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THE ROLE OF CYCLIC AMP AND CYCLIC GMP IN MITOGEN INDUCED CELL PROLIFERATION IN LEUKOCYTES

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INTRODUCTION

ONE of the intriguing questions in immunology today is the understanding of the mechanism by which lymphocytes are activated by mitogenic lectins and antigens (Parker, Snider and Wedner, 1974). Greaves and Bauminger (1972) have shown that lectins can stimulate DNA synthesis without itself entering the cell. This situation is similar to the Two Messenger Hypothesis of hormone action as proposed by Sutherland, Oye and Butcher (1965). It is thus logical to suspect that cyclic nucleotides might similarly be involved.

A report on the possible inhibitory effect of cyclic AMP on cell proliferation was first published by Burk in 1968. Recent evidences confirm a relationship does exist between cyclic AMP and cell proliferation but the precise character of that relationship is far from being clear. Rapidly growing cells have been found to have low cyclic AMP levels and slow growing cells have high cyclic AMP levels (otten, Johnson and Pastan, 1971). There is considerable evidence that indicates cyclic AMP to be involved in certain biochemical events that take the cell through its mitotic cycle.

MITOGEN INDUCED PROLIFERATION

Mature human lymphocytes cultured *in vitro* generally do not synthesize DNA or divide; however, addition of PHA (phytohaemagglutinin; a mitogen) results in a morphological transformation to a lymphoblastoid cell capable of DNA synthesis and eventually mitosis (Nowell, 1960; Robbins, 1964). Lymphocytes are cells whose normal function include quick multiplication upon exposure to antigens. Mitogens or plant lectins mimic the effect of antigen; however unlike antigens they stimulate a large population of predominantly thymus-dependent lymphocytes to divide. Smith, Steiner and Parker (1971) demonstrated that cyclic AMP or agents which stimulates its generation, when introduced before or concomitant with PHA prevents mitogen-induced proliferation. The concentration of cyclic GMP in lymphocytes following PHA-induced mitogenesis was observed to have increased over 10 folds (Hadden *et. al.*, 1972).

YIN—YANG HYPOTHESIS

Goldberg *et. al.* (1973) proposed the "dualism" theory of biological regulation through the opposing actions of cyclic AMP and cyclic GMP. He described the concept of biological regulation through opposing actions of the two cyclic nucleotides by an ancient oriental term, Yin-Yang, which symbolizes a dualism between two opposing natural forces.

MATERIAL AND METHOD

Mature guinea pigs (Central Animal House, Faculty of Medicine, University of Malaya) were sacrificed by injecting 2-3 mls. of Nambutol intravenously. The spleen and appendix were removed aseptically and the organs immersed in RPMI 1640; (Flow Lab., U.K.) with 10% fetal calf serum (Gibco, N.Y.) and 200 units per ml. each of penecillin and streptomycin. Single cell suspension of the organs were prepared and the cell concentration adjusted to $1x10^6$ cells/ml. using a hemocytometer. Cells of 1×10^6 per ml. were cultured in Falcon 16 x 125 mm plastic tubes containing 10% fetal calf serum, mitogen and the modulating agents at various concentrations wherever required, 100 U/ml. of penicillin and 100 ug/ml. of streptomycin, and RPMI 1640 with 1mM glutamine supplemented made up to a total of 2 mls. per culture. The cultures in triplicates were incubated at 37°C in a

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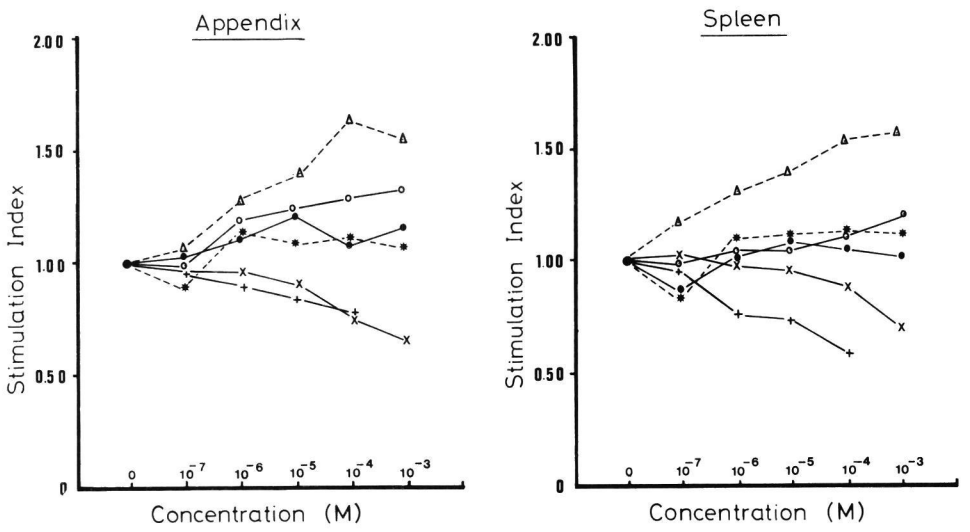

