ing substance) concentration in the allantoic fluid can be observed, while the potassium level is noticeably lowered. These alternations in electrolyte and sugar elimination may have some bearing upon the high mortality rate of such embryos. Addition of Tyrode's solution, consisting of physiological saline and glucose, to cortisone injected embryos raises slightly their survival rate. The results indicate a compensation therapy effect of the solution on embryos under conditions of increased sodium, chloride and sugar excretion.

Summary. 1) Single injections of 1 mg egg of cortisone on the chorio-allantoic membrane of chick embryos produce a striking and specific syndrome: mortality of the embryos is highly increased, growth of surviving embryos is markedly retarded and characteristic developmental modifications are produced: electrolytes and sugar metabolisms are disturbed. 2) Simultaneous administration of Tyrode's solution, consisting of saline and glucose, to such embryos brings about a slight rise in survival rate of embryos, owing probably to its compensation therapy effect upon loss of sodium chloride and sugar (reducing substance). 3) Treatment of cortisone-injected embryos with growth hormone (STH) results in striking alleviation of most of the cortisone caused changes. Mortality rate is lowered almost to the level of that occurring spontaneously during incubation; inhibition of growth as well as many deleterious modifications are successfully counteracted. 4) Various aspects of the interaction of hormones applied are discussed and the conclusion is reached that the action of growth hormone is antagonistic to that of cortisone.

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Received December 18, 1957. P.S.E.B.M., 1958, v97.

Metabolism of Lactic Acid and Glycerol in C^{14}-Glyceryl Lactopalmitate.* (23786)

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The metabolism of acylated glycerol to carbon dioxide and water, in the light of current knowledge depends upon the intermediate formation of free or deacylated glycerol. Similarly, metabolism of fatty acids esterified with glycerol is dependent upon hydrolysis. The hydrolysis rates of glyceryl esters thus become a variable, along with absorption rates and possible transesterifications, determining the catabolism of glycerol. Karnovsky and Gidez(1,2) compared the appearance of CO_2 from the metabolism, in rats, of C^{14}-glycerol esterified with various acids. Esterification with oleic acid decreased the rate at

* A preliminary report was presented at meeting of Am. Soc. Pharm. and Exp. Therap., Chicago, Ill., Apr. 16, 1957. C^{14}-compounds were obtained under U. S. Atomic Energy Commission License 45481.

which carbon dioxide was eliminated from the labelled glycerol. No other essential difference in metabolism was reported. The rapidity with which carbon dioxide is eliminated from ingested glycerol(1,2) affords then, a convenient method for determining whether or not synthetic or natural glycerides are effectively utilized for energy yielding reactions. When glycerol is esterified by fusion with equimolar quantities of lactic acid and palmitic acid an ester preparation with emulsifying properties is formed. The preparation, glyceryl lactopalmitate, would be expected to contain glycerol-lactic acid ester linkages, which have never been identified as naturally occurring, although there is some evidence that pancreatic enzymes can form esters from lactic acid(3). In consequence, a study of glyceryl lactopalmitate has been conducted *in vitro,* and *in vivo* with the aid of glycerol-1,3-C^{14} and lactic acid-2-C^{14}.

