the samples incubated with the trypsin or tyrosinase were assayed on the hemibladders from the same frogs. The culture media from day 28 were pooled, and antidiuretic activity was determined after incubation with trypsin and tyrosinase as described above. The antidiuretic activity was reduced by trypsin and tyrosinase incubation from 18 μunit/ml to less than 5 μunit/ml. The culture media from day 33 were also pooled, and antidiuretic activity was determined after reductive inactivation with sodium thioglycollate. Thioglycollate solution in Sorensen's phosphate buffer (0.1 ml) was added to 0.9 ml of culture media. Controls (0.9 ml) were incubated with 0.1 ml of phosphate buffer at the same temperature and *p*H without sodium thioglycollate. The antidiuretic activity of culture media incubated with 0.01*M* and 0.05*M* sodium thioglycollate for 3 hours at *p*H 7.4 and 37°C was reduced from 20 μunit/ml to 8 μunit/ml and less than 5 μunit/ml, respectively. Since sodium thioglycollate affects bladder permeability (*11*) no hydroosmotic reductive inactivation experiments were performed. Neither in the culture media from brain tissue of the same fetuses, nor in the control media, could antidiuretic or hydroosmotic activities be detected (hydroosmotic activity of < 500 μunit/ml and antidiuretic < 5 μunit/ml). Antidiuretic activity was quantified in three samples (days 13, 28, and 33); but hydroosmotic activity was not estimated on the same samples. If we assume that the hydroosmotic activity of the days 13, 28, and 33 was about that measured in the three samples shown in Table 1, we estimate the ratio of hydroosmotic to antidiuretic activity to be 181. This does not differ significantly from the activity ratio of 195 that we found for synthetic AVT by the same assays. The total amount of the AVT-like peptide released into the culture media during 38 days (as deduced from the biological activities assayed every 5 days) is about ten times greater than the amount contained in the nonincubated pineal glands of the same age [the hydroosmotic activity from the six nonincubated glands being equivalent to 2600 ± 825 (standard error) U.S.P. μunit per gland]. From day 40 onward, both antidiuretic and hydroosmotic activities of culture media progressively decreased. The antidiuretic and hydroosmotic activities of the culture media from pineal glands from human fetuses, the ratio of these activities as well as their susceptibility to tryptic digestion, to specific oxidative inactivation by tyrosinase, and to reductive inactivation by sodium thioglycollate indicate the presence of a peptide containing at least one basic amino acid (*12*), a tyrosine residue (*13*), and a disulfide bond (*14*). These characteristics are consistent with the specific pharmacological profile of AVT (*15*). The total amount of the AVT-like peptide released into the culture media during 38 days of incubation is about ten times greater than the amount contained in the nonincubated glands of the same age, which suggests that pineal ependymal cells can synthesize in vitro a basic peptide presumably identical with AVT. On the other hand, as deduced from the ratio of biological activities in conjunction with the enzymatic degradation results, there is no evidence for the presence of vasopressin or oxytocin in the culture media, suggesting that ependymal cells are not able to synthesize the natural analogs of AVT. If chemical analysis substantiates our results, our study demonstrates for the first time the biosynthesis of AVT by secretory specialized ependymal cells. If ependymal cell secretion represents the first and the most primitive secretory activity of the brain (*8*) and ontogenetically precedes neurosecretion (*16*), then the synthesis of AVT in a primitive ependymal cell would represent the first step in the ontogenetic molecular evolution of the brain octapeptides.

