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HEADQUARTERS DEPARTMENT OF THE ARMY
Washington 25, D.C.

GEN SESSION 4

TITLE: Problems and Progress in the Study of Oral Toxicity of Bacterial Toxins

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ABSTRACT: Food poisoning is caused by the consumption of harmful chemical products produced by the growth of bacteria. A distinction will be made between oral poisons depending on whether or not their harmful action is directly on the alimentary tract. The term entertoxin should be limited to the direct acting toxins. Chemically the orally poisonous bacterial toxins have been identified as proteins. This raises serious questions as to how such toxins can escape the digestive processes of the alimentary tract and still remain poisonous, and how such large-size molecules can cross the intestinal barrier and penetrate the blood stream. Absorption from the intestine into the blood stream takes place by way of the lymphatic system draining the intestine. Evidence will be presented for the concept that even the normal intestine presents no absolute barrier to systemic absorption of protein by way of the lymphatics. The high potency of bacterial toxins accounts for their oral toxicity. Only fantastically small amounts of toxins need escape digestion and be absorbed in order for them to still remain poisonous upon consumption and exposure to digestive juices. A hypothesis will be presented that relates food poisoning by bacterial toxins to accidental circumstances of contact with these poisons rather than any unusual chemical properties that permit them to escape the vicissitudes normal to proteins in the gut. Bacterial toxins need not have any special characteristics other than high potency in order to be capable of acting as oral poisons.

Data will be presented which demonstrate it to be scientifically fallacious to record potency in terms of weight of toxin per unit weight of the poisoned animal. The classical modes of expression of potency in terms of dose per kilogram can be misleading.

GEN SESSION 2

TITLE: Laser Progress and Applications
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ABSTRACT: The intensive laser development which is now taking place is based on a consideration of Schwalow and Townes[1] who determined that optical stimulation could occur when the difference in energy states exceeded a certain minimum value: $n > h \nu / 2\pi Q$. As a unique optical source, the laser has captured the interest of researchers and imagination of equipment developers. The output develops through emission as the population of a higher energy state is stimulated to return to the ground level. The condition of oscillation is controlled by reflectivity of cavity ends, the temperature and the effective volume. The modification of the "Q" by changes in reflectivity during laser stimulation permits operation in a single pulse having a peak power more than 3 megawatts with half power time less than 50 nanoseconds. The "pink" ruby doped with ≈.05% chromium has proved most useful operated as the three level laser. Improved efficiency and quality may be achieved by use of other materials. The laser source is unique optically characterized by its coherence, monochromaticity and high energy desity. It may be useful in range finding, special illumination and communication and guidance control; as a source for special scientific investigations; it has already found uses in microwelding and for restoring detached retinas in the eye.

1. A. L. Schwalow and C. H. Townes, Phys. Rev.112, 1940(1958).

PROBLEMS AND PROGRESS IN THE STUDY OF ORAL TOXICITY OF BACTERIAL TOXINS

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In common parlance, the term food poisoning is associated with the consumption of food in which harmful bacteria have grown and produced products which, when ingested, are upsetting to the unsuspecting victim. Caveat consumere - let the consumer beware. A large number of different and phylogenetically unrelated bacteria have been implicated in food poisoning, some frequently, and others so infrequently that their capacity to cause food poisoning remains suspect.

The signs of food poisoning should be traceable to the effects of particular compounds. In other words, one aspect of basic scientific investigations of food poisoning should be the isolation and characterization of specific compounds which, by their biological effects, can account for the signs and pathology of food poisoning. It is surprising that the number of such compounds from bacteria which have been so specifically identified is small. Most interesting is that the few such materials which are characterizable as to their general nature are proteins. As such, they are properly classifiable as exotoxins, since they appear to be secreted, excreted or otherwise released into the medium in which the bacteria grow, are antigenic, and can be neutralized by specific antibody. These are properties traditionally associated by the bacteriologist with the substances he calls exotoxins.

