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Research Section

A Study of Orally-administered Degraded Carrageenan in the Baboon

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Abstract—No urinary metachromasia was observed when adult baboons were given degraded carrageenan (number-average mol wt 16,000–19,000) in a single dose of 240 mg/kg, which is more than three times the therapeutic dose in man. Even at a dose level of 3 g/kg, more than forty times the therapeutic dose, only 3–6 mg/kg were excreted in the urine over 24 hr, and carrageenan could not be detected in the blood up to 18 hr after dosing, despite the fact that the limit of detection was 5 μg/ml of blood. Urinary metachromasia was determined quantitatively by a spectrophotometric method, and the sulphated polysaccharides responsible for the metachromatic colour were isolated and shown to contain a high proportion of carrageenan, which was characterized by specific rotation, content of ester sulphate and 3,6-anhydrogalactose, infra-red analysis, and paper chromatography of the hydrolysis products. Methods have been developed for the isolation and characterization of degraded carrageenan added *in vitro* to blood, down to a concentration of 100 μg/ml of blood, and for detecting metachromasia down to 5 μg/ml. In the studies undertaken, no detectable amounts of degraded carrageenan were found in the urine of the baboons given three times the therapeutic dose and only very small quantities were detected in specimens of 24-hr urine from animals given the massive dose of 3 g/kg. These results indicate that absorption from the gastro-intestinal tract is unlikely to occur at the levels of degraded carrageenan used therapeutically.

INTRODUCTION

The mechanism of the protective effect of sulphated polysaccharides on experimental ulceration in animals and on peptic ulceration in man is still not definitely understood. Anderson & Soman (1966) showed that oral administration of massive doses (750 mg–4·5 g/kg) of degraded carrageenan to guinea-pigs resulted in detectable urinary metachromasia, suggesting limited gastro-intestinal absorption and excretion of carrageenan.

Eagleton, Watt & Marcus (1968 & 1969) reported that a single subcutaneous injection (400 mg/kg) of degraded carrageenan in guinea-pigs reduced both the volume and the total acid concentration of the gastric juice induced by administration of histamine, and this effect was accompanied by a marked reduction in the incidence and severity of duodenal ulceration. These workers concluded that this inhibition of acid gastric secretion by systemic carrageenan is a major factor in preventing histamine-induced duodenal damage in the guinea-pig.

The object of the present study was to determine whether degraded carrageenan administered orally to the baboon at levels approximating to the human therapeutic level (70 mg/kg) was absorbed from the gut and excreted in the urine.

EXPERIMENTAL

Materials. The degraded carrageenan was prepared from *Eucheuma spinosum* by Laboratoires Glaxo, Paris, (code no. C16/L1927) and gave the following analysis on an anhydrous basis: Total ester sulphate, 37·9% (calculated as SO_3Na); 3,6-anhydrogalactose, 18·1% (as $C_6H_8O_4$); specific rotation ($[\alpha]_D$), + 46·8° (*c* 1·05 g/dl in water); inherent viscosity (η_{inh}), 0·41 dl/g in 0·1 M-sodium chloride solution (*c* 0·21 g/dl, 25°C). The number-average mol wt (M_n) was in the 16,000–19,000 range (W. R. Blakemore & E. T. Dewar, unpublished results), and the weight-average mol wt (M_w, by light-scattering) was 20,000–30,000 (Anderson & Soman, 1966). The material was non-dialysable in Visking dialysis tubing.

Chondroitin sulphate A (chondroitin 4-[potassium sulphate]) and chondroitin sulphate C (chondroitin 6-[potassium sulphate]), prepared by Professor K. S. Dodgson from ox and shark cartilage respectively, were kindly supplied by Dr. D. A. Rees.

Toluidine blue was a George T. Gurr Ltd., London, reagent. A stock solution of this was prepared by dissolving 0·1 g in methanol (70 ml) and diluting to 100 ml with water. A standard solution was prepared by diluting 10 ml of the stock solution to 100 ml with water to give a solution of 0·01% toluidine blue.

Cetyltrimethylammonium bromide (CTAB) was supplied by BDH Chemicals Ltd., Poole, Dorset.

Whole ox blood, obtained from the slaughterhouse in Edinburgh, was citrated (9:1, v/v) with 3·8% (w/v) sodium citrate solution and stored at 0°C.

