eliminated by respiratory $^{14}CO_2$ the 1st week ranged from 0.5 to 2.0%. The major reason for these low levels was that this material was only slowly mobilized from the site of injection, and its conversion to fatty acids and subsequent CO_2 was limited by the rate of mobilization. There was little or no difference in the rate of mobilization from subcutaneous and intramuscular injections.

Some radioactivity from 1-^{14}C-*n*-hexadecane was eliminated in the urine and feces during the 1st week after administration; however, in both groups of animals, the total amount for the entire week was less than 0.01%.

All animals received an unlabeled dose of the emulsion in the opposite leg. Upon death, each of the control legs was immediately examined by gross necropsy by a veterinary pathologist. At no time was there any indication of any type of infection or a sterile abscess due to the injection.

SUMMARY

This investigation has shown that the *n*-hexadecane component and most likely other straight-chain hydrocarbon components of a mineral oil adjuvant emulsion are very slowly mobilized from the site of injection in monkeys and rats. Results have been presented which show that as much as 25–30% of the ^{14}C-*n*-hexadecane tracer remains at the site of injection after 10 months. The *n*-hexadecane tracer, which is mobilized from the site of injection, was readily metabolized to naturally occurring lipids.

Gross necropsy did not reveal any evidence of any pathological states at the sites of injection.

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ACKNOWLEDGMENTS AND ADDRESSES

Received September 15, 1969, from the *Southwest Research Institute, San Antonio, TX 78228*

Accepted for publication February 26, 1970.

This investigation was supported by the Division of Biologics Standards, National Institutes of Health, Contract No. PH 43-67-1104.

The technical assistance throughout the program of Dennis Schmidt was appreciated.

Metabolic Fate of Mineral Oil Adjuvants Using ^{14}C-Labeled Tracers II: Mannide Monooleate

JAMES N. BOLLINGER

Abstract □ This investigation was undertaken to determine the metabolic fate of mannide monooleate when employed in a mineral oil emulsion. Female white rats and female squirrel monkeys were injected subcutaneously or intramuscularly with an emulsion made with mineral oil and surfactant and including either 1-^{14}C-oleate or UL-^{14}C-mannide-labeled mannide monooleate tracer preparations. It was shown that 30–40% of the surfactant mixture is removed from the site of injection after 24 hr. After 1 week, 40–60% of the surfactant is removed from the site of injection; while after 3 months, 10–30% of the surfactant still remains. The 1-^{14}C-oleate-labeled mannide monooleate was largely incorporated into the various lipid classes, while the UL-^{14}C-mannide-labeled mannide monooleate preparation was largely eliminated in the urine. There was some indication that the inguinal lymph nodes of monkeys may have contained unusually large amounts of radioactivity.

Keyphrases □ 1-^{14}C-Oleate-labeled mannide monooleate—metabolic fate □ UL-^{14}C-Mannide-labeled mannide monooleate—metabolic fate □ Mineral oil–mannide monooleate emulsion—subcutaneous, intramuscular injection □ Metabolic fate—mannide monooleate in mineral oil emulsion □ TLC—identity

Freund (1) found that "mannide monooleate"[1] was an effective agent in combining antigens and mineral oils in the form of a water and oil emulsion. However, subsequent use has indicated an infrequent occurrence of cysts following injection of vaccines and allergens (2) made with certain lots of this surfactant. Berlin (3) performed studies which showed that the toxic reactions of a mineral oil–"mannide monooleate" emulsion may be, in part or in whole, related to the level of ester hydrolysis and oxidative changes of this material. Recently, Hardegree and Pittman (4) have shown that the tetanus vaccines which caused sterile abscesses in humans contained free fatty acids and that a variety of these antigens were capable of releasing free fatty acids from a water emulsion of "mannide monooleate."

This study was undertaken to obtain information on the mobilization and metabolism of a mannide monooleate surfactant preparation when it is received as an injected mineral oil emulsion.

