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SUBACUTE SCLEROSING ENCEPHALITIS IN ADULT HAMSTERS INFECTED WITH LANGAT VIRUS

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Summary.—Langat virus, passaged i.c. twice in suckling hamsters, became virulent for adult hamsters and caused progressive subacute encephalitis with a high degree of mortality spread out over the period of 3 months that the animals remained under observation.

The lesions in the brain during the first few days after infection were purely inflammatory in character, but from the second week onwards became also degenerative and subacute and gave rise to widespread granulomatous perivascular cellular infiltrations and a specific astrocytic transformation and proliferation. The unchecked progress of the pathological process led to severe sclerosis and atrophy of many parts of the brain, especially of the hippocampus.

The use of an immunosuppressant did not alter the mortality after i.c. inoculations but enhanced the virulence and invasiveness of Langat virus after intradermal inoculations. In addition, immunosuppression gave rise to an unprecedented increase in the severity of the astrocytic reaction.

In a previous communication (Zlotnik, 1972) a progressive subacute sclerosing encephalitis was described in suckling hamsters infected peripherally (i.p. and i.d.) with Langat virus. Not only were lesions of the CNS produced in hamsters infected with the virulent strain of virus (TP 21), but also in those inoculated with the attenuated strain (TP 21–9). The unusual feature in these experiments was the fact that in spite of disappearance of virus from the CNS by 14 days after infection, the lesions in the CNS progressed and gave rise to a protracted subacute condition with sclerosis and atrophy of parts of the brain, especially the hippocampus and the cerebral cortex. The present study was undertaken in order to ascertain whether the prolonged course of the disease and the subacute brain lesions in suckling hamsters were due only to infection in early post-natal life with a virus of reduced virulence for the particular species, or whether the condition represented a specific disease response of hamsters to infection with Langat virus.

MATERIAL AND METHODS

Animals.—Golden hamsters weighing 40–150 g, aged between 4 weeks and 22 weeks, were used in all experiments. In addition, 3-day old suckling hamsters were inoculated in order to adapt the virus to hamsters, 3-day old mice were used for virus assays, and 3-week old mice of the Porton colony were employed for serum virus neutralization tests.

Inocula.—(a) Virulent Langat virus (Smith, 1956) passaged 9 times in suckling mice TP 21/M9; (b) virulent Langat virus passaged 9 times in suckling mice and twice in suckling hamsters TP 21/M9/H_2; (c) attenuated Langat virus TP 21–9 (Price *et al.*, 1963).

Routes of inoculation.—Intracerebral (i.c.), intradermal (i.d.) and intraperitoneal (i.p.).

The dose for adult hamsters was 0·05 ml for i.c. inoculations, 0·2 ml for i.p. and 0·1 ml for i.d. injections. Suckling hamsters and mice received invariably 0·02 ml i.c. in all the tests.

The titre of the inoculum was 10^7 MLD_{50} (i.c.) for both virulent and hamster adapted Langat virus but only 10^6 MLD_{50} (i.c.) for the attenuated TP 21–9.

Immunosuppression was carried out by means of i.p. inoculations of cyclophosphamide (Endoxana–Ward, Blenkinsop & Co. Ltd.). In each case 100 mg/kg body weight was either given only on the first day after virus inoculation, or was followed by similar doses on the seventh and fourteenth days.

Histological methods.—As a rule all animals were anaesthetized with ether before destruction by decapitation. Brains were removed as soon as possible and some were divided sagittally. Whole or half brains were fixed in 10% formol saline for paraffin embedding and conventional histology, while the other half, when applicable, was used for virus assay. In addition, one block of tissue from each brain was cut on the freezing microtome and formol fixed sections were stained according to a modified Cajal's method for the demonstration of astrocytes (Zlotnik, 1968).

RESULTS

Although suckling hamsters proved to be susceptible to both virulent and attenuated strains of Langat virus, adult animals did not develop any infection as a result of i.p. inoculations and only limited lesions and virus multiplication after i.c. inoculations. In order to study the effects of Langat virus in hamsters, it became necessary therefore to adapt the virus by passaging it twice in 3-day old hamsters. The resulting virus became virulent for hamsters only after i.c. inoculations and gave rise to virus multiplication and bilateral brain lesions.

I. The pathogenesis of mouse adapted Langat virus (TP 21/M9) in adult hamsters

Two groups of hamsters were used: one consisted of animals 7 weeks old and the other of 22 weeks old. Each group was then subdivided, one half receiving i.p. inoculation and the other half i.c. injections of mouse adapted virus (TP 21/M9). There was no mortality and none of the animals developed signs of disease during the 3 weeks following inoculations; they were then destroyed and their brains examined histologically.

As a rule none of the i.p. inoculated hamsters developed lesions in the CNS, but the i.c. infected animals had unilateral changes mainly in the vicinity of the needle track. However, whereas the majority of animals (80%) of both age groups had only minimal inflammatory perivascular reactions, a smaller proportion (20%) had lesions that were unilateral and originating from the needle track but spread out and engulfed the whole hippocampus of the affected side.

The lesions consisted of several perivascular cuffings, proliferation of microglia and astrocytes, degeneration of some pyramidal cells and partial atrophy and distortion of the whole hippocampus.

II. The pathogenesis of hamster passaged Langat virus (TP 21/M9/H2)

Three large groups of hamsters of different ages, 4 weeks old, 7 weeks old and 5 months old, were used in a series of experiments as follows. In each group about one third received i.p. inoculations and two thirds i.c. injections. Following i.p. inoculations there were no signs of disease and all sacrificed animals had no lesions in their brains. After i.c. inoculations, however, there were clinical cases with mortality spread out over the whole period that these animals remained under observation, *i.e.* 42 days for 4 weeks old and 98 days for the older hamsters. The first signs were noted after 6 days and the first death occurred on the seventh day after infection. Animals that died proved unsuitable for examination;

however a large proportion was destroyed *in extremis* and their brains were removed for both histological examination and virus assay. In addition to hamsters exhibiting signs of disease, groups of animals that did not show any clinical involvement were also destroyed and their brains examined.

