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# Ornithine Decarboxylase and Polyamines in Liver and Kidneys of Rats on Cyclical Regimen of Protein-Free and Protein-Containing Diets

## RELATIONSHIP TO DEOXYRIBONUCLEIC ACID SYNTHESIS IN LIVER

By DONALD C. FARWELL, JOSE B. MIGUEZ and EDWARD J. HERBST
*Department of Biochemistry, University of New Hampshire, Durham, NH* 03824, *U.S.A.*

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1. The activity of ornithine decarboxylase in the liver and kidneys of rats maintained on a cyclical regimen of protein-free and protein-containing diets was investigated. There was a daily activation of the enzyme in response to the feeding of protein after 3 days feeding of protein-free diet. 2. The activation of ornithine decarboxylase in the liver and kidneys of rats re-fed on protein was demonstrable throughout 16 cycles of alternating 3-day periods of protein-free and protein-containing diets. The magnitude of the activation in the kidneys diminished from 20-fold stimulation in the first cycle to 5-fold stimulation (compared with animals fed with protein-free diet) in the later cycles of protein re-feeding. The activation of the enzyme in liver was decreased from 20-fold stimulation in the first cycle to approx. 10-fold stimulation in later cycles. 3. The concentration of spermidine was increased by approx. 50% in the liver of animals during cycling from protein-free to protein-containing diets. Spermine was unchanged, and putrescine was maintained at a low concentration approx. one-fifth to one-tenth that of spermidine after protein re-feeding. 4. The incorporation of [$^3$H]thymidine into liver DNA was increased 10-fold in animals re-fed with protein compared with animals receiving protein-free diets. 5. The activation of ornithine decarboxylase by re-feeding of protein was inhibited 90% by the injection of propane-1,3-diamine during re-feeding. The stimulation of DNA synthesis was inhibited 60% by multiple injections of propane-1,3-diamine during the re-feeding of protein.

The feeding of protein-supplemented diets after the maintenance of rats on protein-free diets has been reported to increase the activity of ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) in the liver (Gaza *et al.*, 1973). Similar responses were observed after re-feeding of rats with high-protein diets (60%, w/w, casein) for 2h after starvation for 22h (Pariza *et al.*, 1976) or re-feeding of pellet diets (30%, w/w, protein) after starvation for 16h (Hopkins *et al.*, 1972). Hayashi *et al.* (1972) studied diurnal changes of liver ornithine decarboxylase in rats fed on rat chow *ad libitum* and in starved rats. Enzyme activity was very low in starved rats at all times, but there was a slight activity peak 4h after the beginning of the dark period of the lighting cycle in fed rats. The diurnal activation of the enzyme was protein-dependent, since it was not induced in rats fed on a protein-free diet, and the extent of enzyme activation increased as the dietary protein was increased from 0 to 50%.

The induction of ornithine decarboxylase in cultured rat hepatoma cells also occurs after the addition of amino acids to nutritionally depleted cultures (Hogan, 1971), and a similar stimulation of the enzyme has been reported after the addition of fresh medium to other cell lines in culture (Bachrach, 1976*a*). Rat hepatoma-cell cultures were induced to proliferate by the addition of fresh medium, and the stimulation of DNA synthesis in the cells was prevented by α-methylornithine, an inhibitor of ornithine decarboxylase (Mamont *et al.*, 1976). The inhibition of hepatoma cell division by α-methylornithine could be reversed by the addition of putrescine, spermidine or spermine to the cell cultures. The reversal by spermidine or spermine of the inhibition of DNA synthesis by methylglyoxalbis(guanylhydrazone) {1,1′ - [(methylethanediylidene)dinitrilo]diguanidine} in concanavalin A-activated lymphocytes implicated another enzyme of polyamine biosynthesis, i.e. *S*-adenosylmethionine decarboxylase (*S*-adenosyl-L-methionine carboxy-lyase, EC 4.1.1.50) in DNA replication (Fillingame *et al.*, 1975).

