silt-clay couplet an annual deposit. We therefore believe that the classic interpretation of varves as annual deposits applies to the varves deposited in glacial Lake Barlow-Ojibway.

Antevs measured some 2027 varves, beginning with varve 1 at the base of a section at the mouth of Montreal River, where it enters Lake Timiskaming. Some 58 varves have since been measured above varve 2027, bringing the total to 2075 (*7*). The Cochrane ice readvance, recorded by widespread clay till overlying disturbed varved clay (there is no terminal moraine) is judged to have culminated in year 2025 of the varve chronology (*7, 8*)—that is, 2025 years elapsed from the beginning of deposition of varve 1, when the ice margin stood just north of the mouth of Montreal River, to attainment of the Cochrane maximum. Antevs calculated that the ice margin retreated 454 ft/yr in the southern part of the Barlow-Ojibway basin (*8*, p. 143). Extrapolation of this rate southward to cover retreat across the interval of 57 miles between North Bay and the mouth of Montreal River gives a result of 670 years. Thus, the time interval between the opening of the North Bay outlet and the attainment of the Cochrane maximum was about 2025 plus 670 years, or 2695 years, and between the opening of the North Bay outlet and the end of the varve record, 2075 plus 670 years, or 2745 years.

A bog bottom sample collected by Ignatius (*5*) and Elson from south of the limit of the Cochrane readvance (*9*) yielded radiocarbon ages of 6730 ± 200 and 6970 ± 310 years B.P. (sample Y-222). The sample was of a shell-rich layer 10 to 15 cm thick, overlain by 3.5 m of peat and underlain by gray homogeneous clay; the same succession overlies the uppermost varves of the standard sequence along the shores of the nearby Frederickhouse Lake. This date gives a minimum age of 6970 years B.P. for deposition of varve 2075, and 6970 plus 2745 years or 9715 years B.P. for the opening of the North Bay outlet.

Sample W-136, collected by T. N. V. Karlstrom (*10*), provides a minimum age of 6380 ± 350 years B.P. for deglaciation of the vicinity of Cochrane, Ontario, after the Cochrane readvance. There is no record of the retreat of the Cochrane ice lobe after its advance to a position 21 miles south of Cochrane. If 200 years are allowed for the uncovering of the Cochrane site, then we may calculate a minimum date of 6380 plus 200 plus 2695 years, or 9275 years for the opening of the North Bay outlet.

Even earlier opening of the North Bay outlet is implied by dates on shells from near the upper limits of the marine submergence along Opasatika and Missinaibi rivers. The respective radiocarbon ages of 7280 ± 80 (sample GRO-1698) and 7875 ± 200 years (sample I GSC-14), agree fairly well with each other. The older date is taken as a minimum age for the beginning of the marine episode. If 400 years are allowed for ice retreat from the Cochrane maximum to the opening of James Bay Lowland to marine invasion, we arrive at a date of 7875 plus 2695 plus 400 years, or 10,970 years B.P. for the opening of the North Bay outlet (*11*).

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Experimental Production of Mongoloid Hamsters

Abstract. Hamsters injected at birth with fractions or cell-free filtrates of transplantable human tumor cells as well as certain tissues derived from human beings and rats carrying spontaneous cancers have developed a mongoloid deformity.

This report describes an experimentally induced deformity in hamsters that resembles mongolism (*1*). It is characterized by small size, flat face or microcephalic domed head, protruding eyes and tongue, abnormal teeth or absence of teeth, and bone fragility (Figs. 1–4). The animals are less pugnacious than normal hamsters, live amicably with one another, and can be handled readily.

The phenomenon was observed incidentally during a series of experiments wherein fractions of transplantable human tumor cells (*2*), prepared by ultracentrifugation or sucrose gradient techniques, were utilized for antigen studies (*3*) and injected into rats, mice, and hamsters of various ages. None of the rats or mice, whether newborn or older, ever exhibited abnormalities, although more than 50 litters were treated in each species. However, among the 100 litters (Table 1) of newborn hamsters, (comprising 932 babies) that received the fractions, 81 mongoloid animals appeared. They were evenly divided as to sex (41 males, 40 females), a ratio that has continued in further experiments. The single injection given to the babies, immediately after birth, was 0.03 ml, or less, of material suspended in 0.25*M* or 0.88*M* sucrose (*3*). All the fractions produced some mongoloid animals. Though the injections were usually given subcutaneously, the results were the same if an intraperitoneal route was employed. Hamsters that received control injections of 0.25*M* sucrose, 0.88*M* sucrose, Locke-Ringer's solution, or distilled water remained normal. The mongoloid effect could be produced up to 2 and, occasionally, 3 days after birth but not later. Twenty pregnant mothers given 0.5-ml doses intraperitoneally 1 to 4 days before delivery produced large litters that were normal. At the present writing, mothers are being treated earlier in their pregnancy and a large number of babies are being injected *in utero* at various periods before birth.

