# Behavior of $C^{14}$-labeled Particulate Plasma Triglyceride and "RE Test Emulsion" in RE Hyperfunctional Rats.* (29445)

N. R. Di Luzio and E. L. Bierman
*Division of Physiology, University of Tennessee Medical Units, Memphis, and Department of Medicine, University of Washington, Seattle*

Artificial, finely dispersed fat emulsions are poor tracers for chylomicrons since they contain foreign surfactants(1), larger particles(2) and are removed primarily by reticuloendothelial (RE) rather than parenchymal cells(3-5).

Recently, an *in vitro* method has been developed for dispersing radioactive triglyceride in plasma to form a complex of lipid and plasma protein which has the physical characteristics of particulate fat derived from lymph(6). With this method, high specific activity trace doses of $C^{14}$-labeled particulate triglyceride can be administered intravenously to any species as a tag for native fat particles in plasma. Since the recipient's plasma is used for complexing and foreign surfactants are avoided, it has been assumed that these labeled fat particles are handled normally.

The demonstration that fat emulsions and isologous lymph chylomicrons given intravenously to rats with a hyperfunctional RES are handled differently(4,7,8) provides a means to test whether the transport of this $C^{14}$-labeled triglyceride-plasma complex is influenced by RE function. Similar to previous observations with lymph chylomicrons and in contrast to results obtained with synthetic emulsions(4,7,8), the initial plasma clearance and organ distribution of the $C^{14}$-triglyceride plasma complex is not altered by RE hyperfunction.

*Methods.* RE hyperfunction and hyperplasia were induced in fed male rats (Holtzman) by intravenous administration of a saline suspension of glucan† in the amount of 1 mg/100 g/day for 5 days(9). Control rats received saline. All experiments were conducted on the 6th day.

The radioactive triglyceride-plasma complex was prepared by the method previously described(6). Tripalmitin-1-$C^{14}$, specific activity 82.0 mc/mM (New England Nuclear), was dissolved in petroleum ether (B.P., 30°-60°C) and extracted once with 3 volumes of methanol: 0.15 N NaOH (2:1 V:V) to remove traces of fatty acids. The petroleum ether phase was removed, evaporated to dryness, and the labeled tripalmitin residue redissolved in 0.5 ml warmed acetone. The solution was taken up in a microsyringe and slowly added to 20 ml fresh plasma, pooled from fed control rats, during continuous mechanical stirring in a Vortex mixer at room temperature. For completeness of transfer, the residue was washed further with two 0.25 ml portions of acetone and similarly added to the plasma.

The triglyceride-plasma mixture was layered under a 1:10 dilution of unlabeled control rat plasma in isotonic saline, centrifuged at $6 \times 10^6$ *g* min in the Spinco SW 25.1 swinging bucket rotor, and the top fraction harvested by careful aspiration of the packed particles. With the use of a nomogram based on Stokes' law(10), the fat particles were estimated to have a minimum diameter of 75 mu (Sf 400).

Trace doses (0.5 mg triglyceride; $3 \times 10^6$ CPM) of radioactive particulate fat were given to 7 saline-injected (control) and 7 glucan-injected (RE hyperfunctional) rats by rapid intravenous injection. Blood samples (0.01 ml) were obtained from the tail vein at 1, 3, 5, 7, and 9 min after injection.

The "RE test emulsion" consisting of glycerol, corn oil labeled with tripalmitin-$C^{14}$, and soya lecithin was prepared as previously described(4,5,7,11). This emulsion was administered intravenously to saline- and glucan-treated rats and repeated blood samples obtained.

The blood samples were added to methanol and appropriate volume of chloroform added

* Aided by grants from Nat. Inst. Health.

† Glucan was generously furnished by Standard Brands Inc., Stamford, Conn.

