while in the second week the explants gradually became smaller. When measuring the amount of DNA, RNA and protein, the curve indicated the same decline during the second week (unpublished results). The action of db-cAMP might therefore be explained by the inhibition of cell death/or the stimulation of cell proliferation. We must bear in mind that the growth-promoting effects of cAMP are sometimes claimed to be due to the restoration of the purine nucleotide pool and not to a specific influence[10].

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Differences in cytochalasin D-induced surface alterations between chronic lymphocytic leukaemic and normal lymphocytes[1]

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Summary. Cytochalasin D (CD) causes an unusual surface alteration in normal lymphocytes consisting of the formation of focal irregular club-shaped cell processes. Lymphocytes from chronic lymphocytic leukaemic cases did not show this change on exposure to CD. There was either no surface change or, in some cases, clear double-membrane lined vesicles were formed and appeared to be discharged from the cell. This difference in response may be related to the changes in cell membranes known to occur in malignant transformation.

The effect of cytochalasins on mammalian cells are numerous and are thought to be brought about by an alteration of the cell microfilament function[2–5]. Studies with tritiated cytochalasin D (CD) support this concept as it is taken up and bound to the subplasmalemmal microfilaments or cell membrane[6]. Previous ultrastructural study on the effects of CD on the morphology of cultured tumour cells showed cytoplasmic projections thought to be similar to zeiotic blebs[7]. The present study of the effects of CD on normal human peripheral blood lymphocytes show the morphological surface alterations to consist of unusual cytoplasmic processes. These processes were focal and did not undergo repeated protrusion and retraction in contrast to the phenomenon of zeiosis[8]. The change seen was in keeping with the concept of the action of the cytochalasins on the microfilaments. In the cases of chronic lymphocytic leukaemia (CLL) there was either no marked surface change or, in a few cases, there was formation of double membrane lined vesicles at the cell surface. The reason for the different morphological response in the chronic lymphocytic leukaemic lymphocytes was not apparent from this study.

Materials and methods. CD was dissolved in dimethylsulfoxide (DMSO). Lymphocytes were obtained from normal donors and from cases of CLL. The following lymphocyte preparations were used. 1. Lymphocytes in leucocyte-rich plasma without further manipulation, 2. lymphocytes separated on a Ficoll-Hypaque gradient, washed and suspended in growth medium (GM) consisting of Eagle's medium with 10% fetal calf serum, 3. lymphocytes separated on a Ficoll-Hypaque gradient, washed with GM and resuspended in plasma. Cell suspensions at a concentration of 1 million cells per ml were incubated for 40 min at 37 °C with a final concentration of 1. CD, 1 µg/ml and 2. CD, 15 µg/ml. The concentration of DMSO was 0.5%. Controls with and without 0.5% DMSO were also prepared. Unfixed wet preparations were studied immediately at the end of the 40 min incubation by phase microscopy. The cell suspensions were then fixed in ice-cold 2% gluteraldehyde for scanning electron

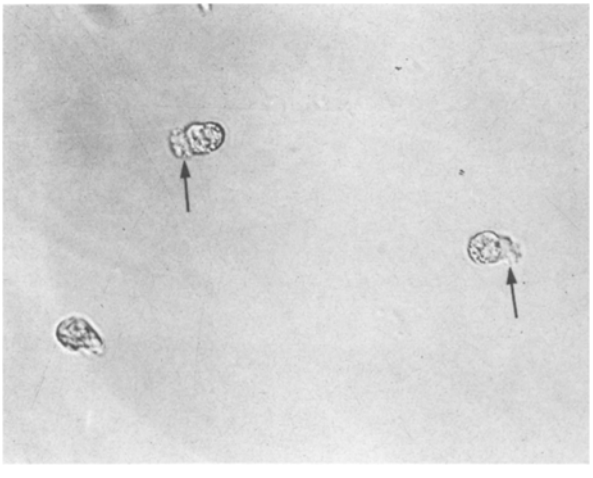

Fig. 1. Arrows point to focal cytoplasmic irregularities at 1 pole of normal lymphocytes exposed to CD 15 µg/ml, unfixed wet specimen. ×550.

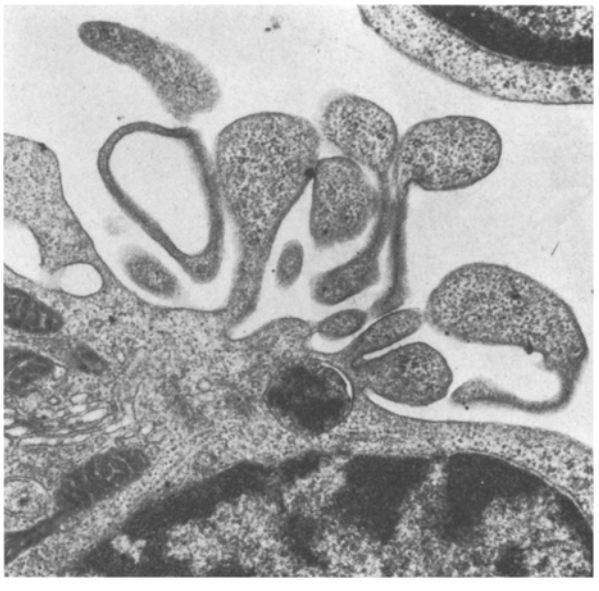

Fig. 2. TEM of above preparation showing surface irregularities to be cytoplasmic, club-shaped processes. × 21,000.

microscopy (SEM) and in gluteraldehyde with postfixation in 2% osmium tetroxide buffered with cacodylate for transmission electron microscopy (TEM). The critical point drying method with CO_2 was used for the scanning electron microscopy preparations. For TEM the material was dehydrated in increasing concentrations of alcohol, cleared in propylene oxide and embedded in epoxy resin.

