the lymph node by means of another polyethylene catheter of no specific length, with an external diameter such as to permit a tight coupling with the other one. In our experiments the syringe was worked by a Palmer perfusor and each radioopaque substance tested was injected at the rate of 1 ml in 2, 4, 8, 16 or 32 min. The maximum total quantity of contrast medium injected at one time is 2 ml in the case of oily media and 4 ml for water-soluble contrast media.

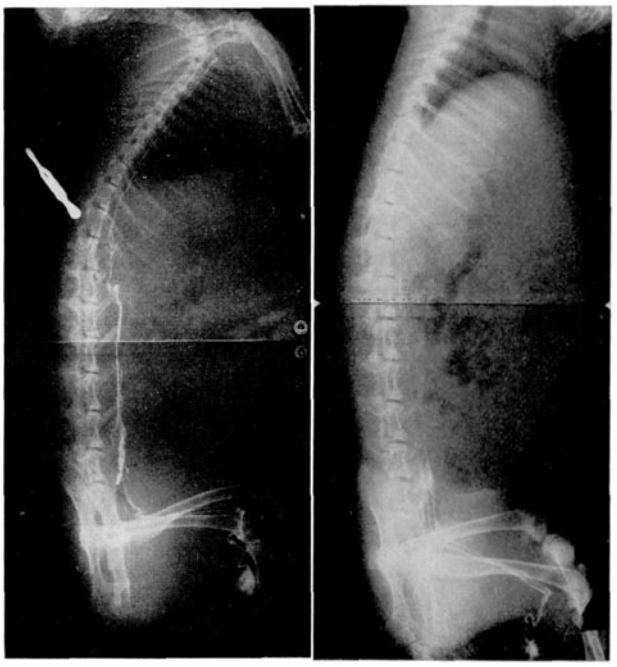

Fig. 1 Fig. 2

Fig. 1. 1 ml of contrast medium in oily solution (480 mg iodine/ml). Speed of injection: 1 ml in 16 min.

Fig. 2. 3 ml of contrast medium in aqueous solution (iodipamide 50%). Speed of injection: 1 ml in 8 min.

The operative wound is then sutured with silk; the polyethylene catheters and the surgical instruments are sterilized by leaving them in 0.5% dodecarbonium chloride for 20 h.

The radiograms have been taken using Philips Practix equipment and Osray-Gevaert film placed right under the animal. Focus-film distance 70 cm, 75 kv, exposure time 0.5 sec.

Results. The method used proved to be completely and always suitable for the purpose in question. We have obtained direct lymphograms with visualization of the lymphatic vessels and lymph nodes, using both oily and water-soluble contrast media. These lymphograms were completely identical to those obtained by cannulization of the efferent vessel (Figures 1 and 2).

Diffusion of the contrast medium through the capsule of the cannulated lymph node never occurred in the case of oily medium while it was a usual occurrence with the water-soluble contrast media used, but with intensity and times completely similar to those observed in lymph nodes belonging to the lymph node stations distant from the site of introduction.

In conclusion, we may state that introduction of radioopaque substances through the lymph node, according to the procedure we have perfected, is exactly equivalent to introduction performed by cannulization of the lymphatic vessel. Moreover, the present method has the great practical advantage of being much easier and quicker to perform and, all in all, of also being less traumatizing to the animal.

Zusammenfassung. Es wird eine Methode der Lymphographie beim Kaninchen mittels Kanüleneinführung in den Popliteallymphknoten beschrieben. Der Vorgang erweist sich als bedeutend einfacher als die unmittelbare Einspritzung in die Lymphgefässe und verkürzt merklich die zur Durchführung notwendige Zeit.

G. Rosati and M. G. Poletto

Laboratori Ricerche Bracco Industria Chimica S.A., Milano (Italy), November 13, 1964.

Influence of Age, Sex and Glandular Extirpation on Muscle Carcinogenesis in Rats

Methandrostenolone, an anabolic and myotrophic steroid, was previously shown to promote muscle tumorigenesis in rats injected with nickel sulphide[1]. The incidence of such tumors was found to be higher in female rats, but castration failed to increase the susceptibility of males[2]. With the object of further investigating the sex factor and the endocrine status in the genesis of these muscle tumors, experiments were carried out in both male and female intact rats of different ages and also following the extirpation of gonads and of the pituitary gland.

Materials and methods. 106 Sprague-Dawley rats maintained on Purina Laboratory Chow and tap water ad libitum were used in these experiments. The distribution of groups with regard to the sex and the age of the animals is given in the appropriate section of the Table. The glandular extirpations were performed under light ether anaesthesia. The gonads were removed through a suprapubic or a lumbar incision for males and females, respectively. The parapharyngeal route was used for hypophysectomy, the animals then being kept under observation for a period of 12 days. Hypophysectomy was judged complete on the basis of body-growth arrest and the fluffy aspect of the fur. After the intervention the animals received Pablum Mixed Cereals as a dietary supplement.

[1] G. Jasmin, Brit. J. Cancer *17*, 681 (1964).

[2] G. Jasmin, E. Bajusz, and A. Mongeau, Rev. Canad. Biol. *22*, 113 (1963).

