H-1 Virus Viremia in the Human.* (30278)

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The H viruses which were originally isolated from transplantable human tumors(1,2) and subsequently directly from human tumors and embryos(3) are a group of small agents(4,5) that in common with Kilham's RV(6) produce a "mongoloid-like" deformity when injected into newborn hamsters. The abnormality is apparently not a true mongolism associated with chromosome aberrations but instead is the result of an osteolytic effect of the viruses on the developing facial bones of the young infected hamster (7). Because of their unique osteolytic property, it was hoped that the H-viruses might be of value in the control of rapidly growing tumors of the bone where other more conventional methods of treatment had failed. Accordingly, 2 young patients with advanced disseminated osteogenic sarcoma were inoculated with H-1 virus, an agent serologically distinct from RV, and blood levels of alkaline phosphatase studied in combination with X-ray pictures to determine whether any changes in the tumor growth pattern occurred. No ameliorating effect on the tumor was observed. At the same time, no untoward result of the virus injection was noted. A viremia and subsequently high titers of both neutralizing and hemagglutination-inhibiting (HA-I) antibodies to H-1 developed in both patients, and in one patient where complete blood studies were made, the virus was recovered from early blood samples in a noteworthy cyclic fashion which will be described. Electron microscope examination of such blood samples confirmed the presence of virus particles. It was apparent that the H-1 virus could produce an extensive viremia in the human.

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Materials and methods. Virus. Virus used for inoculation of Patient #1 was originally isolated from the transplantable human tumor HEp 1(1), passed serially for 3 generations in newborn hamsters and subsequently for 4 passages in rat embryo tissue culture. Hamster passages were prepared as cell-free, distilled water filtrates of a 10% homogenate of liver from 5-day-old hamsters injected at birth with virus. The H-1 virus used for Patient #2 was derived from the same original source as that used for the first patient, but had had 4 hamster passages only, after which the last filtrate was stored at 4°C until used. It was this latter strain that had not been associated with rat tissue after its original isolation which was used for the detailed blood and electron microscope studies to be described here. Both of the virus strains employed had hemagglutination titers of more than 1:2560 with guinea pig cells(8) at the time of use.

Hemagglutination-inhibition tests. These tests were done according to methods previously described by Moore(8). All sera were absorbed with Kaolin prior to use in order to remove non-specific inhibitors(9).

Electron microscopy. For examination in the electron microscope, blood from the patients was processed as follows: One drop of heparinized whole blood was mixed with 3 ml of 0.85% saline and centrifuged at 1500 rpm for 20 minutes. Most of the supernatant was decanted, and the cells were resuspended in approximately 0.2 ml of the remaining supernatant. One drop of this suspension was mixed with 3 drops of 2% phosphotungstic acid neutralized with KOH(PTA). A drop of the cell/PTA suspension was deposited on a Formvar-coated copper grid which had been stabilized with carbon, and after 30 seconds the excess fluid was withdrawn. The specimens were immediately examined in an RCA electron microscope. The accelerating poten-

tial was 100 kv and a 50 μ aperture was used in the objective lens. Micrographs were taken at a magnification of 36,000× and further enlarged photographically.

