A COMPARATIVE STUDY OF THE EFFICIENCY OF CERTAIN GERMICIDES IN THE PRESERVATION OF BIOLOGICS

By

WILBUR G. George MALCOLM III

Thesis submitted to the Faculty of the Graduate School of the University of Maryland in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

1930

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

A COMPARATIVE STUDY OF THE EFFICIENCY OF CERTAIN GERMICIDES IN THE PRESERVATION OF BIOLOGICS.

The matter of preservation is of the greatest importance in the manufacture of biologics that are to be employed in the treatment of human diseases. The use of phenol and trikresol in the capacity of biologic preservatives is almost universal. These chemicals, however, are not entirely satisfactory. With the comparatively recent development of a number of germicidal substances, it seemed appropriate to make a study of some of them to determine whether or not they might be employed to advantage.

There are five distinct features of major importance which must be known in order to judge the potentialities of a germicide as a biologic preservative. Therefore, the preservatives were studied with a view to determining the following:

(1) The germicidal potency upon spores and vegetative cells of bacteria in different media.

(2) The effect upon the immunizing power of the various products.

(3) Toxicity of the germicide, with special reference to the margin of safety including intravenous, intramuscular and intraspinal toxicity tests.

(4) Effect upon the appearance and the precipitation of protein substances.

(5) The histologic changes produced in the animal tissues.

Method of Procedure.

The problem was divided into two distinct parts with different methods of approach. The first portion was a study of the germicidal power of the various chemical substances together with the physical effect upon the basic media employed. Only substances found satisfactory in the first phase of the problem were given further study. The second division of the work was a study of the toxicity of the preservative; its effect upon the potency of the biologics; and the histologic changes produced.

The following germicides were chosen:

1. Phenol (crystals)
2. Hexyresorcinal (S.T.37)(1-1000)
3. Colloidal chlorine cresol - 25% strength
4. Colloidal chlorine thymol - 25% strength
5. Colloidal chlorine cresol - 50% strength
6. Colloidal chlorine thymol - 50% strength
7. Trikresol
8. Yatren (powder)
9. Mercurophen (powder) - 2% aqueous solution
10. Metaphen (1-500) 2% aqueous solution

GERMICIDAL STUDIES.

Determination of germicidal power in salt solution, boullion, and serum, together with coloration and precipitation of protein substances.

A sterile physiological salt solution was used. Plain boullion was employed made from beef infusion to which 1% peptone and .5% sodium chloride had been added. The titration was adjusted to a pH. value of 6.8. The medium was checked for sterility after having been autoclaved for one half hour at 15 pounds pressure.

Horse serum was employed, the blood being taken aseptically, defibrinated and the serum carefully checked for sterility. It was then placed in a refrigerator at 40°F. until used.

Method of Making Dilutions.

Two procedures were employed in making dilutions. In the first method, 1% standard solutions of the chemicals were made, using distilled water as the diluent. From the original 1% solutions, the higher dilutions were made by the addition of the basic media employed in each particular study. The total amount of the chemical and media was made up to 5 c.c. An example is as follows:

Original strength of germicide 1%.

Amt. of chemical	Amt. of media	Per cent dilution	Total c.c.
2.5 c.c.	2.5 c.c.	0.5	5
3.0 c.c.	2.0 c.c.	0.6	5
3.5 c.c.	1.5 c.c.	0.7	5
4.0 c.c.	1.0 c.c.	0.8	5
4.5 c.c.	0.5 c.c.	0.9	5

The dilutions were made in sterile test tubes by means of sterile calibrated pipettes, measuring in tenths.

This method was discarded after a number of determinations had been made. There was not sufficient organic matter present in the media to furnish nutritive material for the bacteria which had survived the bacteriostatic action of the chemical substance. An excessively high efficiency was obtained for all the germicides giving a false interpretation of the results. The second method of dilution insured a sufficient amount of basic media for the bacteria to grow in and approximated more closely the condition found in the biologics.

The dilutions were made so that the total volume of chemical and media was 5 c.c. The germicide was added directly to the media without dilution with distilled water. It may therefore be seen that the manner in which the dilutions were made influences to a great extent the bacteriostatic action of the germicide.

Organisms Employed.

Four species of bacteria were employed in each dilution of the germicide; namely, Bacillus coli, Staphylococcus pyogenes albus, Bacillus mycoides, and Bacillus subtilus. It has been demonstrated that many germicides are more effective against some species of bacteria than others. Other reasons for the choice of these four species were the frequency with which they are found in contaminated biologics. The first two species are vegetative cells only and the latter two are spore forming bacteria.

Inoculations and Controls.

One tenth of a c.c. of each of the cultures was added to 10 c.c. of plain broth and the whole incubated for 24 hours. At the expiration of this time the cultures were filtered to remove the clumps and to produce as uniform a distribution of the bacteria as possible. One tenth of a c.c. of each of these cultures was added to the specified dilutions of the germicide requiring four tubes for each dilution; i.e., one dilution for B. coli, one dilution for Staphylococcus pyogenese albus, one dilution for B. mycoides and one for B. subtilus. After inoculating the various dilutions, they were allowed to stand at room temperature for 24 hours.

Determining the Germicidal Action of the Chemicals.

The tubes were next examined for visible growth and the results were recorded. Then each dilution was plated separately on plain agar media. One c.c. of each of the dilutions was seeded over the surface of plates that had previously been poured, allowed to harden, and incubated for sterility. The inoculated plates were then placed in a 37°C. incubator for 24 hours and examined for bacterial growth. The presence of growth on the plates is indicated in the tabulations by a plus sign and the absence of growth by a minus sign.

Physical Effect of the Germicides upon the Media.

(a)Careful attention was paid to the coloring action of each germicide upon the media employed, (b) Change in the turbidity

of the salt solution, (c) Precipitation of the albuminous material of the boullion and serum and, (d) Miscibility of the germicides with the media. These factors were considered in each of the dilutions made.

Study of Germicidal Action.

Phenol (C_6H_5OH).-

Phenol, due to its antiseptic properties, has been employed as a preservative in biologic products. Chemically it is C_6H_5OH, with the structure 

Results

Chart #1.

Preservative		%	B. coli 1	Staph. albus 2	B. mycoides 3	B. subtilus 4	Media	Physical Effects
Phenol	1-200	0.5	-	-	-	-	Salt Sol.	None
"	1-250	0.4	-	-	-	-	" "	"
"	1-333	0.3	-	-	-	-	" "	"
"	1-500	0.2	-	-	x	x	" "	"
"	1-1000	0.1	-	-	x	x	" "	"
"	1-200	0.5	-	-	-	-	Boullion	Precipitate
"	1-250	0.4	-	x	x	x	"	"
"	1-333	0.3	x	x	x	x	"	"
"	1-500	0.2	x	x	x	x	"	Very light ppt.
"	1-1000	0.1	x	x	x	x	"	" " "
"	1-200	0.5	-	-	-	-	Serum	Precipitate
"	1-250	0.4	-	x	-	-	"	"
"	1-333	0.3	-	x	x	x	"	"
"	1-500	0.2	-	-	x	-	"	Light ppt.
"	1-1000	0.1	x	x	x	x	"	" "

Phenol killed all four species of bacteria in salt solution 1-[illegible] parts; boullion 1-200 parts; serum 1-200 parts.

It caused no discoloration of the salt solution, boullion, or serum. There was a precipitation of the protein in the boullion and serum at the dilutions in which it was germicidal. This precipitate formed almost immediately and increased with agitation. In a comparatively short time it settled to the bottom of the tube leaving the media practically clear. It was also found to be miscible with the medias at the dilutions employed.

Trikresol

This is a coal tar derivative, extensively used as a biologic preservative, because of its antiseptic properties. Chemically it is

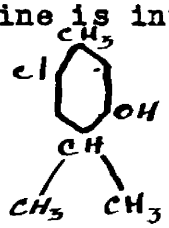
Results.
Chart #2.

Preservative		%	B. coli	Staph. albus	B. mycoides	B. subtilus	Media	Physical Effects			
Trikresol	1-200	0.5	-	-	-	-	Salt sol.	Water	clear	no	ppt
"	1-250	0.4	-	-	-	-	" "	"	"	"	"
"	1-333	0.3	-	-	-	-	" "	"	"	"	"
"	1-500	0.2	-	-	-	-	" "	"	"	"	"
"	1-1000	0.1	-	x	x	x	" "	"	"	"	"
"	1-200	0.5	-	-	-	-	Boullion	Light ppt.			
"	1-250	0.4	-	-	-	-	"	"	"		
"	1-333	0.3	-	-	-	-	"	"	"		
"	1-500	0.2	-	-	x	x	"	Very light ppt.			
"	1-1000	0.1	-	x	x	x	"	None			
"	1-200	0.5	-	-	-	-	Serum	Water	clear	no	ppt
"	1-250	0.4	-	-	-	-	"	"	"	"	"
"	1-333	0.3	-	-	x	-	"	"	"	"	"
"	1-500	0.2	-	x	-	-	"	"	"	"	"
"	1-1000	0.1	x	x	x	x	"	"	"	"	"

Trikresol leaves the salt solution quite clear. It did not cause any discoloration of the serum or boullion. There was a precipitation of the protein material in the germicidal dilutions in both the bouillion and serum. In salt solution bacteria were killed in 1-500 parts; bouillon 1-333 parts; and serum 1-250 parts.

Colloidal chloro-thymol - 25%

This is an amber-yellow solution, water soluble with a weak thymol odor and containing chloro-thymol up to 25%. Thymol is a methyl-isoprophyl-phenol of the formulae C_6H_3-CH_3(1)-C_3H_7(4)OH(3). In this preparation by a substitution of the hydrogen atom the halogen chlorine is introduced into the benzol nucleus. Graphically it is



It was expected that by the introduction of a halogen in the benzol nucleus it would cause a further increase in disinfection value by a decrease in its toxicity[14].

Results.
Chart #3.

Preservative	Dil.	B.coli 1	Staph. albus 2	B. mycoides 3	B.subtilus 4	Media	Physical effects
Colloidal-thymol 25%	1-200	-	-	-	-	Salt Sol	Cloudy, bluish-white pearl
	1-250	-	-	-	-	" "	Cloudy, pearl-grey color
	1-333	-	-	-	-	" "	Cloudy, pearl-grey color
	1-500	x	x	x	x	" "	Cloudy, pearl-grey color
	1-1000	x	x	x	x	" "	Cloudy, pearl-grey color
	1-200	-	-	-	-	Bouillon	Milky-white color, fine ppt. which stays in suspension.
	1-250	x	-	-	-	"	Same as above
	1-333	x	-	x	-	"	Grey color, ppt. in suspension
	1-500	x	x	x	x	"	Grey color, ppt. in suspension
	1-1000	x	x	x	x	"	Grey color, light ppt
	1-200	-	- c	-	-	Serum	Very heavy coagulan some held in suspension, greater share settling rapidly
	1-250	x	x	-	-	"	Straw colored, heavy ppt. that settles rapidly
	1-333	x	x	x	-	"	Serum normal color, very slight ppt. that settles
	1-500	x	x	x	x	"	Negligible ppt. color normal
	1-1000	x	x	x	x	"	Negligible ppt. color normal

This substance was miscible with the media.

It gave the salt solution a cloudy bluish-white appearance, and was effective in 1-333 parts. The germicide killed in 1-200 parts in bouillon and serum. In both of the latter media there was a very heavy precipitation together with a marked turbidity.

Colloidal chloro-cresol - 25%

This also is an amber-yellow solution, water soluble, with a weak odor of cresol and contains chloro-cresol up to 25%. Cresol chemically is $CH_3C_6H_4OH$. In this preparation as with colloidal chloro-thymol, the hydrogen atom is substituted by the halogen chlorine.

Results.
Chart #4.

