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URIDINE TRANSPORT IN CONCANAVALIN A- AND LIPOPOLYSACCHARIDE-ACTIVATED MOUSE LYMPHOCYTES*

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Abstract—Uridine transport is an important factor in regulating uridine accumulation in lymphocytes. Differences in uridine transport rates between T- and B-lymphocytes have been suggested to result in preferential uptake of uridine into T-lymphocytes versus B-lymphocytes. In the present study, a rapid sampling procedure was used to measure within 20 sec the high affinity–low capacity transport of [^{3}H]uridine into concanavalin A (Con A)- and lipopolysaccharide (LPS)-activated lymphocytes. Changes in uridine transport were correlated with the degree of B- or T-lymphocyte stimulation which was determined by monitoring changes in cell size and long-term (2 hr) [^{3}H]uridine and [^{3}H]thymidine accumulation. The results indicated that uridine transport increased with the general activation state of lymphocytes and no preferential uridine transport was observed between Con A or LPS cultures which were maximally acitivated. We concluded that differences in uridine accumulation into mouse lymphocytes should not be attributed solely to differences in transport between T- and B-lymphocyte since uridine transport is substantially influenced by lymphocyte metabolic activity or activation state.

Uridine uptake into lymphocytes has traditionally been used as an indicator of RNA synthesis [1]. Some investigators [2–5] have reported that T-lymphocytes have higher rates of uridine uptake than B-lymphocytes. As a result of this observation, studies have utilized this differential uptake of radiolabeled uridine as a marker for T-lymphocytes [2–8]. Other research, however, has shown that B-lymphocytes from "nude" mice [9] and from human tonsils [10] can accumulate more uridine than corresponding T-lymphocyte populations. Staub *et al.* [10] concluded that alterations in ongoing cellular metabolic activity or functional state accounted for the differences in the amount of uridine accumulated between the two major lymphocyte populations. As pointed out by Staub and coworkers [10], uridine uptake into lymphocytes is influenced not only by the rate of RNA synthesis, but by permeation of uridine into the cell (transport), rate of phosphorylation, and nucleoside pool sizes. Changes in any one of these parameters could result in alteration in uridine uptake. In particular, a difference in the rate of uridine transport into lymphocytes has been suggested to be the most important factor in regulating uridine and other nucleoside accumulation [3, 10].

Previous studies have reported that the transport of various nutrients increases in activated lymphocytes [1]. However, as recently pointed out by Strauss *et al.* [11, 12] and Wohlhueter *et al.* [13], what was measured was not transport *per se* but uptake which is influenced by intracellular metabolism. Strauss and coworkers [11, 12] demonstrated with a rapid sampling procedure that thymidine transport increased in activated lymphocytes, whereas adenosine transport did not. With a similar procedure, Plagemann *et al.* [14] demonstrated that uridine transport is completed within 10–20 sec in uridine kinase-deficient Novikoff cells and in mouse L cells, P388 cells, Chinese hamster ovary cells, and human HeLa cells.

The purpose of the present study was to determine whether mitogen activation, *in vitro*, increases uridine transport into T- and B-lymphocytes. It was anticipated that results would provide insight into the regulation of uridine accumulation in these two lymphocyte subpopulations. An adaption of the rapid sampling technique of Strauss *et al.* [11] was employed to examine the actual translocation event.

MATERIALS AND METHODS

Mice. Female BDF$_1$ (C57BL/6 X DBA/2) mice were obtained from Jackson Laboratories, Bar Harbor, ME. Mice used in these studies were 12 to 24 weeks-old.

Cell preparation for transport studies. Phosphate-buffered saline (with calcium and magnesium) containing 0.1% bovine serum albumin and 180 mg/100 ml of glucose was used for all transport studies (referred to as PBS‡). Splenic nonadherent lymphocytes were obtained by the following procedures. Spleen cell suspensions in cold (4°) PBS were separated by Ficoll-Isopaque [12 parts of 9% (w/v) Ficoll purchased from Pharmacia, Uppsala, Sweden, and 5 parts of 34% (w/v) sodium metrizoate purchased

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‡ Abbreviations: PBS, phosphate-buffered saline; Urd, uridine; dThd, thymidine; MEM, Eagle's minimum essential medium; Con A, concanavalin A; LPS, lipopolysaccharide; and PHA, phytohemagglutinin.

from the Accurate Chemical and Scientific Corp., Hicksville, NY] density gradient centrifugation. The mononuclear fraction was incubated in a plastic culture dish at 37° for 40 min and the nonadherent cells were diluted to 4×10^6 cells/ml; viability was greater than 95 per cent as assessed by trypan blue exclusion. Greater than 95 per cent of the cells were small to medium lymphocytes with the remainder being red blood cells. Preliminary studies revealed that this small contamination with red cells did not significantly alter uridine transport kinetics.

Radioactive chemicals. [6-^{3}H]Uridine ([^{3}H]Urd) with a specific activity of 14 Ci/mmole was obtained from Amersham (Arlington Heights, IL). [Methyl-^{3}H]thymidine ([^{3}H]dThd) with a specific activity of 65 Ci/mmole was obtained from ICN Pharmaceuticals, Inc. (Irvine, CA) and subsequently adjusted to 14 Ci/mmole by addition of cold thymidine.