[1] Arlacel A, Atlas Chemical Industries, Inc., Wilmington, DE 19899. Quotation marks in text of article indicate the impure mixture; absence of quotation marks in text of article indicates pure mannide monooleate.

Table I—Distribution of Radioactivity among the Various Components of UL-^{14}C-Mannide Oleate and 1-^{14}C-Oleate Mannide

Plate Area[c]	Plate I[a] Surfactant[b] Mannide UL-^{14}C, %[d]	Plate I[a] Surfactant[b] Oleate 1-^{14}C, %[d]	Plate II[a] Surfactant[b] Mannide UL-^{14}C, %[d]	Plate II[a] Surfactant[b] Oleate 1-^{14}C, %[d]
a	12.0	6.0	2.9	0.5
b	19.0	2.0	0.8	0.0
c	3.4	7.0	5.7	3.0
d	3.5	8.4	17.9	1.0
e	6.6	7.9	1.2	2.0
f	2.5	8.1	3.8	6.4
g	1.2	2.9	3.9	7.4
h	4.9	3.2	4.1	9.3
i	5.9	9.6	3.1	8.6
j	11.7	24.0	1.3	2.7
k	7.0	7.8	25.2	38.1
l	21.1	13.1	30.1	20.9
m	1.0	0.0	—	—

[a] Refers to systems used in Fig. 1. [b] ^{14}C-Labeled preparations received from Atlas Chemical Industries. [c] Refers to TLC plate areas in Fig. 1. [d] Percentage based on the amount applied to the plates and subsequent serial scrapings of I_2 vapor visualized separation.

Table II—Percentage of ^{14}C-Tracer Remaining in Monkeys and Rats at the Site of Injection after Administration of Mineral Oil–"Mannide Monooleate" Emulsion Containing the 1-^{14}C-Oleate Mannide as a Tracer

Route and Time after Injection	Average Percentage Remaining[a] Monkeys	Rats
IM,[b] 1 day	(3)[c] 78.3	(3)[c] 80.0
Sub Q,[b] 1 day	(3) 82.5	(3) 74.0
IM, 2 days	(3) 77.2	(3) 70.9
Sub Q, 2 days	(3) 77.7	(3) 73.5
IM, 7 days	(3) 41.2	(3) 58.8
Sub Q, 7 days	(3) 41.3	(3) 59.0
IM, 1 month	(2) 49.4	(2) 30.0
Sub Q, 1 month	(2) 37.4	(2) 17.6
IM, 2 months	(2) 46.1	(2) 49.6
Sub Q, 2 months	(2) 30.7	(2) 20.8
IM, 3 months	(2) 35.1	(2) 20.7
Sub Q, 3 months	(2) 22.0	(2) 9.3

[a] Average percentages based on the amount injected. [b] IM, intramuscular; Sub Q, subcutaneous. [c] Numbers in parentheses indicate the number of experimental animals used to determine the percentage ^{14}C-tracer remaining.

EXPERIMENTAL

Adjuvant Components—The adjuvant emulsion used consisted of mineral oil,[2] "mannide monooleate," and water in proportions of 9 parts mineral oil, 1 part surfactant, and 9 parts water.

The surfactant preparation used was obtained from Hilltop Laboratories, Inc., Cincinnati, Ohio. TLC analysis showed the material to contain at least 12 components (Fig. 1). Identification of the various components is tentative in this investigation; however, O'Neill and Yamauchi (5), who recently identified many of the components present in this particular surfactant, indicated that a neutral fraction from the "mannide monooleate" consisted of at least 12 components characteristic of peracylated carbohydrate structures. The intermediate or moderately polar material contained 14 components of the mannide monooleate class. The polar fraction contained predominantly carbohydrate polymers, conjugated dienes, and cyclic fatty acids.

The emulsion used in this study was prepared as described previously (6), using either sonic vibration or a double-hubbed needle, double-syringe method.