Preservative	Dil.	B. coli 1	Staph. albus 2	B. mycoides 3	B. subtilus 4	Media	Physical effects
Colloidal Cresol 25%	1-200	-	-	-	-	Sol. Salt	Very pale pearl-grey color
"	1-250	-	-	-	-	" "	Very light bluish tint
"	1-333	-	-	-	-	" "	Very light pearl-grey color
"	1-500	-	-	-	-	" "	Very light pearl-grey color
"	1-1000	x	x	x	x	" "	Normal color
"	1-200	-	-	-	-	Bouillon	Bluish-white color with formation of flocculant ppt. which settles rapidly
"	1-250	-	-	-	-	"	Light pearl color with ppt. remaining in suspension
"	1-333	-	-	-	-	"	No change in color, fine ppt. held in suspension
"	1-500	-	-	+	+	"	No change in color. No ppt.
"	1-1000	-	x	x	-	"	No change in color. No ppt.

Chart #4 (cont'd)

Preservative	Dil.	B.coli	Staph. albus	B. mycoides	B. subtilus	Media	Physical effects
Colloidal		1	2	3	4		
Cresol 25%	1-200	-	-	-	-	Serum	Heavy ppt. of serum which is held in suspension
"	1-250	-	-	-	-	"	Light straw color, ppt. with rapid settling
"	1-333	-	x	-	-	"	Serum normal color, ppt. in bottom of tube
"	1-500	x	x	-	-	"	Very slight ppt. normal color
"	1-1000	x	x	x	x	"	Normal color, negative ppt.

Colloidal creol (25%) in the salt solution was effective in 1-500 parts giving a very pale pearl-grey color to the solution. In the boullion and serum there was a heavy precipitation of the proteinous material, with a rapid settling of the precipitate. The germicide was effective in boullion in 1-333 parts and serum in 1-250 parts.

Colloidal cresol - 50%

Colloidal thymol - 50%

This strength of the above substances was recommended by the manufacturer after the failure of the 25% solution to prove

satisfactory in this work due to the heavy precipitation of the albuminous material. Dilutions were made in salt solution, bouillon, and serum from 1% to 0.1%, in both the colloidal cresol and colloidal thymol. Although there was an increase in the germicidal action over the 25% colloidal chloro-cresol and thymol, there was also increased precipitation.

Ether as a carrying agent of preservatives in biologics is quite effective for phenol and so was tested in the above colloids in both the 25% and the 50% strengths with the following results:

Chart #5.

	Dilution in %	Precipitation of Bouillon	Serum
Chlorine thymol (25%)	0.9	x	x
	0.8	x	x
	0.7	x	x
	0.6	slight	x
	0.5	slight	x
	0.4	slight	x
	0.3	-	x
	0.2	-	x
	0.1	-	-
Chlorine cresol (25%)	0.9	x	x
	0.8	x	x
	0.7	x	x
	0.6	x	x
	0.5	x	x
	0.4	slight	x
	0.3	slight	slight
	0.2	-	-
	0.1	-	-

The results in the 50% concentration were the same as those in the 25% except in the case of the chlorine thymol. Here the bouillon was precipitated quite heavily in 0.4% and slight in 0.2% with a precipitation of the serum in 0.1%.

The chlorine cresol precipitated slightly in 0.3% bouillon and 0.3% serum. From the results obtained in the precipitation of the proteinous materials, the colloidal crésol and colloidal thymol in both the 25 and 50% strengths; ether would not prevent precipitation to such an extent as to make their employment practicable.

Yatren

This is a German preparation. It is a brown powder which has a maximum solubility in water of 4%. It was not germicidal in 1-50 parts. The media were colored from a deep brown to a light amber depending upon the dilution. There was no precipitation of the protein materials in a 4% solution. The chemical structure of this product was not learned.

Results.
Chart #6.

Preservative	Dil.	B. coli 1	Staph. albus 2	B. mycoides 3	B. subtilus 4	Media	Physical effects
Yatren	1-50	x	x	x	x	Salt sol., bouillon, and serum	Gives the media a color ranging from a deep brown to a light amber, the intensity decreasing with the dilution.
	1-52	x	x	x	x		
	1-55	x	x	x	x		
	1-58	x	x	x	x		
	1-62	x	x	x	x		
	1-66	x	x	x	x		
	1-71	x	x	x	x		
	1-76	x	x	x	x		
	1-83	x	x	x	x		
	1-90	x	x	x	x		
	1-100	x	x	x	x		
	1-111	x	x	x	x		
	1-125	x	x	x	x		
	1-143	x	x	x	x		
	1-170	x	x	x	x		
	1-200	x	x	x	x		
	1-250	x	x	x	x		
	1-333	x	x	x	x		
	1-500	x	x	x	x		
	1-1000	x	x	x	x		

Hexylresorcinal (S.T.37)(1-1000)

A water clear substance prepared in a 1-1000 solution. It was quite miscible with the media and has the formulae $C_6H_3(OH)_2(C_6H_{13})$.(1) This was not germicidal in 1-50 parts with any of the basic media. It caused no discoloration and no precipitation of the boullion and serum.

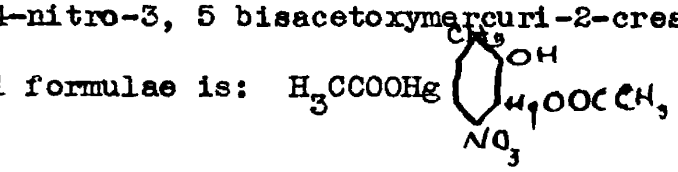
Results.
Chart #7.

Preservative	Dil.	B. coli 1	Staph. albus 2	B. mycoides 3	B. subtilus 4	Media	Physical effects
Hexylresorcinal (S.T.37)(1-1000)	1-50	x	x	x	x	Salt sol., boullion, and serum	No change in any of the three media in color. No precipitation.
	1-52	x	x	x	x		
	1-55	x	x	x	x		
	1-58	x	x	x	x		
	1-62	x	x	x	x		
	1-66	x	x	x	x		
	1-71	x	x	x	x		
	1-76	x	x	x	x		
	1-83	x	x	x	x		
	1-90	x	x	x	x		
	1-100	x	x	x	x		
	1-111	x	x	x	x		
	1-125	x	x	x	x		
	1-143	x	x	x	x		
	1-170	x	x	x	x		
	1-200	x	x	x	x		
	1-250	x	x	x	x		
	1-333	x	x	x	x		
	1-500	x	x	x	x		
	1-1000	x	x	x	x		

As outlined in the salt solutions, there was growth in all dilutions of the boullion and serum. No change was noted in the physical appearance of the boullion or serum, with no precipitation.

Metaphen 2%, diluted in a 2% aqueous NaOH solution.

It is an organic mercurial. Chemically it is known as 4-nitro-3, 5 bisacetoxymercuri-2-cresol.(2) Its structural formulae is:



The solution is amber colored in a 2% concentration. It mixed readily with the media employed. It was necessary to make an initial dilution of 1-1000 using distilled water before making the desired dilutions with the media.

Results.
Chart #8

Preservative	Dil.	B.coli 1	Staph. albus 2	B.mycoides 3	B. subtilus 4	Media	Physical effects
Metaphen 2%	1-12500	x	x	x	-	Salt sol.	Very light amber; no ppt.
	1-10000	-	-	-	-	"	Very light amber; no ppt.
	1-8333	-	-	-	-	"	Very light amber; no ppt.
	1-7142	-	-	-	-	"	Very light amber; no ppt.
	1-6250	-	-	-	-	"	Very light amber; no ppt.
	1-12500	x	-	x	-	Bouillon	Light amber; no ppt
	1-10000	x	-	-	-	"	" " " "
	1-8333	-	-	-	-	"	" " " "
	1-7142	-	-	-	-	"	" " " "
	1-6250	-	-	-	-	"	" " " "

Chart #8 (cont'd)

Preservative	Dil.	B. coli 1	Staph. albus 2	B.mycoides 3	B. subtilus 4	Media	Physical effects
Metaphen 2%	1-12500	x	-	-	x	Serum	Light amber; no ppt.
	1-10000	x	-	x	-	"	Light amber; no ppt.
	1-8333	x	-	-	-	"	Light amber; no ppt.
	1-7142	x	-	-	-	"	Light amber; no ppt.
	1-6250	x	-	-	-	"	Light amber; no ppt.
	1-5555	x	-	-	-	"	Light amber; no ppt.
	1-5000	-	-	-	-	"	Light amber; no ppt.

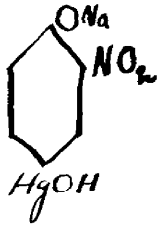
Metaphen gave the media a very light amber colored appearance, but one that was not easily discernible. With a concentration as high as 1-200 parts there was no precipitation of the protein substances in either the bouillon or serum. It was germicidal in dilutions of 1-10000 parts of salt solution; 1-8333 parts bouillon; and 1-5000 parts serum.

As a further study of the germicidal action of this substance a number of bottles were prepared, some containing serum, and some containing bouillon. These were preserved with 1-8000 parts bouillon and 1-5000 parts serum. Each was inoculated with about one-half a gram of dust particles. These cultures showed no growth when placed in direct sunlight for several weeks, after this time there appeared in the

serum a growth of air type bacteria. At the end of 6 weeks the bouillon showed the presence of molds and air types of bacteria. One hundred c.c. of a 1-8000 dilution of tetanus toxin was placed in each of five bottles. Also five bottles containing a similar amount of a 1-8000 dilution of diphtheria toxin were inoculated with dust and incubated for two weeks at 37°C. At the end of this time they were examined and all showed no growth. From these, five samples were placed in the sunlight and five in the dark. At the end of a month all five samples kept from the sun rays were sterile while three of the five samples exposed to the action of sunlight showed growth. While no definite conclusions are drawn as to the effect of sunlight in causing a loss of the germicidal properties of metaphen, it does seem to have some apparent destructive action.

Mercurophen 2% aqueous solution.

This is an organic mercurial. Chemically it is sodium-hydroxymercuri-nitro-phenolate. The 2% aqueous solution is quite orodless. It has a brick red color but in higher dilutions is amber colored. In preparation for the germicidal tests, due to its ability to kill bacteria in high dilutions, a preliminary dilution of 1-1000 parts in distilled water was made before completing the final dilutions by the addition of the basic media.(3) Structurally it is:



Results.
Chart #9.

Preservative	Dil.	B. coli	Staph. albus	B. mycoides	B. subtilus	Media	Physical effects
		1	2	3	4		Sol.
Mercurophen	1-50000	x	-	x	-	Salt/	No precipitation
	1-25000	x	-	-	-	"	" in any of the dilu
	1-16666	-	-	-	-	"	" tions; color of
	1-12500	-	-	-	-	"	" the suspension
	1-10000	-	-	-	-	"	" changes from
	1-8333	-	-	-	-	"	" dark to a light amber, decreasing with the dilution.
	1-25000	x	-	x	-	Bouillon	Amber colored;
	1-16666	-	-	x	-	"	same physical
	1-12500	-	-	-	-	"	effects as
	1-10000	-	-	-	-	"	described above.
	1-8333	-	-	-	-	"	
	1-10000	x	x	-	-	Serum	No precipitation.
	1-8333	x	x	-	-	"	Same color as
	1-7142	-	-	-	-	"	observed in the
	1-6250	-	-	-	-	"	above.
	1-5555	-	-	-	-	"	
	1-5000	-	-	-	-	"	

This substance was miscible with the media, and was germicidal in salt solution in dilutions of 1-16500 parts; boullion 1-12500 parts; and serum 1-8500 parts. It imparts a color of an amber hue not unlike that of normal serum and boullion, but a trifle more intense. There was no precipitation of proteins in serum in dilutions as concentrated as 1-200 parts.

A series of preparations were made as in metaphen under identical conditions, with exposure to sun light to ascertain if it caused a loss of germicidal power. Boullion was preserved with 1-12000 parts; mercurophen and serum 1-8000 parts. After a four months exposure at room temperature the serum and boullion were found to be sterile. After inoculating five samples of tetanus toxin, and five samples of diphtheria antitoxin each containing 1-10000 parts mercurophen and incubating at 37°C. for three weeks, all were found to be sterile. These were then divided into two lots, one exposed to the air and sun light, and the other exposed to the air but protected from sun light. At the end of a month both lots were sterile. It was assumed from this experiment that light does not affect the germicidal power of mercurophen.