Trichloroacetic acid "for protein precipitation" was obtained from BDH Chemicals Ltd., Poole, Dorset.

General experimental methods. Infra-red spectra of polysaccharides were measured in potassium chloride discs with Perkin–Elmer 137 and 257 spectrophotometers.

Estimations of ester sulphate and 3,6-anhydrogalactose were carried out as previously described (Black, Blakemore, Colquhoun & Dewar, 1965). The sulphate content of the carrageenans B1 and B2 isolated from urine was determined with 4-chloro-4′-aminodiphenyl reagent (Jones & Letham, 1954), the absorbance being measured at 254 nm in a Uvispek spectrophotometer using 1-cm cells.

Concentration of solutions was carried out in all cases under reduced pressure below 40°C. Samples were dried at 20°C/0·01 mm over phosphoric oxide and all weights are on an anhydrous basis.

Hydrolysis of polysaccharides was carried out with 50% formic acid at 100°C for 18 hr and was followed by paper chromatography using *n*-butanol/ethanol/water (40:11:19, by vol.) as solvent on Whatman no. 1 paper. Sugars were detected with aniline hydrogen phthalate solution (Wilson, 1959) or with silver nitrate–sodium hydroxide reagents (Hough & Jones, 1962).

Animals

Urinary excretion. In the urine experiments, three mature male baboons (*Papio cynocephalus*), weighing 16–18 kg, were starved overnight, sedated with phencyclidine hydrochloride (Sernylan, Parke-Davis, Hounslow, Middx) given in an intramuscular dose of 1 mg/kg, and restrained in a primate chair. A narrow polyethylene sleeve was strapped on to the penis with elastic tape. A piece of sterile polyethylene tubing, adjusted to allow a free flow of urine, was connected to a "Portex" urine-collection bag. The degraded carrageenan, in aqueous solution (100 ml) at 37°C, was given as a single dose by stomach tube. The

animals were restrained in the primate chairs for 24 hr after dosing, during which period they were allowed drinking water *ad lib.* but no food. After the initial sedation, the animals were given further small doses of phencyclidine where necessary to provide continuous light sedation. The 24-hr specimens were analysed immediately, as described below. Control samples of urine were obtained under the same conditions from each animal before treatment with carrageenan. Baboon no. 1 received both the high (3 g/kg) and low (240 mg/kg) dose levels with a 14-day rest period between doses. Baboon no. 2 received only the large dose and no. 3 only the small dose.

Blood level determinations. One male baboon, weighing 6·2 kg, was used for all experiments including the control. The animal was starved overnight and sedated with an intramuscular dose (1 mg/kg) of phencyclidine hydrochloride (Sernylan) and the carrageenan (18·6 g in 40 ml water) was given in a single dose by stomach tube. During the period of the experiments, the animal was allowed drinking water *ad lib.* but no food. A 7-day rest period was allowed between experiments. In one experiment, a 20-ml sample of blood was withdrawn from the femoral vein into a citrated syringe after 3, 6 and 9 hr. In a second experiment, samples were withdrawn at 12, 15 and 18 hr after dosing. The samples were analysed as described below.

Biochemical determinations

Qualitative metachromasia test. A small aliquot of the fresh urine specimen was acidified to pH 4–5 with 2 N-acetic acid, filtered through a sintered glass crucible (porosity 4), and the filtrate (1 ml) was diluted with water (9 ml) and 0·01% toluidine blue solution (1 ml) was added from a grade 'A' pipette. The absorbance was measured against a water blank at 630 nm in a Uvispek Spectrophotometer using 1 cm cells. A control, in which water (1 ml) replaced the urine, gave an absorbance of 0·71. A test reading of 0·71 ± 0·01 was taken as negative, while an absorbance of less than 0·70 was taken as positive. The test will detect less than 5 μg carrageenan/ml urine.

Quantitative estimation of degraded carrageenan in baboon urine. This colorimetric method is essentially the same as that developed to follow the urinary excretion of sodium laminaran sulphate after injection into experimental animals (Dewar, 1955). A calibration curve was drawn up by treating 10 ml standard solutions, containing 0–100 μg degraded carrageenan, with exactly 1 ml 0·01% toluidine blue solution and measuring the absorbances at 630 nm in a Uvispek Spectrophotometer using 1-cm cells. The following results were obtained:

Carrageenan (μg):	0	10	20	30	40	50	100
Absorbance:	0·706	0·608	0·502	0·401	0·292	0·190	0·168

The graph of carrageenan concentration against absorbance was a straight line over the range 0–50 μg.