^{14}C-Labeled Emulsion—Since the mannide monooleate preparation is not pure but a mixture of monooleate, dioleate, trioleate, *etc.*, and various positional isomers of these esters plus various isomeric forms of dehydrated mannitol and oleic acid, it was decided that the most representative tracer would be ^{14}C-labeled surfactant prepared in the same manner as that used for the preparation of the normally unlabeled material.

Two preparations[3] were used in this study. One was made from 1-^{14}C-oleic acid and unlabeled mannitol, while the other was made from UL-^{14}C-mannitol and unlabeled oleic acid. The specific activity of the 1-^{14}C-oleate mannide was 2.2 μc./mg., and that of the UL-^{14}C-mannide oleate was 2.1 μc./mg. Results of TLC analysis of "mannide monooleate" and radioactivity distribution analysis of the separated components of the two tracers are shown in Fig. 1 and Table I, respectively. The relative amounts of each of the components present in the ^{14}C-labeled material were roughly (visual analysis) the same as those present in the unlabeled material. The data indicate that about 50% of the labeled surfactant is esterified, while the remainder is nonesterified material (chemically altered mannitol and oleic acid).

Dose and Administration—The radioactive tracers were thoroughly mixed with unlabeled surfactant and the mineral oil emulsion prepared as described (6) and used immediately (within 30 min. after preparation).

The size of the dose was 0.1 ml. (approximately 5 μc.) of the emulsion for rats and 0.3 ml. (approximately 15 μc.) for monkeys. The dose was administered with a 1-ml. disposable syringe equipped with a 23-gauge needle. The dose was administered in the right thigh either subcutaneously or intramuscularly, depending upon the phase of the experiment. A total of 60 female white rats and 60 female squirrel monkeys were used in this investigation. Prior to injection, several 1-μl. samples were removed from the ^{14}C-labeled emulsions, and a check was made on the uniformity of distribution of radioactivity within the emulsion. An unlabeled emulsion preparation of the same volume was injected into the left thigh for pathological evaluation by a veterinary pathologist.

After injection of the radioactive emulsions, the animals on the short-term studies (1–7 days) were immediately housed in Plexiglas metabolism cages. In long-term studies (1–3 months), the animals

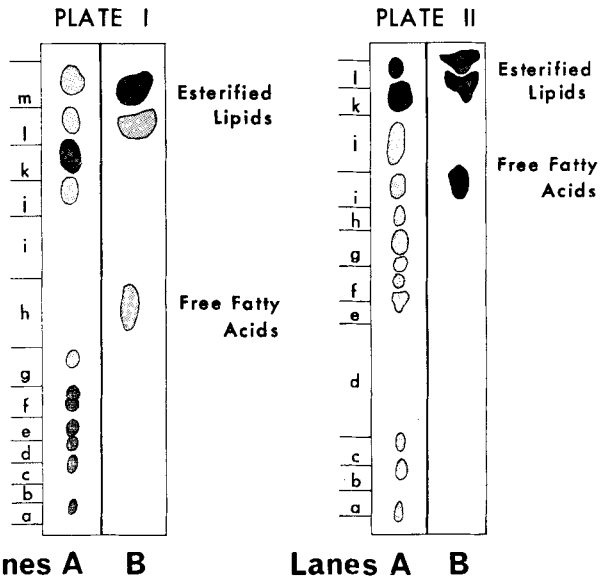

Figure 1—*Plate I shows the distribution of the components of "mannide monooleate" (Lane A) and the relative location of free fatty acids and esterified naturally occurring lipids (Lane B). The letters a–m indicate the components isolated for radioactivity determinations (Table I). The TLC system consisted of 100-μc. silica gel-coated plates developed in a chloroform–diethyl ether–acetic acid (95:5:1) system. Plate II depicts the same materials as Plate I but was developed in a more polar chloroform–diethyl ether–acetic acid (80:20:1) system. The letters depict the areas isolated for radioactivity determinations.*

[2] Drakeol 6-VR, Pennsylvania Refining Co., Butler, Pa.