Results. Patient #1 was a 13-year-old female who had had a right hip disarticulation and subsequently a right hemipelvectomy for an osteogenic sarcoma approximately one year prior to being admitted to the hospital with extensive and inoperable metastases of her tumor. In the hope that the osteolytic virus, H-1, might be of some value in controlling this neoplasm, 0.8 ml of this virus was injected intramuscularly into the right deltoid muscle and 0.8 ml directly into the tumor of the right hip area. No further virus or other treatment was given. Four to 10 days after the injection, the patient developed a step-wise but modest fever to a maximum of 101°F. Since the patient also had a slight urinary tract infection during this period, it was not possible to attribute the fever to virus infection alone. The serum alkaline phosphatase did not rise during the 10 days following virus inoculation (levels were between 24 and 27 units) but did rise rapidly thereafter. This might have been a reflection of an effect of the virus on osteoblasts but there was no convincing evidence of tumor regression. The patient was discharged without fever to home care 15 days after the virus injection and readmitted to the hospital in poor condition 23 days after this discharge. She died as a result of neoplastic disease the next day. During the 40 days that the patient was observed, a number of small samples of blood were obtained—primarily for phosphatase studies. Some of these samples were available for H-virus antibody titration as recorded in Table I (Patient #1). As will be noted, hemagglutination-inhibiting (HA-I) antibodies to H-1 virus were first found in the blood in the day-10 sample after which the antibody titers increased rapidly and remained high until death of the patient. No HA-I antibodies to H-3 or RV were seen at any time. The sera with HA-I antibodies to H-1 also neutralized H-1 virus. Undiluted and 10^{-1} dilutions of these sera mixed with an equal volume of H-1 virus suspension, completely protected newborn hamsters injected with this material. In contrast, control animals injected with virus mixed with undiluted pre-injection blood from the patient, or with 6-day serum, or with two normal human sera, died in 5 days. Similar neutralization tests done with H-3 or RV viruses showed no protection against these agents with any of the serum samples. Complete titrations for neutralizing antibodies were not done, due to the number of newborn hamster litters required. The 3-day blood sample was also injected directly into 2 litters of newborn hamsters. Two of 4 babies in litter #1, and 5 of 8 in litter #2 developed the characteristic "mongoloid-type" deformity associated with H viruses. All these injected hamsters, bled as weanlings, had HA-I and neutralizing antibodies to H-1 whether they were deformed or not. The patient's 3-day blood, therefore, had an H-1 viremia.

TABLE I. Hemagglutination-Inhibition Titers of Patients' Sera to H-1, H-3, and RV Viruses.

Day of treatment	Patient #1			Patient #2		
	H-1	H-3	RV	H-1	H-3	RV
Pre	0	0	0	0	0	0
Day 0		—		0	0	0
1		—		0	0	0
2		—		0	0	0
3	0	0	0	0	0	0
4		—		0	0	0
5		—		0	0	0
6	0	0	0	0	0	0
7		—		0	0	0
8		—		0	0	0
9		—		0	0	0
10	320	0	0	0	0	0
11		—		0	0	0
12		—		0	0	0
13		—		0	0	0
14	1280	0	0	80	0	0
18	2560	0	0		—	
25	2560	0	0	2560	0	0
27		—		2560	0	0
30	2560	0	0	2560	0	0
38	2560	0	0		Died	

Guinea pig cells were employed for these procedures. Results are expressed as the highest serum dilution which completely inhibited hemagglutination.

Patient #2, a 12-year-old girl, was admitted to the hospital for the second time with a diagnosis of osteogenic sarcoma with generalized metastases. The tumor had first been diagnosed in the left leg 6 months previously. Amputation had been refused and radiation

therapy employed without success. One month prior to this second admission, she had had a short 4-day course of actinomycin D and nitrogen mustard therapy both of which were poorly tolerated and therefore discontinued. Three weeks before admission to the virus study ward, she had received a single radiation treatment. Two days later she developed a follicular tonsilitis concurrent with a drop in her white count and hemoglobin. She was given a transfusion that day and a course of penicillin treatment, and discharged afebrile 48 hours later. One week after this discharge, the patient was readmitted to the hospital and, since all previous treatment had failed, she was given a single subcutaneous injection of 1.0 ml of H-1 virus in the deltoid muscle of the right arm. No other treatment was subsequently given. Small samples of 2-3 ml of heparinized blood were obtained just prior to the virus injection and daily thereafter for 2 weeks. The patient then went home for 11 days during which time no blood samples were taken. She returned to the hospital for a terminal stay of 1 week during which 3 additional blood samples were examined. In contrast to patient #1, the serum alkaline phosphatase of patient #2 rose progressively and a plateau was not noted. No ameliorating effect of the virus on the tumor growth was observed. As with patient #1, this second patient developed high HA-I antibody titers specific for H-1 (Table I, Patient #2). That the antibodies appeared somewhat later than those of the first patient might have been due to the radiation and/or chemotherapy received by this individual, to the smaller dose of virus given, or to other factors. The samples of blood with HA-I titers to H-1 also specifically neutralized this virus when tested in the manner previously described. In this patient a complete study for possible viremia was done. Every specimen of whole blood was injected into two newborn hamster litters, each baby receiving 1 drop of blood. Five days after injection, 2 babies from each litter were killed and a cell-free filtrate prepared from their livers. The filtrate was then injected into another litter of newborn hamsters and the procedure repeated for 3 passages or a total of 4 litters. An example of such passage is given in Table II. It represents the treatment and results from the blood of day 2. As will be noted, in this instance, though no evidence of virus was observed in the original litters injected, passage through several litters built up the titer of virus until isolation of H-1 virus could be made. Throughout these experiments, the findings for the two litters of any one day blood sample were remarkably in agreement with one another. At the same time that these tests were done, several drops of the original blood were prepared for electron microscope (EM) study. Results of the hamster tests and EM findings were then compared and found to correlate well (Table III). Isolations of H-1 were made in the hamsters from the blood samples of days 1 through 5 with the maximum virus estimated at days 3 and 4. No isolations could be made

