Table 1. Complement-fixation titer of rabbit antisera with various tissue-culture cell antigens. TC, tissue culture.

Antigen* and dilution	TC passage	Antiserum†				
		HeLa	Human embryo intestine	Cynomolgus monkey heart	Rhesus monkey	
					Kidney	Heart
		200	37	63	1	0
HeLa (1:8)	6	128‡	512	1024	0	4
Human embryo intestine (1:4)	36	64	512	1024	16	32
Cynomolgus monkey heart (1:8)	73	128	512	1024	0	8
Rhesus monkey kidney (Und.)	1	16	8	32	32	4
Rhesus monkey heart (1:16)	0	4	0	8	0	32
		Controls				
Tissue culture medium and antibiotics		0	0	0	0	0
Horse serum (1:64)		0	0	0	0	0
Calf serum (1:4 to 1:1024)		0	0	0	0	0
Human serum (1:1024)		4	0	4	0	0
Monkey serum (1:1024)		0	0	0	4	4

* All cells for CF antigens were grown in calf serum and medium 199 and were standardized to contain 2.5×10^6 washed cells per milliliter and diluted as indicated to obtain two units of antigen in the dilution showing greatest complement fixation with its homologous antiserum as shown by a block titration.
† All reactions were carried out in the presence of two exact units of complement titered in the presence of antigen. Cells for preparation of antisera were grown in horse serum.
‡ Endpoints read from highest dilution tubes showing 3 + or 4 + fixation.

growth in tissue culture is an intriguing concept which would readily explain the observed partial or complete antigenic cross reactions as well as the morphologic similarities of long-term tissue-culture cells and possibly their relationship to malignant cells. That is, if long-term tissue-culture cells from various species gradually acquire a preponderance of a common antigen or antigens as they adapt to independent growth in vitro as well as the property of unrestrained growth in vivo when transplanted back to cortisone- and x-ray-treated rats (*7*), then a new laboratory experimental approach to study of malignancy may be at hand.

This last hypothesis can be approached in two ways: (i) by ruling out all other causes of cross antigenicity and (ii) by starting new cell lines from normal tissue under rigidly controlled conditions to prevent contamination and observing whether these new lines also revert to the common antigenic cell. Such studies are now under way.

3) The third and most likely explanation for common antigenicity of long-term tissue-culture lines derived originally from different animal species is contamination with a common virus or bacterium. To escape detection, such a contaminant would have to be resistant to the antibiotics in the culture medium and should not cause obvious turbidity of the medium, should fail to grow in routine bacteriological culture media, and should cause no extensive cytopathogenic changes in the tissue-culture cells. We have searched for bacterial and viral contaminants by the usual methods, but it is not possible to rule out the presence of an unknown noncytopathogenic virus or bacterium by means of negative tests. A slow-growing diphtheroid-like organism in one of our tissue-culture lines did not share common antigens with the long-term cell lines. Pleuropneumonia-like organisms (PPLO) have been reported in tissue-culture cell lines by Robinson, Wichelhousen, and Raizman (*8*) and are present in our HeLa cell lines. Extensive search by Morton and Rothblat (*9*) has revealed PPLO in several but not all long-term cell lines maintained in this laboratory and in cell lines from other laboratories. CF tests with the PPLO antigen, T5, isolated from our HeLa culture and antisera against the long-term tissue-culture cells were all positive. Similar tests with a PPLO antigen prepared from a laboratory strain of human genital origin, O7, were all negative. These tests suggested that the contaminating PPLO may be one common antigen shared by some long-term cell lines. However, adsorption of the long-term tissue culture antisera with PPLO-T5 did not remove the common antibody for long-term tissue-culture antigens. This, combined with the fact that some of the cell lines did not contain PPLO, suggests that these contaminating organisms (PPLO) are not the explanation of the common antigens in long-term cell lines. However, cell lines may contain different antigenic types of PPLO, and this has not been investigated.

These studies raise more questions than they answer, but two points seem to be fairly well established: (i) The acquisition of common antigens by long-term tissue-culture lines occurs frequently and crosses species lines. The explanation for this is not clear although several possibilities are offered. (ii) Modern tissue-culture media plus antibiotics provide an ideal medium for PPLO, viruses, and some fastidious bacteria and their presence may not be revealed by routine bacteriological sterility tests (*10*).

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12 February 1958

Phosphorescence in Liquid Scintillation Counting of Proteins

Alcoholic solutions of the quaternary base Hyamine have been reported by Vaughan *et al.* (*1*) as being suitable solvents for amino acids and proteins in liquid scintillation counting systems. While investigating this Hyamine system for the counting of proteins and tissues, we encountered, when working with larger weights of such substances, a phosphorescence phenomenon, which, if it is not eliminated, can cause considerable trouble in the counting of samples of this magnitude. Fortunately methods are available whereby such interference can be eliminated or circumvented.

The substances used were dried rat bile, rat liver, and horse serum; whole rat muscle; egg albumin, gelatin, Bactopeptone, and trypsin. As much as 100 mg, and in some cases more, of these substances can be dissolved in 3.0 ml of

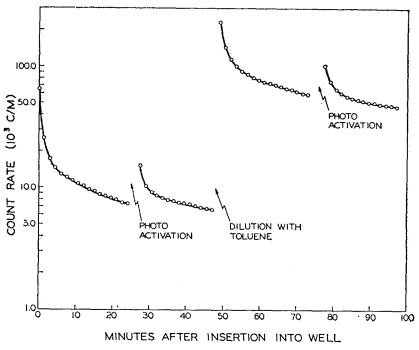

Fig. 1. Count rate of a trypsin-Hyamine solution showing the effects of exposure to light and of dilution with toluene.

