887

712311

February 17, 1982

Richard G. Besha
GC-42 Patents MS6F-055, FORSTL
1000 Independence Avenue, S.W.
Washington, D.C. 20585

S.N. 755,100(70)

We have examined the Board of Appeals decision affirming the Examiner's decision finally rejecting all remaining claims in the subject case and are of the opinion that further action by DOE is unwarranted in view of the close art and the non-energy-related subject matter of the application.

[illegible] SIGNED BY [illegible]

Stephen D. Hamel
Assistant Patent Counsel
for Patent Prosecution
Oak Ridge

CC-101:SDH:djr

August 31, 1979

MEMORANDUM FOR R. V. Lupo
Deputy Assistant General Counsel
for Patent Operation
Germantown, CXXI, A2-3018

APPLICATION SERIAL NO. 755,100(70)

Transmitted herewith is a Reply Brief in the above-identified application.

This Reply Brief must be filed by September 10, 1979.

ORIGINAL SIGNED BY
STEPHEN D. HAMEL
Stephen D. Hamel
Assistant Patent Counsel
for Patent Prosecution
Oak Ridge

CC-101-AHU:dlm
Attachment

Department of Energy
Washington, D.C. 20545

August 23, 1979

Stephen D. Hamel
Assistant Patent Counsel for
Patent Prosecution
Office of Patent Counsel
Oak Ridge Operations Office

U.S. PATENT APPLICATION S.N. 755,100 "AGENT AND METHOD FOR EARLY DETECTION OF PREGNANCY"

Attached is the Examiner's Answer in the above-identified case.

If you wish to file a Reply Brief, it is due on September 10, 1979.

R. V. Lupo
Deputy Assistant General Counsel
for Patent Operations

Attachment

March 30, 1979

MEMORANDUM FOR Dean E. Carlson, Chief
Prosecution Branch
Patents, HQ
Germantown, CXXI, A2-3018

APPL. SER. NO.: 755,100
FILED: December 28, 1976
FOR: AGENT AND METHOD FOR EARLY DETECTION OF PREGNANCY

Enclosed is an Appeal Brief in the above docket.

The Appeal Brief is due in the Patent Office by April 13, 1979.

ORIGINAL SIGNED BY
STEPHEN D. HAMEL

Stephen D. Hamel
Assistant Patent Counsel
for Patent Prosecution
Oak Ridge

CC-101:AHU:br
5 Attachments

Department of Energy
Washington, D.C. 20545

December 21, 1978

Stephen D. Hamel
Assistant Chief for Prosecution
Office of Patent Counsel, Oak Ridge

U. S. APPLICATION S.N. 755,100 - S-47,844
FILED: DECEMBER 28, 1976
FOR: AGENT AND METHOD FOR THE EARLY DETECTION
OF PREGNANCY

Enclosed herewith for your information and file is a copy of an Advisory Action dated December 14, 1978 in the above-identified application.

At the present we plan to file a Notice of Appeal early in February.

Dean E. Carlson

Dean E. Carlson, Chief
Prosecution Branch, Patents

Enclosure:
Advisory Action

November 7, 1978

Dean E. Carlson, Chief, Prosecution Branch, Patents, HQ
Germantown, CXXI, A2-3018

Case No.: S.N. 755,100(70)
Filed : December 28, 1976
For : AGENT AND METHOD FOR THE EARLY DETECTION OF PREGNANCY

Enclosed is a Request for Reconsideration in the above docket. An allowance is not expected, however, in view of previous responses and a telephone interview.

The subject pregnancy test has stimulated enormous interest since the inventor first published his results. He has received over two hundred requests for reprints and several inquiries concerning patent licenses or assignments from representatives of pharmaceutical firms including Hyland Laboratories and Parke-Davis.

While the art is fairly close, it is believed that an appeal is warranted. Before filing an appeal, however, we would prefer that headquarters review the prosecution file in light of the references in order to give us an independent assessment of the merits of the case. Copies of the references are enclosed. We regret that we have no formal translations of the German references, however, their content is believed to be fairly represented in the prosecution history.

ORIGINAL SIGNED BY
STEPHEN D. HAMEL

Stephen D. Hamel
Assistant Patent Counsel
for Patent Prosecution
Oak Ridge

MCP:AHU/br

Enclosures:
1. Request for Reconsideration
2. References

Department of Energy
Washington, D.C. 20545

October 25, 1978

Stephen D. Hamel
Assistant Chief for Prosecution
Office of Patent Counsel, Oak Ridge

U. S. APPLICATION S.N. 755,100 - S-47,844
FILED: DECEMBER 28, 1976
FOR: AGENT AND METHOD FOR THE EARLY DETECTION
OF PREGNANCY

Enclosed herewith for your consideration is a copy of an Office Action dated October 17, 1978 in the above-identified application.

Please note that the Response will be due in the U. S. Patent and Trademark Office by January 17, 1979.

