Biochemical Pharmacology, Vol. 26, pp. 723–728. Pergamon Press, 1977. Printed in Great Britain.

SELECTIVE EFFECTS OF METHYLGLYOXAL-BIS-(GUANYLHYDRAZONE) ON THE DEVELOPMENT OF ANTIBODY-FORMING CELLS IN MICE*

JAMES BENNETT,† JANE EHRKE, CHANDRAKANT DAVE and ENRICO MIHICH

Department of Experimental Therapeutics, Grace Cancer Drug Center, Roswell Park Memorial Institute, New York State Department of Health, Buffalo, NY 14263, U.S.A.

(*Received* 29 *June* 1976; *accepted* 27 *September* 1976)

Abstract—The effects of methylglyoxal-bis(guanylhydrazone) on selected cellular immune functions and on *S*-adenosylmethionine decarboxylase activity were studied in spleen cells from mice given sublethal doses of this drug. The development of antibody-forming cells was markedly inhibited after administration of methylglyoxal-bis(guanylhydrazone) whereas the functions of cytotoxic effector cells and phagocytes were not affected. The activity of *S*-adenosylmethionine decarboxylase was found to be greatly increased in spleen cells from mice given the drug, and this increase was 4- to 5-fold greater in nylon wool-adherent cell populations as compared to that in non-adherent cell populations. The uptake of methylglyoxal-bis(guanylhydrazone) was 1.5- to 2-fold greater in nylon wool-adherent cells than in non-adherent cells. These studies suggest that methylglyoxal-bis(guanylhydrazone) selectively affects antibody-forming cells among mouse spleen cell populations.

Methylglyoxal-bis(guanylhydrazone) (CH_3-G) is active against acute myelocytic leukemia and certain solid tumors in humans [1, 2]. Its use, however, has been limited by host toxicity, most of which stems from the antiproliferative actions of the compound. One of the major expressions of the toxicity of CH_3-G is immunosuppression as reflected in the bone marrow depression and bacterial infections seen in humans [3] and in the inhibition of hemagglutinin production and prolongation of allograft survival seen in mice [4].

Although the exact mechanism of cytotoxicity of CH_3-G is still not understood, several lines of evidence suggest that it may be related to the ability of the drug to interfere with polyamine biosynthesis. Increase in intracellular polyamine levels has been correlated with increase in cell proliferation [5]. In proliferating systems such as rapidly growing tumors, the administration of CH_3-G resulted in decreased spermidine levels [6]. This was presumably due to the specific inhibition by the drug of putrescine-activated *S*-adenosylmethionine decarboxylase (SAMDC) [7], a key enzyme in the biosynthesis of spermidine. Similarly, the conversion of labeled putrescine into spermidine was markedly inhibited in rat liver and kidney after CH_3-G administration [8]. However, considerable increase in SAMDC activity was found in dialyzed extracts of tissues obtained from animals treated with CH_3-G [9]. This paradoxical enhancement of enzyme activity was presumably due to enzyme stabilization by CH_3-G *in vivo* leading to an increase in the apparent half-life of the enzyme [9]. Since spermidine synthesis is inhibited in animals treated with CH_3-G, it is unlikely that this accumulated SAMDC is functional *in vivo*.

In light of the immunosuppressive effects of the drug mentioned above, the purpose of this study was to investigate the selectivity of action of CH_3-G during the response of mice to antigenic stimulation. After immunization with sheep red blood cells (SRBC), mice were treated with CH_3-G, and the function of various cell populations within mouse spleen was studied. The CH_3-G related increase in SAMDC which was described above was followed in these cell populations as a possible marker for drug selectivity. These effects *in vivo* of CH_3-G were correlated with drug uptake *in vitro*. A preliminary report of this work has been published [10].

