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FURTHER OBSERVATIONS ON SUBACUTE SCLEROSING ENCEPHALITIS IN ADULT HAMSTERS: THE EFFECTS OF INTRANASAL INFECTIONS WITH LANGAT VIRUS, MEASLES VIRUS AND SSPE-MEASLES VIRUS

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Summary.—Passage by i.c. inoculations of suckling hamsters enhanced the virulence for adult hamsters of Langat virus (TP21), neurotropic strain of measles virus (HNT) and SSPE-measles virus (HBS), not only for i.c. infections but also for intranasal instillations. The various viral strains passaged in hamsters showed a great similarity of behaviour including the ability of producing in a proportion of apparently unaffected survivors a subacute sclerosing encephalitis, leading to atrophy of parts of the brain especially the rhinencephalon. When large groups of animals were used for transmission experiments it became obvious that within one week after intranasal exposure, all the hamsters either died or became clinically affected, or did not show signs of disease but developed acute inflammatory brain lesions. Later on, between 2–6 weeks following inoculations only 90% of hamsters were affected with either overt signs of disease or subacute brain lesions, suggesting that in about 10% of hamsters the initial infection did not progress further and that in these animals the early brain lesions disappeared. Passage levels, irrespective of the virus used, did not influence the total numbers of infected hamsters but showed a significant effect on the mortality in TP21 and HNT infections where the number of dead and clinically affected increased in the higher passes. In these higher passes the number of survivors with subacute brain lesions decreased. In SSPE-measles virus the number of clinically affected hamsters and those surviving but developing brain lesions remained constant throughout.

Vacuolated neurons were present in the brains of hamsters that survived one of the above 3 viral infections. They were seen beginning from 6 weeks after infection only in animals that developed subacute sclerosing lesions and were most commonly found in the amygdaloid nuclei and in the pyriform cortex. There was a dramatic increase in the number of brains with vacuolated neurons in hamsters infected with the high viral passes; however, in the 36th hamster passage of TP21 no vacuolated neurons were present but the total number of survivors was small, the majority had no brain lesions and none had subacute sclerosing changes.

IN PREVIOUS PAPERS a progressive subacute sclerosing encephalitis was described in adult hamsters, either infected peripherally when 10 days old with the mouse adapted strain of Langat virus or when weaned with a hamster adapted strain of the same virus after i.c. inoculations (Zlotnik, 1972; Zlotnik *et al.*, 1973). Further work with a neurotropic strain of measles virus passaged in hamsters (Burnstein, Jensen and Waksman, 1964) revealed a similar tendency for producing subacute encephalitis in hamsters giving rise to subacute sclerosing lesions indistinguishable from those after Langat virus infection (Zlotnik and Grant, 1975). Later work revealed a condition in hamsters with lesions resembling those in Langat virus and HNT-measles virus after i.c. inoculations with the HBS strain of SSPE-measles virus isolated from brain cell tissue cultures of patients with SSPE

(Horta-Barbosa *et al.*, 1969*a*, *b* and 1970) and subsequently adapted to hamsters (Byington, Castro and Durnstein, 1970; Byington and Johnson, 1972). All the 3 viral strains showed not only a similarity in producing in hamsters a subacute sclerosing inflammatory condition, but also neuronal vacuolation in well defined regions of the brain suggesting also a slow-virus-like effect.

The original strains obtained in this laboratory did not exhibit a noticeable virulence for adult hamsters when infected by a peripheral route including intranasal inoculations. Further passage in suckling hamsters increased the virulence of these viruses, not only when inoculated i.c. but also by intranasal instillations and thus producing a disease process not unlike that after natural infection and avoiding the physical damage to the brain during i.c. injections. The present paper consists therefore of a study of the effects of these viruses on adult hamsters after a number of passages in suckling hamsters.

MATERIALS AND METHODS

Golden hamsters aged between 4 days and 47 weeks were used in the various experiments. Titrations of the inocula were carried out in 3-week old white mice of the Porton strain.

The initial inocula consisted of the following 3 viruses passaged i.c. in 4 days old suckling hamsters: (1) Langat virus (TP21) passaged 9 times in mice (Smith, 1956) and twice in suckling hamsters (Zlotnik *et al.*, 1973); (2) HNT-neurotropic strain of measles virus passaged in suckling hamsters 115 times (Burnstein *et al.*, 1964); (3) HBS-neurotropic strain of SSPE-measles virus passaged in suckling hamsters 11 times (Byington and Johnson, 1972).

Further i.c. passages of the above 3 viral strains were carried out in suckling hamsters and although all the passes were studied, the bulk of the work was centred around the following hamster passes: TP21—2nd, 10th, 19th and 36th passes; HNT—127th, 142nd, 149th and 162nd passes; HBS—18th, 26th and 40th passes. The dose for i.c. inoculations was 0·02 ml for suckling hamsters, 0·03 ml for weaned mice and 0·05 ml for adult hamsters. The intranasal dose was 0·05 ml for suckling hamsters and 0·1 ml for adult hamsters, divided equally between the 2 nostrils. The titre of the inocula, unless otherwise stated, was 10^7 MLD_{50}/ml.

Infected hamsters were destroyed, either when showing signs of disease or at various intervals after inoculation. All the animals were anaesthetized with ether before destruction and brains and spinal cords were removed as soon as possible after death. Brains were usually fixed whole in 10% formol-saline except when material was removed for transmission experiments or virus isolation; a sagittal section divided the brain in 2 halves, one half was fixed in formol and the other stored at −70°. Paraffin embedded sections were stained with H. and E. or Luxol fast blue and frozen sections were stained with Cajal's gold-sublimate for the demonstration of astrocytes.

RESULTS

Intracerebral inoculations

Preliminary studies revealed a great similarity in the pathogenesis of the diseases caused by the 3 viruses and showed that i.c. passage in suckling hamsters increased the virulence of the viral strains not only for adult hamsters but also for 3-week old mice, thus enabling the titration of the various inocula in mice. The increase in the virulence for adult hamsters after passage was expressed by a greater mortality, shortening of the incubation period from 1–3 weeks to between 6–12 days and the appearance of brain lesions in survivors (Table I).

