small molecules. Further studies are being undertaken to substantiate this hypothesis and to determine the nature of the forces which are responsible for the association.

The influence which slight structural modification of the small molecule has on the degree and strength of binding is also rather intriguing. The large differences which were found among the 8-substituted caffeines are particularly interesting. Variations in biological half-lives and in pharmacological activities might be partially explainable on this basis and studies are planned to test this possibility.

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Effects of Tranquilizers on Bacterial Toxemias III

Chlorpromazine

By LEO GREENBERG and JAMES W. INGALLS

Previous papers from this laboratory have indicated the role of reserpine and meprobamate in overwhelming bacterial toxemias experimentally induced in laboratory animals. The present study was primarily concerned with chlorpromazine. In rats and mice, pretreatment with chlorpromazine was shown to prolong significantly the survival of animals inoculated with lethal doses of tetanus or botulinus toxins. Similar prolongations following chlorpromazine pretreatment were demonstrated with *E. coli, S. typhosa,* and *S. marcescens* endotoxins. At identical doses, neither hydroxyzine nor methaminodiazepoxide pretreatment consistently provoked similar responses to toxin challenges.

SINCE 1957, our laboratories have been primarily concerned with the role of tranquilizing drugs in experimental stress induced in laboratory animals through the use of cultures of virulent microorganisms and by various potent microbial products, both of exotoxic and endotoxic type. We have found that reserpine exerted a highly significant life-prolonging effect in tetanus and botulinus exotoxemias and pneumococcal septicemia of mice (1). Similar prolongation of survival time in exotoxemias was found with meprobamate treatment in rats of both sexes as well as mice, although no influence on pneumococcal septicemia was observed (2). Further, the life-prolonging properties of meprobamate were shown to extend to animals inoculated with lethal doses of purified lipopolysaccharide endotoxins of *Escherichia coli* and *Salmonella typhosa* (2). In all cases, the prolongation of survival time was associated with a specific set of experimental conditions, namely the administration of a relatively large dose of tranquilizer approximately 1 hour prior to challenge with an overwhelmingly lethal dose of the toxic agent.

The present study summarizes the results of our investigation of chlorpromazine within this framework of bacterial stress. In the case of chlorpromazine, unlike that previously found with reserpine and meprobamate, the literature is replete with references, both clinical and experimental, indicating an influence of the drug in disease processes. Unfortunately, the direction and magnitude of the influence reported has sometimes been contradictory. Thus, it has been noted that chlorpromazine shortened survival time from pneumococcal (3, 4) or *Salmonella*

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enteritidis infection (2, 5), but did not influence *Salmonella typhimurium* infection (2). It shortened survival time from nasally inoculated streptococci but not from intraperitoneally inoculated streptococci (3). It either depressed (6,7) phagocytosis or enhanced (8) phagocytosis, depending upon the technique used. It had no apparent influence on *Plasmodium berghei, Trypanosoma Congolese,* or *Trypanosoma brucei* infections (3). In low doses, the drug shortened survival from *Trypanosoma cruzi* infections, but did not do so in moderate doses (9). In high doses, chlorpromazine treatment increased survival from *Trypanosoma evansi* infection (9).

In experimentally induced endotoxemias, chlorpromazine treatment is usually reported to exert a favorable influence on survival time (10–14) although no protection against *Aerobacter aerogenes* endotoxin was found (13). With *Salmonella typhosa* endotoxin, Noyes, *et al.* (13), have reported prolongation of survival time with chlorpromazine treatment in two experiments, and decreased survival time in one experiment. Little information exists concerning the role of the drug in exotoxemias. An increased mortality from *Clostridium perfringens* toxin but no influence on botulinus, tetanus, or *Pasteurella pestis* intoxication with chlorpromazine treatment has been noted (13).

Our investigation has been concerned primarily with the influence of chlorpromazine on exotoxemias and in stress induced by purified endotoxins. In almost all cases, hydroxyzine[1] and methamiondiazepoxide[2] were used as comparisons to chlorpromazine since, to the best of our knowledge, no previous study has investigated the possible role of these two potent tranquilizers in bacterial stress.

