[12] Spirin, A. S., *Progr. Nucleic Acid Res.*, **1**, 301 (1963).
[13] Weiss, S. B., and T. Nakomoto, these PROCEEDINGS, **47**, 1400 (1961).
[14] Magasanik, B., and E. Chargaff, *Biochim. Biophys. Acta*, **7**, 396 (1951).
[15] Markham, R., and J. D. Smith, *Biochem. J.*, **52**, 565 (1952).
[16] Sato, K., and E. Egami, *J. Biochem.*, **44**, 753 (1957).
[17] Rushizky, G. W., and H. A. Sober, *J. Biol. Chem.*, **237**, 2883 (1962).
[18] Bather, R., *Brit. J. Cancer*, **11**, 611 (1957).
[19] Cocito, C., and P. Laduron, *Anal. Biochem.*, **7**, 429 (1964).
[20] Crawford, L. V., and E. M. Crawford, *Virology*, **13**, 227 (1961).
[21] Bonar, R. A., R. H. Purcell, D. Beard, and J. W. Beard, *J. Natl. Cancer Inst.*, **31**, 705 (1963).
[22] Boedtker, H., *J. Mol. Biol.*, **2**, 171 (1960).
[23] Strauss, J. H., and R. L. Sinsheimer, *J. Mol. Biol.*, **7**, 43 (1963).
[24] Schaffer, F. L., in *Basic Mechanisms in Animal Virus Biology*, Cold Spring Harbor Symposia on Quantitative Biology, vol. 27 (1962), p. 89.
[25] Holland, J. J., B. H. Hoyer, L. C. McLaren, and J. T. Syverton, *J. Exptl. Med.*, **112**, 82 (1960).
[26] Hausen, P., and W. Schäfer, *Z. Naturforsch.*, **17b**, 15 (1962).
[27] Strohmaier, K., and M. Mussgay, *Science*, **130**, 217 (1959).
[28] Schäfer, W., in *The Viruses*, ed. F. M. Burnet and W. M. Stanley, (1959), vol. 1, p. 475.
[29] Ada, G. L., and B. T. Perry, *Australian J. Exptl. Biol. Med. Sci.*, **32**, 453 (1954).
[30] Frisch-Niggemeyer, W., and L. Hoyle, *J. Hyg.*, **54**, 201 (1956).
[31] Hirst, G. K., in *Basic Mechanisms in Animal Virus Biology*, Cold Spring Harbor Symposia on Quantitative Biology, vol. 27 (1962), p. 303.
[32] Sokol, F., and S. Schramek, *Biochem. Biophys. Res. Commun.*, **12**, 21 (1963), and *Acta Virol.*, **8**, 193 (1964).
[33] Gomatos, P. J., and I. Tamm, these PROCEEDINGS, **49**, 707 (1963).
[34] Bonar, R. A., and J. W. Beard, *J. Natl. Cancer Inst.*, **23**, 183 (1959).

---

*THE EFFECT OF STREPTOMYCIN ON ANTIBODY SYNTHESIS IN VITRO**

BY ROBERT G. KRUEGER

DEPARTMENT OF MICROBIOLOGY, UNIVERSITY OF CHICAGO

Communicated by W. H. Taliaferro, May 17, 1965

Streptomycin inhibits protein synthesis in bacteria by interfering with ribosomal function. SM inhibited the incorporation of amino acids in undefined cell-free systems from *Escherichia coli*[1, 2] and decreased polyphenylalanine synthesis in *E. coli* ribosomes when poly U was used as messenger.[3–5] It was suggested that SM acts by interfering with either the attachment or functioning of poly U on the ribosome. SM did not prevent attachment of poly U to ribosomes, but the presence of poly U on ribosomes made the SM binding site less accessible to the antibiotic.[6] This binding site is on the 30*S* ribosomal subunit, the one to which messenger also binds.[7] Davies *et al.*[8] showed that in *E. coli* ribosomes with poly U as messenger, SM decreased incorporation of phenylalanine and increased incorporation of isoleucine. Thus, SM altered the translation process whereby the information of the messenger was decoded. Davies *et al.*[8] suggested that SM acts on intact bacteria, not by inhibiting protein synthesis, but by causing the synthesis of nonfunctional proteins with altered amino acid sequences. Pestka *et al.*[9] have recently

demonstrated the effect of SM upon RNA codon recognition by poly- and trinucleotide templates.