MATERIALS AND METHODS

Materials. All chemicals were reagent grade. Radioactive sodium chromate (^{51}Cr) was purchased from Amersham/Searle, and labeled *S*-adenosyl-L-methionine-[^{14}COOH] (SAM [^{14}COOH]) (10 mCi/m-mole) was purchased from New England Nuclear. SRBC, guinea pig complement, and fetal calf serum were purchased from Grand Island Biological Co. Methylglyoxal-bis(guanylhydrazone) labeled with ^{14}C in the glyoxal portion of the molecule (^{14}C-CH_3-G) (50 μCi/m-mole) was synthesized and kindly supplied by Dr. S. F. Zakrzewski of this department.

Treatment of animals. Three- to four-month-old C57Bl/6Ja female mice were used. Mice were immunized with 5×10^8 SRBC given i.p. on day 0. Three hr after administration of antigen, daily i.p. treatment with CH_3-G (85 mg/kg) was begun and continued for 5 days skipping day 4 after antigen. Mice were sacrificed by cervical dislocation on day 7 after antigen, and their spleens were used as a

* This study was supported in part by USPHS Project Grants CA 15142 and CA 15143 and Core Program Grant CA 13038 from the National Cancer Institute.

† The research described in this report was done in partial fulfillment of the requirements for the Ph.D. degree in Pharmacology at the Roswell Park Division of the SUNY at Buffalo Graduate School. Present address: Yale University School of Medicine, Section of Medical Oncology, 333 Cedar St., New Haven, CT 06510.

source of immunocompetent cells. This schedule was chosen because administration of a single dose of drug on day 0, 1 or 2 after antigen did not result in suppression of the immune response on the optimal day of assay, which was day 7. Furthermore, administration of drug was stopped 2 days before assay to eliminate residual drug, which could interfere with the determination *in vitro* of SAMDC activity.

Spleen cell suspensions. Upon removal, spleens were immediately immersed into ice-cold RPMI 1640 medium supplemented with 5% heat-inactivated fetal calf serum and 100 units/ml of penicillin G and 100 μg/ml of streptomycin. Four spleen from each group were pooled and suspensions of single cells were obtained [11], washed twice in saline and once in media, counted microscopically, and then used in the various assays. In all the cases involving different groups of mice, the spleen cell suspensions were found to be more than 90 per cent viable by trypan blue dye exclusion technique [12], and the total recovery of cells was similar in all the groups.

Target cells. SRBC were washed with saline, and 5×10^7 cells in 0.1 ml saline were incubated with 50 μCi ^{51}Cr for 3 hr at 37° in an atmosphere of 10% CO_2 in air. The cells were then washed, resuspended in RPMI 1640 medium, and adjusted to 1×10^6 cells/ml. Target SRBC for complement-dependent and complement-independent cytotoxicity assays (see below) were obtained by incubating aliquots of this SRBC suspension for 1 hr at 37° followed by centrifugation to remove any unbound or loosely bound ^{51}Cr. Antibody-coated target SRBC for antibody-dependent cytotoxicity and phagocytosis assays were obtained by incubating aliquots of ^{51}Cr-labeled SRBC suspension in the presence of day 11 C3H anti-SRBC antisera (dilution 1:100) for 1 hr at 37°, again followed by thorough washing to remove unbound or loosely bound ^{51}Cr or antibody.

Cytotoxicity assays. Five separate assays were used to determine the immunological functions studied. In each case the release of ^{51}Cr from target SRBC was used as the index of cellular lysis [13].

(a) Complement-dependent cellular cytotoxicity (CDCC) was determined by incubating in a volume of 0.2 ml 1×10^6 spleen cells with 1×10^5 ^{51}Cr-labeled target cells in 12 × 75 mm plastic tubes (Falcon) at 37° in an atmosphere of 10% CO_2 in air. After 45 min of this incubation, 0.4 ml of a 1 to 40 dilution of guinea pig complement was added and the incubation was continued for another period of 45 min. In this test, antibody to SRBC is secreted by sensitized spleen cells, binds to target SRBC, and marks them for complement-mediated cytotoxicity [14].