Immediately after the qualitative test for metachromasia was carried out, the urine sample was concentrated to dryness at 35°C in a rotary film evaporator, dried at 20°C/0·01 mm over phosphoric oxide, and the dry urinary solids were stored in the deep-freeze at −20°C and subsequently analysed and processed for the isolation of carrageenan.

For the quantitative estimation of carrageenan, the dry urine sample (*c.* 250 mg) was dissolved in water, the pH was reduced to 5 with a few drops of 2 N-acetic acid, and the solution was made up to 100 ml with water. A 10-ml aliquot of this solution A was treated

with 1 ml 0·01% toluidine blue solution and the absorbance was measured as described above. The carrageenan content was then read off from the calibration curve. Various 10-ml solutions were then prepared by taking aliquots of 1–9 ml solution A and diluting to 10 ml with water, and the absorbances of each diluted solution were measured after the addition of toluidine blue (1 ml). The effect of dilution on carrageenan concentration is shown for the urinary solids (259 mg) from baboon no. 2:

Volume of solution A (%):	10	20	30	40	50	60	70	80	90	100
Carrageenan (μg):	60	65	64	64	58·5	55	49	45	41	38

A graph of volume % against carrageenan concentration was plotted, and the maximum concentration was found to be 65 μg, which was taken as the carrageenan content in 10 ml of solution A. Hence solution A contains 650 μg carrageenan in 100 ml and therefore the carrageenan content of urinary solids equals 0·65 × 100/259 or 0·25%. Because of this dilution effect, which was due to some interference with colour development from urinary constituents, it was necessary to plot the graph of volume of solution A against carrageenan concentration for each estimation and the maximum reading was taken in each case.

Isolation of polysaccharide sulphate B1 from baboon no. 1 dry urinary solids. The dry solids (5·45 g) from baboon no. 1, dosed at the 3 g/kg level, were dissolved in water (150 ml), and the solution was dialysed in Visking tubing against running tap water for 20 hr and then concentrated to dryness at 35°C.

The resultant brown glass (380 mg) was taken up in water (10 ml), the insoluble residue was centrifuged and washed with water (2 × 10 ml), and the clear supernatant and washings were stirred with Amberlite resin IR-120-H (20 ml) for 1 hr to remove all metal ions. After filtration, the resin was washed with water (4 × 30 ml) and the acidic filtrate and washings were neutralized to pH 6 with 0·1 N-lithium hydroxide (11·3 ml). The neutral solution was concentrated to a glass (240 mg). This was redissolved in water (5 ml) and acetone (25 ml) was added. Lithium chloride (240 mg) was added to the colloidal solution to coagulate the precipitate, which was then centrifuged, washed with acetone (2 × 30 ml) and ether (30 ml), and isolated as a light-brown powder (154 mg).

The powder was dissolved in water (10 ml), 20% (w/v) CTAB solution was added dropwise until precipitation was complete, and the polysaccharide sulphate-CTAB complex was centrifuged and washed with water (2 × 10 ml). The complex was dispersed in saturated potassium chloride solution (5 ml), and after 1 hr the insoluble residue was centrifuged and washed with potassium chloride solution (5 ml). The supernatant and washings were treated with ethanol (10 ml), and the copious white precipitate was centrifuged, washed with ethanol (2 × 10 ml) and redissolved in water (40 ml). After dialysis against tap water for 20 hr, the solution was concentrated to dryness, the glass (24 mg) was redissolved in water (5 ml), and acetone (30 ml) was added to give a colloidal solution. The colloid coagulated on the addition of one drop of saturated potassium chloride solution, and the precipitate was centrifuged, washed with acetone (30 ml) and ether (30 ml), and dried at 20°C/0·01 mm over phosphoric oxide to give carrageenan B1 as a cream-coloured powder (21·1 mg, 0·39% of dry urine).

In the same way, dry solids (3·05 g) from baboon no. 2 after dosing at the 3 g/kg level yielded carrageenan B2 (6·6 mg, 0·22%).

