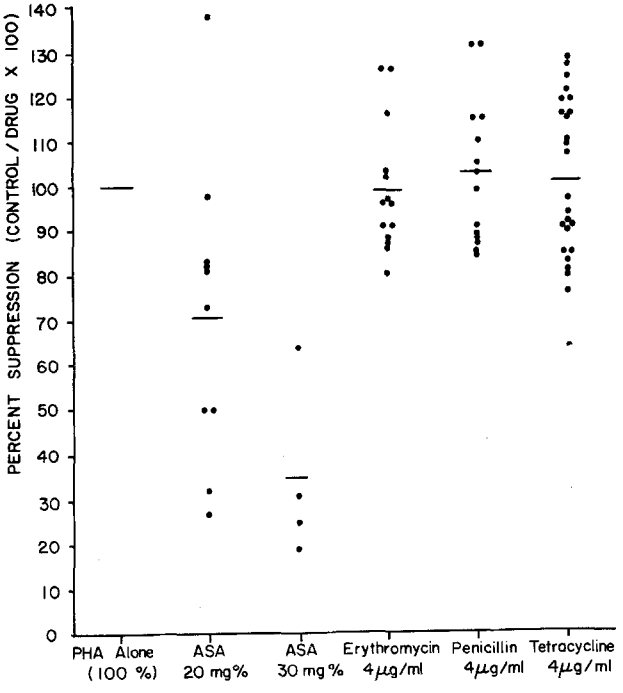
Discussion. Many antibiotics such as chloramphenicol, streptomycin, gentamicin, tetracycline and rifampicin act by interfering with various steps of protein synthesis, translation, transcription or RNA function within bacteria[2,5–8]. Evidence is strong that anti-neoplastic antibiotics as well as chloramphenicol exert an influence on host cell protein synthesis and subsequent immunologic responsiveness[1,2]. Rifampicin has been shown to suppress PHA responsiveness of human lymphocytes in culture[9] and there is a possibility that it may have immunosuppressive effects in vivo[10]. The PHA - stimulated lymphocyte culture system is a widely used measure of one aspect of immunologic competence. Within hours of incubation with PHA, increased RNA and protein synthesis begins in the lymphocyte, followed in about 24 h by increased DNA synthesis[11]. The system thus offers a broad range of synthetic steps for the study of potential inhibitory effects. The long-term use of tetracycline for conditions such as acne and hidradentitis suppurativa, as well as its use for presumptive, though empirical, anti-inflammatory effects in rosacea and certain forms of panniculitis made its investigation for possible immunosuppressive activity seem reasonable.

The concentration of tetracycline used approximates that found in human blood samples after an oral dosage of 500 mg q.i.d.[12]. The erythromycin and penicillin concentrations utilized are also similar to the higher blood levels achieved by standard dosage administration of these antibiotics. The findings in this experiment imply that protein, RNA and DNA synthesis in lymphocytes are not impaired by the antibiotics tested in therapeutic concentrations, since PHA-induced transformation is dependent on all of these metabolic processes. No studies of the metabolites of these antibiotics were investigated.



The effects of antibiotics and acetylsalicylate upon PHA-induced blastogenesis of human lymphocytes. Erythromycin, penicillin and tetracycline failed to demonstrate any inhibitory effect.

Zusammenfassung. Therapeutische Konzentrationen von Penicillin, Erythromycin und Tetracycline wurden Lymphozytenkulturen zugefügt um mögliche Ähnlichkeiten zu der immunsuppressiven Wirkung antineoplastischer Antibiotika zu bestimmen. Eine Hemmung der PHA-induzierten Blastogenese konnte nicht beobachtet werden.

W. C. DAM, F. D. MALKINSON and H. GEWURZ[13]

Departments of Dermatology and Immunology, Rush-Presbyterian-St. Luke's Medical Center, 1725 West Harrison Street, Chicago (Illinois 60612, USA), 13 November 1974.

[5] H. KAJI and A. KAJI, Proc. natn. Acad. Sci., USA *54*, 213 (1965).
[6] J. DAVIES, L. GORINI and B. D. DAVIS, Molec. Pharmac. *1*, 93 (1965).
[7] D. S. FEINGOLD, New Engl. J. Med. *269*, 900 and 957 (1963).
[8] M. R. BLUNDELL and D. G. WILD, Biochem. J. *121*, 391 (1971).
[9] B. S. NILSSON, Lancet *2*, 374 (1971).
[10] M. G. MARIJA, Lancet *2*, 930 (1971).
[11] H. L. COOPER, in *The Cell Cycle and Cancer* (Ed. R. BASERGA; Marcel Dekker, Inc., New York 1971), p. 206.
[12] L. WEINSTEIN, in *The Pharmacological Basis of Therapeutics* (Eds. L. S. GOODMAN and A. GILMAN; MacMillan Co., New York 1968), p. 1242.
[13] Supported by grants from the Fay-Hunter Trust.