Measurement of transport. Rapid separation of lymphocytes from medium and measurement of transport were performed according to the method of Strauss *et al.* [11]. A 0.4-ml aliquot of prewarmed (37°) PBS containing 1.6 million cells was rapidly mixed with 0.1 ml of prewarmed substrate at five times the desired concentration and 5 to 7.5 μCi tritiated substrate per ml. Two aliquots, 0.2 ml each, were removed and layered into 400-μl microfuge tubes (Beckman Instruments, Inc., Creve Coeur, MO). Each microfuge tube contained 50 μl of 3 N potassium hydroxide as the bottom layer and a 150-μl layer of oil which was a mixture of 8 vol. silicone oil at a density of 1.05 g/ml (Aldrich Chemical Co., Milwaukee, WI) and 2 vol. heavy mineral oil (Nujol, from a local drug store) for a final density of 1.03 g/ml. After the appropriate incubation, the microfuge tubes containing the 0.2-ml samples were spun at 10,000 rpm for 30 sec by means of a Beckman microfuge. The cells passed through the silicone oil and pelleted in the potassium hydroxide layer. No radioactivity was found in this layer if cells were excluded. The cell pellets were digested in the potassium hydroxide layer for 2 hr at room temperature before slicing the tip of the tubes into scintillation vials containing scintillation mixture (Research Products International Corp., Elk Grove Village, IL). This solution was neutralized with 50 μl of 3 N $HClO_4$ and allowed to cool to 4° for 3 hr before counting. Samples were counted in a Packard Tri-Carb liquid scintillation counter (Packard Instrument, Inc. Downers Grove, IL). Counting efficiencies ranged between 20 and 25 per cent.

Tissue culture. Tissue culture medium consisted of concentrates of Eagle's minimum essential medium (MEM) which were obtained from Gibco Laboratories, Grand Island, NY. MEM was supplemented with (mg per liter) glutamine, 116; sodium pyruvate, 100; serine, 21; and asparagine, 20 (all obtained from the Sigma Chemical Co., St. Louis, MO). Fetal calf serum (Kansas City Biological Co., Lenexa, KS) was added to a final concentration of 7.5%. Antibiotics were added to give a final concentration of 100 units/ml of penicillin and 0.1 mg/ml of streptomycin. Spleen cells were harvested and diluted to 2 million cells/ml with MEM plus serum and cultured in aliquots of 75–125 ml in 900-ml flat-bottomed glass tissue culture flasks. Cells were incubated at 37° in 7.5% CO_2 in air in a humidified Lab Line incubator (Lab-Line Instruments, Inc., Melrose Park, IL). These mitogens were added at initiation of the cultures and included Concanavalin A (Con A, Sigma Chemical Co.) and *Salmonella typhosa* lipopolysaccharide (LPS, Sigma Chemical Co.) at final concentrations of 2.5 μg/ml and 10 μg/ml respectively.

Cell sizing. Size distribution was determined with a Coulter channellizer II coupled with a Coulter counter model Z_{B1} (Coulter Electronics, Inc., Hialeah, FL). Calibration of the Coulter counter was performed with polystyrene microspheres of 453 μm^3 volume to ensure that each channel represented a cell volume of 6.75 μm^3.

Assay for uridine and thymidine uptake. One-milliliter aliquots were removed from the main tissue culture vessel and placed in twenty-four well culture plates (FB-16-24-TC, Linbro Chemical Co., New Haven, CT) at time intervals specified in Results. Triplicate samples were then pulsed with 1 μCi each of either [^{3}H]Urd or [^{3}H]dThd with the specific activity for both radiolabeled compounds adjusted to 14 Ci/mmole. After 2 hr of incubation, each well was triturated with a Pasteur pipette and 0.2 ml-aliquots were taken from each well and placed in Linbro microculture plates. These cell suspensions were subsequently harvested onto glass-fiber paper discs (Arthur H. Thomas Co., Philadelphia, PA) with saline using a Multiple Automated Sample Harvester (Microbiological Associates, Bethesda, MD). The filters containing radioactive material were placed in vials containing scintillation fluid and counted.

Assay for uridine and thymidine incorporation. Cell suspensions were handled in the same manner as described above for the uptake studies. However, instead of a saline wash, 3–4 ml of 7.5% trichloroacetic acid followed by 1 ml of 95% ethanol per filter disc was used as a wash. This method was adapted from the filter paper method described by Uyeki *et al.* [15] and traps only the acid insoluble cell fraction into which tritiated uridine or tritiated thymidine has been incorporated.

Statistics. Values obtained from separate runs during the transport experiments were pooled, and the apparent K_m, V_{max} and slope values were computed by the method of least squares from Lineweaver–Burk plots. Each plot was then analyzed for lack of fit and in most cases it was determined that further weighted regression analyses were not necessary because of variance homogeneity in the original plots. Differences in slopes among the different treatments were tested for significance using an extension of the *t*-test described by Dixon and Massey [16]. All analyses were performed by computer with programs furnished by Dr. Gary Clark, Department of Biometry, University of Kansas Medical Center. For Figs. 6 and 7, the standard errors of the means for each point ranged between 3 and 11 per cent of the mean.

Data from the cell sizing experiments were represented as number of cells sized versus channel number or as normalized cumulative frequency histograms versus channel number. The Kolomogorov–Smirnov test as modified by Young

[17] was employed to determine statistical differences between cell distributions.

