duction was measured by method of Singh et al.[4] in which labelled live mixed bacterial cells were used. 2 sets of experiments were made using 2 feeds namely green maize and berseem (tables 1 and 2). The production rates of bacteria in animals fed green maize on an average were 153.0 g and 153.7 g/3 kg dry matter intake per day when estimated by injecting untreated and treated labelled cells respectively. In the second set of experiments on berseem diet, the average daily growth was 240.8 g and 238.8 g/4 kg dry matter intake when measured by using untreated and treated cells respectively. The variations observed in the bacterial growth between different animals when fed the same ration were due to difference in quantity of feed consumed by individual animals, and those between 2 feeding regimes may be due to the quality and quantity of the rations consumed by the animals. The difference in the production rates became less when the rumen bacterial growth rate was calculated on equal feed intake.

There was no significant difference in the rates of growth estimated by using either treated or untreated cells. These experiments confirm the earlier assumption that the labelled bacteria produced by in vitro incubation are, after injection, mixed with the rumen microorganisms and are not treated as foreign material. The normal metabolism of labelled cells in the rumen does not appear to influence the growth rate measurements. The present experiments also suggest the possibility of using any other source of labelled proteins (not necessarily of rumen origin) after treatment for protection from degradation in the rumen as a marker for the estimation of microbial growth.

Some characteristics of urokinase released in organ culture of human kidney[1]

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Summary. Plasminogen activator produced in organ culture of human kidney, i.e. in the histotypical arrangement of the tissue, was partially purified by affinity chromatography on para-aminobenzamidine coupled to Sepharose by a 6-carbon spacer, followed by gel chromatography on Sephadex G-100. The molecular weight of 2 active peaks were 27,000 and 52,000 daltons respectively. It was inhibited by DFP and by IgG antiurokinase.

Urokinase has been prepared and purified from human urine[2–5], and commercial preparations are available. In kidney cell cultures, an activator of plasminogen is released[6–10]. There is evidence that in organ culture of human kidney, urokinase is not only released, but also synthetized[11]. In urinary preparations, urokinase has been found to exist in various molecular forms. There are no reports available concerning the forms of urokinase produced in organ culture of the kidney, i.e. in its histotypical arrangement. We report here partial purification by affinity and gel chromatography and some characteristics of urokinase produced in organ culture of human kidney (KA).

Material and methods. Fetal kidneys were obtained at legal abortion of 14–20-week-old foetuses removed by abdominal hysterotomy. The kidneys were divided into pieces about 1 mm³. These explants were cultured in

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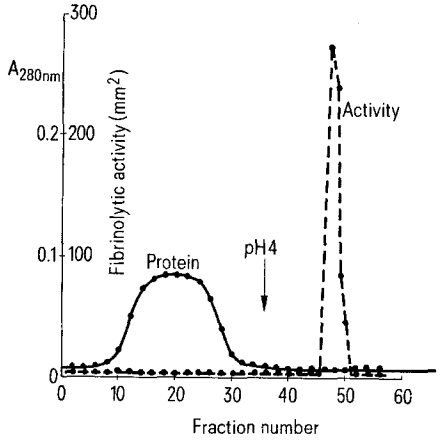

Fig. 1. Elution profile of KA at affinity chromatography on para-aminobenzamidine coupled to CH-Sepharose 4 B with a 6-carbon spacer (see text). Flow rate 45 ml h^{-1}, fraction volume 3 ml.

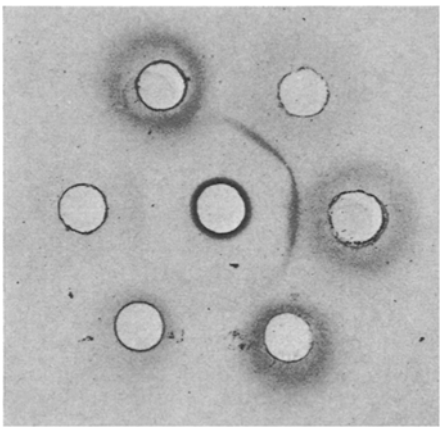

Fig. 2. Immunodiffusion of KA against UK antiserum. UK antiserum was deposited in the central hole and concentrated medium of different kidney cultures in the outer holes.

Neutralisation of the fibrinolytic activity of KA by antiurokinase antibodies

Dilution	KA	KA + normal IgG	KA + antiurokinase IgG
1/2	672	708	116
1/4	510	602	85
1/8	420	458	72
1/16	340	320	36
1/32	300	300	0

Dilution series of KA incubated in proportion 1:1 over night at +4 °C with IgG prepared from normal rabbit serum resp. from rabbit urokinase antiserum. The fibrinolytic activity was tested on fibrin plates and given in mm^2 of the lytic zones.