Materials and methods. Glyceryl lacto-2-C^{14}-palmitate was prepared by fusion of glycerol (1M) and lactic acid (1M) and palmitic acid (1M). Sodium lactate (specific activity 0.82 mc/mM, Isotope Specialties, Inc.); was equilibrated with 80 percent lactic acid prior to esterification to introduce the carbon label. The resulting ester preparation had a saponification number 269, acid number 1.8, and a radioactivity 3.86 x 10^6 disintegrations per minute per g.† Glyceryl-1,3-C^{14}-lactopalmitate was prepared from glycerol-1,3-C^{14} (Research Specialties Company) after equilibration with 95 percent glycerol prior to formation of the ester (saponification number 255, acid number 8.1, 4.17 x 10^{10} disintegrations per minute per g). The radioactive esters dissolved in warm olive oil were administered by intubation to mongrel dogs and albino (Wistar strain) rats. Expired carbon dioxide was collected in sodium hydroxide scrubbers from rats which were maintained in glass metabolism cages. Lymph and blood samples were assayed for lactic acid by method of Barker and Summerson(4). Radioactive lactate was oxidized to acetaldehyde by a modification of the method of Lucus(5) and then collected as the dimedon derivative(6) which is conveniently counted. Samples of blood or lymph were analyzed before and after incubation (as noted in the tables). The acetaldehyde from oxidation of lactate (and that present before oxidation) was obtained as follows: One hundred ml of aliquot from the deproteinization of whole blood or lymph (1 ml of blood diluted to 10 ml) was placed in a 500 ml Kjeldahl flask with cold finger reflux condenser and addition tube drawn to a capillary tip. To this was added 10 ml of solution containing carrier (100 mg lithium lactate) and 5 ml of catalyst (0.05M iodine monochloride). The solution was brought to a boil, and 20 ml of N/5 ceric sulfate in N/2 sulfuric acid was added dropwise over a 15-20 minute period. Air was slowly aspirated through the flask during the period, and the entrained acetaldehyde was collected in 2 absorption towers containing 100 ml each of 0.4% dimedon adjusted to pH 6.0(6). After a total absorption time of ½ hour, the solutions in the towers were combined and then acidified to pH 3.9 with acetic acid. The precipitated dimedon addition product was removed by filtration, washed with water, dried at 80°C and weighed. Radioactivity was then determined. Confirmation of the radioactivity of isolated samples was achieved by recrystallization (m.p. 137-139°C) and conversion to 2,2,7,7,9-pentamethyl-4,5-dioxo-octahydroxanthene. Fecal radioactivity was determined on oven dried (99°C) samples. Urinary radioactivity was determined on dried samples made alkaline with an excess of sodium hydroxide before evaporation. Nonenzymatic hydrolysis of glyceryl monolactate (acetyl value 574, acid number 10.7, saponification number 350) was determined by dissolving 0.1263 g of ester in 300 ml of water at pH 7.3. The pH of the solution was maintained automatically at 7.3 by additions of 0.04593 N sodium hydroxide from a Beckman automatic titrator in an atmosphere of nitrogen. Concentrations of ester were calculated from alkali consumption during the reaction, at 30°C. Enzymatic hydrolysis of glyceryl lactopalmitate was determined by shaking a mixture containing 0.1211 g glyceryl lactopalmitate, 0.05 g lipase (Steapsin, Nutritional Biochemicals Corp.), 10

† Counts were obtained on infinitely thick samples using a Libby anticoincidence counter, *cf.* Libby, W. F., *American Scientist,* 1946, v44, 98.

TABLE I. Distribution of Lactate-2-C^{14} following Administration of Glyceryl Lacto-2-C^{14}-Palmitate* to 20 kg Male Dog.

Time, hr	Thoracic vol lymph, ml	Duct lymph, % admin. lactate in lymph collected
0 – ½	21	.041
½–1¼	38	.10
1¼–4	60	.034
	Blood (after 4½ hr incubation), % admin. lactate in total body blood†	
2¾	2.43	
4	1.31	
24	.10	

* 10 g glyceryl lactopalmitate in 30 g olive oil by stomach tube.

† Calculated from relation Blood vol = .78 × body wt.

ml hexane. 35 ml 2M sodium acetate in 125 ml pyrex g.s. bottle at 29-30°C. At the end of the incubation period, 125 ml of neutral 95% ethanol was added to the reaction mixture which was then titrated to the phenophthalein end point with 0.05 N sodium hydroxide. The percentage hydrolysis was calculated from the alkali consumption less control titrations of mixtures incubated without substrate.

Results. In preliminary studies with dogs, attempt was made to correlate the appearance of lactic acid and its esters in the lymph from the thoracic duct‡ with the intestinal absorption of the administered unlabelled glyceryl lactopalmitate. Polyethylene or siliconized glass cannulas were employed in these experiments. Minor adjustments in the position of the cannula, such as may be influenced by the postural habits of the dog, led to considerable variations in flow rate. Thus, effluent samples may represent lymph that is considerably changed in its chemical composition due probably to some stasis in the duct or cannula. In a duplicate determination, a heparinized sample of lymph from the thoracic duct (initial lactic acid content 101-102 mg/100 ml) after incubation at 37.5° for four and one-half hours increased its lactic acid content to 315-316 mg 100 ml; these results indicated that a variety of artifacts would make interpretation of lactic acid content of lymph difficult unless isotopically labelled material were employed.

A male dog (2.0 kg) was given 10 g of glyceryl lacto-2-C^{14}-palmitate in 30 g of olive oil by stomach tube. Samples of blood and lymph were collected and analyzed for radioactive lactate. The results of these determinations (Table I) indicate that some digestion and absorption of glyceryl lactopalmitate (presence of radioactive lactate in lymph) had taken place within one-half hour after administration of the ester. Since the radioactivity levels in both blood and lymph rose during the first one-two hour period and then fell, metabolism of the ester was indicated. Confirmation of this point was then sought on rats where collection of expired air is easily accomplished.

Following administration of glyceryl-lacto-2-C^{14}-palmitate to rats, elimination of radioactive carbon dioxide was detected for periods as long as forty-eight hours (Table II). The dose of fat employed seems to have been well-tolerated in view of the small (1.4%) amount of fecal loss and absence of diarrhea.

