

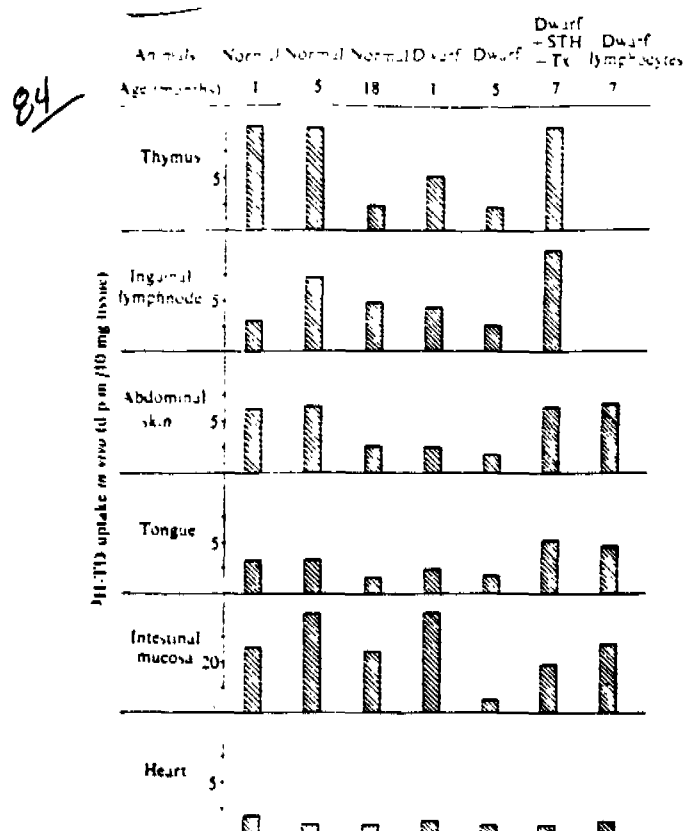


Fig. 3 ³H-thymidine (³H-TD) uptake in different tissues of mice injected with 0.8 μ Ci/g body weight 24 h before sacrifice. Radioactivity was measured in a Tricarb liquid scintillator spectrometer and counts were corrected for quenching and expressed as d.p.m./10 mg tissue. Old Snell-Bagg mice show a decreased thymidine incorporation compared with adult or young animals and a similar decrease is present in 5-month-old dwarf mice when compared to the 1-month-old group. Dwarf mice treated in their postweaning period for 30 days with 250 μ g growth hormone and 1 μ g thyroxine behave at 7 months as do adult normal animals.

1 μ g L-thyroxine for 30 days. Their life-span was prolonged to 12–14 months instead of the usual 5 months (Fig. 1) and their external appearance was similar to that of normal littermates. ³H-thymidine uptake at 7 months of age (Fig. 3), growth potential and the percentage of mitosis in spleen and kidney fragments cultured *in vitro* were also normal.

The delay in the onset of ageing symptoms and the prolongation of life-span in hormonally reconstituted dwarf mice was not achieved when thymectomy in young adult age preceded the hormonal therapy (Fig. 1).

The lymphoid system of the dwarf mice, because of the endocrine deficiency, is not fully developed and the life-span is drastically shortened. The full development of the thymus and the thymus-dependent lymphoid cells which can be induced by hormonal treatment in the postweaning period results in the prevention of early ageing and considerable prolongation of life. Adult normal lymph node lymphocytes transplanted into young dwarf mice are similarly effective. From these data one could deduce that intrinsic environmental factors, such as hormones, determine the functional level of the thymus and through its main cellular product, the lymphocyte, control the ageing processes. Therefore we wish to propose with Burnet² that the thymus might be considered as an important organ for ageing control of body tissues. One could predict then that the longer the thymus functions at its optimal level either as producer of humoral factors and/or lymphocytes, the longer will be the life-span.

The way in which lymphocytes might prolong life and

prevent ageing is far from clear. The most obvious but in our view insufficient explanation is that these thymus-dependent cells act in a strictly immunological sense in recognizing and reacting with foreign matter such as viruses, bacteria or modified self components. While such immunological surveillance is likely to play an important part in life prolongation it does not explain prevention of ageing parameters by lymphocytes. The interpretation we prefer at the present is that lymphocytes can recognize, besides foreign matter, microenvironmental changes, represented by modified host cells or their products. This could result in the cleaning up of such cells and in the release of stimulatory mediators, related to lymphokines, leading to normalization of the area. This view is supported by the experiments on the impairment of the regeneration of liver after adult or neonatal thymectomy^{14–16}.

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Received May 4; revised August 7, 1972.

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Tumours of the Nervous System in Mice treated Neonatally with N-Ethyl-N-nitrosourea

TUMOURS of the nervous system are readily induced in rats by a single transplacental or neonatal exposure to certain chemical carcinogens. This suggests that such tumours in man could well be the result of exposure to chemical carcinogens during foetal or early life. Druckrey *et al.* have studied nervous system carcinogenesis in rats using the nitrosamide N-ethyl-N-nitrosourea, $C_2H_5N(NO)CONH_2$ (ENU), administered both transplacentally^{1,2} and to newborn or very young animals³. Our own experiments with ENU administered to day-old rats⁴ agreed with these results in that a single 10 mg kg⁻¹ dose was sufficient to induce neural tumours in nearly 100% of treated animals, but differed in that in our rats a much higher proportion of tumours occurred in the brain rather than in the peripheral nervous system.

