R1630

#1461

Gellin, Gerald A.

084-30-1786

2-R01-OH-00714-07

Supplemental Information

PATHOMECHANISMS OF CHEMICALLY INDUCED DEPIGMENTATION

50272-101

REPORT DOCUMENTATION PAGE	1. REPORT NO.	2.	3. PB88-247077
4. Title and Subtitle Pathomechanisms of Chemically Induced Depigmentation			5. Report Date 6.
7. Author(s) Gellin, G. A.			8. Performing Organization Rept. No.
9. Performing Organization Name and Address Supplemental Information			10. Project/Task/Work Unit No. 11. Contract(C) or Grant(G) No. (C) (G) OH-001714-07
12. Sponsoring Organization Name and Address			13. Type of Report & Period Covered 14.

15. Supplementary Notes

16. Abstract (Limit: 200 words)

High pressure liquid chromatographic analyses were made of skin from albino-mice and pigmented-mice, and melanoma cells (B16 transplantable hamster melanoma). Under the conditions of system-A, which detects dopa, cysteinyldopas and related compounds, the melanoma cells demonstrated six peaks. Pigmented ear skin showed four of these peaks and albino ear skin showed only one. Using system-B, which detects indole derivatives, two peaks were observed. Enzyme studies demonstrated that three glutathione metabolizing enzymes were elevated after exposure to 4-tertiary-butyl-catechol (98293) (TBC), an antioxidant. The food additive butylated-hydroxytoluene (128370) (BHT) was added to the food given to C57BL/6N-mice. Four weeks after feeding BHT the pigment was sparse and irregular. Electron microscopy studies were carried out on human skin from patients before and after treatment with PUVA. After 2 weeks of treatment many melanosomes demonstrated irregular deposition of pigment which was ultrastructurally interpreted as the start of pheomelanogenesis. Skin biopsy of a black woman with depigmentation who wore rubber gloves while working at a hotel showed no melanocytes. Tests revealed melanosomes with a pheomelanogenesis like ultrastructure. The author concludes that the basic methods needed to investigate chemically induced depigmentation have been developed.

17. Document Analysis a. Descriptors

b. Identifiers/Open-Ended Terms

NIOSH-Publication, NIOSH-Grant, Grant-Number-OH-001714-07, Chromatographic-analysis, Laboratory-animals, Skin-exposure, Pigmentation-disorders, Skin-disorders

c. COSATI Field/Group

18. Availability Statement	19. Security Class (This Report)	21. No. of Pages
	20. Security Class (This Page)	22. Price

PROGRESS REPORT

A. Period: August 1982 to July 1983

B. Publications, Reprints and Preprint previously not included are attached.

C. Personnel engaged on the project and time/effort.

Gerald A. Gellin, M.D., Principal Investigator - 10% time.

Kimie Fukuyama, M.D., Ph.D., will supervise daily progress of research activities, particularly the coordination of different methodologies and findings accumulated by 6 investigators, students and technicians. Electron microscopy of animal skin and tissue culture of melanoma cells are used in this study. 10% time.

Magnus Halldin, Ph.D., Postdoctoral Research Fellow. His HPLC analysis of intermediates of both eumelanin and pheomelanin has allowed us to progress significantly since he joined our group. Preliminary results are shown on Page He will take the position that Dr. Kohzoh Yonemoto held previously and a copy of his C.V. is attached. 100% time.

Neal Castagnoli, Ph.D., conducts chemical analysis of melanin intermediates by mass spectrometers. This segment of the project will become more meaningful when we obtain different intermediates from HPLC columns. 5% time.

Takeshi Kawashima, M.D. He has been appointed to fill the additional 50% position indicated in the original proposal. He has been conducting enzymatic studies of this project. Glutathione metabolizing enzymes are found to be activated in the chemically depigmented melanoma cells (paper accepted for publication in J Invest Derm: attached). He will continue biochemical studies on the skin of mice treated with antioxidants and the methodology developed will be used for human skin as well. He has conducted preliminary studies with butylated hydroxytoluene (BHT), a food additive and antioxidant. The findings are summarized on page 100% time (additional 50% salary is being sought from other sources).

William L. Epstein, M.D. Performs electron microscopy of depigmented skins as shown on page We have recently found 2 patients (one black and another Mexican) proven (by patch test) to suffer from TBC depigmentation. Biopsies showed pheomelanogenesis in the lesions. 5% time.