Mitogenic Responses of Thymus Cell Subpopulations

The central role of the thymus gland in the immune system has been well documethod[1]. This organ provides the peripheral lymphoid system with a heterogenous population of immunologically competent cells (T cells) which function in humoral immunity[2,3] and are paramount in the cellular immune response[4,5]. T cells and their progenitors (thymocytes) exhibit distinctive responses to specific plant mitogens. Thymocytes exhibit marked DNA synthesis when stimulated with concanavalin A (Con A) while phytohemagglutinin (PHA) induces only a slight stimulation[6]. However, T cells within the spleen respond almost equally to both Con A and PHA[7]. The present study investigates the mitogenic responses of subpopulations of murine thymus cells separated on albumin discontinuous gradients to the thymus dependent mitogens Con A, PHA, and the thymus independent mitogen *E. coli* lipopolysaccharide (LPS).

Materials and methods. Thymus glands were removed aseptically from 6–10-week-old male and female C57Bl/6

[1] J. F. A. P. MILLER and I. OSOBA, Physiol. Review *47*, 437 (1967).
[2] B. ANDERSSON and H. BLOMGREN, Cell. Immun. *1*, 362 (1970).
[3] J. F. BACH and M. DARDENNE, Cell. Immun. *6*, 603 (1973).
[4] J. F. A. P. MILLER, Proc. R. Soc., Lond. Ser. B, *156*, 415 (1962).
[5] I. M. ROITT, M. F. GREAVES, G. TORRIGIANI, J. BROSTOFF and J. H. R. PLAYFAIR, Lancet, *2*, 367 (1969).
[6] J. D. STOBO, A. S. ROSENTHAL and W. E. PAUL, J. Immun. *108*, 1 (1972).
[7] J. D. STOBO and W. E. PAUL, Cell. Immun. *4*, 367 (1972).

mice with special care taken to remove parathymic lymph nodes and other adhering tissue from the surface of the organ. Viable cell suspensions were prepared as described previously[8]. Thymus cells were separated on bovine serum albumin (BSA) (fraction V powder, lot No. 201, Miles Laboratories) discontinuous gradients prepared according to the method of RADIT et al.[9] with the modification of COLLEY et al.[10]. Briefly, thymus cells ($1–1.3 \times 10^9$) were suspended in 5.5 ml of 30% BSA, layered in the bottom of 35 ml centrifuge tubes and carefully overlaid with 7 ml each of 27%, 24%, 20%, and 5.5 ml of 10% BSA. Centrifugation was carried out at 4 °C for 30 min at $20{,}000 \times g$ employing a Spinco SW 25.1 swinging bucket rotor (Beckman Instruments). The cell bands which formed at the density interfaces and the pellet were removed, diluted with media 199 (M199), washed 3 times, and the number of viable cells determined by the trypan blue exclusion method. The cell bands were identified as follows: band A, cells collected above 20% BSA; band B, cells collected above 24% BSA; band C, cells collected above 27% BSA; band D, cells collected above 30% BSA; and the pellet.

Mitogenic stimulation of separated or nonseparated thymus cells (1×10^6) was carried out in 1 ml of M199 with L-glutamine and Earle's Modified Salts supplemented with 20% heat inactivated fetal calf serum and 100 units of penicillin per ml and 100 µg of streptomycin per ml. All cell cultures were incubated at 37 °C in a 5% carbon dioxide atmosphere. PHA-M (General Biochemicals, lot No. 230372) was utilized at a concentration of 2 µl which induced maximum stimulation in nonseparated thymus cells incubated for 48 h with the mitogen. Con A (Miles-Yeda, lot No. 78) was reconstituted in phosphate buffered saline (PBS) (pH 7.2), sterilized by filtration, and stored at −20 °C. Maximum stimulation of nonseparated thymocytes occurred when 10 µg Con A was utilized with an incubation period of 72 h. *E. coli* lipopolysaccharide 055:B5 (Difco) was reconstituted in sterile PBS and stored at 4 °C. Maximum stimulation of nonseparated thymus cells occurred when 10 µg of LPS was employed with an incubation time of 48 h.

One µCi of tritiated thymidine (New England Nuclear, specific activity 20 Ci/mmole) was added to each tube for the last 24 h of culturing and the cells were processed for liquid scintillation counting[8]. The data is expressed as mean counts per minute (cpm) of at least 4 replica samples and converted to a stimulation index which is defined as the ratio of cpm stimulated cells to cpm unstimulated cells.