Although SM has not been reported to affect protein synthesis in mammalian cells, the present paper demonstrates that it alters the *in vitro* synthesis of antibody to phage MS-2 in spleen and lymph node cells from immunized rabbits in a manner that appears to be similar to the effect of the antibiotic on bacterial systems. A preliminary description of this work has been presented elsewhere.[10]

Materials and Methods.—E. coli strain AB261 was supplied by Dr. Irving Rappaport. The RNA phages MS-2, M-12, R-17, Fr, and B were obtained from Mr. David Scott, and their origin has been described by Scott.[11] SM and other antibiotics were standard commercial preparations. All antibiotic concentrations are given in terms of the free base.

Phage MS-2 was chosen as an antigen because it has only a single species of coat protein[12, 13] and because it stimulates the synthesis of relatively large amounts of antibody that can be assayed precisely. Purified suspensions of MS-2 were prepared by a modification of the method of Strauss and Sinsheimer.[14] Phage was precipitated from a broth lysate of *E. coli* AB261 with ammonium sulfate, extracted with a fluorocarbon, and banded in a cesium chloride gradient. The other phages were prepared in a similar fashion but were not banded in cesium chloride.

Albino rabbits (2–5 kg) were immunized with an emulsion of 1 part purified MS-2 suspension containing 10^{12} PFU/ml and complete Freund's adjuvant. Each rabbit was given 1-ml inoculations at weekly intervals by the following routes: subcutaneous, intramuscular, intramuscular, footpad. After a rest of 4–6 weeks, the rabbits were secondarily stimulated by injecting 0.5 ml MS-2 suspension intravenously and into the footpad.

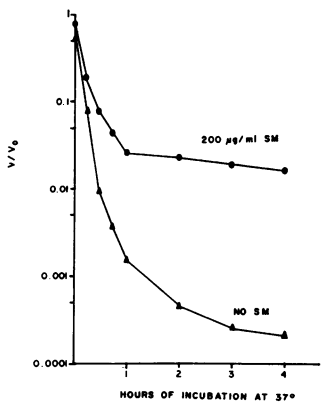

FIG. 1.—Neutralization of bacteriophage MS-2 in 5-day spleen cultures maintained on 200 μg/ml SM before assay.

Five days later, the rabbits were bled by cardiac puncture to obtain serum samples and then sacrificed with ether. The spleens and popliteal lymph nodes were perfused with EBSS,[15] removed aseptically, and placed in chilled EBSS. After the connective tissue capsules were removed, the tissues were diced into small fragments, placed in 20 ml EBSS, and agitated with a magnetic stirrer at 4° for 2–3 hr. The resulting cell suspension was filtered through 200-mesh stainless steel gauze, collected in chilled tubes, centrifuged at 200 × *g* for 10 min, and the supernatant discarded. The cell pellet was resuspended in EBSS containing 0.13% gelatin and centrifuged at 180 × *g* for 5 min. The cell pellet was then resuspended in gelatin-EBSS and centrifuged at 100 × *g* for 15 min. The cells were then suspended in a small volume of EBSS, and the cell count and per cent viable cells were determined by hemocytometer count and the trypan blue exclusion test.[16]

The cells were diluted to a concentration of 2×10^6 cells/ml in Eagle's minimal essential medium[17] (minus serum) with or without SM. Two ml of this suspension were placed in 35 × 10 mm plastic tissue culture dishes and incubated for 12–14 days at 37° in an atmosphere of 5% CO_2–95% air. The cells were washed and resuspended in fresh medium with or without phage at 3–4-day intervals. The cells did not multiply but, after 12–14 days' incubation, 85–90% still excluded trypan blue. Cultures of spleen and lymph node cells gave identical results in all experiments.

