A VIRUS ASSOCIATED WITH TRANSPLANTABLE HUMAN TUMORS*

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APPROXIMATELY two years ago, we were engaged in a series of experiments wherein fractions of transplantable human tumor cells, prepared by ultracentrifugation or sucrose gradient techniques, were used for the immunization of rats, mice, and hamsters of various ages. None of the rats and mice, whether newborn or older, ever developed abnormalities though more than 50 litters were injected with the fractions in each species. However, in this early series, among 100 litters of newborn hamsters comprising 932 babies that had received the fractions at birth, 81 mongoloid-type animals appeared.[1] The deformity was most specific and was characterized by small size, a flattened foreface or microcephalic domed head, protruding eyes and tongue, abnormal or absent teeth and bone fragility (Figures 1 and 2). It was in no way similar to the "runting" often described in rats or mice. Of these first 81 mongoloid hamsters, 41 were males and 40 females, an even distribution according to sex, that has continued. The single injection, given to the hamsters immediately after birth, was less than .03 ml. It was usually given subcutaneously but could be injected intraperitoneally with similar results. These early materials were prepared in 0.25 M or 0.88 M sucrose. Later preparations have also been made in normal saline, Ringer's solution, or distilled water. The type suspending medium does not appear to be of moment. None of these solutions used alone as controls have produced abnormalities. This, of course, is important *per se* but it is also of significance as regards the technique of handling and isolation employed, i.e., that infection did not occur from other sources than the injections. It was noted that the abnormality could be produced occasionally as late as 48 hours after birth. Later, when a higher titer of the effective agent was obtained, deformities were produced with injections

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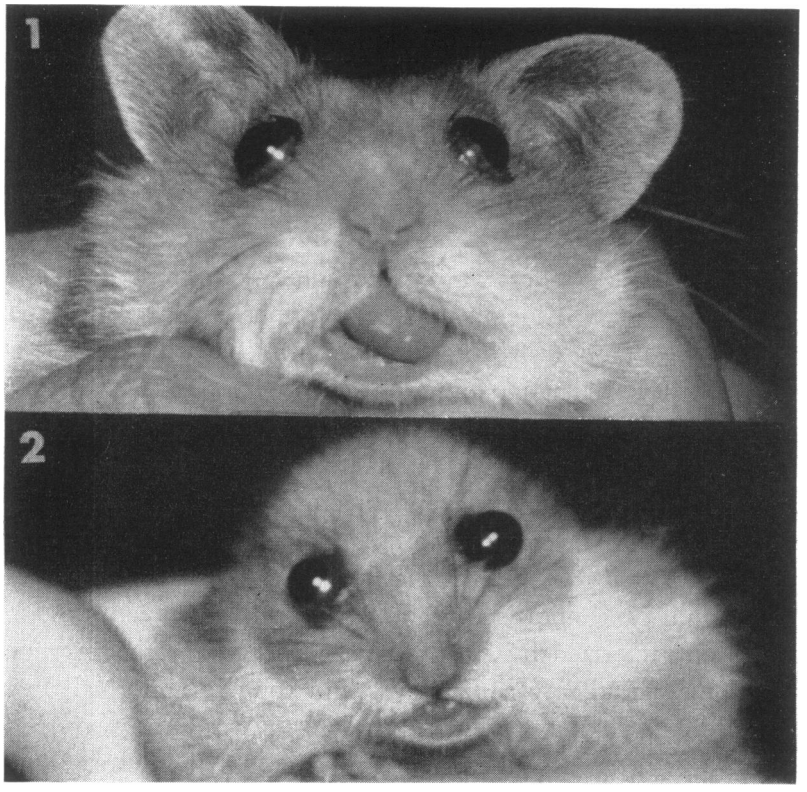

Figures 1 and 2. "Mongoloid" adult hamsters, injected when newborn with a filtrate prepared from one of the transplantable human tumors. Both have flattened forefaces, prominent eyes and tongues. The head of the animal in Figure 2 is microcephalic.

made four days, and occasionally six days, after birth. Hamsters injected with such high titer material when less than four days of age, could transmit the disease to non-injected animals in the same litter. The deformity was also produced by injecting hamsters *in utero*, and here the most interesting finding was that the same abnormality resulted regardless of the period in the mother's gestation that the babies were injected. The hamster has a very short, 16-day gestation period, so that seven days prior to birth is about as early as one can make adequate injections. The embryos are then very small. Yet whatever stage they were in, the abnormality was the same with one exception; in several of the animals injected *in utero*, there were extra digits on the hind

feet. A considerable number of the mothers, whose babies were injected *in utero*, aborted or gave birth to dead young. When 30 pregnant mothers were injected intraperitoneally, with care that the babies they were carrying were not touched, only two so treated had abnormal babies and then only one per litter. These mothers usually remained in good health with no evidence of disease.

The first thought that came to mind when these animals were seen was that some endocrine imbalance had been created. Yet careful study of the endocrine organs, in particular the pituitary and thyroid glands, failed to reveal abnormalities. It was concluded at that time that the protruding eyes and tongue resulted from diminution of skull capacity and not from endocrine disturbance.

These first animals presented a problem in handling. Many were lost because they failed to get enough food. Either their bizarre teeth made the mother reject them or their absence of teeth made eating of solid food pellets difficult. Once this was appreciated and a regimen set up where teeth were cut once a week and a soft mush diet provided, the little animals thrived, developed good coats, and remained in good health for periods comparable to normal hamsters. Over 2,000 have now been maintained for a two-year period.

