The Medical Research Center

Brookhaven National Laboratory

Upton, L. I., New York

R

The Committee on Clinical Investigations and Uses of Radioisotopes hereby approves the program with the following title: 725517

Human Catabolism of Radioiodinated Plasma Proteins in Neoplasia

CIRC # 1 has been assigned to this program.

Walton W. Shreeve

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

E. P. Cronkite

Eugene P. Cronkite, M.D.

E. Schackow

Eckart Schackow, M.D.

M. H. Van Woert

Melvin H. Van Woert, M.D.

J. S. Robertson

James S. Robertson, M.D., Ph.D. (ex officio)

REPOSITORY Records Holding Area, Bldg 494

COLLECTION Committee-Clinical Investigations and Uses of Radioisotopes

BOX No. 4

FOLDER CIRC #1

Date 2 Jan 64

Place Medical Research Center
Brookhaven National Laboratory
Upton, New York

1179337

FORM FOR INITIATION OR REVIEW OF CLINICAL

INVESTIGATIVE PROGRAMS

(Submit original only to Department Chairman)

A. Title of the proposal: Human Catabolism of Radioiodinated Plasma Proteins in Neoplasia

B. Sponsoring physician(s): J. S. Robertson

C. Responsible investigator(s): J. E. Jesseph, J. L. Bateman, S. H. Cohn

D. Brief description of the study, including its general goals and purpose, and pertinent information on past studies: (Attach additional sheets if necessary.)

This study is intended to extend observations already made in protein turnover in neoplastic diseases (Project H-51). In 46 patients with cancer of the breast, for example, there is evidence that there is a progressive shortening of half-life of labeled γ-2 globulin (as measured by whole-body counting) with progression of the malignant process. In patients with chronic cystic mastitis, and in normal controls, on the other hand, the biologic half-life is longer and less variable. It is hoped to confirm and amplify this observation, and further to determine whether the shortening of turnover time can be modified by therapy appropriate to the malignancy (i.e., radiation, chemotherapy, hormones). Together with basic laboratory investigations, this clinical project is intended to illuminate some aspects of the role of protein in the metabolic response to malignancy.

E. Reasons why the investigation(s) are to be performed on human subjects.

Vide supra.

F. Type of patient in which the study is to be done (including approximate number of subjects, if known; special restrictions or requirements; method of obtaining consent; etc.):

It is planned to accept patients with established malignancies of all stages of clinical advancement, with preference for those which traditionally respond best to systemic or local therapy (e.g., breast, lung, etc.). Approximately 50 to 100 patients will be needed, judging from past experience. About 12 control subjects with either low-grade chronic non-neoplastic diseases or with no disease will be needed for comparative purposes. Consent will consist of the patient's or subject's agreement to participate, having been fully apprised of nature, purposes and goals of the investigation.

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G. 1. Are drugs not in the U. S. Pharmacopoeia (USP) or the NNR being used or contemplated for use? Yes____ No x

2. Is an unusual use of a drug(s) accepted by the USP or NNR contemplated? (An example would be the use of an accepted drug in dosages far exceeding the recommended limits or for purposes distinctly different from the usual indications cited.) Yes____ No x

3. Are any biological products to be administered that do not bear on their containers or labels notation of approval by the Biological Control Division of the National Institutes of Health? (SEE ATTACHED SHEET) Yes x No____

4. Is external or internal radiation other than accepted diagnostic or therapeutic procedures to be administered? Yes____ No x

5. Are any (other) unusual procedures being performed or proposed which in your judgment may entail a special hazard - particularly a hazard above and beyond any imposed by accepted diagnostic and therapeutic measures for that patient? Yes____ No x

6. Are any radioisotopes to be administered to human beings? Yes x No____

a. If yes, are the radioisotopes to be used solely within the limits of procedures, specifically described in the USP? Yes____ No x

Describe the radioisotopic preparation(s):

b. Or are the radioisotopes to be used only in accordance with a project previously approved by the former Radioisotope Committee of this Department? Yes x No____

Note the project number: H-51

IF ANY OF QUESTIONS 1 THROUGH 5 ARE ANSWERED AFFIRMATIVELY, a detailed analysis of the potential hazards must be appended, including pertinent bibliographic citations and other relevant information.