(*a*) *Infection of 4-week old hamsters.*—The earliest signs of disease were observed in i.c. inoculated hamsters on the sixth day (one out of 36 infected animals and none out of a similar group of 20 hamsters). Further hamsters developed the disease on the seventh, eighth day and later during the intervening 4 weeks. The course of the disease lasted from 1 to 3 days and all the affected animals either died or were killed *in extremis*, a total of 15 hamsters from one group of 36 and 8 hamsters from another group of 20 (Table I).

TABLE I.—*Mortality in Hamsters Infected i.c. with Langat Virus*

	Group A	Group B	Group C	Group D
Age of hamsters at the time of inoculation (in weeks)	4	4	7	22
Number of inoculated hamsters	20	36	30	30
Mortality 6–21 days	8 (40·0%)	13 (36·0%)	6 (20·0%)	5 (16·6%)
Mortality 21–42 days	—	2 (5·0%)	Nil	Nil
Mortality 42–98 days	—	—	5 (16·6%)	5 (16·6%)
Total mortality	8 (40·0%)	15 (41·0%)	11 (36·6%)	10 (33·2%)

Beginning from the third day until the seventh day after infection, appreciable amounts of virus were isolated from all brains, but in clinical cases virus was also isolated later until the fourteenth day following inoculation. Titration of brain homogenates from 3 hamsters, chosen daily at random from Groups A and B without regard to clinical signs, between the fourth and seventh day after infection yielded from 6 to 9 logs of virus per g of brain (Table II).

TABLE 2.—*Virus Content of the Brains of Three Hamsters Killed Daily Between 4 and 7 Days After Infection*

	Hamster (1)		Hamster (2)		Hamster (3)	
Days after infection	Titre of* virus	Clinical signs	Titre of* virus	Clinical signs	Titre of* virus	Clinical signs
4	6·27	Nil	6·82	Nil	8·5	Nil
5	6·63	Nil	7·72	Nil	9·32	Nil
6	6·67	Present	7·46	Nil	7·46	Nil
7	6·53	Nil	6·96	Present	7·29	Nil

* MLD_{50} (i.c.) per g of brain.

The highest levels of virus in non-clinical cases were recorded on the fourth and fifth days, and after 7 days there was a decline until the fourteenth day when no virus whatsoever was isolated from 6 examined brains. In the 2 clinical cases, on the sixth and seventh day virus levels were not as high as in some of those without signs on the fourth and fifth day; virus was present in 5 clinical cases occurring even 14 days after infection, but could not be found in one case killed *in extremis*. After 14, 21 and 28 days no virus was isolated in spite of the fact that on the last date 2 of the 9 hamsters examined showed very severe signs of disease and had to be killed. Similarly, no virus was detected in any of the hamsters destroyed 42 days after infection.

Histological examination of the brains revealed a bilateral distribution of lesions in all cases, from the earliest recognizable until the most advanced changes. The

first lesions appeared in all the hamsters of a random sample on the fourth day after infection, none of the animals showing any signs of disease. The lesions ranged from only very slight infiltration of lymphoid cells in the chorioid plexus, or minute perivascular cuffings in the olfactory lobe, to more pronounced changes in various parts of the brain with the exception of the cerebellum and brain stem. They consisted of perivascular cuffings composed of either a single layer or several layers of infiltrating cells, slight microglial proliferation and foci of spongy degeneration in the thalamus. On the fifth day the lesions became more acute and more widespread in all the hamsters. At this stage one of 3 brains also had marked degenerative and limited necrotic changes in the cells of the pyramidal layer of the hippocampus. On the sixth day the hamster showing clinical signs had similar lesions to those in non-clinical cases, except that amongst the degenerating cells of the pyramidal layer cell debris was clearly visible (Fig. 1). By the seventh day in both clinical and non-clinical cases the inflammatory perivascular cuffings assumed huge proportions, consisting of several layers of mononuclear cells which had a tendency to occlude the lumina of the blood vessels especially of capillaries (Fig. 2). At this stage the astrocytes in the hippocampus, and occasionally also in the temporal and pyriform cortex, became greatly hypertrophied and there was evidence of early proliferation as well (Fig. 3).

EXPLANATION OF PLATES

FIG. 1.—Degeneration in the pyramidal layer in the hippocampus of a clinically affected hamster. H. and E. ×180.

FIG. 2.—Huge perivascular cuffings in the hippocampus 7 days after infection. H. and E. ×180.

FIG. 3.—Severe astrocytic reaction in the hippocampus 7 days after infection. Cajal ×180.

FIG. 4.—Subacute granulomatous cell accumulations and diffuse astrocytosis in the pyriform cortex 14 days after infection. H. and E. ×75.

FIG. 5.—Diffuse astrocytic transformation and a cell nest in the hippocampus 14 days after infection. H. and E. ×180.

FIG. 6.—Giant cell nest, surrounded by perivascular cuffings in the hippocampus 14 days after infection. H. and E. ×180.

FIG. 7.—Dense astrocytic network around cell nests in the hippocampus 14 days after infection. Cajal ×180.

FIG. 8.—Severe astrocytic proliferation and focal microglial infiltrations in the hippocampus 14 days after infection. H and E. ×180.

FIG. 9.—Pyriform cortex 14 days after infection; astrocytic proliferation, meningeal and peri-capillary round cell infiltrations. H. and E. ×75.

