heptane mixture to 2 parts of tissue-culture material was used. Since it was the aim, however, to remove undesirable reactivities and not to attain a high degree of purification of the viruses, the smallest amount of the fluorocarbon-heptane mixture was used that would serve this purpose—that is, 1 part per 10 parts of the tissue-culture material.

The virus-fluorocarbon mixtures were homogenized in a Servall Omnimixer at 14,500 rev/min for 3 to 4 minutes while the treatment vessels were submerged in ice. The homogenates were centrifuged at 1000 rev/min for 5 to 10 minutes to separate the aqueous from the organic layers. The aqueous phase revealed little, if any, loss of infectivity, as was observed also by Manson *et al.* (*4*). Complement-fixation tests were performed by the standard technique employed in our laboratory (*5*).

Table 1 summarizes the results of an experiment with poliomyelitis antigens derived from HeLa cells, using type-specific serums obtained from monkeys following immunization with viruses grown in monkey renal cells. The first three crude antigens were anticomplementary; hence, specific reactions could not be discerned. After fluorocarbon treatment, the anticomplementary effects were lost, and specific reactions were readily apparent. Exposure to fluorocarbon of crude antigens, which were not anticomplementary, failed to show a decrease in specific reactions, as is shown, for example, in the last antigen included in Table 1. Other tissue-culture antigens (adeno group and Coxsackie B) and antigens derived from chick embryo chorioallantoic membranes (herpes simplex, mumps soluble antigen) or allantoic fluids (mumps and influenza virus antigens) were treated with fluorocarbon without loss in antigen titers. Anticomplementary preparations of these antigens were not available.

In the experiment shown in Table 2, serums were employed which were collected from guinea pigs after they had been immunized with poliomyelitis viruses grown in HeLa cells. The test antigens, likewise, were derived from HeLa cultures with the exception of one of the poliomyelitis type-I preparations, which was obtained from monkey renal cells. The latter reacted only with its homologous serum. All other crude antigens gave strong reactions with all of the antiserums, clearly based on interaction of host antigens with corresponding antibodies present in the serums. Fluorocarbon treatment removed these host antigens so that thereafter only the homologous serum reacted with the type-I antigen.

The technique has made it possible to salvage anticomplementary antigens for routine diagnostic tests and to employ, where unavoidable, antiserums prepared by immunization with virus preparations of the same kind as those used for test antigens.

Table 1. Removal of anticomplementary activity by fluorocarbon without loss of specific antigens. *a*, Antigen anticomplementary; no specific reaction obtained. All titers are expressed as the reciprocal of the highest serum dilution that reacted with the given concentrations of antigen.

Poliomyelitis antigens		Untreated antigens				Treated antigens			
		Saline control	Antipoliomyelitis serum type			Saline control	Antipoliomyelitis serum type		
Type	Diln.		I	II	III		I	II	III
I	1/1	4	a	a	a	0	128	< 32	< 32
I	1/2	4	a	a	a	0	64		
I	1/4	3	a	a	a	0	32		
I	1/8	0	< 32	< 32	< 32	0	< 32		
II	1/1	4	a	a	a	0	< 32	256	< 32
II	1/2	4	a	a	a	0		256	
II	1/4	2	a	a	a	0		128	
II	1/8	0	< 32	< 32	< 32	0		< 32	
III	1/1	4	a	a	a	0	< 32	< 32	256
III	1/2	4	a	a	a	0			256
III	1/4	3	a	a	a	0			128
III	1/8	±	a	a	a	0			< 32
I	1/1	0	256	< 32	< 32	0	256	< 32	< 32
I	1/2	0	128			0	128		
I	1/4	0	64			0	64		
I	1/8	0	< 32			0	< 32		

Table 2. Removal of host antigen by fluorocarbon. All titers are expressed as the reciprocal of the highest serum dilution that reacted with the given concentration of antigen.

Antigens		Guinea pig serums			
		Versus poliomyelitis (HeLa) type			Normal
Virus	Treated	I	II	III	
Poliomyelitis type I (monkey renal cells)	−	128	< 16	< 16	< 16
Poliomyelitis type I (HeLa cells)	−	256	64	256	< 16
Poliomyelitis type I (HeLa cells)	+	128	< 16	< 16	< 16
Coxsackie B_5 (HeLa cells)	−	512	256	512	< 16
Coxsackie B_5 (HeLa cells)	+	< 16	< 16	< 16	< 16
Uninoculated HeLa cells frozen and thawed	−	64	32	64	< 16
Uninoculated HeLa cells frozen and thawed	+	< 16	< 16	< 16	< 16

KLAUS HUMMELER
VINCENT HAMPARIAN
Division of Virology, Department of Public Health and Preventive Medicine, School of Medicine, University of Pennsylvania, Philadelphia, and *Children's Hospital of Philadelphia*

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27 November 1956

Ribosidation as a Means of Activating 6-Azauracil as an Inhibitor of Cell Reproduction

6-Azauracil (*as*-triazine-3,5-dione), an analog of uracil, is an inhibitor of the growth of certain microorganisms (*1*) and of several experimental tumors, including sarcoma 180 (S-180), in mice (*2*). In contrast, in tissue culture, 6-azauracil has no inhibitory activity on HeLa cells or mouse fibroblasts in the highest concentration tested (*3*). In addition, in our experiments with sarcoma 180 cells (in Eagle's medium containing 5 percent dialyzed horse serum, *4*), 6-azauracil in concentrations as high as 5 m*M*, was completely inactive as an inhibitor of cell reproduction. These findings suggested that the inhibitory activity

Table 1. Inhibition of growth of sarcoma 180 by 6-azauridine in tissue culture.

