Effect of Neonatal Thymectomy on Tumour Induction by Adenovirus Type 12 in Hamsters*

Tumour induction by adenovirus type 12 in hamsters is limited to neonatal infection[1,2]. The age-dependent barrier may consist in an altered susceptibility of the target-cells or in a developing system of the organism which is opposed to the tumour development. Since there is increasing evidence that the adenovirus type 12-induced tumour cells contain 'new' tumour-specific antigens[3-7], the immune system, at birth immature, may represent the mechanism which in the mature animal controls tumour growth. Impairment of the immune system should result, therefore, in a higher susceptibility of the animal to the oncogenic activity of the virus. In hamsters as in other rodents neonatal or early thymectomy is known to impair the development of the immune system[8-12].

In various tumourigenic systems, such as polyomavirus-rat[13], polyomavirus-mouse[14-19], polyomavirus-hamster[20-22], adenovirus-mouse[23], and chemical carcinogen-mouse[24-27], neonatal thymectomy indeed favours tumour development. Nevertheless negative results are also reported[28]. In the adenovirus-hamster system, YOHN et al.[29] found a correlation between sex and tumour incidence: in males tumour incidence was one half that of females. Thymectomy at 3 weeks of age increased tumour incidence only slightly in males.

The present study was undertaken to determine the influence of neonatal thymectomy on tumour induction by adenovirus type 12 in both male and female hamsters.

Materials and methods. Randombred Syrian hamsters were used in this experiment as follows: of each litter $^2/_3$ of the hamsters were subjected to thymectomy[12] within 24 h of birth, the remaining animals were shamoperated. At 1 week of age the animals were injected s.c. in the dorsal region with an inoculum of 0.2 ml containing 200 $MTCID_{100}$ (as determined by 6 day reading of KB tubes for cytopathic effect) of adenovirus type 12 (strain Huie). The virus was originally purchased from the American Type Culture Collection and propagated serially in this laboratory on KB cells. The used lot was harvested after the 5th passage and stored in amounts of 1 ml at $-20\,°C$[30].

The animals were weaned at 4–5 weeks. At the time crucial for solid tumour growth, they were examined every other day by careful palpation. Tumours were first detected at match-head size. The day of tumour appearance and the day of death by tumour were noted. Dead hamsters were autopsied, and absence of thymic remnants in thymectomized animals was ascertained by histological examination. The few animals showing remnants are not listed in the following results.

Results. The number of hamsters thymectomized or shamoperated considerably exceeded the number of those surviving the first days. Most losses were due to maternal rejection or cannibalism. Wasting disease never occurred. At 1 week, when the animals were injected, 60 animals were alive: 30 thymectomized and 30 shamoperated (littermates from 16 litters). The thymectomized and the shamoperated group consisted each of 16 males and 14 females.

The final tumour incidence at 4 months is summarized in the Table. Within the shamoperated controls, the incidence in females was slightly higher than in males. Through thymectomy the incidence was increased, in both sexes together more than doubled, in females achieving 100%.

The average time of tumour appearance was not significantly different between thymectomized and shamoperated animals. The tumours grew similarly, except that most of the thymectomized females developed from the beginning a greater number of single s.c. tumours (5 and more), an observation which was confirmed at

Final tumour incidence at 4 months in hamsters injected with adenovirus type 12 in function of operation and sex

Operation	Sex		
	Male	Female	Male + female
Sham	6/16[a]	6/14	12/30
Thymectomy	13/16	14/14	27/30

[a] No. of tumour developing hamsters over No. of injected hamsters.

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autopsy. The average age at death was 59 days in thymectomized and 79 days in shamoperated females. This difference was found to be significant by the t test at a probability of < 0.01. In males the difference was only 7 days and not significant at that probability. The Figure illustrates the time course of death by tumour.

