STUDIES ON PSEUDORABIES (INFECTIOUS BULBAR PARALYSIS, MAD ITCH)

II. Routes of Infection in the Rabbit, with Remarks on the Relation of the Virus to Other Viruses Affecting the Nervous System

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(Received for publication, March 6, 1934)

In a previous communication (Hurst, 1933) the histology of pseudorabies in the rabbit has been considered. While clearly possessed of neurotropic affinities, in this animal the virus does not behave as a strict neurotrope, but produces intranuclear inclusions in cells derived from any embryonic layer. It is interesting now to compare, from the experimental standpoint, the manner of spread of this virus with that of the more purely neurotropic viruses (poliomyelitis, rabies, Borna).

Technique

The incubation periods in four rabbits inoculated subcutaneously with 1 cc. of the supernatant fluid from a 10 per cent unglycerinated brain suspension lasted 51, 52, 54 and 57 hours respectively. With amounts of suspension near the minimal infecting dose, or with very old glycerinated material, the incubation period may be 8 days or longer. In all the passage experiments listed in the following tables fresh material was employed, and a long incubation period was taken as indicating the presence of a minimal amount of virus; except where otherwise stated 1 cc. of a 10 per cent suspension constituted the test dose.

The minimal infecting dose of the Iowa strain of virus was determined for rabbits by Shope (1933) to be not greater than 0.01 mg. wet brain for intracerebral inoculation, and 0.1 mg. for subcutaneous inoculation. Recent tests indicate that the latter dose lies between 0.1 and 0.01 mg., and is often nearer the second figure. Where exact amounts of virus are mentioned in the ensuing experiments the inoculum was titrated for potency at the time of use and the quantities are stated in terms of the minimal dose for subcutaneous inoculation. Dr. M. H. Merrill has found the minimal subcutaneous infecting dose of the Aujeszky virus employed to lie between 0.05 and 0.025 mg. wet brain.

EXPERIMENTAL RESULTS

Evidence of Neural Transmission of the Virus

Iowa Strain.—In infections following subcutaneous, intradermal or intramuscular inoculation (Table I), virus may sometimes be detected in the defibrinated blood provided that sufficient quantity be tested.

Injecting several small samples of blood, Shope (1931) was unable to detect the presence of virus; it is now obvious that even in relatively enormous quantities virus is inconstantly found. Its absence from

TABLE I

Distribution of Iowa Virus Following Intramuscular, Subcutaneous or Intradermal Inoculation into Rabbits

No.	Inoculated	First symptom	Killed or died	Presence of virus in							
				Cerebral cortex	Cervical cord	Dorsal cord	Lumbar cord	Sciatic nerve	Lung	Spleen	Defibrinated blood (6–10 cc.)
		hrs.	*hrs.*								
1	Intramuscular, leg	50	K 50	0	—	—	+72	+144	+90	+120	+124
2	Subcutaneous, flank	52	D 72	+120	—	+52	—	—	—	—	+124
3	Subcutaneous, leg	68	K 74	0	+116	—	+52	+54	+140	0	0
4	Intradermal, leg	?	D 96	0	0	+122	+55	+54	0	—	—
5	Subcutaneous, flank	190	K 190	0	+73	+50	+120	—	—	—	—

+ = development of mad itch with incubation period in hours. — = not tested. 0 = no take.

the blood constitutes the chief point of distinction between it and the classical Aujeszky virus, which is easily detected in minute amounts of blood or serum (Table VI).

In view of its scanty virus content, in the case of positive inoculations with various organs the contained blood cannot be held responsible; small amounts of virus are inconstantly detected in the lungs and spleen, sometimes in its apparent absence from the blood (R 3). Shope found virus in the liver in one of three cases.

By sacrificing the animals at the appropriate time, close relation can be demonstrated between the position of the inoculated area

and the virus content of various parts of the central nervous system. After injection into the flank, abundant virus may exist at the corresponding level of the spinal cord, less in the cervical and lumbar cords (R 5) and little or none in the cerebral cortex (R 2 and 5). With inoculation in the leg, virus is present in greatest quantity in the sciatic nerve and lumbar cord (R 1, 3, 4), and in diminishing amounts at higher levels. As already recorded (1933), death occurs with a comparatively low concentration of virus in the medullary centers. While in the cord the differential distribution of virus is, of course, most evident when itching first begins, *i.e.* soon after the virus has reached the spinal ganglia and segments corresponding to the local

TABLE II

Distribution of Iowa Virus Following Intracerebral Inoculation into Rabbits

No.	Killed or died	Symptoms	Presence of virus in							
			Cerebral cortex	Gasserian ganglion	Cervical cord	Lumbar cord	Sciatic nerve	Lung	Spleen	Defibrinated blood (6–10 cc.)
	hrs.									
8	K 34	None	+50	+60	+60	+70	0	0	+90	+70
9	D 46	Typical—1 hr.	+52	+66	+53	+73	0	+120	+120	0
10	D 92	Typical—4 hrs.	+52	—	+72	+144	0	+88	0	0

lesion, the cerebral cortex may still be uninfected at death (R 4). These findings forcibly suggest an ascending infection by the nervous pathway.

