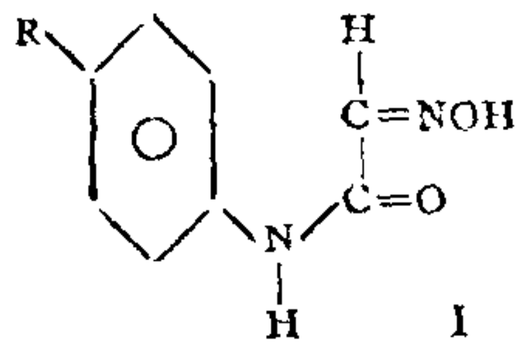
LETTERS TO THE EDITOR

SYNTHESIS OF ISONITROSOACETANILIDES AS ANTIMICROBIAL AGENTS

RECENTLY Maksudov reported isonitrosoaceto-*p*-chloroaniline as a highly effective fungicide[1]. The literature abounds with various types of biological responses including antimicrobial activity associated with different isatin derivatives. In view of these observations and the fact that isonitrosoacetanilide is the precursor of isatin, it was expected that isonitrosoacetanilides may also exhibit antimicrobial activity and thus it was considered of interest to synthesise a number of substituted isonitrosoacetanilides incorporating electron withdrawing halogeno or sulfonamido or carboxyalkyl groups which often enhance the biological activity. As such a number of substituted isonitrosoacetanilides (I) have been synthesised and screened for their antibacterial activity. The synthesis of (I) was achieved by reacting 4-aminobenzoic acid and its methyl, ethyl and propyl esters, a number of substituted anilines and sulfonamide derivatives with chloral hydrate, hydroxylamine hydrochlotide and sodium sulfate (anhydrous).

Isonitrosoacetanilides from 4-aminobenzoic acid and its methyl, ethyl and *n*-propyl esters, sulfanilamide and sulfadiazine were prepared in good yields. Corresponding isonitrosoacetanilides from *n*-butyl-4-aminobenzoate, sulfathiazole, elkosin, sulfadimidine and sulfaphenazole could not be obtained and the unreacted substances were recovered in place of (I). Sulfanilamide gave a better yield in comparison to sulfadiazine.

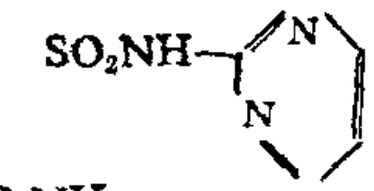


I

Biological data: All compounds listed in Table I have been screened against two organisms, *Bacillus* subtilis and *Staphylococcus aureus* for their inhibitory effects by agar diffusion technique[2]. Six compounds (2, 3, 5, 6, 8 & 9) inhibited the growth of *Bacillus subtillis* and five compounds (1, 4, 7, 10 & 11) were effective against both the organisms. Bacterial cultures maintained at Public Analyst Laboratory to U.P. Government, Lucknow, were used.

TABLE I

Substituted isonitrosoacetanilides (I)

Sl. No.	R	Yield %	Molecular formula	M.P. °C	% N-analyses Calc.	Found
1.	$-CO_2H$	85	$C_9H_8N_2O_4$	>255	13·45	13·12
2.	$-CO_2Me$	90	$C_{10}H_{10}N_2O_4$	227	12·60	12·66
3.	$-CO_2Et$	85	$C_{11}H_{12}N_2O_4$	185	11·85	11·76
4.	$-CO_2Pr$	60	$C_{12}H_{14}N_2O_4$	150	11·19	11·48
5.	SO_2NH– 	85	$C_{12}H_{11}N_5O_4S$	245	21·79	21·53
6.	SO_2NH_2	80	$C_8H_9N_3O_4S$	208	17·27	17·35
7.	Me	90	$C_9H_{10}N_2O_2$	161[3]	..	..
8.	OMe	85	$C_9H_{10}N_2O_3$	184[4]	..	..
9.	Cl	95	$C_8H_7N_2O_2Cl$	172[5]	..	..
10.	Br	90	$C_8H_7N_2O_2Br$	172[5]	..	..
11.	I	90	$C_8H_7N_2O_2I$	178–79[6]	..	..