Intracerebral inoculations of hamsters of various ages revealed that irrespective of passage level the 3 viruses, including the original TP21, passaged in mice, gave rise to 100% mortality in young suckling hamsters aged 1–10 days. In older hamsters, however, the TP21 did not cause overt signs of disease but after 2 passages in suckling hamsters, the TP21/H2 became virulent for adult hamsters killing 33–40% after i.c. inoculations. The HNT strain of measles in its 115th hamster pass and the HBS strain of SSPE-measles virus in its 11th pass proved already adapted to adult hamsters at the time that they were received by us. After additional 2–3 passages, they became even more virulent and HNT/118 and HBS/13 resembled Langat virus in its second hamster pass

TABLE I.—*The Effect of Viral Passage on the Mortality of Hamsters after i.c. Inoculations*

Age of animals (days)	Langat virus		Measles virus		SSPE-measles virus	
	Passage* of viral strain	Mortality (%)	Passage* of viral strain	Mortality (%)	Passage* of viral strain	Mortality (%)
1–10	TP21	100	HNT/118	100	HBS/13	100
15	TP21	nil	HNT/118	95	HBS/13	95
22	TP21	nil	HNT/118	—	HBS/13	83
35	TP21	nil	HNT/118	70	HBS/13	33
1–10	TP21/H2	100	HNT/122	100	HBS/14	100
15	TP21/H2	95	HNT/122	100	HBS/14	76
22	TP21/H2	—	HNT/122	100	HBS/14	52
28	TP21/H2	40	HNT/122	100	HBS/14	64
35	TP21/H2	—	HNT/122	73	HBS/14	—
42	TP21/H2	36	HNT/122	—	HBS/14	—
154	TP21/H2	33	HNT/122	—	HBS/14	—
1–10	TP21/H10	100	HNT/127	100	HBS/17	100
15	TP21/H10	100	HNT/127	100	HBS/17	100
22	TP21/H10	92	HNT/127	100	HBS/17	57
28	TP21/H10	89	HNT/127	100	HBS/17	72
42	TP21/H10	—	HNT/127	80	HBS/17	95
65	TP21/H10	—	HNT/127	55	HBS/17	—
1–10	TP21/H36	100	HNT/149	100	HBS/26	100
28	TP21/H36	100	HNT/149	100	HBS/26	100
42	TP21/H36	100	HNT/149	100	HBS/26	100

* i.c. passage in suckling hamsters.

(TP21/H2). In further hamster passages the 3 strains became progressively more virulent for adult hamsters, causing death in older hamsters until TP21/H36, HNT/149 and HBS/26 caused death in all i.c. inoculated hamsters aged 1–42 days (Table I). Owing to this very high mortality during the first 2 weeks after inoculation with the above high viral passes, none of the hamsters developed subacute sclerosing encephalitis, all of them dying during the acute stage of the disease. Thus, in order to study the effects of passage of the viral strains on the incidence and course of subacute encephalitis a peripheral route of infection had to be investigated.

As already mentioned previously, the passage in hamsters increased the virulence of the viral strains also for weaned mice (Table II). This is especially obvious in Langat virus where the hamster adapted strain showed consistently a titre about 1·5 log higher than the original mouse strain. The HNT/118-measle virus was not highly virulent for weaned mice but killed 2–3 day old suckling mice. After 9 passes in hamsters it became highly virulent for mice. Finally the HBS/13 did not cause mortality in mice but the HBS/18 showed a titre of $10^{11\cdot3}$. It is worth noting that after reaching a maximum at 10–11 log, successive hamster passages did not alter the titre of the virus in the brain when tested in mice. The similarity of titres after a number of hamster passes of the 3 viral strains is very striking, the average being 10^{11} MLD_{50} i.c.g. (Table II).

TABLE II.—*Titrations in 3-week old Mice of Hamster Passaged Viral Strains*

Virus	Passage	Titre (per g of brain)
Langat	TP21/M9	$10^{9\cdot9}$
	TP21/H2	$10^{11\cdot3}$
	TP21/H10	$10^{11\cdot1}$
	TP21/H36	$10^{11\cdot7}$
Measles	HNT/118	$10^{3\cdot3}$
	HNT/127	$10^{10\cdot5}$
	HNT/149	$10^{11\cdot1}$
	HNT/162	$10^{11\cdot1}$
SSPE-measles	HBS/13	nil
	HBS/18	$10^{11\cdot3}$
	HBS/26	$10^{11\cdot1}$
	HBS/40	$10^{11\cdot1}$

Intranasal infections

Langat virus.—Intracerebral passage in suckling hamsters gave rise to an increased virulence for adult hamsters, not only after i.c. inoculations but also after i.n. instillations. Hitherto, however, none of the viral strains became sufficiently virulent to cause clinical signs and death in all inoculated animals, a significant proportion surviving without even showing signs of disease. As a whole, there was a progressive increase in the virulence after i.n. infection in the 3 viruses. Most spectacular was Langat virus, which in its 9th mouse pass, after i.n. instillations gave rise neither to mortality nor to brain lesions, slowly became increasingly virulent to hamsters by the i.n. route until the 36th pass (TP21/H36) caused 84% mortality.

In order to study the effects of passage in suckling hamsters on the virulence of the 3 viruses for adult hamsters when inoculated by the intranasal route, 3 passage levels were chosen of each virus and used for intranasal infections of large groups of 6-week old hamsters. Each group, inoculated with the same passage, was further subdivided into 4 smaller sub-groups of at least 50 hamsters each intended for destruction at regular intervals, of, 1, 2, 3 and 6 weeks after infection in order to ascertain mortality in each group and the pathological changes in the brains of survivors. In Langat virus experiments, the following 3 passes were chosen: TP21/H2, TP21/H10 and TP21/H36. Irrespective of the passage level, mortality started on the 7th day and the bulk of animals that developed clinical signs and died did so between 7–12 days. A small number of animals became ill at a later stage between 18 and 21 days after infection. Recoveries from the disease in clinically affected were very rare and all recovered animals showed various impairments which persisted until death. The most common sequelae were twisted or bent necks and partial paralysis and atrophy of the limbs. In additional experiments where infected animals were kept up till one year it was shown that deaths occurred even after 6 weeks and continued throughout the year reaching in both 2nd and 10th hamster passes 70–80% by the end of one year.

The results of the experiment in which animals were destroyed at interval until 6 weeks after infection are summarized in Table III, where it is shown clearly that the lowest mortality (14%) occurred in the second hamster pass (TP21/H2) and increased only moderately (40%) in the 10th pass (TP21/H10), but reached 84% in the 36th pass (TP21/H36). At the same time only few survivors escaped without developing brain lesions, suggesting inapparent infections. The importance of this observation is the fact that at any stage of the experiment, irrespective of the inoculum used, whether high passage or low passage, the sum total of animals affected, whether dead after showing clinical signs of developing brain lesions as a result of inapparent infection, remained constant and similar in all the 3 passes. Of special significance is the fact that all animals killed after the first week had acute inflammatory lesions, indicating a 100% susceptibility and infection. In the second and subsequent weeks a number of survivors had either no lesions or small involuting changes; however, beginning from the second week the number of animals with severe, usually subacute lesions was in an inverse ratio to the number of clinically affected (Table III). Thus TP21/H2 with a very low mortality had the biggest number of animals with severe or very severe subacute lesions; TP21/H10 with a moderate mortality had a somewhat lower number of survivors with subacute brain lesions and finally TP21/H36 with the highest mortality had only few hamsters with severe lesions in the 2nd and 3rd week after infection, but by the 6th week, when 84% of hamsters died, none of the remaining survivors had subacute sclerosing lesions.