S. PAVEL, M. DORCESCU
RUXANDRA PETRESCU-HOLBAN
ELENA GHINEA
Institute of Endocrinology, Bucharest, Rumania

References and Notes

1. P. G. Katsoyannis and V. du Vigneaud, *J. Biol. Chem.* **233**, 1352 (1958).
2. W. H. Sawyer, in *Neurohypophysial Hormones and Similar Polypeptides*, B. Berde, Ed. (Springer, New York, 1968), p. 734.
3. S. Pavel, *J. Clin. Endocrinol. Metab.* **31**, 369 (1970).
4. ———, *Endocrinology* **77**, 812 (1965).
5. ——— and S. Petrescu, *Nature* **212**, 1054 (1966).
6. D. W. Cheesman, *Biochim. Biophys. Acta* **207**, 247 (1970).
7. S. Pavel, *Endocrinology* **89**, 613 (1971).
8. R. Olsson, *Proc. Int. Congr. Zool. 16th Washington* (1963), vol. 3, p. 38.
9. Ch. Owman, *Acta Morphol. Neerl. Scand.* **3**, 367 (1961); E. Anderson, *J. Ultrastruct. Res.* **8** (Suppl.), 5 (1965).
10. R. Olsson, *Gen. Comp. Endocrinol.* **1**, 117 (1961).
11. S. Pavel and M. Coculescu, *Endocrinology* **91**, 825 (1972).
12. V. du Vigneaud, H. C. Lawler, K. A. Popenoe, *J. Amer. Chem. Soc.* **75**, 4880 (1953).
13. G. W. Bisset, *Brit. J. Pharmacol.* **18**, 405 (1962).
14. R. G. Ames and H. B. van Dyke, *Proc. Soc. Exp. Biol. Med.* **76**, 576 (1951).
15. W. H. Sawyer, *Pharmacol. Rev.* **13**, 225 (1961).
16. G. Sterba, in *Zirkumventrikuläre Organe und Liquor*, G. Sterba, Ed. (Fischer, Jena, 1969), p. 20.

19 March 1973; revised 9 July 1973

Thyroid Hormone Action: A Cell-Culture System Responsive to Physiological Concentrations of Thyroid Hormones

Abstract. *Cells from a rat pituitary tumor cell line will respond in vitro to physiological concentrations of* L-*thyroxine and* L-*triiodothyronine. The cells are grown in a culture medium that contains serum from a hypothyroid calf. Dose-response relationships of a variety of thyronine derivatives indicate that this system has a specificity of response which is similar to that observed in vitro.*

Thyroid hormones affect the growth, development, and metabolism of virtually all tissues of higher organisms. In spite of extensive studies in vivo, the mechanism of action of the thyroid hormones remains to be defined. Although studies in vivo are necessary in order to evaluate the effects of the hormones on the entire organism, an in vitro system offers significant advantages for the study of the action of the thyroid hormones at the molecular level.

Investigations in vitro of the mechanisms of action of L-thyroxine (T4) and L-triiodothyronine (T3) at physiological concentrations (*1*) have not been successful. The hormone concentrations required to induce biological effects in subcellular components (such as mitochondria) range from 10^4 to 10^6 times greater than physiological free hormone concentrations (*2*). Although other investigators have used cell-culture systems to study the action of the thyroid hormones, the cells responded only minimally, even at high hormone concentrations (*3*). In addi-

tion, when these investigators described the hormone concentrations in the culture medium, they did not consider either the hormone content of the serum in the medium or the interaction of the added hormones with the serum component of the medium (*1*).

Both T3 and T4 exist in serum in bound and free forms. Only a very small fraction exists in the free form but it is the free fraction which correlates best with biological activity (*4*). Theoretical considerations as well as experimental studies indicate that as the serum is diluted, bound hormone dissociates to form free hormone and the free hormone concentrations remain relatively constant in spite of serum dilution (*5*). Therefore, unless one determines the concentrations of free T4 or T3, the biological effects of the thyroid hormones would be expected to be correlated with the hormone concentrations in the serum fraction of the medium rather than with the hormone concentrations in the complete medium. Likewise, at identical total hormone concentrations, expressed as moles per liter of cell culture medium, the free hormone concentration and the biological effect would be expected to be inversely proportional to the serum content of the medium. This has been demonstrated by Siegel in human kidney cells (*2*). In previous cell culture studies commercial preparations of serum containing physiological concentrations of T4 and T3 were used (*1*). Therefore, in spite of serum dilution in preparing culture mediums, the concentrations of free hormones would be expected to remain in the physiologic range. Since control cell cultures were not depleted of the thyroid hormones, the regulatory effects of physiologic concentrations of T3 and T4 could not be effectively studied.

