falling. The right side was not used voluntarily. The right side of the face drooped, eyes were tonically deviated to the left, and a right hemianopsia field defect to light and threat was noted. Marked tremors were noted bilaterally with intention. No fasciculations were seen. Deep tendon jerks were 4+ with tap clonus of patella. No Babinski was elicited. The jerks and general state continued downhill, with the animal in a flexed position on the bottom of the cage. During the 5th week of clinical disease myoclonic jerks intensified and a resting continuous tremor of the lips, lower jaw, and head was noted. The animal was killed on 7 July 1969.

Neuropathologic and electron microscopic examination of the brains of the first three animals that we killed after they developed disease revealed a vacuolation of the dendritic and axonal processes in neurons and in the astroglial cells, as well as the presence of large rounded neurons containing a pale cytoplasmic inclusion body (*5, 6*).

Although the same suspensions, from human and affected chimpanzee brains, that have caused disease in the chimpanzees have been inoculated into several other species of primates and other small laboratory animals, including suckling mice and hamsters, no disease has developed in these animals after over 1 year of observation. Since kuru has also been successfully transmitted to the spider monkey (*7*), this species has been used for these inoculations. A chimpanzee, A124, has received a suspension of infectious human brain from patient 2 (M.W.) only by peripheral (intravenous, intraperitoneal, or intramuscular), not intracerebral, routes of inoculation; another animal, A125, has been inoculated in third passage with a filtrate of brain suspension from chimpanzee A82 at 1:1000 dilution, which had been passed through a cellulose acetate (Millipore) membrane, (average pore diameter, 220 nm).

The spongiform encephalopathies include principally a group of subacute presenile dementias, usually called Creutzfeldt-Jakob disease. The basic pathological change is a vacuolization in the dendritic and axonal processes of neurons, and to a lesser extent, in glial elements (*7*). Kuru in New Guineans is associated with the same spongiform degeneration of neurons. Alpers disease, or progressive diffuse cerebral degeneration of infants, may also possibly belong to the group, in view of its pathological similarities. In animals, scrapie and mink encephalopathy show the same process, but in natural scrapie it progresses only to neuronal vacuolation, and not to a full status spongiosis of cerebral gray matter. In scrapie, experimentally transmitted to sheep, goats, mice, rats, hamsters, or gerbils, however, as in experimentally transmitted mink encephalopathy, the spongiform degeneration becomes the dominant picture. The same is true of the spongiform degeneration in experimental kuru and experimental Creutzfeldt-Jakob disease, which exceeds that seen in the natural diseases.

These successful transmissions of Creutzfeldt-Jakob disease with spongiform encephalopathy of gray matter suggest that the disease of these patients should be included with the virus infections which may be designated the subacute spongiform viral encephalopathies: Creutzfeldt-Jakob disease, kuru, scrapie, and mink encephalopathy.

Note added in proof: Since this report was submitted, two of the five chimpanzees referred to above (A106 and A114), inoculated with brain suspension from a Canadian patient and a third British patient, respectively, have developed clinical signs similar to those observed in chimpanzees already killed in advanced stages of induced Creutzfeldt-Jakob disease. Transmission has thus been successfully accomplished with brain suspension from each of six of the eight patients referred to in this report. Further, a variety of tissues obtained at surgical biopsy and autopsy from an additional ten patients, from the United States, Canada, and Great Britain, with Creutzfeldt-Jakob disease, are under study.

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Erythropoiesis in the Dog: The Periodic Nature of the Steady State

Abstract. *In at least six of 11 normal dogs serial measurement of reticulocyte counts showed oscillation with a period of approximately 14 days. The phase of oscillation could be altered by bleeding followed by retransfusion. The observations suggest that canine erythropoiesis is an example of a physiological rhythm which has its origin in homeostatic control.*

Many body parameters are known to be actively controlled in such a way as to oppose disturbances and result in a more or less steady state. A commonly assumed and expressed corollary to this concept of active regulation is that a perfectly steady state results when no external disturbances are acting. However, clear exceptions to this corollary exist. The steady states of the pituitary-adrenal axis and, in the female, the pituitary-gonadal axis are sustained oscillations. On a cellular scale the activities of a number of biochemical intermediates have been shown to oscillate constantly (*1*). Recently, evidence was produced which showed that granulopoiesis is another physiological system whose steady state is one of sustained oscillation (*2*). It was suggested that it is controlled by a negative feedback circuit containing a time-delay and that periodicity arises because this type of circuit is inherently prone to oscillate.