RESULTS

When lymphocytes are stimulated by mitogens, there is an increase in DNA synthesis associated with a concomitant blastogenesis [18, 19]. Figure 1 demonstrates the increase in cell size that occurred in the present study over a 2-day period in the presence of Con A or LPS. As shown, each mitogen induced a progressive increase in cell size with time, but this process occurred at a faster rate in the Con A-stimulated cultures. If the cultures were maintained for 72 hr the size distributions for both the LPS and Con A cultures were approximately the same (data not shown), and each resembled the 48 hr size distribution for Con A (see Fig. 1).

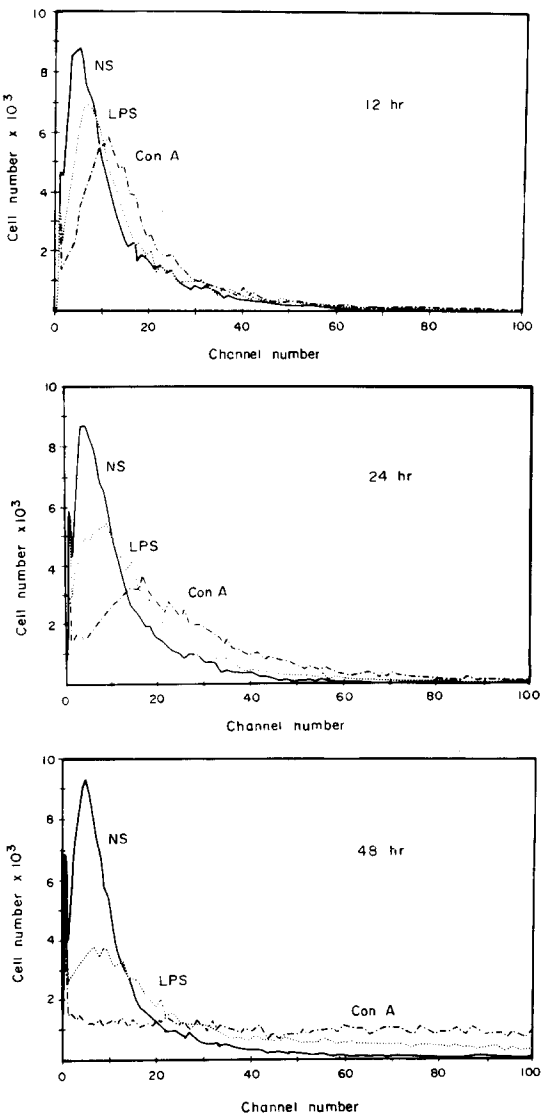

Fig. 1. Changes in cell volume in lymphocyte cultures after 12, 24 or 48 hr of stimulation with Con A or LPS. Spleen cells were cultured at 2×10^6 cells/ml in 100 ml of MEM + serum in glass tissue culture flasks. Con A or LPS was added at the beginning of culture in concentrations of 2.5 μg/ml or 10 μg/ml respectively. Aliquots were subsequently removed at times indicated for sizing. Each graph represents cell volume as a function of cell number versus channel number.

To measure unidirectional influx or transport, it is necessary to measure the process when it is linear with time. As depicted in Fig. 2, total uridine uptake was linear with time at lower concentrations for at least 40 sec, with the period of linearity increasing with increases in substrate concentration. This period of linearity was also observed in stimulated lymphocytes (Fig. 3). Thus, in subsequent experiments, cells were incubated at 20-sec intervals using a modification of the rapid sampling procedure of Strauss *et al.* [11] to work within the time of initial rates of uridine transport. Results shown in Fig. 2 also demonstrate the saturability of the uridine uptake system. By increasing the concentration of cold uridine, the amount of radiolabeled uridine (constant at 5 μCi/ml) taken up at 10^{-2} M remained constant although not totally eliminated. Activated lymphocytes had a greater capacity for total uridine uptake as indicated by saturation between 4 and 5 min at 5×10^{-5} M and no saturation at 5×10^{-6} M when compared with unactivated spleen cells in which the system was saturated within the first minute at both concentrations (Fig. 3). This nonsaturable component of total uridine uptake was found to be temperature-independent (no change at 4°), whereas the initial transport observed between 10 and 60 sec was abolished at 4° (data not shown).

The contribution of this linear, unsaturable component to uridine transport was determined by the method of Pletscher [20], as modified by Tuomisto [21]. This procedure is illustrated in Fig. 4 by a comparison of uridine transport (20 sec) into bulk spleen lymphocytes with stimulated lymphocytes. In these experiments the linear portion of uptake at high concentrations (determined by the method of least squares) was extrapolated to zero concentration. This line was utilised to calculate the linear process at different concentrations as described by Stahl and Meltzer [22]. The calculated nonsaturable uptake was then subtracted from total uptake. The resultant net uptake exhibited a typical saturation curve for uridine transport, as shown in Fig. 4, which was used for kinetic analysis.