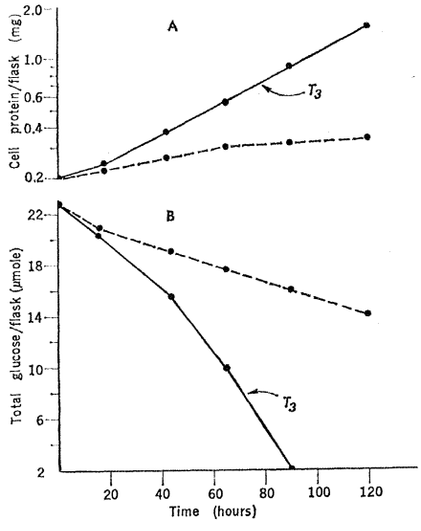

Fig. 1. Effect of triiodothyronine on (A) Cell growth and (B) glucose utilization. Each of 24 flasks was inoculated with 10^6 cells, and the cells were incubated in growth medium for 72 hours. The cells were washed with serum-free medium and were then incubated at 37°C in either hypothyroid medium or medium containing T3 ($5 \times 10^{-8}M$, in the serum fraction of the medium). The cells and mediums were harvested at the times indicated. We determined in each flask the total cell protein and DNA and the amount of glucose in the medium. Only cell protein is illustrated; the ratio of DNA to cell protein remained constant. Each point represents the average of a pair of flasks and the results of each pair agreed within 5 percent.

We describe the development of a cell-culture system in which cellular growth and metabolism are regulated by physiologic concentrations of T4 and T3 as well as by concentrations of T4 and T3 present in thyrotoxic man. The cells are a somatotrophic, rat pituitary tumor cell line, clone GH_1, originally derived by Yasumura, Tashjian, and Sato (*6*). Central to our experimental design was the use of hypothyroid calf serum obtained from a thyroidectomized calf. The hormone concentrations in this serum were $0.8 \times 10^{-9}M$, (50 ng/100 ml) for T3 and $3 \times 10^{-9}M$, (0.233 μg/100 ml) for T4, determined by radioimmunoassay and gas-liquid chromatography, respectively (*7*). In all experiments we used Ham's F-10 medium (Gibco) supplemented to a final concentration of 10 percent hypothyroid serum (hypothyroid medium). Because of the considerations discussed above, we have expressed all hormone concentrations as those prepared in hypothyroid serum prior to the tenfold dilution with medium. Therefore, execpt for estimated levels of free hormone, the concentrations are expressed as those in the serum fraction of the medium. The hormone concentration in the complete medium is one-tenth of this value.

L-Triiodothyronine (T3), L-thyroxine (T4), D-triiodothyronine (D-T3), and D-thyroxine (D-T4) were obtained from Sigma. We investigated the purity of these compounds by gas-liquid chromatography and by radioimmunoassay (*7*). Our T4 was contaminated by T3 (1.6 percent) and purification by chromatography reduced the T3 contamination to a constant 0.3 percent (*8*). The D-T4 was contaminated by 7.0 percent D-T3. The 3,3′,5′-triiodo-DL-thyronine (reverse T3) was a gift from Warner Lambert. L-Thyronine and 3,5-diiodothyronine were obtained from Sigma.

The GH_1 cells (American Type Culture Collection, Rockville, Maryland) were grown in 75-cm² T flasks (Falcon) with Ham's F-10 medium supplemented with horse serum (15 percent) and fetal calf serum (2.5 percent) (growth medium). In general, the medium was replaced twice weekly and the cells were routinely subcultured after dispersion with an ethylenediaminetetraacetic acid (EDTA) salt solution (*9*). For growth and metabolic experiments, the cells were harvested in the late stages of exponential growth with EDTA salt solution and were inoculated with growth medium and incubated in 25-cm² T flasks or in the wells of a micro culture plate (Linbro). The

