OXIDATIVE RATES OF HOMOGENATES OF RAT SMALL INTESTINAL EPITHELIUM. EFFECTS OF FASTING AND INTESTINAL LEVEL[1]

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The transport of certain sugars by small intestinal epithelium depends upon a vigorous oxidative metabolism (Darlington and Quastel, 1953; Ponz and Lluch, 1955). Therefore, knowledge of effects of various nutritional states upon rates of oxidation in this tissue is basic to understanding certain effects of nutrition upon at least one of the specialized functions of the epithelium. This paper describes effects of fasting upon oxidative rates of fortified homogenates derived from essentially the entire small intestinal epithelium. Oxidative rates of epithelial homogenates from 3 different levels of the small intestine in fed rats are given also.

METHODS

Mature male Sprague-Dawley rats weighing 196 to 456 g. were maintained on a ration of Rockland Rat Pellets (Complete) and tap water. For trials with fasted rats, pellets (but not water) were withdrawn for 24 hours. The rats were stunned and bled. The entire small intestine was freed from mesentery, excised, and placed in ice-cold 0.25 M mannitol, cleaned (Hele, 1953) and weighed. The entire organ was treated in a homogenizer and the epithelium was freed from other layers as described by Hele (1953) except that ice-cold 0.25 M mannitol solution (5 ml./g. intestine) was used for homogenization. The homogenates contained some whole cells of columnar epithelium, occurring both singly and in sheets, as well as broken cells. The volumes of epithelial homogenates were measured, and aliquots were taken for nitrogen determination by micro-Kjeldahl and for tests of oxidative rate by direct Warburg manometry. The homogenates contained 1.4 to 2.1 mg. nitrogen/ml.

For tests at different levels of intestine, the entire small intestine from rats weighing 347 to 374 g. was laid out and the length measured. The small gut averaged 113 cm. (range: 109–119 cm.) in length. This total length was cut into 3 sections (upper, middle, and lower) of equal length. Each section was cleaned separately to prevent possible cross-contamination by microorganisms, and separate epithelial homogenates were prepared and tested as described above for the whole intestine. These homogenates averaged 2.2, 2.1, and 2.1 mg. nitrogen/ml. in the upper, middle, and lower thirds, respectively; thus, any differences found in simultaneous comparison of the 3 segments could not be due to use of unequal concentrations or amounts of total tissue.

Each Warburg flask contained 1.0 ml. of homogenate and the following (amounts in μmoles): 80 tris (hydroxymethyl) aminomethane·HCl buffer at pH 8.5, 20 potassium phosphate at pH 8.1, 10 $MgCl_2$, 0.03 cytochrome c (Sigma), 12 potassium adenosine triphosphate (Pabst), 2 potassium diphosphopyridine nucleotide (Sigma, 90% pure); α-ketoglutarate (30 μmoles) was added to $\frac{1}{2}$ of the flasks for 6 rats in each group in the fed *vs.* fasted comparison. The total volume was made to 3.0 ml. with 0.15 M KCl. NaOH was used in the center well. The gas phase was air. The cold flasks containing the reaction mixtures were brought to 35° C

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by shaking in a bath for 10 minutes before starting readings. Expressions of oxygen uptakes, both/mg. nitrogen/hr. and/total epithelium/hr., are based on uptake for each entire hour rather than on initial rate.

Differences were tested statistically by the "U" test of Mann and Whitney (1947) using the hypothesis that there was no difference between groups and the alternative that there was a difference.

RESULTS

Effects of fasting.—(Table 1). There was no significant difference between fed and fasted rats in total nitrogen content of the small intestinal epithelium. Fortified epithelial homogenates from fed rats slightly exceeded those from fasted rats in endogenous oxygen uptake/mg. nitrogen; this difference probably has

TABLE 1

Nitrogen content and respiration rate of homogenates of small intestinal mucous epithelium from fed and fasted rats

Group	No. of rats	Total epithelial nitrogen, mg.	Oxygen uptake in first hour			
			μl./mg. nitrogen/hr.		ml./total epithelium/hr.	
			Endogenous	Increase with α-ketoglutarate	Endogenous	Increase with α-ketoglutarate
Total groups						
Fasted	7	71.8 (53.9–85.1)	51 (43–61)		3.6 (2.8–4.4)	
Fed	10	66.1 (53.6–94.1)	59 (43–65)*		3.9 (2.4–5.4)	
Subgroups†						
Fasted	6	74.7 (59.9–85.1)	51 (43–61)	9 (3–14)	3.8 (3.4–4.4)	0.7 (0.3–1.1)
Fed	6	70.0 (55.4–94.1)	59 (43–65)	30 (10–53)‡	4.1 (2.4–5.4)	2.1 (0.6–3.5)‡

Figures given are averages; ranges in parentheses.

