Table 2. Contraceptive trial with ethynodiol diacetate.

Cycle No.	No. of women	Percent reporting "reactions"	Percent with breakthrough bleeding
1	124	16.9	6.5
2	119	9.3	4.2
3	103	10.7	1.9
4	75	1.0	5.0
5–6	99	1.0	5.0
7–8	88	6.8	0.0
9–10	64	6.2	0.0
Total	662	8.6	3.2

San Juan initiated contraceptive trials with the 5th- to 25th-day oral medication regime by taking a tablet containing 2 mg of ED plus 0.1 mg of EEME. At the beginning of the experiment (July and August 1961), each subject was given a thorough physical examination. Four to six months later, most of the women of this first group were given a second examination. Approximately 70 additional volunteers were added to the study, and the record of the first ten cycles of use is analyzed in Table 2. During this period, no detectable conception occurred. As was the case when other 19-norsteroids were tested, the volunteers reported "reactions" in turns of comments or complaints which were noted during the medicated cycles. More objective was the occurrence of bleeding or spotting on one or more days while the medication was being taken. This we call breakthrough bleeding. As previously noted for other 19-norsteroids (*8, 9*), the first medication cycle was the period of maximum occurrence of these phenomena. The data on breakthrough percentage resemble those found in trials with the daily administration of 10 mg of norethynodrel plus 0.15 mg of EEME, whereas the "reaction" frequencies are similar to those reported for dosages of 5 mg of norethynodrel plus 0.075 mg of EEME. Thus this combination of ED and EEME would appear to yield a lower reaction incidence and a better control of menstrual bleeding than the combination of norethynodrel and EEME.

Table 3. Responses to questioning of ED users after 4 to 6 months of test.

Concerning	Percent claiming		
	Increase	No change	Decrease
Weight	23	40	37
Breast size	7	88	5
Menstrual pain	10	76	14
Menstrual flow	7	61	32
Libido	2	95	2

Amenorrhea, which here means no menstruation upon withdrawal of the medication, occurred in 1.2 percent of the 662 cycles. This is about the incidence which was observed with Enovid (*8*). Analysis of menstrual cycle lengths discloses a mean of 27.4 days per cycle, with 3.4 percent of the cycles less than 24 days. Practically all of these "short" cycles were reported by individuals who omitted the dose for several days. These data emphasize the relative regularity of the imposed cyclicity.

The physical examinations of 45 of the original volunteers have disclosed no notable effect other than menstrual regulation and contraception already noted. Endometrial biopsies disclosed a similar, typical sequence of early progestational change in the endometrial glands, with continuing stromal stimulation so characteristic of 19-norsteroid administration (*8, 10*). Papanicolaou smears were of classes I and II only; before medication 67 percent were class I, during medication 77 percent were class I. The uterine size as measured by palpable fundal area was unchanged in 53 percent of the women after medication. It was decreased in 40 percent and increased in 7 percent.

Table 3 summarizes the replies to questioning of the volunteers at the time of the second examination. No very remarkable change was recorded, although 32 percent of the women who used ED reported some reduction in the amount of menstrual discharge; this reduction had also been observed with other 19-norsteroids. Otherwise, the increases reported by some subjects are balanced by the decreases reported by others (*11*).

In conclusion, we find the ED plus EEME combination a potent antifertility agent, when it is administered orally in low daily dosage. By analogy with our experience with Enovid, contraceptive effectiveness may be expected at a low daily dose (1 mg/day) and presumably with minimal "side effects." Menstrual cyclicity with no untoward effects is adequately controlled. Because of the low dosage regimen, this drug may be not only physiologically safer than others but also more economical.

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Localizing Tritiated Norepinephrine in Sympathetic Axons by Electron Microscopic Autoradiography

Abstract. Following intravenous infusion of tritiated norepinephrine, rat pineals were prepared for combined autoradiography and electron microscopy. Concentrations of photographic grains were observed only over regions of preterminal autonomic axons containing granulated vesicles, thereby directly demonstrating uptake of norepinephrine into these axons and strongly suggesting that their granulated vesicles contain norepinephrine.

