Pourcentage de mitoses marquées en fonction du temps dans les différents stades

	1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h	9 h	10 h	11 h	12 h	13 h
E 16	–	3:113 (2,65%)	–	78:142 (54,93%)	–	117:131 (89,31%)	–	119:125 (95,20%)	–	39:117 (33,33%)	–	33:104 (31,73%)	–
E 18	0:110 (0%)	7:123 (5,69%)	51:121 (42,15%)	69:109 (63,30%)	114:118 (96,61%)	103:107 (96,26%)	125:140 (89,28%)	–	33:112 (29,46%)	–	28:119 (23,53%)	–	–
E 20	0:121 (0%)	–	62:123 (50,41%)	–	87:113 (76,99%)	–	95:106 (89,62%)	–	52:110 (47,27%)	–	74:170 (43,52%)	–	37:151 (24,50%)
E 22	0:184 (0%)	–	74:160 (46,25%)	–	110:132 (83,33%)	–	104:116 (89,65%)	–	34:108 (31,48%)	–	30:114 (26,32%)	–	26:105 (24,76%)
E 24	0:192 (0%)	16:152 (10,53%)	176:394 (44,67%)	–	103:125 (82,40%)	–	176:198 (88,89%)	–	60:197 (45,69%)	–	43:174 (26,43%)	–	31:122 (25,41%)

sont marqués, c'est donc dans cette position là qu'ils réalisent la duplication du ADN. Egalement il n'y a aucun noyau marqué dans la proximité de la lumière.

En fonction du temps écoulé après la période de la captation, les mitoses marquées commencent à se montrer dans une position luminale et au bout de 2 h, le pourcentage de celles-ci est de 2 à 10% (tableau). Entre 3 et 8 h il monte à 96%.

Pendant les heures suivantes il y a une diminution brusque au nombre de noyaux radioactifs dans la région luminale de l'épithélium (tableau). Les figures 1 et 2 (stades 18 et 24) ont été réalisés avec les données du tableau.

Les courbes obtenues permettent de calculer graphiquement la durée des phases G_2, M et S du cycle cellulaire par la méthode de Quastler et Sherman[9], dans lequel le pourcentage des figures mitotiques après 1 h de captation de thymidine H^3 se représente en fonction du temps. Les valeurs obtenues sont de 2 h G_2, entre 5 et 6 h S et 2 h M.

Discussion. Les différentes phases étudiées du cycle cellulaire (G_2, M et S) ne se modifient pas dans l'épithélium olfactif chez l'embryon de stades 16 à 24[7], et il n'existe pas de prolongement du même en fonction de l'âge de l'embryon, comme il a été montré dans le système nerveux[3].

Les noyaux font la synthèse du ADN, pendant 5 à 6 h, dans la portion basale de l'épithélium pseudostratifié, comme dans d'autres ébauches ectodermiques[3,10,6]. Après la duplication du ADN les noyaux radioactifs émigrent vers la lumière, ce qui nous explique l'apparition dans cette position de mitoses marquées.

La durée de cette migration est de 2 h, temps égal à la phase G_2 et semblable à celle qui a éte trouvée par d'autres auteurs[3,6]. Les différentes phases de la mitose se font toujours au niveau de la lumière de l'ébauche, leur durée est de 2 h.

La non-modification des différentes phases du cycle cellulaire, dans les stades étudiés, nous a permis du vérifier qu'au debut, la morphogènese de la placode nasale est un problème de croissance alométrique de ses parois, ce qui nous explique sa morphologie[11].

D'une autre part, la signification de cette migration intercinétique a été mise en relation avec les mécanismes morphogénétiques qui conduisent à l'invagination du tube nerveux[3] et de la placode optique[10,12,13].

La diminution de mitoses marquées à partir de 9 h indique que les noyaux, une fois la mitose réalisée, retournent à leur situation périphérique, où ils restent en repos intercinétique ou bien commencent un nouveau cycle.

