treatment did not affect the development of the embryos or their hatching from eggs of normal hens. In addition, the acetone alone did not affect the poor hatchability of the eggs under study (Table 2). The acetone solutions were aseptically placed near the yolk with a Hamilton syringe and the eggs sealed with collodion or Duco cement. All eggs were placed in the same incubator and the results are shown in Table 2. Injection of vitamin D_3 itself markedly improved the hatchability of the eggs. Although injection of either 25-OH-D_3 or 1,25-$(OH)_2D_3$ improved hatchability of the embryos, at the doses used, hatchability did not return to normal. Nevertheless there is no doubt that injection of all the vitamin D compounds markedly improved embryo development and hatching. These results demonstrate the importance of vitamin D and its metabolites in chick embryonic life and development. They also strongly suggest that 1,25-$(OH)_2D_3$ is not transferred in adequate amounts to the yolk from the maternal circulation.

Exactly why vitamin D deficiency causes a failure in mandible development is unknown, but improper calcium transport or defective collagen synthesis may be involved. In addition, chick embryos from eggs produced by hens maintained on 1,25-$(OH)_2D_3$ may provide an important experimental approach to some of the functions of vitamin D. It is also likely that hens maintained on 1,25-$(OH)_2D_3$ may be used to provide vitamin D deficient embryonic tissue for tissue culture experiments. Certainly these experiments demonstrate that 1,25-$(OH)_2D_3$ cannot satisfy all of the functions of vitamin D in the laying hen.

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Viremia in Experimental Creutzfeldt-Jakob Disease

Abstract. *Inoculation of the buffy coat of blood from guinea pigs infected with Creutzfeldt-Jakob disease resulted in passage of this disease to recipient animals. This demonstrates that there is a viremia in experimental Creutzfeldt-Jakob disease. These findings suggest that the hematogenous route may be implicated in the human infection and that the disease may possibly be transmitted by blood transfusions.*

The occurrence of viremia in encephalitides caused by conventional viruses is a well-recognized event, and its importance in the pathogenesis of these diseases has been emphasized (*1*). However, in spongiform virus encephalopathies, which include scrapie of the sheep, transmissible mink encephalopathy, and kuru and Creutzfeldt-Jakob disease of man (*2*), no viremia has been reported in the diseases affecting humans (*3*). Similarly, no stages of viremia have been detected in large-scale timed experiments dealing with the pathogenesis of scrapie. Thus, in experiments on the distribution of the scrapie virus in tissues and body fluids during the course of the infection in goats (*4, 5*) and in mice (*6*), no virus was found at any time in the circulating blood. However, in a few isolated instances and contrary to these pathogenesis studies, it has been claimed that blood or serum did contain the agent. Thus, serums and blood from mice taken up to 18 hours after inoculation (*7*), serum of a ram with natural scrapie (*8*), and serums of mice and rats (*9*) in the terminal stages of scrapie have been reported to be infective. In one study, the presence of scrapie agent in the blood in a small percentage of mice was thought most likely to result from tissue contamination (*10*).

We have transmitted Creutzfeldt-Jakob disease to guinea pigs and serially propagated it (*11, 12*). Using the guinea pig model, we undertook a series of timed experiments in order to study the pathogenesis of experimental Creutzfeldt-Jakob disease by virological and light and electron microscopic techniques. Our findings indicate the presence of viremia in this disease.

We inoculated 140 approximately 3-month-old guinea pigs (Hartley strain) with 0.1 ml intracerebrally of a 10^{-2} suspension of brain in normal saline from two guinea pigs that developed Creutzfeldt-Jakob disease during the fifth serial passage. Starting with the 1st week and at weekly intervals, up to the 28th week after inoculation, two inoculated guinea pigs (donors) were anesthetized with ether and the thorax was opened sterilely; then 8 ml of blood was removed, with a heparinized syringe, from the heart of each animal. After the blood was removed, the animals were killed and, in addition to the blood, various tissues including the central nervous system (CNS) were removed for virological and microscopic examinations. In our attempt to demonstrate the presence of viremia, we used the technique described by Horstmann (*13*). The blood removed from each animal was placed in a sterile polyallomer tube within a larger sterile polypropylene tube, centrifuged at 1000 rev/min for 15 to 20 minutes and frozen at −90°C overnight. The following day, each frozen tube was cut with a sterile blade approximately 0.5 cm beyond each side of the buffy coat. The section of the blood containing the entire

