Chromosomes of "Mongoloid" Hamsters.* (31039)

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Newborn Syrian hamsters given an intracerebral inoculation of various strains of Rat Virus(1) or H-1 Virus(2) develop a runting syndrome associated with brain, tooth, facial and skeletal abnormalities(3-8). The resemblance of this condition to Down's syndrome (human mongolism) has led to the suggestion that similar etiologic mechanisms might be involved in the two disorders. Since Down's syndrome is associated with a chromosome anomaly (trisomy $G_1(21,22)$), the karyotype of "mongoloid" hamsters was analyzed for possible aberrations.

Materials and methods. Induction of "mongolism." Syrian hamsters (*Mesocricetus auratus*) were obtained from the Lakeview Hamster Colony, Newfield, N. J. Newborn hamsters were inoculated by the intracerebral route, as outlined in Table I. Two viruses were employed, the H-1 virus of Toolan(2) and several isolations of Rat Virus (OLV, 43P RV, Zhdanov and HHP)(1,9,10). Rat Virus (RV) was propagated in rat embryo tissue culture, and infectivity titrations were performed according to methods described previously(1,11). The strains of RV in preparations used had infectivity titers of 10^{-5} to 10^{-6}.

The 8 diseased adult hamsters selected for chromosome study were clearly "mongoloid" in appearance. Uninoculated hamsters from the same source and of roughly similar ages provided the normal chromosome material for comparative purposes. Chromosome preparations were obtained from both the diseased and control hamsters at approximately the same time.

Karyotype analysis. 672 metaphase preparations were obtained from the 8 adult "mongoloid" hamsters, either directly from bone marrow following hemolytic stimulation(12) or after short-term culture of explants of granulation tissue from the bed of skin autografts (Table I). The method of Billingham and Medawar(13) was used for skin grafting. On the fourth postoperative day, fragments of the granulation tissue underlying the graft were set up in tissue culture in Eagle's basal medium with 20% calf serum and subsequently prepared for chromosome analysis by the method of Basrur *et al*(14). Since the Syrian hamster is resistant to the mitotic arresting action of colchicine(15) an increased dose of colchicine was used in the intact animal to secure mitotic arrest in the bone marrow (200 mg/kg) and to terminate the explant cultures (0.5×10^{-3} M; 200 μg/ml). In both procedures, the cells were swollen by exposure to hypotonic saline and then fixed in chilled acetic acid:methanol (1:3). Chromosomes were spread by air-drying bone marrow cell suspensions on chilled slides. Spreading of chromosomes in the explant cultures was facilitated during air-drying by gently warming the coverslips to which the cells were attached. Chromosomes were stained with 1% acetic orcein and photographed by phase contrast microscopy at a magnification of 400 on Kodak high contrast copy film. 385 karyotypes were prepared from 5″ × 7″ prints on Kodabromide F-4 or F-5 paper by arranging matching pairs of chromosomes in order of decreasing size on 4 lines(16). Six pairs were placed on the top and bottom lines and 5 pairs on each of the 2 middle lines. The sex-chromosome pair was placed first followed by the 21 pairs of autosomes.

204 karyotypes were prepared from 4 healthy control hamsters, two of each sex, by the same methods.

Results. Table II gives the frequency distribution of chromosome counts among the 644 metaphase preparations of exact-count quality from "mongoloid" hamsters. The corresponding count distribution among the 204 metaphases from normal uninoculated ham-

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TABLE II. Chromosome Count Frequency Distribution.

Chromosome count		<42	42	43	44	45	46	>46	Total
"Mongoloid" hamsters	No. of cells	43	44	93	439	11	9	5	644
	%	6.7	6.8	14.4	68.2	1.7	1.4	.8	100%
Normal hamsters	No. of cells	7	9	29	148	8	3	0	204
	%	3.4	4.4	14.2	72.5	3.9	1.5	0	99.9%

regularities as the control material. Chromosome deletions in the hypodiploid cells again involved most chromosomes. The extra chromosomes in hyperdiploid metaphases also occurred at random. A gross size inequality of autosome pair 20 existed in 9 karyotypes (2.3%). Size inequality of other autosome pairs occurred with roughly the same frequency as in the control material. Chromatid breaks were rare; none were present in the largest 3 pairs of autosomes. No other abnormalities were present.

Discussion. These findings lend support to the concept that the syndrome of "mongolism" induced by Rat Virus and related agents in hamsters is a cell depletion phenomenon rather than the result of a chromosome anomaly. The causative virus, although introduced intracerebrally, is disseminated throughout the body(19). Since cells undergoing DNA synthesis prior to division are most vulnerable, viral effects are especially pronounced in certain receptive tissues possessing the greatest proliferative activity. The ultimate death of infected cells coupled with the development of immunity(4,5) leads to specific cellular deficits, disappearance of histologic evidence of cytopathic effects, and elimination of the virus. Death of irreplaceable vital cells is responsible for the definitive lesions such as brain damage, tooth aplasia and skeletal defects, while losses in less critical areas such as the skin, intestine and bone marrow may be compensated for by extra proliferative activity of unaffected cells.

