Besides the bovine tongue epithelium, the mucous membrane of the rumen is very susceptible to this virus. This suggested to the first-named author the use of this epithelium for cultivation. We have, therefore, applied a similar method to that used for growing the virus on explanted epithelial bovine tongue tissue.

As a nutrient medium, Baker's solution[5] was used. The mucous membrane was removed from the ruminal wall after it had been washed with warm saline, treated with penicillin and streptomycin in order to prohibit as much as possible growth of micro-organisms present on this tissue, thoroughly minced with scissors and incubated at 37° C. The nutrient medium was aerated with carbogen (95 per cent oxygen and 5 per cent carbon dioxide) to secure sufficient respiration of the tissue.

Not all the various types of epithelial tissue of the rumen are suitable for use. It seems, according to what is observed in the circumstances of the spontaneous disease, that only the smooth part of the ruminal mucosa (pillars) show lesions. This tissue should be used for cultivation of the virus.

In our first attempt, we succeeded in cultivating the virus in twenty-eight successive passages, after which it was inactive for some unexplained reason.

After continuing on the rumen epithelium with virus grown on explanted bovine tongue epithelial tissue, we have propagated it thus far in eight passages, the last two passages showing a titre on bovines of 1 : 279,000 and 1 : 450,000 respectively. This last passage was a large-scale culture (2,000 ml.) in a stainless steel container.

One rumen yields about 75 gm. of epithelial tissue. We obtained with the tissue from two rumens 2 litres of foot and-mouth disease virus of a titre of 1 : 450,000. If we suppose that the average quantity of virus material (vesicles and lymph) of one artificially infected bovine (intradermal infection of the lingual mucosa) is 50 gm. and the average titre of this virus is 1 : 2,000,000, we easily can see that the cultivation in epithelial tissue of one rumen gives us 1 litre with a titre of 1 : 450,000 or about 200 c.c. with a titre of 1 : 2,000,000, which is equal to the yield of four bovine tongues infected during life.

We believe that the use of explanted epithelium from the rumen for the cultivation of foot-and-mouth disease virus to be of high importance. We are convinced that only cultivated foot-and-mouth disease virus can be used in the production of vaccine against this disease. The advantages are: (1) no risk of spreading the disease through mass infection of living animals; (2) great reduction in the costs of virus production; (3) simplification of the method of preparation of the vaccine; and (4) as the result of these advantages, a subsequent lowering of the cost of the vaccine, which should favour a wider usage of the vaccine in the battle against foot-and-mouth disease.

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[1] Maitland, M. C., and Maitland, H. B., 4th Progress Report of the Foot-and-Mouth Disease Research Committee.

[2] Hecke, F., *Z. Bakt.*, **128**, 336 (1933).

[3] Frenkel and van Waveren, "Algemeene Landsdrukkery" (The Hague, 1934–35).

[4] Frenkel, H. S., Office International des Epizooties, **28** (1947).

[5] See Parker, "Methods of Tissue Culture", 76 (Paul Boeber, Inc.).

Triphenyl Tetrazolium Chloride as a Reagent for the Histological Demonstration of Subcutaneous Fat

Subcutaneous fat at the site of injection is stained specifically in five to ten minutes when 0·5 ml. of a 1 per cent aqueous solution of triphenyl tetrazolium chloride is injected into mice. Neutral fat in the deeper layers is stained a bright orange-red, while the secretions of the sebaceous gland cells are stained a brick-red colour.

Similar results are obtained when fresh unfixed strips of skin are immersed in the aqueous solution. The staining reaction is inhibited by most of the common histological fixatives; but these may be used subsequent to the development of colour in the tissues, without deleterious effect.

A variety of contrast stains may be injected in admixture with the triphenyl tetrazolium chloride solution so as to produce simultaneous staining of the various tissue elements. Good results were obtained with solutions containing Janus Green B or Brilliant Cresyl Blue with triphenyl tetrazolium chloride, each at a concentration of 0·5 per cent.

The stained tissues, after removal of hair, may be transferred to any fixative which does not contain fat solvents, and afterwards embedded in gelatine prior to sectioning. The gelatine blocks containing the stained material may be stored for months in 5 per cent formaldehyde solution without loss of colour.

Other mammalian tissues containing deposits of fat, for example, mesentery and lactating mammary gland, stain rapidly and intensely on immersion in aqueous solutions of triphenyl tetrazolium chloride. Particularly intense staining of fatty deposits in the liver of a cat, which had been kept in the laboratory, was observed after immersion in a 1 per cent solution for 1 hour.

When an alcoholic extract of the stained tissues is shaken with an equal volume of arachis oil or animal fat, the red colour passes rapidly into the oil phase. This ready solubility in fats suggests a probable explanation of the staining reaction in fresh tissues, namely, reduction of the triphenyl tetrazolium chloride with precipitation of formazan and re-solution of the formazan in the fatty deposits.

Triphenyl tetrazolium chloride is extremely toxic when injected intravenously into mice, 0·5 mgm. being sufficient to kill instantaneously.

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Brightness and Saturation of Colours in Red-Green Defectives

For theories of colour vision it is important to consider the relationships between the saturation and photopic brightness-levels of certain colours for anomalous and red-green blind subjects.

In a recent series of experiments[1], 23 protanopes, 33 deuteranopes and 31 green anomalous subjects were given tests of saturation of red, green and yellow, and of the photopic brightness-levels of these colours, in a colorimeter which used the Ilford