6. MacLeod, C. M., and Avery, O. T., *ibid.*, 1941, v73, 183.

7. ———, *ibid.*, 1941, v73, 191.

8. Anderson, H. C., and McCarty, M., *Am. J. Med*, 1950, v8, 445.

9. Stollerman, G. H., Glick, S., and Patel, D. J., *ibid.*, 1953, v15, 645.

10. Kroop, I. G., Heffer, E. T., and Shackman, N. H., *Clinical Research Proc.*, 1954, v2, 39.

11. Master, A. M., Dack, S., Grishman, A., Field, L. E., and Horn, H., *J. Mt. Sinai Hosp.*, 1947, v14, 8.

12. Blumgart, H. L., Schlesinger, M. J., and Davis, D., *Am. Heart J.*, 1940, v19, 1.

13. Losner, S., Volk, B. W., and Wilensky, N. D., *A. M. A. Arch. Int. Med.*, 1954, v93, 231.

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A Simplified Method for Determination of Lipide-C^{14} in Liver.* (21020)

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The present methods for determining lipide-C^{14} of tissues, in experiments in which slices are incubated with a C^{14}-labeled precursor, are time-consuming, and suffer from tedious manipulations, such as prolonged extraction and hydrolysis and repeated transferring of extracts from one vessel to another. These disadvantages have been completely eliminated in the simplified procedure described here. The entire analysis can be completed in less than 2 hours.

Procedure. The incubation flask is a 50-ml Erlenmeyer flask with a center well 10 mm in diameter and 20 mm high fused into the bottom. A self-sealing rubber cap is used as stopper. The general details of the incubation procedure have been described elsewhere(1). At the end of the incubation period, 0.25 ml of 30% KOH is introduced into the center well for absorption of CO_2 and, immediately thereafter, 0.25 ml of 5 N H_2SO_4 are added to the medium for inactivation of the tissue. Both alkali and acid are introduced by means of a syringe with a long hypodermic needle that is inserted through the rubber cap. Sufficient time (20-30 minutes at room temperature) is allowed for the absorption of CO_2 by the alkali, and the rubber cap is then removed. The contents of the center well are siphoned directly into a volumetric flask. The incubation medium is carefully filtered through Whatman No. 1 filter paper, so that the tissue slices are retained in the main compartment of the flask. The slices are thoroughly washed with several portions of distilled water, each of which is decanted onto the same filter paper. To avoid disintegration of the slices, the above procedure should be carried out no later than one hour after the reaction is stopped. The medium and washings are discarded unless a component of this fraction, such as glucose, ketone bodies, or the like, is to be analyzed. The filter paper is then washed with 5-10 ml of an alcohol-ether (3:1) mixture, and the washings are returned to the incubation flask. Two ml of 1 M sodium ethylate are then added to the tissue slices in the flask which is covered with a bubble

TABLE I. Test of Extraction Procedure.
To 500 mg ± 5 mg of liver slices was added one of the labeled compounds, and immediately thereafter the mixture was hydrolyzed. Extraction of the acidified hydrolysate was carried out as described in the text. Aliquots of the chloroform phase were directly mounted on aluminum discs and counted in the usual manner.

Labeled compound added to tissue slices	No. of exps.	% of added C^{14} recovered in chloroform phase (range)
*Fatty acid-C^{14}	6	99–101
Tripalmitin-1-C^{14}	3	98–100
Cholesterol-4-C^{14}	3	97–101
Alanine-1-C^{14}	3	0
Acetate-1-C^{14}	3	0
*Glycerol-C^{14}	6	0

* Biologically synthesized.

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stopper. The mixture is then hydrolyzed for 40 minutes on a steam bath. It is important not to allow the hydrolysate to become dry; if the volume becomes reduced during the heating, alcohol is added. At the end of this period, the bubble stopper is removed, 3 ml of water are added, and the heating is continued until the odor of alcohol is no longer detectable. The contents are then cooled, and acidified with 5 N H_2SO_4. Exactly 10 ml of chloroform are added from an automatic burette, an aluminum foil-covered stopper is inserted into the flask, which is shaken vigorously for about 2 minutes. The entire contents of the flask are then transferred to a 15-ml centrifuge tube. (This last transfer need not be quantitative. Since all lipides are now in the chloroform phase, measurements are made on aliquots of the original 10 ml of chloroform.) The tubes are centrifuged for about 5 minutes. Centrifugation separates the contents of the tube into an aqueous upper phase containing the water-soluble components and a lower chloroform phase containing the lipides. These 2 phases are separated by a thin plaque that is soluble in neither phase. Aliquots of the chloroform phase are removed by means of a syringe and a long hypodermic needle. For determination of lipide-C^{14}, a one-ml aliquot is mounted directly on an aluminum disk, and gentle heat is applied to evaporate the solvent. Aliquots of the chloroform phase may be withdrawn in a similar manner for determination of cholesterol or total fatty acids.

TABLE II. Test for Completeness of Hydrolysis. For each hydrolysis experiment, aliquots of an ether solution of tripalmitin or palmitic acid were delivered to duplicate flasks. Hydrolysis described in text. The mixture was acidified and cooled, and lipides extracted with chloroform as described in text. Aliquots of chloroform extract were evaporated to dryness in an atmosphere of CO_2, and fatty acid ester determinations were carried out in duplicate according to the method of Bauer and Hirsch (2).

