tein uptake into the oocytes, as has recently been demonstrated in a cockroach (*9*). Thus, vitellogenin synthesis which is dependent on juvenile hormone appears as an integrated part of insect oogenesis, and the lack of hormonal control exhibited by certain Lepidoptera may be regarded as an adaptive exception to the more general pattern.

M. L. PAN
G. R. WYATT

Department of Biology,
Yale University,
New Haven, Connecticut 06520

References and Notes

1. W. H. Telfer and D. S. Smith, in *Insect Ultrastructure*, A. C. Neville, Ed. (Blackwell, Oxford, 1970), p. 117.
2. W. J. Bell, *J. Insect Physiol.* **15**, 1279 (1969); V. J. Brookes, *Develop. Biol.* **20**, 459 (1969); F. Engelmann, *Science* **165**, 407 (1969); and other papers. For review, see G. R. Wyatt, in *Biochemical Actions of Hormones*, G. Litwack, Ed. (Academic Press, New York, in press), vol. 2.
3. W. H. Telfer, *Annu. Rev. Entomol.* **10**, 161 (1965). Allatectomy on early 5th instar cecropia silkmoth larvae does not prevent the appearance of vitellogenin in the blood of this species at the normal time after spinning, nor does allatectomy of diapausing pupae alter the pattern of [^{3}H]leucine incorporation into this protein during late pharate adult development (M. L. Pan, unpublished observations).
4. T. Wu and F. Quo, *Acta Entomol. Sinica* **12**, 411 (1963); S. Fukuda and S. Kondo, *Zool. Mag.* **74**, 393 (1965); A. Karlinsky, *C. R. Acad. Sci. (Paris)* **264**, 1735 (1967); K. Endo, *Develop. Growth Differ.* **11**, 297 (1970); G. Benz, *Experientia* **26**, 1012 (1970); M. T. El-Ibrashy and I. Z. Boctor, *Z. Vergl. Physiol.* **68**, 111 (1970).
5. We thank Dr. Lincoln P. Brower of Amherst College for providing us with rearing stock and milkweed.
6. W. H. Telfer, *J. Gen. Physiol.* **37**, 539 (1954).
7. W. S. Herman and J. F. Barker, personal communication. We thank them for communicating their results to us.
8. See (*2*). Also, in *Blattella germanica* 0.16 to 0.66 μg of juvenile hormone induces vitellogenin synthesis (J. G. Kunkel and G. R. Wyatt, in preparation).
9. W. J. Bell and R. H. Barth, *Nature* **230**, 220 (1971).
10. J. F. Kennedy, *Experientia* **25**, 1120 (1969).
11. Supported by grant HD-02176 from the U.S. Public Health Service and by Whitehall Foundation.

1 June 1971

Anemia in Sleep-Deprived Rats Receiving Anticoagulants

Abstract. *Independent groups of rats were deprived of sleep and treated with the anticoagulant drugs phenylindanedione or dicoumarol for 1 to 8 days. These animals developed an extremely severe anemia which was accelerated by p-chlorophenylalanine. The red cell count and amount of hemoglobin decreased to half of normal values. No decrease occurred in animals subjected to any one single treatment. Histological examination indicated hemolysis, hypoplasia of hemopoietic organs, slight hemorrhage, but no evidence of stress. The severity of the anemia was inversely related to the amount of sleep permitted during sleep deprivation. This new syndrome demonstrates marked effects of sleep deprivation on both maturation and destruction of red blood cells. Depletion of serotonin by injection of parachlorophenylalanine blocked the increase in amount of brain waves of the type commonly seen in slow wave sleep but did not eliminate the production of these waves. This result is at variance with the theory that serotonin is the neurochemical responsible for the "priming" of slow wave sleep.*