The histological changes in the brains of hamsters were not unlike those described

TABLE III.—*The Effect of Passage Level on the Virulence of Langat Virus (TP21) for Adult Hamsters after Intranasal Infections*

Virus (i.c. passage in suckling hamsters)	No. of weeks from inoculation until death	No. of clinical cases (dead and affected) (%)	Survivors without clinical signs: Total No. of survivors (%)	Survivors without clinical signs: No. of survivors without brain lesions (%)	Inapparent infections: survivors with brain lesions: Total no. (%)	Inapparent infections: survivors with brain lesions: No. of survivors with mild or moderate lesions (%)	Inapparent infections: survivors with brain lesions: No. of survivors with severe or very severe lesions (%)	Total no. of affected hamsters: Clinical cases and survivors with brain lesions (%)	Total no. of affected hamsters: Clinical cases and survivors with only severe or very severe brain lesions (%)
TP21/H2	1	10	90	Nil	90	54	36	100	46
	2	14	86	Nil	86	14	72	100	86
	3	6	94	54	40	10	30	46	36
	6	6	94	10	84	26	58	90	64
TP21/H10	1	2	98	Nil	98	64	34	100	36
	2	10	90	8	82	14	68	92	78
	3	40	60	12	48	10	38	88	78
	6	22	78	16	62	20	42	84	64
TP21/H36	1	40	60	Nil	60	22	38	100	78
	2	76	24	2	22	20	2	98	78
	3	76	24	10	14	10	4	90	80
	6	84	16	10	6	6	Nil	90	84

previously (Zlotnik *et al.*, 1973) after i.c. inoculations, except that the distribution of the lesions was somewhat different. The first pathological changes occurred always in the olfactory lobes, where by the 7th day moderate or moderately severe inflammatory lesions were observed in hamsters infected with the 2nd or the 10th hamster pass (TP21/H2 and TP21/H10) but severe or very severe acute encephalitis was seen in animals infected with the 36th pass, TP21/H36. The moderate lesions consisted of perivascular cuffings composed of one or 2 layers of infiltrating cells, surrounded by variable microglial zones and occasionally neuronal degeneration was also observed. The severe lesions, on the other hand, consisted of very numerous, usually large perivascular cuffings composed of several layers of infiltrating cells surrounded by dense zones of microglial cells. The latter were also invading neuronal layers; at the same time the microglia was undergoing transformation into elongated forms and stab cells. Many neurons were affected by degeneration or necrosis. Early calcification of areas of severe necrosis was not uncommon. In many brains, by the end of the first week the astrocytes were beginning to undergo severe proliferation and transformation leading to complete replacement of normal constituents of the area by astrocytes (Fig. 1).

The distribution of lesions after the first week followed a certain pattern where the olfactory lobe was always affected and whilst in some animals (about 10%) the process did not progress further. In the majority of animals, however, it spread to the pyriform cortex, entorhinal cortex, paraterminal body thalamus, hippocampus and occasionally also to the midbrain, brain stem and cerebellum. It is worth noting that the incidence of lesions in the cerebellum was related to the inoculum that was used, so that 2% of animals infected with TP21/H2 had cerebellar lesions, 6% of those given TP21/H10 and 37% in hamsters inoculated with TP21/H36. The most commonly affected part of the brain after the olfactory lobes was the pyriform cortex (85–90%), less often lesions were present in the entorhinal cortex (about 70%) and in the hippocampus (about 60%).

Two weeks after infection subacute sclerosing changes with severe proliferation and transformation of astrocytes became obvious in the majority of brains. At the same time granulomatous accumulations of cells resembling microglia were also formed, giving rise to conspicuous cell nests, but the microglial diffuse reaction became less marked (Fig. 2). These changes were usually present in the olfactory lobes, pyriform and entorhinal cortex and less often in the hippocampus and thalamus and rarely only in the midbrain. The sequence of events 3 and 6 weeks after infection was the same as described before for i.c. infections (Zlotnik *et al.*, 1973) but only in hamsters infected with the 2nd and 10th pass, where the brunt of the sclerosing process affected the olfactory lobes, pyriform and entorhinal cortex and only in about 60% of animals also the hippocampus, leading to atrophy cavitation and distortion (Fig. 3, 4). Only rarely were advanced subacute lesions present 3 weeks after infection in hamsters infected with TP21/H36, where the process was usually accompanied by widespread degeneration and necrosis. None of the surviving hamsters destroyed 6 weeks after infection with the above 36th passage had subacute sclerosing lesions in their brains.

Isolation of virus from infected brains by cultural means on chick embryo fibroblasts or by i.c. inoculations of suckling hamsters or mice revealed 10^6–10^9 MLD_{50} i.c.g. of virus but only between the 4th and 7th day after infection, irrespective of whether hamsters were 18 or 25 days old at the time of inoculation with virus. Later on, the amount of virus that could be isolated and identified declined sharply and by the 10th day only 3 of 5 infected 18-day old and 4 of 5 infected 25-day old hamsters had small amounts of virus in their brains; however,

