whereas the uptake of biotin, which has been demonstrated to occur by an active process as well as thiamine[7], was not affected by the dye at all under the same conditions. Furthermore, it was found that the effect of methylene blue is exerted on growing yeast. As shown in table 3 the growth inhibition of *S. cerevisiae* by pyrithiamine was abolished by the addition of methylene blue to the growth medium. The abolition of the inhibition by the dye was apparently competitive with pyrithiamine and the ratio of the concentration of methylene blue to pyrithiamine for a half maximal growth inhibition was 35–40.

From the results described above, methylene blue appears to be a specific inhibitor of thiamine transport in baker's yeast. Although the mechanism of its inhibition is unknown it might be suggested that there may be competition between a quaternary nitrogen atom of methylene blue and that of thiamine for a negatively charged group of some surface component of the cell membrane which is involved in thiamine transport in baker's yeast.

Table 3. Effect of methylene blue on growth inhibition of *Saccharomyces cerevisiae* by pyrithiamine

Addition to growth medium		Growth
Pyrithiamine (μM)	Methylene blue (μM)	(optical density at 560 nm)
0	0	0.350
1.0	0	0.015
1.0	10	0.015
1.0	20	0.065
1.0	40	0.170
1.0	100	0.350
2.5	100	0.200
5.0	100	0.100
10.0	100	0.040

Growth studies were carried out using Wickerham's synthetic medium with thiamine omitted as previously described[3]. Growth was measured turbidimetrically at 560 nm. The cells grown with methylene blue were washed twice with water, resuspended and measured.

1 J. Suzuoki, J. Biochem. *42*, 27 (1955).
2 A. Iwashima, H. Nishino and Y. Nose, Biochim. biophys. Acta *330*, 222 (1973).
3 A. Iwashima, Y. Wakabayashi and Y. Nose, Biochim. biophys. Acta *413*, 243 (1975).
4 A. Iwashima, Y. Wakabayashi and Y. Nose, J. Bact. *131*, 1013 (1977).
5 A. Iwashima, H. Nishimura and K. Sempuku, Experientia *36*, 385 (1980).
6 A. Iwashima and Y. Nose, Biochim. biophys. Acta *399*, 375 (1975).
7 T.O. Rogers and H.C. Lichtstein, J. Bact. *100*, 557 (1969).

Protein anabolism in endometrium and myometrium during the growth of induced deciduoma in rats

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Summary. Protein anabolism in the endometrium and myometrium was studied during the growth of induced deciduoma in terms of incorporation of [^{14}C]-leucine into proteins. The data show that the specific activity of the proteins was highest 2 days after decidualisation. Protein synthesis in the endometrium studied on that day as a function of time after injection of the labelled amino acid showed a steady increase during the first 2 h. Cycloheximide (2 mg/kg b.wt) administration produced nearly 95% inhibition of protein synthesis in endometrium as well as in myometrium.

A variety of biochemical changes precede nidatory response in the uterus[2]. Proliferation and transformation of endometrial stromal cells occur as a consequence of the entry of blastocysts. The artificially induced decidual cell reaction forms a convenient model for observing changes during implantation[3]. This reaction in a primed uterus, however, is not dependent on the blastocyst alone but can be elicited by uterine trauma[4]. With a view to understanding some of the changes associated with biochemical transformations during the morphogenesis of deciduoma, a study on protein anabolism in the endometrium and myometrium was carried out. The present paper reports the results of in vivo incorporation of a labelled amino acid into proteins of endometrium and myometrium during growth and regression of deciduoma. In addition, data on the sensitivity of endometrium and myometrium to cycloheximide, a potent inhibitor of protein synthesis, are presented.

Materials and methods. 75-day-old virgin female Wistar rats weighing approximately 280 g were allowed to mate with proven males of the same strain. The presence of spermatozoa in the vaginal smear the next morning was taken as an indication of successful mating and was designated as day 1 of pregnancy. Deciduoma was induced with arachis oil on day 5 of pregnancy, as reported earlier[5]. Animals were maintained on a balanced laboratory diet and water ad libitum.

