same results. In addition it is well known that vit. K-deficient rats are not "starved" animals.**

The transport of electrons, NADPH-cytochrome c reductase to menadione or to other artificial acceptors as 2,6 dichlorophenolindophenol(5,17) and cytochrome c was not inhibited in microsomes from vit. K-deficient rats. Vitamin K_1 when added to the microsomal systems appeared to act as menadione, *i.e.*, as an artificial acceptor, although to a much lesser extent. This may be attributed to not only the relative ease with which menadione can attain an active site compared to vit. K_1, but also to the rapidity with which the semiquinone of menadione can be reoxidized by molecular oxygen.

The effect of vit. K deficiency appears only in the autocatalytic process itself. Thus, the immediate possibilities for investigation are changes in the composition of lipids or the production or increase of an inhibitor to lipid peroxidation.

Summary. Oxygen consumption relative to lipid peroxidation was decreased in microsomes from vitamin K-deficient, warfarin-treated, and dicumarol-treated rats. Vitamin K_1, when added to the microsomal system, appears to function similarly to the artificial acceptor menadione and does not alleviate the differences between control and vitamin K-deficient animals observed in lipid peroxidation.

** A rat on a vit. K-deficient diet will gain weight at a rate almost equal to that of a control. Twenty-four to 48 hours before death due to hemorrhage, the animal will stop eating.

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Gas-Liquid Radiochromatography of Intact Labeled Cholesterol Esters.* (31031)

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Gas-liquid radiochromatography is an excellent technique for simultaneous determination of mass and radioactivity of labeled components emerging from gas chromatographic columns(1-3). It offers several advantages over conventional techniques such as the direct determination of specific activity of one or more components and rapid analysis. It is particularly suited for metabolic studies where a number of components

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can be analyzed (mass and radioactivity) in a given mixture. Absolute and relative specific activity of each component can then be determined. With a properly designed system it is also possible to measure C^{14} and H^3 in a dual labeled component with no isotopic overlap.

The technique has been applied principally to the determination of radioactive fatty acids (1-3). Of practical importance is whether this procedure can be utilized for determination of high boiling compounds such as sterols, sterol esters, and steroids. The present report describes a system for simultaneous measurement of mass and radioactivity of C^{14} and/or H^3 labeled intact cholesterol esters and its application to esters isolated from biological material.

Methods and materials. Gas-liquid chromatography was carried out on a Barber-Colman Series 5000 dual glass column gas chromatograph with flame ionization detectors. Cholesterol esters were separated on a 24 inch × 4 mm glass column packed with 3% QF-1 on 100/120 mesh gas-chrom Q (Applied Science Laboratories). Both the glass column and support were treated with dimethyl-dichlorosilane. The operating conditions were: column bath temperature 290°C, detector bath temperature 300°C, injector block temperature 320°C, argon flow rate 50 ml/min with an inlet pressure of 30 lb/square inch. Under these conditions cholesterol palmitate had a retention time of 4.5 minutes. Appropriate highly purified cholesterol esters were used as standards. The unlabeled cholesterol esters were obtained from Applied Science Laboratories except cholesterol arachidonate which was prepared enzymatically(4). A modified Barber-Colman radioactive monitoring system was utilized to measure the C^{14} or H^3 activities of the cholesterol esters emerging from the column(5). Simultaneous mass and radioactivity were determined on column effluents by splitting the stream emerging from the column. One portion of the stream (1/10) went to the flame ionization detector while the remaining portion (9/10) was transferred at 300°C to a combustion tube filled with CuO or CuO + Fe. The compounds were combusted at 630°C to produce $C^{14}O_2 + H_2O$ (CuO) or $CO_2 + H_2^3$ (CuO + Fe). If dual labeled samples were injected, traps were used to remove one of the labeled components. A magnesium perchlorate was used to remove $H_2{}^3O$ and ascarite (8-20 mesh) absorbed $C^{14}O_2$. The radioactive gases were then led through a proportional detector. The efficiency of the radioactive detector system for C^{14} was 90% and 60% for H^3. Standard labeled cholesterol esters (single and dual labeled) were prepared enzymatically(4) and purified by thin-layer chromatography(6).