These carrageenans were analysed for ester sulphate and 3,6-anhydrogalactose content and characterized by their specific rotation and infra-red spectra and by paper chromatography of their hydrolysis products.

Isolation and characterization of carrageenan from ox blood (in vitro). Degraded carrageenan (50·2 mg) in water (5 ml) was added to citrated ox blood (55·5 ml, containing 50 ml blood), followed by supersaturated potassium chloride solution (500 ml), with stirring, and the mixture was stirred overnight. Trichloroacetic acid (10%, w/v) in saturated potassium chloride solution (150 ml) was added slowly and the mixture was stirred for a further 4 hr. The precipitate of blood proteins was centrifuged and washed with 3% (w/v) trichloroacetic acid in saturated potassium chloride solution (2 × 250 ml), and the clear supernatant and washings were neutralized to pH 8 with solid sodium hydrogen carbonate. After 3 days' dialysis against running tap-water, the solution was concentrated to dryness. The yellow solid (1·48 g) was redissolved in water (40 ml) and centrifuged, and 20% (w/v) CTAB was added to the supernatant until precipitation was complete. The complex was centrifuged, washed with water (2 × 40 ml), dissolved in saturated potassium chloride solution (10 ml) and reprecipitated with ethanol (10 ml). The polysaccharide sulphate–potassium chloride precipitate was centrifuged, washed with ethanol (2 × 40 ml), dissolved in water (40 ml) and dialysed overnight against running tap-water to remove potassium chloride. The dialysed solution A was concentrated to 5 ml, acetone (30 ml) was added, and the colloid was coagulated with one drop of saturated potassium chloride solution. The final precipitate, containing the carrageenan, was centrifuged, washed with acetone (40 ml) and ether (40 ml), and dried at 20°C/0·01 mm over phosphoric oxide to a powder (37·8 mg; 75·3%, based on weight of carrageenan). Found: 3,6-anhydrogalactose, 15·1%; ν_{max} (cm^{-1}), *inter alia*, 940–930 (3,6-anhydrogalactose), 850–840 (axial sulphate), 810–800 (3,6-anhydrogalactose 2-sulphate).

In a control experiment, 50 ml blood gave no precipitate with CTAB in the absence of degraded carrageenan. A similar control of 50 ml baboon blood also gave no precipitate.

Isolation and estimation of carrageenan added to blood in low concentrations. When the concentration of added carrageenan in the blood was reduced below 10 mg/100 ml, the quantity of carrageenan recovered by the above procedure was too small (<3 mg) for identification by chemical and infra-red analysis. Consequently, the final dialysed solution A containing the carrageenan (see above) was concentrated to dryness, the residue was redissolved in a suitable volume of 0·1 N-hydrochloric acid and the sulphated polysaccharide content was estimated with toluidine blue using the method of MacIntosh (1941).

The *in vitro* determinations of the baboon blood levels were carried out similarly except that 20 ml volumes of blood were used instead of 50 ml.

RESULTS AND DISCUSSION

Urinary excretion

The qualitative test of the 24-hr urine specimen from two baboons given 3 g degraded carrageenan/kg showed the presence of sulphated polysaccharides in both specimens. Urinary metachromasia was then determined quantitatively and the results are shown in Table 1, which also includes details of the two experiments carried out with the much lower dose level (240 mg/kg).

Thus at the 3 g/kg level, the urinary excretion of carrageenan amounted to 0·19% of the total oral dose (50·6 g) in baboon no. 1 and 0·1% in baboon no. 2, i.e. between 3 and 6 mg/kg body weight. This is an exceedingly small amount, when one considers that this dose level is more than forty times the human therapeutic dose (*c.* 70 mg/kg). When the degraded carrageenan was given at a dose level of 240 mg/kg, about 3–4 times the therapeutic dose, no

Table 1. *Study of urinary excretion of carrageenan in the baboon*

Baboon no.	Baboon weight (kg)	Total carrageenan dose (g)	Dose level (g/kg)	Total volume of urine (ml)	Qualitative metachromasia reaction	Urinary solids (g)	Carrageenan in dry solids (%)	Concn in urine (μg/ml)
1	16·5	50·6	3·1	570	Positive	21·8	0·43	168
2	15·7	50·6	3·2	875	Positive	20·9	0·25	60
1	15·8	3·8	0·24	410	Negative			
3	18·3	4·2	0·23	1075	Negative			

