with antiserums to different known skeletal filaments of cells support the idea that the autoantibody found in some CJD and kuru patients is directed against the neurofilaments. Furthermore, the absence of a reaction of the positive serums with the skeletal structure of dendrites in our neuron culture strongly suggests that the autoantibody is specific for a form of filament protein restricted to axons. That this human autoantibody reacts with normal rat, mouse, or hamster neural proteins shows that it is not species-specific.

Johnson *et al.* (*10*) in 1965 showed that patients with active chronic hepatitis developed an autoantibody against cytoskeletal proteins (*11, 12*); these autoantibodies (smooth muscle autoantibodies) were later found in patients with other diseases (*13–15*). Toh *et al.* (*16*) demonstrated the transient presence of autoantibodies to 10 n*M* filaments in the serum of children in the acute stage of common viral diseases; these autoantibodies decreased during convalescence. Linder *et al* (*17*) showed that complement can be activated in the absence of antibody and bound specifically to 10 n*M* filaments in a wide range of cells and noted the possible role of this mechanism in virus-infected cells. Dales and Chardonnet (*18*) reported evidence that another cytoskeletal structure, microtubules, may serve as the pathway used by viruses on their vectorial travel from the cellular surface toward the nucleus. Hiller (*19*) has demonstrated an intimate association of viral particles with the cytoskeleton of virus-infected cells, and their interaction with microfilaments. Allison (*20*) postulated that virus infection can lead to T-cell sensitization, and through a carrier effect can stimulate B cells to produce autoantibodies against cellular components.

The failure of the viruses of the transmissible spongiform encephalopathies to induce an immune response, either during the evolution of the natural or experimental disease or in animals hyperimmunized with high concentrations of virus, has been one of their most "unconventional" characteristics (*21*). Although previous serological tests to detect immune reactions in patients and animals with kuru, CJD, and scrapie were consistently negative, it was early postulated by us that the procedures employed, that is, complement fixation and Ouchterlony double-diffusion and precipitation tests, were not sensitive enough to detect a response (*22*). The autoantibody described in this report, although not specific for kuru and CJD, is the first evidence of immune system involvement in these diseases. However, it is too early in our investigations to speculate about the possible role of this autoantibody in the pathogenesis of CJD and kuru or the clinical value of the estimation of this antibody in the course of these diseases. Its occasional presence in patients with other neurological disorders and in healthy subjects must be evaluated.

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Endorphin-Mediated Increases in Pain Threshold During Pregnancy

Abstract. *Maternal pain thresholds in rats were determined during various stages of pregnancy and parturition by measuring the intensity of electric shock that elicited reflexive jumping. There was a gradual rise in the pain threshold between 16 and 4 days prior to parturition and a more abrupt rise 1 to 2 days before that event. This increase was abolished by long-term administration of the narcotic antagonist naltrexone. The endorphin system is thus an important component of intrinsic mechanisms that modulate responsiveness to aversive stimuli. The data also demonstrate the activation during pregnancy of an endorphin system that is apparently quiescent in nonpregnant female rats treated the same way.*

The biochemical demonstration of specific opiate receptors (*1*) and the discovery of endogenous ligands (endorphins) (*2*) for these receptors indicate that in addition to mediating the analgesic effects of alkaloids, the receptors are involved in mediating physiological processes. Since pregnancy, labor, and parturition are states that require adaptation to stress, considerable attention has been focused on the possible involvement of endorphins in the physiological sequelae of pregnancy. Although there is much circumstantial evidence that endorphin systems are activated during pregnancy (*3*), the specific behavioral and physiological consequences of this activation have not yet been elucidated. This report describes experiments showing that normal modulation of responses to painful stimuli during pregnancy can be blocked by administration of naltrexone, a pure, potent narcotic antagonist. The results indicate that endorphin systems are involved in raising maternal thresholds to pain.

