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(73) Titulaire(s) :
THE ROGOSIN INSTITUTE
505 East 70th Street
NEW YORK
New York 10021 (U.S.A.)

(72) Inventeur(s) : 1- LEVINE, Daniel M
430 East 63 Street
New York, New York 10021 (USA)
2- PARKER, Thomas S. (U.S.A.)
3- RUBIN, Albert L. (U.S.A.)
4- GORDON, Bruce R. (U.S.A.)
5- SAAL, Stuart D. (U.S.A.)

(74) Mandataire : CABINET J. EKEME
B.P. 6370
YAOUNDE - Cameroun

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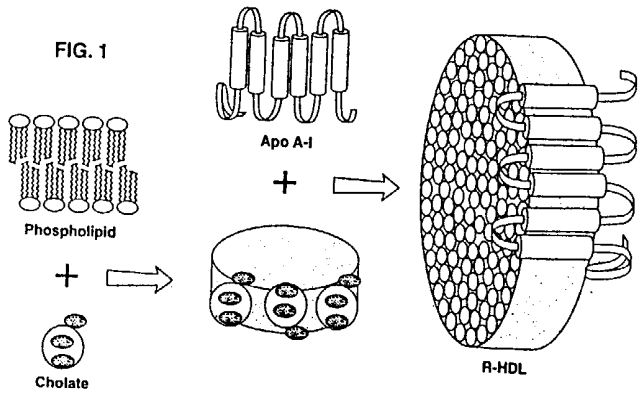
Treatment and prophylaxis of endotoxin cause toxicity is disclosed. This is accomplished by administering phospholipid containing compositions to the subject. The compositions are protein and peptide free, and may contain triglycerides, other polar neutral lipids, bile acids, or bile acid salts.

FIG. 1



METHODS AND COMPOSITIONS USEFUL IN PROPHYLAXIS AND THERAPY OF ENDOTOXIN RELATED CONDITIONS

FIELD OF THE INVENTION

This invention related to the treatment of endotoxin related endotoxemia. More particularly, it relates to the treatment of such poisoning via administration of various compositions which act to neutralise and/or remove endotoxins from the organism, as well as prophylaxis utilising these compositions.

BACKGROUND AND PRIOR ART

Endotoxic shock is a condition, often fatal, provoked by the release of lipopolysaccharide (LPS) from the outer membrane of most gram negative bacterial (e.g., Escherichia coli; Salmonella tymphimurium). The structure of the bacterial LPS has been fairly well elucidated, and a unique molecule, referred to as lipid A, which is linked to acyl chains via lipid A molecule's glucosamine backbone. See Raetz, Ann. Rev. Biochem. 59: 129-170 (1990) in this regard.

The lipid A molecule serves as membrane anchor of a lipopolysaccharide structure ("LPS") and it is the LPS which is implicated in the development of endotoxic shock. It should be pointed out that LPS molecules are characterised by a lipid A type structure and a polysaccharide portion. This latter moiety may vary in molecular details in different LPS molecules, but it will retain the general structural motifs characteristic of endotoxins. It would be incorrect to say that the LPS molecule is the same from bacteria to bacteria (see Raetz, supra). It is common in the art to refer to the various LPS molecules as "endotoxins", and this term will be used hereafter to refer to LPS molecules collectively.

In U.S. Patent No. 5,128,318 the disclosure of which is incorporated by reference, it was taught that reconstituted particles containing both an HDL associated apolipoprotein and a lipid capable of binding an endotoxin to inactivate it could be used as effective materials for alleviating endotoxin caused toxicity.

In the parent and grandparent applications cited in the Related Application section and incorporated by reference herein, it was disclosed that various other materials may be used to treat endotoxin caused toxicity. Specifically, it was found that apolipoproteins are not required in reconstituted particles, and that the reconstituted particle may contain a peptide and a lipid wherein the peptide is not an apolipoprotein.

It was also found that at least some individuals possess native levels of apoliprotein which are higher than normal levels such that effective endotoxemia therapy may be effectuated by administering reconstituted particles containing no apolipoprotein or peptide, but containing the lipid of the disclosure.

In addition, the invention in these applications involved the use of the reconstituted particles and the components discussed herein for prophylaxis against endotoxin caused toxicity, by administering prophylactically effective amounts to subjects in need of prophylaxis. Such subjects include patients suffering from infections or recovering from surgery. These patients sometimes have very low plasma HDL levels, sometimes as little as 20% of normal levels. It is highly desirable, in these cases, for early prophylaxis with HDL, so as to compensate for these drops.

It has now been found, quite surprisingly, that phospholipids may be used alone, or in combination with additional materials, such as neutral lipids, cholates, etc., as effective agents to alleviate and/or prevent endotoxemia. it is especially preferred to use phosphatidylcholines ("PC" hereafter), either alone, or in combination with other phospholipids, such as sphingolipids, in compositions which are essentially free of peptides and proteins, such as apolipoproteins or peptides derived therefrom. Neutral lipids such as mono-, di-, and triglycerides may be combined with the phospholipids, as long as the total amount of neutral lipids is below certain weight percents when the compositions are used in the form of an intravenous bolus. When used in other forms of administration, such as intravenously for example, by continuous infusion, the weight percents are not so critical, but are desirable.

Particularly preferred embodiments of the invention include emulsions where a bile acid, or a bile acid salt, is used together with a phospholipid and a neutral lipid.

The efficacy of bile acids and bile acid salts, which are cholates, in the treatment of endotoxemia is shown herein. These bile acids may be used alone, or in combination with one or more phospholipids, and/or neutral lipids, such as a phosphatidylcholine, and/or a triglyceride.

The invention is described in greater details in the disclosure which follows.

BRIEF DESCRIPTION OF THE FIGURES

Figures 5A and 5B show results obtained when various compositions were tested in a model which determined the neutralisation of endotoxin via determining TNF release in a human whole blood model. Figure 5A shows the role of protein, and 5B that of phospholipid. The compositions tested included natural lipoproteins (VLDL, LDL, HDL), reconstituted HDL ("R-HDL"), and INTRALIPID® compositions, as well as emulsions containing phospholipid and protein.

Figures 6A and 6B compare the role of triglyceride (a neutral lipid), and phosphatidylcholine, a phospholipid, in the same model.

Figure 7 presents information on toxicity associated with administration of various PC and PC/TG compositions in a mouse model, using a 55% lethality model where E. coli LPS is administered.

Figure 8 shows data comparable to that secured for the human whole blood assay, supra, but using phospholipid with unesterified cholesterol, sphingomyelin, or mixtures of both, in pace of triglycerides.

Figures 9A and 9B show results comparable to those shown in figures 5A and 5B, except that in these new figures, phospholipid, unesterified cholesterol and/or sphingomyelin are mixed with triglycerides or esterified cholesterol as the neutral lipid.

Figure 10 compares results obtained from cholesterol ester and triglyceride containing emulsions, in the in vivo mouse model.

Figure 11 graphs the theoretical amounts of triglycerides released into the blood following administration of various TG containing compositions, with toxicity thresholds. "TPN" stands for "total parenteral nutrition" while "RI" stands for compositions in accordance with the invention.

DETAILED DESCRIPTION OF THE EMBODIMENTS

Example 2

Factors which affect the LPS-mediated stimulation of TNF-∝ while preserving the integrity of interaction between plasma proteins, and cellular elements of blood, can be appropriately studies in an in vitro, human whole blood system. Such a system was used to determine which of the components of lipoproteins is important in neutralising LPS.

Materials tested were reconstituted high density lipoprotein (R-HDL), natural plasma lipoproteins (VLDL, LDS, HDL), lipoprotein deficient serum (LPDS), and the triglyceride rich emulsion 20% INTRALIPID® (a mixture of triglycerides and phospholipids).

Blood was collected in a heparinized tube, diluted with Hank's Balanced Salt Solution ("HBSS" hereafter), or the material to be tested, dissolved in HBSS. The resulting material was transferred to Starstedt tubes (250 ul/tube). LPS was dissolved in pyrogen free saline containing 10 mM HEPES, and added (2.5 ul) to a final concentration of 10 ng/ml. After incubation for four hours at 37°C, tubes were chilled to 4°C, followed by centrifugation at 10,000xg for 5 minutes. Supernatant was collected, and assayed for determination of TNF-∝, using a commercially available ELISA.

Table 1, which follows, compares the compositions of the materials tested. Figures 5A and 5B present the results. Data are plotted as amount of TNF-∝ produced, plotted against concentration of added protein (figure 5A), and phospholipid (figure 5B). Logarithmic scales were used, in order to display the wide range of concentrations used, with 10^0 equal to 1 mg/ml. All whole blood

incubations contained 10 ng/ml of E. coli 0111:B4 LPS, supplemented with one of the compositions, as the key for figures 5A and 5B show.

The fact that the materials differ in effectiveness when protein content is plotted (figure 5A), while being very similar when phospholipid content is plotted (figure 5B) suggest that the phospholipid is the important component. This is confirmed by the finding that a protein free lipid emulsion is more effective than is natural HDL, but less effective than R-HDL. Protein does not appear important to the neutralisation.

Composition of natural lipoproteins and reconstituted HDL

Lipoprotein		TC	TG	PC	Protein
Class	Density (g//ml)	Weight %			
VLDL	<1.006	22	53	18	7
LDL	1.007-1.063	48	11	22	20.9
HDL	1.063-1.21	18	8	22	52
R-HDL	1.063-1.21	-	-	79	21
LPDS	>1.21	0	0	2	98
Intralipid	-	1	93	6	0

Example 3

As the next step, protein free lipid emulsions, containing different amounts of neutral lipid, were tested in human whole blood. The same in vitro human whole blood assay as set forth in example 2 were used.

All particles described herein were made via the same protocol, which involved mixing a phospholipid, sphingomyelin or phosphatidylcholine, triolein, and/or unesterified cholesterol ester, dissolved in chloroform, and weighing it into a flask. Vitamin E (0.02% w/v) was added as antioxidant. A dry lipid film was then prepared by blowing nitrogen or argon gas over the sample. A volume of non pyrogenic saline was then added to the flask, followed by mixing on a vortex mixer until all lipid was suspended. The solution was then homogenised in a high pressure homogenise. Samples containing phosphatidylcholine (PC), with or without triolein, were cycled through the homogenise 10 times at 20,000 psi. Samples containing cholesterol ester with one or more other lipids were cycled through 15-20 times at 30,000 psi. Homogenised solutions were filtered through 0.45 μm syringe filters, and the filtrate was stored at room temperature until used (within three days). Figures 6A and 6B present these results. In these studies, LPS-dependent, TNF-α production is plotted against concentration of added triglyceride (figure 6A), or phospholipid (figure 6B). The compositions, as indicated by the key, contained (by weight) 7% triglyceride ("TG"), 45% TG, 89% TG, 94% TG, R-HDL, or phospholipid without TG, (shown in figure 6B only). An 89% TG composition is a 10% INTRALIPID® formulation,

while 94% TG refers to 20% INTRALIPID. In all other tests, egg phosphatidylcholine (PC), and triolein were used.

These results show that the protein free compositions when compared via triglyceride content, are very different. They are very similar when tested via phospholipid (PC) content. This confirms the role of phospholipid, especially since phospholipid alone is effective, but less so than emulsions containing up to 45% TG.

Example 4

The work then proceeded to in vivo experiments in a mouse model, which is accepted as a reliable system for predicting human efficacy.

In these experiments, mice were injected, in bolus form, with sufficient amounts of the formulations described in example 3 as well as others (pure phosphatidylcholine, 7% TG, 25% TG, 45% TG, 71% TG, 81% TG, 89% TG, 94% TG), or a saline control, to provide does of phospholipid (either 200 mg/kg or 400 mg/kg), together with 25 mg/kg of E. coli 0111:B4 LPS. The control group received intravenous physiological saline in a volume sufficient to match the volume of emulsion. Survival after 72 hours is presented in figure 7. Of 344 animals in control groups, 155 survived.

PC alone had a modest protective effect, not statistically significant at the 95% confidence level, while 7%, 45% and 71% TG compositions significantly improved survival. The 80% and 89% TG compositions were marginally effective, while the 94% TG decreased survival.