Scientific nomenclature contributes to clear thinking by insistence upon the use of precise definitions to identify objects and phenomena. It is in this spirit that I propose the limitation of the term enterotoxin to a certain kind of oral poison. When an exotoxin is swallowed, its harmful effect can be due to direct action on the tissue of the alimentary tract, in which case the toxin is properly spoken of as an enterotoxin: one which by direct contact specifically affects the behavior of intestinal cells. The other possibility is that the toxin does not act directly on the gut, but

rather is absorbed frc. the alimentary tract and acts specifically at sites remote from the intestinal lumen. In this situation, we can speak of the toxin as being an oral poison, since it causes harm when swallowed, but should not speak of it as an enterotoxin. Any effects observed on the alimentary tract would be the secondary consequences of action at some distant primary site: an extra-alimentary or extra-lumen site. Such a situation poses an interesting critical problem missing for the case of a true enterotoxin, namely, how can a protein escape the intestinal barriers to the absorption of large-sized molecules?

If a protein can act as an oral poison, we are inclined to make certain common sense inferences as to the properties that are responsible for its being an oral poison. We may reason that either the molecule as a whole, or some smaller specific piece or toxophore, must have some special resistance to the intestinal environment which is harmful to the maintenance of the structural integrity of a protein, for example the actions of proteolytic enzymes. If the toxin can act at a distance from the intestinal tract, we may also be led to suppose that the toxin must possess special properties to account for its transport across the intestinal permeability barriers. It is the validity of these two inferences, when tested against facts, that I will discuss, using botulinal toxin as the model of an orally poisonous exotoxin, and one which has its primary action remote from the digestive system. When I can, I shall consider the biochemical problems encountered. The goals of biochemistry are to relate toxicity to the chemical structure of the toxin molecule and to identify those factors of susceptibility of the host to the toxin which have a biochemical basis.

In botulism, the harm done to the animal victim is the result of a toxemia following ingestion of poisoned food and does not involve an infectious process. Thus we are not involved with considerations of the inflammatory process which complicate problems of toxin absorption and action.

Botulinal toxins appear to be simple proteins. It is not possible to say how the property of toxicity arises in this protein. The search for localized toxophoric groups within the structure of the protein molecule has been negative to date. A variety of unrelated physical agents and chemical reactions cause loss of toxicity. A recent hope that fluorescence of the toxin at 3300 Å after activation at 2900 Å is associated with toxicity has not been substantiated, since detoxification, for example by urea, can be accomplished without an accompanying loss of fluorescence (1). The available facts do not prove, but do support, a hypothesis that attributes toxicity to the maintenance of the structural integrity of the protein molecule as a whole.

Being willing to accept the guess that the toxic structure of botulinal toxin is a complete protein molecule raises the

question of how such a protein can escape detoxification in running the gamut of digestive juices. While the older literature generally reports botulinal toxin to be resistant to detoxification, our own work employing quantitative techniques, adequate numbers of experimental animals, and type A toxin of varying degrees of purity, has shown detoxification by trypsin and chymotrypsin to occur. Results with pepsin have been conflicting. The conclusion that must be drawn is that the capacity to act as an oral poison cannot be a matter of absolute resistance by the toxin to the activity of proteolytic enzymes in the intestinal environment (2). For lethality to be expressed, or any other action of the ingested toxin, it is only necessary for the smallest harmful amount or threshold effective dose of toxin to escape across the alimentary tract barriers before detoxification in the intestine has had time to proceed to completion.

