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In vitro and *in vivo* stimulation of neutrophil migration and lymphocyte transformation by thiamine related to inhibition of the peroxidase/H_2O_2/halide system

A. THERON,* R. ANDERSON,* G. GRABOW* & J. L. MEIRING† * *The Immunology Section, Department of Medical Microbiology, and* † *The Department of Chemical Pathology, Institute of Pathology, University of Pretoria, Republic of South Africa*

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SUMMARY

The effects of thiamine on neutrophil functions and mitogen-induced lymphocyte transformation were investigated *in vitro* and *in vivo* in adult volunteers following the injection of 50 mg thiamine intramuscularly. Thiamine caused stimulation of neutrophil motility *in vitro* and *in vivo* and increased lymphocyte transformation *in vivo*. Enhancement of these functions was related to inhibition of neutrophil post-phagocytic iodination of *Candida albicans* by the MPO/H_2O_2/halide system. The horseradish peroxidase/-H_2O_2/^{125}I-mediated iodination of bovine serum albumin was also inhibited by thiamine concentrations which caused increased neutrophil motility. It was found that preincubation of neutrophils and lymphocytes with the horseradish peroxidase/H_2O_2/halide system caused considerable inhibition of the migratory and proliferative responses respectively. Inclusion of thiamine at concentrations which were found to inhibit the peroxidase/-H_2O_2/halide system protected the neutrophil migratory and lymphocyte proliferative responses from inactivation by this system. It is suggested that thiamine may cause increased neutrophil migration and lymphocyte transformation by protecting these cells from toxic oxidative products generated by the peroxidase/H_2O_2/halide system.

INTRODUCTION

Investigation of the effects of nutritional factors, especially vitamins and minerals, on immunological responsiveness as measured in nutritionally deficient individuals or in normal individuals receiving high daily supplements of the nutritional factor under investigation is a field of considerable interest. We have recently observed (unpublished findings) that a single injection of a vitamin B complex preparation in three individuals with reduced neutrophil motility and lymphocyte mitogen-induced transformation caused a transient normalization of the abnormal leucocyte functions. We have therefore undertaken a study to assess which components of the vitamin preparation possess immunostimulatory activity and in this study we report our findings on thiamine (vitamin B_1).

MATERIALS AND METHODS

Thiamine. For *in vitro* studies, thiamine hydrochloride was used (Sigma Chemical Co., St Louis, Missouri, USA), dissolved in the appropriate cell-suspending medium and investigated over a

Correspondence: Dr R. Anderson, Institute of Pathology, PO Box 2034, Pretoria 0001, RSA.

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concentration range of 10^{-6}–10^{-1} M. For *in vivo* studies, injectable thiamine was used (obtained as a gift from Merck (Pty.) Ltd, South Africa, as 50-mg ampoules). A single dose of thiamine was injected intramuscularly into six normal adult volunteers and neutrophil and lymphocyte functions were assessed prior to injection and 2, 24 and 48 hr after injection.

Neutrophil functions

Motility studies. Neutrophils were obtained from heparinized venous blood (5 units/ml) and resuspended to a final concentration of 6×10^6/ml in HEPES (1 g/litre; Sigma) buffered Hanks's balanced salt solution supplemented with 0·05% bovine serum albumin (BSA) (HBSS; Grand Island Biological Co., New York, USA) as previously described (Anderson & Van Rensburg, 1979).

Two leucoattractants were used:

(a) Fresh autologous serum activated with 100 μg/ml of bacterial lipopolysaccharide (*E. coli*: 0127:B8; DIFCO, Detroit, Michigan, USA), which was diluted eight-fold with HBSS prior to use.

(b) The synthetic chemotactic tripeptide, *N*-formyl-L-methionyl-L-methionyl-L-phenylalanine (F-Met-Met-Phe; Miles Laboratories, Indiana, USA). This agent was used at a final concentration of 5×10^{-7} M (previously found to be the optimal leucotactic concentration) in BSA-supplemented HBSS.