C^{14}, after administration of glyceryl-1-3-C^{14}-lactopalmitate to rats, was also eliminated predominantly in the form of respiratory carbon dioxide. This, together with the similar elimination from glycerol administered in the unesterified form, serves to confirm previous data(1,2). A definite conclusion on comparative rates of oxidation of free and esterified glycerol would require studies on a larger series of animals. Fecal losses are of the same order of magnitude (Table III) irrespective of the type of radioactivity administered. Since the percent of radioactivity recovered in the feces is essentially the same in animals receiving radioactive glycerol as in the animals receiving equimolar doses of glycerol-1,3-C^{14}-lactopalmitate, good absorption of the synthetic fat is again indicated.

Since both glycerol and lactic acid were metabolized following administration of glyceryl lactopalmitate it is presumed that hydrolysis of the ester linkages from both acids has taken place. Hydrolysis of glyceryl lactopalmitate was carried out *in vitro* with commercial samples of calf lipase. In a 4-hour period hydrolysis had taken place to the extent of 32%. In 24 hours, seventy-five per-

‡ The authors thank Dr. L. E. Edwards for his kind assistance.

TABLE II. Metabolism of Glyceryl Lacto-2-C^{14}-Palmitate in Female Rats.

		Percent of dose eliminated in expired air							
		Between hr intervals					Final	Final	
Wt, g	Dose,*ml	0–6	9	12	21	48	urine	feces	Total
240	3		12.2	7.2					19.4
240	3		21.4				16.7	1.4	39.5
250	3		20.4						20.4
		5 hr							
263	3	11.2							11.2
275	3.3		18.9						18.9
				13 hr					
290	3.4			24.6			7.9		32.5
300	3.6				41.6	9.2	3.9	1.4	56.1
390	4.7				51.3	4.7	4.4		60.4

* 10 g of glyceryl lacto-2-C^{14}-palmitate dissolved in 30 ± g of olive oil. 1 ml of solution assayed 2.01×10^6 dpm.

TABLE III. Metabolism of Glyceryl-1,3-C^{14}-Lactopalmitate in Male Rats.

		Percent of dose eliminated in expired air							
		Between hr intervals					Final	Final	
Wt, g	Compound	0–6	9	21	31½	48	urine	feces	Total
						51½ hr			
190	Glyceryl-1,3-C^{14}-lactopalmitate*		46.4	19.6	7.5	4.2	6.2	1.4	85.3
180	*Idem*	27.5		31.6		9.2	3.8	.91	73.0
243	.88 ml olive oil, 50 mg glycerol	43.1		35.3		4.6	4.3	1.4	88.7
213	*Idem*†	43.8		27.0		4.5	3.6	1.7	80.6

* Radioactive ester (10 g) was dissolved in 30 g olive oil, as in previous series. One ml of solution contained by assay 7.77×10^6 dpm.

† 3.83×10^7 dpm glycerol. Total free glycerol present equals amount of glycerol present as ester in the dose of glyceryl lactopalmitate.

cent hydrolysis was achieved. This latter hydrolysis demonstrates the cleavage of both palmitic and lactic acid ester linkages and confirms the interpretation made from the tracer studies. In the absence of enzyme no significant hydrolysis was observed. Hydrolysis of glyceryl lactate proceeds to a significant degree at pH 7.3 in the absence of enzyme. In the titration study, 50% of the product underwent hydrolysis in 550 minutes. Further experimentation would be required to determine whether or not the hydrolysis of glyceryl lactate ester linkages is enzymatically controlled *in vivo*.

Summary. 1. The fate of lactic acid-2-C^{14} following administration of glyceryl lacto-2-C^{14}-palmitate was studied by converting lactate from blood or lymph to acetaldehyde which was counted as the dimedon derivative and as 2,2,7,7,9-pentamethyl-4,5-dioxo-octahydroxanthene. 2. The lactic acid content of lymph samples from the thoracic duct of the dog is subject to variation which arises from the speed with which samples are obtained through a cannula. 3. Appearance of radioactive CO_2 in the expired air of rats following the administration of glyceryl lacto-2-C^{14}-palmitate or glyceryl-1,3-C^{14}-lactopalmitate, as well as the low fecal radioactivities, indicates a high degree of utilization of the fat. 4. In comparison with glycerol and glyceryl esters the metabolism of glyceryl-1-3-C^{14}-lactopalmitate, as determined by the radioactivity of expired CO_2, follows a path consistent with that expected from a normal ester of glycerol.

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Received September 5, 1957. P.S.E.B.M., 1958, v97.