been effectively modified by serial propagation in dog-kidney cultures followed by adaptation to and serial cultivation in swine-embryo tissue. The modified virus is an effective immunizing agent, and produces in the vaccinated animal no adverse reaction except for an occasional case of light and transient opacification of the eye following massive peripheral injection of the virus. The modified ICH virus may spread through the urine of vaccinated animals to intimate contact animals, but although these develop antibodies they show no signs of disease. A combined vaccine prepared by mixing modified CD and ICH viruses elicited good protection against both diseases in doubly susceptible animals, and in puppies already immune to ICH did not interfere with the production of immunity to CD.

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Synthesis of Cholesterol by a Strain of Human Uterine Fibroblasts Propagated *in vitro*. (24243)

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It is generally accepted that fibroblasts constitute a high proportion of cells in loose connective tissue(1). The metabolic capabilities of fibroblasts appear to be related in some fashion to their high degree of resistance to the action of antiphlogistic steroids(2). These observations might be related to the fact that fibroblasts appear to play an important role in extrahepatic metabolism of steroids(3,4). It has also been demonstrated that strains of U12 fibroblasts isolated from human myometrium and propagated *in vitro* for many years have the capacity to metabolize cortisol and progesterone to a variety of steroid products(5). These observations raised the question as to whether U12 cells might also be able to synthesize cholesterol and possibly other steroids from low molecular weight precursors such as acetate. The synthesis of cholesterol could not be evaluated on the basis of nutrition since the least fastidious strain of U12 (6) will not proliferate in media devoid of dialyzed horse serum. The data to be presented indicate that strain U12-79 is capable of synthesizing cholesterol as judged by appearance of isotope in this compound when the cells are grown in a medium containing C^{14} acetate.

Experimental. Stock cultures of U12-79 were propagated in a chemically defined solu-

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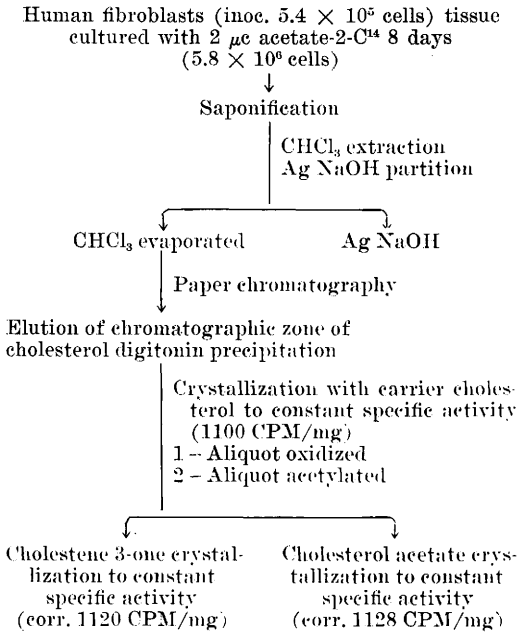

FIG. 1. Procedure for the identification of C^{14} cholesterol U12-79.

tion, S103(7), supplemented with 5% by volume of dialyzed horse serum according to methods described by Swim and Parker(8). To ascertain whether cholesterol was synthesized by U12-79, the cells were propagated in this medium supplemented with 0.17 μM/ml of acetate-2-C^{14} (specific activity, 1 μc/μM) as follows. Ten T60 flasks were planted with 5.4 x 10^5 cells/flask. On the 4th day the fluid was removed from each flask and replaced with fresh medium. On 8th day the medium was again removed and cells were washed twice with Earle's saline(9). At this time, the average number of cells/flask was 5.8 x 10^6. The medium from 4th and 8th day and the Earle's saline were combined. The pooled medium and the cells were analyzed separately for cholesterol as outlined in Fig. 1. The cells were saponified and the resulting mixture extracted 3 times with chloroform. The combined chloroform extract was evaporated and the residue was chromatographed directly on paper, using a modification of the hexane system of Zaffaroni (10), in which the formamide is not applied to the paper. That is, the sample is placed directly on the paper in hexane, and the chromatogram is developed with this solvent as the mobile phase. Under these conditions, cholesterol has an R_f of 0.65. The chromatographic zone corresponding to cholesterol was eluted with MeOH=$CHCl_3$(1:1). Non-radioactive carrier cholesterol was added to the eluate and cholesterol was precipitated with digitonin. The digitonin complex was hydrolyzed and the resulting cholesterol was crystallized to constant specific activity of 427 cpm/μM. A portion of this cholesterol was converted to cholestene-3-one by treatment with chromic acid. The cholestene-3-one after crystallizing to constant specific activity contained 435 cpm/μM. A second sample of purified cholesterol was acetylated and the resulting cholesterol acetate upon crystallizing to constant specific activity contained 428 cpm/μM. C^{14}-cholesterol was also isolated from the medium in this experiment and identified by the same procedures. The total C^{14} found in the cholesterol isolated from the cells and medium was approximately 0.8% of that added to the medium initially as acetate-2-C^{14}.

