of the SP analog is consistent with specificity of its action on the SP receptor. In this context, it is pertinent to point out that another SP analog, (D-Pro^2,D-Trp^{7,9})-SP, has a partial agonistic effect on taenia coli isolated from guinea pigs (*12*).

The finding that the noncholinergic transmissions of cells of guinea pig inferior mesenteric ganglia are specifically inhibited by an SP analog should be considered in the light of the observations that nerve fibers of these ganglia exhibit SP immunoreactivity (*3*), that SP immunoreactivity can be released by solutions high in potassium in a calcium-dependent manner (*7*), and that the membrane depolarization induced by exogenous SP resembles the noncholinergic depolarization elicited synaptically (*5, 6*). All this evidence strongly supports the hypothesis that SP or a closely related peptide is the transmitter responsible for the generation of the noncholinergic depolarization. Furthermore, the demonstration that SP-containing fibers in the inferior mesenteric ganglia originate from sensory neurons in the dorsal root ganglia (*4, 7, 8*) raises the possibility that SP is involved in the transmission of sensory signals from the gastrointestinal tract to the spinal cord as well as to the prevertebral ganglia (*3*); this provides a mechanism for a peripheral reflex regulation of the gastrointestinal activity. Finally, the evidence indicating that SP acts as a sensory transmitter in autonomic ganglia is consistent with its proposed function as a transmitter released from primary afferent fibers in the spinal cord (*13*).

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10. Inferior mesenteric ganglia with their left and right hypogastric nerves and superior cervical ganglia with their cervical sympathetic trunks were rapidly removed from male guinea pigs and rabbits, respectively. The ganglia were continuously superfused with a Krebs solution of the following composition (m*M*): NaCl, 117; KCl, 4.6; $CaCl_2$, 2.5; $MgCl_2$, 1.2; $NaHCO_3$, 25; NaH_2PO_4, 1.2; and glucose, 11.5. The solution was equilibrated with 95 percent O_2 and 5 percent CO_2 and maintained at 35° to 36°C. Microelectrodes filled with 3*M* KCl and having a tip resistance of 30 to 50 megohms were used for intracellular recording and stimulating. Presynaptic stimulation was accomplished by the suction electrode method, in which the hypogastric nerves or the cervical sympathetic trunks were drawn into a small glass pipette for orthodromic stimulation. The potential changes were recorded on a Tektronix oscilloscope and on a Gould Brush pen recorder (model 2200). The figures were reproduced from the tracings of the pen recorder. The peptides, substance P, (D-Pro^2,D-Phe^7,D-Trp^9)-SP and LRF were purchased from Peninsula Laboratories.
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Rabbit Hepatic Progesterone 21-Hydroxylase Exhibits a Bimodal Distribution of Activity

Abstract. *Progesterone 21-hydroxylase activity varies extensively among liver microsomes prepared from individual New Zealand White (NZW) rabbits. The 21-hydroxylase activities are distributed between two groupings that differ by more than tenfold in mean activity. Both male and female animals are represented in the two groupings. However, females exhibited the higher activity more frequently than males. The 21-hydroxylation of progesterone is catalyzed by one of the liver microsomal cytochrome P-450 isozymes, form 1, and these differences in activity are suggestive of differences in the occurrence of this isozyme among NZW rabbits.*

The product of the 21-hydroxylation of progesterone is deoxycorticosterone (DOC), a hormone that affects sodium retention. This reaction is catalyzed by a microsomal form of cytochrome P-450 which occurs most prominently in the adrenal cortex where DOC serves as a precursor for the formation of other adrenal steroid hormones (*1*). Plasma concentrations of DOC increase in humans during pregnancy. However, this has been attributed to the extra-adrenal formation of DOC from circulating progesterone, a hormone that is secreted in large amounts during the latter part of pregnancy (*2, 3*). We have recently identified a specific form of rabbit liver microsomal cytochrome P-450, form 1, that catalyzes this reaction (*4*). Form 1 is one of several forms of cytochrome P-450 that metabolize a wide variety of endogenous and exogenous compounds including drugs and carcinogens as well as steroid hormones (*5*). Many factors are known to alter the expression of the multiple forms of cytochrome P-450 and thereby alter the metabolism of drugs, carcinogens, and steroid hormones (*6*). In the case of progesterone, differences in the expression of cytochrome P-450 form 1 could affect the extent of DOC formation from progesterone. Since DOC can affect sodium retention, increases in the production of DOC from progesterone in the liver could exacerbate hypertensive conditions that arise frequently during pregnancy. In this report, we describe extensive differences in the initial rate of hepatic microsomal 21-hydroxylation among New Zealand White rabbits. These variations are suggestive of differences in the occurrence of cytochrome P-450 form 1 among these animals.

