nol have higher venous blood alcohol concentrations than those given alcohol alone. Ethacrynic acid has been demonstrated to be a weak *in vitro* inhibitor of alcohol dehydrogenase. Mechanism of action and possible clinical significance have also been discussed.

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Physical and Chemical Properties of H-1 Virus. I. pH and Heat Stability of the Hemagglutinating Property.* (30020)

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H-1 virus, one of a group of small spherical agents, was isolated by Toolan(1) from fractions of human tumor tissue (HEp-1) grown in conditioned rats. At least 2 other H-viruses are known: H-3, derived from another human tumor, HEp-3, also grown in rats, and H-B, isolated directly from human placental tissue. H-3 is similar to Kilham's RV(2). All the H-viruses produce a specific deformity in hamsters injected when newborn, but they can be distinguished from one another antigenically and by their different agglutination patterns with red cells from various species of animals. Since its first isolation, H-1 virus has been obtained repeatedly from human tumors received from the operating room and from products of human conception, when 3 to 4 blind passages in newborn hamsters are done(3). It is also this virus that is most active in production of deformities in hamsters infected *in utero*(4).

The activity of the H-viruses can be measured in 2 ways: infectivity in newborn hamsters and agglutination of guinea pig red cells. Data(5,6) have indicated that the virus itself is the hemagglutinating agent. The present paper reports on the stability of the hemagglutinating property of H-1 virus to ranges of heat and pH.

Materials and methods. Source of virus for these experiments was the liver of hamsters injected within 3 days after birth with the sixth hamster passage of H-1 virus. Livers from moribund hamsters were excised, minced, diluted to 10% (w/v) with sterile distilled water, homogenized in a Waring blendor, and filtered through a Selas porcelain candle filter, porosity 0.03. The filtrate was partially purified by centrifugation in a Beckman-Spinco ultracentrifuge in a 40 rotor at 30,000 rpm for 5 hours. The pellet, resuspended in distilled water, contained more than 67% of the initial hemagglutinating activity and about 8% of the original protein, as determined by the Lowry method(7). This final virus preparation had a titer of 1280 hemagglutinating units (HA units) with guinea pig red cells.

Hemagglutination assays were run as follows: equal parts of dilutions of virus and 0.4% guinea pig red cells were mixed. Phosphate-buffered saline (PBS), pH 7.2, was used as the diluent and suspending fluid. The mixtures were stored overnight in the cold room at 5°C, and the end-point was read the

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TABLE I. pH and Heat Stability of H-1 Virus Hemagglutinating Activity.

	Temperature (°C)									
pH	4	25	37	45	55	65	70	75	80	85
1	0	0	0	0	0	0	–	–	–	–
2	1280	1280	640	640	0	0	0	–	–	–
3	1280	1280	640	640	640	160	0	0	0	0
4	1280	1280	640	640	640	640	640	160	0	0
5	1280	1280	320	320	320	320	640	640	160	0
6	1280	1280	160	160	640	640	640	640	80	0
7	1280	1280	640	640	640	640	640	0	0	0
8	1280	1280	1280	1280	640	160	0	0	–	–
9	1280	1280	1280	1280	640	0	–	–	–	–
10	1280	1280	640	320	80	0	–	–	–	–
11	1280	1280	640	0	0	0	–	–	–	–
12	0	0	0	0	0	–	–	–	–	–

Partially purified virus was mixed with pre-heated buffer and kept at the temperature of observation for 30 min. PBS at room temperature was added to adjust the conditions to those of the hemagglutination assay. Data recorded are the titers in HA units remaining after treatment; they represent at least 2 separate runs at each temperature and 3 to 4 where critical areas were noted.

next morning as that dilution of virus which completely agglutinated all the red cells. This was a conservative criterion of virus activity since some hemagglutination was detectable at levels diluted two-fold or more than the reported titers.

MacIlvaine's sodium phosphate-citric acid buffer and Sørensen's glycocoll-sodium chloride-sodium hydroxide or borate-sodium hydroxide buffer were used for the acid and alkaline ranges, respectively(8). One part of virus and 9 parts of pre-incubated buffer were mixed and stored at the temperature of observation for 30 minutes. Equal parts of PBS at room temperature were added to adjust the samples to the conditions of the hemagglutination assay. Thus, only irreversible inactivation was studied. Control samples treated with PBS were run with each test.