In clinical cases of food poisoning, toxin is swallowed with a variety of different foods. It is conceivable that ingested foods can affect the oral potency of toxin by influencing the rate of intestinal detoxification, for example, by competition for or inhibition of proteolytic enzymes. We have been able to establish that the state of alimentation and the kinds of foods ingested with toxin do influence oral toxicity as measured by changes in LD_{50} lethal oral dose values (3). Foods and toxin were given to a mouse in separate per os injections. This procedure was adopted in preference to mixing food and toxin in vitro in order to insure that all results were the consequence of in vivo interactions exclusively. Foods may act to increase or decrease oral toxicity of a fixed quantity of ingested toxin. In TABLE 1, for example, it is demonstrated that olive oil and egg albumin can increase oral toxicity both in terms of increasing the rapidity of deaths and total number of individuals succumbing to a given quantity of toxin. This is unexpected if we have been thinking in terms of food competing for proteolytic enzymes in which case we would not predict an increase in toxicity in the presence of olive oil, a substance which does not react with proteolytic enzyme. Probably different foods can affect toxic potency by different mechanisms

Incidentally, it is a part of the mythology of botulism that those who partake of alcoholic beverages at the fatal feast suffer less serious consequences than those who do not indulge. A few experiments, therefore, have been performed to test this belief. As can be seen in TABLE 2, ingestion of mixtures of brandy and egg albumin, a reasonable simulation of human experience, particularly at Xmas and New Year's parties, and mixtures of brandy with olive oil tended only to reverse slightly, if at all, the enhancing effect on toxicity of the food without reducing potency below the level experienced upon ingestion of toxin alone. Thus, the consumption of alcoholic beverages has no remarkable prophylactic value in botulism except insofar as the true imbiber eats less food. Taking into

account the weight difference between man and mouse at higher doses of brandy than those employed in the experiments performed, one is subject to the risk of drinking lethal quantities of brandy. In such an event, the anticipated cure might be more pleasant than the disease, but equally fatal.

Insufficient data are at hand to permit generalization and prediction of how particular kinds of foods will act. We do not know by what mechanisms the foods affect the toxic dose. But five possibilities worthy of investigation are self evident: foods might protect toxin against destructive intestinal influences; they might act to increase or decrease the secretion of digestive juices; they might combine with the toxin to form larger particles less able to penetrate the gut wall than is free toxin; they might modify the physiological bases of intestinal permeability; or they might have an effect on the rate of peristalsis with a consequent decrease or increase in the sojourn of the toxin in the part of the intestine offering the maximum opportunity for systemic absorption. No one of these possibilities has been adequately explored.

I suspect the effect of food is less by direct action on the toxin than on mechanisms influencing peristalsis and the permeability of the small intestine to whole protein. If foods did interfere with detoxifying proteolysis in the gut, one might hope to show an enhancement of oral toxicity by the use of specific inhibitors of enzyme, trypsin. Soybean and egg albumin trypsin inhibitors have been tried and both fail by their presence in the alimentary tract to affect the oral toxicity of the tetanus and crystalline botulinal toxins (TABLE 3) under the conditions of our tests. The total number of mice succumbing to varying doses of orally administered toxin was not found to be significantly different in the presence and absence of the trypsin inhibitor when a twofold dilution series of toxin was employed at dilutions somewhat above and below the oral LD_{50} dose. The inability of trypsin inhibitor to increase oral potency of the toxin was disappointing, since one would guess that a significant fraction of the great difference in the amount of toxin required for an oral lethal dose relative to a parenteral dose would be the result of destructive tryptic proteolysis in the gut which should be reversed by a specific enzyme inhibitor.

We will now turn our attention to the problems of permeability. How does the toxin go from the intestine to the blood stream? There is good evidence that the route taken is a lymphatic one (4,5), and that the lymphatic route is the only avenue (5). If the lymph draining the intestine is diverted from the body by cannulization of the thoracic duct so that none of the lymph can be spilled over into the blood stream, botulinal toxin fed an animal does not cause poisoning (5). This observation means two things, one, that absorption from the gut is limited to the lymphatic route and second, that the toxin poisons exclusively by direct action on extraintestinal tissue. These observations return a dividend of general scientific usefulness.