IF QUESTION 6 IS ANSWERED AFFIRMATIVELY, a completed supplementary form for Radioisotope Administration to Human Beings must be appended. However, this form need not be filed provided that question 6a or 6b is also answered affirmatively. A separate form must be submitted for each radioisotopic species to be administered.

7/25/63 J. S. Robertson
Sponsoring Physician

Committee on Clinical Investigations and Uses of Radioisotopes

Approval recommended ✓ Date 1/2/64

Disapproval_____ Date_____

V. P. Bond JUN 29 1964
V. P. Bond, M. D.
Chairman, Medical Department

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RESPONSE TO G. 3.:

The human plasma protein fractions to be used in this investigation are those which have been in use for this purpose in this Center since 1957. The plasma is derived from normal volunteer donors and from a patient with a high level of myeloma (γ-2) globulin. The collection, fractionation, storage, assay, preservation and administration have been and are rigidly controlled. No adverse reaction has been observed in any of 400 administrations to nearly 200 individual subjects and patients. The details of methods used have been documented in a previous project request (H-51, dated and approved 6-15-67) and are unchanged with a single exception. We now use the iodination method of Helmkamp et al. (Univ. of Rochester Atomic Energy Project Report UR-568, Mar. 1960) rather than that of Hughes and Straessle (enclosure D, H-51).

Quality control of labeled protein fractions is carefully maintained. All fractions are lyophilized and have been stored in the dry frozen state for a minimum of two years. During the iodination process, antiseptic precautions are observed and the final step in preparation is sterile Seitz filtration into sealed, refrigerated vials. A sample from each preparation is cultured on thioglycollate agar.

To date, there has been no instance of positive culture, pyrogenic reaction, hypersensitivity phenomena, hepatitis or any other clinical or laboratory evidence of impurity of any of the preparations.

BROOKHAVEN NATIONAL LABORATORY

MEMORANDUM

DATE: 24 March 1971

TO: A. Harrison

FROM: E. P. Cronkite, M.D. E P Cronkite

SUBJECT: CIRC Numbers

The following CIRC #s are inactive. Except for completing old records, they should not be used for identifying patient activity until reactivation is formally approved.

Principal Investigator	CIRC #
Dr. Cronkite	3
	4
	20
	23
	35
	35A
Dr. Dahl	5
	6
	14
	25
Dr. Jesseph	1
Dr. Robertson	9
	10
	16
	28
Dr. Schiffer	22

EPC/ck
cc: CIRC Committee
Mr. Finn
Dr. Dahl

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BROOKHAVEN NATIONAL LABORATORY

MEMORANDUM

DATE: 15 June 1957

TO: Lee E. Farr, M.D.

FROM: S. W. Lippincott, M.D.

SUBJECT: Signatures of Isotope Committee for Approval of Accompanying Request Concerning I-131 Labeled Human Protein Fractions - Project H-51

Lee E. Farr
L. E. Farr, M. D.

E. Stickley
E. E. Stickley, Ph. D.

J. S. Robertson
J. S. Robertson, M. D.

E. P. Cronkite
E. P. Cronkite, M. D.

L. K. Dahl
L. K. Dahl, M.D.

V. P. Bond
V. P. Bond, M. D.

R. A. Love
R. A. Love, M. D.

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BROOKHAVEN NATIONAL LABORATORY

MEMORANDUM

DATE: 15 June 1957

TO: Dr. Lee E. Farr

FROM: Stuart W. Lippincott, M.D.

SUBJECT: Permission from Isotope Committee to Administer I-131 Labelled Human Protein Fractions to Clinically Investigated Normal Subjects and to Patients with Neoplastic Disease. Project H-51

Investigators: S. W. Lippincott, W. Wolins, C. T. O. Fong, E. E. Stickley and W. L. Hughes

Enclosures:

A. Turnover values reported in the literature for radioactive labelled (I^{131}) human serum albumin and gamma globulin in man.

B. Chart from: Preparation and properties of serum and plasma proteins. IV. A system for the separation into fractions of the protein and lipoprotein components of biological tissues and fluids. J. Am. Chem. Soc. 68, 459, 1946.

C. Chart from: Biophysical studies of blood plasma proteins. VII. Separation of gamma globulin from the sera of various animals. J. Am. Chem. Soc. 70, 80, 1948.

