calcification is imminent, and its intensity is perhaps related to the rapidity of the calcification that follows.

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Histotopochemistry of Ascorbic Acid in Tendon Fibres

From investigations with the electron microscope, it has been reported that the precursors of collagen fibrils are formed within the cytoplasm of fibrocytes[1]. It follows that the sites of formation of the fibrils and those of the synthesis of collagen are identical. Hydroxyproline, which is a characteristic acid of collagen protein, originates from proline under the action of L-ascorbic acid[2].

For this reason we believe that determination of the distribution and concentration of ascorbic acid in the connective tissue, for example in the tendon, would suggest where hydroxylation of proline takes place and where the tropocollagenous cells, which are the basic elements of the fibrils, are formed.

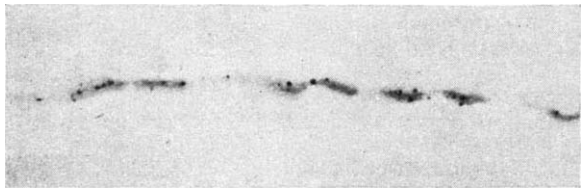

Fig. 1. Black granules of metallic silver in the tendon cells indicate L-ascorbic acid, which takes part in the conversion of proline to hydroxyproline

In this work L-ascorbic acid was determined in isolated fibres from the tail tendon of male albino Wistar rats, 4 months or 28 months old. The method of Giroud and Leblond, applying silver nitrate to metallic silver, was used. The granules occurring in the positive reaction are not granules of vitamin C but granules of metallic silver[3].

It has been found that in isolated tendon fibres of both the younger and the older rats the granules of silver are deposited only in the cytoplasm of fibrocytes; the remaining fibrillar structure is negative.

The occurrence of L-ascorbic acid in the tendon cells which intensively produce collagen supports the suggestion that conversion of proline to hydroxyproline occurs within the cytoplasm of the cells, and that a collagen molecule may there be built up from the individual amino-acids.

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PATHOLOGY

An Unidentified Virus which causes the Rapid Production of Tumours in Mice

In the course of routine passage of Moloney s leukæmogenic virus (MLV) plasma was collected from a leukæmic rat (Chester Beatty Institute outbred albino strain) which had been inoculated with MLV-containing mouse plasma when new-born. After storage at −70°C for three months the rat plasma was diluted 1 in 30 with Hanks's saline and passed through a 'Selas 02' filter, tested and found impervious to *Esch. coli*. The filtrate was injected into 15 new-born *BALB/c* mice, as a test of potency. Only 6 survived to weaning. On the 32nd day 5 had tumours at or near the injection site, and all had grossly enlarged spleens. Usually mice inoculated with MLV remain symptomless until they show the characteristic signs of leukæmia after 8 weeks.

Further samples of the same stored plasma filtrate were injected into new-born *BALB/c* mice, and Chester Beatty albino (outbred) and hooded (inbred) rats. Twenty-eight of 35 mice, and 1 of 13 rats, killed and examined *post mortem* had tumours, and all had splenomegaly.

The pathology of the disease is being examined: the result of early observations are as follows. The animals commonly die of splenic rupture, as in Friend or Rauscher disease. The histology of the spleen and the blood smears are similar to those of Friend disease. There is gross proliferation of reticulum cells in the spleen, and reticulum cells and erythroblasts appear in the blood. The tumours are of two main types, either solid and firm, or cystic and filled with blood. The former arise in the subcutaneous tissue and peritoneum, sometimes attached to the muscle of the abdominal wall, thorax, or diaphragm; their position suggests development at or near the site of injection or along the needle track. They are anaplastic sarcomata consisting of pleomorphic and spindle cells, some very large, invading and disrupting adjacent tissues. The latter may occur anywhere in the subcutaneous space or peritoneal cavity, and often in relation to lymph nodes. They are angiomatous tumours consisting of multiple dilated sinuses which are filled with blood and debris, and lined with cuboidal cells which often form ingrowths into the lumen.