After intracerebral inoculation (Table II) virus is again present inconstantly and in small amount in the blood, spleen and lungs. Diminishing quantities down the nervous axis and absence from the sciatic nerve reverse the distribution indicated in Table I. It is now evident that the sterility of the cerebral cortex in the preceding experiments does not betoken insusceptibility of this part of the nervous system to the action of the virus.

Further evidence in favor of spread by the nervous route is afforded in Tables III and IV. The salivary glands are often infective after intracerebral inoculation or after subcutaneous inoculation into the

base of the ear, and rarely so if injection is practised subcutaneously in the flank or foot. The adrenals are frequently infective after subcutaneous inoculation into the flank, but not after injection into

TABLE III

Infectivity of Adrenal and Salivary Glands at Death Following Inoculation of Iowa Virus at Various Sites in Rabbits

Route of inoculation	No. of animals	Presence of virus in	
		Adrenal	Salivary
Subcutaneous, base of ear	5	0, 0, 0, +83, 0	+70, +86, 0, +68, 0
Subcutaneous, flank	8	+66, +180, —, —, +68, +82, +65, 0	0, 0, 0, 0, +98, 0, —, —
Subcutaneous, dorsum of foot	4	0, 0, 0, 0	0, 0, 0, 0
Intracerebral	4	0, 0, +106, 0	+144, +85, +122, 0

TABLE IV

Duration of Incubation Period Following Subcutaneous Inoculation at Various Sites of Iowa Virus into Rabbits

Dose of virus in M.I.D.	Inoculation into		
	Base of ear	Dorsum of foot	Dorsum of foot after removal of sciatic nerve
5,000	+58, +62, +68	+68, +71, +72	+75, +86, +89
25	+85	+92, +115	+122, +144, +161
			Removal of sciatic and femoral nerves
100	+62	+70	+102, +126
250	+55	+65	+80, +86

Four different samples of virus were used in these experiments, but the data in each horizontal row were obtained with one sample and on one occasion.

the leg or ear. If we admit that spread occurs by whatever nervous paths are available, these observations are readily explainable. In a disease of superlatively rapid progression there might be time for virus

infecting primarily, say, the dorsal cord, to migrate to the adrenals, whereas arriving soon before death in the brain stem it would not have time to pass out to the salivary glands. Conversely, virus reaching the brain stem from the ear would infect the salivary glands but not the adrenals. In the case of primary lumbar infection neither would be affected. Again, as shown in the left hand columns of Table IV, the

TABLE V

Distribution of Iowa Virus during Incubation Period Following Intradermal, Subcutaneous or Intramuscular Inoculation into Rabbits

No.	Killed	Route of inoculation	Amount of 5 per cent suspension	Presence of virus in		
				Site of inoculation	Blood (10–13 cc.)	Spinal cord and ganglia
	min.		*cc.*			
11	30	Subcutaneous	5	—	0*	—
12	90	Subcutaneous	5	—	0*	—
	hrs.					
13	6	Intramuscular	2	—	0†	—
14–15	12	Intradermal	1	+84, +96	0*	0
16–17	12	Intradermal	3	—	0*	0
18	16	Intramuscular	1	+53	0†	0
19–20	18	Intradermal	1	+60, +120	0*	0
21–22	24	Intradermal	1	+50, +66	0*	0
23	28	Intramuscular	1	+144	0†	0
24	36	Intradermal	1	+60	0*	0
25	40	Intramuscular	1	+64	0†	0
26	48	Intradermal	1	+62	0*	+82
27	52	Intramuscular	1	+54	+210†	+72

* Inoculation of defibrinated blood.

† Direct inoculation of whole blood.

Controls developed symptoms at 49 and 53 hours (intradermal) and 55 hours (intramuscular) respectively.

duration of the incubation period following subcutaneous inoculation increases materially with greater length of the nervous pathway. In another experiment a single animal was inoculated at the same moment in the ear, the flank and the foot. An interval of several hours separated the onset of itching in the ear and flank, affected in this order, while the rabbit died before itching began in the foot; here the intervention of individual variations in susceptibility was fully excluded.