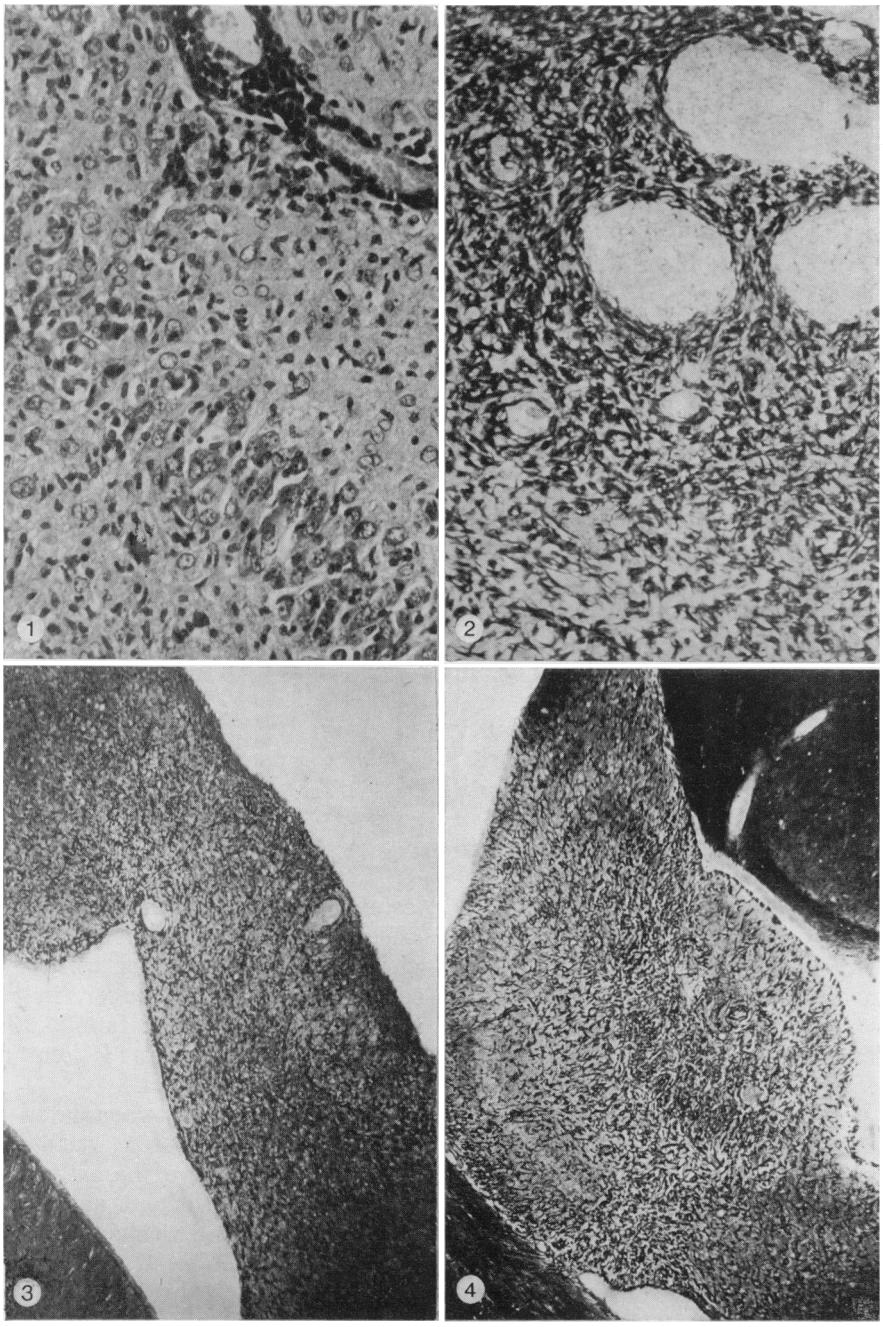

FIG. 1.—Transition between acute and subacute encephalitis. Note perivascular cuffings; severe microglial proliferation and infiltration into pyramidal layer of neurons; and early astrocytic transformation. Hippocampus, 1 week after Langat virus infection. H. and E. × 200.

FIG. 2.—Hippocampus: dense astrocytic subacute reaction and large (unstained) cell nests. 2 weeks after Langat virus infection. Cajal. × 85.

FIG. 3.—Subacute sclerosing changes with atrophy of the pyriform cortex. 3 weeks after Langat virus infection. Cajal. × 33.

FIG. 4.—Advanced subacute sclerosis and atrophy of the hippocampus. 6 weeks after Langat virus infection. Cajal. × 33.

TABLE IV.—*The Relationship between the Virus Content of the Brain, the Appearance of Cerebral Lesions and the Age of Hamsters after i.n. Inoculations with Either Measles or Langat Virus*

			Measles virus (HNT/142)			Langat virus (TP21/H19)		
			Virus content of the brain			Virus content of the brain		
No. of inoculated animals	Age of hamsters (days)	Days after i.n. inoculation	More than 6 log (No. of animals)	1–2 log (No. of animals)	Cerebral lesions (No. of animals)	More than 6 log (No. of animals)	1–2 log (No. of animals)	Cerebral lesions (No. of animals)
5	18	7	5	Nil	5	5	Nil	5
5	18	10	Nil	Nil	5	Nil	3	4
5	25	7	5	Nil	5	5	Nil	5
5	25	10	2	Nil	5	Nil	4	5

with the exception of one 18-day old hamster all the infected hamsters had brain lesions when examined 7 and 10 days after infection. The hamster that had no lesions in the brain had also no virus and it is impossible to know whether the inoculum in this case reached the brain (Table 4).

The effect of age on the susceptibility of hamsters to intranasal infection with Langat virus was studied only in the 19th hamster pass (TP21/H19). Because of the size of the nares, it was difficult to carry out intranasal instillations in hamsters less than 14 days old. The results of a number of experiments, using hamsters of various ages, ranging from 2–47 weeks revealed that at 2 weeks of age hamsters were very susceptible to infection, the virus causing 96% mortality. In older hamsters, aged 3–7 weeks, there was a progressive resistance, mortality being only 31% dead in 6 weeks old. However, in hamsters older than 36 weeks there was again an increased susceptibility that reached 70% mortality in 47 week old (Table V).

Vacuolated neurons as described before (Zlotnik and Grant, 1975) were found most frequently in the area amygdaloidea anterior and nucleus amygdaloideus centralis, less often in the pyriform cortex and occasionally in the corpus striatum. In the amygdaloid nuclei vacuoles were as a rule situated outside the subacute

TABLE V.—*The Relationship Between the Age of Hamsters and the Mortality after Intranasal Inoculations of Virus*

	Mortality after i.n. inoculations (%)		
Age of hamsters (weeks)	Langat virus (TP21/H19)	Measles virus (HNT/142)	SSPE-measles virus (HBS/26)
2	96	100	86
3	54	—	84
4	40	53	80
5	40	52	58
6	31	42	60
36	48	56	66
47	70	90	95

lesions, within the brain substance unaffected by inflammation either in the vicinity or even further away from the sclerotic lesions. However, in the pyriform cortex they were found either very close to the subacute lesions or even within the actual lesions.

The incidence of vacuolated neurons in the brains of hamsters was studied in a very large number of hamsters that survived infection, without showing clinical signs for at least 6 weeks after intranasal inoculations. Only 4 single transverse sections of each brain were examined through the following regions: pyriform cortex-area parolfactoria; anterior thalamus-pyriform cortex; posterior thalamus-amygdala; anterior midbrain-entorhinal cortex. The result of the examination proved that vacuolated neurons were present in 27% of brains of animals

inoculated with TP21/H2 and 36% of brains after TP21/H10 infection. No vacuolated neurons were found in the survivors after TP21/H36 infection (Table VI). The vacuoles were present only in brains that contained well established subacute sclerotic lesions; however, none of the brains after TP21/H36 contained any such lesions. The majority of survivors after the 36th pass had either no lesions in the brain or insignificant involuting changes.

TABLE VI.—*The Occurrence of Vacuolated Neurons in the Brains of Surviving Hamsters Six Weeks after Infection*

Strain of virus	No. of brains with vacuolated neurons after infection with virus (%)		
	Langat virus	Measles virus	SSPE-measles virus
TP21/H2	27		
TP21/H10	36		
TP21/H36	Nil		
HNT/127		4	
HNT/149		25	
HNT/162		28	
HBS/18			5
HBS/26			26
HBS/40			32

Measles virus.—The 115th hamster passage of the HNT strain of measles obtained by us did not cause infection after intranasal instillation in adult hamsters. Further passage in suckling hamsters increased the virulence of the virus for adult hamsters after i.c. inoculations so that HNT/118 killed 70% of 35-day old and HNT/122 killed 100% of 28-day old and 73% of 35-day old, but neither of these 2 passages caused mortality after intranasal inoculations and none of the brains of exposed animals had any lesions (Table I). The first evidence of infectivity by the intranasal route was obtained with the 127th hamster pass (HNT/127) that caused about 40% mortality and gave rise to brain lesions in a large proportion of survivors. In further i.c. passages the virulence of the virus by i.n. instillations of adult hamsters was further enhanced and reached about 76% mortality in HNT/149. The effect of passage level on the virulence of the HNT strain of measles virus was studied in a large scale experiment using 6-week old hamsters, similar to that described for Langat virus. Three passage levels were used as follows: HNT/127, HNT/149 and HNT/162, 200 hamsters being inoculated with each passage and divided into 4 groups of 50 to be observed for signs of disease and mortality and survivors desstroyed after intervals of 1, 2, 3 and 6 weeks (Table VII).