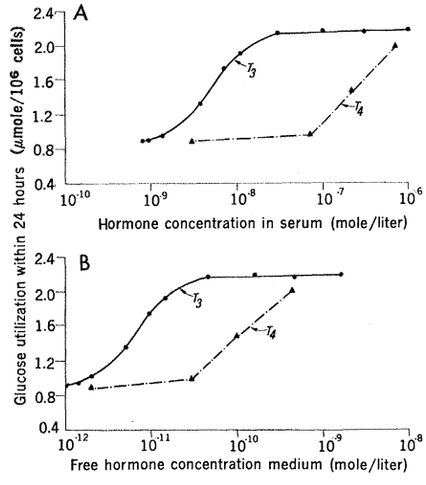

Fig. 2. Relation of glucose utilization to (A) hormone concentration (mole/liter in the serum fraction) and (B) free hormone concentration (mole/liter of total medium). Cells (2×10^5) were inoculated into each chamber of a Linbro culture plate. The initial cell density was 1.5×10^5 cell/cm². After 3 days of incubation, each well was washed with serum-free medium and then filled with a medium containing a specified concentration of T3 or T4. The cells were incubated at 37°C and samples of the mediums were removed every 12 hours for 3 days. The rates of glucose utilization were determined (corrections being made for serial media sampling) and found to be linear throughout the experiment. Results of each duplicate pair agree to within 5 percent. The results are expressed as micromoles of glucose utilized per 24 hours per 10^6 inoculated cells.

final monolayer cell density was 40,000 to 100,000 cell/cm^2. After 24 to 48 hours at 37°C (95 percent air, 5 percent CO_2), the medium was aspirated and replaced with serum-free Ham's F-10 medium. Each culture was incubated at 37°C for an additional 30 to 60 minutes. The serum-free medium was then replaced with hypothyroid medium. In some experiments, T3 or T4 was added when hypothyroid medium was added. In other experiments, the cells were incubated for 24 to 48 hours with hypothyroid medium before the addition of T3 or T4 in order to produce a "hypothyroid state" at the beginning of the experiment. Cells that were incubated with hypothyroid medium alone served as controls.

The concentrations of glucose were determined in samples of the medium by either a glucose oxidase procedure (Worthington) or with *o*-toluidine reagent (Dow). In some experiments, we studied glucose utilization by sampling the medium from the same set of flasks every 10 to 24 hours. We calculated the rate of glucose utilization by correcting for the volume of the medium and for the number of micromoles of glucose removed because of the sampling technique. In other experiments, particularly those which were associated with growth studies, we prepared a series of flasks and harvested the cells and mediums from those flasks every 15 to 24 hours for 4 to 6 days; we calculated the glucose utilization rates from the change in the total of glucose in each flask as a function of time.

Cell growth was quantified either by cell count with a hemocytometer or by determining the amount of cellular DNA (*10*) or cell protein (*11*). One million cells are equivalent to 13 μg of DNA or 130 μg of cell protein. Free hormone concentrations (*12*) were determined by equilibrium dialysis of cell culture medium against serum-free medium at 37°C in a CO_2 incubator. We calculated the free T3 and T4 concentrations by multiplying the percentage of unbound hormone by the hormone concentration in the serum prior to dilution.

The mean generation time for cells incubated with T3 was 39 hours (Fig. 1), whereas cells incubated with hypothyroid medium had a mean generation time of 120 hours. During the first 45 hours, the glucose utilization rates were linear and three times greater in the cultures incubated with T3. This value was similar to the relative difference in the rates of cell growth. At longer incubation times, the glucose utilization rates become nonlinear. This may be due to the fact that glucose utilization reflects processes related both to cell growth (*13*) and to basal metabolic activity, and, at high cell densities, the effect of basal activity becomes significant.

