one would expect that the strips which were more easily stretched, and hence longer, would be more sensitive to norepinephrine. However, we were unable to confirm this in these experiments. Ideally each strip should receive a stretch that is exactly proportional to its length but with our apparatus this was not possible because we could not establish a resting length until the experiment had been completed. It may be, therefore, that the discrepancy between our findings and those of Speden are due, at least in part, to differences in technique. With further modification of the apparatus, to facilitate measurement of length changes during an experiment, we hope to find such a correlation.

Summary. A technique for studying the pharmacology of vascular smooth muscle has been described. This technique utilizes pulsed tension rather than the static tension customarily employed and has permitted the following observations: Contraction responses to the catecholamines have shown accelerated onset and recovery with increased uniformity of response. A differential sensitivity to norepinephrine has been shown to exist in segments taken from different parts of the same aorta. A progressive reduction in sensitivity to norepinephrine was observed from the arch to the origin of the superior mesenteric artery.

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Biological and Chromatographic Evidence for the Storage of Ethynylestradiol-3-Cyclopentyl Ether in Rat Brain. (30249)

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Unlike ethynylestradiol,* the oral administration of its 3-cyclopentyl ether is followed by storage of a considerable amount of estrogenic material in the body fat of the rat(1). The uterine growth-stimulating activity of orally administered estrogenic material stored

* The following trivial names and abbreviations are used in this paper: ethynylestradiol (EE) = 19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17 β-diol; ethynylestradiol-3-cyclopentyl ether (EECPE) = 3-cyclopentyloxy-19-nor-17 α-pregna-1,3,5(10) -trien-20-yn-17 β-ol.

in the fat was similar to that of the standard when the slopes of the two dose-response curves were compared(1). This observation suggested that the substance stored in the fat may have been similar to or identical with the administered compound. One of the objects of this report is to present evidence which supports this hypothesis.

Furthermore, the observation(2) that ethynylestradiol-3-cyclopentyl ether is more effective than the parent compound in inhibiting pituitary gonadotrophin hypersecretion in parabiotic rats prompted us to investigate the possible storage of this compound in the brain. Data are presented which indicate that, unlike ethynylestradiol, oral administration of its 3-cyclopentyl ether is followed by storage of the unaltered estrogen in the brain of the rat.

Materials and methods. A. *General procedure.* Female albino rats (Hemlock Hollow) weighing 160-180 g each received a single oral dose of 2 mg ethynylestradiol (EE) or its 3-cyclopentyl ether (EECPE)† in 1.0 ml sesame oil. Twenty-four or 72 hours after administration, the rats were killed and the entire brains (excluding cerebellum) and perirenal fat pads were removed, weighed and used for biological or chromatographic analysis.

B. *Biological determination of the amount of estrogenic material stored in brain.* The brains of each group (30 rats) of treated or control animals were pooled together in a 40 ml Tenbroek grinder containing 7-8 ml methylene chloride. The brains were ground with successive aliquots of methylene chloride up to a total volume of 300 ml. The mixture was filtered and 10 ml sesame oil dissolved in each filtrate. Next the methylene chloride was removed under water vacuum at 40-45°C on a flash evaporator. The residues (brain substances plus 10 ml sesame oil) constituted the mother solutions. The mother solutions obtained from brains of EECPE-treated rats were diluted with additional sesame oil to provide 3 desired activity concentrations for bioassay work (estimated from preliminary results). Mother solutions from brains of EE-treated or control rats were administered without dilution.

Immature female rats (Hemlock Hollow) weighing 30-35 g were used for bioassay in groups of 10 per dose. The extracts, as well as scalar doses of standard preparations of EE or EECPE, were administered orally in 0.2 ml volumes once daily for 3 days. On the morning of the 4th day, the rats were killed and the wet weight of the uterus (after pressing out the intrauterine fluid) was determined to the nearest 0.1 mg on a torsion balance.