TABLE II. H-1 Virus Viremia in Patient #2. Test of day-2 blood in newborn hamsters.

	Original litters inj	1st pass	2nd pass	3rd pass
Litter #1	7 (0)	→ 5 (0)	→ 4 (4)*	→ Dead at 4 days*
#2	8 (0)	→ 5 (0)	→ 4 (2)*	→ *Idem**

Number in parentheses = No. of hamsters with H-virus deformity compared to total No. of animals surviving.

* Isolation of H-1 virus from livers of baby hamsters.

TABLE III. Viremia in Blood of Patient #2 Injected with H-1 Virus.

Day of treatment	Hamster test*	EM†	Day of treatment	Hamster test*	EM†
Pre	0	0	Day 6	0	0
Day 1	+	P	7	0	0
2	+	P	8	+	P
3	+++	P	9	+++	P
4	++++	P	10	+	P
5	+	0	11	0	0

12, 13, 14, 25, and 27 day tests = negative for both hamsters and E.M. Tests of tumor, liver and kidney at autopsy also negative.

* Hamster tests are graded as follows: + = Isolation of H-1 virus after more than one passage; +++ = one original litter showed immediate isolation, the other isolation on 1st passage; ++++ = both original litters gave immediate isolation. 0 = negative.

† No attempt is made to grade the number of particles in the EM study; they are designated as present (P) or absent (0).

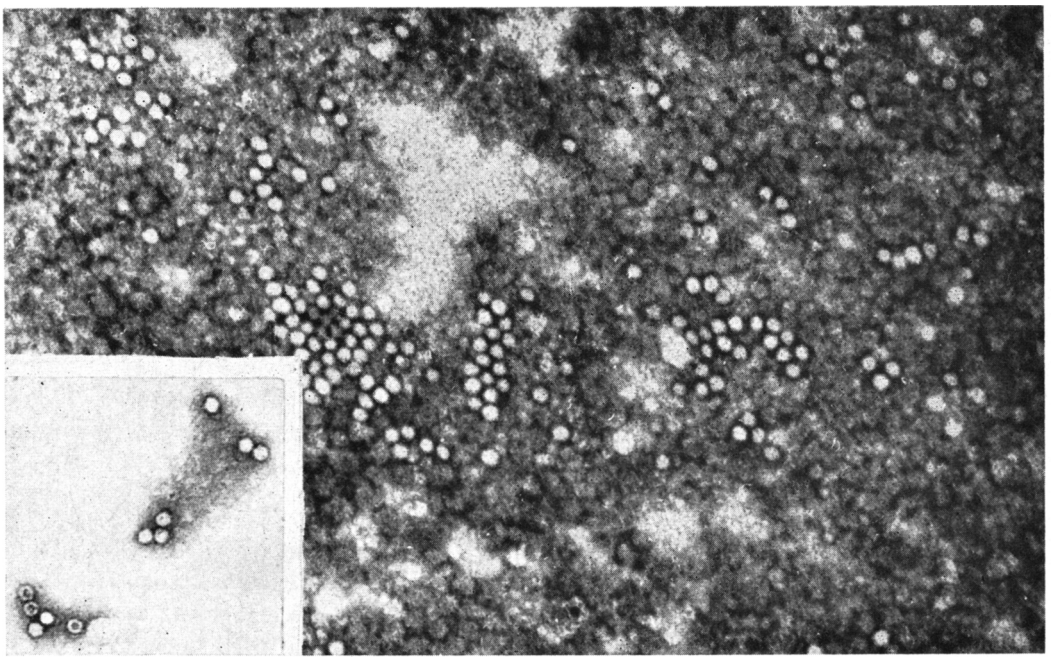

FIG. 1. A group of negatively stained particles found in the blood of Patient #2, 4 days after injection of H-1 virus. × 90,000. Insert. A group of negatively stained particles isolated from tissues of baby hamsters infected with H-1 virus and purified by centrifugation in a CsCl gradient. × 90,000.