The cyclical dietary regimen adopted in this investigation, i.e. the re-feeding of rats with a protein-containing diet after 3 days on a protein-free diet, was introduced by Short *et al.* (1973) as a means of inducing DNA replication in the liver. The stimulation of DNA synthesis in the liver by the nutritional shift and the increased DNA replication after partial hepatectomy occurred on similar time courses, and

Gaza *et al.* (1973) speculated that the early pre-replicative increases in ornithine decarboxylase activity in both intact and regenerating liver were related to hepatic DNA synthesis. Repeated injections of propane-1,3-diamine into rats after partial hepatectomy inhibited the stimulation of ornithine decarboxylase activity and prevented the increase in liver putrescine and spermidine concentrations that occurred after the operation (Pösö & Jänne, 1976). The inhibition of polyamine biosynthesis was accompanied by a sharp decrease in the synthesis of DNA in the regenerating liver. Heller *et al.* (1976) obtained evidence supporting the induction of a protein inhibitor of ornithine decarboxylase in the liver of rats after single or multiple injections of putrescine or spermidine. It seems likely that the inhibitory protein or 'ornithine decarboxylase antizyme' was involved in propane-1,3-diamine inhibition of the decarboxylase, and the concomitant inhibition of DNA synthesis, cited by Pösö & Jänne (1976), and the putrescine inhibition of ornithine decarboxylase in regenerating rat liver previously reported by the same laboratory (Jänne & Hölttä, 1974).

There exists an extensive literature (for reviews see Herbst & Bachrach, 1970; Russell, 1973; Tabor & Tabor, 1976; Clô *et al.*, 1976; Bachrach, 1976*b*) on the possible involvement of polyamines in normal and neoplastic growth processes. A specific role for ornithine decarboxylase and/or polyamines in the promotion by phorbol esters of skin tumours induced in mice by chemical carcinogens was proposed by O'Brien *et al.* (1975*a*,*b*). More recently, the induction of ornithine decarboxylase and the stimulation of DNA synthesis in epidermal skin cultures was demonstrated after the addition of phorbol esters to the culture medium (Yuspa *et al.*, 1976), and there was a good correlation between the 'promoter' activity of the phorbol esters and the stimulation of ornithine decarboxylase and DNA synthesis.

The stimulation of ornithine decarboxylase in liver and possibly other organs might have serious implications in the promotion of systemic tumours after exposure to environmental carcinogens. Intermittent stimulation of the decarboxylase over extended periods could be especially significant in view of the effectiveness of repetitive applications of phorbol esters in the promotion of skin tumours (O'Brien *et al.*, 1975*a*,*b*). The effects of repeated protein cycling on the ornithine decarboxylase activity and polyamine content of liver and kidney are described in the present paper.

## Experimental

### *Animals and diets*

Male Sprague–Dawley rats (80–100g) were obtained from Gofmoor Farms, Westboro, MA, U.S.A. The animals were maintained on pelleted diet [Charles River Mouse–Rat–Hamster Formula, 26% (w/w) protein] supplied *ad libitum* for 1 week before the initiation of the experimental feeding regimens. The lighting cycle was reversed (dark period 06:00–18:00h; light period 18:00–06:00h) to facilitate re-feeding and killing of animals in the dark period during the working day. Animals were housed in individual wire-bottomed cages for re-feeding and inhibitor studies in which short experimental periods (0–24h) were used. For long-term protein-cycling studies, four animals were maintained in each large wire-bottomed cage. The animal room was air-conditioned, humidity-controlled and windowless.

The protein-free diet consisted of (w/w) 89% dextrose, 5% Phillips–Hart Mineral Mixture (Teklad, Madison, WI, U.S.A.), 5% corn oil and 1% Vitamin Fortification Mix (Teklad). The protein-supplemented diet contained 25% casein (Teklad) substituted for dextrose in the above formula. (In some experiments, as indicated in Figure and Table legends, pelleted Charles River diet containing 26% protein was used instead of the defined 25%-casein diet.) Protein cycling was performed essentially as recommended by Gaza *et al.* (1973). The animals received protein-free diet *ad libitum* for 3 days. On day 4 at 06:00h (beginning of the dark period) protein-supplemented diet was provided for 8h, followed by starvation for 16h. The 8h fed–16h starved protocol was repeated each day for 3 days (this constituted one protein cycle) in experiments in which extended periods of protein cycling were used. Thus the protein-free diet for 3 days followed by the '8h–16h' protein diet protocol for 3 days etc. was followed throughout the experimental period of long-term feeding experiments.

