those described in human and experimental Wernicke's disease where the mammillary bodies, anterior cerebellar vermis, gracile nuclei, and peripheral nerves are involved. It seems unlikely that 2,5-hexanedione damages these areas because of an induced thiamine deficiency, since many of the principal abnormalities of Wernicke's disease (macrophage response and periaqueductal and periventricular degeneration) were not present.

Pathological changes in visual nuclei and mammillary bodies probably accompany many human toxic neuropathies of the distal axonopathy type, yet there has been scant documentation of visual or mental state deterioration. Two factors may explain this discrepancy. (i) Few of the clinical reports included detailed analysis of visual function (none has reported visual evoked responses) or serial mental status evaluation. (ii) Many of the case reports involved short, high-level exposures and the clinical manifestations were mild and readily reversed (*1*).

The importance of these findings may lie in the field of public health. *n*-Hexane is widely used as an industrial solvent and is one of the many hydrocarbon components of gasoline. From this study it seems likely that prolonged, low-level industrial or environmental exposure to *n*-hexane or to the other environmentally prominent compounds that evoke distal axonopathy (acrylamide, carbon disulfide, cresyl phosphate) will provoke subtle changes in areas of the nervous system which may be vital to memory and vision. As the neuronal function and population decline in the course of the normal aging process, individuals exposed to such compounds might experience premature or accelerated deterioration in vision and mentation.

Note added in proof: Pilot studies have shown that acrylamide monomer, a chemical widely used in the plastic industry, also produces in rats distal axonal degeneration in the hypothalamus, optic tract, and anterior cerebellar vermis concurrent with a peripheral neuropathy.

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8. Four young adult cats were intoxicated and two were used as controls. All animals were housed in cages provided with smooth floors designed to prevent trauma to plantar nerves. The neurotoxin was administered by mouth by allowing the animals free access to drinking water containing 0.5 percent 2,5-hexanedione for periods up to 136 days. Control cats drank water as desired. All animals were weighed periodically, examined for signs of physical or neurological deterioration, and perfused through the aortic arch with 4 percent paraformaldehyde followed by 5 percent glutaraldehyde, each in 0.1*M* phosphate buffer (*p*H 7.4). After perfusion, tissue was removed from the peripheral and central nervous systems, postfixed with 2 percent Dalton's chrome osmium, embedded in epoxy resin, and processed for light and electron microscopy.
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Estrogen Formation in the Early Rabbit Embryo

Abstract. *Androgen formation (3β-hydroxysteroid dehydrogenase activity) was detectable in the rabbit blastocyst on day 5 of gestation (before implantation); estrogen formation was first detectable on day 7. The capacity to form estrogen on the day of implantation suggests that estrogen formation in the blastocyst may play a role in the implantation process.*

Indirect evidence suggests that estrogen action is involved in establishing direct contact between mother and embryo at implantation. In the rat, progesterone and estrogen in the maternal circulation are required for the initiation of blastocyst implantation, and estrogen appears to facilitate implantation even in species (such as the rabbit) that apparently lack an absolute requirement for maternal estrogen (*1*). The observation that antiestrogen inhibits implantation in intact (*2*) and ovariectomized, progesterone-treated rabbits (*3*) suggests that small amounts of estrogen are essential even in this species. Although the origin of estrogen for implantation of the blastocyst in the ovariectomized rabbit has not been defined, embryos of several species are capable of a variety of steroid hormone transformations before and after implantation (*4–6*). On the basis of histochemical studies of 3β- and 17β-hydroxysteroid dehydrogenase (E.C. 1.1.1.51) activities and the measurement of estradiol content in rabbit blastocysts, Dickmann *et*

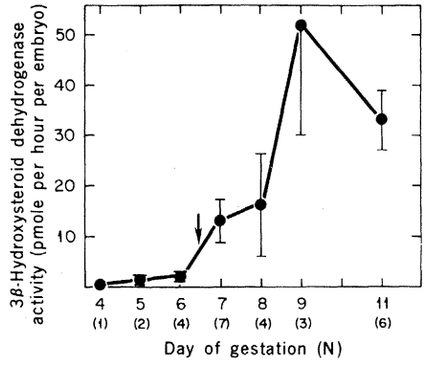

Fig. 1. Developmental pattern of 3β-hydroxysteroid dehydrogenase activity in the early rabbit embryo. Rabbit blastocysts and early embryos with membranes were recovered at various times during gestation and incubated in the presence of 5 μ*M* [7-^{3}H]dehydroepiandrosterone (30 c/mmole) for 2 hours at 37°C. At the end of the incubation, the reactions were stopped with chloroform; methanol (in a ratio of 2:1) and the reaction products, androstenedione and testosterone, were isolated and quantified (*8*). Six to eleven embryos (days 4 and 5), three embryos (day 6), or one embryo (days 7 to 11) were used for each determination. The data are presented as the mean ± the standard error of the mean for the number of determinations shown in parentheses, with the exception of day 4, which is a single determination, and day 5, which is the mean with the range of two observations. The arrow indicates the approximate time of implantation.

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al. have proposed that blastocyst estrogen has a primary role in implantation (*5*). Furthermore, they have shown that estrogen-enriched eggs transferred to uteri of pseudopregnant rabbits cause a local increase in capillary permeability similar to the onset of the decidual reaction (*7*). However, the question of whether the blastocyst of the rabbit is itself capable of synthesizing estrogen or if it merely absorbs estrogen from the milieu has not been resolved.

