give bright fluorescence. In the rat metaphase cell these areas included portions of the long arms of chromosomes 2, 3, and 7, a paracentric band on the long arm of chromosome 14, and one arm of chromosome 15. The Y chromosome appeared polymorphic in intensity of staining.

Interphase nuclei from species in which the Y chromosome stains intensely with fluorescent dyes have characteristic, bright fluorescent spots (9). We examined rat interphase nuclei from tissue cultures and peripheral blood cultures of male origin (approximately 1000 cells per culture). We found bright spots in 3 to 5 percent of such cells but most of the nuclei exhibited only a somewhat uniform granular staining. Occasionally, large, but poorly defined, masses within the nucleus stained with greater intensity.

Most of the hepatomas examined had a bimodal population of dividing cells, with varying proportions of diploid cells and the remainder clustered about a hypotetraploid mode (6). The fluorescent banding patterns in the diploid tumor cells were comparable to those of diploid cells from normal sources, and we could find no evidence of structural rearrangements.

Structural rearrangements were observed in the hyperploid cells and in some instances the chromosomes that contribute to large marker chromosomes could be identified. For example, a dicentric chromosome could be identified, by its banding pattern, as the fusion product of a No. 1 chromosome with a smaller acrocentric chromosome, probably a No. 8 (Fig. 2B). In other tumor cells, however, we observed abnormal chromosomes that did not exhibit the fluorescent banding patterns of normal chromosomes. For example, a pair of large telocentric markers were found in most metaphase spreads from one of the tumors. In one such cell with 86 chromosomes the four No. 1 chromosomes showed the expected banding patterns, but the marker chromosomes showed a repeating pattern of broad bands of fluorescence not seen in normal cells (Fig. 2B).

Much of the previous difficulty in identifying specific chromosomes in the rat karyotype has been overcome by use of the flourescence-banding technique.

Recognition of the normal fluorescent banding pattern of rat chromosomes can serve to identify translocation exchanges that would otherwise be undetected and to identify those chromosomes that contribute to the formation of new mark-

er chromosomes in malignant cells. The former is of great interest for analysis of diploid and "pseudodiploid" tumors. The importance of the latter (identification of the origin of marker chromosomes) relates to the survival value of such chromosomes in that we have seen markers in many chemically induced hepatomas (6) and they have also been reported in Rous sarcomas (10).

By using a fluorescent staining technique, we have been able to describe the normal chromosome banding-pattern for the rat and to define two types of abnormal chromosomes in rat hepatomas. One of these abnormal types is clearly the result of fusion of normal chromosomes but the origin of the other is unexplained.

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6,7-Dihydroxytetrahydroisoquinoline: Uptake and Storage by Peripheral Sympathetic Nerve of the Rat

Abstract. 6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinoline is a pharmacologically active alkaloid that can be formed by condensation of dopamine with formaldehyde. We used fluorescence microscopy to study in vitro the uptake and storage of this compound by sympathetic nerves of the rat iris. Rats were treated with reserpine or with the methyl ester of α-methyl-p-tyrosine in order to deplete the endogenous catecholamine stores. Accumulation of the alkaloid was about one-tenth that of norepinephrine. Uptake was completely blocked by $10^{-5}M$ desmethylimipramine. These results offer some explanation for the sympathomimetric properties of the alkaloid. Similar results can be expected for similar tetrahydro-isoquinolines that may be formed in vivo from endogenous catecholamines during ingestion of alcoholic beverages.

1,2,3,4-Tetrahydroisoquinoline (TIQ) alkaloids are synthesized in isolated intact adrenal glands from cows as a result of a condensation reaction between endogenous catecholamines and perfused acetaldehyde (1). Similar TIQ products are formed from dopamine (DA) and acetaldehyde in rat tissue homogenates (2) or from endogenous catecholamines during perfusion of cow adrenal glands with formaldehyde (1). In vivo, TIQ's are synthesized in the adrenal medullas of rats during administration of methanol (3), which is a metabolic precursor of formaldehyde. It has been suggested that TIQ alkaloids can be biosynthesized in the adrenals and the adrenergic neurons of man during ingestion of alcoholic beverages and that these alkaloids may alter adrenergic function (4). We re-

port that 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (6,7-dihydroxy-TIQ), the alkaloid formed by condensation of DA with formaldehyde, is taken up and accumulated in vitro by sympathetic nerves of the rat iris. The amount of alkaloid accumulated was less than that for norepinephrine (NE) but greater than that for DA. Uptake was blocked by low concentrations of desmethylimipramine (DMI). Thus, 6,7-dihydroxy-TIQ has properties akin to those of the catecholamine neurotransmitters and may be capable of altering activity of sympathetic nerves.