### *Ornithine decarboxylase assay*

Ornithine decarboxylase was assayed on 100000*g* supernatants of liver and kidney 10% (w/w) homogenates prepared in 0.3M-sucrose containing 0.3mM-EDTA at 4°C. The enzyme activity was determined by measuring the release of $^{14}CO_2$ from DL-[1-$^{14}$C]-ornithine. Incubations were carried out in 12ml centrifuge tubes equipped with a rubber septum–polyethylene centre-well assembly (Kontes Glass Co., Vineland, NJ, U.S.A.) containing 50$\mu$l of NCS Solubilizer (Amersham/Searle Corp., Arlington Heights, IL, U.S.A.) on a 1cm$^2$ folded filter paper as a $CO_2$-trapping agent. Assays contained 50mM-Tris/HCl buffer, pH7.2, 10mM-EDTA, 1mM-dithiothreitol, 0.04mM-pyridoxal phosphate, 1mM-L-ornithine monohydrochloride containing 0.1$\mu$Ci of DL-[1-$^{14}$C]ornithine monohydrochloride (30mCi/mmol; New England Nuclear Corp., Boston, MA, U.S.A.) and 0.2ml of tissue supernatant in a final volume of 0.25ml. Incubations were carried out for 1h at 37°C, and the reaction was terminated and $CO_2$

was released from the assay medium by the addition of 0.25ml of 0.2M-$H_2SO_4$; the incubation was continued for a further 30min to ensure complete absorption of the $^{14}CO_2$ by the NCS trapping agent on the filter paper. The filter paper was transferred to scintillation vials containing 5ml of toluene/Omnifluor (New England Nuclear) and radioactivity was measured in a Packard Tri-Carb scintillation counter at 80% efficiency. All enzyme activities were corrected against a blank heated at 100°C for 10min and assayed as above.

*Polyamine determinations*

The analysis of polyamines was performed on 0.2M-$HClO_4$ extracts of liver and kidney. Homogenates of tissues in $HClO_4$ were extracted overnight in the cold, centrifuged for 10min at 2000*g* and the supernatants were removed and frozen until the analyses were carried out. The analyses of polyamines were performed by the modification of Seiler's dansyl procedure (Seiler & Wiechmann, 1967) previously described (Herbst & Dion, 1970; Wyatt *et al.*, 1973).

*[$^3H$]Thymidine incorporation into DNA*

The labelling of tissue DNA with [$^3$H]thymidine was determined after the intraperitoneal injection of 10–20$\mu$Ci of [*Me*-$^3$H]thymidine (New England Nuclear; 6.7Ci/mmol) in 0.5ml of sterile 0.9% NaCl at 1h before the animals were killed. Tissue (2g) was homogenized in 8ml of 0.2M-$HClO_4$, and the DNA was extracted from the precipitate after removal of RNA by a modified Schmidt–Thannhauser procedure (Threlfall *et al.*, 1966). The DNA content of the extracts was determined by the diphenylamine reaction (Burton, 1956) and radioactivity was measured in Aquasol counting fluid (New England Nuclear) in a Packard Tri-Carb liquid-scintillation counter.

*Protein determination*

The protein content of tissue supernatants in which ornithine decarboxylase was assayed was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard.

