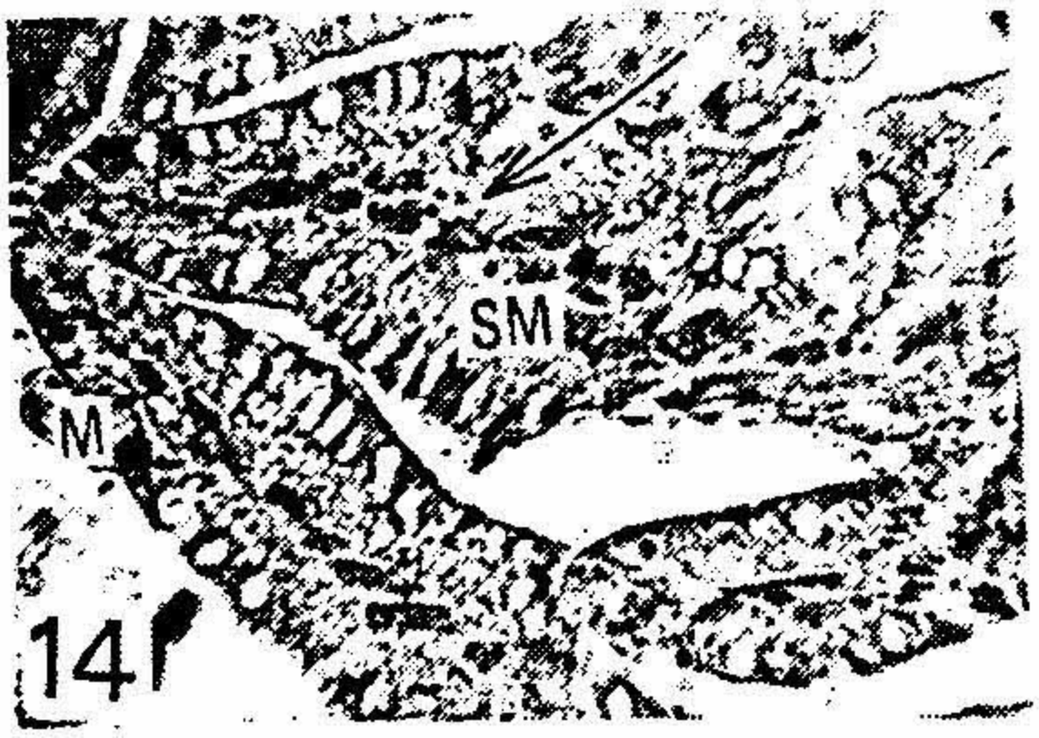


14

FIGS. 11 and 12. Middle intestine of *E. danricus* at 16 and 24 hr respectively. × 150 and × 320. FIG. 13. Middle intestine of *C. batrachus* at 8 hr. × 80. FIG. 14. Middle intestine of *C. striatus* at 8 hr. × 320. FIG. 15. Middle intestine of *C. batrachus* at 16 hr. × 80. FIG. 16. Middle intestine of *C. striatus* at 16 hr. × 320. FIG. 17. Middle intestine of *C. batrachus* at 24 hr. × 320. FIG. 18. Middle intestine of *C. striatus* at 24 hr. × 320. FIGS. 19 and 20. Posterior intestines of *E. danricus* at 8 and 16 hr., respectively, × 320.

3.2. *Middle intestine*

In this region, the absorbed lipids, 8 hr after feeding, were moderate in *E. danricus* and *C. batrachus* (figs. 10 and 13). On the contrary, the intensity was weak in *C. striatus* (fig. 14) and was not restricted towards the basal portions of the villi but was uniform throughout the submucosa. In *E. danricus* and *C. batrachus* the submucosal lymph spaces and blood vessels along the basal regions of villi showed positive but moderate absorption. The epithelial cells in all the three species of fishes were negative for lipid absorption but in *E. danricus*, the goblet cells showed characteristically positive results for lipids at 8 hr, particularly along their margins (fig. 10). However, 16 hr after feeding, the mucosal epithelial cells of *E. danricus* showed positive results (fig. 11). The submucosal region at this stage was devoid of absorbed lipid. In *C. batrachus* and *C. striatus* (figs. 15 and 16), the condition at this stage was almost similar to that observed at 8 hr.