For this study, New Zealand White rabbits (12 males and 11 virgin females, 3 to 4 kg) were purchased from commercial rabbitries in the San Diego area. The animals were given continuous access to food and water. The food was then withdrawn, and after a 24-hour fast the animals were killed by intravenous administration of lethal doses of sodium pentobarbital. Microsomes were prepared (*7*), suspended in 50 m*M* potassium phosphate, *p*H 7.4, containing 20 percent glycerol, and stored at −60°C. Protein and cytochrome P-450 concentrations were determined as described by Bensadoun and Weinstein (*8*) and by Omura and Sato (*9*), respectively.

Each 1.0-ml reaction mixture con-

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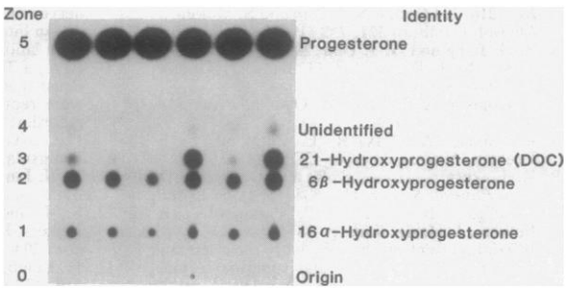

Fig. 1. Autoradiographic image of the separation of progesterone and its metabolites by thin-layer chromatography. Each lane displays metabolites extracted from reaction mixtures containing equal amounts of liver microsomes prepared from individual New Zealand White rabbits. Three of the principal metabolites are identified by the legend on the right. Assay procedures are described in the text.

tained 50 μmole of potassium phosphate, *p*H 7.4, 4.0 μmole of glucose 6-phosphate, 1.4 μg of glucose-6-phosphate dehydrogenase, 2.0 μmole of $MgCl_2$, 30 to 50 μg of microsomal protein, and specified amounts of [^{14}C]progesterone (Amersham; 56 mCi/mmole) added in 10 μl of methanol. After addition of the substrate, the reaction mixture was incubated for 3 minutes at 37°C before the reaction was initiated by the addition of 0.5 μmole of reduced nicotinamide adenine dinucleotide phosphate (NADPH). The reaction was terminated after 5 minutes by the extraction of the substrate and products into 9 ml of chloroform. A portion of the organic phase was evaporated to dryness, and the residue was dissolved in 25 μl of ethyl acetate and applied to a thin-layer chromatography plate coated with silica gel (Bakerflex IBF-2) by means of an Atlas semiautomatic applicator (Analytichem International). The plates were developed by means of the sequential application of two solvent systems as described by Menard *et al.* (*10*). Portions of the silica gel plate that contained radioactive material as visualized by autoradiography were removed for scintillation counting. The total recovery of progesterone and its major metabolites was 85 ± 10 percent. The addition of cold metabolites to the incubation mixture prior to extraction with chloroform did not affect recoveries.

Rabbit liver microsomes have been shown to hydroxylate progesterone at carbons 6, 16, and 21, although no single study has clearly documented the simultaneous formation of all three metabolites (*11*). As shown in Fig. 1, the thin-layer chromatographic procedure used in this study resolves three major metabolites that were seen to comigrate with authentic 6β-, 16α-, and 21-hydroxyprogesterone. Antibody to NADPH-cytochrome P-450 reductase inhibited the production of all three of these metabolites but did not alter the rate of formation of the unidentified metabolite. The identification of the product of 21-hydroxylation, DOC, was corroborated by mass spectral analysis. The amount of DOC produced under similar assay conditions by the five preparations of microsomes varied considerably (Fig. 1). When microsomes exhibiting the highest activities were mixed with microsomes displaying the lowest 21-hydroxylase activities prior to the reaction, the rate of catalysis was additive. This finding suggested that these differences cannot be attributed to the presence of an endogenous, diffusible inhibitor or to secondary metabolism of the DOC by those microsomes exhibiting the lower apparent rate of 21-hydroxylation. When the dependence of the initial rate of 21-hydroxylation of progesterone on the initial concentration of progesterone was examined for several preparations of microsomes exhibiting widely differing rates of metabolism, they displayed similar apparent Michaelis constants of 2 μ*M* but varied extensively in apparent maximum velocity.

