though in a somewhat extreme form. He presumed narrow channel openings to the cell exterior, while in *Blasia* the nucleus is broadly "exposed."

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8. I thank J. B. Hanson for suggestions.

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Growth Retardation in Offspring of Female Rats Treated with Morphine Prior to Conception

Abstract. *Treatment of female rats with morphine sulfate for 5½ or 10 days, prior to drug withdrawal for 5 days and subsequent mating, results in retarded growth of offspring. The effect is not present at birth but appears at 3 to 4 weeks of age. It occurs even though offspring are not exposed to morphine in utero or postnatally. It is not eliminated by cross-fostering and is apparently of prenatal origin.*

Recent studies of tolerance to opiates (*1*), and in particular the persistence of tolerance to the analgesic effect 11 months after a single injection of morphine (*2*), suggest that some transferable factor induced by morphine treatment is involved in the development of tolerance to narcotic analgesics (*2*, *3*). Results of passive transfer of serum from tolerant to nontolerant animals, however, have been equivocal (*4*). To further characterize this elusive morphine factor, we sought to determine whether treatment of females with morphine prior to conception affects the responses of their offspring to opiates (*5*). Since, in both tolerant and nontolerant rats, total recovery of injected morphine occurs by 48 hours after the last injection (*6*), our animals were not mated until 5 days after morphine treatment was terminated.

In the course of this work we noted a transient but significant depression in body weight in the progeny of morphine-treated females in comparison with the body weight of offspring of saline-treated mothers, although the young had not been previously exposed to the drug either in utero or after birth. Growth retardation did not occur until after weaning at 25 days of age and was no longer apparent by 8 weeks.

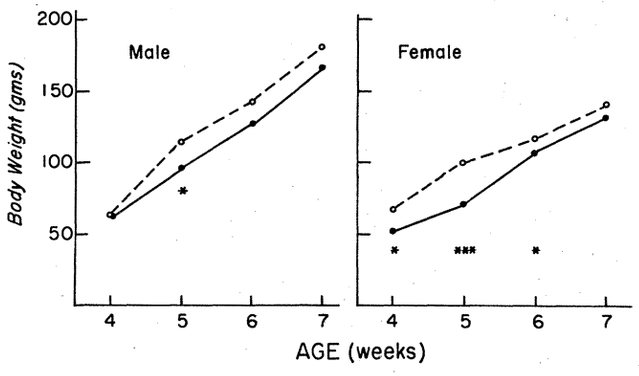
Ten Holtzman female rats were injected subcutaneously for 5½ days with increasing doses of morphine sulfate: starting dose was a single 10 mg/kg injection; thereafter rats received 15 mg/kg twice daily on days 2 to 5 and 22 mg/kg once on the final day of treatment. Controls were similarly injected with physiologic saline. Five days after the last injection, the females were mated with drug-free males. Neonates were weighed at birth and weekly thereafter. Mean body weights were similar for the two groups of offspring at birth and for the period up to weaning. Figure 1 presents the results of a typical experiment illustrating the body weight changes in 4- to 7-week-old progeny. "Experimental" refers to the offspring of morphine-sulfate-injected females; "control" signifies offspring of saline-injected mothers. Each point represents mean body weight (in grams) of 13 to 27 rats. The decrease in growth rate among offspring of morphine-treated mothers appeared consistently in similar studies with 28 breeder females, although some variation occurred, in individual experiments, between sexes in both time of onset (4 to 5 weeks) and extent of growth depression.

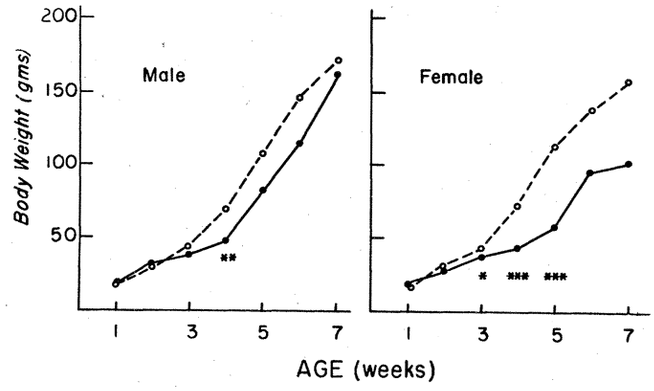
In addition, differences in viability among female offspring were observed between control and experimental groups of offspring. Although such differences were not evident prior to weaning, the incidence of deaths among 4- to 7-week female offspring of drug-treated mothers (29 percent) was significantly higher than in the corresponding control group (3 percent; a chi-square test gave a $P < .01$).

