Clin. exp. Immunol. (1973) **15,** 203–212.

THE EFFECT OF HYDROCORTISONE ON THE INCORPORATION OF TRITIATED THYMIDINE BY HUMAN BLOOD LYMPHOCYTES CULTURED WITH PHYTOHAEMAGGLUTININ AND POKEWEED MITOGEN

DOROTHY H. HEILMAN, MARGARET R. GAMBRILL AND J. P. LEICHNER

Cellular Immunology Research Laboratory, Veterans Administration Hospital, Washington, and Department of Microbiology, School of Medicine, George Washington University, Washington, U.S.A.

(*Received* 8 *March* 1973)

SUMMARY

Methods based on [^{3}H]thymidine incorporation and morphology were used for further studies on the effect of hydrocortisone on the transformation of human lymphocytes by phytohaemagglutinin (PHA) and pokeweed mitogen (PWM). Result obtained with both methods showed inhibition of PHA stimulation in cultures preincubated with 0·01–10 μg/ml of hydrocortisone. [^{3}H]thymidine incorporation due to PWM in vertical tube cultures was depressed by 1·0 μg/ml of hydrocortisone in cultures harvested at 48 hr, but not in similar cultures incubated for 3, 4 and 5 days. In vertical cultures 10 μg/ml of hormone depressed the level of uptake in most experiments. By contrast [^{3}H]thymidine incorporation was enhanced in horizontal PWM cultures by 0·1 and 1·0 μg/ml of hydrocortisone, and 10 μg/ml caused stimulation or a return to the normal PWM level.

Microscopic and time-lapse observations on living cells showed that typical PWM blasts first appeared on the third day of incubation and after 5 days were numerous in corticosteroid-treated as well as in untreated cultures. Ten micrograms per millilitre of hydrocortisone increased the fragility of PWM blasts in both kinds of cultures and caused degeneration of variable numbers of blasts in 5-day-old vertical tube cultures. The depression of [^{3}H]thymidine uptake in vertical cultures was thought to be due to a combination of enhanced toxicity of corticosteroid hormone in deep cultures, and loss of incorporated [^{3}H]thymidine due to increased cellular fragility. Results obtained with the isotopic and morphologic methods indicated that the transformation of B lymphocytes by PWM is relatively resistant to the action of hydrocortisone *in vitro*.

Correspondence: Dr Dorothy H. Heilman, Cellular Immunology Research Laboratory, Veterans Administration Hospital, 50 Irving St., N.W. Washington D.C. 20422, U.S.A.

INTRODUCTION

In a recent study it was shown that pre-incubation of human leucocyte cultures with hydrocortisone, which depresses lymphocyte transformation by phytohaemagglutinin, did not inhibit typical B blasts in cultures stimulated by pokeweed mitogen (Heilman, 1972). In previous reports horizontal Leighton tube cultures were employed in preference to vertical tube cultures in which cells became enmeshed in fibrin and debris, and were difficult to disperse for cinematography and for careful quantitation of blastogenesis by the morphologic method (Heilman & Leichner, 1972). With the quantitative technique employed it was possible to detect a small proportion of hydrocortisone—sensitive cells responding to PWM early in incubation (Heilman, 1972). The morphologic method, however, is time consuming for observing the kinetics of cellular responses to different mitogens. In the present study the technique of [^{3}H]thymidine uptake described by Moorhead & McFarland (1966) was used along with the morphologic method to extend observations on interactions between leucocytes, mitogens and hydrocortisone. Discrepancies in results obtained with the two techniques were partially resolved by microscopic and time-lapse observations of cultured cells.

MATERIAL AND METHODS

Test materials

PHA-P (Difco lot 526500) and PWM (Gibco lot 1003N) were prepared and stored as previously described (Heilman, 1972). PHA-P was diluted in Tyrode's solution as indicated in individual experiments.