Table 1. Concentrations of various thyronines that induce a half-maximal effect upon glucose utilization in GH_1 cells. As illustrated for T3 and T4 in Fig. 2, dose-response relationships were determined for L-thyronine and thyronine derivatives: D-thyroxine (D-T4), D-triiodothyronine (D-T3), 3,5-diiodo-DL-thyronine (T2), and 3,3′,5′-triiodo-DL-thyronine (reverse T3). The concentrations indicated are those in the serum fraction of the medium which induce a half-maximal effect. The highest concentration studied was $5 \times 10^{-5} M$. Therefore, the values listed for thyronine, T2, and reverse T3 are based on the maximal effect determined for T3.

Thyronine	Concentration (mole/liter)
T3	6.5×10^{-9}
T4	3.5×10^{-7}
D-T3	6.0×10^{-8}
D-T4	4.0×10^{-6}
T2	2.2×10^{-5}
Reverse T3	1.5×10^{-5}
L-Thyronine	1.7×10^{-5}

Since early glucose utilization rates correlate directly with rates of cell growth, we have used this parameter to determine a dose-response relationship for T3 and T4 (Fig. 2). The results are expressed as micromoles of glucose utilized per 24 hours by 10^6 inoculated cells during the first 48 hours of incubation.

Figure 2 illustrates a dose-response curve relating glucose utilization to hormone concentration in the serum fraction of the medium (Fig. 2a) and to free hormone concentration (Fig. 2b). For T3, the glucose utilization rates increase with increasing hormone concentrations to a maximal rate of 2.2 μmole of glucose utilized within 24 hours per 10^6 inoculated cells (*14*). The hormone concentrations in the serum fraction of the medium which induced a half-maximal effect were $6.5 \times 10^{-9} M$ for T3 and $3.5 \times 10^{-7} M$ for T4. On the basis of the free hormone concentrations, a half-maximal response was induced by $8 \times 10^{-12} M$ T3 and $1 \times 10^{-10} M$ T4. Since T3 is a contaminant of T4 (0.3 percent), we corrected for the biological activity of the T3. We then estimated that T4 had 2 percent of the intrinsic activity of T3 on the basis of the concentrations in the serum fraction of the medium (Table 1) and 8 percent of the activity on the basis of the free hormone concentrations. In contrast, T3 is three to four times as active as T4 in stimulating oxygen consumption in vivo (*15*). Recent studies indicate, however, that T4 is converted to T3 to a significant degree in vivo (*16*) and, therefore, the extent of the intrinsic biological activity of T4 is not established. The lower degree of activity of T4 relative to T3 in this system compared to activity in vivo may be due to a low rate of T4 to T3 conversion or may reflect low intrinsic T4 activity. To differentiate between these possibilities, detailed T4 to T3 conversion rates as well as dose-response relationships would have to be determined.

In order to evaluate the specificity of the cell culture response and to relate the response to thyroid hormone effects in vivo, we determined dose-response relationships for a variety of thyronine derivatives in addition to T3 and T4 (Table 1).

Table 1 indicates the concentrations of T3 and T4 in the serum fraction of the medium which induce a half-maximal response as well as the serum concentrations of the derivatives D-thyroxine (D-T4), D-triiodothyronine (D-T3), 3,3′,5′-triiodo-D,L-thyronine (reverse-T3), 3,5-diiodothyronine, and L-thyronine which induce a half-maximal response. On an equimolar basis, D-T3 has one-tenth of the activity of T3. The specific optical rotation for the D-T3 studied was -21.6 (National Research Council standard L-T3 is $+21.5$) ($c = 2$ in 1*N* HCl/EtOH, 1 : 2). This indicates that the biological activity of D-T3 is likely to be intrinsic and not related to T3 contamination. After correction for D-T3 contamination (7.0 percent), D-T4 has approximately one-tenth the activity of T4. Reverse T3, 3,5-diiodothyronine and thyronine have less than 0.5 percent the activity of T3. This may, however, reflect T3 contamination. The relative response of the GH_1 system to the iodothyronine derivatives (Table 1) correlates well with their observed effects in vivo on oxygen consumption and goiter prevention (*15*). We have examined several times the response of this cell culture system to T3, T4 and to the

other thyronines described in this report. It has remained essentially unchanged after more than 1 year of cell culture. These cells also continue to elaborate growth hormone (*18*).

