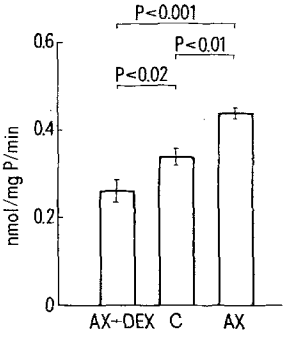
into ice-cold 0.25 moles sucrose and homogenized in a glass-Contes homogenizer. We used the assay for MAO decribed by Wurtman and Axelrod[6]. The incubation mixture consisted of 25 µl tryptamine C^{14} (6.25 nmoles, specific activity 2.7 mCi/mmole, Radiochemical Centre Amersham) 250 µl 0.1 moles potassium phosphate buffer (pH 7.4) and 25 µl of 2% tissue homogenate. The mixture for enzyme assay was incubated in a water bath at 37 °C for 20 min. The reaction was stopped by adding 0.2 ml of 2 N HCl, and the radioactive products were extracted into 10 ml of toluene. 4 ml samples of toluene extract were measured for radioactivity in 10 ml of Bray's solution by a Packard scintillation counter. Triplicate determinations and 2 series of experiments were performed.



MAO activity as C^{14}-indolacetic acid production in 0.5 mg thyroid incubated for 20 min. *AX* Adrenalectomy, *C* controls, *AX + DEX* adrenalectomized + dexamethasone treatment. Means of 10 values ± SE (results of experiments 1 and 2 were pooled). The statistical significance was calculated by the Student t-test.

Results. The activity of MAO in the thyroid gland is shown in the figure. In the adrenalectomized rats, the activity was significantly higher than in controls ($p < 0.01$). The administration of dexamethasone to adrenalectomized rats not only inhibited this increase in MAO activity, but depressed this activity well below levels found in intact controls. The differences are statistically significant.

Discussion. The data of the present study show that MAO activity in the thyroid gland is strongly influenced both by adrenalectomy and dexamethasone treatment. The effect of adrenalectomy is consistent with that on other organs, such as liver and in the heart[4, 6], where significant increases in MAO activity were found. Moreover dexamethasone administration to adrenalectomized rats decreased enzyme activity as compared to controls or adrenalectomized rats. Its increase in adrenalectomized rats, and its depression after dexamethasone, support the finding that corticosteroids which have been found to stimulate some of thyroid functions may do it through influencing MAO activity in the thyroid gland.

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Comparison of the production rates of bacteria in the rumen estimated by using labelled live and formaldehyde treated mixed bacterial cells

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Summary. The bacteria production rates in the rumen have been estimated by injecting ^{14}C- and ^{35}S-labelled mixed rumen bacteria, either live or killed by treatment with formaldehyde, into the rumen and applying isotope dilution technique. The rate of bacteria production when estimated by using either live- or dead-(protected-)labelled bacterial cells were comparable.

An experimental approach was described for the measurements of bacteria and protozoa production rates in the rumen by isotope dilution technique using ^{35}S- and ^{14}C-labelled mixed cells[1–5] and ^{14}C-Streptococcus bovis[6]. In this paper ^{14}C- or ^{35}S-labelled mixed bacterial cells of rumen origin, treated with formaldehyde to protect their being metabolized in the rumen, were used to estimate bacteria production in the rumen, and comparison was made of the growth obtained by injecting labelled mixed rumen bacterial cells used earlier[4].

Materials and methods. Animals and feeding regime. 3 male Murrah buffalo (Bos bubalis) of about $2^1/_2$ years of age with permanent rumen cannulae were used in these experiments. 2 sets of experiments were done. In the first set of experiments each animal was offered 15–20 kg green chopped maize daily and in the second set 35–40 kg berseem (Trifolium alexandrinum) was fed to each animal. The residue was weighed daily to assess their intake. The animals were kept on a pre-experimental feeding period of 4 weeks during which they received their ration once daily, and thereafter the animals received their daily ration in 12 equal amounts at 2-h-intervals for a period of 3 weeks. The residue, if any, at the end of each 2-h-interval was removed and weighed. The samples of feed offered and of residue were collected daily for analysis.

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Preparation of labelled bacteria. Bacteria from the rumen of animals used later for the in vivo experiments were labelled with ^{14}C or ^{35}S by incubating a sample of rumen contents at 39 °C under an atmosphere of CO_2 as described earlier[4].

Treatment of bacteria with formaldehyde. ^{14}C- or ^{35}S-labelled mixed bacteria of rumen origin were separated from coarse feed particles and protozoa[4] and were treated with 10 volumes of commercial formaldehyde (37% w/v) for 1 h. Thereafter the bacterial suspension was centrifuged and the bacterial pellet was resuspended in 250 ml of centrifuged rumen liquor (supernatant of 20,000 × g for 15 min) and an aliquot was taken for estimating the amount of radioactivity added. The bacterial suspension was injected into the rumen through the cannula in a single dose. The contents of the rumen were mixed simultaneously by hand.

Sampling and processing of bacterial cells. Samples (35 ml) from the rumen were drawn at various time intervals up to 10 h from 4 different sites and were processed for estimating the radioactivity and calculations of the bacteria production rates[4].