Discussion. The foregoing data indicate that the sequence of reactions leading to synthesis of cholesterol from acetate is operative in strain U12-79. Although the present experiments do not provide information on the extent to which net synthesis occurs, they indicate that these cells are capable of synthesizing cholesterol. Labeled cholesterol is found in a variety of tissues after intact animals are administered isotopic acetate(11); however, it is difficult to distinguish the cellular site of origin in tissues due to the mixed cell population. In this connection, it is of interest that cells of mesenchymal origin are capable of synthesizing cholesterol after 5 years *in vitro*. In view of the difficulties and uncertainties involved in studying the factors concerned with accumulation of cholesterol in various mammalian cells in the intact animal, the U12-79 system appears to offer a number of advantages as a model system for investigations of this type. In the course of these experiments other labeled compounds were isolated, some of which appear to be steroidal in nature. Experiments are under way at the present time to identify the sterol type com-

pounds in an attempt to relate some of the anabolic and catabolic aspects of steroid metabolism by strain U12-79.

Summary. Strain U12-79 of human uterine fibroblasts propagated *in vitro* for almost 5 years was grown in a medium containing acetate-2-C^{14}. C^{14}-cholesterol was isolated from both the cells and the medium.

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Rate of Flow and Cell Count of Thoracic Duct Lymph in the Mouse. (24244)

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This report describes a method for collection of thoracic duct lymph in anesthetized mice, and presents data on rate of flow, white cell count, and total lymphocyte output of lymph obtained. These data are compared with similar measurements of thoracic duct lymph in other animal forms.

Technic. Male mice (40 days old) of NIH-Webster strain, unfasted, 17-23 g B.W. were anesthetized by intraperitoneal injection of 6% sodium pentobarbital (7 mg/100 g B.W.). An incision was made through the abdominal wall, following the usual dorsal approach for left adrenalectomy. Manipulations and lymph collection were performed in a bloodless field under a dissecting microscope. The incised body wall was retracted to expose the abdominal aorta. Viscera within the field were carefully retracted, care being taken to avoid entering the peritoneal cavity. The aorta, cranial to the mesenteric lymph node (Aselli), was displaced gently away from posterior abdominal muscles caudal to the diaphragm. This procedure exposed the thoracic duct, contiguous to posterior aspect of the aorta. The dissection, carefully executed, resulted in formation of a depression or cup, bounded by the aorta on the left side and paravertebral muscles on the right. The superior border of this cup was demarcated by the aorta as it traversed the diaphragm; the inferior border of the cup was formed by juncture of the aorta, mesenteric lymph node, and paravertebral muscles. The floor of the cup was formed primarily by the left side of the thoracic duct.

The point of a 27 G hypodermic needle was used to make 2 slits in the exposed thoracic duct. The first opening was cephalic to a segmental artery crossing the duct. To insure complete drainage of the thoracic duct lymph into the structural cup, a second opening was made caudal to this segmental artery. After incising the wall of the duct, a free flow of clear or milky lymph drained into the anatomical cup or depression. The tip of a fine glass sequestrene treated cannula of small bore was then placed in the cup adjacent to the duct incisions. Lymph was drained through the cannula into a small glass vial under slight negative pressure. Lymph was collected for half hour periods, measurements being made at intervals during collection.

Results. Table I presents pertinent data