The validity of assessing changes in intestinal absorption mechanisms for dietary sugars with non-metabolizable analogues (glucalogues)

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1. Transfer potentials were obtained from everted jejunal sacs prepared from fed euthyroid, fasted euthyroid, fed hypothyroid and fasted hypothyroid rats by addition of serial concentrations of the dietary sugars glucose and galactose and the glucalogues 3-o-methyl glucose, α-methyl glucoside and 6-deoxy-D-glucose to the incubation fluids. The kinetic parameters of 'apparent Michaelis constant' (apparent K_m) and maximum transfer potential difference (pd_{max}) obtained from the results were used to characterize the changes in the electrogenic transfer mechanisms for these substrates.

2. Analysis of the significant differential changes in values for 'apparent K_m' and pd_{max} for the two dietary sugars and the three glucalogues indicated heterogeneity in the mechanisms for sugar transfer across the intestine and suggested a minimum of four possible carriers.

3. The validity of using glucalogues to characterize changes in the transfer mechanisms for the dietary sugars in different dietary and hormonal states was assessed. None of the kinetic parameters for electrogenic glucalogue transfer matched those for the dietary sugars in all the experimental conditions. The employment of glucalogues to assess changes in electrogenic transfer mechanisms for dietary sugars can thus lead to invalid conclusions.

4. Fasting decreased the 'apparent K_m' of the dietary sugars and the glucalogues. However, the pd_{max} values for glucose, galactose, and α-methyl glucoside decreased whereas those for 3-o-methyl glucose and 6-deoxy-D-glucose did not.

5. Hypothyroidism showed different effects in fed and fasted intestine. Because hypothyroidism induced a reduction in food intake, separation of the direct effects of the condition on electrogenic transfer from reduced food intake effects was not possible.

Non-metabolizable analogues of sugars ('glucalogues') have been used extensively to study the mechanisms for the transfer of the dietary sugars, glucose and galactose, across the small intestine. Recent investigations, however, have shown the existence of multiple transfer mechanisms for hexoses in both rat (Levin & Syme, 1971; Debnam & Levin, 1975*b*, 1976; Reiser *et al.* 1975; Berman *et al.* 1976; Levin, 1976) and hamster (Honegger, 1973; Honegger & Semenza, 1973; Honegger & Gershon, 1974; Wiseman, 1977). In order to assess the validity of the use of glucalogues to characterize the transfer mechanisms for the two dietary sugars we have compared the effects of hormonal and dietary conditions on the operational parameters of electrogenic transfer of glucose and galactose and the glucalogues 3-o-methyl glucose, α-methyl glucoside and 6-deoxy-D-glucose. A preliminary account of part of this work has been given (Levin & Syme, 1971).

EXPERIMENTAL

Materials and Methods

Animals. White male rats of the Sheffield strain (bred at University of Sheffield) weighing 230–250 g were used. Control animals were allowed free access to the food (Diet 86; Burnhill, Cleckheaton, W. Yorks.) and water. When animals were fasted the food was removed for 3 d but free access to water was allowed.

Production of hypothyroid rats. Animals were given alkaline (pH 8·5) 0·5 mM-6-*n*-propyl-2-thiouracil in their drinking water for at least 28 d. Rectal temperature, thyroid and adrenal

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weights were routinely measured as in previous studies (Levin & Syme, 1975; Syme & Levin, 1976) to confirm the efficacy of the goitrogen.