D. Chart from: Preparation and properties of serum and plasma protein. XXIV Iodination of human serum albumin. J. Am. Chem. Soc. 72, 452, 1950.

E. Patients from whom blood has been drawn for fractionation following clinical study.

F. References

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Introduction:

The present research to be undertaken is an extension of clinical investigations that have been in progress for several years (1 - 9). The original (and current) objective was and still is a thorough study of the properties (and function) of the serum proteins in neoplastic diseases. Should any distinctive characteristic(s) exist for any given protein fraction distinguishing it from a similar fraction in normal subjects or those with various non-neoplastic diseases, a basis might be established for a diagnostic test for cancer. (More specifically, a test establishing site of origin would obviously be of greater clinical value than a single screening test, although the latter would have a true value in establishing or ruling out a diagnosis of cancer). To date, the principal approach has been a detailed investigation of the physico-chemical nature of crystalline albumin and the globulins utilizing technics involving; (1) immunochemical procedures, (2) electrophoresis, (3) ultracentrifugation and (4) infrared spectroscopy. The results of these studies have been reported in the references cited (1 - 9).

Objective:

The purpose of the projected research is to determine whether the normal individual, when administered I^{131} labelled protein fractions, has similar or different turnover rates from the patients with various neoplastic diseases receiving identical fractions. Further experiments are designed to determine whether these same fractions, when administered to rabbits, have the same or different turnover rates. The final series of experiments has been devised to determine whether <u>in vivo</u> localization of $gamma_2$ globulin carrying anti-cancer antibody occurs at the site(s) of tumor formation and whether this is absent when only normal (no antibody) $gamma_2$ globulin is tested.

In animal neoplasia, we have shown that in the case of the rabbit Brown-Pearce tumor, the latter may be used as a source of antigen in a complement fixation test that is 95% sensitive and 100% specific. The antibody is absent in the albumin fraction and present in the globulin fraction (5 fold compared to whole serum and the greatest amount is in the $gamma_2$ globulin fraction). In unpublished data, with far less sensitivity and specificity, we have demonstrated complement fixation from the sera of man with various histologic types of carcinoma. In these experiments, it is assumed that the neoplasm in the patient will act as the antigen and that the injected $gamma_2$ globulin carrying antibody will unite with it. Such an antigen - antibody union might therefore occur with the antibody containing $gamma_2$ globulin but not with the normal $gamma_2$ globulin. This event might then be detected by <u>in vivo</u> counting and differences in turnover of normal and "cancer" $gamma_2$ globulin are also conceivable. This concept is entirely different from the non-specific concentration of I^{131} labelled albumin used in brain tumor localization (22,23) where concentration is 10-15% more in tumors than in normal tissue. Some increased concentration by the latter technique has also been found in liver metastases (24). In actuality, the groups of experiments will be as follows:

A. Autologous radioactive iodinated albumin and $gamma_2$ globulin turnover in clinically investigated normal subjects.

B. Homologous radioactive iodinated $gamma_2$ globulin turnover in normal subjects and those with carcinoma.

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C. In vivo localization of radioactive iodinated $gamma_2$ globulin (from normal subjects and patients with carcinoma) in patients with known metastatic carcinoma.

D. Turnover of albumin and $gamma_2$ globulin (from normal subjects and patients with carcinoma) in rabbits.

Methodology:

Thus far no autologous study of turnover of albumin or $gamma_2$ globulin in man has been reported in the literature. Such information should be as valuable, if not more so than homologous studies. In our plan, both autologous and homologous studies will be carried out. The review of the literature summarized in enclosure A pointed out a number of undesirable conditions in previous experiments that should be avoided. In general, previous investigators have utilized a commercially prepared product from a pool (I^{131} albumin or globulin, no $gamma_2$ fraction) so that the source of the sera is unknown. Presumably these sera came from healthy individuals but this is not an established fact. The turnover studies have been made on such groups as medical students and ambulatory laboratory workers. No observations have been recorded concerning the actual health status of these subjects, so that at best, they are only presumably healthy. In some instances, convalescent hospital patients have been combined with the so-called normals. In reporting the half life, some papers have not shown by graph or table any data, and in others, frequently only a minimum number of blood samples have been taken. This makes it difficult to estimate the validity of the observations with reference to compartments and interpretation of the findings.