Hans Rorsman, M.D. He continues to collaborate with us in human studies dealing with induction of pheomelanogenesis in patients treated with PUVA. All PUVA treated skins will be sent by him.

Ching Chung Wang, Ph.D. He will be consulted regarding chemical determination of melanin intermediates.

Elaine Vander-Beugle, Technician. Assists in tissue culture and electron microscopy including tissue preparation, staining of the sections and daily maintenance of animals and tissue culture cells. 100% time.

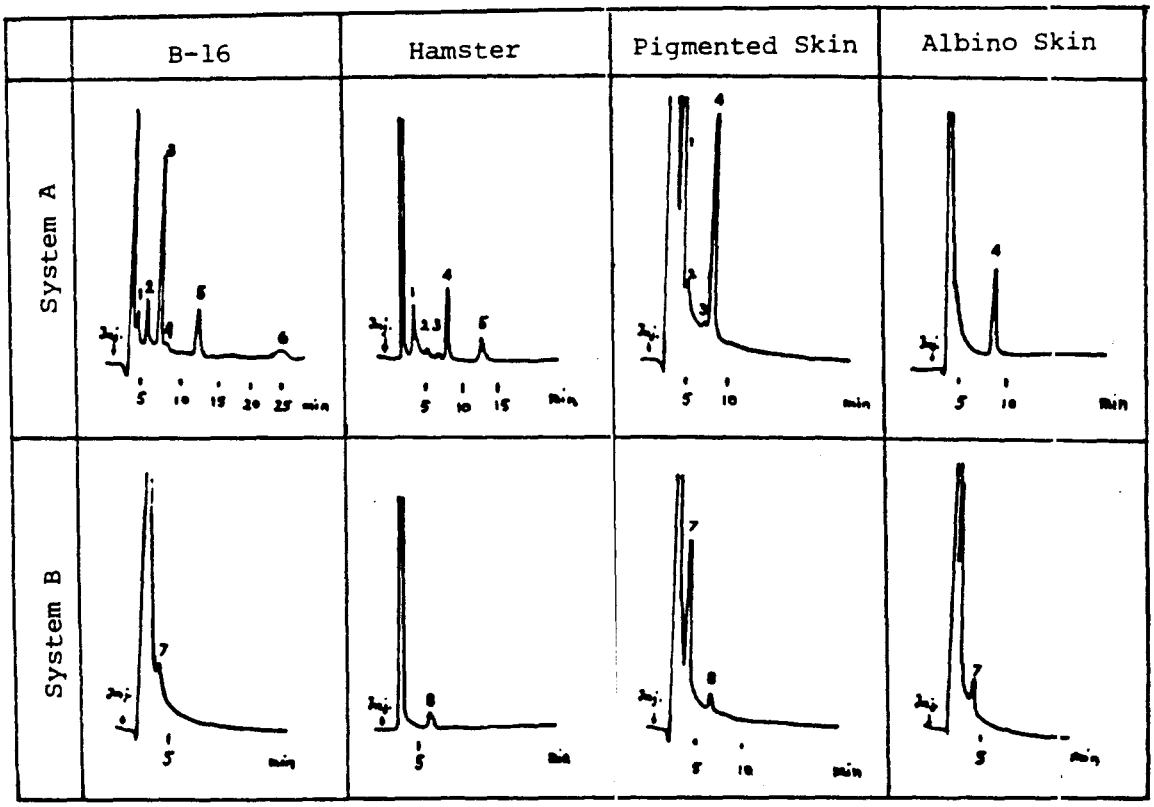
Marci Yellin, Sr. Clerk Typist. Takes care of all purchase orders and keeps current inventory of supplies. She types manuscripts, research progress report and correspondence among investigators. 10% time.

D. Research Plan: There are no significant changes in direction of research as proposed in September, 1982.

E. Accomplishments

1) HPLC Analysis of Catechols in Skin and Melanoma Cells.

Ears of mice, both albino and pigmented, and melanoma cells (B16, transplantable hamster melanoma) were homogenized separately in 0.4 N HCl with a polytron and ultrasound sonicator. Samples were ultracentrifuged and the supernatant was applied on a 10 µm C-18 LiChrosorb column in order to separate electrochemically detectable compounds, most probably catechols, by HPLC. An amperometric detector (BAS Inc., West Lafayette, In.) whose potential was +750 mV vs the Ag/AgCl reference electrode was used and glassy carbon served as the working electrode. Two different mobile phases were used: A) 6.0 g methanesulfonic acid and 3.0 g phosphoric acid per liter MilliQ purified water, pH 3.0, at a flow rate of 1.5 ml/min. B) 3.0 g phosphoric acid per liter 20% aqueous methanol, pH 4.0, at a flow rate of 1.6 ml/min. System A detects dopa, cysteinyldopas and related compounds, while system B detects indole derivatives. L-dopa, L-5-S- and D-5-S-cysteinyldopa, 5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid synthetically made and identified by NMR and mass spectral analysis were used as reference compounds.