Dean E. Carlson

Dean E. Carlson, Chief
Prosecution Branch, Patents

Enclosure:
Office Action

July 21, 1978

Dean E. Carlson, Chief, Prosecution Branch, Patents, HQ
Germantown, CXXI, A2-3018

APPL. SER. NO.: 755,100(70)
FILED: December 28, 1976
FOR: AGENT AND METHOD FOR THE EARLY DETECTION OF PREGNANCY

Enclosed is an amendment in the above docket. The amendment

(X ) adds no claims in excess of those previously paid for.

( ) adds ____ additional independent claims at $10 each $_____

( ) adds ____ additional claims at $2 each _____

Total Fee . . . _____

Response is due in the Patent Office by August 6, 1978.

ORIGINAL SIGNED BY
D. S. ZACHRY

Stephen D. Hamel
Assistant Patent Counsel
for Patent Prosecution
Oak Ridge

MCP:AHU/br

Enclosure:
Amendment (in dupl.)

Department of Energy
Washington, D.C. 20545

April 10, 1978

Stephen D. Hamel
Assistant Chief for Prosecution
Oak Ridge Patent Group

U. S. APPLICATION S.N. 755,100 - S-47,844
FILED: DECEMBER 28, 1976
FOR: AGENT AND METHOD FOR THE EARLY DETECTION OF PREGNANCY

Enclosed herewith for your consideration is a copy of an Office Action dated April 6, 1978 in the above-identified application, together with the necessary references.

Please note that the Response will be due in the U. S. Patent and Trademark Orrice by July 6, 1978.

Dean E. Carlson

Dean E. Carlson, Chief
Prosecution Branch, Patents

Enclosures: 6 K
Office Action
References (2)

January 11, 1978

Dean E. Carlson, Chief, Prosecution Branch, Patents, HQ
Germantown, CXXI, A2-3018

APPL. SER. NO.: **755,100(70)**
FILED: **December 28, 1976**
FOR: **AGENT AND METHOD FOR THE EARLY DETECTION OF PREGNANCY**

Enclosed is an amendment in the above docket. The amendment:

( X) adds no claims in excess of those previously paid for.

( ) adds _____ additional independent claims at $10 each $_____

( ) adds _____ additional claims at $2 each _____

Total Fee . . .$_____

Response is due in the Patent Office by **January 19, 1978.**

Stephen D. Hamel,
Asst. Chief for Patent Prosecution
Oak Ridge Patent Group

MCP:

Enclosure:
Amendment (in dupl.)
**Declaration (in dupl.)**
**Prior Art Letter (in dupl.)**
**References**

January 10, 1978

The Commissioner of Patents
Washington, D. C. 20231

Sir:

Applicant : David M. Goldberg
Serial No.: [illegible]
For : [illegible] AND METHOD FOR THE EARLY DETECTION OF PREGNANCY

The following references have recently been discovered by applicant's attorney and are believed to be relevant to the claimed invention.

1. Wald, Nancy J., "Comparative Value of Immunologic and Non-Immunologic Tests for Pregnancy Diagnosis," Am. Clin. Path. (1968), 50, pp. [illegible].

This article compares the accuracy of several prior art pregnancy detection methods. It is clear that of the tests studied, all had reduced accuracy when used in the early stages of pregnancy. It is clear from page 82, second column, that the tests were based on HCG, rather than a mixture of proteins as the claimed invention.

2. Horwitz, Charles A., et al., "Evaluation of a New Pregnancy Test," Obstet. and Gynec., 40 (Oct. 1972), pp. 503-508.

This article shows in Table I that the subject test was only 32% accurate at 31-40 days from LMP and 92% accurate at 41-50 days from LMP.

3. Lamb, Ernest J., "Immunologic Pregnancy Tests," Obstet. and Gynec., 39 (May, 1972), pp. 595-598.

This article describes several tests based on HCG. Of the tests described, at least one gave false negative in 13 of 50 samples and false positive in 11 of 50 samples. The factors which account

for false positives or false negatives are described on pp. 670-671. The claimed test which detects a plurality of pregnancy-specific antigens would avoid these difficulties since the presence of additional specific antigens would tend to confirm a positive result.

4. Dietrich, Michael, et al., "Evaluation of four slide test kits for the detection of human chorionic gonadotropin in urine.", CMA Journal, August 3, 1974/Vol. 111, pp. 235-237.

This article describes pregnancy tests using HCG antiserum. The sensitivities of the tests are given in Table III and the production of HCG during pregnancy is shown in Fig. 1. It is clear that tests capable of detecting only one HCG would be less reliable in the early stages, and that enhanced reliability would result from applicant's test which is capable of detecting several specific antigens early in pregnancy.

5. U. S. Patent 4,055,449, issued December 27, 1977 to Kohn, et al., for "Pregnancy Specific β1-glycoprotein and process for isolating it."