A similar disease, granuloprival cerebellar hypoplasia, is produced in neonatal hamsters by intracerebral inoculation of Rat Virus(19). If the inoculation is deferred until the hamster is 6 days old, "mongolism" develops instead(5). This critical age-response relationship is strikingly reminiscent of the importance of timing in the experimental production of congenital defects in the fetus and probably reflects the unusually primitive development of the newborn hamster after a gestation period of only 16 days.

Although some viruses are capable of producing chromosome changes, stable alterations of the karyotype are usually associated with neoplastic change or transformation in tissue culture(20). In several human viral exanthemata the chromosomal aberrations which accompany the acute phase of the disease disappear following recovery and elimination of the virus(21). Chromosomal changes might occur in the primary viral target tissues during the induction period of hamster "mongolism." However, since the presence of virus in the cell is probably lethal to the cell, such abnormalities would be transient and would presumably play no role in the manifestation of the disease.

Human mongols may inherit the extra chromosomal material characteristic of this condition from a parent. In most cases, however, the extra chromosome is derived presumably by non-disjunction, either during meiosis or shortly after fertilization(22). The cause of non-disjunction is unknown but the possibility of an infectious mechanism is suggested by the occurrence of a cyclical incidence of births of mongol infants(23). The tentative implication of a virus comes from the association of epidemics of infectious hepatitis with an increased number of mongol births 9 months later(24). The apparent susceptibility of newborn mongols to hepatitis (25-27) may be a related phenomenon.

The invariable and unique association of a specific chromosome anomaly with Down's syndrome implies that the chromosomal disorder is responsible for the manifestations of the human disease(22). Our failure to detect any abnormality of the karyotype in the "mongoloid" hamster suggests that different etiologic mechanisms are operative in the two conditions and indeed this conclusion is re-

TABLE I. "Mongoloid" Hamsters: Methods of Viral Infection and of Karyotype Analysis.

Hamster No.	1	2	3	4	5	6	7	8	Totals
Sex	Male	Male	Male	Male	Male	Female	Female	Female	5 males; 3 females
Age	5 wk	13 wk	22 wk	28 wk	50 wk	5 wk	13 wk	28 wk	
Weight	44 g	74 g	102 g	97.5 g	101 g	30.5 g	60 g	90.5 g	
Virus	OLV (RV)	H-1	ZHDANOV (RV)	OLV (RV)	43P RV (RV)	HHP (RV)	H-1	OLV (RV)	
Dilution*	10^{-1}	10^{-4}	10^{-2}	10^{-1}	10^{-2}	10^{-4}	10^{-4}	10^{-1}	
Route	i.c.	i.c.	i.c.	i.c.	i.c.	i.c.	i.c.	i.c.	
Hamster age at inoc.	6 days	<1 day	10 days	6 days	6 days	<1 day	<1 day	6 days	
Chromosome analysis: Procedure	Bone marrow	Autograft bed granulation tissue culture	Bone marrow	Autograft bed granulation tissue culture	Bone marrow	Autograft bed granulation tissue culture	Autograft bed granulation tissue culture	Autograft bed granulation tissue culture	3 direct; 5 tissue culture
No. of metaphases photographed	79	94	70	53	82	59	205	30	672
No. of karyotypes prepared	66	32	51	30	53	40	108	5	385 (232 ♂; 153 ♀)

* Dose: .02-.04 ml.

sters is given for comparison. There are no significant differences between the two groups. Detailed analysis was undertaken of the 385 karyotypes prepared from metaphases of "mongoloid" hamsters and of the 204 karyotypes prepared from all the metaphases obtained from normal hamsters. Particular attention was directed toward the possible occurrence of abnormalities of individual chromosomes, such as chromatid breaks, translocations and other unusual forms, and in addition a catalog was compiled of the frequency of involvement of individual chromosomes in apparent deletions and reduplications. Since the X-chromosomes and the smallest 2 pairs of autosomes (#s 20 and 21) are the only unequivocally recognizable chromosomes in the hamster karyotype, the identification of chromosomes by number is given as a guide and is not intended to imply exact recognition. In the *control material,* apparent chromosome duplications of autosome pairs 3, 6, 13, 15 and 21, accounted for the hyperdiploid metaphases. Chromosome losses, usually involving only one member of a pair, were evident in most autosome pairs in hypodiploid metaphases. The occurrence of size inequality of allegedly homologous chromosome pairs has been described as a feature of the normal hamster karyotype(17). Similar inequalities were encountered in the present control material but little significance can be attached to comparisons of this phenomenon from cell to cell since there is no independent method for establishing the identity of homologues. However, striking size inequalities were present in autosome pairs 20 and 21 which can be positively identified. One member of pair 20 was at least twice the size of the other in 8 karyotypes (3.9%). Pair 21 exhibited a difference of similar magnitude in 10 karyotypes (4.9%). Chromatid breaks were present in the long arm of one of the largest chromosomes (autosome pair 2 or 3) in only 2 of the control karyotypes (1%). This compares with a frequency of 4.5% observed in normal hamster embryo tissue culture(18). No other abnormalities were encountered in the control karyotypes.