DL-Leucine-[1-^{14}C] (sp. act. 53.7 mCi/mmole, obtained from the Isotope Division, Bhabha Atomic Research Centre) was injected i.p. at a dose of 0.2 μCi/g b.wt. Cyclohex-

Effect of cycloheximide on incorporation of [^{14}C]-leucine into proteins of endometrium and myometrium on day 7 of pseudopregnancy

Status	Endometrium cpm/mg protein	% inhibition	Myometrium cpm/mg protein	% inhibition
Control	7584 ± 209 (5)*	–	4717 ± 99 (10)	–
Cycloheximide-treated	377 ± 30 (7)	95.3	237 ± 26 (7)	94.08

* Mean ± SEM (n).

imide was dissolved in 0.9% NaCl and injected i.p. at a dose of 2 mg/kg b.wt 1 h before administration of the labelled leucine.

Animals were sacrificed by cervical fracture and the deciduoma was quickly excised and placed on ice. Pieces of whole uterus of day 5 and 6 and parts of separated endometrium and myometrium of later stages were taken for radioanalysis as reported earlier[6]. Endometrium was separated from myometrium by squeezing[5], and this was effective only from the 2nd day following deciduoma induction. Protein was estimated[7] and the results were expressed as specific activity: cpm/mg protein. The data were analyzed statistically by Student's t-test.

Results. Data on growth and regression of deciduoma following oil stimulus on day 5 of pregnancy are presented in figure 1. The rate of growth of endometrium is more pronounced during the initial stages and progresses up to day 12 and declines thereafter. Myometrium also grows gradually and the weight increases rapidly from day 11 reaching a peak on day 15. This phase of growth from day 11–15 also includes the development of the metrial gland.

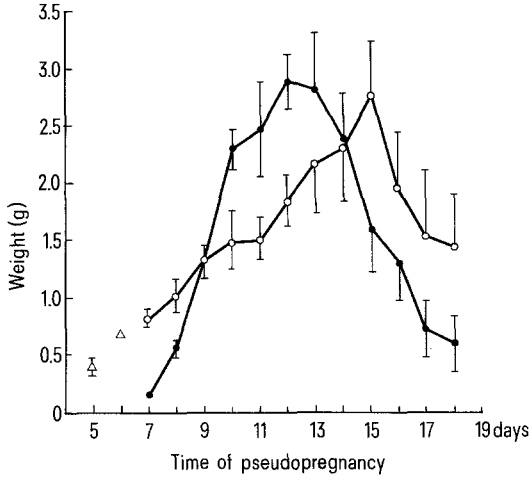

Fig. 1. Growth and regression of rat deciduoma following stimulation on day 5 of pregnancy with arachis oil. Each point represents mean ± SD of fresh weight from 8–14 animals. Points without bars include SD. Whole uterus (△), endometrium (●——●) and myometrium (○——○).

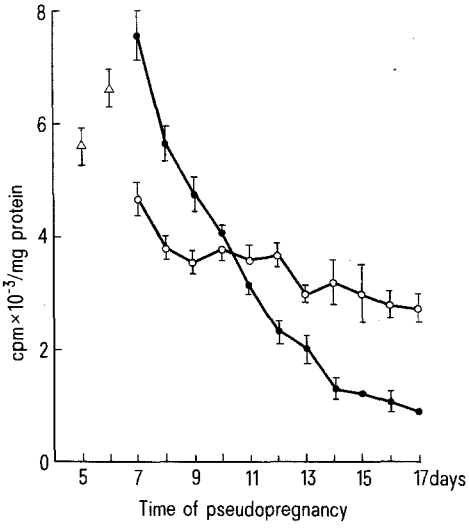

Fig. 2. Incorporation, in vivo, of [^{14}C]-leucine into proteins of rat deciduoma for 1 h. Each point represents mean ± SD of specific activity from 8–12 animals. Points without bars include SD. Whole uterus (△), endometrium (●——●) and myometrium (○——○).

Results on incorporation of the labelled leucine for 1 h into proteins of endometrium and myometrium as a function of growth and regression of deciduoma are presented in figure 2. The data show that the specific activity of proteins of endometrium and myometrium is highest at 2 days post-induction. The rate of protein synthesis falls steadily and markedly in the endometrium as the deciduoma ages. Myometrial protein synthesis, on the other hand, shows a reduction initially but remains fairly steady subsequently. Labelling of endometrial and myometrial proteins of deciduoma (2 days post-induction) studied as a function of time after injection of the [^{14}C]-leucine shows that the incorporation increases steadily up to a period of 2 h (figure 3).

