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ISSN 0014-4827

Experimental Cell Research 106 (1977) 95–104

COLCEMID AND RELATED ALKALOIDS INHIBIT LECTIN-MEDIATED STIMULATION OF RNA SYNTHESIS IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES

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SUMMARY

Colcemid, colchicine and vinblastine inhibit two of the early events occurring during lectin-mediated lymphocyte activation, namely the stimulation of [^{3}H]uridine incorporation into acid-soluble nucleotides and into cellular RNA, as well as later events such as morphological transformation and [^{3}H]TdR incorporation. Colcemid also inhibits the activation of lymphocytes through the mixed lymphocyte reaction.

The effect of colchicine and related vinca alkaloids on living cells has generally been thought to be mediated by the action of these compounds on microtubules. More recently a series of reports demonstrating effects of colchicine on plasma membranes or plasma membrane related functions have appeared. Ukena & Berlin have shown that the topographical distribution of membrane proteins involved in phagocytosis and in adenine and lysine transport is altered by treatment of polymorphonuclear leukocytes with colchicine [1], and that concanavalin A (ConA)-mediated agglutination of these cells is decreased after treatment with vinblastine or colchicine [2]. Yin et al. showed that colchicine, colcemid and vinblastine decreased the ConA-induced binding of red blood cells to SV3T3 cells [3].

Colchicine has been shown to competitively inhibit the transport of nucleosides into several mammalian cells [4] and Berlin has reported that incubation of rabbit alveolar macrophages in the presence of colchicine alters the affinity of a transport system for nucleosides [5].

Edelman et al. [6] and Yahara & Edelman [7] have reported that colchicine and related compounds at concentrations of 10^{-6} M and greater reverse the concanavalin A (ConA) induced inhibition of "capping" of "membrane immunoglobulin" by anti-immunoglobulin, in mouse spleen lymphocytes. Medrano et al. [8] and Wang et al. [9] have shown that these same compounds decreased the mitogenic response to ConA of human peripheral blood lymphocytes and mouse spleen lymphocytes as tested by [^{3}H]TdR incorporation. The results that we present here confirm and extend their findings. Our results show that colchicine, colcemid and vinblastine, at a concentration of 10^{-6} M, inhibit an early event in lectin-induced lymphocyte activation, as tested by [^{3}H]uridine incorporation into acid-soluble nucleotides and into cellular RNA. In addi-

tion, we have found that the activation of lymphocytes through the mixed lymphocyte reaction is also inhibited by colcemid.

MATERIALS AND METHODS

Chemicals

All chemicals used were of reagent grade, and were purchased from commercial sources. Colchicine (Sigma Chemical Co., St Louis, Mo.). Colcemid (Calbiochem, La Jolla, Calif.) and vinblastine (Velban, Eli Lilly & Co., Indianapolis, Ind.) were dissolved freshly before each experiment. Colcemid was dissolved in 95% ethanol, and in the experiments in which its effect was tested, a similar amount of ethanol was added to the control cultures. Addition of ethanol did not have any significant effect on the control or lectin-stimulated cultures. Colchicine and vinblastine were dissolved in phosphate-buffered saline (PBS). 2-Mercaptoethanol (Matheson, Coleman & Bell, Norwood, Ohio) was diluted freshly before use.

Lectins-phytohemagglutinin

Lectins-phytohemagglutinin (Bacto PHAP, Difco Laboratories, Detroit, Mich.) was purified by the method of Weber et al. [10]. The material eluted from SP Sephadex C50 (Pharmacia, Uppsala, Sweden) with 1/15 M KH_2PO_4–Na_2PO_4 (pH 8.0) was dialysed against PBS, and concentrated by pressure dialysis to a concentration of 1.5 mg/ml, as determined by the method of Lowry et al. [11], using bovine serum albumin as a standard. Good cell viability and maximum [^{3}H]TdR incorporation at 72 h were consistently obtained with 15 μg/ml of this material; this concentration of PHA was used in all experiments, unless otherwise stated. ConA (Calbiochem) was used without further purification.