In *E. danricus* and *C. batrachus*, 24 hr after feeding, there was progressive decrease in the intensity of absorbed lipid in the middle intestine (figs. 12 and 17); the activity was, however, restricted to the epithelial cells of mucosa in the former and to the submucosal region of the basal portions of the villi in the latter. On the other hand, the mucosal epithelial cells, especially towards the apical portions of the villi in *C. striatus*, exhibited moderate absorption at this time (fig. 18), while the intensity was reduced in the submucosal region.

3.3. *Posterior intestine*

The columnar epithelial cells of the mucosa in *E. danricus* (fig. 19) and *C. striatus* (fig. 23) exhibited weak absorption 8 hr after feeding. The goblet cells and the submucosa, however, gave negative results for lipid absorption in *E. danricus* whereas in *C. striatus*, a considerable decrease in the intensity of lipid absorption was observed in the submucosal region along the apical portions of the villi (fig. 23). In *C. batrachus* the submucosal lymph space, along the bases of the villi showed moderate absorption 8 hr after feeding (fig. 22). The intensity was, however, less in comparison to the anterior and middle regions of the intestine of the same stage. Mucosal epithelial cells, however, showed positive results in contrast to middle intestine of the same stage.

Moderate absorption was observed in the basal portions of the columnar epithelial cells of mucosa in *E. danricus*, 16 hr after feeding (fig. 20). On the contrary, a progressive decrease in the intensity of absorption was noted in *C. batrachus* at this time interval along the basal portions of the villi in the submucosa (fig. 24), while in *C. striatus*, moderate absorption was seen in the submucosal lymph spaces along the apical portions of the villi (fig. 25). The columnar epithelial cells of mucosa in both *C. batrachus* and *C. striatus* were devoid of absorbed lipid.

Marked decrease in the intensity of absorption was noted, 24 hr after feeding, in this region of the intestine in all the three species of fishes (figs. 21, 26 and 27). The sites of decreased absorption varied in the three species of fishes, being the mucosal epithelial cells, the submucosal lymph spaces at the bases of villi and submucosal region towards the apical portions of the villi in *E. danricus*, *C. batrachus* and *C. striatus* respectively.

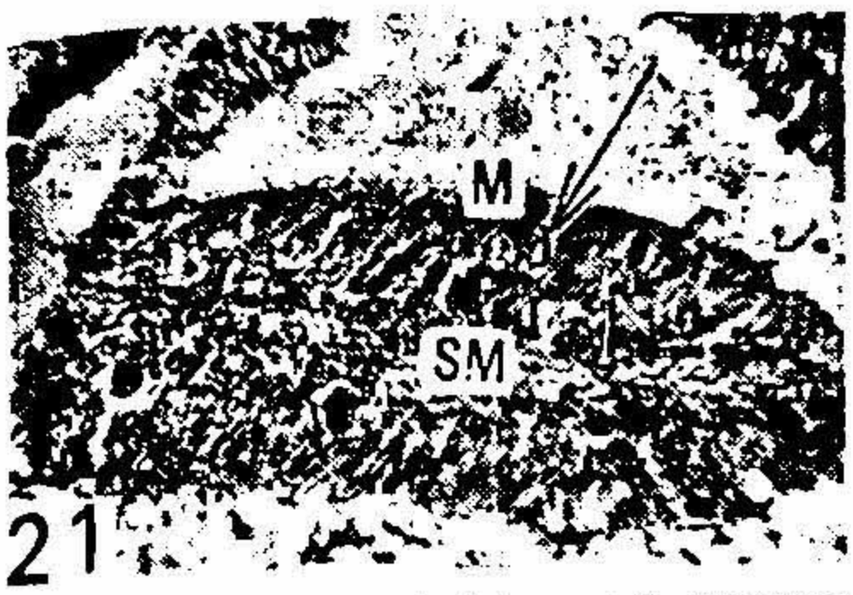
3.4. *Pyloric caeca*

The pyloric caeca of *C. striatus*, 8 hr after feeding, showed moderate absorption along the free border and the basal portions of the mucosal epithelial cells (fig. 28). The blood vessels and the capillaries of the submucosa of the apical portions of the villi depicted weak absorption. A significant increase in the intensity of absorption was noted 16 hr after feeding in the submucosal lymph spaces and blood vessels towards the apical portions of the villi (fig. 29). The mucosal epithelial cells showed fairly moderate absorption at 16 hr. However, the absorption was seen to be maximum in the pyloric caeca, 24 hr after feeding (fig. 30). The intensity was quite strong throughout the submucosal region. The mucosal epithelial cells also depicted fairly good intensity of absorption at this stage. The goblet cells, however, showed negative results.