We used fluorescence microscopy to study the accumulation of DA, NE, or 6,7-dihydroxy-TIQ in the sympathetic nerves of the iris of the rat. In this well-known method (5), moist formaldehyde gas is used to transform the

catecholamines into strongly fluorescent quinoidal tautomers of dihydroisoquinolines. As seen in Fig. 1, 6,7-dihydroxy-TIQ (compound 2) is an intermediate in the reaction sequence for DA. Although it is not itself fluorescent, 6,7-dihydroxy-TIQ can be made fluorescent (compound 4) by heating it with moist formaldehyde gas (5). Heating with formaldehyde forms the basis for the histochemical visualization of both 6,7-dihydroxy-TIQ and the catecholamines in the rat iris (6).

We used 44 male albino Columbia-Sherman rats, weighing about 150 g each. In order to deplete the sympathetic nerves of endogenous catecholamines, the animals were treated either with reserpine, which interferes with catecholamine storage mechanisms (7), or with the methyl ester of α-methyl-p-tyrosine, which blocks synthesis of catecholamines at the step catalyzed by tyrosine hydroxylase (8). The same approach has been used (9) to study the uptake of catecholamines into rat irises or heart atria. The rats were injected intraperitoneally with reserpine (10), 5 or 10 mg/kg at 20 hours before decapitation; or with α-methyl-p-tyrosine methyl ester (11), 500 mg/kg at 20 hours before decapitation and again with 300 mg/kg at 2 hours before decapitation. Each excised iris was incubated separately in 1 ml of Krebs Ringer phosphate buffer, pH 7.4, that contained 6,7-dihydroxy-TIQ (12), NE (13), or DA (14) in various concentrations. Ascorbic acid (0.2 mg/ml) was added to the medium to prevent aerobic oxidation of the catechol compounds. Irises were incubated for 30 minutes in a 37°C oven. They were then transferred to fresh Krebs Ringer medium and rinsed by reincubation for another 15 minutes at 37°C. Stretch preparations of the irises were allowed to dry for 1 hour in a closed chamber containing Drierite. They were then treated with formaldehyde vapor (relative humidity, 70 percent) at 80°C for 1 hour and were examined under the fluorescence microscope (15).

In early experiments, one iris from each animal was a control to determine how much of the endogenous stores of catecholamines had been depleted by each drug. Reserpine always produced complete depletion, but the degree of depletion varied when α-methyl-p-tyrosine methyl ester was used. The uptake of 6,7-dihydroxy-TIQ, DA, or NE in one iris was measured by comparing fluorescence in this
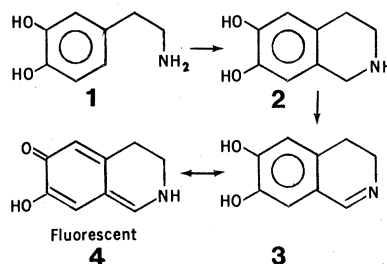

Fig. 1. Conversion of dopamine (compound 1) to the fluorescent quinoidal tautomer (compound 4) of 6,7-dihydroxy-1,2-dihydroisoquinoline (compound 3) by formaldehyde gas and heat (5). 6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinoline (compound 2), the alkaloid we studied, is an intermediate in the formation of compound 3. Norepinephrine, which is dopamine hydroxylated at the β position, undergoes the same reactions to yield the quinodial tautomer of 4,6,7-trihydroxy-1,2-dihydroisoquinoline.

iris to that in the control iris from the same rat. In later experiments with animals treated with reserpine, the uptake of 6,7-dihydroxy-TIQ by sympathetic nerves of one iris was compared to the uptake of either DA or NE in the opposite iris. This permitted a direct comparison of the relative uptake and storage of the TIQ and the catecholamines.

Reserpine depletes the endogenous stores of catecholamines by interfering with uptake and storage mechanisms (that are dependent on Mg^{2+} and adenosine triphosphate) of the neuronal granules (7). A catecholamine uptake mechanism that is insensitive to reserpine exists over the whole catecholamine neuron (16). Also, isolated amine storage granules can take up catecholamines after reserpine treatment if the catecholamine concentration is high (17).