Leucocyte cultures

Cell-rich plasma was obtained from healthy donors. Care was taken to avoid specimens from donors who had experienced stress during venipuncture. The method used for preparation of cultures for thymidine uptake has been described in detail (Heilman, Thornton & Baetz, 1970). Ten millilitres aliquots of culture were prepared from a master mix consisting of 20% autologous plasma, 1% L-glutamine, medium 199 (Gibco) and unpurified leucocytes to provide $3{\cdot}3 \times 10^5$ lymphocytes/ml. Thirty minutes before the addition of mitogens 0·1 ml of hydrocortisone was added to test aliquots which were thoroughly mixed and maintained at room temperature. Solutions of PHA-P and PWM were added in the amount of 0·1 ml/10 ml. After thorough mixing each aliquot was used to prepare triplicate 3 ml cultures in 16×125 mm unsiliconized glass tubes fitted with siliconed rubber stoppers. In tests with PHA corks were replaced by closures after 24 hr of incubation at 37°C. Cultures with PHA were harvested at 3 days and those with PWM at 5 days of incubation. [^{3}H]thymidine (1 μCi/ml of culture) was added 4 hr before harvest. Cells were washed in cold 0·9% NaCl solution. Protein was precipitated with trichloracetic acid, solubilized with NCS solubilizer and suspended in scintillation fluid prepared with toluene. Tritium activity was assayed in a Beckman liquid scintillation counter and the results were expressed as disintegrations per min.

Cultures were also prepared in 28×110 mm short Leighton tubes as previously described (Heilman, 1972). Each 5-ml culture contained 5×10^5 lymphocytes/ml, 20% fresh autologous plasma, 1% L-glutamine and medium 199. Solutions of hydrocortisone and mitogens were added to cultures in 0·05 ml amounts. Cultures were sealed with siliconed rubber

stoppers and were incubated horizontally at 37°C. For some experiments [^{3}H]thymidine was added to cultures before harvest. Four hours later cells were scraped from the glass and each culture was well mixed and was transferred to a 16 × 125 mm tube. Cells were prepared for scintillation counting as described above. Leighton tube cultures were also used for estimating the degree of blastogenesis by the morphologic method. Stained films were prepared and examined as previously described (Heilman, 1972). For the examination of living cells horizontal cultures were placed at a 45° angle for 1 hr to allow cells to settle. A drop of culture removed from the bottom of horizontal or vertical tubes was placed between large coverslips and the preparation was sealed with paraffin. Coverslip cultures were observed with an inverted phase microscope enclosed in a 37°C incubator and fitted with a 16 mm time-lapse ciné camera. Photographs were taken at the rate of thirty frames per minute.

RESULTS

Optimal concentrations of PHA-P and PWM in vertical tube cultures

Two-fold dilutions of both mitogens were tested by the method of thymidine uptake. Because of variations in the dry weight of different lots of comparable potency, concentrations were expressed in terms of standard doses which were roughly equivalent of 15 μg/ml of PHA-P and 50 μg/ml of PWM. In preliminary experiments cultures incubated with PHA which were corked throughout incubation became acid early and showed a reduction in thymidine incorporation compared with cultures in which corks were replaced by closures at 24 hr. Dose response curves for both mitogens are shown in Fig. 1. Optimal concentrations of PHA were restricted to a fairly narrow zone (1/2–2 standard doses). The activity of PWM was lower than that of PHA, but was maintained at a plateau in response to widely different dosages. In this experiment and in two similar tests variations within each group of three cultures were increased when less than one-fourth of the standard dose of

