UNCLASSIFIED

AD NUMBER

ADB226818

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies only; Proprietary Info.; May 97. Other requests shall be referred to U.S. Army Medical Research and Materiel Command 504 Scott St., Ft. Detrick, MD 21702-5012.

AUTHORITY

USAMRMC ltr dtd 26 Jan 2000

THIS PAGE IS UNCLASSIFIED

AD_____

MIPR NUMBER 96MM6720

TITLE: Role of Progesterone in the Etiology of Breast Cancer

PRINCIPAL INVESTIGATOR: Gopalan Shyamala, Ph.D.

CONTRACTING ORGANIZATION: Department of Energy
Berkeley, California 94720

REPORT DATE: May 1997

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Distribution authorized to U.S. Government agencies only (proprietary information, May 97). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

DTIC QUALITY INSPECTED 4

19970728 069

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	May 1997	Annual (1 May 96 - 30 Apr 97)

4. TITLE AND SUBTITLE
Role of Progesterone in the Etiology of Breast Cancer

5. FUNDING NUMBERS
96MM6720

6. AUTHOR(S)
Gopalan Shyamala, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Department of Energy
Berkeley, California 94720

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution authorized to U.S. Government agencies only (proprietary information, May 97). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

One of our research goals is to examine the effects of age on mammary development analogous to that accompanying pregnancy and determine if this differs between normal mice and mice carrying an imbalance in the natural ratio of the two forms ("A" and "B" forms") of progesterone receptor; progesterone receptor is essential for pregnancy dependent mammary development. Our studies conducted during the past grant period reveal that with increasing age mammary glands acquire a greater sensitivity to estrogen and progesterone, which becomes dramatically evident at ~8 months of age. This is particularly significant since ~8 months of age in mice roughly corresponds to a peri-menopausal human female. This means that in the aging female who is known to be at a higher risk for breast cancer, one of the contributing factors may be an increased sensitivity to sex steroids. We also find that (a) with increasing parity more epithelial cells are present in mammary glands which, once again, is very dramatic after four pregnancies and (b) in transgenic mice carrying an excess of the "A" form of progesterone receptor and hence an imbalance in the normal ratio of the two forms of progesterone receptor, mammary morphogenesis is altered.

14. SUBJECT TERMS
Breast Cancer

15. NUMBER OF PAGES
17

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Limited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

____ Where copyrighted material is quoted, permission has been obtained to use such material.

____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

____ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

✓ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

G. Shur June 9th 1997

PI - Signature Date

TABLE OF CONTENTS

INTRODUCTION

Background and Significance of the Research

Numerous epidemiological studies spanning nearly two decades have clearly established that, excluding the genetic background, reproductive history is an important and consistent "natural" risk factor associated with breast cancer. In particular, both early menarche and late age at menopause have been shown to be associated with an increased risk. Epidemiological studies have also shown that early (before or around age 20) full term pregnancy offers about a 50% protection against breast cancer but this protection is greatly reduced when pregnancy occurs at a later age and no longer apparent if it takes place after age 34. Taken together, these studies indicate that it is the total length of time between menarche and menopause and/or the total length of time between menarche and first pregnancy which is associated with the risk factor (1-3).

A distinguishing physiological characteristic of the female between the onset of menarche and menopause is the presence of functional ovaries which, during each menstrual cycle synthesize and secrete the female sex steroids, estrogen and progesterone (4,5). Accordingly, the total length of time between menarche and menopause can be translated as the total years to which the normal breast is exposed to estrogen and progesterone. Similarly, estrogen and progesterone are also the principal hormones of pregnancy and progesterone is absolutely essential for the maintenance of pregnancy to full term (6). This taken together with the fact that these ovarian steroids have been implicated in mammary cancer for more than half a century, argues the need to target estrogen and progesterone as key factors responsible for the observed relationships between the reproductive history and the breast cancer risk.

Extensive studies spanning over two decades have clearly established that almost all the effects of steroid hormones, including estrogen and progesterone, are mediated through their respective receptors present in the cells which are responsive to these hormones (7,8). There is also extensive documentation that in female sex organs the synthesis of progesterone receptors (which are required for progesterone action) require estrogen (8). In the human female, the growth of the breast accompanying each menstrual cycle is associated with the luteal phase or the progesterone dominant phase of the cycle (9-14). Similarly the extensive growth of the breast which accompanies pregnancy also co-relates with the rising levels of progesterone (15). Based on all these observations, we had proposed a few years ago that, among the two ovarian steroids, it is the action of progesterone mediated through its receptor which was ultimately responsible for the growth of normal breast, i.e. within the context of the two steroids, the action of estrogen was indirect in so far that it was required for the synthesis of progesterone receptors (16). Unequivocal in vivo evidence to support our earlier proposal is the fact that in genetically engineered mice lacking progesterone receptors (mice in which progesterone receptor gene has been "knocked out") there is an arrested development of the breast; in particular, in the mice lacking progesterone receptors, the breast does not exhibit any lobulo-alveolar development or extensive lateral branching of ducts (17-19). This

arrested development persists despite the administration of estrogen and progesterone (at doses and length of time) known to be sufficient to cause development in normal mice, analogous to that seen during pregnancy. These recent data obtained with the mice lacking progesterone receptors not only confirm our earlier proposal with regard to the primary importance of progesterone in the growth of normal breast but also provide absolute proof that the action of progesterone is mediated through its receptor.

