	Titre of AN Ab[a]		Thymosin liver activity[b]		Crystals in epithelial cells[c]	
	Swiss	SWAN	Swiss	SWAN	Swiss	SWAN (%)
$3^1/_2$-month-old	0	8–64	128	16–32	0	10
5-month-old	0	64–2048	128	8–16	0	80

[a] Obtained by immunofluorescence. [b] Obtained by the technique of BACH et al.[3–9]. [c] % of epithelial cells with crystals.

the development of epithelial cells in NZB and (NZB × NZW) F1. A deficit in T cell functions has been observed moreover in both humans and animals with auto-immune disease[6,7], along with a definite reduction of thymosin-like activity in the serum[3,8,9].

The Table shows the presence of crystals, the level of serum thymosin-like activity and the titre of AN Ab for the Swiss and SWAN mice. In SWAN mice the level of serum thymosin-like activity falls between $3^1/_2$ and 5 months, whereas the number of cells with crystals increases over the same period, as does the number of mice positive in AN Ab. We suggest that the cytoplasmic crystals represent an intracellular build up of thymosin, or a precursor which cannot be secreted by the cell, perhaps due to lack or modification of an enzyme necessary for the activation of this hormone. Other examples of intracellular crystals representing storage of unsecreted protein have been documented[10–12].

This hypothesis will be tested by a more detailed investigation of the age at which the crystals first appear in the SWAN mice and by looking for similar formations in other autoimmune animals, such as NZB mice and perhaps in old mice of normal strains. In observations made on a few SWAN mice at an age of 1 year, crystals were present though less abundantly than in the 5-month mice.

Résumé. L'étude en microscopie électronique du thymus de souris autoimmunes SWAN de 5 mois dont le taux d'activité «thymosine like» est très bas, montre la présence d'inclusions cristallines dans le cytoplasme des cellules réticuloépithéliales. Ces cristaux évoquent la possibilité d'un défaut d'excrétion de l'hormone thymique par les cellules épithéliales expliquant la faible activité hormonale trouvée dans le torrent circulatoire de ces animaux.

D. M. SCHMITT[13] and J. C. MONIER[14]

Laboratoire d'Immunopathologie, Pavillon R, Hôpital Edouard Herriot, F–69374 Lyon (France); and Laboratoire d'Hygiène, Université Claude Bernard, 8, Avenue Rockefeller, F–69008 Lyon (France),
14 May 1974.

[6] B. C. LEVENTHAL and N. TALAL, J. Immun. *104*, 918 (1970).
[7] J. C. MONIER and M. ROBERT, Ann. Immun., in press (1974).
[8] J. F. BACH, M. DARDENNE and M. PAPIENIK, Lancet *2*, 1056 (1972).
[9] J. F. BACH, M. DARDENNE and J. C. SALOMON, Clin. exp. Immun. *14*, 247 (1973).
[10] J. P. THIERY, Revue Hémat. *13*, 61 (1958).
[11] D. W. FAWCETT, *The Cell* (Saunders Co., London 1969), p. 319.
[12] J. C. CAWLEY, C. R. BARKER, R. D. BRITCHFORD and J. L. SMITH, Clin. exp. Immun. *13*, 407 (1973).
[13] Laboratoire d'Immunopathologie, Pavillon R, Hôpital Edouard Herriot, F–69374 Lyon, France.
[14] Laboratoire d'Hygiène, Université Claude Bernard, 8, Avenue Rockefeller, F–69008 Lyon, France.

Antiandrogenic Suppression of Lymphocytic Blastogenesis: in vitro and in vivo Observations

The androgenic dependence of prostatic cancer and its treatment by antiandrogenic therapy by the administration of estrogen has been well documented since the classical studies of HUGGINS et al.[1]. However, the potential effects of such therapy on the immunologic responsiveness of the host to malignancy have not been delineated. Recently, ABLIN[2] alluded to the possibility that as estrogens result in a generalized stimulation of the reticuloendothelial system leading to what appears to be suppression of cell-mediated hypersensitivity reactions and enhancement of circulating antibody production that pallative hormonal therapy in patients with advanced

[1] C. HUGGINS, R. E. STEVENS JR. and C. V. HODGES, Arch. Surg. *43*, 209 (1941).
[2] R. J. ABLIN, in *Symposium on Normal and Abnormal Growth of the Prostate* (Ed. E. R. AXELROD; Charles C. Thomas, Springfield 1975), in press.