Preparation of cell cultures

Human peripheral blood lymphocytes were drawn from healthy volunteers in the presence of 2 U/ml of Heparin (Bioheprin, Ries Biologicals, Inc., Costa Mesa, Calif.), and were purified by a method derived from that of Böyum [12]: 5 ml of heparinized blood were layered over a 5 ml solution of 6.35% w/v Ficoll (Pharmacia, Uppsala), and 10.03% w/v Hypaque (Winthrop Labs) and centrifuged for 50 min at 500 g at 4°C. The mononuclear cells at the plasma/Ficoll-Hypaque interface were collected and washed twice with PBS containing 0.3 mM EDTA. The cells were then resuspended at a density of 1–1.5×10^6/ml in incubation medium consisting of 80% RPMI 1640 (GIBCo, Grand Island, N.Y.) and 20% heat-inactivated (56°C, 30 min) autologous serum, and containing 100 μg/ml streptomycin (Pfizer, New York), and were distributed in 1 ml fractions in glass test tubes (Pyrex, No. 9826). The cells were preincubated for 18–24 h prior to any experimental manipulation. All incubations were carried out at 37°C, in a 95% air, 5% CO_2 atmosphere. The method gave 96–98% mononuclear cells, 95–99% were viable, as assayed by their capacity to exclude the dye Trypan blue. 10–20% of the cells were monocytes. In one experiment, in order to remove monocytes, mononuclear cells were purified using Ficoll-Hypaque as described, resuspended in RPMI 1640 at a concentration of 1×10^6 cells/ml, and passed through a nylon wool column (7 cm×2 cm) at room temperature. This procedure removed 90% of the mononuclear phagocytes. 0.5–1% of the recovered cells were able to spread on a glass coverslip.

Incorporation of radiolabeled nucleosides

[^{3}H]Uridine (29 Ci/mM) and [^{3}H]TdR (24 Ci/mM) (Schwartz-Mann, Orangeburg, N.Y.) were used at a concentration of 12.5 μCi/ml.

In the experiments in which incorporation of radiolabelled nucleosides into acid-soluble and -insoluble cellular materials was studied, the cells were washed three times with 5 ml cold PBS and resuspended in 0.3 ml 0.25 N cold perchloric acid (PCA), and centrifuged at 800 g for 10 min at 4°C. The supernatant fluid containing the acid-soluble nucleotides was removed and a 100 μl aliquot was placed in 10 ml Bray's scintillation fluid and counted in a Packard liquid scintillation counter.

The precipitate, containing radiolabelled macromolecules, was washed three times with 5 ml 0.25 N cold PCA, dissolved in 0.3 ml 1 N NaOH, and counted in the same manner as the acid-soluble fraction. Results are expressed in cpm/culture.

In the experiments in which incorporation of labelled nucleotides into acid-insoluble macromolecules only was examined, the cells were harvested on Millipore filters (0.45 μm pore size) (Millipore Corp.). The filters were washed with 15 ml cold PBS, and 25 ml 0.25 N cold PCA, dried and counted in 10 ml Liquifluor-toluene (New England Nuclear Corp.).

The degree of phosphorylation of [^{3}H]TdR in the acid-soluble fraction was measured as follows: the acid-soluble supernatant was neutralized with 1 M KOH, centrifuged to remove precipitated potassium perchlorate, and the clear supernatant was spotted on DEAE filter paper (Whatman DE81). Radioactivity bound to the filters was counted in Liquifluor-toluene either directly (total acid-soluble radioactivity), or after washing the filters successively with 10 ml each of ammonium formate (10^{-3} M), water and 95% ethanol (acid-soluble [^{3}H]TdR nucleotides).

All experiments were done in triplicate and the results are plotted graphically as the arithmetic mean of the radioactive counts incorporated per culture. The bars in the graphs indicate the maximal variation of incorporation in each experiment.

Preparation of lumicolchicine

Colchicine (1 mg/ml) in ethanol was irradiated by long wave UV for various periods of time, and the UV absorption spectra were recorded. After 4 h irradiation the 350 nm absorption, characteristic of colchicine, had completely disappeared, and a new peak at 265 nm

had appeared, as has been described for the photoisomerization of colchicine into β lumicolchicine [13]. This indicated that 4 h of UV irradiation gave quantitative isomerization of colchicine to β-lumicolchicine. Thin-layer chromatography of this preparation showed that the compound had an R_f value different from that of colchicine and similar to that of lumicolchicine as described by Sagorin et al. [14]. No antimitotic effect of this lumicolchicine preparation was found when the compound was tested on L strain mouse cells at a concentration of 10^{-6} M.