As a whole, measles virus infection in hamsters inoculated with the HNT strain run a similar course to that of Langat virus. Mortality seldom was observed before 7 days after intranasal inoculation and the majority of infected animals developed the disease and died between the 7th and 22nd day after infection. Almost all the hamsters that developed signs of disease died; recoveries were an exception. The brain lesions in both clinically affected hamsters and survivors after inapparent infections were indistinguishable from those after Langat virus inoculations. There was a similar diffuse inflammatory reaction after one week, which became very severe in all clinically affected hamsters (Fig. 5). In the 2nd and 3rd week the acute reaction was superseded by subacute sclerosing lesions, giving rise to severe astrocytic proliferation and transformation in areas such as olfactory lobes, pyriform and entorhinal cortex and hippocampus. Large cell nests, being granulomatous accumulations of transformed microglial cells, were abundant especially in areas showing severe astrocytic changes. Degeneration necrosis and calcifications were also present (Fig. 6, 7). The distribution of the lesions was also similar to that after Langat infection, the first lesions appearing in the olfactory lobes and spreading from there to the pyriform and entorhinal cortex, thalamus and hippocampus. Other areas were also affected, but only in the acute stages of the disease. After 3 weeks, lesions were

TABLE VII.—*The Effect of Passage Level on the Virulence of Measles Virus (HNT) for Adult Hamsters after Intranasal Infections*

			Survivors without clinical signs						
					Inapparent infections: survivors with brain lesions			Total no. of affected hamsters	
Virus (i.c. passage in suckling hamsters)	No. of weeks from inoculation until death	No. of clinical cases (dead and affected) (%)	Total no. of survivors (%)	No. of survivors without brain lesions (%)	Total no. (%)	No. of survivors with mild or moderate lesions (%)	No. of survivors with severe or very severe lesions (%)	Clinical cases and survivors with brain lesions (%)	Clinical cases and survivors with only severe or very severe brain lesions (%)
HNT/127	1	6	94	4	90	28	62	96	68
	2	28	72	10	62	8	54	90	82
	3	8	92	8	84	32	52	92	60
	6	46	54	18	36	12	24	82	70
HNT/149	1	28	72	Nil	72	18	54	100	82
	2	48	52	4	48	8	40	96	88
	3	76	24	10	14	6	8	90	84
	6	76	24	10	14	6	8	90	84
HNT/162	1	26	74	8	66	18	48	92	74
	2	58	42	8	34	6	28	92	86
	3	68	32	10	22	12	10	90	78
	6	72	28	12	16	2	14	88	86

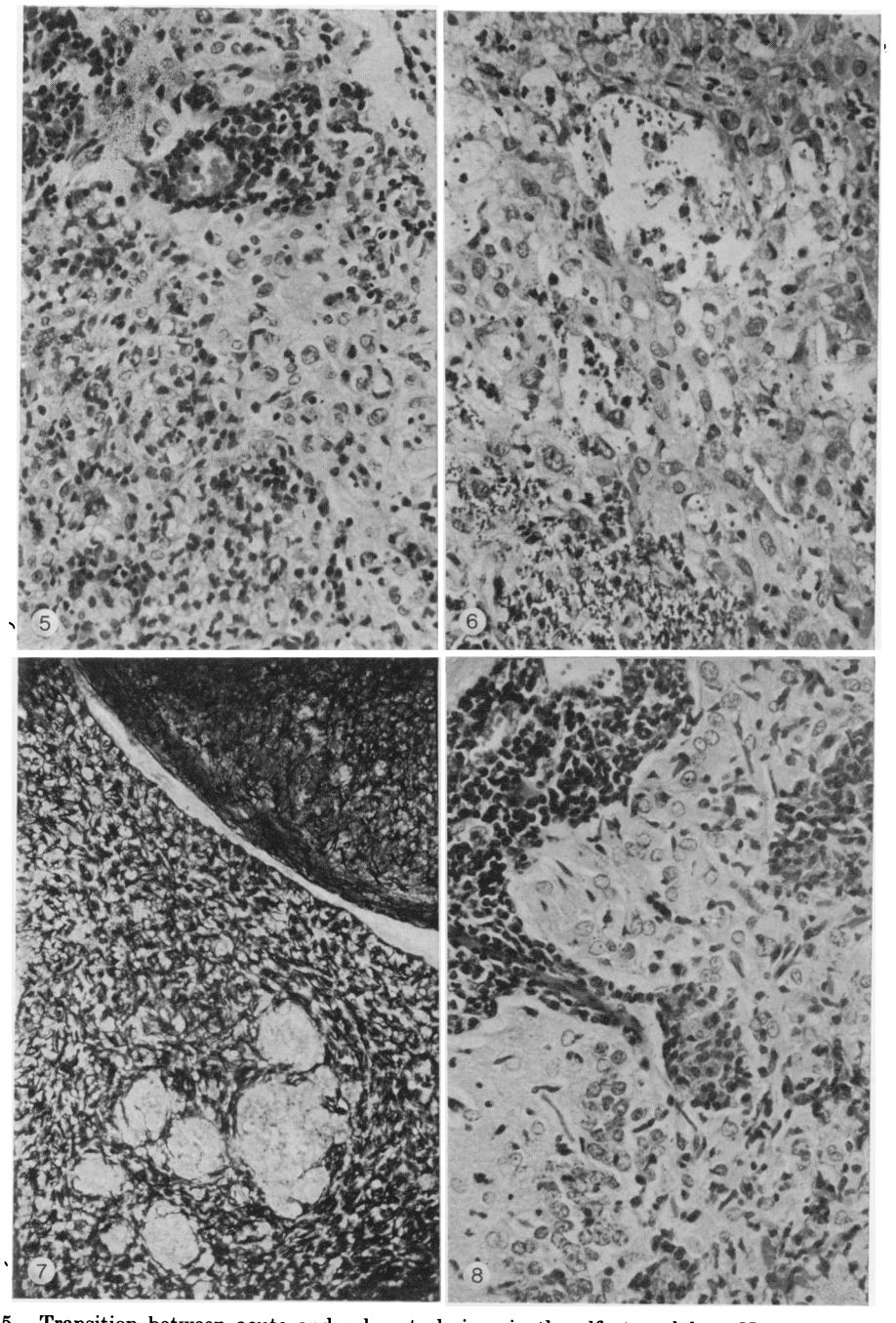

FIG. 5.—Transition between acute and subacute lesions in the olfactory lobe. Note perivascular cuffings; severe microglial infiltrations and the formation of cellular accumulations, and astrocytic proliferation and transformation. Measles virus (HNT strain) infection. H. and E. × 200.

