Type Ia and type Ib ganglion cells also differ in the diameters of their dendritic fields at a given retinal eccentricity. This disparity exists throughout the retina but is marked in the central retina (Fig. 2). Dendritic field diameter of both types increases with increasing retinal eccentricity, but type Ia cells are consistently larger than type Ib cells (Fig. 2A).

The morphological differences (and differences in connections) between class I and class II ganglion cells probably underlie the physiological distinction between "transient" and "sustained" cells and perhaps also between "Y" (nonlinearly summing) and "X" (linearly summing) cells, respectively (*6, 9, 10*). If we assume a relationship between dendritic field diameter and receptive field center size (*11*), we can compare the dimensions of class I cells in Golgi preparations with Cleland and Levick's data (*9*) on field center size of "brisk" transient cells (Fig. 2B). Both the dendritic fields of class I cells and the field centers of transient cells increase in diameter with retinal eccentricity (*6, 9*). OFF-center transient cells are larger than ON-center transient cells, just as type Ia cells are larger than type Ib cells at a given retinal eccentricity. Hammond's more extensive physiological data (*9*), from units with eccentricities up to 35°, also support this finding. Finally, some central, tufted, broadly stratified, type Ib cells have particularly small dendritic field diameters (Fig. 2A) as do some ON-center transient cells (Fig. 2, A and B). This comparison supports the hypothesis that ganglion cells branching in sublamina *a* will be OFF-center and those branching in sublamina *b*, ON-center.

Our hypothesis pertains to the responses of ganglion cells with direct cone bipolar input but implies nothing concerning the response polarity of either of the two types of cone bipolar cells. ON- or OFF-center responses of ganglion cells could be determined by any of several disynaptic sequences of excitatory and inhibitory synapses. Nevertheless, there is ultrastructural evidence that flat cone bipolar cells receive excitatory synapses from cone photoreceptors [which hyperpolarize to photic stimulation (*12*)], but invaginating cone bipolar cells do not (*13*). The simplest interpretation of these findings is that the flat cone bipolar cell is hyperpolarizing to photic stimulation, or OFF-center, when the flow of excitatory transmitter from the photoreceptor is diminished, and the invaginating cone bipolar cell is depolarizing, or ON-center, under the same conditions of illumination. The presence of ON/OFF dualism in cone bipolar cells of the cat is at least plausible, for such ON/OFF symmetry has been found among bipolar cells in nonmammalian vertebrates (*14*). If both flat and invaginating cone bipolar types make excitatory synapses on ganglion cells, as Naka has found in catfish for both ON- and OFF-center bipolar cells (*15*), then inferences made from the synaptic ultrastructure (*13*) are consistent with our hypothesis that sublamina *a* contains the processes of OFF-center cells and sublamina *b* the processes of ON-center cells.

It has been supposed that stratification of the IPL [so prominent in typical nonmammalian vertebrates (*16, 17*)] is of little functional significance in mammals (*18*). This opinion stands in contrast to Cajal's view that the IPL is similarly organized in all vertebrates (*16*). More study is needed to determine the precise meanings of multiple stratification in the IPL of the vertebrate retina. It is now evident, however, that a bisublaminar organization of the IPL is characteristic of the mammalian retina, that it may obscure but never obliterates Cajal's many-tiered stratification, and that it is designed to serve ON/OFF symmetry in retinal ganglion cells.

E. V. FAMIGLIETTI, JR.
Laboratory of Vision Research, National Eye Institute, Bethesda, Maryland 20014

HELGA KOLB
Laboratory of Neurophysiology, National Institute of Neurological and Communicative Disorders and Stroke, Bethesda