RESULTS

Viability

Unstimulated cells, and cells stimulated by concentrations of PHA up to 15 μg/ml, were always more than 80%, and usually more than 90% viable after 24 and 48 h of culture. Cell viability in cultures incubated with colchicine and related compounds, at concentrations of 10^{-6}–10^{-8} was about 75% after 24 h and 70% after 48 h; less than 40% of the cells incubated in 10^{-4} M colchicine were viable at 48 h. In some experiments, particularly in those where 3×10^{-5} M mercaptoethanol was added to the cultures, the viability of colchicine treated cells was over 95%, and equivalent to that of control cells.

Agglutination

The extent of lectin-induced agglutination of the cells, as estimated by phase contrast microscopy, did not appear to be grossly decreased in cultures treated with colchicine. In fact, in the presence of colchicine, colcemid or vinblastine, cell aggregates were often larger. Also, whereas in absence of colchicine and related alkaloids macrophages appeared to be "radiating" from some of the aggregates, this was not observed in the presence of these compounds.

Morphology

Cells were examined by light and electron microscopy 48 and 72 h after the addition of PHA and/or colchicine to the cultures. In presence of 15 g/ml PHA, 60–75% of the cells were converted to lymphoblasts as measured by increased size (a diameter of 15 μm or more), basophilic cytoplasmic staining and nuclear enlargement (as compared with unstimulated lymphocytes), and very often one or more prominent nucleoli; electron microscopy of these blast-like cells showed increased cytoplasmic polysomes, nuclear enlargement, abundant heterochromatin and prominent nucleoli. If the cells were preincubated 2 h in 10^{-6} M colchicine and incubated in the presence of this compound together with PHA (15 μg/ml) for an additional 48 h almost no blasts were found. The morphology of these cells could not be distinguished from that of unstimulated lymphocytes. In experiments where cells were incubated for 72 h in presence of PHA (15 μg/ml) and colchicine (10^{-6} M), 10–15% of the cells were identified as blasts by light and electron microscopy.

Effect of colcemid on PHA-stimulated RNA synthesis

Most of the experiments described here utilized as the index of lectin-induced stimulation the uptake and incorporation of [^{3}H]-uridine during a 2 h pulse given 22 h after PHA addition. This parameter was chosen for two reasons: (1) Lectin-induced stimulation of RNA synthesis precedes that of DNA synthesis by 12–24 h. Thus RNA synthetic rates are a measure of one of the "early" events in blast transformation. (2) Virtually no lectin-stimulated cells have entered mitosis by 22 h. Thus the population of cells examined consists exclusively of the cells originally present in the culture and the results are not influenced by progeny lymphocytes.

Colcemid concentrations of 10^{-9}–10^{-7} M had no detectable effect on the incorporation of [^{3}H]uridine into cellular RNA; col-

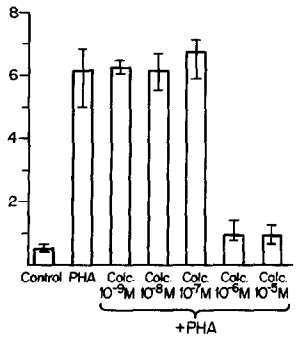

Fig. 1. Ordinate: [³H]uridine acid-insoluble cpm $\times 10^{-4}$.

Dose effect of colcemid on PHA-stimulated [³H]-uridine incorporation. Cultures of 1×10^6 primary human mononuclear cells in 1 ml of medium were incubated for 2 h with various amounts of colcemid. PHA (15 μg/ml) was then added to the appropriate samples, and the cells were incubated for 22 h. 12.5 μCi of [³H]uridine were then added to each sample. The cells were incubated for 2 h, harvested on Millipore filters, and the radioactivity present in acid-insoluble RNA was determined as described in Methods.

cemid concentrations of 10^{-6} and 10^{-5} inhibited the incorporation of [³H]uridine by PHA-stimulated cells by more than 90% (fig. 1). Similar results were obtained using ConA as a mitogen (data not shown). To be certain that the inhibitory effect of colcemid on cellular RNA synthesis reflected a qualitative alteration in the cellular response to lectins, and not merely a decreased response to a given concentration of PHA, cells were incubated with concentrations of PHA ranging from 0 to 60 μg/ml, for 24 h in the presence of colcemid (10^{-6} M). Colcemid had no inhibitory effect on the uptake or incorporation of [³H]uridine in unstimulated lymphocytes. However, in stimulated lymphocytes the uptake of [³H]uridine into acid-soluble nucleotides and into RNA was inhibited by colcemid at all concentrations of PHA used (fig. 2), indicating that high doses of PHA cannot reverse the effect of colcemid in this system. Since 10^{-6} M colcemid and 15 μg/ml PHA, respectively, gave maximal inhibition and stimulation of RNA synthesis, these compounds were used at these concentrations in all subsequent experiments.