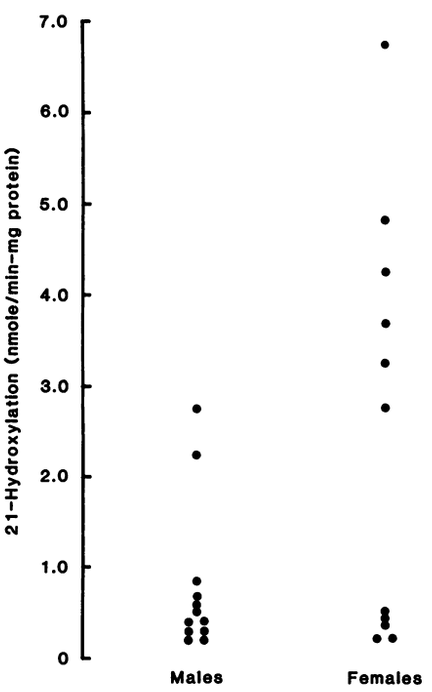

Fig. 2. Scatter-diagram showing the distribution of initial rates of progesterone 21-hydroxylase activity exhibited by liver microsomes prepared from individual male and female New Zealand White rabbits. Assay procedures are described in the text.

Preparations of liver microsomes from 12 male and 11 female animals were screened by using an initial concentration of 10 μ*M* progesterone to examine the range and the distribution of 21-hydroxylase activity. The initial rate of formation ranged from 0.18 to 6.8 mU, where a milliunit is 1 nmole of DOC formed per minute per milligram of microsomal protein. A bimodal distribution of initial rates was observed (Fig. 2). Microsomes prepared from 8 of the 23 animals exhibited high 21-hydroxylase activity, with an average rate of 3.8 ± 1.5 mU, whereas the remaining preparations exhibited a tenfold lower average rate of 0.36 ± 0.22 mU. The average specific content of cytochrome P-450 in the group of microsomes exhibiting the high 21-hydroxylase activity was roughly 25 percent greater than that of the microsomes exhibiting the lower activity, 2.3 ± 0.2 as opposed to 1.8 ± 0.4 nmole of P-450 per milligram of protein ($P < .01$; Student's *t*-test). Males and females were represented in both groupings. Although the high 21-hydroxylase activity was seen more frequently for female (50 percent) than for male (20 percent) rabbits, the statistical significance of this difference could not be established with the number of animals examined in the present study (*12*). Sex-specific differences in a number of monooxygenase activities associated with the cytochrome P-450 system have been noted in the rat but rarely observed in other species (*13*). However, since differences in 21-hydroxylase activity can be seen for both sexes in the rabbit, any sex-related factors that might affect the expression of the 21-hydroxylase would probably differ from those leading to sex-specific differences for the rat liver monooxygenases.

The bimodal distribution of progesterone 21-hydroxylase activity reported here is suggestive of differences in the occurrence or induction of a specific form of cytochrome P-450. Recent work in our laboratory (*4*) has demonstrated that one of the multiple forms of cytochrome P-450 prepared from rabbit liver microsomes, form 1, catalyzes this reaction, and the identification of microsomes with elevated levels of this cytochrome has greatly facilitated its isolation. The differences in 21-hydroxylase activity that we have observed among New Zealand White rabbits may mimic a situation that occurs in the human population. Winkel *et al.* (*3*) have noted tenfold differences in the fractional conversion of progesterone to DOC among both

male and female human individuals. The rabbit may therefore provide a useful animal model in which differences in the extra-adrenal formation of DOC during pregnancy can be studied.