4. Discussion

Fats are absorbed almost exclusively in the small intestine[5,6], there being two pathways for absorbed lipids, namely, (i) blood vessels including the capillaries in the walls of the intestine, particularly those in the villi, and (ii) through lymph spaces and lymph vessels[7]. According to Bykov[5], about 75% of the fat passes into the lymph while only 25% passes into the blood.

In the present investigation, the whole intestine has been observed to take part in the absorption of the lipids, but the three different regions, *viz.*, anterior, middle and posterior showed variations in the intensity and distribution of absorbed lipids. Although the maximum absorption in all the three species of fishes was in the anterior regions of the intestine, there is some absorption in the other regions also. The anterior intestine exhibited overall maximum intensity of absorbed lipids, 8 hr after feeding. This is in conformity with the findings of Baker[8], Dawes[9], Hewitt[10], Sastry and Garg[1] and Senior[11]. The maximum intensity of neutral fats in the anterior intestine at 8 hr is probably due to two reasons, *viz.*, firstly the anterior intestine receives bile which according to Bykov[5] enhances lipid absorption and secondly, this region is the first to receive the food and thus starts absorption early. However, from the present observations, it is evident that the rates of absorption of lipid are variable, being slower in herbivorous *E. danricus* as the staining reactions for lipid absorption were mainly restricted to the surface layers, *i.e.*, mucosal epithelial cells. Further, the submucosal regions showed weaker stain intensity indicating thereby that the absorbed lipid had not reached this region in sufficient quantity at 8 hr stage. On the contrary, in



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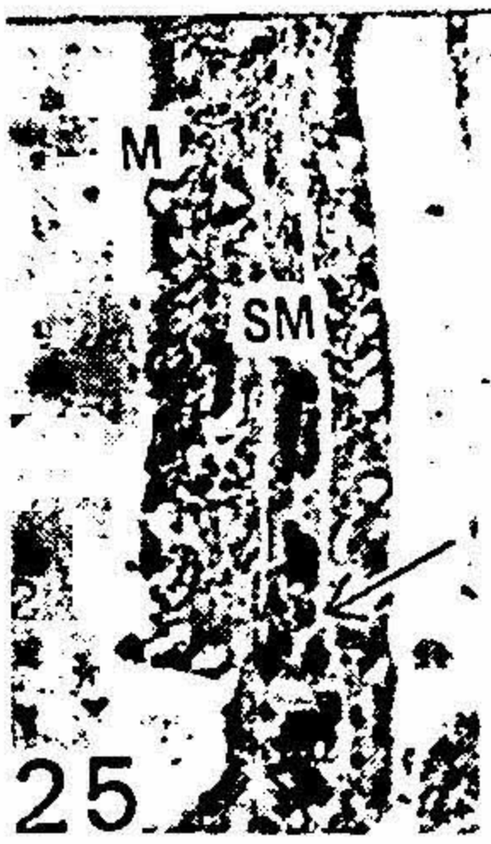

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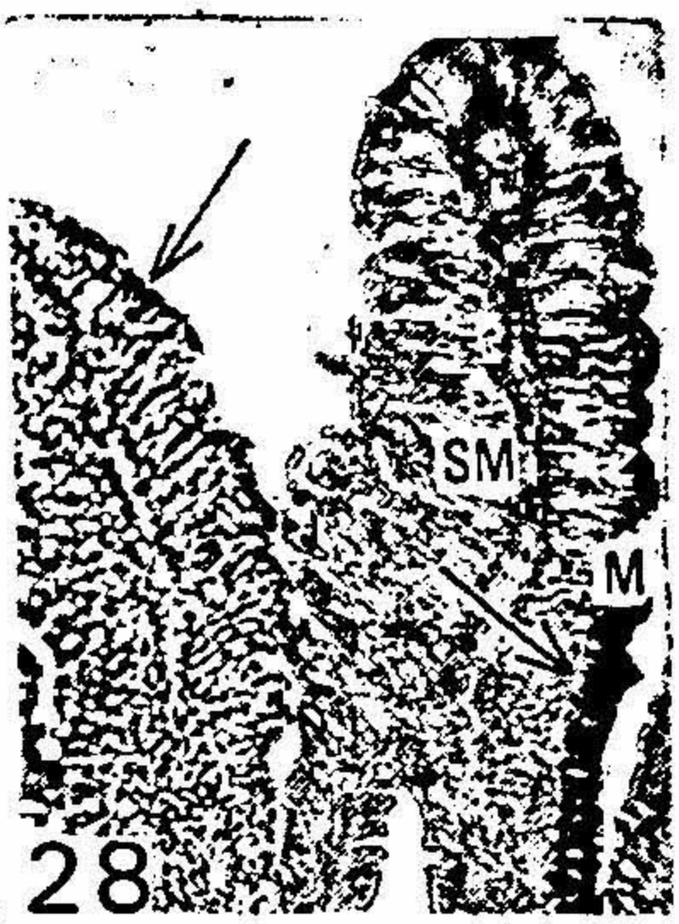