Erythropoiesis is controlled by a hormone erythropoietin whose plasma level is a function of circulating red cell hemoglobin and whose principal action is to induce erythropoietin-sensitive stem cells to differentiate into recognizable erythroid precursors (*3*). These precursors must proliferate and mature before they can enter the blood

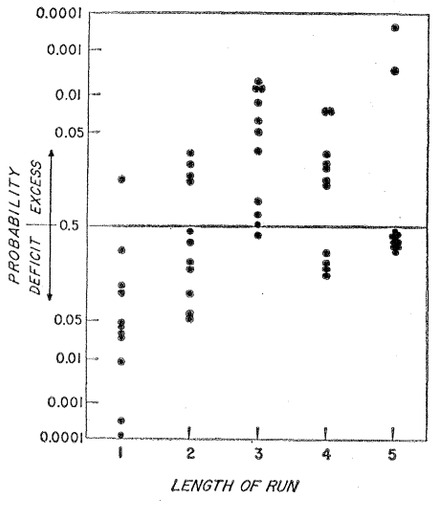

Fig. 1. Analysis of reticulocyte runs. The number of runs of each length was determined in each dog. The probability that it would have arisen by chance alone is shown. If the number observed was greater than the number expected the probability is shown in the top or "excess" half of the figure; and if less, the probability is shown in the bottom or "deficit" half of the figure. The term "run" is defined in the text.

as reticulocytes. Because of this, when any disturbance changes the number of circulating red cells, there is a substantial time-delay before this disturbance can be corrected by the marrow. Erythropoiesis is therefore a negative feedback circuit containing a time-delay and might well show steady state oscillation. Occasional reports in the literature point to the possibility (see *4*).

To test this hypothesis we bled 11 healthy, adult, male, mongrel dogs (0.3 to 0.5 ml of blood three times weekly), measured the red cell counts and reticulocyte percentages by standard methods, and used the absolute reticulocyte counts as a measure of the rate of erythropoiesis. Visual inspection of the results of the serial counts strongly suggested oscillation in at least six of the dogs. At times, in two of the six dogs the red cell count seemed to show low-amplitude oscillation with a similar period to that of the reticulocyte count, but this was inconstant and was not seen in the other animals. The serial reticulocyte counts were analyzed by applying the test based on runs suggested by Levene and Wolfowitz (*5*). This test primarily detects nonrandomness in a series of observations and, in the absence of a progressive change in the mean with time, points to periodicity as the cause of the nonrandomness. A run of length n is defined as following an observation when the next n observations either increase or decrease progressively. If a sample is random, then the numbers of runs of various lengths should also be random, having probability distributions determined both by the length of the run and the total number of observations. The data from each dog were analyzed separately by noting the number of runs of each length and determining the probability that this number would have arisen by chance alone assuming the data were random. Figure 1 shows that in seven of the 11 dogs there was a significant deficit of runs of length 1, indicating that in these dogs the reticulocyte counts were not randomly distributed in time. There was also a significant excess of runs of length 3 in six of the dogs. Since there was no evidence of overall regression with time and since a run of length 3 represents 7 days, the results further suggest that the nonrandomness was due to a cycle with a period of approximately twice 7, that is, 14, days. There are two possible reasons why a reticulocyte cycle was not detected in all the dogs. It may be that oscillation was present in all but had too low an amplitude to be detected in some; alternatively, it is possible that erythropoiesis in the dog is a system close to the borderline between oscillation and constancy, so that stable oscillation occurs only in some animals. The same reasons probably also account for the failure to detect oscillation of the red cell count.