In random migration systems the leucoattractant was replaced with an equal volume of BSA-supplemented HBSS (0·8 ml). Cell suspensions were preincubated with the various thiamine concentrations for 15 min. The vitamin remained in the cell compartment throughout the incubation period. The final cell concentration was 3×10^6/ml for all motility studies. Assays of motility were performed in modified Boyden chambers (Wilkinson, 1971) using 5-μm-pore-size Sartorius filters (Sartorius-membranfilter, Göttingen) and a 2-hr incubation period. Results are expressed as cells, which have completely traversed the filter, per microscope high power field as an average of triplicate filters.

Phagocytic studies. In studies of post-phagocytic metabolic activity the cell-suspending medium used was 0·15 M phosphate-buffered saline (PBS), pH 7·2. These activities were stimulated using *Candida albicans* at concentrations which in our hands give maximal stimulation of post-phagocytic metabolic activity. Pure neutrophil suspensions prepared as previously described (Anderson & Van Rensburg, 1979) of >90% purity and viability were used in these studies.

Hexose monophosphate shunt (HMS) activity. This was measured according to the method of Wood, Katz & Landau (1963) with minor modifications (Anderson & Van Rensburg, 1979) by potassium hydroxide absorption of $^{14}CO_2$ derived from glucose radiolabelled in the 1-C position ([1-^{14}C]D-glucose, New England Nuclear, Boston, Massachusetts, USA). The reaction mixture contained 2×10^6 PMN (0·1 ml), 0·1 ml autologous serum, 1×10^7 *C. albicans* (0·1 ml), 0·1 ml of the thiamine concentration and 0·6 ml radiolabelled glucose containing 0·06 μCi. Incubation was for 1 hr after which the reaction was terminated and CO_2 released by the addition of 2 ml of 1 N HCl. After a further hour, the radioactivity associated with KOH was determined in a liquid scintillation counter. Results are expressed as nmol glucose oxidized per 2×10^6 PMN.

Measurement of H_2O_2 production. In these experiments the effects of thiamine on the release of H_2O_2 into the extracellular medium following ingestion of opsonized *C. albicans* were determined. Opsonization was performed at 4°C using 1 ml of fresh pooled serum per 10^8 organisms for 2 hr. The organisms were washed twice and resuspended to a concentration of 5×10^9/ml. Each experimental tube contained 1×10^7 PMN (0·2 ml), 0·1 ml *C. albicans* (to give a PMN : *C. albicans* ration of 1 : 50), 0·1 ml appropriate thiamine concentration, 0·1 ml of 10 mM sodium azide and 0·4 ml of PBS supplemented with 10 mM glucose. Tubes were preincubated for 15 min prior to the addition of *C. albicans*. After a 30-min incubation period the tubes were centrifuged at 5,000 *g* for 10 min and the supernatant fluid assayed for H_2O_2 according to the method of Root *et al.* (1975) by reduction of scopoletin fluorescence. Each assay tube contained 2·5 ml distilled H_2O, 50 μl of horseradish peroxidase (Sigma) at a stock concentration of 13·8 units/ml, 20 μl of 200 mM scopoletin (Sigma) and 0·5 ml of the cell-free supernatant. Standard curves were constructed in the range 0·1–10 nmol of H_2O_2. The H_2O_2-dependent reduction in fluorescence was monitored in a Perkin–Elmer model

204 Hitachi fluorescence spectrophotometer at an exciter wavelength of 390 nm and an analyser wavelength of 460 nm. Results are expressed as nmol $H_2O_2 \cdot (10^7 \text{ PMN})^{-1} \cdot \text{min}^{-1}$.

Neutrophil superoxide production was assayed as described by Curnutte & Babior (1974). The reaction mixtures contained 5×10^6 PMN in 0·5 ml, 0·1 ml preopsonized *C. albicans* to give a cell:micro-organism ratio of 1:50, 0·1 ml of 1 mM horse heart ferricytochrome *c* (Cyt. *c* type VI, Sigma) and 0·1 ml of appropriate thiamine concentration or PBS in controls. The final reaction volume was 1 ml. Tubes were incubated in a waterbath at 37°C for 45 sec (reaction linear to 90 sec), after which the reaction was terminated with 1 ml ice-cold PBS and tubes centrifuged and the supernatants assayed for reduced Cyt. *c* in a Unicam SP 1700 ultraviolet spectrophotometer at 550 nm. The amount of reduced Cyt. *c* was calculated using the absorbance coefficient of 15·5 mM at 550 nm (Margoliash & Frohwirt, 1959). Superoxide-dependent reduction of Cyt. *c* was expressed as the difference in Cyt. *c* reduction between reaction mixtures containing no superoxide dismutase (Sigma) and those containing 200 units/ml superoxide dismutase.