(b) Complement-independent cellular cytotoxicity (CICC) was determined by incubating as above 5×10^6 spleen cells with 1×10^5 target cells for 20 hr in the absence of complement. This test involves the secretion of antibody from sensitized spleen cells, the binding of antibody to target SRBC, and the lytic action of a killer spleen cell on the antibody-coated target cells [14].

(c) Antibody-dependent cellular cytotoxicity (ADCC) was determined by incubating for 20 hr 5×10^6 spleen cells as in (b) above with 1×10^5 antibody-coated targets (see Target cells). This test, somewhat similar to the CICC above, has been originally used for determining the lytic action of 'killer' cells normally present in the spleen of non-immunized mice [15]. The difference between CICC and this test is that an excess of exogenously prepared target antiserum, rather than endogenously secreted antibody, is added for the immune reaction to occur. In the conditions of experiments, immunization with SRBC did not result in any significant enhancement of this reaction. The CDCC, CICC and ADCC reactions were stopped by diluting the incubation mixtures with 2 ml of ice-cold media.

(d and e) Phagocytosis was measured based on the protection from osmotic lysis of macrophage-engulfed SRBC using the method described by Hersey [16]. Briefly, 5×10^6 spleen cells were incubated for 20 hr with 1×10^5 target SRBC in a final volume of 0.2 ml. For both conditions 1 ml of distilled H_2O was then added to the tubes for 15 sec followed by the addition of 1 ml of twice concentrated RPMI 1640 medium. Phagocytosis was measured as the difference after hypotonic shock between ^{51}Cr released from SRBC in the presence of sensitized spleen cells or normal spleen cells plus mouse antiserum to SRBC and the ^{51}Cr released in the presence of normal spleen cells or normal spleen cells plus normal mouse serum.

For determination of release of ^{51}Cr in each test, cell suspensions were centrifuged at 500 g for 5 min and supernatants were poured off into 12 × 75 mm disposable glass tubes. Radioactivity in both pellet and supernatant was obtained as counts per minute in a Packard Auto-Gamma spectrometer. The per cent of ^{51}Cr released from the target cells was calculated in the following way:

$$\%\ ^{51}Cr \text{ release} = \frac{\text{supernatant cpm}}{\text{pellet cpm} + \text{supernatant cpm}} \times 100\%$$

The percentage of specific release was the difference between the values obtained with effector cells from immunized animals and those obtained with effector cells from non-immunized animals.

Cell separation techniques. Subpopulations of spleen cells were obtained by two different methods, namely by passage of cell suspensions through nylon wool columns or by layering of spleen cells on SRBC monolayers coated with anti-SRBC antibody. In the case of nylon wool columns [17], 0.6 g of nylon wool was packed in a 12 cm^3 syringe. Columns were saturated with Dulbecco's phosphate-buffered saline (PBS) containing 5% heat-inactivated fetal calf serum, sealed to prevent drying and allowed to equilibrate at 37° for 45 min. Approximately 4×10^8 spleen cells in 3 ml PBS were layered on the column and allowed to incubate as above for 45 min. At the end of incubation the non-adherent fraction was eluted by dropwise addition of PBS to the column until the eluate measured 25 ml. The remaining adherent cells were removed from the nylon wool by agitation in RPMI 1640 medium. Using the fluorescence-labeled anti-mouse IgG antibody technique, 70 per cent of the adherent cells and 15 per cent of non-adherent cells were found labeled (Dr. C. Porter, personal communication). This is also consistent with the results reported earlier from this laboratory [11]. In the

other fractionation procedure [18], SRBC monolayers were formed on plastic tissue culture dishes through a poly-L-lysine bridge and then coated with mouse hyperimmune anti-SRBC antibody. Twenty million spleen cells in 2 ml media were placed on the monolayer and allowed to incubate for 10 min at 37°. The cell suspensions were then centrifuged at 100 *g* for 5 min at 24°. After centrifugation, supernatants containing non-adherent cells were collected. For elution of adherent cells the SRBC monolayer was lysed by hypotonic shock for 60 sec and cells were collected in RPMI 1640 medium. In both fractionation procedures, fractions were washed thoroughly and adjusted to the appropriate cell density.