References and Notes

1. D. Baylor, M. G. F. Fuortes, P. O'Bryan, *J. Physiol. (London)* **214**, 265 (1971).
2. S. Kuffler, *J. Neurophysiol.* **16**, 37 (1953).
3. B. B. Boycott and H. Kolb, *J. Comp. Neurol.* **148**, 91 (1973).
4. L. Missoten, *The Ultrastructure of the Human Retina* (Arscia, Brussels, 1965); H. Kolb, *Philos. Trans. R. Soc. London Ser. B* **258**, 261 (1970).
5. H. Kolb and E. V. Famiglietti, Jr., *Science* **186**, 47 (1974); E. V. Famiglietti, Jr., and H. Kolb, *Brain Res.* **84**, 293 (1975); H. Kolb, E. V. Famiglietti, Jr., R. Nelson, *Jpn. J. Ophthalmol.*, in press.
6. B. B. Boycott and H. Wässle, *J. Physiol. (London)* **240**, 397 (1974).
7. D. F. Wann, T. A. Woolsey, M. L. Dierker, W. M. Cowan, *IEEE Trans. Bio-Med. Electron.* **20**, 233 (1973). Dendrites were sampled at 2- to 3-μm intervals and logged into an image-processing computer which drives the stage and focus of an optical microscope. Images of the dendritic trees could be rotated about any of three axes.
8. E. Ramón-Moliner, *J. Comp. Neurol.* **119**, 211 (1962). The "tufted" branching pattern of retinal ganglion cells is a consequence of "pinnate" branching (many small stems emerging along the length of a single thick stalk) and "palmate" branching (sprays of terminal branches arising from the end of a thicker stalk), both grafted onto the basic dichotomous pattern of "radiate" branching.
9. C. Enroth-Cugell and J. Robson, *J. Physiol. (London)* **187**, 517 (1966); B. Cleland, M. W. Dubin, W. R. Levick, *ibid.* **217**, 473 (1971); B. G. Cleland and W. R. Levick, *ibid.* **240**, 421 (1974); ———, H. Wässle, *ibid.* **248**, 151 (1975); P. Hammond, *ibid.* **242**, 99 (1974); Y. Fukada, *Vision Res.* **11**, 95 (1971); J. Stone and Y. Fukuda, *J. Neurophysiol.* **37**, 722 (1974).
10. E. V. Famiglietti, Jr., in preparation; ——— and H. Kolb, in preparation; ———, annual meeting of the Association for Research in Vision and Ophthalmology, Sarasota, Fla., 28 April to 2 May 1975; H. Kolb and E. V. Famiglietti, Jr., *ibid.*
11. A. Gallego, *An. Inst. Farmacol. Esp.* **3**, 31 (1954).
12. T. Tomita, *Cold Spring Harbor Symp. Quant. Biol.* **30**, 559 (1965); D. Baylor and M. G. F. Fuortes, *J. Physiol. (London)* **207**, 77 (1970); R. Nelson, *J. Comp. Neurol.*, in press.
13. E. Raviola and N. B. Gilula, *J. Cell Biol.* **65**, 192 (1975).
14. F. S. Werblin and J. E. Dowling, *J. Neurophysiol.* **32**, 339 (1969); A. Kaneko, *J. Physiol. (London)* **207**, 623 (1970).
15. K.-I. Naka, *Invest. Ophthalmol.*, in press.
16. S. Ramón y Cajal, *The Structure of the Retina* (Thomas, Springfield, Ill., 1972).
17. J. Y. Lettvin, H. Maturana, W. Pitts, W. S. McCulloch, in *Sensory Communication*, W. Rosenblith, Ed. (MIT Press, Cambridge, Mass., 1961), p. 757. Seeking a morphological counterpart of their "bug detector" in Cajal's work (*16*), Lettvin *et al.* selected the bistratified cell (their H type) as a prototype, suggesting that such ganglion cells achieved feature detection by differencing the inputs in two individual strata of the IPL [see also J. Schipperheyn, *Acta Physiol. Pharmacol. Neerl.* **13**, 231 (1965)]. It is not clear, however, that they regarded the IPL as fundamentally bisublaminar. Their proposals will be more fully discussed (E. V. Famiglietti, Jr., in preparation).
18. B. B. Boycott and J. E. Dowling, *Philos. Trans. R. Soc. London Ser. B* **225**, 109 (1972).
19. We thank R. Nelson for his helpful discussion and P. Gouras for his support. We are grateful to W. M. Cowan for permission to use the image-processing computer developed under NIH contract NEI 71-2289. We thank T. A. Woolsey and M. L. Dierker for help with its use. For a part of this study, E.V.F. was supported by PHS postdoctoral fellowship 1 F22 EYO 1488-01.