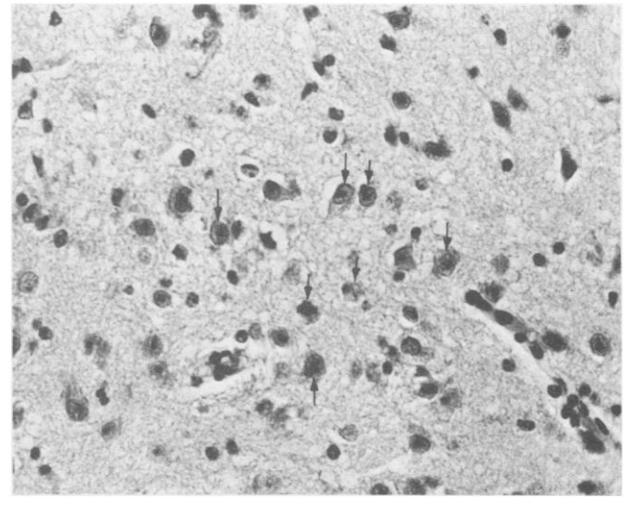

Fig. 1. Thalamus from animal inoculated with blood drawn at week 25. Several disintegrating neurons are seen (arrows) in a field showing spongiform changes of the surrounding neuropil. Hematoxylin-eosin (× 640).

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buffy coat and 0.5 ml each of plasma and red blood cells were placed in a sterile glass tube and thawed. The buffy coat was used for inoculations undiluted. Three normal guinea pigs (recipients) were inoculated with the blood of each donor animal every week; each guinea pig received 0.1 ml intracerebrally, subcutaneously, intramuscularly, and intraperitoneally.

Virus was present in the circulating blood drawn from donor guinea pigs at the 1st, 2nd, 3rd, 12th, 13th, 15th, 20th, 24th, 25th and 26th weeks after inoculation (Table 1). The brains of recipients inoculated with these bloods showed microscopic evidence of a spongiform virus encephalopathy, which is characteristic for experimental Creutzfeldt-Jakob disease. The fate of many recipient animals is still pending; thus it cannot yet be decided conclusively whether there is an eclipse of the virus from the blood of the donor guinea pigs—for example, between the 3rd and 12th weeks. By the 28th week all remaining donor animals showed clinically and microscopically advanced stages of experimental Creutzfeldt-Jakob disease.

In contrast to signs in the donor animals that were inoculated with infected brain, the clinical signs of the disease in the recipient guinea pigs inoculated with the buffy coat were very subtle, consisting of ruffled fur, sluggish or uncoordinated movements of the extremities and head, lack of interest in food, and generalized weakness. Ten of the positive recipient animals showed these clinical signs (*14*), and, with the exception of the CNS lesions, no pathological findings were present in the visceral organs. The long incubation periods in these animals suggest that the titers of virus in the blood were low. In the remaining four positive recipient animals, none of the above clinical signs could be detected; within a day or two these animals became prostrated and moribund without any detectable prodromal signs (*15*).

The recipient animals were defined as positive by the presence of a spongiform encephalopathy. The most conspicuous and prominent change was a widespread destruction of the nerve cells, which was present predominantly in the cerebral cortex, and to a lesser degree in the basal nuclei, thalamus, and hypothalamus. In some cortical regions only a few well-preserved neurons were present, many nerve cells had disappeared, and in others the only structure still remaining was the swollen, pale, lytic nucleus. Status spongiosus was seen in the neuropil (Fig. 1), and to a lesser degree vacuoles were found in the cytoplasm of the nerve cells in the cortex and in the subcortical gray structures. A moderate increase of astrocytes and mild increase of microglia cells were present along with the neuronal destruction. No inflammatory perivascular or diffuse infiltrates of any type were encountered. Control animals inoculated similarly with blood of normal healthy guinea pigs have not shown any spongiform virus encephalopathy.

