shown that defined media (balanced salt solutions enriched with glucose, amino-acids and vitamins) fail to support neural cultures unless supplemented biological fluids (various fetal or adult sera, embryonic extracts). The role of these biological supplements is to provide not only greater nutritional support, but also some special agents necessary for the survival, proliferation and/or expression of differentiated properties of the cultured cells or organs.

In our experimental conditions, it appears that the 2 BME media involve important modifications of the SCG morphology, that Krebs' solution is less toxic because ultrastructure is conserved, and finally that the NCTC 109 with newborn calf serum permits the best conservation of the SCG structures.

In conclusion, these results show that NCTC 109 medium with 20% newborn calf serum could be used preferentially for the biochemical, electrophysiological and pharmacological analysis where the SCG must stay in artificial medium.

1 We are indebted to Miss M. Vio, Mrs J. Bottini, Mr C. Cataldo and Mr P. Cau for their helpful assistance.
2 M. Dolivo and M.G. Larrabee, J. Neurochem. *3,* 72 (1958).
3 M. Dolivo. Fed. Proc. *33,* 1043 (1974).
4 P. Keen and M.W.G. McLean, J. Neurochem. *22,* 5 (1974).
5 D.A. McAfee and P. Greengard, Science *178,* 310 (1972).
6 P. Keen and W.G. McLean, Br. J. Pharmac. *46,* 529 (1972).
7 P. Kalix, D.A. McAfee, M. Schorderet and P. Greengard, J. Pharmac. exp. Ther. *188,* 676 (1974).
8 S. Varon, Exp. Neurol. *48,* 93 (1975).
9 A.M. Glauert, in: Practical methods in electron microscopy, p. 1–110. Ed. A.M. Glauert. North-Holland Publ., Amsterdam 1975.
10 G. Majno, in: The liver, morphology, biochemistry, physiology, p. 267. Ed. Ch. Rouiller. Academic Press, New York 1964.

Formation of lung colonies by mouse ascitic teratocarcinomas

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Summary. Intravenous transplantation of teratocarcinomas resulted in the formation of colonies only in the lung. The lung colony system would be advantageous for the quantitative studies of differentiation of teratocarcinoma stem cells.

Recently, mouse teratocarcinomas have been shown to be useful for studying embryonic development in mice[2]. But few quantitative studies have been reported. Williams and Till[3] have reported that virus-transformed rat embryonic cells form colonies in the lung when they were injected i.v. This method provided the basis for a quantitative assay for malignancy of transformed cell lines. Recently, we also induced colony formation of teratocarcinomas in the lung by i.v. injection of embryoid bodies (EBs)[4,5]. In this paper, we show that this method may be useful for the quantitative study of differentiation of teratocarcinoma stem cells.

Materials and methods. Embryoid bodies (teratocarcinoma OTT6050), serially transplanted into mice (129/Sv) every 3 weeks, were taken from the peritoneal cavity of the mouse and washed 3 times with phosphate buffered saline (PBS). EBs suspended in 0.25 ml of PBS were injected through the tail vein of syngeneic mice. At the same time, 0.25 ml aliquots taken from the same suspension were placed in culture dishes, dried and stained with Giemsa's solution for counting EBs in the dish, thereby estimating the number of EBs injected. For counting the number of colonies, the mice wer killed and their lungs were excised. These lungs were fixed in the Bouin's fixative for 1 day and were transferred into 70% alcohol. Colonies visible on the lung surface were counted.

Results and discussion. When EBs were injected through the tail vein, they formed teratomatous colonies in the lung (figure 1). These colonies could be identified on the surface of unfixed lung about 2 weeks after injection. When the lungs were fixed in the Bouin's fixative and thereafter dipped into 70% alcohol, even about 7-day colonies on the lung surface were visible with ease. In the experiment, surface colonies that could be seen only with the naked eye were counted. The teratomatous colonies have never been found on the surfaces of the other organs.

Colonies often contained embryonal carcinoma cells as well as various types of tissues and these colonies were transplantable (figure 2).

The number of colonies formed on the lung surface increased in proportion to the number of EBs injected

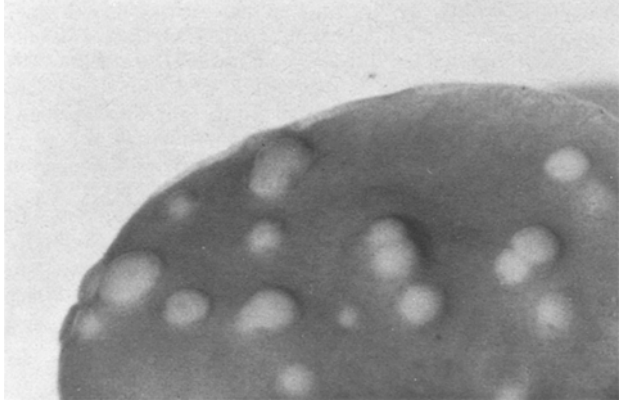

Fig. 1. The teratomatous colonies, which were derived from embryoid bodies, are seen on the surface of lung 20 days after injection. × 30.

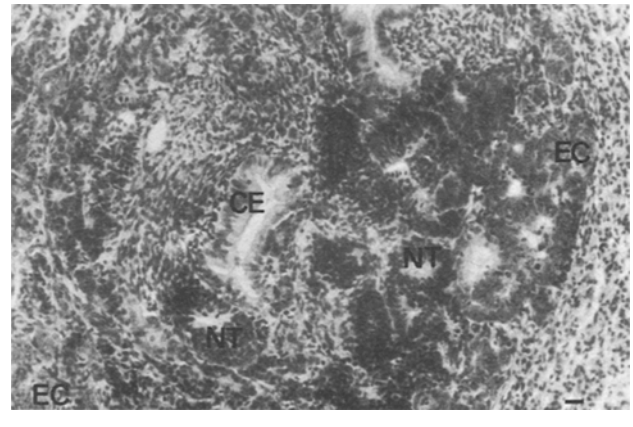

Fig. 2. The histological section of a teratomatous colony of 30 days old. EC, teratocarcinoma stem cells (embryonal carcinoma cells); NT, neural tube; CE, ciliated epithelia. Bar = 20 μm.