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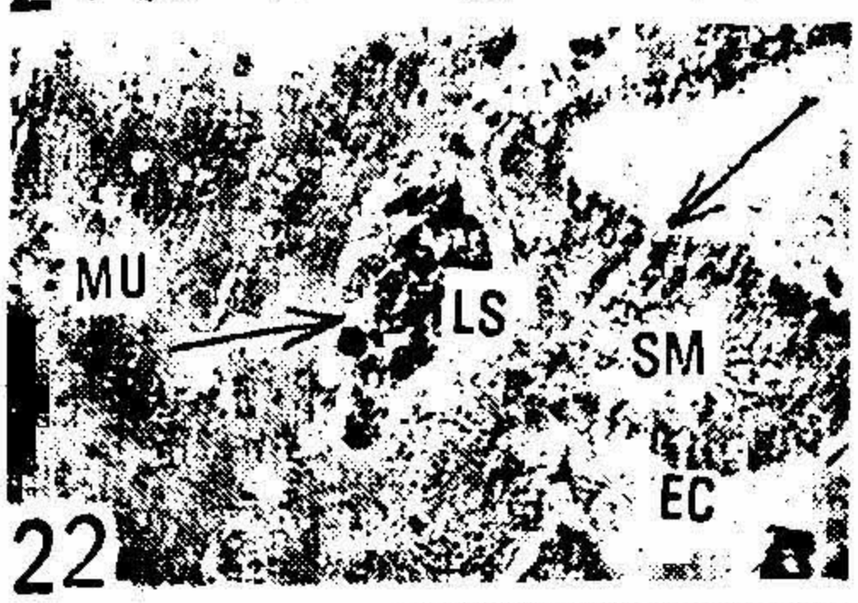

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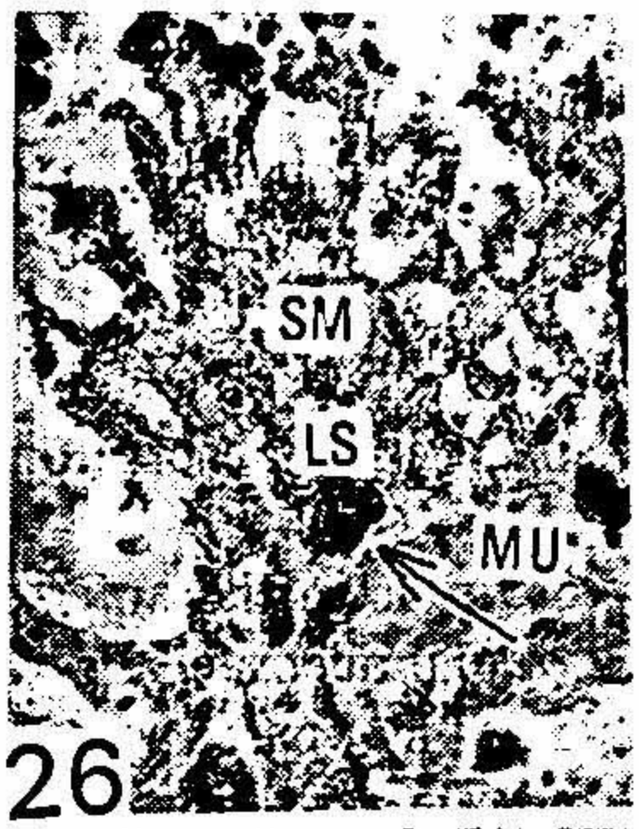