Figure 3 résume les mouvements de la migration nucléaire intercinétique que réalisent les noyaux des éléments cellulaires de la placode olfactive.

10 J. Zwaan, P. R. Bryan et T. L. Pearce, J. Embryol. exp. Morph. *21*, 71 (1969).
11 E. Campelo, Thesis Doctoral, Univ. Valladolid (1976).
12 J. Zwaan et R. W. Hendrix, Am. Zool. *13*, 1039 (1973).
13 R. W. Hendrix et J. Zwaan, Nature *247*, 145 (1974).

Effect of propylthiouracil on intestinal tumor formation by azoxymethane in rats[1]

D. V. Singh[2,3], R. L. Campbell, Y. N. Lin and N. D. Nigro

Department of Surgery, Wayne State School of Medicine, Detroit (Michigan 48201, USA), 10 January 1977

Summary. Treatment with propylthiouracil (PTU) resulted in a significant decrease in azoxymethane-induced intestinal tumors, total concentration of fecal bile acid as well as the fecal neutral steroids, cholesterol and coprostanol. Thus, a hypothyroid state induced by PTU treatment may affect intestinal carcinogenesis in this animal model by lowering the concentration of fecal bile acids and neutral steroids.

There has been speculation for many years that thyroid hormone may affect neoplastic transformation[4–7]. Thyroid hormone is also known to affect cholesterol metabolism[8]. It has been shown that when rats are given thyroxin, the secretion of bile acids from the liver is increased[9–12]. Hypothyroidism, on the other hand, diminishes the secretion of bile acids[11,12].

It has been shown that high concentrations of bile acids in feces enhances intestinal tumor formation in rats given 1,2-dimethylhydrazine or azoxymethane[13,14]. Rats in an induced hypothyroid state might develop fewer intestinal tumors when given one of these carcinogens due to decreased amounts of luminal bile acids. The purpose of this experiment was to test this hypothesis by inducing

intestinal tumors with azoxymethane in rats given propylthiouracil, a hypothyroid inducing agent.

Material and methods. 40 male Sprague-Dawley rats, 8–10 weeks old, purchased from Sparton Research Animals, Inc., Haslett, Michigan, and weighing 250 g, were used. They were divided in 2 groups, 1 group A of 20 rats were fed a normal diet (Purina rat chow) and the other group B of 20 rats were fed the same diet containing 0.05% 6-N-propyl-2-thiouracil (PTU, Sigma Chemical Co.) by weight of the feed.

All rats were caged individually, and given water ad libitum. Food consumption of individual rats was measured once for a 5-day period during the experiment. Metabolism cages were constructed to prevent coprophagy and food waste. Azoxymethane was obtained from Ash Stevens Company of Detroit and was prepared as an aqueous solution. All animals were injected s.c. 8 mg/kg per week until they were sacrificed. The experiment was terminated at 24 weeks. A complete necropsy was done on all rats, and the size and location of intestinal tumors was recorded. A total of 60 tumors was examined histologically, 30 from each of the 2 groups of animals. For comparison, 15 small tumors, each about 0.5 cm in diameter, and 15 large ones, each about 1.0 cm in diameter, were chosen from representative levels of the intestinal tract. The tissues were fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin and eosin. Blood was collected from the orbital fossa at different time intervals and the level of L-Thyroxin (L-T_4) in the serum was determined colormetrically using an L-T_4 kit (Oxford Laboratories). Feces were collected from each animal in each group over a 48-h period 2 weeks before the end of the experiment. The material from 2 animals was combined for each sample and frozen until analyzed. The bile acid and neutral steroid composition was determined as previously described[15]. The data are expressed as mean $\pm$ SE and were analyzed with the use of the Student's t-test[16].