This GH_1 somatotrophic cell system is the first in vitro system which responds significantly to T3 and T4 at physiological concentrations determined in man and other species, as well as to concentrations which have been observed in thyrotoxic man (*1, 7, 17*).

Pituitary somatotrophs also appear in vivo to be a sensitive target cell for thyroid hormone (*19*). The somatotrophic pituitary population decreases in vivo from 40 percent to less than 5 percent of the total cell population in a period of 2 to 3 weeks after thyroidectomy (*20*). Therefore, the thyroid hormone regulation of growth and metabolism of GH_1 cells likely reflects its action on pituitary somatotrophs in vivo.

On the basis of the dose-response relationships for T3 and T4 and other iodothyronines, the GH_1 cell system, as described, appears to reflect certain of the biological actions of the thyroid hormones in vivo and has promise as a model system for study of the regulatory effects of physiological concentrations of thyroid hormone on mammalian cells.

HERBERT H. SAMUELS
JIR S. TSAI
RAQUEL CINTRON
Endocrine Division, Department of Medicine, New York University School of Medicine, New York 10016

References and Notes

1. The hormone concentrations present in fetal calf, calf, and horse serum are approximately $2 \times 10^{-9}M$ (130 ng/100 ml) for T3 and $1 \times 10^{-7}M$ (7.77 μg/ml) for T4, as determined by radioimmunoassay, competitive binding analysis, and gas chromatography (*7*).
2. L. R. Mandel and F. A. Kuehl, Jr., *Biochem. Biophys. Res. Commun.* **28**, 13 (1967); M. P. Primack, D. F. Tapley, J. Buchanan, *Endocrinology* **91**, 840 (1972); E. C. Wolf and J. Wolf, in *The Thyroid Gland*, R. Pitt-Rivers and R. W. Trotter, Eds. (Butterworth, London, 1964), vol. 1, p. 237.
3. I. Leslie and R. Sinclair, *Exp. Cell Res.* **17**, 272 (1959); S. Halevy and L. Avivi, *ibid.* **20**, 458 (1960); E. Siegel and C. A. Tobias, *Nature* **212**, 1318 (1966); M. Hamburgh, *Develop. Biol.* **13**, 15 (1966); J. M. Pawelek, *ibid.* **19**, 52 (1969); E. Kohen, C. Kohen, B. Thorell, *Exp. Cell Res.* **59**, 307 (1970); E. Siegel, *Endocrinology* **91**, 580 (1972).
4. K. A. Woeber, in *The Thyroid*, S. C. Werner and S. H. Ingbar, Eds. (Harper & Row, New York, 1971), p. 256.
5. J. H. Oppenheimer and M. I. Surks, *J. Clin. Endocrinol.* **24**, 785 (1964); G. C. Schussler and J. E. Plager, *J. Clin. Endocrinol. Metab.* **27**, 242 (1967).
6. Y. Yasumura, A. H. Tashjian, G. H. Sato, *Science* **154**, 1186 (1966).
7. T. Mitsuma, N. Nihei, M. C. Gershengorn, C. S. Hollander, *J. Clin. Invest.* **50**, 2679 (1971). We thank Drs. T. Mitsuma, N. Nihei, and C. S. Hollander for doing these determinations.
8. D. Bellabarba, R. E. Peterson, K. Sterling, *J. Clin. Endocrinol. Metab.* **28**, 305 (1968).
9. J. Paul, in *Cell and Tissue Culture* (Williams & Wilkins, Baltimore, 1970), p. 218.
10. K. Burton, *Biochem. J.* **62**, 315 (1956).
11. O. H. Lowry, N. J. Rosebrough, A. I. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 264 (1951).
12. K. Sterling and M. A. Brenner, *J. Clin. Invest.* **45**, 153 (1966).
13. V. J. Cristofalo and D. Kritchevsky, *Proc. Soc. Exp. Biol. Med.* **118**, 1109 (1965).
14. Although not definitively illustrated in this experiment, other studies utilizing higher concentrations of T4 indicate that T3 and T4 induce the same maximal effect.
15. W. L. Money, S. Kumaoka, R. W. Rawson, *Ann. N.Y. Acad. Sci.* **50**, 512 (1960).
16. L. E. Braverman, S. H. Ingbar, K. Sterling, *J. Clin. Invest.* **49**, 855 (1970); H. L. Schwartz, M. I. Surks, J. H. Oppenheimer, *ibid.* **50**, 1187 (1971).
17. B. E. P. Murphy, C. J. Pattee, A. Gold, *J. Clin. Endocrinol.* **26**, 247 (1966).
18. H. H. Samuels, J. S. Tsai, R. Cintron, unpublished data.
19. J. Solomon and R. O. Greep, *Endocrinology* **65**, 158 (1959); H. Brauman and J. Corvilain, *J. Clin. Endocrinol.* **28**, 301 (1968).
20. R. A. Schooley, S. Friedkin, E. S. Evans, *Endocrinology* **79**, 1053 (1966).
21. We thank C. S. Hollander, S. Kammerman, L. Shenkman, and J. Ross for their comments on this manuscript. This work was supported by American Cancer Society grant P-595 and by Public Health Service research career development award AM 46546-03 (H.H.S.).