A standard for mass and radioactivity was routinely prepared for each component to be determined. The standard contained a known amount of mass and DPM per unit volume. This was generally 5 γ and 2000 DPM H^3 or C^{14} per microliter hexane solution. The DPM were accurately determined on a Nuclear Chicago liquid scintillation system. Standardization was carried out each day the instrument was used or when the combustion tube or column was changed. The overall reproducibility of the method for mass and radioactivity was ± 5%. Quantitative specific activity determinations were carried out on labeled rat liver cholesterol esters by the radiochromatographic procedure and compared to a liquid scintillation counting-chemical assay technique(7). A normal rat received 15 μc cholesterol-4-C^{14} orally in a test meal(7) and 50 μc cholesterol 7α-H^3 dispersed in rat serum. The animal was sacrificed at 6 hours, and the blood, liver, and adrenals removed. The cholesterol esters were isolated as described earlier(7). The liver cholesterol esters were then separated into saturated, monounsaturated, linoleate, and arachidonate on silica gel G glass coated plates impregnated with $AgNO_3$ (6), and then analyzed by both procedures.

Results and discussion. The gas chromatographic separation of a series of cholesterol esters is shown in Fig. 1. Excellent resolution was obtained between cholesterol esters differing by 2 carbons. The mass of each component was approximately 5 γ. Separation could not be achieved on the basis of saturation; preliminary separation of the esters by thin-layer chromatography followed

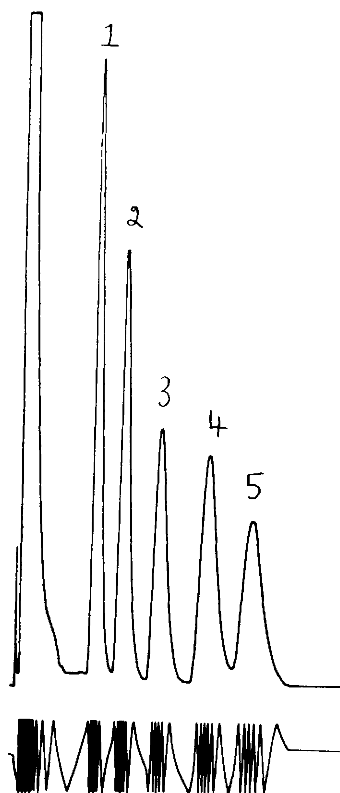

FIG. 1. Separation of cholesterol esters by carbon number. QF-1 column, 290°C. Each peak represents 5γ. Cholesterol ester peaks as follows: 1 laurate, 2 myristate, 3 palmitate, 4 stearate, 5 arachidonate.

by gas-liquid chromatography afforded a means of determining the saturated, monounsaturated, linoleate, and arachidonate esters. Kuksis(8) also reported the separation of intact cholesterol esters by carbon number on short thermally stripped SE-30 columns. The presently described system with QF-1 as a stationary phase gave good results without special treatment of the phase or temperature programming.

The simultaneous determination of mass and C^{14}-activity of a mixture of synthetic C^{14}-cholesterol esters is shown in Fig. 2. Resolution of the radioactive peaks was excellent considering the small differences in retention time between the various components. The procedure was applied to labeled cholesterol esters isolated from the serum, liver, and adrenal of rats administered labeled cholesterol (Fig. 3, 4, 5). Varying amounts of H^3-activity were found to be associated with the cholesterol esters of the various tissues. The serum and liver cholesterol esters contained fatty acids of 16, 18, and 20 carbons while the adrenal cholesterol esters in addition to these acids had a high proportion of acids with 22 carbons. This acid was previously shown to be a C_{22} tetraenoic acid(9). There is also present in the adrenal cholesterol ester fraction, another acid (between the C_{20} and C_{22} acids) which could not be identified.

Gas-liquid radiochromatography of the total cholesterol ester fraction affords an opportunity to ascertain the pattern of labeling of the cholesterol esters by carbon number. When this procedure is coupled with thin-layer chromatography, quantitative mass and specific activity data can be obtained on the saturated, monounsaturated, dienoic, tetraenoic, and other polyunsaturated cholesterol esters. This is shown in Table I. The specific activities of dual labeled rat liver cholesterol esters are in good agreement with those results obtained by the standard liquid scintillation-chemical assay procedure. However, the gas-liquid radiochromatographic procedure is more rapid, much smaller amounts of cholesterol ester can be detected and fewer separate analytical determinations are needed. The procedure is particularly useful for metabolic *in vitro* studies where small amounts of labeled metabolites and end products can be detected and quantitated in a given sample.