Recent experiments have shown that of a number of animals injected with sarcoma virus (SV) when new-born 30/46 *CB* hooded rats developed gross splenomegaly, and 16 had sarcomas of the diaphragm or other sites. 4/9 cream and 5/19 golden hamsters have developed sarcomas, also mainly situated on or near the diaphragm. Many of the rats and hamsters had large thin-walled cysts at lymph node sites containing either clear or blood-stained fluid.

Two of 5 attempts to transplant sarcomas in *BALB/c* mice were successful, and the cell line is now in its fifth passage. All recipients have developed splenomegaly.

Tissues of tumour-bearing mice and plasma of injected rats were tested by inoculation into *BALB/c* mice for the presence of tumour-producing virus (Table 1).

'Selas 02' filtrates of tumour homogenates or plasma induced rapid development of tumours and splenomegaly in mice inoculated when 1–7 days old, in some cases as early as 12 days after injection. As the age of injection increased, the incidence of tumours declined, but that of splenomegaly remained high. Fifteen mice inoculated at 112 days all developed splenomegaly, but only two developed tumours.

One to 2 day-old Chester Beatty albino rats were injected with the original plasma filtrate. Their plasma was collected at 8, 30, and 54 days, and injected into 1–3-day-old *BALB/c* mice. Mice which received the 30- and 54-day samples developed tumours and splenomegaly in 12–15 days. Those which received the 8-day sample

Table 1

Inoculum	Age injected (days)	No. recorded *post mortem*	Mice with tumours	Mice with splenomegaly	Latent period to death (days)
(I) Plasma from tumour-bearing mice	1–3	12	10	10	28–66 (av. 38)
(II) As (I) 'Selas 02' filtered	1–3	20	11	20	24–42 (av. 30)
	11?	15	2	15	35–116 (av. 68)
(III) 'Selas 02' filtrate of tumour tissue	1–3	18	17	18	12–17 (av. 13)
	5–7	19	17	19	12–30 (av. 16)
(IV) 'Selas 02' filtrate of pooled infected rat plasma	1–3	13	13	13	18–20
Rat plasma 8 days after injection	1–3	6* (5 survive)	0	2	70–100
Rat plasma 30 days after injection	1–3	7	7	7	12–15
Rat plasma 54 days after injection	1–3	6 (2 survive)	6	3	13–?

* These mice died with generalized lymphatic leukæmia.

remained healthy for 70 days, when they developed the typical signs of MLV-induced leukæmia—an indication that Moloney virus was still present in the virus preparation (Table 1).

The possibility that the tumours produced might be due to polyoma virus was considered. Certain differences were already apparent: for example, no parotid or kidney tumours were seen in the injected mice, and polyoma tumours have never been reported as early as two weeks after inoculation. Certain specific tests were performed. (1) Active preparations of SV produced no cytopathic effect in mouse embryo tissue cultures, and did not agglutinate guinea-pig red cells in the cold. (2) The sera of mice bearing sarcomata did not inhibit hæmagglutination by polyoma virus. (3) In two separate tests samples of SV were found to be ether-sensitive. These results make it very unlikely that the tumours produced are due to polyoma, or any closely related virus.

The presence of other viruses in the SV preparations is being considered. As indicated above, MLV is almost certainly still present. The type of splenomegaly, and its short latent period, suggests this may be due to Friend, or possible Rauscher, virus. In this connexion it is noteworthy that *BALB/c* mice may carry the Rauscher virus in a latent form[1]. Since none of these leukæmogenic viruses has been shown to produce sarcomata of the type reported here it appears likely that they are due to a hitherto unidentified virus. The Schmidt–Ruppin derivative of Rous sarcoma virus rapidly produces tumours in small mammals, and is ether-sensitive—in these respects it resembles SV. This virus is being tested for. Another possibility is that the observed effect may be the result of some kind of synergism between two or more viruses (as, for example, between the Moloney or Friend virus and mouse hepatitis virus[2], and this possibility is also being investigated.