Quantitative myeloperoxidase assay. MPO was assayed by the increase in fluorescence at 470 nm which accompanies the oxidation of guaiacol. In these experiments, 10^8 PMN (1 ml) were sonicated in an MSE ultrasonic disintegrator (3×20 sec bursts) at an amplitude of 10 μm peak-to-peak and the sonicate centrifuged at 5,000 ***g*** for 10 min. The supernatant was used as a source of MPO and 0·1-ml volumes were incubated with 0·1 ml of the appropriate thiamine concentration for 30 min. Following incubation, 0·1-ml aliquots of the mixtures were then assayed for MPO according to the method of Paul, Selvaraj & Sbarra (1978) with minor modifications. The assay system consisted of 1 ml glycine–NaOH buffer, pH 10, 1 ml of 3×10^{-2} M guaiacol, 1 ml of 1×10^{-2} M H_2O_2 and 0·1 ml of the reaction mixture. The rate of oxidation was measured spectrophotometrically at 470 nm. Results are expressed as enzyme units/10^8 PMN, calculated from a standard curve in the range 0·83–27·5 units of horseradish peroxidase (Sigma) per ml.

MPO-mediated iodination of ingested protein. This was determined by the method of Root & Stossel (1974) with minor modifications. To 0·1 ml of PMN suspension (1×10^7/ml) was added 0·1 ml of *C. albicans* (1×10^8/ml), 0·1 ml of fresh autologous serum, 0·1 ml of the various thiamine concentrations, 0·1 ml of a ^{125}I solution (0·6 μCi/ml) (New England Nuclear, sodium iodate), and 0·5 ml of PBS. Incubation was for 60 min at 37°C on a turntable after which the extent of incorporation of ^{125}I into acid-precipitable protein was determined by solid scintillation counting. Results are expressed as nmol ^{125}I in the protein precipitate.

Effects of the drugs on the horseradish peroxidase/iodide/H_2O_2 system. To investigate the effects of thiamine in a cell-free system, the effects on the horseradish peroxidase (HRP) iodination of bovine serum albumin were assessed. Each reaction system contained 0·27 units of HRP (HRP type VI, Sigma), 10 μM H_2O_2 (Merck Chemical Co.) and 0·06 μCi ^{125}I, 5 mg BSA and the appropriate thiamine concentration in a total reaction volume of 1 ml. Tubes were incubated and processed as above for the MPO system.

Exposure of neutrophils to the peroxidase/iodide/H_2O_2 system. In this series of experiments the effects of the peroxidase/iodide/H_2O_2 system in the presence and absence of the drugs on neutrophil motility were investigated. Neutrophils (5×10^6) in 0·5 ml were preincubated with 0·27 units of horseradish peroxidase, 1 nM hydrogen peroxide, 1 mM sodium iodide and drug concentrations which caused maximal stimulation of motility in a final reaction volume of 1 ml HBSS without BSA. Control systems contained no drug. The reaction systems were incubated at 37°C for 15 min after which the supernatants containing the reactants were removed following centrifugation and the cell pellet resuspended to 5×10^6/ml in HBSS supplemented with 0·05% BSA. The differently processed neutrophils were then tested for reactivity to EAS.

Activation of the MPO/H_2O_2/halide system by F-Met-Met-Phe and EAS. To measure the effects of the leucoattractants on activation of the MPO/H_2O_2/halide system, 1×10^6 neutrophils (0·1 ml) were incubated with 5×10^{-5} M, 5×10^{-6} M and 5×10^{-7} M F-Met-Met-Phe and 10% EAS in the presence of 0·06 μCi ^{125}I in a total reaction volume of 1 ml containing 2 mg BSA. Reaction systems were incubated for 1 hr at 37°C after which the effects of the leucoattractants on activation of the MPO/H_2O_2/halide system were investigated according to the extent of ^{125}I incorporation into acid-precipitated BSA.