For assay of the phage-neutralizing antibody produced in the *in vitro* system, 2×10^6 cells were removed from the main culture, washed, resuspended in 1.9 ml Eagle's medium, and incubated at 37°. Then from 10^6 to 10^8 PFU of MS-2 in 0.1 ml EBSS were added. At appropriate intervals 0.1-ml samples were removed and assayed for viable phage particles by the plaque assay method with *E. coli* AB261.[18] Cells that had been incubated in the continuous presence of phage were washed 3 times before being used for antibody assay.

Figure 1 describes the neutralization of MS-2 by cells incubated with and without SM. For routine assay the PFU/ml was measured initially and at a single time following incubation of cells

and phage at 37°. Since the differences in extent of neutralization between uninhibited and SM-inhibited cultures remained more or less constant after 60 min, the phage-neutralizing activity of cell cultures was expressed as the plaque reduction number (*PRN*) which was calculated from the equation:

$$PRN = \frac{1}{t} \ln \frac{V_t}{V_0},$$

where V_0 is the initial PFU/ml, V_t is the PFU/ml at time t which is always taken to be 60 min. This is the same equation used to calculate K, the neutralization constant, describing the rate of neutralization of a given antiserum in terms of the slope of the neutralization curve. However, the values obtained as just described were not true neutralization constants because they were not taken from the linear portion of the neutralization curve. Therefore, the noncommittal *PRN* expression has been used instead.

For measurement of complement fixation in the presence of phage and antibody synthesized *in vitro*, cells were removed from the main culture, washed in Eagle's medium, and resuspended to a concentration of 2×10^6 cells/ml in a volume of 2 ml. To this suspension was added 50 $C'H_{50}$ of guinea pig complement in 4.5 ml Veronal buffer.[19] After 1 hr at 37°, the reaction mixture was centrifuged for 5 min at $5100 \times g$. Then 2 ml of a 1:5 dilution of the supernatant in chilled Veronal buffer were added to 4 ml buffer and 1 ml 2.5% sheep red cells optimally sensitized with rabbit hemolysin. After 1 hr at 37°, the reaction mixture was centrifuged 10 min at $5100 \times g$, and the oxyhemoglobin in the supernatant determined by its absorption at 541 mμ. Controls, including buffer plus complement, phage plus complement, cells plus complement, red blood cell blank, and complete lysis blank, were diluted 1:10 before addition of sensitized red blood cells. Complement fixation was equal to the difference between the number of $C'H_{50}$ remaining in control and experimental reaction mixtures.

Results.—Characteristics of the antibody-forming system: The ability to form neutralizing antibody *in vitro* could be demonstrated only in cells from rabbits that had previously been immunized with phage MS-2 and possessed serum neutralizing antibody to it. The antibody measured *in vitro* was specific for MS-2. Unrelated phage T1 and T4 were not neutralized.

Cells from immunized rabbits produced 100 times as much antibody when incubated in the presence of MS-2 as in its absence (Table 1). This effect could have been produced by the phage stimulating the cells either to release previously synthesized antibody or to synthesize antibody *de novo*. Two kinds of experiments support the concept of *de novo* synthesis.

TABLE 1

PRODUCTION OF NEUTRALIZING ANTIBODY DURING *in vitro* INCUBATION IN THE PRESENCE AND ABSENCE OF PHAGE MS-2

Condition of incubation	Cell Culture: Spleen	Cell Culture: Lymph node
MS-2 present	29.30*	26.00
MS-2 absent	0.12	0 11

* *PRN.*

Duplicate samples of cells were removed from the main spleen culture 2 days after its initiation and 8 days after the initiation of the lymph node culture. Phage was added to one sample which was then held at 37° for 1 hr and assayed for neutralization. The other sample was held at 37° for 1 hr without phage, and the cells were removed by centrifugation. The supernatant was then incubated with phage for 1 hr at 37° and also assayed for neutralization.