In order to make the current study as meaningful as possible, a normal subject is defined as a person feeling in good health and found free of disease after being hospitalized (on a voluntary basis) and investigated clinically as thoroughly as any patient suspected of having a neoplastic disease. A donor (from whom blood fractions are prepared) is a normal subject so defined. (Pairing by sex in age groups is made). Such a person is bled and the serum proteins are separated by one of two technics e.g. enclosures B and C. The degree of homogeneity of the fractions is established by determining the electrophoretic pattern and sedimentation constant. Iodination is done by the method of Hughes and Strassle, enclosure D.

As indicated, I^{131} has been selected for labelling although other labels may be used such as N^{15} and S^{35}, depending principally on whether one elects a technique dependent on incorporation or upon rate of loss of label from protein. For this study, endogenous labelling of the plasma protein (labelled amino acid administered to patient orally or parenterally) or endogenously labelled homologous plasma protein fractions tranferred from a donor to a recipient (so-called standard method of Marger and Tarver) are unsuitable. Technically and for chemical reasons, iodination with I^{131} in vitro is most satisfactory. Both half life in days and ratio of circulation to total albumin may be determined at intervals.

The tagged fractions are to be tested for sterility (by filtering and culture) prior to administration to patients. The amount of such protein given will be 10 milligrams so that it cannot have any significant effect on the albumin or $gamma_2$ globulin concentration in the subject or upon protein metabolism. The

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preparations will be given intravenously and the samples taken over a 21-day period for in vitro counting. The decrease in radioactivity in successive samples of sera will be plotted so that radioactivity is on the ordinate and time on the abscissa. This curve may then be divided into three segments: (1) immediate mixing within the blood stream; (2) equilibration - the mixing of the iodinated fraction with the extravascular fluid (exchangeable pool); and (3) the metabolic degradation (turnover) of the isotopically labelled protein.

Certain routine procedures will be carried out on all subjects: (1) stable diet throughout experiment; (2) body weight, albumin and globulin levels: before, during and at end of experiment; (3) Lugol's solution (15 drops daily) two days prior to and during the studies (to prevent thyroid uptake and promote excretion of I^{131} liberated on degradation of the iodo-albumin); (4) external in vivo survey (count) over thyroid to confirm that no significant thyroid uptake occurs and; (5) since the number of microcuries of I^{131} per milligram of administered protein fraction will be known, the method of reporting results will be a semilogarithmic plot of count per minute representing the known number of milligrams of labelled fraction versus tissue in hours and days.

Dosimetry and counting considerations:

In calculating whole-body radiation dose from I^{131}, the customary figure has been 0.284 rep (25,26) from one microcurie per kilogram body weight, assuming no excretion; when an effective half life of 4.63 days is used, the figure becomes: $0.164 \frac{rep}{\mu c/kg}$.

Current practice prefers the use of the rad as unit of radiation dose. The appropriate factor for use in calculation becomes: $0.1525 \frac{rads}{\mu c/kg}$ from which the amount of I^{131} to deliver the allowed level of 0.200 rads for a tracer investigation is found to be 1.31 µc/kg. A subject of 50 kg body weight may be given 65 µc as a tracer under the above assumptions and stipulations.

For an approach to the present problem, we can use the 4.63 day half life reported as the effective half life of RISA. Thus, at the end of 21 days, about 5% of the initial levels can be expected to persist. Minimum counting requirements of about 500 cpm per sample of 4 ml require an initial activity of:

$$\frac{500 \text{ cpm}}{4 \text{ ml}} \times 5 \times 10^3 \text{ ml} \times \frac{1 \mu c \ I^{131}}{0.8 \times 10^6 \text{ cpm}} \times \frac{1}{5\%} = 16 \ \mu c \ I^{131}$$

This is the quantity needed for the initial administration to provide minimum counting (estimated) levels at 3 weeks in a 70 kg subject. The plasma dose (total) will be:

$$0.1525 \frac{rads}{\mu c/kg} \times \frac{16 \ \mu c}{70 \text{ kg}} = 0.035 \text{ rads}$$

Customary tracer levels are five times greater; permitted values could be ten or twenty times the figure computed here.