Discussion. Tumour induction by adenovirus type 12 in hamsters depends on age and dose[31]. The dose used in this experiment at 1 week had led to tumour development in 40% of the shamoperated controls. Neonatal thymectomy increased the tumour incidence to 90%, in females taken separately, to 100%. Since a diminishing effect was not to be expected, a lower incidence in the controls would have been more appropriate. The virus was given at 1 week but not later, since thymectomy in hamsters impairs the homo-[11] and heterograft-rejection[10] only gradually. The average time of tumour appearance was not significantly different between thymectomized and shamoperated tumour developing animals. Under the conditions of this experiment, the immune system interacted with tumour development at an early stage, i.e. before the tumours became palpable. The tumours once established were not affected. The earlier death of thymectomized females is explained by the fact that in these animals much more single tumours developed.

The antigen(s) escaping rejection in the immunologically impaired animal may be identical with the antigen(s) presumed to be responsible for the virus-induced tumour immunity[32–34]. Their relation to the 'new' antigens demonstrable in adenovirus type 12-induced tumour cells[3–7] is not yet known.

Tumour incidence within thymectomized and shamoperated animals was slightly higher in females than in males. The sex-difference in tumour susceptibility was first observed by YOHN et al.[29] and confirmed recently by HOOK and KIRK[35] in transplantation experiments. YOHN et al.[36] have further shown that ovariectomy at 3 weeks of age reduces the susceptibility of females, thus suggesting that the different behaviour is based on the action of estrogens.

The results presented here give evidence that the development of adenovirus type 12-induced hamster tumours is impaired by the immune system, and correspond well with the above-cited finding that it is enhanced by the female hormonal system, therefore indicating that at least 2 developing systems of the host exert an influence.

Zusammenfassung. Die Tumoranfälligkeit von Hamstern, die am Ende der ersten Lebenswoche eine Injektion von Adenovirus Typus 12 erhielten, wurde erhöht, wenn den Tieren unmittelbar nach der Geburt der Thymus entfernt worden war. Die bei der gewählten Virusdosis erzeugte Tumorträgerrate von 40% bei scheinoperierten Kontrolltieren stieg nach neonataler Thymektomie auf 90%, bei Weibchen allein auf 100% an.

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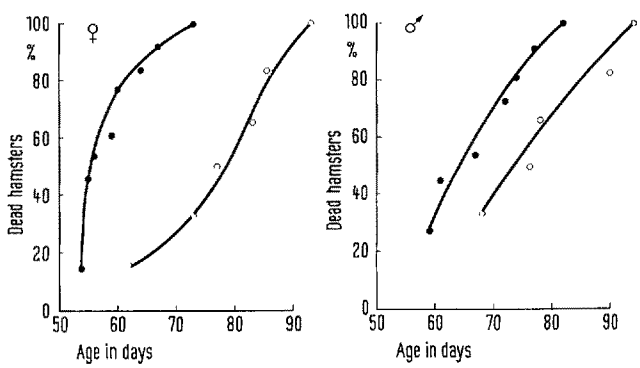

Cumulative percentage of death by adenovirus type 12-induced tumours in function of operation (• thymectomy, o sham) and sex. In each group the number of animals developing tumours (shown in the Table) is taken as 100%.

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LDH Isozyme Pattern in Induced Muscle Disease (Coxsackie Group A Virus Infection)

A question of the pathognomonic specificity of the atypical LDH isozyme pattern of muscle in Duchenne type muscular dystrophy[1–6] is raised by occasional reports of its occurrence in non-dystrophic muscle disease[7], by its induction by denervation in other species[6] and by the normal pattern in muscle from dystrophic mice[4]. We were prompted to examine the LDH pattern in other induced muscle disease with the expectation of relating it to the species, etiology and time of insult. Coxsackie Group A virus infection of newborn mice was chosen for its predictable incubation period[8] and selective muscle lesions[9,10]. That muscle metabolism of mice, as well as cell structure, is likely to be altered by Coxsackie Group A virus infection has been well demonstrated[10].

LDH isozyme patterns were examined in saline extracts of muscle from newborn mice sacrificed at daily intervals after i.p. injection of Coxsackie Group A viruses, types 20 and 21, and from non-infected mice of the same age. Extracts rather than homogenates were made to minimize chance of infection; the patterns in both kinds of preparations from normal mouse muscle were similar. The infected mice were paralyzed on the 4th day, and were dead by the 5th (type 20) or 7th (type 21). Muscle