TABLE 2

INTRACELLULAR AND EXTRACELLULAR NEUTRALIZING ANTIBODY BEFORE AND AFTER INCUBATION WITH PHAGE MS-2

Antibody measured	Cell Culture: Spleen	Cell Culture: Lymph node
Intracellular		
Before incubation with MS-2	0.42*	3.29
After incubation with MS-2	0.19	0.81
Extracellular	124.70	98.80

* *PRN.*

Cells from cultures maintained for 5 days without phage were incubated with phage for 1 hr at 37° and then assayed for neutralization to give the extracellular *PRN*. At the beginning of the incubation period, another sample of each culture was disrupted by 2-min exposure in a 20-kc ultrasonic generator and centrifuged 30 min at $12,000 \times g$. The supernatant was assayed to give the initial intracellular *PRN*. At the end of the incubation with phage, a third cell sample was washed to remove phage and the final intracellular *PRN* was obtained as just described.

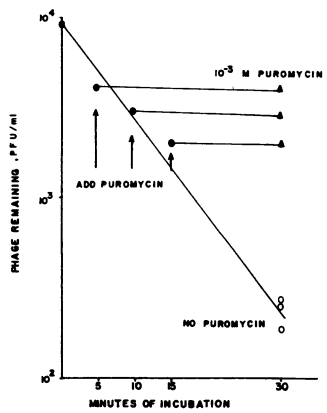

FIG. 2.—Effect of puromycin on the synthesis of neutralizing antibody in 5-day spleen cultures.

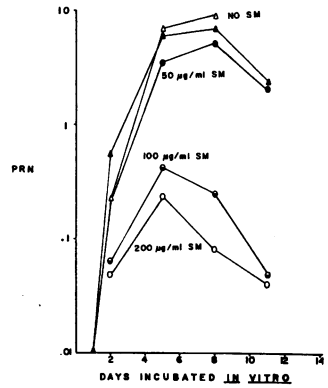

FIG. 3.—Effect of various concentrations of SM on neutralization of MS-2 by lymph node cells incubated continuously with 2×10^8 PFU/ml MS-2 with or without SM.

First, the amount of intracellular antibody before and after incubation with phage was compared with the amount of antibody released extracellularly (Table 2). Although there was a slight decrease in intracellular antibody after incubation with MS-2, the amount of antibody released by the cells exceeded this decrease by 100–200-fold.

Second, cell cultures were stimulated to produce antibody by the addition of MS-2, and then puromycin, an inhibitor of active protein synthesis,[20] was added 5, 10, and 15 min after phage. Figure 2 shows that 10^{-3} *M* puromycin immediately inhibited further neutralization of MS-2, thus giving strong evidence that this neutralization resulted from *de novo* synthesis of antibody. Other experiments demonstrated that 10^{-3} *M* puromycin had no effect on the neutralization of MS-2 by previously synthesized antibody.

Effect of SM on antibody synthesis: Lymph node cells were cultured in the continuous presence of MS-2 and varying concentrations of SM. The ability of uninhibited cells to synthesize neutralizing antibody in the standard assay system rose sharply between the 1st and 5th day of culture and declined slowly after the 8th day (Fig. 3). Fifty μg/ml SM had no effect on production of neutralizing activity, but 100 and 200 μg/ml of the antibiotic reduced the *PRN* by 10–100-fold. The highest SM concentration used had no adverse effect on the cultured cells as judged by their morphological appearance and their ability to exclude trypan blue and had no effect on the neutralization of MS-2 by specific antiserum.

Figure 4 describes the synthesis of antibody by spleen cells exposed to MS-2 only at the time of assay. Continuous exposure of the cell culture to the phage was not required for attaining high levels of antibody synthesis. Figure 4 also shows that cells treated with SM during the 1-hr assay period only were just as strongly inhibited in production of neutralizing antibody as were cells cultured in the continuous presence of the antibiotic. Conversely, cells maintained in the presence of SM and then assayed in its absence produced nearly normal amounts of neutralizing antibody (Fig. 4). These observations demonstrate that SM acts quickly to inhibit production of neutralizing antibody and that its effect is reversible.

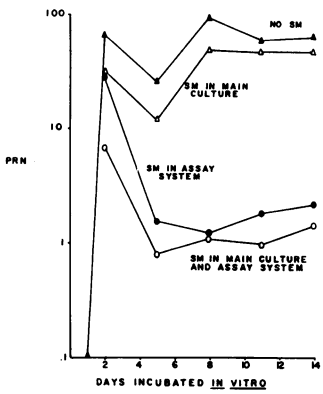

FIG. 4.—Effect of 200 μg/ml SM in the main spleen cell culture and in the assay system on the neutralization of MS-2.