Inhibition of peroxide availability. To determine if thiamine bound or inactivated H_2O_2, it was

incubated at 10^{-3} M with 2 μM peroxide for 30 min and the mixture assayed for peroxide by the reduction of scopoletin fluorescence as described above.

Effects on the xanthine–xanthine oxidase system. To assess if thiamine had superoxide scavenging properties the effects on xanthine–xanthine oxidase-mediated reduction of ferricytochrome *c* were assessed. Each reaction system contained 1·7 mM xanthine, 100 μM Cyt. *c*, the appropriate drug concentration and 0·12 units xanthine oxidase (Sigma, xanthine oxidase grade 1, from buttermilk) in a reaction volume of 3 ml 0·05 M PBS, pH 7·2. Superoxide-mediated reduction of Cyt. *c* was assessed after 3 min (still on the linear portion of the reaction curve) at 22°C spectrophotometrically at 550 nm.

Lymphocyte transformation. Blood for these studies was defibrinated and fractionated by density-gradient centrifugation (Ficoll–sodium metrizoate gradients) at 400 ***g*** for 25 min. The mononuclear cell layer was removed and washed twice in TC 199 (GIBCO), pH 7·2, supplemented with HEPES (Sigma), 2 g/litre, and 10% heat-inactivated autologous serum. The cell suspension was adjusted to 4×10^6 mononuclear cells/ml. Aliquots of 50 μl (2×10^5 cells) were placed in wells of 5-mm Linbro tissue culture plates (Flow Laboratories, Inglewood, California, USA) together with 100 μl of serum-supplemented TC 199. The mitogens used in this study were phytohaemagglutinin (PHA; Wellcome Reagents Ltd, Beckenham, England) and concanavalin A (Sigma) at concentrations of 25 and 50 μg/ml. Mitogens were added in 20-μl volumes to triplicate wells and unstimulated controls received 20 μl of TC 199. The different concentrations of thiamine (10^{-6}–10^{-2} M) were added in 20-μl volumes to triplicate wells. The final volume in each well was brought to 200 μl by the addition of serum-supplemented TC 199. The plates were mixed and incubated for 48 hr in a humidified atmosphere of 3% CO_2 in air after which 20 μl of tritiated thymidine (^{3}H-TdR, [methyl-^{3}H]thymidine, New England Nuclear) containing 0·2 μCi was added to each well and the plates incubated for a further 18 hr. Harvesting was performed using a multiple automated sample harvester (Mash II, Microbiological Associates, Bethesda, Maryland, USA). Incorporation of ^{3}H-TdR was assessed in a liquid scintillation spectrophotometer.

Effects of the HRP/H_2O_2/iodide system on transformation. These were assessed as above by incubating the lymphocytes (in *serum-free* PBS) with the same HRP, H_2O_2 and iodide concentrations as above. After incubation, in the presence and absence of the various thiamine concentrations, the lymphocytes were washed, resuspended to 4×10^6 ml in serum-supplemented TC 199 and tested for responsiveness to the mitogens.

RESULTS

Calculation and expression of results

Results are expressed as the mean value with standard error of six separate experiments for each investigation. Statistical analysis was performed by the Student *t*-test (*t*-statistic for two means).

Neutrophil motility

Preliminary studies with the leucoattractants F-Met-Met-Phe and EAS showed that thiamine at concentrations of $> 10^{-3}$ M caused stimulation of motility to EAS. These studies were repeated at an increased number of thiamine concentrations between 10^{-3} and 10^{-1} M. Results of studies of random motility and migration to EAS and F-Met-Met-Phe are shown in Fig. 1. Progressive stimulation of migration to EAS was observed at thiamine concentrations of $> 10^{-3}$ M which was maximal at 5×10^{-2} ($P < 0{\cdot}005$). Slight but insignificant stimulation of motility to F-Met-Met-Phe and of random motility at 10^{-2} M thiamine was observed. Thiamine at concentrations of 10^{-2} M and 5×10^{-2} M possessed no intrinsic leucotactic activity (results not shown).