They provide us with an experimental means for deciding whether or not an oral toxin acts directly on the gut and legitimately can be considered a true enterotoxin. This question is not always easily answered. A case in point is the staphylococcal enterotoxin which traditionally has been thought to act on the intestinal tract directly, a point of view which has been placed in doubt by investigators at the University of Chicago. Since the staphylococcus toxin is a protein, it seems possible that a definitive answer should be forthcoming by observing animals orally fed the toxin and cannulated to prevent intestinal lymph from flowing into the general circulation.

Does the toxin that escapes from the alimentary tract and enters the blood stream actually have the dimensions of a protein? Such a question is related to the biochemical one of the size of the ultimate toxic particle.

Since in the natural situation botulism results from food poisoning, one might hope Nature to be parsimonious and to permit only the toxicologically active fragment of the protein particle to escape from the intestine into the general circulation. Such a possibility would be reinforced by any normal tendency of the alimentary tract's permeability barriers to refuse passage to whole protein. Heckly, Hildebrand and Lamanna (4) have studied this question. They have found the systemically absorbed toxin which appears first in the lymph and then in the blood to have the dimensions of a protein. By ultracentrifugal analysis, the sedimentation value (S_{20}) of the toxin appearing in the lymph draining from the small intestine of the rat was found to be 7.9±3.5 which is within the size range of protein. Defeat has followed an attempt to find toxicity resident in a particle smaller in size than a protein.

The data available indicate that the toxin in lymph has dimensions within the range for proteins and need not be broken down to smaller non-protein elements in order to pass through the intestinal barriers into the lymphatic system. While the observed sedimentation coefficient of the absorbed toxin in lymph was significantly less than of crystalline toxin, it is probable that a small percentage of the crystalline toxin can dissociate to the smaller protein particle size, and it was this portion of toxic protein whose passage into the lymph was favored.

There is no evidence that crystalline toxin in the intestine is "digested" into smaller-sized toxic particles. This statement is based on sedimentation coefficients determined for crystalline toxin both before and after exposure to residence in the small intestine of the rat for a period of 2 hours. The particle size of the bulk of the toxin was not demonstrably reduced by exposure of the crystalline toxin to the digestive process in the living intestine. The sedimentation coefficient of the batch of crystalline toxin employed was in good agreement with the 19.3 value or 900,000 molecular weight reported for this material (6).

There is no reason to believe that the toxin crosses the small intestine as smaller than protein particles which are reaggregated in lymph to the dimensions of a protein. Rather our view is that the small intestine does not present an absolute barrier to the passage of protein. Botulinal toxin is but one among many whole proteins which can be absorbed from the small intestine in small quantities.

That the true particle size of toxin in lymph is not determinable by sedimentation studies because of absorption of the toxin to albumin is most unlikely. Since albumin is the most abundant and highly charged of the lymph proteins one might infer the toxin to be absorbed above pH 7 to albumin rather than to globulin. The fact that the toxin present in lymph migrates electrophoretically at the same rate as crystalline toxin, rather than at a rate corresponding to some value intermediate to toxin and albumin or to the value for albumin argues against the existence of a small molecular weight toxophore adsorbed to albumin. In addition, toxic lymph when dialyzed against serum albumin does not release toxic material able to pass across the walls of dialysis tubing. By placing mixtures of crystalline toxin and proteolytic enzymes in dialysis tubing one does not find toxic material escaping from the bag. This result might be expected to follow if proteolytic enzymes could chop off pieces of the protein molecule, and thus permit the escape of smaller-sized dialyzable toxophoric pieces. The conclusion to be drawn from these experiments (4) is that the measured sedimentation coefficients of toxin which has passed from the intestine into lymph are values for toxin unassociated with a carrier protein.

In another effort to settle the question of the size of the ultimate toxic particle, we have determined the sedimentation coefficient of type A botulinal toxin in lymph and blood after intravenous injection in rabbits (7). Such exposure of toxin to the in vivo extra-alimentary environment for as long as two hours did not reveal the occurrence of toxic materials with dimensions smaller than that of a protein. Thus residence in the body fluids did not demonstrably result in any disassociation or breakdown of the toxin to low molecular weight non-protein toxic moieties.