Increased incorporation of [³H]uridine into RNA is noted in the first 4 h after lectin stimulation [15]. We examined the effects of colcemid on this process as a measure of the effect of this drug on early events in lectin-induced lymphocyte stimulation. As shown in fig. 3, colcemid markedly inhibited the PHA-induced burst of [³H]uridine incorporation during the 4 h following the addition of the lectin.

To determine whether the time of addition of colcemid to the cultures affected the nature and extent of colcemid-mediated inhibition of [³H]uridine uptake by PHA-stimulated lymphocytes, the drug was

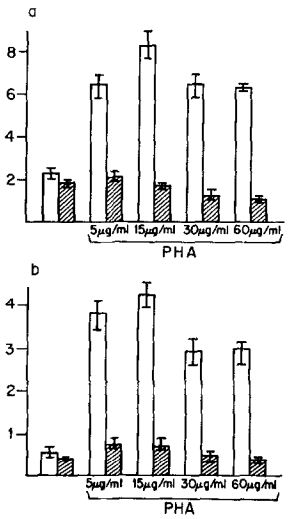

Fig. 2. Ordinate: [³H]uridine (*a*) acid-soluble; (*b*) acid-insoluble cpm $\times 10^{-4}$.

Dose effect of PHA on [³H]uridine incorporation into colcemid-treated lymphocytes. Cultures of 1×10^6 primary human mononuclear cells in 1 ml of medium were incubated for 2 h with (shaded bars) or without (open bars) colcemid (10^{-6} M). Various amounts of PHA were then added to the cultures, and the cells were incubated for 22 h. 12.5 μCi of [³H]uridine were then added to each sample. The cells were incubated for 2 h, harvested and processed for determination of radioactivity in acid-soluble nucleotides and acid-insoluble RNA as described in Methods.

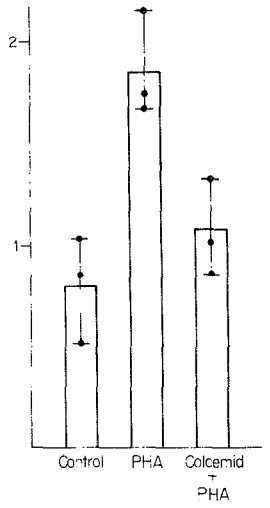

Fig. 3. Ordinate: [^{3}H]uridine acid-insoluble cpm $\times 10^{-3}$.

Effect of colcemid on [^{3}H]uridine incorporation during the 4 h following addition of PHA. Same protocol as for fig. 1, except that 12.5 μCi [^{3}H]uridine were added to the cultures at the same time as PHA, and the cells were harvested 4 h later.

added to the cells at various times before or after the addition of PHA. Twenty-two hours after addition of PHA [^{3}H]uridine was added to the cultures and its incorporation into acid-soluble nucleotides and into RNA was assayed 2 h later (fig. 4). Addition of colcemid up to 6 h after the addition of PHA completely inhibited the uptake of [^{3}H]uridine into acid-soluble nucleotides and acid-insoluble RNA as assayed at 22 h. Addition of colcemid 12 h after PHA partially inhibited [^{3}H]uridine incorporation. Addition of colcemid at the time of [^{3}H]-uridine labeling (i.e., 22 h after the addition of PHA) had only a moderate inhibitory effect on the uptake of [^{3}H]uridine into acid-insoluble RNA. Two conclusions may be drawn from these data: (1) The PHA-mediated stimulation of lymphocyte RNA synthesis, whether measured at 4 h or at 22 h, can be completely inhibited by colcemid. In the latter case, treatment with colcemid up to 6 h after the addition of PHA resulted in complete inhibition of lectin-stimulated [^{3}H]uridine incorporation into RNA. (2) The colcemid-mediated inhibition of [^{3}H]uridine incorporation is not a result of inhibition of transport of the labelled nucleoside, since addition of colcemid together with [^{3}H]uridine does not decrease the amount of [^{3}H]uridine in the acid-soluble nucleotide pool.