## Results

*Ornithine decarboxylase and polyamines in tissues after protein cycling*

The time course of the stimulation of ornithine decarboxylase activity in the liver and kidneys of rats after the feeding of protein-free diet for 3 days was investigated at 2h intervals after re-feeding of the protein-containing diet. The results obtained over an experimental period of 16h were compared with the enzyme activity in tissues of animals continued on the protein-free diet and in control rats fed on protein-containing diet *ad libitum*. The latter series of animals provided data from which the magnitude of the diurnal stimulation of ornithine decarboxylase (Hayashi *et al.*, 1972) could be compared with the stimulation of the enzyme by the protein-cycling regimen. The data are summarized in Fig. 1. The

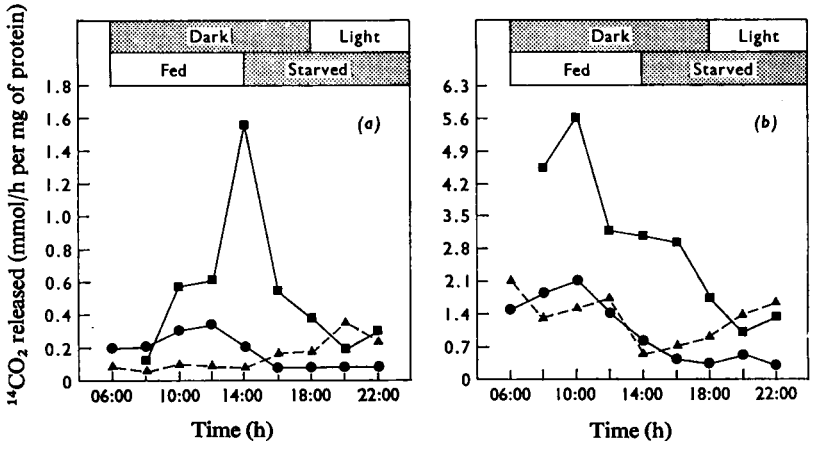


Fig. 1. *Time course of ornithine decarboxylase stimulation after re-feeding of protein*

The rats were cycled twice from protein-free to protein-containing diet (commercial Charles River pelleted diet, 26% protein) as described in the Experimental section. At the beginning of the second cycle of protein re-feeding (06:00h, in the dark period) and for 16h thereafter, animals from the group re-fed with protein (■), from the group continued on the protein-free diet (▲) and from control animals fed on the protein-containing diet *ad libitum* without cycling (●) were killed at 2h intervals and ornithine decarboxylase activity was measured in the liver (*a*) and the kidneys (*b*). The points are the mean activities of ornithine decarboxylase in tissues from four to nine animals.

enzyme activity was highest (8-fold higher than the diurnal stimulation) at 8h in the liver, whereas in the kidneys the activity peak occurred earlier (4h) and was 3-fold higher than the diurnal stimulation in rats fed on rat chow *ad libitum*.

Polyamines were determined on liver tissue of animals fed on protein-free diet and on tissue of animals re-fed with protein-supplemented diet, and these results are summarized in Table 1. There are increases of 50–100% in the tissue content of spermidine in animals fed on the protein diet, but these changes are difficult to interpret, since there is considerable variability in the spermidine concentration at different times. Spermine is essentially unchanged by the protein-cycling regimen and only traces of putrescine could be detected in the animals on the protein diet. The turnover of putrescine pools is apparently quite rapid and the diamine is converted into spermidine, as reflected in the higher polyamine concentration in the re-fed animals, in which traces of putrescine were detected.

There is an elevated activity of ornithine decarboxylase on each day of the 3-day period of re-feeding of protein after the 3-day protein-free diet cycle. The ornithine decarboxylase activity in liver is maintained at 8–10 times the activity in animals on the protein-free diet on each day of the protein-containing diet regimen (Table 2). The enzyme activity remains elevated in the kidneys on day 2 of protein re-feeding (8 times the activity in animals on the protein-free diet), but on day 3 it falls to approx. 4 times the activity in animals on protein-free diet. It might therefore be expected that daily oscillations of ornithine decarboxylase activity would occur in these tissues during each 3-day period of protein re-feeding in animals cycled for extended periods.

