

KBr. The purity of the compounds was checked on T.L.C.

Isonitrosoaceto-*p*-carbethoxyaniline: In a 100 ml conical flask were placed 1.8 g of chloral hydrate and 24 ml of water. To this solution, there was then added 2.6 g of crystalline sodium sulfate, a solution of 1.65 g of ethyl-4-aminobenzoate in 6 ml of water to which 1 ml of concentrated hydrochloric acid had been added to dissolve the amine and finally a solution of 2.2 g of hydroxylamine-hydrochloride in 10 ml of water. After 5 min. of vigorous boiling, the flask was cooled and the crystals of isonitrosoaceto-*p*-carbethoxyaniline were filtered and air dried and recrystallised from ethanol, yield 85%; m.p. 185°.

Other substituted isonitrosoacetanilides (I) prepared by this method are listed in Table I.

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AMPLIFICATION OF IMMUNE RESPONSE BY CYCLIC AMP

INTERACTION of an antigen with specific "receptors" on lymphocyte surface is the first membrane event in the development of immune response¹. Cyclic 3', 5'-adenosine monophosphate (cyclic AMP), the product of a membrane bound enzyme adenylate cyclase, has been widely implicated in a number of biological functions of cells² including the immune reactions^{3,4}. The purpose of the present study is to explore the effect of cyclic AMP on the immunological expression in mice challenged with sheep red blood cells. Preliminary results are recorded in this communication.

Method

8.7×10^8 sheep red blood cells were injected intraperitoneally into AIIMS strain of mice (25 g body weight). To some of them, 20 μ g of cyclic AMP in saline was injected 15 min. later. After 48 h animals were anaesthetized, heart blood and spleen collected. The number of plaque forming cells was assayed by JERNE's technique⁵.

Results and Discussion

Data presented in Fig. 1 show that intraperitoneal injection of 20 μ g of cyclic AMP into mice just after a challenge with sheep red blood cells results in doubling of the number of plaque forming cells in spleen and a higher haemagglutination titre in serum. In these experiments, immunological measurements were made 48 h after the antigen challenge. A time course experiment revealed that the immuno-enhancing effect of cyclic AMP was noticeable on subsequent days also, without drastically altering the pattern of immune response (Fig. 2). Cyclic AMP thus appears to have amplified the antigen effect over a stretch of time.