Measurement of transfer potential differences (pd). Everted sacs 50 mm long were prepared from the mid jejunum, filled with 0·6 ml of bicarbonate saline (Krebs & Henseleit, 1932) containing 164 mM-mannose and incubated in vessels similar to those used by Barry *et al.* (1964). The transfer potential differences were measured by two agar salt bridges placed in the serosal and mucosal solutions. These bridges were connected to two calomel half-cells arranged back-to-back across the input terminals of a Vibron electrometer. The sacs were left until a stable pd was recorded (approximately 10 min after their removal from the rat). Serial additions of D-glucose, D-galactose, 3-O-methyl glucose, α-methyl glucoside or 6-deoxy-D-glucose were made to the mucosal solution bathing the sacs. The concentrations used were 2, 4, 8, 16 and 32 mM unless otherwise stated. The mucosal solutions were gassed vigourously with oxygen–carbon dioxide (95:5, v/v). The bubbles of gas flowed up and over the surface of the suspended sacs as this was found to give the lowest values for 'apparent K_m' (Ibrahim, 1978). The experiments lasted a maximum of 30 min. Details of how corrections were made for osmotic effects and of the graphical procedures used to derive the transport parameters of electrogenic active transfer (the 'apparent Michaelis constant' ('apparent K_m') and the maximum pd developed (pd_{max})) can be found in Levin & Syme (1975).

Pair-feeding experiment. Euthyroid rats and those chemically thyroidectomized of a similar weight were paired. During 1 week the quantity of food eaten by the hypothyroid rat each day was given to its euthyroid partner the next day. At the end of the week the animals were anaesthetized, their intestines removed and the electrogenic transfer of α-methyl glucoside studied in vitro.

Chemicals. The sugars and glucalogues used were all of A.R. quality; D-glucose and D-galactose were obtained from May and Baker, α-methyl glucoside from British Drug Houses Ltd., and 3-O-methyl glucose and 6-deoxy-D-glucose from Koch Light Laboratories.

Barry *et al.* (1964) showed that glucose was well metabolized by rat jejunum, galactose was poorly metabolized whilst α-methyl glucoside and 3-O-methyl glucose were not metabolized at all. Crane (1960) discussed the non-metabolizable nature of 6-deoxy-D-glucose.

Expression of results. Results are expressed as the mean values with their standard errors. Where a comparison is made between two sets of values, an unpaired Student's t test was used to establish the significance of the difference between the means. The difference was taken as significant when $P \leqslant 0{\cdot}05$. The results were also assessed by analysis of variance and the differences between groups located by the least significant range test as described by Sokal & Rohlf (1969). The details of this test have been described in a previous paper (Syme & Levin, 1977). The test was applied at the $P = 0{\cdot}05$ level.

RESULTS

The actual values for the 'apparent K_m' and pd_{max} for the two dietary sugars and three glucalogues are shown in Table 1. In Table 2 comparisons are made between different groups of animals; the numerical values are the difference between the two means in each comparison.

Effects of hypothyroidism on electrogenic transfer

Fed rats. Hypothyroidism caused significant decreases in the 'apparent K_m' values for glucose (41%) galactose (24%) and α-methyl glucoside (56%) concomitant with the decreases in their pd_{max} values, approximately 22% for glucose, 15% for galactose and 18% for α-methyl glucoside. Hypothyroidism, however, had no significant effect on the

Table 1. *The 'apparent Michaelis constant' ('apparent* K_m') *(mM) and maximum potential difference (*pd_{max}*; mV) for glucose, galactose, 3-O-methyl glucose, α-methyl glucoside and 6-deoxy-D-glucose in fed and fasted euthyroid and hypothyroid rats*

(Mean values with their standard errors; nos. of animals given in parentheses)

Sugar ...	Glucose				Galactose				3-O-Methyl Glucose				α-Methyl Glucoside				6-deoxy-D-glucose			
	Apparent K_m		pd_{max}		Apparent K_m		pd_{max}		Apparent K_m		pd_{max}		Apparent K_m		pd_{max}		Apparent K_m		pd_{max}	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Euthyroid Fed	6·09	0·49 (9)	14·36	0·44 (9)	9·91	0·83 (13)	14·52	0·64 (13)	20·43	1·22 (8)	9·15	0·57 (8)	6·79	0·49 (5)	11·76	0·38 (5)	5·92	0·48 (7)	6·84	0·50 (7)
Euthyroid Fasted	2·50	0·20 (8)	9·97	0·34 (8)	6·14	0·38 (12)	10·85	0·68 (12)	10·72	1·41 (9)	7·81	0·58 (9)	3·42	0·29 (8)	9·37	0·78 (8)	4·10	0·35 (10)	6·73	0·36 (10)
Hypothyroid Fed	3·62	0·44 (9)	11·23	0·44 (9)	7·54	0·89 (12)	12·37	0·85 (12)	17·10	2·81 (8)	8·15	1·04 (8)	3·00	0·47 (8)	9·59	0·28 (8)	4·99	0·52 (10)	7·03	0·39 (10)
Hypothyroid Fasted	1·60	0·23* (8)	10·32	0·86* (8)	3·76	0·45 (10)	9·49	0·76 (10)	13·71	1·88 (9)	10·24	0·98 (9)	3·06	0·27 (8)	9·49	0·28 (8)	3·61	0·35 (9)	6·88	0·49 (9)

