raphy and alkali reaction may be applied, omitting the special pre-extraction. In premixes the NFZ will be recovered with an accuracy of 99.8 ± 0.42%.

Summary

In the determination of NFZ in feed the main problem is purification of the extracts. Suitable conditions for color reactions are obtained only by pre-extraction using 60° C. heptane, carbon tetrachloride, and hexane, followed by absorption of the NFZ on Al_2O_3 chromatography columns and elution with 80% (v./v.) ethanol. If this method is applied, the determination of blanks by analysis of unmedicated feed mixtures is not necessary.

For colorimetric determination the reaction with alkali is preferable. The method allows detection of 99.5 ± 0.83% of NFZ. Reaction with phenylhydrazine is not strictly quantitative in samples where the overlapping error due to unknown interfering substances cannot be exactly controlled.

Literature Cited

(1) Beckman, H. F., J. AGR. FOOD CHEM. **6,** 130 (1958).
(2) Brüggemann, Johs., Bronsch, K., *Z. anal. Chem.* **169,** 88 (1959).
(3) Buzard, J. A., Ells, V. R., Paul, M. F., *J. Assoc. Offic. Agr. Chemists* **39,** 512 (1956).

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FOOD ADDITIVE SAFETY

Metabolism of Glyceryl Lactate-C^{14} Palmitate by Rats

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Glyceryl lactate palmitate has been found useful in baking, and therefore studies to prove its safety were conducted. The ester was readily hydrolyzed in vitro by a lipolytic enzyme to its components: lactic acid, palmitic acid, and glycerol. When administered orally to rats in different vehicles, the lactate moiety of glyceryl lactate (2,3-C^{14}) palmitate was metabolized as rapidly as and in a manner similar to 2,3-C^{14} lactic acid, which was largely absorbed, readily stored and oxidized by the liver, and randomly distributed throughout the body without high localization in any organ when equilibrium was attained. This ester was easily hydrolyzed to compounds which are natural and accepted as safe, and since the lactate moiety of the ester was metabolized like free lactic acid, this glyceryl lactate palmitate is believed safe for use in shortening.

GLYCERYL LACTATE palmitate has been useful in shortening for cake mixes and other bakery goods.

Lactic acid, palmitic acid, glycerol, and glyceryl monopalmitate are accepted as safe for use in foods. If glyceryl lactate palmitate hydrolyzed readily to these materials and the fate of the lactate moiety of the ester was metabolically similar to that of free lactic acid, its use in shortening would be safe. To establish its safety, the hydrolysis of the nonlabeled ester by a lipolytic enzyme was studied.

Two metabolic balance studies in fasted rats were conducted with both 2,3-C^{14} lactic acid (L.*A) in the presence of glyceryl palmitate (GP) and glyceryl lactate palmitate (GL*P) containing the comparable labeled lactic acid. In one study (series 1), the labeled ester was intubated as an emulsion in water–propylene glycol (PG) containing carboxymethylcellulose; the labeled lactic acid was given in water, which was preceded immediately by warmed glyceryl palmitate, administered in a water–propylene glycol suspension containing carboxymethylcellulose. During the analytical determination, oxidation was performed by wet combustion and activity of a Hyamine solution was counted by liquid scintillation. In the other metabolic study (series 2), the tagged ester or the labeled lactic acid and glyceryl palmitate were given as an aqueous emulsion that contained sodium caseinate and sucrose. In series 2a, one rat was given the ester in the vehicle employed for series 1. For analyses, oxidation was by dry combustion and activity of barium carbonate was counted by a flow gas counter.

Although the quantity of lactic acid or ester given in both series was of the same order of magnitude, the concentration of activity was much greater in the first series (Table I).

Methods

Enzymatic Study. To 200 mg. of nonlabeled glyceryl lactate palmitate and 50 mg. of sodium taurocholate were added 200 ml. of water. This mixture was heated to 65° C. and shaken vigorously to emulsify the GLP. After the emulsion had been cooled (with shaking) to about 40° C. 2.0 ml. of a buffer solution (66 ml. of 1*N* NH_4OH and 134 ml. of 1*N* NH_4Cl) and 50 mg. of a lipase enzyme (a whole hog pancreas preparation, Viokase, Viobin Corp., Monticello, Ill.) were added. The final mixture was vigorously shaken for about 1 minute and then placed immediately in a shaking water bath at 37° C. Upon completion of incubation (1 to 22 hours), the flask was removed from the bath and diluted with 100 ml. of 3A alcohol (95.2% ethyl alcohol; 5 gallons of commercially pure methanol added to every 100 gallons of ethyl alcohol).

The hydrolyzed mixture was made acidic and extracted with 25 ml. of *n*-hexane. The hexane phase was washed three times with 25 ml. of water. Each water phase was extracted with 25 ml. of *n*-hexane. All of the hexane phases were combined and evaporated to dryness over a steam bath. To determine free palmitic acid, the residue was dissolved in alcohol, and titrated with dilute alcoholic KOH, using phenolphthalein as an indicator. (Good recovery of a known amount of palmitic acid from a synthetic mixture approximating that of the enzyme hydrolyzed mixture had been shown previously.)

The free lactic acid was determined on the above combined three water phases. The lactic acid was oxidized to acetaldehyde with a potassium permanganate–manganous sulfate system.

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Table I. Conditions of Giving C^{14}-Labeled Compounds Orally to Rats

Lactic Acid-C^{14}

	Series 1					Series 2		
Rat No.	L-1	L-4	L-2	L-5	L-3	L-1	L-2	L-3
Rat wt., g.	146	148	168	159	164	133	112	215
Sex	M	M	F	F	F	M	M	M
Compds. given	GP + L*	GP + L*	GP + L*	GP + L*	GP + L*	GP + L*	GP + L*	GP + L*
Vehicle	GP, as aqueous propylene glycol dispersion. Lactic acid, as aqueous solution					GP + L*, as dispersion in aqueous solution of caseinate and sucrose		
Fasted, hours[a]	20 to 27					16 to 18		
Lactic acid, mg./kg.	150	166	142	68	136	128	359	77.2
Total activity, μc.	89.3	96.6	97.1	43.96	91.1	4.3	9.4	5.9
Activity, μc./kg.	612	675	578	276	555	32.3	84.3	27.4
Killed, hours[b]	48	48	48	48	24	26.5	26.5	26.5

Glyceryl Lactate-C^{14} Palmitate

	Series 1						Series 2		Series 2a
Rat No.	GLP-2	GLP-4	GLP-1	GLP-5	GLP-6	GLP-3	GLP-1	GLP-2	GLP-3
Rat wt., g.	156	144	158	159	145	151	193	318	114
Sex	M	M	F	F	F	F	M	M	M
Compds. given	GL*P	GL*P	GL*P	GL*P	GL*P	GL*P	GL*P	GL*P	GL*P
Vehicle	GL*P, as aqueous propylene glycol dispersion with CMC						GL*P, as dispersion in aqueous solution of caseinate and sucrose		GL*P, as aqueous propylene glycol dispersion with CMC
Fasted hours[a]	20 to 25						16 to 18		17
GLP, mg./kg.	938	1129	1051	640	499	1021	1151	985	1368
Total activity, μc.	65.7	86.4	72.7	31.1	22.4	66.5	14.9	17.5	9.14
Activity, μc./kg.	421	600	460	196	154	440	75.5	55.0	80.2
Killed, hours[b]	48	48	48	48	48	24	26.5	26.5	26.5

[a] Hours prior to ingestion.
[b] Hours after ingestion.

