

Auxin Effects on Tracheal Differentiation in Root Callus Tissues in vitro

In this communication, the effects of auxin concentration on the cellular organization and tissue differentiation in root callus tissues of tomato (*Lycopersicum esculentum* Mill. var. Sutton's 'Best of All') and flax (*Linum usitatissimum* L.), grown on WHITE's medium¹ supplemented with coconut milk (10%) and a range of 2,4-D (for tomato) and NAA (for *Linum*) concentrations, are presented.

The growth of the tomato callus tissues grown on a medium containing 10.0 mg/l 2,4-D was slow, and was almost entirely the result of meristematic activity in the central core of tissue. Little obvious cellular differentiation occurred in the callus, the cells presenting a quite uniform appearance. In the callus tissues grown on the medium containing 2.0 mg/l 2,4-D there was a marked degree of differentiation. Groups of tracheal elements were found scattered throughout the peripheral layers of the callus tissue. Cells on the outside of these groups of tracheal elements showed clear evidence of meristematic activity giving rise to new cells towards the periphery by periclinal division walls. Active cell division at such points gave the callus a pronounced lobed appearance in this medium. When the callus tissues were grown on a 0.2 mg/l 2,4-D medium, the tracheal elements, instead of being differentiated in loose, scattered patches, were formed in compact groups, designated 'vascular nodules'. Radiating rows of cambium-like cells differentiated on the outer margin of such vascular nodules, which looked like well-defined growth centres.

The most noticeable difference on account of changes in NAA concentration in the medium supporting *Linum* callus tissues was in the degree of tissue organization. The irregular planes of divisions in the meristematic layers and uneven enlargement of underlying cells in the callus tissues grown in the presence of more than 4.0 mg/l NAA, produced an unorganized mass of cells; more regular divisions in the meristematic regions and approximately equal radial extension of the newly formed cells produced the fan-shaped lobes in media containing 2.0 mg/l NAA and less. The vascular nodules found in the above callus

tissues were, however, not identical. In higher auxin media, they were irregular in outline and deep-seated in the callus tissue; but in lower auxin medium they were more superficial and spherical, with concentric layers of meristematic cells. In callus tissues grown on media containing 0.1 mg/l NAA, root primordia differentiated close to the vascular nodules.

The results indicate that there was a marked correlation between the concentration of auxin in the medium and the extent of tracheal differentiation in the callus cultures of both tomato and *Linum*. With a very high concentration (10.0 mg/l) of auxins in the media, relatively few tracheal elements appeared in the callus; but at a lower concentration (2.0 mg/l) of auxin, the differentiation into tracheal elements was considerable. Further lowering of the auxin concentration (to 0.1 mg/l) in the medium resulted in the development of distinct vascular nodules in the callus body. Development of such vascular nodules appeared to be a prerequisite for the initiation of root primordia in *Linum* cultures².

Zusammenfassung. Organisation und Differenzierung trachealer Elemente im Kallusgewebe von Wurzeln von *Lycopersicum esculentum* Mill. und *Linum usitatissimum* L. hängen von der Auxinkonzentration der Nährlösung ab. Der Effekt der untersuchten Auxine in Gewebekulturen (2,4-D, NES) deutet auf eine negative Korrelation zwischen Auxinwirkung und Differenzierung hin.

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¹ P. R. WHITE, in *The Cultivation of Animal and Plant Cells* (Ronald Press Co., New York 1953).

² I wish to acknowledge the help and interest of Professor H. E. STREET in the present investigation, which was carried out in the Department of Botany, University College of Swansea, Wales. Sincere thanks are also due to Dr. G. G. HENSHAW for his encouragement.

The Influence of Antiserum on the Metastasis of Ascites Cells from the Yoshida Sarcoma

At present human tumours cannot be influenced by antiserum^{1,2}. However, passive immunization was found to be effective on animal tumours, especially ascites cells³ and leukemia^{4,5}.

Our studies are concerned with the influence of antiserum against the Yoshida sarcoma on the metastasis of ascites cells from this tumour. We used the following method: Ascites cells from the Yoshida sarcoma were injected subcutaneously into Wistar rats. 8 days after the application an extract was prepared from the solid tumour that had developed. The tissue was homogenized with 0.9% sodium chloride (1:10). In order to produce the antiserum against the tumour, we injected the homogenate intramuscularly into rabbits (4–5 kg). Details concerning the isolation of the antiserum will be described

separately⁶. The titre of the antiserum was determined by the ring test and the method of OUCHTERLONY⁷. For the experiments reported here the titre was adjusted to 1:160. The serum of the rabbits was injected intra-

¹ C. M. SOUTHAM, *Cancer Res.* 21, 1302 (1961).

² H. ISLIKER, J. C. CEROTTINI, J. C. JATON, and G. MAGNENAT, in *Chemotherapy of Cancer* (Ed., P. A. PLATTNER; Elsevier Publishing Company, Amsterdam-London-New York 1964).

³ M. H. FLAX, *Cancer Res.* 16, 774 (1956).

⁴ P. A. GORER, M. A. TUFFREY, and J. R. BATCHELOR, *Ann. N.Y. Acad. Sci.* 101, 5 (1962).

