The enhanced enzyme specific activity suggests that ChE is synthesized by the fibroblasts in the presence of hypercholesterolemic serum. The increased enzyme activity does not appear to be derived from the incubation media since addition of horse serum ChE (1 mg/ml) to this medium did not result in increased enzyme activity in the cultured cells. The presence of ChE in the atheroslcerotic intima could also be due to synthesis of this enzyme by intimal cells stimulated by increased cholesterol ester and fatty acid synthesis. It has been suggested[5] that the principal biological function of ChE in the liver is to remove toxic butyrylcholine formed from butyryl CoA, an intermediate in fatty acid metabolism in mammals. A similar role for ChE in the reticuloendothelial system has also been proposed[15]. This proposed function of ChE is further supported by our findings of decreased cholesterol ester synthesis by neostigmine in the fibroblasts. It has also been suggested[16] that ChE acts as a tissue growth factor by maintaining ideal conditions for fatty acid metabolism. In the atherosclerotic intima, ChE may play a similar role in relation to lipid metabolism and smooth muscle cell proliferation.

Cholinesterase activity and 1-^{14}C acetate incorporation into the cholesterol ester in fibroblast cultures in presence of normal serum (NS); NS + cholinesterase (ChE); Hypercholesterolemic serum (HCS) and HCS + neostigmine (N)

Type and No. of experiments	Cholesterol ester (cpm/mg protein)	Cholinesterase units*/ mg protein/h
A NS (3)	1150± 230 (SEM)	370±98
B NS+HChE[1] (3)	1310± 150	498±30
C HCS (4)	12150± 950	792±77
D HCS+N (3)	7830±1370	454±56

* ChE units. nmoles of thiocholine formed/mg protein/h. [1]HChE-horse serum cholinesterase. Statistical analysis by Student's t-test. Cholesterol ester C vs A and B, $p < 0.001$; C vs D, $p < 0.01$. Cholinesterase - C vs A, B and D, $p < 0.001$.

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Induction of lymphomas by urethane in combination with diethylstilboestrol in CFLP mice[1]

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Summary. The combination of urethane and the non-steroidal oestrogen diethylstilboestrol induced lymphomas in CFLP mice.

It is known that synergism can be demonstrated in the leukemogenic effect of oestradiol and urethane repeatedly administered to C57BL mice[2]. In the present paper it is reported that urethane in combination with the non-steroidal oestrogen diethylstilboestrol (DES) induces lymphomas in CFLP mice of closed bred colony.

Materials and methods. 6–8 weeks old male CFLP, as well as newborn CFLP, BALB/c, C3H/He-mg, CBA/Ca and AKR mice, obtained from the Laboratory Animals Breeding Centre of Hungary (Gödöllö) were used. The adult CFLP mice were treated with a single dose of 1000 mg/kg urethane (Reanal, Budapest) i.p. and of 50 mg/kg DES (Gedeon Richter Ltd., Budapest) s.c. at 14 days interval or simultaneously. The autopsy of those animals which seemed to be ill was performed immediately, whereas the survivors were killed within 300 days. To transplant the lymphomas induced by combined treatment, the thymomas and the pathologic spleens were carefully homogenized with Potter-Elvehjem homogenizer in M 199 medium at 37 °C and filtrated through a 10-layered gauze. In all cases, 5×10^6 cells were transplanted i.p. into 10/10 newborn mice. For the light microscopy formalin fixation, paraffin embedding and hemalaun-eosin staining, whereas for electron microscopy glutaraldehyde and OsO_4 fixation, araldite embedding and uranyl acetate and lead citrate staining were applied. The ultrathin sections were studied with JEOL 100 B electron microscope.