Table II. Rate of Exhaled $C^{14}O_2$ Following Ingestion of Labeled Compounds

[Percentage of dosage (cumulative)]

Lactic Acid-C^{14} (Series 1)

Terminal Hour of Sample	(M) L-1	(M) L-4	(F) L-2	(F) L-5	(F) L-3	Av.
1	2.31	2.38	2.32	4.25	1.93	2.64
3	17.20	16.04	19.72	23.34	14.22	18.10
6	29.41	26.50	37.95	32.58	28.82	31.05
9	36.73	31.47	43.37		36.27	36.96
10				37.04		
12	38.72	33.67	45.60		39.67	39.39
15	39.86	34.73	46.73	41.52	42.43	41.05
18	41.48	35.81	49.40		44.02	42.68
21	42.97	37.02	50.74	42.70	45.14	43.71
24	44.55	38.21	51.81		45.87	45.11
28				45.88		
30	46.60	43.44	55.32			48.45
36	51.39	50.69	62.27	52.82		54.29
48	56.29	56.67	69.22	61.40		60.90

Glyceryl Lactate-C^{14} Palmitate (Series 1)

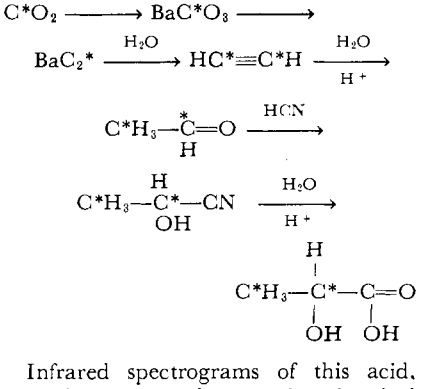
	GLP-2	GLP-4	GLP-1	GLP-5	GLP-6	GLP-3[a]	
1	1.74	1.69	1.14	1.68	2.07	1.44	1.63
3	12.11	16.04	12.61	12.13	17.03	12.55	13.75
6	22.17	28.42	24.80	29.72	30.20	24.35	26.61
9	29.46	35.30	41.03			31.30	34.27
10				40.59	36.37		
12	31.32	38.84	48.32			34.32	38.20
15	32.75	41.25	50.44	49.27	40.55	35.84	41.68
18	33.60	43.38	52.71			37.57	41.82
21	34.42	44.60	54 53	55.93	44.05	38.92	45.41
24	35.39	45.48	56.33			40.74	44.49
25				58.77	47.35		
30	37.58	49.27	62.60				49.82
36	41.46	53.96	65.36	61.09	55.21		55.42
48	46.74	57.88	66.52	64.40	62.84		59.68

[a] (F).
M = male.
F = female.

The acetaldehyde was distilled into an excess of sodium bisulfite solution. After the excess sodium bisulfite was measured by titration with iodine, the solution was made alkaline to decompose the bisulfite-aldehyde complex and this portion of the bisulfite measured by titration with iodine.

The data presented in Figures 2 to 3 are based upon the above determinations. The sum of the two acids, determined independently, agreed well with the total free acid determined directly on the enzyme-hydrolyzed alcoholic mixture by titration with 0.17*N* alcoholic KOH, employing 1% of thymophthalein as an indicator and comparing with a blank using all reagents except the GLP.

Metabolic Study. 2,3-C^{14}-DL-lactic acid was prepared by the following steps:



Infrared spectrograms of this acid, radiochromatographs, and chemical analyses, prepared from paper chromatograms of the labeled lactic acid, showed it to be free of impurities. The

lactate ester was prepared by esterification of 2,3-C^{14}-DL-lactic acid with nonlabeled glyceryl palmitate. The purity of the labeled ester was ascertained by analytical determinations which included acid number, hydroxyl number, saponification number, α-glyceryl palmitate content, and "free glycerol" content.

In series 1, the aqueous solution of lactic acid represented 245 mg. of the acid per 1000 μc., and 1 ml. of the solution contained 185 μc. Just prior to intubation of the rat with labeled lactic acid, a dosage of warmed glyceryl palmitate suspension equivalent to that in the ester for the stated amount of lactic acid was administered. This suspension consisted of (weight per cent): glyceryl palmitate 10, water 44, propylene glycol 44, and carboxymethylcellulose 2. Five rats were given labeled lactic acid in series 1.

In series 1 and 2a (rat 3), an individual suspension was prepared for each rat. This contained (weight per cent): glyceryl lactate-C^{14} palmitate 10, water 44, propylene glycol 44, and carboxymethylcellulose 2. To ascertain the activity of the dosage, in each instance, a weighed sample was collected from the syringe employed for intubation before and after administration of the material to the rat. The labeled ester was given to six rats in series 1 and to one rat in series 2a.

In series 2, the suspensions consisted of (weight per cent): labeled glyceryl lactate palmitate 10 (or 10% L*A + GP), sucrose 4.50, sodium caseinate 2.25, and water 83.25. Three rats were given the free labeled acid and two were given the ester.

The amount of material given to each rat was determined gravimetrically (Table I).

In both instances, rats were fasted prior to administration of the material. Rats from the 30-year-old Food and Drug Research Laboratories' colony of randomly bred Wistar strain rats were used in series 1 and Charles River Wistar rats in series 2 and 2a. The rats in series 1 and one rat in series 2a were permitted 5 grams of Purina laboratory chow 2 hours after being placed in a metabolic chamber. The remaining rats in series 2, given the material in association with sucrose and sodium caseinate, were not given added feed in the chamber. In both cases, water was permitted ad libitum. After intubation, the rats were maintained in a glass metabolic chamber and manifold which provided for collection of the exhaled air, feces, and urine. In both series, the animals were terminated by intraperitoneal injection of Nembutal, followed by cyanide to stop aerobic oxidative metabolism. In series 1, the rats were killed by intraperitoneal injection (cyanide) 24 or 48 hours after administration of the material; in series 2 they were killed by inhalation (cyanide) 26.5 hours after intubation.

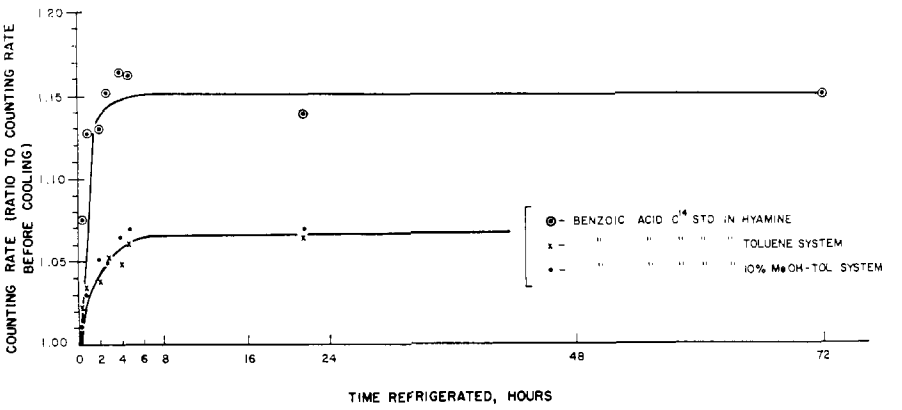


Figure 1. Counting rate (scintillation) as related to time of refrigeration

In series 1, the carbon dioxide scrubbers consisted of 700 × 40 mm. glass cylinders containing 200 ml. of 10% aqueous sodium hydroxide, through which 0.5 to 1 liter per minute of respired air was delivered by means of a fritted-glass cylinder. Although in a control run, in which respired air was passed through three serial cylinders, 99.9% of the total activity of the respired air was found in the first scrubber, in practice two serial scrubbers were employed. The scrubbers were replaced frequently during the collection of respired air (Table II). For analyses, carbon dioxide was evolved from the alkali by addition of concentrated H_2SO_4 and collected into 5 ml. of a Hyamine system. This was stored in a deep freeze (−6° C.) overnight, after which the activity was determined by liquid scintillation (efficiency about 33%) employing a Packard Tri-Carb unit (*7*). Maximum efficiency of counting was not obtained until the Hyamine system had been cooled for several hours (Figure 1).