Summary. Gas-liquid radiochromatography has been successfully applied to the simul-

TABLE I. Specific Activities of Dual Labeled [C^{14} and H^3] Rat Liver Cholesterol Ester Classes.

	Cholesterol esters,† DPM/mg × 10^3			
Label*	Saturated	Monounsaturated	Linoleate	Arachidonate
	Gas-liquid radiochromatography			
H^3	730	760	687	992
C^{14}	218	285	140	105
	Liquid scintillation counting—chemical assay			
H^3	723	811	760	1065
C^{14}	200	262	157	99

* Rats received 50 μc cholesterol-7α-H^3 intravenously and 15 μc cholesterol-4-C^{14} orally; animals killed at 6 hr after receiving labeled cholesterol.

† Specific activities of esters expressed in terms of free cholesterol.

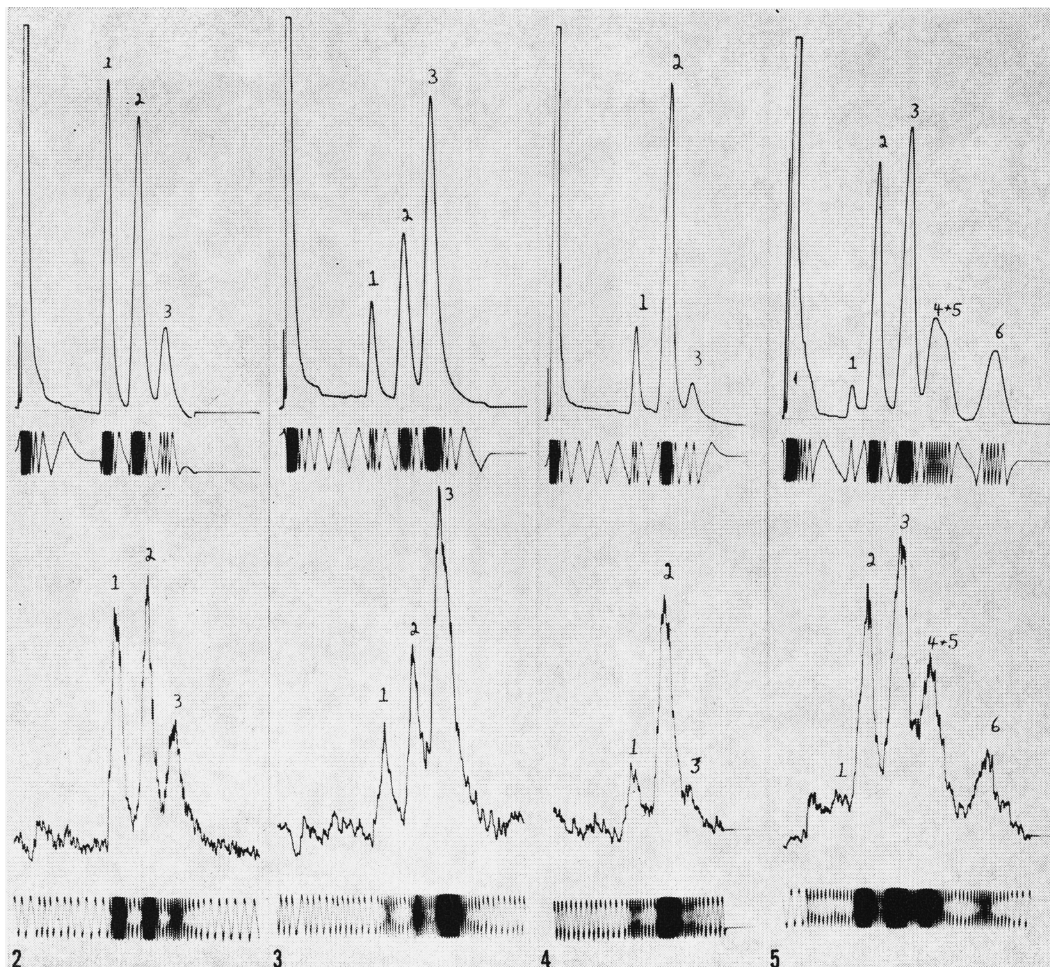

FIG. 2. Separation of a mixture of C^{14}-cholesterol esters. QF-1 column 290°C. Cholesterol ester peaks as follows: 1 palmitate, 2 oleate, 3 arachidonate. Mass load of palmitate and oleate 10 γ each, and 3 γ for arachidonate. Radioactivity load palmitate 3600 DPM, oleate 4500 DPM, arachidonate 2100 DPM.