Discussion.

The germicidal power refers to the destruction of all the species of bacteria employed in the experiments.

Yatren gives the media a color ranging from a deep brown to a light amber, the intensity decreasing with the increased dilution.

There is no precipitation of media, but the germicidal action is not efficient at 1-50 parts, so the volume of the chemical needed for the killing of the bacteria would be too great.

Hexylresorcinal (1-1000) was not effective in 1-50 parts and although it is non-precipitating, the quantity of the germicide employed indicated that the killing power was not great enough to use in the capacity of a biologic preservative. Hexylresorcinal is colorless and caused no change in the physical condition of any of the products.

Colloidal chloro-thymol, 25%, was germicidal in salt solution at 1-333 parts, bouillon 1-200 parts, and serum 1-200 parts. It had such a marked precipitating action upon the protein substances that it should not be employed.

Colloidal chloro-cresol, 25%, destroyed bacteria in 1-500 parts salt solution, 1-333 parts bouillon and 1-250 parts serum. Heavy precipitation of the bouillon and serums made its use prohibitive. A study of the 50% chloro-thymol and 50% chloro-cresol proved them to be powerful germicidal substances, miscible with the media but precipitated so heavily that they could not be used in the capacity of biologic preservatives.

Phenol is germicidal in a 0.3% salt solution, 0.5% boullion and 0.5% serum. There was no discoloration of the salt solution. The bouillon and serum showed a precipitation in a concentration of 1-200 parts.

Trikresol had a germicidal potency in salt solution of 1-500 parts, bouillon 1-333 parts, and serum 1-250 parts. There was no discoloration of the media with some precipitation of the protein substances.

Metaphen was germicidal in salt solution in 1-10000 parts, bouillon 1-8333, and serum 1-5000. This substance gave salt solution, bouillon and serum a light amber color. There was no precipitation of protein substances in dilutions of 1-200 parts. Direct exposure to sun light seemed to lessen the germicidal power of the substance.

Mercurophen killed bacteria in salt solution in 1-16500 parts, bouillon 1-12500 parts, and serum 1-8500 parts. It was non-protein precipitous in dilutions of 1-200 parts. This substance gave the media a light amber-red color but was not objectionable.

The germicidal power of each substance decreased with an increasing amount of organic material present in the media employed. There was also evidenced a selective action of the chemical substances, the organic mercurials being particularly effective against Staphylococcus pyogenes albus. There were certain discrepancies manifested in this series of experiments. However, the tests were repeated a number of times and the germicidal and physical effects were found to be as stated.

Of the chemicals studied those best suited for biologic preservatives from the standpoint of the germicidal efficiency, the physical effect, and the appearance of the product were; namely, mercurophen, metaphen, trikresol, and phenol. These four were further studied.

TOXICITY TESTS.

This phase of the work was undertaken chiefly to determine the toxicity of metaphen and mercurophen but included in part, the toxicity of phenol and trikresol. The toxicity of these substances has been determined by Leake[4], Hale[5] and a number of other workers with comparable results.

Leake in 1917 using mice as experimental animals undertook some experiments to determine the toxicity of certain disinfectants used in biologic products. His results indicated that assuming the M.L.D. to be the amount necessary to kill 80 per cent of the mice in a given dose, phenol has an M.L.D. of .00037 gms. per gm. weight of mouse. Trikresol has the same M.L.D. as found for phenol. The toxicity of trikresol and phenol was not lessened when diluted with normal horse serum. These results agree with the findings of Hale.

Methods.

The experimental animals employed in this part of the problem were rabbits and albino rats. Two routes of injection were followed, one intravenous and the other intramuscular.

The phenol was prepared by weight and diluted to the required volume with double distilled H_2O. Trikresol was in the liquid state and diluted by volume as were also mercurophen and metaphen. The quantity of each germicide to be injected into each animal

was found by multiplying the weight of the rat or rabbit by the dose in grams of the germicide and dividing the total by the grams of germicide per c.c. of solution. In the rat, intravenous injections were made in the saphenous vein. Intramuscular injections were made in the fleshy portion of the hind leg. With rabbits intramuscular injections were made in the hind leg and intravenous injections were made in the marginal ear veins. The rats used were between 100 and 150 gms. in weight, the weight being taken before a heavy feeding. Rabbits were not less than 1½ kilograms in weight and were apparently in excellent physical condition. The amounts injected were measured in Luer syringes, a 2 c.c. graduated in 1/10 c.c. and a 1 c.c. graduated in 1/100 c.c. The quantity to be injected governed the choice of the syringe being used.

Animals surviving 14 days after the time of injection were discharged and considered as having survived the dosage.

Dilutions of Germicides.

In order to test the effect of dilution upon the toxicity of the germicidal substances, mercurophen and metaphen were administered intravenously in rats in 0.1 and 0.5% solutions, and intramuscularly in 1 and 2% solutions. All injections in rabbits both intravenously and intramuscularly were made in 2% solutions.

TOXICITY TEST PROTOCOLS

The following detailed protocols represent the results of the toxicity tests of metaphen and mercurophen. The route of injection; test animal employed; weight of animal; mgs. dosage; percent survival; and the Maximum Tolerated Dose (M.T.D.) for the dilutions of each mercurial studied are given in tabular form.

Metaphen - intramuscular

1% solution

Test Animal - Albino Rat

Rat No.	Weight gms.	Mg. dosage	Result	Time
101	150	16.6	Survived	Discharged
102	130	16.6	"	"
103	122	16.6	"	"
104	115	16.6	"	"
105	155	16.6	"	"
106	120	20.0	"	"
107	97	20.0	"	"
108	118	20.0	"	"
109	125	20.0	"	"
110	147	20.0	"	"
111	132	24.0	"	"
112	158	24.0	"	"
113	135	24.0	Lethal	9 days
114	155	24.0	Survived	Discharged
115	152	24.0	Lethal	6 days
116	146	28.0	"	1 day
117	157	28.0	"	5 days
118	112	28.0	"	3 days
119	135	28.0	"	3 days
120	154	28.0	"	3 days

Mgs. dosage	Percent survival
16.6	100%
20.0	100 %
24.0	60%
28.0	0

M.T.D.= 24

Metaphen - intramuscular

2% solution

Test animal - Albino rat

No. of Rat	Mgs. Dosage	Percent survival
4	14.0	100%
4	16.6	100%
4	20.0	75%
4	24.0	0

M.T.D.= 20

Metaphen - intravenous

0.1% solution

Test animal - Albino rat

Rat No.	Weight gms.	Mg. dosage	Results	Time
140	123	4	Survived	Discharged 14 days
141	108	4	"	"
142	105	4	"	"
143	127	4	"	"
144	108	4	"	"
145	107	6	"	"
146	119	6	Lethal	8 days
147	120	6	Survived	Discharged
148	111	6	"	"
149	110	6	Lethal	8 days
150	120	8	"	5 days
151	122	8	"	6 days
152	110	8	"	6 days
153	132	8	"	7 days
154	100	8	"	6 days

Mgs.dosage	Percent survival
4	100%
6	60%
8	0

M.T.D.=6

Metaphen - intravenous

0.5% solution

Test animal - Albino rat

No.of rat	Weight gms.	Mgs. dosage	Result	Time.
220	139	2.6	Survived	Discharged 14 days
221	124	2.6	"	"
222	115	2.6	"	"
223	130	2.6	"	"
224	160	4.0	Lethal	1 day
225	138	4.0	"	1 day
226	132	4.0	Survived	Discharged
227	120	4.0	"	"
228	130	6.0	Lethal	1 day
229	160	6.0	"	1 day
230	130	6.0	"	1 day
231	125	6.0	"	1 day

Mgs. dosage	Percent survival
2.6	100%
4.0	50%
6.0	0

M.T.D. = 2.6

Metaphen-intramuscular

2% solution.

Test animal - rabbitt.

No. of Rabbit	Weight gms.	Mgs. dosage	Result	Time
55	2300	6	Survived	
56	1960	6	"	
57	2075	6	"	
58	2745	6	"	
59	2975	8	"	
60	2530	8	"	
61	1975	8	"	
62	2840	8	"	
63	2445	10	Lethal	9 days
64	2730	10	Survived	
65	2840	10	"	
66	2570	10	"	
67	2070	12	"	
80	1625	12	Lethal	7 days
81	2000	12	Survived	
82	1925	12	Lethal	6 days
83	1620	14	"	6 days
84	1810	14	"	7 days
85	2200	14	Survived	
86	2150	14	Lethal	5 days
87	2020	16	Survived	
88	2480	16	Lethal	4 days
89	1650	16	Lethal	4 days
90	2020	16	Lethal	2 days

Mgs. Dosage	Percent Survival
6	100%
8	100%
10	75%
12	50%
14	25%
16	25%

M.T.D.= 10.

Metaphen - Intravenously

2 % solution

Test animal - rabbit

No. of rabbit	Weight gms.	Mgs. dosage	Result	Time
18	1210	3	Survived	
19	2570	3	"	
20	2830	3	"	
21	2170	3	"	
22	2160	4	"	
23	2150	4	"	
24	2250	4	"	
25	1600	4	"	
26	2385	4	Lethal	8 days
27	2050	5	Survived	
28	2315	5	Lethal	8 days
29	1750	5	"	8 days
30	1860	5	"	9 days
31	1375	6	"	2 days
32	1875	6	"	4 days
33	1700	6	"	7 days
34	1400	6	"	3 days

Mgs. dosage	Percent survival
3	100 %
4	80 %
5	25 %
6	0

M.T.D.= 4

Mercurophen - intramuscular

1.0% solution

Test animal - Albino rat

No. of rat	Weight gms.	Mgs. dosage	Result	Time in days
170	122	16.6	Survived	
171	148	16.6	"	
172	124	16.6	"	
173	130	16.6	"	
174	115	16.6	"	
175	145	20.0	"	
176	108	20.0	"	
177	90	20.0	Lethal	12 days
178	136	20.0	Survived	
179	140	20.0	"	
180	178	24.0	"	
181	132	24.0	"	
182	118	24.0	Lethal	3 days
183	130	24.0	"	8 days
184	151	24.0	Survived	
185	145	28.0	Lethal	3 days
186	116	28.0	"	3 days
187	123	28.0	"	3 days
188	126	28.0	"	2 days
189	142	28.0	"	3 days

Mgs. dosage	Percent survival
16.6	100%
20.0	80%
24.0	60%
28.0	0

M.T.D.= 24.

Mercurophen - intramuscular

2% solution

Test animal - Albino rat

No. of rat	Mgs. dosage	Percent survival
4	14.0	100%
4	16.6	80%
4	20.0	20%
4	24.0	0

Mercurophen - intravenous

0.1% solution

Test animal - Albino rat

No. of rat	Weight gms	Mgs. dosage	Result	Time
28	155	8	Survived	Discharged 14 days
29	137	8	"	"
30	195	8	"	"
31	102	8	"	"
32	140	8	"	"
33	109	10	"	"
34	120	10	Lethal	1 day
35	147	10	"	1 day
36	195	10	"	1 day
37	125	10	"	1 day
38	107	12	"	1 day
39	115	12	"	1 day
40	147	12	"	1 day
41	145	12	"	1 day
42	130	12	"	3 days

Mgs. dosage	Percent survival
8	100%
10	20%
12	0

M.T.D. = 8

Mercurophen - intravenously

0.5% solution

Test animal - Albino rat

No. of rat	Weight gms	Mgs. dosage	Result	Time
155	110	4	Survived	Discharged
156	115	4	"	"
157	135	4	"	"
158	137	4	"	"
159	120	4	Lethal	6 days
160	142	6	"	1 day
161	157	6	"	1 day
162	110	6	"	1 day
163	147	6	"	3 days
164	147	6	"	1 day
165	148	8	"	1 day
166	152	8	"	1 day
167	117	8	"	1 day
168	127	8	"	1 day
169	115	8	"	1 day

Mgs. dosage	Percent survival
4	80%
6	0
8	0

M.T.D.=4

Mercurophen - Intramuscular

2 % solution

Test animal - rabbit

No. of rabbit	Weight gms.	Mgs. dosage	Result	Time
43	1775	6	Survived	
44	2350	6	"	
45	2340	6	"	
46	2060	6	"	
47	1860	8	"	
48	2370	8	Lethal	7 days
49	2370	8	Survived	
50	2370	8	"	
51	2410	8	"	
52	2200	10	"	
53	1530	10	"	
54	2240	10	"	
67	2480	10	Lethal	8 days
68	1710	10	Survived	
69	1660	12	Lethal	7 days
70	2165	12	Survived	
71	1830	12	Survived	
72	1970	12	Survived	
73	2275	14	Lethal	4 days
74	2000	14	Survived	
75	2140	14	Lethal	3 days
76	1820	14	"	5 days
77	1680	16	"	2 days
78	1930	16	"	5 days

Mgs. dosage	Percent survival
6	100 %
8	80 %
10	80 %
12	75 %
14	50 %
16	0 %

M.T.D. = 12

Mercurophen - Intravenous

2 % solution.