To determine whether the colcemid-mediated inhibition of [^{3}H]uridine labelling detected during relatively short (24 h) periods of PHA stimulation persisted in the case of longer incubations, cells were incubated in the presence of PHA and colcemid for 24, 48 and 72 h prior to labeling with [^{3}H]uridine. As indicated in fig. 5, col-

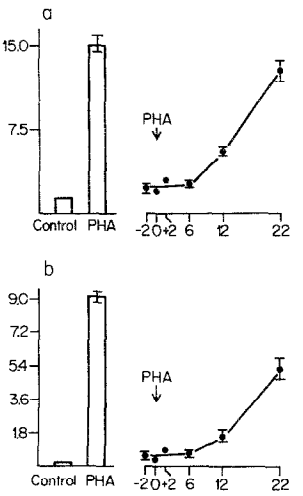

Fig. 4. Abscissa: (right) time of colcemid addition (hours); *ordinate:* (*a*) [^{3}H]uridine acid-soluble cpm $\times 10^{-4}$; (*b*) [^{3}H]uridine acid-insoluble cpm $\times 10^{-4}$.

Effect of the time of addition of colcemid on PHA-stimulated [^{3}H]uridine incorporation. Cultures of 1×10^6 primary human mononuclear cells in 1 ml medium were incubated with PHA (15 μg/ml). At various times before, simultaneously with, or after the addition of PHA, colcemid (10^{-6} M) was added to the cultures where appropriate. Twenty-two hours after the addition of PHA, each sample received 12.5 μCi [^{3}H]-uridine, and was incubated for an additional 2 h. The cells were then harvested and processed for determination of radioactivity in acid-soluble nucleotides and acid-insoluble RNA, as described in Methods.

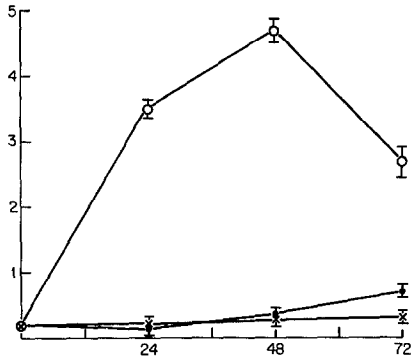

Fig. 5. Abscissa: time (hours); *ordinate:* [^{3}H]uridine acid-insoluble cpm$\times 10^{-5}$. ×—×, Control; ○—○, PHA; ●—●, colcemid+PHA.

Time course of PHA-stimulated RNA synthesis in presence or absence of colcemid. Cultures of 1×10^6 primary human mononuclear cells in 1 ml medium were incubated for 2 h with or without colcemid (10^{-6} M). PHA (15 μg/ml) was then added to the cultures as appropriate. Two hours prior to harvesting, 12.5 μCi [^{3}H]uridine were added to the cultures. Cells were harvested on Millipore filters 0, 24, 48, and 72 h after the addition of PHA, and the radioactivity present in acid-insoluble RNA was determined as described in Methods.

cemid completely inhibited the incorporation of [^{3}H]uridine into RNA at 24 and 48 h. At 72 h a small but consistent increase in incorporation was seen, and this can perhaps be correlated with the observation that a small number of cells treated with colcemid and PHA were morphologically blastlike at this time.

Effect of colchicine and lumicolchicine on PHA-stimulated RNA synthesis

Colcemid and colchicine at concentrations of 10^{-6} M inhibit nucleoside transport into mammalian cells only minimally [4, 5]. In our experiments the simultaneous addition of colcemid and labelled nucleoside did not decrease [^{3}H]uridine incorporation into the acid-soluble nucleotide pool (fig. 4). Nevertheless, we compared the effects of several concentrations of colchicine and of lumicolchicine on [^{3}H]uridine incorporation to determine what contribution, if any, the nucleoside transport inhibiting activity of these compounds makes to the overall inhibition of [^{3}H]uridine incorporation into PHA-stimulated lymphocytes. Lumicolchicine is a colchicine analog that does not bind to microtubular protein [16], but is as effective as colchicine in equimolar concentrations in inhibiting nucleoside transport into animal cells [4, 5]. Lumicolchicine at concentrations of 10^{-6} and 10^{-7} M did not inhibit the PHA-mediated stimulation of [^{3}H]uridine incorporation into acid-insoluble RNA (fig. 6), while the same concentrations of colchicine and vinblastine (data not shown) inhibited [^{3}H]uridine incorporation by 85%. (Similar results were obtained when ConA (20 μg/ml) was used in place of PHA (data not shown). It should be noted that inhibition of [^{3}H]uridine incorporation by 10^{-7} M colchicine was not always observed; on some occasion this concentration of colchicine was only minimally inhibitory.