Localization studies will be attempted with the cancer patients included in this study series. A spectrometer type scintillation detector system will be

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utilized, based on the whole-body scanning equipment of the in vivo counting room. Since the quantity of radioactivity needed for this procedure is much higher, it is proposed to use the maximum allowable levels with these subjects, the localization measurements at the site of known metastases taken at 1/4, 1/2, 1 ... to 6 hours.

Summary:

Albumin and $gamma_2$ globulin are being prepared from normal subjects and patients with neoplastic diseases. Following labelling with I^{131} and sterilization, turnover and in vivo localization studies will be performed. Normal subjects will be injected only with fractions from normal subjects, but patients with neoplastic diseases will receive fractions prepared from normal subjects and patients with carcinoma. The amount of protein injected will be 10 milligrams. To obtain minimum counting at 3 weeks for the turnover studies in a 70 kg subject, an initial activity of 16 μc I^{131} will be needed. The plasma dose from this will be 0.035 rads. Customary tracer doses are up to five times greater and may be desirable in these studies. For the in vivo studies the upper permissible value, up to 20 times the suggested minimal dose, a total of 320 microcuries I^{131} may be necessary to obtain satisfactory in vivo counts because of the very small amount of antibody, percentage-wise, contained in the $gamma_2$ globulin fraction prepared from the patients with neoplastic diseases.

Encls. A, B, C, D, E, and F

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Enclosure A

TURNOVER VALUES REPORTED IN THE LITERATURE FOR RADIOACTIVE-LABELLED (I^{131}) HUMAN SERUM ALBUMIN AND GAMMA GLOBULIN IN MAN

REFERENCES	CONTROLS (NORMALS) Number	Age	Types of Individuals	HALF-LIFE (DAYS) Albumin	Gamma Globulin	VARIOUS DISEASES Number	Age		HALF-LIFE (DAYS) Albumin	Gamma Globulin
Sterling, K. J. Clin. Invest. 30, 1228 (1951)	21		Medical Students	10.5 ± 1.5						
Vaughn, J.H., Armato, A., Goldthwaite, J., Brachman, P., Favour, C.B. and Bayles, T.B. - J. Clin. Invest. 31, 668 (1952) - Abstract only	4		"Normals"		7.0 - 8.8					
Ibid						4		Rheumatoid Arthritis		1.8
Dixon, F.J., Talmage, D.W., Maurer, P.M. and Diechmiller, M. J. Exper. Med. 96, 313 (1952)	14	32-72	Not Indicated	13.1 ± 2.8						
Berson, S.A., Yalow, R.S., Schreiber, S.S. and Post, J. J. Clin. Invest. 32, 746 (1953)						46	22-69	Variety of Non-Neoplastic Diseases	9 - 24	
Freinkel, N., Schreiner, G.E. and Athens, J.W. J. Clin. Invest. 32, 138 (1953)	10	18-35	Medical Students & Convalescent Hospital Patients	9.2 ± 1.2						
Havens, W.P., Dickensheets, J., Bierly, J.N. and Eberhard, T.P. J. Clin. Invest. 33, 136 (1954)	9		Normal Subjects & Convalescent Patients		10.6 - 16.6 Av. 13.5	6		Hepatic Cirrhosis	Av. 7 5.2 - 10.8	
Dixon, F.J., Maurer, P.M. and Deichmiller, M.P. Proc. Soc. Exper. Biol. & Med. 83, 287 (1953)						11		10 Non-Neoplastic Diseases and 1 Post-operative carcinoma bladder	15	
Eisenmenger, W.J. and Slater, P.J. J. Clin. Invest. 32, 564 (1953) - Abstract only			No Subjects Mentioned but Reference made to Normal Values	3.5 - 11						
Ibid	8		"Normals"	XXXXXXX	11.5 - 19	25		Cirrhosis	9.5 - 11	6 - 13
Armstrong, S.H., McLeod, K., Wolter, J. and Kukral, J. J. Lab. & Clin. Med. 43, 918 (1954)	3		"Initially Normal"	11 - 19		2		Cirrhosis and Amyloid Nephrosis	11 - 15	
Sterling, K., Lipsky, S.R. and Freedman, J. Metabolism 4, 343 (1955)	8		Medical Students	11 (Mean)		10		Post-operative non-neoplastic cases	4.6 - 7.3	
Schwartz, E. J. Lab. & Clin. Med. 45, 340 (1955)						2		Myxedema	(About) 27	
Marger, S. and Tarver, I. J. Clin. Invest. 35, 1161 (1956)	7	20-45	Ambulatory Laboratory Workers	9-13 (short term study)						
	8	20-45	Ambulatory Laboratory Workers	8-10 (long term study, fast component) 15-25 (long term study, slow component)						