TABLE I. Vascular Removal of Labeled Triglyceride-Plasma Complex in Normal and RE Hyperfunctional Rats.

| Time after inj, min | % ID*/ml plasma Control N | Mean ± S.D.† | Glucan N | Mean ± S.D. | p‡ |
|---|---|---|---|---|---|
| 1 | 7 | 2.1 ± .42 | 7 | 1.7 ± .30 | N.S. |
| 3 | 7 | .66 ± .17 | 7 | .71 ± .18 | N.S. |
| 5 | 7 | .38 ± .16 | 7 | .53 ± .15 | N.S. |
| 7 | 7 | .26 ± .14 | 7 | .45 ± .16 | N.S. |
| 9 | 7 | .18 ± .11 | 7 | .35 ± .14 | N.S. |

* % ID = % of injected radioactivity.
† S.D. = Standard deviation.
‡ Probability that differences between values for control and glucan-treated rats are significant (N.S. = p >.05).

to give a final volume ratio of 1:2. The lipids were extracted and purified(12) and radioactivity determined with a Nuclear-Chicago automatic liquid scintillation system by previously described procedures(5).

All rats were killed with ether 10 minutes following injection and organ weights determined, and radioactivity assayed.

*Results.* Trace doses of the $C^{14}$-labeled triglyceride-plasma complex were very rapidly removed from circulation in both control and glucan-stimulated rats. At no time during the initial clearance did the rats with RE hyperfunction demonstrate significantly greater removal of radioactivity from plasma (Table I).

The previously observed effect of glucan in inducing RE hyperplasia is reflected by significant increases in weights of organs such as liver, spleen and lung (Table II). However, uptake of radioactivity in these organs was similar in both groups (Table III).

In contrast to the unaltered removal rate of $C^{14}$-labeled particulate lipid, the vascular clearance of a comparable amount of the "RE test emulsion" was significantly increased in RE hyperfunctional rats. The mean and standard error of the half-time was 2.13 min ± 0.45 in control group, contrasted to 1.14 ± 0.19 min in the RE hyperfunctional group ($P<.05$). The organ distribution of the "RE test emulsion" at 10 min was not significantly different between groups (Table IV). Independent of RE activity, liver manifested a greater uptake of the "RE test emulsion" than the triglyceride plasma complex, while lung uptake was significantly reduced.

TABLE II. Organ Weights in Normal and RE Hyperfunctional Rats.

| | Organ wt, % body wt Control N | Mean ± S.D. | Glucan N | Mean ± S.D. | p |
|---|---|---|---|---|---|
| Liver | 7 | 4.12 ±.16 | 7 | 4.69 ±.32 | <.02 |
| Spleen | 7 | .25 ±.01 | 7 | .68 ±.21 | <.01 |
| Lung | 7 | .52 ±.07 | 7 | 1.31 ±.40 | <.01 |

TABLE III. Organ Uptake of Radioactive Triglyceride-Plasma Complex in Control and RE Hyperfunctional Rats.*

| | % ID/organ Control N | Mean ± S.D. | Glucan N | Mean ± S.D. | p |
|---|---|---|---|---|---|
| Liver | 7 | 52.97 ±7.54 | 7 | 56.41 ±11.18 | N.S. |
| Spleen | 7 | 1.51 ± .39 | 7 | 1.45 ± 1.07 | N.S. |
| Lung | 7 | 6.26 ±1.27 | 6 | 8.35 ± 2.69 | N.S. |

* Values were obtained 10 min following intravenous administration of the particulate lipid tracer.

*Discussion.* Glucan previously has been shown to produce a marked hyperplasia and hyperfunction of the RES(4,5,8,9). Liver, spleen and lung weights uniformly increase, in the absence of any alteration in total body weight, and the clearance of colloidal carbon from the circulation increases more than 10-fold(5,7,9). Animals pretreated with glucan removed most artificial fat emulsions from plasma more rapidly than do control animals and a greater proportion of the injected dose is found in the liver(5), almost exclusively in phagocytic cells(4). On the other hand, the removal of isologous lymph chylomicrons is unaffected by RE hyperfunction, and he-

TABLE IV. Organ Uptake of "RE Test Emulsion" in Control and RE Hyperfunctional Rats.*

| | % injected dose/organ N | Saline | Glucan | p |
|---|---|---|---|---|
| Liver | 5 | 68.3 ±3.68 | 67.8 ±8.10 | N.S. |
| Spleen | 5 | 2.9 ± .87 | 2.1 ± .69 | N.S. |
| Lung | 5 | 1.3 ± .99 | 1.6 ± .51 | N.S. |

* Values expressed as means ± standard deviation.

patic uptake of chylomicrons has been localized to parenchymal cells(4,8).

