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.

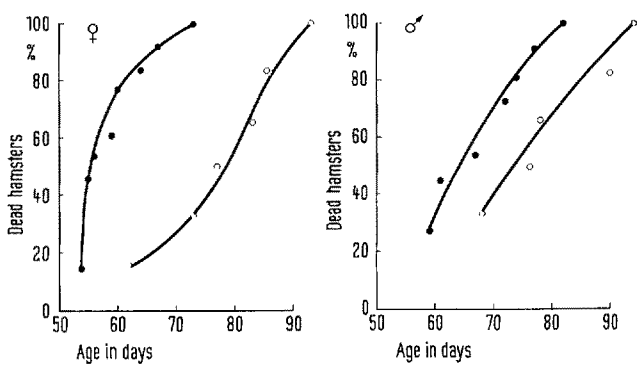
Tumour incidence within thymectomized and shamoperated animals was slightly higher in females than in males. The sex-difference in tumour susceptibility was first observed by YOHN et al.[29] and confirmed recently by HOOK and KIRK[35] in transplantation experiments. YOHN et al.[36] have further shown 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.

M. GASSER and H. LOEFFLER

Institut für Mikrobiologie und Hygiene der Universität Basel (Switzerland), 10th April 1967.



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%.

[31] Y. YABE, J. J. TRENTIN and G. TAYLOR, Proc. Soc. exp. Biol. Med. *111*, 343 (1962).
[32] B. E. EDDY, G. E. GRUBBS and R. D. YOUNG, Proc. Soc. exp. Biol. Med. *117*, 575 (1964).
[33] J. J. TRENTIN and E. BRYAN, Proc. Soc. exp. Biol. Med. *121*, 1216 (1966).
[34] G. C. SCHILD, C. W. POTTER and J. S. OXFORD, Nature *213*, 519 (1967).
[35] R. R. HOOK JR. and B. E. KIRK, Proc. Am. Ass. Cancer Res. *7*, 32 (1966).
[36] D. S. YOHN, C. A. FUNK and J. T. GRACE JR., Proc. Am. Ass. Cancer Res. *6*, 70 (1965).

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

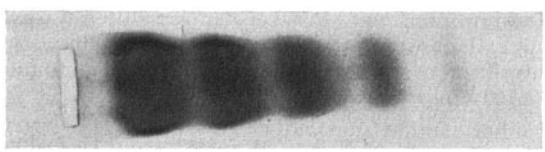
A question of the pathognomonic specificity of the atypical LDH isozyme pattern of muscle in Duchenne type muscular dystrophy[1–6] is raised by occasional reports of its occurrence in non-dystrophic muscle disease[7], by its induction by denervation in other species[6] and by the normal pattern in muscle 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

lesions consisted of diffuse myositis[11]. The isozymes in 10 or 20 λ aliquots of the extracts (200 mg mouse leg/ml 0.85% saline at 5 °C for 24 h) were separated by agar gel electrophoresis with barbital buffer, pH 8.6, and a current of 10 M.A./slide for 1.5 or 2.5 h in a Beckman Durrum-type electrophoresis cell. They were developed in a substrate containing lactate, DPN and nitrotetrazolium blue[12] cleared with an acid-alcohol wash, dried overnight at 37 °C on Mylar, and the relative enzyme activity measured in a Beckman Analytrol at 500 nm.

The isozymes separated into 5 anodal bands. The slowest moving band was predominant with the fastest band, LDH-1, the least prominent[4]. The same pattern was found in the extracts from infected and from non-infected mice; there was no difference with age (from 0–4 days), nor with type or length of virus infection (Figure). Total and relative activities of the enzyme fractions were similar in all the preparations.

Our failure to elicit a change in LDH pattern of mouse muscle by Group A Coxsackie virus infection and the normal pattern reported in dystrophic muscle disease of mice suggest that decreased LDH-5 is as much a species, as a disease specific expression.



LDH isozyme pattern in saline extract of leg muscles of 1-day-old normal mouse. Similar patterns were found in tissues of 2- and 4-day-old normal mice and in mice of the same ages after injection with Coxsackie virus Group A, types 20 and 21.

Résumé. Le zymogramme des LDH du muscle de souriceau nouveau-né souffrant de myosite diffuse causée par le virus de Coxsackie Groupe A est semblable à celui du souriceau sain de même âge, suggérant qu'un zymogramme anormal des LDH dans les affections musculaires n'est pathognomonique que chez certaines espèces.