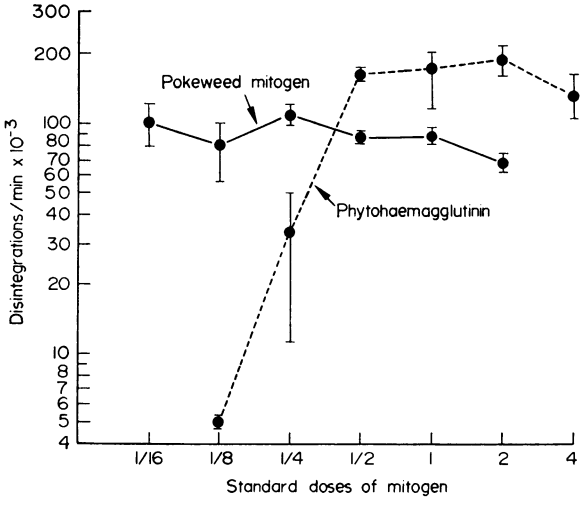

FIG. 1. ^{3}[H]thymidine uptake in human lymphocytes stimulated by different doses of PHA-P (3-day-old cultures) and of PWM (5-day-old cultures). Represented are the mean DPM and range of values in triplicate upright tube cultures.

PWM was used. As a result subsequent experiments with the isotopic method employed the standard dose of stock solution of PHA-P (0·1% v/v) and two concentrations of PWM, the standard dose (1·0% v/v) and one-fourth of the standard dose (0·25% v/v).

Effect of different concentrations of hydrocortisone on [^{3}H]thymidine incorporation due to PHA and PWM

Results obtained with PHA cultures pre-incubated with varying amounts of hydrocortisone are presented in Fig. 2. In this test and in a duplicate experiment a progressive

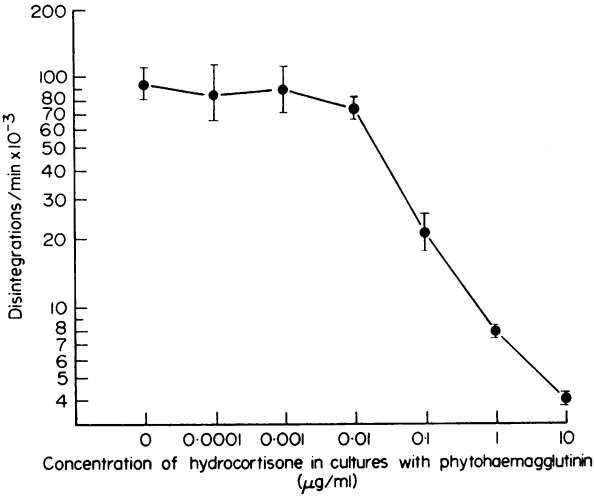

FIG. 2. [^{3}H]thymidine uptake in 72-hr cultures of human lymphocytes stimulated by PHA-P after incubation for 30 min with 0·0001–10 μg/ml of hydrocortisone. Represented are the mean DPM and range of values in triplicate upright tube cultures.

decrease in thymidine uptake was observed with 0·01 μg/ml to 10 μg/ml of hydrocortisone. Similar results were obtained with the morphologic method employing Leighton tube cultures (Table 1). Concentrations of 0·1 μg/ml or more of hormone enhanced the survival of macrophages. The effect of ten-fold increments of hydrocortisone on [^{3}H]thymidine uptake in PWM-stimulated cultures is shown in Table 2. Different amounts of hydrocortisone were tested in single Leighton tube cultures as well as in triplicate upright tubes. Variable results with overlapping of the data were obtained with upright tube cultures, but [^{3}H] thymidine uptake appeared to be significantly reduced by 10 μg/ml of hydrocortisone in three out of four experiments. In horizontal cultures 0·1 and 1·0 μg/ml of hormone enhanced [^{3}H]thymidine uptake, whereas 10 μg/ml caused stimulation in one instance and in two experiments uptake was similar to that due to PWM alone.

Effect of hydrocortisone on PWM stimulation at different period of incubation

To avoid cumulative toxicity resulting from high doses of PWM and hydrocortisone, vertical tube cultures for thymidine uptake were preincubated with 1 μg/ml of hormone and stimulated with 1/4 dose of PWM. Cultures were harvested at 48, 72, 96 and 120 hr (±1·5 hr). The results, shown in Table 3, demonstrated partial inhibition at 48 hr, but not at 3, 4, and 5 days of incubation.