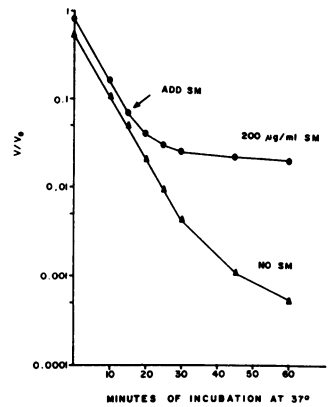

FIG. 5.—Time required for SM to initiate inhibition of synthesis of neutralizing antibody. Spleen cells were maintained for 5 days without either SM or MS-2 before being used in the assay.

The rapidity with which SM cuts off synthesis of neutralizing antibody is emphasized by Figure 5. Reduction in the rate of appearance of neutralizing antibody was apparent within 5 min of addition of SM to cells actively synthesizing antibody.

Antibiotics closely related to SM in structure and mode of action on bacteria inhibited synthesis of neutralizing antibody almost as well as SM itself (Table 3). The effectiveness of dihydrostreptomycin indicates that the action of SM on antibody synthesis cannot be ascribed to its comparatively nonspecific ability to cross-link and precipitate polyanions because dihydrostreptomycin does not have this property.[21]

TABLE 3

EFFECT OF ANTIBIOTICS RELATED TO SM ON SYNTHESIS OF NEUTRALIZING ANTIBODY IN SPLEEN CELLS

SM in main culture, 200 μg/ml	Antibiotic in assay system, 200 μg/ml	*PRN*
+	None	57.70
+	Streptomycin	0.92
+	Dihydrostreptomycin	0.62
+	Neomycin	1.44
+	Kanamycin	1.72
0	None	57.70
0	Streptomycin	1.83
0	Dihydrostreptomycin	1.63
0	Neomycin	3.39
0	Kanamycin	2.03

Spleen cells were removed from the main culture on the 5th day of incubation without phage.

In other experiments, coliphage T4 was used as the antigen. Essentially the same results were obtained in both uninhibited and SM-inhibited cell cultures as have just been described for phage MS-2.

Does SM alter the translation of a messenger for antibody synthesis?: Davies *et al.*[8] demonstrated that in *E. coli*, SM alters the translation of poly U. They suggested

TABLE 4

FAILURE OF SM TO PREVENT THE APPEARANCE OF COMPLEMENT-FIXING ANTIBODY IN SPLENIC CELL CULTURES

Day of culture	Complement Fixed, $C'H_{50}$ Units		Phage Neutralized, *PRN*	
	No SM	SM, 200 μg/ml	No SM	SM, 200 μg/ml
2	19.9	19.1	2.5	0.1
5	10.9	8.4	27.2	0.5
8	14.3	10.0	38.6	0.7
11	15.5	15.1	24.8	0.6
14	16.3	25.3	2.8	0.7

Cells in the main culture were incubated without phage in the continuous presence of SM.

that miscoding in the presence of SM causes the synthesis of altered protein molecules lacking in biological activity. According to their hypothesis, SM might not totally inhibit antibody synthesis in spleen and lymph node cell cultures but might instead cause the synthesis of antibody molecules so altered in structure that they no longer neutralize MS-2. Evidence for this hypothesis could be obtained if the altered antibody still possessed some reactivity toward the MS-2 antigen and could be identified by other immunological methods.

Therefore, *in vitro* synthesis of antibody to MS-2 was followed by simultaneous determination of both neutralization and complement fixation. Table 4 gives the results of such a dual estimation of antibody carried out on a single cell culture on 5 different days. SM exerted its usual strong inhibition of phage neutralization but it had no effect on the fixation of complement in the presence of MS-2. All controls indicated that this complement fixation resulted from the appearance of antibody specific for MS-2. Since this phage appears to have only one kind of coat protein[12, 13] which could act as a single antigen, the only simple interpretation of this experiment is that SM alters the specificity of antibody against MS-2 in such a way that it still combines with the phage and can fix complement but can no longer bring about the neutralization of phage infectivity in the plaque assay.

