(10) found that only 1/10 as many viable organisms are required to produce a given thermogenic response in rabbits when the organisms are grown in rabbit serum for 2-14 days. It is evident that growth in serum is not a necessary factor for augmentation since it also occurred in our experiments when killed *E. typhosa* organisms were suspended 12-18 hours in homologous plasma.

Summary and conclusions. The effects of intravenously administered typhoid vaccine upon the leukocyte population and the temperature of the rabbit were studied.

The response of the temperature and of the leukocyte count was dependent upon the amount of typhoid vaccine given. Small amounts of the vaccine caused little or no fever, a heterophile leukocytosis, and a mononuclear leukopenia. When larger doses of vaccine were given, the rabbits responded with high fever, an initial heterophile leukopenia followed by a secondary leukocytosis, and a sustained mononuclear leukopenia. Ten times the number of organisms were required in saline suspensions to produce a thermogenic and leukocytic response comparable to that produced when the vaccine was given in homologous plasma. The plasma-typhoid and saline-typhoid reaction differed only in degree except for the earlier onset of fever when the organisms were given in plasma. These experiments on rabbits with typhoid vaccine indicate that when leukocyte changes are used as a test for pyrogens, it is necessary to employ a series of total and differential counts on each animal.

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The Absorption and Distribution of Labeled Fatty Acids in the Rat.* (18299)

JOHN G. CONIGLIO,† CARL E. ANDERSON,‡ AND C. S. ROBINSON.
From the Department of Biochemistry, Vanderbilt University, School of Medicine, Nashville, Tenn.

The immediate distribution and deposition of ingested fatty acids into the lipid fractions of tissues have not been extensively studied. Various investigators(1-7) observed the passage of unsaturated fatty acids into tissues. Cavanagh and Raper(8) found the concentration of deuterium from "deuterated" linseed oil to be greater in the glyceride fatty acids of rat livers and less in the acetone-insoluble phospholipids. Similar technics were used by Sperry *et al.*(9) who showed signifi-

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† Holder of an A.E.C. predoctoral fellowship.

‡ Present address, School of Medicine, The University of North Carolina, Chapel Hill, N.C.

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cant concentrations of deuterium in liver and intestinal fatty acids and much smaller concentrations in depot fat. Using C^{14}-labeled sodium acetate, Pihl and Bloch(10) found that liver neutral fat was regenerated more rapidly than liver phospholipids in contrast to the fat in the mesentery and other tissues in which the isotope concentration was higher in the phospholipid fraction than in the neutral fat.

At the time this work was begun, pure radioactive fatty acids were not available. We prepared radioactive lipids by injecting C^{14}-labeled sodium acetate into a white rat, extracted the lipid from the carcass and fed this to other rats. These were killed, the lipid extracted from various organs and examined for radioactivity.

Experimental. Carboxyl-labeled acetic acid was prepared from CH_3MgBr and $C^{14}O_2$. 16.56 mg of the sodium salt and 400 mg of sodium pyruvate(11,12) were injected in 2 nearly equal doses. The interval between doses was 20 hours and the rat was decapitated 48 hours after the second dose. The total number of counts injected into the rat was 6.4×10^7, and of this amount 1.14×10^6 c/m or 1.78% of the administered dose was found in the fatty acids isolated from the tissues studied. The liver and carcass contributed almost all of this activity, only 0.14% coming from the mesentery and intestines. Approximately 90% of the counts administered was found in the expired CO_2. The C^{14}-labeled fatty acids were isolated by alkaline alcohol extraction from an ether solution of the pooled fatty acids obtained from the liver, intestines, mesentery and carcass and were purified by the method described by Armstrong(13). Water-soluble, short chain fatty acids were extracted from the mixture by thorough washing with hot water. Because of the small amount of labeled material available, fractionation of the fatty acids was not attempted. Acetic acid has been shown(11, 14) to serve as a precursor of the higher fatty acids. Because of this and the fact that short chain fatty acids were removed, the material fed presumably consisted mostly of long chain fatty acids.