25 March 1976; revised 18 June 1976

Cytochrome P-450 and Drug Metabolism in *Trypanosoma cruzi*: Effects of Phenobarbital

Abstract. *The epimastigotes of* Trypanosoma cruzi *hydroxylate drugs at substantial rates. The activity, which is of the mixed-function oxidase type, is increased by phenobarbital and is inhibited by CO, SKF 525-A, and metyrapone. The hydroxylation is paralleled by increases in free and membrane-bound ribosomes.*

Although Chagas' disease, which is caused by *Trypanosoma cruzi*, is a major health problem, no chemotherapeutic agents have been found that will cure the disease in animals or man. The failure of drugs to affect the parasite may be due to a permeability barrier in the flagellate (*1*) or to rapid conversion of the drugs to nontoxic compounds. We now present data indicating that the epimastigote forms of *T. cruzi* possess an active detoxifying system whose character-

istics are those of a monooxygenase system linked to P-450, and whose activity is increased by phenobarbital. Cytochrome P-450 is the terminal oxidase of a reduced nicotinamide adenine dinucleotide phosphate (NADPH) system that converts a number of lipid soluble compounds of both endogenous and exogenous origin into more polar compounds (*2*). Cytochrome P-450 appears to serve both as the substrate-binding site and as the site of oxygen activation (*3*) of the enzyme system.

Stock cultures of *T. cruzi* were maintained in a monophasic (liquid) medium (*4*) at 27°C in the presence of 7.5 μM hemin (controls). Experimental cultures were grown in a diphasic (solid medium with liquid overlay) medium (*5*) in the absence of hemin. Phenobarbital (2 to 5 m*M*) was added to the growth medium 2 days after the inoculum, and the parasites were collected at the end of the exponential phase of growth (day 7) by centrifugation. They were washed three times with 100 volumes of Ringer-phosphate solution (*p*H 7.4), and resuspended in the same medium at a final concentration of about 1×10^9 cells per milliliter. The monooxygenase activity of *T. cruzi* toward aniline, *p*-nitroanisole, naphthalene, and aminopyrine was measured as follows: Cells in a final volume of 5 ml were incubated at 27°C with the corresponding substrate, and at zero time and timed intervals, the cell suspension was homogenized in a French press cell at 14.2 atm. Portions (1.0 ml) were pipetted into test tubes containing 0.1 ml of concentrated HCl for naphthalene and 0.1 ml of 100 percent trichloroacetic acid for aminopyrine and aniline. The formation of 1,2-dihydro-1,2-dihydroxynaphthalene and 1-naphthol, and formaldehyde and *p*-aminophenol was estimated as described (*6*). *p*-Nitrophenol was determined by its absorbance at 420 nm at *p*H 7.85 (*7*).