Results. The initial body weights in groups A and B were same, but the mean final body weights in group A (control) was 434.0 $\pm$ 17.2 g, whereas in group B (experimental) it was 236.0 $\pm$ 5.6 g, significantly lower ($p < 0.0005$). Group B ate 16.99 $\pm$ 0.94 g per rat/day whereas group A consumed 26.28 $\pm$ 1.75 g per rat/day ($p < 0.005$). However, on a body weight basis group B consumed more food, 7.22 $\pm$ 0.30 g/100 g b.wt as compared to group A, 5.74 $\pm$ 0.34/100 g b.wt ($p < 0.025$).

Composition of bile acids and neutral steroids in feces

Bile acid	Normal diet (group A)	Normal diet + PTU (group B)	P*
Lithocholic	0.15 $\pm$ 0.2**	0.13 $\pm$ 0.02	NS
Hyodeoxycholic	1.14 $\pm$ 0.09	0.37 $\pm$ 0.04	< 0.0005
Deoxycholic	1.35 $\pm$ 0.12	0.98 $\pm$ 0.15	< 0.05
Chenodeoxy	0.22 $\pm$ 0.03	0.11 $\pm$ 0.02	< 0.01
12-ketolithocholic	0.39 $\pm$ 0.04	0.33 $\pm$ 0.05	NS
Cholic	0.64 $\pm$ 0.06	0.21 $\pm$ 0.04	< 0.005
Total	3.89 $\pm$ 0.25	2.13 $\pm$ 0.26	< 0.0005
Neutral Steroid			
Cholesterol	1.40 $\pm$ 0.16**	1.01 $\pm$ 0.14	< 0.05
Coprostanol	1.77 $\pm$ 0.20	1.11 $\pm$ 0.15	< 0.025
Coprostanone	0.02 $\pm$ 0.004	0.01 $\pm$ 0.003	NS
Total	3.19 $\pm$ 0.33	2.13 $\pm$ 0.22	< 0.01

* Student's t-test, level of significance. ** Bile acid and neutral steroids expressed in mg/g dry feces.

At the beginning of the experiment the concentration of serum L-T_4 was 1.71 $\pm$ 0.22 µg/100 ml in group A and 1.50 $\pm$ 0.17 µg/100 ml in group B. After 8 weeks this concentration was 2.81 $\pm$ 0.24 µg/100 ml in group A, 1.79 $\pm$ 0.11 µg/100 ml in group B ($p < 0.01$), while at 16 weeks, it was 0.97 $\pm$ 0.02 µg/100 ml in group A and 0.83 $\pm$ 0.04 µg/100 ml in group B ($p < 0.01$). At the end of the experiment of the concentration of L-T_4 was lower in both groups as compared to the beginning of the experiment, but there was no significant difference between the groups. The thyroid glands of the PTU-treated rats were 4–8 times larger than those of the control animals. This is a further indication of hypothyroidism in group B animals. All rats developed intestinal tumors except 1 animal in group B. The total number of tumors in the 20 rats of group A was 167 (average 8.35 $\pm$ 1.08 tumors/rat. The 20 rats in group B developed 68 intestinal neoplasms, an average of 3.40 $\pm$ 0.55 tumor/rat ($p < 0.005$).

The average number of tumors in the small intestine in group A was 2.50 $\pm$ 0.51, whereas it was 0.65 $\pm$ 0.22 ($p < 0.005$) in group B. The average number of tumors in the large intestine was 5.85 $\pm$ 0.82 in group A and 2.70 $\pm$ 0.42 in group B ($p < 0.005$). Abdominal carcinomatosis was present in 8 animals in group A whereas there were only 3 in group B. There was 1 rat with an ear tumor in group A but none in group B.

All 60 intestinal tumors examined histologically were adenocarcinoma regardless of size. Both the cellular changes and the degree of penetration of the tumor through the bowel wall were similar. In both groups the penetration through the bowel wall was much more frequent in the larger tumors as would be expected. However, PTU-treated rats had not only fewer tumors but they were smaller in size, and they consequently had less involvement of the peritoneal cavity.