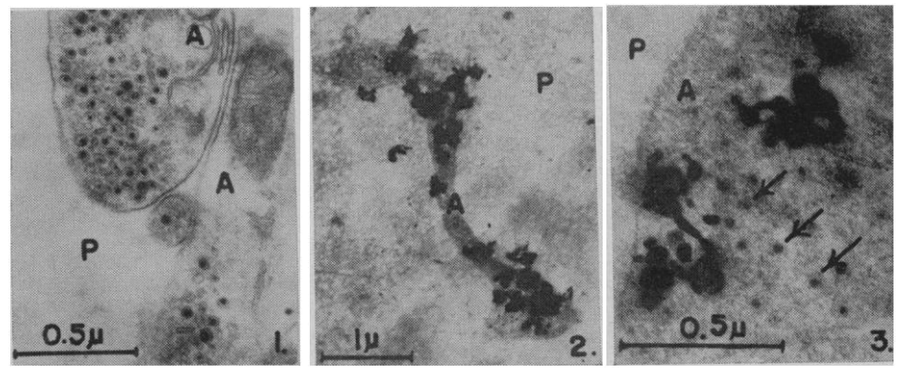
Electron-microscope studies (*1–5*) have established the presence of characteristic "granulated vesicles" in many autonomic axons. These granulated vesicles are 40 to 50 mμ wide, contain a 20 to 30 mμ electron-dense core, and seem to be concentrated in preterminal axoplasm (Fig. 1). It has been suggested that granulated vesicles contain serotonin (*1*), norepinephrine (*2*), or one of several "reducing amines" (*3*). These suggestions rest upon such circumstantial evidence as the morphological analogy between granulated vesicles and chromaffin cell granules possessing a limiting membrane and a dense core, the known concentration of norepinephrine in sympathetic nerves (*6*), and the evidence from centrifugation studies of splenic nerve homogenates that at least 20 percent of the total norepinephrine is associated with "particles" somewhat similar to catecholamine-containing granules obtained from adrenal homogenates (*7*). From one brief

reference to an electron microscopic examination of splenic nerve fractions containing particle-associated norepinephrine (*8*), it is uncertain whether the structures observed are identical with the granulated vesicles described above. Electron-microscope studies of autonomic nerves in reserpinized rats (*3, 9*) have not established a definite alteration in the population of granulated vesicles; and, in general, reserpine is too nonspecific a releasing agent to yield precise chemical information about the structures it affects. Thus there is no unequivocal evidence that granulated vesicles in autonomic axons contain norepinephrine, or that they are present only in adrenergic sympathetic fibers.

Recent studies show that tritiated norepinephrine (H^3-NE) is rapidly concentrated and retained in certain tissues (*10*). This uptake of H^3-NE is prevented by sympathetic denervation (*11*). Once bound in a tissue, H^3-NE can be released by sympathetic nerve stimulation and by various sympathomimetic agents (*12*). These findings suggest that H^3-NE is taken up into adrenergic sympathetic axons and/or into some anatomically separate structure requiring the presence of sympathetic axons to maintain its capacity to bind H^3-NE, such as chromaffin cells (*13*). To decide between these alternative explanations and to obtain more definite information about granulated vesicles in autonomic axons are major aims of the present study. The association of H^3-NE with physically separable cytoplasmic particles, and the tendency of catecholamines to form insoluble compounds with fixatives, suggest that the amount of H^3-NE lost during fixation, dehydration, and embedding for electron microscopy is small, although direct measurements have not been performed.

Successful localization of H^3-NE by the present methods, however, merely requires the preservation of sufficient quantities of tritiated material to yield unequivocal autoradiographs on thin sections of tissue. The attribution of autoradiographic grain clusters specifically to the presence of H^3-NE in the underlying tissue section is justified by the demonstration (*10*) that more than 90 percent of a tissue's radioactivity following injection of H^3-NE is due to that compound, while its major metabolite, tritiated normetanephrine, contributes an amount of radioactivity that is negligible in the present investigation.