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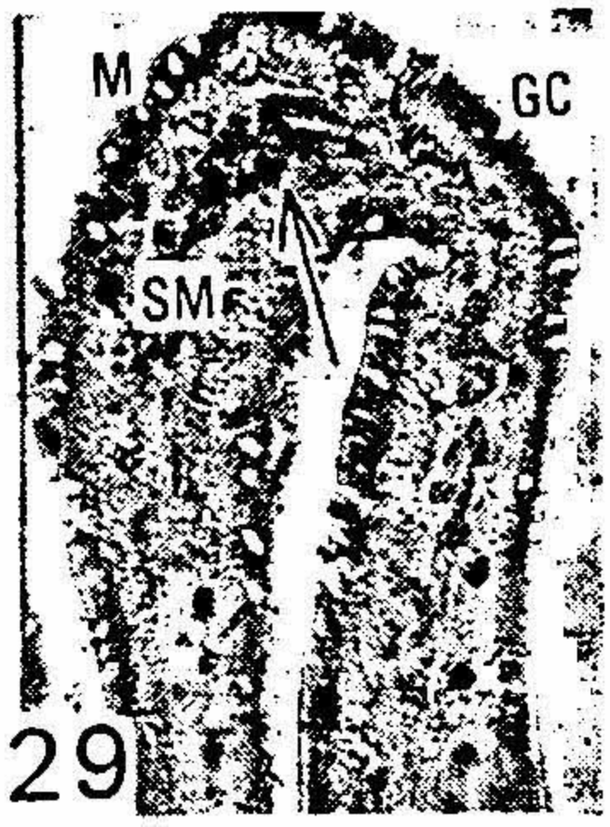

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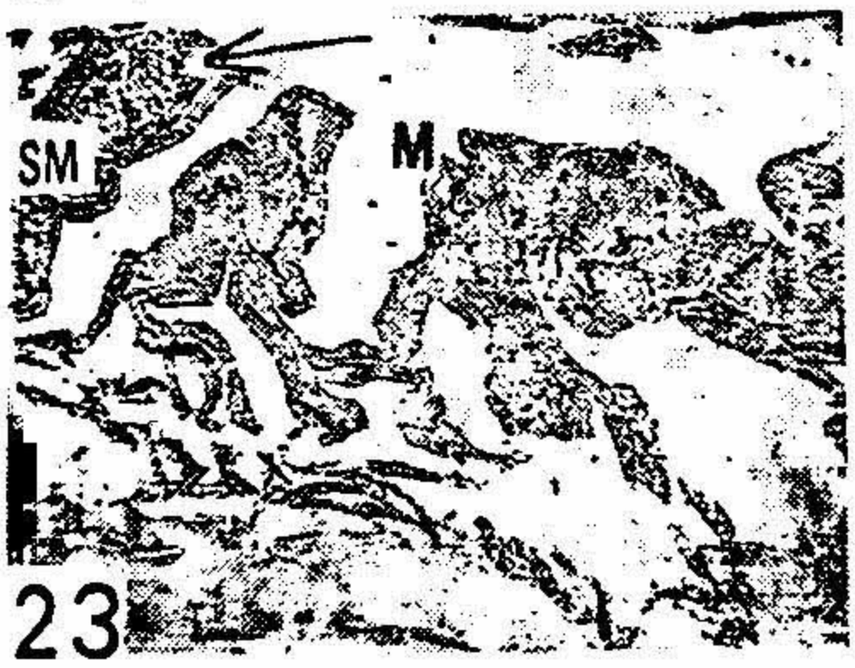