FIG. 10.—Distortion and atrophy in the hippocampus 3 weeks after infection; note huge cell nest and destruction of the pyramidal layer. H. and E. ×30.

FIG. 11.—Atrophy and sclerosis of the hippocampus 3 weeks after infection; note dense astrocytic network and giant cell nests (unstained). Cajal ×30.

FIG. 12.—Very severe atrophy and sclerosis of the temporal cortex 98 days after infection. Cajal ×75.

FIG. 13.—Very severe sclerosis and atrophy of the hippocampus 98 days after infection; note cell nests undergoing calcification and the extension of the pathological process to the cerebral cortex and midbrain. H. and E. ×30.

FIG. 14.—Very severe atrophy of the hippocampus 98 days after infection. Cajal ×30.

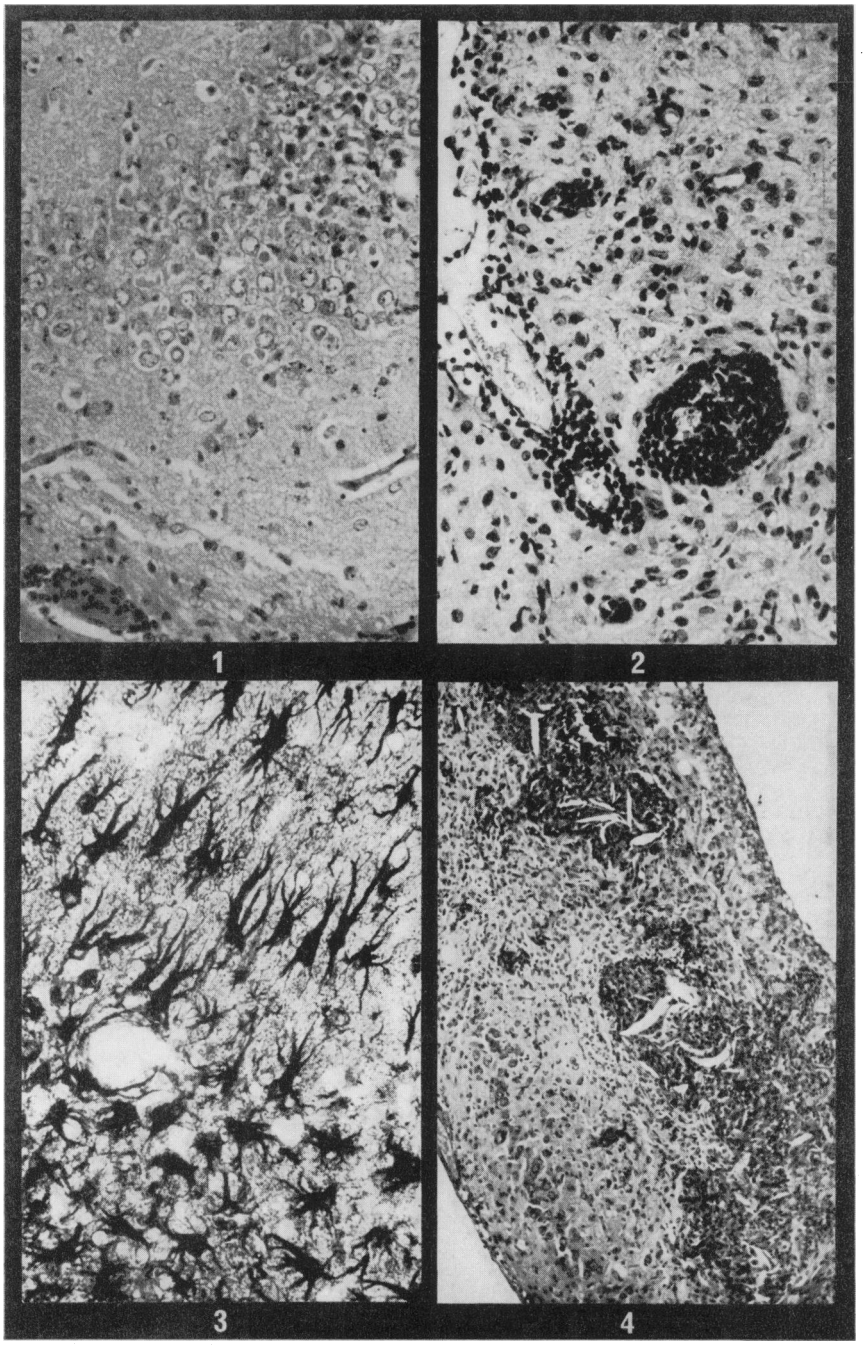
FIG. 15.—Diffuse microglial reaction in a sclerotic hippocampus 98 days after infection. H. and E. ×30.

FIG. 16.—Severe destruction of pyramidal cells in the hippocampus 98 days after infection. H. and E. ×75.

FIG. 17.—A very dense astrocytic network in a sclerotic hippocampus 98 days after infection. Cajal ×75.

FIG. 18.—Widespread necrosis amongst the proliferating astrocytes in the hippocampus of a hamster dying 80 days after infection. Cajal ×75.

