tion at a faster rate, our results indicate that the changes accompanying a stimulus in the uterine lumen also trigger protein synthesis in the myometrium. Our data showing increased fresh weight of the myometrium along with the enhanced incorporation of labelled amino acid should be viewed in the light of the recent report of Martin[15] on the enhanced muscular activity of mouse myometrium soon after receiving an oil stimulus.

Cycloheximide is a well known inhibitor of mammalian protein synthesis. Barkai and Kraicer[16] used a lethal dose of 50 mg cycloheximide/kg b.wt to inhibit ornithine decarboxylase and total protein syntheses in the stimulated uterus. However, cycloheximide has been shown to inhibit protein synthesis at considerably smaller doses[17,18]. Although the effect of this inhibitor on the actual process of stromal cell differentiation remains to be elucidated, results of the present investigation show that proliferating cell populations of deciduoma are sensitive to cycloheximide at a sublethal dose of 2 mg/kg b.wt. Further studies which are in progress on individual differentiating cell populations of endometrium would throw more light on the biochemistry of trasnformation of stromal cells to decidual ones.

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Digestive enzymes of some earthworms

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Summary. 4 species of tropical earthworms differed with regard to enzyme activity. The maximum activity of protease and of cellulase occurred in the posterior region of the gut of the earthworms. On the average *Octochaetona surensis* shows maximum activity and *Drawida calebi* shows minimum activity for all the enzymes studied.

The feeding biology and food preferences of earthworms have been studied and their digestive enzymes have been qualitatively surveyed[1-10]. Tracey[7] demonstrated qualitatively the presence of cellulase in 17 species and chitinase in 12 species of earthworms. The nature of the intestinal protease in *Pheretima elongata* (Steph)[8] and of the process of digestion in *Eisenia foetida* (Savigny)[9] have been reported. Nielsen[10] made a survey of carbohydrases in some 30 soil invertebrates including 3 species of earthworms with a wide range of substrates. The present investigation reports the occurrence and activity of protease, amylase, invertase, cellulase and urease in the guts of 3 species of earthworms and in whole tissue of *Dichogaster bolaui.*

Materials and methods. Lampito mauritii (Kinberg), *Octochaetona surensis* (Michaelson), *Drawida calebi* (Gates) and *Dichogaster bolaui* (Michaelson) were collected from a pasture soil. Except in the case of *D. bolaui* the guts of the earthworms were dissected free after anesthetization in 8% ethanol. The alimentary canal was washed to free it from gut contents, and homogenates of different regions of the guts of 10 specimens were made (anterior; up to 35 segments, middle: 35-70 segments and posterior; from 70-last segment) in cold distilled water. For *D. bolaui,* because of its smaller size, homogenates of the entire worm was made taking 10 specimens at a time. Homogenates were then centrifuged at 2000 rpm for 20 min in a refrigerated centrifuge. The supernatant fluid was collected and adjusted to a known volume, and the Folin-lowry method[11] was used for protein estimation. Protease activity was determined following the method of Speir and Ross[12] except that the incubation mixture contained 1 ml of enzyme preparation and 1 ml Tris-HCl buffer (0.1 m, pH 8.1) containing sodium caseinate (1% w/v). The activity is expressed as µg of tyrosine released per mg of protein per h. The method of Burton et al[13] was followed for the determination of carbohydrase activity except that 1 ml of enzyme preparation was incubated with 1 ml of substrate (1% soluble starch, 6% sucrose and 3% carboxymethyl cellulose for amylase, invertase and cellulase respectively) and 1 ml Sorensen's buffer (pH 6.5) plus 0.2 ml toluene for 24 h. The activities are expressed in µg of reducing sugar (glucose) formed per mg of protein per h. The method for the measurement of urease activity involves the determination of ammonia[14] released by incubation of the extract (1 ml) with Tris-HCl buffer (1 ml. pH 9.0), urea solution (1 ml of 1% w/v) and toluene (0.2 ml) at 37 °C for 96 h. The activity is expressed in µg of ammonia per mg of protein per h. For each species 5 replicates were taken and 3 readings were made for each enzyme in each replicate.