* Concentration range of 0·5–8 mM.

Table 2. *Values for 'apparent Michaelis constant' ('apparent* K_m') *(mM) and maximum potential difference (*pd_{max}*; mV) for fed (F) and fasted (FA) euthyroid (E) and hypothyroid (H) rats presented in Table 1 analysed by the least significant range test (Sokal & Rohlf, 1969) which compares the computed difference with the actual difference between two particular means*

Sugar ... Group Comparison	Glucose		Galactose		3-O-Methyl glucose		α-Methyl glucoside		6-Deoxy-D-glucose	
	Apparent K_m	pd_{max}	Apparent K_m	pd_{max}	Apparent K_m	pd_{max}	Apparent K_m	pd_{max}	Apparent K_m	pd_{max}
EF *v.* HF	2·47*	3·13*	2·37*	2·15*	3·33	1·00	3·79*	2·17*	0·93	0·19
EF *v.* EFA	3·59*	4·39*	3·77*	3·67*	9·71*	1·34	3·37*	2·39*	1·82*	0·11
HF *v.* HFA	2·02*	0·91	3·78*	2·88*	3·39	2·09	0·06	0·10	1·38	0·15
EFA *v.* HFA	0·90	0·35	2·38*	1·36	2·99	2·43	0·36	0·12	0·49	0·15

* $P=0·05$.

'apparent K_m' or pd_{max} values for either 3-o-methyl glucose or 6-deoxy-D-glucose.

Fasted rats. In the fasting state hypothyroidism affected only the 'apparent K_m' for galactose (39% decrease). Remarkably, none of the parameters of electrogenic transfer for the other compounds was affected.

Effect of fasting on electrogenic transfer

Euthyroid rats. Fasting significantly reduced the 'apparent K_m' values for both dietary sugars (glucose by 54%, galactose by 38%) and for the three glucalogues (3-o-methyl glucose by 48%, α-methyl glucoside by 50% and 6-deoxy-D-glucose by 31%). In the instance of the pd_{max}, while fasting significantly decreased the value for glucose (31%), galactose (25%) and α-methyl glucoside (20%), the small decrease of 15% for 3-o-methyl glucose and the even smaller decrease of 2% for 6-deoxy-D-glucose were not significant.

Hypothyroid rats. In this condition, fasting caused a significant decrease in the 'apparent K_m' for glucose (56%), and for galactose (50%) but had no significant effect on that for 3-o-methyl glucose, α-methyl glucoside and 6-deoxy-D-glucose. It should be noted that the decrease in 'apparent K_m' for glucose was so large that a lower range of concentrations (0·5–8 mM instead of 2–32 mM) had to be used to obtain saturation kinetics. The pd_{max} for glucose showed only a small and insignificant decrease (8%) but that for galactose was significant (23%). The pd_{max} values for the glucalogues were not significantly altered.