Test animal - rabbit.

No. of rabbit	Weight gms.	Mgs. dosage	Result	Time
9	1010	3	Survived	
10	2600	3	"	
11	1775	3	"	
12	1380	3	"	
13	2300	4	"	
14	2040	4	"	
15	2175	4	Lethal	3 days
16	2225	4	Survived	
17	1325	4	"	
35	1575	5	Lethal	3 days
36	1550	5	Survived	
37	3970	5	Lethal	5 days
38	1770	5	"	3 days
39	2015	6	"	3 days
40	2040	6	"	4 days
41	1465	6	"	3 days
42	1780	6	"	5 days

Mgs. dosage	Percent survival
3	100 %
4	80 %
5	25 %
6	0

M.T.D. = 4

Results of Phenol and Trikresol Experiments.

The toxicity tests of these two substances was carried only far enough to compare their reactions with those produced by metaphen and mercurophen.

The M.T.D. in rats was 250 mgs. per kilo intravenously, and 350 mgs. intramuscularly for both phenol and trikresol. Further tests for the M.T.D. were discontinued because of the extreme tremors manifested at as low dosages as 30 mgs. intravenously and 70 mgs. intramuscularly. Many times there was an onset of these symptoms before the injection was completed.

Toxicity of Metaphen and Mercurophen.

In determining the M.T.D. of these substances and to properly evaluate them a number of factors were considered.

Of first importance was the strain of rats used in the tests. The albino rats employed in the standardization of arsphenamine at the United States Hygienic Laboratory were used exclusively in obtaining the results which are given in the protocols. Parallel experiments carried out on another strain of rats showed a lower tolerance for the germicides than is reported, sometimes as much as 40 per cent on identical dosages. Also during periods of intense heat animal tolerance for the mercurials was very much lessened.

Metaphen.

The M. T. D. dose is as follows:

Method of injection	Animal	% of solution	M.T.D.
Intravenously	Rat	0.1	6 mgs.
"	"	0.5	2.6 mgs.
"	Rabbit	2.0	4 mgs.
Intramuscularly	Rat	1.0	24 mgs.
"	"	2.0	20 mgs.
"	Rabbit	2.0	12 mgs.

Mercurophen.

The M. T. D. dose is as follows:

Method of injection	Animal	% of solution	M.T.D.
Intravenously	Rat	0.1	8 mgs.
"	"	0.5	4 mgs.
"	Rabbit	2.0	4 mgs.
Intramuscularly	Rat	1.0	24.0 mgs.
"	"	2.0	16.6 mgs.
"	Rabbit	2.0	12.0

It is quite evident that the M.T.D. is not a fixed value. As shown by the effect of dilution and the tolerance of individual animals, there is a broad range of susceptibility. In some preliminary experiments an individual animal would occasionally tolerate a dosage as great as 40 mgs. per kilogram. The higher concentrations may produce thrombosis and cause sudden death. It will be noted in the intravenous experiments in rats that when $\frac{1}{2}$% solutions were employed death occurred generally within 24 hours, giving a dosage toleration of 50% less than that of the 0.1% solution. So from this data it may be inferred that dilution influences the susceptibility of the animals. This is particularly true with intravenous injections.

The M.T.D. dose in rabbits was found to be 4 mgs. per kilogram intravenously and 12 mgs. per kilogram intramuscularly.

Mercurophen showed 100% greater tolerance intravenously for the 0.1% solution than for the 0.5% solution. Rabbits had a lower tolerance than was exhibited by rats both intravenously and intramuscularly.

If the toxicity of metaphen and mercurophen were placed upon a comparative basis, there is little difference between them. Mercurophen appeared to be somewhat less toxic than metaphen when used intravenously in rats but both had the same M.T.D. in rabbits

intramuscularly and intravenously. Both substances when injected intramuscularly in doses of 12 mgs. per kilogram or more produced some irritation, which was manifested for several minutes. It was stated by Kolmer[6] that the solubility in the tissue, percentage of mercury, and rate of dissociation of mercury influence toxicity and therapeutic activity.

McNider[7] pointed out that animals poisoned by bichloride of mercury, depending upon the reaction, may be divided into several groups. In the first group were animals which developed an intense gastroenteritis and died within 48 hours from the early development of the shock state, with its associated disturbed circulation; preventing the mercury from reaching and seriously damaging the kidneys. The second group of animals recovered from the gastroenteritis and associated shock, and died within seven days. The kidneys of these animals showed no marked injury to the glomeruli but a diffuse Wiegert's like necrosis of the epithelium of the tubules without swelling of the cells. A final group showed a delayed acute renal injury. These animals had a severe type of acid intoxication and intense swelling of renal epithelium, followed by necrosis. McNider states that it appears that this type of renal injury is not due to the mercury but is in some way dependent upon the disturbances of the acid base equilibrium of the blood.

INTRASPINAL TESTS.

To Test the Action of Metaphen, Mercurophen, Phenol, and Trikresol, when injected Intraspinally.

In 1913, Kramer[8] observed a number of cases of sudden death after the treatment of cerebro-spinal fever with a specific antiserum and advanced the hypothesis that the preservative trikresol was the cause of the symptoms. That the toxic action of the serum when introduced into the subarachnoid space of dogs and cats was caused by the preservative trikresol was later proven in an experimental manner by Hale[9]. In 1914, Voegtlin[10] confirmed the results of Kramer and Hale and found that trikresol and phenol were equally toxic when injected in like quantities, and stated, as a general rule, "The effect on blood pressure increases in proportion to the rate of injection and the concentration of the phenol preservative."

The fact that phenol and trikresol possess such toxic properties when injected intraspinally made it necessary to know the effects produced by the mercurials metaphen and mercurophen upon the central nervous system.

Procedure.

The experiments were carried out on dogs. The anaesthetic employed was ether and the degree of angesthesia obtained was quite constant by performing tracheotomy and introducing the vapors from

a bottle directly into the trachea. The blood pressure was taken from the carotid artery, and tracings were obtained by using a mercury manometer. Respiratory rates were recorded graphically by inserting a canula into the pleural cavity just above the diaphragm and recordings made on a tambour.

Methods of Injection.

Two methods of injection were employed; namely, pressure and gravity.

Several vertebrae in the lumbar region were exposed and injections made by introducing a needle directly into the spinal canal. Spinal fluid was permitted to escape. The needle was then connected with a Luer type of syringe or graduated burette depending upon the method used.

Antimeningococcus serum was preserved with mercurophen and metaphen in 1-2500 parts and phenol 1-200 parts. This permitted a margin of more than double the quantity needed for preservation purposes for metaphen and mercurophen. Sera preserved with each of the above substances were injected alternately using both the pressure and gravity methods of administration.

Protocols.

Dog #1, 12.3 kilograms, anaesthetic - ether

11:50 to 12 M. 5 c.c. serum preserved with 1-2500 parts of mercurophen, administered by gravity method. No change in respiration or blood pressure.

12:06 to 12:09 p.m. 5 c.c. of mercurophen serum 1-2500 parts, administered by gravity method. Slight rise in blood pressure, respiration normal.

12:14 to 12:15 p.m. 2 c.c. 1-2500 parts mercurophen administered by gravity method. Respiration and blood pressure constant.

12:25 to 12:30 p.m. 3 c.c. $\frac{1}{2}$% phenol serum administered by gravity method. Slight rise in blood pressure followed by a gradual decrease; respiration normal.

12:40 to 12:45 p.m. 5 c.c. of 1-200 parts phenol serum, administered by pressure method. Drop in blood pressure, respiration became shallow and checked before the pressure was released after which time respiration and blood pressure again became normal.

12:55 to 12:58 p.m. 1.7 c.c. 1-100 phenol in normal salt solution, administered by gravity method. An immediate drop in blood pressure; respiration normal.

1:00 to 1:01 p.m. 2 c.c. 1-100 phenol in normal salt solution, administered by pressure method. An immediate drop in the blood pressure, until it was released when it became normal; respiration normal.

1:06 to 1:10 p.m. 4 c.c. of normal salt solution, administered by gravity method, blood pressure and respiration normal.

1:18 to 1:26 p.m. 5.3 c.c. of metaphen serum 1-2500 parts, administered by gravity method. No change in blood pressure or respiration.

1:33 to 1:34 p.m. 3 c.c. 1-2500 parts metaphen serum, administered by pressure method.

1:36 p.m. pressure released. Blood pressure and respiration normal.

1:39 to 1:44 p.m. 7 c.c. of 1-100 phenol in normal salt solution administered by pressure method.

1:46 p.m. pressure released; immediate drop in the blood pressure, respiration became shallow but after the pressure had been released there was no recovery and the animal succumbed.

Dog #2, 8.2 kilograms, anaesthetic - ether

10:32 a.m. withdrew 1.5 c.c. spinal fluid - clear

10:41 to 10:50 a.m. 10 c.c. of serum preserved with metaphen 1-2500 parts by gravity method.

10:59 a.m. pressure released and serum permitted to flow out. Blood pressure and respiration normal.

11:10 to 11:12 a.m. 4.1 c.c. of ½% phenolyzed serum administered by gravity method. A drop in blood pressure with respiration shallow and uneven. Immediately before releasing pressure coarse tremors were manifested. After the pressure was released there was an immediate rise in blood pressure to normal and respiration became constant.

11:27 to 11:30 a.m. 4 c.c. of 1-2500 parts mercurophen serum administered by gravity method.

11:30 to 11:33 a.m. 1.5 c.c. of additional serum.

11:34 a.m. pressure released. Blood pressure normal, respiration normal. No change in respiration and blood pressure after the release of the preserved serums.

11:45 to 11:52 a.m. 4 c.c. mercurophen in 1-2500 parts serum administered by gravity method. Blood pressure and respiration normal.

12:01 to 12:06 p.m. 4.2 c.c. of ½% phenol serum administered by gravity method. A slight rise in blood pressure followed by a gradual lowering. When pressure was released blood pressure became normal.

12:10 to 12:11 p.m. 2.5 c.c. of ½% phenol serum; rapid decrease in blood pressure, respiration normal.

12:19 to 12:23 p.m. 9 c.c. of 1-2500 parts metaphen serum administered under pressure injection. Blood pressure and respiration normal.

12:27 to 12:29 p.m. 7.3 c.c. of 1-2500 parts mercurophen. Respiration and blood pressure normal.

12:37 to 12:39 p.m. 2.9 c.c. of ½% phenol serum administered by gravity method. There was a gradual decrease in blood pressure but it returned to normal when pressure was released.

12:45 to 12:47 p.m. 7 c.c. ½% phenol serum administered under pressure. There was a marked decrease in blood pressure; respiration became shallow and finally stopped; pressure was released after which the respiration and blood pressure became normal.

12:54 to 12:58 p.m. ½% phenol in normal salt solution administered by pressure method. There was a marked decrease in blood pressure, tremors were shown, respiration became shallow and finally stopped.

The Effect of Phenolyzed Serum.