from the blood of days 6 and 7 but virus was again recovered from days 8 through 10. All the remaining blood samples were completely negative nor could virus be recovered from the tumor or two tissue samples (liver and kidney) obtained at autopsy. As noted, the EM studies agreed with these findings. A maximum number of particles was found at day 4 (Fig. 1). These negatively stained particles had an average size of 245 Å, ranging from 230 Å to 260 Å; a few showed empty central zones measuring 115 Å. Surrounding the central zones was a coat 55 Å to 65 Å in thickness. For comparison the insert of Fig. 1 shows particles isolated from the tissues of a baby hamster infected with H-1 virus, and purified by centrifugation in a CsCl gradient. All these particles are similar to those previously described in H-1 infected tissues(4,5). No particles were seen in the EM on days 5, 6, or 7 while those found on days 8 to 10 had a "motheaten" appearance. It is not known whether this latter finding was due to an artifact or was the possible result of an antigen-antibody reaction. Additional blood was not available for re-examination.

Discussion. The findings show that human beings are susceptible to the H-1 virus since they can develop viremia and also both HA-I and neutralizing antibodies to this agent after injection. This is in agreement with the previously recorded observation that high titers of HA-I and neutralizing antibodies to H-1 were found in the sera of 2 laboratory personnel as well as in a few cancer patients(10). It is of interest that the virus appears to have an eclipse phase. After subcutaneous injection, the virus appeared in the blood stream on day 1 and increased in amount until the 4th day, decreased greatly on the 5th day and could not be recovered on the 6th and 7th days post-injection. It reappeared on the 8th, 9th and 10th days and then disappeared permanently. By the 10th day, it seems likely that some antibodies to the virus were present in the patient even though they were not detected by the means employed, and that therefore our "superficial" findings may not have truly reflected the underlying antigen-

antibody situation. The odd appearance of the virus seen in the EM during the 8-10-day period may have been a corroboration of this speculation. That the "cyclic" pattern of the viremia is a bona fide observation is indicated by similar findings in a study of H-1 viremia in adult hamsters to be reported elsewhere (11).

Summary. A viremia and subsequently HA-I and neutralizing antibodies were found in each of 2 human subjects injected with H-1 virus, indicating that the human is susceptible to, and can support, the proliferation of this agent. Studies of blood samples from one of the 2 individuals indicated that the viremia had a cyclic pattern.

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H-1 Virus Viremia in the Adult Hamster.* (30279)

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A previous paper(1) reported that H-1 virus can produce a viremia in the human host as well as the formation of both hemagglutination-inhibiting (HA-I) and neutralizing antibodies. An interesting finding was the "cyclic" pattern of the viremia observed. In one individual where daily blood samples were studied, it was possible to isolate H-1 virus from the whole blood from days 1 through 5 after injection and again from days 8 through 10. No virus could be recovered from the blood of days 6 and 7. Electron microscope observations corroborated these findings. The present paper describes a similar cyclic pattern of H-1 virus viremia in adult hamsters and also the appearance of titratable HA-I and neutralizing antibodies for this agent while the viremia was still present.

Materials and methods. The procedures employed for these experiments are the same as those previously described(1).

Virus. The H-1 virus used for injection of adult hamsters had a hemagglutination titer of 1:2560 with guinea pig red cells. It had originally been isolated from the transplantable human tumor, HEp 1(2), passed serially for 4 passages in newborn hamsters, and then injected into a human(1) from whom it was isolated 2 days after injection.

Animals. Young adult female hamsters, 2 months of age, were employed for the present report. (A repeat of this work with male animals of the same age or older hamsters of either sex gave the same results.) Twenty-eight animals were given a single subcutaneous injection of ¼ ml of virus. The hamsters were bled from the heart daily thereafter in groups of 2 until day 12. Two further bleedings were done on days 14 and 21. The blood obtained was pooled and tested for H-1 viremia by injection of the whole blood into newborn hamsters. Each baby received 1 drop. If virus is present, such injected babies either

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