Effect of pair feeding on electrogenic transport parameters for α-methyl glucoside

Because hypothyroidism causes a decreased food intake in rats of approximately 27% (Syme & Levin, 1976) it was important to see whether the effects of hypothyroidism on the parameters of electrogenic transport was mediated partially through this dietary action. A pair-feeding experiment was undertaken to assess the effect, if any, a reduction in food intake had on the electrogenic transfer of α-methyl glucoside. Giving the same amount of food eaten by a hypothyroid group of rats to a group of euthyroid controls had a significant effect on the electrogenic transfer of α-methyl glucoside. The 'apparent K_m' of the five, pair-fed controls ($4·5 \pm 0·46$ mM) showed a significant 30% decrease ($P < 0·05$) compared to that of the normal fed controls ($6·79 \pm 0·49$ mM). The pd_{max} was also significantly decreased by 15% ($P < 0·05$) from $11·76 \pm 0·38$ mV to $9·94 \pm 0·58$ mV.

DISCUSSION

Before the analysis and interpretation of the results it will be useful to discuss briefly two important aspects of electrical measurements of sodium-coupled hexose transfer mechanisms: (1) the relationship between the 'apparent K_m' and pd_{max} obtained from electrical studies and the 'apparent K_m' and maximum rate of substrate transfer (J_{max}) obtained from chemical measurements; (2) the mechanisms by which fasting and hypothyroidism induce changes in these parameters of electrogenic hexose transfer.

Whilst changes in the pd_{max} of hexose transfer mechanisms measured in vivo do not always correlate with changes in their J_{max} measured chemically (Debnam & Levin, 1975*a*, *b*) this divergency has not been observed in vitro. Studies under in vitro conditions (see Syme & Levin, 1976) have shown that under specific conditions changes in pd_{max} mirrored changes in J_{max}. Other workers (Hoshi *et al.* 1976) have also reported that values for transfer pd and chemical transfer are closely related as long as the conductivity of the incubating medium is maintained constant. In the instance of 'apparent K_m' it has already been shown (Syme & Levin, 1977) that for neutral amino acids the values obtained electrically agree well with those estimated from values for chemical transfer. The present study shows that

Table 3. *Comparison of the estimates of 'apparent Michaelis constant' ('apparent* K_m*') values (mM) obtained for fed and fasted euthyroid rats from chemical measurements of transfer undertaken in vitro and from electrical measurements of transfer potential differences*

Sugar or glucalogue	Type of experimental study	'Apparent K_m' Other authors: Fed	Source	Fasted	Source	Present study: Fed	Fasted
Glucose	Chemical	9	Fisher & Parsons (1953a)	—	—	—	—
		14	Hopfer (1977)	—	—	—	—
	Electrical	4	Asano (1964)	4·3	Lyon & Sheerin (1971)	6·1	2·5
		—	—	0·8	Lyon & Crane (1966)	—	—
Galactose	Chemical	1·4	Berman *et al.* (1976)	10	Barnett *et al.* (1968)	—	—
		14·3	Berman *et al.* (1976)	—	—	—	—
		35	Fisher & Parsons (1953b)	—	—	—	—
	Electrical	14	Barry & Eggenton (1972)	6·8	Lyon & Sheerin (1971)	9·9	6·1
		10	Debnam & Levin (1970)	5	Debnam & Levin (1970)	—	—
				4	Lyon & Crane (1967)	—	—
3-o-Methyl glucose	Chemical	—	—	42	Barnett *et al.* (1968)	—	—
		—	—	5·5	Ravis & Feldman (1978)	—	—
	Electrical	—	—	15·4	Lyon & Sheerin (1971)	20·4	10·7
α-Methyl glucoside	Chemical	—	—	—	—	—	—
	Electrical	—	—	—	—	6·8	3·4
6-Deoxy-D-glucose	Chemical	—	—	—	—	—	—
	Electrical	—	—	0·9	Lyon & Crane (1966)	5·9	4·1

for hexoses and the glucalogues there is reasonable agreement between the 'apparent K_m' values obtained and those published by other authors (Table 3).