Epimastigotes of *T. cruzi* metabolize *p*-nitroanisole, aminopyrine, and aniline at appreciable rates (Table 1); however, the substrates are slightly toxic, as judged by a moderate decrease in cell mobility. Naphthalene, which is highly toxic (no mobility), is not metabolized. Cells that are grown in the presence of phenobarbital show marked increases in hydroxylating activity and essentially normal mobility, which suggests that increased metabolism results in protection of the cells. Phenobarbital-treated cells still do not metabolize naphthalene at measurable rates, but some mobility is restored. Hydroxylation of *p*-nitroanisole is inhibited by CO, SKF 525-A, and metyrapone, all known inhibitors of microsomal monooxygenases (*2*) and is dependent on O_2 (Table 2). The inhibitory effects of SKF 525-A and metyrapone appear to be specific since they are not correlated with changes in cell mobility. Maximum effects are obtained with 0.5 to 5 m*M* phenobarbital. The addition of 5 m*M* phenobarbital to *T. cruzi* cultures increases the number of ribosomes in the cytoplasm and those associated with the endoplasmic reticulum and nuclear envelope (Fig. 1, a and b). Concomitantly, the density of the nucleoplasm and mitochondrial matrix increases, possibly because the amount of ribosomal protein increases in these two organelles. Compared with Fig. 1b, Fig. 1a (arrow) shows an increase in the density of the mitochondrial matrix. These changes correlate well with changes in the hydroxylating activity of *T. cruzi* cells. Difference spectroscopy (*8*) indicates that untreated cells contain 0.07 nmole of cytochrome P-450 per milligram of protein. This value increases to 0.34 nmole/mg in cells grown in 5 m*M* phenobarbital. Chemical analysis of microsomes from control cells shows an RNA to protein ratio of 0.088 and a lipid to protein ratio of 0.73. Treatment with phenobarbital increases the RNA to protein and the lipid to protein ratios to 0.18 and 2.14, respectively. The latter ratios are characteristic of the rough endoplasmic reticulum.

The hydroxylation system is localized in the microsomal fraction, which corresponds to 10 percent of the total protein. Thus, control and phenobarbital-

Table 1. Hydroxylation of drugs by *T. cruzi* epimastigotes in the presence (experimental) or absence (control) of 2 m*M* phenobarbital. The signs in parentheses indicate the mobility of the cells after the incubation period: +++, normal; ++, reduced; +, severely reduced; −, no mobility.

Substrate (4 m*M*)	Hydroxylation (nmole/mg protein per 3 hours)		
	Control cultures	Experimental cultures	Increase (%)
p-Nitroanisole	1.65 (++)	4.27 (+++)	258.7
Aminopyrine	1.65 (++)	3.31 (+++	200.6
Aniline	3.95 (++)	9.9 (+++)	253.8
Naphthalene	0.0 (−)	0.0 (+)	

Table 2. Effect of inhibitors on the *O*-demethylation of *p*-nitroanisole by *T. cruzi* epimastigotes grown in the absence of phenobarbital. Signs in parentheses indicate mobility as in Table 1.

Conditions	Hydroxylation (nmole *p*-nitrophenol/mg protein per 3 hours)	Percent
Control, air (atmospheric pressure)	1.7 (++)	100
CO atmosphere	0.0 (−)	0.0
N_2 atmosphere	0.0 (++)	0.0
SKF 525-A (0.042 μM)	0.0 (+)	0.0
Metyrapone (33.1 μM)	0.132 (−)	7.7
Metyrapone (66.2 μM)	0.076 (−)	4.4

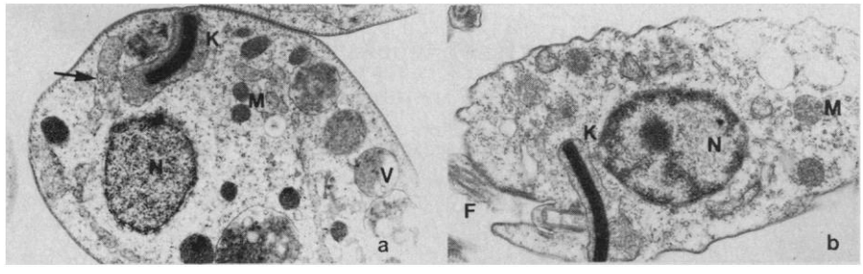

Fig. 1. Thin sections of *T. cruzi* grown (a) in the presence and (b) the absence of phenobarbital (5 m*M*). Cells were fixed in buffered 1.75 percent glutaraldehyde and then in buffered 2 percent osmium tetroxide; they were stained with uranyl acetate and lead citrate. (a) The dense mitochondrial matrix (arrow), kinetoplast (*K*), nucleus (*N*), microbodies (*M*), and endocytotic vacuoles (*V*) are indicated (× 14,500). (b) In this section the emergent flagellum (*F*) is indicated, the cisternal matrix of the mitochondrion is electron translucent, and the number of ribosomes per unit area of cell appears to be less than in the phenobarbital-treated cells (×15,000).