This recent patent would appear to demonstrate the prior art use of a single protein for immunological tests. A copy is available of this reference.

6. Hodgen, Gary D., et al., "Pregnancy Diagnosis by a Hemagglutination Inhibition Test for Urinary Macaque Chorionic Gonadotropin (MCG)", J. Clin. Endocrinol Metab. 38: 927 (1974).

This reference indicates that even in primates, the chorionic gonadotropin tests are prone to error in the early stages of pregnancy.

7. Lin, Tzue-Ming, et al., "Characterization of four human pregnancy-associated plasma proteins", Amer. J. Obstet. and Gynec., 119 (1974) 225-236.

This article describes the presence of pregnancy-associated [illegible] proteins, however there is no teaching that the proteins were present in pregnancy serum or urine sufficiently early during gestation to be useful in a pregnancy test.

8. Lin, et al., "Measurement of pregnancy-associated plasma proteins during gestation and their immunological identification", Intersociety 448.

This reference describes several pregnancy-associated proteins, however it is not indicated whether they are present sufficiently early in gestation to be useful in a pregnancy test.

9. Berne, Bernard M., "Alpha-2 Pregnoglobulin Levels - A Test of Placental Function in Early Pregnancy," Intersociety, 490.

This reference describes a protein which is also present in males and apparently non-pregnant females.

10. Bohn, Von Hans, "Isolierung und Charakterisierung des schwanger-schafts-spezifischen β-1-Glykoproteins," Gynec., Band XXIV, Seite 292-302 (1972).

This reference describes the isolation of a β-glycoprotein. There is no suggestion to prepare antiserum from a mixture of placental pregnancy-specific proteins.

11. Hofmann, R., et al., "Immunochemical Investigations on the Problem of the 'Pregnancy Zone'," Arch. Gynak. 208, 286-294 (1970) (translation).

This article deals with the presence of pregnancy antigens and the use as antiserum of a mixture of absorbed antipregnancy and antiplacenta immune serum. There is no teaching that any of the detected antigens were present sufficiently early in pregnancy to be useful in a pregnancy test.

12. Hofmann, R., et al., "Immunochemical Investigations on the Problem of the 'Pregnancy Zone'," Arch. Gynak. 208, 187-195 (1969) (translation).

This article describes the production of antiplacental antisera. In the method described, there was no attempt to separate pregnancy-specific antigens from non-specific antigens, prior to injection into the host. There was no teaching that the antiserum prepared would be useful for detecting a plurality of antigens which appear in early pregnancy.

Sincerely,

Allen H. Ostoff
Attorney for applicant
Oak Ridge Patent Group

HCP:AHO/br

Enclosures:
References

**MEMO ROUTE SLIP**
Form ERDA-93 (1-75) ERDAM 02

☐ See me about this. ☐ Note and return. ☐ For concurrence. ☐ For signature. ☐ For action. ☐ For information.

| TO (Name and unit) | INITIALS | REMARKS |
|---|---|---|
| Dean Carlson<br>Office of Asst. General Counsel for Patents<br>Germantown, CXXI, A2-3018 | DATE | Attached is a Request for Extension of Time in S.N. 755,100(70) - Holladay |
| TO (Name and unit) | INITIALS / DATE | REMARKS |
| TO (Name and unit) | INITIALS / DATE | REMARKS |
| FROM (Name and unit)<br>Allen Uzzell<br>Oak Ridge Patent Group | | REMARKS |
| PHONE NO. | DATE | |

USE OTHER SIDE FOR ADDITIONAL REMARKS

GPO : 1975 O—577-369

**MEMO ROUTE SLIP**
Form ERDA-93 (1-75) ERDAM 0240

☐ See me about this. ☐ Note and return. ☐ For concurrence. ☐ For signature. ☐ For action. ☐ For information.

| TO (Name and unit) | INITIALS | REMARKS |
|---|---|---|
| Mr. David S. Zachry, Jr., Chief<br>Oak Ridge Patent Group<br>Energy Research and Development Administration | DATE | |
| TO (Name and unit) | INITIALS / DATE | REMARKS |
| TO (Name and unit) | INITIALS / DATE | REMARKS |
| FROM (Name and unit) | | REMARKS |
| PHONE NO. 23-35093 | DATE 9/21/77 | |

USE OTHER SIDE FOR ADDITIONAL REMARKS

GPO : 1975 O--577-369

1075749

Don Clair

Please show this to David Holladay and ask whether this man or his group inquired into his pregnancy test results or asked for a reprint of the May 3, 1977 - Knoxville Journal article.

Allen

# Pregnancy test nearly perfect

By The Associated Press

BUFFALO, N.Y. — A refined pregnancy test has been found to be 99.5 per cent accurate in 544 cases evaluated at Deaconess Hospital here.

