

Unlike previous experience with the *MtT/F4*, most of the animals in which the tumour grew had some, and usually extensive, growth of tumour in the thorax; this included both the Wistar/Fu and Fisher/Fu rats. This unusual pattern of growth is probably due to a technical error whereby the tumour was inadvertently introduced directly into the thorax; a subsequent investigation in which the tumour was transplanted as a single small fragment by trocar into the subcutaneous tissues, has resulted in local subcutaneous growth only. For this reason it was not possible to make observations on the latent period or rate of growth in relation to thymectomy.

The tumour killed, or severely debilitated, most of the hosts by extensive intra-thoracic growth, seven to eight weeks after transfer. At this time, all the debilitated animals were killed and it was found that the tumour had taken in 2/5 female and 2/5 male thymectomized Wistar/Fu rats. Ten weeks after transfer of the tumour, five male and five female Wistar/Fu, intact littermates of the thymectomized rats, were killed and none was found to have growth of the tumour; seventeen weeks after the transfer, an additional four male and four female Wistar/Fu, intact littermates, were alive without detectable growth of tumour.

As expected, all five of the intact, and all four of the thymectomized, Fisher/Fu rats had successful growth of the tumour when they died or were killed, seven to eight weeks after the transfer. In all the rats in which the tumour grew, both Wistar/Fu and Fisher/Fu, the adrenals were approximately ten times normal size and the mammary glands were greatly enlarged.

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Effect of pH on Human Complement Activity

WHILE investigating the effect of acetylsalicylic acid (aspirin) on complement (*C'*) activity, it was observed that incorporation of 40 mg per cent of the drug in a well-buffered reagent diluent, 0.15 M triethanolamine buffered salt (TBS) solution¹, significantly increased the haemolytic activity of human complement (Hu*C'*)². Since it was also noted that the drug slightly reduced the pH of the reaction mixture, the studies were extended to investigate the effect of pH change on Hu*C'* activity.

Pooled human serum freshly collected and stored not longer than one month in a mechanically operated freezer (−60° C) provided the source of Hu*C'*. Assays of *C'* were carried out using a precise spectrophotometric method as

previously described³. Haemolytic activity was expressed as 50 per cent haemolytic units per ml. (*C'H*₅₀/ml.) of serum. TBS, pH 7.2, containing magnesium and calcium ions in concentrations optimal for immune haemolysis, was used as the standard reagent diluent. For experiments in which it was desired to alter the pH of the system, required volumes of 0.1 N hydrochloric acid or 0.1 N sodium hydroxide were added to a stock concentration of TBS and then diluted so that a constant ionic strength was maintained. The influence of pH on the haemolytic activity of Hu*C'* was investigated over the pH range 5.55–8.20.

Table 1. EFFECT OF pH ON THE HAEMOLYTIC ACTIVITY OF Hu*C'*

pH	5.55	6.14	6.53	6.77	7.20	7.55	7.80	8.20
Hu <i>C'</i> titres* (<i>C'H</i> ₅₀ /ml.)	<30	88	226	279	239	197	186	166

* Arithmetic means of three independent determinations.

The results of this investigation are summarized in Table 1. Maximum haemolytic activity of Hu*C'* was observed at pH 6.77 and not at the pH level (7.2) usually used. Adjustment of pH to either the acid or alkaline side of pH 6.77 resulted in reduced Hu*C'* titres. These observations paralleled the findings of Ibe and Wardlaw⁴ in which it was demonstrated that in the case of guinea-pig *C'* (Gp*C'*), maximum haemolytic activity was manifested at pH 6.8. Both studies corroborate and extend the observations of Kabat and Mayer⁵, who noted that the haemolytic activity of Gp*C'* progressively increased as the pH of the system was reduced from 8.52 to 7.15. However, the latter investigators did not present data concerning the effects of lower pH values on immune haemolysis. In view of the present findings, consideration should be given to the potential value of conducting tests involving guinea-pig and human *C'* at pH 6.8 rather than using the more generally used pH 7.2.

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Adenovirus 12 Virion-free 'T' Antigen from Infected Cells inhibited with Cytosine Arabinoside

RAPP *et al.*¹ described a method for producing SV 40 'T' antigen free of virion by using cytosine arabinoside (CA) to block the growth of virus in tissue cultures of cercopithecus kidney cells. We found that cytosine arabinoside could also be used to produce virus-free adenovirus 12 'T' antigen in human cell tissue cultures as follows: It was found that the pH of the media and the condition of the human cell lines (*HEK*, *HeLa* or *KB* monolayers) used to produce adenovirus 12 'T' antigens could be best controlled in test-tubes and that when this was done more uniform production of 'T' antigen was obtained. About three hundred test-tubes were used for each experiment. Evenly growing cell monolayers were drained of fluid and washed with Eagle's basal medium containing 4 mM glutamine plus antibiotics. Each culture was then inoculated with 0.2 c.c. virus mixture (virus diluted with Eagle's basal medium containing 4 mM glutamine plus antibiotics) at multiplicities between 1 and