Among the various classes of steroid receptors, progesterone receptors are somewhat unique in that they exist in two molecular forms commonly referred to as the 'A' and 'B' form (20). In the same cell the 'A' and 'B' forms can have different functions and also the activity of the individual form of the receptor can vary between different types of cells (21,22). Furthermore, depending on the cell, the 'A' form can either inhibit or enhance the activity of the 'B' form receptor (21). Therefore, to the extent age is a critical factor which influences the reproductive capability of the female, the epithelial cell of the breast in a young female represents a different phenotype from that of the older female. As such the relative activities of the 'A' and 'B' forms of the receptor and the modulation of the activity of the 'B' form by the 'A' form can vary between the "young" and the "old" cells; this, in turn will lend to differences in the type of genes expressed and their magnitude of expression between the "young " and "old" cells. In this context, it is important to note that the 'A' and 'B' forms of progesterone receptor have also been shown to modulate estrogen receptor and estrogen dependent gene expression (23-25). Thus, an alteration in the relative activities of the 'A' and 'B' forms of progesterone receptor can conceivably affect both estrogen and progesterone dependent gene expression. We believe that such differential gene expression may in fact be responsible for the age dependency associated with the beneficial effect of pregnancy on breast cancer and the risk associated with the total length of time between menarche and menopause.

We further hypothesize that the relative activities of either the 'A' or the 'B' form or both change due to age and reproductive status and such alterations result in an imbalance in the relative activities of the growth factors and growth inhibitors, a phenomenon often associated with carcinogenesis or initiation of tumor formation. In this context it is noteworthy that while both pregnancy and carcinogenesis represent extensive epithelial cell proliferation, initiated in the relatively quiescent breast of the adult female, only the growth accompanying pregnancy is followed by terminal differentiation of the epithelial cells. Thus, it is possible that carcinogens can mimic the effects of endogenous cellular factors responsible for the epithelial growth occurring during pregnancy but fail to achieve terminal differentiation, a process perhaps intrinsic only to cells replicating in response to progesterone. Therefore, an investigation of progesterone and hence, progesterone receptor action on normal breast is essential to understand the mechanisms involved in the growth and differentiation of normal breast and this is also a pre-requisite for understanding the role of progesterone in the etiology of breast cancer. This is because cancer arises from the normal breast and its hall mark is unrestricted growth resulting from derangement in the balance between normal growth and differentiation. Therefore, studies on tumor cells, while being informative with respect to their behavior, cannot allow an identification of pathways responsible for giving rise to tumor. To this end, the purpose of the research project is as follows:

Purpose of the research:

1. To examine the effect of age on the ability of normal mammary glands to undergo DNA synthesis in transgenic mice carrying either the 'A' or the 'B' form of progesterone receptor and determine if these exhibit differences from that of normal (wild type) mice.

2. To examine the effect of age on the ability of normal mammary glands to achieve pregnancy dependent differentiation and determine if this differs between the normal (wild type) mice and transgenic mice carrying either the 'A' or the 'B' form of progesterone receptor.

3. To examine the pattern of gene expression in mammary glands of transgenic mice carrying either the 'A' or the 'B' form of progesterone receptor and compare this with that of normal (wild type) mice and also determine if these change as a function of age.

BODY

During this past year, we have devoted our efforts primarily to examine the effect of age on the ability of mammary glands to achieve pregnancy dependent differentiation and determine if these differ between the normal (wild type) and transgenic mice carrying either the "A" form of progesterone receptor. To meet this objective, we had proposed to breed separate groups of nulliparous females beginning at ~twelve weeks of age and, therefore, at eight week intervals up to fifty weeks of age. To assess differentiation, we proposed to examine wholemount preparations and histological sections of lactating mammary glands from these various groups. However, once funding was obtained and we began to plan our experiments, it occurred to us that with increasing age, the ability to become pregnancy may be compromised which, in turn, may jeopardize the outcome of our experiments. Therefore, we decided to use an alternate experimental protocol which has been widely used to elicit differentiation analogous to that observed during pregnancy i.e. to ovariectomize the animal and then administer estrogen and progesterone for twenty-one days (26).

(a) Experimental Methods:

Nulliparous females of different ages were ovariectomized and after two weeks, administered one microgram of estrogen and one milligram of progesterone per day, for a total period of twenty-one days. Mammary epithelial growth and differentiation which occurs during pregnancy, in response to estrogen and progesterone, is characterized by outgrowths from terminal and lateral branches, and lobulo-alveolar structures begin to fill the interductal spaces. Therefore, to assess pregnancy type differentiation, we estimated the degree of lobulo-alveolar development in wholemount preparations, prepared as described previously (27) except for using carmine instead of hemotoxylin.