Hyodeoxycholic, deoxycholic, lithocholic, 12-ketolithocholic, chenodeoxycholic, and cholic acids were identified as major fecal bile acids by gas-liquid chromatography. The total concentration (mg/g dry feces) of these bile acids was significantly decreased in group B as compared to group A ($p \pm 0.005$). Of the individual bile acids only the fecal concentrations of lithocholic and 12-ketolithocholic acids were the same in each group (table).

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2 To whom reprint requests should be sent.

3 Present address: Division of Toxicology (HFF-152), Food and Drug Administration, 200 'C' Street, S.W., Washington, D.C. 20204, USA.

4 F. Bielschowsky, and W. H. Hall, Br. J. Cancer *7*, 358 (1953).

5 S. C. Newman and R. C. Moon, Endocrinology *80*, 896 (1967).

6 W. C. Newman and R. C. Moon, Cancer Res. *28*, 864 (1968).

7 J. G. C. Spencer, Br. J. Cancer *8*, 393 (1974).

8 D. Kritchevsky, Giorn. Arterioscl. *2*, 175 (1967).

9 W. T. Beher, M. E. Beher and G. Semenuk, Metabolism *15*, 181 (1966).

10 A. Vanlyle, J. Endocr. *16*, 213 (1957).

11 J. C. Thompson and H. M. Vars, Am. J. Physiol. *179*, 405 (1954).

12 O. Strand, J. Lipid Res. *4*, 305 (1963).

13 C. Chomchai, N. Bhardrachari and N. D. Nigro, Dis. Colon Rectum *17*, 310 (1974).

14 N. D. Nigro, N. Bhadrachari and C. Chomchai, Dis. Colon Rectum *16*, 438 (1973).

15 N. D. Nigro, R. L. Campbell, D. V. Singh and Y. N. Lin, J. nat. Cancer Inst. *57*, 883 (1976).

16 W. Beyer, Handbook of Tables for Probability and Statistics, 2nd ed. Chemical Rubber Company, Cleveland, Ohio 1968.

The total concentration of fecal neutral steroids (cholesterol + coprostanol + coprostanone) in group A was 3.19 ± 0.33 mg/g dry feces and in group B, 2.13 ± 0.22 mg/g dry feces ($p < 0.01$, table). Furthermore, the concentrations of coprostanol and cholesterol were significantly lower in group B than group A. There was no significant difference between the groups in fecal coprostanone concentration.

Discussion. A number of reports have been presented concerning the depression of thyroid function with advancing age of laboratory animals[17]. Our results are similar to those reported that the serum concentration of L-T_4 declined during the period of the experiment in both animal groups. During the first 4 months of the experiment, however, animals given PTU were hypothyroid with respect to the control group.

The administration of PTU for 24 weeks to rats given weekly doses of azoxymethane significantly decreased the carcinogenic effect of this compound. The hypothyroid rats developed less intestinal cancers, smaller in size and less malignant as indicated by the decreased metastatic spread of the disease. A decrease in thyroid hormone level may affect intestinal carcinogenesis in a variety of ways. The amount and nature of an animal's diet have been found to influence the genesis of chemically induced and transplanted tumors[18]. In our experiment less total food was consumed per rat and body growth was virtually arrested in rats ingesting PTU. Thus the reduced carcinogenic effect of azoxymethane may be due to a restriction in total calories in the PTU-treated rats. However, these animals consumed more food per 100 g b.wt than did the normal group. The relationship between tumor incidence and total and/or relative calorie-intake is a concept that requires much further study. On the other hand, recent evidence has indicated that L-T_4 is directly mitogenic to certain cell lines in vitro[19], while a reduction of growth by lowered serum L-T_4 can be demonstrated for an estrogen-dependent rat pituitary tumor[20]. Thus, it could be that the hypothyroid state has affected the intestinal cell proliferation in a manner reducing the susceptibility of the mucosa to neoplastic transformation[21] or the growth rate of the developing tumors.