FIG. 3. Labeled (H^3) rat serum cholesterol esters. QF-1 column 290°C. Cholesterol ester peaks according to fatty acid carbon number: 1 C_{16}, 2 C_{18}, 3 C_{20}.

FIG. 4. Labeled (H^3) rat liver cholesterol esters. QF-1 column 290°C. Cholesterol ester peaks according to fatty acid carbon numbers: 1 C_{16}, 2 C_{18}, 3 C_{20}.

FIG. 5. Labeled (H^3) rat adrenal cholesterol esters. QF-1 column 290°C. Cholesterol ester peaks according to fatty acid carbon number: 1 C_{14}, 2 C_{16}, 3 C_{18}, 4 and 5 C_{20} + unknown, 6 C_{22}.

taneous analysis of mass and radioactivity of labeled intact cholesterol esters. Representative curves are presented for labeled cholesterol esters isolated from rat serum, liver, and adrenal gland. Quantitative mass and radioactive measurements were carried out on the labeled cholesterol esters isolated from the liver of rats given cholesterol-4-C^{14} and cholesterol-7α-H^3. The results indicate that gas-liquid radiochromatography coupled with thin-layer chromatography affords an excellent means for determining the specific radioactivity of labeled cholesterol ester classes.

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An Epithelial-Like Cell Line in Continuous Culture from Normal Adult Human Gingiva.* (31032)

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There have been several reports of the continuous cultivation *in vitro* of adult human epithelial-like cells obtained from abnormal tissue(1-4). Continuous cultivation of epithelial-like cells derived from normal adult human tissue is comparatively uncommon(5, 6).

This report deals with the isolation and growth in continuous culture of an epithelial-like cell line derived from an area of clinically normal adult human attached gingiva.

Source of tissue. The tissue was obtained from a 19-year-old white female in apparent good health. The attached gingiva, labial mucosa, tongue, cheeks and oropharyngeal regions were clinically normal.

Using mandibular block anesthesia (lidocaine HCl 2% with epinephrine, 1:100,000) with no local infiltration into the biopsy areas a strip of attached gingiva measuring 2 × 6 mm was removed in the mandibular anterior region (Fig. 1). At the same time a small fragment of labial mucosa (2 × 3 mm) was also excised.

Material and method. The tissue was washed in Hanks' salt solution containing 0.05% $NaHCO_3$ to remove adherent blood and saliva.

The gingival specimen was cut into 3 sections and the labial specimen was cut in half (Fig. 2). The fragments were placed in separate sterile 60 mm petri dishes with the cut surface against the glass. The fragments were held in position with a small strip of gelatin film(7) until outgrowth occurred at which

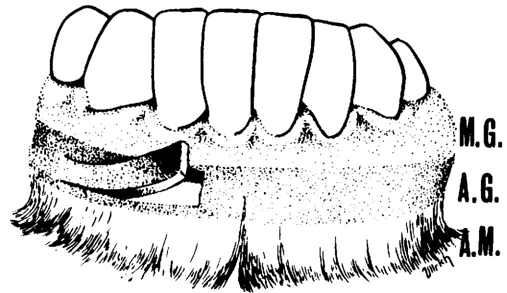

FIG. 1. Diagram showing area where gingival specimen was obtained. M.G., marginal gingiva; A.G., attached gingiva; A. M., alveolar mucosa.

time the gelatin film was removed. The medium consisted of Eagle's basal solution supplemented with 15% calf serum.† The Eagle's solution was modified by the addition of inositol(8) and the use of Hanks' salt solution containing 0.05% $NaHCO_3$. Penicillin, streptomycin and amphotericin B were added to the medium to give final concentrations of 100 units, 100 μg and 4 μg, per ml, respectively. Two ml of medium were placed in each petri dish. The culture dishes were then incubated at 36-37°C in an atmosphere of approximately 5% CO_2 in air. Cultures were examined twice weekly when the medium changes were made.

Results. Epithelial-like outgrowth was noted from 2 gingival and one labial explant by the third week. One section of labial mucosa showed only fibroblast-like outgrowth and one fragment of gingiva did not grow. During the fourth week a few small separate colonies of compact epithelial-like cells were noted in the 4 plates showing growth. These

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