12 February 1973; revised 1 June 1973

Behavior of Free-Ranging Macaques after Intraventricular 6-Hydroxydopamine

Abstract. *Macaques* (Macaca mulatta) *observed in a free-ranging colony on Guayacan Island, Puerto Rico, were significantly different in their social interactions, initiatives, facial expressions, and postures after intraventricular 6-hydroxydopamine compared with sham-treated and field controls. This study extends the known effects of 6-hydroxydopamine and catecholamine depletion to the social interactions of a higher primate species under free-ranging conditions.*

We recently reported that intraventricular 6-hydroxydopamine (2,4, 5 - trihydroxyphenethylaminehydrobromide) produced decreases in important positive social behaviors of *Macaca speciosa* observed in an artificially composed social group in a laboratory enclosure (*1*). The changes in social behavior and reductions in brain catecholamines persisted until the animals were killed 16 days after treatment. This suggested that it might be possible to study the effects of central catecholamine depletion on the normal behavioral repertoire, including familial and social relationships, which could be observed most naturally in free-ranging individuals. We now report the first such study of the effects of a specific brain biochemical lesion in free-ranging primates.

Six adult females and two adult males were trapped from two existing colonies of free-ranging macaques (*M. mulatta*) living on Guayacan Island. The females had been living in one social group of about 35 individuals for many years. Four had infants that remained in the group during the captivity period (9 to 21 days). Two large males of identical weight who appeared to be dominant and central animals in their respective social groups were trapped and paired experimentally. The females were paired on the basis of known or probable family relationships or by frequent social interactions or social position in the groups (*2*). The animals, weighing 7 to 12 kg, were implanted with permanent cannulas placed stereotaxically in the lateral ventricle (*1*). After recovery from surgery each pair of awake animals was injected intraventricularly with 6-hydroxydopamine or the carrier (*3*) as follows: 2 mg on the first day, then 4 mg, 8 mg, 8 mg, and 8 mg at 12-hour intervals for a total of 30 mg. On a similar schedule 31 mg produced whole brain norepinephrine depletions of 69 percent in the laboratory (*1*). The animals were observed for adequate oral intake and general condition. On the fourth day after the final injection all monkeys were released across the 80-acre (~ 32-hectare) island from their usual territory. They were followed and observed for an average of 5 hours daily for 10 days from their release. An experienced observer who was blind to their treatment condition recorded the total time during which each individual could be seen, and the frequency or duration of major social interactions (social grooming, self-grooming, threats, attacks, sexual presentations, copulation) and responses to social interactions (*4*). In addition to the four treated and four sham-treated animals, three animals who were not trapped served as field controls.

The monkeys tolerated the cannula