Results. The hemagglutinating ability of H-1 virus proved to be stable to wide ranges of pH and heat (Table I). No loss of titer was seen from pH 2 to pH 11 at 4° and 25°C. Heating at 37° and 45°C resulted in reduced activity over the entire pH range, except at pH 8 and pH 9, with a minimum at pH 5 and pH 6. At 45°C, hemagglutinating ability was inactivated at pH 11.

Heating at higher temperatures progressively reduced the pH range over which the virus retained its hemagglutinating activity. The rate of fall was approximately one pH unit for each 10°C rise in temperature, starting at 37°C for the alkaline and at 45°C for the acid range, up to 85°C at which point no further activity was detectable. Hemagglutinating ability persisted longest at pH 5 and pH 6 where it was still present at 80°C. It was in this same pH range that activity dropped farthest at moderate temperatures (see above). At pH 8 and pH 9, complete activity was retained up to 45°C after which it dropped rapidly.

Discussion. Previous, unpublished work using crude infected liver filtrates showed that the hemagglutinating ability of 3 of the H-viruses, H-1, H-3, and H-B, were stable to changes of pH and temperature(5). The results reported here with partially purified H-1 virus confirmed the hardiness of this viral property. Stability to widely varied heat and pH conditions are characteristic of, but not exclusive to, the H-viruses. Ginsberg(11) has shown that adenoviruses retain their infectivity over a range of pH 2 to pH 11 at 22° to 23°C. Bachrach and Schwerdt(12) have demonstrated that poliovirus infectivity is stable for at least one day from pH 1.5 to pH 10.5.

In our studies, although there was generally a gradual loss in hemagglutinating ability with increasing temperature at the different pH levels, 2 areas at moderate temperatures (37° and 45°C) were noteworthy exceptions. These were pH 5 and pH 6 at which levels hemagglutinating activity was minimal and pH 8 to pH 9 where it was maximal. At the former pH values, activity

persisted and even increased at higher temperatures of incubation, whereas at the latter pH levels, hemagglutinating ability fell rapidly as temperature rose. The altered hemagglutinating activity may indicate a change in the protein coat. This point is under study.

Absence of hemagglutinating ability does not necessarily mean that infectious virus is not present, since a titer of 10^3 newborn hamster infectious doses is required before hemagglutinating activity is demonstrable(5, 6,9,10). The agent of hemagglutination appears to be the virus particle itself as determined from density gradient ultracentrifugal and electron microscopic studies. No other particles have been seen even when agglutinated red cells are used as the source of materials for electron microscopy(5,6,13). Therefore the stability of the hemagglutinating property of H-1 virus probably indicates the great stability of the virus particle itself, a corroboration of laboratory observations (*e.g.*, H-virus stored for 3 years at refrigerator temperature has retained its original HA and infectivity titers).

Summary. The hemagglutinating property of H-1 virus is stable from pH 2 to pH 11 at 4°, 25°, and 37°C. With increasing temperature, loss of titer gradually occurs, particularly at the extremes of the pH ranges. Hemagglutinating activity is still detectable after treatment at 80°C at pH 5 and pH 6.

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Use of Cell-Banked Monkey Kidney Cells for the Isolation of Respiratory Viruses.* (30021)

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For primary isolation and growth of many of the myxoviruses in cell culture systems, kidney cells of Old World monkeys appear to have an advantage in sensitivity when compared with other practically available cells. There are, however, certain problems which attend the use of primary monkey kidney (PMK) cell cultures, such as lack of a convenient and flexible source of supply, and uncertain status of various lots in respect to growth characteristics and latent viruses. In an effort to circumvent these problems, our laboratory has made use of secondary monkey kidney cell cultures (SMK) prepared from freezer-banked PMK cells which have been grown, frozen, and stored after the method of Stulberg(1). This report will describe the procedure for preparing these cells, our experience using them in studies of respiratory viruses over a period of 4 years, and the results of tests which indicate that such SMK cells match sensitivity of PMK cells when used for the primary isolation of parainfluenza viruses types 1, 2, and 3.

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Materials and methods. Source of MK cells. Two lots of cells were prepared from