Dr. Jack Lippes, chairman of the obstetrics at the hospital, said the new blood test can confirm pregnancy in a woman as early as one day after menstruation was to have begun.

Furthermore, the test can indicate at that early date if the pregnancy is abnormal, he said.

The most accurate test now used is 98 per cent accurate, said Lippes, who is best-known for his invention of the Lippes Loop birth control device.

Like other pregnancy tests, the new one is based on the presence of a hormone called human Chorionic Gonadotrophin. The hormone is produced by the human placenta beginning early in pregnancy.

The refinement involves a quantitative analysis of hCG in the patient's blood, Lippes said. The test is based on the work of Dr. Om P. Bahl, chairman of cell molecular biology at the State University at Buffalo, who first isolated and purified the hCV molecule in 1972.

Because the amount of hCG produced in early pregnancy multiplies geometrically, periodic readings that show a lesser increase would indicate an abnormal fetus developing, Lippes said.

In the 11-month study of the new test, 184 women tested positively and 360 negatively. There were no cases where the test said a woman was pregnant when, in fact, she was not. False positive readings sometimes occur in tests now used.

Three false negative readings were recorded, Lippes said. In all three cases the women miscarried within 48 hours, indicating that the fetuses may have already been dead when tested, he said.

To: Mr. G. Wilson Horde
Union Carbide Corporation
Law Department
Oak Ridge, Tennessee

Date: February 10, 1977

FILING NOTICE

Subject: AEC Case S 47,844 Your Docket(s) CNID 3520

A patent application was filed December 28, 1976 in the U. S. Patent Office on the above invention, under the title "Agent and Method for the Early Detection of Pregnancy",

and assigned Serial Number 755,100(70).

D. S. Zachry
Chief, Oak Ridge Patent Group

Enclosure:
Cy. application

1075751

UNITED STATES
ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION
WASHINGTON, D.C. 20545

February 8, 1977

David S. Zachry, Chief
Oak Ridge Patent Group

CASE S-47,844 - HOLLADAY, DAVID W.

The application on the above-identified docket was filed in the United States Patent and Trademark Office December 28, 1976 and assigned Serial Number 755,100.

The Patent and Trademark Office has initially assigned this case to Group 125.

This case does not require a Secrecy Order.

for Dean E. Carlson, Chief
Prosecution Branch, Patents

AMERICAN REVOLUTION BICENTENNIAL 1776-1976

TO: Dean E. Carlson
Office of Assistant General
Counsel for Patents
Century XXI, Germantown

DATE: December 29, 1976

Enclosed are patent assignments in the cases listed below:

ERDA Case S-47,844

D. S. Zachry cad

MCP:DSZ:cad

D. S. Zachry, Chief
Oak Ridge Patent Group

Enclosure:
Patent Assignment (in dupl.)

1075753

UNION CARBIDE CORPORATION
NUCLEAR DIVISION
P. O. BOX Y, OAK RIDGE, TENNESSEE 37830

December 28, 1976

United States Energy Research and Development
Administration, Oak Ridge Operations
Post Office Box E
Oak Ridge, Tennessee 37830

Attention: Mr. D. S. Zachry, Chief
Oak Ridge Patent Group

Gentlemen:

ERDA Case S-47,844 (CNID 3520)

Enclosed is the executed Assignment in the subject case. This Assignment has been approved by Mr. R. F. Hibbs on behalf of Union Carbide.

Our investigation reveals that the use and publication status are essentially the same as cited in the invention disclosure.

The executed application was returned on December 16, 1976.

Sincerely,

M. J. Skinner
Patent Agent

MJS:ha

Enclosure

December 16, 1976

Dean E. Carlson, Chief, Prosecution Branch, Patents, HQ

ERDA CASE S-47,844

Enclosed are an application in the above case and the following additional papers:

( ) Bristol Board Drawing(s) ( ) Record of Invention

( ) Prior Art Letter (in dup.) ( )

( ) Assignment (in dup.) ( )

Fees payable are:

Basic Fee . . . . . $65

Additional Fees:
Total claims in excess of ten, times $2 . . . . . _____

Number of independent claims minus one, times $10 10

Total Filing Fee . . . . . $ 75

Filing prior to January 1, 1977 is necessary.

Publication status:
Published January 1, 1976, in Immunological Communications.

Foreign filing [is] ~~[is not]~~ XXXXXXX recommended. The following countries should be considered.
Canada and other countries permitting filing after above publication.

15/5 DJH

Stephen D. Hamel,
Asst. Chief for Patent Prosecution
Oak Ridge Patent Group

MCP: AHU:cad

Enclosures:
As stated above

1075755

December 14, 1976

Union Carbide Corporation
Law Department
ATTN: Mr. G. Wilson Horde
Post Office Box Y
Oak Ridge, Tennessee 37830

Gentlemen:

ERDA CASE S47,844 (CNID3520 )

Enclosed are a patent application and formal papers for execution by the appropriate Carbide employees.