Treatment with PTU also resulted in a significant decrease in the total concentration (mg/g dry feces) of the major fecal bile acids as well as in the neutral steroids, cholesterol and coprostanol. Earlier investigations have indicated that increased fecal concentrations of bile acids enhance the carcinogenic affect of azoxymethane[13,14]. Certain bile acids have been found to promote the carcinogenic effect of N-methyl-N'-nitro-N-nitrosoguanidine in colonic mucosa[22,23]. On the other hand, fewer tumors were observed in non-functional large bowel segments where luminal bile acids were absent[24].

It cannot be ascertained from the present data whether lower thyroid hormone levels affected intestinal carcinogenesis directly or if the effect were mediated by the influence of the hormone upon other physiological functions. In view of previous findings, however, the effect of the thyroid hormone on liver bile acid metabolism, fecal bile acid concentrations or intestinal tumor growth rates may be the most important factors to explore in this animal model.

17 P. Kumaresan and C. W. Turner, Proc. Soc. Biol. Med. *124*, 752 (1967).
18 D. B. Clayson, Cancer Res. *35*, 3292 (1975).
19 W. L. Kirtland, J. M. Sorrentino and D. A. Sirbasku, J. nat. Cancer Inst. *56*, 1159 (1976).
20 J. M. Sorrentine, W. L. Kirkland and D. A. Sirbasku, J. nat. Cancer Inst. *56*, 1155 (1976).
21 W. Oehlert, Cell Tissue Kinet. *6*, 325 (1973).
22 T. Narisawa, N. E. Magadia and J. H. Weisburger, J. nat. Cancer Inst. *53*, 1093 (1974).
23 B. S. Reddy, T. Narasawa, J. H. Weisburger and E. L. Wynder, J. nat. Cancer Inst. *56*, 441 (1976).
24 R. L. Campell, D. V. Singh and N. D. Nigro, Cancer Res. *35*, 1369 (1975).

Changes in the thermal denaturation profiles of DNA from different developmental stages of the newt *Triturus vulgaris*

K. Lohmann and Lore Schubert

Zoologisches Institut der Universität Köln, Experimentelle Morphologie, Weyertal 119, D–5 Köln 41 (Federal Republic of Germany), 12 April 1977

Summary. The melting profiles of DNA samples from the early gastrula and early neurula of *Triturus vulgaris* are essentially the same, whereas DNA from mid to late gastrula possesses higher T_m values and shows a deviation from the regular sigmoidal shape at temperatures above T_m. The plot on normal probability paper indicates a second DNA fraction which melts at higher temperatures and, consequently, it has a higher GC-content than the bulk DNA. These facts confirm our idea that differential DNA replication occurs during gastrulation.

In early amphibian development, the RNA content of the embryo remains essentially constant. At the onset of gastrulation, a progressive enhancement of gene activity sets in[1]. In the same period, short-term variations in the nuclear DNA content in various regions of *Triturus vulgaris* embryos have been detected by cytophotometric measurements[2–4]. There are 2 facts which lead us to the conclusion that there might be a gene amplification process during gastrulation. 1. The above-mentioned changes in DNA content are correlated with the beginning of ribosomal RNA synthesis. 2. The increase in DNA content is accompanied by a considerable increase in nuclear RNA content[5]. In order to prove whether the genes for ribosomal RNA (rDNA), whose base composition differs from the bulk DNA, are amplified, we analyzed the melting behaviour of DNAs, which were isolated from different developmental stages.

Materials and methods. All studies were carried out with *Triturus vulgaris* embryos from early gastrula to early neurula stage (Harrison stage 10/11a, 12a/b, 15) which

1 E. M. Deuchar, Adv. Morph. *10*, 175 (1973).
2 K. Lohmann and W. Vahs, Experientia *25*, 1315 (1969).
3 K. Lohmann, Wilhelm Roux' Arch. *169*, 1 (1972).
4 K. Lohmann, Wilhelm Roux' Arch. *177*, 285 (1975).
5 K. Lohmann and U. Jansen, Experientia *32*, 380 (1976).