The data presented in Table V also apparently rule out the possibility of blood transmission, though later experiments will show that these results cannot be accepted at their face value. Even with considerable volumes of inoculum (over 12,000 M.I.D.) no transient leakage into the blood stream is detectable during the first hour or two, and apparent sterility as concerns the virus is maintained to almost the end of the incubation period. To be more precise, the experiments indicate the presence at one time in the whole circulating fluid of less than about 10 M.I.D. virus. The spinal cord and ganglia may show virus before the blood, while at the site of inoculation active increase is occurring.

TABLE VI

Distribution of Aujeszky Virus Following Subcutaneous Inoculation into Rabbits

No.	Inoculated	First symptom	Killed or died	Presence of virus in										
				Cerebral cortex	Cervical cord	Dorsal cord	Lumbar cord	Sciatic nerve	Lung	Spleen	Adrenal	Salivary	Defibrinated blood	
													0.25 cc.	5.0 cc.
		hrs.	*hrs.*											
70	Flank	—	K 30	0	0	+72	—	—	+84	+110	—	—	0	+149
71	Flank	—	K 33	0	0	+96	—	—	+120	+140	—	—	0	+180
72	Flank	—	K 42	0	0	+86	—	—	+80	+86	—	—	+86	+72
6	Flank	47	K 48	+78	—	+66	—	—	+47	+47	+49	+66	+47	+49
7	Leg	46	D 53	+65	—	—	+60	+48	+45	+48	+50	+48	+74	+74

Finally, the histological findings already reported (1933) point also to nervous transmission of the virus. It seems, therefore, that indubitable evidence of spread of the Iowa virus by the nervous route is forthcoming.

Aujeszky Strain.—Similar study of the course of infection with the Aujeszky virus (Table VI) is complicated by the infectivity, towards the end, of even minute amounts of blood, rendering difficult the correct evaluation of positive inoculations with the various viscera. If, however, examination is made sufficiently early, the blood is infective only in large doses, and the recovery of virus from lung and spleen can be accepted as significant. Experiment showed

that using 5 cc. as the test inoculum the blood is uninfective at 1/2, 6 and 12 hours after subcutaneous inoculation; from about 24 hours onwards uniformly positive results are obtained. The detection of virus, at a rather later stage, in only that part of the nervous system directly connected with the site of inoculation (R 70–72) suggests that, as is the case with the Iowa strain, nervous infection is brought about by the neural route, and not, as previous observers (Schmiedhoffer, 1910; Remlinger and Bailly, 1933) have held, by the circulation. This conclusion is supported by the histological findings. It will be noted that the virus appears in the cord at a much earlier period than does the Iowa strain.

Effect of Nerve Section on the Course of Infection Following Subcutaneous Inoculation

If in the case of the Iowa virus introduced subcutaneously spread is possible only by the nervous route, ablation of the nerve supply beforehand should prevent infection. Bertarelli and Melli (1913) claimed that in some cases tying or removal of a part of the nerve protected against inoculation of the peripheral segment.

In the present investigation six rabbits underwent removal of over an inch of the sciatic nerve of one side, and four of the sciatic and femoral nerves of one thigh. Ether was the anesthetic employed. Immediately after closure of the wound (sealed with collodion), or after an interval of 3 weeks from the date of excision, virus was inoculated subcutaneously in the dorsum of the denervated foot. In all cases a lengthened incubation period was noted, but finally every animal died (Table IV). One scratched the site of operation in the thigh, four scratched areas unrelated to the excised nerve (opposite flank or opposite hind limb) and five died suddenly without having scratched. Three of the last group were examined for the presence of virus in the cord and medulla, with positive results; virus was also present in the lungs, spleen and blood.

The conclusion seems unavoidable that in the absence of nervous connections the Iowa virus may reach the nervous system through the medium of the blood. The following experiments reveal, however, the interesting fact that this spread is not direct.

Results of Intravenous Inoculation of Virus

Infection regularly ensues after intravenous introduction of even small doses of virus; in contrast to the figures for most neurotropic viruses (poliomyelitis, rabies, etc.), in the present instance the minimal infecting dose is only 3–10 times that for subcutaneous inoculation. The incubation period is a few hours longer than after subcutaneous or intramuscular injection of the same quantity into the flank. The symptoms vary. Rather less than half the animals die suddenly, or exhibit only some restlessness with rapid shallow breathing before exitus. The remainder itch; in each of thirteen cases this itching occurred on some part of the trunk or limbs (thrice on a fore limb, twice on a hind limb, eight times on one or both flanks and never on the head).