Evidence that SM causes the synthesis of antibody molecules with altered specificity was also obtained by studying the heterologous neutralization of other RNA phages. Scott[11] has shown that the phages M-12, R-17, B, and Fr are closely related to MS-2 and are neutralized by MS-2 antiserum in that order. In the absence of SM, substitution of these phages for MS-2 in the neutralization assay system caused the synthesis of neutralizing antibody in proportion to the closeness of their relation to MS-2 (Table 5). This strongly suggests that the antibody

TABLE 5

ABILITY OF HETEROLOGOUS PHAGES TO ELICIT NEUTRALIZING ANTIBODY SYNTHESIS IN THE PRESENCE AND ABSENCE OF SM

Day of culture	Cell culture used	SM, 200 μg/ml	MS-2	Heterologous Phages			
				M-12	R-17	β	Fr
1	Spleen	0	26.7*	0.53	0.30	0.25	0.14
1		+	10.0	0.66	0.69	5.1	1.1
2	Lymph node	0	20.3	0.13	0.09	0.05	0.004
2		+	0.52	0.09	0.30	0.12	0.20
5	Spleen	0	84.0	2.3	0.20	0.16	0.09
5		+	0.09	0.37	0.19	0.60	3.4
6	Lymph node	0	27.6	18.5	15.5	6.1	0.70
6		+	0.24	11.2	11.9	20.3	0.19
13	Spleen	0	22.4	6.8	1.5	0.11	0.013
13		+	1.1	10.8	36.6	10.9	49.6

* *PRN*.

Cells in the main culture were incubated *in vitro* without phage and with 200 μg/ml SM. The cells were from animals immunized with MS-2; neutralizing antibody was elicited *in vitro* by heterologous phages.

elicited by the heterologous phages was antibody against MS-2 itself. When the same assays were conducted in the presence of SM, new and random orders of relatedness were observed. In the uninhibited system, trials on different days of culture consistently ranked the heterologous phages in the same order of effectiveness in stimulating synthesis of neutralizing antibody, the identical order obtained by neutralization by serum antibody against MS-2. In contrast, successive trials in the presence of SM gave entirely unpredictable results (Table 5). First one and then another heterologous phage was the most effective stimulator of neutralizing antibody synthesis. On one occasion (day 13), the most distantly related phage, Fr, elicited the synthesis of more neutralizing activity than MS-2 itself.

Discussion.—These experiments demonstrate that spleen and lymph node cells from rabbits immunized with the RNA phage MS-2 were stimulated *in vitro* to active synthesis of antibody that neutralizes the phage and fixes complement. The way in which SM altered the serological activity of the antibody synthesized in its presence suggests that the effect of SM on the synthesis of antibody protein is the same as its effect on the synthesis of bacterial protein. A second suggestion following inevitably from the first is that the mechanism of antibody synthesis is the same as for other protein molecules and, specifically, that it occurs on the ribosome and that it involves the participation of a messenger. This conclusion is in accord with other recent investigations on the mechanism of antibody synthesis.[22-25]

The differential effect of SM on the appearance of neutralizing and complement-fixing activity could be explained if the MS-2 particle contains two distinct antigens, one engendering neutralizing antibody, the other, complement-fixing antibody. SM might then inhibit the synthesis of the first antibody but not the second. However, present evidence[12, 13] indicates that MS-2 has only a single coat protein and thus only a single antigen. Even if the neutralizing and complement-fixing activities that appear in MS-2-stimulated cultures do reside in different antibody molecules, it still seems unlikely that the action of SM would be so exquisitely specific that it would inhibit the synthesis of one antibody molecule and not the other.

The alternate explanation is that SM binds to the ribosomes of antibody-synthesizing cells and changes the reading of the antibody messenger so that the reactive sites of the antibodies synthesized on SM-bound ribosomes are subtly altered—enough to destroy their ability to neutralize MS-2 but not enough to destroy their ability to fix complement in its presence. It is possible that the antibodies synthesized in SM-treated cells are still able to bind to MS-2 particles and thus can fix complement but that they dissociate from the phage particles when diluted in the plaque assay test and thus fail to neutralize their infectivity. The postulated alteration in antibody structure could be a single amino acid substitution in the reactive site itself, but substitution elsewhere in the antibody molecule could also affect the reactive site by changing secondary or tertiary structure.