Recent developments in the theoretical analysis of the kinetics of transfer mechanisms (Geck & Heinz, 1976) have made it increasingly difficult to regard changes in 'apparent K_m' as indicative of similar changes in the real K_m of the carrier. However, while such parameters must be used with caution to infer actual changes in carrier affinity they can be used to reveal whether transport mechanisms for different hexoses respond in a similar manner to dietary or hormonal changes. If all the sugars utilize a single carrier then experimental hormonal or dietary manipulations should cause common increases or decreases in 'apparent K_m' and J_{max}. If, however, these parameters respond differently for different sugars then this can be taken as presumptive evidence for multiple carriers or transfer mechanisms. Experiments employing this interpretation (Syme & Levin, 1977) revealed that neutral amino acids utilize at least three carrier systems. Confirmation of a multiple carrier system for neutral amino acids, using a chemical uptake technique, has been reported (Sepulveda & Smith, 1978).

A further complication that needs discussing is the influence of changes in J_{max} (of which pd_{max} is an index) on 'apparent K_m'. The current concept is that changes in J_{max} influence the 'apparent K_m' because of the unstirred layer of fluid that covers enterocyte transport sites (Wilson & Dietschy, 1972). Read *et al.* (1977) used a computer simulation based on the equation of Wilson & Dietschy (1972) to elucidate the influences that unstirred layer thickness and variations in J_{max} have on the 'apparent K_m' and pd_{max} for electrogenic glucose transfer. Changes in either parameter profoundly altered the 'apparent K_m' but had less effect on the pd_{max}. If pd_{max} is used as an index of J_{max} then whenever pd_{max} increases or decreases, the effects of the increased or decreased J_{max} acting via the unstirred layer would

be to increase or decrease respectively, the 'apparent K_m'. This interaction makes it difficult to interpret changes in 'apparent K_m' that are of the same direction as the changes in pd_{max} as evidence of alterations in K_m *per se*. However, when the 'apparent K_m' and pd_{max} for a transfer process do not show changes in the same direction, or one changes and one does not, then it is justified to infer that the changes observed in the 'apparent K_m' must be due to factors other than the J_{max}-unstirred layer effect. This reasoning has been applied to the changes in 'apparent K_m' and pd_{max} found in the present study in order to analyse and interpret the values for hexoses and glucalogues in hypothyroid and fasting rats (Table 4).

The results will be discussed first in terms of the advisability of using glucalogues to characterize the electrogenic hexose transfer mechanisms for the dietary sugars and second, for investigating the effects of fasting and hypothyroidism on the intestinal electrogenic transfer mechanism.

Characterization of dietary electrogenic transfer systems by using glucalogues

The results show clearly that fasting and hypothyroidism have different actions on the kinetic parameters for the electrogenic transfer of the two dietary sugars and the three glucalogues. The results in Table 4 can be analysed in two ways, vertically and horizontally. If analysed vertically one compares the effects of hypothyroidism, fasting and their combination on the 'apparent K_m' and pd_{max} for glucose with their effects on the kinetic parameters for galactose and the three glucalogues. Assuming that glucose is transported by a carrier or transfer mechanism (A) then it is obvious that the kinetic parameters for galactose behave differently from those for glucose indicating the necessity for another carrier or transfer mechanism (B). Similarly, the changes in kinetic parameters for 3-o-methyl glucose and α-methyl glucoside are different from those for glucose and galactose and from each other. Thus two further carriers (C and D respectively) need to be postulated. The behaviour of the parameters for 6-deoxy-D-glucose is identical to those of 3-o-methyl glucose therefore the carrier is C also. A minimum of four carriers are thus needed to characterize the behaviour patterns of the various 'apparent K_m' and pd_{max} values. If the information in Table 4 is analysed horizontally the assumption has to be made that if there were only one carrier or mechanism for the sugars and glucalogues then hypothyroidism (euthyroid fed *v.* hypothyroid fed), for instance, should always have the same effect on their kinetic parameters. In fact two different effects are observed, one where the 'apparent K_m' and pd_{max} decrease and one where they remain unchanged. To explain these effects two carriers (1 and 2 respectively) need to be postulated. Similarly fasting euthyroid rats has two different effects on the kinetic parameters (both decreasing, or the 'apparent K_m' decreasing with no change in the pd_{max}) two further carriers 3 and 4 must be created. By such reasoning nine differences can be identified. However, each difference may not indicate a separate carrier or transfer mechanism because the change in the 'apparent K_m' could be due to a similar change in the pd_{max}, as previously described. If those instances where both 'apparent K_m' and pd_{max} decrease together are removed then a minimum of six responses are seen. Unlike the previous analysis for the vertical columns it cannot be assumed that each of these six responses is due to a separate carrier for clearly we have studied only five sugars. The reason for the less vigourous nature of the horizontal analysis is that it is not possible to differentiate the six responses as unique unlike the situation of the vertical analysis i.e. it is not known whether the two carriers (1 and 2) assumed from the comparison of euthyroid fed *v.* hypothyroid fed are similar to or different from those (3 and 4) assumed from the comparison of euthyroid fed *v.* euthyroid fasted.