When the dose was increased to provide 400 mg/kg of PC both the 89% and 94% TG emulsions significantly decreased survival time, probably due to TG poisoning, as explained infra.

Example 5

The work described in examples 2-4 established that phospholipids are in active agent useful in inhibiting endotoxemia. The fact that non-polar lipids other than triglycerides may form emulsions with phospholipids other than PC suggested that others may be tried. Exemplary are spingomyelin (another phospholipid),

and unesterified cholesterol (a polar neutral lipid), and mixtures of these. So, too, esterified cholesterol (an nonpolar ester), squalene (a hydrocarbon), and vitamin E (a nonpolar antioxidant) may be used. A series of experiments were designed to test these, using the human whole blood assay of example 2, supra, and the mouse survival assay of example 4.

Emulsions were prepared, in the manner described supra, using pure phosphatidylcholine, phosphatidylcholine with 10% (wt/wt) unesterified cholesterol, 10% (wt/wt) sphingomyelin, or 10% total of a mix of both. Emulsions were added to whole blood, at a concentration of 100 mg/dl, with reference to PC, and 10 ng/ml of LPS. The mixture was incubated, and TNF-α release measured.

The results are shown in Figure 8. TNF-α production was substantially reduced with PC alone. Emulsions containing unesterified cholesterol, sphingoyelin, or the mix of both, were also suppressive of TNF-α release.

Example 6

The whole blood assay was also used to determine the effect of unesterified cholesterol and/or sphingomyelin to neutral lipid containing emulsions. Again, the emulsions were added at 100 mg/dl PC. The various compositions (wt/wt) are set forth in the following table.

Emulsion	Composition
PC with 45% TG	55: 45
PC + TG + C	54.4: 45.3: 0.3
PC + TG + SP	51.6: 43.0: 5.4
PC + TG + C + SP	51.4: 42.9: 0.3: 5.4
PC + CE	54.5: 45.5
PC + CE + C	54.4: 45.3: 0.3
PC + CE + SP	51.6: 43.0: 5.4
PC + CE + C + SP	51.5: 42.9: 0.3: 5.4

Figures 9A and 9B display the results. PC emulsions made with either neutral lipid, with our without additional polar lipids, demonstrated inhibition. Again, the LPS concentration used 10 ng/ml, which is a clinically relevant concentration of

endotoxin. The cholesterol ester containing emulsions are less effective than are TG containing emulsions, while those emulsions containing unesterified cholesterol did not suppress TNF-∝ production.

Example 7

Cholesterol ester containing emulsions were tested in an in vivo model (i.e., that used in example 4), with a lethal dose of endotoxin. Emulsions were prepared with PC and TG, or PC and cholesterol ester (CE), and were administered to provide a single bolus dose of 200 mg/kg of PC, together with 25 mg/kg of E. coli O111: B4 LPS (a lethal dose), through the tail vein. Control groups received intravenous physiological saline in a volume to match the volume of emulsion.

In figure 10, the data compare the results from the CE and TG containing emulsions. Each emulsion was tested in a minimum of two experiments, using a total of 16 or more animals.

As shown, emulsions containing 7% or 45% CE (wt%) significantly improved survival. These results, taken wit those of example 6, show that CE can be substituted for TG to create emulsions that neutralise endotoxin.

Example 8

Protein-free emulsions of phospholipid with triglyceride effectively block TNF-∝ production in whole blood stimulated with LPS. In theory, these emulsions might also be effective in vivo if they can be administered safely in doses that provide protective concentrations of phospholipid in plasma. Our previous experiments with R-HDL suggest that the minimum dose of phospholipid is approximately 200 mg/kg. Using this dose and a plasma volume of 4.5% of body weight, one can calculate the concentration of triglyceride expected in plasma following administration of a series of emulsions, with increasing triglyceride content. The result is shown in figure 11 as a smooth line curving upward with increasing weight percent TG. Plasma TG concentrations rarely rise above 1000 mg/dl in healthy adults even after a fatty meal. Pancreatitis is reported in patients with plasma TG above 2000 mg/dl (Farmer, et al., Amer. J. Med. 54: 161-164 (1973); Krauss, et al., Amer. J. Med. 62: 144-149 (1977); Glueck, et al., J. Lab. Clin. Med. 123: 59-61). Plasma TG above 4000 mg/dl is

extremely rare and cause for serious concern. The last two thresholds are shown by horizontal lines in the figure above. Administration of either 10% or 20% INTRALIPID® in a dose to provide 200 mg/kg phospholipid is expected to raise plasma TG concentrations (see the two open circles) well above the safe limits. By contrast, administration of emulsions containing, 7%, 45%, 71% or 78% (solid squares left to right) raises plasma TG to 136, 477, 1300 or 2000 mg/dl respectively. Emulsions with TG content up to ~50% are expected to be free of toxicity from TG.

Example 9

The efficacy of combinations of phospholipid and bile acid, i.e., sodium cholate, was tested in the same type of experiment set forth in the previous examples.

The procedure by which the formulations administered to the test animals was prepared, however, differed.

In this example, and in the examples which follow, formulations were prepared using a Microfluidiser high pressure homogeniser. This apparatus facilitates scale up.

Either of liquid triolein or liquid so triglyceride was weighed into an appropriate amount of water, or water plus 9 mM, 18 mM or 36 mM sodium cholate. Solid granular phophatidylcholine was weighed onto weighing paper, and then added, slowly, t the solution, while stirring. It requires anywhere from 3-5 minutes to disperse the lipid. following dispersion, the materials were poured into the microfluidiser. The device uses hydraulic pressure to actuate a pump which, in turn, directs two opposing jets of sample at each other. The pressure can be as high as 25,000 pounds per square inch. Upon collision, the jets are forced through a plus sign shaped orifice, thereby homogenising the sample.

Sample was recirculated through the microfluidiser, with "one pass" being defined as the amount of time it takes to pump all sample through the machine. The sample was circulated for 20 passes, to produce a homogenised sample. Dextrose was added to a final concentration of 5%.

Endotoxin purified from E. coli 0111:B4 (40 mg/kg), and emulsion, as discussed infra (200 mg phosphatidylcholine/kg), via intravenous injection through the tail vein. Those mice which received cholate alone were given a volume of sodium cholate equal to the cholate/EML (emulsion) preparation, at the same cholate concentration. The control mice received the same volume of 5% dextrose, so as to match plasma osmolality.

The results are set forth in the Table which follows immediately. The emulsion is the phosphatidylcholine/7% triglyceride emulsion described in the preceding examples. Sodium cholate, when used, was added at the indicated concentration, to the raw materials, prior to the emulsification of the materials.

Table 1. Protection of Mice from Lethal Endotoxin Challenge

			7% TG + CA			18mM CA	
Time	Control	7% TG	9mM	18mM	36mM	no PC or TG	+PC
hrs	Survivors (N)						
0	28	28	8	16	8	8	8
24	9	12	4	15	8	8	8
48	5	10	2	15	8	8	8
72	2	5	1	15	8	8	8
96	1	0	1	15	8	7	8

For convenience, weight percentage of the emulsions are as follows. When 9mM cholate was used, the percentages by weight relative to the emulsion are 7% cholate, 6.1% triglyceride, and 86.9% phosphoatidylcholine. At 18 mM cholate, the weight percentages are 13.1% cholate, 5.7% triglyceride, and 81.2% phosphatidylcholine. At 36 mM cholate, the relative values are 23.2% cholate, 5% triglyceride, and 71.8% phosphatidycholine.

It should be noted that the amount of LPS administered in these experiments (40 mg/kg), is much higher than the amount used for lethality studies in the prior experiments. The intent of these higher doses is to overwhelm any protective effect attributable to phosphatidylcholine and/or triglyceride. Thus, the

conclusion to be reached following these experiments is that there is a protective effect attributable to the bile acid salt, sodium cholate.

Not presented herein are studies carried out using other bile acid salts and taurine containing bile salts. Additional examples of bile acids include allodeoxycholic acid, lithocholic acid, hyodeoxycholic acid, hyocholic acid, α, β, ω-muricholic acids, murodeoxycholic acid, ursodeoxycholic acid, ursocholic acid, and all of the salts of these, such as their sodium salts or taurine or glycine conjugates. See Hoffman, supra.

Example 10

Further studies were then carried out, the first of which was a survival study, using mice as the subject animals.

In the survival study, the subject animas were divided into four groups. The first group received a 5% dextrose solution, and acted as a control. The second group received an emulsion of 93% (by weight) phophatidylcholine and 7% (by weight) triglyceride, prepared as described , supra. The emulsion contained 5% dextrose, and soy phospholipids at approximately 50 mg/ml of lipid.

In the third and fourth group, the animals received an emulsion similar to that given to the second group, supplemented with either 18 mM sodium cholate, or 18 mM sodium deoxycholate. In this experiment, the protocol used was identical to that set forth in example 9.

Survival was measured 72 hours after challenge, and is summarised in the following table:

Table 1. Effect of Adding Bile acid to 7% Triglyceride Emulsion on 72 hr Survival in Mice

Group		N	Survival	p value Vs group		
			%	1	2	3
1	5% Dextrose	28	4	-	-	-
2	7% TG	64	8	NS	-	-
3	Sodium Cholate + 7%TG	16	94	0.00001	0.00001	-
4	Sodium Deoxycholate + 7% TG	8	75	0.0001	0.00001	NS

Note that the statistical significance of between group survival comparisons was tested by the generalised Wilxox method, using a computer program. Comparisons against group I controls are listed under "1", comparisons against group 2 animals, treated with 7% emulsion are listed under "2", and comparison against group 3 animals, treated with emulsion plus sodium cholate, are listed under "3".

Both the percentage of survival, and the statistical analysis show that the clear, unexpected superiority of the bile acid salt containing formulations.

Example 11

A second set of experiments used a rabbit model. In this model, release of TNF (tumor necrosis factor)-α was determined.

The rabbits were divided into three goups, and received 5% dextrose solution, the emulsion of phospholipid and triglyceride (93%/7%), discussed supra, or a 93%/7% emulsion which also contained 18 mM cholic acid. All emulsions were adjusted to 5% dextrose, as in example 10. The rabbits received a priming bolus of emulsion and, two hours later, were challenged with 100 ug of E. coli 0111:B4 LPS. Following the priming bolus, the formulations were administered to provide a continuous maintenance infusion, via intravenous administration, of 50 mg of lipid per kilogram of body weight per hour. The intravenous administration was continued for three hours following challenge.

Blood was taken from the rabbits at base line, 30 minutes after the administration of primary bolus, and every hour over the five hours of administration.

In the table which follows, the peak TNF-α values are presented. These occurred two hours after administration of the endotoxin.

Statistical significance was determined, using the well know Student's test. As the table shows, the TNF-α values were significantly reduced following the administration of 18 mM cholic acid.

Table 2. Effect of Emulsions on TNF-α Production in Rabbits

Emulsion	TNF-	Significance	
	ng/ml	N	P
5% Dextrose Control	134 ± 70	9	
7% TG Emulsion	68 ± 5	5	<0.05
7% TG Emulsion + 18 mM Cholic acid	39 ± 20	4	<0.01

The foregoing examples detail the invention which involves, in one aspect, the alleviation or prevention of endotoxemia in a subject via administering an effective amount of a phospholipid with which an endotoxin associates. The association of phospholipid and endotoxin is then removed from the subject via standard biological processes well known to anyone familiar with processes via which lipoprotein particles are removed. Association of the endotoxin with the phospholipid inactivates it.

The examples also show that administration of a member of the family of cholanoic acids or cholanoic acid salts, such as a bile acid or a bile acid salt can also be used to achieve the same end as the phospholipids, i.e., the alleviation or prevention of endotoxemia. Thus, peptide and protein free compositions containing one, or both of a bile acid/bile acid salt and a phospholipid may be used to treat endotoxemia. Cholanoic acids are described by, e.g., Hofmann, Hepatology 4 (5): 4S-14S (1984), incorporated by reference. Attention is drawn in particular to page 5S, figures 1 and 2, incorporated by reference, showing the structures characteristic of the cholanoic acids.