There has been little work in other species, but neural tumours have been induced in the rabbit⁵ and the dog⁶ using repeated administrations of N-methyl-N-nitrosourea. Treatment with these carcinogens has not induced tumours of the nervous system in mice. Methylnitrosourea administered to adult mice gave rise to thymic lymphomas⁷. Mice treated neonatally developed lymphomas⁸ or lymphosarcomas⁹, and intracerebral inoculation resulted only in leukaemia and lung tumours¹⁰. ENU administered to female mice at about the 16th day of pregnancy resulted in multiple lung tumours in the offspring but no neural tumours¹¹, though evidence of CNS damage was noted.

We have examined this apparent resistance of the murine nervous system to nitrosamide carcinogens. The effects of administering a range of doses of ENU to newborn mice of the four pure-line strains at present maintained in our laboratories, A, C57BL, IF and DBA Bcr, were studied. We have now observed a number of nervous system tumours which are believed to be the first induced in mice by a systemically acting carcinogen.

Sixteen day-old litters of each strain were injected subcutaneously with a saline solution of freshly dissolved ENU at doses of 10 to 160 mg kg⁻¹ body weight. The volume injected was 0.02 ml. g⁻¹ body weight, and controls were treated similarly with normal saline. The final number of mice used were 113 of strain A, 94 C57BL, 88 IF and 104 DBA Bcr. Mice were weaned at 3-4 weeks old (or later if necessary) and were maintained on 'Thompson cube diet 42' and tap water *ad lib*. Whenever possible animals showing evidence of tumours were killed for histological examination. Material, including the nervous systems of the affected animals, was fixed in formalin: acetic acid: methanol mixture (1:1:8), embedded in paraffin wax, and sections were stained by routine staining techniques.

Nervous system tumours have been found in ten mice, particulars of which are summarized in Table 1. These tumours gave rise to less visible disturbance than those seen in rats despite the considerable size of some of the lesions, as only four of the ten mice showed any degree of hind limb paralysis, and in two of these it was only slight.

The tumours can be allocated to two groups, those in the hindbrain arising between 20 and 28 weeks, and those in the peripheral nervous system, of which the first was found in a high-dose mouse at 40 weeks. There may be tumours in the animals still surviving, and a detailed report will be submitted later.

Although comparable numbers of mice of each strain were used, the ten mice with neural tumours belonged to only two of these, five IF and five DBA. Even these tumours have developed in only a small proportion of the animals at risk but thymic or splenic lymphomas have occurred in larger numbers. At this stage, A and C57BL mice appear to be resistant to neural carcinogenesis by nitrosamides, like those strains used by other workers⁷⁻¹¹.

Table 1 Nervous System Tumours in Mice treated Neonatally with N-Ethyl-N-nitrosourea

Strain	Sex	Mouse Dose mg kg ⁻¹	Age at death in weeks	Location	Tumours Type
DBA	M	80	20	Cerebellum	Oligoastrocytoma
IF	M	80	20	Cerebellum	Medulloblastoma
IF	M	80	27	Cerebellum	Medulloblastoma
IF	M	80	28	Cerebellum	Medulloblastoma
IF*	M	120	40	Cranial nerve	Schwannoma
				Brachial nerve	Schwannoma
IF	F	120	41	Sciatic nerve	Neurofibrosarcoma
DBA	F	40	50	Cranial nerve	Neuroblastoma
					Schwannoma
DBA	F	40	57	Spinal nerve	Schwannoma
DBA	M	10	69	Spinal nerve	Schwannoma
DBA	M	20	69	Spinal nerve	Schwannoma

* This mouse also had tumours of hepatocarcinoma in the liver

The location of the four brain tumours is interesting. All were situated in the cerebellum, three of these being medulloblastomas. All these were male mice which had received ENU at 80 mg kg⁻¹. This high proportion of cerebellar tumours again contrasts sharply with results obtained in rats¹⁻⁴ and suggests that IF or DBA mice, or their hybrids, may provide a useful experimental model for the study of these tumours. We believe the experimental medulloblastomas obtained will help to clarify the controversial question of the origin of these tumours in man.

We thank Professors D. G. Harnden and W. T. Smith for their interest, Misses M. Trumper and V. Nash for technical assistance, and the Cancer Research Campaign for financial support.

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Received September 4, 1972.

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The Suppression of Non-specific Esterase Activity in Mouse Skin Sebaceous Gland by "CS" Gas

In spite of the widespread use of the compound *o*-chlorobenzylidenemalononitrile (CS)¹⁻³ as a riot control agent there appears to be no published data on its carcinogenic activity⁴⁻⁷, although the usefulness of CS as a riot control agent has been stressed because of the relatively wide margin between effective and toxic doses⁸.

We report here testing of CS with a rapid bio-assay system which has been used as a predictive screening test for carcinogenesis. This bio-assay system involves the application of the test compound to mouse skin, followed by the evaluation of the area of skin sebaceous gland non-specific esterase activity⁹⁻¹⁰ which can be accurately and rapidly obtained with an image analysing computer. The application of well-known carcinogens and tobacco condensates to mouse skin causes suppression of sebaceous gland non-specific esterase activity whereas the non-carcinogens and irritants, so far tested, do not possess this ability. The degree of suppression has also been found to correlate with the known potency of the carcinogenic compounds.

CS is known to react readily with thiol groups at nearly neutral pH (refs. 10-12). As this reaction could account for the suppression of non-specific esterase activity, the related $\alpha\beta$ -unsaturated compounds cinnamonnitrile and cinnamaldehyde were also included in the experiments. The reactivity of these $\alpha\beta$ -unsaturated compounds with the thiol, glutathione, was in the order CS $\geq$ cinnamaldehyde $\geq$ cinnamonnitrile.

Groups of eight CFLP female mice, a hysterectomy-derived strain of Swiss origin 50 day old at start of treatment, when the hair growth cycle is in the telogen phase, were painted on