The total amount of steroid present in each residue was determined on the basis of data shown in Fig. 1. This was done by: (a) calculating the amount of original brain tissue necessary to produce a uterine weight increase comparable to that of 0.15 μg of the standard; (b) assuming that this amount of brain tissue contained 0.15 μg of the steroid; and (c) multiplying 0.15 by the dilution coefficient of the residue. Since the weight of the brains was quite constant (1.2 $\pm$ 0.1 g), the total amount of steroid stored in each brain was estimated by dividing the total amount of steroid found by the number of rats in the group.

C. *Preparation for TLC of estrogenic material stored in brain and body fat.* The brains of each group (10 rats for the 24-hour and 30 rats for the 72-hour determinations) of treated and control animals were removed, weighed, pooled by group and transferred to a 40 ml Tenbroek grinder containing 7-8 ml of methylene chloride. The brains were ground with successive aliquots of methylene chloride up to a total volume of 100 ml for the 24-hour and 300 ml for the 72-hour determinations. The total volume was filtered and to 2 control samples (10 brains each) 50 μg of either EE or EECPE were added (control on recovery of steroids). The methylene chloride was flash evaporated under water vacuum at 40-45°C preparatory to purification and chromatography.

The perirenal fat of each group (10 rats) of experimental and control animals was excised, pooled by group, weighed and transferred to a 15 ml Tenbroek grinder containing 4-5 ml methylene chloride. The fat was

† This compound which has the non-proprietary name of Quinestrol, was kindly supplied by Prof. Ercoli, Vismara Terapeutici, Como, Italy.

TABLE I. Steroid Concentration in Brain of Female Albino Rats Following Single Oral Administration of 2 mg/Animal of EE or EECPE.

Hr after administration	No. of animals	Total amt of steroid recovered (μg)			Avg amt of EECPE stored in each brain (μg)
		Controls	EE	EECPE	
24	30	0	0	69.4	2.3
72	30	0	0	15.0	.5

ground with successive aliquots of methylene chloride up to a total volume of 100 ml. The extracts were filtered and flash evaporated as above, leaving an oily residue. To 2 ml of residue from control animals, 50 μg of either EE or EECPE were then added (control on recovery of steroids). Two ml of each oily residue was then transferred to 50 ml centrifuge tubes preparatory to purification and chromatography.

D. *Purification and chromatography of estrogenic material stored in brain and body fat.* The residues of the brain and fat extracts were suspended with gentle warming in 50 ml 70% methanol and the suspensions stored overnight at —45°C to precipitate lipids(3). After centrifugation (—15°C at 2500 rpm), the methanolic supernatants were filtered through sintered glass filters (fine porosity) which were kept cold by dry ice packs surrounding them. The filtrates were flash evaporated under vacuum at 40-45°C. If a significant amount of residue appeared, the entire methanol precipitation was repeated. The final fat-free extracts were taken up in 0.5 ml benzene-methanol (1:1). The extracts and authentic standards for EE and EECPE were spotted 2 cm from the edge of glass plates covered with 250 μ layer of activated (100°C for 30 min) silica gel G (Brinkmann). The chromatograms were developed in an ascending system of either chloroform-methanol-water (485:15:1) or benzene-ethanol (90:10) in a saturated atmosphere. After ½ hour the solvent front was marked, the plates were dried, sprayed with 2% H_2SO_4 in 50% ethanol(4) and placed in a preheated oven at 100-105°C for 20 minutes to develop the colored estrogen spots. Preliminary chromatograms of extracts where EE or EECPE had been added to brains of control animals showed (visually assessed) recoveries of about 90% for the former and 40-50% for the latter. Recoveries from fat were about 90% for EE and only 20-30% for EECPE. The poor recovery of EECPE was due to its relatively low extractability from fat into 70% methanol. However, the fat precipitation by 70% methanol was an unavoidable prerequisite to subsequent chromatography. In view of this, we made no effort to quantitate our TLC results. (Quantitation was achieved by the bioassay, which did not employ fat precipitation, while TLC provided qualitative identification.)

Results. A. *Biological determination of amount of estrogenic material stored in the brain.* The results are shown in Table I and Fig. 1. Approximately 2.3 μg of steroid were present in each brain 24 hours after administration of EECPE. The 72nd hour determination showed that approximately 75-80% of the original amount of steroid stored in the brain had disappeared. Administration of EE was not followed by storage of steroid material in the brain.