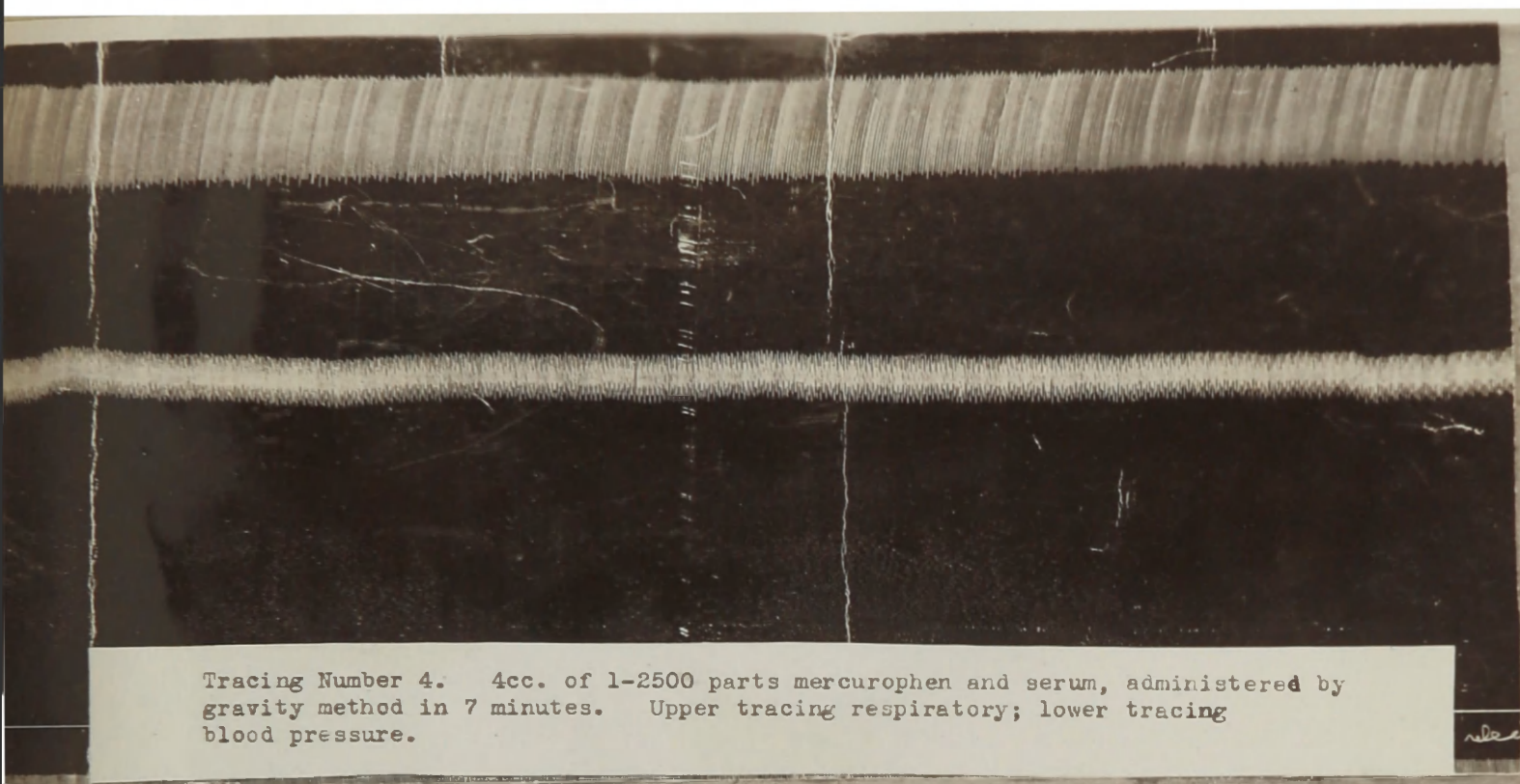
The results obtained from a 1-200 parts phenolyzed serum were in general analogous to those of Voegtlin.(10) In all cases there was an immediate and marked drop in the blood pressure with a slowing up and finally, checking of the respiration if the pressure was not released. Serum injected under pressure gave a more precipitous drop than when injected by the gravity method. The amount of phenolyzed serum necessary to cause these effects was quite small, 2 c.c., within several minutes time. The respiratory and blood pressure changes are shown in the tracings 1 and 2.

The extreme symptoms manifested in the vasomotor and respiratory centers by the use of phenol and trikresol, eliminate these substances as preservatives in biologics which are to be administered via the spinal canal.

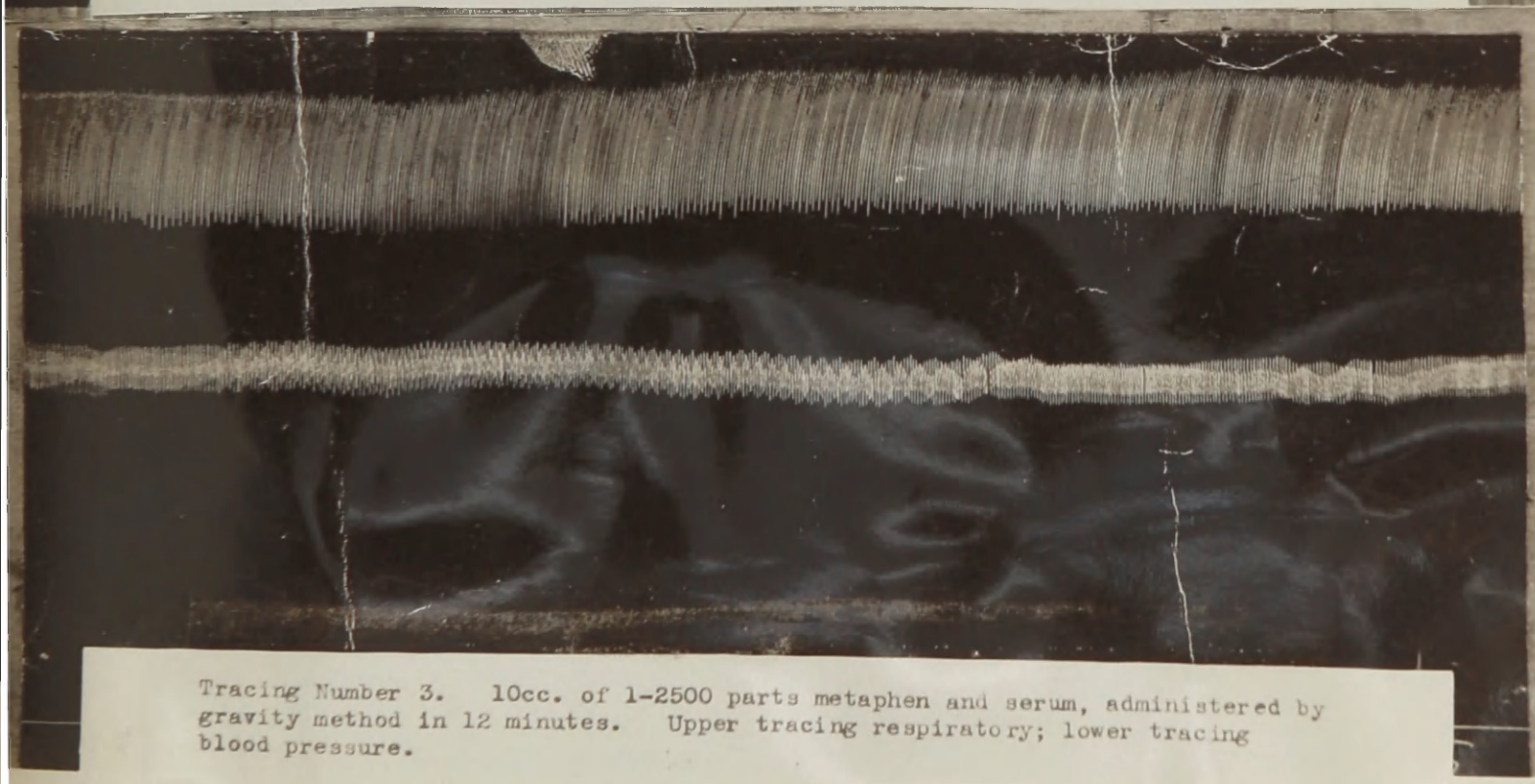
Metaphen and Mercurophen.

A review of the protocols and the respiratory and blood tracings for the mercurials will show an absence of the severe symptoms that were manifested in the phenol experiments. Injections

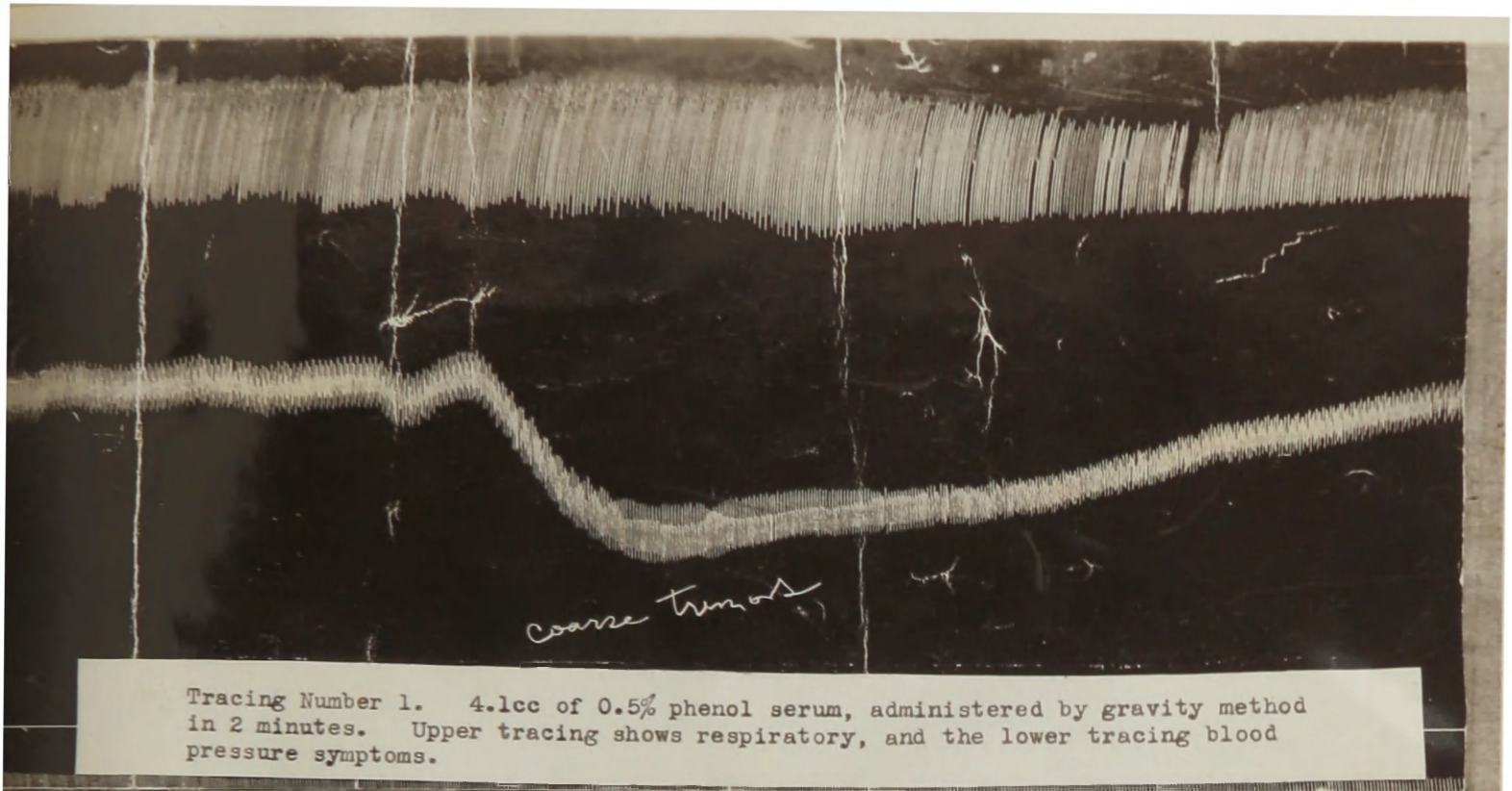
of large amounts of serum preserved with metaphen and mercurophen had no noticeable effect upon the blood pressure or respiratory movements as shown in tracings 3 and 4. The pressure method of injection produced a slight increase in the blood pressure which was not dangerous. It may, therefore, be assumed that neither metaphen nor mercurophen may be expected to have any detrimental effects upon the vasomotor or respiratory centers in the dosages used.