Feeding experiments. The C^{14} fatty acids were dissolved in olive oil. One ml of the solution was administered by stomach tube to each of 5 young white rats weighing between 100 and 130 g, which had been fasted for 24 hours. The labeled fatty acids received by rats 2 and 3 assayed 671 c/m/mg; those received by rats 4, 5 and 6 assayed 654 c/m/mg. Rats 2, 3 and 4 and rats 5 and 6 were decapitated 5 and 7 hours after feeding, respectively. During the absorption period the animals were kept in individual metabolism cages and water was furnished *ad libitum*. Each cage was contained in an airtight "dry box" furnished with an inlet tube for introducing CO_2-free air and an outlet tube for removing the expired CO_2. Urine and feces were collected and expired CO_2 was collected in traps containing NaOH solution.

Isolation of lipids. The tissues were cut into small pieces and washed with a minimum of physiological saline to remove excess blood. Tissue lipids were extracted from the liver and mesentery by methods similar to those described by Bloor(15) and by Entenman and Chaikoff(16). After the combined ether and alcohol extracts had been evaporated to dryness in a stream of nitrogen and under reduced pressure, the residue was dissolved in petroleum ether and clarified by filtering through fat-free filter paper. The extracts were made to volume with petroleum ether and aliquots taken for the analyses listed below. Free fatty acids in the tissue extracts

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were separated by alkaline alcohol extraction and determined before the lipid extracts were fractionated. Total fatty acids and fatty acids obtained from the various fractions were determined by the method of Kelsey(17). Phospholipids were isolated by precipitation with acetone and $MgCl_2$ as described by Bloor(15,18) and determined by measuring the phosphorus content(19). The acetone-soluble fraction was used for the determination of the activity of the combined fatty acids derived from triglycerides and cholesterol esters and also for the enzymatic hydrolysis of the triglycerides with castor bean lipase(17). The fatty acids thus obtained by the selective hydrolysis of neutral fat were analyzed for activity and compared with the mixture of triglycerides and cholesterol ester fatty acids.

Radioactivity determinations.§ The fatty acids in the fractions listed above were converted to CO_2 by wet combustion, using the combustion fluid of Van Slyke and Folch as prepared by Lindenbaum *et al.*(20) and using the method of the latter with modification to suit our combustion apparatus, as previously described(21). CO_2-free NaOH was used for the absorption of the $C^{14}O_2$. The radioactive carbonate was precipitated as $BaC^{14}O_3$, centrifuged, washed with CO_2-free water, and transferred as a slurry in methyl alcohol to shallow cups. They were dried initially under an infra-red lamp and finally in an electric oven. All samples were counted with a thin window (1.64 mg cm^2) Geiger-Müller tube. The results were corrected for self absorption, and the counting time for each sample was long enough to insure a reliable error of less than $\pm$ 5%. Background was determined before and after each series of samples analyzed and more often when samples of activity very close to background were encountered. Duplicate determinations were done where possible.

Discussion. Satisfactory absorption from the intestine occurred, ranging from 73 to 90% and less than 1% of the C^{14} was expired as CO_2. The greatest C^{14} activity was found in the liver and mesenteric fat. Significant activity was also present in peri-renal and gonadal fat though it could be demonstrated only qualitatively by mounting the fatty acids directly. Feces were excreted by only 2 of the rats during the experimental period. Radioactive carbon was present in both instances. Four rats excreted urine and all samples contained C^{14}. Blood total lipids showed a high activity.