Fig.(1) Control (without any mitogen added)

—•—•— 3' AMP —x—x— c AMP --*----*-- 3' GMP

—o—o— 5' AMP —+—+— DBC --Δ----Δ-- c GMP

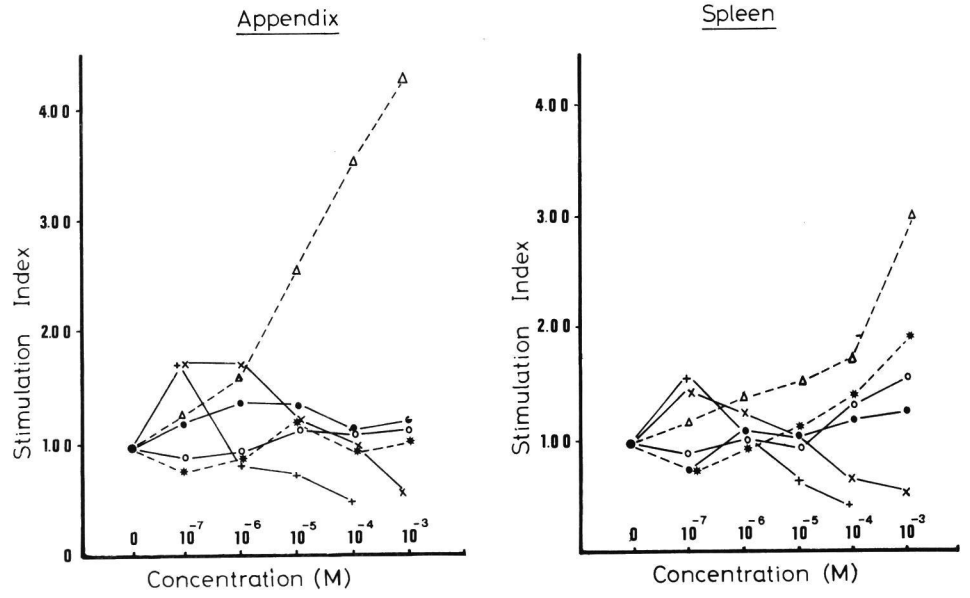

Fig.(2) 10 μg/ml. Phytohemagglutinin (PHA)

—•—•— 3' AMP —x—x— c AMP --*----*-- 3' GMP

—o—o— 5' AMP —+—+— DBC --Δ----Δ-- c GMP

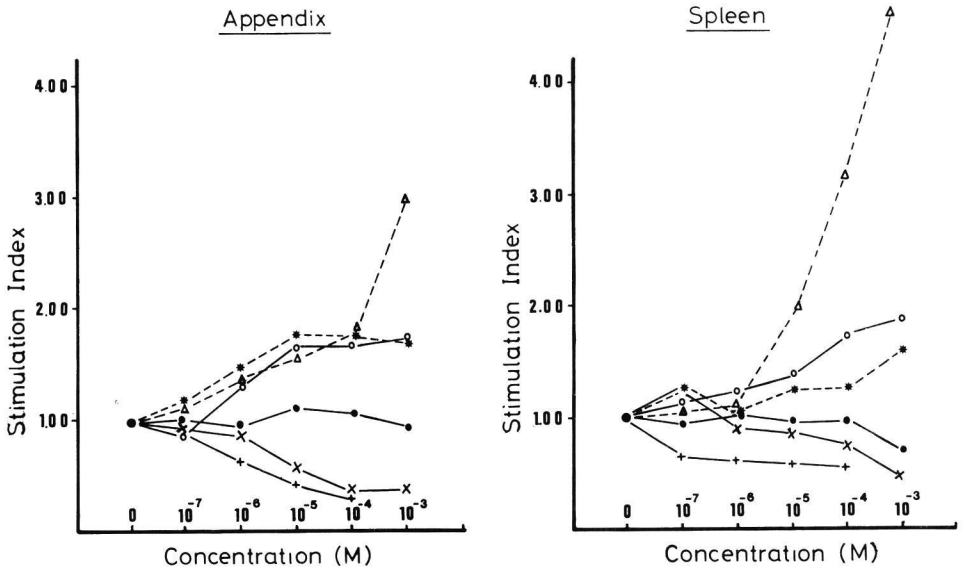

Fig.(3) 5 µg/ml Pokeweed Mitogen (PWM)