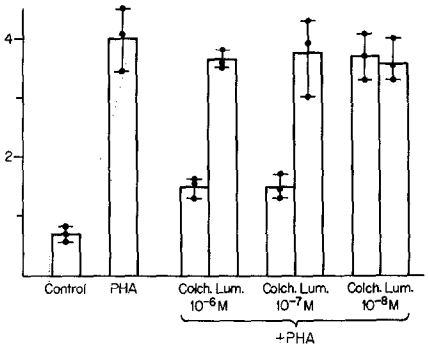

Fig. 6. Ordinate: [^{3}H]uridine acid-insoluble cpm $\times 10^{-4}$.

Dose effect of colchicine and lumicolchicine on PHA-stimulated [^{3}H]uridine incorporation. Cultures of 1×10^6 primary human mononuclear cells in 1 ml medium were incubated for 2 h after addition of various amounts of colchicine or lumicolchicine. PHA (15 μg/ml) was then added to the appropriate samples, and the cells were incubated for 22 h. 12.5 μCi of [^{3}H]uridine were then added to each sample. The cells were incubated for 2 h, harvested on Millipore filters, and the radioactivity present in acid-insoluble RNA was determined as described in Methods.

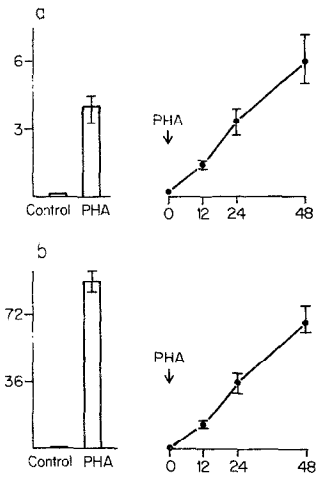

Fig. 7. Abscissa: (*right*) time of colcemid addition (hours); *ordinate:* (*a*) [^{3}H]TdR acid-soluble cpm $\times 10^{-4}$; (*b*) [^{3}H]TdR acid-insoluble cpm $\times 10^{-4}$.

Effect of the time of addition of colcemid on PHA-stimulated [^{3}H]TdR incorporation. Cultures of 1×10^6 cells in 1 ml medium were incubated with PHA (15 μg/ml). Simultaneously with, or at various times after, the addition of PHA, colcemid (10^{-6} M) was added to the cultures where appropriate. Fortyeight hours after addition of PHA, all cultures received 12.5 μCi [^{3}H]-TdR, and were incubated for 4 h. The cells were then harvested and processed for determination of radioactivity in acid-soluble nucleotides and acid-insoluble DNA, as described in Methods.

Influence of macrophages

Although the major proportion of cells isolated by the Ficoll-Hypaque method are lymphocytes, 10–20% of the cells in the culture are mononuclear phagocytes. Since these cells have been reported to exert "trophic" effects on cultured lymphocytes, we wished to be certain that the colchicine-mediated inhibition of lymphocyte stimulation was not related in some way to an effect of this compound on the macrophages in the culture. Mononuclear cells were therefore passed through a column of glass wool, a procedure known to retain mononuclear phagocytes on the column, and the eluted cells were placed into culture. Mononuclear phagocytes composed less than 1% of the eluted cells. These column purified lymphocytes were preincubated as appropriate with colchicine, stimulated with PHA, and labelled with [^{3}H]uridine from 22 to 24 h after addition of PHA. 3×10^{-5} M β-mercaptoethanol, a compound known to increase the viability and the plaque-forming capacity of purified mouse lymphocytes [17] and to enhance the capacity of purified human lymphocytes to respond to PHA in the absence of macrophages (P. Wernet, personal communication) was added to half of the cultures. Neither the removal of more than 90% of the macrophages, nor the addition of 3×10^{-5} M β-mercaptoethanol restored the capacity of PHA-stimulated lymphocytes to incorporate [^{3}H]uridine in the presence of colchicine. It is of interest that over 95% of the cells cultivated with β-mercaptoethanol and colchicine were viable, whereas only 75% of the cells that had been incubated with colchicine but without mercaptoethanol were viable.