I thank Dr. M. H. Salaman and Dr. K. E. K. Rowson of this Department, and Dr. R. J. C. Harris of the Imperial Cancer Research Fund Laboratories, for their advice. I also thank Dr. F. C. Chesterman (also of the Imperial Cancer Research Fund) for his advice concerning the histological preparations. Mr. R. Evans helped with the tissue culture and Miss J. M. Burke with histology and hæmatology.

This work was partially supported by the British Empire Cancer Campaign for Research.

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Ink Blue as a *p*H Indicator for Certain Biological Systems

THE observation that commercial blue-black ink poured on to the uncolonized agar of a Petri dish culture of *Trichophyton mentagrophytes* became discoloured in the region of the dermatophyte, but not of a contaminating mould, led to further investigation of this phenomenon. A solution of one of the major constituents of blue-black ink—ink blue (supplied by Imperial Chemical Industries as 'Ink Blue AS' crystals)—behaved similarly, and it seemed probable that the clearing was caused by either *p*H or *Eh* conditions around the colony. The response of Ink Blue to changes of this kind was initially examined in aqueous solution.

The absorption maximum of a 0·005 per cent solution of ink blue in water, measured on a Unicam '*SP*600' spectrophotometer, was 610 mμ. It was found that a standing period of at least 5 min was required before the solution attained stability of optical density, and 25 min was necessary when 1 ml. of a 0·05 per cent solution was added to 10-ml. buffer at *p*H 7·8. Thereafter the absorption remained constant.

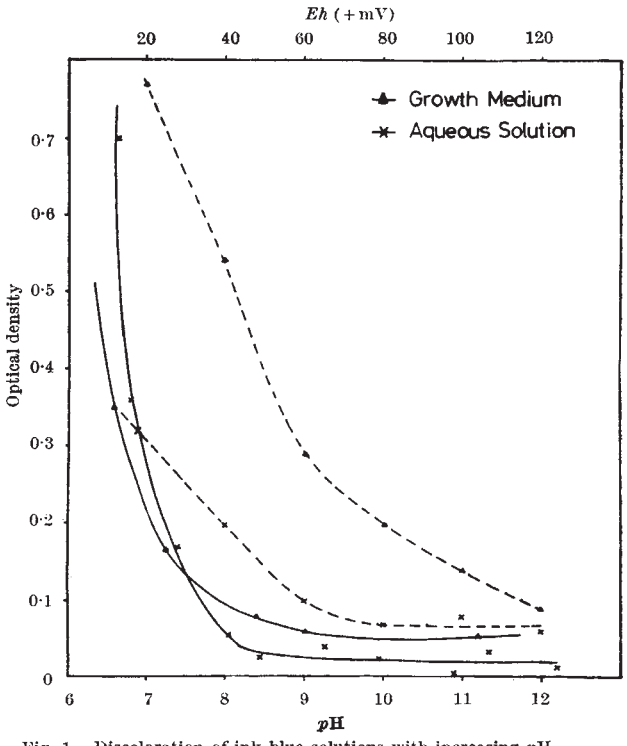

Fig. 1. Discoloration of ink blue solutions with increasing *p*H, ——, and *Eh*, – – – –

The behaviour of ink blue solution over the *p*H range 3·5–12·21 was investigated by adding 1 ml. of a 0·05 per cent solution to 5 ml. of an appropriate buffer. After it had stood for 30 min the optical density at 610 mμ was measured against a distilled water blank. The *p*H values were checked on a direct-reading *p*H meter (E.I.L., model '23*A*') using reference electrode '*RJ*23' and glass electrode '*GG*23'. The change from blue to colourless occurred between *p*H 7·38 and 8·04.

The influence of *Eh* on the discoloration of ink blue was examined by adding 1-ml. quantities of a 0·05 per cent solution to 5 ml. of appropriate dilutions (0·01–0·08 M) of molar sodium thioglycollate solution, giving a range of *Eh* of −100 mV to +120 mV, and the optical density of each sample was measured at 610 mμ against a distilled water blank (Fig. 1). *Eh* was measured on the E.I.L. *p*H meter using the platinum metal electrode '*EPT*23' and reference electrode '*RJ*23'. There was a progressive clear-