Prolonged wakefulness produces widespread effects, but very few other than those on brain and behavior have been studied. In experiments designed to test the effect of sleep deprivation combined with anticoagulant drugs, it was discovered that rats receiving anticoagulants of the indirect type, when deprived of sleep for 8 days, developed an extremely severe anemia. A number of rats were then subjected to these treatments for various lengths of time (*1*). On each day, ten animals for each treatment were killed, and blood and tissue samples were obtained (*2*). Some rats received the anticoagulants phenindione or dicoumarol for this period, some were deprived of sleep, and others were deprived of sleep and treated with anticoagulant. Others received no treatment and served as control animals. There was no change in the mean amount of hemoglobin (Fig. 1) in animals given single treatments or in control animals. However, there was a marked decrease in the mean amount of hemoglobin in those groups receiving combined treatments on days 7 and 8, with the decrease beginning on day 6. These values were significantly low, relative to the values for the groups that received a single or no treatment [$P = .001$ (*3*)]. Since the chemical structure and side effects of dicoumarol and phenindione are quite different, the fact that anemia develops similarly in animals deprived of sleep and treated with these drugs suggests that the contribution of these drugs to the phenomenon must be due to their anticoagulant properties. Additional groups of rats received an intraperitoneal injection of *p*-chlorophenylalanine (PCPA, 316 mg/kg) every 72 hours. The first injection was given 24 hours before the start of sleep deprivation and treatment with anticoagulant. Treatment with PCPA in addition to sleep deprivation and treatment with anticoagulant had an accelerating effect on the development of anemia; the anemia began to develop by day 3 (Fig. 1).

The anemia developed in these animals was very severe and could easily be seen on gross examination. The tissues such as the liver, kidneys, skin, and lungs were extremely pale. At times it was even possible to detect an anemic rat before its internal organs were inspected. The ears were very pale, the eyes were barely pink, and their feet seemed to have lost circulation. Symptoms of hemorrhage such as those observed in other studies (*4*) were minimal. Hence, the anemia could not be accounted for in terms of hemorrhagic disturbance. The hematologic examination revealed a decline in hemoglobin to 50 percent of normal values (Fig. 1), with a proportionate decline in red cell count and hematocrit values, indicating a normocytic anemia.

All animals were weighed just before the beginning and again at the end of their respective experimental program. Most animals had lost weight. However, the anemia could not be attributed to loss of weight, since weight loss was similar in anemic and nonanemic rats. Weighing of food showed that the animals were eating the same amount of food regardless of treatment group. Prothrombin times demonstrated that all rats fed anticoagulant were hypoprothrombinemic.

To determine the contribution of sleep to the appearance of anemia, we altered conditions to increase sleeping time. Ten rats deprived of sleep and treated with phenindione were fed in their home cages for eight continuous hours instead of three times a day for 2 hours, 40 minutes each time. This group, which could sleep a certain amount of time, had hemoglobin values almost within normal limits. Ten rats were placed for 8 days in the same containers with a larger platform (11 cm diameter) and fed phenindione in the usual schedule. The blood tests in this group were totally normal. One animal had slight hemorrhage around the testicular area. These experiments support the conclusion that the deprivation