B. *Identification by TLC of estrogenic material stored in brain and body fat.* The results obtained by chromatography of methylene chloride extracts of brain and fat were very similar. A typical chromatogram is shown in Fig. 2. Reddish-staining spots corresponding to EECPE were observed in chromatograms of extracts of brains and fat of rats treated with EECPE 24 hours previously, extracts of fat of rats treated with EECPE 72 hours previously and extracts of brains or fat of control rats to which EECPE had been added. EE-like spots were only detected when EE had been added to control brain or fat extracts. No EE appeared in chromatograms of extracts of fat or brains of EE or EECPE-treated animals. No other spots were observed which were not also evident in chromatograms of extracts of control brains or

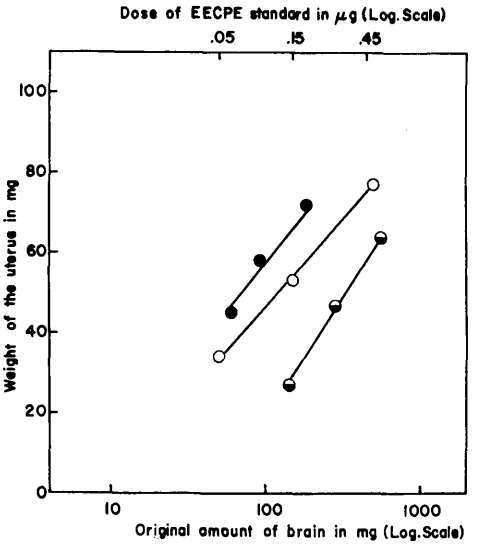

FIG. 1. Uterotrophic assay of extracts of brains of rats treated 24 or 72 hr previously with EECPE. EECPE standard ○—○; extract of brains of rats treated with EECPE 24 hr previously ●—●; extract of brains of rats treated with EECPE 72 hr previously ◒—◒. Each point represents the mean uterine weight of 10 rats. Statistical analysis showed that there is no significant difference in parallelism between the 3 dose-response curves. Control uterine weight (20 rats) was 13.4 mg. Uterine weights for EE standards (not shown on graph): 23.3 mg (0.05 μg dose); 39.8 mg (0.15 μg dose); 54.8 mg (0.45 μg dose); 10 rats per dose level. Uterine weight for undiluted extracts of brains of rats treated with EE (not shown on graph): 14.2 mg (30 rats).

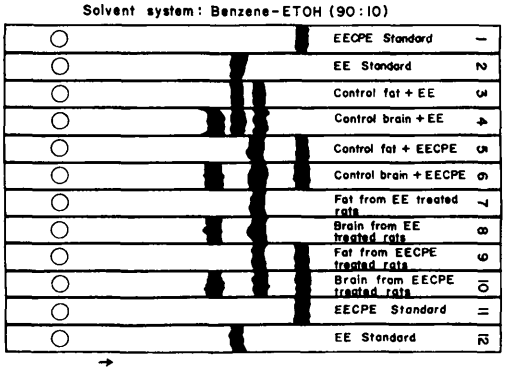

FIG. 2. Thin layer (silica gel G) chromatogram of EECPE and EE, and of extracts of brain and fat of rats treated with these compounds. In ascending order the spots are: Rf 0.56, blackish color, unknown material present in all brain extracts; Rf 0.62, bright red, EE; Rf 0.69, grayish color, unknown material present in all fat and brain extracts; Rf 0.83, bright red, EECPE.

fat with no steroid added. The results were similar whether the chromatograms were developed with chloroform-methanol-water (485:15:1) or with benzene-ethanol (90:10). The Rf values for EE, EECPE and the various extracts are reported in Table II.