FIG. 19.—Very severe and very widespread astrocytosis in the temporal cortex of a hamster treated with cyclophosphamide after i.c. infection. Cajal ×75.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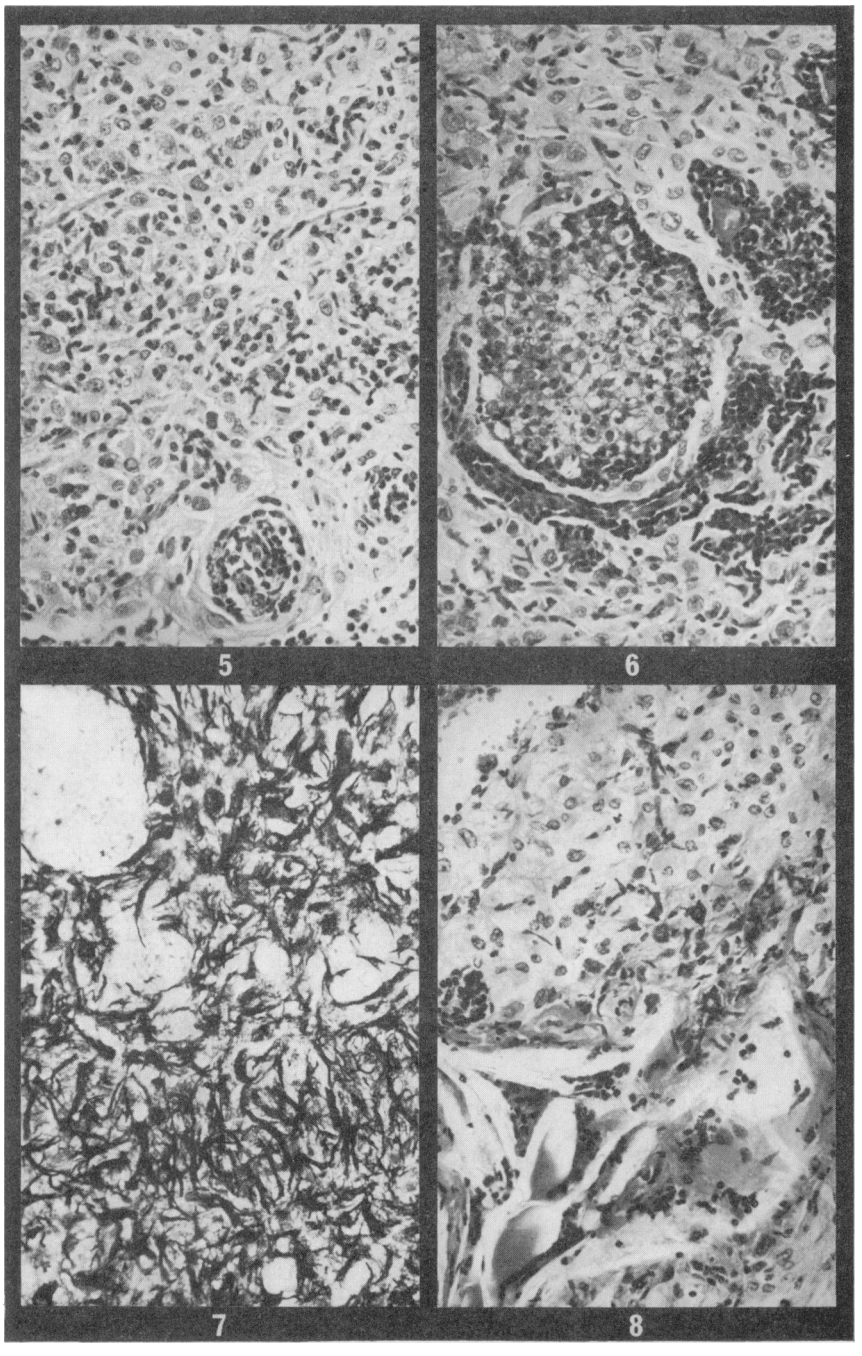

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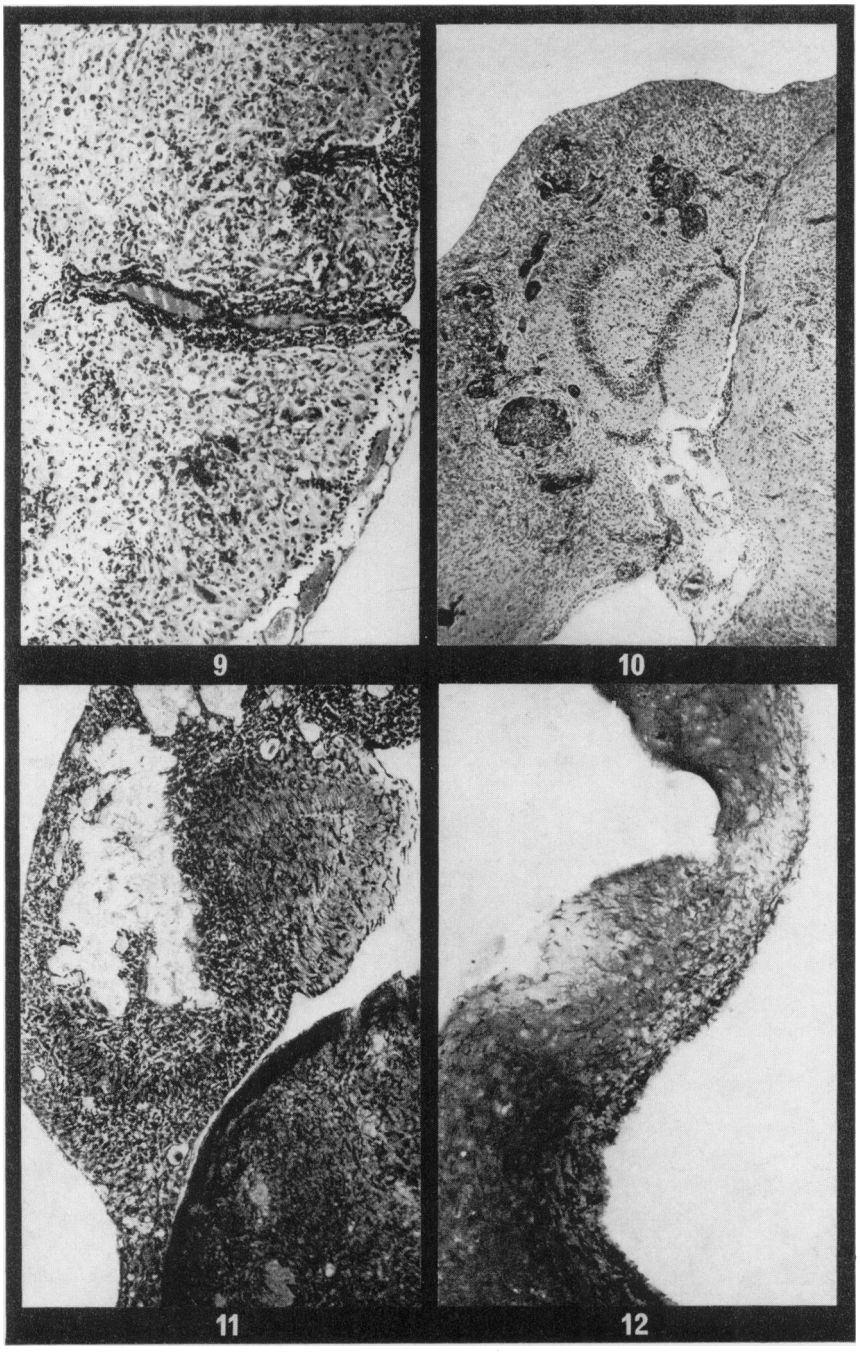