The effects of extended periods of protein cycling have been investigated during 16 cycles of alternate 3-day regimens of protein-free and protein-containing diets. The stimulation of ornithine decarboxylase activity in the liver and kidneys of rats 8h after the feeding of 25% casein diet is summarized in Fig. 2. The stimulation of the enzyme is highest in the two tissues after the first cycle, but there is substantial elevation of the activity at each cycle throughout the

Table 1. *Polyamine content of the liver of rats after protein cycling*

The rats were cycled twice from protein-free to protein-containing diet (commercial pelleted diet, 26% protein) as described in Fig. 1. The livers of rats re-fed with protein and from animals maintained on the protein-free diet were analysed for polyamines as described in the Experimental section. The polyamines were determined on pooled tissues of four rats at each time.

| Time (h) | Animal diet | Polyamines* (μmol/g of liver) Spermine | Spermidine |
|---|---|---|---|
| 06:00 | Protein-free | 0.643 | 0.955 |
| 10:00 | Protein-free | 0.667 | 0.875 |
| | 26% Protein | 0.883 | 1.229 |
| 12:00 | Protein-free | 0.519 | 0.582 |
| | 26% Protein | 0.504 | 0.770 |
| 14:00 | Protein-free | 0.586 | 0.840 |
| | 26% Protein | 0.556 | 1.160 |
| 16:00 | Protein-free | 0.443 | 0.619 |
| | 26% Protein | 0.574 | 1.061 |
| 18:00 | Protein-free | 0.413 | 0.567 |
| | 26% Protein | 0.555 | 1.018 |
| 20:00 | Protein-free | 0.439 | 0.928 |
| | 26% Protein | 0.565 | 1.055 |
| 22:00 | Protein-free | 0.508 | 0.755 |
| | 26% Protein | 0.546 | 0.939 |

* Traces of putrescine, insufficient for accurate quantitation by the analytical procedure, were detected in tissues of animals fed on the protein-containing diet.

Table 2. *Daily changes in ornithine decarboxylase and polyamines after re-feeding of protein*

The rats were cycled twice from protein-free to 25%-casein diet. At the beginning of the second cycle of protein-containing diet, animals were killed and tissues were analysed for ornithine decarboxylase and polyamines. Animals were killed 8h after re-feeding on days 1, 2 and 3 of the protein-re-feeding cycle (8h feeding and 16h starvation). The numbers of animals are indicated in parentheses. Ornithine decarboxylase activity is the mean±S.E.M. Polyamine values were obtained on pooled tissues of animals in each group.

| | Ornithine decarboxylase (pmol of $CO_2$ per h/mg) Liver | Kidney | Polyamines (μmol/g of liver) Spermine | Spermidine | Putrescine |
|---|---|---|---|---|---|
| Day 1 | | | | | |
| Protein-free (6) | 12±4.7 | 540±175 | 0.382 | 0.598 | — |
| 25% Casein (6) | 1240±174 | 5260±492 | 0.285 | 0.549 | 0.028 |
| Day 2 | | | | | |
| 25% Casein (4) | 1170±221 | 4620±234 | 0.336 | 0.772 | 0.067 |
| Day 3 | | | | | |
| 25% Casein (4) | 924±171 | 2180±878 | 0.509 | 0.741 | 0.176 |