That the periodicity of the reticulocyte count was a function of the circulating red cell was, however, shown by a further experiment. Four dogs whose reticulocyte count showed periodicity were bled 1.5 percent of their body weight and retransfused 2 or 3 days later. This procedure, which provided a pulse stimulus to erythropoiesis, was carried out when the reticulocyte count was at a peak. No effect should have been observed on the reticulocyte cycle if periodicity were due to some factor external to the marrow but imposing its own periodicity on the marrow. On the other hand, if the reticulocyte cycle were due to a feedback loop controlling erythropoiesis, the effect of the pulse stimulus would have depended on its size. If small, it would have had no effect; if larger, it might have brought erythropoiesis to its mean level and temporarily abolished oscillation; but if still larger, it might have permanently altered the phase of oscillation to one determined by the timing of the pulse. In three of the four dogs, bleeding and retransfusion seemed to produce a permanent shift of about 180° in the phase of oscillation. Figure 2 shows the results in one of them. In the fourth dog, which had the smallest stimulus to erythropoiesis

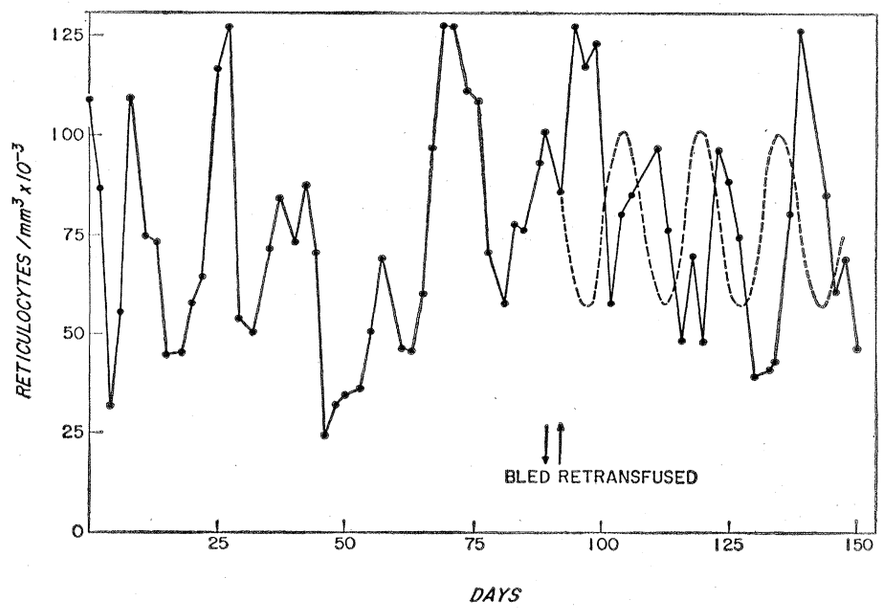

Fig. 2. Serial reticulocyte counts showing a reticulocyte cycle. As indicated by the arrows this dog was bled and 3 days later was retransfused. The dashed line is an extrapolation of the reticulocyte cycle present before bleeding. The experimental procedure seemed to shift the phase of oscillation approximately 180° but did not affect the period of oscillation.

as judged by the smallest fall in red cell count after bleeding, oscillation seemed to disappear after bleeding and retransfusion.

These results, therefore, suggest that the steady state of canine erythropoiesis is one of sustained oscillation and that this oscillation is inherent in the nature of the feedback circuit controlling erythropoiesis. There is no direct evidence from our experiments that erythropoietin comprised part of the feedback loop, although this is a reasonable hypothesis. At least for the physiological system of canine erythropoiesis, the term "steady state" appears to be somewhat of a misnomer and might be better replaced with some other term such as "controlled state." Many experimental or therapeutic manipulations of erythropoiesis or other biological systems are based on the assumption that the system being studied does not show time-dependent variation. However, if the system is known to be actively controlled then the possibility of periodicity should be considered and the assumption re-examined. Unsuspected time-dependent variation will merely contribute to the overall variation if the responses of groups are being measured but may seriously confound interpretation if individuals are being studied. On the other hand, the known existence of periodicity in a biological system might suggest the presence of unsuspected feedback control, and consideration of the characteristics of the oscillation might indicate the physical basis for that control. Finally, the periodicity of many periodic diseases may have its origin in an unrecognized physiological rhythm which in turn results from the action of feedback control.