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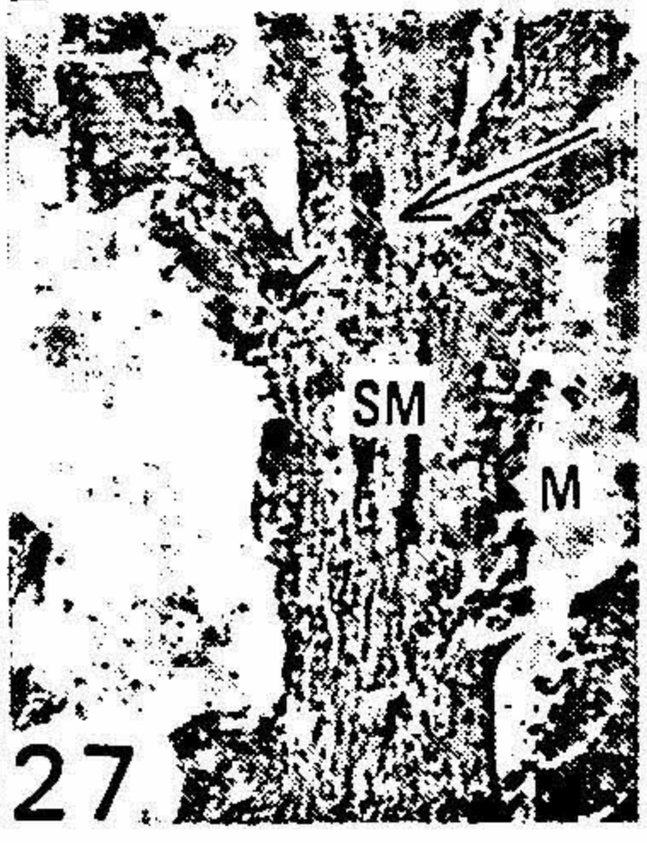

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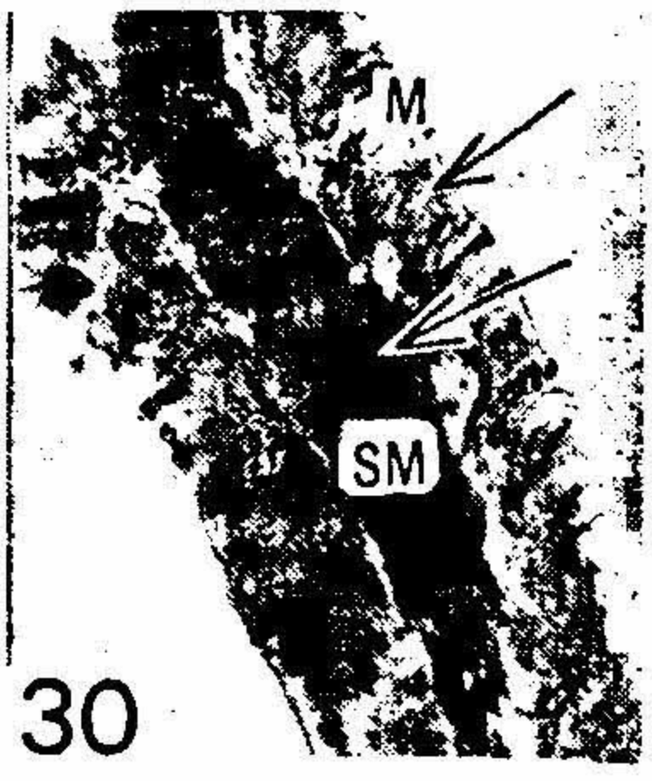

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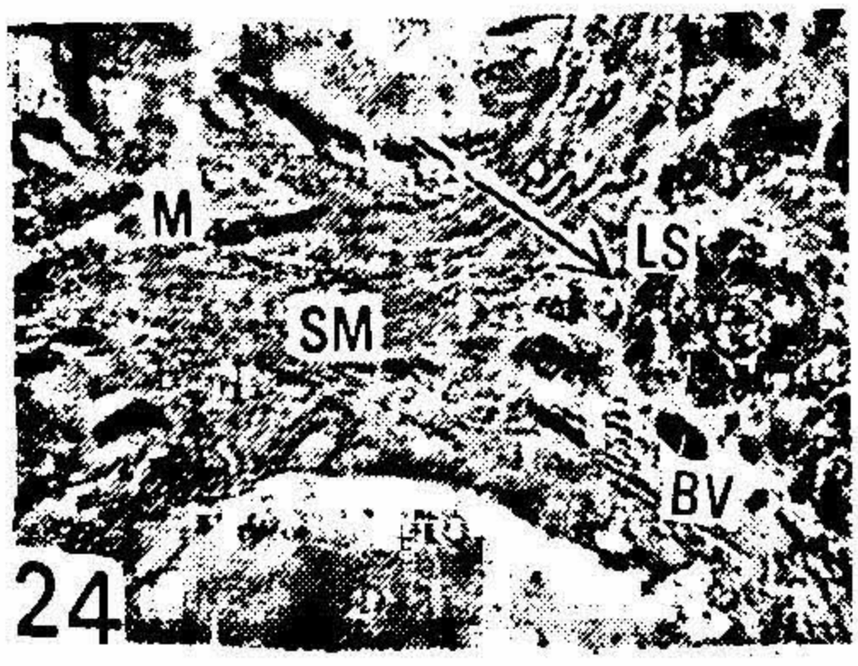