Results and discussion. Total cell recovery for all gradients ranged between 49 and 62% with band C containing the largest proportion of recovered cells (49.2%). The remaining cells were distributed in bands, A, B, D, and the pellet (A, 0.8%; B, 20.0%; D, 18.5%; P, 11.5%). The cell viability of all bands was greater than 90% while the viability of cells in the pellet was only 50%. Although all bands exhibited some heterogeneity, band A contained the largest concentration of large cells (> 12 µm). Band B contained about half small cells (< 8 µm) and half medium (8–12 µm) to large cells. Band C was predominately small cells with a few medium cells. Band D was made up almost entirely of small cells. The pellet contained predominately small cells plus erythrocytes and cellular debris.

The mitogenic stimulation of thymus cell subpopulations to PHA is illustrated in Table I. The cells recovered from band B showed a moderate increase in their stimulation index. Nonseparated control cells and the separated cells from bands A, C, D, and the pellet were not stimulated by PHA. The mitogenic responses of thymus cell subpopulations to Con A is shown in Table II. Cells obtained

[8] R. J. MARTIG and J. L. TRIBBLE, Infection Immunity *10*, 1018 (1974).
[9] I. J. RADIT, R. I. MISHELL and R. W. DUTTON, J. exp. Med. *128*, 681 (1968).
[10] D. G. COLLEY, A. Y. SHIH WU and B. H. WAKSMAN, J. exp. Med. *132*, 1107 (1970).

Table I. Response of C57Bl/6 mouse thymus cells separated on BSA discontinuous gradients to PHA

Cell fraction	Unstimulated cells (counts/min)	Stimulated cells (counts/min)	Stimulation index
Control[a]	157 ± 12[b]	152 ± 10	0.97
	188 ± 18	151 ± 37	0.80
	198 ± 29	199 ± 15	1.00
Band A	619 ± 51	629 ± 04	1.02
	1924 ± 401	1596 ± 16	0.80
	637 ± 28	545 ± 129	0.86
Band B	399 ± 59	571 ± 72	1.43
	358 ± 12	531 ± 20	1.48
	125 ± 12	168 ± 34	1.44
Band C	120 ± 09	138 ± 06	1.15
	210 ± 22	168 ± 20	0.80
	222 ± 10	140 ± 08	0.63
Band D	212 ± 53	144 ± 20	0.68
	254 ± 20	254 ± 17	1.00
	102 ± 16	103 ± 29	1.00
Pellet	103 ± 32	116 ± 04	1.12
	211 ± 43	163 ± 24	0.77
	188 ± 18	151 ± 37	0.80

[a] Nonseparated thymocytes. [b] Data for 3 individual experiments is given. Values listed are the mean cpm of 4 replica tubes plus the standard deviation.

Table II. Response of C57Bl/6 mouse thymus cells separated on BSA discontinuous gradients to Con A

Cell fraction	Unstimulated cells (counts/min)	Stimulated cells (counts/min)	Stimulation index
Control[a]	419 ± 34[b]	1063 ± 185	2.54
	134 ± 28	250 ± 16	1.87
	116 ± 12	144 ± 08	1.23
Band A	430 ± 20	413 ± 32	0.96
	263 ± 21	283 ± 39	1.08
	245 ± 40	242 ± 25	0.08
Band B	398 ± 52	1562 ± 203	3.92
	162 ± 31	932 ± 201	5.75
	117 ± 15	507 ± 31	4.33
Band C	381 ± 52	385 ± 75	1.01
	130 ± 27	154 ± 05	1.18
	149 ± 10	116 ± 09	0.78
Band D	433 ± 29	385 ± 20	0.89
	151 ± 16	119 ± 16	0.78
	109 ± 03	75 ± 08	0.69
Pellet	292 ± 46	367 ± 14	1.25
	203 ± 85	149 ± 21	0.73
	99 ± 06	119 ± 21	1.20

[a] Nonseparated thymocytes. [b] Data for 3 individual experiments is given. Values listed are the mean cpm of 4 replica tubes plus the standard deviation.

Table III. Response of C57Bl/6 mouse thymus cells separated on BSA discontinuous gradients to LPS

Cell fraction	Unstimulated cells (counts/min)	Stimulated cells (counts/min)	Stimulation index
Control[a]	370 ± 49[b]	398 ± 50	1.08
	116 ± 09	173 ± 44	1.48
	224 ± 04	243 ± 30	1.08
Band A	1522 ± 225	1336 ± 108	0.88
	4405 ± 359	1994 ± 451	0.45
	702 ± 99	295 ± 62	0.42
Band B	253 ± 19	528 ± 15	2.09
	158 ± 20	311 ± 78	1.96
	166 ± 35	395 ± 32	2.38
Band C	93 ± 18	99 ± 12	1.09
	48 ± 06	74 ± 15	1.55
	173 ± 13	221 ± 40	1.28
Band D	136 ± 24	167 ± 30	1.23
	84 ± 11	89 ± 22	1.05
	249 ± 52	184 ± 29	0.74
Pellet	276 ± 105	192 ± 07	0.69
	160 ± 21	159 ± 48	0.99
	129 ± 14	124 ± 08	1.09