Table 2. *Analyses of carrageenans B1 and B2*

Polysaccharide sulphate	Yield (% of dry urine)	Carrageenan content (%)	$[\alpha]_D$ (*c.* 0·5 in H_2O)	SO_3Na (%)	3,6-Anhydrogalactose (%)	Important bands in infra-red spectrum (cm^{-1})	Sugars on hydrolysis
B1	0·39	88	+ 34±4°	26·4	9·6	940–930 850–840 810–800	Gal, GA R_{gal} 1·3 R_{gal} 1·5
B2	0·22	87	—	33·0	10·8	940–930 850–840 810–800	Gal, GA R_{gal} 1·3 R_{gal} 1·5

Gal = galactose; GA = glucuronic acid; R_{gal} = distance travelled on chromatogram relative to galactose.

urinary metachromasia was detected, although the toluidine blue test will detect less than 5 μg carrageenan/ml urine.

The polysaccharide sulphate responsible for the metachromatic colour was isolated from both samples of urine at the 3 g/kg dose level and found to contain a high proportion of carrageenan (Table 2). The yields of both sulphated polysaccharides were in good agreement with those determined colorimetrically (Table 1). The specific rotation of B1 was less than that (+47°) of the original carrageenan but was still clearly positive, in marked contrast to the negative rotations of chondroitin sulphates A, B and C (Brimacombe & Webber, 1964). The quantity of B2 isolated was too small for the measurement of optical rotation. The ester sulphate content of B1 was less than that (37·9%) of the original carrageenan, indicating some contamination, although the sulphate content of B2 was appreciably higher; chondroitin sulphates A, B and C (as their dipotassium salts) have a theoretical sulphate (as SO_3Na) of only 19·3%.

The 3,6-anhydrogalactose contents of B1 and B2 indicate that the polysaccharide sulphates contain 53–60% of carrageenan. The presence of 3,6-anhydrogalactose in B1 and B2 is definite evidence of carrageenan, for it was found that colour formation with chondroitin sulphate A (and presumably other mucopolysaccharides) was negligible in this test.

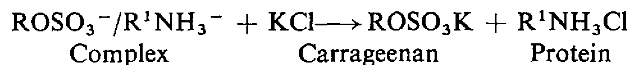
Further evidence of carrageenan was obtained from the infra-red spectra. The spectra of B1 and B2 (Table 2) were characteristic of carrageenan from *E. spinosum* (Black *et al.* 1965) with peaks at 940–930, 850–840, and 810–800 cm^{-1}, the last being due to 3,6-anhydrogalactose 2-sulphate (Anderson, Dolan, Penman, Rees, Mueller, Stancioff & Stanley, 1968). Chondroitin sulphate A showed bands at 940–930 and 860–850 cm^{-1} (axial sulphate), but gave no absorption at 810–800 cm^{-1}. Chondroitin sulphate C absorbs at 820 (equatorial sulphate) but not at 810–800 cm^{-1}.

Hydrolysis of the two polysaccharides with 50% formic acid and subsequent paper chromatography showed the main monosaccharide component to be galactose, which is absent from hyaluronic acid, chondroitin sulphate and heparin. Several other components, however, were also present, notably a sugar with R_{gal} 1·5 and another with R_{gal} 1·3, which is probably 2-acetamido-2-deoxy-D-galactose derived from chondroitin sulphates (probable contaminants of both B1 and B2). Glucuronic acid was a minor component.

Blood levels

If it is assumed that all the carrageenan excreted in the urine over 24 hr, i.e. 3–6 mg/kg body weight, was contained in the blood volume (*c.* 60 ml/kg body weight) of the baboon at one time, then the maximum concentration of carrageenan in the blood would be 5–10 mg/100 ml. Obviously, this concentration is probably an upper limit but it formed a useful basis for our *in vitro* experiments with both ox and baboon blood.