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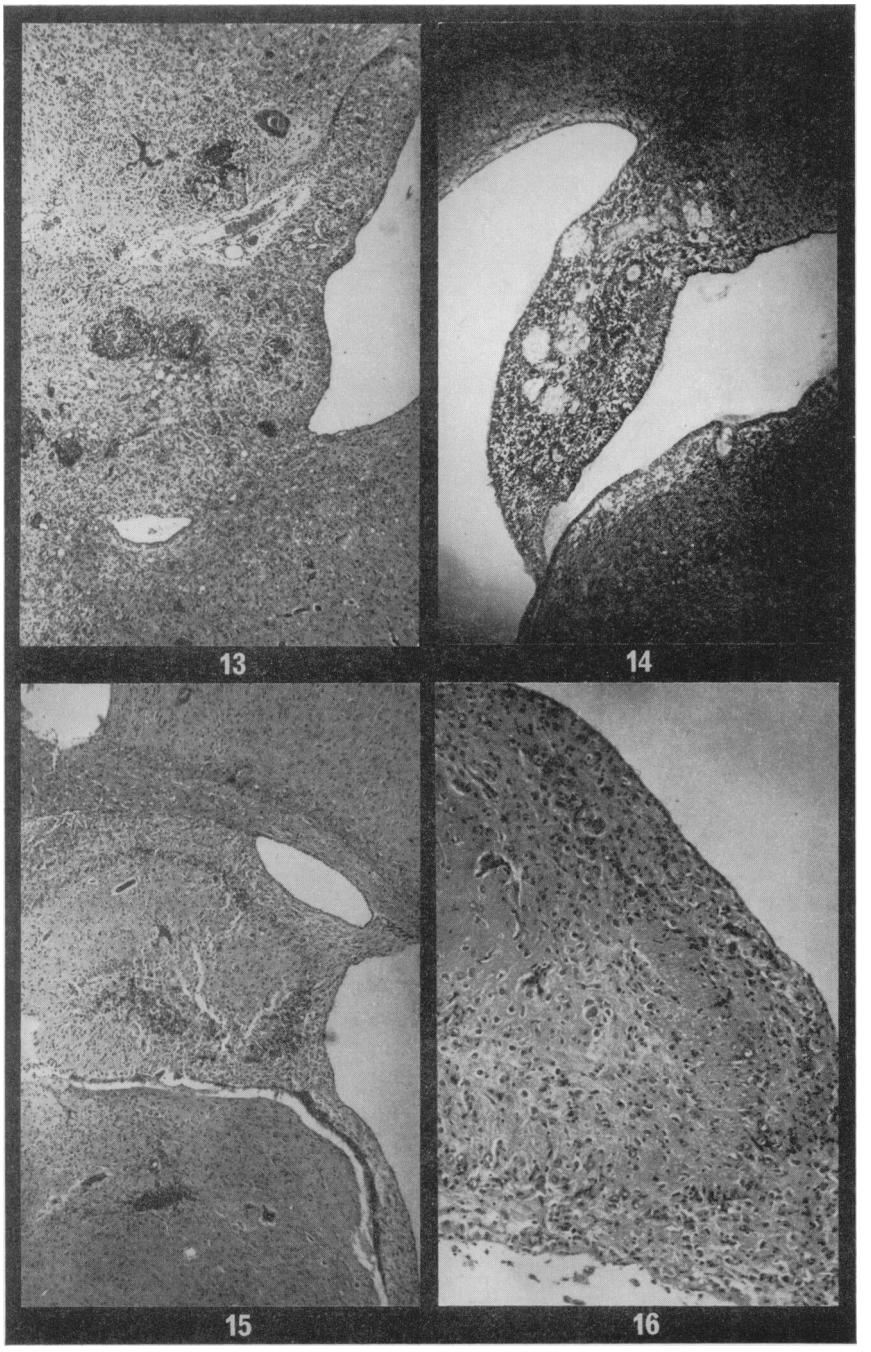