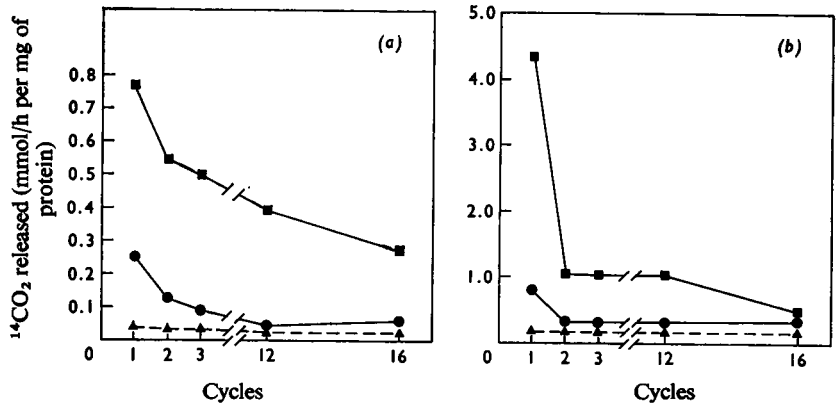


Fig. 2. *Activation of ornithine decarboxylase during cycles of protein re-feeding for an extended period*
Rats were cycled from protein-free to 25%-casein diet. Each cycle included 3 days *ad libitum* feeding of protein-free diet, followed by 3 days feeding of protein-containing diet on the '8h fed–16h starved' schedule illustrated in Fig. 1. At the end of the 8h protein re-feeding period in day 1, animals were killed and ornithine decarboxylase was assayed in the liver (*a*) and kidneys (*b*) of re-fed rats (■), in tissues from animals continued on the protein-free diet (▲) and in tissues from control rats fed on 25%-casein diet *ad libitum* (●). Each point is the mean of the enzyme activity in the tissues from four rats.

experimental period. The enzyme activity is extremely low in the tissues after each cycle of protein-free diet regardless of the duration of the nutritional cycling regimen. Animals fed on the 25%-casein diet *ad libitum* were included throughout the experimental period to correct for any diurnal elevation of ornithine decarboxylase activity at the time of death (8h after the beginning of the dark cycle). Tissues from these diurnal controls contained higher enzyme activity than tissues from animals on the protein-free diet but only 25% of the activity of the tissues from animals re-fed with the protein-containing diet.

*Inhibition of ornithine decarboxylase in protein-cycled animals by injection of putrescine or propane-1,3-diamine*

The inhibition of ornithine decarboxylase in regenerating liver by the injection of propane-1,3-diamine has been reported (Pösö & Jänne, 1976). In a related investigation, the induction of a specific inhibitory protein, ornithine decarboxylase 'antizyme', in the liver of rats injected with putrescine or spermidine was reported by Heller *et al.* (1976). The 'antizyme' was induced in both unstimulated control rats and in dexamethasone-stimulated rats. It was therefore decided to investigate the effect of the injection of a diamine on the induction of ornithine decarboxylase activity by protein cycling. The injection of propane-1,3-diamine (750μmol/kg) into 'unstimulated' animals (on protein-free diet for 3 days), or into animals in which the enzyme was stimulated by re-feeding of protein, severely inhibited the enzyme activity in tissues of animals from both experimental groups (Table 3). The effect of injections of putrescine was less pronounced, but this diamine also decreased ornithine decarboxylase activity in tissues of rats from both groups.

Table 3. *Inhibition of ornithine decarboxylase activity in rat tissues by diamines*
Animals fed on protein-free diet for 3 days or cycled from protein-free diet to 25%-casein diet were injected with 0.9% NaCl, putrescine (750μmol/kg) or propane-1,3-diamine (750μmol/kg) in 0.9% NaCl intraperitoneally. Injections were made at 2h and at 6h after re-feeding of the protein-containing diet at the beginning of the dark period, and the animals were killed at 8h after re-feeding. The animals on the protein-free diet were injected with 0.9% NaCl, putrescine or propane-1,3-diamine at comparable times (2 and 6h after the start of the dark period) and were killed at 8h. The numbers of animals are indicated in parentheses and the ornithine decarboxylase activity is the mean±S.E.M.

| | Ornithine decarboxylase (pmol of $CO_2$/h per mg) | |
|---|---|---|
| | Liver | Kidney |
| Protein-free (0.9% NaCl) (4) | 7±3 | 620±202 |
| Protein-free (putrescine) (4) | 2±0.4 | 25±11 |
| Protein-free (propane-1,3-diamine) (4) | 0 | 0 |
| 25% Casein (0.9% NaCl) (10) | 467±57 | 3900±884 |
| 25% Casein (putrescine) (8) | 269±56 | 863±266 |
| 25% Casein (propane-1,3-diamine) (4) | 51±28 | 82±18 |