TABLE 1. The effect of different concentrations of hydrocortisone on the percentage of blasts and macrophages in cultures with phytohaemagglutinin (PHA-P)

	Concentration of hydrocortisone in culture (μg/ml)				
	0	0·01	0·1	1·0	10
Blasts (%)	91	77	39	20	9
Macrophages (%)	6	8	14	17	19

TABLE 2. Effect of preincubation of lymphocyte cultures with different concentrations of hydrocortisone on transformation by pokeweed mitogen (PWM)

Experiment No.	Kind of culture	Disintegrations/min × 10^{-3}/1 × 10^{6} lymphocytes						
		Control cultures	Amount of hydrocortisone added to PWM cultures (μg/ml)					
			0	0·001	0·01	0·1	1·0	10
1	Vertical	<1	88* (84–93)	79 (73–84)	77 (66–87)	93 (90–95)	102 (96–108)	76 (74–80)
	Horizontal	<1	121	120	125	155	155	
2	Vertical	1	134 (117–152)	125 (121–133)	129 (121–133)	130 (123–135)	134 (133–136)	109 (104–113)
	Horizontal	2	111	131	134	266	270	107
3	Vertical	<1	66 (59–77)	— —	— —	— —	63 (55–72)	40 (19–74)
	Horizontal	<1	43	—	72	97	104	85
4	Vertical	<1	44 (41–46)	43 (40–45)	49 (46–55)	55 (47–59)	57 (56–58)	44 (42–48)
	Horizontal	<1	64	68	62	71	74	64

* Mean of triplicate cultures with range of values in parentheses.

TABLE 3. Incorporation of [^{3}H]thymidine at different periods of incubation by human lymphocytes preincubated with 1·0 μg/ml of hydrocortisone and stimulated with one-fourth dose of pokeweed mitogen. Average DPM × 10^{-3} with range of values in parentheses

Cultures	Days of incubation			
	2	3	4	5
Controls	<1	—	—	<1
PWM	25(23–26)	102(98–105)	114(105–119)	93(80–102)
Hydrocortisone + PWM	16(15–17)	98(89–114)	120(106–133)	98(97–103)

Addition of divided doses of hydrocortisone to cultures throughout incubation

In order to compensate for possible inactivation of hydrocortisone by leucocytes during the 5-day incubation period, divided doses of hormone were added to Leighton tube cultures 30 min before the addition of PWM and again on days 2, 3 and 4. A total of 10 μg/ml of hydrocortisone in divided amounts resulted in marked toxicity in control cultures and in those with mitogen. Severe toxicity was not observed in a similar test with 4 μg/ml of hormone in which a single dose resulted in 45% blasts and divided doses in 41% blasts. Transformation by PWM in the absence of hormone was 43%.

The effect of 10 μg/ml of hydrocortisone on blastogenesis and thymidine incorporation due to PWM and PHA

Inhibition of PWM and PHA stimulation by a high concentration of corticosteroid hormone was explored in parallel tests employing morphology and thymidine uptake. The results of a typical experiment are summarized in Table 4. A marked decrease in PHA-

TABLE 4. Comparison of the morphologic and isotopic methods for determining the effect of 10 μg/ml of hydrocortisone on lymphocyte transformation by phytohaemagglutinin (PHA) and pokeweed mitogen (PWM)

Test materials in culture	Leighton tube cultures*		Vertical tube cultures (DPM × 10^3)
	Blasts (%)	Macrophages (%)	
None	0·5	7·7	<1
Hydrocortisone	0	16·4	<1
PHA	90·0	3·6	66 (45–83)
Hydrocortisone + PHA	28·8	11·9	18 (6–30)
PWM	50·2	1·3	88 (71–130)
Hydrocortisone + PWM	50·7	0·2	36 (29–41)

* The percentage of blasts was in proportion to the total number of lymphocytic cells and the percentage of macrophages to the total number of mononuclear cells.

transformation by the hormone was shown by both methods. Hydrocortisone did not affect the relative number of blasts in PWM cultures, or enhance the survival of macrophages, but caused an apparent drop in thymidine uptake in vertical tube cultures.

