other agents. Thirdly, the separation is achieved at a single passage level. This eliminates the need for additional passages which could result in further changes in the virus. Our ALV-free primary and secondary vaccine seeds are at the same passage levels as the current ALV-contaminated primary and secondary vaccine seeds.[8]

Comparative studies of the ALV-contaminated and ALV-free vaccine seeds with respect to neurovirulence tests in monkeys and immunogenicity in monkeys and man are underway.

Summary. An avian leukosis virus (ALV) free primary yellow fever vaccine seed has been developed by ridding the 17D virus vaccine seed of its ALV contaminant. By differential filtration through Millipore membrane filters of different pore sizes, yellow fever virus was separated from its contaminant by physical means. The ALV could not be detected in the new primary and secondary vaccine seeds by RIF, COFAL, and FAB tests.

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[8] An updated passage history of the YF vaccine virus will be published elsewhere. In brief, the passage history of the ALV-contaminated primary seed is: lot 145-3 (passage 229) → lot YF-1 (P230) → lot YF 10 (P231) → lot AB237 (232); for the ALV-free primary seed the history is: lot 145-3 (P229) → lot 145-3A1 (P230) → lot 145-3A1-50 (P231) → lot 17D-51 (P232).

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A Note on the Intestinal Absorption of Cholesteryl Ethers in the Rat* (32886)

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It has been reported that cholesteryl-2,2-methyl ethyl caproate, the ester bond of which is not split by cholesterol esterase *in vitro* is not absorbed by the intestinal tract of the rat (1). This evidence was based on the absence of this ester in the thoracic duct lymph when fed to rats. The above finding was interpreted by Vahouny and Treadwell (1) to show what they call an "absolute requirement for free sterol for absorption by rat intestinal mucosa."

Napier (2) found "no significant" absorption of cholesterol esters of 2-methyl laurate or myristate in balance studies in the rat. Significant amounts of the cholesterol moiety of these esters (maximally 3.6%) were,

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however, recovered from the carcass of these rats.

This note demonstrates that cholesteryl ethers are absorbed by the intestinal tract of the rat and largely transported in the ether form in the thoracic duct lymph the extent of absorption being inversely related to the chain length of the component aliphatic alcohol.

Materials and Methods. Cholesteryl-4-^{14}C ethers of methyl-, ethyl-, propyl-, amyl-, and decylalcohols were prepared as described by McKennis (3). The ethers were purified to better than 99% purity by thin-layer chromatography, Cholesterol-4-^{14}C was a product of Radiochemical Centre, Amersham, England.

Cholesterol and the cholesteryl ethers were dissolved in triolein, 10 mg/ml and 250 μl of this solution were given to rats with a thoracic duct fistula via a gastric catheter. Lymph was collected for 24–48 hours, the lipids were extracted with chloroform–methanol 2:1 and saponified. The nonsaponifiable fraction was used for activity determination and thin-layer chromatography using silica gel plates. The plates were developed with 20% ethyl ether in light petroleum. The spots were visualized by exposure to iodine and identified by standards. The spots were scraped off and eluted with chloroform. An aliquot of the eluate was again used for activity determination.

The cholesteryl methyl ether could be well separated from the intact cholesterol esters of lymph by thin-layer chromatography using 10% diethyl ether in light petroleum as developing phase. This method was used to characterize the radioactivity of the lymph lipids after feeding this ether.

Radioactivity was assayed by liquid scintillation counting using a Packard 4000 Spectrometer.

Results and Discussion. Table I shows the results of the lymph experiments. It is quite clear that cholesteryl ethers are absorbed as such by the intestinal mucosa and are found in the thoracic duct lymph largely in this form. Some activity is also found in the cholesterol fraction (or cholesterol ester fraction if unhydrolyzed lymph fat is used).

In these experiments between 40 and 50% cholesterol is recovered in the lymph and about 33% cholesteryl methyl ether. With an increase of one carbon atom in the chain length of the aliphatic alcohol, the percentage appearing in the lymph decreases by a third. Further increase with one or two carbons has little effect, while the ether of decylalcohol (C 10) is recovered to around 4% in the lymph (1.2–8.8%). Even for these ethers most of the recovered activity was in the ether form.