[^{3}*H*]*TdR incorporation*

To determine whether PHA-induced DNA synthesis is also blocked by colcemid, this compound was added together with or at various times after PHA. Forty-eight hours later the cells were assayed for their capacity to incorporate [^{3}H]TdR. If colcemid was added together with PHA, [^{3}H]TdR incorporation into acid-soluble nucleotides and into DNA was blocked completely (fig. 7). Addition of colcemid at later times caused partial inhibition of [^{3}H]TdR incorporation. Addition of colcemid at the same time as the labelled nucleoside (48 h) slightly enhanced the incorporation of label into the acid-soluble pool, while incorporation into macromolecular DNA was somewhat decreased. This situation could arise from a decrease in the phosphorylation of [^{3}H]-TdR. To test this possibility, the acid-

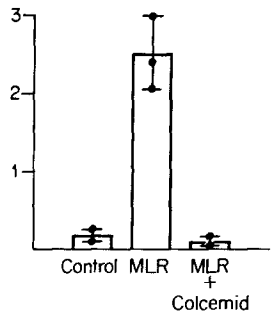

Fig. 8. Ordinate: [^{3}H]TdR acid-insoluble cpm$\times 10^{-5}$. Effect of colcemid on the mixed lymphocyte reaction. 5×10^5 lymphocytes from each of two donors were mixed in 1 ml medium containing 20% heat-inactivated serum from one of the donors, in the presence or absence of colcemid (10^{-6} M). As an unstimulated control, 1×10^6 cells from one of the donors were cultivated in 20% autologous serum in absence of colcemid. After 96 h of incubation, 12.5 μCi of [^{3}H]TdR were added to all the cultures; the cells were incubated for 17 h, harvested on Millipore filters, and the radioactivity present in acid-insoluble DNA was determined, as described in Methods.

soluble material was assayed for its content of TdR nucleotides. Eighty to 90% of the radiolabelled TdR in the acid-soluble fraction was recovered as TdR nucleotides, regardless of whether the cells were labelled in the presence or absence of colcemid. Since the phosphorylation of [^{3}H]TdR was unaffected by colcemid, it seemed unlikely that the inhibition of [^{3}H]TdR incorporation into DNA was mediated by an effect of colcemid on thymidine kinase activity.

Mixed lymphocyte reaction

Lectin stimulation is only one of a variety of ways in which lymphocytes can be stimulated to synthesize DNA and to divide. The mixed lymphocyte reaction (MLR) can also be used for this purpose. To determine whether colchicine inhibits the MLR, lymphocytes from two unrelated donors were mixed and incubated in the presence and absence of colchicine for 96 h prior to labelling with [^{3}H]TdR. Seventeen hours later the cells were harvested and assayed for their content of acid-insoluble radioactivity. As an unstimulated control, lymphocytes from one of the donors were cultivated in the absence of colcemid. The level of DNA synthesis in the mixed lymphocyte culture incubated in colcemid-containing medium was comparable to that in the unstimulated control culture (fig. 8). Although the cells were incubated in culture for more than 4 days, over 80% of the colcemid-treated cells and 90% of the control cells remained viable.

DISCUSSION

Colchicine, colcemid and vinblastine inhibit the lectin-induced stimulation of human lymphocytes, as estimated by [^{3}H]uridine and [^{3}H]TdR incorporation into acid-soluble and acid-insoluble cellular materials, and by cellular morphology. These results confirm those of Medrano et al. [8] and of Wang et al. [9], and extend them in that the stimulation of uridine uptake and of RNA synthesis, events that occur relatively early in the response to mitogenic lectins, have now been shown to be also inhibited by these drugs. In contrast to our results, Betel & Martijnse [18] have found that colchicine and vinblastine, at concentrations of 10^{-6} M and lower, do not inhibit the activation of rat lymph node cells by ConA. A difference in the sensitivity of human and rodent cells to these effects of antimitotic drugs might account for these apparently conflicting results; it is of interest to note that Edelman et al. used 10^{-4} M colchicine to inhibit the ConA stimulation of mouse spleen cells [6], whereas, in accord with our results, they found that 10^{-7}–10^{-6} M colchicine was sufficient to inhibit the stimulation of human peripheral blood lymphocytes [9]. Such a species dif-