Phagocytosis of *C. albicans* and post-phagocytic HMS activity and superoxide production were unaffected by thiamine at concentrations up to 10^{-2} M. Likewise, no effects on H_2O_2 production at concentrations up to 5×10^{-3} M were observed (results not shown). Thiamine at concentrations up to 10^{-2} M had no effect on the xanthine–xanthine oxidase-mediated reduction of ferricytochrome *c* with values of 306 ± 17 and 312 ± 21 nmol reduced ferricytochrome *c*/min for control systems and

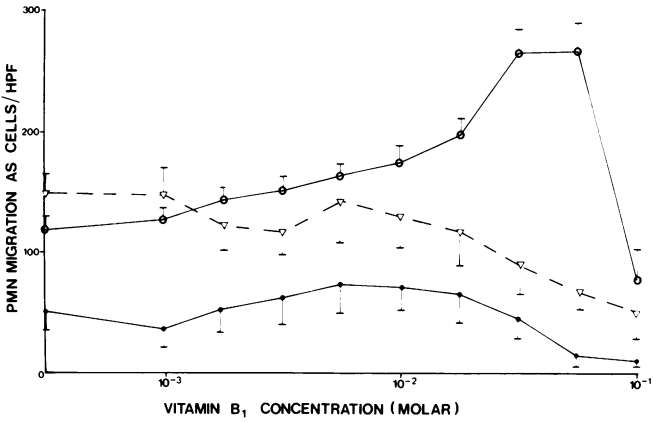

Fig. 1. The effects of vitamin B_1 (thiamine) on neutrophil migration to EAS (o——o), F-Met-Met-Phe (▽——▽) and on random motility (●——●).

systems containing 10^{-2} M thiamine respectively. Thiamine at 10^{-3} M had no binding or deactivating effects on formed H_2O_2 (results not shown).

Post-phagocytic MPO-mediated iodination was progressively inhibited by thiamine concentrations of $>10^{-4}$ M (Table 1). Likewise, progressive inhibition of the cell-free HRP-mediated iodination of BSA was observed over the same concentration range (Table 1). Thiamine at concentrations up to 10^{-2} M had no effect on the halide-independent MPO-mediated oxidation of guaiacol with values of $30{\cdot}2 \pm 4{\cdot}3$ and $29{\cdot}6 \pm 3{\cdot}9$ enzyme units/10^8 neutrophils for controls and experimental systems respectively.

Incubation of neutrophils with the HRP/H_2O_2/iodide system caused considerable inhibition of the neutrophil migratory response to autologous EAS. The mean percentage inhibition was $81 \pm 5{\cdot}4\%$. However, inclusion of thiamine at concentrations which inhibit activity of the peroxidase/H_2O_2/halide system and cause stimulation of motility protected the neutrophil migratory responsiveness to EAS (Fig. 2).

The effects of F-Met-Met-Phe and 10% EAS on activation of the MPO/H_2O_2/halide system are shown in Table 2. Both leucoattractants caused activation of the MPO/H_2O_2/halide system, which was related to the concentration of F-Met-Met-Phe (a fixed EAS concentration was used).

Table 1. *In vitro* effects of thiamine on the MPO-mediated iodination of *C. albicans* and on the HRP-mediated iodination of BSA

Thiamine concentration (molar)	MPO-mediated iodination of *C. albicans**	HRP-mediated iodination of BSA†
Control	$0{\cdot}85 \pm 0{\cdot}19$	$0{\cdot}72 \pm 0{\cdot}12$
1×10^{-4} M	$0{\cdot}65 \pm 0{\cdot}11$ (24)‡	$0{\cdot}69 \pm 0{\cdot}07$ (4)‡
5×10^{-4} M	$0{\cdot}50 \pm 0{\cdot}08$ (41)	$0{\cdot}59 \pm 0{\cdot}06$ (18)
1×10^{-3} M	$0{\cdot}48 \pm 0{\cdot}07$ (44)	$0{\cdot}32 \pm 0{\cdot}06$ (55)
5×10^{-3} M	$0{\cdot}26 \pm 0{\cdot}03$ (70)	$0{\cdot}07 \pm 0{\cdot}01$ (90)
1×10^{-2} M	$0{\cdot}08 \pm 0{\cdot}01$ (91)	$0{\cdot}03 \pm 0{\cdot}01$ (96)

* Results as mean nmol ^{125}I precipitated with standard error of five experiments.