Please return the executed material at your earliest opportunity so that we may secure the benefits of an early filing date in the U. S. Patent Office.

Sincerely,

D. S. Zachry, Chief
Oak Ridge Patent Group

MCP:AHU:cad

Enclosures:
1. Application
2. Assignment

November 12, 1976

Union Carbide Corporation
Law Department
ATTN: Mr. G. Wilson Hords
Post Office Box Y
Oak Ridge, Tennessee 37830

Gentlemen:

ERDA CASE S-47,844 (CRID 3520)

Enclosed is a draft of an application for patent in the subject case.

It is understood that the inventors are presently located at X-10, and it will be appreciated if you will submit the case to the inventors for their consideration and comments.

Your cooperation in this matter is greatly appreciated.

Sincerely,

ORIGINAL SIGNED BY
D. S. ZACHRY

D. S. Zachry, Chief
Oak Ridge Patent Group

MCP/AHU
cwh
Enclosure:
Draft Application

October 20, 1976

Dean E. Carlson, Chief, Prosecution Branch, Patents, HQ

ERDA CASE S-47,844 DISCLOSURE UNDER CONTRACT W-7405-ENG-26

Transmitted herewith is a disclosure in the above case involving an early pregnancy detection method. The method makes use of the discovery that placentas and possibly other pregnancy associated structures contain isolatable proteins which are also present in the very early stages of pregnancy.

According to the disclosed method, these proteins are isolated and injected into an animal which produces an anti-serum. This anti-serum can be purified and used to detect trace quantities of the protein present in women as little as 18 days after conception.

The serum is readily producible by well established techniques. It is expected that the cost of an early pregnancy test would not exceed $5. A publication was made of the development January 5, 1976. There has already been substantial inquiry into the development and it is expected to have extensive licensing possibilities.

Due to the demonstrated interest in the private sector filing is recommended. Because of the impending statutory bar, the case will be handled under the expedited procedure. A P-2 priority is recommended.

IRVING BARRACK
EOR

D. S. Zachry, Chief
Oak Ridge Patent Group

MCP:ANU:cd

Enclosures:
1. ERDA - 711
2. Disclosure

cc: Robert J. Marchick w/o encl.

P-2

1075158

PATENT INFORMATION WORKSHEET

Section 1

| "S" No. | OP. | Pat. No. | Appl.No. | Yr. | Mo. | Day |
|---|---|---|---|---|---|---|
| 47,844 | | | | | | |

| Inventor's Last Name | First Name | M.I. |
|---|---|---|
| Holladay | David | W |

Source

Section 2 (Will be completed at Headquarters)

Section 3

Keywords

ORI C730000 Protein + Detection + Method + Animal + Biology + Agent

UCND

Invention Title

Method and Serum For the Detection of Early Pregnancy

Brief Description of Invention

An early pregnancy detection method comprises isolating serum from placentas proteins specific to pregnancy, preparing an antiserum to the proteins, and using the antiserum to detect the presence of the original proteins in the early stages of pregnancy.

| Check Priority | Page No. | Total No. Pages |
|---|---|---|
| P1 P2 ✓ P3 P0 | | |

OAK RIDGE NATIONAL LABORATORY
OPERATED BY
UNION CARBIDE CORPORATION
NUCLEAR DIVISION

POST OFFICE BOX X
OAK RIDGE, TENNESSEE 37831

'975

United States Energy Research and Development
Administration, Oak Ridge Operations
Post Office Box E
Oak Ridge, Tennessee 37830

Attention: Mr. D. S. Zachry, Chief
Oak Ridge Patent Group

S.N. 841,844

Gentlemen:

Subject: Disclosure - CNID No. 3520

Enclosed are two copies of the subject disclosure entitled "Early Pregnancy Detection Method", Inventor: David W. Holladay.

This development was published in Immunological Communications 5 (1&2), 1-11 (January 1976).

Sincerely,

H F McDuffie

H. F. McDuffie, Director
Information Division

HFM:ha
Enclosures
cc: H. Postma
J. B. Storer
K. H. Pierce - RC

1075760

DISCLOSURE

FROM

UNION CARBIDE CORPORATION, NUCLEAR DIVISION

ERDA CASE NO.: S- CNID NO. 3520

INVENTOR: David W. Holladay

SUBJECT: EARLY PREGNANCY DETECTION METHOD

ABSTRACT: A unique antisera specific to pregnancy-induced antigens has been developed in goats and rabbits using prenatal extracts in combination with immunoadjuvants over a prolonged injection schedule. This antisera may be used to reliably detect pregnancy as early as 18 days after conception.

DESCRIPTION: Background of the Invention

The subject development relates generally to antisera development techniques and more specifically to the development of an antisera specific to pregnancy-induced proteins in human sera.