EXPERIMENTAL

Materials and Methods.—The animals used in this study were 200–300-Gm. male and female CFN rats and 20–25-Gm. CF_1 male mice (Carworth Farms), caged in small groups and kept in the thermostatically controlled animal house for several days prior to use. In all experiments, food was withheld after the toxic challenge but water was allowed *ad libitum.*

All drugs[3] were diluted with sterile, isotonic saline. In all experiments, matched groups of 10 animals were used. Tranquilizer injections were made intraperitoneally in a volume of 0.2 ml. 1 hour prior to the toxin challenge. Control animals received 0.2 ml. of sterile saline injection. The control and experimental animals in any given series were inoculated within a few minutes of one another.

Clostridium botulinum type A toxin[4] diluted with two parts of glycerol and having a mouse LD_{50} of approximately 5×10^6/ml. was used as a challenge, as was tetanus toxin[5] in 0.85% saline with 0.3 *M* glycine, an Lf. titer of 870/ml., and a mouse LD_{50} of 112 million. Toxins were stored in the refrigerator, the botulinus material at −20° to maintain potency.

Escherichia coli, Salmonella typhosa, and *Serratia marcescens* endotoxins were purchased as powdered lipopolysaccharides from Difco Laboratories. Powdered *Salmonella equi* endotoxin was graciously sent by Dr. Otto Westphal of the Dr. A. Wander Forschungsinstitut of Freiburg-Zahringen, Germany. All toxins were diluted with sterile saline and warmed to body temperature prior to intraperitoneal injection in a volume of 0.1 ml.

For the single diphtheria experiment, large male guinea pigs were purchased locally. Diphtheria toxin[5] partially purified from broth, with an LD_{50} of approximately 3×10^5/ml. was used as the challenge.

RESULTS AND DISCUSSION

The data comparing chlorpromazine with hydroxyzine and methaminodiazepoxide in various exotoxic and endotoxic conditions are summarized in Table I. From these data, a consistent pattern of response with chlorpromazine pretreatment is evident. In all cases, a moderate dose of the drug administered intraperitoneally 1 hour prior to challenge either by exotoxins or endotoxins resulted in a marked increase in survival time. This response is apparently unrelated to the general tranquilizing properties of chlorpromazine since neither of the other two tranquilizing drugs tested, hydroxyzine and methaminodiazepoxide, showed similar propensities.

Although the data are not included in Table I, preliminary studies showed that marked increases in survival time were not induced by relatively small doses of chlorpromazine (on the order of 2.5 mg./Kg.); and, as in our previous findings with reserpine (1) and meprobamate (2), no influence on survival was noted if the drug was administered after the toxin challenge.

It will be noted that of the three major exotoxins of bacterial origin, only tetanus and botulinus toxins were employed in this study. Mice and rats are notoriously refractory to diphtheria toxin, and the effects of various tranquilizers, including chlorpromazine, on survival from diphtheric intoxication in guinea pigs has been presented earlier (15). It may be of interest to mention that since this earlier study did not include data on methaminodiazepoxide, 11 guinea pigs were pretreated with 25 mg./Kg. of the drug prior to challenge with diphtheria toxin. Eleven untreated control animals showed a mean death time of 12.9 ± 0.5 hours while the treated animals died in 13.1 ± 0.5 hours, a difference of no statistical significance.

In speculating on the mode of action by which chlorpromazine manifests its antitoxic properties,

[1] Marketed as Atarax by J. B. Roerig and Co.
[2] Marketed as Librium by Roche Laboratories.
[3] Chlorpromazine supplied as Thorazine (Smith Kline and French), hydroxyzine supplied as Atarax (J. B. Roerig and Co.), methaminodiazepoxide supplied as Librium (Roche Laboratories), and reserpine supplied as Serpasil (Ciba) through the courtesy of their manufacturers.
[4] Supplied through the courtesy of Matteo Cardella, Immunology Branch, U. S. Army Biological Warfare Laboratories, Fort Detrick, Frederick, Md.
[5] Supplied through the courtesy of Dr. H. A. Dettwiler, Eli Lilly and Co., Indianapolis, Ind.