Observations on living cells

Cultures representative of different experimental groups were prepared for morphologic examination. Differences between T-blasts and B-blasts were detected optimally with time-lapse photographs of coverslip cultures which were thin enough to show intracellular detail without interfering with the characteristic motion of the cytoplasm. PWM blasts seen during the first 2½ days of incubation could not be distinguished from PHA blasts. The first typical PWM blasts in horizontal cultures were identified late on the third day of incubation. In addition to characteristics already described (Heilman & Leichner, 1971) a useful feature

was the relatively rapid 'brush-fire' type of anterior cytoplasmic motion in typical PWM blasts which contrasted with the swimming type of motion exhibited by T-blasts.

At 5 days of incubation PWM blasts in horizontal and vertical cultures preincubated with 1·0 μg/ml or less of hydrocortisone were numerous and similar in appearance to those in cultures with PWM alone. B-blasts were also numerous in cultures pretreated with 10 μg/ml of hydrocortisone, but on continued incubated in coverslip preparations cytoplasmic bubbling and other degenerative changes appeared earlier than in untreated PWM cultures. In some instances moderate numbers of degenerated blasts were seen in 5-day-old vertical cultures with 10 μg/ml of hydrocortisone and PWM, even before incubation in thin preparations. Degenerated blasts were not noted initially in comparable horizontal cultures. After 24 hr of incubation macrophages were scarce in hormone-treated and untreated PWM cultures.

DISCUSSION

These studies demonstrate some of the problems encountered in experiments with adrenal cortical hormones *in vitro*. Pokeweed blasts incubated with high concentrations of hydrocortisone were especially sensitive to changes in the environment. In order to interpret discordant results obtained with different techniques it was necessary to examine cultured cells in a number of ways. These experiments provide another example of discrepancies that can occur between data obtained with the morphologic and isotopic methods for estimating lymphocyte transformation. In general the inhibitory effect of hydrocortisone on PHA stimulation was similar with both *in vitro* methods.

The importance of dosage of PHA in leucocyte cultures was recognized early (Ling, 1968) The dose response curve and narrow range of optimal activity observed in the present study were similar to those described by Rigas, Tisdale & Hecht (1970) for PHA-P and by Tormey & Mueller (1965) for PHA-M. Further assays were not done in this study to establish optimal concentrations of PHA-P because of differences reported by other investigators in tests employing cells from different donors (Naspitz & Richter, 1968). In the present study PWM was effective over a wide range of concentrations, which was in agreement with reports of Chalmers *et al.* (1967) and Naspitz & Richter (1968).

With the exception of vertical tube cultures incubated with 10 μg/ml of hydrocortisone, data obtained with the isotopic method agreed in general with those previously reported with the morphologic method (Heilman, 1972). In both studies hydrocortisone selectively inhibited a large part of the T-cell population stimulated by PHA, and a small proportion of lymphocytes responding to PWM early in incubation. Evidence from histochemical and morphologic studies has shown that most of the blasts appearing during the first 2½–3 days of incubation with PWM were indistinguishable from T-blasts in PHA cultures (Chessin *et al.*, 1966; Barker & Farnes, 1967; Douglas *et al.*, 1967; Jones & Roitt, 1972). Our finding that some lymphocytes responding early to PWM were sensitive to hydrocortisone further associated that segment with the T-cell population. Chessin *et al.* (1967) first observed typical PWM blasts after 60 hr of incubation which were identified as plasmablasts by fine structural examination (Douglas *et al.*, 1967). In studies with experimental animals it has been demonstrated that PWM stimulates T-cells as well as B-cells (Janossy & Greaves, 1971, 1972; Jones & Roitt, 1972; Jones, 1972). In these and other studies with lymphocytes from experimental animals cultures were harvested at 3 days, and in one