† Results as mean nmol ^{125}I precipitated with standard error of three experiments.

‡ Percentage inhibition of the control value.

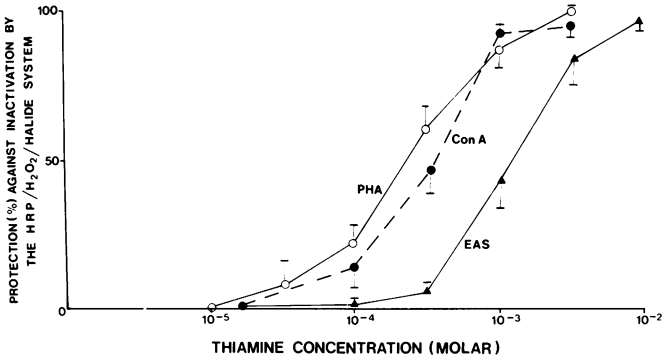

Fig. 2. Effects of thiamine on HRP/H_2O_2/iodide-mediated inhibition of neutrophil migration to EAS (▲——▲) and lymphocyte transformation to 25 μg/ml PHA (○——○) and 25 μg/ml Con A (●- - -●).

Lymphocyte functions

Slight but insignificant stimulation of lymphocyte mitogen-induced transformation was observed at 10^{-4} M thiamine. The value for 25 μg PHA was 50,250 ± 3,036 and 59,695 ± 2,692 c.p.m. for control systems and systems containing 10^{-4} M respectively.

Incubation of lymphocytes with the HRP/H_2O_2/iodide system caused considerable inhibition of the proliferative response to both mitogens at both concentrations. The mean percentage inhibition was 89 ± 6·2%, 93 ± 4·3%, 99 ± 1·1% and 91 ± 3·2% for 25 and 50 μg PHA, and 25 and 50 μg Con A respectively. However, inclusion of thiamine at concentrations which inhibit activity of the peroxidase/H_2O_2/halide system protected the lymphocyte proliferative response to both mitogens. Results for 25 μg PHA and 25 μg Con A are shown in Fig. 2.

Injection of thiamine was associated with increased neutrophil motility to both leucoattractants, decreased MPO-mediated iodination of *C. albicans* and increased lymphocyte transformation to PHA (Table 3). Phagocytosis of *C. albicans* and post-phagocytic HMS activity were unaffected.

DISCUSSION

This study has shown that thiamine causes inhibition of the peroxidase/H_2O_2/halide system which is related to enhanced neutrophil motility *in vitro* and *in vivo* and lymphocyte transformation *in vivo*. The inhibition of the MPO/H_2O_2/halide system was not due to inhibition of MPO *per se* as shown by lack of effects of thiamine on the halide-independent oxidation of guaiacol. It is possible that the

Table 2. Effects of F-Met-Met-Phe and EAS on activation of the MPO/H_2O_2/halide system

Leucoattractant	Extent of activation of the MPO/H_2O_2/halide system (nmol ^{125}I precipitated)
Control (no leucoattractant)	0·07 ± 0·01*
5×10^{-5} M F-Met-Met-Phe	0·23 ± 0·03
5×10^{-6} M F-Met-Met-Phe	0·16 ± 0·01
5×10^{-7} M F-Met-Met-Phe	0·09 ± 0·01
10% EAS	0·21 ± 0·04

* Results as mean and standard error of five separate experiments.

Table 3. The *in vivo* effects of thiamine on neutrophil motility and post-phagocytic hexose monophosphate shunt activity and MPO-mediated iodination of *C. albicans* and on lymphocyte transformation

	Neutrophil migration to:		Neutrophil post-phagocytic activity		Lymphocyte transformation to PHA	
Time of testing	F-Met-Met-Phe*	EAS*	HMS activity†	MPO-mediated iodination‡	25 μg/ml§	50 μg/ml§
Before injection of 50 mg thiamine	124 ± 24	174 ± 15	15·9 ± 2·6	1·0 ± 0·10	57,051 ± 3,400	77,684 ± 10,348
After 2 hr	226 ± 19	264 ± 24	16·7 ± 4·0	0·083 ± 0·09	81,895 ± 16,522	88,550 ± 13,987
After 24 hr	226 ± 16	248 ± 28	18·1 ± 2·9	0·086 ± 0·05	58,109 ± 6,268	54,671 ± 6,997

* Results as mean cells/microscope high power field with standard error of six separate experiments.
† Results as μmol glucose metabolized/2×10^6 neutrophils with standard error of six separate experiments.
‡ Results as nmol ^{125}I precipitated/1×10^6 neutrophils with standard error of six separate experiments.
§ Results as mean radioactive counts per minute with standard error of six separate experiments.

thiamine inhibits the peroxidase/H_2O_2/halide system by scavenging potentially toxic oxidative intermediates generated by this system.