In series 2, 1 liter per minute of air was drawn through the metabolic chamber, then through a drying system, and into a continuous radioactive gas analyzer. This analyzer consisted of an ionization chamber, which determined and recorded the total activity by integration. The air was then drawn through an infrared analyzer which determined the total carbon dioxide. The specific activity of the carbon dioxide in the respired air was also recorded automatically (*3*). The respired air was finally drawn through four serial traps containing 2*M* aqueous sodium hydroxide. The activity of the total respired air was determined thereon and provided a good check on the operation of integrator. An aliquot of the alkaline solution was treated with 10% of aqueous barium chloride prior to counting by a flow gas counter (Nuclear-Chicago D-47).

In both series, samples of blood were drawn and stored at 30° F. until they could be burned and counted, individually. Samples of lactic acid*, glyceryl lactate* palmitate, urine, and respired air were stored in a refrigerator until further processing.

In series 1, the excised tissues from each rat were homogenized individually with 2 or 3 parts of alkaline water in a Teflon homogenizing tube. In series 2, the individual samples were dried in a vacuum oven at 70° C. for 24 hours and then ground to a homogeneous powder with a mortar and pestle.

In series 1, the remaining carcass (after individual tissues had been excised) was partially solubilized by heating with 3 parts of 50% aqueous alkali on a steam bath for 2 days. The preparation, containing the softened bones, was then homogenized in a Waring Blendor. In series 2 the entire remaining carcass was placed in a stainless steel Waring Blendor, which was half filled with water and autoclaved at 15 p.s.i. for 1 hour. After the homogenate had been spread on a tray, it was dried in a Stokes freeze dryer for 24 hours. The dried frozen material was ground in a ball mill for 24 hours.

All samples of tissue were frozen until they were burned. Including the GL*P, all samples, except those of respired air in both series and the lactic acid* in series 1, were burned.

In series 1, aliquots in duplicate of all individual samples, except total adrenals, were dried in vacuum using a Rinco evaporator. The distillate was collected in a trap in dry ice–acetone and transferred to a methanol-toluene system, where the activity was counted by the Tri-Carb unit. The solid from the evaporation was burned to CO_2 by the wet method of Van Slyke and Folch (*8*), employing the combustion solution described by Van Slyke, Plazin, and Weisiger (*9*). The $C^{14}O_2$ was collected in 5 ml. of Hyamine solution (*7*), 4.0 ml. of which were counted at an efficiency of about 35% after being retained in the deep freeze overnight. At least two counts were performed on a single analysis. For each count either 10,000

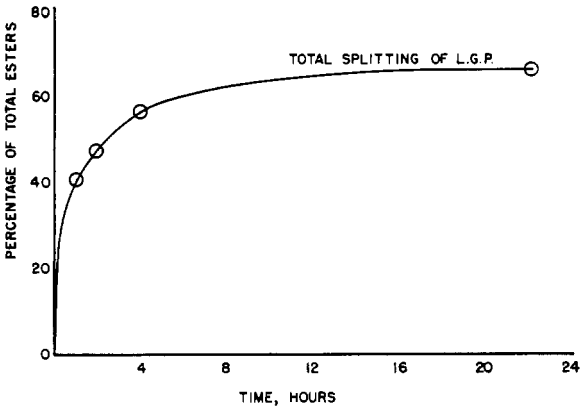


Figure 2. Experimental data on lipolytic enzyme–catalyzed hydrolysis of glyceryl lactate palmitate

Total splitting of GLP

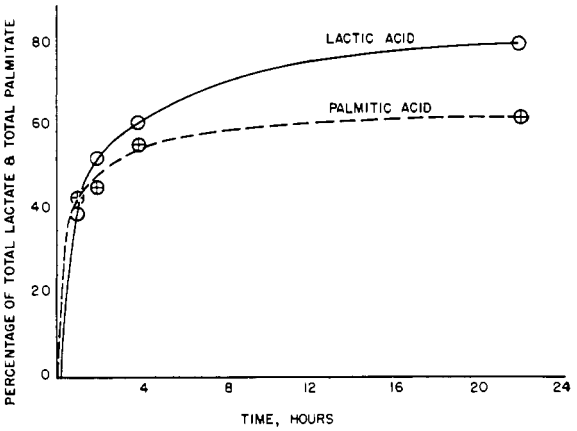


Figure 3. Relative rates of splitting by lipolytic enzyme–catalyzed hydrolysis of lactic acid and palmitic acid from glyceryl lactate palmitate

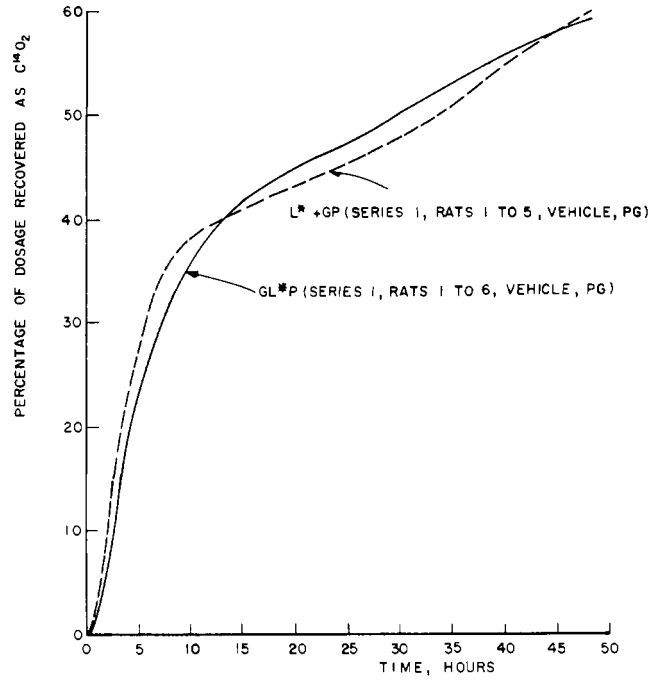


Figure 4. Average cumulative radioactivity of respired $C^{14}O_2$

Series 1

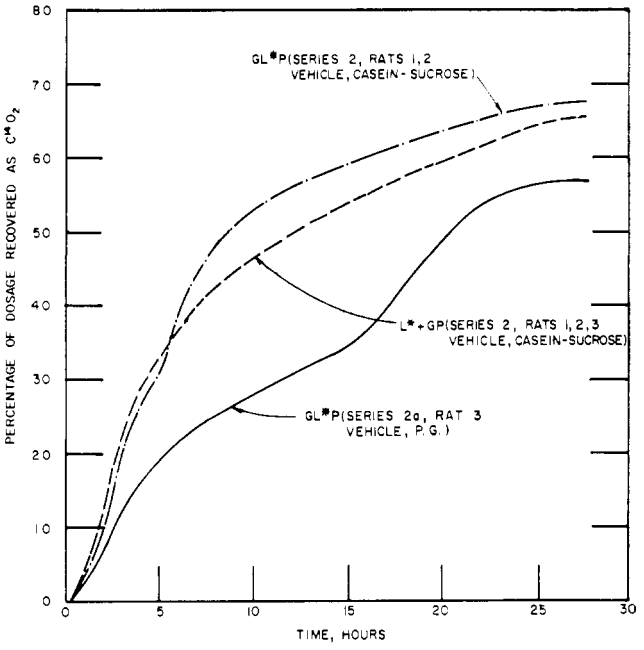


Figure 5. Average cumulative radioactivity of respired $C^{14}O_2$

Series 2

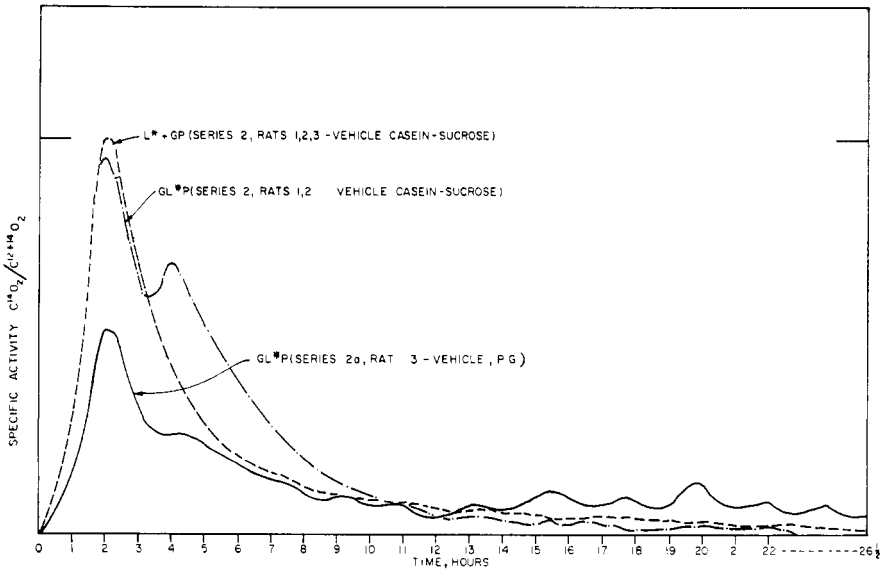


Figure 6. Specific activity of respired $C^{14}O_2$ *vs.* time following administration of radioactive GL*P or L* + GP