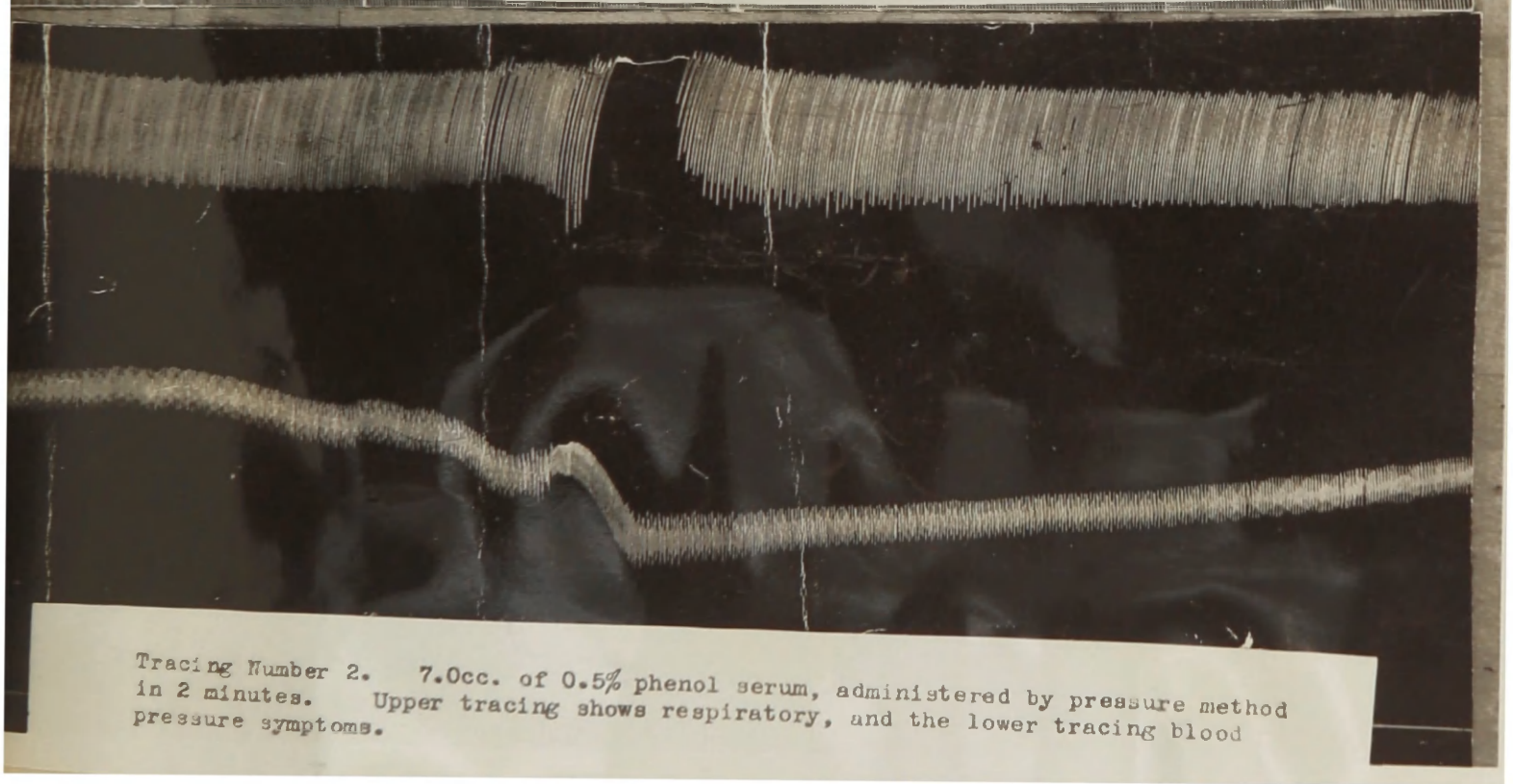
Tracing Number 4. 4cc. of 1-2500 parts mercurophen and serum, administered by gravity method in 7 minutes. Upper tracing respiratory; lower tracing blood pressure.



Tracing Number 3. 10cc. of 1-2500 parts metaphen and serum, administered by gravity method in 12 minutes. Upper tracing respiratory; lower tracing blood pressure.



Tracing Number 1. 4.1cc of 0.5% phenol serum, administered by gravity method in 2 minutes. Upper tracing shows respiratory, and the lower tracing blood pressure symptoms.



Tracing Number 2. 7.0cc. of 0.5% phenol serum, administered by pressure method in 2 minutes. Upper tracing shows respiratory, and the lower tracing blood pressure symptoms.

POTENCY TESTS.

Method of Procedure.

Unpreserved sera were collected from various biologic houses for this phase of the study. Tetanus toxin and antitoxin, diphtheria toxin and antitoxin, were used to determine the effect of the germicidal substances on their immunizing powers. To the above toxins and antitoxins were added the quantities of each preservative which had been found to be germicidal. The substances used and quantities added were:

Phenol, in antitoxins ½%, toxins ½%.
Trikresol, .4% in toxins and antitoxins.
Metaphen, antitoxins 1-5000 parts, toxins 1-10000 parts.
Mercurophen, antitoxins 1-8000 parts, toxins 1-12000 parts.

These were then placed in a refrigerator at 40°F. together with the controls of unpreserved serum. A preliminary potency test was made on the diphtheria and tetanus toxins and antitoxins to determine the exact unitage. They were then placed in an incubator at 37°C. to hasten any action of the preservatives. At intervals potency tests were made on preserved and unpreserved sera to note any lessening of the unitage.

Potency Tests (proper)

The methods employed in testing the potency of the biologics studied is essentially the same as that used at the United States Hygienic Laboratory in testing the potency of standard commercial diphtheria and tetanus antitoxins. These tests may be found in a

pamphlet of the United States Hygienic Laboratory entitled "Tests of Standard and CommercialDiphtheria and Tetanus Antitoxin".(15).

Testing the Antitoxins.

Standard diphtheria toxin.-

A standard toxin was employed which had been properly aged and ripened and the M.L.D. determined. Each standard toxin was tested against a standard antitoxin and each L plus dose of toxin was used on four animals. The desirable quantity of diluted toxin should be in the neighborhood of 2 c.c. and the dilutions made accordingly.

Determination of Dilutions of the Antitoxins.

This was done so that 1 c.c. of a final dilution was represented by one unit of antitoxin. Not less than 1 c.c. of the sample was used in the preliminary dilution. The required amount of salt solution was pipetted into a flask, the antitoxin sample was drawn in slight excess into a 1 c.c. wash-out pipette. The meniscus was then brought down to the proper calibration mark by wiping the tip of the pipette with sterile gauze. After delivery each pipette was washed out a number of times. If secondary dilutions were required salt solution was placed in immunity flasks in an amount determined by the dilution desired, and the primary dilution delivered from wash-out capacity pipettes of 1 or 2 c.c. capacities.

Filling the Syringes.

Two syringes were set up for each sample. Four additional syringes were set up for controls, each containing the L plus dose of standard toxin and one unit of standard homologous antitoxin.

Rosenau syringes without the side arm were attached to sterile vaselined needles by means of short heavy rubber tubing. The standard toxin was first pipetted into the syringe by means of graduated delivery pipettes, then followed the addition of 1 c.c. of the antitoxin sample measured by 1 c.c. delivery pipettes. As each c.c. of the diluted antitoxin was added to the L plus dose of toxin, the syringe was briskly rotated to insure proper and thorough mixing.

Injection.

The injections were made into guinea pigs one hour after the antitoxin had been placed in the syringes, remaining during the interim at room temperature. The guinea pigs employed conformed as nearly as possible to a 250 gram weight. Weights of less than 240 or more than 280 grams made the animals unsuitable. Any variance in weights inside these limits were corrected for by distributing the animals in such a manner that each group was represented by heavy and light animals. Before injections were made the abdominal wall of the guinea pig was closely clipped but not disinfected. Injections were made subcutaneously in the abdominal region, the needle being inserted its entire length, passing through the linea alba to prevent leakage upon withdrawal. Following the injection of the toxin-antitoxin mixture a volume of physiological salt solution sufficient to bring the total volume to

about 4 c.c. was swirled through the syringe and injected.

Interpretation of Results.

If the antitoxin had not lost any of its potency, the animals injected with the L plus dose of toxin died in approximately four days. (This could not be strictly adhered to and some leaway for the interpretation of results had to be given due to the biologic factor of individual resistance of the animals). Four control animals employed in the testing of the standard antitoxin and toxin died upon the fourth day after injection.

Testing of Tetanus Antitoxin.

The treatment of the standard tetanus toxin and antitoxin was similar to the methods employed in diphtheria standard toxin and antitoxin with these differences. The mixing of tetanus toxin and antitoxin was done by the light of a carbon filament incadescent lamp, and secondly, the dilutions were carried one decimal further than with diphtheria antitoxin and toxin.

Interpretation of Results.

Due to the fact that autopsies yield not definite information as to the cause of death from tetanus it was necessary to make observations of the animals for tetanic symptoms. These were observed on the second, third, and fourth days by making a thorough inspection of the animals. An expression of the degree of tetanic symptoms was recorded as follows: Hypertonicity, slight symptoms, symptoms, marked

symptoms and severe symptoms. Allowance for the individual variance of the animal to response was made and evaluated as in the diphtheria antitoxin tests.

Testing of Diphtheria Toxins and Tetanus Toxins.

With the testing of these substances a preliminary potency test was conducted to determine the exact M.L.D. per c.c. After this determination dilutions were made in exactly the same manner as in the case of the antitoxins, except in the final dilutions one M.L.D. was contained in 2 c.c. The method of injection into guinea pigs was the same as that used with antitoxins and the results were interpreted in the same manner. Animals used in the testing of tetanus toxins and antitoxins were on the average 100 gms. heavier in weight than those used in diphtheria toxin and antitoxin. The weights were within the limits of 350 and 380 grams in order to be used.

RESULTS OF POTENCY TESTS.

Diphtheria Antitoxin Protocols.

Protocol A.

Diphtheria antitoxin.

Antitoxin held at 40°F. for 16 days, and subsequently incubated for 14 days at 37°C.

Units per c.c. - 1300

Standard toxin potency - 0.162 L plus dose diluted to 2.27 c.c.

Serum without any preservative -	animal #1 died 7 days after injection, showed diphtheritic lesions;
	animal #2 died 7 days after injection, showed diphtheritic lesions.
Serum plus 0.5% phenol -	animal #3 discharged, no lesions;
	animal #4 died in 5½ days, lesions.
Serum plus 0.4% trikresol -	animal #5 died 7 days, diphtheritic lesions;
	animal #6 died 9 days, diphtheritic lesions.
Serum plus 1-5000 parts metaphen -	animal #7 died 7 days, diphtheritic lesions;
	animal #8 died 7 days, diphtheritic lesions.
Serum plus 1-5000 parts mercurophen -	animal #9 died 8 days, diphtheritic lesions;
	animal #10 died 8½ days, diphtheritic lesions.

The L plus dose of standard toxin administered in this experiment was just a little low.

Protocol B.

Diphtheria antitoxin.

Antitoxin held at 40°F. for 16 days and then incubated for 22 days at 37°C.

Units per c.c. - 1300

Standard toxin potency - 0.165 L plus dose in 2.27 c.c.

Serum without any preservative -	animal #1 died 3½ days, diphtheritic symptoms; animal #2 died 4 days, diphtheritic symptoms.
Serum plus 0.5% phenol -	animal #3 died 5 days after injection with symptoms; animal #4 died 4 days after injection with symptoms.
Serum plus 0.4% trikresol -	animal #5 died 6 days after injection with symptoms; animal #6 died 5 days after injection with symptoms.
Serum plus 1-5000 parts metaphen -	animal #7 died 4 days after injection; symptoms animal #8 died 4 days after injection; symptoms
Serum plus 1-5000 parts mercurophen -	animal #9 died 5 days after injection; symptoms animal #10 died 5½ days after injection; symptoms

Protocol C.