The nonsaturable uptake component could be ascribed to either a low affinity–high capacity transport system or to a simple diffusion. However, the temperature independency and the inability to saturate the system with any uridine concentrations in the experimental range argue against the first possibility and, for the second possibility, it is uncertain how much diffusion actually contributes to this process in light of the hydrophilic nature of uridine in solution. Wohlhueter and coworkers [13] failed to detect any significant contribution from "simple diffusion" in thymidine transport studies in Novikoff cells. However, nonsaturable uptake could result from mechanical trapping or adsorption, especially when one compares the increase in the nonsaturable component of uptake in lymphocytes stimulated for 48 hr with Con A (Fig. 4) with the increased cell size at 48 hr (Fig. 1). As shown in Fig. 4, the rate of total 20-sec uptake was greatest in cells that had been

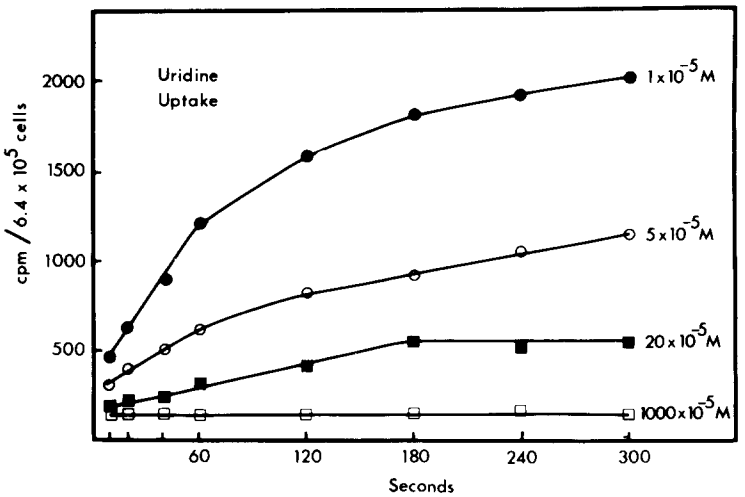

Fig. 2. Time dependence of uridine uptake in spleen lymphocytes from normal BDF_1 mice. For each sample, a 0.4 ml-aliquot containing 1.6×10^6 cells was mixed with 0.1 ml of [^{3}H]uridine (5.0 μCi/ml) at five times the final concentrations indicated. Duplicate 0.2-ml samples were taken at the indicated times, centrifuged, and counted as described in Materials and Methods. The concentration of the nonsaturable component was not subtracted from these values.

stimulated for 48 hr with Con A. These cultures contained a large number of blast cells (Fig. 1). Thus, linear uptake of uridine at high concentrations could result from increased nonspecific adsorption of label onto the lymphocytes caused by the increased cell surface area, as well as a concomitant increase in extracellular water trapped in the cell pellet. In any event, the linear, nonsaturable component of the transport process was subtracted from each experiment and the resulting curve represented only the high affinity–low capacity transport event in the present studies.

After determining that a 20-sec incubation period was within the linear period of uridine transport, a series of experiments was performed to determine if preferential accumulation of radiolabeled uridine occurred in activated T- or B-lymphocytes. Advantage was taken of the ability of Con A and LPS to selectively activate T- and B-lymphocytes. The degree of lymphocyte activation in culture was monitored by assessing [^{3}H]dThd and [^{3}H]Urd uptake and incorporation as well as changes in cell volume; there is a general increase in all parameters after mitogen activation and they are generally accepted as indicators of lymphocyte activation [1].

To determine the degree of mitogen-induced lymphocyte activation, the amount of [^{3}H]dThd and [^{3}H]Urd accumulated after a 2 hr pulse was assessed at different intervals during a 48-hr incubation period (Table 1). The *uptake* in Table 1 is the amount of nucleoside taken up by intact lymphocytes as assessed by saline wash onto glass-fiber filters,

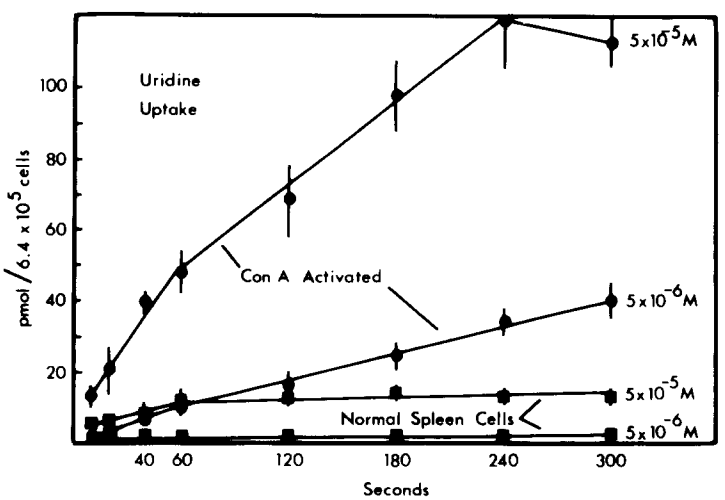

Fig. 3. Time dependence of total uridine uptake (two concentrations) in Con A-stimulated (48 hr) and normal spleen lymphocytes. For each sample, a 0.4-ml aliquot of 1.6×10^6 cells was mixed with 0.1 ml of [^{3}H]uridine (7.5 μCi/ml) at five times the final concentrations indicated. Duplicate 0.2-ml samples were taken at indicated times, centrifuged, and counted as described in Materials and Methods. The contribution of the nonsaturable component was not subtracted. Each point represents the mean of four values. S.E. of the mean is also shown when greater than 1 pmole.

