Faecal excretion of degraded and native carrageenan by the young rat

Hawkins & Yaphe (1965) have shown that when native, undegraded carrageenan is fed to young rats, at levels of 2 to 20% in the diet, it is 90–100% excreted in the faeces. No comparable results appear to be available for degraded carrageenan in the 16 000–19 000 number-average molecular weight range, and this report deals with the faecal excretion of both degraded and native iota-carrageenan by the young rat at a concentration of 5% in the diet. We have recently shown (Beattie, Blakemore & others, 1970) that when adult baboons were given a single large dose of 240 mg/kg of degraded carrageenan, which is more than three times the human dose in peptic ulcer treatment, no urinary metachromasia could be detected.

Carrageenans. The degraded carrageenan (code no. C16/L1927) was prepared from *Eucheuma spinosum* seaweed by Laboratoires Glaxo, Paris, and the native iota-carrageenan was a commercial product from Société Auby, Neuilly-sur-Seine, France. The analyses on the anhydrous basis are given in Table 1. The number-average molecular weight of C16 was in the 16 000 to 19 000 range (Blakemore & Dewar, 1970) and the weight-average molecular weight (by light scattering) was between 20 000 and 30 000 (Anderson & Soman, 1966). The molecular weight of the native carrageenan was unknown, but the inherent viscosity (η_{inh} in 0·1M NaCl solution) was about 18 times that of the degraded material.

Table 1. *Analyses of carrageenans*

Carrageenan	Inherent viscosity (η inh) (dl/g)	$[\alpha]_D$ in water	Total sulphate (SO_3 Na), %	Sulphated ash, %	3,6-Anhydro-galactose ($C_6H_8O_4$), %
Cl6 degraded	0·41	+46·8°	37·9	29·7	18·1
Native	7·43	40	34·8	30·4	21·9

Animal experiments. Fifteen young male albino rats (Charles River C.D. strain) about 115 g, were randomly allocated to three treatment groups: 5% native carrageenan in the diet, 5% degraded carrageenan in the diet, and control diet without carrageenan. Spillers Laboratory Small Animal Diet (SG1/41B) in powdered form was mixed with the powdered degraded or native carrageenan, and 10 g balls were prepared by mixing with a known quantity of water and then drying at less than 60° to remove the water.

The animals were caged singly in suspended metal cages with a wire mesh floor through which scattered food and excreta could fall on to absorbent material beneath. Scatter of food or of faecal pellets between cages was prevented by placing folded sheets of blotting paper beneath each cage. Two balls (20 g) of each diet were placed in the food hopper daily as the sole source of food and the animals had water freely. After each 24 h, food consumption was measured. The feeding experiment lasted ten days, and on the tenth day, the 24 h faecal samples from each animal were collected, dried in a vacuum over phosphoric oxide to constant weight, and analysed for 3,6-anhydro-galactose (Black, Blakemore & others, 1965). The carrageenan content was calculated from this result after correcting for a mean "3,6-anhydrogalactose" value for the control faeces. The validity of the method was confirmed for 2 animals by estimation of total ester sulphate (Black & others, 1965).

The food intakes (Table 2) for the first seven and last three days and the weight gains for each animal in the three groups, indicated that there were no meaningful differences between animals receiving native and degraded carrageenan, although the mean weight

gain of the control group was significantly higher than that of the test groups. The mean daily consumption of each rat over the last three days was taken as the mean food intake over the 24 h period of faecal collection. Again (Table 3), there were no significant differences in any parameters between animals receiving native carrageenan and those receiving degraded carrageenan. The presence of carrageenan in the diet was readily detected by the increased weight of faeces compared to that of the control group. The faecal excretions of both native and degraded carrageenans were less than 100%, and the low figures may be partly attributed to small errors in measuring the

Table 2. *Food intake and weight gain in groups of young rats fed native and degraded carrageenan in the diet*