Determination of SAMDC. Approximately 1×10^8 spleen cells in 0.5 ml of ice-cold 25 mM Tris buffer, pH 7.5, containing 0.1 mM EDTA and 5 mM dithiothreitol were disrupted in a Potter–Elvehjem homogenizer with a motor-driven Teflon pestle. Homogenates were centrifuged at 100,000 *g* for 60 min in a Beckman L 2-65B ultracentrifuge and supernatants were used as a source of enzyme. SAMDC activity was determined by measuring the release of $^{14}CO_2$ from SAM [^{14}COOH] as previously described [19]. The standard incubation mixture contained in a final volume of 0.2 ml: 0.5 μmole putrescine; 1 μmole dithiothreitol; 20 μmoles of sodium potassium phosphate buffer, pH 7.2; 0.04 μmole SAM[^{14}COOH] (~30,000 dis./min); and 50 μl of the cell extract. Incubations were carried out for 60 min at 37° in glass tubes (16 × 100 mm) fitted with a rubber stopper equipped with a disposable polypropylene center well (Kontes). The released $^{14}CO_2$ was trapped into 1.0 ml phenethylamine placed in the center well. The reaction was stopped by injecting 0.5 ml of 20% H_2SO_4 through the rubber stopper into the reaction mixture, and after all $^{14}CO_2$ had been absorbed, the center well and its contents were plunged into a counting vial containing 3 ml of counting fluid (toluene containing 0.6% 2,5-diphenyloxazole, 0.06% dimethyl 1,4-bis-[2-(4-methyl-5-phenyloxazole)]benzene, and 15% Beckman Biosolv-3) and counted in a Packard scintillation counter. The results were corrected for cell extract which had been heated at 100° for 10 min and thereafter assayed as above. The reaction was linear with time and enzyme concentration, and the activity was expressed as nmoles $^{14}CO_2$/mg of protein/60 min. Protein concentration was determined by the method of Lowry *et al.* [20].

Uptake and efflux determinations. Spleen cell suspensions (1×10^7 cells/ml) in RPMI 1640 medium containing 10% dialyzed fetal calf serum were incubated with 10 μM ^{14}C-CH_3-G for 15 min at 37°. At the end of the incubation period, 5-ml aliquots of cell suspension were pipetted into tared centrifuge tubes and centrifuged at 4° for 5 min at 500 *g*. Uptake of ^{14}C-CH_3-G/mg of wet cells was determined as the ^{14}C radioactivity remaining after corrections for extracellular space. The extracellular ^{14}C counts were estimated on the basis of data obtained with [^{3}H]inulin [21]. For efflux determinations, cells were preincubated with 100 μM ^{14}C-CH_3-G for 15 min at 37° as described for uptake. Cells were then centrifuged, resuspended in drug-free medium, and incubated at 37° for various periods of time, and the amount of ^{14}C-CH_3-G remaining was determined as described above for intake.

All assays in this investigation (immune, enzyme and uptake) were carried out in duplicate and each experiment was repeated at least three times; similar results were obtained each time. Unless otherwise stated, results from representative experiments are shown.

RESULTS

Immunosuppressive effects of CH_3-G. As shown in Table 1, CH_3-G caused marked inhibition of the CDCC, CICC and phagocytosis with endogenous antibody. The CDCC response with spleen cells from mice treated with CH_3-G was reduced to $\frac{1}{3}$ of that found with cells from mice which had not received drug. The CICC response was reduced to background levels with spleen cells from mice treated with CH_3-G.