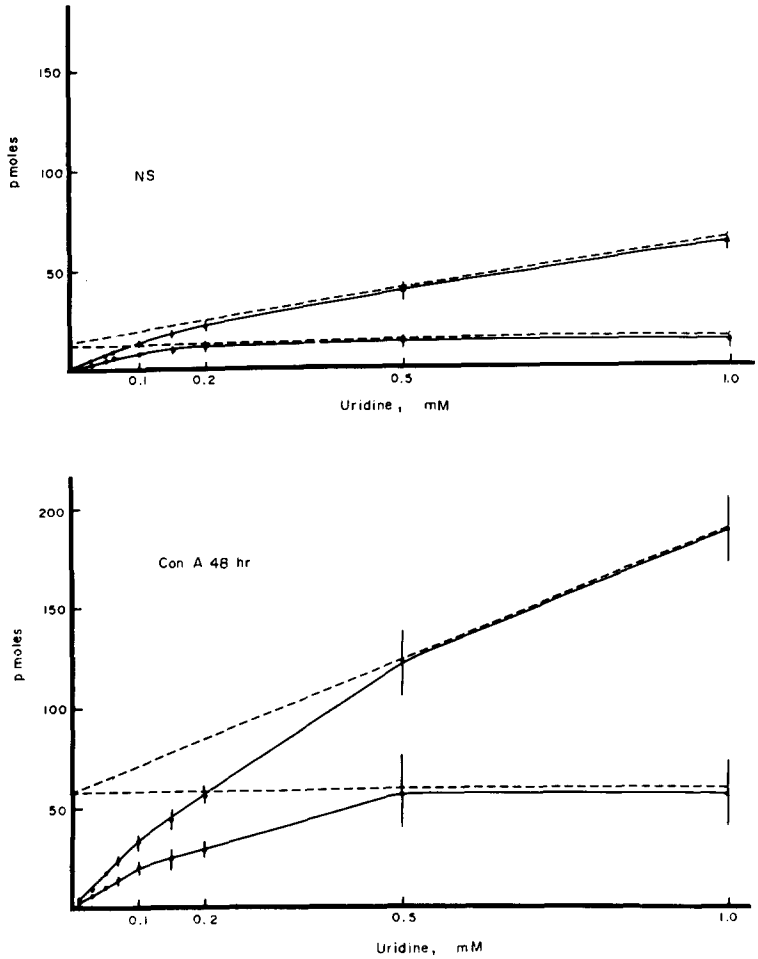

Fig. 4. Uridine uptake as a function of concentration in stimulated and unstimulated lymphocytes (upper diagram, normal spleen lymphocytes; bottom diagram, 48-hr Con A-stimulated lymphocytes). The upper black line in each diagram represents the total uptake of uridine. The dotted lines confine the linear, nonsaturable part of uptake which is then subtracted from the total uptake at each concentration, and the bottom dark line shows the saturable uptake which presumably represents the uridine transport event. Values from this saturable process were used for kinetic analysis. The uptake is presented as pmoles per 6.4×10^5 cells per 20-sec incubation. Each point represents the mean ± S.D. for four values.

Table 1. Time course of nucleoside accumulation (2-hr pulse) into mitogen-activated lymphocytes

		Uptake in cpm per 10^6 cells				
		Number of hours				
		0	6	12	24	48
None	Thd	1,793 ± 73	807 ± 114	320 ± 23	812 ± 118	1,679 ± 277
	Urd	2,453 ± 267	3,866 ± 149	2,750 ± 390	3,548 ± 185	3,531 ± 230
Con A	Thd	2,066 ± 160	1,071 ± 216	1,236 ± 275	23,660 ± 1,955	39,644 ± 5,552
	Urd	2,491 ± 73	16,220 ± 1,754	15,040 ± 1,761	37,615 ± 5,230	27,698 ± 1,556
LPS	Thd	1,207 ± 236	669 ± 119	941 ± 113	5,451 ± 389	15,763 ± 1,083
	Urd	1,878 ± 500	8,353 ± 150	5,159 ± 513	6,258 ± 471	15,754 ± 734
		Incorporation in cpm per 10^6 cells				
		Number of hours				
		0	6	12	24	48
None	Thd	1,276 ± 38	488 ± 26	276 ± 11	602 ± 67	943 ± 236
	Urd	1,861 ± 241	3,199 ± 146	2,314 ± 372	3,016 ± 494	3,261 ± 324
Con A	Thd	1,342 ± 193	877 ± 34	913 ± 7	17,934 ± 816	26,135 ± 2,700
	Urd	1,974 ± 345	15,104 ± 4,116	14,551 ± 2,304	37,250 ± 6,689	26,780 ± 481
LPS	Thd	842 ± 110	522 ± 31	838 ± 56	3,922 ± 304	11,943 ± 1,149
	Urd	1,590 ± 423	7,200 ± 291	4,546 ± 269	5,399 ± 422	13,169 ± 2,117