Results and discussion. The table summarises the enzyme activities in the different regions of the alimentary canal of 3 species of earthworms and in whole tissue of *D. bolaui.* Amylase activity is uniform throughout the gut of *O. surensis* and *D. calebi* and highest in the middle region of *L. mauritii.* Urease activity is uniform throughout the gut of *L. mauritii* and *D. calebi;* no activity could be detected in

Enzyme activity in the guts of earthworms Mean ± SEM

	L. mauritii				*O. surensis*				*D. calebi*				*D. bolaui*
	Ant.	Mid.	Post.	Av.	Ant.	Mid.	Post.	Av.	Ant.	Mid.	Post.	Av.	
Protein (mg)	1.06 ±0.22	0.68 ±0.14	0.59 ±0.12	0.78 ±0.10	0.58 ±0.23	0.46 ±0.08	0.17 ±0.11	0.40 ±0.09	0.38 ±0.03	0.19 ±0.02	0.14 ±0.03	0.24 ±0.03	0.14 ±0.03
Protease*	23.8 ±7.96	32.28 ±13.58	7.71 ±2.40	21.26 ±5.56	12.16 ±3.60	28.69 ±13.25	57.68 ±15.59	32.86 ±8.15	7.88 ±0.84	8.56 ±0.74	10.67 ±0.87	9.04 ±0.56	32.89 ±3.49
Amylase*	75.49 ±6.31	142.45 ±29.42	135.5 ±27.57	117.81 ±14.28	108.38 ±44.69	202.25 ±56.68	284.19 ±112.04	198.27 ±45.39	30.99 ±9.33	43.93 ±17.81	59.68 ±22.67	44.08 ±9.99	258.54 ±60.02
Invertase*	37.98 ±19.04	32.05 ±15.99	41.47 ±8.73	33.84 ±8.28	123.55 ±15.79	209.09 ±18.79	389.05 ±21.64	240.85 ±31.23	36.38 ±8.16	119.18 ±27.23	29.83 ±1.97	61.79 ±14.97	137.01 ±30.53
Cellulase*	16.66 ±5.30	29.48 ±3.51	37.39 ±9.46	27.84 ±4.13	42.10 ±18.82	78.95 ±9.15	119.83 ±19.03	87.69 ±10.75	12.51 ±2.03	20.98 ±3.28	37.76 ±5.31	23.75 ±3.73	141.36 ±29.79
Urease*	0.96 ±0.24	0.77 ±0.29	0.87 ±0.17	0.87 ±0.14	NA	NA	NA	NA	0.22 ±0.09	0.17 ±0.04	0.29 ±0.09	0.23 ±0.04	0.73 ±0.14

*µg/mg protein/h. NA, no activity; Ant., anterior region; Mid., middle region; Post., posterior region; Av., average.

O. surensis. Protease and cellulase activity are maximal in the posterior region of *O. surensis* and of *D. calebi* and in the middle region of *L. mauritii*. On the average, *O. surensis* shows maximum activity and *D. calebi* shows minimum activity for all the enzymes in the gut. Cellulase activity in whole tissue of *D. bolaui* is maximal compared with the gut enzyme activity of the above species. The differential enzyme-activity is perhaps related to the type of food and rate of eating of each species. In *Lumbricus terrestris* cellulase activity in the anterior half of the gut is 10-fold higher than in the posterior half[7] and the mucosa of crop, gizzard and fore-intestine (up to a portion corresponding to the 60th segment) secrete proteolytic enzymes[6]. The activity decreases sharply in the mucosa of the middle and hind intestine. Nielsen[10] observed on the basis of his qualitative study that of the 3 species of earthworms only *Dendrobaena octaedra* showed evidence of digesting cellulose and chitin and the other 2 species did not have the requisite enzymes to digest cellulose, pectin, xylan and chitin. The question whether cellulase present in various animals is produced by the gut flora or by the animals themselves is not very important from the ecological point of view as long as the possible association between animals and cellulotypic microbes is constant[10]. The finding that cellulase activity is maximal in the posterior region of the gut of the worms supports the view that microorganisms present in the fore and mid gut might be helping in the partial digestion and processing of the complex plant remains containing cellulose, xylan, mannan, pectin etc. This study indicates that tropical earthworms play an active part in the decomposition process in forest and grassland litter as they possess cellulase.

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Removal of polyethylene glycols from immunoglobulin samples by adsorption chromatography on polystyrene beads

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Summary. A chromatographic method is described for 80% removal of polyethylene glycol of different types, 600–6000, from human immunoglobulin preparations. Bio-beads SM-2 were used in batch procedures or packed on a 16 × 100 mm column. The polyethylene glycols were desorbed with 75% ethanol.

The use of high molecular weight polyethylene glycols (PEG) for the fractionation and purification of proteins from human plasma, as an alternative to cold ethanol precipitation techniques, is now well documented[1–5]. However, no reliable method has yet been described for effective removal of the trace contaminating polymers in such protein preparations. When preparing plasma proteins by PEG fractionation procedures the remaining traces of polymers are now of the order of 0.15% (w/v). In gel filtration, for example, the linear polyethylene glycols behave like molecules much larger than expected[6,7]. Because of this, the effective hydrodynamic radii of the