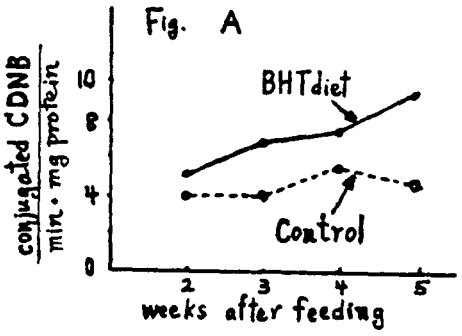


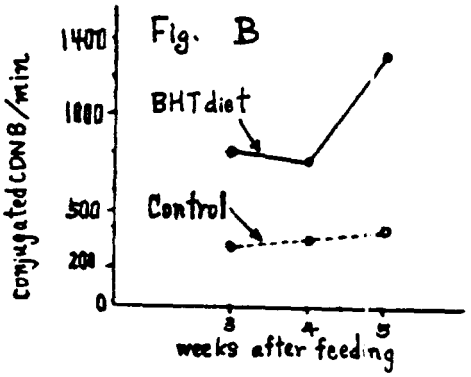
By system A B-16 melanoma cells showed 6 peaks (Peak 1 - 6): Peak 3 showed the same retention time as L-dopa. No peaks corresponded to the cysteinyldopa references. Chemical determination of each peak is in progress. Hamster melanoma cells showed a similar spectra, although there was no detectable peak 6 and the ratio between the peaks was different from that of B-16 melanoma cells. Pigmented ear skin showed Peaks 1, 2, 3 and 4 but albino ear skin showed only peak 4.

By system B we have so far detected 2 peaks (Peak 7 and 8) which are present in varying degrees in the samples tested.

2) Assay of Enzymes Considered to be Involved in Pheomelanogenesis.

Studies with TBC in mouse ear skin and melanoma cells showed that 3 glutathione metabolizing enzymes were elevated after exposure to TBC, an antioxidant (see publications). In order to determine if other antioxidants stimulate pheomelanogenesis and activities of glutathione metabolizing enzymes, we elected to use Butylated Hydroxytoluene (BHT). Since this antioxidant is used as a food additive we made 0.4% (w/w) BHT-containing chow and fed C57BL/6N mice (body weight 25-28 g) for 5 wks. Livers were removed weekly and glutathione S-transferase (GST) measured by the same method used previously by us. Both specific (Figure A) and total (Figure B) activities were increased significantly by the BHT diet.

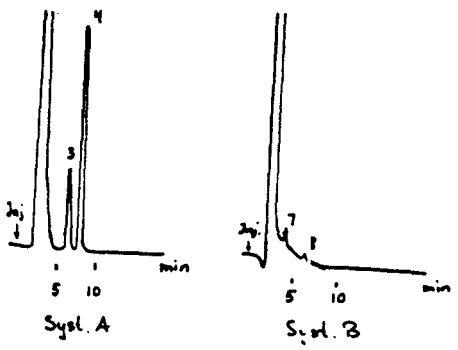




At 4 wks after BHT feeding hair of the back skin became grayish while that of controlled diet mice remained black. Biopsies were prepared for paraffin sectioning and stained with Fontana-Masson stain for pigment. The color of growing hair is dependent upon the degree of pigment in the medulla. The pigment appeared as a regular banding in hairs of mice on the controlled diet, while it was sparce and irregular in mice on the BHT-containing diet.