[3] Both of these tracers were prepared by Atlas Chemical Industries, Inc., Wilmington, DE 19899, with a scale-down of procedures normally employed in the preparation of Arlacel A.

Table III—Percentage of ^{14}C-Tracer Remaining in Monkeys and Rats at the Site of Injection after Administration of Mineral Oil–"Mannide Monooleate" Emulsion Containing the UL-^{14}C-Mannide Oleate as a Tracer

Route and Time after Injection	Average Percentage Remaining[a] Monkeys	Rats
IM, 1 day	(3)[b] 55.0	(3)[b] 67.2
Sub Q, 1 day	(3) 59.5	(3) 68.1
IM, 2 days	(3) 57.3	(3) 56.4
Sub Q, 2 days	(3) 65.7	(3) 58.6
IM, 7 days	(3) 46.1	(3) 54.1
Sub Q, 7 days	(3) 48.5	(3) 52.3
IM, 1 month	(2) 42.1	(2) 30.7
Sub Q, 1 month	(2) 30.7	(2) 19.7
IM, 2 months	(2) 44.4	(2) 39.5
Sub Q, 2 months	(2) 31.9	(2) 17.8
IM, 3 months	(2) 12.3	(2) 13.0
Sub Q, 3 months	(2) 17.1	(2) 8.9

[a] Average percent of the administered radioactivity remaining at the site of injection. [b] Numbers in parentheses indicate the number of experimental animals used to determine the percentage of ^{14}C-tracer remaining.

Table IV—TLC Separation and Liquid-Scintillation Counting of the Radioactivity Remaining at the Site of Injection of Rats and Monkeys 3 Months after Injection of a Mineral Oil–"Mannide Monooleate" Emulsion Containing 1-^{14}C-Oleate Mannide and UL-^{14}C-Mannide Oleate

TLC[a] Fraction	1-^{14}C-Oleate Mannide Rats, %[b]	Monkeys, %	UL-^{14}C-Mannide Oleate Rats, %	Monkeys, %
a–b	4.3	2.6	9.2	3.4
c–g	8.8	5.6	11.1	5.7
h–i	6.3	2.5	6.1	1.2
j–k	21.7	17.7	19.5	12.0
l–m	57.7	71.0	52.1	77.3
Solvent front	1.6	1.2	2.4	0.4

[a] TLC system used consisted of silica gel-coated plates developed in a chloroform–diethyl ether–acetic acid (95:5:1) system. Fractions were scraped from the plates as indicated on Plate I, Fig. 1. [b] Average percentages obtained from the total amount of radioactivity applied to the plates.

were housed in metal metabolism cages which allowed monitoring of urine and feces but not CO_2. Collection of $^{14}CO_2$ was carried out as described in a previous study (6). The determination of mobilization and degree of metabolism were monitored as previously described (6).

RESULTS AND DISCUSSION

Table II shows the average percentage of 1-^{14}C-oleate mannide tracer preparation remaining at the site of injection 1 day to 3 months after administration. Approximately 20–25% of this tracer was removed from the site of injection of rats and monkeys the 1st day after both intramuscular (IM) and subcutaneous injection (Sub Q). Seven days after injection, however, 40% (rats) to 60% (monkeys) had been removed from both sites of injection. After 3 months, 22% (Sub Q) to 35% (IM) still remained at the site of injection in monkeys; in rats the average amount that remained ranged from 9% (Sub Q) to 20% (IM).