Two groups of seven pregnant rats and two groups of seven nonpregnant rats were housed individually and given free access to food and water. Pain thresholds were determined for one group of pregnant animals beginning 16 days before parturition and continuing until 41 days afterward. One group of nonpregnant rats was tested in parallel with the pregnant group an equal number of times with the same interval between tests. To determine whether endorphins are involved in modulating responsiveness to aversive stimuli during preg-

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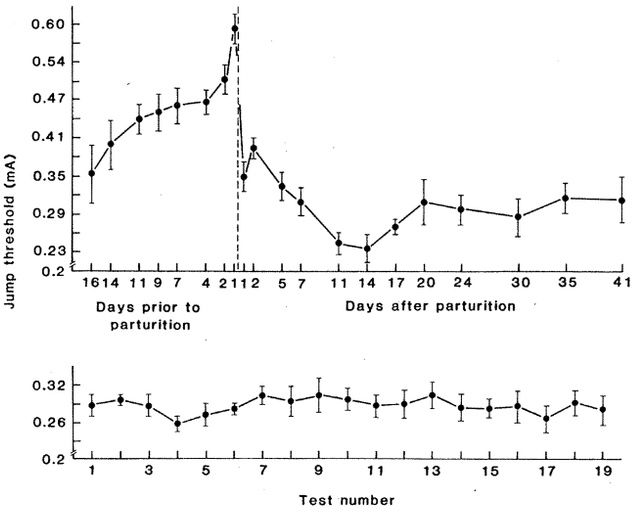

Fig. 1. (A) Mean jump threshold in pregnant rats as a function of days before and after parturition (dashed line). Each point represents the mean threshold of eight rats ± standard error (S.E.). (B) Mean jump threshold in nonpregnant females as a function of repeated tests. These determinations were made in parallel with the schedule shown in (A). Each point represents the mean threshold of seven rats ± S.E.

nancy, the remaining pregnant and nonpregnant groups were implanted with long-acting naltrexone pellets (*4*) and tested in the same way, except that testing was discontinued after parturition. Also, before pellet implantation and testing, five baseline pain thresholds were determined for the nonpregnant group.

Jump (pain) thresholds were determined by subjecting the animals to successively more intense shocks (*5, 6*). The jump threshold was defined as the lower of two consecutive intensities that elicited simultaneous withdrawal of both front paws from the grids (*7*). Each trial was begun by giving the animal a 300-msec foot shock at a current intensity of 0.1 mA. Subsequent shocks were increased in 0.05-mA steps at 20-second intervals. After each trial, the current intensity was reset to 0.1 mA for the next trial until eight determinations of jump threshold were completed for each animal (intertrial intervals, 30 seconds). The mean jump threshold for each animal was then calculated.

Figure 1 shows the alterations in pain threshold during pregnancy and parturition for the unimplanted pregnant group and thresholds for the unimplanted nonpregnant group during repeated testing over the same time span. Two-way analysis of variance indicates that before parturition the jump thresholds were significantly different between groups [$F(1, 13) = 29.36$, $P < .001$] and over days [$F(6, 78) = 5.65$, $P < .001$]. There was also a significant interaction between treatment and days [$F(6, 78) = 4.13$, $P < .001$]. Scheffé post hoc comparisons showed that while none of the thresholds for the nonpregnant animals differed significantly from those of the first test day ($P > .01$), all of the thresholds for the pregnant animals prior to parturition were significantly different ($P < .01$) from those of the first test day. The thresholds for the pregnant and nonpregnant animals on the first day of testing were not significantly different [$t(13) = 1.35$, $P > .05$]. However, there was a gradual increase in jump threshold between 16 and 4 days before parturition and a more abrupt increase 1 to 2 days before that event. The mean threshold increased to 0.51 mA 2 days and 0.59 mA 1 day before birth, compared with 0.35 mA on the first day of testing.

Analysis of variance of jump thresholds after parturition indicates that there was no significant difference in threshold between the pregnant and nonpregnant groups [$F(1, 13) = 1.06$, $P < .05$], while there was a significant change in threshold over days [$F(11, 143) = 36.32$, $P < .001$] and a significant interaction between days and groups [$F(11, 143) = 13.09$, $P < .001$]. One day after parturition there was an abrupt decrease in jump threshold followed by a more gradual decline over the next 13 days (the mean jump threshold 14 days postpartum was 0.24 mA, compared to 0.59 mA 1 day before parturition).

In seven of eight animals, the jump thresholds 11 days postpartum were actually less than the thresholds measured on day 7; this was also observed on day 14 for all but two of the eight animals. Moreover, on days 11 and 14, six of the eight animals had lower thresholds than on day 17. After day 17 there did not appear to be any systematic changes in jump threshold. Essentially the same pattern of change was demonstrated when five additional pregnant rats were tested 15, 13, 11, 8, 6, and 2 days before parturition and 2, 6, 8, 10, 13, 15, 17, and 21 days afterward. In all, jump thresholds for 13 of 13 pregnant rats were higher during pregnancy than in the period after they gave birth.