(b) Results:

(i) Effect of age on the ability of mammary glands to undergo lobulo-alveolar development in response to estrogen and progesterone.

In all age groups tested, administration of estrogen and progesterone resulted in lobulo-alveolar development while the control mice treated with oil had virtually no lobulo-alveolar development (Figure 1; compare Panels A,C, and E with Panels B, D, and F). The degree of lobulo-alveolar development was similar among mice of twelve weeks and twenty weeks of age (Figure 1; Compare Panels B and D). However, in 30-32 week old mice, there was a significantly greater degree of lobulo-alveolar development (Figure 1, Panel F). To verify this and further discriminate the differences between the 20 week and 30-32 week old mice, we repeated the experiment with the modification such that estrogen and progesterone were administered only for ten days. As expected, with shorter duration of steroid administration, there was a lesser degree of lobulo-alveolar development. However, once again, as compared to 20 week old mice, there was a greater degree of lobulo-alveolar development in 30 week old mice (Figure 2).

(ii) Effect of age and parity on the ability of mammary glands to undergo lobulo-alveolar development in the absence of exogenous estrogen and progesterone.

From the data shown in Figures 1 and 2, it appeared that with increasing age, mammary glands might acquire a greater sensitivity to estrogen and progesterone. However, with increasing age, there is a decrease in ovarian function which results in lower levels of estrogen and progesterone as compared to that present in younger females. Therefore, if with age, mammary glands do acquire an increased sensitivity to estrogen and progesterone, it should be apparent (perhaps to a lesser degree) even in the absence of any exogenous estrogen and progesterone administration. To determine if this was true, we examined the mammary glands of old mice (~55-68 weeks) for their degree of lobulo-alveolar development. In old nulliparous mice (55 weeks), the degree of lobulo-alveolar development in mammary glands was comparable to that seen in young (20 weeks) old nulliparous mice (Figure 3; compare Panel A with Panel B). On the other hand, in older mice which had undergone pregnancies, with increasing parity there was an increase in lobulo-alveolar development which was quite dramatic in mice having undergone four pregnancies (Figure 3; compare Panel C with Panel D). The effect of parity on lobulo-alveolar development is summarized in Figure 4.

(iii) Studies on transgenic mice:

Our studies on transgenic mice have not progressed as rapidly as we had anticipated because of the shortage of these mice (due to lengthy breeding protocols), coupled with the fact that aging studies require longer time. Nevertheless, we have obtained evidence that in young mice carrying an excess of the "A" form of the progesterone receptor as transgene, as compared to age matched controls, there is an increase in sidebranching and alveolar buds (Fig. 5; compare Panel A with Panel B). Our preliminary results examining the progression of these structures to lobules also reveal that this process may be slower in transgenic mice as compared to wild type. We are currently performing experiments to determine the effect of age on this process.

CONCLUSIONS

With increasing age, there is an increase in the degree of lobulo-alveolar development in response to estrogen and progesterone which is dramatically apparent in mice beginning at ~8 months of age. This suggests that at around 8 months of age, mammary glands may acquire a greater sensitivity to estrogen and progesterone. These findings have important implications to mammary carcinogenesis for several reasons. To begin with, the age (~8 months) in mice (when an increased sensitivity to estrogen and progesterone is seen) corresponds to the age of a peri-menopausal human female. As such, an increased sensitivity to ovarian steroids resulting in relatively higher proliferation of epithelial cells can increase the chances of both intrinsic mutagenesis and that resulting from exposure to carcinogens; they, in turn, can lead to cancer. An increased sensitivity to endogenous steroids will also result in an increased sensitivity to exogenous steroids e.g. environmental estrogens. Therefore, in the aging female who is at a higher risk for breast cancer, an increased sensitivity to estrogen and progesterone may be a contributing risk factor. To test our hypothesis, during the next grant period, we will examine the effects of these steroids on DNA synthesis in mice of different ages.

It is known that mammary glands of multiparous non-pregnant females have more epithelial cells than their nulliparous counterparts. However, our finding that there is a dramatic increase after four pregnancies suggests that a complex phenomenon may underlie the relationship between parity and mammary morphology, perhaps once again related to altered sensitivity to estrogen and progesterone.

Our observation that in transgenic mice carrying an excess of the "A" form of progesterone receptor mammary morphogenesis may differ from the wild type mice lends support to our hypothesis that an imbalance in the ratio and hence, the activities of the two forms of progesterone receptor may have important consequences to mammary development and hence, carcinogenesis.

REFERENCES

1. Staszewski, J. (1971), Age and menarche and breast cancer. *J. National Cancer Institute* **47**, 935-94.

2. Key, T.J.A. and Pike, M.C. (1988), The role of estrogens and progestine in the epidemiology and prevention of breast cancer. *Eur. J. Cancer Clin. Oncol.* **24**, 29-34.