The amount of UL-^{14}C-mannide oleate preparation remaining at the site of injection of monkeys and rats for 1 day to 3 months after injection is summarized in Table III. During the first 24 hr. after administration, as much as 35–45% of the radioactivity injected into rats and monkeys had been removed from both IM and Sub Q sites of injection. Two to seven days after injection, the amount remaining in both monkeys and rats changed very slowly, an average of 50% being removed from the site of injection the 1st week. After 1 month, intramuscularly injected animals lost approximately 60%, and subcutaneously injected animals lost 70–80% from the site of injection. Essentially, no change was noted after 2 months. By the 3rd month after injection, both the intramuscularly and subcutaneously injected animals had lost, from both sites of injection, approximately 88% of the radioactivity associated with the UL-^{14}C-mannide oleate preparation.

In general, one-half of the ^{14}C-labeled "mannide monooleate" was removed from the site of injection the 1st week. It took 3 months, however, to remove roughly one-half to three-quarters of the material remaining after 1 week.

In an effort to define the nature of the radioactive material left at the site of injection, the chloroform–methanol leg extracts (6) were applied to TLC plates. The extracts were developed in a chloroform–diethyl ether–acetic acid system (95:5:1), an example of which is shown in Fig. 1. The distribution of radioactivity on the TLC plate (Table IV) indicated that the material remaining at the site of injection of both monkeys and rats was the esterified components of mannide monooleate preparation. The free fatty acids, free sugar, and their altered forms apparently were removed from the site of injection. The nature of the material removed and of that left at the site of injection was not verified chemically.

Distribution of Radioactivity among the Major Organs—The average levels of radioactivity (counts/min./100-mg. wet tissue) of the major organs of monkeys and rats after the administration of 1-^{14}C-oleate and UL-^{14}C-mannide surfactant preparations are presented in Tables V and VI, respectively. All organs of both monkeys and rats incorporated radioactivity within the first 24 hr. However, the level (c.p.m./100-mg. wet tissue) was less with UL-^{14}C-mannide oleate than with the 1-^{14}C-oleate mannide preparation. The depot of fat of those animals receiving 1-^{14}C-oleate-labeled surfactant consistently contained the highest specific activity of any organ except the inguinal lymph nodes. In those animals receiving UL-^{14}C-mannide oleate tracer, the depot fat contained comparatively little radioactivity.

Approximately 75% of the inguinal lymph nodes analyzed had a fairly large amount (200–2000 c.p.m./20-mg. wet tissue) of radioactivity, regardless of the tracers administered. These data seem to indicate that the lymph was a major route in the transport of the surfactant and not necessarily a site of accumulation.

Nature of the Radioactivity in the Major Organs—Attempts were made to evaluate the distribution of radioactivity within the major organs of monkeys 3 months after administration of a mineral

Table V—Average Level of Radioactivity in the Major Organs of Monkeys and Rats after Administration of a Mineral Oil–"Mannide Monooleate" Emulsion Containing 1-^{14}C-Oleate Mannide as the Tracer

Time after Injection	Number[a] of Animals	Liver M	Liver R	Fat M	Fat R	Spleen M	Spleen R	Kidney M	Kidney R	Small Intestine M	Small Intestine R	Ovary M	Ovary R	Lung M	Lung R	Inguinal Lymph Node M
		Average Counts per Minute per 100-mg. Wet Tissue[b]														
1 day	6	153	102	348	405	118	59	149	103	135	59	129	73	65	48	826
2 days	6	104	52	614	316	142	43	149	57	150	36	90	96	103	30	635
7 days	6	399	64	1732	544	199	70	197	74	161	34	92	73	119	54	522
1 month	4	232	49	1977	509	73	29	144	25	55	23	69	38	111	20	469
2 months	4	131	9	1233	612	36	10	46	10	26	11	27	32	20	9	875
3 months	4	331	23	1679	1105	54	28	119	22	38	48	44	80	38	18	253

[a] Number of monkeys (M) and number of rats (R) used in this study. [b] Average counts/min./100-mg. wet tissue except for inguinal lymph nodes, which weighed approximately 20 mg.