In our preparations, reserpine treatment totally depleted the endogenous catecholamine stores (Fig. 2a). Uptake of NE or 6,7-dihydroxy-TIQ was dependent on concentration in the range of 0.1, 1.0, 3.0, and 10.0 μg of free amine per milliliter. The uptake of NE was much greater than that of 6,7-dihydroxy-TIQ, as judged by fluorescence intensity. For example, the nerve plexus appeared intensely fluorescent after incubation with NE (0.1 μg/ml), while only a few weakly fluorescent fibers were seen after incubation with the TIQ at this concentration. With 1.0 μg of NE per milliliter (Fig. 2, e and f), there was a smooth appearance of the majority of the terminals, and preterminal axons were evident. Similar results with NE have been reported

(9). On the other hand, the TIQ at 1.0 μg/ml (Fig. 2, b and c) gave a different morphologic appearance. Fluorescence was localized in the varicosities, which were quite large and very often separated from one another; preterminal axons were rarely seen when the TIQ was used at this concentration. With the TIQ at 10 μg/ml, fluorescence was smoother but remained varicose. We estimate that the uptake affinity for NE may be ten times greater than that for 6,7-dihydroxy-TIQ, as judged by fluorescence intensity and population of nerve terminals.

In other experiments, the accumulation of 6,7-dihydroxy-TIQ and that of DA were compared; both compounds were tested at 0.1, 1.0, and 3.0 μg/ml. Their morphologic appearances were similar, but the TIQ appeared more strongly fluorescent.

The compound DMI (18) inhibits the axonal uptake of NE but does not affect the uptake into storage granules except at much higher concentrations (7, 16). We studied the effect of low concentrations of DMI (10^{-5} and $10^{-7}M$) in our system. With $10^{-5}M$ DMI, there was total blockade of uptake of 6,7-dihydroxy-TIQ into rat irises (Fig. 2d). Uptake of NE was similarly inhibited, in agreement with results by others (16). At a concentration of $10^{-7}M$, DMI partially inhibited uptake of NE or the TIQ. Uptake of the TIQ appeared to be much more strongly inhibited, but this was difficult to judge with certainty since estimations were made from different base values (that is, the uptake of NE was greater to begin with, and its residual fluorescence after DMI may have been more discernible to the eye).

In another series of experiments, rats were treated with α-methyl-p-tyrosine methyl ester. The morphologic appearance of the irises after uptake of NE (1 μg/ml) was less smooth and more studded with varicosities than in preparations from rats treated with reserpine. Similar results with NE have been reported (9). Uptake of the TIQ was also evident in these preparations. Depletion of endogenous catecholamines by α-methyl-p-tyrosine was variable. The degree of depletion appeared to correlate well with the degree of sedation exhibited by the animals. In several heavily sedated animals, the uptake of NE was compared to uptake of 6,7-dihydroxy-TIQ. Uptake of NE was more pronounced, as in the reserpine experiments. These results show that

uptake and storage of 6,7-dihydroxy-TIQ are not related to some special property of animals treated with reserpine but are evident as well after depletion of catecholamines by inhibition of synthesis.

A strong uptake mechanism for 6,7-dihydroxy-TIQ exists in peripheral sympathetic nerve terminals of the rat iris. Granular binding and storage of the alkaloid are suggested by (i) the very intense localization of fluorescence in the varicosities of the adrenergic plexus (Fig. 2, b and c) and (ii) the retention of the alkaloid during a 15-minute rinsing of the irises with fresh medium. The alkaloid achieved intraneuronal concentrations estimated to be about 100 μg/ml $(6 \times 10^{-4}M)$ in the cell cytoplasm and about 1 mg/ml $(6 \times 10^{-3}M)$ or higher in the varicosities; these concentrations are required to visualize the fluorescent structures $(5, 19)$. These concentrations represent accumulations of 100- to 1000-fold over the $6 \times 10^{-6}M$ alkaloid (1 μg/ml) originally added to the medium. The pronounced inhibition of uptake by DMI at concentrations as low as $10^{-7}M$ shows that the alkaloid utilizes the same uptake system as do the catecholamines.

Uptake and accumulation of other radioactive TIQ alkaloids by the synap-tosomal fraction of whole rat brain have been observed in vitro (20); however, uptake by synaptosomes was not very much affected by DMI at concentrations up to $10^{-3}M$. The DMI is similarly ineffective in preventing uptake of DA in the striatal regions of brain (21).