*Stimulation of DNA synthesis in liver by protein cycling*

Short *et al.* (1973) have studied the stimulation of DNA synthesis in the liver of intact rats after a nutritional shift from a protein-free to a protein-containing diet. At least 3 days on a protein-free diet were required for a maximal response to the re-feeding of protein, and the greatest incorporation of [$^3$H]-thymidine into liver DNA occurred at 16–17h after the shift to the protein diet. We have studied the time course of the incorporation of [$^3$H]thymidine into liver DNA under our conditions of protein cycling. The magnitude of the response of DNA replication to the feeding of protein for 8h followed by 16h starvation is very similar to the stimulation of DNA synthesis reported by Short *et al.* (1973) in animals fed on protein-containing diet *ad libitum* after 3 days on a protein-free diet (Fig. 3). The incorporation of [$^3$H]thymidine into liver DNA was increased approx. 10-fold over the low rate of DNA replication in animals on the protein-free diet. Animals fed on the commercial pelleted diet (26% protein) *ad libitum* incorporated more [$^3$H]thymidine into DNA at all times compared with controls on the protein-free diet, but the incorporation by the protein-cycled animals was 4 times as much at 17h.

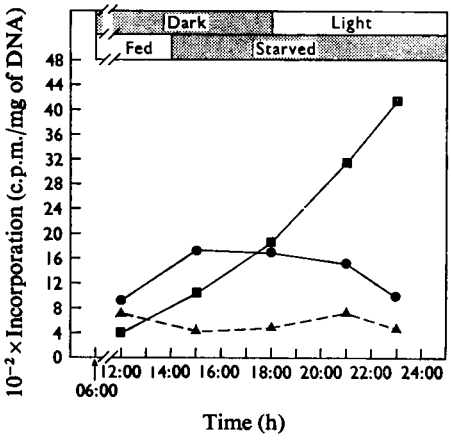


Fig. 3. *Time course of DNA synthesis in liver after re-feeding of protein*

Rats were cycled twice from protein-free to protein-containing diet (commercial pelleted diet, 26% protein) as described in Fig. 1. During the first day of the second cycle of protein re-feeding, animals from the group re-fed with protein (■), from the group continued on the protein-free diet (▲) and from control animals fed on the protein-containing diet *ad libitum* without cycling (●) were killed at intervals. At 1h before the death of each animal, 10$\mu$Ci of [$^3$H]thymidine in 0.5ml of 0.9% NaCl was injected intraperitoneally. The liver DNA was prepared and incorporation of [$^3$H]thymidine into DNA was measured as described in the Experimental section. Each point is the mean of the incorporation of [$^3$H]-thymidine into the liver DNA of four rats.

Table 4. *Inhibition of DNA synthesis in rat liver by propane-1,3-diamine*

Animals were cycled twice from protein-free to 25%-casein diet. At intervals of 3, 6, 9 and 12h after re-feeding at the beginning of the dark period at 06:00h (or at the same times for animals continued on the protein-free diet) animals were injected with 0.9% NaCl. At 15h after the beginning of the dark period, all animals received 20$\mu$Ci of [$^3$H]thymidine in 0.5ml of 0.9% NaCl intraperitoneally. At 1h after the injection of [$^3$H]thymidine, all animals were killed, liver DNA was prepared and the incorporation of [$^3$H]-thymidine was measured as described in the Experimental section. The incorporation data are the means±S.E.M. for four animals.

| | [$^3$H]Thymidine incorporated (c.p.m./mg of DNA) |
|---|---|
| Protein-free (0.9% NaCl) | 2853±359 |
| 25% Casein (0.9% NaCl) | 12380±706 |
| Protein-free (propane-1,3-diamine) | 1514±415 |
| 25% Casein (propane-1,3-diamine) | 4817±1167 |

*Inhibition of DNA synthesis in the liver of protein-cycled animals by propane-1,3-diamine*

Pösö & Jänne (1976) were able to decrease the incorporation of [$^3$H]thymidine into the DNA of regenerating liver by the injection of propane-1,3-diamine at 3h intervals after partial hepatectomy. The injections also prevented the stimulation of ornithine decarboxylase and decreased the enrichment of the regenerating liver with spermidine that accompanied the elevation of the decarboxylase activity. The decrease in liver spermidine was the result of the decreased production of putrescine and the inactivity of propane-1,3-diamine as a polyamine precursor. We have tested the experimental procedures of Pösö & Jänne (1976) as a means of inhibiting DNA synthesis in the liver of protein-cycled rats. The animals were injected with propane-1,3-diamine at 3, 6, 9 and 12h after cycling from a protein-free to a protein-containing diet. The stimulation of DNA synthesis in the liver after the re-feeding of protein was decreased by about 60% by the administration of the inhibitor of ornithine decarboxylase (Table 4).