In addition to the lesions previously described as occurring after peripheral or intracerebral inoculation, the adrenals of rabbits receiving large doses of virus show, with fair regularity, considerable areas of acute necrosis in both cortex and medulla; these closely resemble the similar areas in herpes (Smith, 1931). Hemorrhage and infiltration with polymorphonuclear leucocytes and a few eosinophils accompany the destruction. Acidophilic nuclear inclusions, more coarsely granular than those in the nervous tissues and very like those of herpes, exist in cells surrounding the necrotic areas, while the sympathetic ganglia on the surface of the glands show typical alterations in nerve cells, capsule cells and neurilemmal nuclei.

When itching occurs the corresponding spinal ganglia are typically affected, but, as with subcutaneous inoculation, at death no definite lesions are discernible in the cerebral cortex or brain stem.

The progress of infection as revealed by experimental study is as follows:

The distribution of the Iowa strain after the administration of massive doses is set forth in Table VII. At the end of 1/2 hour a small quantity of virus is still present in the blood, but after 2 hours all, or almost all, has disappeared. Using the usual test dose of 1 cc. of a 10 per cent emulsion, no virus is now detectable in any of the viscera. In the belief that this result could not represent the true state of affairs, another rabbit was given 5000 M.I.D. (*i.e.* roughly 3 M.I.D. per gm. body tissues) and larger amounts of the various organs emulsified; where possible, doses of 1, 5 and 20 cc. of the supernatant fluid

TABLE VII

Distribution of Iowa Virus Following Intravenous Inoculation of Massive Doses into Rabbits

No.	Dose in M.I.D.	First symptom	Killed or died	Presence of virus in											
				Cerebral cortex	Medulla	Cervical cord	Dorsal cord	Lumbar cord	Lung	Spleen	Liver	Kidney	Adrenal	Testis or ovary	Defibrinated blood(10–12 cc.)
		hrs.	*hrs.*												
53	5,000	—	1/2	—	—	—	—	—	—	—	—	—	—	—	+133
54	5,000	—	2	—	—	—	—	—	0	0	0	0	0	0 (O)	0
55	10,000	—	2	—	—	—	—	—	0	0	0	—	—	—	+232
56	2,500	—	16	—	—	—	—	—	0	0	0	—	—	—	0
57	2,500	—	24	0	0	0	0	0	+110	0	0	+140	+70	0 (T)	0
58	2,500	—	24	0	0	—	—	0	+79	0	—	—	—	—	+165
59	2,500	—	40	0	0	0	+130	0	+116	0	0	+72	+72	+64 (T)	+117
60	2,500	—	48	0	0	—	—	+113	+60	+101	—	—	—	—	+85
61	2,500	—	48	0	0	+122	+92	+76	+79	+135	0	—	—	—	+90
62	2,500	58*	58	+91	+69	—	—	+69	+72	+91	+120	+72	+80	—	+72
29	1,000	64†	72	0	+182	—	—	+75	+88	+130	0	+126	+59	+100 (T)	+108
31	1,000	?*	90	0	+72	—	+130	+126	+135	0	—	—	+91	—	—

* Did not itch.

† Bit and scratched.

from a 10 per cent suspension were injected into fresh animals. Positive results were now obtained with the lung, spleen, kidneys and ovaries, and negative with the blood, urine, liver, adrenals, brain and cord, and mixed subcutaneous and muscular tissues. By comparing the weights of the organs and skeletal tissues with the results obtained the following quantitative distribution of virus was determined. Lung $> 22 < 110$ M.I.D., spleen $> 2\frac{1}{2} < 12$ M.I.D., kidneys $> 25 < 125$ M.I.D., ovaries $> 2 < 6$ M.I.D., blood < 6 M.I.D., liver < 40 M.I.D., adrenals < 2 M.I.D., brain and cord < 6 M.I.D., skeletal tissues < 600 M.I.D. The length of the incubation periods in the passage animals indicated that the actual figures were probably much less than the maxima suggested. When, to eliminate the possibility of virus being already firmly bound to the cellular elements of the viscera, two experiments were performed, similar save for the fact that whole emulsions were substituted for the supernatant fluids, definitely lower figures were obtained. This is probably an example of the inhibitory effect exercised by thick tissue suspension on the action of a virus, a phenomenon already known in the case of poliomyelitis and other viruses. At the most generous estimate, therefore, four-fifths of the virus introduced could not be recovered within 2 hours of injection. Even if the higher limits indicated for the virus content of the lung, spleen and kidney were the correct ones, they represented in these organs only an approximately threefold concentration over what should obtain with uniform distribution; in the case of the ovaries the concentration was possibly sixfold.

This loss of virus may possibly be due to inactivation or destruction by the elements of the blood, for titration of a mixture of proportional quantities of virus and freshly drawn heparinized or defibrinated blood, incubated for 2 hours at 37°C., showed roughly a 90 per cent decrease in activity. This observation makes it very unlikely that pseudorabies virus multiplies in the blood.