3. Henderson, B.E., Ross, P.K. and Pike, M.C. (1991), Toward the primary prevention of cancer. *Science* **254,** 1131-1138.

4. Sitteri, P.K. and Febres, F. (1979), Ovarian hormone synthesis, circulation and mechanism of action. *In: Endocrinology* **3,** 1401-1417. (Eds: L.J. De Groot, G.F. Cahill, Jr., W.D. Odell, L. Martini, J.T. Potts, Jr., D.H. Nelson, E. Steinberger and A.J. Winegrad), Grune and Stratton Publishers.

5. Odell, W.D. (1979), The reproductiove system in women. *In: Endocrinology* **3**, 1383-1400 (Eds: L.J. De Groot, G.F. Cahill, Jr., W.D. Odell, L. Martini, J.T. Potts, Jr., D.H. Nelson, E. Steinberger and A.J. Winegrad). Grune and Stratton Publishers.

6. Buster, J.E. and Marshell, J.R. (1979), Conception, Gamete and Ovum transport, Implantation, Fetal - Placental Hormones Hormonal preparation for parturition and parturition control. *In: Endocrinology* **3**, 1595-1612. (Eds: L.J. De Groot, G.F. Cahill, Jr., W.D. Odell, L. Martini, J.T. Potts, Jr., D.H. Nelson, E. Steinberger and A.J. Winegrad) Grune and Stratton Publishers.

7. Evans, R.M. (1988). The steriod and thyroid hormone receptor super family. *Science* **240**:889.

8. Tsai, M.J. and O'Malley, B.W. (1994). Molecular mechanisms of action or steroid/thyroid receptor super family members. *Ann. Rev. Biochem.* **63**:451-486.

9. Masters, J.R.W., Drife, J.O. and Scarisbrick, J.J. (1977), Cyclic variation of DNA synthesis in human breast epithelium. *J. Natl. Cancer Inst.* **58**:1263.

10. Meyer, J.S. (1977). Cell proliferation in normal human breast ducts, fibroadenomas and other ductal hyperlasias measured by nuclear labeling with tritiated thymidine: Effects of menstrual phase, age and oral contraceptive hormones. *Human Path.* **8**:67.

11. Going, J.J., Anderson, T.J., Battersby, S., Macintyre, C.C.A. (1988), Proliferative and secretory activity of human breast during natural and artificial mentrual cycles. *Am. J. Pathol.* **130.**

12. Anderson, T.J., Battersby, S., King, R.J.B., McPherson, K., Going, J.J. (1989). Oral contraceptive use influences resting breast proliferation. *Human Path.* **20**:1138.

13. Potten, C.S., Watson, R.J., William, G., et al. (1988). The effect of age and menstrual cycle upon proliferative activity of the normal human breast. *Brit. J. Cancer* **58**:163-170.

14. Ferguson, D.J.P., Anderson, T.J. (1981). Morphological evaluation of cell turnover in relation to the menstrual cycle in the 'resting' human breast. *Brit. J. Cancer* **44**:177-181.

15. Topper, Y.S. and Freeman, C.S. (1980), Multiple hormone interactions in the developmental biology of the mammary gland. *Physiol. Rev.* **60**: 1049.

16. Shyamala, G. (1987), Endocrine and other influences in the normal development of breast, In: Fundamental Problems in Breast Cancer (AHG Paterson Ed.) Martinus Nijhoff Publishing, pp 127-137.

17. Lydon, J.P., DeMayo, F.J., Shyamala, G., Conneely, O.M. and O'Malley, B.W. (1995). Characterization of a progesterone receptor null mutant mouse model. In: Proceeding of the 77th Annual meeting of the Endocrine Society, Abstract #P-2531.

18. Conneely, O.M., Lyndon, J.P., DeMayo, F.J., Shyamala, G. and O'Malley, B.W. (1995). Physiological consequences of progesterone receptor ablation in mice In: Proceeding of the 77th Annual meeting of the Endocrine Society, Abstract #S5-2.

19. Lydon, J.P., De Mayo, F.J., Funk, C.R., Mani, S.K., Hughes, A.R., Montgomery, Jr., C.A., Shyamala, G., conneely, O.M. and O'Malley, B.W.. Mice lacking progesterone receptor exhibit pleiotropic reproductive abnomalities. *Genes and Development* (In Press).

20. Wei, L.L. and Horwitz, K.B. (1986). The structure of progesterone receptors. *Steroids* **46**:677.

21. Tora, L., Gronmeyer, H., Turcotte, B., Gaub, M.P., Chambon, P. (1988). The N-terminal region of the chicken progesterone receptor specifies target gene activation. *Nature* **333**:185.

22. Vegeto, E., Shabaz, M.M., Wen, D.X., Goldman, M.E., O'Malley, B.W. and McDonnell, D.P., (1993). Human progesterone receptor A form is a cell and promoter specific repressor of human progesterone receptor B function. *Mol. Endocrinol.* **7**:1244.