Series 2 and 2a

Table III. Fate of Ingested Lactic Acid-C^{14}

	Series 1							
Rat No.	L-1	L-4	Av.	L-2	L-5	Av.	Av.	L-3
Sex	M	M	2 males	F	F	2 females	4 rats	F
Hour	48	48	at 48 hours	48	48	at 48 hours	at 48 hours	at 24
Vehicle	H_2O-PG	H_2O-PG	...	H_2O-PG	H_2O-PG	...	...	H_2O-PG
Total activity given, μc.	89.3	96.6	92.95	97.1	43.96	70.53	81.74	91.1
	Percentage of Dosage Recovered							
Not absorbed								
Feces	2.30	1.32	1.81	0.80	0.96	0.88	1.35	0.30
Contents of G.I. tract	0.75	0.68	0.72	0.82	2.12[a]	1.47	1.09	0.18
Total	3.05	2.00	2.53	1.62	3.08	2.35	2.44	0.38
Absorbed and excreted								
Breath	56.29	56.67	56.48	69.22	61.40	65.31	60.90	45.87
Urine	7.18	6.94	7.06	6.54	4.66	5.60	6.33	3.51
Total	63.47	63.61	63.54	75.76	66.06	70.91	67.23	49.38
Absorbed and unexcreted								
Remainder	25.70	27.44	26.57	20.81	19.43	20.12	23.34	33.10
Liver	1.59	1.85	1.72	1.72	1.93	1.82	1.77	16.84
Heart	0.16	0.08	0.12	0.08	0.07	0.08	0.10	0.12
Kidneys	0.36	0.31	0.34	0.33	0.25	0.29	0.32	0.30
Adrenals	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Spleen	0.37	0.30	0.34	0.15	0.07	0.11	0.21	0.12
Gonads	0.19	0.31	0.25	0.02	0.02	0.02	0.14	0.03
Fat[b]	0.04	0.16	0.10	0.02	0.11	0.07	0.10	0.10
Blood[b]	0.29	0.25	0.27	0.50	0.11	0.30	0.29	0.36
Muscle[b]	0.04	0.10	0.07	0.05	0.34	0.20	0.13	0.10
Brain	...	...	...	...	0.36	0.18	0.09	...
Total	28.75	30.81	29.78	23.69	22.70	23.20	26.50	51.08
Grand total	95.27	96.42	95.85	101.07	91.84	96.46	96.17	100.84
Absorbed and unexcreted (summarized)								
Liver	1.59	1.85	1.72	1.72	1.93	1.82	1.77	16.84
Five selected tissues[c]	1.09	1.01	1.05	0.59	0.42	0.51	0.78	0.58
Remainder	26.07	27.95	27.01	21.38	20.35	20.87	23.95	33.66
Total	28.75	30.81	29.78	23.69	22.70	23.20	26.50	51.08

	Series 2			
Rat No.	L-1	L-2	L-3	Av.
Sex	M	M	M	3 males
Hour	26.5	26.5	26.5	26.5 hours
Vehicle	H_2O, casein, sucrose	H_2O, casein, sucrose	H_2O, casein, sucrose	
Total activity given, μc.	4.3	9.4	5.9	6.5
	Percentage of Dosage Recovered			
Not absorbed				
Feces	0.35	0.20	0.35	0.30
Contents and G.I. tract	3.72	4.37	4.31	4.13
Total	4.07	4.57	4.66	4.43
Absorbed and excreted				
Breath	69.89	58.85	67.86	65.53
Urine	3.37	5.68	3.31	4.12
Total	73.26	64.53	71.17	69.65
Absorbed and unexcreted				
Remainder	20.68	16.30	17.00	17.99
Liver	2.89	7.63	4.08	4.87
Heart	0.07	0.13	0.10	0.10
Kidneys	0.44	0.40	0.62	0.49
Adrenals	0.03	0.01	0.03	0.02
Spleen	0.13	0.10	0.13	0.12
Gonads	0.50	0.37	0.78	0.55
Fat[b]	...	0.53	0.12	0.22
Blood[b]	0.08	0.05	...	0.04
Muscle[b]	0.19	0.16	0.39	0.25
Brain	0.59	0.12	0.74	0.48
Lungs	0.20	0.28	0.44	0.31
Skin	0.22	0.12	0.13	0.16
Total	26.02	26.20	24.56	25.60
Grand total	103.35	95.30	100.39	99.68
Absorbed and unexcreted (summarized)				
Liver	2.89	7.63	4.08	4.87
Five selected tissues[c]	1.17	1.01	1.66	1.28
Remainder	21.96	17.56	18.82	19.45
Total	26.02	26.20	24.56	25.60

[a] Includes G. I. tract as well as contents. [b] Aliquot. [c] Heart, kidneys, adrenals, spleen, and gonads.

counts were made, or in the case of low activity, counts were performed for at least 10 minutes.

In series 1, the activity of the dosage of lactic acid was determined directly by adding an aliquot of the lactic acid solution to a methanol-toluene system. After being stored at −6° C., an efficiency of 46.7% was attained with the Tri-Carb counter. Two analyses were performed on each of two samples for each dosage of the ester in both series.

In series 2, combustion was performed by dry oxidation on duplicate aliquots of each individual tissue.

The combustion apparatus consisted of a furnace (Multiple Unit, Hevi Duty Electric Co.) preheated to about 650° C., combustion tubes (Vycor 10, 5 × 520 mm. in O.D.), and a trap containing 2*M* sodium hydroxide. The tubes were packed with CuO wire held in place by asbestos plugs. Oxygen was passed through the system for about 30 minutes. An aliquot of the NaOH containing $C^{14}O_2$ was precipitated with 5 ml. of 10% aqueous barium chloride while swirling rapidly. The precipitated barium carbonate was transferred, over vacuum, to a weighed 7/8-inch (diameter) Whatman No. 42 filter paper. A filter tower (Tracerlab No. E-29) was employed. Samples were dried on the tower base of the filter apparatus under an infrared lamp for 12 minutes. During drying, the filters were covered and air passing over them was filtered through a Seitz filter to remove any filterable radioactive contamination from the atmosphere.

These samples of $BaCO_3$ were then placed in aluminum planchets and the activity was counted by a flow gas counter (Nuclear-Chicago D-47) with the automatic sample changer and related equipment. Most of the samples were counted for 3000 counts at least twice; in a few samples of low activity, 1000 counts were made and in some of the very active samples, 10,000 counts. A correction of 15 counts per minute was made for the average background activity. The count rate was corrected for self-absorption by the following formula (*4*):

$$I_0 = \frac{I \alpha d}{1 - e^{-\alpha d}}$$

where

I = count rate corrected for background

I_0 = count rate corrected for self-absorption

α = absorption coefficient for C^{14} in $BaCO_3$ (0.286)

d = density of sample, mg./sq. cm. (wt. of sample)/(area of paper)

Efficiency of counting was about 25%.