The experiments in which heterologous phages were used to stimulate antibody synthesis in cells from rabbits immunized with MS-2 may be interpreted in a similar fashion. It appears that SM, by causing the insertion of spurious amino acids into the polypeptide chain of the antibody molecule, caused the synthesis of antibodies that reacted more strongly with one or more heterologous antigens than with the homologous antigen that elicited their synthesis. The misreading of the antibody

messenger by SM-bound ribosomes must occur in an unpredictable fashion, because successive trials with heterologous phages always produced different results.

The minimum concentration of SM required to affect the course of antibody synthesis in cell cultures was about 100 times higher than that required to inhibit the growth of bacteria or the synthesis of protein in cell-free bacterial systems.[4, 5] Mammalian cells are generally regarded as relatively impermeable to SM[26] and this may explain the difference. However, levels of SM comparable to those required to kill bacteria are capable of inhibiting the growth of intracellular bacteria such as brucellae within their mammalian host cells[27-30] and preventing the phagocytosis of typhoid bacilli by macrophages.[31] The rapidity with which SM inhibited the synthesis of neutralizing antibody and the ease with which its inhibitory action could be reversed by washing the cell cultures show that SM quickly passed in and out of the mammalian cells used here. Since these experiments implicate the ribosome as the site of action of SM in mammalian cells, it may be that their relative resistance to SM lies not at the level of the whole cell, but at the level of the ribosome itself. Such a situation would have a parallel in the differential effect of chloramphenicol on mammalian cells and on bacteria—a greater concentration of this antibiotic is needed to inhibit protein synthesis in mammalian ribosomal systems than in bacterial ones.[32]

Summary.—Spleen and lymph node cells from rabbits immunized with the RNA phage MS-2 actively synthesized specific neutralizing and complement-fixing antibody. In the presence of 200 μg/ml SM, the appearance of neutralizing activity was reversibly inhibited, but the production of complement-fixing activity was unchanged. Antibody synthesized by untreated cells consistently neutralized closely related RNA phages in the same order observed with serum antibody against MS-2. In the presence of SM the related phages were not neutralized in proportion to the closeness of their relation to MS-2, and the relative order of neutralization varied randomly from trial to trial. It is suggested that SM causes the synthesis of antibody with altered serological specificity in a manner similar to its effect on protein synthesis in bacterial extracts.

The author wishes to thank Drs. J. W. Moulder, R. Haselkorn, and L. Gorini for many helpful discussions.

The following abbreviations were used: SM, streptomycin; poly U, polyuridylic acid; PFU/ml, plaque-forming units per ml; EBSS, Earle's basic salt solution; $C'H_{50}$, 50% hemolytic units.

* This investigation was supported by U.S. Public Health Service graduate training grant 1T1-AI-238.

[1] Erdos, T., and A. Ullmann, *Nature*, **192**, 633 (1959).

[2] Mager, J., M. Benedict, and M. Artman, *Biochim. Biophys. Acta*, **62**, 202 (1962).

[3] Flaks, J. G., E. C. Cox, and J. R. White, *Biochem. Biophys. Res. Commun.*, **7**, 385 (1962).

[4] Flaks, J. G., E. C. Cox, M. L. Witting, and J. R. White, *Biochem. Biophys. Res. Commun.*, **7**, 390 (1962).

[5] Speyer, J. F., P. Lengyel, and C. Basilio, these PROCEEDINGS, **48**, 684 (1962).

[6] Davies, J. E., these PROCEEDINGS, **51**, 659 (1964).

[7] Cox, E. C., J. R. White, and J. G. Flaks, these PROCEEDINGS, **51**, 703 (1964).

[8] Davies, J. E., W. Gilbert, and L. Gorini, these PROCEEDINGS, **51**, 883 (1964).

[9] Pestka, S., R. Marshal, and M. Nirenberg, these PROCEEDINGS, **53**, 639 (1965).