23. McDonnell, D.P. and Goldman, M.E. (1994). RU486 exerts anit-estrogenic activities through a novel progesterone receptor A-form mediated mechanism. *J. Biol. Chem.* **269**:11945.

24. Chalbos, D. and Galtier, F. (1994). Differential effect of Forms A and B of human progesterone receptor on estradiol dependent transcription. *J. Bio. Chem.* **269**:23007.

25. Kraus, W.L., Weis, K.E., and Katzenellenbogen, (1995) Inhibitory cross-talk between steroid hormone receptors: Differential targeting of estrogen receptors in the repression of its transcriptional acctivity by agonist and antagonist occupied progestin receptors. *Mol. Cell. Biol.* **15**:1847-1857.

26. Ichinose, R.R. and Nandi, S. (1966) Influence of hormones on lobulo-alveolar differentiation of mouse mammary gland in vitro. *J. Endcrinol.* **35**:331.

27. Silberstein, G.B., VanHorn, K., Shyamala, G., and Daniel, C.W. (1994) Essential role of endogenous estrogen in directly stimulating mammary growth demonstrated by implants containing pure anti-estrogens. *Endocrinology* **134**:84-90.

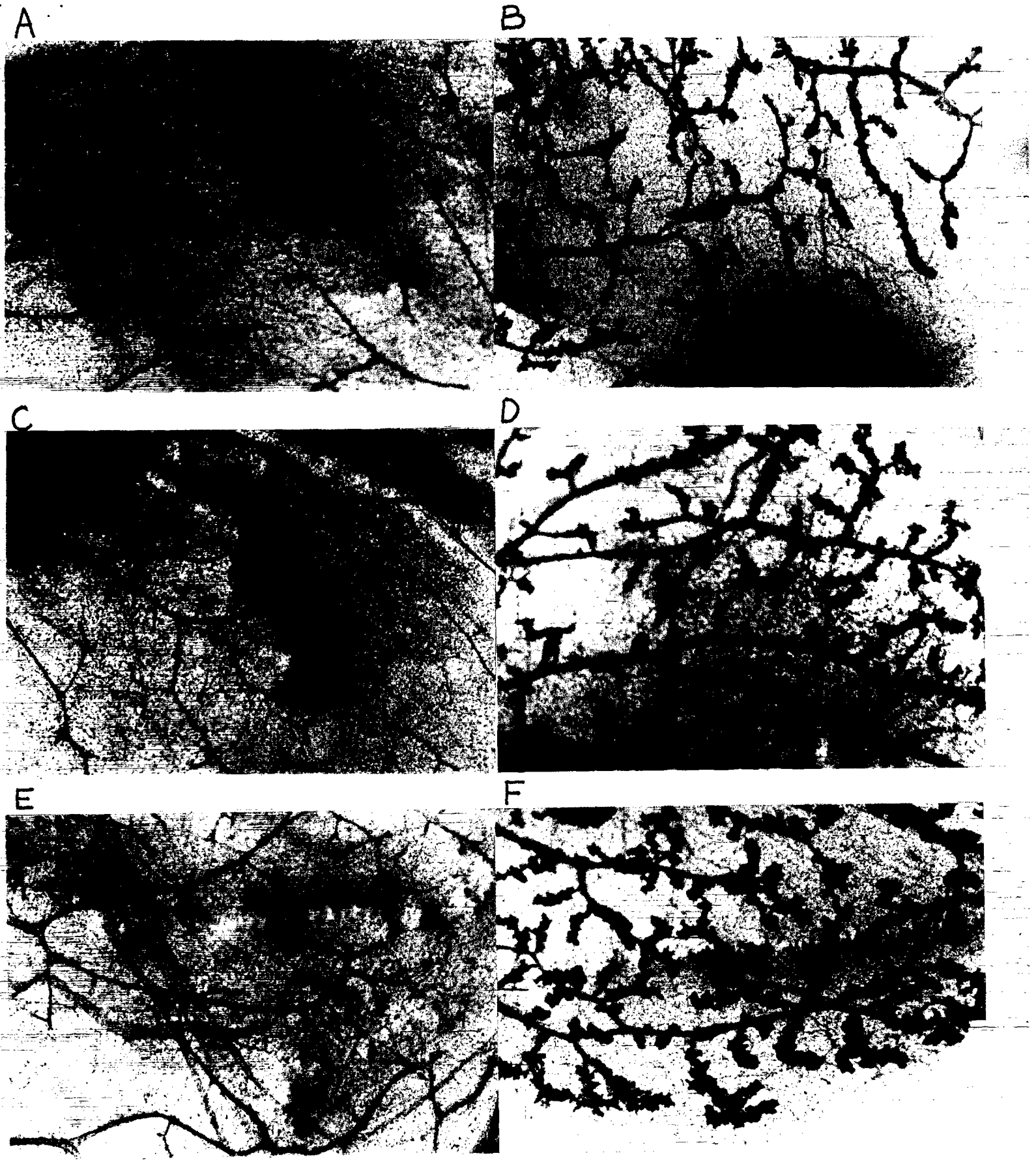

Figure 1: Wholemount preparations of mice ovariectomized at indicated ages and administered with either oil (A, D and E) or estrogen and progesterone (B, D, and F) for twenty-one days. (A and B): 12 weeks; (C and D): 19 weeks; (E and F): 32 weeks.