Results

Enzymatic Study. Nonlabeled glyceryl lactate palmitate was split easily, in vitro, by the lipolytic enzyme in the

Table IV. Fate of Ingested Glyceryl Lactate-C^{14} Palmitate (Series 1)

Rat No.	GLP-2	GLP-4	Av.	GLP-1	GLP-5	GLP-6	Av.	Av.	GLP-3
Sex	M	M	2 males	F	F	F	3 females	5 rats	F
Hour	48	48	at 48 hours	48	48	48	at 48 hours	at 48 hours	24
Vehicle	H_2O-PG	H_2O-PG		H_2O-PG	H_2O-PG	H_2O-PG			H_2O-PG
Total activity given, $\mu c.$	65.7	86.4	76.0	72.7	31.1	22.4	42.1	55.7	66.5
	Percentage of Dosage Recovered								
Not absorbed									
Feces	4.60	8.48	6.54	5.20	6.24	5.58	5.67	6.02	2.60
Contents of G. I. tract	0.43	0.32	0.38	0.52	2.28[a]	3.35[a]	2.05	1.38	0.09
Total	5.03	8.80	6.92	5.72	8.52	8.93	7.72	7.40	2.69
Absorbed and excreted									
Breath	46.74	57.88	52.31	66.52	64.40	62.84	64.59	59.68	40.74
Urine	4.19	6.25	5.22	6.04	1.48	5.00	4.17	4.59	4.96
Total	50.93	64.13	57.53	72.56	65.88	67.84	68.76	64.27	45.70
Absorbed and unexcreted									
Remainder	32.45	15.08	23.76	26.59	14.34	13.75	18.23	20.44	30.30
Liver	2.62	1.43	2.02	1.75	1.99	1.92	1.89	1.94	11.95
Heart	0.08	0.08	0.08	0.07	0.06	0.09	0.07	0.08	0.08
Kidneys	0.24	0.29	0.26	0.25	0.03	0.31	0.20	0.22	0.33
Adrenals	0.02	0.01	0.02	0.01	<0.01	0.01	0.01	0.01	<0.01
Spleen	0.12	0.38	0.25	0.12	0.06	0.13	0.10	0.16	0.24
Gonads	0.11	0.14	0.12	0.01	<0.01	0.02	0.01	0.06	0.03
Fat[b]	0.08	0.08	0.08	0.03	0.16	0.09	0.09	0.09	0.03
Blood[b]	0.33	0.54	0.44	0.08	0.19	0.09	0.12	0.25	0.09
Muscle[b]	0.06	0.13	0.10	0.07	0.19	0.13	0.13	0.12	0.06
Brain	...	...	...	...	0.39	0.31	0.23	0.14	...
Total	36.11	18.16	27.13	28.98	17.41	16.85	21.08	23.51	43.11
Grand total	92.07	91.09	91.58	107.26	91.81	93.62	97.56	95.18	91.50
Absorbed and unexcreted (summarized)									
Liver	2.62	1.43	2.02	1.75	1.99	1.92	1.89	1.94	
Liver	2.62	1.43	2.02	1.75	1.99	1.92	1.89	1.94	11.95
Five selected tissues[c]	0.57	0.90	0.73	0.46	0.15	0.56	0.39	0.53	0.68
Remainder	32.92	15.83	24.38	26.77	15.27	14.37	18.80	21.04	30.48
Total	36.11	18.16	27.13	28.98	17.41	16.85	21.08	23.51	43.11

[a] Includes G. I. tract as well as contents.
[b] Aliquot.
[c] Heart, kidneys, adrenals, spleen, and gonads.

presence of sodium taurocholate. Total ester bonds split were: 42% at 1 hour, 48% at 2 hours, 58% at 4 hours, 60% at 6 hours, and 68% at 22 hours (Figure 2). In a replicate, the values were: 38% at 1 hour, 58% at 3 hours, and 72% at 21.5 hours. Even without sodium taurocholate in the enzymatic system, 58% of the ester bonds were split after 21.5 hours. The presence of sodium taurocholate, calcium chloride (0.5 ml. of a 1% solution), and albumin (0.5 ml. of a 3% solution) in the enzymatic system increased the rate of splitting to 77% at 21.5 hours. It is significant that the incorporation of additives, in vitro, increased the rate and amount of splitting, since more of such substances are present in vivo.

The relative rates of splitting of the total available lactate and palmitate ester bonds (Figure 3) for the first hour were rapid and about equal. As the reaction continued, the rates were reduced. Equilibrium conditions appeared to be reached slightly faster for the palmitate than for the lactate bond. After 22 hours, 79% of the total lactate and 61% of the total palmitate bonds were split.

These results indicated that, in the mammalian digestive tract where more active enzymes are present and where absorption of products prevents equilibrium with reaction products, rapid and complete hydrolysis of all ester linkages of the glyceryl lactate palmitate probably would occur, yielding lactic and palmitic acids and glycerol as sole primary products.

Metabolic Study. Although other investigators (*2, 5, 6, 10*) have studied the fate of lactic acid and a glyceryl lactate palmitate (*6*), their materials were labeled differently from those in this study. Brin, Olson, and Stare (*1*) showed that D-lactate is not oxidized as rapidly as L-lactate in the animal tissue of two species. None of these studies attempted to provide either a balance or the distribution among the various tissues.

Respired Air. Table II indicates that 2,3-C^{14} lactic acid was absorbed readily from the gastrointestinal tract and rapidly oxidized, as indicated by the $C^{14}O_2$ in the exhaled air. An average of 60.9% of the dosage of activity of lactic acid given in aqueous propylene glycol was recovered as $C^{14}O_2$ in the exhaled air of four rats within 48 hours (series 1). The variation in rate among the five rats does not appear to be related to sex, although the females oxidized lactic acid slightly faster than the males (Table II). The average cumulative activity, in terms of percentage of dosage, in the exhaled air of five rats is presented graphically in Figure 4.

When the lactic acid* was intubated with sucrose and sodium caseinate (series 2), the rate of absorption and oxidation was faster than in series 1. In series 2, the average cumulative radioactivity recovered in the exhaled air of three male rats within 26.5 hours was 65.5% (Figure 5). The early absorption and oxidation of lactic acid were illustrated further by the specific activity of $C^{14}O_2$ in the exhaled air at various periods (Figure 6). The peak of activity occurred at about 2 hours with both the free and esterified lactic acid.

The comparably labeled lactate moiety of glyceryl lactate palmitate was also readily absorbed from the gastrointestinal tract and rapidly oxidized. An average of 59.7% of the dosage of activity of the ester given in aqueous propylene glycol (series 1) was recovered as $C^{14}O_2$ in the exhaled air of five rats within 48 hours (Table II). As with lactic acid, the females may oxidize the

Table V. Fate of Ingested Glyceryl Lactate-C^{14} Palmitate

(Series 2 and 2a)

Rat No.	GLP-1	GLP-2	Av.	GLP-3
Sex	M	M	2 males	M
Hour	26.5	26.5	at 26.5	26.5
Vehicle	H_2O, casein, sucrose	H_2O, casein, sucrose	Hours	H_2O-PG
Total activity given, μc.	14.9	17.5	16.2	9.14
	Percentage of Dosage Recovered			
Not absorbed				
Feces	0.95	0.12	0.54	5.80
Contents and G.I. tract	3.39	3.90	3.64	3.36
Total	4.34	4.02	4.18	9.16
Absorbed and excreted				
Breath	69.94	64.90	67.42	58.07
Urine	6.38	7.56	6.97	5.54
Total	76.32	72.46	74.39	63.61
Absorbed and unexcreted				
Remainder	14.30	19.70	17.00	19.30
Liver	1.65	3.05	2.35	3.09
Heart	0.06	0.15	0.10	0.10
Kidneys	0.31	0.53	0.42	0.50
Adrenals	0.11	0.02	0.06	0.02
Spleen	0.02	0.24	0.13	0.12
Gonads	0.18	0.45	0.32	0.31
Fat[a]	0.06	0.10	0.08	0.06
Blood[a]	0.23	0.09	0.16	0.02
Muscle[a]	0.06	0.12	0.09	0.05
Brain	0.13	0.35	0.24	0.35
Lungs	0.18	0.25	0.22	0.19
Skin	0.05	0.17	0.11	0.05
Total	17.34	25.22	21.28	24.16
Grand total	98.00	101.70	99.85	96.93
Absorbed and unexcreted (summarized)				
Liver	1.65	3.05	2.35	3.09
Five selected tissues[b]	0.68	1.39	1.03	1.05
Remainder	15.01	20.78	17.90	20.02
Total	17.34	25.22	21.28	24.16

[a] Aliquot.
[b] Heart, kidneys, adrenals, spleen, and gonads.

ester slightly faster than the males. The average cumulative activity in the exhaled air of six rats given GL*P in aqueous propylene glycol is shown in Figure 4.