The subject being treated is preferably a human, but the practice of the invention is equally applicable in a veterinary context as well.

"Alleviation" as used herein refers to treatment to ease the burden of endotoxemia caused by any of the various endotoxins produced by, e.g., gram negative bacteria (S. tymphimurium, E. coli, etc.). Prophylaxis may be accomplished by administering the agent at a point where the subject is in or about to be in, a situation where endotoxin exposure may result. Classically, this occurs during surgery. Thus, a subject who is about to experience a surgical procedure may have the active ingredient administered preparatory to the procedure.

The effective amount of phospholipid and bile acid combination necessary for treatment of the subject can vary. In generally, a dose up to from about 200 total mg to about 800 mg of phospholipid per kilogram of body weight of the subject is preferred, although the amount may drop, or increase, depending upon the severity of the endotoxemia or the degree of risk in the context of the prophylaxis. For cholanoic acids and salts, such a s the bile acids and their salts, a dose of from about 10 mg to about 300 mg/kg of body weight, more preferably 15 mg to about 275 mg per kg of body weight is used.

It is desirable to administer the bile acid/bile acid salt and phospholipids in compositions which also contain neutral lipids, but this is not necessary, as neutral lipid free emulsions of phospholipids are also envisioned. The desirability of combined administration of the phospholipids results from the fact that the neutral lipids and phospholipids associate into particles which resemble the lipoproteins, but differ therefrom in that they contain no protein of peptide components, which are of course, always present in the lipoproteins.

Especially desirable forms of treatment are those where the phospholipid is a phosphatidylcholine, such as egg yolk phosphatidylcholine, soy based phosphatidylcholine or a sphingolipid. For the bile acid/bile acid salt, preferred are cholic acid and/or its salts, such as sodium cholate, sodium deoxycholate, and sodium chenodeoxycholate. With respect to the neutral lipids, it is preferred to use cholesterol ester or triglyceride, but other neutral lipids, such as squalene

or other hydrocarbon oils, di- and mono-glycerides and antioxidants such as vitamin E may also be used.

The form in which the compositions may be administered can vary, with a bolus or other intravenous forms being especially preferred. When a bolus form is used, the composition contains triglyceride, e.g., some care must be given in dosing. It is fairly well known that triglycerides are toxic if administered in too large an amount. The artisan of ordinary skill, however, can easily formulate the compositions so that the risk of triglyceride poisoning is reduce, or eliminated. In generally, when a bolus form is used, the compositions should contain no more than about 80 weight percent by weight of triglyceride or other neutral lipid, preferably no more than 70 weight percent. Most preferably, the compositions should contain no more than about 50 weight percent , of neutral lipid, when a bolus is administered.

When non-bolus forms are employed, however, such as other intravenous forms, the risk of poisoning is decreased. Nonetheless, the ranges delineated supra are preferred for intravenous, or other forms of administration, although it must be understood that they are not required. For bile acids and bile acid salts, doses are preferably from about 25 mg/kg of body weight up to about 500 mg/kg of body weight with especially preferred doses being from about 50 mg/kg of body weight to about 100 mg/kg of body weight. for phospholipids, a dose of from about 100 mg/kg of body weight up to about 1000 mg/kg of body weight are preferred. Doses are generally, however, and will vary depending on the subject and the mode of administration.

As indicated, supra, the protein and peptide free formulations require that at least one phospholipid or bile acid/bile acid salt be present. For phospholipids, it is preferred that at leas tone neutral lipid, such as a triglyceride, diglyceride, or monoglyceride be present. The compositions may include additionally material such as sterols (e.g., cholesterol, β-sitosterol), esterified or unesterified lipids (e.g., cholesterol ester or unesterified cholesterol), hydrocarbon oils such as squalene, antioxidants such as vitamin E but these are not required. Of course, more than one phospholipid, and/or more than one neutral lipid may be used in any such formulation. When combinations of neutral lipid and phospholipid are

used, the neutral lipid should be present at from about 3% up to about 50% by weight relative to the total amount of lipid in the composition.

In the case of the bile acid/bile acid salts, these may be used separately, or in combination a phospholipid, a neutral lipid, or both. With respect to these additional materials (e.g., phospholipids and neutral lipids), preferred species are those discussed and mentioned supra. Optional additional ingredients include those listed supra.

Also as part of the invention are compositions useful in treating endotoxemia. One embodiment of his feature of the invention is a composition containing at least one of each of a bile acid/bile acid salt, a phospholipid, and a neutral lipid, wherein the composition as a whole contains an endotoxemia alleviating amount of active ingredient. This composition preferably contains, by weight percent, from about 5% to about 30% by weight bile acid/bile acid salt, from about 3% to about 50% by weight neutral lipid, and from about 10% to about 95% by weight of phospholipid. Especially preferred are compositions containing from about 10-15% by weight of bile acid/bile acid salt, from about 5% to about 10% by weight of neutral lipid, and the balance of the composition being phospholipid.

It should be noted that these weight percentages are relative to compositions consisting of three components. When the three component system is combined with, e.g., a carrier, adjuvant, optional ingredients such as those discussed supra, the percentage by weight relative to the entire composition will drop. It is to be borne in mind that such therapeutic compositions are always protein free and peptide free.

In the case of compositions which do not contain a bile acid or a bile acid salt, such protein free, peptide free compositions contain, preferably, at least about 3% by weight of a neutral lipid, up to about 50% by weight neutral lipid, the balance being at least one phospholipid. Preferably, the neutral lipid is a triglyceride, but may be any of the additional neutral lipids discussed supra. Also, the phospholipid is preferably a phosphatidylcholine.

Other aspects of the invention will be clear to the skilled artisan and need not be reiterated here.

It will be understood that the specification and examples are illustrative but not limitative of the present invention and that other embodiments within the spirit and scope of the invention will suggest themselves to those skilled in the art.

CLAIMS

1. A protein free and peptide free pharmaceutical composition comprising a cholanoic acid or cholanoic acid salt, a phospholipid, and a neutral lipid.

2. A pharmaceutical composition according to Claim 1 where the cholanoic acid or cholanoic acid salt is a bile acid or bile acid salt.

3. A pharmaceutical composition according to Claim 2 where the bile acid salt is selected from sodium cholate, sodium deoxycholate, and sodium chenodeoxycholate.

4. A pharmaceutical composition according to Claim 2 where the bile acid salt is sodium cholate.

5. A pharmaceutical composition according to any one of Claims 1-4 where the phospholipid is a phosphatidylcholine.

6. A pharmaceutical composition according to any one of Claims 1-4 where the phospholipid is a sphingolipid.

7. A pharmaceutical composition according to any one of Claims 1-6 where the neutral lipid is a triglyceride.

8. A pharmaceutical composition according to any one of Claims 1-6 where the neutral lipid is a cholesterol ester.

9. A pharmaceutical composition according to any one of Claims 1-8 comprising up to 70% by weight of neutral lipid.

10. A pharmaceutical composition according to any one of Claims 1-9 comprising up to 50% by weight of neutral lipid.

11. A pharmaceutical composition according to any one of Claims 1-10 comprising from about 5% to about 10% by weight of neutral lipid.

12. A pharmaceutical composition according to any one of Claims 1-11 comprising a phospholipid : neutral lipid weight ratio of about 93 : 7.

13. A pharmaceutical composition according to any one of Claims 1-12 comprising from about 5% to about 30% by weight of cholanoic acid or cholanoic acid salt.

14. A pharmaceutical composition according to any one of Claims 1-13 comprising from about 10% to about 15% by weight of cholanoic acid or cholanoic acid salt.

15. A protein free and peptide free pharmaceutical composition comprising a cholanoic acid or cholanoic acid salt, a phospholipid, and a neutral lipid having a total weight ratio of about 13 : 81 : 6 respectively.

16. A pharmaceutical composition according to Claim 15 where the phospholipid is a phosphatidylcholine, the neutral lipid is a triglyceride, and the cholanoic acid or cholanoic acid salt is sodium cholate.

17. A pharmaceutical composition according to any one of Claims 1-16 for use in therapy.

18. The use of a pharmaceutical composition according to any one of Claims 1-16 in the manufacture of a medicament for the treatment or prophylaxis of a human or animal suffering from endotoxemia.

19. The use of a pharmaceutical composition according to any of claims 1-16 for the manufacture of a medicament for the treatment of prophylaxis of a human or animal suffering from endotoxemia in an amount sufficient to provide up to about 400mg, suitably 200mg. preferably 100mg. of phospholipid per kilogram of body weight of said subject.

20. A protein free and peptide free pharmaceutical composition comprising:
(a) at least one neutral lipid at an amount from about 3% to about 50% by weight of total lipid in said composition, and
(b) at least one phospholipid.

21. A pharmaceutical composition according to Claim 20 where the phospholipid is a phosphatidylcholine.

22. A pharmaceutical composition according to Claim 20 where the phospholipid is a sphingolipid.

23. A pharmaceutical composition according to Claims 20-22 wherein the neutral lipid is a triglyceride.

24. A pharmaceutical composition according to any one of Claims 20-22 where the neutral lipid comprises a cholesterol ester.

25. A pharmaceutical composition according to any one of Claims 20-24 where the composition further comprises sphingosine.

26. The use of protein free and peptide free pharmaceutical composition comprising at least one phospholipid and at least one neutral lipid for the manufacture of a medicament for the treatment or prophylaxis of a human or mammal suffering from endotoxemia.

27. The use of a pharmaceutical composition according to any one of claims 22-26 for the manufacture of a medicament for the treatment or prophylaxis of a human or mammal suffering from endotoxemia.

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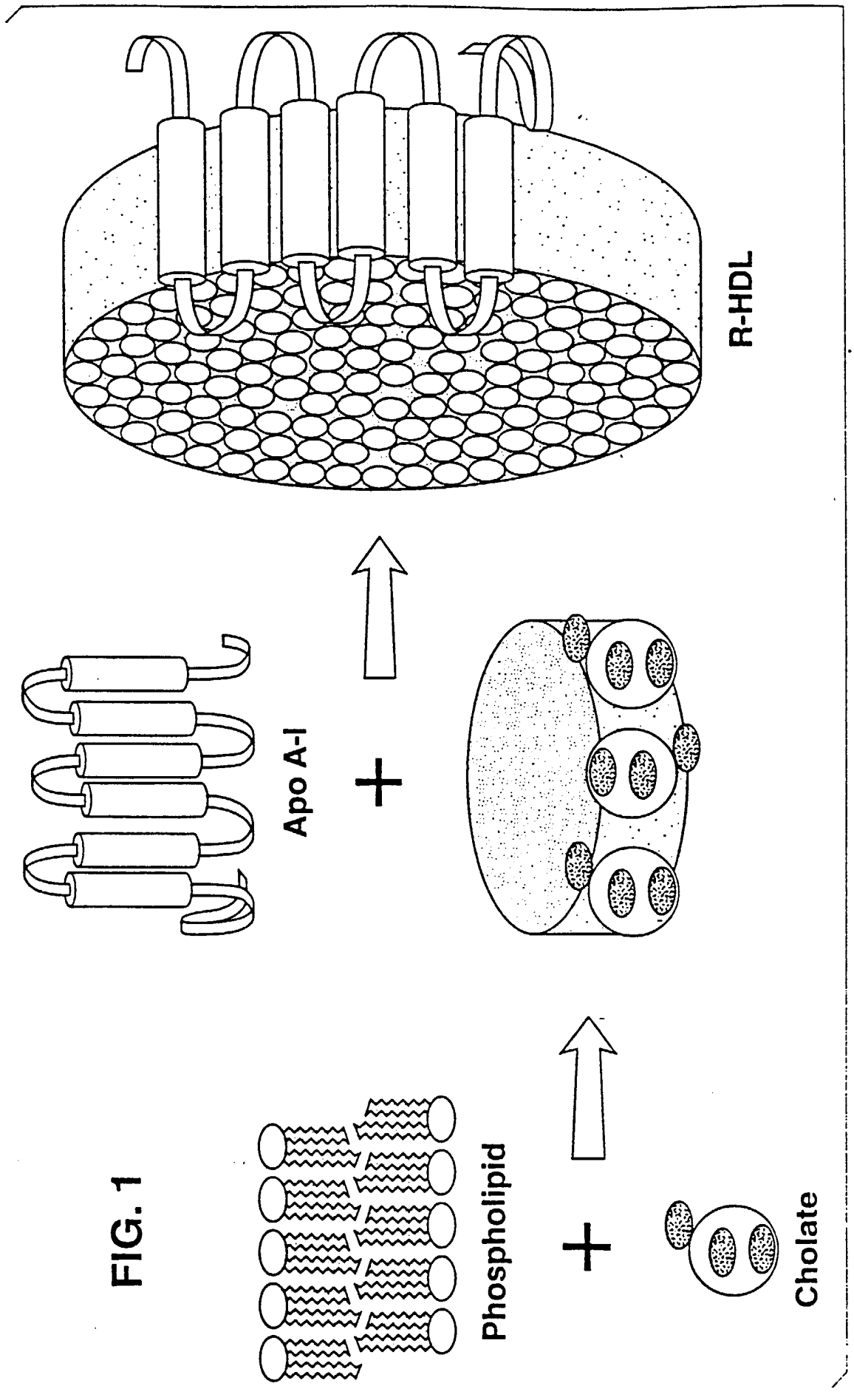