Table 1. Effects of CH_3-G on immune functions of mouse spleen cells*

Treatment		% ^{51}Cr release				
					Phagocytosis	
CH_3-G	SRBC	CDCC	CICC	ADCC	Endog. Ab	Exog. Ab
−	−	7	7	44	90	50
+	−	4	5	53	94	58
−	+	75	44		47	49
+	+	22	7		83	54

* C57BL/6 Jacobs mice were immunized (+) i.p. with a single dose of 5×10^8 SRBC on day 0, and their spleen cells were tested on day 7. Some groups of mice, as indicated in the table (+), were also injected i.p. with CH_3-G (85 mg/kg), on day 0, 3 hr after antigen administration, and again on days 1, 2, 3 and 5. Spleen cells were incubated with ^{51}Cr-labeled SRBC at an effector cell to target cell ratio of 10:1 for 1.5 hr in the CDCC test (with added guinea pig complement for the last 45 min) and at a ratio of 50:1 for 20 hr in the other tests. Antibody released into the incubation mixture by sensitized spleen cells is referred to as endogenous antibody (endog. Ab). When the SRBC target cells are precoated with anti-SRBC antiserum raised in a separate batch of mice, the antibody is referred to as exogenous antibody (exog. Ab).

Table 2. Effects of CH_3-G on immune functions of nylon wool-separated mouse spleen cells*

Spleen cell population	CH_3-G	% Specific ^{51}Cr release				
		CDCC	CICC	ADCC	Phagocytosis Endog. Ab	Phagocytosis Exog. Ab
NA	−	3	2	34	89	63
NA	+	3	0	33	92	66
A	−	55	41	40	47	44
A	+	32	8	41	82	43

* Mice were treated and their spleen cells were tested according to the schedule outlined for Table 1. Per cent specific release is obtained by subtracting the values obtained with cells under appropriate control conditions from those obtained with cells under indicated experimental conditions. Spleen cells were separated on nylon wool columns into nylon wool non-adherent (NA) and nylon wool adherent (A) fractions, as outlined in Materials and Methods.

In contrast, the ADCC response was approximately the same with spleen cells from both untreated and drug-treated mice. In the phagocytic system, a decrease in the per cent of ^{51}Cr released indicates an increase in the fraction of labeled SRBC which became engulfed by the phagocytes and thereby was protected from hypotonic shock. Antibody has been shown to be necessary for this protection [16]. In these systems, antibody to SRBC is supplied either by spleen cells from immunized mice (endogenous antibody) or is contained in the antiserum added to the assay mixture (exogenous antibody). In the case of endogenous antibody, water lysis was significantly reduced with cells from mice not treated with drug, indicating protection of target consequent to phagocytosis. However, with sensitized spleen cells from mice treated with CH_3-G, very little phagocytic protection of target cells was seen. In the case of exogenous antibody, there was a significant phagocytic acitivity in all the groups and no differences were observed regardless of treatment of spleen donor mice with CH_3-G.

Results obtained with spleen cells separated through nylon wool columns into non-adherent (NA), T cell-enriched, and adherent (A), non-T cell-enriched fractions are shown in Table 2. Here control values obtained with cells from non-immunized mice were subtracted from those obtained with cells from immunized mice and the per cent of specific ^{51}Cr release is reported. In the case of both CDCC and CICC, there was only background release of ^{51}Cr when non-adherent cells were used as effectors. However, with adherent cells, a good response was obtained in both CDCC and CICC, and this response was significantly inhibited when cells from mice treated with CH_3-G were used. In the phagocytic system which depends on endogenous antibody, that is antibody which is secreted by effector spleen cells during incubation, phagocytic protection from hypotonic lysis was evident only with adherent cells and was reduced by drug treatment. However, when exogenous antibody was added to the incubation mixture, there was no drug-related effect on either ADCC or phagocytosis.

Activity of SAMDC in mouse spleen cells. As shown in Table 3, administration of CH_3-G to mice resulted in a dramatic increase in the activity of SAMDC in the extracts from spleen cells. This increase occurred only upon drug administration and appeared not to be affected by immunization. After fractionation of spleen cells through nylon wool columns, an even more striking difference in enzyme activity was seen. In the drug-treated groups there was consistently a 4- to 5-fold greater activity of SAMDC in the adherent cells as compared to that in non-adherent cells. Again these differences appeared not to be affected by immunization.