Alone among the classical bacterial exotoxins, botulinal toxin has been considered to be an oral poison. If botulinal toxin is truly unique in this respect, exploration of this situation might provide clues of a biochemical nature to the biological properties of the toxin. Unfortunately, oral toxicity is not a characteristic unique to botulinal toxin (8, 9). Both diphtheria and tetanus toxins, materials not associated with clinical cases of food poisoning, can act as oral poisons and at the very most are only one order of magnitude less toxic orally than botulinal toxin in terms of the number of intraperitoneal LD_{50} doses equivalent to one oral LD_{50} dose (TABLE 4). This finding suggests that oral toxicity of the bacterial exotoxins is not an expression of intrinsic qualities of chemical

structure of the toxins, but rather is a consequence of a physiological fact. This fact is the inability of the alimentary tract of the so-called normal animal to prevent the escape of small quantities of different kinds of whole proteins into the general circulation by way of the lymphatic route. The alimentary tract does not present an absolute barrier to the systemic absorption of whole protein, a fact which allergists have long recognized (10). The prime avenue of escape is the small intestine, probably the jejenum chiefly. Intrarectal instillation of toxin in monkeys (11) and rabbits (12) is slower than oral administration in causing deaths.

Potentially, any toxic protein is an oral poison if its potency is high enough for the minute amounts crossing the intestinal wall to exceed the threshold values for physiological activity at locations distant from the intestine. I emphasize the term minute quantity, since a lethal dose of botulinal toxin for the mouse involves fantastically small weights of material, the order of 1 to 10 thousandths of a millionth of a gram. The fact of escape of toxic protein through the wall of the large and small intestines should not shock us in spite of the classical teaching of physiology that the intestine is a formidable barrier to passage of protein. Hogben (13) has neatly stated a philosophy relevant to the problem of the penetration of tissue barriers by large-sized molecules such as microbial toxins: "Passage across cell membranes must be considered in statistical terms of likelihood and unlikelihood. Given a sufficiently sensitive method, any substance can be shown to cross a boundary". Even objects as large as non-pathogenic bacteria and yeast can pass from the intestine of normal rats to lymph though the numbers are extremely small (14). There is a possible correlation between the size of a particle and the number penetrating the gut wall since fewer yeasts escape than bacteria, and fewer of these large particles than toxin. Is such experience indicative of a similar path and mechanism of escape from the intestine for these qualitatively different kinds of particles, namely, diffusion from the intestine through "holes" in the intestine varying statistically in diameter in a normally distributed manner?

With bacterial toxins, for example the neurotoxins, extremely small rates of passage of proteins across tissue barriers can have pathological consequences. This means we cannot divert the passage of toxic proteins in the same vein as the physiologist, who, in considering permeability of tissues to proteins, is generally focusing his attention on orders of magnitude of penetration considerably beyond those of concern to the bacteriologist, immunologist and pathologist. Incidentally, the bacterial toxins can serve as useful tools to monitor the specificity of action of substances changing the permeability of the intestine to particular classes of compounds. For example, disodium ethylenediaminetetraacetic acid (EDTA) increases absorption of heparin and heparinoids (15), highly charged anionic substances, but does so without increasing

passage of botulinal t xin in mice (16) or non-pathogenic bacteria in rats (14).

I should now like to turn my attention to the question of the oral dose of toxin required for poisoning the individual.

The opportunity for harm to befall the host, is a relative matter arising from the interaction of host factors and the harmful agent. In botulism the biochemical substrate of the peripheral nervous system of the host affected by the toxin may be in quantity independent of the body weight of the poisoned animal. This can follow from the fact that the number of nerve cells in an individual is fixed at birth and so does not increase with size and age of the individual. In mice lack of a relationship between body weight and the quantity of toxin required for a fatal parenteral dose has been found (17). The weight of toxin required for a fatal dose is the same for the small and large mouse. This is not a finding peculiar to botulism. We have found the same fact to be true for tetanus toxin (TABLE 5). Similar reports exist for Shigella paradysenteriae endotoxin in mice (18), β -naphthyl-thiourea in rats (19) and histamine in mice (20).