FIG. 1

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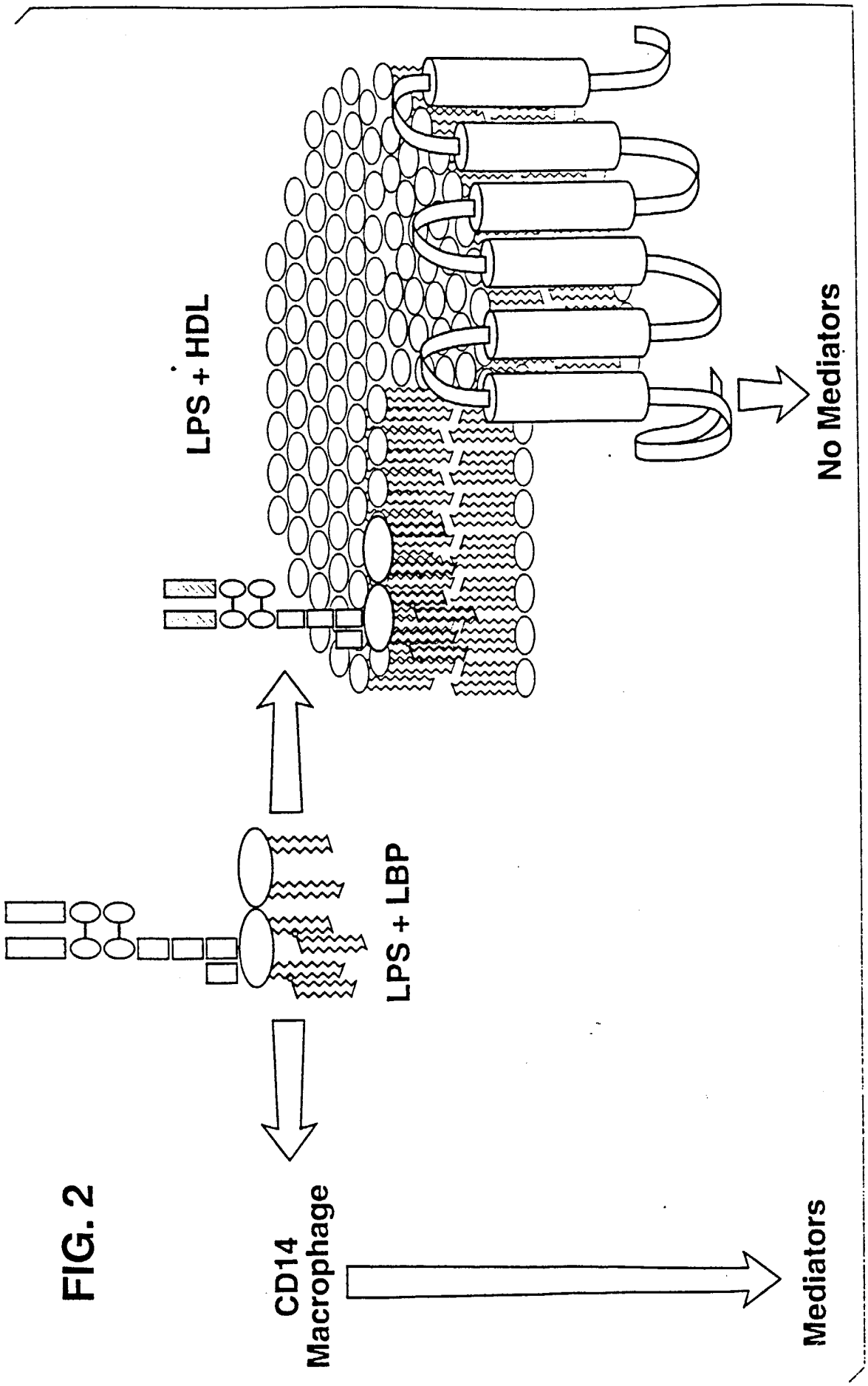

FIG. 2

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FIG. 3

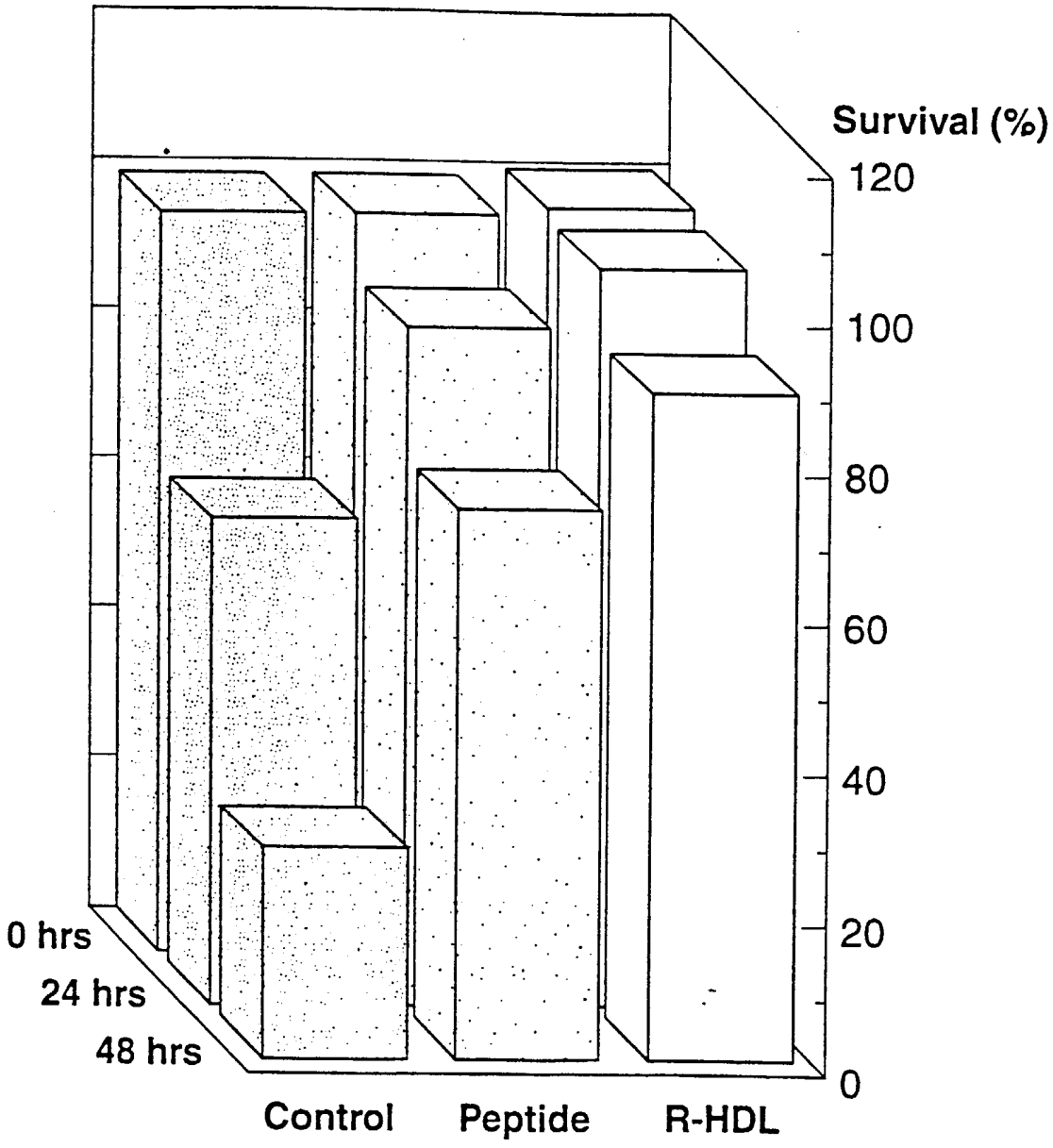

Survival (%)
120
100
80
60
40
20
0
0 hrs
24 hrs
48 hrs
Control
Peptide
R-HDL

FIG. 4A

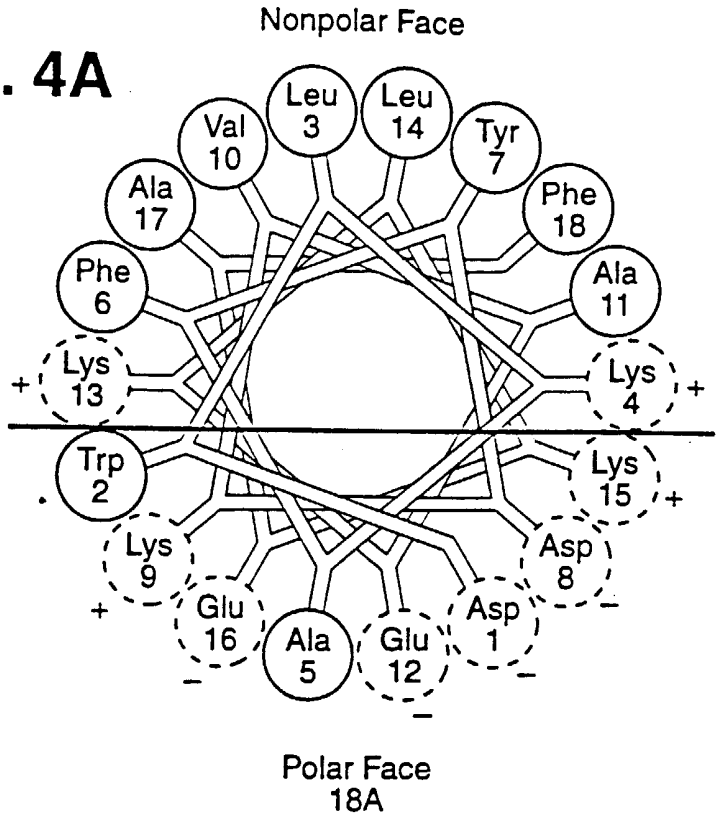

Nonpolar Face
Leu 3
Leu 14
Tyr 7
Phe 18
Ala 11
Lys 4
Lys 15
Asp 8
Asp 1
Glu 12
Ala 5
Glu 16
Lys 9
Trp 2
Lys 13
Phe 6
Ala 17
Val 10
Polar Face
18A