Neutrophil motility to EAS and lymphocyte transformation were inhibited by the HRP/H_2O_2/-halide system *in vitro*. This inhibition was prevented by concentrations of thiamine which decreased peroxidase-mediated iodination. Thiamine-induced stimulation of migration and inhibition of the MPO/H_2O_2/halide system are probably related since interaction of leucoattractants with neutrophils causes generation of superoxide and H_2O_2 and degranulation with release of MPO (Becker *et al.*, 1974; Becker, Sigman & Oliver, 1979). The presence of MPO and H_2O_2 with a halide in the extracellular milieu could be expected to inhibit neutrophil migration. This inhibition could be decreased by thiamine. Impairment of neutrophil functions as a consequence of auto oxidation and stimulation by agents which inhibit superoxide production has been described previously by Boxer, Allen & Baehner (1978). However, these authors attributed the toxic effects to superoxide. We have been unable to demonstrate increased neutrophil responsiveness to chemoattractants in the presence of concentrations of superoxide dismutase up to 2,000 units. It is possible that agents which inhibit superoxide production increase neutrophil motility by restricting the availability of H_2O_2 for the MPO/H_2O_2/halide system. The stimulation of neutrophil motility *in vitro* by thiamine was observed only when EAS was used as the leucoattractant. Experiments performed to measure the effects of F-Met-Met-Phe and EAS on activation of the MPO/H_2O_2/halide system showed that F-Met-Met-Phe at 5×10^{-7} M caused only slight activation of this system which may explain the absence of thiamine-mediated stimulation of motility when 5×10^{-7} M F-Met-Met-Phe was used as the leucoattractant. EAS and higher concentrations of F-Met-Met-Phe caused activation of the MPO/H_2O_2/halide system. It is probable that thiamine would have caused stimulation of motility to F-Met-Met-Phe had these higher concentrations of the formyl peptide been used. It has previously been reported that although some leucoattractants cause stimulation of neutrophil oxidative metabolism, the leucotactic potential of a chemoattractant and its ability to stimulate oxidative events associated with microbial killings are dissociable activities (Anderson, Glover & Rabson, 1978; Bass, De Chatelet & McCall, 1978). Enhanced motility was observed *in vivo* following injection of 50 mg thiamine. The duration of stimulation was 24–48 hr. These findings indicate that the thiamine concentrations required to stimulate neutrophil motility *in vivo* are considerably less than those required to achieve the same effects *in vitro*.

Co-culturing of lymphocytes with neutrophils or neutrophil product(s) released by sonication of neutrophils inhibits mitogen-induced lymphocyte transformation (Hsu, Wu & Rivera-Arcilla, 1979). We have recently observed that mitogens cause stimulation of neutrophil HMS activity and release of MPO *in vitro* (unpublished findings). It is possible that MPO and H_2O_2 release by neutrophils inhibits lymphocyte transformation *in vivo* and *in vitro* which could be prevented by inhibitors of this system. The absence of significant effects of thiamine on lymphocyte transformation *in vitro* may be related to the use of pure lymphocyte preparations.

The most probable mechanism of thiamine-induced stimulation of neutrophil motility *in vitro* and *in vivo* and lymphocyte transformation *in vivo* is protection of these functions due to inhibition of the peroxidase/H_2O_2/halide system. Oxidizable groups on the thiamine molecule may compete with similar groups on neutrophil and lymphocyte membranes for toxic oxidative products of the peroxidase/H_2O_2/halide system. *In vivo* stimulation by thiamine of neutrophil motility and lymphocyte transformation to mitogens suggests that this agent may have immunotherapeutic potential.

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