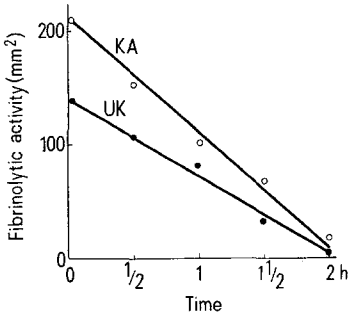

Fig. 3. Inhibition of KA and UK incubated with equal amounts of DFP in a concentration of 2.5×10^{-5} M in the reaction mixture.

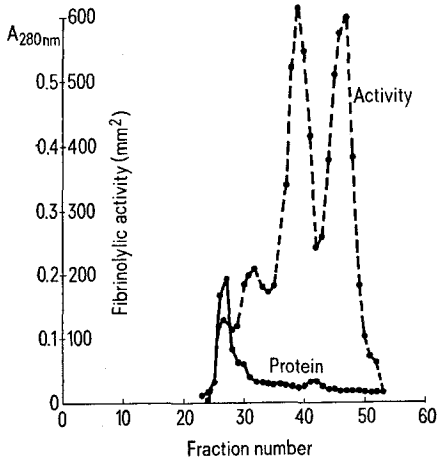

Fig. 4. Elution profile of KA previously purified by affinity chromatography on Sephadex G-100. Flow rate 4.6 ml h^{-1}, fraction volume 2.3 ml (see text).

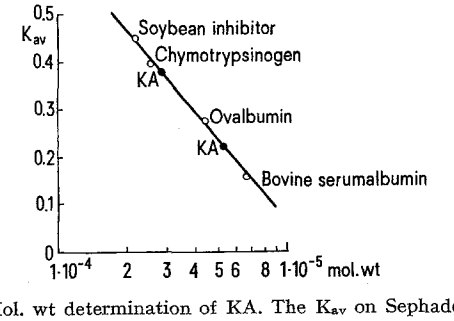

Fig. 5. Mol. wt determination of KA. The K_{av} on Sephadex G-100 for the 2 peaks were 0.224 and 0.379.

Petri dishes on gel foam slices (Spongostan®, Ferrosan, Malmö, Sweden). As culture medium, the purely synthetic medium Parker 199 (SBL, Stockholm, Sweden) was used. Survival of the explants was checked by serial sectioning and histological examination of randomly selected explants. Good survival was found for 3 weeks of culture. During this period, the culture medium was collected every 3rd day.

The culture medium was centrifuged and then dialysed against 0.1 M phosphate buffer, pH 7.0, 0.4 M NaCl at + 4 °C for 48 h. Affinity chromatography was performed with a column 1.6×40 cm packed to a height of 12 cm in which p-amino-benzamidin was carbodiimide-coupled to CH-Sepharose-4 B (Pharmacia Fine Chemicals, Uppsala, Sweden).

The column was equilibrated with 0.1 M phosphate buffer, pH 7.0, 0.4 M NaCl. Elution was performed by shift of pH to 4. The UV-absorption of the fractions was read at 280 nm. The fibrinolytic activity of the fractions was tested on plasminogen-containing plates[12] as well as on plasminogen-free fibrin (Poviet Production, Oss, Netherlands) plates.

Gel filtration was performed on a column of Sephadex G-100, 1.6×100 cm (Pharmacia Fine Chemicals, Uppsala, Sweden) in 0.05 M Tris-HCl, pH 7.8, 0.3 M NaCl. The column was calibrated with Blue Dextran (V_0) and K_2CrO_4 (V_t). UV-absorption was read at wave-lengths 620 and 410 nm respectively. Soya bean inhibitor, chymotrypsinogen, ovalbumin and bovine serum albumin read at 280 nm were used as reference substances. Neutralisation experiment: Dilutions of KA and urokinase (UK) (Leo Pharmaceutical, Denmark) were incubated over night at 4 °C with IgG prepared from normal rabbit serum and with IgG prepared from rabbit urokinase antiserum. IgG was prepared according to the method of Steinbuch and Audran[13]. Double diffusion in agarose was performed according to Ouchterlony[14]. Inhibition by diisopropylfluorophosphate (DFP): Solutions of UK and KA were incubated with equal amounts of DFP at a final concentration of 2.5×10^{-5} M in the reaction mixture (stock solution of 10^{-2} M DFP in propylene glycol diluted in Tris buffer 0.075 M, pH 7.8) for 0–2 h. Nonreacted DFP was removed by gel filtration on small columns filled with Sephadex G-25 (Pharmacia Fine Chemicals, Uppsala, Sweden), after which the solutions were tested for their fibrinolytic activity on fibrin plates.