FIG. 4B

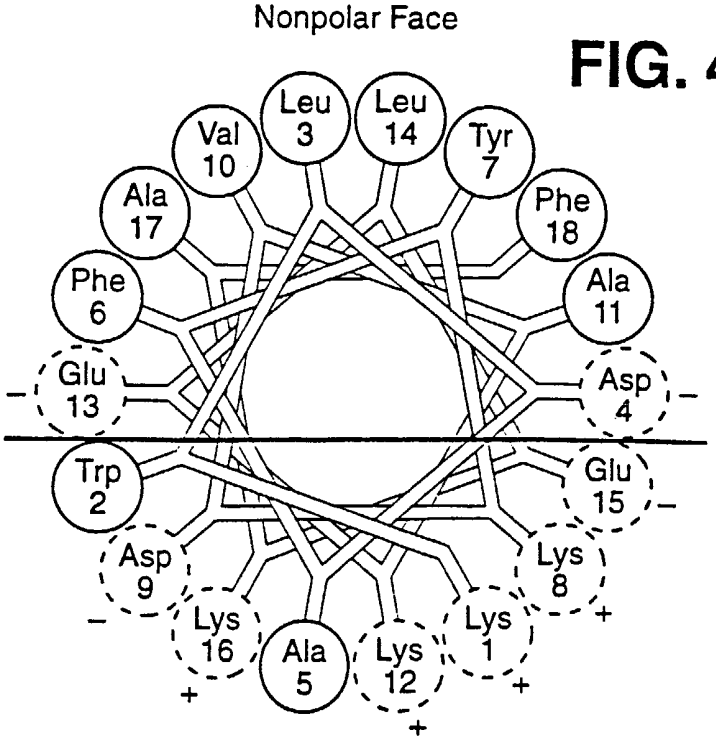

Nonpolar Face
Leu 3
Leu 14
Tyr 7
Phe 18
Ala 11
Asp 4
Glu 15
Lys 8
Lys 1
Lys 12
Ala 5
Lys 16
Asp 9
Trp 2
Glu 13
Phe 6
Ala 17
Val 10
Polar Face
Reverse 18A

FIG. 4C

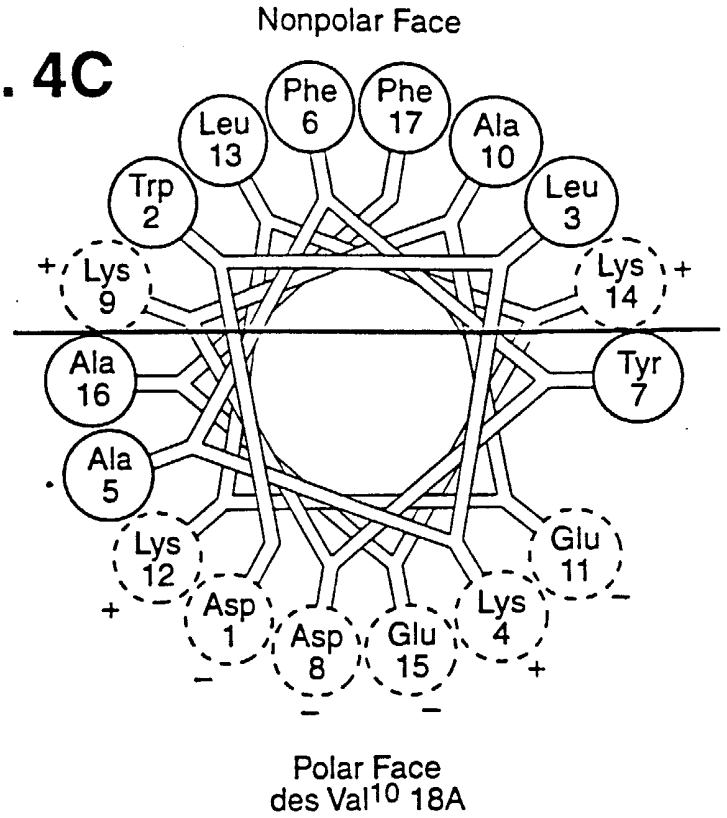

Nonpolar Face
Phe 6
Phe 17
Leu 13
Ala 10
Trp 2
Leu 3
Lys 9
Lys 14
Ala 16
Tyr 7
Ala 5
Lys 12
Glu 11
Asp 1
Asp 8
Glu 15
Lys 4
Polar Face
des Val10 18A

FIG. 4D

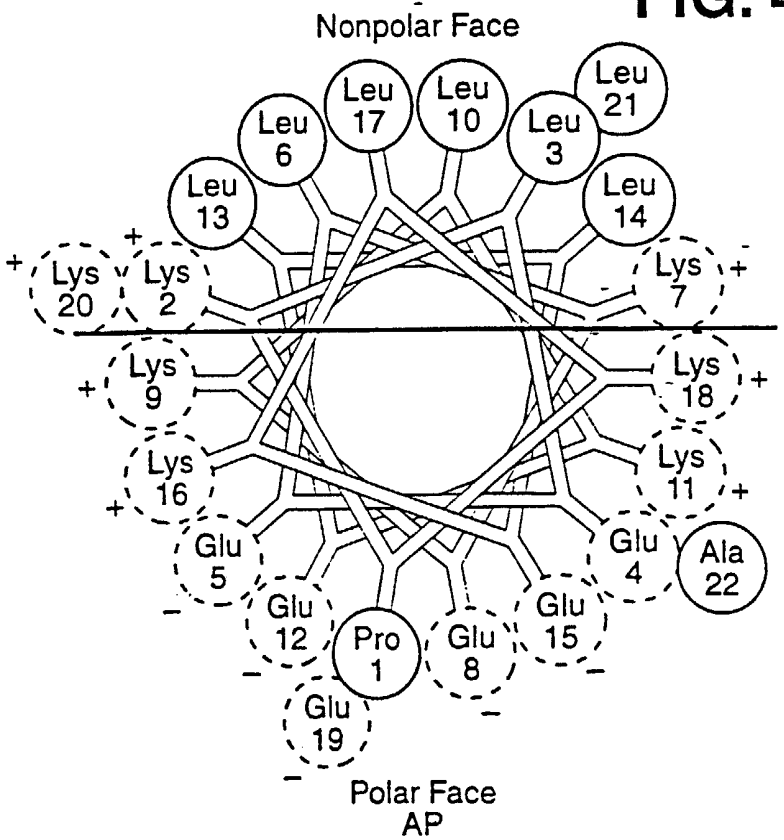

Nonpolar Face
Leu 17
Leu 10
Leu 21
Leu 6
Leu 3
Leu 13
Leu 14
Lys 20
Lys 2
Lys 7
Lys 9
Lys 18
Lys 16
Lys 11
Glu 5
Glu 4
Ala 22
Glu 12
Pro 1
Glu 8
Glu 15
Glu 19
Polar Face
AP

FIG. 4E

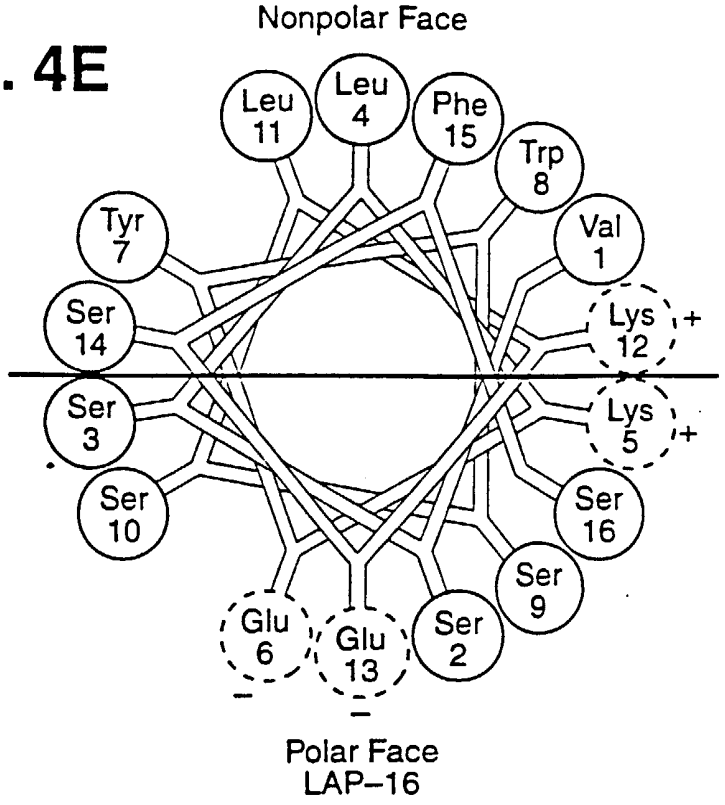

Nonpolar Face
Leu 11
Leu 4
Phe 15
Trp 8
Val 1
Tyr 7
Ser 14
Lys 12
+
Ser 3
Lys 5
+
Ser 10
Ser 16
Ser 9
Glu 6
Glu 13
Ser 2
Polar Face
LAP–16

FIG. 4F

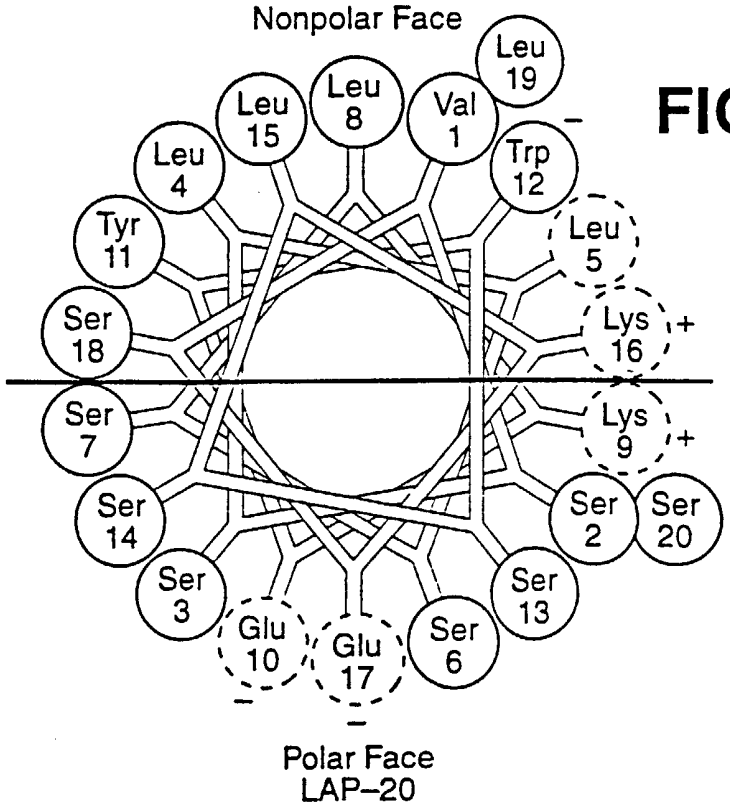
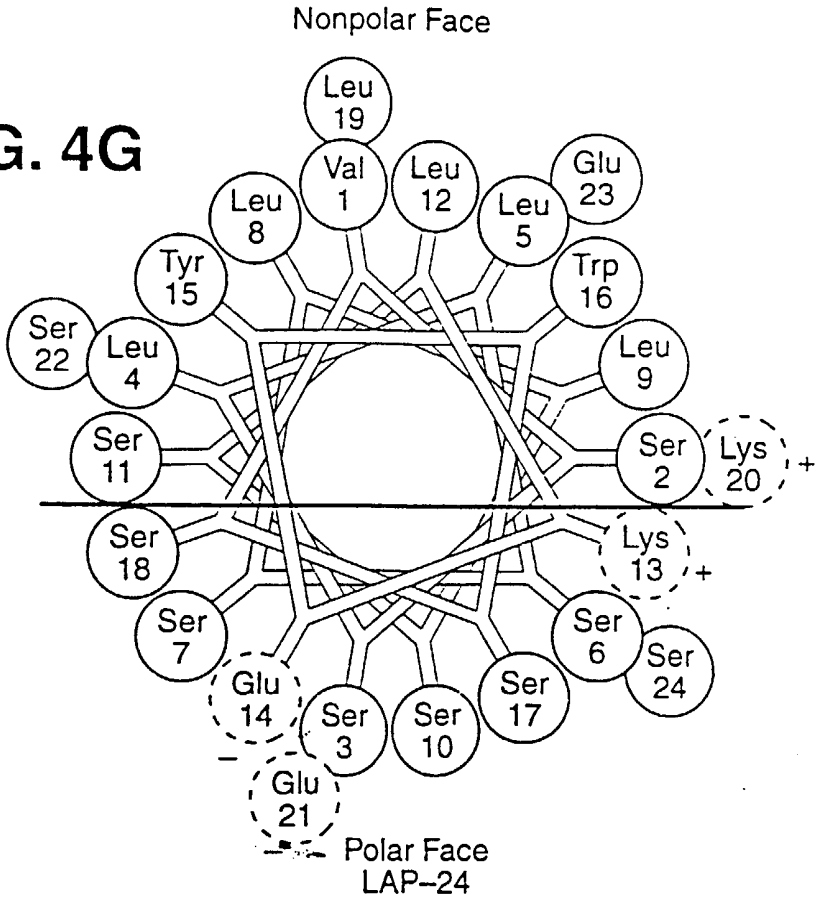