With the usual test doses, from 24 hours onwards virus is found constantly in the lungs, kidney and adrenals. The spleen is usually infective towards the end of the incubation period and during the symptomatic stage. At a similar stage virus is present in the testis, but only inconstantly in the liver. Virus returns to the blood within 24 hours, and thereafter persists in the circulation; this is in contrast

to the sterility obtaining for a much longer time after subcutaneous inoculation.

Virus is usually absent from the nervous system until late in the incubation period. In three rabbits killed in the presymptomatic stage (R 59, 60, 61) the cord was infective before the medulla or cerebral cortex. In two during the period of the developed disease (R 62, 31) the infectivity of the medulla was equal to or greater than that of the cord; these animals did not itch. In one case (R 29) the reverse obtained; this animal itched. In two fatal cases (R 29, 31) the cerebral cortex was still uninfected at death.

This irregular distribution of virus throughout the nervous system does not suggest infection directly from the blood. It has already been seen that at the 2nd hour no virus has, apparently, settled in the nervous tissues. Moreover, if infection of the nervous system occurred at the time of introduction of the virus, in addition to the lesions constantly found after intracerebral inoculation we should expect a shorter incubation period, since minute amounts of virus must be inoculated intracerebrally to give an incubation period of over 50 hours. If longer incubation were attributable to delay in passage of virus through, say, the capillary walls, lesions in the endothelial cells might be expected; the absence of any lesions in the brain or brain stem has already received comment.

On the other hand, if from the foci of infection obviously established in various viscera during the first few minutes after inoculation virus were to spread by the nervous route, the findings are readily explainable. In the majority of cases, owing to proximity to the viscera, the spinal cord and ganglia would be first infected, lesions produced here and referred itching evoked. Thus we might explain the observations in the case of R 29. In other cases spread to the medulla might occur (perhaps from the lungs) by the vagus; if this were sufficiently rapid the animal might die of medullary paralysis before the cord and ganglia were sufficiently affected to give rise to peripheral irritation (*e.g.*, as in R 62 and 31). That the lesions observed in the spinal ganglia of itching animals result from the ascent of virus deposited in the subcutaneous or muscular tissues is unlikely for several reasons. In the first place no considerable quantity can be detected here at the 2nd hour; amounts so minute as to escape detection would be incapable

of producing the disease with the length of incubation noted in the experiments. Secondly it would be anticipated that a certain number of animals would itch on the head or neck, whereas in each of thirteen instances itching was confined to the trunk or limbs. Thirdly, in two of five cases the area bitten contained no detectable amount of virus; in the other three the incubation periods in the passage animals were 73, 93 and 96 hours respectively. These figures contrast with those

TABLE VIII

Distribution of Iowa Virus Following Intravenous Inoculation of Small Doses into Rabbits

No.	Dose in M.L.D.	First symptoms	Killed	Presence of virus in											
				Cerebral cortex	Medulla	Cervical cord	Dorsal cord	Lumbar cord	Lung	Spleen	Liver	Kidney	Adrenal	Testis or ovary	Defibrinated blood (10–12 cc.)
63	60	—	24	—	—	0	0	0	0	0	0	0	0	0 (T)	0
64	60	—	36	—	—	0	0	0	0	0	0	0	0	0 (T)	0
65	60	—	48	—	—	0	0	0	+124	0	0	0	0	0 (T)	0
66	60	—	60	—	—	0	0	0	0	0	0	0	0	0 (T)	0
67	60	—	64	0	0	0	0	0	+108	0	0	+69	+133	0 (O)	0
68	20	—	66	—	—	+87	0	0	0	0	0	+65	+87	0 (T)	+120
69	60	70*	71	0	—	0	—	+120	+122	0	0	0	0	0 (T)	0

Controls for Nos. 63–66 and 69: Subcutaneous +68; intravenous +71.
Control for No. 67: Intravenous +64.
Controls for No. 68: Subcutaneous +64; intravenous +74.
* Bit and scratched.

for the local lesion at a corresponding period after subcutaneous or intramuscular inoculation (Table V); they would probably be in keeping with the presence of a small amount of virus passed centrifugally from a central nervous reservoir. Finally, three rabbits were inoculated, one directly into the kidney, one into the liver and one into the lung; before death each scratched the flank or shoulder. (The organ inoculated showed in each case acute necrotic lesions with abundant nuclear inclusions in the cells characteristic of the particular organ; *i.e.*, in cells derived from mesoderm and entoderm.) It would

seem, therefore, that itching after intravenous inoculation is a referred sensation from a central lesion determined by virus originating from some source other than the area scratched.