Lippincott, Wolins, Fong, Stickley and Hughes

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Enclosure B

PREPARATION AND PROPERTIES OF SERUM AND PLASMA PROTEINS. IV. A SYSTEM FOR THE SEPARATION INTO FRACTIONS OF THE PROTEIN AND LIPOPROTEIN COMPONENTS OF BIOLOGICAL TISSUES AND FLUIDS

Cohn, E.J., Strong, L.E., Hughes, W.L., Jr., Mulford, D.J., Ashworth, J.N., Melin, M., and Taylor, H.L.

J. Am. Chem. Soc. 68: 459-475 (1946)

Method 6

Plasma

ethyl alcohol = 8%
temperature = -3°C
protein = 5.1%
pH = 7.2
r/2 = 0.14

Supernatant I
ethyl alcohol = 25%
temperature = -5°C
protein = 3%
pH = 6.9
r/2 = 0.09

Ppt I
(mainly fibrinogen)

Supernatant II + III
ethyl alcohol = 18%
temperature = -5°C
protein = 1.6%
pH = 5.2
r/2 = 0.09

Ppt II + III
(mainly Beta and Gamma Globulins)

Supernatant IV -1
ethyl alcohol = 40%
temperature = -5°C
protein = 1.0%
pH = 5.8
r/2 = 0.09

Ppt IV -1
(mainly alpha globulins)

Supernatant IV -4
ethyl alcohol = 40%
temperature = -5°C
protein = 0.8%
pH = 4.8
r/2 = 0.11

Ppt IV -4
(Mainly alpha and beta globulins)

Supernatant V

Ppt V
ethyl alcohol = 10%
temperature = -3°C
protein = 3%
pH = 4.5
r/2 = 0.1

Supernatant
ethyl alcohol = 40%
temperature = -5°C
protein = 2.5%
pH = 5.2
r/2 = 0.01

Impurities

Supernatant

Albumin

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BIOPHYSICAL STUDIES OF BLOOD PLASMA PROTEINS.
VII. SEPARATION OF GAMMA GLOBULIN FROM THE SERA OF VARIOUS ANIMALS

Nichol, J.S. and Deutsch, H.F.
J. Am. Chem. Soc. 70, 80 (1948)

Serum: 1 vol. diluted with 3 vols. H_2O
0.05 M HAc or 0.05 M Na_2HPO_4 to pH 7.7; 50% EtOH
20%

↓ Ppt A (Crude Gamma Globulins)
Suspended in dil. NaCl
to give 0.5 to 1% protein suspension
0.05 M HAc to pH 5.1
50% ETOH to 15% temp. 0 to -5°C
μ2 0.01

↓ Supernatant A

↓ Ppt B (largely Beta + $Gamma_1$ Gloublins)

↓ Supernatant B

Either
0.05 M Na_2HPO_4 to pH 5.6 to 6.0
50% ETOH to 12%
μ = 0.005 to 0.01 temp. -3.5°C

↓ Ppt C_1 (largely $Gamma_1$ Globulins)

Supernatant

or

0.05 M Na_2HPO_4 to pH 7.2 to 7.4
50% ETOH to 25% temp. -10°C
μ = 0.007 to 0.015

Centrifuge

↓ Ppt C_2 ($Gamma_2$ Globulin)
Suspended, frozed dried in vacuo

↓ Supernatant

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Enclosure D

PREPARATION AND PROPERTIES OF SERUM AND PLASMA PROTEIN XXIV

IODINATION OF HUMAN SERUM ALBUMIN

W. L. Hughes and R. Straessle
J. Am. Chem. Soc. 72, 452 (1950)

Iodination with I^{131}

The object of the iodination is to put in the protein molecule, one or two atoms of iodine with the purpose of preparing a protein derivative which could be followed in its course of metabolism.