Table VI—Average Level of Radioactivity in the Major Organs of Monkeys and Rats after Administration of a Mineral Oil–"Mannide Monooleate" Emulsion Containing UL-^{14}C-Mannide Oleate as the Tracer

Time after Injection	Number[a] of Animals	Liver M	Liver R	Fat M	Fat R	Spleen M	Spleen R	Kidney M	Kidney R	Small Intestine M	Small Intestine R	Ovary M	Ovary R	Lung M	Lung R	Inguinal Lymph Nodes, M
		Average Counts per Minute per 100-mg. Wet Tissue[b]														
1 day	6	33	7	29	8	34	8	46	8	54	9	38	9	20	5	120
2 days	6	25	11	37	12	27	7	39	10	58	12	36	11	27	7	132
7 days	6	43	13	31	13	22	10	30	14	27	10	17	11	55	10	38
1 month	4	44	15	26	18	20	13	70	16	17	10	11	9	20	7	9
2 months	4	12	9	10	8	12	5	38	9	19	6	9	7	6	4	277
3 months	4	31	10	18	13	28	7	161	20	14	10	14	10	12	8	1889

[a] Number of monkeys (M) and number of rats (R) used in the study. [b] Average counts/min./100-mg. wet tissue except for inguinal lymph nodes which weighed approximately 20 mg.

Table VII—Distribution of Radioactivity within Various Organs of Monkeys 3 Months after Administration of a Mineral Oil–"Mannide Monooleate" Emulsion Containing 1-^{14}C-Oleate Mannide as the Tracer

TLC Fraction[b]	Tissues[a]: Liver, %	Fat, %	Spleen, %	Kidney, %	Small Intestine, %	Ovary, %	Lung, %	Brain, %	Inguinal Lymph Nodes, %
a–b	0.0	0.9	60.5	0.0	0.0	35.6	0.0	20.8	10.9
c–e	0.0	0.0	0.0	70.7	0.0	0.5	0.0	14.9	0.0
f–g	2.1	6.5	0.0	29.1	62.9	6.5	44.9	15.8	0.0
h–i	4.2	17.3	16.9	0.0	0.0	35.4	0.0	0.0	7.8
j–k	49.9	11.7	10.8	0.0	37.3	9.5	33.0	19.8	4.2
l–m	31.2	0.0	11.8	0.0	0.0	4.0	22.0	28.7	40.6
Solvent front	12.5	63.6	0.0	0.0	0.0	8.3	0.0	0.0	36.4

[a] Pooled tissue extracts. [b] Probable contents of fractions: (a–b) phospholipids and chemically altered oleic acid; (c–i) chemically altered oleic acid, oleic acid, and monoglycerides; (j–solvent front) esterified surfactant and esterified naturally occurring lipids (triglycerides, sterol esters, and free sterols).

oil–"mannide monooleate" emulsion containing either UL-^{14}C-mannide or 1-^{14}C-oleate-labeled surfactant. The results (Tables VII and VIII) of the serial TLC scrapings and liquid-scintillation counting of the fractions obtained from organ extracts varied considerably. This variation was interpreted as an indication of metabolism once these tracers had reached the various tissues. For example, the depot fat contained some radioactivity associated with free fatty acids and a large amount (64%) associated with newly synthesized triglycerides when the 1-^{14}C-oleate mannide preparation was used. The spleens of the same animals, on the other hand, contained large amounts of radioactivity (60%) at the origin of the TLC plates, indicating that this was probably newly synthesized phospholipids.

The distribution of radioactivity in those monkeys receiving the UL-^{14}C-mannide oleate was completely different from those receiving the 1-^{14}C-oleate mannide preparation. Since no definite chemical studies were performed, these results can best be explained on the basis that the metabolism of the labeled oleate and the labeled mannitol tracers would be expected to be different. The nature of the radioactivity found in the lymph nodes also, unfortunately, remained undetermined. However, it was suspected to be esterified material of the surfactant.

