725203

The Medical Research Center
Brookhaven National Laboratory
Upton, L. I., New York

BROOKHAVEN NATIONAL LABORATORY

MEMORANDUM

REPOSITORY Records Holding Area Bldg 494
COLLECTION Protocols - Clinical
BOX No. 4
FOLDER Human Protocols 1950-1963

R

DATE: 13 July 1959

TO: Ctte on Use of Radioactive Isotopes in Patients

FROM: W. L. Hughes, A. Scanu, W. Wolins

SUBJECT: Interactions of serum lipoproteins labeled with I^{131}. Project H-55.

Permission is requested from the Medical Department Committee on Use of Radioactive Isotopes in Patients to continue the use of I^{131}-labeled serum lipoproteins according to Project H-37 with the following modifications.

Objective:

Primary objective will be to explore the physiological interactions between human serum lipoproteins, including chylomicrons, with the intention of learning more about their function and fate. A technique of preparation of undenatured lipid-free protein from α_1-lipoproteins has been recently described by Scanu et al. (Arch. Biochem. Biophys. 74, 390, 1958). In studies carried out in part in this Laboratory, it has been shown that this α-protein recombines in vitro with serum lipids to reform lipoproteins (unpublished observations). In the present investigation I^{131}-labeled human α_1-lipoproteins and I^{131}-labeled human lipid-free α_1-protein will be injected into a normal subject and their turnover rate and distribution in the other serum lipoprotein classes will be studied.

In previous experiments by D. Gitlin (J.C.I. 37: 172, 1958) it has been shown that α- and β-lipoproteins have much shorter half-lives than other serum proteins. It has been reported also that I^{131}-labeled α- and β-lipoproteins are removed from blood faster than γ-globulin or iron-binding protein (J.C.I. 35:44, 1956). Studies on in vitro interactions of serum lipoproteins have been reported by Florsheim and Morton (J. Appl. Physiol. 10: 301, 1957).

1176389

Methodology:

a) Source of lipoproteins. Normal human donors will be used as a source of lipoproteins. 500 ml. of blood will be withdrawn and after clotting, serum will be separated by centrifugation. Alpha- and beta-lipoproteins will be prepared by ultracentrifugation at d 1.063 and d 1.21. Removal of lipids from alpha-lipoproteins will be performed by the technique of Scanu et al (Arch. Biochem. Biophys. 74: 390, 1958).

b) Labeling procedure. Iodination will be performed according to McFarlane (Biochem. J. 62: 135, 1956). Removal of free iodine will be accomplished by the use of Ioresin (Abbott).

c) Tests of sterility. Prior to the study of the turnover of the iodinated lipoproteins tests will be performed to assure the sterility of the preparations (filtration and cultures).

d) Dosage of protein. From 1 to 2 milligrams of protein will be injected.

e) Patients. These will be patients in a variety of diseased states with associated evident or presumed abnormalities in lipoprotein metabolism.

f) Authority. Responsible physician: W. Wolins.

g) Counting. By means of whole body counting the rate of degradation of the administered radioiodinated proteins will be determined. Blood samples will be also withdrawn at regular time intervals to determine the distribution of radioactivity in the various serum lipoprotein classes. A well-type scintillation detector will be used for the purpose.

Dosimetry:

From Bertinchamps-Cotzias nomogram the dose rate at the center of a 70 Kg. man-equivalent cylinder containing a uniform concentration of I^{131} of 1 $\mu c/Kg$. is 0.021 rads/day or the total dose (at $T = \infty$) is 0.270 rads. Assuming a biological half-life

1176390

for the α-lipoprotein of 5 days (Gitlin reported 4 to 5 days in children) gives an effective half-life of 3 days and a resultant total dose of 0.08 rad.

Minimum counting requirements of about 500 cpm per sample of 4 ml. require a total plasma activity of

$$\frac{500 \text{ cpm}}{4 \text{ ml}} \times 5 \times 10^3 \text{ ml} \times \frac{1\mu c \ I^{131}}{0.8 \times 10^6 \text{cpm}} = 0.8 \ \mu c \ I^{131}$$

We will want to continue measurement for about 16 days (i.e. 2 physical half-lives of I^{131}). In addition at least half of the plasma activity will be lost by extravascular distribution. In order to assess the biological homogeneity of our material we should follow it until at least 90% has been catabolized. Therefore, we need to inject 4 x 2 x 10 = 80 times more activity than is needed for immediate counting, i.e. 64 μc I^{131}, or about 1 μc/Kg. As shown above this load should give a total dose of only 0.08 rad or less than half of that permitted in tracer investigations.
(see Dr. Lippincott, H-51).

Summary:

1. Alpha-lipoproteins and their delipidated derivative will be prepared from sera of normal human donors.

2. Following labeling with I^{131} and sterilization, turnover in vivo and distribution of the radioiodinated protein in the other lipoprotein classes will be studied.

3. The human subjects will be injected with protein fractions separated from sera of normal subjects.

4. The amount of protein injected will be 1 - 2 mg.

5. To follow activity when it has decreased to only a few per cent of the injected dose for the turnover studies in a 70 Kg. subject, an initial activity of 1μc/Kg. of I^{131} will be needed, from which the estimated dose will be 0.08 rad.

6. Lugol's solution will be administered beginning 3 days prior to injection of radioactivity to block I^{131} uptake by the thyroid.

1176391

Request approved:

Elmer E. Stickley, Ph.D., Chairman

Walton W. Shreeve

Walton W. Shreeve, M.D., Ph.D.

Donald C. Borg, M.D.

J. S. Robertson

James S. Robertson, M.D., Ph.D., Ex Officio

Lee E. Farr

Lee E. Farr, M.D., Ex Officio

1176392