The possible roles and importance of the heterogeneity in the transfer mechanisms for hexoses and the glucalogues have been discussed by Levin (1976) who suggested that they may be examples of glucose isocarriers; the isocarrier being analogous to the isoenzyme.

Table 4. *The effects of fasting, hypothyroidism and their combination on the parameters of electrogenic sugar and glucalogue transfer ('apparent Michaelis constant' ('apparent* K_m') *and maximum potential difference* (pd_{max}))

(The Table represents a summarized version of the data presented in table 2 so as to show the number of differences needed to satisfy the range of changes)

Sugar ...	Glucose			Galactose			3-o-Methyl glucose			α-Methyl glucoside			6-Deoxy-D-glucose		
Group comparison	Apparent K_m		pd_{max}	Apparent K_m		pd_{max}	Apparent K_m		pd_{max}	Apparent K_m		pd_{max}	Apparent K_m		pd_{max}
EF *v.* HF	↓	I A	↓	↓	I B	↓	o	2 C	o	↓	I D	↓	o	2 C	o
EF *v.* EFA	↓	3 A	↓	↓	3 B	↓	↓	4 C	o	↓	3 D	↓	↓	4 C	o
HF *v.* HFA	↓	5 A	o	↓	6 B	↓	o	7 C	o	o	7 D	o	o	7 C	o
EFA *v.* HFA	o	8 A	o	↓	9 B	o	o	8 C	o	o	8 D	o	o	8 C	o

E, euthyroid; H, hypothyroid; F, fed; FA, fasted; ↓, significant decreases; o, insignificant change; 1–9, no. of responses from horizontal analysis; A, B, C, D, no. of mechanisms from vertical analysis.

To summarize, the most important conclusions from the results are: (1) that there is further evidence of a heterogeneity in the response of the jejunal electrogenic transfer mechanisms for hexoses and glucalogues to dietary and hormonal manipulations; (2) that the patterns of change in the parameters of electrogenic jejunal transfer for dietary sugars cannot always be obtained by using a glucalogue; (3) that a minimum of four (but possibly up to six) electrogenic transfer mechanisms (or 'carriers') is needed to explain the observed changes in fasting, hypothyroidism and their combination; (4) studies that employ a glucalogue to assess absorption following dietary or hormonal alterations cannot assume that the change observed will be true for glucose and galactose, especially if 3-o-methyl glucose and 6-deoxy-D-glucose are used. In our conditions α-methyl glucoside appeared to be the best glucalogue to use, but even it did not match exactly the changes for galactose or glucose.

Effects of fasting and hypothyroidism on sugar and glucalogue electrogenic transfer parameters

The results can also be used to assess the influence of fasting and hypothyroidism and their combination on sugar and glucalogue electrogenic transfer. In the instance of fasting (Table 4) the results extend the in vitro and in vivo studies of Debnam & Levin (1975*b*, 1976) who showed that in euthyroid rats the 'apparent K_m' and maximum velocity (measured chemically) fell for glucose, galactose and α-methyl glucoside. The present study shows that a similar decrease in 'apparent K_m' occurs for 3-o-methyl glucose and 6-deoxy-D-glucose and since the pd_{max} did not decrease, these reductions in 'apparent K_m' on fasting are probably indicative of a real change.