Results and discussion. A summary of the morphological changes in the lymphocytes in GM is shown in the table. In the unfixed preparations of normal lymphocytes with CD, focal cytoplasmic processes involving usually about $^1/_6$ to $^1/_4$ of the cell circumference were seen (figure 1). The processes and the cells themselves were stationary with no evidence of the continual protrusion and retraction as seen in zeiosis and we feel this term is inappropriate for the cytochalasin-induced processes. The cells themselves. were also nonmotile. By TEM focal areas of club-shaped, cytoplasmic processes were seen (figure 2). By SEM the covering of the entire surface of the control lymphocytes with small microvilli differed markedly from those exposed to the cytochalasin where the focal, irregular club-shaped processes were present at 1 pole of the cell (figure 3). Washing the cytochalasin from the cells also allowed a return to normal shape and as evidenced by the trypan blue dye exclusion test there was no loss of viability.

In the chronic lymphocytic leukaemia cases when exposed to the cytochalasins only occasional cells had the focal cytoplasmic processes, the majority appearing normal and round (figure 4). By TEM 9 of the cases showed no surface abnormality. In 6 cases some cells had clear,

1 The study was supported by the National Cancer Institute of Canada.

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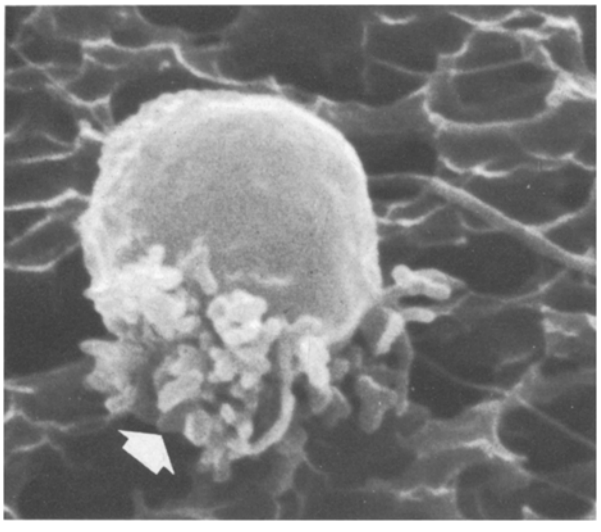

Fig. 3. SEM picture of these cells shows focal nature of these cytoplasmic processes (arrow). Note smoothness of remainder of cell surface. ×9500.

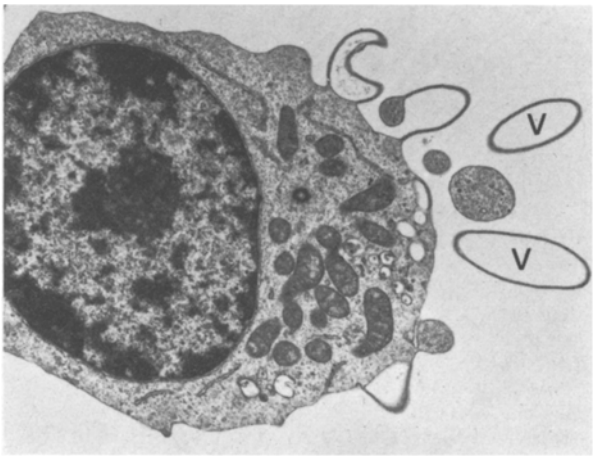

Fig. 5. TEM of one of the cases of chronic lymphocytic leukaemia with blebbing. Note apparent extrusion of double membrane-lined vesicles (V). ×9000.

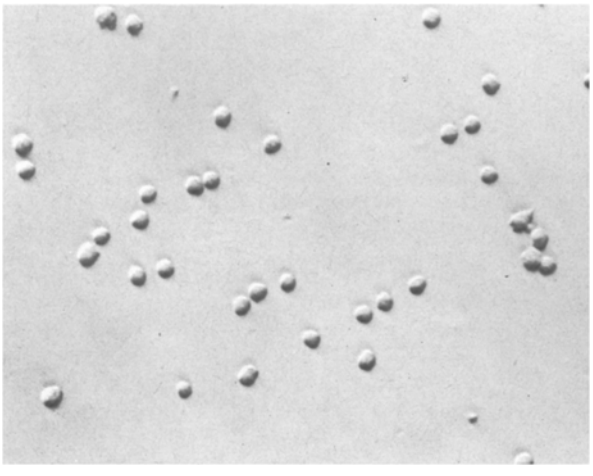

Fig. 4. Chronic lymphocytic leukemic lymphocytes exposed to CD 15 μg/ml. Note normal shape in contrast to figure 1. ×350.