FIG. 6.—Pyriform cortex; severe astrocytic proliferation and transformation, foci of degeneration with calcification and microcavitation. 3 weeks after intranasal infection with measles virus (HNT strain). H. and E. × 200.

FIG. 7.—Hippocampus; very dense subacute astrocytic proliferation and transformation and the formation of large cell nests (unstained). 2 weeks after measles virus (HNT strain infection). Cajal. × 85.

FIG. 8.—Hippocampus; Transition from acute to subacute inflammatory reaction. Note huge perivascular cuffings, microglial infiltration, cell nests formation and astrocytic proliferation. SSPE-measles virus (HBS strain) infection. H. and E. × 200.

seldom, if ever, found in the cerebellum or brain stem. Finally, after 6 weeks the olfactory lobes, pyriform cortex and in about 50% of cases the hippocampus showed advanced sclerosis, atrophy and distortion of various parts.

The inverse ratio between survivors with brain lesions and those developing clinical signs was as obvious in measles virus infections as in Langat virus irrespective of the passage level (Table VII). The sharp rise in mortality from 46% in HNT/127 to 76% in HNT/149 was not, however, maintained in further passages as no further increase in the virulence was noted in HNT/162.

The size of the infective dose did not affect the overall susceptibility of hamsters to the HNT/149 strain of measles to a large extent, but seemed to have influenced the ratio between the number of survivors with brain lesions and those developing signs of disease. An increase in mortality was accompanied by a drop of inapparent infections and *vice versa*. Thus, 3 log of virus (10^3 MLD_{50} i.c.) gave rise to the highest mortality (63%) but the lowest number of survivors with brain lesions (8%). Seven log of virus caused 51% mortality and 33% of survivors with brain lesions. Five log of virus, although giving rise to a mortality not significantly different from that after 7 log, caused the appearance of brain lesions in only 18% of survivors (Table VIII).

Age seemed to have had a similar effect on the susceptibility to measles virus as to Langat virus. The 142nd pass of HNT strain caused 100% mortality in 2-week old hamsters but in 4-week old only 53% died; the lowest mortality was recorded in 6-week old (42%). Older hamsters, however, became more susceptible so that in 47 weeks old animals there was 90% mortality (Table V).

Live virus (over 6 log) was isolated from brains of infected hamsters 7 days after infection irrespective of the age of the hamsters, whether 18 days old or 25. However, 10 days after intranasal instillations of virus, no virus was found in 18-day old hamsters and only in 2 of 5 inoculated at 25 days old. Brain lesions, on the other hand, were present in all hamsters whether inoculated at the age of 18 or 25 days (Table IV).

The appearance of vacuolated neurons in the brains of infected hamsters followed the same pattern as that after Langat virus inoculations. However, in measles virus very small numbers of vacuolated neurons were occasionally found even 3 weeks following infection. A more consistent occurrence of vacuoles was observed 6 weeks after infection but the numbers were very small (4%) after HNT/127 infection and reached 25% only after inoculations with HNT/149 and 28% after HNT/162 (Table VI). The distribution of vacuoles was more or less similar in measles virus infections as in Langat virus. The majority of vacuoles were found in the neurons of the amygdala some distance from the sclerotic lesions or in the pyriform cortex close to the subacute changes.

TABLE VIII.—*The Relationship between the Dose of Virus and the Susceptibility of Adult Hamsters to i.n. Infection: A Comparison between HNT/149 Strain of Measles Virus and HBS/26 Strain of SSPE-measles Virus*

	Measles virus (HNT/149)			SSPE-measles virus (HNS/26)		
i.n. dose of virus (log)	Mortality at 48 days (%)	No. of survivors with brain lesions (%)	Total no. affected (%)	Mortality at 48 days (%)	No. of survivors with brain lesions (%)	Total no. affected (%)
7	51	33	84	24	59	83
5	49	18	67	73	18	91
3	68	8	76	73	9	82

SSPE-measles virus

The 11th hamster pass of the HBS neurotropic strain of the SSPE-measles virus, received here from Dr Byington, did not give rise to infection after intranasal inoculations of adult hamsters. Similar results were obtained with HBS/12, but HBS/13 gave rise to occasional infection with mortality but none of the survivors showed any evidence of infection or brain lesions. A decisive change in the virulence of the virus occurred at the 18th pass with a mortality of 60% after intranasal instillations. Further passes did not alter the actual death rate which remained constant from HBS/18 to HBS/40. However, whereas the majority of animals developed signs of disease and died during the first 2–3 weeks after infection with HBS/18 the increase of the passage level tended to concentrate the mortality during the first 2 weeks after infection with HBS/26 and only during the first week after infection with HBS/40 (Table IX).

In a large-scale experiment involving 200 6-week old hamsters similar to that described for Langat and measles viruses and including an additional group of 100 hamsters that were inoculated with HBS/26 and left without interference for 28 weeks, it became obvious that although mortality did not increase during the successive hamster passages the number of survivors without brain lesions declined in the higher passes. A further point of interest was the fact that both mortality and inapparent infections with brain lesions after 28 weeks were almost the same as after 6 weeks (Table IX).

The clinical signs in hamsters infected by the i.c. route with the initial 4 passes of SSPE-measles virus (HBS/11–HBS/14) differed from those observed in Langat or HNT-measles virus. The animals developed early in the disease spasticity of limbs and marked myoclonal jerks, especially when touched or roused. These signs were less noticeable in 15th and 16th passes and when inoculated with HBS/18, irrespective of the route of inoculation, myoclonal spasms were absent.

The cerebral lesions in hamsters infected intranasally with the 3 passes of the HBS strain of virus (18th, 26th and 40th) were as a whole indistinguishable from those infected with Langat or measles virus. All hamsters had acute lesions of encephalitis after the first week following i.n. instillations with very severe perivascular infiltrations and microglial proliferations (Fig. 8). The most severe lesions were usually in the olfactory lobes and in some hamsters these were the only lesions. However, in the majority of animals lesions spread out from the olfactory lobes to other cortical and subcortical regions as in Langat and measles virus infections. By the end of the 1st week there was in many areas a very severe astrocytic reaction that consisted of an acute proliferation and hypertrophy of astrocytes (Fig. 9). By the end of the 2nd week or during the 3rd week the astrocytic reaction became subacute, engulfing whole areas of the brain such as olfactory lobes, pyriform and entorhinal cortex, hippocampus and parts of the thalamus. This reaction was accompanied by the formation of large cell nests consisting of granulomatous proliferations of microglial cells, necrosis, microcavitation and calcification (Fig. 10, 11). After 6 weeks severe sclerosis with atrophy of selected areas was often seen, the most usually affected areas being the olfactory lobes, the pyriform and entorhinal cortex and in about 40% of cases also the hippocampus. The sclerotic and atrophic changes became very advanced in hamsters that survived for 28 weeks after i.n. infection (Fig. 12).