The failure to observe any significant difference in distribution of the "RE test emulsion" in normal and RE hyperfunctional rats is due to the trace dose of triglyceride employed. With higher doses of triglyceride, the intravascular removal rate is decreased 10-fold in the control group. The rapid removal rate presently observed in the RE hyperfunctional group is not altered by the dose employed and the rapid vascular clearance is associated with enhanced Kupffer cell uptake(4,5).

The very rapid exponential removal of trace doses of the $C^{14}$-labeled triglyceride-plasma complex in both groups of rats was observed previously in man(6). This rate is much faster than the removal of lymph particles in the rat(5,7,13,14). Presumably, this is due to the marked difference in lipid dose since it has been shown in numerous studies that the rate of removal of lymph particles from plasma is inversely proportional to the load(14).

Initial removal of the particulate lipid from plasma and its organ distribution was not altered in RE hyperfunctional rats. Hypertrophy of liver, lung and spleen of the RE hyperfunctional rats was of the same order of magnitude observed previously in glucan-treated animals(4,5,8,9). In addition, RE hyperfunction was confirmed by the more rapid clearance of a "RE test emulsion"(4,5,7) in the glucan-treated group when compared to controls.

Previous observations have demonstrated that the rate of removal of the triglyceride-plasma complex is significantly inhibited by simultaneous administration of unlabeled lymph, indicating a similar behavior(6). Similarly, the clearance of the "RE test emulsion" is impaired by administration of colloidal carbon(8), while chylomicron removal is not significantly altered(5). These findings suggest that chylomicrons and the triglyceride plasma complex are not removed by a phagocytic mechanism. This study indicates that the particulate triglyceride tracer prepared from plasma *in vitro* is handled in the same manner as native lipid particles and unlike that of the phagocytizable "RE test emulsion." The further use of radioactive particulate triglyceride in the evaluation of lipid disorders appears warranted.

*Summary.* The behavior of a radioactive triglyceride-plasma complex prepared *in vitro* was compared to that of an "RE test emulsion" in normal and RE hyperfunctional rats. The removal rate and organ distribution of the particulate fat tracer was not influenced by RE hyperfunction. However, the corresponding dose of the "RE test emulsion" was removed more rapidly from plasma in RE hyperfunctional rats. Thus the particulate fat tracer does not appear to be removed by a phagocytic process and therefore behaves more like native lipid particles than foreign emulsified material.

The capable technical assistance of Mrs. Betty Lyons and Miss Jackie Dempsey is gratefully acknowledged.

1. Waddell, W. R., Geyer, R. P., Saslaw, I. M., Stare, F. J., *Am. J. Physiol.*, 1953, v174, 39.

2. Pinter, G. G., Zilversmit, D. B., *Biochim. Biophys. Acta,* 1962, v59, 116.

3. Murray, R. G., Freeman, S., *J. Lab. Clin. Med.*, 1951, v38, 56.

4. Ashworth, C. T., Di Luzio, N. R., Riggi, S. J., *Exp. Molecular Path.*, 1963, Suppl. 1, 83.

5. Di Luzio, N. R., Riggi, S. J., *J. Reticuloendothelial Soc.*, 1964, v1, 248.

6. Bierman, E. L., Hamlin, J. T., III, PROC. SOC. EXP. BIOL. AND MED., 1962, v109, 747.

7. Salky, N. K., Di Luzio, N. R., P'Pool, D. B., Sutherland, A. J., *J. Am. Med. Assn.*, 1964, v187, 744.

8. Di Luzio, N. R., Riggi, S. J., *J. Reticuloendothelial Soc.*, 1964, v1, 136.

9. Riggi, S. J., Di Luzio, N. R., *Am. J. Physiol.*, 1961, v200, 297.

10. Dole, V. P., Hamlin, J. T., III, *Physiol. Rev.*, 1962, v42, 674.

11. Blickens, D. A., Di Luzio, N. R., *J. Reticuloendothelial Soc.*, 1964, v1, 68.

12. Sperry, S. M., *Methods of Biochemical Analysis,* 1955, v2, 83. Interscience Publishers, New York.

13. French, J. E., Morris, B., *J. Physiol.*, 1957, v138, 326.

14. Belfrage, P., Borgstrom, B., Olivecrona, T., *Acta Physiol. Scand.*, 1963, v58, 111.

Received May 4, 1964. P.S.E.B.M., 1964, v116.