Results and discussion. The autopsy of mice treated with urethane and DES revealed 2 tumour types. On the one hand, the combination of urethane + DES induced malignant lymphomas in some of the mice. Results are shown in the table. The difference of the frequency of lymphomas in experimental groups against the controls proved to be significant when DES was administered simultaneously with or after urethane treatment (χ^2-test, $p < 0.01$ and $p < 0.001$, respectively). However, between the effects of the sequential combinations, no significant difference was found in respect to frequency and average latency period of lymphomas. On the other hand, multiple lung tumours developed in all animals treated with urethane, irrespective of the administration of DES. The development of lung tumours was expected according to our previous results

Induction of lymphomas by urethane in combination with diethylstilboestrol in adult male CFLP mice

Treatment*	n	Animals with lymphomas	Average latency period of lymphomas (days ± SD)**
DES and after 14 days urethane	22	4 (18.1%)	172 ± 15.8
DES and urethane simultaneously	25	8 (32.0%)	161 ± 46.7
Urethane and after 14 days DES	25	11 (44.0%)	186 ± 70.3
Urethane	48	No lymphomas within 300 days	
DES	40	2 (5.0%)	On the 223rd and 229th day
Untreated controls	45	1 (2.2%)	On the 270th day

*Dose of urethane: 1000 mg/kg i.p., dose of DES: 50 mg/kg s.c.; **the survivors were killed and examined on the 300th day of the experiment.

which had shown that 1000 mg/kg of urethane could induce lung adenomas in CFLP mice in 11 weeks with a mean tumour number of 7.43 per animal[3].

The transplantation of the induced lymphomas into newborn CFLP mice was successfully performed in 6 cases in spite of the genetic heterogenicity of CFLP mice. The successive passage of lymphomas from CFLP mice into newborn BALB/c, C3H/He-mg, CBA/Ca and AKR mice was also positive.

All the induced and transplanted lymphomas seemed histologically to be lymphoblastic lymphosarcoma, a frequent type of malignant diseases of hematopoietic organs in mice[4,5]. The original structure of the thymus and lymph nodes completely disappeared due to tumour proliferation. The tumour cells were relatively monomorphic atypic lymphoblastic elements with large and rounded nuclei and prominent nucleolus, the cytoplasm margin was narrow. Several mitosis also occurred. Primarily in the thymus tumours, sporadic cells with wide clear cytoplasm reminescent of the 'starry sky' were observed. The tumour cells infiltrated the liver, spleen, kidney, bone marrow and even the lung adenomas sometimes. Electron microscopy also showed that the tumour cells correspond to lymphoblasts. In the induced thymomas, intracellular A type and extracellular C type virus particles could be observed in the transplanted lymphomas C type particles. Our morphological findings showed a good agreement with that of other chemically induced lymphomas in mice[6,7].

According to our results, urethane in combination with not only natural oestradiol but also with synthetic DES induced lymphomas in a low-leukemia colony of mice. Separately, the carcinogenic effect of urethane[8] and DES[9] in animals is well known. Our results indicate that in combination their carcinogenic potential may be enhanced. Human epidemiological data show that DES and urethane can be considered certain and possible carcinogens in man, respectively[10]. However, on the basis of a single experimental observation, the carcinogenic risk of their combination to man cannot be estimated yet.

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Quantitative histological study of spinal afferent innervation on the ventral surface of the cat stomach by horseradish peroxidase (HRP) method

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Summary. Retrograde axonal transport of horseradish peroxidase (HRP) was applied to the ventral surface of the cat stomach. We investigated the number, size and distribution of HRP-positive cells in spinal ganglia. The unexpected finding was the wide distribution of these cells from T3 down to L3. This would result in a diffuse pattern of referred pain.

Much of the work on visceral afferent innervation has been based on electrophysiological activation of afferent fibres[1] and histological anterograde degeneration method[2]. These methods gave us the information qualitatively, but exact quantitative observation is still obscure. Recently retrograde axonal transport of horseradish peroxidase (HRP) was demonstrated in peripheral afferent fibres and its usefulness was revealved[3-5]. In our study HRP was applied to the ventral surface of the cat stomach. We investigated the number, size and distribution of HRP-positive cells in spinal ganglia.

Material and methods. The cats weighing about 2-3 kg were laparotomized and injected 200 mg of HRP (Type II: Sigma Chemical Co.) diluted to 33.3% in physiological saline, into the ventral wall of the stomach via multiple penetrations with Hamilton syringe under Nembutal anesthesia. Figure 1 shows the injection sites of HRP. We took care not to leak HRP out of the wall of the stomach at the