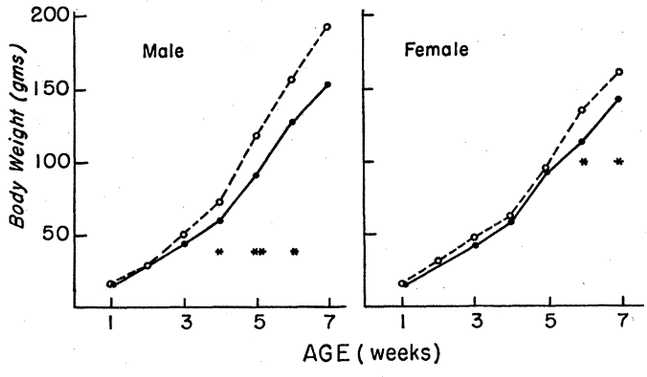
To assess the effect of an increase in both the duration and extent of morphine treatment of mothers, female rats were injected with morphine sulfate or saline twice daily for 10 days before drug withdrawal and mating. Starting dose was 10 mg/kg per injection; the dose was increased daily, in 5 mg/kg increments, to a maximum total daily dose of 60 mg/kg on the fifth through tenth day of treatment. Although there were again no weight differences between groups at birth, growth retardation occurred prior to weaning in progeny from mothers treated with higher doses of the opiate (Fig. 2). There was also a marked increase in deaths among the pups of morphine-treated females (62 percent), so that statistical analysis of differences beyond 5 weeks of age was not feasible.

To assess the role of postnatal influences on the observed growth effect, cross-fostering experiments were performed. As in the first experiment, ten rats were treated for 5½ days prior to drug withdrawal and subsequent mating. At 1 to 3 days after birth, offspring of the two groups of females were exchanged; progeny of morphine-treated females were placed with saline-injected foster mothers, and offspring of the control females were placed with mothers which had previously received morphine. The neonates remained with their foster mothers until weaning. Although there is some modification of the growth effect among cross-fostered animals both in time of onset and in sex differences, the main effect of treatment is still evident and significant (Fig. 3). Weight differences between progeny of treated and control mothers remain and are not eliminated by the cross-fostering procedure. Since manipulation of infant rodents has residual effects both on behavior and on response to drugs (*7*), the additional handling required by the cross-fostering procedure itself may have contributed to the differences observed. An additional comparison, between nonfostered offspring of the saline-injected mothers and similar control offspring cross-fostered to morphine-treated foster mothers, revealed no detrimental effect of nursing by the morphine-treated mothers on growth of the young.

It thus appears that postnatal effects,







Figs. 1 and 2 (top). Mean body weight of offspring of female rats treated with saline (dash lines) and morphine (solid lines). Significant differences (t-test for independent groups) between control and experimental groups are indicated by asterisks. Fig. 1 (left). Total dose in 5½ days, 36.5 mg; $*P < .05$; $***P < .001$. Fig. 2 (right). Total dose in 10 days, 106 mg; $*P < .05$; $**P < .01$; $***P < .001$.

Fig. 3 (left). Mean body weight of cross-fostered offspring of saline- and morphine-treated female rats. Total dose in 5½ days, 36.5 mg. Significant differences (t-test for independent groups) between control (dash lines) and experimental groups (solid lines) are indicated by asterisks: $*P < .05$; $**P < .01$.

such as maternal care of the young or some factor transmitted through the milk, fail to explain the difference in rate of growth. In evaluating prenatal influences, we considered several possibilities. Morphine, by its ability to inhibit ovulation (*8*), may alter the gestation period or the reproductive cycle of the mothers. Any maternal debility and weight loss induced by the drug may likewise affect the gestation period or the size and weight of litters. However, we observed no differences in the gestation period or in litter size and weight between treated and control mothers. Moreover, no gross behavioral discrepancies between treated and control females were noted during the mating or gestation periods, although specific measures of behavioral activity were not determined. Nor were there any differences in body weight between the two groups of mothers during mating or gestation. Thus, factors implicating possible prenatal debility were absent.

It is difficult to postulate a mechanism whereby morphine exerts such a prolonged effect on animals not previously exposed to the drug. Numerous experiments have demonstrated that drugs may affect fetus development either by a direct chemical effect on the fetus or by an alteration of the maternal environment during the gestation period. The stage of embryonic development is critical for determining embryonic sensitivity to the drug and the pattern of abnormality induced. Defects resulting from the action of teratogens may be functional as well as morphologic and may be expressed at several stages: in early development, in the perinatal period, or during various postnatal periods (*9*). Doses of morphine approaching the maternal 50-percent lethal dose are teratogenic to mice when given during the 8th or 9th day of gestation (*10*). Treating gravid rats with various tranquilizers may also affect the later behavior of their developing offspring (*11*). However, these studies are not comparable to ours since they all involve exposure of the fetus to the drug.

In a recent study which reported that chronic exposure to a drug prior to pregnancy altered the behavior of offspring (*12*), the maternal-offspring behavioral interaction—which may be an important variable in evaluating the influence of any drug effect—was not considered. In addition, although there is no evidence that opium alkaloids are mutagenic, such a possibility cannot be ruled out. The possible selection, by the drug, of certain clones of gametes must also be considered. The many reported effects of opiates referable to the hypothalamus (*8*, *13*) could suggest a direct action of morphine on the hypothalamic-hypophyseal axis resulting, in some way, in retarded growth of the offspring. Although these interpretations are speculative, it is clear that morphine may exert a profound and long-lasting effect on animals never exposed to the drug.