Appendix 2

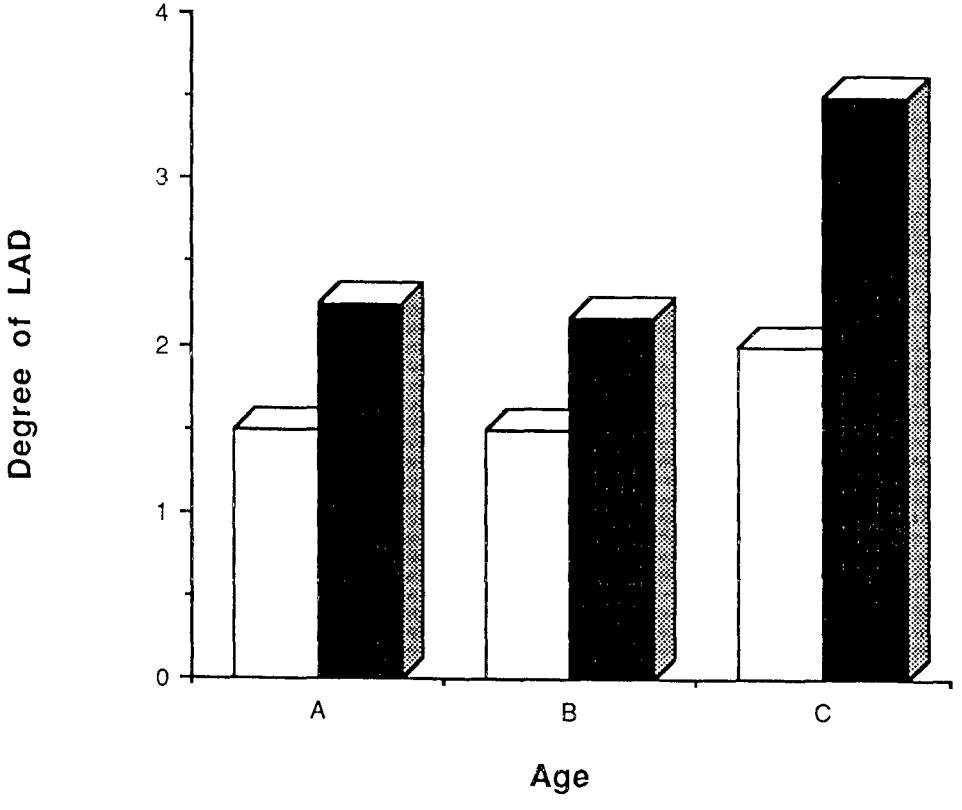

Figure 2: Effect of age on mammary lobulo-alveolar development: Adult mice at indicated ages were ovariectomized and administered with estrogen and progesterone for either 10 days (☐) or twenty-one days (■) prior to killing and processing of mammary glands. (A) 11-12 weeks; (B) 18-20 weeks; (C) 30-32 weeks. Units of LAD are arbitrary with #4 being equivalent to 100% of mammary tissue composed of lobulo-alveolar epithelial cells.

