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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TABLE I. Absorption and Metabolism of Uniformly Labeled C^{14},H^3-β-Carotene and 6,7-C^{14}-Retinol in the Rat over a Period of Six Hours.

	C^{14} (dpm)	H^3 (dpm)	Ratio C^{14}:H^3
β-Carotene injected (1.22 mg)	1,559,300	6,147,750	0.25
Retinol injected (0.214 mg)	3,350,250	—	—
Total radioactivity injected	4,909,550	6,147,750	0.80
Radioactivity remaining in intestine + contents	3,134,150	4,298,800	0.73
Radioactivity absorbed	1,775,400	1,848,950	0.96
Radioactivity in liver	146,550	20,800	7.05

loris and 10 inches below the ligament of Treitz. A suspension was prepared containing 1.25 mg of doubly-labeled C^{14},H^3-β-carotene and 0.217 mg of singly-labeled 6,7-C^{14}-retinol in 200 mg of corn oil and 1.5 ml of 5% albumin and injected into the ligated intestinal segment of each rat. At the end of 6 hours the animals were sacrificed by decapitation and their ligated intestinal segments and livers removed. Lipid extracts were made of the ligated intestinal segments plus its content and of the livers according to the method of Bligh and Dyer(4).

All radioactivity was measured on lipid extracts using a Packard Tri-Carb Liquid Scintillation Spectrometer. Chemical and color quenching were corrected by means of either dilution or spiking techniques.

The amount of radioactivity absorbed at the end of 6 hours was calculated by subtracting the activity found in the ligated intestinal segment (including contents) from the total radioactivity injected.

TABLE II. Absorption and Metabolism of Uniformly Labeled C^{14},H^3-β-Carotene and 6,7-C^{14}-Retinol in the Rat over a Period of Six Hours.

	μMoles of labeled carbon from	
	β-Carotene	Retinol
Injected	91.20	14.99
Remaining in intestine plus contents	63.77	9.14
Absorbed	27.43	5.85
Liver	0.31	0.62
Calculated amount in liver if β-carotene were converted to retinol	2.96	

Results and discussion. Table I summarizes the results obtained from a typical rat 6 hours after simultaneous administration of uniformly-labeled C^{14},H^3-β-carotene and 6,7-C^{14}-retinol. It may be noted that the ratio of C^{14}:H^3 absorbed was 0.96 while the ratio found in the liver was 7.05. Since the specific activity of the C^{14} and H^3 of the administered β-carotene and retinol was known, it was possible to calculate the amount of labeled carbon in the various fractions derived from β-carotene and retinol.

Table II summarizes such calculations from the data presented in Table I. Here one finds that 5.85 μmoles of labeled carbon from retinol was absorbed and 0.62 μmole appeared in the liver while 27.43 μmoles of labeled carbon from β-carotene was absorbed and only 0.31 μmole appeared in the liver. If β-carotene were only converted to retinol, then the retinol so formed should have been metabolized in the same manner as the retinol injected and a proportionality should exist between the amount of labeled carbon absorbed and that stored in the liver from β-carotene and retinol. Using this proportionality one calculates that 2.96 μmoles of labeled carbon should have been found in the liver from β-carotene instead of the observed 0.31 μmole. This evidence strongly suggests that β-carotene was converted to product(s) other than retinol and that some β-carotene was metabolized by a process other than central fission.

The validity of this experiment may be questioned on the grounds that the C^{14}:H^3 ratio may be altered during absorption and metabolism of β-carotene. This possibility was tested by administering doubly-labeled β-carotene (C^{14}:H^3 = 0.25) in the same manner as previously described. Six hours after injecting this material similar C^{14}:H^3 ratios were found for the radioactivity of the intestinal segment (0.30), absorbed radioactivity (0.25) and the radioactivity of the liver (0.32). Therefore, the C^{14}:H^3 ratio was not significantly altered during its absorption and metabolism.

Summary. Evidence has been presented

through the use of doubly-labeled β-carotene and singly-labeled retinol that β-carotene is converted to product(s) other than retinol.

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Protection Against Junin Virus by Immunization with Live Tacaribe Virus. (30251)

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A new and important group of South American viruses has been recently recognized. It comprises Junin virus, the causative agent of Argentinian hemorrhagic fever(1,2), Tacaribe virus, which was recovered from bats in Trinidad(3) and Machupo virus, the causative agent of Bolivian hemorrhagic fever (4). The 3 viruses are almost indistinguishable by the complement-fixation (CF) test (4,5). However, they can be clearly differentiated by the plaque neutralization test(4,6) with a slight, but consistent, one way reaction between Tacaribe antibody and Junin virus.

Tacaribe virus is not known to be pathogenic for any adult laboratory animal; however, Junin virus is pathogenic for the adult guinea pig. By immunizing adult guinea pigs with Tacaribe virus and then challenging them with Junin virus, the immunological relationship between these two viruses could be further clarified. The results of this experiment and of serological tests with bloods obtained from both immunized and control animals are the subject of this report.

Materials and methods. Tacaribe virus, prototype strain TRVL 11573. Infectious stock virus used for immunization of guinea pigs and in the plaque neutralization test was prepared from suckling mouse brains (SMB) harvested 7 days after intracerebral inoculation, and made up as a 20% suspension in phosphate buffered saline (PBS)(7) with 0.5% bovine plasma albumin (BPA). This virus has had 22 serial passages in suckling mice (NIH-General Purpose); the virus used in the preparation of sucrose-acetone extracted(8) CF antigen had undergone one additional suckling mouse passage.

Junin virus, XJ strain. The infectious stock virus used in the challenge experiments and the plaque neutralization tests was prepared from a suspension of newborn guinea pig (NIH-Hartley) spleens harvested as animals sickened after intraperitoneal inoculation, and made up as a 20% suspension in PBS with 5% BPA. Previously this virus was passaged twice in guinea pigs, 13 times in newborn mice and 23 times more in guinea pigs. The CF antigen was prepared by sucrose-acetone extraction(8) of SMB harvested 7 days after intracerebral inoculation; this virus had undergone 2 passages in guinea pigs and 17 in newborn mice.

Immunization and challenge. Forty adult female guinea pigs (300-400 g) were immunized with a single intramuscular inoculation of 0.1 ml of stock Tacaribe virus diluted 1:20 in medium 199; this contained 3×10^5 plaque forming units (PFU) of virus. A similar number of control animals was inoculated with 0.1 ml of 20% normal SMB which was also diluted 1:20 in medium 199. Complement-fixation and plaque neutralization tests were performed on unpooled samples of sera obtained from individual control and immunized guinea pigs which were bled out by cardiac puncture prior to immunization and on days 14, 21, 32 and 49. Groups of immunized and control animals were challenged intraperitoneally with 0.1 ml of serial 10-fold