[a] Nonseparated thymocytes. [b] Data for 3 individual experiments is given. Values listed are the mean cpm of 4 replica tubes plus the standard deviation.

from band B exhibited the greatest mitogenic response. The mitogenic responses of non-separated cells was higher than the responses of bands A, C, D, or the pellet. Thymus cell subpopulations were stimulated with the thymus independent mitogen *E. coli* LPS (10 μg). This data (Table III) shows that cells from band B exhibited the highest degree of stimulation. Cells in the other bands and pellet did not respond to LPS. The cell subpopulation isolated from the lowest density area of the gradient (band A) was rich in large blast-like cells displaying an abundant amount of cytoplasm and large round nuclei. These cells were not stimulated by any of the mitogens but did display the highest basal uptake of tritiated thymidine (Tables, I, III) when incubated without mitogen. Due to the higher level of tritiated thymidine uptake in this cell population it is felt that these cells correspond to the highly mitotic cells of the outer cortext of the intact thymus[11].

There was a significant enrichment of cells in band B responding to mitogenic stimulation when compared to the other bands and unseparated cells. This reactive subpopulation was not, however, morphologically homogenous. It is not clear at the present time which of the morphological cell types respond to the mitogens. Previous investigations have shown that cortisone resistant thymocytes are responsive to PHA and Con A. Therefore, it is probable that the cells isolated from band B correspond to the cortisone resistant immunologically competent cells of the thymus medulla[12]. The morphological heterogeneity of this reactive subpopulation may be due to contamination with nonreacting cells. However, a more plausible explanation would be that this subpopulation contains cells that are in various stages of immunological maturation and thus appear morphologically dissimilar.

Our data (Table III) show a small number of thymus cells isolated from band B that are capable of responding to the thymus independent mitogen *E. coli* LPS. However, unseparated thymus cells exhibited no mitogenic response to LPS, which is in agreement with the data of ANDERSSON et al.[13]. Density gradient separation of thymus cells allowed the isolation of mitogen responsive cells in a single subpopulation. Speculation on the functionality of the thymus cells responding to LPS may lead to the following suppositions. First, the cells may be B cells involved in humoral immunity and correspond to the antibody producing cells described by VITETTA et al.[14]. On the other hand they may represent B cells that are destined to become T cells under the influence of the thymus. A third possibility is that the LPS reactive cells are contaminants from the circulation.

Zusammenfassung. Fraktionen von Thymuslymphozyten der Maus, die im diskontinuierlichen BSA-Dichtegradienten aufgetrennt worden waren, wurden auf ihre Stimulierbarkeit durch die «thymusabhängigen» Mitogene Concanavalin A und Phythämagglutinin sowie durch das «thymusunabhängige» Lipopolysaccharid von *E. coli* untersucht. Eine Lymphozytenfraktion niederer Dichte konnte mit allen Mitogenen stimuliert werden. Dieser Befund wird im Rahmen der Funktion einzelner Subpopulationen der Thymuslymphozyten diskutiert.

S. E. NIELSON and J. L. TRIBBLE

Section of Microbiology, Immunology and Plant Pathology, School of Life Sciences, University of Nebraska, Lincoln, Nebraska (68508, USA), 17 October 1974.

[11] G. SAINTE-MARIE and C. P. LEBLOND, Proc. Soc. exp. Biol. Med. *97*, 263 (1958).
[12] M. ISHIDATE and D. METCALF, Aust. J. exp. Biol. *41*, 637 (1963).
[13] J. ANDERSSON, G. MÖLLER and O. SJÖBERG, Cell. Immun. *4*, 381 (1972).
[14] E. S. VITETTA, J. W. UHR and E. A. BOYSE, Proc. natn. Acad. Sci., USA *70*, 834 (1973).

Absence of a Circadian Rhythm of Corticosterone Secretion in Monolayer Cultures of Adult Rat Adrenocortical Cells

We have shown that adult rat adrenocortical cells from the zona fasciculata-reticularis retain their structural and functional differentiation in monolayer culture and secrete corticosterone at an apparently steady overall daily rate when maintained with ACTH[1-3]. The intact adrenal cortex, however, shows a circadian rhythm of responsivity to ACTH in vitro[4], and circadian rhythms of several physiological and biochemical parameters including oxygen consumption and corticosteroid secretion have been reported in organ-cultured adrenal glands[5-7]. It has therefore been suggested[8] that the adrenal cortex possesses intrinsic rhythmicity of function, since rhythms persisted for many days in organ cultures maintained under constant conditions. Because our previous experiments did not exclude the possibility of circadian variations in monolayer cultures, we have examined the secretion of corticosterone by freshly-prepared cultures on a 3 and 6-hourly basis. This is the first time, to our knowl-