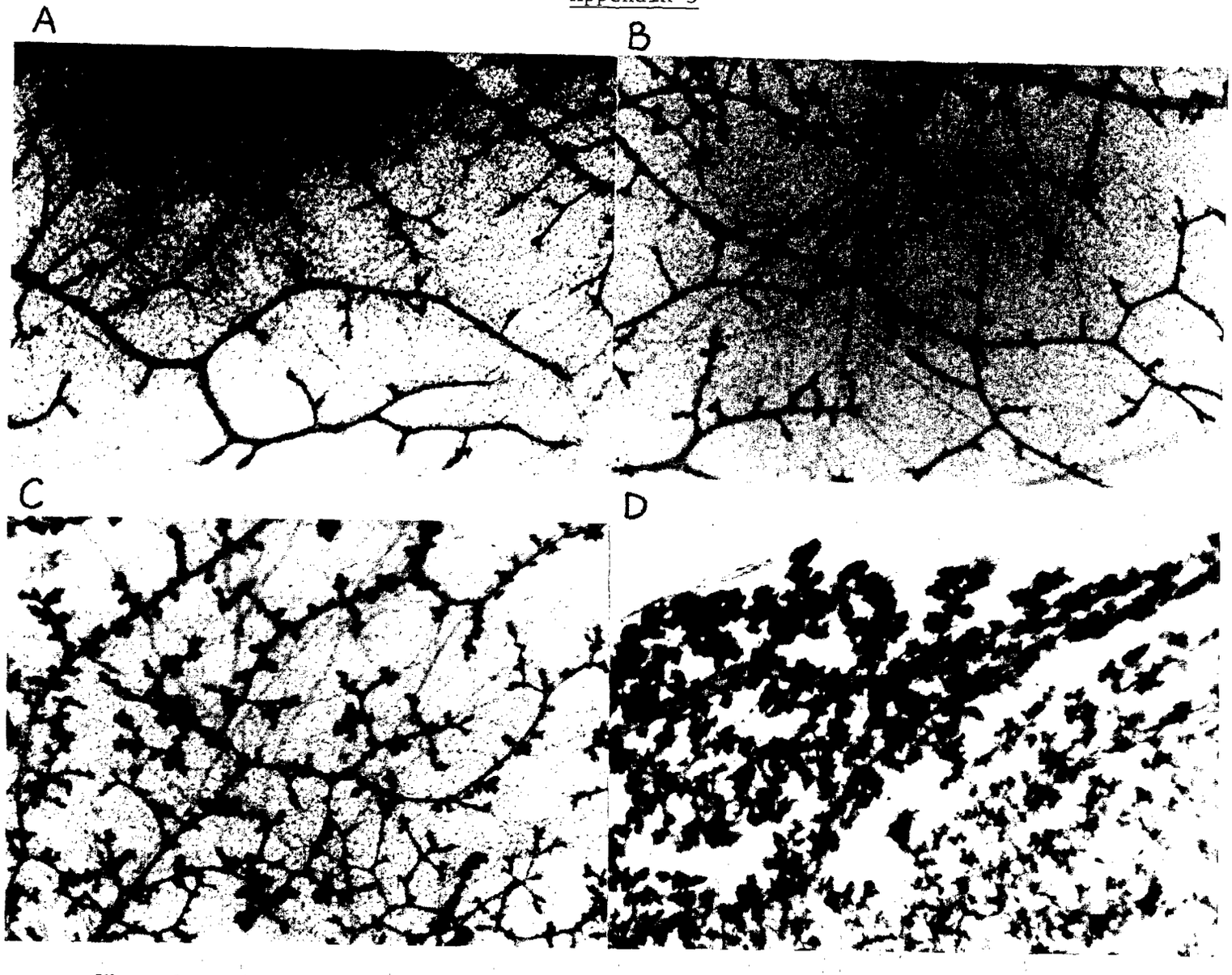

Figure 3: Wholemount preparation of mammary glands of adult non-pregnant mice at indicated ages and parity. (A) 20 week old nulliparous (B) 55 week old nulliparous (C) 68 week old multiparous, three pregnancies (D) 68 week old multiparous, four pregnancies

Appendix 4

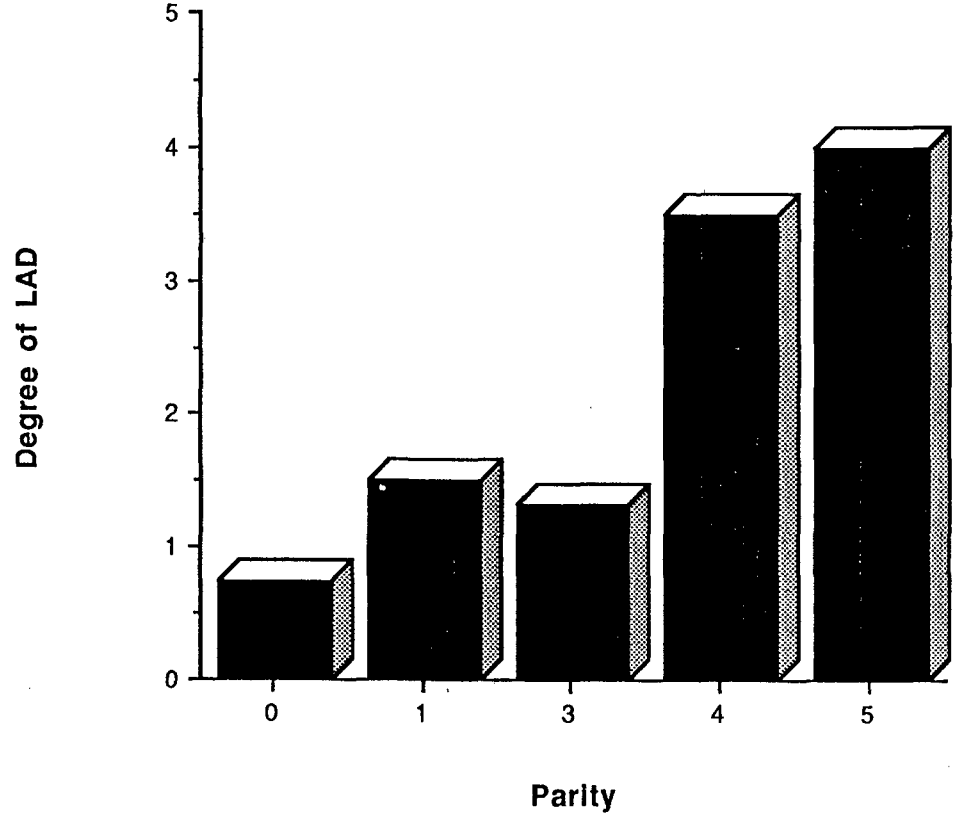

Figure 4: Degree of mammary lobulo-alveolar development in old, non-pregnant mice (55-66 weeks of age) as a function of parity. Units of LAD are arbitrary with #4 being equivalent to 100% of mammary tissue composed of lobulo-alveolar epithelial cells.

