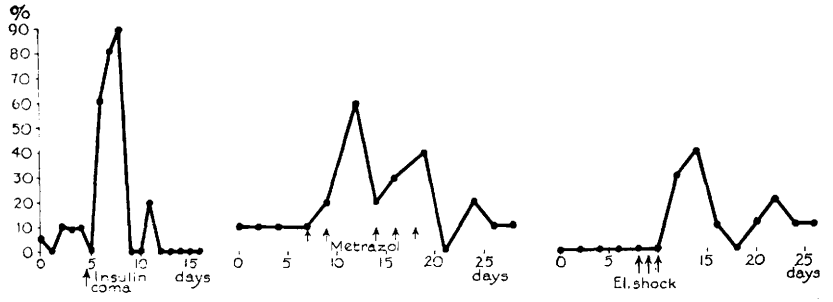

FIG. 1.

The effect of insulin coma, metrazol and electric shock on the restoration of inhibited conditioned reflexes. All rats were conditioned to the bell and after a conditioned reflex was established it was inhibited by lack of reenforcement. The records show that these inhibited reflexes are restored by insulin coma (5 units insulin per kilo intraperitoneally and 5 units subcutaneously), metrazol convulsions (45 mg per kilo) and electric shock applied to the head.

conditions may be responsible for these cortical effects. It appears to be significant for the understanding of the mechanism of shock therapy to point out that the procedures studied in this paper presumably act on the cortex by removing inhibitory processes. Rose and collaborators[3] described a single sheep which for unknown reasons had lost its previously established C.R. and found that repeated insulin comas restored the C.R. The present work confirms and extends this observation, suggests the mechanism involved and indicates that convulsions as well as insulin coma may restore inhibited C.R.

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Antagonism of Anti-sulfonamide Effect of Methionine, and Enhancement of Bacteriostatic Action of Sulfonamide by Urea.*

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Holder and MacKay[1] have recently reported the use of a mixture of strong urea with sulfonamides topically applied to infected

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* Supported by a grant from the General Research Fund of the University of Minnesota.

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wounds. Urea markedly enhances the solubility of sulfonamides and could thus increase their action.

Urea offers certain advantages over other substances which have been described as having antisulfonamide-inhibitor activity, such as azochloramid,[2, 3] and certain purines.[4] Such inhibitors are methionine,[5] para-amino benzoic acid, peptone, tissue extracts, purulent exudates, and so forth.[6] These advantages are that urea is relatively nontoxic;[7] it is a strong peptizing agent, exerting marked solvent action on necrotic tissue, pus and debris, and thus chemically debrides contaminated wounds and mechanically removes inhibitors; it lyses bacteria; it deodorizes foul smelling wounds rapidly; it renders sulfonamides more soluble; and it is very inexpensive. These properties have been reviewed elsewhere.[1]

In addition, it would be highly important if an antisulfonamide-inhibitor action, or sulfonamide-enhancing action could be demonstrated for urea.

Experimental. A strain of *Escherichia coli* was selected which acclimated itself to a synthetic medium of the following composition.

NH_4NO_3	5.0 g
Na_2SO_4	5.0 g
$MgSO_4$	0.1 g
K_2HPO_4	2.0 g
Glucose	10.0 g

Tap water to make volume to 1.0 liter.

Glucose in itself, in concentrations of 0.6 to 2.6%, has been claimed to exert some antisulfonamide activity,[8] although other reports have disagreed with this view.[5] In our experiments this possible effect of glucose was controlled throughout by always using the same concentration and proper controls.

In this preliminary study, sodium sulfadiazine† was selected as the sulfonamide, and dl-methionine‡ as the sulfonamide inhibitor.

Preliminary experiments were performed to find the tolerance range of *E. coli* in our basal medium to methionine and urea. Experi-

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† Kindly supplied by Dr. Edward F. Roberts, of Lederle Laboratories, Inc.

‡ Supplied through the courtesy of Dr. D. F. Robertson, of Merck and Co., Inc.

ments were also conducted to find that concentration of sodium sulfadiazine which would give partial bacteriostasis. Further inhibition was not desired because complete inhibition would conceal any enhancing effect of urea.

In concentrations below 1.8%, urea may act as an additional source of nitrogen for *E. coli,* but the experiments reported are not sufficient to demonstrate this possibility. 1.6% urea did not seem to show any marked effect on the growth of the test organism, and therefore, this concentration was used.

In concentrations between 1.0 to 100.0 mg %, methionine had no effect on the size of the populations in our medium, in 24 hours.

Sodium sulfadiazine was used in 1 mg % concentration in our tests because it caused an inhibition of 80% under the conditions of our experiments.

After autoclaving, the pH of the basal medium was approximately 7.0 as measured with a glass electrode. It was unaltered by the addition of the concentrations of sodium sulfadiazine, methionine and urea used.