Of interest is the fact that the experience with a parenteral route of injection cannot be generalized to include the oral route. With type A crystalline botulinal toxin and tetanus toxin which we have tried, the youthful mouse required more toxin than did the older heavier mouse for a lethal dose (TABLES 6 and 7). While common sense might dictate a skeptical attitude toward such a finding, a fact of anatomy may justify the finding. In length the small intestine averages 40 cm in the young 12-14 g mouse, and 57 cm in the old 40-43 g mouse. There is, roughly speaking, 50 per cent more intestinal surface area provided for the systemic absorption of toxin in the large than the small mouse under comparison. Since the small and large mouse require the same minimum parenteral dose for lethality, the lesser oral dose for the large mouse could merely reflect the greater opportunity for systemic absorption before peristalsis removes ingested toxin from the bounds of the small intestine where absorption is most prominent.

No evidence has been developed for pinpointing the chemical molecular basis for toxicity in botulism, staphylococcal food poisoning and in infectious diarrheas. Oral toxicity for botulinal and other recognized food poisoning toxins can hardly be considered an unusual property because diphtheria and tetanus toxins, bacterial exotoxins not ordinarily thought of as oral poisons, will cause toxemia when ingested in sufficient quantity. Perhaps in diphtheria this fact has some role to play in the natural infection since the organisms growing in the naso-pharyngeal area are producing toxin which must in part be ingested as an inevitable consequence of the swallowing reflex. In some clinical cases of cryptic tetanus it

would be wise to seek for an unsuspected source of intestinal absorption of toxin.

Oral toxicity of any toxin would appear to be affected by any factor which can influence the length of residence of active toxin in the small intestine or the permeability of any portion of the gut to whole protein. Our understanding of these factors is still at the stage of development of fundamental descriptive data. We require detailed knowledge of the anatomical and physiological bases for toxicity by the oral route before we can achieve biochemical understanding of toxicity at the molecular level.

What general conclusions about bacterial exotoxins as oral poisons can we draw which has relevance to clinical medical experience? Unless a toxic protein produced by a bacterial species can directly adversely affect the normal activity of the alimentary tract, food poisoning should not be attributed to any unique intrinsic chemical properties which account for oral toxicity and are absent for other kinds of poisoning by bacterial toxins. The actual assessment of the capacity of a bacterial exotoxin to act as an oral poison must rest on an understanding of ecological circumstances: the relationship of food consumption to food preparation, and environmental factors influencing bacterial production of toxins in foods. These circumstances determine whether or not a particular organism will occur in a food and can grow to produce sufficient toxin to survive food preparation procedures such as cooking and to avoid total destruction in the intestine so that small quantities escaping the alimentary tract barriers by way of the lymphatic route are above the threshold values needed for pathological effects to manifest themselves. In this light it is sanitation, bacterial ecology, and the feeding habits of animals, and not biochemistry which have the stronger light to cast upon the calculation of the possibilities for the actual occurrence of clinical cases of food poisoning. This concept expands the list of harmful organisms which potentially can cause food poisoning. We can expect on rare occasions the proper interconnection of events which will result in cases of food poisoning by organisms not ordinarily believed to be food poisoning organisms.

The basis for oral toxicity would seem to rest on high potency of a toxin associated with a lack of capacity of the small intestine to prevent, in an absolute sense, the systemic absorption of proteins in small quantities. My basic hypothesis then, is that oral poisoning by a bacterial exotoxin is an accident of immediate circumstance. The historical biological origin of oral toxicity is not in an orderly evolution of proteins specifically directed toward the acquisition of unique properties conferring the character of oral toxicity.