Sally Kelly and Arlene Belorit

Division of Laboratories and Research, New York State Department of Health, and Institute of Preventive Medicine, Albany Medical College, Albany (New York 12201, USA), 17th February 1967.

[1] J. C. Dreyfus, J. Demos, F. Schapira and G. Schapira, C. r. hebd. Séanc. Acad. Sci. Paris *254*, 4384 (1962).
[2] R. J. Wieme and J. E. Herpol, Nature *194*, 287 (1962).
[3] A. E. H. Emery, Nature *201*, 1044 (1964).
[4] M. G. Lauryssens, M. J. Lauryssens and H. A. Zondag, Clinica chim. Acta *9*, 276 (1964).
[5] C. M. Pearson, N. C. Kar, J. B. Peter and T. L. Munsat, Am. J. Med. *39*, 91 (1965).
[6] I. A. Brody, Neurology, Minneap. *14*, 1091 (1964).
[7] A. E. H. Emery, J. Génét. hum. *14*, 318 (1965).
[8] G. Dalldorf, G. M. Sickles, H. Plager and R. Gifford, J. exp. Med. *89*, 567 (1949).
[9] R. Gifford and G. Dalldorf, Am. J. Path. *27*, 1047 (1951).
[10] G. Dalldorf and J. L. Melnick, in *Viral and Rickettsial Infections of Man* (Ed. F. L. Horsfall jr. and I. Tamm; J. B. Lippincott Co., Philadelphia 1965), p. 480.
[11] We are most grateful to Dr. Hildegard Plager and her staff for the mouse tissue and to Dr. Doris Collins for the tissue section interpretations.
[12] H. J. van der Helm, Lancet *2*, 108 (1961).

Meiotic Consequences of a Combined Treatment Fast Neurons-FUDR in *Vicia faba*

Some chemicals, like the bifunctional alkylating agent Myleran and the pyrimidine analogue 5-fluorouracil deoxyriboside (FUDR), have been reported to increase the effects of ionizing radiations in post treatment[1–4].

The synergistic effect obtained is higher after Co^{60} γ-rays than after fast neutrons[2–4]. Although the theory has been advanced that FUDR exerts its chromosome breaking effect by inhibition of the enzyme thymidilate synthetase, the mechanism by which the effects of radiations are modified is not yet clear but should be different from the previous one. At some Co^{60} γ-rays doses, it was found that all classes of chromosome aberrations are enhanced. Since it is well known that neutron effects are far more difficult to modify, see e.g. oxygen effect, it was in the scope of the present experiment to see if treatments by FUDR after fast neutron irradiations result in observable effects at meiosis.

Material and technique. Vicia faba dry seeds ssp. *minor* from Gembloux (Belgium) were irradiated by fast neutrons (fission) in the following experimental conditions: ITAL reactor (Wageningen, the Netherlands). Reactor power: neutron flux density was 2.10^7 $n/cm^2/sec$; neutron fluence applied was $2.2 \cdot 10^{10}$ n/cm^2 corresponding approximately to an absorbed neutron dose of 125 rad; γ contamination was about 140 rad/h. After irradiation, half of the seeds were treated by a solution of FUDR (concentration: 0.1 mg/100 ml). A control set (not irradiated) was also treated by FUDR and compared with a untreated set. All seeds were sown on perlite medium.

After 2 weeks, seedlings were transplanted in liquid medium (Hoagland) under bubbling conditions. They were grown in the following conditions: light intensity: 15.000 lux; photoperiod: 16 h light. Under these conditions they reached the flowering period in 2 months. At that time flower samples were collected for cytological investigation (5 samples in each series). They were fixed with Carnoy (2 h) then transferred into 70° alcohol. They were stained according to Feulgen technique and mounted in Depex.

Results. The criteria on which the present analysis is based have different meanings according to the stage investigated. The stages going from diplotene to metaphase I are generally suitable to identify translocations. Anaphase I allows us to observe the consequence of trans-

[1] M. Moutschen-Dahmen, J. Moutschen and L. Ehrenberg, Radiat. Bot. *6*, 251 (1966).
[2] M. Moutschen-Dahmen, J. Moutschen and L. Ehrenberg, Radiat. Bot. *6*, 425 (1966).
[3] J. Moutschen and N. Degraeve, Experientia *22*, 581 (1966).
[4] J. Moutschen, M. K. Jana and N. Degraeve, Caryologia *19*, 4 (1966).