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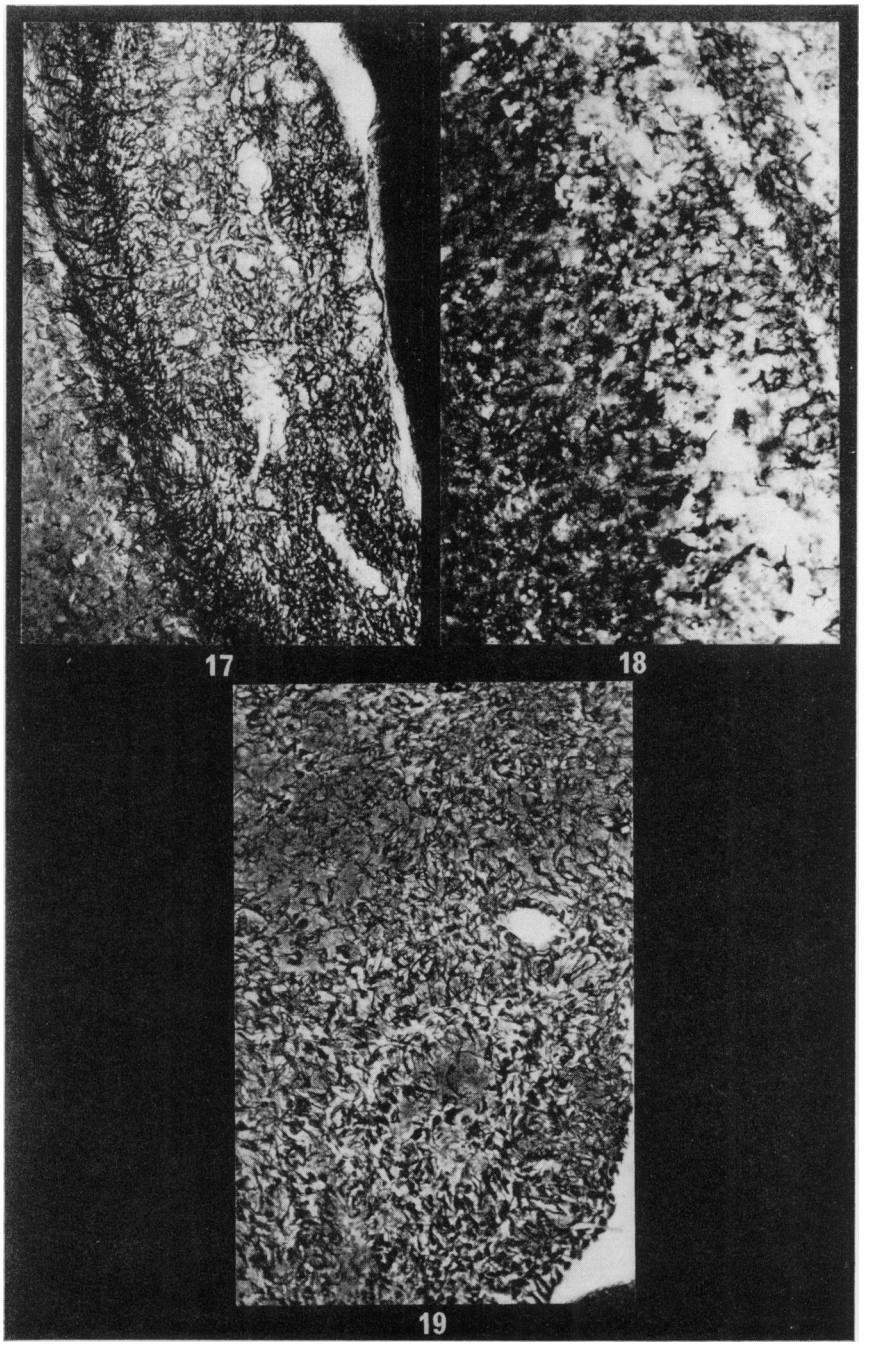
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Fourteen days after infection all the hamsters showing clinical signs of disease had very extensive lesions spreading from the olfactory lobe to the midbrain, the cerebellum and midbrain being only rarely affected. Although changes were usually present in many parts of the brain, the hippocampus and cerebral cortex were always affected. At this stage the lesions assumed a subacute character, and many huge perivascular cuffings after complete occlusion of the lumina appeared as large granulomatous cell accumulations or smaller cell nests. Other perivascular cuffings, resembling those seen in allergic encephalitis, being composed of several layers of infiltrating large mononuclear cells, remained usually in the vicinity of the cell nests (Fig. 4, 5, 6). The astrocytes in affected areas were undergoing progressive proliferation and transformation into large cells with a clear cytoplasm, easily stained in H and E preparations. The proliferating astrocytes showed a tendency to form epithelium-like cell accumulations that were spreading out and replacing existing structures such as the pyramidal neuronal layer of the hippocampus (Fig. 4, 5, 6). In preparations stained according to Cajal's method the greatly hypertrophied astrocytes of the hippocampus, or cerebral cortex, formed a very thick network and very large tangles around the granulomatous cell nests (Fig. 7). Microglial proliferation was either in the form of dense focal accumulations within the mass of transformed astrocytes (Fig. 8) or as diffuse infiltrations, especially in areas of spongy degeneration. At this stage some of the cell nests began to show signs of degeneration or partial calcification. At the same time there was also evidence of atrophy of the hippocampus and the temporal cerebral cortex, with a marked reduction in size and hydrocephalus. The anterior parts of the brain, such as the olfactory lobe, pyriform and frontal cortex, were often surrounded by a copious meningeal exudate that was composed entirely of large mononuclear cells which extended along the capillaries and other blood vessels into the brain parenchyma (Fig. 9).

Lesions similar to those seen in clinically affected hamsters were present also in 3 of the 10 brains from hamsters that did not exhibit any signs of disease during the first 2 weeks after infection. The other 7 brains had only occasional perivascular cuffings in the olfactory lobe, corpus striatum or the cerebral cortex(Table III).