Nonpolar Face
Leu 19
Leu 15
Leu 8
Val 1
Leu 4
Trp 12
Tyr 11
Leu 5
Ser 18
Lys 16
+
Ser 7
Lys 9
+
Ser 14
Ser 2
Ser 20
Ser 3
Ser 13
Glu 10
Glu 17
Ser 6
Polar Face
LAP–20

FIG. 4G


Nonpolar Face
Leu 19
Val 1
Leu 12
Glu 23
Leu 8
Leu 5
Tyr 15
Trp 16
Ser 22
Leu 4
Leu 9
Ser 11
Ser 2
Lys 20
+
Ser 18
Lys 13
+
Ser 7
Ser 6
Ser 24
Glu 14
Ser 3
Ser 10
Ser 17
Glu 21
Polar Face
LAP–24

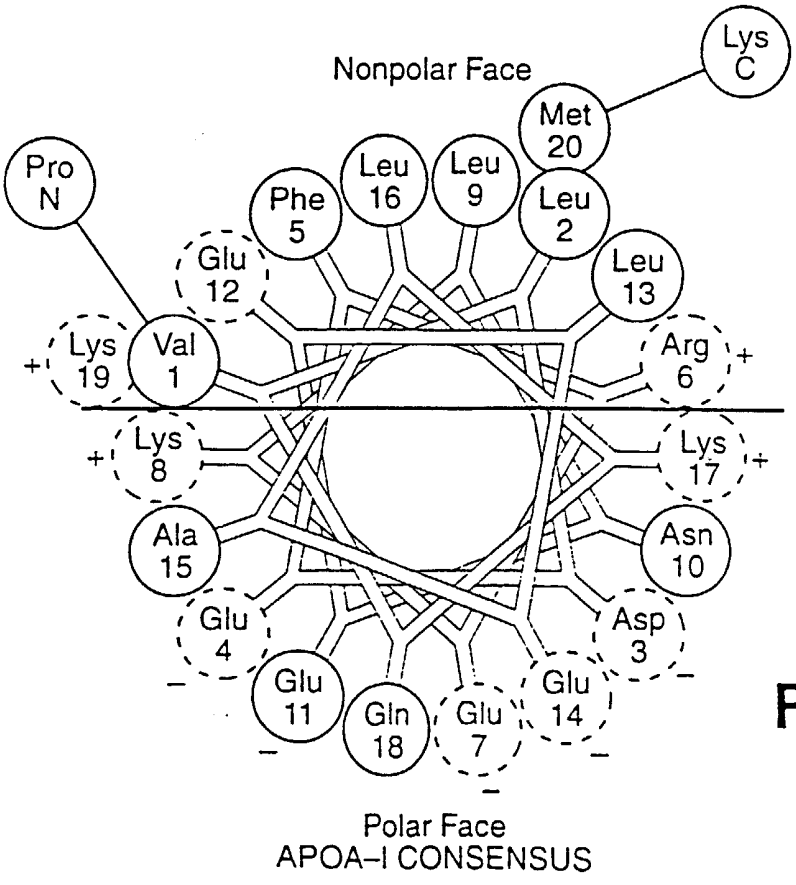

Nonpolar Face
Lys C
Met 20
Pro N
Leu 16
Leu 9
Phe 5
Leu 2
Glu 12
Leu 13
Lys 19
+
Val 1
Arg 6
+
Lys 8
+
Lys 17
+
Ala 15
Asn 10
Glu 4
Asp 3
Glu 11
Gln 18
Glu 7
Glu 14
Polar Face
APOA–I CONSENSUS