The successful demonstration of viremia in our pathogenesis experiments is attributed to the technique used. In studying the temporal distribution of the virus in tissues and body fluids on animals inoculated with scrapie, no virus was found in the circulating blood, when whole blood (*4*), blood clot, or serum (*5, 6*) were studied for infectivity. On the basis of the results of Eklund *et al.* (*6*) which showed that scrapie replicates in "lymphocytic tissues" (spleen, lymph nodes, thymus) after subcutaneous inoculation of mice, we postulated that maximal infectivity should reside in the buffy coat (white blood cells) rather than with the serum or with red blood cells. Eklund *et al.* showed high virus titers in the lymphocytic tissues by the 8th week and before the virus was detected in the brain by the 16th week after subcutaneous inoculation (*6*).

In experimental Creutzfeldt-Jakob disease, the demonstration of viremia indicates that this agent may be disseminated by the hematogenous route. Thus, this route appears to be important for the pathogenesis of subacute spongiform virus encephalopathies as it is in encephalitides caused by conventional viruses. Our studies do not exclude the possi-

Table 1. Preliminary data on viremia experiments in Creutzfeldt-Jakob disease.

Blood taken after infection	Guinea pigs inoculated with blood	Subsequent condition	Spongiform encephalopathy
1st week	No. 1	Day 443, killed	+
	No. 2	Pending	
	No. 3	Pending	
2nd week	No. 1	Day 429, killed	+
	No. 2	Day 488, found dead	+
	No. 3	Pending	
3rd week	No. 1	Day 2, found dead	
	No. 2	Day 2, found dead	
	No. 3	Day 308, killed	+
4th to 11th weeks	Three animals each week	All alive (pending)	
12th week	No. 1	Day 438, found dead*	
	No. 2	Day 504, killed	+
	No. 3	Pending	
13th week	No. 1	Day 379, killed	+
	No. 2	Pending	
	No. 3	Pending	
14th week	Three animals each week	All alive (pending)	
15th week	No. 1	Day 52, found dead*	
	No. 2	Day 138, found dead*	
	No. 3	Day 463, killed	+
16th to 19th weeks	Three animals each week	All alive (pending)	
20th week	No. 1	Day 396, killed	+
	No. 2	Day 447 killed	+
	No. 3	Pending	
21st to 23rd weeks	Three animals each week	All alive (pending)	
24th week	No. 1	Day 1, found dead	
	No. 2	Day 307, killed	+
	No. 3	Pending	
25th week	No. 1	Day 147, killed	+
	No. 2	Day 184, killed	+
	No. 3	Day 343, killed	+
26th week	No. 1	Day 210, killed	+
	No. 2	Pending	
	No. 3	Pending	
27th and 28th weeks	Three animals each week	All alive (pending)	

*Autolysis.

bility of a pluripotential spread of the virus in subacute spongiform virus encephalopathies. However, the available results in scrapie—namely, spread of the infection after intracerebral inoculation to visceral tissues (*4, 5*) and the reverse, spread of the infection from the periphery to the brain (*5, 6*)—can most easily be explained by viremia rather than by propagation of the virus along neural pathways, either centrifugally and centripetally from and to the brain. The demonstration of viremia in experimental Creutzfeldt-Jakob disease may also have implications for the disease afflicting man.

Although it is not known how the infection spreads in human spongiform virus encephalopathies, the virus of Creutzfeldt-Jakob disease has been found in the liver, kidney, lung, lymph nodes, and cerebral spinal fluid, and the virus of kuru has been found in lymph nodes, kidney, and spleen of humans (*16*). It is conceivable that the hematogenous spread of the infection is also implicated in man. The presence of virus in the blood in experimental Creutzfeldt-Jakob disease suggests that this may be true and that there may well be a danger of transmitting this disease via blood transfusions from humans harboring the agent during the incubation period, when the clinical disease is not readily apparent. Gajdusek mentioned that two humans harboring Creutzfeldt-Jakob disease were professional blood donors until shortly before the onset of their symptoms (*3*).