In reviewing what has been said, one must be impressed with the predominance of questions raised rather than solidly established facts offered. I have adopted this mode of presentation consciously. We are living in an age when it has become the habit of scientific institutions to sell themselves to the public. As a result the popular press is bombarding us with a continuous succession of scientific triumphs. This must sometimes have a discouraging effect upon the uninitiated scientist and students. By constantly praising ourselves, the rising generation may come to the feeling that it has grown up too late to participate in the real progress of science. The administrator may tighten his purse strings against the true need for research unless he can be promised a materialization of the breakthroughs he has been reading about. And so I chose my emphasis with an eye to satisfying the need to reassure our neighbors that we have not achieved true wisdom in all things. There remains many a thing both elementary and subtile to be learned even in such an old-fashioned subject field as is represented by the bacterial toxins.

In conclusion, I hope I have reassured you that if the food you eat poisons you, it is an accident and not a diabolical plot against you planned by Mother Nature. Good day and good eating to you.

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Table #1

Effect of per os injection of egg albumen & olive oil on the toxicity of botulinal toxin

Cumulative Deaths after Receiving Toxin per Os									
Toxin Dilution	Hr	Sham	Albumen	Olive Oil	Toxin Dilution	Hr	Sham	Albumen	Olive Oil
1:2	12	1	6	9	1:8	12	2	4	3
	24	7	16	15		24	6	9	8
	36	10	20	18		36	7	14	12
	48	12	20	20		48	9	15	13
	48	17	20	20		48	11	20	16
1:4	12	0	5	3	1:16	12	2	1	0
	24	1	12	6		24	3	1	4
	36	2	14	9		36	3	2	6
	48	4	16	10		48	4	2	6
	48	9	18	14		48	5	12	6
Total Deaths.....							42	70	56

(From 3)

Table #2

Effect of brandy (96 proof) on the per os toxicity of botulinal toxin in the presence of food

(a) Presence of olive oil*

Cumulative Deaths after Ingestion of Toxin									
Hr	Undiluted			1:2			1:4		
	Control	Olive Oil	Olive Oil & Brandy	Control	Olive Oil	Olive Oil & Brandy	Control	Olive Oil	Olive Oil & Brandy
6	2	4	3	2	0	3	1	4	1
12	5	15	8	3	1	4	3	4	2
18	12	17	12	4	4	5	5	4	2
24	15	19	12	9	9	9	6	6	3
30	18	20	16	12	12	10	8	10	7
36	19		19	15	12	11	9	12	8
42	19		20	16	12	12	9	14	10
48	20			16	13	13	10	15	13
60				17	15	14	11	15	14
72				18	15	14	11	15	13
72					16	15	13	17	17
Total Deaths.....							51	53	52

(b) Presence of egg albumen

Continued on next page

TABLE #2 Continued

Hr	Cumulative Deaths at Hr Indicated after Toxin Ingestion — Dilution of Toxin								
	1:1.5			1:2			1:4		
	Sham	Albumen	Albumen & Brandy	Sham	Albumen	Albumen & Brandy	Sham	Albumen	Albumen & Brandy
12	1	3	1	1	2	0	1	1	0
24	2	6	6	2	2	2	1	2	2
36	2	12	8	3	5	2	2	3	3
48	4	14	10	3	5	3	2	4	4
60	5	15	10	3	7	4	2	4	4
72	5	15	10	4	7	4	2	4	4
72	5	15	13	4	9	5	2	4	4
Total deaths							11	28	22

(From 3)

Table #3

NUMBER OF MICE SUCCUMBING TO ORALLY ADMINISTERED CRYSTALLINE TYPE A BOTULINAL TOXIN IN THE PRESENCE AND ABSENCE OF ORALLY ADMINISTERED TRYPSIN INHIBITOR

Experiment	1	1	2	3	1	2	1
	Soybean trypsin inhibitor						
Inhibitor present per mouse	0.2mg.11/32*	0.5mg.	3/12	2/12 9/20	1mg	23/32 9/32	1.5mg 12/32
Inhibitor absent	12/32		3/12	4/12 11/20		25/32 8/32	13/32
	Eggwhite trypsin inhibitor						
Inhibitor present per mouse	0.5mg.8/32	1mg.	22/32	10/32 1/32	2mg.		
Inhibitor absent	11/32		21/32	8/32 4/32		8/32	

*Dead mice/number tested.