The possibility of using placental extract to detect pregnancy at an early stage arose from an investigation by the Molecular Anatomy Program at the Oak Ridge National Laboratory to attempt to determine whether placental and other fetal antigens are re-expressed in diseased states, particularly cancer. It has long been known that levels of existing serum proteins change in pregnancy, and new proteins appear which are produced by either the mother or the fetus. Previous studies of pregnancy sera, for example, by gel precipitation,[1] gel electropherograms,[2] and immunoelectrophoresis,[3] have detected and identified many pregnancy-associated marker molecules. Generally, these studies use

third trimester sera from the mother and are directed toward identifying previously unknown pregnancy-associated marker molecules rather than developing techniques for detecting these marker molecules in the early stages of pregnancy.

The need for a reliable method of detecting pregnancy in the very early stages has become increasingly important for a variety of reasons. For example, the risk to women seeking abortions is greatly reduced when abortions are performed in the earliest stages of pregnancy. It is also desirable for potential carriers of genetic diseases to determine if they are pregnant as early as possible in order to commence the necessary corrective prenatal treatment. Also, increasing numbers of women in the child bearing age employed in government and private industry are exposed to both potential fetal radiation hazards and chemical mutagens and would therefore be interested in detecting pregnancy at the earliest possible stage.

A need therefore exists to develop a reliable method of detecting the presence of a fetus in the earliest stages of pregnancy. The subject development as described hereinbelow was designed, in part, to meet this need.

Summary of the Invention

It is an object of the subject development to determine how early pregnancy-associated antigens can be detected in the sera of pregnant women.

It is a further object of the subject development to provide an early pregnancy detection method which is both reliable and simple to

perform.

It is yet another object of the subject development to provide an early pregnancy detection method which is inexpensive and therefore feasible for routine determinations.

In accordance with the above-stated objectives, the subject invention is the development of an antisera specific to human prenatal proteins for detecting pregnancy in humans comprising separating normal human proteins from pregnancy-associated proteins in prenatal extract to form a concentrate of pregnancy-associated proteins. The pregnancy-associated proteins are then injected as a concentrate into a warm-blooded host to produce antibodies specific to the pregnancy-associated proteins. After the host has had sufficient time to form these antibodies, the sera of the host may be used as an antisera for indicating pregnancy by conventional sera-antisera detection methods.

Brief Description of the Figures

These and other objects, features, and advantages of the subject development will be apparent from the description thereof, taken in conjunction with the accompanying figures in which:

Fig. 1 is a photograph of a typical immunodiffusion plate for serum from three women in third trimester pregnancy, reaction time 24 hours; and

Fig. 2 is a photograph of an immunodiffusion plate for serum from a woman 18-22 days pregnant, reaction time 16 hrs.

Detailed Description of the Preferred Embodiment

As previously stated, the subject development is a by-product from

an investigation directed toward determining whether placental and other antigens are re-expressed in cancer. The investigation ended shortly after the discovery that it would be possible to detect pregnancy in the very early stages. Therefore, the procedures described herein are experimental procedures and could easily be improved without affecting the scope of the invention if it is desired to manufacture the subject antisera on a regular basis.

Any type of human prenatal extract may be used as the source of pregnancy-associated antigens. Placentas were used in the experiments described herein due to their relative ease in procurement. Each placenta is washed to remove a maximum of blood elements and serum proteins. The placenta is then homogenized in a buffer solution to release the pregnancy-associated antigens bound in the cellular structure of the placenta. The homogenate is centrifuged and collection of the supernatant is performed. The homogenization and centrifugation steps may be repeated if it is desired to obtain a maximum of supernatant.

The supernatant is then treated with a bacteriostat (or bacteriocide) and dialyzed to maintain the integrity of the solution. Any material that precipitates during dialyzation can be removed by further centrifugation.

Any normal human serum proteins in the solution are removed by cycling the solution over an immunoadsorbent column of antibodies to normal human serum. The unbound fraction emerging from this treatment containing the pregnancy-associated antigens is still a complex mixture, containing at least 20 different proteins, as judged by gel electrophoresis. Only 10 to 15 of the proteins in the mixture have proved to

be sufficiently antigenic to result in detectable antibody production to them. Therefore, it may be desirable to perform further operations to separate these proteins of interest or it may be desirable to modify the existing extraction procedure to minimize the extraction of proteins of placental or fetal origin that do not appear in early pregnancy sera. For example, a mild acid extraction of diced placenta, without homogenization, may produce an extract having fewer undesired proteins.

The resulting placental extracts are then concentrated for injection purposes. The antigen content of a typical extract at this point is 0.1 to 1.0 mg/ml, based on intensity of reaction in immunodiffusion. The extract is mixed with an adjuvant to enhance the action of the antigen, and the preparation is injected in an appropriately-sized host so that antibodies to the pregnancy-associated antigens may be formed. Experimentally, a rather lengthy injection schedule (7 to 8 months) is required to produce the desired antibodies. High-titer antiserum from the host is adsorbed with lyophilized, pooled serum from normal human males to render it specific for placental antigens.