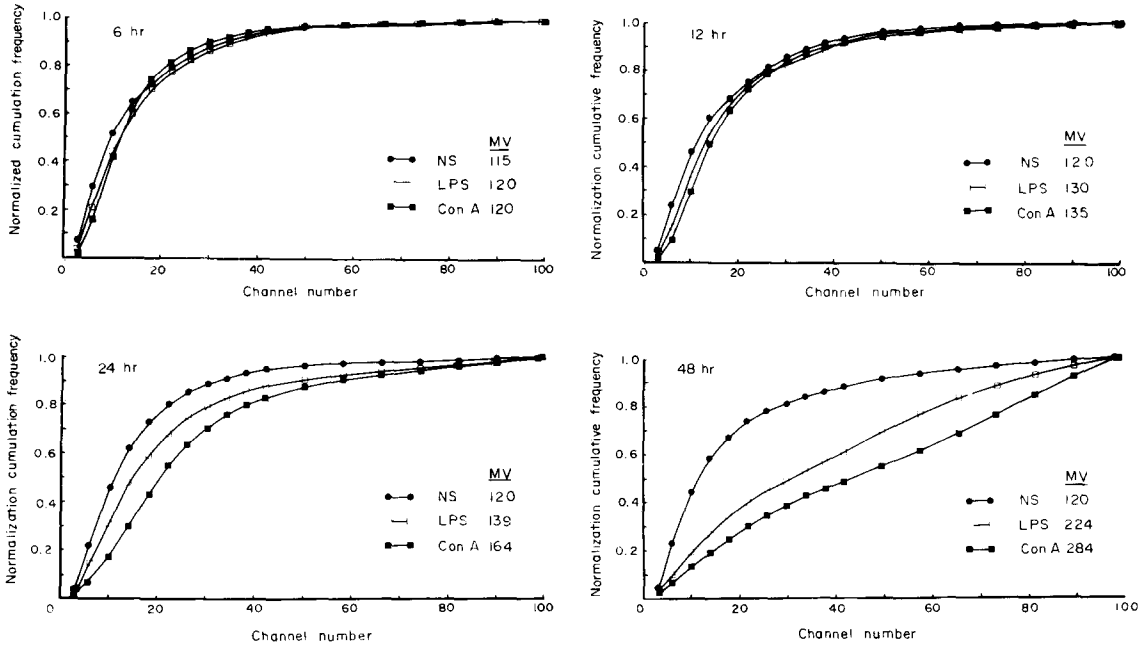

Fig. 5. Changes in cell size distribution in lymphocyte cultures stimulated for 6, 12, 24, or 48 hr with Con A or LPS. Spleen cells were cultured as described in Fig. 1. Each graph represents the normalized cumulative frequency versus channel number assessed at times indicated. Approximately 10,000 cells were sized for each distribution. MV refers to modal volume (μm^3). NS represents nonstimulated cultures.

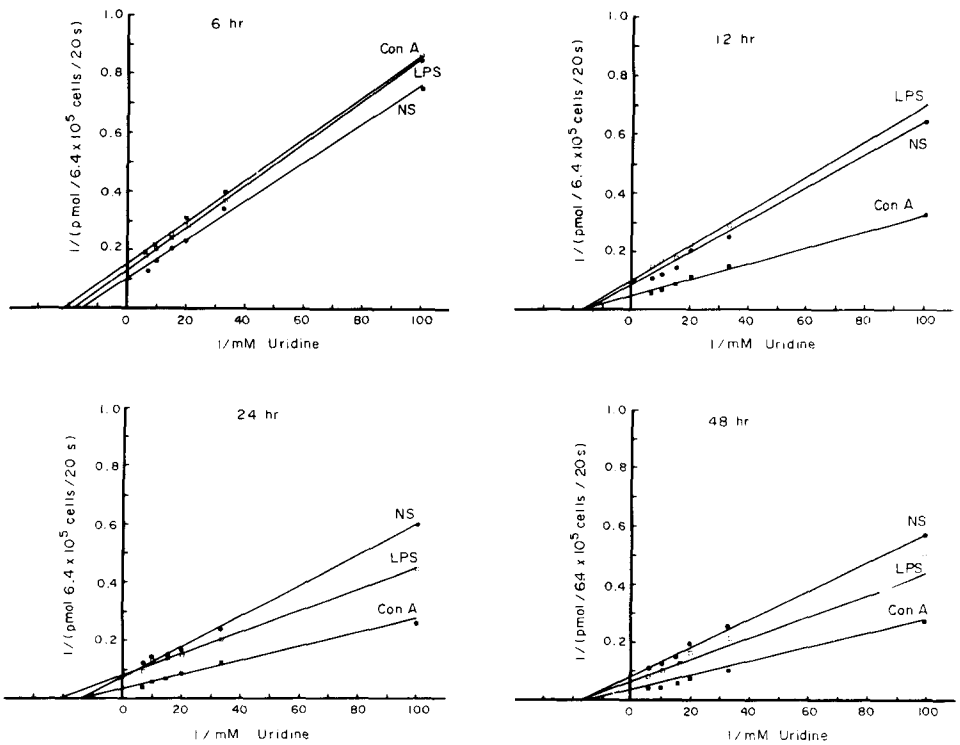

Fig. 6. Lineweaver–Burk kinetics of uridine transport by lymphocytes stimulated 6, 12, 24 or 48 hr with Con A or LPS. Lymphocytes were harvested from culture at the times indicated and suspended in PBS. For each sample, a 0.4-ml aliquot of 1.6×10^6 cells was mixed with 0.1 ml of [^{3}H]uridine (5.0 μCi/ml) at five times the final concentrations indicated. Duplicate 0.2-ml samples were then taken at 20-sec intervals, centrifuged, and counted as described in Materials and Methods. Uridine transport was determined by subtracting the contribution of the nonspecific uptake component as described in Fig. 1. Each point represents the mean of twelve values determined by three separate experiments.

whereas *incorporation* represents the amount of nucleoside incorporated into the nucleic acids as determined by trichloroacetic acid and ethanol washes of the cell pellet on glass-fiber filters. The results show that most of the [^{3}H]nucleosides associated with activated lymphocytes were incorporated into nucleic acids within the 2-hr pulse period. Uridine accumulation began within the first 6 hr after mitogen addition and peaked at 24 hr for Con A-activated cells or 48 hr for LPS-activated cells. Thymidine accumulation began in both mitogen cultures between 12 and 24 hr after activation, with peak uptake observed after 48 hr. These time courses of nucleoside uptake are similar to the time courses of uridine [23, 24] and thymidine [25] uptake in lymphocytes reported by other investigators.