Three weeks after infection all the clinically affected hamsters and 7 of 10 non-clinical cases had very advanced subacute lesions in the brain. The affected brains were macroscopically altered in that the temporal cortex appeared thin, slightly collapsed and wrinkled. Transverse sections of the brain revealed a small atrophied hippocampus and a very thin cerebral cortex around the lateral ventricles. The histological changes resembled those seen after 2 weeks, but atrophy and astroycytic sclerosis were somewhat more advanced and the actual distortion of the hippocampus became very marked (Fig. 10, 11). In one case, however, the most severe lesions were present in the corpus striatum, which became converted into a mass of astrocytes surrounding large granulomatous cell nests.

Six weeks after infection 10 hamsters that did not show any signs of disease were destroyed and brains examined. Six brains had very advanced lesions somewhat similar to those seen after 3 weeks, except that the hippocampus became reduced further to about half its normal size, the cerebral cortex appeared even thinner and many of the cell nests were undergoing calcification. Of the other 4 brains, 3 did not show any lesions but one had marked atrophy and reduction in size of the hippocampus, but without any sign left of the subacute inflammatory reaction (Table III).

TABLE III.—*Presence of Brain Lesions in Non-clinically Affected Hamsters Inoculated i.c. with Langat Virus*

Age of hamsters at the time of inoculation (weeks)	Interval between inoculation and destruction							
	2 weeks		3 weeks		6 weeks		14 weeks	
	No. of brains examined	No. of brains with lesions	No. of brains examined	No. of brains with lesions	No. of brains examined	No. of brains with lesions	No. of brains examined	No. of brains with lesions
4	10	3	10	3	10	6	—	—
7	—	—	10	8	—	—	10	8
22	—	—	10	9	—	—	10	7

(*b*) *Infection of mature hamsters.*—Two groups of hamsters were used in these experiments: one group consisted of hamsters 7 weeks old and the other was composed of animals aged 22 weeks. All these animals were inoculated i.c. with the same Langat virus passaged in hamsters as the 4 weeks old. The sequence of events resembled in many respects that in 4-week old hamsters except that some survivors were kept for 98 days following infection.

The first signs of disease appeared in both groups after 7 days and further cases with mortality occurred between 7 and 21 days (Table II). There was no mortality nor clinical cases between 21 and 45 days, but further hamsters developed signs of illness and died between 45 and 98 days. A comparison between the mortality in the 3 age groups showed that between 6 and 21 days, there was 20·0% mortality in 7-week old, 16·6% in 5-month old, but 36·0% in 4-week old hamsters. During the fourth, fifth and sixth weeks after infection there were 2 additional cases among the 4-week old animals, but no cases or mortality occurred in older hamsters. However, in the latter age groups a further 5 animals developed the disease in each group at between 45 and 98 days.

The occurrence of brain lesions in clinically affected older hamsters was very similar to that in the 4-week old animals, except that in the clinical cases destroyed 7 days after infection brain changes were more severe and advanced and in addition to the inflammatory and granulomatous lesions, astrocytic transformation and proliferation were very prominent in all affected areas.

At 3 weeks following infection 2 groups of 10 hamsters each, none of them showing any signs of disease, were destroyed. One group consisted of animals infected when 7 weeks old and the other when 22 weeks old. Symmetrically bilateral lesions, similar in every respect to those seen in 4-week old hamsters, were found in 8 out of 10 7-week old, and in 9 out of 10 22-week old groups (Table III). At 98 days after infection a further 2 groups of hamsters not showing signs of disease were destroyed and brains examined. Again 8 out of 10 7-week old had very advanced changes, but in the 5-month old hamsters only 7 brains had lesions (Table III). In addition to the above 20 brains, the brain of a 7-week old hamster that developed clinical signs following inoculation 3 weeks after infection, but later recovered, was also examined. The lesions in the brain of the recovered hamster did not differ from those non-clinically affected and destroyed 98 days after infection.

As a rule, all the brains that revealed histological lesions also had marked hydrocephalus, severe reduction in size of the hippocampus and thinning of the cerebral cortex. The cerebellum and brain stem were not usually affected, although a few brains had perivascular cuffings in the white matter of the cerebellum. The most commonly affected parts of the brain were the hippocampus and the cerebral cortex; however, in one brain the thalamus showed the most advanced changes while the cerebral cortex became reduced to a thin membrane (Fig. 12). The hippocampus was atrophied in most brains and reduced to about one third of its original size. The general pattern of its histological structure was greatly disturbed, the organ consisting of a dense network of astrocytes, necrosis or calcification of cell nests and almost complete destruction of neurons in both pyramidal and granule layers (Fig. 13, 14). In some brains, however, the subacute process was replaced by a diffuse microglial reaction centred in the pyramidal layer of the hippocampus and causing infiltrations around degenerate neurons and calcified cell nests (Fig. 15, 16). The astrocytes at this stage formed

a very dense network of long fibres, giving rise to shrinkage and sclerosis of the affected part (Fig. 17). In one clinical case dying at about 80 days after infection the astrocytic reaction assumed enormous proportions in the hippocampus. The astrocytes became greatly hypertrophied, stellate in shape and at the same time large numbers of cells were also undergoing necrosis (Fig. 18).

III. Immunological studies

In a number of experiments, studies were made of the effects of immunization against Langat virus and of immunosuppression on the development of the subacute forms of encephalitis in hamsters. For this purpose a large group of adult hamsters aged 7 weeks was immunized by means of a single i.p. dose of attenuated Langat virus (TP 21–9) and subsequently groups of immunized hamsters were challenged i.c. at intervals of 7, 14 and 21 days with the hamster passaged virus (TP 21/M9/H_2). Two other control groups were used, one receiving only the immunizing inoculation of attenuated virus and the other being given only the i.c. challenge. None of the immunized hamsters, whether challenged i.c. or not, developed any signs of disease during the initial period of 21 days after challenge, but 2 of 10 control hamsters that were inoculated i.c. only with hamster passaged Langat virus died. Histological examination of brains of immunized hamsters destroyed between 21 to 29 days after i.c. challenge and control animals that received only the immunizing dose revealed no lesions whatsoever, but in non-immunized animals challenged i.c. severe subacute lesions similar to those described previously were noted in 6 of 8 brains.