Pregnancy detection tests may be performed by a variety of serum/antiserum methods using the subject antisera. Experimental determinations were made using gel precipitation, however, other methods such as immunoelectrophoresis, block gel precipitation or cross immunoelectrophoresis may prove to be more useful to enhance the sensitivity of the reaction or when time is of the essence. As can be seen in Figs. 1 and 2, pregnancy-associated antigens can be clearly detected by gel precipitation 18 to 22 days after conception as well as in the third trimester of pregnancy.

Because of regular visits associated with routine prenatal care, it was a simple task to obtain serum samples of pregnant women in the second and third trimesters from staff obstetricians at a nearby hospital. However, it was very difficult to obtain candidate females for very early pregnancy tests since there were no "fertility" clinics in the area at the time of ongoing research of the subject development. As a result, only five samples were obtained from candidates in the earliest stages of pregnancy. The table below summarizes the results of those tests.

Early Detection of Pregnancy Using the Subject Antisera

| Candidate | Estimated Time Since Conception (days) | Standard Time Interval Necessary for Definitive Pattern (hr) | Bands of Identification |
|---|---|---|---|
| M | 18-22 | 16 | 2 |
| J | 22-26 | 16 | 3 |
| P | 28-32 | 16 | 2 |
| P | 32-36 | 16 | 2 |
| B | 42-46 | 40 | 4 |

For the first four early pregnancy cases, the antiserum used had been obtained from host animals for short periods ranging from one to five months before testing. For sample B above, the antiserum had been stored for about three years at 4-8°C. Although the time required to obtain a definitive pattern with this aged antiserum was twice that of pervious tests, its pattern was very comparable to those obtained with the much fresher antiserum.

## Example

Term placentas were obtained at delivery from the Oak Ridge Hospital. The placentas were chilled in 0.85% sodium chloride solution to be transported to the MAN Program laboratories. The placentas were then washed for 1 hour in tap water and excess fat cut away. The placenta was then cut into pieces ∿1 cm on a side and frozen to be batch processed at a later date. Subsequently, the frozen material was homogenized in three volumes of 0.1 M, pH 7, phosphate buffer. The homogenate was centrifuged at 17,000 x g for 10 min. in an angle head centrifuge, the supernatant was collected, the pellet was rehomogenized in two volumes of the same buffer, and a second centrifugation at 17,000 x g for 10 min. was performed. The proteins of interest were precipitated from the pooled supernatants by adding solid ammonium sulfate to 55% saturation. Following centrifugation, the material was dissolved in a minimal amount of 0.1 M, pH 7, phosphate buffer containing 0.1% sodium azide, the solution was dialyzed against the same buffer, and any material that precipitated was removed by centrifugation.

Normal serum proteins left in the solution were removed by affinity chromatography wherein immunoadsorbent columns were prepared by covalent bonding of antibodies against normal serum proteins to cyanogen-bromide activated Sepharase.

The placental extracts were concentrated to about $15A_{280}$ units per ml and mixed with an equal volume of Freund's complete adjuvant. The preparation was injected subcutaneously, either in the hips or behind

the neck of the host (4 ml for goats, 1 ml for rabbits). Thereafter, the same amounts of extract without adjuvants were injected subcutaneously at weekly intervals. Every fourth week, injection was done with antigen plus adjuvant. First bleeding was at 5 weeks (40 ml for rabbits and 150 ml for goats) and at biweekly intervals thereafter. High-titer antiserum was obtained, usually in about 7 to 8 months. The antiserum was absorbed with lyophilized, pooled serum obtained from normal human males (100 mg/ml of antiserum) to render it specific for placental antigen.

RELATED ART: References:

1. Thornes, R. D., M. D. Thesis, University of Dublin, 1957, cited in Ref. 2. See also MacLaren, J. A., Thornes, R. D., Roby, C. C. and Reid, D. E., Am. J. Obstet. Gynecol., Vol. 78 (1959), p. 939.

2. Smithies, O., Adv. Protein Chem., Vol. 14 (1959), p. 65.

3. Hirshfeld, J. and Söderberg, U., Nature, (London), Vol. 187 (1960), p. 332.

There are no other known methods to detect pregnancy which use human placental extracts as the basis for their reagents. The only other known pregnancy detection method which is reliable in the first 60 days of gestation is a radioreceptor assay method for human chorionic gonadotropin (HCG) developed by B. B. Saxena, et al., at Cornell University Medical College. This method has been described in Science, Vol. 18 (1974), pp. 793ff, "Radioreceptor Assay of Human Chorionic Gonadotropin: Detection of Early Pregnancy". The Cornell radioreceptor detection technique is rather expensive to perform (~$75) due to the complexity involved in

using radioactive substances and is therefore prohibitive on a large scale basis. It is estimated that the subject method could be performed for less than $5.