It was soon learned that fractions from all eight of the transplantable human tumors regularly carried in conditioned rats and/or hamsters in our laboratory, could produce the specific abnormality described. The effective agent derived from these tumors, which varied widely in their type, was filterable and very stable in cold, either as a filtrate at 4° C. or in the frozen state at −79° C. It was, however, destroyed by heat and ultraviolet irradiation. It could be neutralized by normal rat serum, even after this was heated at 56° C. for 1 hour, and to a lesser degree by sera from other animals, such as rabbits, mice, calves, horses, guinea pigs, and chickens. In contrast to the neutralizing effect of the animal sera mentioned, human and hamster sera were unique in that they offered no protection.

Our next concern was whether we had picked up a contaminant agent from the host animals. This problem was approached in two ways: First, normal tissues and tumors from hamsters, rats, and mice were repeatedly tested and passed in baby hamsters with negative results, with the possible exception of one animal injected with an extract of a spontaneous rat tumor.[1] Spontaneous and transplantable rat, mouse, and

hamster tumors were also grown in conditioned rats, and again filtered and tested in newborn hamsters. No abnormalities resulted. Second, normal tissues and tumors from cancer patients as well as livers and spleens of individuals dying from accident, congenital heart disease, or other heart conditions, as well as pneumonia, were tested. This presented some problems as very fresh autopsies are hard to come by, and tissues other than tumor can seldom be removed at operation. The tissues from 8 "normal" individuals yielded nothing, whereas fractions of the livers and spleens of three out of four cancer patients produced a few mongoloid hamsters. The tumors of these patients did not. Our studies on the fresh human tissues as well as animal tissues are being continued with the ever-present problem of contamination being kept in mind.

When Dr. Dalldorf entered the investigation, he made an extensive study of the pathology of the adversely affected baby hamsters, and it was his observation that the abnormality we had seen was actually the end result of an infection in which the agent preferentially attacked the bones and teeth of the newborn animals.[2] The destruction of tooth odontoblasts and the enamel organ in young hamsters is the pathology which gives us our toothless adults with their appealing features of permanent childhood. Long bones show similar pathology, graded in degree according to the severity of infection.

About this time, work on passage of cell-free filtrates made from the livers and spleens of infected baby hamsters was intensified. Such filtrates were passed into new groups of newborn animals and an immediate increase of titer was produced, especially if the filtrate was made about the 6th to 9th day of life. Whereas previously half to all of the animals in an injected litter became mongoloid, they now died at the 7th to 12th day after birth, and it was necessary to dilute the filtrate in order to obtain survivors and mongoloid adults. As an example, in one such series, 10 out of 10 newborn hamsters became abnormal when injected with a filtrate directly derived from the HEp 1 tumor, one-half of a litter odd when this filtrate was diluted (1:10), and none odd at 10^{-2} dilution. A first passage filtrate prepared from livers and spleens of the infected babies showed a rise in titer to 10^{-4}, the next passage to 10^{-6}, and the third produced mongoloid animals when diluted out to 10^{-8}.[3]

One of the most interesting observations concerned with this agent is that, unlike the polyoma virus, it does not produce tumors. Instead it induces a deformity unlike any reported for the hamster previously.

Whether there is any relation between the deformity seen in hamsters and human mongolism where an extra autosome has been recorded is unknown. It has not been possible to do a good chromosome study as yet on these Syrian-strain abnormal animals, and the Chinese hamster with its small number of large, well-studied chromosomes is no more susceptible to the agent than are rats or mice. It is rather difficult, however, to envision a change in chromosome configuration occurring *after* birth. In passing, it may be noteworthy to mention the high per cent of leukemia recorded among mongoloid children.

Electron microscope studies have been made with the collaboration of Dr. Satish Chandra on the tumors themselves, on infected tissues of baby hamsters, and on pellets prepared from centrifuging filtrates. They have revealed a preponderance of a small virus, unlike any known to us, that has a small nucleoid less than 125Å in diameter. These studies are in their preliminary stage and are being continued.

A large series of investigations on the biochemical nature of this agent are being done in collaboration with Drs. Marion and Ralph Barclay of the Sloan-Kettering Institute. It has been learned that DNA prepared from the transplantable human tumors as well as bones of infected babies is highly infective and can produce the typical "mongoloid" abnormality in hamsters. The titer rises very rapidly on passage. Purified RNA from the same source has no effect whatsoever.

Immunological studies with this virus are also in their early stages. It has been found that the progeny of mongoloid babies can be completely protected by their mothers which have a high titer of protective and specific antibody in their sera. Litters of such mothers are completely normal in appearance whether or not they have been injected at birth with the agent. The babies apparently have had only a temporary passive immunity, for they offer their own offspring no protection. On the other hand, normal female hamsters that have had one litter of infected babies may or may not develop antibodies and thus a second litter that is infected may or may not be protected. If, however, the mother has had a litter injected *in utero*, she usually reacts strongly to the agent and develops antibodies that protect subsequent litters. Such mothers have occasionally died with a hemorrhagic liver which, when filtered, proved infective. Studies on agglutination and possible differences between specific and nonspecific neutralizing antibodies are also in progress.

In sum, then, a filterable agent has been found with the properties of a DNA-virus that is associated with eight human tumors transplanted in conditioned rats. This virus produces an infection with an attendant pathology and deformity, if injected into newborn hamsters. It has also been found in the livers and spleens of cancer patients but not, to date, with one possible exception, in the tissues of rats, mice, hamsters, or the tissues of human beings dying of other causes.

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