[10] Krueger, R. G., *Bacteriol. Proc.* (1965), p. 59.

[11] Scott, D. W., *Virology*, **26**, 85 (1965).

[12] Nathans, D., G. Notani, J. H. Schwartz, and N. D. Zinder, these PROCEEDINGS, **48**, 1424 (1962).
[13] Schwartz, J. H., J. M. Eisenstadt, G. Browerman, and N. D. Zinder, these PROCEEDINGS, **53**, 195 (1965).
[14] Strauss, J., and R. L. Sinsheimer, *J. Mol. Biol.*, **7**, 43 (1963).
[15] Earle, W. R., *J. Natl. Cancer Inst.*, **4**, 165 (1943).
[16] Vas, S. I., E. L. Medson, and F. C. Lueng, *Can. J. Bacteriol.*, **10**, 561 (1964).
[17] Eagle, H., *Science*, **130**, 432 (1952).
[18] Adams, M., *Bacteriophages* (New York: Interscience Publ., Inc., 1959).
[19] Kabat, E. A., and M. M. Mayer, *Experimental Immunochemistry* (Springfield: C C Thomas & Co., 1961), 2nd ed., p. 149.
[20] Nathans, D., *Federation Proc.*, **23**, 984 (1964).
[21] Aronson, J., W. F. Meyer, and T. D. Brock, *Nature*, **202**, 555 (1964).
[22] Ambrose, C. T., and A. H. Coons, *J. Exptl. Med.*, **117**, 1075 (1963).
[23] Uhr, J. W., *Science*, **142**, 1476 (1964).
[24] Smiley, J. D., J. G. Heard, and M. Ziff, *J. Exptl. Med.*, **119**, 881 (1964).
[25] Svehag, S. E., *Science*, **146**, 659 (1964).
[26] Brock, T. D., *Federation Proc.*, **23**, 965 (1964).
[27] Freeman, B. A., and L. R. Vana, *J. Infect. Diseases*, **102**, 258 (1958).
[28] Jenkin, C., and B. Benaceraf, *J. Exptl. Med.*, **112**, 403 (1960).
[29] Showacre, J. L., H. E. Hopps, H. G. Dubuy, and J. E. Smadel, *J. Immunol.*, **87**, 153 (1961).
[30] Richardson, M., and J. N. Holt, *J. Bacteriol.*, **84**, 638 (1962).
[31] Morello, J. A., and E. C. Baker, *J. Infect. Diseases*, **115**, 131 (1965).
[32] Weisberger, A. S., and S. Wolfe, *Federation Proc.*, **23**, 976 (1964).

---

*PURIFICATION AND PROPERTIES OF HISTIDINE DECARBOXYLASE FROM LACTOBACILLUS 30a**

BY J. ROSENTHALER, BEVERLY M. GUIRARD, G. W. CHANG, AND ESMOND E. SNELL

DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY

Communicated April 30, 1965

Despite the physiological importance of histamine, the mechanism of its formation by histidine decarboxylase has not been adequately investigated. Three apparently different mammalian enzymes that decarboxylate histidine are known, each of which is reported to require pyridoxal-5′-phosphate (PLP) as coenzyme.[1–3] Even in purified preparations, however, their activities are extremely low. For a source of this enzyme we turned, therefore, to *Lactobacillus* 30a, which produces it in comparatively large amounts.[4, 5] Indirect evidence led to the conclusion that the histidine decarboxylase of this organism[4] and of *Clostridium welchii*,[6] and the histidine and glutamate decarboxylases of *Escherichia coli*[7] did not possess PLP as coenzyme. Purified glutamate decarboxylase has since been shown to contain firmly bound PLP which is necessary for its activity.[8] The nature of the bacterial histidine decarboxylase is not yet clear. Its formation in *Lactobacillus* 30a is dependent upon the presence of vitamin B_6,[5, 9] and some partially purified preparations were activated by PLP and Fe^{+++}.[9] However, these activating effects were subsequently found to be nonspecific, and hence could not be considered evidence for the PLP-dependence of this decarboxylase. We describe herein procedures for preparation of the ap-