Karyotypes prepared from metaphases of "mongoloid" hamsters possessed the same ir-

inforced when the method of neonatal viral inoculation used to procure "mongoloid" hamsters is contrasted with the pre- or immediately post-zygotic event necessary for the development of Down's syndrome. However, the existence of certain parallels between the two conditions promotes hamster "mongolism" as a possible model of the human disease. Hamster "mongolism" is characterized by defects of teeth(7), skeleton(8) and brain (6). Other interesting properties of the causative agents, RV and H-1 strains, which are among the smallest known viruses(28), are their ability to cross the placenta in the hamster(29,30) and rat(31, but see 32), their involvement in spontaneous neonatal hepatitis in rats(31), and their association with leukemia in rats(33).

Although entirely speculative at present, a unifying working hypothesis may be based on the similarities between the two types of mongolism, human and hamster. "Mongolism" in the hamster is clearly a viral disease and it is highly probable that its characteristic features are determined by selective developmental arrest in the primary 'target' tissues and organs. Down's syndrome is presumably the expression of a specific chromosome anomaly and its manifestations are probably the result of developmental disturbance in many of the regions also involved in hamster "mongolism." Since there is a possibility that a virus might be operative in the initial production of the chromosome anomaly in Down's syndrome and in view of the other putative viral stigmata (predisposition to hepatitis neonatorum and to leukemia), the hypothesis is advanced that a virus with roughly the same affinities as RV in the suckling hamster might be the primary etiologic agent in Down's syndrome. In this scheme, the virus is envisaged as the cause of the typical chromosome anomaly, while either the altered genotype or persisting virus produces the characteristic features of the disease.

Summary. The chromosomes were studied in 8 adult Syrian hamsters which had developed "mongolism" following an intracerebral inoculation of virus in the neonatal period. No significant chromosome abnormalities were detected. It is concluded that viral-induced "mongolism" in the hamster is an example of selective postnatal developmental arrest and that the particular manifestations of the condition can be accounted for on the basis of cell depletion due to the lethal effect of virus on dividing cells.

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Base Composition of Ribosomal Ribonucleic Acid in Newborn and Adult Rat Brain. (31040)

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The chemical changes that occur during organ ontogeny may be assumed to reflect the altering metabolic requirements that are a concomitant of morphological development. The recent efforts of a number of laboratories to establish a molecular basis of memory and learning would indicate that chemical changes may also occur as a result of experiential stimuli, and ribonucleic acid (RNA) has been suggested as participating in this process(1-7). In the adult brain if any RNA changes occur after maturation they may be related to some adaptive or mental process, whereas in the newborn animal structural development might also involve changes in RNA. However, it would appear that if no differences in RNA composition could be demonstrated between the young and adult brain the concept of RNA base alterations as a chemical mediator in information processing might be in question.

Hydén has proposed that memory is coded in RNA in a manner analogous to the genetic coding of chromosomal DNA(8). If development and/or learning alters RNA base composition, it should be possible to demonstrate that newborn rat brain, with a minimum of input, is different from the brain of adult animals in this regard. It would not be possible to differentiate between changes due to structural and psychological development, but if no differences were apparent, change in base composition as a mediator in development and learning would necessarily be predicated on equal and opposite changes occurring for these two variables. Another possibility for the lack of any differences resides in localized brain areas undergoing base composition alterations which are opposite and equal irrespective of the nature of the stimulus.

This study is designed to ascertain whether differences in ribosomal-RNA base composition exist between neo-natal and maturated rat brain, as a first approach to the problem of RNA mediating memory and learning processes in brain.

Methods. Newborn rats were obtained as soon as possible post-partum, and at the time of decapitation and removal of the brains were 1-18 hours of age. In some cases the mothers of the litters were used as adult experimental animals. Since the sex of the newborn was not determined, this was deemed desirable so that any sex differences between the two groups could be negated.

The brains of approximately 30-40 newborn rats and 3-5 adult brains were pooled for each analysis. The brains were homogenized in 0.32 M sucrose, .001 M $MgCl_2$, .0004 M KH_2PO_4, .0004 M K_2HPO_4, pH 6.8. The debris, nuclear and mitochondrial fractions were removed by centrifugation in a Spinco model L ultracentrifuge at 18,000 $\times$ *g* for 8 minutes and decantation of the supernatant. The sediment was then washed, recentrifuged; the wash was added to the first supernatant. This was then spun at 90,000 $\times$ *g* for 75 minutes in a #40 rotor. The microsomal pellet obtained was extracted by the method of Kirby(9) as modified by Ding-