Animal no.	Treatment group	Initial wt (g)	Final wt (g)	Over 10 day period on test: Weight gain (g)	Food intake (g/day) over 7 days	Food intake (g/day) over 3 days
1	5% Native carrageenan	118	177	59	17·7	18·7
3		105	167	62	16·0	16·9
6		110	178	68	16·3	19·3
13		107	157	50	14·5	14·9
15		117	180	63	16·9	19·5
Mean		111·4	171·8	60·4	16·3	17·9
± s.e.*		2·6	4·3	3·0	0·5	0·9
4	5% Degraded carrageenan	106	175	69	18·2	18·9
7		114	174	60	18·3	19·1
10		118	185	67	18·0	19·1
12		120	192	72	19·0	19·8
14		126	162	36	18·6	19·4
Mean		116·8	177·6	60·8	18·4	19·3
± s.e.*		3·3	5·1	6·5	0·2	0·2
2	Control	112	196	84	16·9	19·3
5		112	190	78	15·5	16·8
8		127	196	69	16·5	17·2
9		117	198	81	15·6	19·9
11		120	201	81	16·3	17·2
Mean		117·6	196·2	78·6	16·2	18·1
± s.e.*		2·8	1·8	2·6	0·3	0·6

* s.e. = standard error

Table 3. *Analyses of faeces and excretion of carrageenans over 24 h.*

Animal no.	Treatment group	Mean food intake (g/24 h)	Dry faeces (g)	Faeces, % of food intake	Carrageenan in faeces %	Carrageenan excretion %	Excretion range %	Mean excretion %
1	5% native carrageenan in diet	18·7	4·900	26·2	16·7	88		
3		16·8	4·631	27·6	15·5	85		
6		19·3	5·366	27·8	13·9	77	68–88	80
13		14·9	4·545	30·5	13·4	82		±3·5 (s.e.)
15		19·5	4·785	24·5	13·9	68		
4	5% degraded carrageenan in diet	18·9	4·375	23·1	17·8	82		
7		19·0	4·513	23·8	18·4	88		
10		19·1	5·054	26·4	17·9	95	82–95	87
12		19·8	4·398	22·2	18·4	82		±2·4 (s.e.)
14		19·4	4·362	22·5	19·4	87		
2	Control: rat cake only in diet	19·3	3·145	16·3				
5		16·8	2·672	15·9				
8		17·2	2·420	14·1				
9		19·9	4·183	21·0				
11		17·2	2·815	16·3				

daily food intake and to assessing accurately the "3,6-anhydrogalactose" figure for the control faeces blank (0·37% as $C_6H_8O_4$).

The above results show that, when native and partially degraded iota-carrageenans were fed to young rats, at a concentration of 5% in the diet, there were no significant differences in the faecal excretion of the two polymers and the weight gained by the animals.

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Correlation of urinary histamine excretion and 24 h urine volumes in rats and man

Urinary output of free histamine has frequently been used as an indicator of the level of whole body histamine metabolism (Johnston & Kahlson, 1967). The reliability of this measurement has been discussed in relation to the origin of urinary histamine and the very small fraction it represents of the total histamine metabolites (Lindell & Westling, 1966).

We report here a correlation of urinary histamine excretion and the 24 h urine volume in rats and man. This correlation has been the subject of frequent fruitless speculation, being considered unlikely by Anrep (1944) and Duner & Pernow (1956).

Female albino rats, either Wistar (SNR strain) or Sprague Dawley (Charles River strain), 150–250 g, were allowed free access to food and water, the diet being made by mixing Dixon's 41B meal with egg white, casting into sticks and baking. The rats were housed in glass metabolism cages ('Metabowl', Jencon's), and the 24 h urine output collected into 1 ml of 3N hydrochloric acid. Histamine was estimated by the fluorometric method of Oates, Marsh & Sjoerdsma (1962). Successive daily collections of 24 h urines were made. Rats No. 8–11 had 0·1N ammonium chloride solution instead of drinking water, and in addition, on days 4–6 of the experiment, rats 8–11 received 200 mg/kg of histidine hydrochloride subcutaneously.

In 9 of 11 rats there was a good correlation of histamine content and 24 h urine volume (Table 1).

Treatment with ammonium chloride gave a urine of low pH, which would be expected to facilitate the excretion of histamine, but the correlation of histamine excretion and urine volume was not disturbed. Treatment with L-histidine also did not affect the correlation.