Growth recovery to control levels was complete by 2 months of age in our studies; yet differences in the initial analgesic response to morphine between offspring of treated and untreated mothers were observed after that time (*5*). It is conceivable that other, and presently undefined, biochemical or physiologic changes persist even after differences in either growth rate or analgesic response are no longer discernible. Since in these studies the young had had no previous morphine experience either before or after birth, the alterations in both growth pattern and viability of offspring of morphine-treated females suggest a marked influence on the cells or organ systems of the mother.

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Inhibition of the Renin-Angiotensinogen Reaction by Pepstatin

Abstract. *Pepstatin, an* N*-acylated pentapeptide obtained from culture filtrates of actinomycetes and first characterized as an inhibitor of pepsin, produces inhibition of the renin-substrate reaction both in vitro and in vivo.*

Pepstatin has recently been demonstrated to be a specific inhibitor of acid proteases such as pepsin, casein proctase, and hemoglobin proctase (*1*). The compound has been isolated from culture filtrates of various species of streptomycetes and has been chemically characterized as a pentapeptide, whose structure is (*1, 2*): isovaleryl-L-valyl-L-valyl-4-amino-3-hydroxy-6-methylheptanoyl-L-alanyl-4-amino-3-hydroxy-6-methylheptanoic acid.

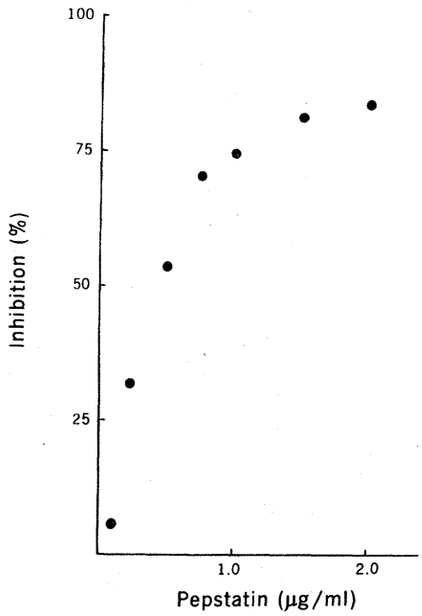

Fig. 1. Inhibition of the renin-angiotensinogen reaction by varying concentrations of pepstatin.

We have observed that pepstatin inhibits the action of the renal enzyme renin (a "neutral" protease) on its substrate (angiotensinogen, found chiefly in the α_2-globulin fraction of plasma) in vitro. The following protocol was used for determining the inhibition. Lyophilized substrate—prepared from nephrectomized rats according to Boucher *et al.* (*3*)—with a specific activity of 65 ng/mg was diluted to 4000 ng/ml with Sörensen phosphate buffer (0.067*M*) *p*H 7.4 (*4*). The actual incubation mixture consisted of 1 ml of substrate solution (4000 ng), 0.01 dog unit of hog renin (Nutritional Biochemical), and either 0.1 ml of 0.9 percent NaCl or 0.1 ml of Sörensen buffer containing varying amounts of pepstatin (0.1 to 2.0 μg). After 20 minutes of incubation at 37°C, the reaction was stopped by the addition of 0.2 ml of 1*N* HCl, and the tubes were placed in boiling water for 5 minutes. Finally, 0.7 ml of 0.067*M* Na_2HPO_4 was added to correct the *p*H. Pressor activity was assayed in the urethan-anesthetized, ganglion-blocked rat nephrectomized 6 to 18 hours previously [see (*4*) for further details].

Incubation without pepstatin yielded 320 ng of angiotensin after 20 minutes. Values for the percentage of inhibition (Fig. 1) were obtained in comparison with this figure (nanograms of angiotensin formed in the presence of pepstatin/320 × 100 percent). As seen in Fig. 1, approximately 0.4 μg of pepstatin per milliliter of the incubation mixture inhibited the renin-substrate reaction by 50 percent. Under these conditions, maximum inhibition asymptotically approached 90 percent.

In the bilaterally nephrectomized rat, the intravenous injection of hog renin produces a sustained increase in blood pressure that is due to the continuous reaction of renin with its substrate (*5*). Our studies indicate that the intravenous injection of pepstatin, in doses of 50 to 200 μg, produces a reduction in the elevation of blood pressure caused by the injection of 0.005 dog unit of renin. This effect is relatively brief, with the blood pressure returning to preinjection levels within 10 to 20 minutes.

Pepstatin apparently has a very low toxicity; LD_{50} (lethal dose, 50 percent effective) values obtained by intraperitoneal injection ranged from 1090 mg/kg for mice to 450 mg/kg for dogs, with the values for rats and rabbits being intermediate (*1*). As such, pepstatin could become a valuable tool for investigating the role of renin in various forms of experimental hypertension. It also has theoretically important applications in the treatment of acute renal failure, in which the intrarenal release of renin is thought to be a principal agent in the production of renal damage. The kinetic characteristics of this unique renin inhibitor and its possible in vivo applications remain to be studied.

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