Muscle tumorigenesis in relation to age, sex, and endocrine status

Status	Age (days)	Sex	Number of rats	Total number of rats with tumors	Average weight of tumors (g ± S.E.)	Time of appearance of least palpable tumors (days)	Overall average time of appearance (days)	Incidence at necropsy 5th month	6th month	7th month
Intact	30	male	14	8	13.4 ± 6.7	139	175	1/2	5/9	2/3
Intact	30	female	10	4	7.4 ± 1.8	158	210	0/5	2/3	2/5
Intact	60	male	10	7	27.0 ± 12.6	165	179		1/2	6/8
Intact	60	female	18	15	47.3 ± 15.9	126	144	4/6	5/5	6/7
Intact	90	male	8	3	3.8 ± 2.6	172	185		0/2	3/6
Intact	90	female	10	4	20.4 ± 9.8	156	172		0/2	4/8
Castrated	30	male	12	4	2.1 ± 0.6	159	169		2/4	2/8
Castrated	60	female	12	8	13.2 ± 3.5	152	160	0/2	3/4	5/6
Hypophysectomized	55	female	12	3	9.9 ± 7.5	176	185		0/2	3/10

On the first day of each experiment, 0.1 ml of a 10% aqueous suspension of nickel sulphide, to which 2000 units of penicillin G were added, were injected into the right gastrocnemius. Additional details relative to the mode of administration of the carcinogen and method of assessment of tumor growth have been given in a previous publication[1]. The animals that did not die spontaneously were killed with a guillotine either when they were found to be in poor condition or after seven months' observation, at which time the experiments were terminated. Tumors were dissected from the gastrocnemius muscle and weighed when fresh and all organs were examined macroscopically before being fixed in Susa solution or frozen in liquid air for histoenzymologic studies. Paraffin sections were stained with hematoxylin-phloxine-saffron and Masson trichrome.

Results. Data given in the Table show both the incidence and progression of tumors in relation to the time of exposure to nickel sulphide. This was made possible by killing groups of rats at different time intervals with a view to carrying out histogenic studies. Malignancy was evaluated according to dedifferentiation of muscle cells and the loss of activity of both succinate and lactate dehydrogenases. The optimal response in intact animals of different ages occurred in the 60-day-old female group as evidenced by the higher incidence, rate of development and weight of the tumors. In male rats of the same age, the incidence was also high, but the development and progression of the tumors was relatively slower. In prepuberal rats 30 days of age, the tumor incidence, especially in females, was markedly lower; similar findings were observed in the 90-day-old group.

A comparison of the susceptibility of intact and castrated animals shows, particularly in males, a marked depression in both the incidence and size of tumors. The difference is perhaps less evident in females as far as the incidence is concerned, but the growth-rate of the tumors in these animals is definitely slower. Hypophysectomy reduced the incidence and delayed the appearance of tumors. Finally, it can be seen that there is a close relationship between the values for the weight of the tumor and those pertaining to the incidence and progression.

Discussion. The present experiments were designed to study the rate of appearance and formation of muscle tumors over a given period of observation, Therefore, all the animals were subjected to an identical dosage of and exposure time to nickel sulphide. It is very likely that longer exposure to the carcinogen would have resulted in a final incidence that would have shown no variation as regards the age, sex and endocrine status of the animal. But under the experimental conditions used here, it seems quite apparent that nickel carcinogenesis is optimal in young female puberal rats of this particular strain, a finding that is in agreement with our previous observations. It is also of interest to note that the earliest signs of malignancy were histologically demonstrable after 4 months of exposure to nickel sulphide in two animals of this same group. In pre-puberal males, tumorigenesis is singularly rapid in comparison with females of the same age; the phenomenon may find its explanation in the fact that the body growth-rate is relatively higher in males. In older post-puberal animals of both sexes, however, there is a significant decrease in the incidence and rate of appearance and formation of tumors. Similarly, the hypophysectomized rats, like the pre-puberal animals, were less prone to develop tumors.

Our observations are reminiscent of those reported by HUGGINS et al.[3] concerning the high susceptibility of 50 to 60-day-old Sprague-Dawley rats to benzanthracene-induced mammary induced tumors and the regulatory effect of hormones upon these experimental tumors. A parallel can also be drawn between our observations and those of SIMPSON and EVANS[4] concerning the delaying action of hypophysectomy in rats on the rate of appearance and the diminution in the weight of tumors induced by benzanthracene[5].

Résumé. Nos recherches sur la carcinogénèse du muscle strié par le sulfure de nickel chez des rats d'âge et de sexe différents et après extirpation des gonades ou de l'hypophyse ont démontré que: (1) la fréquence, l'apparition et la progression des tumeurs étaient au plus haut niveau chez les femelles de 60 jours; (2) la castration chez les mâles, et à un degré moindre chez les femelles, réduisait à la fois la fréquence et la croissance tumorale; (3) l'hypophysectomie exerce une action freinatrice mais n'empêche pas nécessairement la tumorogénèse.

G. JASMIN

Département de Pathologie, Université de Montréal (Canada), Octobre 29, 1964.

[3] C. HUGGINS, G. BRIZIARELLI, and H. SUTTON, J. exp. Med. *109*, 25 (1959).

[4] E. M. SIMPSON and E. S. EVANS, Cancer Res. *19*, 1096 (1959).

[5] This investigation has been supported by a grant from the National Cancer Institute of Canada.