I.R. data (μ)
Compound No. —6. C = O, 5·95; NH_2, 2·94, 3·01, —NH or OH, 3·11, SO_2, 8·40.

Experimental: Melting point were taken in open capillaries and are not corrected. Infrared spectrum was recorded on a Perkin-Elmer Spectrophotometer in

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

Isonitrosodceto-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 response[1]. 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 cells[2] 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

$8{\cdot}7 \times 10^8$ sheep red blood cells were injected intraperitoneally into AIIMS strain of mice (25 g body weight). To some of them, 20 μg 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 technique[5].

Results and Discussion

Data presented in Fig. 1 show that intraperitoneal injection of 20 μg 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.

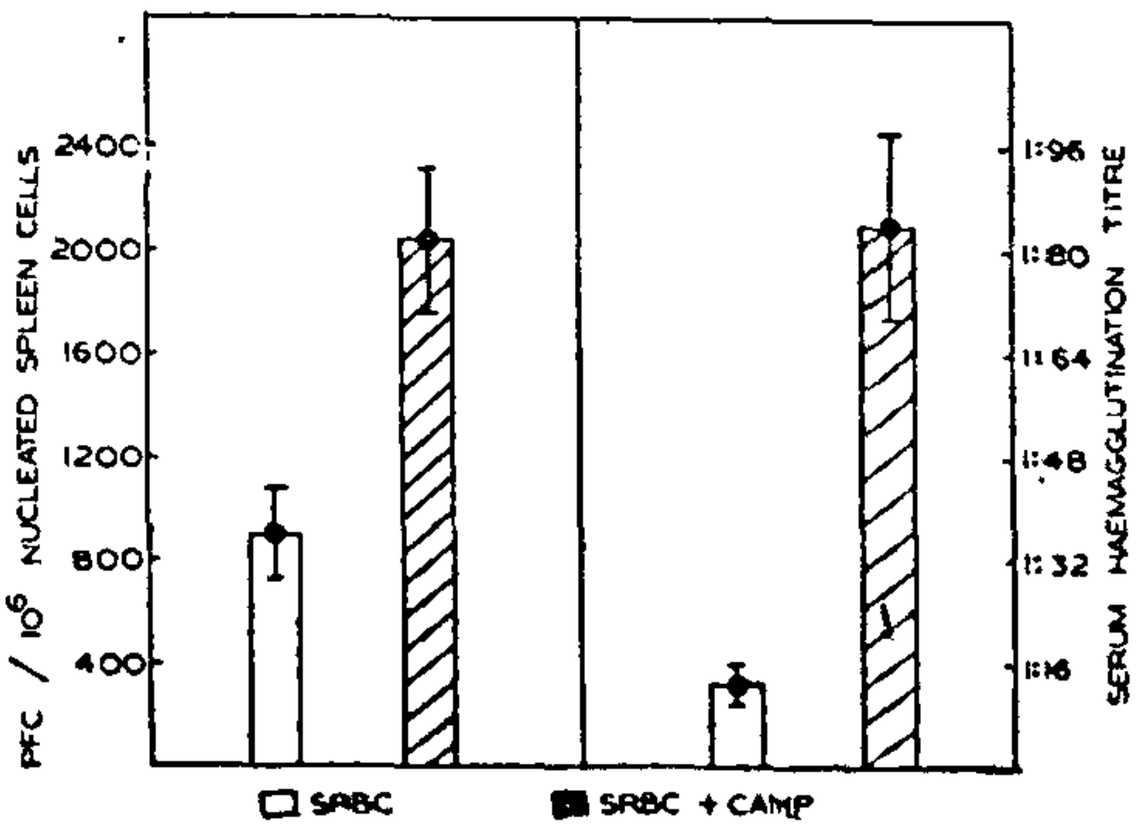

FIG. 1. Effect of cyclic AMP on immune response of mice against sheep RBCs *in vivo*. For details of antigen challenge see "Method". Values represent mean ± S.E. obtained from 4–6 mice in each group.

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