Table I. Effect of diethylstilbestrol diphosphate (DEP-S) on the incorporation of ^{3}H-thymidine of peripheral blood lymphocytes stimulated with phytohaemagglutinin (PHA)

Mean ± S.D. × 10^{-4} Counts/min incorporation of ^{3}H-thymidine of 10^6 peripheral blood lymphocytes incubated with [a]			
PHA	PHA + DES-P	Without PHA	DES-P
7.5 ± 4.6	3.3 ± 2.3	1.3 ± 0.83	0.92 ± 0.38

[a] Data expressed as mean value ± 1 S.D. of triplicate determinations on 7 adult males.

Table II. Effect of serum from patients with prostatic cancer prior to and following estrogen therapy on the incorporation of ^{3}H-thymidine of autologous peripheral blood lymphocytes stimulated with phytohaemagglutinin

Mean $\times 10^{-4}$ cpm incorporation of ^{3}H-thymidine of 10^6 peripheral blood lymphocytes incubated with autologous serum[a]		
Prior to estrogen	Following estrogen	Significance[b] (p)
19.5	9.9	< 0.05

[a] Data expressed as mean value of triplicate determinations on 8 patients with prostatic cancer prior to and following receipt of estrogen.

[b] Probability that difference in mean cpm prior to and following receipt of estrogen are not due to chance as determined by the Student's t-test.

cancer of the breast or prostate may reduce the surveillance efficiency of their immunologic system. As there is evidence suggestive that in vitro reactivity of peripheral blood lymphocytes (PBL) to various mitogens, notably phytohaemagglutinin (PHA), closely parallels cell-mediated immunologic competence, we have as an initial approach to investigating the effects of exogenous estrogen on immunologic competence evaluated the effect of estrogen on PHA induced lymphocytic blastogenesis.

Human PBL from 7 healthy adult males, 18 to 33 years of age, were obtained by centrifugation of the leucocyte-rich plasma of heparinized blood on a Ficoll (Pharmacia Fine Chemicals, Uppsala, Sweden) – Isopaque (Nyegaard and Company, Oslo, Norway) gradient. Triplicate 2 ml cultures containing 5.0×10^5 PBL/ml of 80% RPMI 1640 medium (Grand Island Biological Company, Grand Island, New York) supplemented with 20% foetal calf serum (Grand Island Biological Company, Grand Island, New York) containing 100 U/ml penicillin G and 100 µg/ml streptomycin with and without purified PHA (Burroughs Wellcome, Beckenham, England, Lot K 6165) reconstituted in phosphate buffered saline, pH 7.2, at a concentration of 5 µg protein/ml culture were prepared and incubated for 68 h at 37 °C in a 5% CO_2 in air mixture. Viability was assessed by trypan-blue dye exclusion. 4 µCi of ^{3}H-thymidine (^{3}HTdR, specific activity 66 Ci/mM, ICN Pharmaceuticals, Inc., Irvine, California, Lot No. 613262) was added 4 h before harvesting. Blastogenesis was defined as incorporation of ^{3}HTdR in the trichloroacetic acid-insoluble fraction (DNA) measured by liquid scintillation counting (Mark IV, Searle, Chicago, Illinois) and expressed as cpm per 10^6 PBL.

The effect of estrogen on blastogenesis was evaluated by the addition of 500 µg/ml culture of Stilphostrol (diethylstilbestrol diphosphate, DEP-S, Dome Laboratories, West Haven, Connecticut) determined as the optimal inhibitory doseage from a dose response curve for PHA-stimulated PBL cultured in varying concentrations of DEP-S, to cultured PBL with and without PHA.

The effect of DEP-S on the incorporation of ^{3}HTdR of human PBL from 7 healthy adult males stimulated with PHA is presented in Table I. The data are presented as the mean $\pm$ 1 S.D. of triplicate determinations expressed as cpm/10^6 PBL. As shown, there was a 56% reduction in the mean response of PBL to PHA following the incorporation of DEP-S into the culture medium. Based upon the 'sign test' of the probability under the 'null hypothesis' of this reduction or suppression of lymphocytic blastogenesis occuring by chance was 0.008, indicating a very significant difference in lymphocytic blastogenesis of PHA in the presence and absence of DEP-S.