In order to confirm that these enzymatic differences were indeed an intrinsic property of these cell types and not simply an artifact related to the nylon wool separation, another procedure for separating mouse spleen cells into non-T cell-enriched and T cell-enriched fractions was carried out. Spleen cell suspensions were layered onto monolayers of antibody-coated SRBC, and the spleen cells which adhered (A) to the monolayers were separated from those which did not adhere (NA). The results shown in Table 4

Table 3. Activity of SAMDC in nylon wool-separated spleen cells from normal and immunized mice treated with either saline or CH_3-G*

Treatment		SAMDC activity (nmoles $^{14}CO_2$/mg protein/60 min) in cell populations		
CH_3-G	SRBC	Non-separated	NA	A
−	−	0.2	0.3	0.3
+	−	10.1	2.4	12.3
−	+	0.1	0.2	0.5
+	+	8.4	3.3	14.1

* Mice were treated according to the regimen outlined in Table 1 and their spleen cells were separated on nylon wool columns into nylon wool non-adherent (NA) and nylon wool adherent (A) fractions. Spleen cells were homogenized and centrifuged, and the 100,000 g supernatant was used as a source of enzyme. The *S*-adenosylmethionine decarboxylase (SAMDC) activity was determined in reaction mixtures containing, in a final volume of 0.2 ml: 0.5 μmole putrescine, 1 μmole dithiothreitol, 20 μmoles of sodium potassium phosphate buffer, 0.04 μmole *S*-adenosylmethionine [^{14}COOH], and 50 μl of 100,000 g supernatant. Tubes were incubated for 1 hr at 37 .

Table 4. Activity of SAMDC in SRBC monolayer separated spleen cells from normal and immunized mice treated with either saline or CH_3-G*

Treatment		SAMDC activity (nmoles $^{14}CO_2$/mg protein/60 min) in cell populations		
CH_3-G	SRBC	Non-separated	NA	A
−	−	0.1	0.2	0.2
+	−	5.9	1.3	6.8
−	+	0.2	0.2	0.2
+	+	5.8	1.9	6.2

* Mice were treated according to the regimen outlined in Table 1. The enzyme was obtained and assayed as outlined in Table 3. Spleen cells were separated on antibody-coated SRBC monolayers into non-adherent (NA) and adherent (A) fractions as outlined in Materials and Methods.

are consistent with those reported in Table 3. The administration of CH_3-G brought about a large increase in the activity of SAMDC, and this activity was 4- to 5-fold greater in the non-T cell-enriched fraction as compared to the T cell-enriched fraction.

Uptake and efflux of ^{14}C-CH_3-G in mouse spleen cells. The uptake of ^{14}C-CH_3-G was studied with non-separated as well as nylon wool-separated spleen cells obtained from mice treated as above. After incubating the different groups of cells with ^{14}C-CH_3-G for 15 min the results shown in Table 5 were obtained. There was no consistent change in the cellular uptake of this compound as a result of either immunization or CH_3-G treatment *in vivo*. However, the uptake by the nylon wool adherent spleen cells was consistently greater than the uptake by the nylon wool non-adherent cells.

Similar studies were carried out to measure the efflux of ^{14}C-CH_3-G from these spleen cell populations. Following a 15-min incubation of drug with cells, the rate of efflux measured at 1, 15, 30 and 60 min was found to be approximately the same in both adherent and non-adherent cells (data not shown).

DISCUSSION

Drug selectivity within the immune system has been reported [22, 23]. Recently it has been shown that treatment with methotrexate followed by 5-fluorouracil inhibited 19 S hemolytic antibody production but had no effect on allograft rejection or contact sensitivity to oxazolone [24]. Knowledge of such selectivity is important. Often therapeutic efficacy of a particular agent may be compromised due to debilitating immunosuppression. Since many different cell types are involved in the development of immune responses, the immunological damage done by a particular chemotherapeutic agent cannot be accurately assessed nor can specific replacement therapy be set up until the immunocompetent cells and functions most sensitive to that agent are known.