treated cells contain 0.7 and 3.4 nmole of cytochrome P-450 per milligram of microsomal protein, respectively. Microsomes also contain cytochrome b_5, cytochrome c reductase-NADPH (E.C. 1.6.2.3), and cytochrome b_5 reductase-NADH (E.C. 1.6.2.2) (*8*).

These results suggest that an important factor in the insensitivity of *T. cruzi* to chemotherapeutic agents may be its ability to metabolize foreign compounds at substantial rates and that this ability is increased by phenobarbital.

M. AGOSIN
C. NAQUIRA*
J. PAULIN
J. CAPDEVILA

Department of Zoology, University of Georgia, Athens 30602

References and Notes

1. M. Wittner, R. M. Rosenbaum, Y. Kress, *Ann. Trop. Med. Parasitol.* **69**, 19 (1975).
2. M. Agosin and A. S. Perry, in *The Physiology of Insecta*, M. Rockstein, Ed. (Academic Press, New York, 1974), vol. 5, p. 537.
3. J. B. Schenkman, O. Remmer, R. W. Estabrook. *Mol. Pharmacol.* **3**, 113 (1969); D. Y. Cooper, S. Levin, S. Narasimhulu, O. Rosenthal, R. W. Estabrook, *Science* **147**, 400 (1965).
4. L. S. Diamond, *J. Parasitol.* **54**, 715 (1968).
5. J. F. De Boisso and A. O. M. Stoppanni, *Proc. Soc. Exp. Biol. Med.* **136**, 215 (1971).
6. W. Bleecker, J. Capdevila, M. Agosin, *J. Biol. Chem.* **148**, 8474 (1973); J. Capdevila, N. Ahmad, M. Agosin, *ibid.* **250**, 1048 (1975).
7. K. J. Netter and G. Seidel, *J. Pharmacol. Exp. Ther.* **146**, 61 (1964).
8. M. Agosin, C. Naquira, J. Paulin, J. Capdevila, *Int. J. Biochem.*, in press.
9. Supported by NIH grant AI-12244. We thank C. Mothershed for technical assistance and the University of Georgia for use of the electron microscope facility.
* Present address: Instituto de Medicina Tropical, "Daniel A. Carrion," Universidad de San Marcos, Lima, Peru.

30 March 1976; revised 9 June 1976

Progesterone Binding to Hen Oviduct Genome: Specific versus Nonspecific Binding

Abstract. *Data are presented to explain discrepancies in the literature involving the in vitro binding of steroid receptor complexes to isolated nuclei and chromatin. The type of binding in vitro of the progesterone-receptor complex to nuclei, chromatin, or DNA of hen organs is largely determined by the ionic strength of the medium. Low ionic conditions (0.01 to 0.05 molar potassium chloride) result in a nonspecific, nonsaturable binding, while high ionic conditions (0.15 to 0.20 molar potassium chloride) create a tissue-specific, saturable binding. Pure DNA binds the steroid receptor complex extensively in low salt but very little in the higher salt conditions.*

There have been controversies in the literature concerning in vitro binding of steroid receptor complexes to cell nuclei. These controversies are concerned with the chemical identity of the nuclear binding sites (that is, acceptor sites) (*1–4, 5*) as well as with possible tissue-specific binding (*2, 3, 5–7*). This report explains some possible causes of these discrepancies and presents methods and conditions for assaying for tissue-specific, high-affinity binding.

Steroid hormones enter target cells and bind to receptor proteins with high affinity and stereospecificity. Part, if not all, of these complexes undergo an obscure "activation" process wherein they become capable of migrating and binding to the nuclei (*5, 8*). The result of this nuclear binding is an immediate alteration of nuclear transcription (*5, 9*). Nontarget tissues do not respond to physiological levels of the steroids because they do not contain "active" receptors. The presence in nontarget cells of inactive receptors not capable of binding steroids has not been investigated.