Concomitant with changes in uridine and thymidine accumulation were increases in cell size, as shown by Fig. 5. Beginning with 6 hr after stimulation, there was a statistically significant increase in the size distribution of stimulated lymphocytes. This increase continued through the 48-hr culture period, with the modal volume for the LPS cultures always being less than the modal volume for the Con A cultures. The increase in size represented blastogenesis induced by the mitogens with the peak occurring at the same time as maximum DNA synthesis (Table 1, 48 hr). The increases in size distribution were not due to cell clumping since the size distributions remained the same among Con A-stimulated lymphocytes that had treated previously with 50 mM alpha-methyl mannoside (not shown).

Figure 6 illustrates the kinetics of uridine transport into Con A- and LPS-stimulated lymphocytes. In these experiments, no increase in uridine transport was observed until 12 hr after stimulation, and only in lymphocytes exposed to Con A. The V_{max} values in these cells were 21 ± 1.9 pmoles for the Con A cells, compared with 9.9 ± 0.8 pmoles and 11.6 ± 0.6 pmoles for LPS and unstimulated cells respectively. After 12 hr the V_{max} of transport gradually increased in the mitogen-stimulated cultures up to 48 hr when the cultures were terminated. At this time, the V_{max} values for unstimulated, LPS and Con A cells were, respectively, 9.3 ± 0.9, 13.7 ± 1.6, and 31.6 ± 1.63 pmoles. The K_m values for all experiments ranged between 4.1 and 7.9 × 10^{-5}M. In conjunction with the results of long-term (2 hr) [^{3}H]nucleoside accumulation and cell volume (Table 1), these results indicated that the V_{max} of transport was greater in bulk lymphocytes stimulated by Con A than those stimulated by LPS due to the activation of more cells by Con A. This was more apparent when LPS cultures were cultured an additional 24 hr (72 hr total), at which time the V_{max} for [^{3}H]uridine transport was 28 ± 5.9 pmoles for LPS-activated lymphocytes (Fig. 7). This compares with a V_{max} of 31.6 ± 1.63 pmoles for maximally stimulated Con A cells.

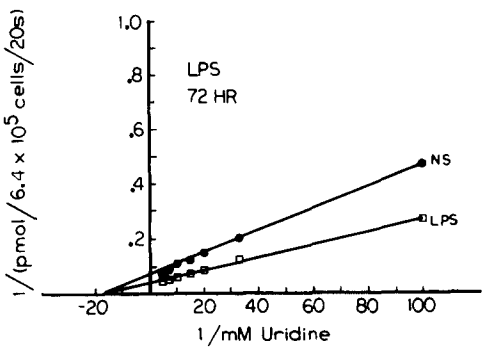

Fig. 7. Lineweaver–Burk kinetics of uridine transport by lymphocytes stimulated 72 hr with LPS. Cells were treated as described in the legend for Fig. 6. Each point represents the mean of eight values determined by two separate experiments.

DISCUSSION

This study demonstrates that increased uridine transport into activated lymphocytes correlated with increased cell size and long-term (2 hr) nucleoside accumulation. Although the amount of uridine transport was always greater in Con A-activated lymphocytes than in LPS-activated lymphocytes, the difference was attributed to a greater degree of cellular activation among stimulated T-cells when compared with stimulated B-cells. The degree of activation *in vitro* was based on cell volume changes and long-term (2 hr) uridine and thymidine accumulation.

Other studies have also observed a dichotomy in activation rates between LPS- and Con A-activated lymphocytes. Freedman [24] examined early calcium fluxes into mitogen activated lymphocytes and, although PHA, Con A and LPS each induced calcium uptake at maximally stimulating doses, Con A and PHA cultures consistently gave higher transformation indexes than LPS cultures after 48 hr. Reasons for this effect were unknown. Pienkowski *et al.* [26] detected a 2-fold increase in [^{3}H]uridine accumulation in mouse spleen cells exposed to Con A when compared with LPS-exposed cultures. They further showed that increased uridine accumulation into lymphocytes was associated with the early metabolite event of RNA synthesis, thus adding more evidence to the association of uridine uptake with RNA metabolism in lymphocytes as made previously by Cooper [27]. This association has also been carried over in determining differences in rates of cellular metabolism between T- and B-lymphocytes. Staub *et al.* [10] have observed that B-cells from tonsils were three times more active than T-cells by virtue of their uridine uptake rates. They further substantiated the metabolic differences by demonstrating that the B-cells had a greater rate of [^{3}H]dThd uptake and DNA polymerase activity. In addition, it was suggested that different rates of uridine transport might influence the amount of uridine eventually accumulated by lymphocytes, based on the premise that transport was rate-limiting [28].