(figure 3). However, when the number of EBs injected was more than 350 per mouse, the number of colonies became saturated at about 130 per lung. In the range that the number of EBs injected was less than 350 per mouse, the efficiency of colony formation was 35%. It may fluctuate by host and the populations of EBs from mouse. It is most likely that each colony in the lung is derived from single EBs so long as the number of EBs injected does not exceed 350 per mouse.

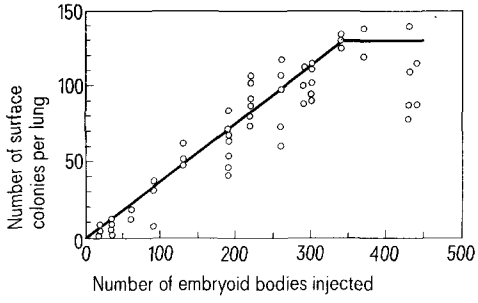

Fig. 3. Relationship between the number of embryoid bodies injected and the number of colonies formed on the lung surface.

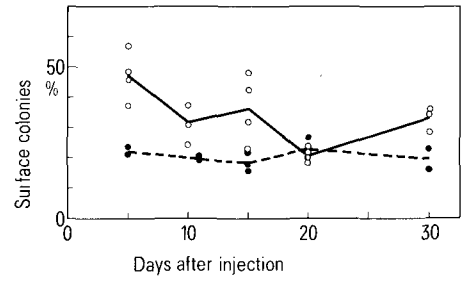

Fig. 4. Number of colonies on the lung surfaces of nonirradiated mice killed at various times after the injection. 2 experimental data are shown.

Figure 4 shows that the efficiency of the surface colony formation was nearly constant through the days after injection. This implies that a population of EBs capable of forming colonies is initially restricted and that the retarded colony formation rarely happens. By this phenomenon, a population of the lung colonies is expected to have the same age of injection, and we are able to compare the developmental stages and the differentiation potency among the colonies in the mice. Figure 4 shows that the teratomatous colonies in the lung were not rejected by host for about a month after injection.

EBs employed in the present experiment were 40–150 μm in diameter. When these large aggregates are injected i.v., they will certainly be first trapped in the capillary vessels of the lung and it will hardly be possible for them to go through the lung into the other organs. This would explain why EBs form colonies exclusively in the lung. We here stress that each colony may be derived from single EBs and may have almost identical environmental conditions. With these advantages, we can extend the utility of the system previously reported by Williams and Till[3]. All of colonies formed in the lung have one or more types of tissues[5]. Therefore, the lung colonies can also be investigated from the standpoint of developmental biology.

1 I thank Prof. M. Yoneda for reading the manuscript.
2 G.R. Martin, Cell *5*, 229 (1975).
3 J.F. Williams and J.E. Till, J. nat. Cancer Inst. *37*, 177 (1966).
4 S. Amano and A. Hagiwara, Dev. Growth Diff. *18*, 95 (1976).
5 T. Ishikawa and A. Hagiwara, Dev. Growth Diff. *19*, 329 (1977).

Histochemistry of some trout respiratory muscles

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Summary. A histochemical study has been made of the main cranial muscles which produce ventilation movements of the rainbow trout. It is shown that a greater proportion of red(aerobic) fibres is present in those muscles known to be active during shallow ventilation than those which become active at greater ventilation volumes. An ordered recruitment of red, pink and white fibres within these muscles is also likely.

The cranial muscles of teleosts function in a co-ordinated manner to produce movements of the jaws, operculi and their associated skeletons during feeding and ventilation. The anatomy of the muscular and skeletal systems of a number of species has been described in some detail[2–4]. Recent electromyographical studies of lightly anaesthetised and free swimming fish has led to some understanding of the co-ordination of these muscles especially during respiration[5–11]. However, little is known about the fibre composition and metabolism of these muscles. In the present histochemical study the types and distribution of muscle fibres has been determined in 5 cranial muscles of the rainbow trout and the results correlated with previous investigations on their function[5,6].

Materials and methods. Rainbow trout (*Salmo gairdneri* Richardson) about 35 cm in length, were obtained from Midland Trout Hatchery, Nailsworth, Gloucestershire, and maintained in tanks at 15 °C. The muscles investigated (figure 1) were chosen because of their accessibility and varied function. A total of 6 fish were used in these investigations and the cranial muscles were dissected within 15 min of death which was by an overdose of MS 222 (Sandoz) anaesthetic. Blocks of muscle were mounted in 'Tyro-M-Bed' (Aerosol Marketing Chemical Co. Ltd, London) on cryostat chucks and rapidly frozen by plunging into liquid freon (Arcton 12, ICI) cooled to its melting point in liquid nitrogen (−159 °C). About 12 serial transverse sections 10 μm thick were cut from each block at −23 °C and mounted directly on coverslips.

Sections were stained for myofibrillar ATPase by a modified method of Guth and Samaha[12,13]. Fibre types were distinguished by preincubation for 1–10 min in a solution of 18 mM $CaCl_2$, 100 mM 2-amino-2-methyl-1-propanol pH 10.3 prior to staining for ATPase activity[13]. In contrast, fast white fibres which have a high myofibrillar ATPase activity biochemically[15] are more alkaline stable and are