FIG. 4H

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FIG. 4I

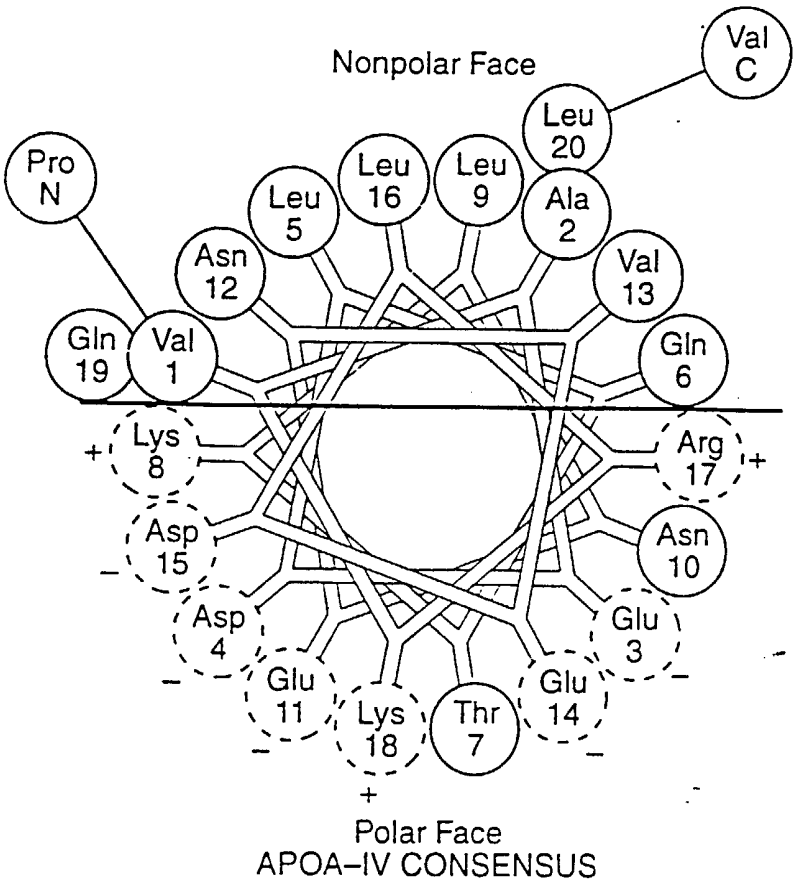

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FIG. 5A

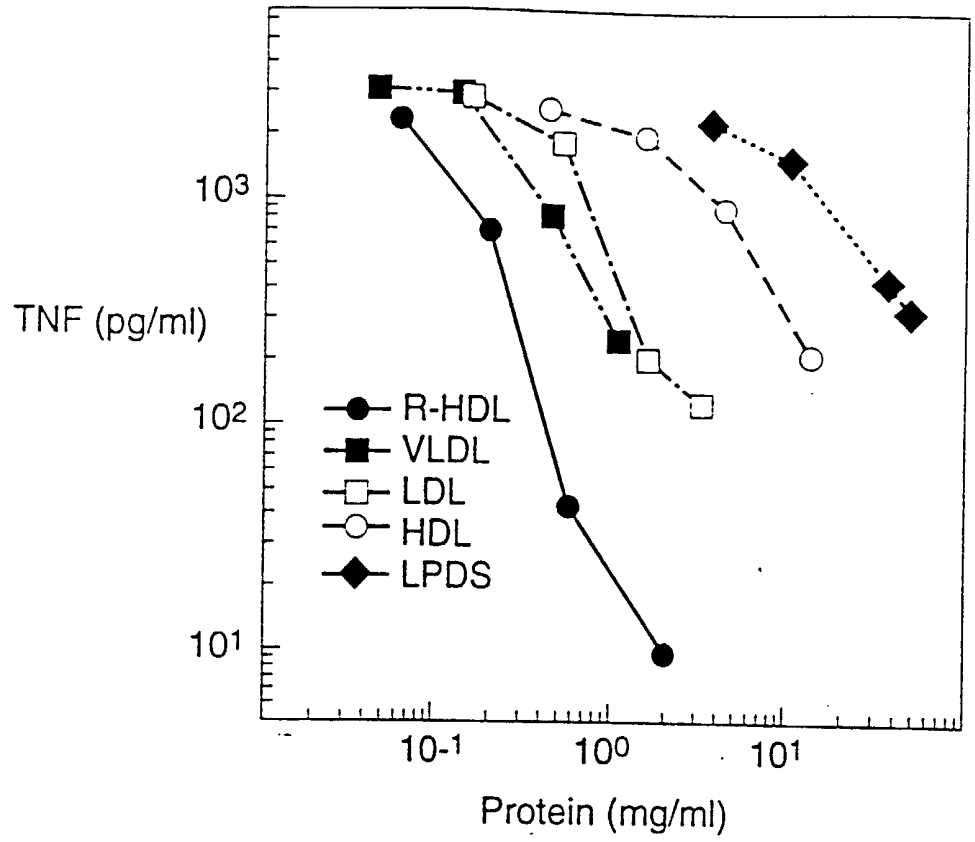

FIG. 5B

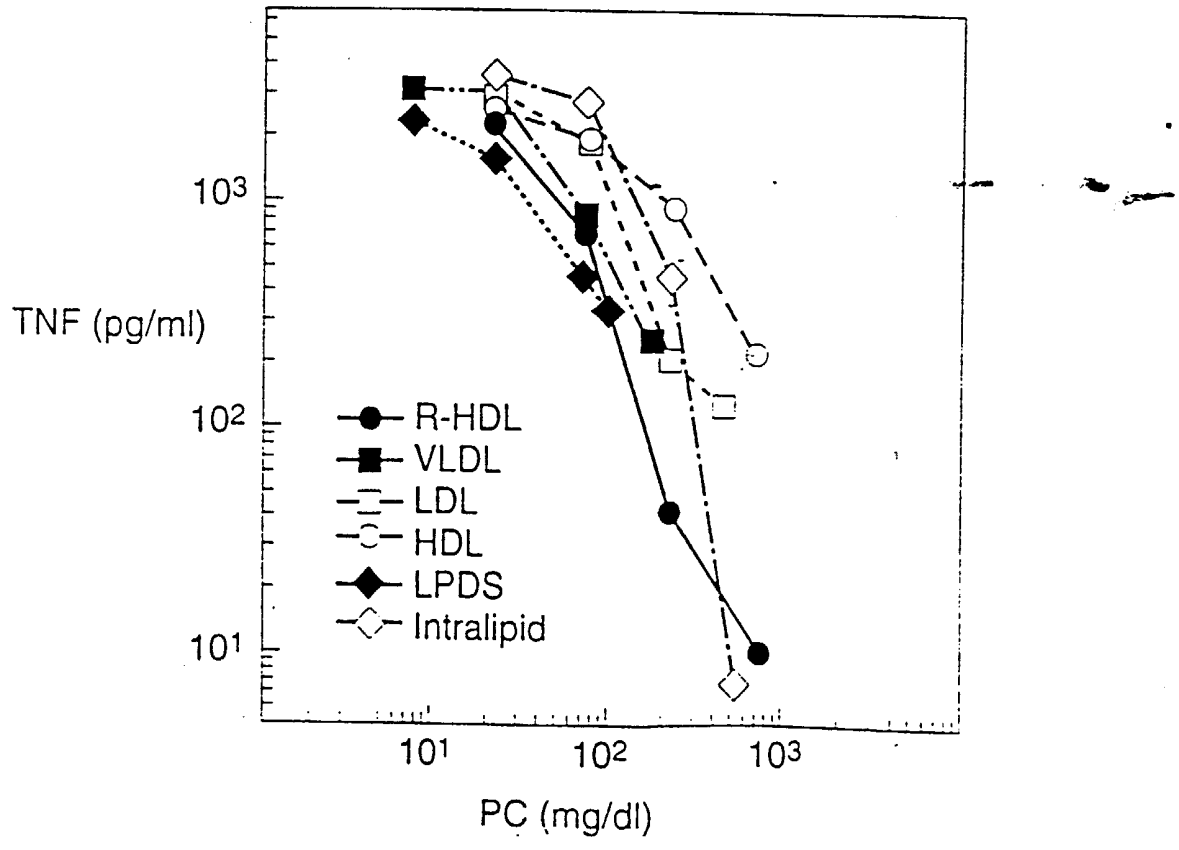

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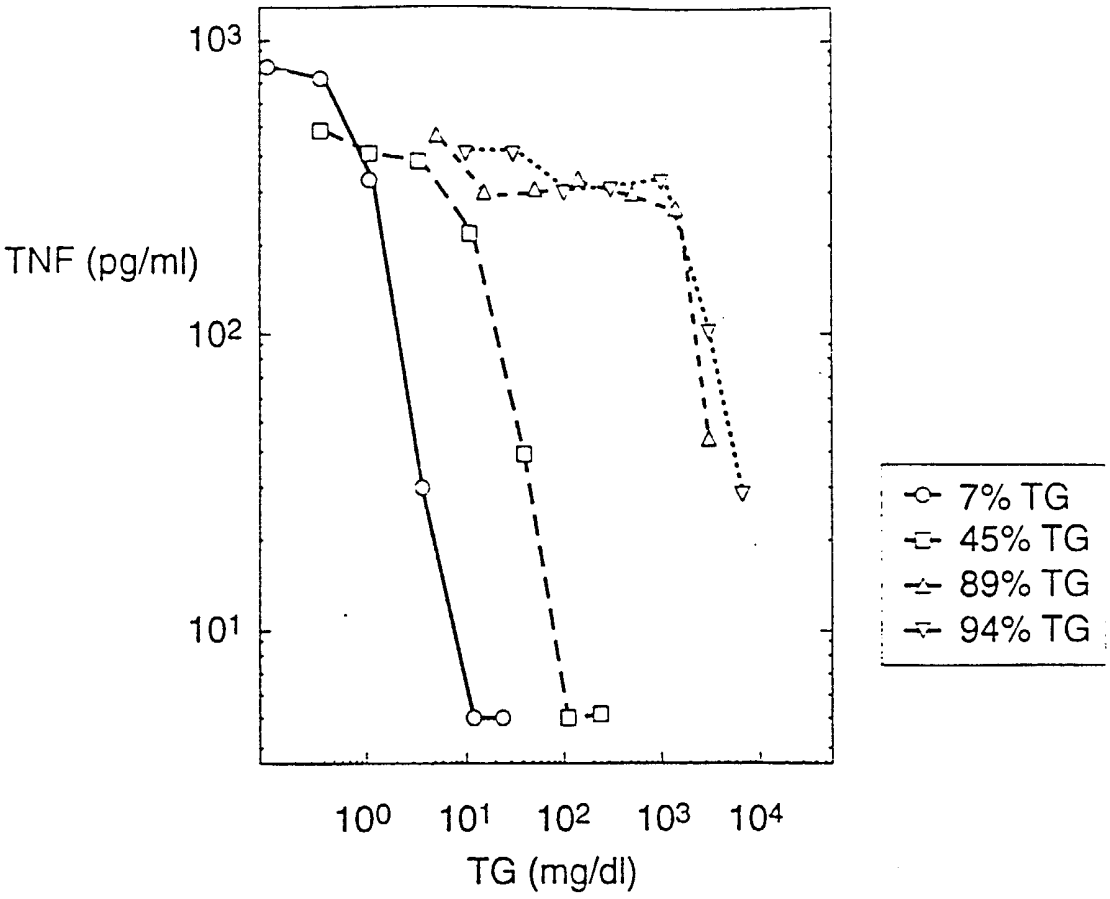
FIG. 6A