The lactate portion of the ester was also oxidized more rapidly in an aqueous solution of sucrose and sodium caseinate. Each of two male rats (series 2) oxidized 67.4% (average) of the dosage within 26.5 hours. The influence of the vehicle was also illustrated by comparing series 2 and 2a, since in series 2a, 58.1% of the dosage given in an aqueous propylene glycol solution was found within 26.5 hours in the respired air of a male rat (Figure 5). This difference in response in series 2 and 2a is manifested also in the specific activity of the carbon dioxide in the exhaled air of the rats given the ester (Figure 6).

An important consideration with respect to safety is the fact that in both series the lactate moiety was oxidized as readily as the free lactic acid, which is a natural metabolite in the human body. Within 48 hours, four rats oxidized 60.9% of the lactic acid and five rats oxidized 59.7% of the lactate portion of the ester (series 1, Figure 4); in the other vehicle within 26.5 hours, three rats oxidized 65.5% of the free acid and two rats oxidized 67.4% of the same moiety when esterified (series 2, Figure 5).

Total Recovery. The over-all recovery of radioactivity within 48 hours in four rats given free lactic acid in an aqueous propylene glycol solution (series 1) ranged from 91.8 to 101.1% with an average of 96.2%. The total recovery in the case of the fifth rat killed after 24 hours was 100.8% (Table III). In three rats in series 2, total recovery ranged from 95.3 to 103.4%, with an average of 99.7% (Table III). The slightly better total recovery in series 2 probably represents differences in techniques and methods and was probably unrelated to the vehicle.

Unfortunately, the gastrointestinal tract and contents were not processed in the same manner in both series. A portion of the "not absorbed" material was probably in the intestinal tissue in the process of being absorbed. This was particularly evident in the case of rat L-5 in series 1 (Table III) and rats GLP-5 and 6 (Table IV), since the values found in the gastrointestinal tract and contents were somewhat higher than those representing only the contents of comparable rats in this series. Nevertheless, only a small portion of the lactic acid was not absorbed from either vehicle.

The smaller urinary activity at 26.5 hours (4.1%) in the case of rats given lactic acid in sucrose and caseinate (series 2) as compared to 6.3% at 48 hours in the other vehicle (series 1) may have been more related to time than to the vehicle, since 3.5% of the activity was present at 24 hours in the urine of one rat given the free acid in the latter vehicle (series 1, Table III and Figure 7).

The amount of lactic acid absorbed but unexcreted in series 1 (water-P.G.) is much greater at 24 than at 48 hours, since 51.1% was recovered at 24 hours, while only 26.5% remained after 48 hours (Table III and Figure 7). A comparable value (25.6%) was absorbed and unexcreted after 26.5 hours when the lactic acid was administered in sucrose and caseinate (Table III and Figure 7).

The bar graphs in Figure 7 represent the over-all pattern with respect to lactic acid. When time was varied and the vehicle (H_2O-P.G.) was maintained constant (series 1), more was oxidized at 48 than at 24 hours. The liver was the major site of storage and oxidation, since it contained 16.8% after 24 hours, but only 1.8% after 48 hours (bars 1 and 2). However, these data do not eliminate the possibility of gradual transfer from the liver to other sites for oxidation. When the time was maintained essentially constant and the vehicle was varied, absorption and oxidation were more rapid in the case of the sucrose and caseinate (bars 2 and 3). Comparison of bars 1 and 3 indicated that the faster absorption and oxidation in the one vehicle were essentially compensated by the longer time in the other vehicle.

The over-all recovery in the case of five rats given the ester in the aqueous propylene glycol suspension and killed at 48 hours (series 1) varied from 91.1 to 107.3%, with an average of 95.2%. Recovery in the case of a sixth rat in series 1 killed at 24 hours was 91.5% (Table IV). As in the case of the free acid, a slightly better total recovery with less variation was attained in series 2—96.9, 98.0, and 101.7%, respectively (average 98.5% Table V).

In the case of the ester, the vehicle had an influence on the amount not absorbed. In the propylene glycol vehicle, an average of 6.0% was found in the feces of five rats at 48 hours, 2.6% in the feces of one rat at 24 hours (series 1, Table IV), and 5.8% in the feces of another rat at 26.5 hours (series 2a, Table V). When the ester was given in sucrose and casein-

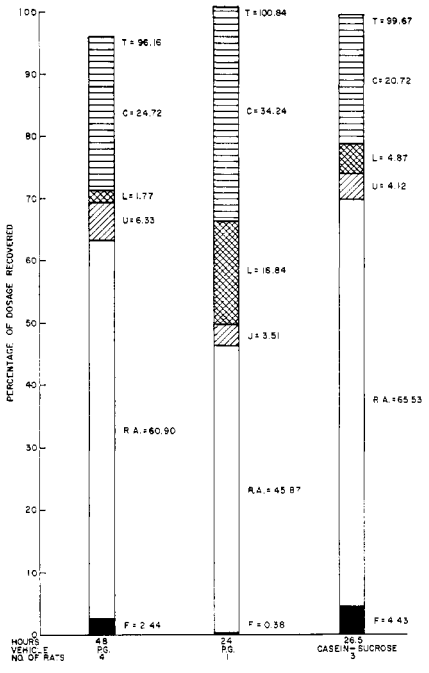


Figure 7. Recovery of lactic acid-C^{14}

Series 1 and 2

T. Total *U.* Urine
C. Carcass *R.A.* Respired air
L. Liver *F.* Feces

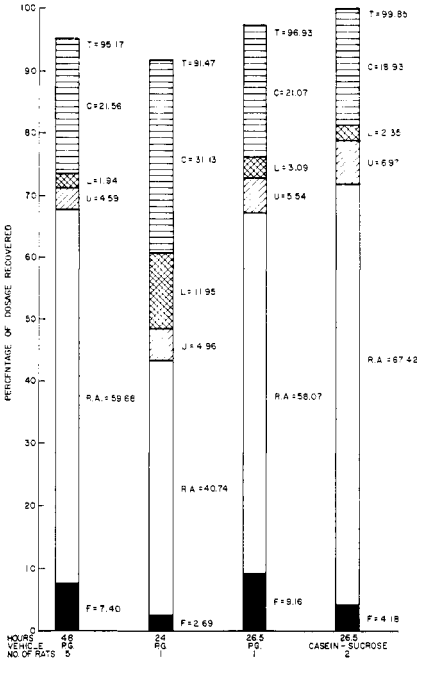


Figure 8. Recovery of glyceryl lactate-C^{14} palmitate

Series 1, 2, and 2a

T. Total *U.* Urine
C. Carcass *R.A.* Respired air
L. Liver *F.* Feces

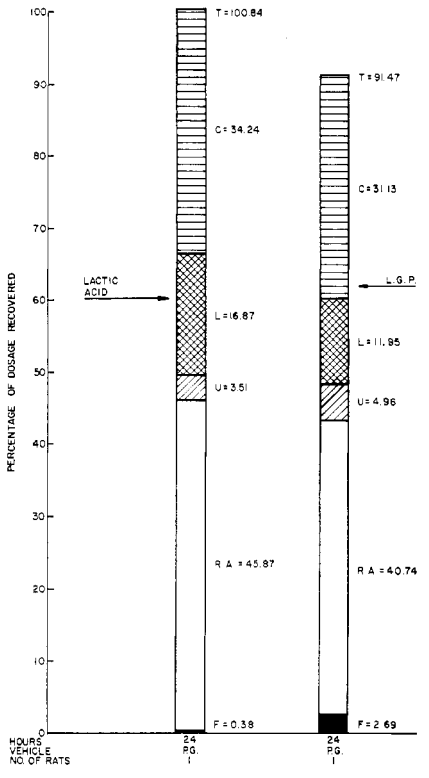


Figure 9. Comparison of recovery of lactic acid-C^{14} *vs.* glyceryl lactate-C^{14} palmitate

24 hours—P.G.