6,7-Dihydroxy-TIQ is a pharmacologically active alkaloid (22). At doses of 0.2 to 4.5 mg/kg, injected intravenously into dogs or cats, this alkaloid exhibits pressor action, and causes increased respiration and a slowed pulse with increased amplitude. When given intraperitoneally to mice at a dose of 600 to 700 mg/kg, this alkaloid causes gen-
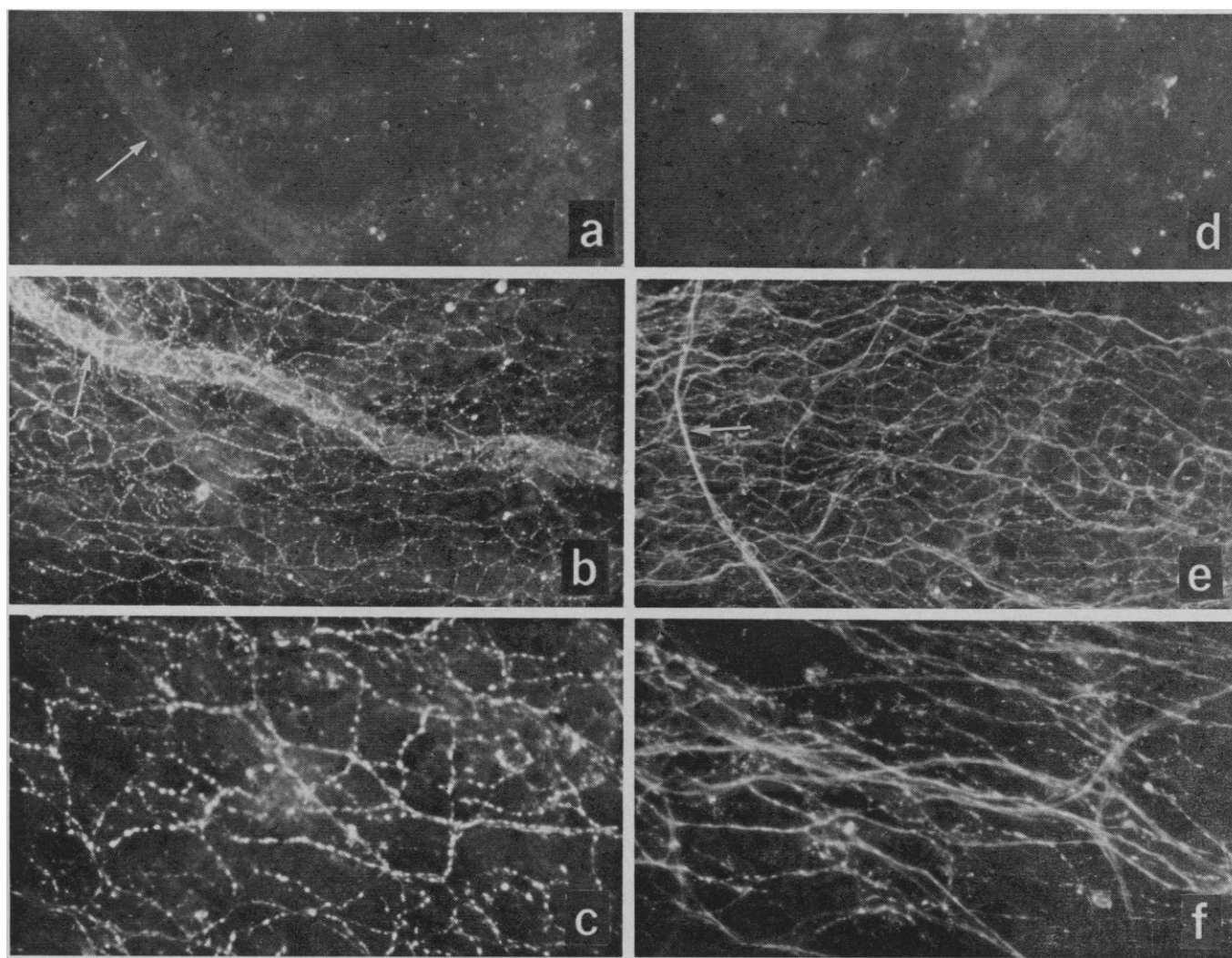