Vacuolated neurons similar to those described in the brains of hamsters after Langat and measles virus infection were also present in SSPE-measles virus infected hamsters, beginning from 6 weeks after intranasal instillations (Table VI). Examination of a very large number of brains from survivors after i.n. inoculations with the 3 passages of the HBS strain of virus

TABLE IX.—*The Effect of Passage Level on the Virulence of SSPE-Measles Virus (HBS) for Adult Hamsters after Intranasal Infections*

Virus (i.e. passage in suckling hamsters)	No. of weeks from inoculation until death	No. of clinical cases (dead and affected) (%)	Survivors without clinical signs					Total no. of affected hamsters	
			Total no. of survivors (%)	No. of survivors without brain lesions (%)	Inapparent infections: survivors with brain lesions			Clinical cases and survivors with brain lesions (%)	Clinical cases and survivors with only severe or very severe brain lesions (%)
					Total no. (%)	No. of survivors with mild or moderate lesions (%)	No. of survivors with severe or very severe lesions (%)		
HBS/18	1	28	72	Nil	72	20	52	100	80
	2	56	44	2	42	16	26	98	82
	3	72	28	Nil	28	16	12	100	84
	6	60	40	30	10	6	4	70	64
HBS/26	1	32	68	Nil	68	16	52	100	84
	2	62	38	Nil	38	16	22	100	84
	3	74	26	2	24	14	10	98	84
	6	62	38	14	24	6	18	86	80
	28	58	42	14	28	12	16	86	74
HBS/40	1	40	60	Nil	60	10	50	100	90
	2	40	60	2	58	16	42	98	82
	3	36	64	10	54	20	34	90	70
	6	56	44	4	40	10	30	96	86

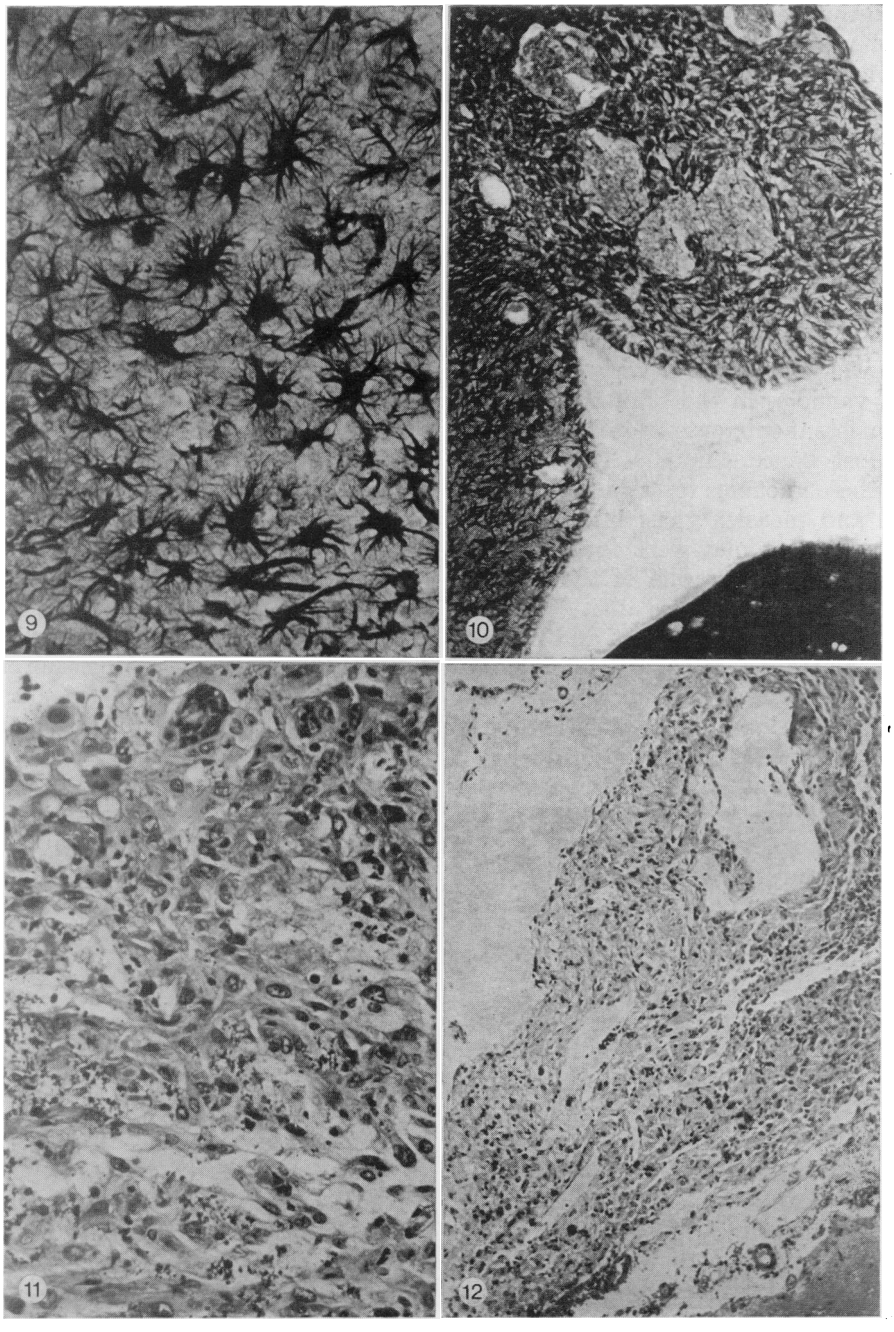

FIG. 9.—Hippocampus; acute stage—severe astrocytic reaction. SSPE-measles virus (HBS strain) infection. Cajal. × 200.

FIG. 10.—Hippocampus; very dense subacute astrocytic proliferation and transformation and cell nests formation. 3 weeks after SSPE-measles virus infection (HBS strain). Cajal. × 85.

FIG. 11.—Pyriform cortex—severe astrocytic proliferation and transformation with foci of degeneration and calcification. SSPE-measles virus infection (HBS strain). H. and E. × 200.

FIG. 12.—Olfactory lobe severe subacute sclerosis with atrophy. 28 weeks after SSPE-measles virus infection (HBS strain). H. and E. × 85.

revealed that only occasionally vacuolated neurons were present in the brain after infection with HBS/18 (5%) but were frequently found after HBS/26 (26%) and HBS/40 (32%) inoculations. It is worth noting that the above figures show the percentage of the total number of survivors after infection, and if one considers the fact that these vacuolated neurons were found only in hamsters showing subacute lesions and that from each of the 4 regions of the brain, only one 5 μm section was examined, a very high incidence of vacuoles in the brain of hamsters with persistent subacute sclerosing encephalitis must be concluded. The distribution of these vacuoles resembled that after Langat and measles virus infections but occasionally vacuoles were found also in the neurons of the raphé of the medulla in the formatio reticularis alba.