24

FIG. 21. Posterior intestine of *E. danricus* at 24 hr. × 320. FIG. 22. Posterior intestine of *C. batrachus* at 8 hr. × 320. FIG. 23. Posterior intestine of *C. striatus* at 8 hr. × 80. FIG. 24. Posterior intestine of *C. batrachus* at 16 hr. × 320. FIG. 25. Posterior intestine of *C. striatus* at 16 hr. × 320. FIG. 26. Posterior intestine of *C. batrachus* at 24 hr. × 80. FIG. 27. Posterior intestine of *C. striatus* at 24 hr. × 320. FIGS. 28, 29 and 30. Pyloric caeca of *C. striatus* at 8, 16 and 24 hr, respectively. × 150, × 320 and × 320.

(BV = Blood vessels, EC = Epithelial cell, GC = Goblet cell, LS = Lymph space, M = Mucosa, MU = Muscularis and SM = Submucosa.

C. batrachus and *C. striatus*, the weaker intensity in the mucosal epithelial cells and stronger intensity in the submucosal lymph spaces and the blood vessels indicate that the lipids in these two species pass quickly as they had reached the deeper layers of the intestine at the same time interval, at which the lipids were restricted mainly to the mucosal epithelial cells in *E. danricus*. From the above it can be concluded that the rate of fat absorption is faster in omnivorous and carnivorous fishes (*C. batrachus* and *C. striatus* in the present study) than the herbivorous fishes (*E. danricus* in the present case).

The main sites of lipid absorption according to Sastry and Garg[1] are the epithelial cells of mucosa, the submucosal lymph spaces and blood capillaries, which have been confirmed by the present findings. The muscularis and the serosa were devoid of lipids in the present investigation which has also been reported by Sastry and Garg[1].

The middle and the posterior intestines of all the three species of fishes depicting overall moderate absorption at 8 hr is probably due to the fact that by the time the food reaches these regions, most of the lipids are already absorbed in the anterior regions of the intestine. The presence of lipids around the goblet cells in the middle intestine of *E. danricus* at 8 hr confirms the observations of Hewitt[10] and probably indicates that the goblet cells also take part in the lipid absorption.

The decline in the intensity of lipids in the anterior intestine of *E. danricus* at 16 hr shows that the transportation of the absorbed lipid had already started at this time interval and that the remaining food had passed on to the lower portions of the intestine for absorption. The middle and the posterior intestine of *E. danricus* and all the regions of intestine of *C. batrachus* and *C. striatus*, at this time interval, also exhibit steady decline in the intensity of lipid absorption which is indicative of the fact that after absorption in the mucosal epithelial cells, the absorbed material passes on to the submucosa, wherefrom it is transported to the general circulation. Al-Hussaini[12] made similar observations in the intestine of cyprinids and according to him the anterior and the middle portions of the intestine are the most active sites for absorption. However, the differences in the sites and the intensity of absorption in the different regions of the intestine of the three present species of fishes can possibly be correlated to the lengths of their intestines. Due to the small length of the intestine in *C. batrachus* and *C. striatus*, the ingested food probably travels to the intestine in a comparatively short duration and eventually the transportation of the absorbed material is quicker than in a herbivorous fish (*E. danricus*) in which the intestinal length is comparatively greater.

The significant decrease in the intensity of lipid absorption, 24 hr after feeding in the various regions of the intestine in all the three species of fishes, is suggestive of the fact that sufficient quantity of the administered diet had already been absorbed by this time.

The pyloric caeca of *C. striatus* showing minimum and maximum absorption at 8 and 24 hr is in conformity with the observations of Brown[13] and Greene[14] who state

that the rate of lipid absorption in pyloric caeca is slower than in the intestine. Nevertheless, the significance of the pyloric caeca as fat absorbing organs cannot be overlooked since they are treated as the main centres of fat absorption by Greene[14].

Acknowledgements

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