TABLE I. Transport of Cholesterol and a Series of Homologous Cholesteryl Ethers in Rat Thoracic Duct Lymph after Their Oral Administration.

The ethers were fed in solution in triolein, 10 mg/ml of triolein; 250 μl of the triolein solution was administered via a stomach catheter.

Compound administered	Recovery in t.d. lymph (%)	Recovered activity as cholesteryl ether (%)
Cholesterol	43.5	—
Cholesteryl methyl ether	33.2, 33.4	96
Cholesteryl ethyl ether	10.6	86
Cholesteryl propyl ether	7.6, 8.4, 11.0	97, 98
Cholesteryl amyl ether	9.4	90
Cholesteryl decyl ether	1.2, 3.1, 3.2, 5.3, 8.8	86, 97

Table II shows the result of a separation by TLC of the total lymph lipids from a rat fed cholesteryl methyl ether. In this experiment 96% of the activity was found as ether.

It is clear from the above results that cholesteryl ethers are absorbed by the intestinal

TABLE II. Thin-layer Chromatogram of Total Lymph Lipids from a Rat Fed Cholesteryl-4-^{14}C Methyl Ether in Triolein.

The lipids recovered from the lymph were applied to a silica gel thin-layer plate developed with 10% diethyl ether in light petroleum. The respective spots localized after exposure to iodine vapors and identified by standards were scraped off and their radioactivity determined by liquid scintillation counting.

	Distribution (%)
Front	0.3
Cholesterol ester	0.9
Blank	0
Cholesteryl methyl ester	96.5
Triglyceride	0.7
Free cholesterol	1.6

tract as such and that cholesterol can be absorbed even if it is not in the form of the free sterol. Such a finding is not surprising considering the fact that many nonpolar lipids such as hydrocarbons have been found to be absorbed to some extent.

The extent of the absorption of the cholesteryl ethers is inversely related to the chain length of the component aliphatic alcohol. Intestinal absorption of the cholesteryl decyl ether is of the same order of magnitude as the absorption of the cholesterol ester of 2-methyllaurate when this is calculated on the retention in the carcass(2).

The finding of Vahouny and Treadwell (1) that the cholesterol esters of 2,2-methyl ethyl caproic acid are not absorbed in measurable quantities may be explained as an effect of the considerable bulk of this branched chain fatty acid ester.

Summary. Cholesteryl ethers of aliphatic alcohols when fed in triolein are absorbed by rat intestinal mucosa and transported in the thoracic duct lymph of rats fed these ethers. The recovery of the ethers in the lymph decreases with the chain length of the aliphatic alcohol moiety of the ether. Around 1/3 of the methyl ether fed being recovered and 4% of the decyl ether. The cholesteryl ethers are recovered in the lymph in general to more than 90% in unchanged form.

The results obtained clearly show that the free form is not the only possible transport form for cholesterol into the intestinal mucosa cells.

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Effect of Intracisternally Administered Insulin-^{131}I in Normal and Vagotomized Dogs* (32887)

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Although it is generally stated that insulin has no direct action on brain metabolism, recent studies suggest that this is not the case (1–8). One of the chief arguments against a direct effect of insulin on the central nervous system is the supposed inability of this comparatively large molecule to pass the blood–brain barrier (9–12).

We have previously demonstrated that insulin is found normally in the cerebrospinal fluid (CSF) and that CSF insulin levels rise following an increase in the plasma insulin concentration (13). This indicates that insulin can cross the blood–CSF (and presumably also the blood–brain) barrier.

Recently, Chowers *et al.* (7,8) have published data in support of the possibility that insulin has direct effects on brain. These workers reported that intracisternal injection of insulin in dogs resulted in a decrease in the glucose concentration of the CSF and of the blood, despite the apparent failure of insulin to pass out of the CSF into the blood. Since vagotomy prior to insulin injection prevented only the peripheral hypoglycemia, they concluded that the injected insulin has a direct effect on parasympathetic centers in the brain to increase the release of endogenous insulin, as well as an effect on glucose utilization by the central nervous system.

These provocative reports have led us to reinvestigate the effects of intracisternally-administered insulin.

Methods. Experiments were carried out on male and female dogs (10–15 kg) fasted for

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