Results and discussion. One of the major problems in applying isotope dilution technique for the measurement of bacterial protein synthesis rate is that the labelled protein used as a marker may not remain as protein once it enters the rumen, since it is attacked by the rumen microorganisms and is degraded into amino acids and some deamination may also take place. This problem was avoided in the method published earlier[4], in which the mixed bacterial population of the rumen was tagged with radioactive carbon or sulphur and was replaced into the rumen to estimate the dilution rate and eventually the production of rumen bacteria. The method was based on the assumption that the labelled bacterial population produced by in vitro incubation on injection is mixed with the rumen bacteria and is not treated as a foreign body by the rumen microbes. It has been reported that E. coli and Bacillus subtilis which are not normally found in the rumen are digested very soon after injection[7]. This assumption has been tested by experimentation on buffaloes. The labelled mixed bacterial cells of rumen origin were treated with formalin, which killed the bacteria and can also protect them from microbial attack and degradation in the rumen[8]. These dead-labelled bacterial cells were injected into the rumen as a marker for estimating bacterial growth. Simultaneously the bacteria pro-

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Table 1. Production rate of rumen bacteria estimate by using either live or formaldehyde treated labelled mixed bacterial cells as marker

Experiment No.	Feed offered	Material injected	Radioactivity injected as labelled bacteria (dpm × 10^6)	Specific radioactivity at time 0 (dpm/mg bacteria)	Pool size of bacteria (g)	Turnover time (min)	Production rate of bacteria (mg/min)
1	Green maize	^{14}C-untreated bacterial cells	33.20	955	34.76	305.3	113.88
2	Green maize	^{35}S-untreated bacterial cells	115.58	3020	38.27	410.4	93.25
3	Green maize	^{14}C-formaldehyde-treated bacterial cells	8.98	40	22.58	201.6	111.98
4	Green maize	^{35}S-formaldehyde-treated bacterial cells	186.90	8318	22.47	214.0	105.00
5	Berseem	^{35}S-untreated bacterial cells	532.23	9285	57.32	320.0	179.14
6	Berseem	^{35}S-untreated bacterial cells	532.23	7190	74.02	381.0	194.30
7	Berseem	^{35}S-untreated bacterial cells	532.23	10460	50.88	301.0	169.05
8	Berseem	^{35}S-formaldehyde-treated bacterial cells	184.26	3548	51.93	299.0	173.68
9	Berseem	^{35}S-formaldehyde-treated bacterial cells	184.26	3827	48.15	272.0	177.00
10	Berseem	^{35}S-formaldehyde-treated bacterial cells	184.26	2897	63.60	350.0	181.72

Table 2. Ruminal bacterial growth calculated on equal feed intake in buffaloes

Experiment No.	Feed offered	Material injected	Feed intake (dry matter g/day)	Production rate of bacteria g/day	g/3000 g DM	g/4000 g DM
1	Green maize	Untreated bacterial cells	3335	164.0	147.53	–
2	Green maize	Untreated bacterial cells	2543	134.4	158.47	–
3	Green maize	Formaldehyde-treated bacterial cells	3256	161.3	148.6	–
4	Green maize	Formaldehyde-treated bacterial cells	2856	151.2	158.8	–
5	Berseem	Untreated bacterial cells	4130	258.0	–	249.8
6	Berseem	Untreated bacterial cells	4410	279.8	–	253.8
7	Berseem	Untreated bacterial cells	4452	243.4	–	218.8
8	Berseem	Formaldehyde-treated bacterial cells	4494	250.1	–	233.0
9	Berseem	Formaldehyde-treated bacterial cells	4274	254.9	–	238.5
10	Berseem	Formaldehyde-treated bacterial cells	4294	261.7	–	243.8

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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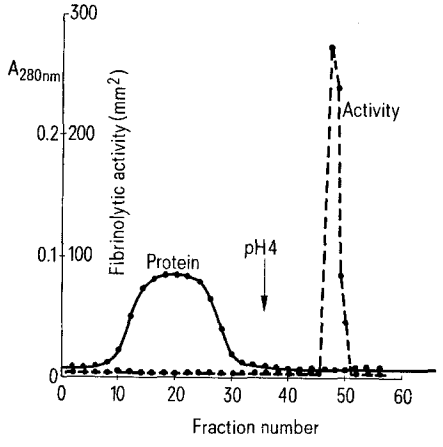

Fig. 1. Elution profile of KA at affinity chromatography on para-aminobenzamidine coupled to CH-Sepharose 4 B with a 6-carbon spacer (see text). Flow rate 45 ml h^{-1}, fraction volume 3 ml.

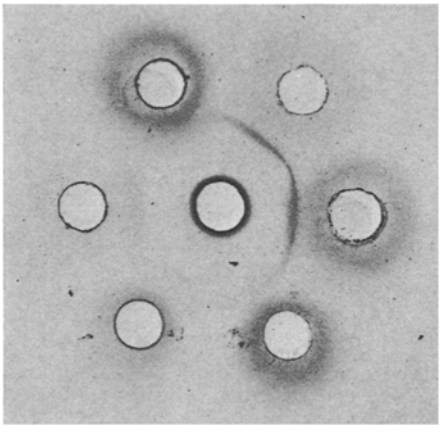

Fig. 2. Immunodiffusion of KA against UK antiserum. UK antiserum was deposited in the central hole and concentrated medium of different kidney cultures in the outer holes.