As in the case of Langat and measles viruses, mortality after i.n. infection of hamsters with the HBS strain of virus depended to a large extent on the age of the hamsters. It can be said that generally mortality after i.n. instillations with HBS/26 was high irrespective of the age of the hamsters. Although at the age of 2 weeks only 86% died, mortality did not decline very much in 3- and 4-week old animals. The lowest mortality was present in 5-week old animals but in older hamsters it became very high reaching 95% in the 47-week old (Table V).

The effect of the size of the i.n. dose of the HBS strain of virus on the mortality and development of brain lesions in survivors was similar to measles (HNT) infections (Table VIII) but the results were much more clearly cut. The lowest mortality was observed after 7 log of virus but the number of survivors with brain lesions was the highest; on the other hand the smallest dose of 3 log of virus gave rise to a very high mortality but the lowest number of survivors with brain lesions. The total number of hamsters affected, irrespective of the size of the infecting dose was similar irrespective of dose.

DISCUSSION

The results of the present work indicate that hamsters have a special predilection for subacute sclerosing encephalitis after infections with either Langat virus, measles virus or SSPE-measles virus. It is especially noteworthy that the tendency for developing subacute changes is particularly manifest in infections with these viruses before they became fully adapted to hamsters. In infections with passages of the above viral strains, sufficiently virulent for hamsters to initiate a disease but before their full adaptation, all exposed animals developed an acute encephalitis which could be either very severe and spread throughout the brain or mild and remain confined only to the olfactory lobes or their vicinity. Not all hamsters, even with severe brain lesions, always developed signs of disease, a large proportion of infected animals that survived infection appearing normal. All clinically affected animals died and those with inapparent infections sometimes developed signs at a later date, mortality being occasionally spread out over a whole year. However, the majority of surviving hamsters with acute brain lesions did not recover from the pathological process but the acute inflammatory reaction in their CNS became subacute. The subacute process progressed slowly, had little tendency for the involution of lesions and gave rise to a subacute sclerosing encephalitis with destruction or atrophy of whole parts of the brain.

Once the viral strains, after a number of hamster passages, became fully adapted to hamsters, there was a 100% mortality after i.c. inoculations and a high death rate after intranasal infections. The initial reaction in the brain was as in less adapted strains an acute inflammatory process that gave rise, however, to widespread degeneration and necrosis. In the brains of survivors after such infections usually limited lesions were present. These showed a tendency for involution and usually disappeared completely 3–4 weeks after exposure to the virus.

Although it might not be advisable to speculate at this stage, it is, however, impossible to avoid making a comparison between the infections of hamsters with neurotropic viral strains, such as Langat or measles or SSPE-measles, not fully adapted to that species with SSPE in man. It seems that there might be the possibility that SSPE is caused as a result of infection with a viral strain of measles or virus not fully adapted to man. This could be either in the form of measles virus that was accidentally passed in animals and partly lost its virulence for man, or that the infecting virus was not human measles but an animal virus immunologically very similar to measles. There were other workers who suggested that SSPE was not caused by measles virus but by a virus very similar to measles or variant of measles virus which had a very strong cell association (Katz *et al.*, 1970). Of the other suggestions as to the origin of SSPE, a very plausible explanation was offered by Burnet (1968), Gerson and Haslam (1971), Horta-Barbosa *et al.* (1971) and Saunders *et al.* (1969) that SSPE is due to the failure of cellular immunity, thus preventing the eradication of intracellular measles virus antigen. Some authors put forward theories, perhaps less plausible but nevertheless based on pathological and virological observations, that SSPE, is the result of a dual infection with measles virus and papova virus (Barbanti-Brodano *et al.*, 1970; Brody and Detels, 1970; Koprowski, Barbanti-Brodano and Katz, 1970). The result of the present work seems to suggest that the neurotropic strain of measles virus passaged in hamsters (HNT) and the neurotropic strain isolated from a case of human SSPE, (HBS) in spite of being passaged several times in hamsters, remained quite different in spite of the fact that antigenically and immunologically they were very similar if not identical.

In the present study attempts to isolate virus from animals with subacute sclerosing lesions in their CNS by means of animal inoculations and tissue culture were not successful but this does not indicate the absence of virus from such lesions in view of the fact that Byington and Johnson (1972) demonstrated cell associated virus from brains of hamsters infected with SSPE-measles virus as long as 81 days after inoculation by means of co-cultivation techniques (Horta-Barbosa *et al.*, 1969*a*, *b*; 1970, 1971).

The intranasal route was shown to be a very effective method of establishing an infection of all, or almost all, inoculated susceptible hamsters and although mortality was lower than after i.c. inoculations, the spread of the infection and the disease process were free from interference and from the initial mechanical damage to the brain substance. It is very probable that intranasal instillations reproduced very accurately natural infections, especially where recovery from the disease process was concerned. This is based on the observation that one week after infection all, or almost all, animals had brain lesions but after 2 weeks a proportion of hamsters had no lesions or only mild involuting changes. The similarity between the 3 viruses discussed in this paper is very striking and is not limited only to the ability of giving rise to subacute sclerosing encephalitis but also to the same clinical signs, identical brain lesions with a tendency to progress towards partial or complete atrophy of parts of the rhinencephalon. In all the 3 viruses the sum total of hamsters infected and going down with clinical signs and those developing inapparent infections with brain lesions was almost constant in various passage levels, mortality being low and inapparent infections very numerous in the lower passes with a high mortality and fewer inapparent infections seldom causing subacute sclerosing changes in the very high passage levels.

The occurrence of vacuolated neurons in selected areas of the brain, especially in the amygdaloid nuclei and pyriform cortex reported previously (Zlotnik and Grant, 1975) was confirmed in this work and it was further shown that in SSPE-

measles virus infections vacuoles of the same type and distribution as in measles virus and Langat virus were also present. However, in the present study, by using considerably larger numbers of infected animals than before, a more correct assessment of the incidence of vacuolated neurons was achieved. These large-scale experiments have shown that in all the 3 virus infections vacuolated neurons were present in the brain already at 6 weeks after intranasal exposure. In the HBS strain of SSPE-measles virus vacuoles were seen as early as 3 weeks after inoculation, admittedly only in very small numbers. As far as Langat virus was concerned (TP21/H2) infection with the 2nd hamster pass gave rise to vacuolated neurons in 27% of survivors and (TP21/H10) the 10th pass in 36% of surviving animals. No vacuolated neurons were found in TP21/H36 infections but here the number of survivors with brain lesions was small and none of them had true subacute sclerosing lesions. The HNT strain of measles virus and the HBS strain of SSPE-measles virus showed a gradual increase in the number of vacuolated neurons from the lower passes to the higher. The higher passes hitherto investigated had the highest incidence of vacuolated neurons; however, unlike Langat virus, these 2 viruses still produced subacute sclerosing lesions in the survivors after infection with the highest passes. It is possible to assume that still further passage will most probably bring about conditions similar to those in Langat virus, with the disappearance of survivors with subacute sclerotic lesions and vacuoles in neurons.

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