The data from the examination of liver and mesenteric lipids are given in Table I. It should be pointed out that the figures in columns 1-4 represent specific activity, not mgs and are directly related to the counts administered. In each experiment the specific activity of the liver total fatty acids exceeded that of the mesenteric fatty acids. In the liver the fatty acids derived from the neutral fat or acetone-soluble fraction contained the highest concentration of radioactive carbon. The specific activity value observed for the liver total fatty acids was lower than that of the neutral fat fatty acids, a result of the dilution of this very active fraction by the less enriched fatty acids of the phospholipids. The ratio between the concentrations of these 2 fractions ranged narrowly about an average of 2.24. This finding is in agreement with those of other investigators(8,10) and indicates that in the liver a mechanism may be active in keeping the ratio of neutral fat and phospholipid constant during the transport of ingested fatty acids. This situation apparently does not exist in the mesentery where the ratios were variable.

The activity of the free fatty acids and phospholipid fatty acids in the liver was identical in 4 instances indicating a common origin of these fractions. That the free fatty acids do not represent preformed fatty acids but originate from the autolytic decomposi-

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TABLE I. Summary of Radioactivity Data of Lipid Fractions of Liver and Mesentery.

Rat No.	Fatty acids fed (mg)	% absorbed	Specific activity* (× 10²) Liver 1	Liver 2	Liver 3	Liver 4	Liver 4/3	Mesentery 1	Mesentery 2	Mesentery 3	Mesentery 4
2	107	83	2.53	1.21	1.08	3.02	2.79	1.15	1.90	0.79	0.68
3	83	85	1.63	1.11	1.15	2.07	1.80	—	0.92	0.06	—
4	275	73	2.81	2.42	1.90	4.30	2.26	0.17	0.80	0.43	0.17
5	258	90	0.91	0.67	0.65	1.35	2.08	0.23	0.67	0.29	0.24
6	127	81	4.73	2.48	2.34	5.34	2.28	1.34	1.54	0.77	1.04
Mean			2.52	1.58	1.42	3.22	2.24	0.72	1.17	0.47	0.53
Stand. error of mean			±0.65	±0.37	±0.30	±0.72	±0.16	±0.31	±0.24	±0.14	±0.20

* Specific activity $= \frac{\%\text{ of administered counts}}{\text{mg C}}$.

1. Total fatty acids; 2. Free fatty acids; 3. Phospholipid fatty acids; 4. Neutral fat fatty acids.

TABLE II. Specific Activity of Liver Fatty Acids Isolated from Triglycerides Alone and from a Mixture of Triglycerides and Cholesterol Esters.

Rat No.	Specific activity (× 10²) Triglyceride fatty acids	Triglyceride + cholesterol ester fatty acids
2	3.02	3.86
3	2.07	2.07
5	1.35	1.54
6	5.34	3.97

tion of the phospholipids of the excised liver is in keeping with Fairbairn's observation(22) of the rapid hydrolysis of phospholipids of extirpated livers of cats and rats. The specific activity of the free fatty acids in the mesentery was always greater than the activity of any other fraction. They must, therefore, be regarded as preformed material, perhaps being deposited as soaps from the chyle.

In Table II a comparison is made of the concentration of C^{14} in the fatty acids of the triglycerides and of the fraction containing both triglycerides and cholesterol esters. Generally speaking, the activity of cholesterol esters was equal to or greater than the activity of the glycerol esters, an indication of the importance of cholesterol in the metabolism of ingested fatty acids.

Summary. Biosynthesized lipids labeled with C^{14} was fed to rats and the lipids from various organs examined for radioactivity. The importance of the observations in studies of lipid metabolism was pointed out.

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Effect of Leucocytes and Bacteria on Glucose Content of the Cerebrospinal Fluid in Meningitis. (18300)

SIDNEY GOLDRING AND CARL G. HARFORD.

From the Departments of Medicine, Neuropsychiatry, and the Oscar Johnson Institute for Medical Research, Washington University School of Medicine, St. Louis.

The subnormal level of glucose in the cerebrospinal fluid of patients with bacterial meningitis is frequently of diagnostic value in differentiating bacterial from aseptic meningitis(1). Reasons for the reduced concentration of glucose in bacterial meningitis are not understood although it is stated that the main factor is utilization of glucose by bacteria growing in the fluid(1). On the other hand, the concentration of glucose in aseptic

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