If a much smaller dose be given intravenously (Table VIII) its distribution during the incubation period, though showing similarities to that in the preceding table, is more erratic. It is to be noted that the condition of the blood more nearly resembles that after subcutaneous inoculation in rarely containing detectable virus.

The distribution of the Aujeszky strain after intravenous inoculation was not fully worked out; it was determined, however, that 2 hours after administration of 5000 M.I.D. the blood in doses of 1, 5 and 20 cc. did not reproduce the disease in passage animals (*i.e.*, contained < 6 M.I.D. virus).

An important fact emerging from these experiments by the intravenous route is that if after subcutaneous inoculation virus were to enter the circulation intermittently, even in considerable amount, its rapid disappearance therefrom would lead to the restitution of sterility. Thus the results charted in Table V are not in themselves sufficient to eliminate the possibility of blood spread; on the other hand the presence of virus in the lung and spleen in its absence from the blood (Tables I and II), and the possibility of infection resulting from inoculation in a denervated area are readily explained. Again, it seems that when virus is detectable in the blood at the end of the incubation period or earlier, according to experimental conditions and virus used, its presence here must be due to one or both of two factors, its continuous emission in considerable amount from the primary or other focus, or failure of the mechanism for its removal.

Appearance of Virus in the Blood after Subcutaneous Inoculation

The possibility that the appearance of virus in the blood is due to failure of some absorptive mechanism is excluded by the following experiment. Two animals were inoculated subcutaneously with Aujeszky virus. At the time when virus was expected to appear in the blood stream (22 and 26 hours), a test bleeding was performed and various amounts of blood inoculated into fresh animals. The original pair of animals was then inoculated intravenously with 5000 M.I.D. virus; 2 hours later they were killed and the blood was titrated

once more. In neither animal was its virus content appreciably higher than before.

Acting on the assumption that the reticulo-endothelial system might be concerned in the removal of virus from the circulation, an attempt was made to demonstrate the Iowa strain in the blood stream at various stages of the incubation period in animals previously treated with India ink.

Higgin's non-waterproof American Drawing Ink was dialyzed against sterile Locke's solution changed each alternate day for 10 days. Doses of 5 cc. ink diluted to 7.5 cc. with sterile Locke's solution were injected intravenously into rabbits on each of 2 successive days. The animals were then subjected to subcutaneous inoculation with the Iowa virus, and the ink injections continued daily. At various periods the rabbits were killed and the heart blood titrated in fresh animals.

About a year had elapsed since the experiments listed in Table V were performed, in which time the virus had been passed on a number of occasions. Control rabbits not receiving India ink now frequently showed small quantities of virus in the blood during the incubation period, and the differences in this respect between them and the animals given ink were not sufficiently marked to be considered significant. These observations probably indicate that with passage the Iowa virus is approximating to the Aujeszky strain in its capacity for invading the blood stream.

Existing records do not, unfortunately, furnish precise information regarding the ease with which newly isolated strains of the Aujeszky virus invaded the blood stream. The following statements seem to indicate that different strains, or the same strain at different times, may vary in this respect. Aujeszky (1902) stated that the virus was almost always in the blood, thus implying a certain number of negative tests; in our experience the Aujeszky virus now used is always present in, and may be detected in minute amounts of the blood of infected rabbits. Isabolinsky and Patzewitsch (1912) found the blood infective only in large doses and after a long incubation period. Sangiorgi (1914) could detect virus in the blood only just before death. It is possible that with repeated passage the Iowa virus may appear in the blood stream in larger amount than heretofore, in which case the only distinguishing feature between it and the Aujeszky strain in common use will disappear and complete uniformity of behavior be established between the two viruses.

DISCUSSION

The more strictly neurotropic viruses (poliomyelitis, rabies, Borna) reach the central nervous system from the periphery by the neural route, and there induce a primary degeneration of nerve cells accompanied or followed, as the case may be, by reactive phenomena in the vascular and interstitial tissues. The evidence presented in this and in a previous paper shows that the same is true of the pseudorabies virus, which is clearly neurotropic. In the rabbit death ensues before the primary attack on the neurons is masked by reactive change. But whereas viruses of the former group are rarely or never detected in the blood stream, if at all only during the period when wholesale destruction and phagocytosis of nerve cells may account for some leakage into the circulation, the capacity of the Aujeszky virus for abundant increase in non-nervous tissue enables it to establish a primary extraneural focus, from which, apparently, considerable quantities of virus are emitted into the blood from an early stage of the incubation period. The differences observed in this respect between the Aujeszky and the Iowa strain indicate that the circulation of virus in the prenervous phase of the disease is, unfortunately, an occurrence insufficiently constant to serve as a means of distinguishing this type of virus from the stricter neurotropes. Moderate amounts of virus can be dealt with by a mechanism of removal leaving the blood free from detectable contamination. Under these circumstances it is even uncertain what the real position regarding the stricter neurotropes may be, except that as far as is known they do not multiply to any extent outside the nervous system.