Tests were undertaken to determine if tissues other than the six tumors first studied (H Ep 3, HS 1, H Ad 1, H Emb Rh 1, A-42, and H Ep 5) contained the factor. As seen in Table 1, 185 newborn hamsters were injected with tumor, liver, and spleen fractions obtained from a patient with carcinoma of the liver. One baby, injected with a fraction of "normal" liver area from this patient, became a mongoloid animal, entirely similar to the others described. In a subsequent series of experiments, it was found possible to induce the deformity in a few hamsters injected with fractions of the livers or spleens of cancer patients, and of rats carrying spontaneous tumors. Spontaneous tumors of human beings, rats, or mice and tissue fractions derived from normal human beings, rats, mice, or hamsters were found to be without activity, as were tissue fractions prepared from adult mongoloid hamsters themselves.

It was not possible to tell whether or not the babies were going to be odd for at least 10 to 14 days after birth. At this time it became evident that the mongoloid babies were smaller than normal babies and that their faces were flatter. Many also had long, needle-like, curved teeth that made the mother refuse to nurse them. When the babies began to supplement their nursing at

approximately 14 days of age, the little mongoloid animals were at a great disadvantage because of their bizarre dentition or, conversely, their total lack of teeth. In the first series of animals (group 1), many were lost because this difficulty was not recognized. After a soft mush diet was provided, the survival rate increased greatly.

At 25 days of age, some of the affected hamsters weighed as little as 15 to 25 gm, while normal animals on the same diet weighed approximately 60 gm. As the animals grew older there was less discrepancy between the two groups. The average weight of the 54 animals that have survived over 1 year from the 82 mongoloid hamsters in group 1 is 90 gm (variation, 68 to 140 gm) as compared with 160 gm for control hamsters of the same age. It has been necessary to feed the mongoloid hamsters a soft diet throughout life, and those with peculiar dentition must have their teeth cut once a week. Their coats compare favorably with those of the normal animals (*4*). Eye infections, probably due to their exophthalmos, have been a common problem. Given good care, however, they live as long as the average laboratory hamster. Of the 28 mongoloid animals no longer surviving from the original 82, only ten died from disease or accident. Eight were used for I^{131} studies, four were used for a thyronine experiment, and six died in shock immediately after a small dose of streptomycin was given for a mild eye infection. No tumors have been observed in any of the animals.

When affected hamsters were autopsied, their internal organs showed few morbid changes. The thyroids were unremarkable, as had been indicated by the I^{131} uptake studies (*5*), which gave results within normal limits. The pituitaries, when examined histologically, also appeared normal. Several of the adrenals were hemorrhagic, with abnormal cortical structure, and some of the male testes were underdeveloped. (Nevertheless, at least ten litters, sired by mongoloid fathers, were delivered and raised by mongoloid mothers; these babies have been entirely normal and have grown to be much larger than their parents.) The most consistent finding was a fragile bone structure and, in particular, paper-thin skulls. The eyes appeared to be of normal size. Indeed, the protrusion of the eyes and tongue may have been an expression of the microcephaly. It is interesting that three of the affected hamsters died with extensive fatty degeneration of the liver, an observation which has been reported in human mongolism (*6*).