TABLE I.—EFFECT OF CHLORPROMAZINE (25 MG./KG.), HYDROXYZINE (25 MG./KG.), AND METHAMINODIAZEPOXIDE (25 MG./KG.) ON SURVIVAL TIME FROM BACTERIAL TOXINS IN MATCHED GROUPS OF RATS AND MICE

Animal	Treatment	Survival, min. ± S.E.	Significance
	Botulinus Exotoxin (1–10)		
Mice (Male)	Control	82 ± 3	....
	Hydroxyzine	78 ± 3	None
	Methaminodiazepoxide	84 ± 3	None
	Chlorpromazine	102 ± 6	$P < 0.01$
Rats (Female)	Control	240 ± 11	....
	Hydroxyzine	254 ± 17	None
	Methaminodiazepoxide	309 ± 6	$P < 0.001$
	Chlorpromazine	459 ± 14	$P < 0.001$
	Tetanus Exotoxin (1–10)		
Mice (Male)	Control	94 ± 2	....
	Hydroxyzine	112 ± 21	None
	Methaminodiazepoxide	123 ± 20	$P > 0.2$
	Chlorpromazine	150 ± 15	$P < 0.01$
Rats (Male)	Control	629 ± 11	....
	Hydroxyzine	687 ± 26	None
	Methaminodiazepoxide	647 ± 13	None
	Chlorpromazine	994 ± 71	$P < 0.001$
	Serratia marcescens Endotoxin (1–50)		
Mice (Male)	Control	995 ± 52	....
	Hydroxyzine	997 ± 77	None
	Methaminodiazepoxide	1041 ± 54	$P > 0.5$
	Chlorpromazine	1387 ± 57	$P < 0.001$
	Escherichia coli 0127:B8 Endotoxin (1–50)		
Mice (Male)	Control	806 ± 40	....
	Hydroxyzine	901 ± 59	None
	Methaminodiazepoxide	850 ± 52	None
	Chlorpromazine	1136 ± 38	$P < 0.001$
	Salmonella equi Endotoxin (1–50)		
Mice (Male)	Control	963 ± 58	....
	Chlorpromazine	1024 ± 65	$P > 0.4$
	Escherichia coli 026:B11 Endotoxin (1–50)		
Mice (Male)	Control	622 ± 38	....
	Chlorpromazine	1152 ± 55	$P < 0.001$
	Salmonella typhosa Endotoxin (1–50)		
Mice (Male)	Control	624 ± 35	....
	Chlorpromazine	1122 ± 78	$P < 0.001$

we noted with interest a suggestion by Boroff (16) that both chlorpromazine and reserpine release serotonin into the peripheral circulation and that both of these drugs should antagonize toxins since serotonin itself has been shown to exert a powerful antitoxic effect in laboratory animals (16, 17). If the underlying assumption concerning serotonin release is true, this is an attractive hypothesis, and our results with chlorpromazine would not be surprising. However, Berger, *et al.* (18), were not able to demonstrate any increase in serotonin metabolites after chlorpromazine administration, although the response was marked after reserpine administration.

Moreover, it has been reported recently (19) that a dose of reserpine known to release considerable serotonin into mouse circulation was ineffective in altering the lethality of an *Escherichia coli* endotoxin. We therefore undertook a large-scale experiment to clarify this point. Using 20 matched groups of animals, high and low doses of reserpine administered at different times prior to challenge with four different *E. coli* lipopolysaccharide preparations were evaluated. Death times were recorded hourly around the clock until the conclusion of the experiment. In no case did reserpine treatment have any significant effect, either for better or worse, on the lethality of the various endotoxins.

Until further information is available, we can only conclude that the relationships among chlorpromazine, reserpine, and serotonin in regard to their effects in endotoxemias are obscure, and we find no evidence for the suggestion that the antitoxic activity of chlorpromazine and reserpine can be explained by serotonin increase in the peripheral circulation of treated animals. In fact, after 4 years of intensive research with reserpine, meprobamate, and chlorpromazine in bacterial stress, we are unable to discern any evidence that the antitoxic properties of these three agents are manifested through a single mode of action.

SUMMARY AND CONCLUSIONS

1. Rats and mice were subjected to overwhelming bacterial stress, using purified exotoxins and various lipopolysaccharide endotoxins.

2. In all cases, a single, moderate dose of chlorpromazine administered 1 hour prior to toxin challenge resulted in a prolongation of survival time.

3. Neither hydroxyzine nor methaminodiazepoxide altered survival time significantly in toxin-challenged animals.

4. Doubt is cast on the probability that the antitoxic properties of chlorpromazine are simply a reflection of the release of serotonin into the circulation of treated animals.

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