It is interesting to note that Axelrad *et al.* (1970) have published results that can be interpreted to indicate that the jejunum from fasted rats transfers, in vitro, more 3-o-methyl glucose into the serosal solution than fed jejunum. This does not correlate well with our finding of an insignificant decrease in pd_{max} observed with 3-o-methyl glucose during fasting. However, Axelrad *et al.* (1970) incubated their everted sacs without any metabolizable substrate for 90 min. As the metabolism of fasted jejunum is greatly reduced compared to fed jejunum (Levin *et al.* 1965) it is possible that intestine from fed animals deteriorates much more quickly in vitro than that from fasted animals. Hence with no metabolic substrate and an excessively long incubation period, fasted gut probably survives longer and transfers better than fed gut. Clearly in the light of our findings it is necessary to re-assess the effects of fasting on 3-o-methyl glucose and 6-deoxy-D-glucose transfer in vitro using shorter incubations with metabolizable substrate supplied from the serosal side as used in our conditions.

The effects of hypothyroidism on transfer are complex especially as the condition causes a decrease in food intake of approximately 27% and the pair-feeding experiment showed that such a reduction in food intake *per se* caused changes in 'apparent K_m' and pd_{max} for α-methyl glucoside. This makes it very difficult to assess whether the changes observed arise from the primary action of the lack of thyroid hormones or from a secondary effect of the decreased food intake or from both. Previous studies (Halliday *et al.* 1962; Bronk & Parsons, 1964, 1965; Gelb & Chalfin, 1967) have not discussed this complication. In the fed state hypothyroidism caused decreases in the 'apparent K_m' for glucose, galactose and α-methyl glucoside concomitant with decreases in their pd_{max}. However, in the case of 3-o-methyl glucose and 6-deoxy-D-glucose (euthyroid fed *v.* hypothyroid fed, Table 4) neither parameter changed. To try and avoid the effects of food intake we studied the action of hypothyroidism in fasted animals (Table 4). Quite different changes were now found; only in the instance of galactose was a significant effect on the electrogenic parameters observed, its 'apparent K_m' was decreased. The influence of fasting on the effects of hypothyroidism can be observed by contrasting hypothyroid fed *v.* hypothyroid fasted with

euthyroid fed *v.* hypothyroid fed in Table 4. Fasting appears to have prevented the decrease in pd_{max} for glucose and the decrease in 'apparent K_m' and pd_{max} for α-methyl glucoside. It is interesting in this context to note that Edmonds *et al.* (1970) reported that fasting inhibited the effect of tri-iodothyronine on the action of aldosterone on the colon pd. Unfortunately, whilst the effect of hypothyroidism in fasted animals obviates the effects of decreased food intake, it is not known whether enterocytes from fasted animals respond to thyroid lack in the same way as those from fed animals. Thus at present, it is not possible in hypothyroidism to separate dietary-induced changes of 'apparent K_m' and pd_{max} from those created by the lack of thyroid hormones *per se*.