Respiratory $^{14}CO_2$—A substantial amount of radioactivity is eliminated in the respiratory CO_2 of animals given the 1-^{14}C-oleate mannide preparation as the tracer (Table IX). In monkeys, the average daily rate of $^{14}CO_2$ eliminated was approximately 0.9%, yielding a total average of 6% of the administered radioactivity eliminated the 1st week. Similar results were obtained with rats; however, the daily rate was slightly higher, and the total amount eliminated during the 1st week averaged close to 7% of the amount given.

On the other hand, a very small amount (0.1–0.2%) of the UL-^{14}C-mannide oleate tracer preparation was eliminated in the respiratory CO_2 the 1st week after injection. No determinations were made after the 1st week.

Urine and Feces—Table X data show that only a very small amount of radioactivity was eliminated in the urine of monkeys and rats when the 1-^{14}C-oleate-labeled surfactant was administered. The total amount eliminated for 1 week after injection ranged from 0.5 to 1%.

When the UL-^{14}C-mannide surfactant was administered, a large amount of radioactivity, roughly 25% of the dose administered, was eliminated in the urine the first 24 hr. During the 2nd day, the

Table VIII—Distribution of Radioactivity within Various Organs of Monkeys 3 Months after Administration of a Mineral Oil–"Mannide Monooleate" Emulsion Containing UL-^{14}C-Mannide Oleate as the Tracer

TLC Fraction[b]	Tissues[a]: Liver, %	Fat, %	Spleen, %	Kidney, %	Small Intestine, %	Ovary, %	Lung, %	Brain, %	Inguinal[c] Lymph Nodes, %
a–b	64.4	2.4	10.3	22.6	10.3	0.0	17.0	13.4	87.5
c–e	10.7	37.6	4.4	7.5	0.0	25.0	12.6	24.4	10.5
f–g	0.0	12.0	0.0	4.9	0.0	37.5	21.5	0.0	1.8
h–i	4.5	4.8	0.0	7.9	18.2	8.7	0.0	6.3	0.0
j–k	4.1	24.0	38.2	46.0	45.6	28.9	0.0	1.6	0.0
l–m	9.5	0.0	20.6	5.3	12.5	0.0	29.6	54.3	0.0
Solvent front	6.6	20.0	26.5	5.7	13.7	0.0	19.3	0.0	0.0

[a] Pooled tissue extracts. [b] Probable contents of fractions: (a–b) phospholipids and chemically altered oleic acid; (c–i) chemically altered mannitol, mannitol, and monoglycerides; (j–solvent front) esterified surfactant and esterified naturally occurring lipids (triglycerides, sterol esters, and free sterols). [c] The lymph extract was hydrolyzed with perchloric acid prior to TLC separation. TLC separation before hydrolysis yielded results similar to Table VII.

Table IX—Elimination as Respiratory $^{14}CO_2$ of 1-^{14}C-Oleate Mannide and UL-^{14}C-Mannide Oleate from Both Rats and Monkeys for 1 Week after Injection of a Mineral Oil–"Mannide Monooleate" Emulsion

	Days after Injection							
	1, %[b]	2, %	3, %	4, %	5, %	6, %	7, %	Total, %
				1-^{14}C-Oleate Mannide				
Rats (6)[a]	1.96	0.98	0.88	1.12	0.79	0.81	0.70	7.24
Monkeys (6)	0.98	0.83	0.94	0.76	0.88	0.89	0.72	6.00
				UL-^{14}C-Mannide Oleate				
Rats (3)	0.16	0.03	0.01	0.02	0.02	<0.01	<0.01	0.23
Monkeys (3)	0.03	0.03	0.02	0.01	0.02	0.02	0.02	0.15

[a] Number in parentheses is the number of animals analyzed. [b] Average percentage based on the total amount of radioactivity given.