Subcutaneously implanted naltrexone pellets markedly reduced the increase in jump threshold observed during pregnancy (Fig. 2A) without altering either the length of gestation or the average litter size (10.3 pups for naltrexone-implanted mothers, 9.8 pups for unimplanted mothers). Naltrexone pellets did not induce significant alteration in the jump threshold of any of the seven nonpregnant females (Fig. 2B). The mean jump threshold, determined over five baseline trials (35 determinations), was 0.29 ± 0.009 mA, and was unchanged over a 15-day period after pellet implantation (0.29 ± 0.007 mA; 56 determinations).

The results indicate that intrinsic mechanisms that modulate responsiveness to aversive stimuli are activated during pregnancy and parturition. Different stages of pregnancy are associated with different degrees of attenuation of pain responsiveness; the highest threshold was observed 1 to 2 days before parturition. Moreover, during the postpartum period, maternal rats apparently go through transient hyperalgesia before the pain threshold returns to baseline levels.

The failure of naltrexone to alter jump threshold in nonpregnant female rats (Fig. 2B) indicates that its ability to markedly reduce the increase observed during pregnancy (Fig. 2A) is due to

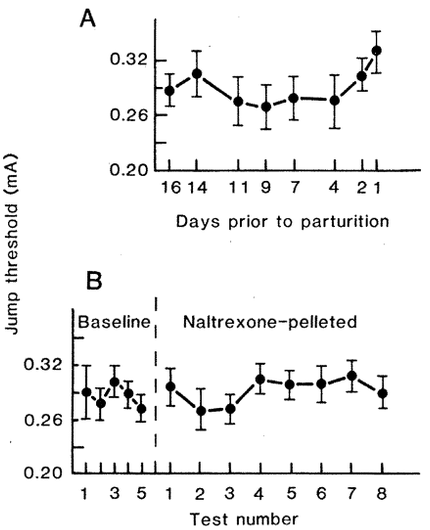

Fig. 2. (A) Mean jump thresholds of pregnant rats implanted with two naltrexone pellets, presented as a function of days before parturition. Testing was begun 1 day after implantation. Each point represents the mean threshold of seven rats ± S.E. (B) Jump thresholds in nonpregnant females before and after implantation. Testing was resumed 1 day following implantation. Thereafter, determinations were made in parallel with the schedule shown in (A). Each point is the mean threshold of seven rats ± S.E.

pharmacological, not physiological, antagonism of endogenous pain-attentuating processes. Thus during pregnancy there is activation of an endorphin system that is apparently quiescent in nonpregnant female rats treated the same way. Endorphins therefore appear to be an important component of intrinsic mechanisms that modulate responsiveness to aversive stimuli during pregnancy.

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4. Cylindrical pellets (diameter, 4.5 mm; length, 9 mm) of naltrexone (30 mg), cholesterol (105 mg), and glyceryl tristearate (15 mg) were prepared as described by A. L. Misra, R. B. Pontanai, and N. L. Vadlamani [*Res. Commun. Chem. Pathol. Pharmacol.* **20**, 43 (1973)], and A. L. Misra and R. B. Pontanai [*Commun. J. Pharm. Pharmacol.* **30**, 325 (1978)]. Two 30-mg pellets were implanted subcutaneously under light ether anesthesia in the dorsal area of each rat 1 day before the start of testing. This was sufficient to block the analgesic effects of morphine in rats (10 mg/kg, intraperitoneally) for 2 to 3 months.
5. Tests were conducted in a standard chamber with a 30 by 24 cm floor grid composed of 14 bars (diameter, 6 cm) spaced 1.8 cm apart. Scrambled electric shock was delivered to the grids by a constant-current shock generator.
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Ornithine Decarboxylase Is Important in Intestinal Mucosal Maturation and Recovery from Injury in Rats