(From 9)

TABLE #4

NUMBER OF INTRAPERITONEAL LD_{50} REQUIRED FOR ONE ORAL LD_{50}*

Species	Toxin	
Mouse, 20 gm.	Botulinal type A	50,000 to 250,000
Mouse, 20 gm.	Tetanus	80,000 to 1,200,000
Guinea Pig 900 gm.	Diphtheria	100,000

*With the mice the toxins were administered orally by the use of a slightly curved blunt-nosed needle on a syringe. Diphtheria toxin was administered by forced feeding of gelatin capsules containing concentrated toxin. These methods appear to successfully introduce toxin into the gut without contamination of the mouth and throat and appear to be well tolerated without evidence of tissue trauma. Deaths, therefore, are thought to be truly representative of absorption of toxin from the normal gut.

(From 9)

TABLE #5

INTRAPERITONEAL LD_{50} OF TETANUS TOXIN SOLUTION WITH DIFFERENT WEIGHT MICE*

Average Weight				
Small Mice (gm)	Large Mice (gm)	Technician	Small mice	LD_{50} Large mice
7.6	39.3	A	1,631,000	1,350,000
9	40	A	189,000	244,000
9.7	40.1	A	364,000	283,000
9.4	39.6	A	389,000	305,000
11	39	A	111,000	55,000
		B	81,000	53,000
9.4	37	A	257,000	259,000
		B	323,000	212,000

* The titration values for the small and large mice are not significantly different, the variation being within the limits of experimental error. The method of Pizzi (1950), which permits the use of the Reed and Muench type of calculation, was employed for determining the standard error of the LD_{50}.

(From 9)

TABLE #6

TITRATIONS BY THE ORAL ROUTE OF CRYSTALLINE BOTULINAL TYPE A TOXIN IN MICE OF DIFFERENT WEIGHTS

Toxin (ml.)	Weight of mice (grams)										
	Experiment 1		Exper. 2		Exper. 3			Exper. 4			
	13-15	38-40	12-14	40-43	13-14	20-22	37-39	13-14	20-22	34-36	
0.08					20/20	17/20		12/20	15/20		
0.04	19/20*	20/20			17/20	13/20	17/20	5/20	9/20	13/20	
0.02	2/20	20/20	7/20	16/20	2/20	8/20	11/20	1/20	1/20	11/20	
0.01	1/20	9/20	1/20	15/20	8/20	5/20	8/20	0/20	2/20	10/20	
0.005	2/20	5/20	3/20	7/20	1/20	3/20	7/20	1/20	1/20	0/20	
0.0025	1/20	3/20	1/20	3/20			2/20			0/20	
0.00125			0/20	2/20							

* Dead mice/number injected. The difference in deaths between the 13-14 gm. and 20-22 gm. mice is probably not significant. On the other hand, the LD_{50} for the largest mice is significantly less (i.e. toxin required for death) than for the smaller mice.

(From 9)

TABLE #7

ORAL TOXICITY OF TETANUS TOXIN FOR MALE MICE OF DIFFERENT WEIGHTS

Toxin (ml.)	Weight of mice (grams)					
	Experiment 1		Experiment 2		Experiment 3	
	13-14	37-39	13-14	38-40	13-14	34-35
0.75			2/12	10/12	4/12	8/12
0.50	5/12*	6/12	2/12	4/12	5/12	3/12
0.25	2/12	1/12	0/12	3/12	1/12	4/12
0.125	0/12	1/12	0/12	4/12	0/12	2/12
0.0625	0/12	0/12				
0.03125	0/12	0/12				

*Dead mice/number injected.

(From 9)