Recent studies by Plagemann and coworkers [13, 14, 28–31], however, have overturned the idea that transport is rate-limiting by demonstrating with a rapid sampling procedure in Novikoff hepatoma cells that the rate of phosphorylation of nucleosides was almost 200-fold slower than transport of nucleosides and, therefore, intracellular phosphorylation

was the rate-limiting step. These investigators also proved that transport was independent of phosphorylation and energy by measuring transport in ATP-depleted hepatoma cells. Since intracellular kinase activity is known to increase in lymphocytes during activation, their results further support the notion that cellular metabolism is linked to uridine accumulation.

In the present study, we determined that uridine transport in Con A-activated lymphocytes was temperature-dependent and linear for at least 40 sec. These factors were assumed to be identical for LPS-activated lymphocytes. However, this assumption is a caveat that could partially account for differences in uridine transport among the mitogen-activated T- and B-lymphocytes. We believe this unlikely, however, because the capacity for *total* uridine uptake in LPS cultures was similarly increased with time in comparison with total uptake in Con A cultures during the 48-hr sampling period. Also, the observation that the V_{max} values for uridine transport were similar in maximally activated LPS lymphocytes (Fig. 7, 72 hr) and in Con A lymphocytes (Fig. 6, 48 hr), as assessed by cell size and total 2-hr nucleoside accumulation, further supports our notion that the actual membrane translocation processes were the same in the two cell types.

Further studies using physically separated or purified T- or B-lymphocytes would aid in determining potential differences in uridine transport between the two subpopulations. A problem with this approach is that by purifying these cells one alters the activation kinetics of mitogens due to the absence of certain accessory cells in culture. This is especially true for T-cells, which require the presence of macrophages for activation. Lymphocytes can be activated *in vivo* by injecting mitogens into mice, but subsequent manipulations necessary to separate these activated T- or B-cells (e.g. separation via nylon wool columns) could perturb the cell membrane and thereby alter nucleoside transport. However, once these technical difficulties are overcome and the effect of physical separation on normal transport is characterized, further studies on nucleoside transport could be performed on these purified subpopulations. At present, activation of T- or B-cells by selective mitogen stimulation *in vitro* offers the simplest method for obtaining large samples of relatively pure populations of activated T- or B-lymphocytes.

The progressive increases observed in the V_{max} for uridine transport after mitogen stimulation suggest that the number of transport sites is increased in activated lymphocytes. This conclusion is partially supported by the work of Peters and Hausen [23, 32], which showed an increase in uptake (V_{max}) into PHA-stimulated lymphocytes (pretreated with cycloheximide and actinomycin D) with no change in K_m values. They interpreted these results as an indication of unmasking of pre-existing sites by PHA stimulation; however, because 10–60 min incubation times were used, these results could be attributed to changes in uridine phosphorylation rates. Marz *et al.* [33] maintain that nucleoside transport rates remain constant and independent of metabolic events, based on studies with cycloheximide and actinomycin D-pretreated Novikoff cells in which uridine and thymidine transport kinetics remained stable while total nucleoside uptake decreased with the loss of inhibited kinase activities. In contrast, our results indicated that uridine transport increases with greater lymphocyte stimulation. It could be argued that 20-sec incubations in the present study were too long and still influenced by rates of intracellular phosphorylation. To test that theory, we have assessed 20-sec uptake of uridine into lymphocytes pretreated 1–9 min with 5 mM NaCN and 5 mM iodoacetate to inhibit intracellular phosphorylation [29]. No significant difference was observed in the amount of uridine taken up between pretreated and control lymphocytes (data not shown). Strauss *et al.* [12] have also noted that spleen lymphocytes must be activated by Con A before any significant thymidine transport can be observed. Collectively, these results point out an important difference with respect to transport kinetics between spleen lymphocytes, which are normally quiescent, and actively growing tumor cell lines. Not all nucleoside transport must necessarily increase in lymphocytes after stimulation, as pointed out by studies with adenosine transport. Strauss *et al.* [12] observed that Con A activation did not alter adenosine transport in lymphocytes. Their experiments also demonstrated that changes in cell surface area *per se* do not affect nucleoside transport since the activated lymphocytes were much larger than normal lymphocytes (modal volumes of 150 μm^3 vs 28 μm^3). This is supported by results from Tsan and Berlin [34] which showed that lysine and adenosine transport into rabbit lung macrophages was not altered even after these cells had internalized about 50 per cent of their plasma membranes during phagocytosis of latex beads.

In summary, these studies showed that, during the first 48 hr of stimulation with Con A or LPS, uridine uptake (2-hr pulse) and transport increased with the general activation state of murine lymphocytes. V_{max} values for uridine accumulation were always greatest in Con A-stimulated lymphocytes, but because more cells were activated by Con A than LPS, the difference could not be attributed to preferential accumulation of uridine by T-lymphocytes. These results also indicated that differences in uridine accumulation into T- or B-lymphocytes observed in some studies should not be attributed to a difference in uridine transport between the lymphocyte subpopulations. A general conclusion from these studies is that uridine accumulation into murine lymphocytes is substantially influenced by cellular metabolic activity which will mask any differential accumulation associated with T- or B-lymphocytes, if such a difference actually exists.

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