Abstract. *A transient increase in ornithine decarboxylase activity and polyamine biosynthesis occurs in the intestinal mucosa of the newborn rat in the third week after birth. During this period, there is a rapid conversion of the mucosa from a fetal to a mature adult status. A similar increase in ornithine decarboxylase activity also accompanies the rapid recovery of the mucosa 1 week after an injury is induced by chemotherapy in adult rats. In vivo, α-difluoromethyl ornithine, a highly selective, enzyme-activated, irreversible inhibitor, suppresses these increases in mucosal ornithine decarboxylase and delays both intestinal mucosal maturation and recovery from injury. Thus increased ornithine decarboxylase activity, with the resultant increase in polyamine content, may play an essential role in intestinal mucosal maturation and regeneration in the rat.*

The decarboxylation of ornithine by ornithine decarboxylase (ODC) (E.C. 4.1.1.17) leads to the formation of putrescine, and this reaction is the initial and rate-limiting step in the biosynthesis of polyamines (*1*, *2*). Marked increases in ODC activity and rapid accumulation of the tissue polyamines putrescine, spermidine, and spermine are characteristically associated with rapid growth (*2*, *3*). Attempts to document in vivo an essential role for this increased polyamine biosynthesis in cell growth and differentiation has proved difficult because many of the available ODC inhibitors are competitive, and hence reversible (*4*).

Recently, a potent, enzyme-activated, irreversible ODC inhibitor, DL-α-difluoromethyl ornithine (DFMO; RMI 71782), has been developed at the Merrell Research Center (*5*). Other than the selective inhibition of ODC, DFMO has no acute pharmacologic activity, and is essentially nontoxic in normal mice and rats (*6*, *7*). DFMO suppresses induced formation of putrescine and arrests the growth of mouse L1210 leukemia cells and rat hepatoma cells in culture (*6*), and completely suppresses the sharp rise in uterine ODC activity accompanying murine embryogenesis, with resultant complete arrest of embryonic development (*7*). DFMO may thus be used to manipulate selectively the polyamine biosynthetic pathway and to delineate the functional role of ODC and the polyamines in tissue growth.

The rapidly proliferating and maturing epithelium of the small intestine offers a model for the study of cell growth and differentiation where polyamine biosynthesis may play a critical role. In the first 3 weeks after birth in the rat, the mucosal crypts elongate, cell proliferation increases, the villi lengthen, and mature mucosal cells bearing disaccharidases increase in number, with the most rapid increases in enzyme activity occurring during week 3 after birth (*8*). The intestinal mucosa of the adult rat also undergoes constant cell regeneration; the process from cell division through maturation and loss into the lumen is completed in 2 to 3 days. This cell growth pattern is altered by injury to the mucosa (*8*). Treatment with arabinosylcytosine (ara-C), a chemotherapy agent that acts specifically against cells in the proliferative phase, or S (synthesis) phase of the cell cycle, is followed by a loss of mature mucosal cells and an increased proliferation of the uninjured crypt cells. During the next 2 to 3 days, new mucosal cells bearing the disaccharidases appear and increase in number, and histologic and biochemical recovery is complete in 1 week (*9*). Our laboratory has previously shown that ODC and diamine oxidase (DAO) (E.C. 1.4.3.6), an enzyme which deaminates the polyamine putrescine, are found in increasing amounts during cell maturation in the intestinal mucosa (*10*). The ontogenic developmental pattern of DAO in the newborn rat is similar to that of the disaccharidases and can be used to monitor the maturity and integrity of the intestinal mucosa (*11*). We therefore studied the dynamics of polyamine biosynthesis and metabolism in the rat small intestine mucosal epithelium during maturation in the newborn and recovery from injury in the adult.

In newborn rats the most rapid increases in the activities of maltase and DAO, enzymes of the mature mucosa, occurred during week 3 after birth, when there was also a rapid increase in polyamine biosynthesis and mucosal ODC activity increased 15-fold from its low basal level (Fig. 1A). The ODC "spike" was accompanied by an increased activity of the second critical enzyme in the polyamine biosynthetic pathway, *S*-adenosylmethionine decarboxylase (SDC) (E.C. 4.1.1.50). SDC catalyzes the decarboxylation of *S*-adenosyl methionine, thus providing propylamino groups for the subsequent biosynthesis of spermidine and spermine. The increases in the polyamine biosynthetic enzyme activities were accompanied by increases in polyamine content (Fig. 1A). These enzyme activities and polyamine concentrations returned to their basal levels by day 24, when the mucosa had achieved its mature status.

A rapid increase in ODC activity and polyamine biosynthesis also accompanied mucosal regeneration and maturation during the first week of recovery

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