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Abnormal Development of Kitten Retino-Geniculate Connectivity in the Absence of Action Potentials

Abstract. *Action potentials were silenced in one eye of neonatal kittens by repeated intraocular injections of tetrodotoxin for 5 to 8 weeks. After tetrodotoxin blockade was allowed to wear off, receptive field properties of individual relay cells in the lateral geniculate nucleus were examined. The many* ON-OFF *and binocular fields found in the layers that receive input from the treated eye suggest that these cells had extremely abnormal retino-geniculate synaptic connections. These effects were different in kind from those seen after deprivation rearing that does not silence action potentials. Lack of action potential activity was concluded to lead to abnormal development in the central nervous system.*

Action potential activity per se has been suggested, mainly on theoretical grounds, as affecting development of central nervous system (CNS) organization (*1*, *2*). Some experiments in the peripheral nervous system support this idea. For example, abolition of impulse activity retards reduction from polyneuronal to mononeuronal innervation of developing rat muscle fibers (*3*). Studies in the CNS show that environmentally induced distortion of neural activity results in abnormal connections in the cat visual system (*4*). None of these experiments abolished action potentials, however, which continue for visual neurons even in the dark. Lack of a role for action potentials is suggested by the result that an axolotl eye sensitive to tetrodotoxin (TTX) and silenced by transplantation to a newt with endogenous TTX can develop an anatomically normal retino-tectal projection (*5*). We now report severe defects in the lateral geniculate nucleus (LGN) of kittens reared with the action potentials from one eye totally abolished by intraocular TTX injections. These defects are different from any reported in other types of deprivation rearing experiments on the kitten visual system and are thus presumed to be caused by the abolition of impulse activity.

Tetrodotoxin, a sodium channel blocking agent, injected into the vitreous humor of an eye reversibly blocks the firing of action potentials in retinal ganglion cells. One intraocular TTX injection in a dose tolerated by the animal produces complete blockade for more than 48 hours. It is possible to maintain blockade for weeks by reinjections at 2-day intervals. Total recovery of ganglion cell activity occurs 5 to 7 days after the final TTX injection (*6*).

Each of seven kittens received unilateral, intraocular TTX injections on alternate days, beginning 1 to 5 days after birth and continuing for 5 to 8 weeks. Animals were set up for single-unit recording 1 to 3 weeks after the final injection, according to standard techniques (*7*, *8*). Units were recorded from both the left and right LGN and on occasion in the retina and optic tract. Receptive fields were characterized with hand-held and flashing spot targets (*8*, *9*), and were all within 25° of the area centralis. No units in the LGN C layers are reported here, although binocular units at the Al-C boundary are included. We studied 70 cells in deprived laminae, 11 binocular cells at laminar boundaries, and 53 cells in laminae innervated by the uninjected eye.

Several hundred recordings of ganglion cells and optic tract fibers from injected eyes failed to show any gross abnormality. This is not too surprising, since the region studied is fairly mature at birth and also because all retinal neurons distal to ganglion cells utilize slow potentials that are unaffected by TTX. Ganglion cell receptive fields were either ON-center or OFF-center, and they had other properties appropriate for kittens of the ages studies (*7*). In contrast, relay cells in the deprived LGN layers were strikingly abnormal. Normally, and also after monocular, binocular, or dark-rearing deprivation (*9–11*), LGN cells have either ON-center or OFF-center receptive fields that result from excitatory inputs from either ON-center or OFF-center retinal ganglion cells, but never from both types (*9*). Nearly 40 percent of cells recorded from in the deprived layers of TTX-treated animals received both ON and OFF excitatory input (ON-OFF cells in Fig. 1) (*12*). The LGN layers innervated by the uninjected eye served as controls, as did the LGN of an additional animal that had received a full series of sham injections (*13*); no ON-OFF cells were found in any of these cases. The fact that nondeprived LGN layers were normal rules out any systemic, nonspecific TTX effects. Further, it is unlikely that TTX has disruptive effects other than action potential blockade; its action is specific, and it neither destroys cells nor blocks axonal transport (*14*). The ON-OFF cells were not an artifact of stimulation characteristics. The ON-OFF responses occurred to a wide variety of stimulus spot sizes and intensities. Stimulus centering in the receptive field was carefully controlled. Despite deliberate stimulus decentering, ON-OFF histograms similar to Fig. 1 could not be generated in normal ON or OFF units.

Retinal ganglion cells can be classified as X and Y types, as well as ON-center and OFF-center types (*15*). In normal animals, inputs to LGN cells are segregated with respect to X and Y types,

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