T. Total *U.* Urine
C. Carcass *R.A.* Respired air
L. Liver *F.* Feces

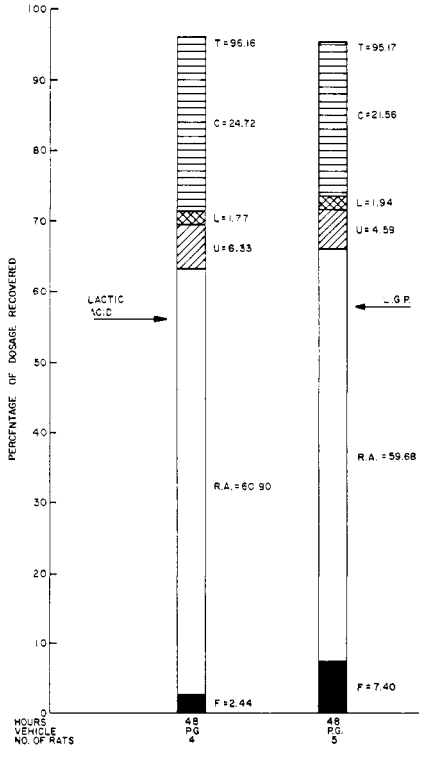


Figure 10. Comparison of recovery of lactic acid-C^{14} *vs.* glyceryl lactate-C^{14} palmitate

48 hours—P.G.

T. Total *U.* Urine
C. Carcass *R.A.* Respired air
L. Liver *F.* Feces

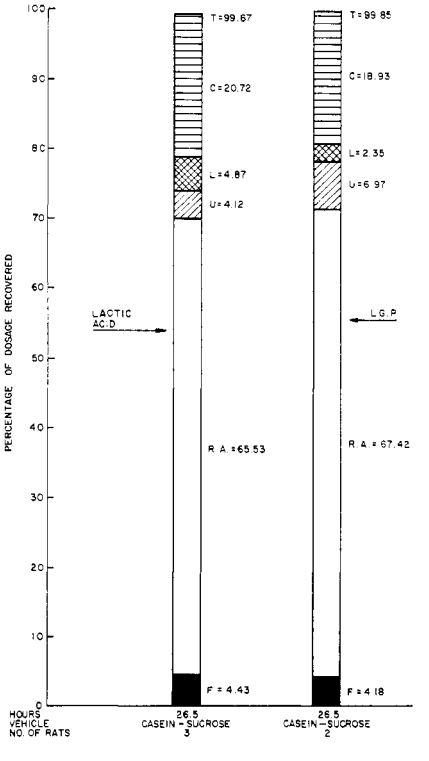


Figure 11. Comparison of recovery of lactic acid-C^{14} *vs.* glyceryl lactate-C^{14} palmitate

26.5 hours—casein and sucrose

T. Total *U.* Urine
C. Carcass *R.A.* Respired air
L. Liver *F.* Feces

ate to two rats, an average of only 0.54% was obtained in the feces at 26.5 hours (series 2, Table V). The presence of carboxymethylcellulose in the water–propylene glycol system may have exerted some small inhibitory effect with respect to absorption from the gastrointestinal tract.

There was no particular urinary pattern; in series 1, 5.0% was found in the case of one rat at 24 hours and an average of 4.6% in the case of five rats at 48 hours; in series 2, 7.0% and in series 2a, 5.5% were found at 26.5 hours (Tables IV and V).

The amount of the lactate moiety that was absorbed but unexcreted in series 1 (water-P.G.) was much greater at 24 than at 48 hours, since 43.1 and 23.5%, respectively, were recovered (Table IV and Figure 8). A comparable amount (21.3%) was absorbed but unexcreted in the case of the other vehicle at 26.5 hours (series 2, Table V and Figure 8). It is not readily apparent why rat GLP-3 (series 2a, Table V) did not have more than 24.2% in this category. At this time it can only be attributed to animal variability. An atypical second rapid rate of oxidation occurred in this rat between the 15th and 22nd hours (Figure 5) followed by a second reduced rate thereafter. Per-

Table VI. Specific Activity in Tissues Resulting from Ingestion of Labeled Acid and Ester

	Lactic Acid-C^{14}[a] (Series 1 and 2)		
Series	1	1	2
Time, hours	48	24	26.5
Vehicle	P.G.	P.G.	Casein, sucrose
No. and sex[b]	2M & 2F	1F	3M
Tissue		*D.P.M.[c]/Mg. Tissue*	
Liver	720	6358	1585
Kidneys	616	547	664
Adrenals	863	859	557
Spleen	498	527	755
Heart	351	327	395
Muscle	264	220	369
Carcass	428	493	384[d]
Fat	226	247	1340[d]
Blood	241	186	270[d]
Testes	286	...	556[d]
Ovaries	746	467	...
Brain	...	...	916
Lungs	...	...	651
Skin	...	...	354

	Glyceryl Lactate-C^{14} Palmitate[a] (Series 1, 2, 2a)			
Series	1	1	2a	2
Time, hours	48	24	26.5	26.5
Vehicle	P.G.	P.G.	P.G.	Casein, sucrose
No. and sex[b]	2M and 3F	1F	1M	2M
Tissue		*D.P.M.[c]/Mg. Tissue*		
Liver	719	4617	1025	517
Kidneys	449	597	441	391
Adrenals	788	618	1683	1670
Spleen	491	424	681	426
Heart	242	281	323	182
Muscle	278	212	234	158
Carcass	366	523	385	187
Fat	163	63	598	112
Blood	189	194	...	211[e]
Testes	302	...	347	207
Ovaries	476	993	...	...
Brain	496[f]	...	452	283
Lungs	...	...	416	272
Skin	...	...	315	222

[a] Corrected to standard dosage of 100 μc.
[b] M = male; F = female.
[c] D.P.M. = disintegrations per minute.
[d] Obtained on 2M rats.
[e] Obtained on 1M rat.
[f] Obtained on 2F rats.

haps a stimulus for oxidation, unlike that in any of the other rats, was induced during this period.

Figure 8 portrays the over-all response with respect to the lactate moiety of the ester. When time was varied and the vehicle (H_2O-P.G.) was constant (series 1), equilibrium was not reached at 24 hours. Once again, the liver appeared to be the major site of storage and oxidation, since 12.0% was recovered after 24 hours, but only 1.9% after 48 hours (bars 1 and 2). When time was constant (26.5 hours) and the vehicle was varied (series 2 and 2a) absorption and oxidation were faster in the casein and sucrose (bars 3 and 4). The difference in results obtained at 24 hours (P.G., series 1) and 26.5 hours (P.G., series 2a) is not readily explainable. The faster absorption and oxidation in series 2 (sucrose and caseinate) than in series 1 (P.G.) were compensated by the longer time in series 1 (bars 1 and 4).