Diphtheria antitoxin.

Antitoxin held at 40°F. for 16 days, and then incubated for 39 days at 37°C.

Units per c.c. - 300.

Standard toxin potency - 0.165 L plus dose.

Serum without preservative -	animal #1 died in 4 days; diphtheritic lesions animal #2 died 5 days; diphtheritic lesions.
Serum plus 0.5% phenol -	animal #3 died in 4 days; diphtheritic lesions, animal #4 died in 4 days; diphtheritic lesions.
Serum plus 0.4% trikresol -	animal #5 died in 5 days; diphtheritic lesions, animal #6 died in 6 days; diphtheritic lesions.
Serum plus 1-5000 parts metaphen -	animal #7 died 4½ days; diphtheritic lesions, animal #8 died 5 days; diphtheritic lesions.
Serum plus 1-5000 parts mercurophen -	animal #9 died in 5 days; diphtheritic lesions, animal #10 died in 5 days;diphtheritic lesions.

RESULTS OF POTENCY TESTS

OF DIPHTHERIA TOXIN.

Protocol D.

Diphtheria toxin.

The toxin was held at 40°F. for 16 days and then incubated for 16 days at 37°C.

M.L.D. per c.c. - 450

Serum without preservative -	animal #1 died in 5 days, diphtheritic symptoms;	
	animal #2 died in 4 days, diphtheritic symptoms.	
Serum plus 0.5% phenol -	animal #3 died in 8 days, diphtheritic symptoms;	
	animal #4 died in 7 days, diphtheritic symptoms.	
Serum plus 0.4% trikresol -	animal #5 died in 5 days, diphtheritic symptoms;	
	animal #6 died in 7 days, diphtheritic symptoms.	
Serum plus 1-5000 parts metaphen -	animal #7 died in 7 days, diphtheritic symptoms;	
	animal #8 died in 7 days, diphtheritic symptoms.	
Serum plus 1-5000 parts mercurophen -	animal #9 died in 8 days, diphtheritic symptoms;	
	animal #10 died in 7 days, diphtheritic symptoms.	

Protocol E.

Diphtheria toxin.

The toxin was held at 40°F. for 16 days and then incubated for 28 days at 37°C.

M.L.D. per c.c. - 450

Serum without preservative	-	animal #1 died 7 days, diphtheritic symptoms; animal #2 died 5 days, diphtheritic symptoms.
Serum plus 0.5% phenol	-	animal #3 died 5 days, diphtheritic lesions; animal #4 died 5 days, diphtheritic lesions.
Serum plus 0.4% trikresol	-	animal #5 died 8 days, diphtheritic symptoms; animal #6 died 6½ days, diphtheritic symptoms.
Serum plus 1-5000 parts metaphen	-	animal #7 died 4 days after injection, diphtheritic symptoms; animal #8 died 7 days after injection, diphtheritic symptoms.
Serum plus 1-5000 parts mercurophen	-	animal #9 died 6 days after injection, diphtheritic symptoms; animal #10 died 7 days after injection, diphtheritic symptoms.

RESULTS OF POTENCY TESTS

OF TETANUS ANTITOXIN

Protocol F.

Tetanus antitoxin.

Antitoxin kept at a temperature of 40°F. for 24 days, then incubated for 7 days at 37°C.

Units per c.c. -225

Standard toxin potency - 0.00065 M.L.D.

Serum without preservative - animal #1, 4 days - symptoms (tetanic) survived;
animal #2, 4 days - symptoms " survived.

Serum plus 0.5% phenol - animal #3, 4 days - symptoms - survived;
animal #4, 4 days - slight symptoms, survived.

Serum plus 0.4% trikresol - animal #5, 4 days - symptoms, survived;
animal #6, 4 days - symptoms, survived.

Serum plus 1-5000 parts metaphen-animal #7, 4 days - symptoms, survived;
animal #8, 4 days - severe symptoms, survived.

Serum plus 1-5000 parts mercurophen-animal #9, 4 days - severe symptoms, survived;
animal #10,4 days - severe symptoms, survived.

Protocol G.

Tetanus antitoxin.

Antitoxin kept at a temperature of 40°F. for 39 days, then incubated for 14 days at 37°C.

Units per c.c. - 500

Standard toxin potency - 0.00068 M.L.D.

Serum without preservative	-	animal #1, died 5 days, tetanic symptoms; animal #2, died 4 days, tetanic symptoms.
Serum plus 0.5% phenol	-	animal #3, died 5 days, tetanic symptoms; animal #4, died 5 days, tetanic symptoms.
Serum plus 0.4% trikresol	-	animal #5, died 4½ days, tetanic symptoms; animal #6, died 6 days, tetanic symptoms.
Serum plus 1-5000 parts metaphen	-	animal #7, died 5 days, tetanic symptoms; animal #8, died 6 days, tetanic symptoms.
Serum plus 1-5000 parts mercurophen	-	animal #9, died 4 days, tetanic symptoms; animal #10, died 5 days, tetanic symptoms.

RESULTS OF POTENCY TESTS OF

TETANUS TOXIN.

Protocol H.

Tetanus toxin.

Toxin held at a temperature of 40°F. for 24 days, then incubated for 14 days at 37°C.

M.L.D. per c.c. - 10000.

Serum without preservative -	animal #1, discharged, no symptoms; animal #2, discharged, no symptoms.
Serum plus 0.5% phenol -	animal #3, discharged, no symptoms; animal #4, discharged, no symptoms.
Serum plus 0.4% trikresol -	animal #5, discharged, no symptoms; animal #6, discharged, no symptoms.
Serum plus 1-5000 parts metaphen -	animal #7, discharged, no symptoms; animal #8, discharged, no symptoms.
Serum plus 1-5000 parts mercurophen -	animal #9, discharged, no symptoms; animal #10, discharged, no symptoms.

Subsequent potency tests were made upon this serum in M.L.D's. of 5000, 1000, and 100 and found that no symptoms were evidenced. The tetanus toxin completely lost its potency upon incubation at 37°C.

DISCUSSION.

The tests were so devised that if the biologics had not lost their potency, the test animals injected with the L plus dose of toxin should die in approximately four days. However, individual guinea pigs vary in their power of response to an antitoxin and toxin and this caused a variation in the death rate of animals on identical dosages; as shown by a review of the protocols. If the biologic factor of individual resistance is taken into consideration in the interpretation of the results the following conclusions seem to be warranted.

Diphtheria antitoxin when preserved with phenol, trikresol, metaphen, and mercurophen showed no loss of immunizing power from that of the unpreserved serum when incubated at 37°C. for sixteen, twenty-two, and thirty-nine days.

The diphtheria toxin showed no decrease in potency from that of the unpreserved toxin after sixteen and twenty-eight days incubation at 37°C.

Tetanus antitoxin retained its immunizing power for the periods studied; seven and fourteen days.

Incubation at 37°C. for fourteen days caused a complete loss of potency in the control and preserved sera of tetanus toxin.

A close analysis of the potency tests showed similar results with no definite evidence of loss of potency due to the action of any of the chemical substances used as preservatives.

HISTOPHATOLOGIC STUDY OF THE EFFECTS OF METAPHEN AND MERCUROPHEN UPON THE TISSUES.

The ability of certain mercuric salts to produece a nephritis has been known for years.

In 1860 Pavy[11], induced an experimental nephritis with mercury. He considered the Malphigian bodies to be the seat of an injury and also noted the necrosis of tubular epithelium. Heinke[12] considered the toxic action of bichloride of mercury in the kidney to be due to vasomotor paralysis, with resultant thrombosis and infarction. At a later date Aschoff[13] reported in detail the renal pathology of mercuric chloride poisoning, which consisted in swelling, hyaline vacuolation, and necrosis of the tubular epithelium.

The present study was undertaken to learn if the mercurials metaphen and mercurophen produced any tissue changes in 25% Maximum Tolerated Doses. This quantity of each substance was chosen as it permits a wide margin of safety in the preservation of the biologics.

Procedure.

For this study albino rats were used as the experimental animals. One hundred and fifty per cent and 25 per cent

of the Maximum Tolerated Doses of metaphen, mercurophen, and mercuric chloride were administered intramuscularly. These dosages were used in order that the lesions produced by a toxic dose and sub-tolerated dose might be learned. Mercuric chloride was also employed so that a comparative study of the lesions produced by metaphen and mercurophen might be made.

The animals were killed at varying lengths of time in an attempt to observe the various stages of the lesions produced. The histologic study was limited to the heart, liver, spleen, and kidneys. No gross lesions were observed in the other organs. The following detailed protocols show the findings in each test animal.

Mercurophen.

150 per cent Maximum Tolerated Dose.

Rat #1.

Died on third day. Post mortem autolysis did not permit a study of the tissues.

Rat #2.

Died three days after injection. Post mortem observations:

Spleen - enlarged

Heart - normal

Liver - normal

Kidney - numerous pale yellow flecks in the cortex.

Microscopic findings:

Heart muscle - some areas of transverse fragmentation. Cross striation is generally well preserved. Some evidence of interstitial fibrosis.

Spleen - engorged with blood with considerable numbers of lymphocytes in the pulp.

Liver - engorged with blood. The periphery of the portal vein shows some fibrosis. The nuclei are well stained. Relatively few liver cells in the peri-portal areas are filled with small fat droplets.

Kidney - in the outer zone of the cortex the epithelial cells in some tubules appear cloudy, others oxyphil and granular, others show coagulation necrosis, some tubules are calcified, some swollen, and in others the epithelium is desquamated. The glomeruli in general appear quite normal, an occasional one showing poor nuclear staining. There is moderate intertubular congestion. The straight collecting tubules are not much involved, a few show granulation and a few calcified patches. Fat droplets are demonstrated in the epithelium of the proximal convoluted tubules. Several patches of fibrinoid degeneration were found in the outer and the intermediate zones of the convoluted tubules.

Rat #3.

Died three days after injection. Post mortem observations:

Heart - normal

Liver - normal

Spleen - enlarged, bluish-black color

Kidney - showed a few white specks in the cortical substance.

Microscopic findings:

Heart - a few areas of transverse fragmentation, otherwise normal.

Liver - no lesions.

Spleen - the Malphigian bodies are enlarged. The pulp is engorged with blood and contains a considerable amount of hemosiderin and many small lymphocytes.

Kidney - in the outer zone of the cortex the convoluted tubules are swollen, the epithelium in some granular, and in others desquamated. There are several large areas of hemorrhage and a generalized small lymphocyte infiltration. The glomeruli are normal in appearance. Calcification is seen in a few of the convoluted tubules. The epithelium of the collecting tubules is granular and swollen. There is some interstitial hemorrhage and small lymphocyte infiltration. There is a complete absence of casts.

Rat #4.

Died three days after injection. Post mortem observations:

Heart - normal

Liver - normal

Spleen - enlarged

Kidney - the cortex is swollen and shows pale yellow patches.

Microscopic findings:

Heart - normal

Liver - congested. There are small patches of granular necrotic liver tissue showing no nuclear staining, bordered by small lymphocytes and situated near the portal venules. Liver cells in the peri-portal areas are filled with small fat droplets.

Kidney - in the cortex some tubules are swollen and show coagulation necrosis, while others are granular. Some tubules show complete desquamation and breaking down of the epithelium to the basement membrane. Calcified tubules are found throughout the cortex. The majority of the glomeruli are normal, several contain some granular exudate in the capsular spaces. Some of the straight collecting

tubules are swollen and others granular, while a few contain hyaline casts. Large areas of hemorrhage are found within the zone occupied by Henle's loops and the collecting tubules. A moderate amount of fibrinoid degeneration is seen in the convoluted tubules in the intermediate zone. Very small fat droplets are demonstrated in the basal portion of the epithelial cells of the convoluted tubules in the intermediate zone.

Rat #5.