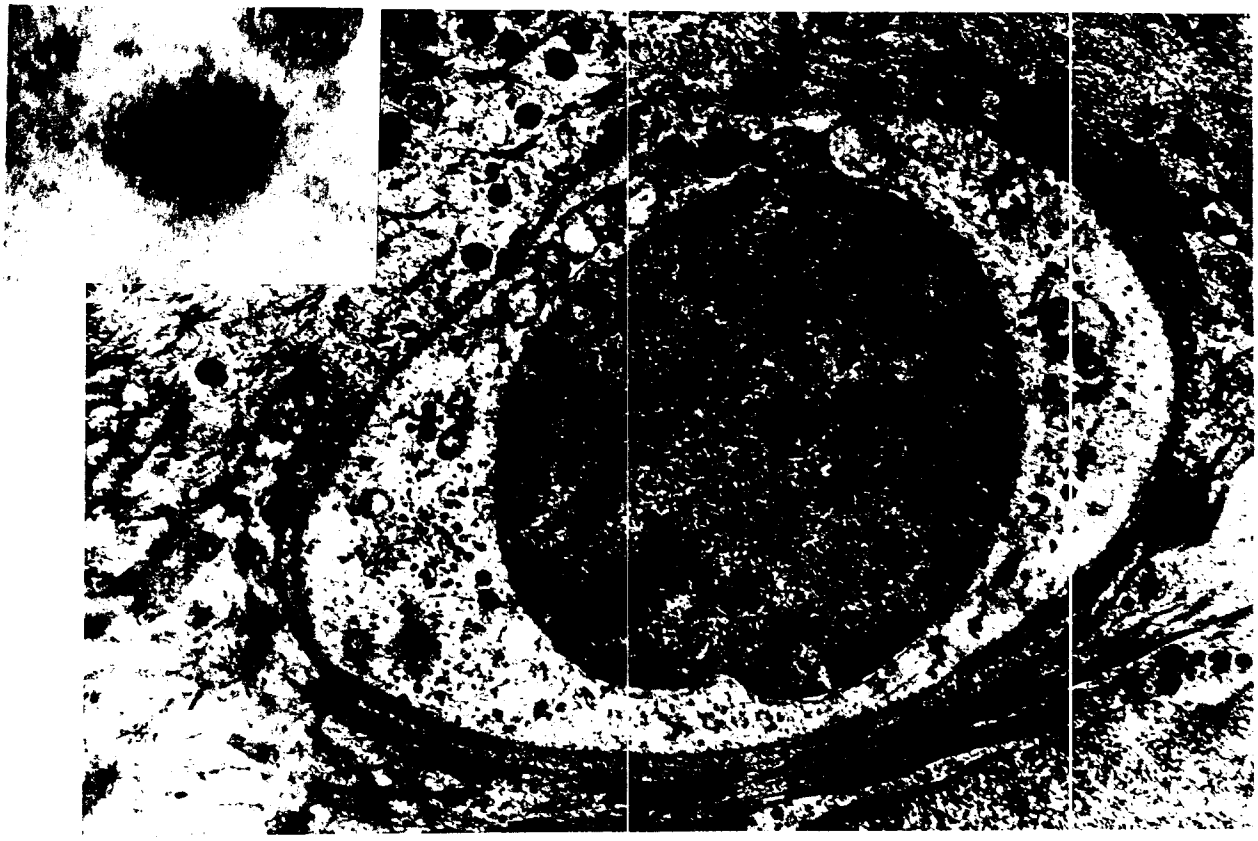
HPLC analysis

Extracts were made from ear skins of BHT treated mice. By system A, peaks 3 and 4 were present, but peaks 1 and 2, seen in pigmented skin was no longer detectable. By system B, reduction of peak 7 occurred but peak 8 was still present in a trace amount.

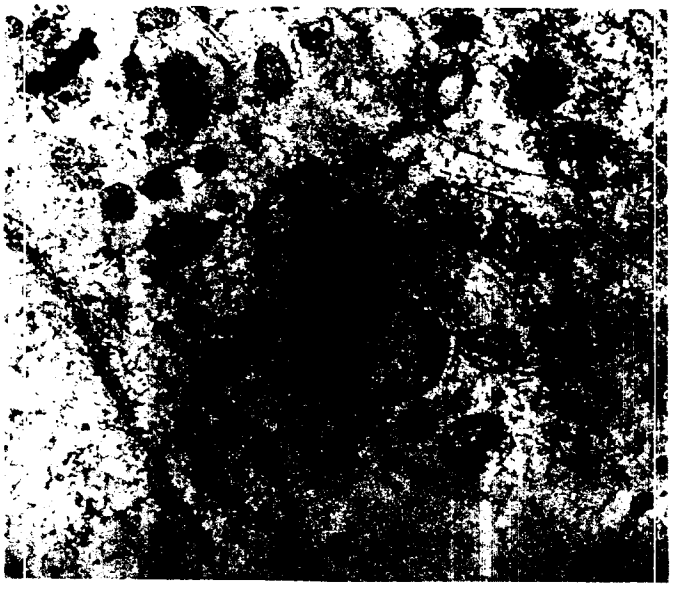


3) Electron Microscopy of Human Skin with Elevated Levels of 2-S-cysteinyldopa.