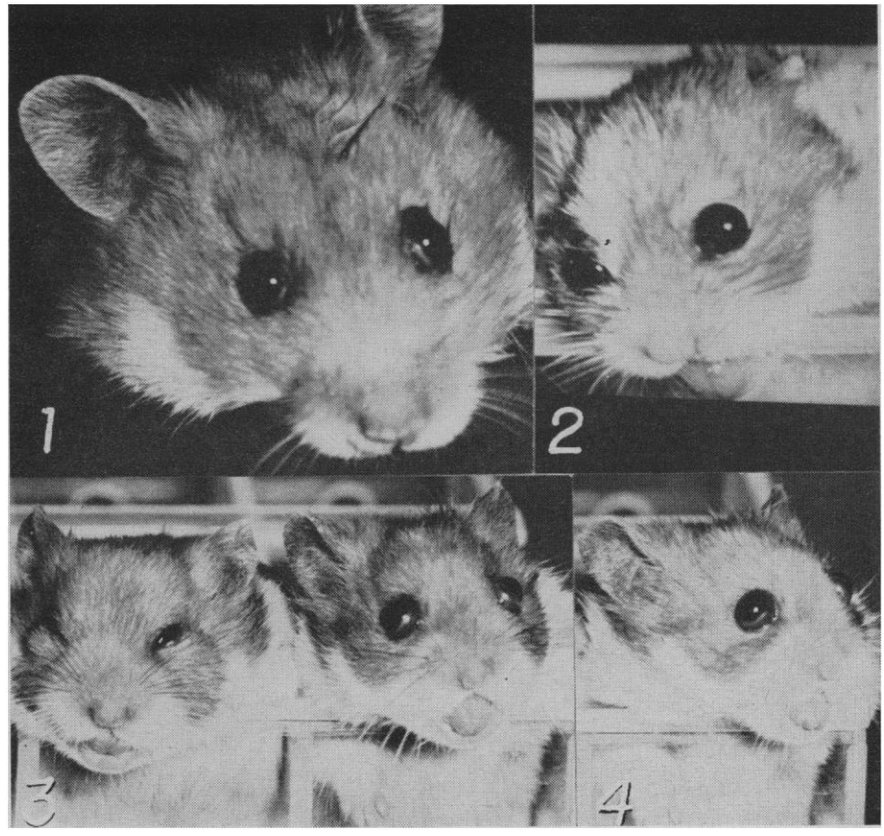

Fig. 1. Normal hamster. Note the pointed face. Figs. 2–4. Mongoloid hamsters. The protruding tongues and extreme exophthalmos are evident. The animal in Fig. 2 is the same age as that in Fig. 1. The animal on the left in Fig. 3 has slanted eyes, which are occasionally seen. The hamster on the right in Fig. 3 is shown again, side view, in Fig. 4.

The effective factor or factors in the fractions used for group 1 was remarkably stable. These materials had been prepared from either fresh or frozen (−79°C) tumor and stored at 4°C, or frozen at −79°C and then thawed and refrigerated. Such preparations remained active for 6 months or more. Refreezing did not damage their efficacy. Heating in boiling water for 15 minutes, however, completely destroyed activity, as a rule.

At the present time, few fractions are being employed for continued studies since cell-free filtrates, prepared by putting a tumor or tissue homogenate, suspended in distilled water, through a 0.03 Selas filter, have proved active. The percentage of mongoloid hamsters produced by filtrates of some tumor tissues (for example, H Ep 3 or A-42) is 100 percent, and thus an ideal model system can be constituted.

Electron-microscope studies of potent fractions and cell-free filtrates have thus far disclosed no foreign or abnormal particulate structures. These studies are being continued. Whatever the factor may be, it can be neutralized completely by normal Wistar rat serum, used in dilutions of 1 part serum to 3 parts effective material. This serum activity was not lost after heating at 56°C for 60 minutes. Rat serum, refrigerated for 6 months or more, and serum from x-irradiated rats were effective also. Fresh or refrigerated sera of other animals (guinea pig, rabbit, mouse, or chicken) were less protective than the rat serum, while a number of human

Table 1. Summary of data on experimental production of mongoloid hamsters.

No. litters	No. babies injected	Injected with	No. survivors (3+ months)	No. mongoloid animals
100	932	Fractions	144	81
19	198	Control solutions	164	0
19	185	Tissues of cancer patient	120	1
138	1315		428	82

and hamster sera were entirely free of inhibitory activity. Lyophilized human serum fractions also failed to inhibit the effect of a potent filtrate.

The data cited in the last two paragraphs were obtained from observations on 5500 hamster babies injected since group 1 was done (*7*).