Iodinating reagent is iodine dissolved in two moles potassium iodide and the following conditions are to be used:

Iodination of the Protein

1. Temperature kept low as possible
2. Protein concentrate kept between 2 - 10%
3. pH kept alkaline around pH 9.5
4. Iodination to be carried out with 2 millicuries I^{131}

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Enclosure F

REFERENCES

1. Studies of Various Tests for Malignant Neoplastic Diseases. (An Editorial)
   Lippincott, S. W. and Dunn, J. E., Jr.
   Am. J. Clin. Path. 20, 655 (1950)

2. Studies of Various Tests for Malignant Neoplastic Diseases. VI. Tests for the Least Coagulable Serum Proteins and the Idoacetate Index.
   Ellerbrook, L. D., Meek, E. D. and Lippincott, S. W.
   J. Nat. Cancer Inst. 12, 49 (1951)

3. Studies of Various Tests for Malignant Neoplastic Diseases. VIII. The Determination of Plasma or Serum Mucoprotein.
   Rhees, M. C., Ellerbrook, L. D. and Lippincott, S. W.
   Am. J. Clin. Path. 24, 774 (1954)

4. The Precipitation of Serum Proteins by Acidified Sodium Orthovanadate in Neoplastic and Other Diseases.
   Eriksen, N., Ellerbrook, L. D. and Lippincott, S. W.
   Cancer Research 14, 145 (1954)

5. Comparison of Physicochemical Properties of Crystalline Human Serum Albumin in Neoplasia.
   Lippincott, S. W., Eriksen, N., Fong, C.T.O. and Thornton, H.
   Proc. Am. Soc. Exp. Biol. and Med. 13, 1431 (1954)

6. Serological Activities of Serum Fractions in Animal Neoplasia.
   Lippincott, S.W., Fong, C.T.O. Thornton, H. and Ellerbrook, L.D.
   J. Nat. Cancer Inst. 15, 1231 (1955)

7. Serum Protein Analysis by Electrophoresis and by the Wolfson-Cohn Chemical Method.
   Eriksen, N., Ellerbrook, L. D. and Lippincott, S. W.
   Clin. Chem. 2, 334 (1956)

8. Infrared Spectroscopy of Crystalline Albumin in Human Neoplasia.
   Fong, C.T.O., Lippincott, S. W. and Eriksen, N.
   J. Nat. Cancer Inst. 18, 271 (1957)

9. Complement Fixation in Animal Neoplasia. VI. Development of the Reaction in Various Breeds of Rabbits Carrying the Brown-Pearce Carcinoma.
   Lippincott, S. W., Thornton, H., Ellerbrook, L. D., Rhees, M.C. and Fong, C.T.O.
   J. Nat. Cancer Inst. 14, 395 (1953)

10. The Turnover Rate of Serum Albumin in Man as Measured by I^{131}-Tagged Albumin.
    Sterling, K.
    J. Clin. Invest. 30, 1228 (1951)

11. Fate of Intravenously Injected Labelled Gamma Globulin in the Normal Subject and in Patients with Rheumatoid Arthritis.
    Vaughn, J.H., Armato, A., Goldthwaite, J., Brachman, P., Favour, C.B. and Bayles, T.B.
    J. Clin. Invest. 31, 668 (1952) - Abstract only

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12. The Half-Life of Homologous Gamma Globulin (Antibody) in Several Species.
Dixon, F.J., Talmage, S.W., Maurer, P.M. and Deichmiller, M.
J. Exper. Med. 96, 313 (1952)

13. Tracer Experiments with I^{131} Labelled Human Serum Albumin: Distribution and Degradation Studies.
Berson, S.A., Yalow, R.S., Schreiber, S.S. and Post, J.
J. Clin. Invest. 32, 138 (1953)

14. Simultaneous Distribution of T-1824 and I^{131} Labelled Human Serum Albumin in Man.
Freinkel, N., Schreiner, G.E. and Athens, J.W.
J. Clin. Invest. 32, 138 (1953)

15. The Half-Life of Normal Human Gamma Globulin in Patients with Hepatic Cirrhosis.
Havens, W.P., Dickensheets, J., Bierly, J.N. and Eberhard, T.P.
J. Clin. Invest. 33, 136 (1954)

16. Half-Lives of Homologous Serum Albumins in Several Species.
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