Compound	Amt added, mg	Treatment	% recovery in $CHCl_3$ phase as fatty acid ester
Tr*	17	2 ml Na ethylate 30 min.†	0
Tr	17	4 ml Na ethylate 30 min.†	0
Tr	17	1 ml 90% KOH in 50% EtOH; 6 hr†	0
P	14	2 ml Na ethylate 30 min.†	0
Tr	17	0	100

* Tr = Tripalmitin; P = Palmitic acid.
† Time on steam bath.

TABLE III. Lipide-C^{14} Recoveries: A Comparison of Values Obtained by the Method Described Here with Those Obtained by Methods Used Earlier. 500 ± 5-mg liver slices were incubated in 5 ml bicarbonate buffer to which 2 μM acetate-1-C^{14} had been added. Incubation was carried out at 37.5° for 3 hr. Duplicate flasks were incubated, and their contents were analyzed separately; average values are reported below.

Rat	Labeled acetate added to flask, μmoles	% of added C^{14} recovered in lipides by: Method described here	Method of Chernick *et al.* (3)	Method of Felts *et al.* (1)
A	2	29		28
B	2	13		13
C*	2	2.8		2.8
D*	2	1.5		1.6
1M	10	4.5	4.4	
2M	10	4.8	4.9	

* Rats C and D were fasted for 48 hr.

Test of reliability of procedure. The reliability of the procedure was tested by the following experiments:

1. A biologically synthesized C^{14}-fatty acid mixture, cholesterol-4-C^{14}, tripalmitin-1-C^{14}, alanine-1-C^{14}, acetate-1-C^{14}, and glycerol-C^{14} were added to separate 500-mg portions of liver slices, and immediately thereafter the mixture was hydrolyzed and extracted with chloroform. The design and results of the experiment are given in Table I. Essentially all of the C^{14} of the added fatty acids, tripalmitin, and cholesterol was recovered in the chloroform fraction, whereas none of the C^{14} of the water-soluble compounds was present in that fraction.

2. The efficacy of the hydrolysis and extraction procedure was further determined with unlabeled tripalmitin and palmitic acid. The details and results of this experiment are shown in Table II. After each of the various periods of hydrolysis tested, none of the fatty acid ester was recovered in the chloroform phase. This shows that hydrolysis is complete under the conditions employed here.

3. The lipide-C^{14} recoveries observed with the method described here were compared with those obtained by methods used earlier in this laboratory, namely, that of Chernick *et al.*(3) and that of Felts *et al.*(1). The design and results of these experiments are given in Table III. It is clear that the lipide-C^{14} yields observed with the abbreviated method described here are in good agreement with those obtained by the laborious procedures employed earlier.

Summary. 1. A rapid method for determination of lipide-C^{14} in experiments in which liver slices are incubated with C^{14}-labeled compounds is described. 2. Data on the reliability of the procedure, and a comparison of values obtained by this simplified procedure with those obtained by the previous laborious methods are presented.

1. Felts, J. M., Chaikoff, I. L., and Osborn, M. J., *J. Biol. Chem.*, 1951, v191, 683.

2. Bauer, F. C., Jr., and Hirsch, E. F., *Arch. Biochem.*, 1949, v20, 242.

3. Chernick, S. S., Masoro, E. J., and Chaikoff, I. L., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 348.

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Antigenicity of Canine Distemper Inclusion Bodies as Demonstrated By Fluorescent Antibody Technic. (21021)

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The fluorescent antibody technic was developed by Coons *et al.*(1) and is a method in which antibody conjugated to fluorescein is employed as a specific histochemical stain for the localization of antigen in cells. The technic has been used to demonstrate antigens of rickettsiae and mumps virus(1), pneumococcus(2,3), Friedländer bacillus(4), leptospira(5) and homologous plasma proteins in tissues(6). The method was recently employed to show viral antigen in the inclusion bodies of canine infectious hepatitis(7). This report deals with the application of the technic to cells containing inclusion bodies of canine distemper.

Materials and methods. Fluorescein isocyanate was prepared and conjugated to protein of anti-canine distemper serum* according to the method of Coons and Kaplan(8). Absorption of the conjugate on liver powder for preventing non-specific fluorescence in tissues was not found necessary in the smear preparations which were used. Smears of urinary bladder epithelium from natural cases of canine distemper were used as a source of inclusion bodies. Smears were made on coverslips which were subsequently dried 30 minutes before staining. The staining procedure was as follows: A smear was treated for 20 minutes with the specific conjugate and the excess shaken off. The smear was washed for a few seconds in buffered saline, pH 9, and then gently agitated in fresh buffered saline for 10 minutes. The coverslip was dried and mounted smear surface down over a square aperture in a slide. *Fluorescence* in the smears was produced with ultraviolet light and observed through a standard microscope. The light source was a Leitz, 8 ampere, carbon arc. Filters consisted of 3.2 cm of $CuSO_4$ (25 g/100 ml) in a pyrex cuvette and Corning filter No. 5840 (½ standard thickness) to remove visible light. The filtered ultraviolet radiation practically free of visible light was directed into an ordinary glass substage condensor by means of a polished mirror of aluminum-magnesium alloy. A protecting filter (Wratten gelatin filter No. 2A) was mounted in the ocular of the microscope. Photomicrographs of fluorescence were taken with Eastman Super Panchro-Press, Type B film with 20-minute exposures. After a suitable area of fluorescence was located in the smear and photographed, the slide was re-

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