The possibility that the observed reduction of lymphocytic blastogenesis to PHA in the presence of DEP-S was due to a lymphocytotoxic effect of DEP-S, was excluded by the observation that the viability (as determined by trypan-blue exclusion) of PBL incubated for 72 h in the supplemented culture medium alone and the supplemented culture medium containing the inhibiting dosage of DEP-S were identical.

The results suggest that DEP-S suppresses the in vitro response of human PBL to PHA. In view of recent studies by BRESCIANI et al.[3] demonstrating the interaction of estrogen receptors with intranuclear cellular components, i.e. chromatin, it is suggested that the observed reduction in the blastogenic response of PBL to PHA in the presence of DEP-S may be due to an intrinsic nuclear alteration of DNA synthesis rather than an actual blockage of lymphocytic receptor transforming sites. This reduction or suppression of blastogenesis in the presence of estrogen suggests, particularly in view of preliminary studies[4] as shown in Table II, demonstrating suppression of blastogenesis of PHA-stimulated PBL in the presence of autologous serum from patients with prostatic cancer following estrogenic therapy, that the pallative effects of estrogen therapy in the clinical treatment of patients with prostatic cancer may be countered by its adverse effect on the host's immunologic responsiveness to malignancy.

[3] F. BRESCIANI, E. NOLA, V. SICA and G. A. PUCA, Fedn Proc. *32*, 2126 (1973).

[4] R. J. ABLIN, P. GUINAN, G. R. BRUNS and I. M. BUSH, Clin. Res. *22*, 599 A (1974).

Subsequent studies presently in progress shall attempt to verify the above hypothesis, i.e. that reduction in the blastogenic response of PBL to PHA in the presence of DEP-S is due to an intrinsic nuclear alteration of DNA synthesis and to evaluated and correlated the level of estrogen in the systemic circulation with lymphocytic reactivity to PHA: a) in patients with prostatic cancer prior to and following estrogen therapy and b) in females prior to conception and during each trimester period in the presence of autologous or isologous serum.

Zusammenfassung. Nachweis, dass die Fähigkeit zur Blastogenese der Lymphozyten gesunder junger Männer nach Reizung mit Phytohämagglutinin im peripheren Blut unterdrückt wird, wenn die Lymphozyten zusammen mit Östrogen (Diethylstilbestrol-Diphosphat) kultiviert werden.

R. J. Ablin, G. R. Bruns, P. Guinan and I. M. Bush

Immunobiology Section and the Center for the Study of Prostatic Diseases, Division of Urology, Cook County Hospital and Graduate School of Medicine; 1825 West Harrison Street, Chicago (Illinois 60612, USA); the Hektoen Institute for Medical Research; Mount Sinai Hospital Medical Center and the Departments of Microbiology and Urology, the Chicago Medical School/University of Health Sciences, Chicago (Illinois, USA), 13 May 1974.

Influence of Chronic Treatment with 2-Bromo-α-ergocryptine (CB-154) on the Reproductive and Lactational Performance of the C3H/HeJ Female Mouse[1]

2-Bromo-α-ergocryptine (CB-154) is an effective suppressor of pituitary prolactin secretion in mice[2,3], rats[4,5], certain domestic animals[6] and man[7–9]. A number of laboratories have provided convincing evidence that prolactin is critically involved in the development and growth of murine mammary tumors[10–13]. Thus, chronic CB-154-induced suppression of prolactin secretion has been shown to virtually prevent the appearance of spontaneous mammary carcinoma in mice[3] and promote regression of carcinogen-induced rat mammary tumors[14–16].

Because of the: 1. striking anti-mammary tumorigenic effects of the drug in rodents[3,14–16]; 2. possible significant role for prolactin in human breast tumorigenesis[17–19] and 3. current and contemplated use of the drug for prolactin suppression in women[8], it is imperative to determine the effects of the drug on other endocrine related processes. Thus, the purpose of this investigation is to determine the effects of chronic treatment with CB-154 on the reproductive and lactational activities of the C3H/HeJ female mouse.