The pineal body was examined because of the richness of its sympathetic innervation (*14*), its known concentration of norepinephrine (*15*), its ability to concentrate H^3-NE in vivo in a particulate fraction similar to the particle-associated norepinephrine obtained from rat heart homogenates (*16*), the availability of electron microscopic descriptions of pineal autonomic nerves (*1, 3, 5*), and the knowledge that at least some of the neurites containing granulated vesicles are axons which terminate on pineal parenchymal cells (*5*). Thirty minutes after a slow intravenous infusion of 250 μc of *dl*-norepinephrine-7-H^3 (20 mc/mg), the pineal bodies of adult Osborne-Mendel rats were fixed by perfusion with osmium tetroxide (*17*) and embedded in Epon 812. Thin sections were prepared for autoradiography and examination in an RCA EMU 3E electron microscope by methods recently described (*18*), with Ilford L4 nuclear research emulsion and exposure times of 4 to 12 days.



Figs. 1–3. Electron micrographs of sympathetic axons (*A*) in perivascular space (*P*) of rat pineal body after injection of H^3-NE. Fig. 1. Clusters of granulated vesicles in sympathetic axons. Fig. 2. Autoradiographic grain concentration over a sympathetic axon. Fig. 3. Specific association of autoradiographic grains with axonal regions containing granulated vesicles (arrows). Figures 2 and 3 are electron microscopic autoradiographs showing opaque, characteristically coiled autoradiographic grains over specimen areas containing H^3-NE. The presence of processed photographic emulsion gives a mottled appearance to these two micrographs.

Electron microscopy revealed a very striking localization of photographic grains to areas overlying nonmyelinated axons situated in the perivascular spaces and occasionally between the pineal parenchymal cells (Fig. 2). These axons were single or in bundles and often were encompassed by a basement membrane without an intervening Schwann cell, like autonomic axons elsewhere in the body (*2*). Grain concentrations occurred only over nonmyelinated axons which contained granulated vesicles in the immediate vicinity of grain aggregation (Fig. 3). No grain concentrations were found over pineal parenchymal cells or their perivascular processes, or over any other cells in the perivascular spaces. Cells with granules resembling those in chromaffin cells were not seen.

From these findings we conclude: (i) Circulating H^3-NE is taken up into nonmyelinated axons. (ii) No anatomically separate entities such as chromaffin cells or Schwann cells are required for the uptake of H^3-NE by these axons. (iii) The autonomic axons incorporating H^3-NE are adrenergic sympathetic axons because this capacity, in autonomic nerves elsewhere in the body, is displayed only by elements possessing the defining pharmacological parameters of adrenergic sympathetic axons (*11, 12*). (iv) The constant association of autoradiographic grain concentration with granulated vesicles directly demonstrates a constant association of H^3-NE with granulated vesicles, thereby providing independent evidence for the hypothesis that norepinephrine in sympathetic axons resides in membrane-limited structures, and strengthening the idea that (v) norepinephrine resides in the electron-dense core of the granulated vesicle. (vi) The presence of granulated vesicles can be used as one criterion for the identification of adrenergic sympathetic axons in electron micrographs. (vii) The absence of autoradiographic grain concentrations over pineal parenchymal cells and the failure to observe typical granulated vesicles in these cells (*1, 5*) suggest that pineal cells neither contain endogenous nor bind exogenous norepinephrine.

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Air Pollution: Photooxidation of Aromatic Hydrocarbons

Abstract. A number of aromatic hydrocarbons participate as effectively as the olefins in atmospheric photooxidation reactions in the presence of nitrogen oxides and ultraviolet light. Judged both on the basis of reactivity and concentrations in the atmosphere, the aromatic hydrocarbons cannot be ignored as contributors to the photochemical type of air pollution.

When an olefin and nitric oxide, NO, in concentrations of parts per million (ppm) are exposed to ultraviolet radiation (>2900 Å) in the presence of oxygen, a rapid oxidation of NO occurs. This reaction is much more rapid than the thermal oxidation of NO by molecular oxygen in the same concentrations. Previous investigators, working with model systems, have usually studied only the hydrocarbons of the olefin series (*1*, *2*).