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4. The abnormality of the hamsters described in this report is physically quite different from the "runting syndrome" described in rats and mice. Baby rats and mice injected with potent fractions developed normally.
5. I am deeply indebted to William Money of the Sloan-Kettering Institute for the I^{131} studies.
6. The relationship of the abnormality described here for hamsters to that of human mongolism is unknown. An extra autosome has been found in the cells of a number of human mongoloid individuals. It does not seem likely that an extra chromosome exists in the animals described here, although this possibility is being investigated. It appears probable that we have here a phenocopy of the mongoloid entity.
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Rapid Induction of Allergic Encephalomyelitis in Rats without the Use of Mycobacteria

Abstract. Rats, in contrast to certain other species of animals reported, have a striking capacity to develop allergic encephalomyelitis within 2 to 3 weeks following one injection of spinal cord antigen combined with Freund's incomplete adjuvant—that is, adjuvant prepared without addition of killed mycobacteria.

The necessity of combining nervous tissue inocula with Freund's complete adjuvant (emulsifying agent, paraffin oil, and killed mycobacteria) for rapid and regular induction of allergic encephalomyelitis (AE) in monkeys and guinea pigs has been reported (*1, 2*). In these studies, injection of nervous tissue emulsions not containing the mycobacteria induced little, if any, disease. Freund and Stone (*2*) have determined the minimal amount of mycobacteria required for induction of characteristic allergic encephalomyelitis in the guinea pig.

Work in our laboratory indicates that mycobacteria are not required for rapid induction of this inflammation in rats. Groups of adult male or female Wistar rats, obtained from two commercial sources, were injected intracutaneously with guinea pig spinal cord (collected aseptically and stored at −20°C for 1 to 7 days) homogenate combined with either complete adjuvant (that is, with added mycobacteria) or incomplete adjuvant (without added mycobacteria) prepared as described by Freund (*3*). Each rat received approximately 115 mg (wet wt.) of spinal cord (an excessive dose used in past work with this host) in 0.7 ml of inoculum distributed among six sites over the upper dorsum and one site on the ventral neck. The animals were given free access to food pellets and water. They were observed daily for neurological signs for 21 to 26 days following injection; then they were killed and their brains and spinal cords were removed for histological studies. A minimum of seven different hematoxylin and eosin stained sections (at levels of thalamus, mesencephalon, cerebellum-pons, medulla, and cervical-thoracic spinal cord) of nervous tissue from each rat were examined microscopically for lesions.

The results of two representative experiments are shown in Table 1. Twenty-four rats received either of two spinal cord homogenates combined with incomplete adjuvant. Nine rats exhibited moderate to severe flaccid paralysis of the hind legs within 14 to 20 days. Numerous and intense lesions were found in these 9 animals as well as in 13 of the remaining 15 rats. As expected, 6 of the 11 control rats which received spinal cord homogenate combined with complete adjuvant developed allergic encephalomyelitis. The data (Table 1) are in agreement with the results of three other experiments, not given in detail here. Additional control rats, which were similarly injected with guinea pig kidney homogenate and incomplete adjuvant or with only the adjuvant, remained clinically well and were subsequently found to have no lesions.

It is of interest that allergic encephalomyelitis may be induced in rats by an intracutaneous injection of the spinal cord antigen alone. For example, 2 of 16 rats used in two experiments were found to have lesions when sacrificed approximately 3 to 4 weeks postinjection. This finding is not unexpected in view of studies of earlier workers (*4*) showing that this inflammation or its equivalent may occasionally be induced in rats, rabbits, and monkeys by often-repeated injections of nervous tissue homogenates or extracts. More recently, Morrison (*5*), Jervis *et al.* (*6*), and Waksman (*7*) have reported that encephalomyelitis occasionally may be induced in rabbits, dogs, and mice after one to several injections of nervous tissue not combined with Freund's adjuvant.

The data (Table 1) indicate that in the rat, emulsifying agent-paraffin oil, without addition of mycobacteria, provides the necessary adjuvant effect for rapid, regular induction of the inflammation. The work reported here has direct bearing on studies of the mode of action of Freund type immunological adjuvants with respect to their capacity to enhance immune responses, of the immediate or the delayed type or both, against a wide variety of antigenic materials, including nervous tissue antigens (*8*).

Table 1. Allergic encephalomyelitis (AE) in rats following an intracutaneous injection of spinal cord combined with Freund's immunological adjuvants.

Expt. No.	Spinal cord antigen	Paraffin oil and emulsifying agent	Killed mycobacteria	No. with signs and lesions of AE	No. with lesions of AE only	Total No. injected
E 7-58	+	+	0	3	9	12
	+	+	+	3	0	6
G11-58	+	+	0	6	4	12
	+	+	+	2	1	5

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