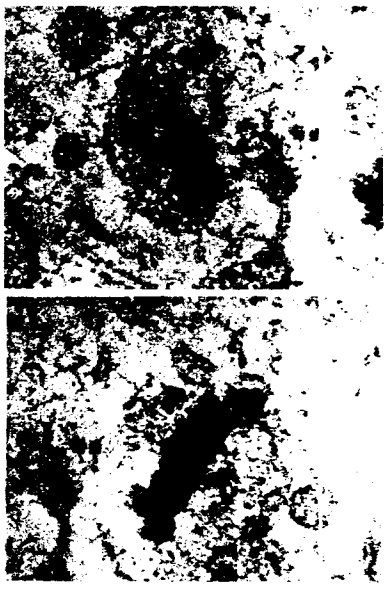
We have studied the ultrastructure of melanocytes of 6 patients before and after PUVA treatment. The figure below shows a typical melanocyte (Mag. x 12,500) containing eumelanosomes (insert, Mag. x 90,000).



After 2 weeks of PUVA treatment many melanosomes demonstrated irregular deposition of pigment which was ultrastructurally interpreted as the beginning of pheomelanogenesis.



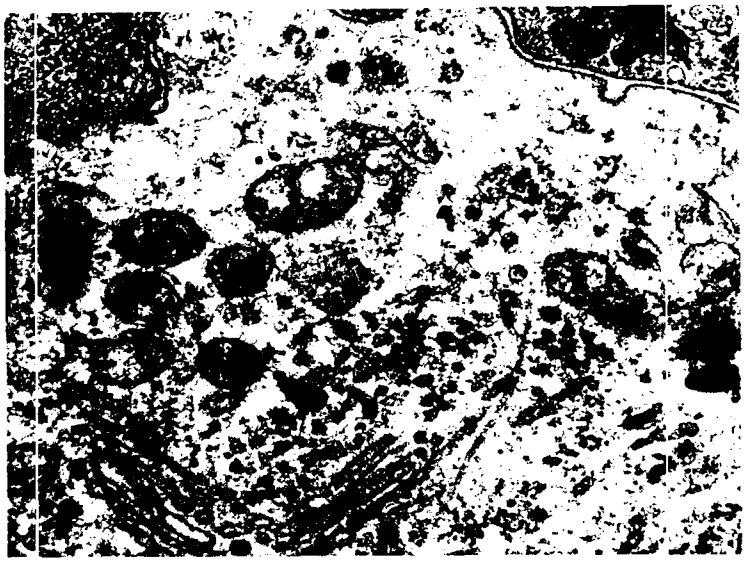
x 45,000



x 90,000

4) A patient seen recently by the P.I.

A black woman working at a hotel in downtown San Francisco exhibited depigmentation of the hands. She wears rubber gloves at work. The skin biopsy from the lesion showed no melanocytes. The P.I. patch tested her with 0.1% TBC on the arm and a biopsy taken 1 week later showed melanosomes with a "pheomelanogenesis-like" ultrastructure.



x 45,000

x 90,000

F. Significance and Future Goals

We believe that our laboratories have achieved the basic methodology necessary to investigate chemically induced depigmentation. Our team is capable of analyzing the conditions resulting from environmental hazardous materials with both chemical and morphological techniques. We will continue to use tissue cultured cells and animals to conduct basic experiments for obtaining dose- and time-dependent information and statistical analysis of findings. However we anticipate that all methodology and information will be useful in the understanding of chemically induced depigmentation in man and in prevention of environmental occupational disease.

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Magnus Halldin, Ph.D.	Postdoctoral Research Fellow	12/15/54

EDUCATION *(Begin with baccalaureate training and include postdoctoral)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
School of Pharmacy, Univ. of Uppsala	B.S.	1973	Pharmacy
School of Pharmacy, Univ. of Uppsala	Ph.D.	1978	Pharmacognosy

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to recent representative publications, especially those most pertinent to this application. Do not exceed 2 pages.

01/78-10/82: Teaching Assistant, Institute of Pharmacognosy, University of Uppsala, Sweden. Within this time period, served one year as a Research Assistant.

11/82-present: Postdoctoral Research Fellow, chemical studies of melanin, University of California, San Francisco.