However, Haagen-Smit (*3*) using rubber cracking as an index of ozone formation investigated the photooxidation of model mixtures of aromatic hydrocarbon and nitrogen dioxide, NO_2, systems. He found that appreciable rubber cracking occurred in mixtures containing xylenes and mesitylene. The photooxidation rates of NO in the presence of these aromatic hydrocarbons were not studied nor were their rates of reaction considered. In the present work both the photooxidation and the reaction rates of the aromatic hydrocarbon nitrogen oxide systems were investigated.

Mixtures in plastic bags containing 3 ppm of nitric oxide and 5 ppm of organic compound in a simulated atmosphere composed of 20 percent oxygen and 80 percent nitrogen were irradiated between two banks or warm-white, black-light, and sunlight type fluorescent lamps at a temperature of 36° to 38°C. The plastic was a copolymer of fluorinated ethylene and propylene. Colorimetric analyses for NO_2 were made at intervals during irradiation, which was continued until the NO_2 passed through maximum concentration and then began to decrease. The reactivity was expressed in terms of the average conversion rate at the time the NO_2 concentration reached one-half of its maximum value (half-conversion-time). The concentrations of ozone and of organic peroxy compounds were determined by the oxidation of iodide ion to triiodide ion in neutral solution. These concentrations were measured after irradiation times when the NO_2 concentration was substantially reduced. Corrections were made for the small amount of oxidation of the iodide solution by that NO_2 which remained in the reaction mixture.

The photooxidation of the mixtures of aromatic hydrocarbon and NO_2 were performed in an infrared cell adjusted for an optical path length of 80 m. A group of 72-inch warm-white and black-light fluorescent lamps lined the inner circumference of the cell. The temperature of the cell was about 45°C. The first order rate constant for NO_2 photolysis (K_d) as measured in the infrared cell was 0.2 per minute.

In this work the thermal rate of oxidation of NO by oxygen was measured at 3 ppm of NO and 0.05 to 0.1 ppm of NO_2 in 20 percent oxygen and 80 percent nitrogen. Values in the range of 0.005 to 0.007 ppm per minute were obtained repeatedly. Evidence for the participation of the organic hydrocarbons in the photooxidation of NO was based on reaction rates in excess of 0.005 to 0.007 ppm per minute.

The effect of ultraviolet light and 5 parts of aromatic hydrocarbons or olefins per million on the rate of oxidation of 3 parts of NO per million is given in Table 1. In the plastic containers, the K_d for NO_2 photolysis in nitrogen was 0.35 per minute. Net oxidant values expressed as parts per million by volume for ozone plus organic peroxycompounds are also listed for the more reactive aromatic compounds.

Table 1. Effect of organic hydrocarbons on the photochemically induced oxidation of nitric oxide to nitrogen dioxide.

NO_2		Oxidant	
Rate of formation*	Time to reach maximum (min)	Concn. (ppm)	Time obtained (min)
	Ethylene		
0.08	45		
	Propylene		
.18	20		
	Isobutene		
.20	23		
	Toluene		
.03	100	0.5	180
	p-*Xylene*		
.07	50	.65	150
	o-*Xylene*		
.07	50	1.0	130
	m-*Xylene*		
.17	25	0.85	90
	Mesitylene		
.22	17	1.1	150
	Isopropylbenzene		
.02	125		

* The rate is expressed as half-conversion time.

Summarizing the results, Table 1 clearly indicates that the most reactive aromatic hydrocarbons studied, such as 1,3,5-trimethylbenzene and *m*-xylene, participate in the photooxidation of NO at about the same rate as 1-alkenes. *O*-xylene and *p*-xylene reacted at about the same rate as ethylene. Monoalkyl derivatives such as toluene and isopropylbenzene participate much less than the dialkyl and trialkylbenzenes. This trend in reactivity for the aromatic hydrocarbons appears to be qualitatively related to their basicities and hyperconjugative order. The appreciable oxidant concentrations obtained strongly confirm the work of Haagen-Smit and Fox (*3*).

Preliminary infrared analyses indicate that the half-conversion times to products of the more reactive aromatic hydrocarbons such as 1,3,5-trimethylbenzene and *m*-xylene are somewhat less than the half-conversion time of about 4 hours for ethylene. Other