instance as early as 48 hr (Wu & Waksman, 1972). Scott (1972) has recently presented dual lines of evidence to show that only a small portion of the blastogenic response to PWM in 3-day-old cultures of peripheral blood lymphocytes from mice is supplied by B-cells. Determinations of lymphocyte transformation by PWM during the first 3 days of incubation probably measure principally the response of T-cells, and do not reflect the potential action of PWM on B-cells, which transform maximally at 4–6 days, and for full morphologic expression require replacement of culture medium and further incubation (Barker, Lutnzer & Farnes, 1969; Barker, 1969).

In tests to determine effective concentrations of hydrocortisone on thymidine uptake (Fig. 2) and on blastogenesis (Table 1) in PHA cultures the bend-point occurred at 0·01 μg/ml, and 0·1 μg/ml caused marked inhibition. Similar results have been reported by Tormey, Fudenberg & Kamin (1967). In previous studies preincubation with 0·1 μg/ml or less of corticosteroid hormones inhibited blastogenesis due to antigens and allogeneic leucocytes (Caron, 1969; Heilman & Leichner, 1972). A number of reports have demonstrated inhibition of mitogenesis due to PHA and antigens by 1 μg/ml of corticosteroid hormones (Nowell, 1961; Stefani & Oester, 1967; May, Lyman & Alberto, 1970). These studies indicate that concentrations effective under controlled conditions *in vitro* are well within physiologic limits.

In a previous report the observed increase in the percentage of PWM-stimulated blasts in cultures with hydrocortisone was thought to be due in part to destruction of a portion of the small lymphocyte population by the hormone (Heilman, 1972). In the present study, however, the total [^{3}H]thymidine incorporation in PWM cultures incubated with 1 μg/ml of hydrocortisone equalled or exceeded that in untreated PWM cultures. The results suggest that an increase in the number of transformed cells was responsible for the augmented production of DNA. The highest concentration of hydrocortisone used in this study was more toxic in upright tubes than in thin horizontal cultures. In general test materials such as tuberculin are more effective and more toxic in deep cultures than in horizontal cultures (Heilman *et al.*, 1970). Observations on living cells indicated that B-blasts had developed in the presence of 10 μg/ml of hormone, but that some of the blasts in deep cultures had degenerated by the fifth day. The increased fragility of PWM blasts due to hydrocortisone seen in coverslip preparations was confirmed by the results of fine structural examination of similar cultures (Heilman & Leichner, 1973). Blasts of the plasmacytic series were present in cultures incubated with 10 μg/ml of hydrocortisone and PWM. In addition there were increased numbers of recently-disrupted blasts and variable quantities of free strands of endoplasmic reticulum. These findings indicate that some of the incorporated [^{3}H]thymidine may have been lost during the washing procedure prior to the precipitation of protein. It was not possible to estimate the extent to which cellular disruption may have influenced the final results.

Kissling, Speck & Goselink (1972) recently reported that concentrations of 10–1000 μg/ml of hydrocortisone added simultaneously to human leucocyte cultures with PHA or PWM depressed thymidine uptake due to both mitogens in cultures harvested at 72 hr. In another group of experiments Kissling *et al.* (1972) added 100 μg/ml of hydrocortisone to cultures 1 min–72 hr after the addition of PHA, and 1 min–96 hr after PWM. Thymidine uptake was unaffected by the addition of hydrocortisone to PHA cultures, but was virtually suppressed in cultures with PWM. The results obtained with PHA agreed with the report of Elves, Gough & Israels (1964) who noticed that 100 μg/ml of prednisolone added before or with PHA produced a significant inhibition of transformation, but was inactive when added