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9 April 1969

Hyperphagia and Polydipsia in Socially Isolated Rhesus Monkeys

Abstract. *Three rhesus monkeys which had been isolated from social contact during their first year of life persistently overate and overdrank during adulthood. These monkeys ingested approximately twice as much fluid and food as the control animals reared normally.*

Evidence regarding the profound effects on social, sexual, and maternal behavior of monkeys removed from their mothers during the first hours of life and raised in total social isolation for the first 6 to 12 months (*1*) indicates that many disturbances persist throughout the animal's adult life.

During an experiment on three isolated monkeys (*2*), it became apparent that they ingested greater quantities of fluid than the controls. To test the limits of this phenomenon we attached 3.8-liter containers of water to their chairs with the intent of measuring consumption under what appeared to be inexhaustible supplies. This procedure was terminated when one of the monkeys (C-2) overdrank and required emergency resuscitation, catheterization of the bladder, and pumping of the stomach. Since the completion of the behavioral experiments and the return of these animals to standard laboratory cage conditions, daily measures of fluid intake and urine output have been obtained.

The three socially isolated monkeys were born in the University of Wisconsin primate laboratories and were separated from their mothers within 24 hours of birth (*3*). They were then kept in total social isolation for 12 months. One of the monkeys, female B-37, was born in July 1961 and the other two, C-2 and C-3, were males born in July 1962. In a series of studies it was determined that these monkeys had suffered severe damage in terms of social relations with other monkeys and that repeated exposure to normal animals in testing situations did not ameliorate their behavior (*4*).

The isolated monkeys were received in our laboratories in May 1966. After a brief period of acclimatization to the new laboratory, they were used, along with three feral males of approximately the same size and age, in an experiment on nonverbal communication (*2*).

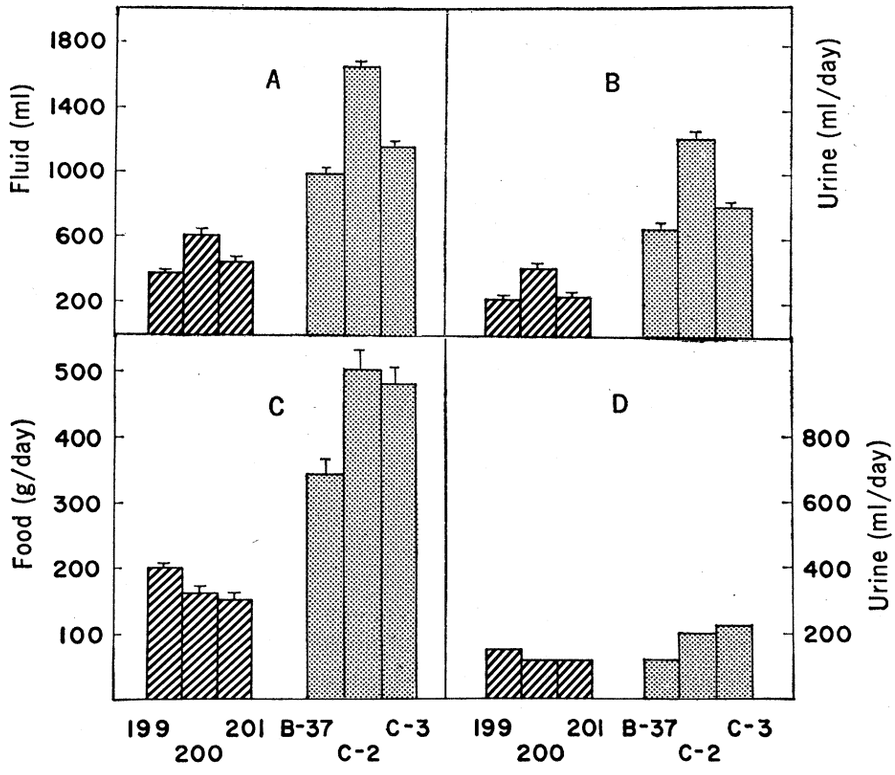

Fig. 1. Mean daily consumption of food and water and urine production for social isolates (dotted regions) and controls (hatched areas). (A) Mean and standard error of daily water consumption over 135 days; (B) mean and standard error of urine output per day during 135 days; (C) mean and standard error of consumption of monkey food pellets in grams per day over 20 days; (D) mean urine production per day during 24 hours of water deprivation.