The results of this study indicate that CH_3-G is selective in its immunosuppressive activity. Administration of CH_3-G to mice was found to inhibit CDCC, CICC and phagocytosis with sensitized spleen cells (namely in the presence of endogenous antibody). The production of antibody by sensitized spleen cells is common to all of these tests and is thus a likely site of inhibition by the drug. The killer and phagocytic cell functions also involved in these tests appear to be spared from the inhibitory effects of the drug. In fact, no reduction of response was seen in drug-treated groups when the killer and phagocytic cell functions were measured in the ADCC and phagocytosis (exogenous antibody) tests, respectively, in which antibody to SRBC is added to the system and is therefore no longer a limiting component. The actual secretion of antibody, the binding of antibody to target cells, and the interaction of effector cells with target cells were also not inhibited by the drug. This conclusion is based on results (not shown) where the above tests were carried out in the presence of 100 μM CH_3-G added *in vitro* to the assay mixture. This is unlike other agents which have been found to inhibit specific effector to target cell interactions [25].

Similar results were found in the cell separation experiments in which a non-T cell fraction enriched in antibody-secreting cells was used in the assays. Even though the inhibition of the sensitive responses observed in this fraction of spleen cells was significant, it was not as great as that seen with non-separated cells. This may be due to selective destruction or retention of drug-damaged cells on nylon wool columns. This is a realistic possibility because recovery from the nylon wool columns of spleen cells from mice not treated with drug was 80–85 per cent, whereas recovery of cells from drug-treated mice was only 60–65 per cent. Since the number of cells as well as the final volume of incubation used for these tests was kept constant, cell loss in the drug-treated groups might reflect the selection of a population of cells less perturbed by the toxic effects of the drug.

The enhancement of SAMDC activity after CH_3-G administration was used as a possible marker for drug effect. The greater activity in non-T cell-enriched as compared to T cell-enriched fractions is suggestive of greater drug effect in non-T cell-enriched fractions. It has been reported that the CH_3-G-related increase in SAMDC is mediated through a stabilization of the

Table 5. Uptake of ^{14}C-CH_3-G into nylon wool-separated spleen cells from normal and immunized mice treated with either saline or CH_3-G*

Treatment		Total uptake in cells		
CH_3-G	SRBC	Non-separated	NA	A
−	−	38 ± 7	27 ± 3	45 ± 4
+	−	41 ± 10	31 ± 8	51 ± 10
−	+	36 ± 4	33 ± 10	47 ± 3
+	+	40 ± 3	26 ± 6	47 ± 5

* Mice were treated as outlined in Table 1. Spleen cells were separated on nylon wool columns into nylon wool non-adherent (NA) and nylon wool adherent (A) fractions as outlined in Materials and Methods. Cells were incubated with 10 μM ^{14}C-CH_3-G at 37 for 15 min and uptake was determined as outlined in Materials and Methods and is shown as pmoles CH_3-G/mg of cells. Each value is an average of three experiments ± the standard deviation except for NA, which represents an average of two experiments ± 0.5 range.

enzyme [9]. However, other factors may also play a role. There are studies [26] which suggest that the biosynthesis of ornithine decarboxylase, another enzyme involved in the synthesis of polyamines, is regulated through the levels of intracellular polyamine pools and that CH_3-G can alter this biosynthesis through a disruption of these pools. A similar phenomenon may exist in the regulation of SAMDC. Studies by Dave and Cheng [27] have shown a further enhancement in the already accumulated SAMDC activity upon removal of CH_3-G from monolayer cultures of HeLa TK$^-$ cells, which suggests factors in addition to enzyme stabilization to be responsible for the increased enzyme activity. However, it should be mentioned here that the CH_3-G-related changes in SAMDC reported in this study seemed specific for SAMDC, for no corresponding changes were seen in ornithine decarboxylase under the conditions of this investigation.