Significant differences between comparable fed and fasted groups:

*0.05> P >0.02.

‡0.02> P >0.01.

†Subgroups made up of those rats in the total groups in which the effect of adding α-ketoglutarate was tested.

little physiological significance since the endogenous respiration of the *total* epithelium was the same in fed and fasted rats. In the presence of added α-ketoglutarate, respiration was increased in homogenates from both fed and fasted rats; however, the increase was about 3 times as great in the fed rats as in the fasted rats. Since this difference with α-ketoglutarate was significant statistically not only in oxygen uptake/mg. epithelial nitrogen but also in uptake/*total* epithelium, it appears to have a possible physiological significance.

Rates at 3 levels of small intestine.—(Table 2). There were no differences in endogenous oxidative rate/mg. nitrogen of fortified epithelial homogenates from the upper and middle thirds of small intestine. In the earlier work of Dickens and Weil-Malherbe (1941), the oxidative rate of mucosal strips was higher in jejunum than in duodenum; the division of the upper small gut differed from the division

in the present work; so the 2 studies are not directly comparable. The rate/mg. nitrogen with our homogenates from the lower third of the small intestine was significantly lower than with either the upper or middle thirds in the early hours of the test; this is consistent with work with strips of ileal mucosa in Ringer's solution or serum (Dickens and Weil-Malherbe, 1941). Table 2 shows also that oxidative rates/mg. nitrogen decreased from hour to hour in about the same way for upper and middle thirds, and a generally low level was reached by the fifth to seventh hour with these 2 segments from most of the rats. The rates with lower gut segments also decreased at first, but then they generally leveled off about the

TABLE 2

Endogenous respiration of homogenates of epithelium from upper, middle and lower thirds of the small intestine

	Upper	Middle	Lower
Total epithelial nitrogen, mg.	28.3 (23.8–34.1)	24.0 (21.6–26.4)*	21.1 (18.6–25.8)*
Oxygen uptake, μl./mg. nitrogen/hour for hours:			
1	62 (51–69)	59 (53–66)	35 (30–43)†‡
2	44 (38–48)	43 (38–50)	28 (26–31)†‡
3	32 (26–35)	32 (28–35)	21 (14–25)†§
4	21 (18–23)	24 (20–26)	18 (13–22)
5	10 (1–16)	15 (3–23)	15 (12–19)
6	6 (2–14)	12 (6–19)	14 (11–20)
7	4 (2– 9)	6 (2–11)	16 (9–20)*
Oxygen uptake of total segment epithelium, ml./hour, for hours:			
1	1.7 (1.4–2.3)	1.4 (1.3–1.5)	0.8 (0.6–1.1)†‡
2	1.2 (1.0–1.6)	1.0 (0.9–1.2)	0.6 (0.5–0.7)†‡

Figures are averages, with ranges in parentheses, for segments from 5 rats, except that only 4 rats are included in hours 3–7 for the middle third, 4–7 for the lower third, and 6–7 for the upper third; data from one of the 5 rats was omitted thus due to very rapid upturning of oxidative rate starting the third hour in the middle third and the fourth hour in the lower third.

*Significantly different from the upper third ($P<0.05$).
†Significantly different from the upper third ($P<0.01$).
‡Significantly different from the middle third ($P<0.01$).
§Significantly different from the middle third ($P<0.02$).

third to fifth hours, and sometimes turned up again about the fifth to seventh hours. This rate pattern in the lower third probably is due to the developing metabolism of microorganisms, as reported in occasional experiments with mucosa from lower ileum and colon (Dickens and Weil-Malherbe, 1941). The sharp rate upturns in middle and lower segments beginning in the third and fourth hours, respectively, in one of the rats must be due to a greater than usual microbial contamination. Thus, only the early, steadily declining respiration may safely be attributed to animal enzymes.