The first task in the present study was to determine whether carrageenan at this concentration could be isolated from blood and characterized, and all the initial *in vitro* experiments were carried out with citrated whole ox blood. The decomposition of any blood protein carrageenate complexes by addition of supersaturated potassium chloride solution to the blood containing the added carrageenan can be represented by:



$$\underset{\text{Complex}}{ROSO_3^-/R^1NH_3^-} + KCl \longrightarrow \underset{\text{Carrageenan}}{ROSO_3K} + \underset{\text{Protein}}{R^1NH_3Cl}$$

Addition of trichloroacetic acid completed the precipitation of proteins, after which exhaustive dialysis was required to free the solution from salts. After precipitation of the

Table 3. *Analyses of carrageenans isolated from ox blood*

Concentration of carrageenan in blood (mg/100 ml)	Yield (% of added carrageenan)	3,6-Anhydro-galactose (%)	Important bands in infra-red spectrum (cm^{-1})		
100	75	15·1	940–930	850–840	810–800
20	69	13·4	940–930	850–840	810–800
10	66	—	940–930	850–840	810–800

polysaccharide sulphate with CTAB, the CTAB complex was decomposed with potassium chloride, and the polysaccharide sulphate was precipitated with acetone and analysed.

In a control experiment, comparable blood without added carrageenan gave no precipitate with CTAB. The results for three concentrations of carrageenan in the blood are shown in Table 3.

The combination of bands in the infra-red recorded in Table 3 is characteristic of this type of carrageenan, which was confirmed at the first two concentrations by the presence of 3,6-anhydrogalactose. The infra-red analysis showed the presence of some contamination, particularly in the material isolated at the 10 mg/100 ml level.

The results of the spectrophotometric estimation of polysaccharide sulphate obtained from carrageenan added to blood in concentrations below 10 mg/100 ml are given in Table 4, for

Table 4. *Spectrophotometric estimation of carrageenan isolated from blood*

Type of blood	Concentration of carrageenan in blood (mg/100 ml)	Recovery of carrageenan (%)
Ox	10	54
	5	54
	2	51
Baboon	2	47
	0·5	*c*.38

three concentrations of carrageenan in ox blood and two concentrations in baboon blood. The lowest level of detection by this procedure, using toluidine blue, is 5 μg/ml blood. This concentration gives a very slight turbidity on the addition of CTAB, while no turbidity is obtained at lower concentrations.

A male baboon was given a single oral dose of 3 g degraded carrageenan/kg body weight, and 20 ml blood was withdrawn from the femoral vein at 3-hr intervals and treated as described for the *in vitro* experiments. In the first experiment, no turbidity was detected after 3, 6 or 9 hr while in the second experiment, there was no evidence of turbidity in the blood samples withdrawn after 12, 15 or 18 hr. The concentration of carrageenan in the blood thus did not reach 5 μg/ml.

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REFERENCES

Anderson, N. S., Dolan, T. C. S., Penman, A., Rees, D. A., Mueller, G. P., Stancioff, D. J. & Stanley, N. F. (1968). Carrageenans. Part IV. Variations in the structure and gel properties of κ-carrageenan and the characterisation of sulphate esters by infrared spectroscopy. *J. chem. Soc.* (*C*) p. 602.

Anderson, W. & Soman, P. D. (1966). The absorption of carrageenans. *J. Pharm. Pharmac.* **18**, 825.

Black, W. A. P., Blakemore, W. R., Colquhoun, J. A. & Dewar, E. T. (1965). The evaluation of some red marine algae as a source of carrageenan and of its κ- and λ-components. *J. Sci. Fd Agric.* **16**, 573.

Brimacombe, J. S. & Webber, J. M. (1964). *Mucopolysaccharides: Chemical Structure, Distribution and Isolation.* B.B.A. Library, Vol. 6, p. 64. Elsevier Publishing Co., Amsterdam.

Dewar, E. T. (1955). Sodium laminarin sulphate as a blood anticoagulant. *Proceedings of the 2nd International Seaweed Symposium*, Trondheim, Norway, p. 55.

Eagleton, G. B., Watt, J. & Marcus, R. (1968). The inhibitory effect of subcutaneously administered degraded carrageenan on gastric secretion. *J. Pharm. Pharmac.* **20**, 970.

Eagleton, G. B., Watt, J. & Marcus, R. (1969). Effect of subcutaneously administered degraded carrageenan on the production of histamine-induced gastric and duodenal ulceration. *J. Pharm. Pharmac.* **21**, 123.

Hough, L. & Jones, J. K. N. (1962). *Methods in Carbohydrate Chemistry.* Vol. 1. p. 28. Academic Press, New York.

Jones, A. S. & Letham, D. S. (1954). A submicro method for the estimation of sulphur. *Chemy Ind.* p. 662.