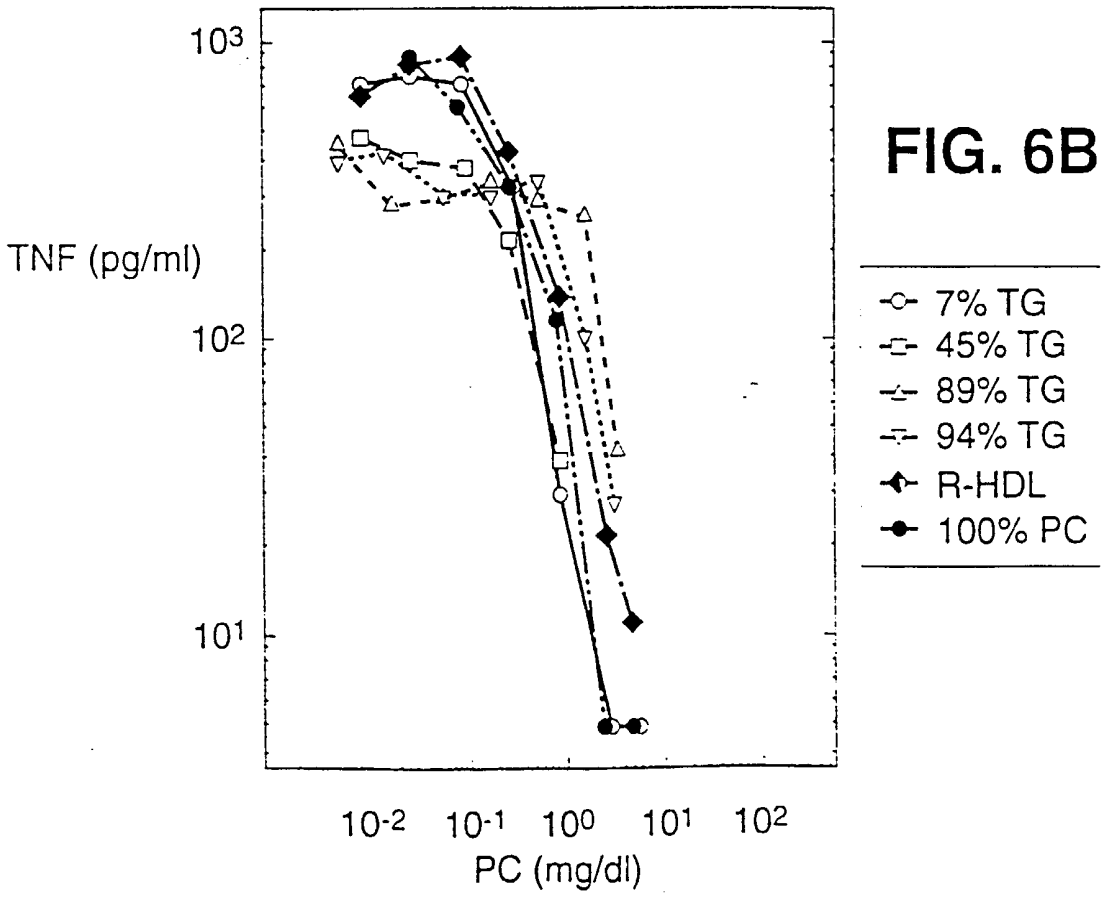


FIG. 6B

FIG. 7

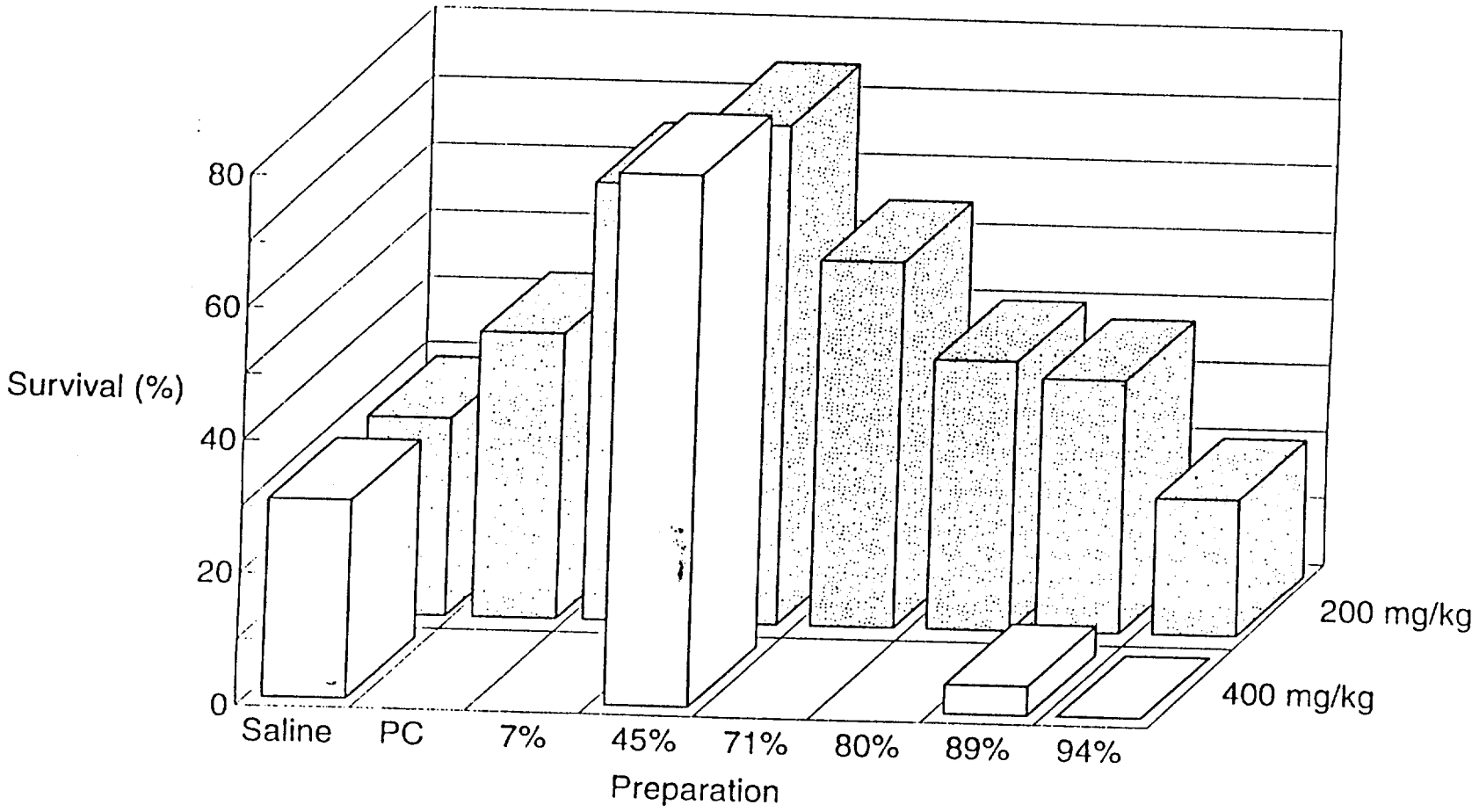


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FIG. 8

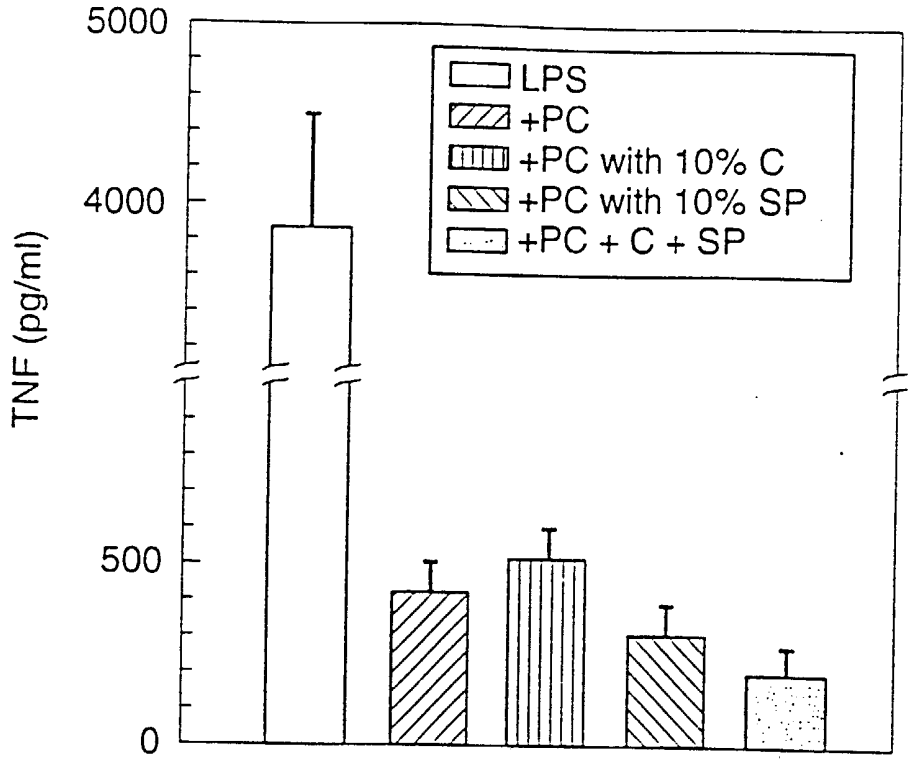


FIG. 9A

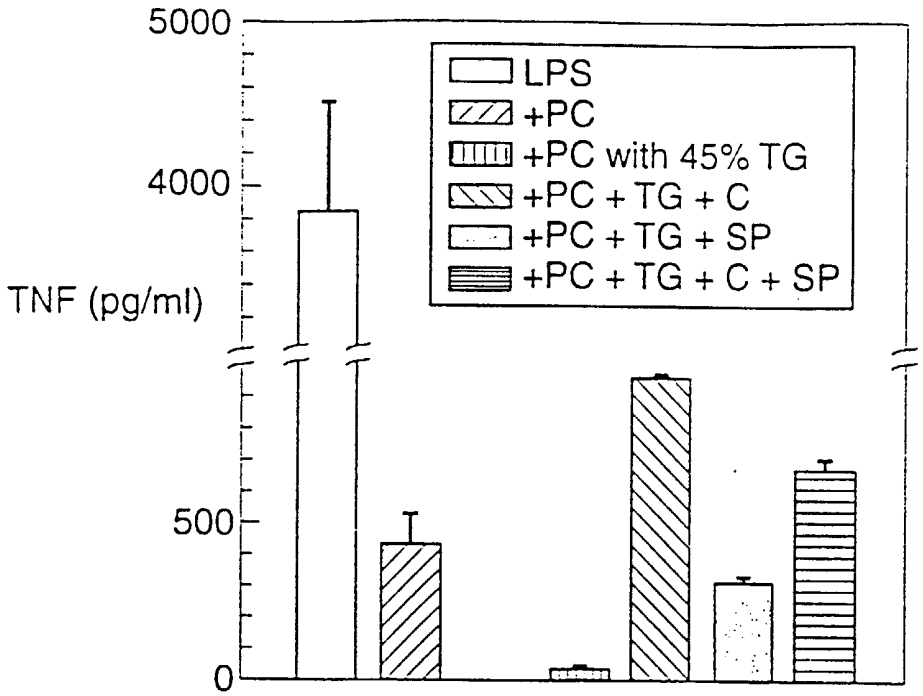

5000
4000
500
0
TNF (pg/ml)
LPS
+PC
+PC with 45% TG
+PC + TG + C
+PC + TG + SP
+PC + TG + C + SP

FIG. 9B

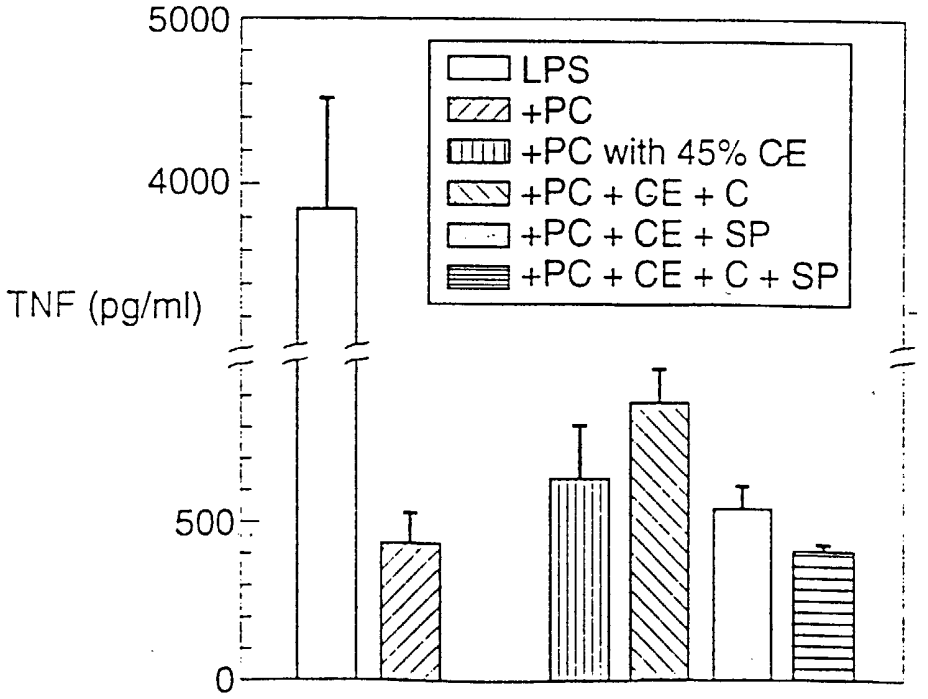

5000
4000
500
0
TNF (pg/ml)
LPS
+PC
+PC with 45% CE
+PC + CE + C
+PC + CE + SP
+PC + CE + C + SP

FIG. 10

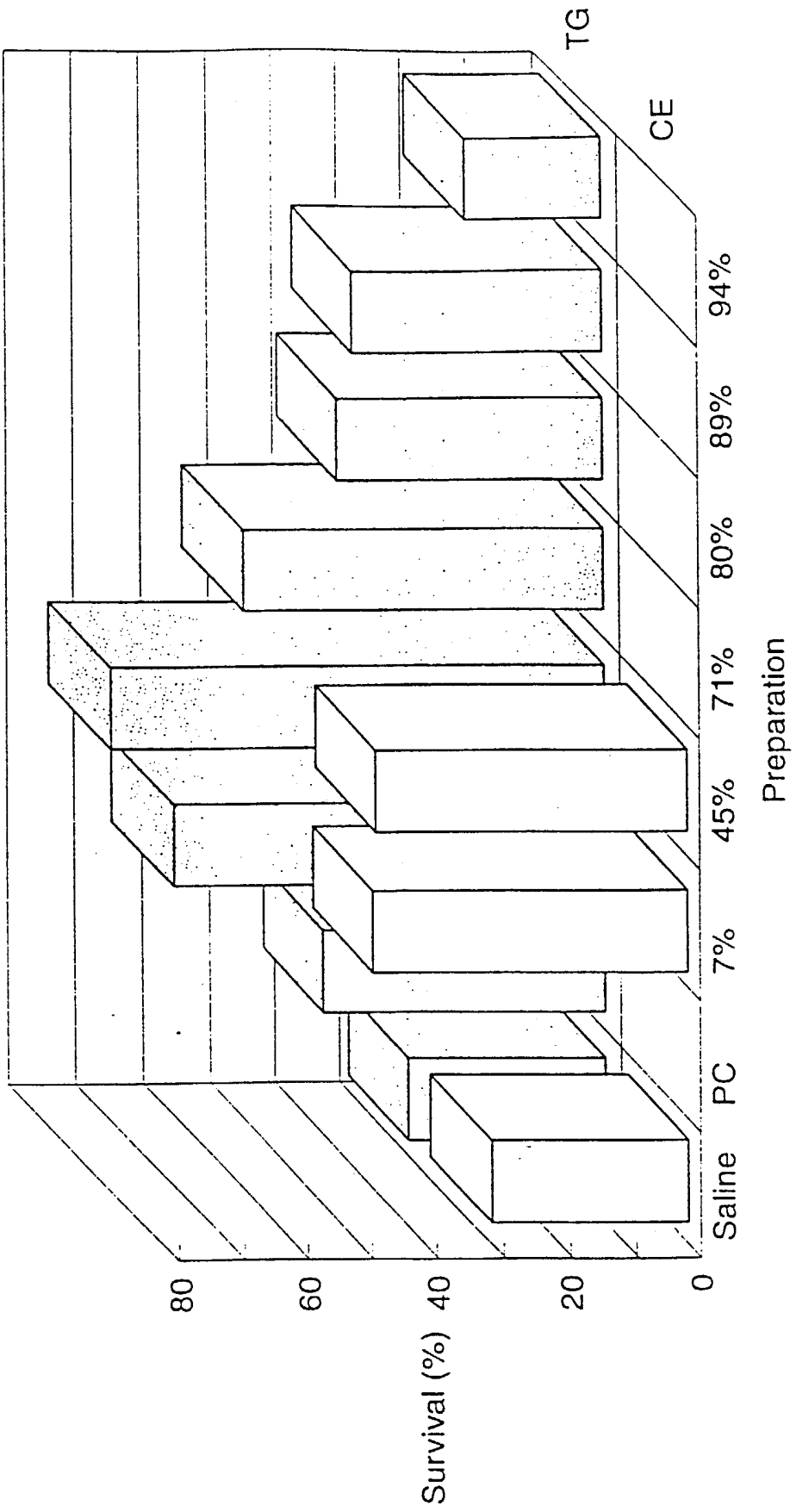
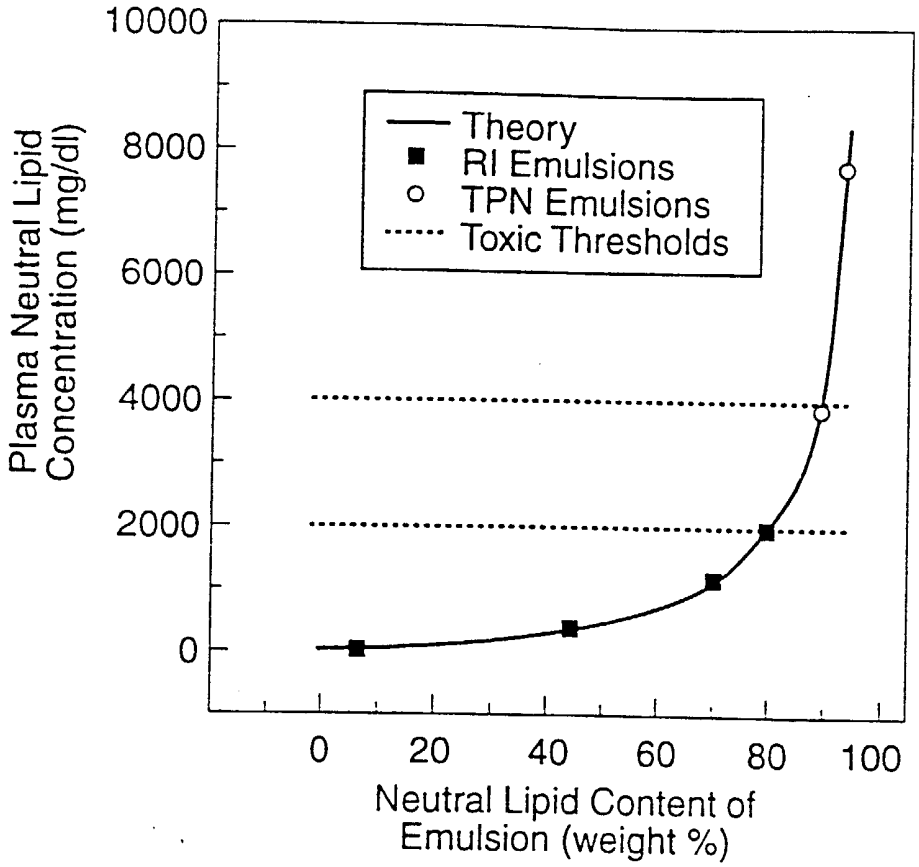


FIG. 15


10000
8000
6000
4000
2000
0
Plasma Neutral Lipid Concentration (mg/dl)
Theory
RI Emulsions
TPN Emulsions
Toxic Thresholds
0
20
40
60
80
100
Neutral Lipid Content of Emulsion (weight %)