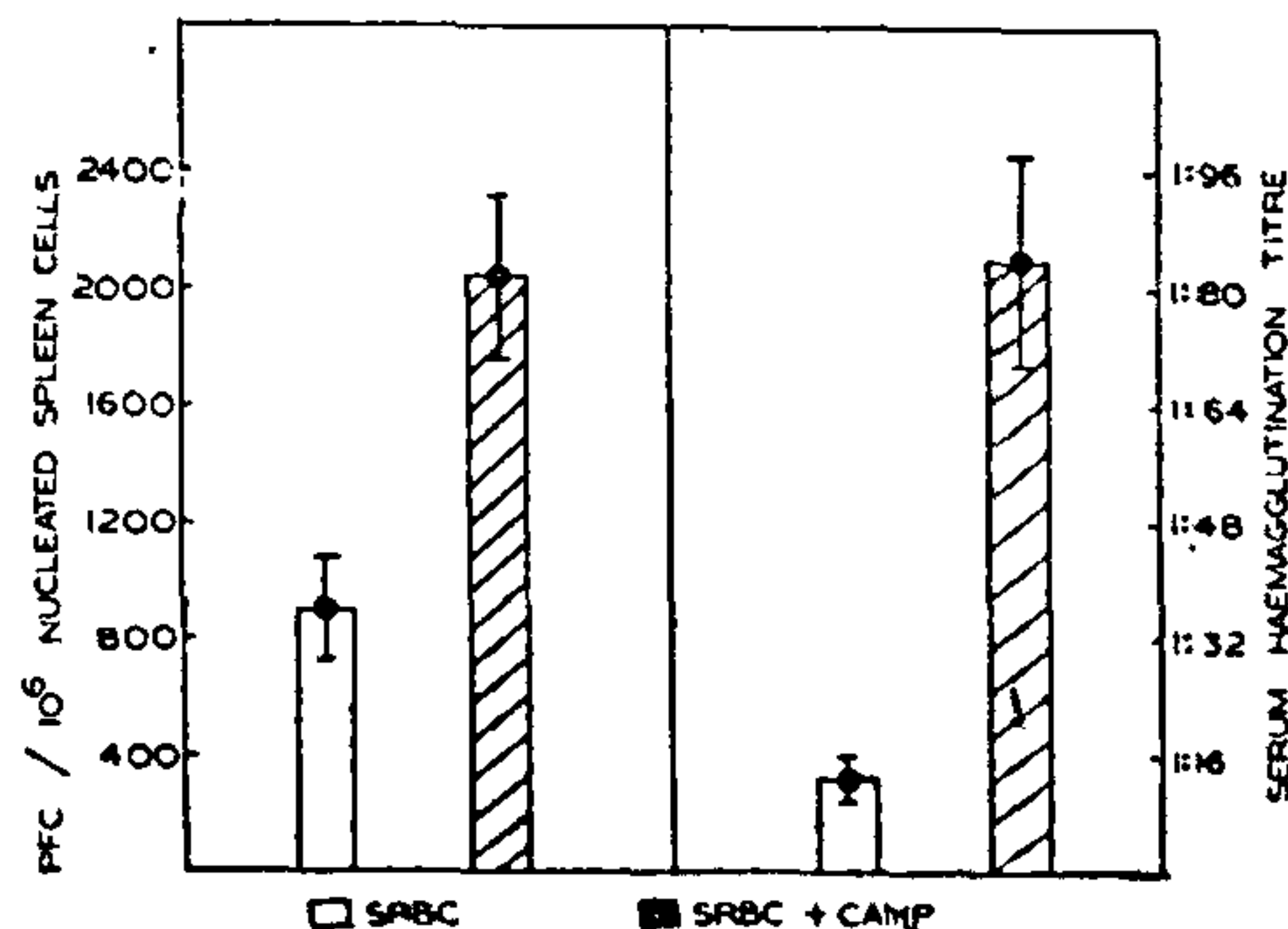


FIG. 1. Effect of cyclic AMP on immune response of mice against sheep RBCs *in vivo*. For details of antigen challenge see "Method". Values represent mean \pm S.E. obtained from 4-6 mice in each group.

Immune response is a complex phenomenon involving interaction of more than one type of cell and it is difficult to define the type of cell influenced by cyclic AMP *in vivo*. The adjuvant-like action of cyclic AMP observed here agrees with the reports showing that agents which had immuno-enhancing effect (e.g., poly A : U, epinephrine, isoproterenol, norepinephrine and aminophylline) could have done so by increasing the cyclic AMP levels in target lymphoid cells^{6,7}. Cyclic AMP treatment had perhaps increased the sensitivity by altering the affinity or had lowered the threshold for reactivity between the antigen and the antigen sensitive cells of spleen. Although

cyclic AMP increased the percentage of mitotic cells in rat bone marrow *in vitro*, a non-specific amplification of the antigenic message is perhaps a better explanation. Cyclic AMP is known to mediate differentiated functions of cells⁹. In fact, isoproterenol, epinephrine and aminophylline were reported to stimulate the overall synthesis and secretion of immunoglobulins in human peripheral lymphocytes¹⁰. It is possible that cyclic AMP had amplified the secretory rate and/or the formation of anti-sheep red blood cell antibody. Since the function measured here represents protein (antibody) synthesis, the possibility exists that cyclic AMP could have affected the general steps such as uptake of precursor aminoacids, (messenger?) RNA synthesis, faster aminoacid assembly or secretion rate of proteins, etc. Moderate stimulation of RNA synthesis¹¹ and DNA synthesis¹² have been recorded before. Preliminary experiments showed that cyclic AMP can stimulate the incorporation of ³H-lysine in human peripheral lymphocytes. Further experiments are required to understand the biochemical basis of modulating effect of cyclic AMP on the immune system.