—•—•— 3' AMP —x—x— c AMP --*----*-- 3' GMP

—o—o— 5' AMP —+—+— DBC --Δ----Δ-- c GMP

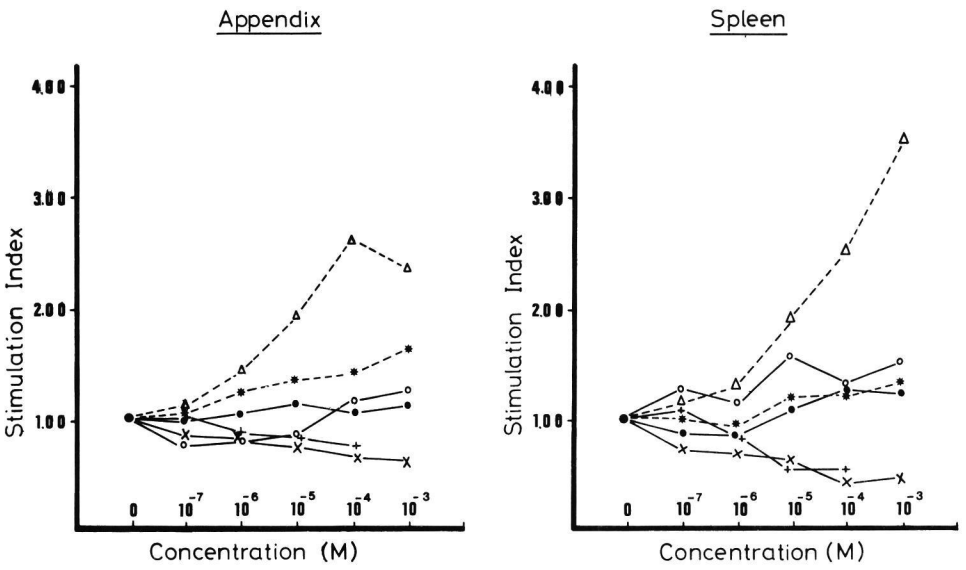

Fig.(4) 7.5 µg./ml. Concanavalin A (Con A)

—•—•— 3' AMP —x—x— c AMP --*----*-- 3' GMP

—o—o— 5 AMP —+—+— DBC --Δ----Δ-- c GMP

humidified atmosphere containing 5% CO_2 (Forma Scientific CO_2 Incubator).

Determination of DNA Synthesis

PHA, Con. A and LPS cultures were terminated on the third day of the experiment, whilst PWM cultures were terminated on the fifth day. 24 hrs. prior to termination to each culture was added 2 uCi of tritiated thymidine (Amersham, England). Harvesting of the cells is by filtration (Bransome and Grower, 1970). The radioactive counts was done by the Beckman LS-100 Liquid Scintillation System. Mean values of triplicate cultures were presented in the results.

Reagents

MITOGEN: Bacto Phytohemagglutinin M (Difco Lab., U.S.A.), Pokeweed mitogen (Gibco, U.S.A.), Concanavalin A and E. coli Lipopolysaccharide (Sigma Chem. Co., U.S.A.).

NUCLEOTIDES AND CYCLIC NUCLEOTIDES: All supplied by Sigma Chem. Co. Adenosine 3'-Monophosphate (A-1377), Adenosine 5'-Monophophate Type IV (A-2002), Guanosine 3'-Monophosphate Grade 1 (G-3628), Adenosine 3':5'-cyclic Monophosphate (A-9501), N^6, $O^{2'}$-Dibutyryl Adenosine 3':5'-cyclic Monophosphate Grade II (D-0627), and Guanosine 3':5'-cyclic Monophosphate (G-6129).

RESULTS

Cyclic AMP, Dibutyryl Cyclic AMP, Cyclic GMP, 3'-AMP, 3'-GMP were added respectively into cell cultures stimulated by the different mitogens. A set of controls was done without any mitogens added to determine whether the nucleotides concerned does affect leukocytes without any mitogenic stimulation. The effect of each nucleotide was observed over a range of concentrations, 10^{-7} to 10^{-3}M.

C.P.M.: Radioactive counts per minute

$$\text{S.I.} : \text{Stimulation Index} = \frac{\text{C.P.M. of Experimental}}{\text{C.P.M. of Control}}$$

DISCUSSION

Lymphocyte populations obtained from various lymphoid tissues differ markedly in their capacity to respond by increased DNA synthesis to T and B cell mitogens (Jacobson and Blomgren, 1974). PHA and Con. A seems to stimulate T-cells almost exclusively, PWM stimulates both B and T cells (Greaves and Roitt, 1968) and lipopolysaccharide triggers non T-cells, predominantly B-cells (Andersson, Moller and Sjoberg, 1972).