MacIntosh, F. C. (1941). A colorimetric method for the standardization of heparin preparations. *Biochem. J.* **35**, 776.

Wilson, C. M. (1959). Quantitative determination of sugars on paper chromatograms. *Analyt. Chem.* **31**, 1199.

Étude du carragène dégradé administré par voie orale au babouin

Résumé—Aucune métachromasie de l'urine n'a été observée chez des babouins adultes après l'administration d'une dose unique de 240 mg/kg de carragène dégradé (moyenne numérique du poids moléculaire: 16.000–19.000). Cette dose est plus que le triple de la dose thérapeutique employée pour l'homme. Même au niveau de 3 g/kg, soit plus de quarante fois la dose thérapeutique, seulement 3 à 6 mg/kg étaient excrétés par l'urine en 24 h et on ne pouvait déceler le carragène dans le sang au cours des 18 h suivant l'administration, ceci nonobstant le fait que la limite de détection était de 5 μg/ml de sang. La métachromasie de l'urine a été déterminée quantitativement par une méthode spectrophotométrique. Les polysaccharides sulfatés responsables de la couleur métachromatique ont été isolés et on y a trouvé une forte proportion de carragène, qui a été identifié par son pouvoir rotatoire spécifique et sa teneur en sulfate estérifié et en 3,6-anhydrogalactose, par analyse infrarouge et par la chromatographie sur papier des produits d'hydrolyse. Des méthodes ont été élaborées pour isoler et identifier jusqu'à un taux de 100 μg par ml de sang le carragène dégradé ajouté *in vitro* au sang et pour déceler la métachromasie jusqu'à 5 μg/ml. Aucune quantité décelable de carragène n'a été découverte, au cours des recherches décrites ici, dans l'urine des babouins qui avaient reçu trois fois la dose thérapeutique et l'on n'en a décelé que de très faibles quantités dans des échantillons d'urine excrétée pendant 24 h après l'administration par des animaux qui avaient reçu la dose massive de 3 g/kg. Ces résultats indiquent qu'aux doses utilisées en thérapeutique, le carragène n'est probablement pas absorbé par le tractus gastro-intestinal.

Eine Untersuchung über die orale Verabreichung von abgebautem Carragenin an Paviane

Zusammenfassung—Bei der Verabreichung von abgebautem Carragenin (durchschnittliches Molekulargewicht 16 000–19 000) an Paviane in einer einzelnen Dosis von 240 mg/kg, dem über Dreifachen der therapeutischen Dosis für Menschen, an erwachsene Paviane wurde im Urin keine Metachromasie beobachtet. Selbst bei der Dosierung 3 g/kg, dem über Vierzigfachen der therapeutischen Dosis, wurden nur 3–6 mg/kg im Urin in 24 Stunden ausgeschieden. Im Blut war Carragenin bis zu 18 Stunden nach der Verabreichung nicht nachweisbar, obwohl die Nachweisgrenze bei 5 μg/ml Blut liegt. Die Metachromasie des Urins wurde quantitative mit einer spektroskopischen Methode bestimmt. Die sulfatierten Polysaccharide, die für die metachromatischen Farbe verantwortlich sind, wurden isoliert und enthielten einen hohen Anteil von Carragenin, das durch die spezifische Drehung, den Gehalt an Estersulfat und 3,6-Anhydrogalactose, Infrarotanalyse und Papierchromatographie der Hydrolyseprodukte identifiziert wurde. Es wurden Methoden für die Isolierung und Charakterisierung von abgebautem Carragenin, das *in vitro* dem Blut zugesetzt wurde, bis hinab zu einer Konzentration von 100 μg/ml Blut und für die Feststellung von Metachromasie bis hinab zu 5 μg/ml entwickelt. Bei den durchgeführten Untersuchungen wurden keine feststellbaren Mengen von abgebautem Carragenin im Urin von Pavianen gefunden, die das Dreifache der therapeutischen Dosis erhalten hatten, und nur sehr kleine Mengen in Proben des 24-Stunden-Urins von Tieren entdeckt, welche die hohe Dosis von 3 g/kg erhalten hatten. Diese Ergebnisse zeigen, dass eine Resorption aus dem Gastrointestinaltrakt bei den therapeutisch verwendeten Dosen von abgebautem Carragenin nicht zu erwarten ist.