Discussion. The TLC evidence supports the view that orally administered EECPE is stored unaltered in the brain and body fat of rats. Orally administered EE could not be detected by TLC in either tissue despite the fact that in control experiments added EE could be much more efficiently recovered from fat or brain than could added EECPE. These findings are supported by the more sensitive biological determination which likewise showed no estrogenic activity in extracts of fat(1) or brains from EE-treated rats. It is also of interest that no EE was detected in extracts of brains or fat of rats treated with EECPE. This suggests that either no hydrolysis occurs in these sites or that any EE so formed is not stored. In this regard it should be emphasized that no other possible metabolic products appeared on the chromatograms. The above data support the hypothesis(1) that the greater biological activ-

TABLE II. Rf Values of Sulfuric Acid Reaction Estrogens in Two TLC Systems.

Sample	Rf (H_2SO_4 reaction) Benzene 90: Ethanol 10	Chloroform 485: Methanol 15: Water 1
EECPE standard	.82-.85	.81
Control fat	—	—
Idem + 50 μg EECPE	.83	.81
Control brain	—	—
Idem + 50 μg EECPE	.85	.81
Extract of fat of EECPE-treated rats	.85	.80
Extract of brain of EECPE-treated rats	.82	.80
Ethynyl estradiol standard	.60-.63	.46
Control fat	—	—
Idem + 50 μg EE	.61	.46
Control brain	—	—
Idem + 50 μg EE	.64	.47
Extract of fat of EE-treated rats	—	—
Extract of brain of EE-treated rats	—	—

ity of the etherified form of EE may be due to prolonged storage in, and sustained release from, body fat depots.

The observed storage of EECPE in brain may reflect an extension of the body fat depot. Further studies with labeled EECPE will be necessary to determine whether this compound is uniformly distributed throughout the brain or localized to specific areas. In either case, the storage of a high concentration of EECPE in the brain conceivably could be related to the potent gonadotrophin-inhibiting properties of this compound.

Summary. The possible storage of estrogenic substances in brains of rats treated with ethynylestradiol (EE) or its 3-cyclopentyl ether (EECPE) was investigated. Twenty-four or 72 hours after a single oral administration of 2 mg of the test steroids, brains were removed and extracted with methylene chloride. The extracts were bioassayed for uterotrophic potency or subjected to thin layer chromatography. Uterotrophic activity, corresponding to 2.3 and 0.5 μg of the standard, was detected 24 and 72 hours after administration of EECPE. This material corresponded to authentic EECPE in two TLC systems. Similar chromatographic analyses showed that the estrogenic substance, previously detected biologically in body fat of rats treated with EECPE(1), likewise corresponded to the unaltered ether. Neither bioassay nor TLC detected any estrogenic substances in brains or fat of rats treated with EE. The evidence supports the view that EECPE, but not EE, is stored unaltered in brain and body fat and that such storage may account for the enhanced antigonadotrophic and uterotrophic potency of the etherified form of the steroid.

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Metabolism of β-Carotene.* (30250)

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It has been well established that β-carotene is converted to retinol in the animal body. Olson has reviewed the evidence supporting the central fission theory and the alternate concept of cleavage of the conjugated chain adjacent to one β-ionone ring(1). The most recent evidence appears to favor the central fission hypothesis but the details of the sequence of reactions are still unknown. If this mechanism is correct and if the only metabolic fate of carotene is to form retinol, then all metabolized β-carotene would form retinol and no extraneous by-products would be formed in this conversion process. The purpose of this study was to determine if in the rat all absorbed carotene were converted to retinol and then metabolized as such.

Materials and methods. Preparation of uniformly, doubly-labeled β-carotene was carried out by growing *Phycomyces blakesleeanus* in the presence of both 1-C^{14}-sodium acetate and H^3-sodium acetate(2) and purified by the procedure of Lotspeich *et al*(3). All radioactive β-carotene used had an E 1%/1 cm of 2700. The 6,7-C^{14}-retinol was obtained commercially from Hoffmann-LaRoche, Inc.,† and according to U.V. absorption was 95% pure.

Six, 24-hour fasted female albino rats of the Wistar strain weighing approximately 200 g were anesthetized with ether and an isolated, intact segment of intestine ligated at the py-

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† We wish to thank Dr. Oswald Wiss of Hoffmann-La Roche, Inc., for sending this material to us.