We assessed more carefully the binding that occurs in vivo as well as in vitro between isolated nuclei or chromatin and isolated progesterone-receptor complex (P-R) (*10*). More than one class of binding sites was observed in vivo and in vitro. The highest affinity class of binding sites was tissue specific, survived high ionic conditions (0.15 to 0.2*M* KCl), and represented the only type of nuclear binding in avian oviducts exposed to physiological levels of the hormone (1 to 17 ng per milliliter of serum). When this class was saturated in vivo with progesterone, the full responses of RNA polymerase I and II activities were observed (*10*). Although a higher level of binding to weaker sites could be achieved in vivo, there was no further alteration in polymerase activities. We feel that the highest affinity class of sites may represent the primary sites for steroid-induced alterations in transcription. We are thus able to selectively analyze (utilizing the high ionic conditions) binding in vitro only to the high affinity class of sites in isolated nuclei. Under low ionic conditions, the P-R binds in a nonsaturable, nonspecific fashion to many nuclear components. Under higher ionic conditions, however, a saturable binding to nuclear material is achieved which is tissue specific. Very little binding to DNA or other components occurs.

Figures 1 and 2 show the patterns of binding of P-R to the nuclear material of target tissue (oviduct) and nontarget tissue (spleen) under varying ionic condi-

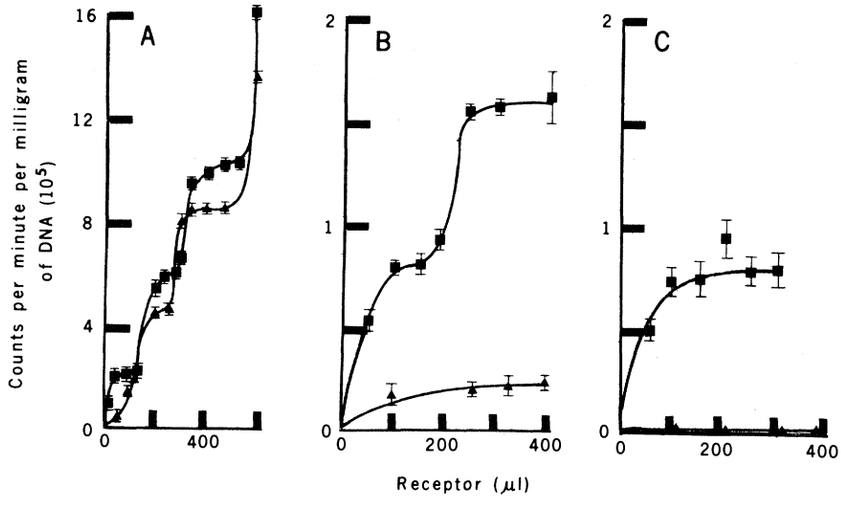

Fig. 1. Binding of isolated [^{3}H]progesterone-receptor complex (P-R) to (■) hen oviduct and (▲) spleen nuclei in (A) 0.05*M* KCl, (B) 0.10*M* KCl, and (C) 0.18*M* KCl. The isolation of partially purified P-R is described elsewhere (*10, 11*). The binding of the P-R to nuclei was performed according to the "standard method" in (A) and (C) and the "streptomycin method" in (B) (*10*). Briefly, 25 to 50 μg of DNA (as nuclei) was incubated at 4°C for 90 minutes with increasing levels of P-R. The incubation period was selected since equilibrium binding is achieved at the ratio of receptor to DNA used in these experiments (*10*). Tubes containing the binding assays were centrifuged, the pellets were washed several times in dilute buffers, and the nuclear material was collected on membrane filters and counted in a scintillation spectrometer. The micrograms of DNA per filter were then estimated by diphenylamine assay and the counts per minute per milligram of DNA were calculated as described (*10*).