Of greater value in differentiating between the two types of virus is the observation that while the stricter neurotropes just mentioned, when introduced into the blood stream in any amount less than an overwhelming one, do not cause infection, the minimal intravenous infecting dose of pseudorabies virus is but little greater than that for subcutaneous or intramuscular inoculation. It is important to recognize that this property of the pseudorabies virus is not due to ability to penetrate directly the hemato-encephalic barrier; rather its pluricellular affinities, abundantly evident on histological study, enable it to establish multiple infective foci from which follows extension by the

neural route. Except when they are of the nature of a general response to infection, the lesions of rabies and Borna disease in organs other than the nervous system occur primarily in connection with the nervous apparatus of the organ, which they reach by centrifugal spread along the nerve trunks. In the case of pseudorabies the results of direct inoculation into a given organ (liver, lung or kidney), together with numerous other observations, clearly demonstrate the capacity of the virus for evoking primary and specific lesions in cells derived from entoderm and mesoderm as well as in those of ectodermal origin.

In many ways remarkable similarity exists between the pseudorabies and the herpes virus. Goodpasture and Teague (1923 *a*, 1925 *a*), Smith (1931) and others have called attention to the pluricellular affinities of the latter. The herpes virus does not appear in the blood (Goodpasture, 1925 *b*), or its presence there is ephemeral during the acute stages of the malady (Levaditi, 1926). Hence no doubt the negative results of intramuscular inoculation following nerve section (Goodpasture and Teague, 1923 *b*; Levaditi, 1926). From our experience with pseudorabies we can, however, envisage the possibility of herpes virus introduced into a denervated area growing locally and entering the blood stream undetected, but, provided that its affinity for the general viscera were less than is the case with the pseudorabies virus, of its frequent failure in low concentration to establish infective visceral foci from which spread to the nervous system might follow. Occasional success in this direction might account for the results of Marinesco and Draganesco (1932) which, they assume, discount Goodpasture's evidence for axonal spread of the virus (1925 *b*). While a few workers (le Fèvre de Arric and Millet, 1925; Levaditi, 1926) have found intravenous inoculation unattended by cerebral trauma rarely effective in inducing encephalitis, many observers (Doerr and Vöchting, 1920; Luger and Lauda, 1921; Remlinger and Bailly, 1925; Goodpasture and Teague, 1923 *b*) seem to have encountered little difficulty in producing infection by this route. The lesions in the adrenals following intravenous injection of herpes virus (Smith, 1931) are similar to those described in the present paper. In all the cases quoted the dosage has been fairly large, but far from comparable with that requisite in the case of poliomyelitis or rabies. Although Good-

pasture and Teague felt that their success with intravenous inoculation did not warrant the categorical assertion that the axis cylinders are the only portal of entry of the virus to the central nervous system, it seems highly probable that an *ad hoc* inquiry would reveal after intravenous inoculation of herpetic material a state of affairs similar to that obtaining in pseudorabies; *viz.*, an ascending neural infection from primary foci set up in various viscera.

At first sight the behavior of certain other neurotropic viruses appears different from that of those thus far dealt with. In horses dying of equine encephalomyelitis[1] animal inoculation demonstrates the existence of virus only in the central nervous system, in which the lesions are typical of an acute neurotropic virus disease. Before nervous symptoms are manifest, however, virus may be present in the blood in considerable quantities. Much the same is true of the guinea pig (Howitt, 1932), in which virus may early be recovered from the blood and viscera, but later only from the nervous system and perhaps the salivary glands and adrenals. That the organism is evidently capable of multiplication in situations outside the nervous tissues follows also from the work of Syverton, Cox and Olitsky (1933).

Almost identical findings are recorded in the case of the neurotropic modification of the yellow fever virus. At death in the mouse (Theiler, 1930; Dinger, 1931), guinea pig (Lloyd, Penna and Mahaffy, 1933; Theiler, 1933) and monkey (Lloyd and Penna, 1933) virus appears to be localized in the central and peripheral nervous system, adrenals and perhaps salivary glands, leaving the blood and other viscera free. The circulation of virus in the blood, immediately following massive intraperitoneal inoculation, may no doubt legitimately be compared with the absorption of a non-specific agent such as ovalbumin (Hughes and Theiler, 1934). But Sawyer and Lloyd (1931) found the blood infective for 4 and possibly 5 days, while after administering minute doses Hughes and Theiler noted a latent period preceding the appearance of virus, which then circulated for from 1–3 days; in the intervening period multiplication must have taken place.