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15. Autopsies of these guinea pigs revealed: hemopericardium (No. 2 of the 2nd week group), stomach torsion and dilatation of stomach (No. 1 of the 13th week group), liver abscesses (No. 1 of the 25th week group), and polycystic kidneys (No. 1 of the 26th week group).
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Search Image for Leaf Shape in a Butterfly

Abstract. *The butterfly* Battus philenor *forms search images for leaf shape when searching for its two larval host plants in southeast Texas. This behavior increases the rate of discovery of host plants and permits females to track changes in relative host plant suitability for larval growth. Apostatic selection resulting from search image formation is a likely explanation for divergence in leaf shape by the two host plants.*

The significance of a plant's leaf shape has been attributed to abiotic environmental factors (*1*). In contrast, Gilbert (*2*) noted that the species in some tropical plant families that support populations of coevolved herbivorous insects differ greatly in leaf shape. He suggested that apostatic selection (*3, 4*) exerted by those herbivores may have produced the observed leaf shape diversity. Central to Gilbert's argument is the assumption that searching insects are able to discriminate leaf shapes and that individuals searching for one leaf type are less likely to respond to another. One mechanism that can lead to such differential response is search image formation. I report here that (i) ovipositing females of the pipevine swallowtail butterfly, *Battus philenor*, search selectively for either broad- or narrow-leaved larval host plants; (ii) females can switch preference from one leaf shape to another on the basis of experience and hence form true search images; (iii) a search image for one leaf shape results in host plants with that leaf shape being discovered in greater proportion than their abundance in the habitat; and (iv) butterflies with strong search images discover larval food plants at higher rates than butterflies with weak search images. In addition, I suggest that *B. philenor* is the primary selective agent responsible for divergence in leaf shape by its two larval host plants in southeast Texas.

I conducted this study between 22 March and 22 May 1977 in the open longleaf pine uplands of the Big Thicket region of southeast Texas. Adults of *B. philenor* are common in the pine upland habitat at that time of year; females can be found searching among the herbaceous vegetation for the two larval food plants *Aristolochia reticulata* and *A. serpentaria* (Aristolochiaceae) (*5*). The two host plants, perennial herbs reaching a maximum height of 40 to 50 cm, are closely related within the genus *Aristolochia* (*6*), yet differ in leaf shape. The more common species *A. reticulata* has the broad, ovate leaves characteristic of the genus (*6*), whereas all *A. serpentaria* plants in areas of sympatry with *A. reticulata* have long, narrow, parallel-sided leaves resembling grass blades.

Ovipositing *B. philenor* can be followed easily in the field, permitting observation of host plant search behavior under natural conditions (*5*). My preliminary observations suggested that females use leaf shape as a visual cue for locating host plants. As a female flies slowly above the herbaceous vegetation, she periodically approaches and lands on a plant and "tastes" it, presumably with tarsal chemoreceptors similar to those present in other insects (*7*). If the plant is not an *Aristolochia*, she immediately resumes search flight. If the plant is an *Aristolochia*, she either lays a small cluster of eggs or resumes search flight without ovipositing. Since the two *Aristolochia* species constitute less than 5 percent of the plants that females approach and "taste," it seems unlikely that the butterflies recognize a plant as an *Aristolochia* by employing long-distance olfactory cues such as are used by some other insects (*8*).

To test the hypothesis that leaf shape is an important cue used in initiating approach to a plant, I observed ovipositing females in an approximately 80-acre (32 ha) area of open longleaf pine upland in the Kirby State Forest, 15 miles (24 km) north of Kountze in Hardin County, Texas. All herbaceous plants and shrubs growing in the area were classified as having either long, narrow leaves or

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