From the standpoint of utilization in foods, the similarity of the over-all pattern of metabolism between free lactic acid and the lactate moiety of the ester is of utmost importance. Recovery in the feces, respired air, urine, liver, and carcass has been compared under three conditions (Figures 9 to 11). In series 1 the materials were given in aqueous propylene glycol and the rats (one in each instance) were killed after 24 hours. The free acid and ester found were: respired air 45.8 and 40.7%; liver 16.8 and 12.0%; and carcass 34.2 and 31.1% (Figure 9). When the free acid was given to four rats and the ester to five rats, killed after 48 hours (series 1), recoveries of lactic acid and ester were: respired air 60.9 and 59.7%; liver 1.8 and 1.9%; and carcass 24.7 and 21.6% (Figure 10). In the third comparison, in which aqueous sucrose and caseinate were employed as the vehicle, three rats were given the free acid and two rats were given the ester (series 2). When these animals were

Table VII. Specific Activity in Tissues Resulting from Ingestion of Lactic Acid-C^{14} or Glyceryl Lactate-C^{14} Palmitate[a]

D.P.M./Mg. Tissues (± S.D.)

Series	No. and Sex	Material	Liver	Kidneys	Adrenals	Spleen	Heart	Muscle	Carcass	Fat	Blood	Testes	Brain	Lungs	Skin
						Conditions. 26.5 Hours. Vehicle, Casein and Sucrose in H_2O									
2	3 M	LA	1585 ± 934	664 ± 45	557 ± 146	755 ± 151	395 ± 183	369 ± 177	384 ± 171[b]	1340 ± 1439[b]	270 ± 132[b]	556 ± 54	916 ± 168	651 ± 174	354 ± 22
2	2 M	GLP	517 ± 108	391 ± 67	1670 ± 1898	426 ± 62	182 ± 28	158 ± 11	187 ± 62	112 ± 25	211[c]	207 ± 50	283 ± 158	272 ± 14	222 ± 20
														Ovaries	
						Conditions. 24 Hours. Vehicle, P. G. and H_2O									
1	1 F	LA	6358	547	859	527	327	220	493	247	186			467	
1	1 F	GLP	4617	597	618	424	281	212	523	63	194			993	
						Conditions. 48 Hours. Vehicle, P.G. and H_2O									
1	2 M, 2 F	LA	720 ± 113	616 ± 116	863 ± 365	498 ± 148	351 ± 184	264 ± 104	428 ± 114	226 ± 199	241 ± 72	286 ± 167		746 ± 416	
1	2 M, 3 F	GLP	719 ± 213	449 ± 31	788 ± 352	491 ± 65	242 ± 35	278 ± 34	366 ± 118	163 ± 74	189 ± 71	302 ± 43		476 ± 32	

[a] Corrected to standard dosage of 100 μc. [b] Obtained on 2 rats [c] Obtained on 1 rat.

killed 26.5 hours later, the acid and lactate portion of the ester found were: respired air 65.5 and 67.4%; liver 4.9 and 2.4%; and carcass 20.7 and 18.9% (Figure 11). There was no outstanding difference between results obtained with the two materials.

Specific Activity of Tissues. Since the amount of activity administered by stomach tube varied among the animals, the specific activity, in terms of disintegrations per milligram of fresh tissue, has been corrected to a standard constant dosage of 100 μc. for all animals. The data in Table VI represent essentially random distribution, except that in series 1, although the other tissues appear to have attained equilibrium at 24 hours, the specific activity of the liver is ten times higher at 24 than at 48 hours. Data in series 2 indicate that the specific activity of the liver may be a more sensitive indicator than the specific activity in the respired air for measuring equilibrium of lactic acid in the body, since the specific activity of the liver had not yet reached equilibrium (Table VI) whereas the specific activity of the $C^{14}O_2$ in the exhaled air had approached equilibrium (Figure 6). Comparable conclusions may be drawn with respect to the lactate moiety of the ester (Table VI). Because of the small size of the adrenals and the difficulty in completely and adequately isolating them from all surrounding tissue, the accuracy of their individual determination is less than that obtained on the other individual samples.

The similarity between specific activities under different conditions is shown in Table VII. These values indicated a close similarity between the free and esterified lactate in series 1. The greater variation in series 2 may reflect the lesser activity administered.

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Literature Cited

(1) Brin, M., Olson, R. E., Stare, F. J., *J. Biol. Chem.* **199,** 467 (1952).
(2) Conant, J. B., Cramer, R. D., Hastings, A. B., Klemperer, F. W., Solomon, A. K., Vennesland, B., *Ibid.*, **137,** 557 (1941).
(3) Domingues, F. J., Gildner, K. J., Baldwin, R. R., Lowry, J. R., *Intern. J. Appl. Radiation and Isotopes* **7,** 77 (1959).
(4) Domingues, F. J., Sarko, A., Baldwin, R. R., *Ibid.*, **1,** 94 (1956).
(5) Lorber, V., Lifson, N., Wood, H. G., Warwick, S., Shreeve, W. W., *J. Biol. Chem.* **183,** 517 (1950).
(6) McKennis, H., Jr., Turnbull, L. B., Haag, H. B., Larson, P. S., *Proc. Soc. Exptl. Biol. Med.* **97,** 498 (1958).
(7) Passman, J. M., Radin, N. S., Cooper, J. A. D., *Anal. Chem.* **28,** 484 (1956).
(8) Van Slyke, D. D., Folch, J., *J. Biol. Chem.* **136,** 509 (1940).
(9) Van Slyke, D. D., Plazin, J., Weisiger, J. R., *Ibid.*, **191,** 229 (1951).
(10) Vennesland, B., Solomon, A. K., Buchanan, J. M., Cramer, R. D., Hastings, A. B., *Ibid.*, **142,** 371 (1942).

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COMPOSITION OF FATS

Fatty Acid Composition of Food Fats

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Gas-liquid chromatography was employed to investigate the nature of the fatty acids present in margarines, spreads, shortenings, and some meat fats. The results obtained from polyester and silicone columns generally agreed, and showed that C_{22} fatty acids were present in 3 of 16 margarines, 6 of 14 spreads, and 1 of 7 shortenings. In these products marine oils appeared to be the main source of the long-chain fatty acids. Fat of animal origin also contained fatty acids of odd-numbered carbons.

An interest in long-chain fatty acids in foods was aroused when rapeseed oil became a possible constituent of the Canadian diet. Since C_{20} and C_{22} acids are also components of marine oils, it was decided to investigate the nature of the fatty acids present in margarines, spreads, shortenings, and some animal fats. Gas-liquid chromatography was employed as an effective means of obtaining quantitative analyses.

Materials and Methods

One-gram aliquots of margarines and spreads were extracted with diethyl ether; the ether extracts were washed with water, dried with anhydrous sodium sulfate, and evaporated in the presence of nitrogen. The method of Bligh and Dyer (*2*) was used to extract the lipide material from meat. By direct transesterification (*5, 8*), the methyl esters of fatty acids were prepared. Approximately 100 mg. of each margarine and spread fat, of each shortening as purchased, and each meat fat were methylated by refluxing in 10 ml. of methanol and 1 ml. of 7% HCl in methanol for 30 minutes. After removal of the HCl and methanol with the aid of a water bath and a stream of nitrogen, 1 μl. of the methyl esters was inserted into a Beckman GC-2 gas chromatograph in which the injector was modified and the gas sampling valve was removed. A 1-mv. recorder was employed.

Methyl esters of fatty acids were chromatographed on a 6-foot, 1/4-inch column packed with butanediol succinate (*5*) of m.p. 97° C. on acid-washed Chromosorb W (1 to 6 parts by weight). The temperatures of the injector and the column were 206° and 232° C., respectively, while the helium flow was 80 ml. per min. For confirmation of the results obtained with the polyester column, the fractions of each chain length were determined with a 2-foot, 1/4-inch column of silicone on C_{22} firebrick (1 to 6 parts by weight) operated