Administration of cycloheximide to rats 2 days post-decidualization results in 95% inhibition of endometrial as well as myometrial protein synthesis within a period of 2 h (table 1).

Discussion. To understand the intricate biochemical mechanisms operating in the uterus during pre-implantation stages, the artificially induced decidual cell reaction serves as a useful tool[3]. This process of decidualisation, encompassing growth and differentiation of endometrium, is hormone-dependent[8]. Accompanying the differentiation of stromal cells into decidual cells are a variety of biochemical changes such as increased RNA[9,10], DNA[11,12] and proteins[11,13]. Results of the present investigation demonstrate that changes with respect to protein synthesis are quite pronounced in the endometrium when compared with those of myometrium during the growth and regression of deciduoma. It was not possible to separate endometrium from myometrium before day 7 of pseudopregnancy (2 days after deciduoma induction). However, it is apparent that the heterogenous endometrial cells are in an enhanced state of proliferation and growth following the stimulus. Fractions of deciduomal cells taken from animals 2 days post trauma employing velocity sedimentation have been shown by Moulton and Blaha[14] to have higher rates of DNA, RNA and protein synthesis. It is noteworthy that the amount of endometrial alkaline phosphatase, one of the markers of decidualisation, is high during this period[5].

Although endometrium plays an important role with respect to ova implantation by undergoing cellular prolifera-

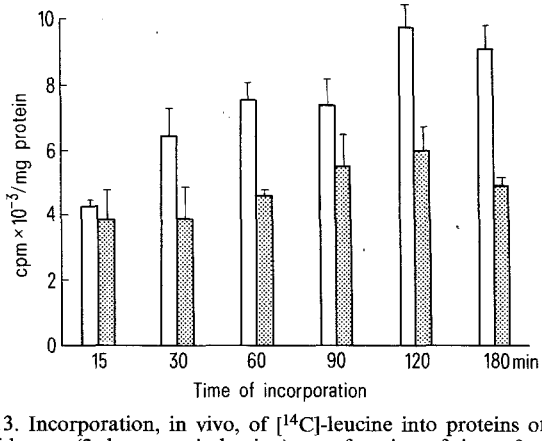

Fig. 3. Incorporation, in vivo, of [^{14}C]-leucine into proteins of rat deciduoma (2 days post induction) as a function of time after the amino acid injection. Histogram represents mean ± SD of specific activity of endometrial (unshaded) and myometrial (shaded) proteins from 5 animals.

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.

1 Permanent address: R. Ruia College, Bombay 400 019.
2 P.J. Heald, J. Reprod. Fert., Suppl. *25,* 29 (1976).
3 V.S. DeFeo, in: Cellular Biology of the Uterus, p. 191. Ed. R.M. Wynn. Appleton, New York 1967.
4 C.A. Finn and D.G. Porter, in: The Uterus. Elek Science, London 1975.
5 U. Tarachand and P.J. Heald, Biol. Reprod. *20,* 617 (1979).
6 U. Tarachand and J. Eapen, Am. J. Obstet. Gynec. *114,* 62 (1972).
7 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193,* 265 (1951).
8 S.R. Glasser and J.H. Clark, in: The Developmental Biology of Reproduction, p. 311. Ed. C.L. Markert and J. Papaconstantinou. Academic Press, New York 1975.
9 M.C. Shelesnyak and L. Tic, Acta endocr. *42,* 465 (1963).
10 B.G. Miller, J. Endocr. *59,* 275 (1973).
11 B.E. Ledford, J.C. Rankin, R.R. Markwald and B. Baggett, Biol. Reprod. *15,* 529 (1976).
12 R.M. Das and L. Martin, J. Reprod. Fert. *53,* 125 (1978).
13 S.C. Bell, Biol. Reprod. *20,* 811 (1979).
14 B.C. Moulton and G.C. Blaha, Biol. Reprod. *18,* 141 (1978).
15 L. Martin, J. Reprod. Fert. *55,* 135 (1979).
16 U. Barkai and P.F. Kraicer, Int. J. Fert. *23,* 106 (1978).
17 L.I. Rothblum, T.M. Devlin and J.J. Ch'ih, Biochem. J. *156,* 151 (1976).
18 U. Tarachand and J. Eapen, Strahlentherapie *147,* 97 (1974).

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