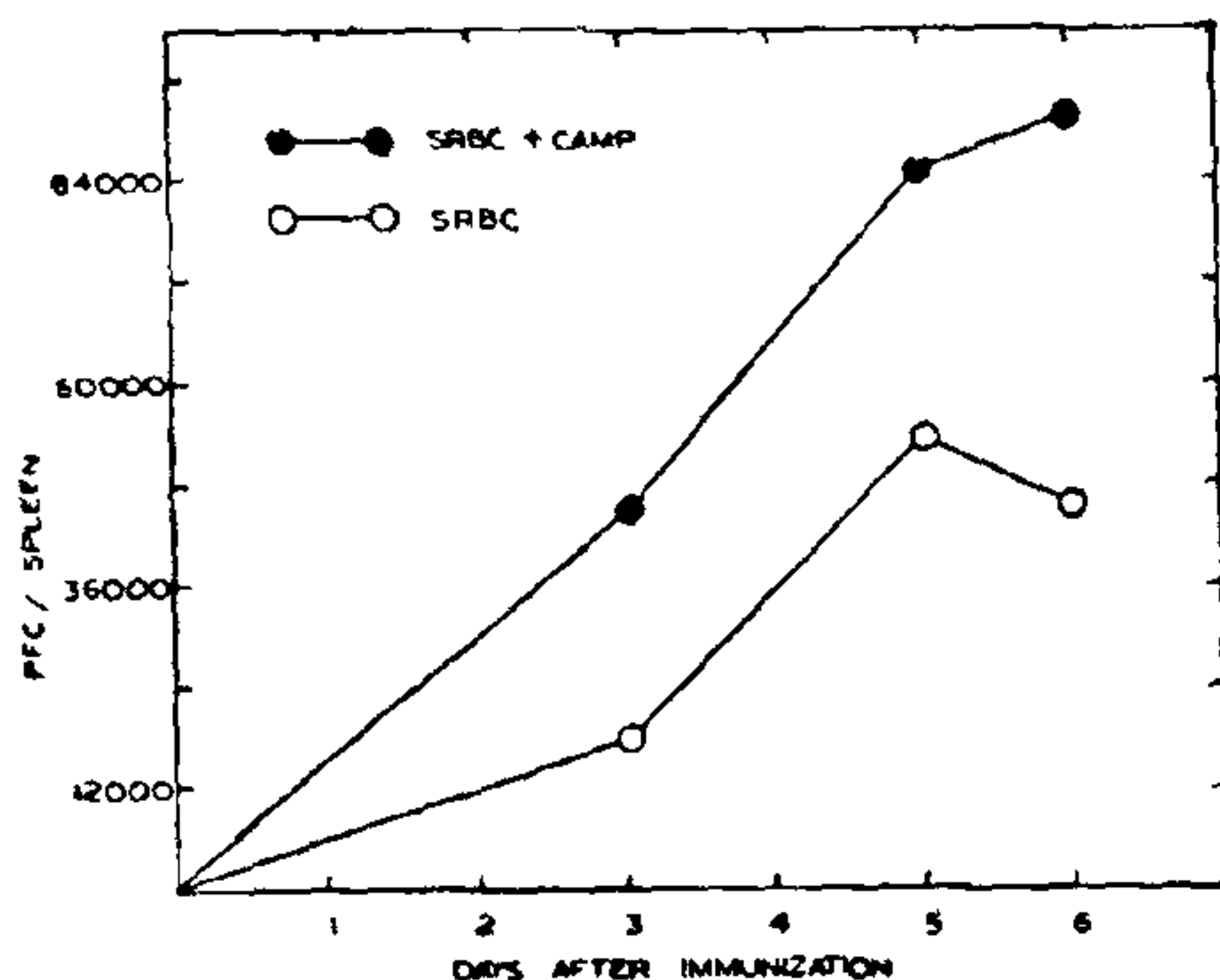


FIG. 2. Kinetics of immune response in control and cyclic AMP treated mice. Conditions were the same as described for Fig. 1 except that 2-4 mice in each group were sacrificed on indicated days.

Abbreviation used is cyclic AMP: cyclic 3', 5'-adenosine monophosphate.

In conclusion it can be stated that injection of cyclic AMP into mice challenged with sheep RBCs augmented the number of plaque forming cells in spleen and haemagglutination titre of serum. Such an amplification of immune response by cyclic AMP is in accordance with the mediatory role of the nucleotide on the differentiated functions of cells in general.

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EFFECT OF COLOURED LIGHT ON EARTHWORMS

THOUGH no definite visual organs (eyes) have been observed on the earthworms, they are known to be sensitive and react immediately to light. It has been reported, for example, that a sudden illumination at night will cause them to quickly rush back to their burrows. This is explained on the basis of the presence, particularly on the prostomium, of lens-like structures which respond to light stimuli. Hegner and Stiles¹ have stated that a "positive reaction to faint light has been demonstrated for the manure worm *Eisenia foetida*; this positive reaction to faint light may account for the emergence of the worms from their burrows at night. It is an interesting fact that although the worms react negatively to sunlight, they respond positively to red light and may be collected at night with the use of such a light". Howell² observed *Pheretima* sp. as completely photonegative and respond in proportion to the intensity of light.