⁵ G. MATHÉ, J. L. AMIEL, L. SCHWARZENBERG, A. CATTAN, and M. SCHNEIDER, in *Chemotherapy of Cancer* (Ed., P. A. PLATTNER; Elsevier Publishing Company, Amsterdam-London-New York 1964).

⁶ H. KRÖGER and E. STUTZ, in press.

⁷ O. OUCHTERLONY, *Ark. Kemi* 26b, 14 (1949).

Influence of serum, antiserum and 6-mercaptopurine (6-MP) on the metastasis of ascites cells from the Yoshida sarcoma

	Control	Antiserum	Antiserum + 6-MP	Normal serum	Normal serum + 6-MP	6-MP
Number of animals	17	17	16	9	8	10
Death by tumour	16	13	7	7	8	10
Without tumour on the 40th day	1	4	9	2	—	—

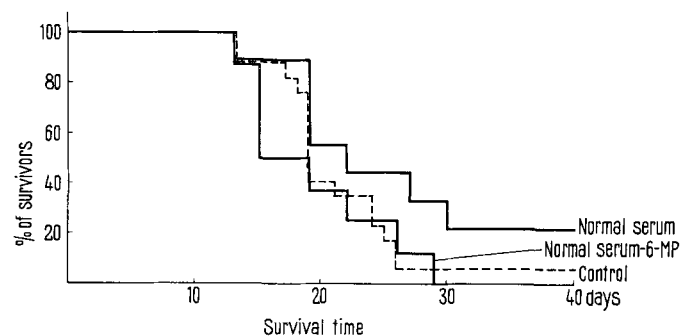


Fig. 1. Influence of normal rabbit serum and 6-mercaptopurine (6-MP) on the metastasis of ascites cells from the Yoshida sarcoma in rats. 30 sec prior to the application of the ascites cells 0.5 ml serum was injected intravenously. The same amount of serum was applied on days 1, 2, 3, 5, 7, 9, 11 and 13. In the cases indicated, 6-MP was injected subcutaneously together with the serum.

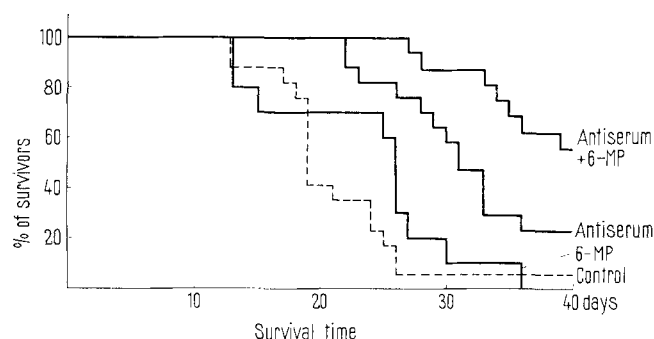


Fig. 2. Influence of antiserum against the Yoshida sarcoma and of 6-mercaptopurine (6-MP) on the metastasis of ascites cells from the tumour in rats. For details see Figure 1.

venously into rats aged 80 to 100 days. 6-Mercaptopurine (6-MP), dissolved in 1N KOH and adjusted to pH 7.0 with HCl, was applied by subcutaneous injection into the groin of the rats; the dose amounted to 0.5 ml of a solution 1 mg/ml. For the final studies the ascites cells from the Yoshida sarcoma were injected intravenously into the rats in a solution which contained $5 \cdot 10^5$ cells in 0.5 ml and also 0.9% sodium chloride and 200 mg% glucose. All the rats were dissected either on the day of death or on the 40th day after the beginning of the experiment.

As can be seen from Figure 1 and the Table, after intravenous injections of $5 \cdot 10^5$ ascites cells from the Yoshida sarcoma into rats only 1 out of 17 animals survives a period of 40 days without developing a tumour. The other 16 rats die within 27 days after the application. Neither simultaneous injection of normal rabbit serum nor of 6-MP shows a remarkable effect on the rate of survival. When the normal rabbit serum is replaced by the antiserum against the Yoshida sarcoma, the result is different (Figure 2 and Table). 4 out of 17 rats survive the period of 40 days and do not show any sign of a tumour. The experiment in which we combined the antiserum with 6-MP gave quite a promising prospect. By this application, 56.2% of the tumour-infected rats survived 40 days, and careful dissection thereafter did not give the slightest indication of a tumour. Long-term experiments are now being performed⁸.

Intravenous injection of ascites cells from the Yoshida sarcoma into rats results in the death of 16 out of 17 animals 27 days after the application. This effect remains unchanged by simultaneous injection of normal rabbit serum as well as by the combination of the serum with 6-MP. However, the application of antiserum against the tumour cells together with 6-MP leads to the remarkable fact that after 40 days almost 60% of the animals show no sign of a tumour.

Zusammenfassung. Intravenöse Injektion von Ascites-Zellen des Yoshida-Sarkoms in Ratten bewirkte, dass 27 Tage nach der Applikation 16 von 17 Tieren gestorben sind. Dasselbe Ergebnis wurde erhalten bei Injektion von normalem Serum oder bei der Kombination dieses Serums mit 6-Mercaptopurin. Dagegen führte gleichzeitige Applikation von Antiserum und 6-Mercaptopurin zu der erstaunlichen Tatsache, dass nach 40 Tagen etwa 60% der so behandelten Tiere keinen Tumor aufwiesen.

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