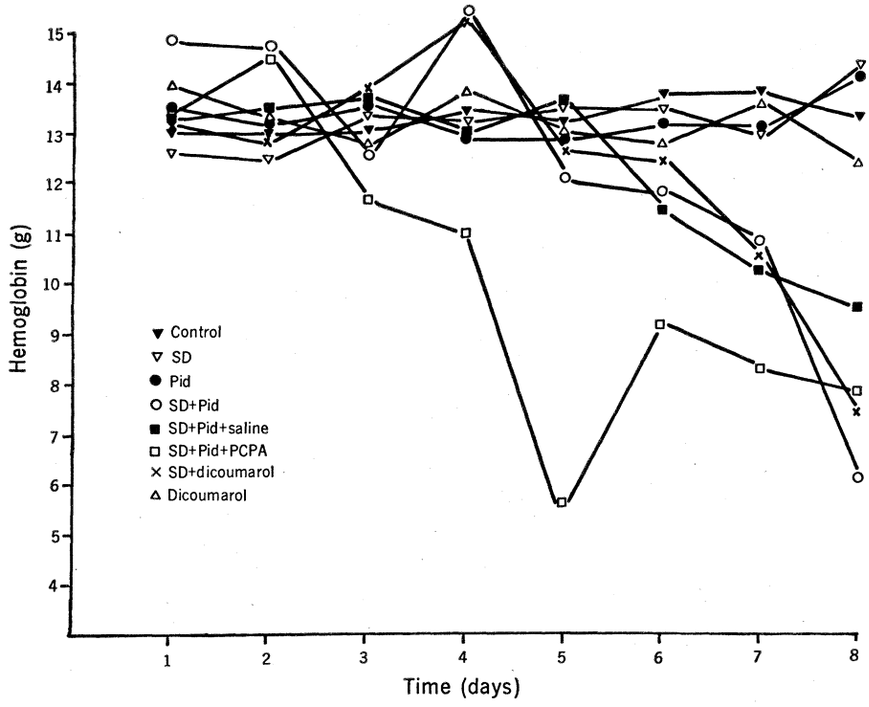

Fig. 1. Mean amount of hemoglobin from 1 to 8 days of treatment; SD, deprived of sleep; Pid, treated with phenylindanedione; PCPA, treated with parachlorophenylalanine.

of sleep is an essential for the appearance of the anemia. Further, they indicate that the procedures used did not produce the hemorrhagic lesions observed in previous studies when rats treated with anticoagulants were exposed to standard stress procedures (*4*).

Portions of liver, heart, testicles, intestine, adrenals, and skin from rats receiving different treatments were prepared for histological study. Microscopic examination indicated very little if any hemorrhage in the tissues. Of all the tissues examined the spleen gave the greatest amount of information. In the spleens of rats deprived of sleep and treated with anticoagulant, normoblastic activity was reduced or absent. This finding suggests that erythropoietic activity had been reduced. Iron deposits were also observed, indicating hemolytic activity had increased.

The results indicate that the profound anemia observed is related to the particular mechanisms affected by sleep deprivation in the presence of the hypoprothrombinemia produced by these anticoagulants. Behavioral observations and electroencephalograms suggested that the rats attempted to sleep and at times approached fast-wave sleep (FWS), this being prevented immediately by the animal's head or body falling into the water. The effect of sleep deprivation was examined in some animals with implanted electrodes. Figure 2A shows typical electroencephalogram records and the integrated pattern (*5*) for control animals and those deprived of sleep for 2 or 8 days. The changes in amplitude reflect an increase in slow waves associated with slow-wave sleep (SWS). These have been calculated from the integrator reset frequency, and the resulting means of the mean integrator reset frequency with standard deviations are graphically represented in Fig. 2B. We can detect a statistically significant change in mean integrator reset frequency of animals deprived of sleep as compared to controls. The latter show little change over the 8 days, whereas, for the former, values increase over the whole 8 days. Although the mean integrator reset reflects only change in amplitude, it may be assumed from this that the animals deprived of sleep had an increased "pressure" for sleep. Behavioral and electroencephalographic observations demonstrated that it was impossible for the rats to attain FWS. Hence, the increased "pressure" to sleep results in more SWS, (Fig. 2), with the mean integrator resets and the amount of slow waves apparently increasing as the