Table X—Elimination of Radioactivity in the Urine of Rats and Monkeys for 1 Week after Administration of a Mineral Oil–Mannide Oleate Emulsion Containing 1-^{14}C-Oleate Mannide or UL-^{14}C-Mannide Oleate as the Tracer

	Days after Injection							
	1, %[a]	2, %	3, %	4, %	5, %	6, %	7, %	Total, %
				1-^{14}C-Oleate Mannide				
Rats (3)[b]	0.27	0.12	0.15	0.15	0.09	0.13	0.09	1.00
Monkeys (3)	0.09	0.12	0.12	0.06	0.07	0.04	0.04	0.54
				UL-^{14}C-Mannide Oleate				
Rats (12)	25.63	2.91	1.98	1.39	1.19	1.15	1.37	35.62
Monkeys (18)	22.87	4.95	3.72	1.90	1.99	1.20	1.73	38.36

[a] Average percentage based on the total amount of radioactivity given. [b] Number in parentheses is the number of animals analyzed.

Table XI—Distribution of Radioactivity in 24-Hr. Urine Samples in Monkeys and Rats 1 Day after Administration of UL-^{14}C-Mannide Oleate as the Tracer

TLC Fraction[a]	Monkeys, %	Rats, %
a–b	46.1	66.2
c–g	53.8	33.4
h–i	0.1	0.2
j–k	0.0	0.2
l–m	0.0	0.0

[a] Probable contents of fractions: (a–g) nonesterified, unaltered, and chemically altered mannitol; (h–m) esterified surfactant.

amount decreased to about 3 and 5% in rats and monkeys, respectively. Three to seven days after injection, the level eliminated in the urine stabilized to about 1% daily. The total amount eliminated the 1st week was about 35–40%. In general, there was no significant difference between monkeys and rats in the total amount eliminated in the urine. The results on the total amount eliminated in the urine of rats agree with those of Porter and Titus (7). However, these investigators studied only the mannide-labeled surfactant. As shown, the 1-^{14}C oleate-labeled material is not eliminated in the urine.

Table XI presents TLC separation and a liquid-scintillation counting analysis of the radioactive components in the urine of those animals receiving the UL-^{14}C-mannide oleate preparation. These data indicate that the material eliminated so rapidly in the urine was the nonesterified sugar components of the surfactant.

No appreciable amount of radioactivity was detected in the feces when either tracer was given.

Pathological Observations—Gross necropsy of all animals indicated that no sterile abscesses, infections, nor other observable pathological conditions were produced at the site of injection. No extensive histological examinations were performed; therefore, it is not possible to state definitely whether any subtle histopathological conditions existed.

SUMMARY

This study has shown that, when using a "mannide monooleate"–mineral oil emulsion, the surfactant was removed from the site of injection faster (40–60% the 1st week) than the mineral oil (1–5% the 1st week) (6). The obvious danger in this phenomenon is that the loss of the emulsifier may allow the mineral oil to coalesce into larger droplet sizes and thereby retard mineral oil mobilization from the site of injection. Those components of the "mannide monooleate" preparation that were retained at the site of injection for longer periods of time (3 months or more) appeared to be the esterified components as opposed to the nonesterified components (free oleic acid, mannitol, and their chemically altered forms), which appeared to be removed within 24 hr. after injection. These data suggest that, had this surfactant been pure mannide monooleate or even its other esterified forms, its longer retention at the site of injection may have also assisted in a more uniform mobilization of mineral oil.

This investigation has yielded additional information on the mobilization and metabolism of the mannide monooleate surfactant preparation when it is received as an injected emulsion. No information was obtained directly which would implicate the surfactant used in this study as a particularly noxious material. Although not tested in this study, this would seem to indicate that the reported toxic effects associated with this material are in some way related to the various antigen preparations.

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ACKNOWLEDGMENTS AND ADDRESSES

Received September 15, 1969, from the *Southwest Research Institute, San Antonio, TX 78228*

Accepted for publication March 13, 1970.

This investigation was supported by the Division of Biologics Standards, National Institutes of Health, Contract No. PH 43-67-1104.

The technical assistance throughout the program of Dennis Schmidt was appreciated.