10 min after PHA. Nowell (1961) had previously described the inability of 10 μg/ml of prednisolone to depress transformation in cultures incubated with PHA for 24 hr. Others have noted a loss of inhibitory action of corticosteroid hormones when they were placed in cultures simultaneously with or subsequent to the addition of PHA (Tormey *et al.*, 1967; Caron, 1969). In the report of Kissling *et al.* (1972) the depression of thymidine uptake by 100 μg/ml of hydrocortisone on PWM stimulation was severe, even in cultures previously exposed to mitogen for 4 days. The high concentration of hormone may have prevented thymidine uptake, even in cultures containing a large number of blasts. If DNA had been produced our results suggest that incorporated thymidine might have been lost at the time of harvest due to increased cellular fragility. Observations on the appearance of cells in culture would be needed to interpret the results.

The present study shows that information gained with a single method for estimating lymphocyte transformation may be incomplete or misleading. Monitoring the appearance of cells from representative cultures during incubation has proved valuable, not only for estimating the total response, but also for observing the participation and fate of different kinds of cells at successive periods of incubation.

REFERENCES

BARKER, B.E. (1969) Phytomitogens and lymphocyte blastogenesis. *In Vitro*, **4**, 64.

BARKER, B.E. & FARNES, P. (1967) Histochemistry of blood cells treated with Pokeweed mitogen. *Nature, (Lond.)*, **214**, 787.

BARKER, B.E., LUTZNER, M.A. & FARNES, P. (1969) Ultrastructural properties of pokeweed-stimulated leukocytes *in vivo* and *in vitro*. *Proceedings of the Third Annual Leukocyte Culture Conference* (Ed. by W. O. Rieke), p. 587. Appleton-Century-Crofts, New York.

CARON, G.A. (1969) The effect of antimetabolites and corticosteroids on lymphocyte transformation *in vitro*. *Proceedings of the Third Annual Leucocyte Culture Conference* (Ed. by W. O. Rieke), p. 287. Appleton-Century-Crofts, New York.

CHALMERS, D.G., COOPER, E.H., EVANS, C. & TOPPING, N.E. (1967) Quantitation of the response of lymphocytes in culture to specific and non-specific stimulation. *Int. Arch. Allergy*, **32**, 7.

CHESSIN, L.N., BÖRJESON, J., WELSH, P.D., DOUGLAS, S.D. & COOPER, H.L. (1966) Studies on human peripheral blood lymphocytes *in vitro*. *J. exp. Med.* **124**, 873.

DOUGLAS, S.D., HOFFMAN, P.F., BÖRJESON, J. & CHESSIN, L.N. (1967) Studies on human peripheral blood lymphocytes *in vitro*. III. Fine Structural features of lymphocyte transformation by pokeweed mitogen. *J. Immunol.* **98**, 17.

ELVES, M.W., GOUGH, J. & ISRAELS, M.C.G. (1964) Place of lymphocyte in reticuloendothelial system: study of *in vitro* effect of prednisolone on lymphocytes. *Acta haemat. (Basel)*, **32**, 100.

HEILMAN, D.H. (1972) Failure of hydrocortisone to inhibit blastogenesis by pokeweed mitogen in human leucocyte cultures. *Clin. exp. Immunol.* **11**, 393.

HEILMAN, D.H. & LEICHNER, J.P. (1971) Comparison of lymphoblasts in cultures stimulated by pokeweed mitogen (PWM) and phytohemagglutinin (PHA). *Fed. Proc.* **30**, 466 (Abstract).

HEILMAN, D.H. & LEICHNER, J.P. (1972) Effect of cortisol on the transformation of human blood lymphocytes by antigens and allogeneic leucocytes. *Proceedings of the Sixth Leucocyte Culture Conference* (Ed. by M. R. Schwartz), p. 581. Academic Press, New York.