Table 2 shows also that the total nitrogen content of the entire epithelium obtained from the upper third was significantly greater than that of the middle or lower thirds. From the nitrogen content and the oxygen uptake/mg. nitrogen, the total endogenous oxygen uptake for the entire epithelium from each segment

was calculated for the first 2 hours of the test (*i.e.*, for the period before bacterial metabolism began to interfere in any of the rats). These total uptakes for the lower segment were significantly lower than the corresponding values for both upper and middle segments in both the first and second hours (table 2). Also, the difference in total uptake between upper and middle thirds was very nearly significant statistically ($P = 0.056$) in the first hour.

DISCUSSION

The decreased ability of epithelium from fasted rats to oxidize added substrate (α-ketoglutarate) *in vitro* perhaps reflects a lesser ability to oxidize, *in vivo*, substrates derived from food, when oral feeding is begun again after a period of food deprivation. If so, rates of certain energy-dependent absorption processes in the intestine might decrease in fasted rats.

There are several possible explanations for the lower initial respiration rate in fasted rats' gut epithelium in the presence of added α-ketoglutarate. One is that in fasted rats there appear to be fewer respiring elements (mitochondria) in intestinal epithelial cells (Miller, 1922). Thus, a limiting level of respiratory catalysts perhaps prevented as great an increase with added substrate. However, the correctness of this explanation for our results is in doubt, since Miller's (1922) results were obtained with rats fasted for longer periods than used herein. Also, Miller's evidence is limited in value by difficulties of technique and by its inconsistency with earlier evidence. (See Macklin and Macklin, 1932, for a summary of the early work on effects of fasting on numbers of mitochondria.) Another possible explanation is suggested by the findings that prolonged underfeeding of rats from weaning resulted in a smaller oxygen consumption of small intestinal mucosa slices with glucose as substrate, and that this lesser repiration was due principally to protein restriction (Vitale *et al.*, 1953). However, the total epithelial nitrogen in our mature rats' small intestines was not decreased after 24 hours of feed deprivation, and so it is uncertain if total protein deficiency was involved in the smaller oxidation of α-ketoglutarate we observed in our fasted rats. Other explanations might be advanced, but there seems to be no evidence for or against any of them.

Evidence given herein that withdrawal of feed for 24 hours produced no detectable change in total nitrogen of rat small gut epithelium is consistent with histological evidence (Leblond and Stevens, 1948) that replacement of gut epithelium in rats continued during a 4-day fasting period. In contrast, fasting periods longer than 24 hours or more (depending on the season) in mice (Sun, 1927) or prolonged underfeeding from birth in rats (Miller, 1927) or underfeeding until death during an Indian famine in humans (Charlton, 1946) produced deterioration of intestinal epithelium. If the turnover time for the entire small gut epithelium is the same in fasted as in fed rats, *i.e.*, about 1½ days (Leblond and Stevens, 1948), then approximately ⅔ of the epithelium (containing about 48 mg. Kjeldahl nitrogen) must have been sloughed off and replaced during the 24-hour feed deprivation period employed herein. Nitrogen and amino acids used in this continuing replacement protein synthesis during the 24 hours without feed perhaps came partly from feed protein present in the gastrointestinal tract at the start of the fast, partly from digested protein of epithelial cells extruded into the lumen high up in the alimentary tract, and partly from rather readily labilized protein from other parts of the body.

The low oxygen consumption/mg. of nitrogen in epithelium from the lower small gut of fed rats could not be due to use in each flask of too little *total* tissue to sustain vigorous oxidation. (See Methods.) However, it could be due to use of too little oxidatively *active* tissue (mainly mitochondria) if, as seems possible, the ratio of mitochondria to total tissue is low in ileal epithelium.

SUMMARY

1. Respiration of fortified homogenates of the entire epithelium from the small gut was studied in rats from which feed had been withdrawn for 24 hours and in fed rats. Endogenous respiration rates differed little in fed and fasted rats; however, the increases in rates when α-ketoglutarate was added were strikingly higher in the fed rats. The nitrogen content of the total epithelium was the same in the fed and fasted rats.

2. The epithelial layers of the upper, middle, and lower thirds of the small gut were compared in fed rats. The upper third exceeded both the middle and lower thirds in total nitrogen content. Fortified homogenates from the upper and middle thirds each exceeded those from the lower third in endogenous oxygen uptake per mg. of nitrogen and in endogenous oxygen uptake per total segment epithelium, during early hours of the test. Later in the test period, other differences developed which were attributed to bacterial metabolism rather than to animal metabolism.

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