Materials and methods. All animals used in this study were C3H/HeJ mice obtained from the Jackson Laboratories, Bar Harbor, ME. They were housed in either groups of 3 (3 females) or groups of 4 (3 females plus 1 male) in a temperature (24° ± 0.5 °C) and light (14 h/day) controlled environment and provided a diet of Wayne Lab Blox (Allied Mills, Inc., Chicago, IL) and water ad libitum.

Treatment of mice with CB-154 prior to mating. 24 nulliparous 2-month-old female mice were given s.c. injections of 0.1 mg CB-154 suspended in 0.9% NaCl solution daily, for 50 days. The CB-154[4] suspension (1mg/ml) was prepared by dissolving the drug initially in a minimal amount of ethanol and diluting to volume with 0.9% NaCl solution.

[1] Supported by NIH research grant No. CA-13777 and American Cancer Society research grant No. ET-59.

[2] R. Yanai and H. Nagasawa, Hormone Res. *5*, 1 (1974).
[3] C. Welsch and C. Gribler, Cancer Res. *33*, 2939 (1973).
[4] E. Flückiger, in *Prolactin and Carcinogenesis* (Eds. A. R. Boyns and K. Griffiths; Alpha Omega Alpha Publishing, Cardiff, Wales, U.K. 1972), p. 162.
[5] C. Brooks and C. Welsch, Proc. Soc. exp. Biol. Med. *146*, 433 (1974).
[6] D. Schams, V. Reinhardt and H. Karg, Experientia *28*, 697 (1972).
[7] J. L. Pasteels, A. Danguy, M. Frérotte and F. Ectors, Annls. Endocr. *32*, 188 (1971).
[8] P. M. Lutterbeck, J. S. Pryor, L. Varga and R. Wenner, Br. med. J. *3*, 228 (1971).
[9] M. Rozencweig, J. C. Hueson, S. Bela, M. L'Hermite and C. Robyn, Eur. J. Cancer *9*, 657 (1973).
[10] O. Mühlbock and L. M. Boot, Cancer Res. *19*, 402 (1959).
[11] J. Furth, in *Hormones and Neoplasia* (Eds. A. Engel and T. Larson; Thule Int. Symp., Stockholm; Nordeska Bokhandelns Förlag, Stockholm 1968), p. 1.
[12] O. H. Pearson, O. Llerena, R. Llerena, A. Molina and T. Butler, Trans. Ass. Am. Phys.ns *82*, 225 (1969).
[13] C. W. Welsch, H. Nagasawa and J. Meites, Cancer Res. *30*, 2310 (1970).
[14] J. C. Heuson, C. Waelbroeck-Van Gaver and N. Legros, Eur. J. Cancer *6*, 353 (1970).
[15] H. Stähelin, B. Burckhardt-Vischer and E. Flückiger, Experientia *27*, 915 (1971).
[16] E. E. Cassell, J. Meites and C. W. Welsch, Cancer Res. *31*, 1051 (1971).
[17] H. Salih, H. Flax, W. Brander and J. R. Hobbs, Lancet *2*, 1103 (1971).
[18] R. P. Dickey and J. P. Minton, New Engl. J. Med. *286*, 843 (1971).
[19] R. M. L. Murray, G. Mozaffarian and O. H. Pearson, in *Prolactin and Carcinogenesis* (Eds. A. R. Boyns and K. Griffiths; Alpha Omega Alpha Publishing, Cardiff, Wales, U.K. 1972), p. 158.

Table I. Effect of daily treatment for 50 days of C3H/HeJ female mice with CB-154 prior to mating on reproductive performance

Treatment	No. of mice	No. and % of mice which became pregnant	Mean[a] latency period of parturition (day)	Mean[a] No. of pups per litter at weanling	Pups surviving to weanling (%)	Mean[a] weight of pups at weanling (g)
Controls	24	24 (100%)	35.0 ± 2.4	4.9 ± 0.7	53	9.5 ± 0.6
CB-154[b]	21	21 (100%)	28.1 ± 3.4	5.2 ± 0.3	62	8.4 ± 0.1

[a] Mean ± standard error. [b] CB-154, 0.1 mg/mouse/day.