HEILMAN, D.H. & LEICHNER, J.P. (1973) Electron microscopic study of the effect of hydrocortisone on human leukocytes cultured with phytohemagglutinin and pokeweed mitogen. (In preparation.)

HEILMAN, D.H., THORNTON, C. & BAETZ, B. (1970) A method for quantitating blastogenesis by tuberculins in cultures of human blood lymphocytes. *Amer. Rev. resp. Dis.* **101**, 569.

JANOSSY, G. & GREAVES, M.F. (1971) Lymphocyte activation. I. Response of T and B lymphocytes to phytomitogens. *Clin. exp. Immunol.* **9**, 483.

JANOSSY, G. & GREAVES, M.F. (1972) Lymphocyte activation. II. Discriminating stimulation of lymphocyte subpopulations by phytomitogens and heterologous antilymphocyte sera. *Clin. exp. Immunol.* **10**, 525.

JONES, G. (1972) Lymphocyte activation. I. Expression of theta, H-2 and immunoglobulin determinants on lymphocytes stimulated by phytohaemagglutinin, pokeweed mitogen, concanavalin A or histocompatibility antigen. *Clin. exp. Immunol.* **12,** 391.

JONES, G. & ROITT, I.M. (1972) Immunoglobulin determinants on lymphoid cells in culture. *Cell. Immunol.* **3,** 478.

KISSLING, M., SPECK, B. & GOSELINK, H. (1972) Effects of hydrocortisone on lymphocytes stimulated by phytohaemagglutinin and pokeweed mitogen. *Vox Sang.* (*Basel*), **23,** 244.

LING, N.R. (1968) *Lymphocyte Stimulation* (Ed. by N. R. Ling), p. 19. John Wiley & Sons Inc., New York.

MAY, C.D., LYMAN, M. & ALBERTO, R. (1970) Effect of compounds which inhibit lymphocyte stimulation on the utilization of glucose of leukocytes. *J. Allergy*, **46,** 21.

MOORHEAD, J.F. & MCFARLAND, W. (1966) Efficiency of scintillation counting of tritium-labelled reaction *in vitro*. *Nature* (*Lond.*), **211,** 1157.

NASPITZ, C.K. & RICHTER, M. (1968) The response of human peripheral lymphocytes to various mitogenic agents *in vitro*. *Int. Arch. Allergy*, **33,** 411.

NOWELL, P.C. (1961) Inhibition of human leukocyte mitosis by prednisolone *in vitro*. *Cancer Res.* **21,** 1518.

RIGAS, D.A.,TISDALE, V.V. & HECHT, F. (1970) Transformation of blood lymphocytes in *Ataxia telangiectasia*. *Int. Arch. Allergy*, **39,** 221.

SCOTT, M.T. (1972) Biological effects of the adjuvant *Corynebacterium parvum*. I. Inhibition of PHA and mixed lymphocyte and GVH reactivity. *Cell Immunol.* **5,** 459.

STEFANI S. & OESTER, Y.T. (1967) Effect of prednisolone on the phytohemagglutinin-induced radioresistance and blastoid transformation of human lymphocytes *in vitro*. *Transplantation*, **5,** 317.

TORMEY, D.C., FUDENBERG, H.H. & KAMIN, R.M. (1967) Effect of prednisolone on synthesis of DNA and RNA by human lymphocytes *in vitro*. *Nature* (*Lond.*), **213,** 281.

TORMEY, D.C. & MUELLER, G.C. (1965) An assay for the mitogenic activity of phytohemagglutinin preparations. *Nature* (*Lond.*), **26,** 569.

WU, AN-YA SHIH & WAKSMAN, B.H. (1972) Cellular differentiation in the thymus. IV. Response of rat thymic subpopulations to anti-thymus serum and other nonspecific mitogens. *Cell. Immunol.* **3,** 516.