Hyamine solution, prepared according to the method of Vaughan (*1*). These 100-mg protein samples usually required heating between 50° and 70° C for periods ranging from ½ hour to several hours. Samples were then cooled to room temperature, diluted with 10 ml of toluene containing 0.5 percent DPO, cooled, and counted. All counting was done with a Tri-Carb liquid scintillation counter operated at 1100 v with channel settings 6–35 and 6–100.

It was observed that neither radioactive isotope nor scintillation phosphor was needed to obtain a tremendous count rate. From 20,000 to 300,000 count/min was observed with Hyamine-protein-toluene solutions of 100-mg portions of the proteins mentioned above. This count rate decreased regularly but slowly. The normal instrumental background was reached only after several days. (For rate studies zero time was taken as start of addition of toluene to the protein-Hyamine solution.) It was also observed that protein-Hyamine solutions containing no added toluene (or radioisotope) exhibited this slow-decay phosphorescence. (For these solutions, zero time was taken as time of insertion of the cooled sample into the counting well of the counter.) In general, the magnitude of the count was not as great (up to 50,000 count/min) as it was when toluene was present. When these protein-Hyamine solutions were diluted with toluene, alcohol, acetone, dioxane, ether, water, or glycerine, the rate was raised; the first five substances yielded a count rate of several hundred thousand counts per minute. The count rate on dilution seemed to be an inverse function of the viscosity of the diluting solvent; the lower the viscosity, the higher the rate.

The count rate of both the protein-Hyamine and protein-Hyamine-solvent solutions could be increased by exposure of the solutions to an incandescent lamp. The entire phenomenon is shown in Fig. 1 for 100 mg of trypsin dissolved in 3.0 ml of Hyamine solution. Here are shown the initial count, a photoactivated count, the count after dilution with 10 ml of toluene, and another activated count. The activation consisted of exposing the sample to the light of a 75-w Reflectorspot at 25 cm for 1 minute. (Empty vials, or vials containing toluene, gave no more than a 2-count/min increase when they were so illuminated for 5 minutes.)

In all cases, the initial count increased as sample weight increased. Below a concentration of about 10 mg of protein per milliliter of Hyamine solution, the phenomenon was negligible so that the phenomenon described does not invalidate the procedure of Vaughan *et al.* for either C^{14} or H^3 counting. In several of the experiments when the protein-Hyamine solution was diluted with toluene or other solvent, the initial rate started at zero and increased within a few seconds to a maximum and decayed in the usual way. In all experiments, the difference in count rate between the two channels soon became constant and small, showing the pulse heights of the counts to be chiefly below the 35 setting. The variation of the rate with photomultiplier voltage was as follows (calling the rate at 1100 v 1.00): 1020, 0.26; 1180, 1.35; 1260, 1.28; 1340, 0.88; 1400, 0.46.

Debye and Edwards showed that proteins in alkaline solution exhibit phosphorescence (*2*). They attributed this chiefly to tyrosine and tryptophan. As a check, 100-mg portions of these two amino acids were allowed to stand with 3.0 ml of Hyamine solution until solution occurred and were then counted after addition of toluene. Both of the two gave a slight, fairly rapidly decreasing count rate: tyrosine, 1500 count/min; tryptophan, 3500 count/min. In about 10 minutes the normal background was obtained. Exposure of the solutions to the Reflectorspot gave no increase in count rate for the tyrosine; the tryptophan rate increased by 300 count/min.

Another sample of trypsin, a substance which in 100-mg portions in Hyamine solution gave a tremendous count rate, was dissolved in 1.0*M* KOH and counted. No count was obtained. Hyamine solutions without protein gave no count either alone or when diluted with toluene or when exposed to light. Hyamine solutions of sterols, or of amino acids other than those mentioned above, failed to provide counts. For a given Hyamine preparation there seemed to be a possible correlation between initial count rate and number of hydrolyzable bonds. The count rate for equal weights of glycine, glycylglycine, glutathione, Bactopeptone, and trypsin increased in the order given.

The precise cause of this phosphorescence is not known. Heating of the sample may be significant. However, the effect can be completely eliminated in several ways. As already mentioned, for small quantities of proteins the effect is inconsequential. The purity of the Hyamine itself is of prime importance. Repeated crystallization of the original quaternary chloride from toluene, until the filtrate is colorless, yields Hyamine which, when it is converted to the quaternary base in methanol solution, with small weights of nonradioactive protein and several hours of sample cooling, produces only normal instrumental background. Large weights of proteins can be used if the solution is acidified prior to counting. Acidification of a high-counting protein-Hyamine-toluene solution reduced the count rate immediately to normal instrumental background. Such acidified solutions can be counted immediately after acidification. Little or no change in counting efficiency results from acidification.

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4 April 1958

Isometric Twitch Tension of Frog Skeletal Muscle as a Function of Temperature

Current work at this laboratory on the action of high-intensity ultrasound on the twitch contraction of excised frog skeletal muscle makes it necessary to distinguish between temperature and nontemperature effects produced by the sound. Therefore a study (*1*) was undertaken to determine the variation in the magnitude of the maximum isometric tension and the action potential of excised frog biceps muscle at a number of temperatures in the range 2° to 35°C. Unfortunately, in the recent literature there appears little data on frog skeletal muscle twitch tension at temperatures above 25°C. Walker (*2*), working at the two isolated temperatures 14.5° and 27.5°C found different results for *in situ* summer and winter frogs. His experiments on excised muscles produced variable results, with an indication that twitch tension is less at the lower temperature. Hill's well-known results (*3*) are restricted to temperatures below 22°C; Buchthal's results (*4*) on single fibers are limited to temperatures up to 26°C. The work of early investigators, referenced later in this report, yielded variable results.

The following procedure was used in a series of 32 experiments. At an initial