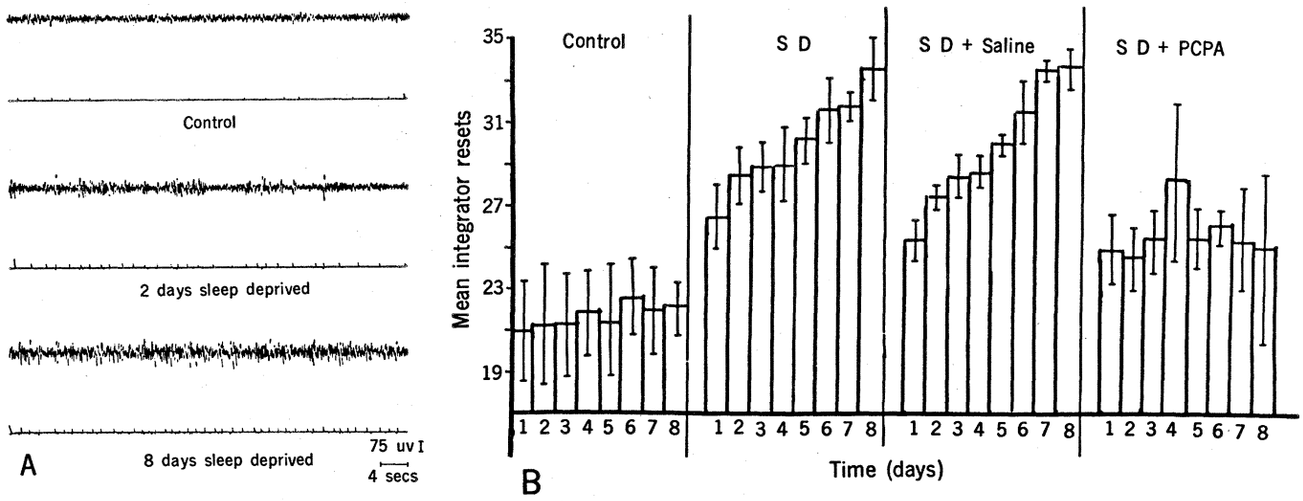

Fig. 2. Electrocorticograms and integrated pattern of electrocorticograms for rats deprived of sleep. (A) Characteristic electrocorticogram and integrated pattern for control rats and rats deprived of sleep for 2 days or for 8 days. (B) Mean and standard deviation of integrated electrocorticograms daily for control rats and rats deprived of sleep (SD), deprived of sleep and given saline (SD + saline), and deprived of sleep and given parachlorophenylalanine (SD + PCPA) for from 1 to 8 days. In each group, $N = 4$.

length of time for deprivation of sleep increases.

When rats are deprived of FWS, there is an accumulation of serotonin (*6*). If SWS is triggered by serotonin, this increased "pressure" for SWS during total FWS deprivation could be the result of the increased accumulation of serotonin. In Fig. 2B are shown the mean integrator resets for the animals which were treated with PCPA when deprived of sleep. Treatment with this compound in the dosage used results in depletion of brain serotonin (*7*). It can be seen that the rise in mean integrator resets over the 8 days of deprivation of sleep did not occur, although animals injected with saline showed the same rise. While PCPA thus prevented the increase in number of mean integrator resets and thus the increase in SWS, it did not suppress SWS completely. The resulting mean integrator reset values were not statistically different from those for the control groups. Hence, a long-term depletion of serotonin by continuous administration of PCPA was not capable of totally eliminating the slow waves from the electroencephalogram. This finding therefore raises the question of whether serotonin is alone responsible for SWS.

The histological analysis suggested that the development of anemia was due to (i) the development of a certain degree of hemolysis of red cells (iron deposits were quite obvious in sections of the spleen) and (ii) the arrest of red cell maturation (normoblastic activity in the spleen seemed to be markedly diminished in the anemic animals). The mechanisms whereby hypoplasia occurred are unknown, but we might assume that sleep deprivation affects the kidney (erythropoietin system). A possible basis for hemolysis of red cells is suggested by the work of Luby *et al.* (*8*), who studied the effects of sleep deprivation on the energy transfer system of the red blood cell in man and reported that the effort to stay awake stimulated high energy turnover in the red blood cell while depleting amounts of adenine phosphates. Since we know that the decrease in adenosine triphosphate in red cells causes sphering (*9*) and we assume that in our rats amounts of erythrocyte adenosine triphosphate were decreased, then we can assume that a certain degree of spherocytosis occurred in the anemic rats, making them more liable to hemolysis. The development of the anemia was also probably related to an additional effect on the platelets and vessel wall. This is suggested by the fact that, in those animals depleted of serotonin by parachlorphenylalanine, the anemia developed faster and the hemorrhages (albeit small ones) increased at about the same time that the anemia developed. Serotonin is a potent vasoactive compound that is liberated at the time of hemorrhage and augments vasoconstriction at the site of injury, thus helping to prevent loss of blood (*10*). It seems reasonable to assume that the faster developing anemic process with PCPA is due as much to the removal of the action of serotonin on the vessel wall as to reduction of brain serotonin and resulting effect on sleep.