Fig. 2. Stretch preparations of irises from rats treated with reserpine (10 mg/kg) 20 hours before killing. Irises are shown before and after incubation in Krebs Ringer solution containing either 6,7-dihydroxy-TIQ or NE, with and without DMI. (a) Untreated iris. No specific fluorescence is seen. A small blood vessel (arrow) crosses the field ($\times$270). (b) Iris incubated in 6,7-dihydroxy-TIQ (1 μg/ml). Distinct fine varicosities can be seen forming a rich nervous plexus which is similar in appearance to the normal. There is a rich perivascular plexus (arrow) in the upper portion of the field ($\times$270). (c) Higher magnification of the tissue as in (b). The discrete varicosities are easily recognized ($\times$675). (d) Iris after incubation with 6,7-hydroxy-TIQ (1 μg/ml) in the presence of DMI ($10^{-5}M$). There is no evidence of specific fluorescence in nerve terminals. The appearance is similar to that in (a) ($\times$675). (e) Iris incubated in NE (1 μg/ml). The plexus of nerve terminals has a smooth appearance due to fluorescence of that portion of the nerve fibers between the varicose enlargements. Discrete varicose terminals can also be seen in many areas. In addition, there is fluorescence of the preterminal axons (arrow, left side of field). These structures do not fluoresce under normal circumstances ($\times$270). (f) Higher magnification of the tissue in (e). Both smooth axons and discrete varicose enlargements are readily seen ($\times$675).

eralized depression, profound exophthalmos, and pilomotor effects. A variety of effects on blood pressure, smooth muscle, and respiration are also exhibited by a large number of *O*- and *N*-alkyl derivatives of 6,7-dihydroxy-TIQ and by the corresponding 5,6-dihydroxy-TIQ analogs (*22, 23*). These actions indicate stimulation of sympathetic nerve function.

Our observation of uptake and storage of 6,7-dihydroxy-TIQ by sympathetic nerves suggests several mechanisms of action for this alkaloid. First, it may be capable of acting as a false transmitter. A number of other amines that are taken up and stored by adrenergic neurons in the periphery or in the brain are believed to function as false transmitters (*24*). Second, 6,7-dihydroxy-TIQ may produce sympathomimetic effects by displacement of catecholamines from storage sites. Release or displacement of catecholamines from rat brain synaptosomes in vitro has been observed for 1-methyl-6,7-dihydroxy-TIQ (salsolinol) (*20*). Lastly, the TIQ's may produce sympathomimetic effects by interfering with reuptake of normally released natural transmitters. In our experiments with the rat iris, there was indication that the alkaloid and NE shared the same uptake mechanisms since they were both blocked by low concentrations of DMI. In studies with rat brain synaptosomes, 1-methyl-6,7-dihydroxy-TIQ blocked the uptake of both DA and NE (*20*). These observations open a number of interesting possibilities for pharmacologic and therapeutic studies with naturally occurring and synthetic TIQ alkaloids.

Biosynthesis of TIQ alkaloids might occur in the catecholamine storage sites in the adrenals, sympathetic nerves, and brain of man during ingestion of alcoholic beverages (*1, 4*). The potential actions of TIQ alkaloids as false transmitters and as agents that release catecholamines and block their uptake are consistent with the hypothesis that these alkaloids may contribute to alterations in activity of the sympathetic or central nervous system during ingestion of alcoholic beverages.

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Coexistence of Two Asexual Strains on a Single Resource

Abstract. A stable equilibrium was obtained for two F⁻ strains of Escherichia coli in a glucose minimal medium. This equilibrium cannot readily be explained by traditional models of population genetics and apparently violates some forms of the ecological principle of competition exclusion. A mechanism involving an inverse relationship between the growth rates of these strains at the exponential and "stationary" phases is suggested as a possible explanation for the observed stable equilibrium.

In the literature on population genetics considerable attention has been given to the conditions necessary for multiple genotypes to maintain stable equilibria in a single population. Ecological literature gives fair consideration to analogous equilibria, those for multiple species in the same environment. The pure frequency models of population genetics predict a number of situations by which the opposing forces of mutation, selection, or both mutation and selection lead to stable equilibria for multiple alleles at the same locus (*1*). Although ecological theory does not predict the precise condition under which stable equilibria will obtain for species at the same trophic level, it seems generally agreed that a necessary condition is a fair amount of environmental heterogeneity and ecologically divergent species (*2*). By combining ecological and genetic theory, additional mechanisms for stable genetic polymorphisms (*3*) and stable states of interspecific coexistence (*4*) may be derived.

While performing some experiments on the partitioning of resources by competing strains of the bacterium *Escherichia coli*, a stable equilibrium was obtained for two F⁻ strains in a glucose minimal medium. This equilibrium cannot be readily explained by traditional models of population genetics. In addition, a stable equilibrium by closely related forms in a very simple environment is unexpected on ecological grounds and is in apparent violation of the sometimes criticized principle of competitive exclusion (*5, 6*). Consequently, I felt this result and a possible explanation for it worthy of a special report.

The culture method used was essentially similar to that of Atwood, Schneider, and Ryan (*7*). An initial inoculum of 0.1 ml of stationary phase cells of two F⁻ strains of *E. coli* was placed in 50-ml Erlenmeyer flasks con-