A dose-response study was previously done on each of the mitogens used. The optimun concentrations were respectively, PHA at 10 ug/ml., PWM at 5 ug/ml., Con. A at 7.5 ug/ml. and LPS at 10 ug/ml.

The range of effectiveness of each of the nucleotides were obtained. In general there was a notable stimulation by cyclic GMP and inhibition by cyclic AMP at 10^{-4} to 10^{-3}M. 3'-AMP and 3'-GMP showed slight or no stimulation at all, and 5'-AMP showed slight inhibition. A fall in cyclic AMP level is apparently important for the initiation of growth because growth stimulation is prevented if cyclic AMP is maintained at high levels following the addition of growth-promoting agents (Pastan, Johnson and Anderson, 1975). Cyclic AMP and 5'-AMP inhibits the growth of cells apparently by a depletion of pyrimidine precursors for DNA and RNA synthesis (Hilz and Kaukel, 1973). Siefert and Rudland (1974) suggest that cyclic GMP acts as a positive signal in the control of cell functions while cyclic AMP represents a negative signal.

SUMMARY

Goldberg's Ying-Yang Hypothesis of Biological Control through cyclic GMP and cyclic AMP was demonstrated in guinea pig leukocyte proliferation. The results distinctly showed enhancement by cyclic GMP and inhibition by cyclic AMP for all the four mitogens and two cell types used. The pattern of response was similar regardless of the type of mitogens used nor the type of cells. This strongly suggest that the effect of cyclic GMP and cyclic AMP were nonspecific in nature.

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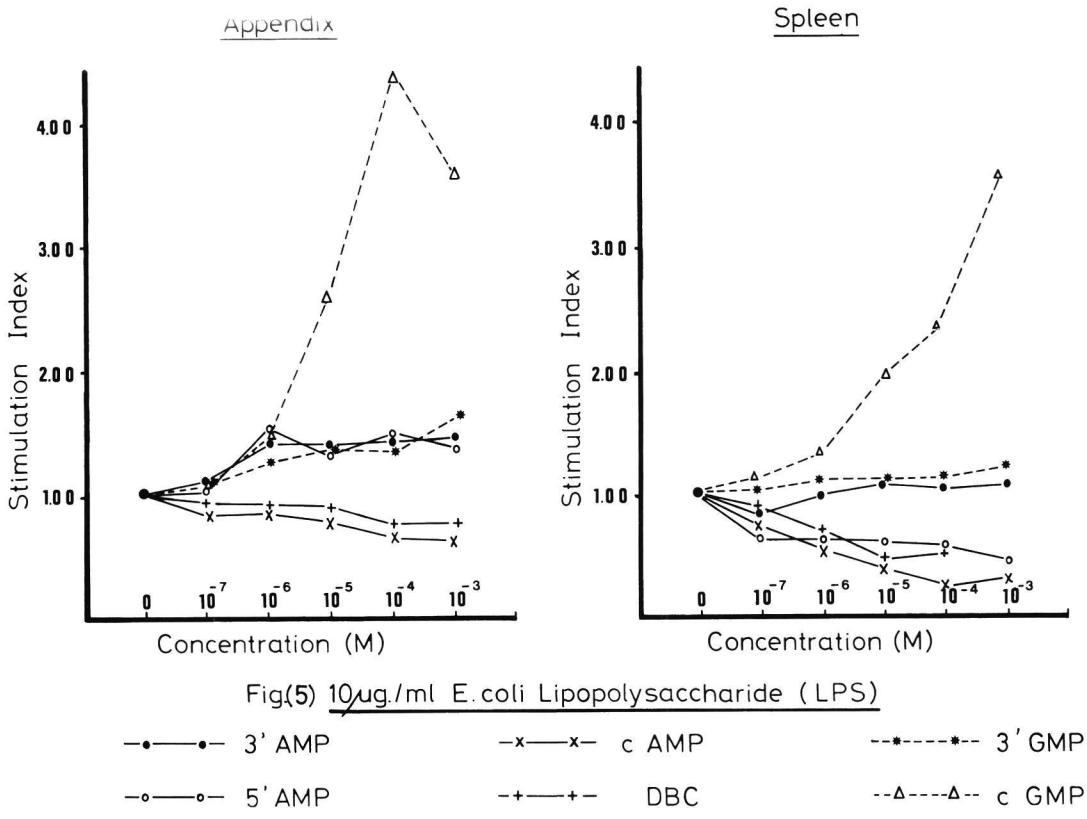

Fig(5) 10µg./ml E.coli Lipopolysaccharide (LPS)

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