R. R. DRUCKER-COLÍN *
L. B. JAQUES
Department of Physiology, College of Medicine, University of Saskatchewan, Saskatoon
G. WINOCUR†
Department of Psychology, University of Saskatchewan

References and Notes

1. Male Wistar rats (250 to 300 g) were housed in individual cages in isolated, air-conditioned rooms at 21° to 22°C, with fluorescent lighting and controlled cycles of 12 hours each of day and night. The principles of animal care of the Canadian Federation of Biological Societies were observed. During 15 days of acclimatization, the rats were given full access to powdered food. When the experiments were begun, the animals were allowed to eat and drink 8 hours during a day, divided into three equal periods. Anticoagulant was given mixed in powdered food at a dosage of 800 ppm for phenindione (Danilone, Frosst) and 200 ppm for dicoumarol. The animals were deprived of sleep by being placed on a small platform (4.5 cm in diameter) surrounded by water inside a plastic container. This prevented total relaxation of the body musculature normally associated with fast wave sleep (FWS) but did not prevent the animals from occasionally drifting into slow wave sleep (SWS).
2. Animals were killed with an overdose of pentobarbital, and blood samples were removed by cardiac puncture. Examination was made for signs of anemia and hemorrhage, both external and internal. Hematological values were obtained with the model C Coulter counter, and prothrombin times were determined with commercial thromboplastin.
3. Differences between mean values for hemoglobin, hematocrit, and red blood cell count of combined control groups versus combined treatment groups were examined by Student's *t*-test. Differences for days 1, 2, 3, and 4 were not significant. Differences for days 6, 7, and 8 were significant ($P = .001$).
4. L. B. Jaques, *Anticoagulant Therapy: Pharmacological Principles* (Thomas, Springfield, Ill., 1965).
5. The electroencephalograms were recorded with model 5, 4-channel Grass polygraph with Grass integrator U 1-1 to give a running record of voltage as a function of time. Recordings were made for 4 hours each day for the duration of the deprivation. For every rat and for each day a mean integrator reset value was obtained by counting the number of pen resets for each 240 minutes of recording and dividing by 240.
6. F. Hery, J. F. Pujol, M. Lopez, J. Macon, J. Glowinski, *Brain Res.* **21**, 391 (1970).
7. M. Jouvet, *Science* **163**, 32 (1969).
8. E. D. Luby, J. L. Grisell, C. E. Frohman, H. Lee, B. D. Cohen, J. S. Gottlieb, *Ann. N.Y. Acad. Sci.* **196**, 71 (1962).
9. M. Nakao, T. Nakao, S. Yamazoe, H. Yoshikawa, *J. Biochem. (Japan)* **49**, 487 (1961).
10. L. B. Jaques and L. M. Fisher, *Arch. Int. Pharmacol. Ther.* **123**, 325 (1960).
11. Complete details are given in R. R. Drucker-Colín, thesis, University of Saskatchewan, 1971 (University Microfilms, University of Michigan, Ann Arbor 48104).
12. Supported by funds to L.B.J. from Medical Research Council of Canada, grant MT-2744. Danilone and dicoumarol were given by Chas. E. Frosst & Co., Montreal, and Abbott Laboratories, Montreal. We thank Dr. T. A. Cunningham and Dr. G. J. Millar for supervision and help in various aspects of the research; and the technical staffs of the Department of Physiology, and the hematology section of University of Saskatchewan Hospital for their assistance.

* Present address: Instituto Miles de Terapeutica Experimental, Calzada Xochimilco No. 77, Apartado Postal 22026, Mexico 22, D.F.
† Present address: Department of Psychology, Trent University, Peterborough, Ontario, Canada.

5 May 1971; revised 10 September 1971

Polyadenylic Acid Sequences: Role in Conversion of Nuclear RNA into Messenger RNA

Abstract. *Polyadenylic acid [poly(A)] segments containing 150 to 250 nucleotides appear to be covalently linked to heterogeneous nuclear RNA (HnRNA) and messenger RNA (mRNA) in eucaryotic cells. The poly(A) is synthesized in the nucleus, and is probably linked initially to HnRNA that is ultimately transported as mRNA to the cytoplasm. Studies with inhibitors of RNA or poly(A) synthesis indicate that synthesis of poly(A) segments is independent of transcription. The poly(A) marker may prove useful to elucidate mRNA modification and transport in eucaryotic cells.*

Rapidly labeled nuclear RNA from mammalian cells can be divided into two major classes (*1*). In the nucleolus there are large ribosomal precursor RNA molecules of two uniform sizes (45*S* and 32*S* which undergo specific cleavage to yield the RNA eventually found in cytoplasmic ribosomes (*2, 3*). The second type of nuclear RNA, found outside the nucleolus, consists of molecules ranging in size up to 20,000 nucleotides. The base composition of