**PROBABLE VALUE:** Preliminary experimental data indicate the subject development can be used as an early pregnancy detection method. Leading obstetric and cancer research centers have indicated tremendous interest in the development due to its expected usefulness and relative simplicity.

**RECOMMENDATION:** The filing of a patent application may be warranted.

PERTINENT FACTS:

Information obtained from: D. W. Holladay and UCN-2687 (12356 9-69)

Verbal reference number: 4736 (ORNL)

Contract involved: W-7405-eng-26

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Date of First Disclosure to Others:

(a) Name: N. G. Anderson Date: October 1972 Recorded in: Lab Nb. #2a, p. 170

(b) Name: J. E. Caton Date: " Recorded in: "

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APPROVED BY: N. E. Woolf DATE: Sept 14, 1976





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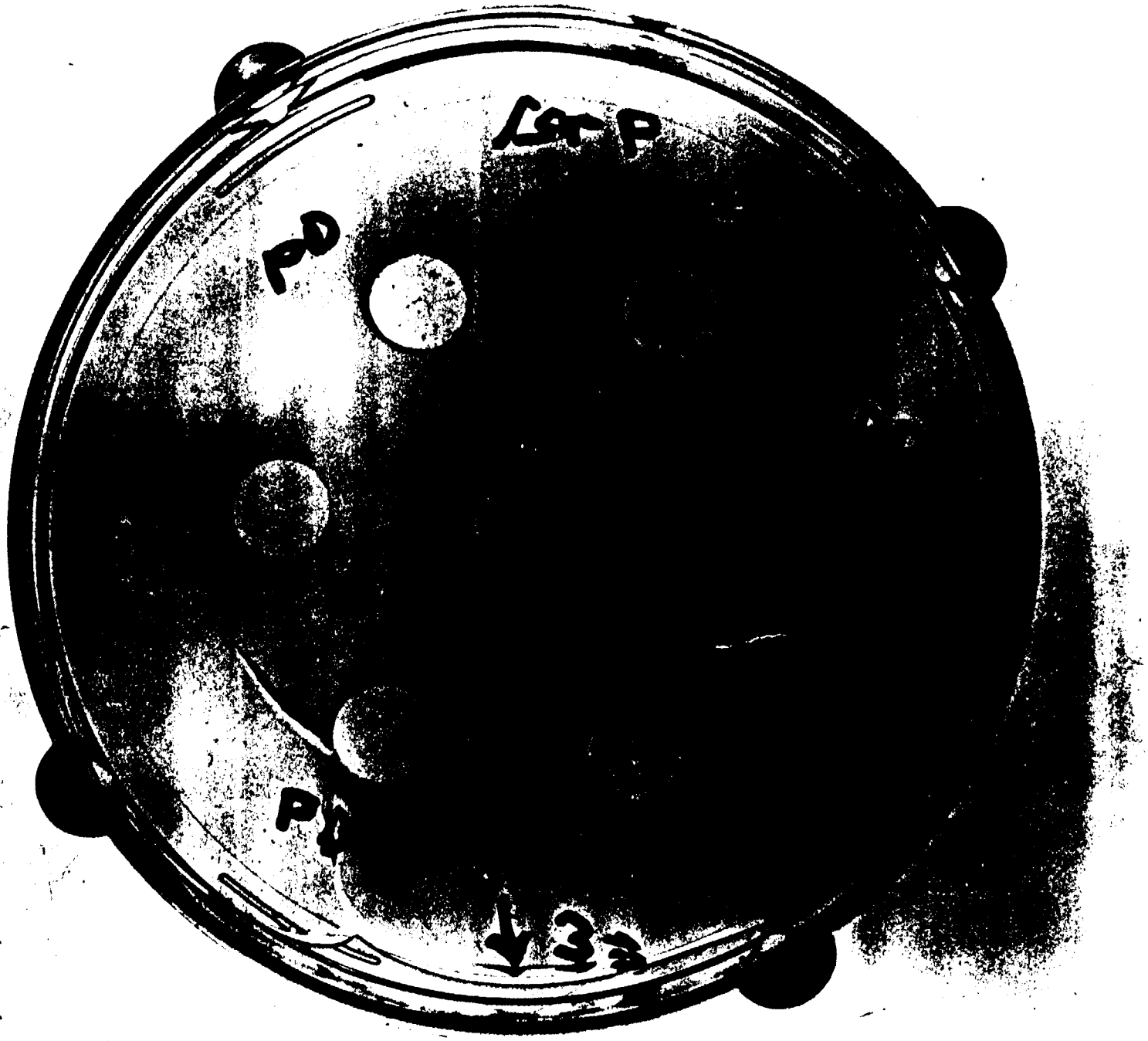


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FIG. 1

FIG. 2