Using sensitive enzymatic assays (*8*), we have quantified the capacity of the rabbit embryo to synthesize androgens from circulating precursors and to convert androgens to estrogen before and after implantation. Sexually mature New Zealand rabbits were mated, and the onset of pregnancy was established as the time of observed copulation. Blastocysts were collected by perfusing the uterus with 10 ml of Eagles minimal essential medium. Implanted embryos were obtained by dissecting the uterine wall.

The conversion of [7-^{3}H]dehydroepiandrosterone to androstenedione and testosterone was used to assess 3β-hydroxysteroid dehydrogenase activity (*8*), the rate-limiting reaction in androgen synthesis in tissues of the rabbit embryo (*9*). For assessing estrogen synthesis, blastocysts or embryos with fetal membranes were incubated with [1,2,6,7-^{3}H]testosterone, and the estradiol formed was isolated and measured (*8*).

The developmental pattern of 3β-hydroxysteroid dehydrogenase activity in the early rabbit embryo is shown in Fig. 1 and that of estrogen synthesis in Fig. 2. Estradiol-17β synthesis first appeared in the day 7 embryo (0.2 pmole per hour per embryo) peaked at a concentration approximately tenfold higher (2.1 pmole per hour per embryo) in the day 8 embryo, thereafter declining to undetectable concentrations (Fig. 2A). This developmental pattern was similar when the data were expressed per milligram of protein (Fig. 2B). While estrogen formation was not detectable in uterine tissue taken from the site of implantation on day 9 of gestation, it was readily detectable by day 11 in placental tissue (3.5 pmole per hour per milligram of protein).

The assumption has been made that, in this tissue as in other rabbit embryo tissues (*9*), 3β-hydroxysteroid dehydrogenase is the rate-limiting enzyme for converting circulating precursors such as dehydroepiandrosterone, the major 19-carbon steroid of the adrenal, to androgens. The appearance of 3β-hydroxysteroid dehydrogenase activity before implantation may be important in supplying androgen substrate for estrogen synthesis. It is of interest that 3β-hydroxysteroid dehydrogenase activity on day 5, although lower than the concentrations obtained later, is sufficient to provide saturating substrate for estrogen synthesis 2 days later. Thus, from day 5 it is likely that sufficient androgen is synthesized by the blastocyst to provide for estrogen formation. Androgens could also reach the blastocyst from the maternal circulation.

The fact that the rabbit blastocyst acquires the ability to synthesize estrogens at the time of implantation suggests that estrogen of embryonic origin may be important in implantation in this species. The findings also provide a possible explanation for the fact that, although estrogen appears to facilitate implantation in the rabbit (*3*), the maternal ovary itself is not essential for the process. Since estrogen formation has also been demonstrated in the blastocyst of the pig (*4*), estrogen synthesis can be inferred to be a common property of the blastocyst at the time of implantation.

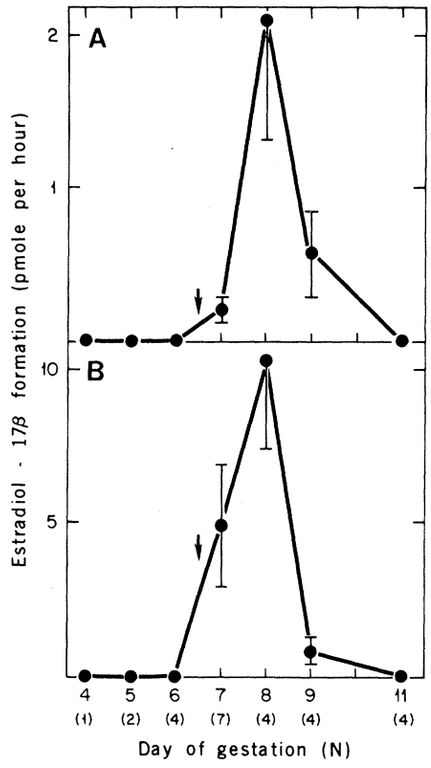

Fig. 2. Developmental pattern of estradiol-17β formation in the early rabbit embryo. Estradiol-17β was measured per embryo (A) and per milligram of protein (B). Rabbit blastocysts and early embryos with membranes were recovered at various times during gestation and incubated in the presence of 0.5 μM [1,2,6,7-^{3}H]testosterone (85 c/mmole) for 1 hour at 37°C. The [1,2,6,7-^{3}H]estradiol formed was purified and analyzed (*8*). On days 4, 5, 6, and 11 all determinations were zero. Proteins were determined by the method of Lowry *et al.* (*11*). The arrow indicates the approximate time of implantation. A total of 40 mg of authenic estradiol were added to the material chromatographing with estradiol after the final thin-layer procedure (*8*) of samples of blastocysts from days 7, 8, and 9. The estradiol was recrystallized five times in ether-petroleum ether (in a ratio of 1:1), and the radioactivity of the various mother liquors and final crystals was monitored. The average change in specific activity was less than 8 percent, which thus confirms that this activity does in fact represent estrogen synthesis.

It is of interest that the capacity of the embryo to aromatize androgens is transient, remaining measurable for only 3 days after the onset of implantation. The rate of estrogen synthesis in day 8 embryos is the highest (when expressed per milligram of protein) that we have observed in any tissue of the fetal rabbit (*8*). In subsequent stages of gestation, estrogen synthesis is demonstrable only in the placenta (from day 11), the ovary (from day 17.5) and the brain (from day 16 (*8, 10*). The anatomical location of the site of estrogen synthesis in the embryo at the time of implantation is unknown, although 3β- and 17β-hydroxysteroid dehydrogenase activities are more prominent in the embryonic disk than in the trophoblast (*5*).

The results indicate that the early rabbit embryo is capable of androgen synthesis from some precursors and of converting the androgen to estrogen. The estrogen formed by the embryo may be of critical importance in implantation.

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