In another series of experiments, hamsters were infected either i.c. or i.d. with hamster passaged Langat virus followed by one or 3 i.p. injections of cyclophosphamide. Although there was no substantial increase in the mortality in the cyclophosphamide treated animals, histological examination of their brains revealed that in both clinical and non-clinical cases the lesions were of much greater severity and wider distribution than in untreated hamsters. Especially prominent were the degenerative changes, with marked spongy degeneration and necrosis in all the regions of the brain with the exception of the cerebellum and brain stem. Granulomatous cell accumulations often undergoing calcification were also abundant. However, the most striking changes were seen in the astrocytes where very severe subacute proliferation and hypertrophy of unprecedented proportions were evident throughout the brain (Fig. 19).

Hamsters infected by the intradermal route did not develop any lesions, but those treated with cyclophosphamide survived the inoculation but developed moderately severe lesions (both degenerative, astrocytic and granulomatous) in many parts of the brain within 4 weeks of infection.

In a third series of experiments, hamsters were immunized by i.p. inoculations of attenuated virus (TP 21–9), followed by immunosuppression with cyclophosphamide, and were challenged later i.c. with hamster passaged Langat virus. Although none of the challenged hamsters developed clinical signs when they were killed 3 weeks later, a small proportion of animals challenged 7 days after the immunizing dose developed limited subacute lesions, mainly in the thalamus. About half of the hamsters challenged i.c. 14 days after immunization had very severe subacute lesions throughout the cerebral cortex, foci of astrocytic proliferation and granulomatous changes in the hippocampus. Finally those challenged 21 days after the immunizing dose escaped without developing any brain lesions.

DISCUSSION

The results of the various inoculations of hamsters with Langat virus provide yet another example of the prolonged effects of viral infection on the central nervous system, especially when the host is only partly susceptible to infection with the virus. It seems obvious that most if not all inoculated hamsters had virus replication in their brains and had at least initial limited inflammatory and astrocytic changes during the 7 days after infection. It must be assumed, therefore, that the pathological process developed further in some hamsters, causing death soon afterwards. By 14 days after infection, virus disappeared from all animals not showing clinical signs, but the encephalitic process continued in the majority of animals, becoming subacute in character, causing occasional overt signs of disease and death during the whole period of over 3 months that these animals remained under observation. Although many affected animals appeared normal on clinical examination, this is doubtful in view of the very severe lesions found in their brains. The inability to recognize less obvious or less advanced signs of disease remains a very serious handicap and a disadvantage in experimental work with animals such as hamsters.

The pathological changes consisted of a mixture of acute and granulomatous inflammatory lesions and a progressive astrocytic reaction, leading to atrophy and sclerosis of parts of the brain. This type of encephalitic process is unprecedented, and especially the almost specific predilection for the hippocampus and the tendency to form these subacute and irreversible changes. The fact that many viruses may cause lesions of encephalitis in the absence of clinical signs has been stressed by some writers (Aguilar, 1970; Binn *et al.*, 1966; Doherty and Reid, 1971; Roca-Garcia *et al.*, 1964; Zlotnik, 1968; Zlotnik *et al.*, 1970*a*, *b*, 1971). However, only recently it has been shown that some attenuated clones of virus, such as WEE or SFV, may give rise to protracted or delayed subacute lesions with astrocytic proliferation and sclerosis (Zlotnik *et al.*, 1972*a*, *b*). The present work brings out still further the hitherto unknown feature that under conditions of reduced susceptibility the virus of Langat gave rise in hamsters to a complicated granulomatous process and caused a fatal disease with progressive sclerosis and atrophy of large areas of the brain.

The subacute progress of the encephalitic process cannot be attributed to the persistence of Langat virus in the brain, nor to an increased immune response to the virus. This has been shown by the fact that virus disappeared from the brain of infected hamsters after 14 days and that immunization with an attenuated clone of virus not only did not enhance the pathological process but prevented it. The limited experiments with immunosuppression by means of cyclophosphamide suggest that Langat virus behaved in hamsters as if they were partly immunosuppressed. Hitherto, it has been shown that cyclophosphamide caused persistence of virus in the CNS of animals infected with group B arboviruses, a decrease in the inflammatory reaction and an increase in the extent of degenerative lesions (Thind and Price, 1969; Nathanson and Cole, 1970; Zlotnik *et al.*, 1970*a*, 1972*a*). The Langat virus infection of hamsters throws further light on the action of the immunosuppressant, in that it caused an unprecedented astrocytic reaction throughout the brain.

To conclude, it must be emphasized that the results obtained in adult hamsters, which proved to be similar in most respects to those of young suckling animals, show clearly that the subacute granulomatous encephalitis with a tendency to

produce atrophy of the hippocampus and other parts of the brain is a specific entity and must be attributed to the effect of Langat virus on the brain of hamsters. The differences between the pathogenesis of the disease in mice (Webb *et al.*, 1968) and in hamsters could not be explained simply by the lesser susceptibility of hamsters to Langat virus, and an immunological involvement is very likely.

The progressive and sclerotic character of the disease caused by Langat virus in hamsters is very different from the delayed type of encephalitis observed in mice passively immunized against Semliki Forest virus (Seamer, Boulter and Zlotnik, 1971) and resembles in some respects S.S.P.E. in man (Zeman and Kolar, 1968; Zu Rhein and Chou, 1968; Meulen, Katz and Müller, 1972).

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