Results and comments. At affinity chromatography of the dialyzed culture medium, most of the protein passed through the column without being bound (figure 1). At elution the activity was found in a sharp peak without any detectable protein. This is in agreement with the use of this method for purification of commercial urokinase preparations[15]. Immunological identity with urokinase prepared from urine (Leo Pharmaceutical, Denmark) was found in the Ouchterlony test (figure 2) and in the neutralisation experiment (table) in which inhibition of activity was found by IgG prepared from rabbit urokinase antiserum[15]. Figure 3 demonstrates inactivation of KA by DFP indicating that it is a serine protease. This characteristic has previously been shown for urinary preparations[16].

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Gel filtration chromatography gave 2 active peaks with molecular weights of 27,000 and 52,000 daltons (figures 4 and 5). We also observed that in early cultures mainly the high molecular KA was released, and in late cultures mainly the low molecular KA. No other mol.wt determinations of preparations from kidney cultures are on record. For urinary preparations, various mol. wts have been reported. Lesuk et al.[3] prepared urokinase with a mol.wt of 54,000. White et al.[4] found 2 fractions with mol.wts of 31,500 and 54,700, respectively, and Kok and Astrup[17] 2 fractions of about 43,000 and 54,000. Using Sephadex separation Doleschel and Auerswald[18] found 3 active peaks for fractions with the mol.wts of 27,000, 54,000 and 104,000. Recently Ogawa et al.[5] reported the mol.wt to be 33,000, Johnson et al.[19] 2 forms *viz* 33,500 and 47,000, and Holmberg et al.[15] 2 forms *viz* 33,000 and 54,000. This indicates the existence of 2 molecular forms of about 30,000 and 50,000 daltons in urinary preparations. In our study, in which the KA was obtained from organ cultures, i.e. with the kidney cells in their histotypical arrangement, the 2 mol.wt fractions thus seem to correspond to those found in the urinary preparations.

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On urea formation in marine mammals[1]

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Summary. Ornithine carbamoyltransferase (EC 2.1.3.3) has been determined in homogenates of liver of the sei whale (Balaenoptera borealis), the bottle-nose dolphin (porpoise) (Tursiops truncatus) and California sea lion (Zalophus californianus). These marine mammals show levels of this ornithine-urea cycle enzyme which are typical of terrestrial mammals.

All terrestrial mammals studied possess in liver the enzymes for the biosynthesis of urea via the ornithine-urea cycle[2]. It is established that terrestrial mammals excrete urea in the urine as the principal nitrogenous waste product[3]. Only a few reports are available on the urinary nitrogen components of marine mammals, however. In some early work, Schmidt-Nielsen and Holmsen[4] made determinations on nitrogenous components of the urine of several sei whales (Balaenoptera borealis) and found that urea was the major nitrogenous material. In one specimen, for example, urea-N constituted 93% of the total urinary nitrogen, while lesser amounts were found partitioned among ammonia-N (3.3%), protein-N (1.9%), uric acid-N (0.58%) and creatine-N (0.45%) (calculated from the original data by Brown[5]). Most of the urinary nitrogen in harbor seals (Phoca vitulina)[6] and in northern fur seals (Callorhinus ursinus)[7] is accounted for by urea. The urea content of the urine of the fasting humpback whale (Megaptera nodosa) reported by Bentley[8] suggests that this marine mammal is also primarily ureotelic.

Ornithine carbamoyltransferase of marine mammals (liver, 38 °C)

System	μmoles citrulline produced in 15 min		
	Balaenoptera borealis*	Tursiops truncatus**	Zalophus californianus***
Complete	6.34 ± 0	2.58	2.99
Less ornithine	0.25	0.01	0.01
Less carbamoylphosphate	0.14	0.07	0
Boiled homogenate	0.21	0.03	0.02

*Homogenate in cetyltrimethylammonium bromide representing 3.0 mg liver; triplicate determination. Male specimen; 14 m body length. **Homogenate in water representing 0.5 mg liver. Male specimen, 114 kg; 3 m body length. ***Homogenate in water representing 0.5 mg of liver. Adult specimen.

One of the 5 enzymes responsible for the biosynthesis of urea by the ornithine-urea cycle is ornithine carbamoyltransferase (EC 2.1.3.3) which catalyzes the following reaction:

Ornithine + carbamoylphosphate = citrulline + P_i.

This report provides evidence for the occurrence of ornithine carbamoyltransferase in the liver of 3 marine mammals: the sei whale, Balaenoptera borealis; the bottle-nose dolphin (porpoise), Tursiops truncatus; and the California sea lion, Zalophus californianus.

Materials and methods. Homogenates of liver (10% w/v in water or in 0.1% cetyltrimethylammonium bromide) were assayed for ornithine carbamoyltransferase as described elsewhere[9]. The complete incubation system contained Na glycylglycine buffer (45 mM), L-ornithine

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