The results of this investigation indicate that CH_3-G selectivity inhibits the development of the antibody-forming cell response *in vivo*. It is possible that CH_3-G acts by blocking the proliferation of antibody-forming cells. Alternatively, the possibility cannot be ruled out *a priori* that the effects of this agent are due to an inhibition of T-helper cells which would then inhibit the antibody response. However, biochemical evidence presented in this study, such as the 4- to 5-fold greater enhancement of SAMDC in non-T cell-enriched fractions as compared to T cell-enriched fractions, as well as the 1.5- to 2-fold greater uptake of ^{14}C-CH_3-G in the non-T cell fraction as compared to the T cell fraction, appears to support the concept of a selective inhibition of antibody-forming cells by CH_3-G.

REFERENCES

1. V. T. Oliverio and C. Zubrod, *A. Rev. Pharmac.* **5**, 335 (1965).
2. B. I. Shnider, J. Colsky, R. Jones and P. P. Carbone, *Cancer Chemother. Rep.* **58**, 689 (1974).
3. R. H. Levin, E. Henderson, M. Koryn and E. J. Freireich, *Clin. Pharmac. Ther.* **6**, 31 (1965).
4. E. Mihich, *Fedn Proc.* **23**, 388 (1964).
5. D. H. Russell, *Life Sci.* **13**, 1635 (1973).
6. D. H. Russell and O. Heby, *Cancer Res.* **34**, 886 (1974).
7. H. G. Williams-Ashman and A. Schenone, *Biochem. biophys. Res. Commun.* **46**, 288 (1972).
8. A. E. Pegg, *Biochem. J.* **132**, 537 (1973).
9. A. E. Pegg, A. Corti and H. G. Williams-Ashman, *Biochem. biophys. Res. Commun.* **52**, 696 (1973).
10. J. Bennett, J. Ehrke, C. Dave and E. Mihich, *Pharmacologist* **17**, 301 (1975).
11. S. A. Cohen, M. J. Ehrke and E. Mihich, *J. Immun.* **115**, 1007 (1975).
12. D. J. Dixon, E. A. Dulmadge and F. M. Schabel, *Cancer Chemother. Rep.* **50**, 247 (1966).
13. C. S. Henney, *J. Immun.* **110**, 73 (1973).
14. C. Mawas, T. Carey and E. Mihich, *Cell Immun.* **6**, 243 (1973).
15. P. Perlmann and H. Perlmann, *Cell Immun.* **1**, 300 (1970).
16. P. Hersey, *Transplantation* **15**, 282 (1973).
17. D. Trizio and G. Cudkowicz, *J. Immun.* **113**, 1093 (1974).
18. E. Kedar, M. O. de Landazuri and B. Bonavida, *J. Immun.* **112**, 1231 (1974).
19. A. E. Pegg and H. G. Williams-Ashman, *J. biol. Chem.* **244**, 682 (1969).
20. O. H. Lowry, N. J. Rosebrough, A. L. Farr and J. R. Randall, *J. biol. Chem.* **193**, 265 (1951).
21. C. Dave and E. Mihich, *Biochem. Pharmac.* **21**, 2681 (1972).
22. E. Mihich, in *Principles of Immunology* (Eds. N. R. Rose, F. Milgrom and C. J. Van Oss), pp. 358–70. MacMillan, New York (1973).
23. E. Mihich, in *Drug Resistance and Selectivity* (Ed. E. Mihich), pp. 391–409. Academic Press, New York (1973).
24. J. A. DiLorenzo, D. E. Griswold, C. R. Bareham and P. Calabresi, *Cancer Res.* **34**, 124 (1974).
25. J. Ehrke, S. A. Cohen and E. Mihich, *Fedn Proc.* **35**, 531 (1975).
26. J. L. Clark and J. L. Fuller, *Biochemistry* **14**, 4403 (1975).
27. C. Dave and Y. Cheng, *Pharmacologist* **17**, 300 (1975).