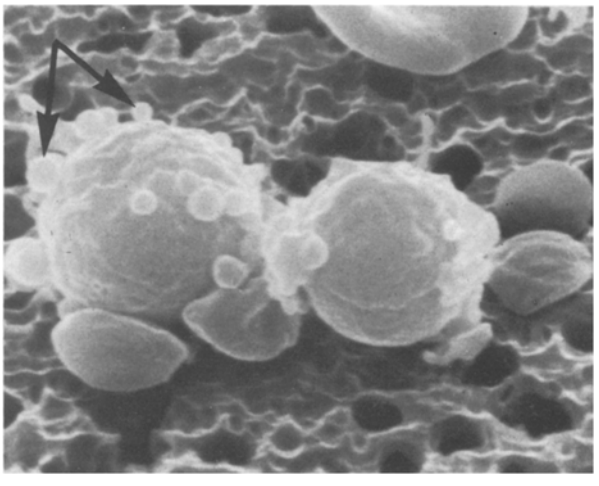

Fig. 6. By SEM spherical blebs of various sizes (arrows) can be seen on the surface of the lymphocytes. ×5600.

Effects of cytochalasin D on cell morphology of normal and chronic lymphocytic leukemic lymphocytes

Diagnosis	Morphological Changes Preparation	No. of cases	Wet film	TEM	SEM
Normal	Lymphs in GM	15	Normal, round	Normal	Small microvilli
	Lymphs in DMSO (0.5%)	4	Normal, round	Normal	–
	Lymphs in GM & CD 15 μg/ml	15	Focal cell processes	Focal club-shaped processes	Focal club-shaped processes
CLL	Lymphs in GM (0.5%)	15	Normal, round	Normal	Small microvilli
	Lymphs in DMSO	3	Normal, round	Normal	–
	Lymphs in GM & CD 15 μg/ml	15 9	Majority (95%) normal, round	Few cells club-shaped processes	Few cells club-shaped processes
		6	Surface blebs	Double membrane lined vesicles	Surface blebs

double membrane lined vesicles extending from the cell surface (figure 5). In some cells they appeared to be discharged from the cell. The SEM confirmed this finding by the demonstration of sausage shaped or spherical blebs on the cell surface of the lymphocytes (figure 6).

The focal surface changes in the normal lymphocytes may be explained on microfilament alteration due to the CD[7,9]. The difference of response in the CLL cells is harder to explain but differences in the cell membranes of malignant and normal cells are well known[10]. Similarly the phenomenon of 'blebbing' in malignant cells on exposure to drugs which affect the cell membrane has also been recognised[11]. The possibility of the 'blebbing' being due to the associated chemotherapy for the CLL is unlikely as in one of the cases with marked blebbing no therapy had been given. Although the change was seen in all preparations, when the cells were examined in growth medium after washing the change was more pronounced. This enhancement may be due to the washing making the cell membrane more susceptible to the drug action or to there being some protective factors in the plasma. The focal nature and asymmetry of the cell processes in the cytochalasin-affected normal lymphocytes is perhaps surprising as cytochalasin has been found to inhibit the 'capping' phenomenon. It is, however, in keeping with previous findings of cytochalasin in various cultured cells[9,12].

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The immunopotentiating effect of thiosulphate in vivo[1]

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Summary. Sodium thiosulphate injected i.v. into mice causes a marked increase in concentration of several serum proteins, particularly immunoglobulins. When given together with antigen, it significantly potentiates the T-dependent humoral responses.

The requirement of thiols for the activation of in vitro immune responses[3,4] warrants the search for active in vivo sulfur-containing immunopotentiators. The suppression rather than potentiation caused by organic thiols in vivo[5,6] turned attention to thiosulphate (TS), which was thought to increase IgM-production by activating thiol-disulfide interchange[7,8], a key process in IgM-pentamerization[9].

Materials and methods. Inbred Balb/c and colony-bred Swiss mice of both sexes, 6–8 weeks old, were used for experiments. The mice were injected i.v. with TS (5%) daily during different periods of time in doses corresponding to the maximal human clinical doses (0.3 mg/g b.wt) or twice as much. Control animals received saline. Blood samples (0.3 ml) were taken for analysis once a week and responses of individual mice were recorded. For chemical analysis of serum proteins, 2 electrophoretic methods were used: a) modified[10] electrochromatographic fractionation[11] and b) isoelectric focusing on polyacrylamide gel.

To test the influence of TS-treatment on the specific humoral immune responses, groups of mice were immunized i.v. either with 5×10^8 trinitrophenyl-chicken red bloods cells (TNP-CRBC), or 1×10^8 sheep red blood cells (SRBC) (T-dependent response)[12]. For studying T-independent response, 10 μg of trinitrophenyl-lipopolisaccharide (TNP-LPS)[13] was injected i.v. Different schedules of TS-administration (0.3 mg/g daily) were used (table 2). The number of plaque-forming cells (PFC) was recorded 4 days after immunization by microscope slide