## Discussion

The nutritional alteration of ornithine decarboxylase activity in the liver and kidneys of experimental animals provides a convenient method to investigate

the significance of this enzyme in the metabolism of these organs in health and disease. There is evidence that repetitive stimulation of ornithine decarboxylase in the skin of mice is a factor in the promotion of tumours initiated by a single subcarcinogenic application of 7,12-dimethylbenz[*a*]anthracene (O'Brien *et al.*, 1975*a*,*b*). The molecular mechanisms of the initiation and promotion of tumours resulting from the exposure of animals to chemical carcinogens are not defined, and a possible linkage between a regulatory enzyme and either stage of tumorigenesis is potentially applicable to the elucidation of these mechanisms. There is currently no firm evidence that the modification of ornithine decarboxylase activity in liver or other organs contributes to the induction or the ultimate development of malignant disease of these tissues. Bachrach (1976*b*) has reviewed evidence suggesting that polyamines are 'chemical markers of malignancy'. Although there are reports showing that ornithine decarboxylase activity is elevated in transplanted rat hepatomas (relative to the activity in the liver) and that the enzyme increases with hepatoma growth rate (Williams-Ashman *et al.*, 1972), there is also evidence that these correlations do not occur consistently. Pariza *et al.* (1976) point out that there are conflicting data from several laboratories in which the same hepatoma, e.g. hepatoma 7800, has been studied and that the combined evidence was not in conformity with the generalization that the activity of ornithine decarboxylase is proportional to tumour growth rate. It is difficult, furthermore, to interpret the apparent differences between the actively proliferating cells of the transplanted hepatomas and the normal non-dividing adult liver cells.

The elevation of ornithine decarboxylase activity in the liver and kidneys after the feeding of protein to animals deprived of dietary protein for relatively short periods provides an experimental model in which the consequences of such nutritional shifts can be tested. The elevation of ornithine decarboxylase activity, accompanied by an increased replication of DNA in the liver induced by protein re-feeding, can be decreased by the administration of putrescine or propane-1,3-diamine. Thus the experimental system includes a means of 'turning on' as well as inhibiting two metabolic phenomena (ornithine decarboxylase activation and DNA replication) that have been implicated in both the proliferation of normal cells in regenerating liver (Pösö & Jänne, 1976) and the proliferation of tumour cells initiated by a chemical carcinogen (O'Brien *et al.*, 1975*a*,*b*). The proliferative stimulus of partial hepatectomy has also been used to increase the incidence of liver tumours in rats that received a single injection of *N*-dimethylnitrosamine (Craddock, 1971), and these results suggest that replicating cells are especially sensitive to the action of carcinogens.

Thus the phenomena of elevated ornithine decarboxylase activity and DNA replication triggered by nutritional cycling of protein might affect the vulnerability of the liver and other organs to carcinogens. Dietary habits that severely restrict protein intake for short periods followed by the ingestion of high-protein meals would probably induce the enzymic changes that have been produced experimentally in rat tissues. It will be of interest therefore to examine carefully the impact of dietary protein cycling on the initiation and promotion of systemic tumours induced by chemical carcinogens that have been detected in the human environment.

Regardless of the relevance of the dietary stimulation of ornithine decarboxylase to carcinogenesis, the modulation of the activity of this enzyme by protein in the diet may affect the biosynthesis of nucleic acids and protein in liver and other tissues. Polyamines increase the activity of RNA polymerases and stimulate protein and DNA synthesis in a variety of experimental systems (Tabor & Tabor, 1976). An increase in the concentration of tissue polyamines would be expected to accompany stimuli to ornithine decarboxylase, the first enzyme in the biosynthetic pathway to polyamines. The stimulation of ornithine decarboxylase activity, in response to protein replenishment after starvation or dieting, might thus be a significant regulatory mechanism in the utilization of dietary protein for growth or tissue repair processes.

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