Webster and Fite state[2] that louping ill virus introduced intra-

[1] Work shortly to be published.

[2] Webster, L. T., and Fite, G. L., personal communication.

nasally, and presumably entering the body in comparatively small amounts, may be detected in appreciable quantities in the blood of mice during the prenervous phase, though when nervous symptoms develop it is absent therefrom. Pool, Brownlee and Wilson (1930), Gordon *et al.* (1932) and MacLeod and Gordon (1932) have already shown that in the natural host, the sheep, the blood is infective during the febrile stages of the disease, that such infectivity is not necessarily followed by the development of nervous symptoms, that the contagium is transmissible by a blood-sucking insect (*Ixodes ricinus* L.), and that intravenous inoculation of virus may be effective in producing the typical disease.

If this third group of neurotropic viruses be regarded as having affinities for tissues other than the nervous system, and at the same time a slower rate of progression along the nerves than the pseudorabies virus, it is possible to imagine that the systemic and nervous phases of the diseases they cause may become temporally dissociated. The visceral reaction occurs and the virus is overcome. But virus which has meanwhile travelled centripetally along the nerves now finds itself in a susceptible tissue isolated by the hemato-encephalic barrier from humoral influences. Encephalitis follows. If this interpretation of the facts is correct, the viruses of this group are distinguishable from that of pseudorabies chiefly by the accident of their slower migration along the nerves.

In the matter of nomenclature we may perhaps refer to these viruses with pluricellular affinities as pantropic. The chief objection to designating them viscerotropic and neurotropic viruses lies in the possible implication that the factors making for general tissue affinity on the one hand and neurotropism on the other are separable and independent, being analogous in this respect to the flagellar and somatic antigens of bacteria. Though of course this may prove to be the case, none of the facts at present ascertained necessitates the assumption. The acquisition of more pronounced neurotropism on brain-to-brain transfer may well indicate a basic alteration in the virus as an entity, impressed on it by the medium of culture; the dissociation of visceral and nervous manifestations of virus action to a slow progression along the nerves coupled with the peculiar physiological semi-isolation of the nervous system.

Levaditi's conception of a group of viruses linked by a selective affinity for skin and nervous tissue is considered by many workers to be based on incomplete observation and undue elasticity in interpretation of fact. Thus poliomyelitis and rabies viruses have no particular affinity for the dermis; herpes virus attacks cells derived from all three embryonic layers; the lesions of cerebral vaccinia are largely meningeal or ascribable to meningeal lesions. If a classification of viruses in terms of their cellular affinities were desired, a primary grouping according as to whether they are neurotropic, viscerotropic or pantropic might supply the framework The supposed characteristics of the first and third classes have been outlined above. Infections with the second type of virus would usually leave the nervous system unscathed, yet under suitable conditions might produce an encephalopathy from interference with the vascular system of the brain. Perhaps in hog cholera encephalomyelitis this factor is far from negligible. It is to be noted that a more or less similar classification is implicit in Seifried's description of the various encephalomyelitides (1931).

SUMMARY

After intramuscular, intradermal and subcutaneous inoculation, the pseudorabies virus reaches the central nervous system by way of the peripheral nerves, although it is circulating in the blood. Centrifugal spread from the infected nervous tissues by the neural route also occurs. After intracerebral inoculation the virus passes in the reverse direction, down the nervous axis. The Aujeszky strain invades the blood stream more readily than does the Iowa strain; but possibly with repeated passage the latter is approximating in this respect more closely the classical Aujeszky strain. After intravenous inoculation, effective with even small doses, virus is rapidly removed from the blood, and multiple infective foci are established in various organs; thence ascent of the virus by the peripheral nerves leads to infection of the central nervous system, the symptomatology differing according to whether the spinal cord or the medulla is first reached. The lack of evidence that the virus can penetrate directly the hemato-encephalic barrier deserves emphasis. When subcutaneous inoculation is practised in an area deprived of its nerve supply, the ability of the virus to invade

the blood stream permits it to establish infective foci in the various viscera, and, after a predictable delay, the coursé of infection resembles that following intravenous injection. The pseudorabies virus is pantropic; *i.e.*, it readily attacks cells derived from any embryonic layer. Lesions in the adrenal gland following intravenous inoculation are very like those due to herpes virus similarly introduced, this being one point of similarity in the pathogenic action of the two organisms. The relation of the pseudorabies virus to other viruses affecting the central nervous system is discussed.

I am greatly indebted to Miss Ruth Kemmerer for much valuable technical assistance during this investigation.

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