Concn. of 6-azauridine (μmole/ml)	Growth as compared with control (%)
0	100
0.01	88
0.03	83
0.1	50
0.3	16

Table 2. Reversal by uridine of the inhibitory action of 6-azauridine (0.2 μmole/ml) on sarcoma 180 in tissue culture.

Concn. of uridine (μmole/ml)	Growth as compared with control (without inhibitor or uridine) %
0	37
0.002	59
0.006	84
0.02	94
0.06	94
0.2	96

on sarcoma-180 *in vivo* might be attributable to a metabolite of the analog formed by the liver or other normal tissues. An attractive possibility for consideration, as a metabolite of 6-azauracil, was its riboside (6-azauridine), since this derivative is formed by certain microorganisms and inhibits the growth of 6-azauracil-resistant strains that emerge when *Streptococcus faecalis* is grown in the presence of 6-azauracil (*5*). The 6-azauridine used was prepared both biosynthetically (using *S. faecalis* 8043) and by chemical synthesis (*6, 7*).

Of the sarcoma-180 cells (*8*), 200,000 were introduced into each culture flask (Earle's T-15) (*9*) in which 2 ml of Eagle's medium containing 10 percent horse serum was present (*4*). After 24 hours, this medium was replaced by Eagle's medium containing 5 percent dialyzed horse serum and various concentrations of 6-azauridine; the medium was renewed daily. After 7 days, the protein content of the cell layer was determined, using the method of Oyama and Eagle (*10*). In the controls, without inhibitor, a three- to four-fold increase in cell protein, as compared with that observed 24 hours after inoculation, was obtained. Both the biosynthetically and the chemically prepared 6-azauridine showed the same high activity in inhibiting the growth of sarcoma-180; the results obtained with the biosynthetic material are shown in Table 1.

The activity of 6-azauridine was antagonized by uridine: as is shown in Table 2, the molar ratio of metabolite to antagonist necessary to abolish almost completely the action of 6-azauridine (at the level tested, 0.2 μmole/ml) was 1/10. Even with a tenfold higher concentration of uridine, under these conditions, no evidence of toxic effects on the cells was observed. Deoxyuridine was also active in reducing the inhibitory activity of 6-azauridine; however, quantitative data concerning its activity and its possible toxicity for the cells have not yet been obtained. Further work will be concerned with the action of related analogs and their nucleosides, the effects of other possible reversing agents and their comparative activity, and the effects of the agents on other cell lines in tissue culture (*11*).

RICHARD SCHINDLER
ARNOLD D. WELCH

Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut

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21 December 1956

Tungsten Microelectrode for Recording from Single Units

An electrode has been developed to fill the need for an easily made, sturdy device capable of resolving single-neuron action potentials at least as well as the commonly used micropipette. It was designed to be used not only in acute animal experiments in the central nervous system, but also in cases where pipettes may be especially prone to break, such as in chronic unrestrained preparations, with muscle, and in the human being during neurosurgical procedures. Early experience made it clear that, while tip diameters of the order of 20 μ may at times be adequate for resolution of unitary spikes recorded extracellularly, tips 5 μ or less are much more satisfactory, and that intracellular recording usually demands tips of less than 1 μ in diameter. Since steel wire becomes too fragile near the tip when thus sharpened and also requires too thick a shaft, tungsten was selected as by far the stiffest, easily available metal.

The electrode consists of an electrolytically sharpened tungsten wire insulated to the tip with a suitable lacquer. A wire 125 μ (5 mils) in diameter and about 1 inch long is bent slightly near one end which is then mounted in a 27-gage hypodermic needle. Because crimping of these needles results in perfectly satisfactory electric contact, no attempt has been made to solder the tungsten. Electropolishing is then carried out by a method analogous to that described by Grundfest *et al.* (*1*) for steel: the terminal few millimeters are immersed in a saturated aqueous potassium nitrite (KNO_2) solution, and an alternating current is passed between the wire and a nearby carbon rod, using 2 to 6 v, which may be conveniently obtained from a 6.3-v filament transformer fed by a Variac (*2*). The optimum voltage is not critical, but currents that are too low or too high tend to cause pitting.

If the wire is kept stationary and if the polishing is allowed to continue until all bubbling ceases, a rather abrupt pencil-like point is obtained which has a tip of ultramicroscopic dimensions (from 0.5 to 0.05 μ in diameter). Such a result is explained by the fact that the meniscus height depends on the diameter of the wire, which decreases as the polishing proceeds. The suddenness of the taper may give rise to excessive dimpling of the tissue to be penetrated. This may be avoided by lowering and raising the wire during all but the final stages of polishing, thus producing almost any degree of taper. A hydraulic drive with two oil-filled syringes and plastic tubing may be used for this, as well as for the coating.

Fig. 1*A* shows an electromicrograph of a wire sharpened as described; the tip measures about 0.4 μ. Tips of this size or less are consistently obtained without particular skill or practice.

Sharpened electrodes are washed in detergent and coated with a clear lacquer (*3*) that has been allowed to thicken to an almost honeylike consistency by exposing to air at room temperature for some hours. Under a dissecting microscope (6 to 40 magnifications), the wire is lowered into a beaker brimful of freshly stirred lacquer, and then slowly raised. When the tip