ference in the sensitivity of lymphocytes to the effects of antimitotic drugs on lectin-induced lymphocyte stimulation might help to define better the cellular target of these drugs in this system; for instance, the inhibitory effects of colchicine on the polymerization of spindle microtubules occurs at the same concentration in rodent and in human cells [14, 19], and this therefore suggests that colchicine does not inhibit lymphocyte activation through its interaction with tubulin from spindle microtubules.

Nucleoside transport into some mammalian cells is decreased by colcemid and colchicine [4, 5]. We do not believe that the results reported in this communication can be explained on the basis of such an inhibition of [^{3}H]nucleoside uptake by these drugs for the following reasons:

(1) A concentration of colchicine sufficient to inhibit lymphocyte stimulation by more than 90% (10^{-6} M) causes only a 10% decrease in nucleoside uptake in systems where such a decrease has been reported [4, 5]. The K_i for colchicine on adenosine transport in various mammalian cells is of the order of 7×10^{-5} M [4]. The initial rate of adenosine uptake in rabbit alveolar macrophages is decreased by less than 20% in presence of 10^{-6} M colchicine [5].

(2) Lumicolchicine inhibits nucleoside transport in systems in which such an inhibition by colchicine is detected [4, 5]; it did not affect lymphocyte stimulation as tested by [^{3}H]uridine uptake.

(3) Vinblastine does not inhibit nucleoside transport [5]; it did block the PHA stimulation of [^{3}H]uridine uptake.

(4) When added at the same time as [^{3}H]-nucleosides, colchicine did not decrease the uptake of these compounds into acid-soluble cellular material (figs 4, 7).

Lymphocyte stimulation can also be achieved by interaction with allogenic cells. Colcemid, at the same concentration that inhibited lectin-mediated lymphocyte stimulation, inhibited a 2-way mixed lymphocyte reaction, as tested by [^{3}H]TdR incorporation into acid-insoluble cellular material after 96 h of culture. Although recruitment and amplification of the responder populations were probably blocked by colcemid acting as a mitotic inhibitor, there should still have been a substantial number of new cells (i.e., cells that were not the progeny of cells that had divided earlier in the culture) entering their first mitosis after 96 h [20]; therefore the inhibition of [^{3}H]-TdR incorporation by colcemid probably represented, in this system also, an inhibition of the stimulation of the lymphocytes. In accord with this interpretation, Wilson et al. have found a significant decrease in the total number of responding cells in such a mixed lymphocyte reaction when performed in presence of colchicine [20].

The inhibition by colcemid of lymphocyte activation was not complete. A late increase in [^{3}H]uridine was detected in colcemid-treated PHA-stimulated cultures (fig. 4); this late increase can be correlated with the appearance in the cultures of 10–15% of blast-like cells, as judged by both light and electron microscopy. A comparable delayed increase in [^{3}H]TdR incorporation and in the proportion of blast-like cells has also been observed by Wang et al. [9]. Whether this represents an escape of some of the cells from the colcemid-mediated inhibition, or whether the effect of colcemid is simply to slow down or delay the process of activation, cannot be answered at this time. It is also of interest to note that colchicine and vinblastine do not inhibit some of the events associated with antigen-induced activation of guinea pig lymphocytes. Bloom et al. [21] have shown that vesicular stomatitis virus (VSV) replicates in thymus-

derived guinea pig lymphocytes only when these cells are stimulated by antigen, and that addition of colchicine or vinblastine from the time of initiation of the cultures does not inhibit viral replication in these cells. These results show that colchicine and vinblastine do not prevent the antigen-induced events that are necessary for VSV infection of guinea pig lymphocytes; they can be taken to indicate that the drugs do not inhibit all of the events associated with lymphocyte activation, or that guinea pig lymphocytes, like those from other rodents, are relatively resistent to the effects of colchicine and vinblastine on lymphocyte stimulation.

This work has been supported by grant AI 08697 from the USPHS. S. C. S. is an Established Investigator of The American Heart Association.

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Received August 2, 1976
Accepted December 8, 1976