Appendix 5

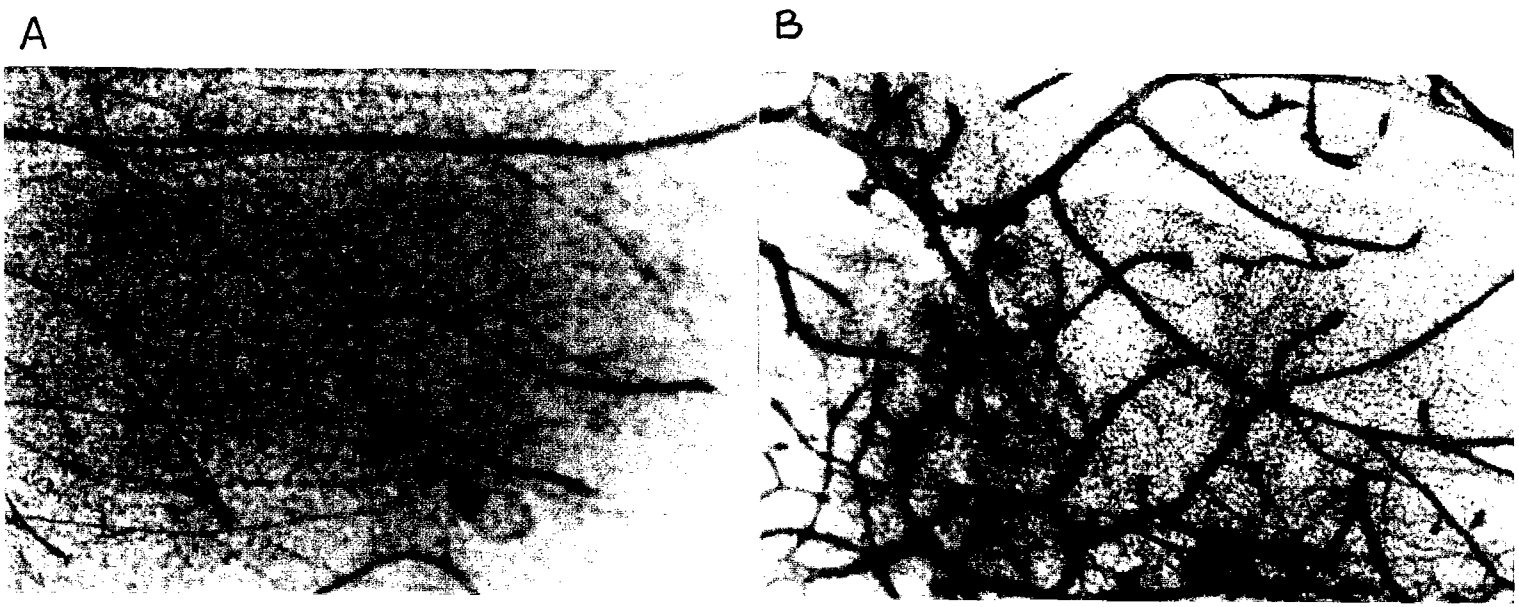

Figure 5: Wholemount preparation of adult (12 week old) transgenic mouse carrying an excess of the "A" form of progesterone receptor gene (Panel B) and her control litter mate (Panel A).

Received 2/8/00

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 26 Jan 00

MEMORANDUM FOR Administrator, Defense Technical Information Center, ATTN: DTIC-OCA, 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for the following Awards.

DAMD17-86-C-6169	ADB116203
DAMD17-94-J-4056	ADB218947
DAMD17-94-J-4394	ADB220575
DAMD17-94-J-4358	ADB236080
DAMD17-94-J-4169	ADB236753
DAMD17-94-J-4049	ADB234453
DAMD17-94-J-4080	ADB218909
DAMD17-94-J-4080	ADB233428
DAMD17-94-J-4431	ADB220348
DAMD17-94-J-4335	ADB234557
DAMD17-94-J-4388	ADB218872
DAMD17-94-C-4081	ADB246577
DAMD17-94-J-4025	ADB238010
DAMD17-94-J-4080	ADB241898
MIPR 96MM6720	ADB240182
MIPR 96MM6720	ADB226818

Request the limited distribution statement for Accession Document Numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Virginia Miller at DSN 343-7327 or by email at virginia.miller@det.amedd.army.mil.

FOR THE COMMANDER:

PHYLIS M. RINEHART
Deputy Chief of Staff for
Information Management