PUBLICATIONS

1) The importance of side-chain hydroxylated metabolites of Δ^6-THC in Rhesus monkey. M. Halldin, M. Widman and B. Martin. Acta Pharm Suec 16, 34-40, 1979.

2) Chemical synthesis and biological occurence of carboxylic acid metabolites of $\Delta^{1(6)}$-THC. S. Agurell, C. Edward, M. Halldin, K. Leander, S. Levy, J.-E. Lindgren, R. Mechoulam, M. Nordquist and A. Ohlsson. Drug Metab Dispos 7, 155-161, 1979.

3) *In vitro* metabolites of THC by Rhesus monkey liver and human liver. M. Widman, M. Halldin and B. Martin. In "Marihuana: Biological Effects" (G.G. Nahas and W.D.M. Paton, Eds.), pp. 101-103, Pergamon Press, New York, 1979.

4) Urinary metabolites of Δ^1-THC in man. M. Halldin, S. Carlsson, S.L. Kanter, M. Widman and S. Agurell. Arzneim-Forsch/Drug Res 32 (II), 7, 764-768, 1982.

5) Identification of *in vitro* metabolites of Δ^1-THC formed by human livers. M. Halldin, M. Widman, C.V. Bahr, J.-E. Lindgren and B.R. Martin. Drug Metab Dispos 10, 297-301, 1982.

6) Further urinary metabolites of Δ^1-THC in man. M. Halldin, L.K.R. Andersson, M. Widman and L.E. Hollister. Arzneim-Forsch/Drug Res 32 (II), 9, 1135-1138, 1982.

7) Studies on the biotransformation of tetrahydrocannabinol in man and animals. M. Halldin. Acta Univ Upsaliensis (Dissertation 77), Faculty of Pharmacy, 1982.

8) Glucuronic acid conjugate of Δ^1-THC identified in the urine of man. M. Halldin and M. Widman. Arzneim-Forsch/Drug Res 33 (1), 1, 177-178, 1983.

9) A comparison between the metabolism of Δ^1-THC by perfused lung and liver of rat and guinea pig. M. Halldin, M. Widman, H. Isaac, E. Nilsson and A. Ryrfeldt. Xenobiotic, submitted for publication.

cont.

publications, cont.

10) Acidic metabolites of Δ^1-THC excreted in the urine of man. M. Halldin, M. Widman, S. Agurell, L.E. Hollister and S.L. Kanter. Proceedings from Cannabis conference in Louisville, KY, August 19-20, 1982.

11) The metabolism of Δ^1-tetrahydrocannabinol in man. M. Widman, M. Halldin and S. Agurell. In "Drugs and the Pharmaceutical Sciences", Vol. 20, Marcel Dekker, Inc., in press, 1983.

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Takeshi Kawashima, M.D.	Postdoctoral Research Dermatologist	06/28/54

EDUCATION *(Begin with baccalaureate training and include postdoctoral)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Tokyo Medical College, Tokyo, Japan	MD	1980	Dermatology

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to recent representative publications, especially those most pertinent to this application. Do not exceed 2 pages.

05/80-08/82: Dermatologist, Tokyo Medical College Hospital, Tokyo, Japan.

09/82-present: Postdoctoral Research Dermatologist, University of California, San Francisco, Department of Dermatology, San Francisco, California.

PUBLICATIONS

1) Kawashima T, Ohi T: Proliferating trichilemmal cyst- a case report and statistical analysis on the reports in Japan. Rinsho Dermatol 24,4:387-389, 1982.

2) Fukuhara S, Kawashima T, Katoh T, Kawasaki O, Tokuda Y, Shirota T: A case of Klippel-Trenaunay-Perkes Weber syndrome. Rinsho Dermatol 24,11:1249-1254, 1982.

3) Ohi T, Kawashima T, Katoh T, Nakano Y, Hokano M: A case report on adult T-cell leukemia. Japan J Dermatol 92,13:1369-1378, 1982.

4) Yonemoto K, Kawashima T, Gellin GA, Epstein WL: Enzyme dynamics in pheomelanogenesis caused by 4-tertiary butyl catechol (TBC) in melanoma cells. Clin Res 31,2:611A, 1983.

5) Kawashima, T, Satoh T, Ohi T, Honda T: A case report on congenital leukemia. Rinsho Dermatol 25,8:730-734, 1983.

6) Kawashima T, Katoh T, Ohi T, Wakashin K, Hachiya T, Fukuhara S: Malignant schwannoma with Recklinghausen disease. Rinsho Dermatol 25,9:857-861, 1983.