

ins. Liposomes can be prepared having within the membrane bilayer lipids suitable for coupling with proteins and other macromolecules. Typically, lipids such as phosphatidyl ethanolamine, phosphatidyl serine or phosphatidyl inositol would be suitable.

#### EXAMPLE VIII

The method described in Example I is employed to prepare liposomes containing alkaline phosphatase. In this case the lipid mixture consists of 25 milligrams of dipalmitoyl phosphatidyl choline, 10 milligrams of phosphatidyl ethanolamine and 8.6 milligrams of cholesterol. These liposomes are purified by repeated centrifugation after which they are resuspended in 2 ml. of 0.1 M borate buffer pH 8.5. To this suspension is added 20 microliter of 25% glutaraldehyde. After 10 minutes at room temperature, the mixture is dialyzed overnight against 2 liters of borate buffer. The activated liposomes are then added to 2.4 milligrams of bovine serum albumin in 1 ml. borate buffer. The mixture is then incubated overnight at 4° C. whereupon the liposomes with protein attached are separated from unbound protein by centrifugation at 25,000 g for 30 minutes. Following the assay method described in Examples III and IV and using rabbit antibody to bovine serum albumin, an immune lysis assay can be prepared which will detect albumin in samples quantitatively in the range of from 0.1 to 2 µg 1 ml.

#### EXAMPLE IX

In order to assay the cardiac glycoside-digoxin was coupled to dipalmitoylphosphatidylethanolamine. To this end mixture containing 0.5 grams (0.64 mmole) of Digoxin in 20 ml. of Ethanol/Dioxane (4:1,v/v) was added to 60 ml. of 0.1 M sodium metaperiodate. The mixture was stirred for 30 minutes at room temperature whereupon 4.5 ml. of ethylene glycol was added. This mixture was stirred for one-half hour at room temperature and evaporated under reduced pressure. The resultant solid was then extracted 3 times with 50 ml. of chloroform. The extracts were pooled (total volume 150 ml.) and solvent evaporated under reduced pressure, producing 0.9 gm of oily residue. Twenty-five mg of this crude product of digoxin dialdehyde in 1 ml. of ethanol/chloroform (1:4) was added to 20 mg of dipalmitoylphosphatidylethanolamine in 1 ml of ethanol/chloroform (1:2). Four drops of triethylamine were added and the reaction mixture (pH 9) was incubated overnight at 37° C. and finally evaporated to a dry residue under reduced pressure. This residue was suspended in 2 ml. of ethanol/chloroform (1:1) and 4 milligram of sodium borohydride was added. This mixture was stirred for 30 minutes and then evaporated to dryness under reduced pressure. This residue was then triturated with ethanol and filtered to give a filtrate which upon evaporation yielded 45 milligrams of dipalmitoylphosphatidylethanolamine-digoxin conjugation product.

#### EXAMPLE X

The conjugate of digoxin and dipalmitoylphosphatidylethanolamine is employed to prepare liposomes with digoxin. In this case 22 milligrams of dimyristoylphosphatidyl choline, 8.6 milligrams of cholesterol, 1.6 milligrams of dicetylphosphate, and 2.5 milligrams of Digoxin-dipalmitoylphosphatidylethanolamine conjugate were dissolved in 3 ml. of chloroform. Solvent was evaporated under reduced pressure and the lipids de-

posited as a thin film on the internal wall of a 100 ml. round-bottomed flask. The lipid film was then dispersed in (solutions as in Example I lines 8-10 and purified also as in Example I).

Assays were performed as in Example VI. Inhibition by digoxin in test samples was observed in range of 0.5-10 ng/ml digoxin in the test sample.

Liposomes can be frozen successfully if they are first suspended in an isotonic medium—0.01 M phosphate buffer containing 0.15 M sodium chloride. Also useful are solutions buffered in the range from pH 4 to pH 10 containing 0.3 M glucose or like carbohydrate. However, liposomes frozen in proteinaceous media e.g. containing bovine serum albumin of rabbit gamma globulins are not preferred as these show elevated levels of enzyme activity in the absence of lytic reagents—detergent or complement plus antibody. Best results are achieved with rapid freezing at a rate of at least 5° C. per minute. Prior to freezing liposomes are suspended in isotonic media at concentrations of 1-10 mg/ml. For example liposomes prepared as in Example I are suspended in 0.01 M sodium phosphate buffer containing 0.15 M sodium chloride. This suspension contains 2.5 mg of total lipid in 1 ml of liquid. 0.1 ml aliquots of this mixture are placed in a 5 ml. vial. These are then frozen to -20° C. at a rate of 5° C. per minute. The liposomes can be thawed and used after long periods of storage.

While specific embodiments of this invention have been shown and described, it will be understood that many variations are possible. Particular concentrations, combinations and materials can vary greatly so long as the signal to noise ratio minimums of the invention are maintained which aids in preventing false readings. A wide variety of materials can be tested in a wide variety of high volume screening test by relatively unskilled personnel. The material to be tested can be body fluids or mixtures of all kinds. When serum is tested it is preferably treated chemically and/or with heat to remove undesirable inhibition. Prior treatment with amino groups is one such chemical method. Typically, 0.1 ml of 2.54 M ammonia is added to 1.9 ml of serum which is then neutralized by the addition of 0.1 ml of 2.54 M hydrochloric acid. Also sulthydryl blocking reagents can be useful. In this case 0.1 ml of 0.2 M mercaptoethanolin phosphate buffered saline is added to 1 ml of serum. Then 1 ml of 0.2 M iodoacetamide is added. Similarly useful is the sulfonic acid azo dye chlorazol fast pink which selectively inhibits human complement activation but not guinea pig complement. Heat treatment of at least about 58° C. for at least 30 and preferably 60 minutes is also useful to prevent unwanted inhibition of the complement reaction.

What is claimed is:

1. An immuno test method comprising forming a mixture of
  - (a) a stable liposome labeled with one of an antigen or its cognate antibody sequestering an enzyme carried within it and having a signal to noise ratio of no less than 5;
  - (b) a substrate for said enzyme
  - (c) a test material to be tested for specific activity of the said one antigen or cognate antibody; and
  - (d) complement,
 and detecting the presence or absence of enzymatic activity in said mixture under conditions which permit an immune reaction to expose said enzyme to said substrate.