Twenty-five ml amounts of medium containing the various test substances were inoculated with 0.1 ml of a 24-hour, synthetic medium culture of *E. coli* so that the initial populations at zero time were approximately 800,000 organisms per ml.

The numbers of bacteria after 24 hours of incubation at 37°C were determined by plating out in nutrient agar medium, five replicate plates per test culture. Counts were made after 48 hours of incubation at 37°C.

Results. The individual plate counts and the averages of the 5 replicate plates are given in Table I. All counts were made in a $1:10^6$ dilution of the original test cultures, except those so indicated. The counts are all expressed as 10^6 organisms per ml of the test culture.

The above figures show that urea overcomes the inhibitory action of methionine on the growth inhibitory effect of sodium sulfadiazine on *E. coli.* Furthermore, urea in conjunction with sodium sulfadiazine displays greater growth inhibitory effect than the latter alone on *E. coli in vitro.*

Discussion and Conclusions. Whether the urea effect is an enhancement of the sodium sulfadiazine effect alone, or a neutralization of the methionine effect, or both, cannot be established from this preliminary data, although we are investigating this point further. However, under the conditions of our experiment, the following conclusions seem to be justified:

1. With the concentration of sodium sulfadiazine used, approximately 80% inhibition of growth of *E. coli* in synthetic medium occurred. 2. In the concentrations used, methionine and urea alone, or in combination, displayed no effect on the growth of the test organism. 3. Methionine almost completely inhibited the bacteriostatic effect of sodium sulfadiazine, thus confirming previous reports. 4. Urea and sodium sulfadiazine together showed a greater growth inhibitory effect than the latter alone. 5. Urea neutralized this inhibitory effect of methionine on sodium sulfadiazine and in conjunction with the sulfonamide, even though in the presence of methionine, displayed a greater growth inhibitory effect than the sulfadiazine alone.

Work is in progress to elucidate the action of urea on the effect of other inhibitors and on other sulfonamides. We are also investigating the action of urea on sulfonamide-fast pathogens in synthetic medium, and on the bacteriostatic action of sulfonamides in experimental and clinical treatment of infected wounds.

TABLE I.

Antagonism of Anti-sulfonamide-Inhibition of Methionine and Enhancement of Bacteriostatic Action of Sulfonamide by Urea.

Exp.	Control		SAD		M	U	U + M		U + SAD		M + SAD		U + SAD + M	
	1	2	1	2			1	2	1	2	1	2	1	2
I	193	231			247									
	207	238			214									
	170	252			242									
	191	249			229									
	183				226									
Avg	189	242			231									
II	186	223	48	40		230			*11.0	* 9.7	159	164	*19.9	*34.8
	197	200	44	35		201			10.0	10.6	164	142	25.0	27.8
	177	207	43	30		233			9.6	11.3	162	162	18.3	27.1
	231	198	46	28		227			11.4	9.9	189	161	20.5	28.8
	226	229	45	31		236			10.6	10.6	179	157	19.3	31.6
Avg	203	211	45	33		225			10.5	10.4	171	157	20.6	30.0
III	184	202	43	41			167	184	*14.6	*16.6	207	145	33	32
	196	190	55	51			207	192	16.5	12.8	214	193	35	35
	218	203	55	57			214	158	15.2	19.0	213	173	34	45
	186	173	30	50			205	164	15.1	13.5	200	157	40	37
	184	192	52	65			188	190	15.4	15.7	221	173	23	43
Avg	193	192	46	53			196	177	15.3	15.5	211	168	33	38

All figures represent 10^6 organisms per ml.
*Counts made on 10^{-5} dilutions. All others made on 10^{-6} dilutions.
SAD—synthetic medium with 1.0 mg% sodium sulfadiazine.
M—synthetic medium with 1.0 mg% methionine.
U—synthetic medium with 1.6% urea.

Clinical experience[9] has confirmed the work of Holder and MacKay, and we feel that the beneficial action of urea in combination with the sulfonamides in the treatment of infections may be due partly to the effects described in this paper.

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Distribution of Vitamin A in Experimental Liver Damage.*

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The vitamin A content of cirrhotic livers is low[1, 2, 3] but in acute human[2, 4] and experimental[5] hepatitis normal amounts are found. The blood vitamin A level in acute hepatitis, however, is low[6, 7, 4] and hemeralopia appears.[8] Histologically in human cirrhosis and hepatitis the vitamin A distribution is markedly altered; the total amount often reduced.[9] To explain these alterations the histologic vitamin A distribution in experimental liver damage was studied.

Sixty-two rats were intoxicated with various doses of carbon tetrachloride. Of these 15 were on stock diet; 15 received supplements of 800 units of vitamin A every other day; 3 each received a high fat, high protein and high carbohydrate diet respectively. Of 23 vitamin A deficient rats, 13 received one dose of 800 units of vitamin A; another 6, 8 mg carotene. The organs were examined by routine histology and for vitamin A by fluorescence micro-

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