REFERENCES

Asano, T. (1964). *Am. J. Physiol.* **207**, 415.
Axelrad, A. D., Lawrence, A. L. & Hazelwood, R. L. (1970). *Am. J. Physiol.* **219**, 860.
Barnett, J. E. G., Jarvis, W. T. S., Munday, K. A. (1968). *Biochem. J.* **109**, 61.
Barry, R. J. C., Dikstein, S., Matthews, J., Smyth, D. H. & Wright, E. M. (1964). *J. Physiol., Lond.* **171**, 316.
Barry, R. J. C. & Eggenton, J. (1972). *J. Physiol., Lond.* **227**, 201.
Berman, W. F., Bautista, J. O., Rogers, S. & Segal, S. (1976). *Biochim. biophys. Acta* **455**, 90.
Bronk, J. R. & Parsons, D. S. (1964). *Nature, Lond.* **201**, 712.
Bronk, J. R. & Parsons, D. S. (1965). *J. Physiol., Lond.* **179**, 323.
Crane, R. K. (1960). *Physiol. Rev.* **40**, 789.
Debnam, E. S. & Levin, R. J. (1970). *J. Physiol., Lond.* **209**, 29*P*.
Debnam, E. S. & Levin, R. J. (1975a). *J. Physiol., Lond.* **246**, 181.
Debnam, E. S. & Levin, R. J. (1975b). *J. Physiol., Lond.* **252**, 681.
Debnam, E. S. & Levin, R. J. (1976). *Gut* **17**, 92.
Edmonds, C. J., Thompson, B. D. & Marriot, J. (1970). *J. Endocr.* **48**, 189.
Fisher, R. B. & Parsons, D. S. (1953*a*). *J. Physiol., Lond.* **119**, 210.
Fisher, R. B. & Parsons, D. S. (1953*b*). *J. Physiol., Lond.* **119**, 224.
Geck, P. & Heinz, E. (1976). *Biochim. biophys. Acta* **443**, 49.
Gelb, A. M. & Chalfin, D. (1967). *Clin. Res.* **15**, 232 (Abstr.).
Halliday, G. J., Howard, R. B. & Munro, A. F. (1962). *J. Physiol., Lond.* **164**, 28*P*.
Honegger, P. (1973). In *Comparative Physiology*, p. 441 [L. Bolis, K. Schmidt-Nielsen and S. H. P. Maddrell, editors]. Amsterdam: North-Holland Publishing Company.
Honegger, P. & Gershon, E. (1974). *Biochim. biophys. Acta* **352**, 127.
Honegger, P. & Semenza, G. (1973). *Biochim. biophys. Acta* **318**, 390.
Hopfer, U. (1977). *Am. J. Physiol.* **233**, E445.
Hoshi, T., Suziki, Y., Kusaki, T. & Igarashi, Y. (1976). *Tohoku J. exp. Med.* **119**, 201.
Ibrahim, K. M. (1978). A study on the effect of unstirred layers and of glycocholate on electrogenic sugar transfer by rat jejunum. MSc Thesis, University of Sheffield.
Krebs, H. A. & Henseleit, K. (1932). *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33.
Levin, R. J. (1976). In *Digestion in the Fowl*, p. 63 [K. N. Boorman and B. M. Freeman, editors]. Edinburgh: British Poultry Science Ltd.
Levin, R. J., Newey, H. & Smyth, D. H. (1965). *J. Physiol., Lond.* **177**, 58.
Levin, R. J. & Syme, G. (1971). *J. Physiol., Lond.* **213**, 46*P*.
Levin, R. J. & Syme, G. (1975). *J. Physiol., Lond.* **245**, 271.
Lyon, I. & Crane, R. K. (1966). *Biochim. biophys. Acta* **112**, 278.
Lyon, I. & Crane, R. K. (1967). *Biochim. biophys. Acta* **135**, 61.
Lyon, I. & Sheerin, H. E. (1971). *Biochim. biophys. Acta* **249**, 1.
Ravis, W. R. & Feldman, S. (1978). *J. Pharmac. Sci.* **67**, 245.
Read, N. W., Barber, D. C., Levin, R. J. & Holdsworth, C. D. (1977). *Gut* **18**, 865.
Reiser, S., Michaelis, O., Putney, J. & Hallfrisch, J. (1975). *J. Nutr.* **105**, 894.
Sepulveda, F. V. & Smith, M. W. (1978). *J. Physiol., Lond.* **282**, 73.
Sokal, R. R. & Rohlf, F. J. (1969). In *Biometry, the Principles and Practice of Statistics in Biological Research*, p. 224. San Francisco: W. H. Freeman & Co.
Syme, G. & Levin, R. J. (1976). *Proc. R. Soc.* B **194**, 121.
Syme, G. & Levin, R. J. (1977). *Biochim. biophys. Acta* **464**, 620.
Wilson, F. W. & Dietschy, J. M. (1972). *J. clin. Invest.* **51**, 3015.
Wiseman G. (1977). *J. Physiol., Lond.* **273**, 731.

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