located at the metacarpal level of the host regenerate. Thus, in the two species of urodele used in these experiments the nature of morphogenesis of the accessory blastemata was directly correlated with their location along the proximo-distal axis of the host regenerate. It is concluded that the *stimulus* for the accumulation of the blastema cells of the accessory regenerates, as distinct from their *morphogenetic* induction, was provided by the transplanted epidermal cap[4].

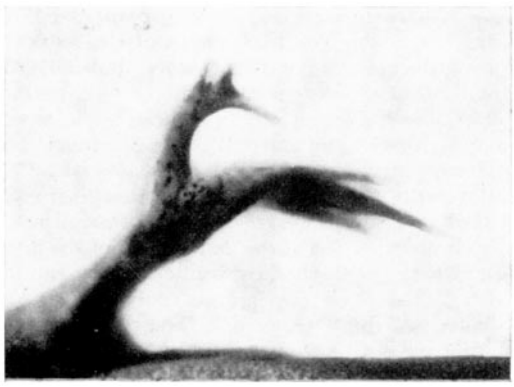

Fig. 5. Regenerated accessory 3-digit limb (*A. talpoideum*) (× 6).

Résumé. Les événements morphogénétiques qui suivent le greffage d'un blastème apical à la face préaxiale d'un membre antérieur en voie régénératrice, chez *Ambystoma*, sont tout à fait normaux, mais le niveau où se localise le régénérat accessoire est différent selon l'espèce utilisé. Ces résultats sont discutés au point de vue du stimulus d'agrégation des cellules appartenant au régénérat accessoire.

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Cannulation of the Popliteal Lymph Node in Rabbits: A Method for Introducing Radio-Opaque Substances into the Lymphatic System

The need to have available an experimental method for the first evaluation of lymphographic contrast media is much felt at present.

A useful method for this purpose must essentially comply with the following practical requirements: (1) be usable in ordinary laboratory animals, possibly of low cost; (2) not offer too great technical difficulties; (3) permit good standardization of the methods of administration, particularly as regards the rate; (4) ensure survival of the animal in order to allow long-term controls.

Two routes of introduction of the contrast medium – the lymphatic vessel and the lymph node – are known in direct lymphography. With the procedures now in use, each of the routes of introduction offers benefits and inconveniences which have been clearly shown by TJERNBERG[1] in his reports of experiments on rabbits and dogs.

On injecting the contrast medium into the lymph node through a hypodermic needle, TJERNBERG observed that the ease with which the substance flows from the site at which the needle is introduced, and the insufficient control of the flow, due to the 'manual' regulation, rather limit the possibilities of examining the lymphatic structures remote from the site of introduction.

He concludes, however, that this is still the simplest method for carrying out direct lymphography and expresses the hope that research will perfect techniques capable of eliminating the above-mentioned inconveniences.

Bearing this suggestion in mind, we have studied a procedure of direct lymphography in rabbits which, although using the lymphatic node as a route of introduction, also offers the advantages obtained with cannulization. This procedure is based on the use of a polyethylene catheter instead of the needle, and on the possibility of ligaturing the lymph node capsule around same.

Method. Adult rabbits of both sexes were used. After pentobarbital anaesthesia, the skin area is shaved and disinfected with alcohol. The skin is incised for 2 cm, following the fold between the femoral and semi-membranous biceps as closely as possible. In order to determine the exact point of the incision, it is always advisable to press the sides of the popliteal fossa to make the lymph nodes stand out better below the skin, forming a hernia between the margins of the above-mentioned muscles.

After incising the skin, we usually continue under a dissection microscope (magnification: 6 ×) in order to free the lymph node from the surrounding connective tissue. The lymph node is generally easily recognized, but it may be of help to make it stand out by subcutaneously injecting Patent blue peripherically, in the quantity of 1–2 ml of 5% solution.

Delicately holding the lymph node between two fingers, a hole is made in the capsule at the inferior pole, using a no. 1 hypodermic needle and penetrating for 2–3 mm into the pulp. A polyethylene PE 100 catheter, 40–50 mm long, is then introduced through the hole, making it penetrate for 4-5 mm, with rotatory movements. At this point, using curved forceps, the catheter is held firm and a circle of lymph node tissue is collected around it. At the same time, applying slight traction, an assistant can carry out a ligature with silk thread immediately below the holding point of the forceps. The ligature fixes the catheter to the lymph node and ensures a perfect fit.

After placing the animal under the radiological apparatus in the required position, the syringe containing the contrast medium is connected to the catheter fixed in

[1] B. TJERNBERG, Acta rad., Suppl. 214 (1962).

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.

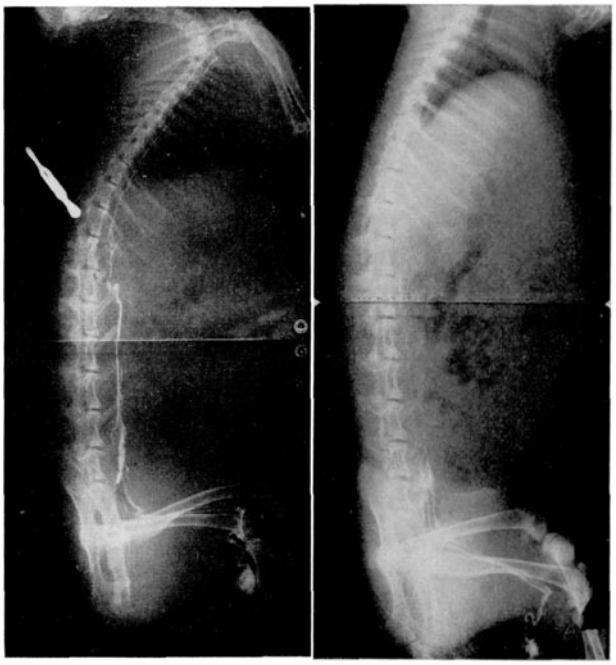

Fig. 1

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.

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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).