Died five days after injection. Post mortem observations:

Heart - normal

Liver - normal

Spleen - normal

Kidney - the cortex is swollen and shows pale yellow patches.

Microscopic findings:

Heart muscle - normal

Spleen - Malphigian bodies are very much enlarged, made up of and bordered by numerous small lymphocytes. There is a moderate amount of interstitial fibrosis, diffuse lymphocyte infiltration, a few reticulum cells, and a number of

plasma cells within the pulp.

Liver - the liver shows a few liver cells in the peri-portal areas filled with small fat droplets.

Kidney - outer zone of the cortex shows areas of hemorrhage. Some tubules show complete coagulation necrosis, others are swollen and granular, while other convoluted tubules show desquamation of the epithelium and filling of the lumen with cellular debris. There is no calcification. A few glomeruli show lymphocytes within Bowman's capsules. The straight tubules are thin walled, a few show cloudy swelling. Otherwise, the collecting tubules appear normal.

Rat #6.

Died five days after injection. Post mortem observations:

Heart - normal

Spleen - normal

Liver - normal

Kidney - the cortex shows small yellow patches.

Microscopic findings:

Heart muscle - normal

Spleen - moderate amount of blood. The pulp shows interstitial fibrosis and diffuse lymphocyte and plasma cell infiltration.

Liver - moderate amount of congestion. In the ~~port~~-portal areas there is an accumulation of endothelial cells. There is slight cloudy swelling of some of the hepatic cells.

Kidney - in the outer zone of the cortex there is complete coagulation necrosis of the majority of the convoluted tubules. In the intermediate zone some tubules show complete desquamation of the epithelium, others are granular. There is no evidence of calcification but considerable interstitial fibrosis is seen. The limbs of Henle's loops show a few patches of cloudy swelling. The collecting tubules show no lesions.

25 per cent Maximum Tolerated Dose.

Rat #7.

Killed five days after injection. Post mortem observations:

Heart - normal

Spleen - normal

Liver - normal

Kidney - normal

Microscopic findings:

Heart - normal

Spleen - pulp engorged with blood and contains many

lymphocytes and plasma cells.

Kidney - in the outer zone of the cortex the convoluted tubules in a few areas were quite granular, others were swollen, and a few showed partial desquamation of the epithelium. The glomeruli appeared normal. Among the straight tubules there was lymphocyte infiltration and some fibrosis.

Liver - the peri-portal connective tissue showed a heavy infiltration with small lymphocytes and plasma cells.

Rat #9.

Killed seven days after injection.

All organs normal on both gross and histologic examination.

Rat #15.

Killed twelve days after injection.

All organs normal on both gross and histologic examination.

Rat #8.

Killed fifteen days after injection.

All organs normal on both gross and histologic examination.

Rat #11.

Killed seventeen days after injection.

All organs normal on both gross and histologic examination.

Rat #12.

Killed twenty-one days after injection.

Heart, spleen, and liver normal.

Kidney - in the outer zone of the cortex some of the proximal convoluted tubules are swollen; the organ is normal in other respects.

Metaphen.

150 per cent Maximum Tolerated Dose.

Rat #16.

Died three days after injection. Post mortem observations:

Heart - normal

Spleen - enlarged

Liver - normal

Kidney - numerous yellow specks scattered throughout the cortex.

Microscopic findings:

Heart - perivascular fibfosis, with a moderate amount of interstitial lymphocyte infiltration.

Spleen - engorged with blood. Malphigian bodies enlarged. Infiltration with lymphocytes and a few plasma cells in the pulp. Considerable deposition of hemosiderin.

Liver - slight cloudy swelling of the hepatic cells with relatively few liver cells in the peri-portal areas filled with small fat droplets.

Kidney - in the outer zone of the cortex some tubules show oxyphil granules, others are swollen, others show complete desquamation of the epithelium and still others are much dilated and contain casts. The glomeruli as a whole are normal, a few are hyperemic, and contain granular exudate within the capsules. The straight collecting tubules show a moderate amount of cloudy swelling in the distal portion. Small fat droplets are demonstrated in the basal portion of the epithelial cells of the convoluted tubules in the outer zone.

Rat #20.

Died three days after injection. Post mortem observations:

Heart - normal

Spleen - greatly swollen

Liver - normal

Kidney - the entire cortex shows yellowish deposits.

Microscopic findings:

Heart - normal

Spleen - the Malphigian bodies are very much enlarged, and poorly defined. The pulp is engorged and contains much hemosiderin. There are many lymphocyte and plasma cells in the pulp.

Liver - in the peri-portal zones there is cloudy swelling of the hepatic cells which are filled with small fat droplets. Moderate fibrosis occurs about the bile ducts in the peri-portal zones.

Kidney - in the outer zone of the cortex a majority of the convoluted tubules are granular and swollen. Some show no nuclear staining, some oxyphil granulation of the epithelium, and still others complete desquamation of the epithelium, filling the lumen with cellular debris. There is a moderate amount of interstitial fibrosis. The glomeruli are generally well preserved, a few show hyperemia. The convoluted tubules in the intermediate zone contain a few casts but there is no calcification. The collecting tubules are thin walled and contain a few hyaline casts.

Rat #19.

Died five days after injection. Post mortem observations:

Heart - normal

Liver - normal

Kidney - normal

Microscopic findings:

Heart muscle - normal

Spleen - Malphigian bodies are enlarged, and contain numerous lymphocytes and plasma cells. The pulp is engorged

and contains hemosiderin and numerous lymphocytes and plasma cells.

Liver - the venules are congested with blood and broken down erythrocytes. Relatively few hepatic cells in the periportal areas contain small fat droplets.

Kidney - in the outer zone of the cortex all the tubules are swollen, some show granular degeneration, others show the epithelium to be mostly desquamated, and others contain hyaline casts. A few glomeruli show fibrosis about the periphery of Bowman's capsules. No lesions are found in the straight collecting tubules.

Rat #18.

Died five days after injection. Post mortem observations:

Heart - normal

Spleen - normal

Liver - normal

Kidney - a few small yellow patches in the cortex

Microscopic findings:

Heart muscle - normal

Spleen - the Malphigian bodies show poor staining, are enlarged and a number show fine collagen fibrils permeating

their structure. The pulp is rich in blood, contains numerous lymphocytes and much hemosiderin.

Liver - normal

Kidney - in the outer zone of the cortex some of the convoluted tubules show oxyphil granular degeneration, others are swollen, others calcified and others contain casts, There is no coagulation necrosis. In the intermediate zone there is calcification of many tubules, while many others show partial desquamation of the epithelium. The glomeruli as a whole are normal, a few showing partial anemia. The straight collecting tubules are sometimes atrophied, and contain a few casts. There is a moderate amount of fibrinoid degeneration in the outer and intermediate zones of the cortex.

Rat #17.

Died five days after injection. Post mortem observations:

Heart - normal

Liver - normal

Spleen - normal

Kidney - normal

Microscopic findings:

Heart - normal

Liver - normal

Spleen - normal

Kidney - the convoluted tubules in the outer zone of the cortex show some swollen, others granular, others partially necrotic, and others partially desquamated. The glomeruli are normal with the exception of a few that contain a granular exudate within the capsular spaces. The straight collecting tubules are normal. Within the cortex are several small foci of fibrinoid degeneration of the epithelium.

Rat #21.

Died seven days after injection. Post mortem observations:

Heart - normal

Spleen - enlarged

Liver - normal

Kidney - a few yellow patches within the cortex

Microscopic findings:

Heart - interstitial infiltration with a few lymphocytes.

Spleen - engorged. The pulp contains considerable hemosiderin.

Liver - normal

Kidney - the convoluted tubules in the outer zone show some swollen, others granular, and a few contain casts. The distal convoluted tubules show partial epithelial desquamation in one small area. There are a number of foci of interstitial infiltration. A few glomeruli show granular exudate within the capsules. Henle's loops and the straight collecting tubules are normal.

Metaphen.

25 per cent Maximum Tolerated Dose.

Rat #27.

Killed five days after injection.

No gross post mortem lesions.

Microscopic findings:

Heart - normal

Spleen - Malphigian bodies enlarged. Pulp engorged with blood.

Liver - normal

Kidney - the outer zone of the cortex shows some swollen convoluted tubules.

Rat #23.

Killed twelve days after injection.

No gross post mortem lesions.

Microscopic findings:

Heart - normal

Liver - normal

Spleen - normal

Kidney - normal

Rat #25.

Killed twelve days after injection.

No gross post mortem lesions.

Microscopic findings:

Liver - normal

Spleen - engorged with blood.

Heart muscle - normal

Kidney - normal

Rat #26.

Killed nineteen days after injection. Post mortem observations:

Heart - normal

Liver - normal

Spleen - normal

Kidney - normal

Microscopic findings:

Liver - normal

Spleen - normal

Heart muscle - normal

Kidney - normal

Rat #24.

Killed nineteen days after injection.

Both the gross and histologic examinations showed the heart, liver, spleen, and kidneys to be normal.

Rat #22.

Killed twenty-two days after injection. Post mortem observations:

Heart - normal

Spleen - normal

Liver - normal

Kidney - normal

Microscopic findings:

Liver - normal

Heart muscle - normal

Spleen - normal

Kidney - patches of interstitial fibrosis among the proximal convoluted tubules of the cortex.

Mercuric Chloride.

150 per cent Maximum Tolerated Dose.

Rat #15a.

Died three days after injection; post mortem autolysis.

Rat #13.

Died five days after injection. Post mortem observations:

Heart - normal

Spleen - enlarged

Liver - enlarged

Kidney - numerous small yellow granules in the cortex.

Microscopic findings:

Heart muscle - small patches of granular degeneration obscuring the cross striation of the muscle fibres in several areas. Slight patchy lymphocyte infiltration among the muscle fibres.

Spleen - the Malphigian bodies are poorly defined, made up of lymphocytes and plasma cells. The pulp is engorged with blood and contains much hemosiderin, numerous lymphocytes and a few plasma cells.

Kidney - in the outer zone of the cortex the convoluted tubules are all swollen, in some to the point of occlusion. A

few tubules show granular degeneration. In the intermediate zone the tubular epithelium is more granular, sometimes partially desquamated. The majority of the glomeruli are normal, a few showing hyperemia. Among the straight tubules there is lymphocyte infiltration and proliferation of young fibroblasts.

Rat #14.

Died seven days after injection. Post mortem observations:

At the site of injection there is a large area of necrosis in the muscular tissue.

Heart - normal

Spleen - greatly enlarged

Liver - normal

Kidney - the cortex is cloudy and slightly swollen.

Microscopic findings:

Heart muscle - slight interstitial lymphocyte infiltration.

Spleen - the pulp is engorged with blood. There is a heavy deposit of hemosiderin and an infiltration with small lymphocytes. The Malphigian bodies are enlarged.

Liver - many hepatic cells show cloudy swelling. There are relatively few liver cells in the peri-portal area filled with minute fat droplets.

Kidney - the convoluted tubules in the outer zone of the cortex show varying phases of necrosis. Some are swollen and granular, some show complete coagulation necrosis, and others calcification. In the intermediate zone there is more extensive coagulation necrosis than in the outer zone. In many tubules the epithelium is desquamated, other tubules are calcified, and still others contain casts. This area shows some interstitial fibrosis. The glomeruli appear normal or occasionally hyperemic. The straight collecting tubules are normal.

Rat #15.

Died seven days after injection. Post mortem observations:

Heart - normal

Liver - normal

Spleen - normal

Kidney - cortex contained numerous yellow patches.

Microscopic findings:

Heart - normal

Spleen - shows enlargement of the Malphigian corpuscles. The spleen pulp contains a moderate amount of blood and some pigment.

Liver - there is cloudy swelling of the hepatic cells about the portal veins with comparatively few cells filled with small fat droplets.

Kidney - in the outer zone of the cortex, some of the tubules show oxyphil granulation, others are swollen, others desquamated and others show coagulation necrosis. There is some calcification of a few of the tubules. Several areas show foci of small lymphocyte infiltration and fibrosis. The glomeruli are normal. A few collecting tubules contain casts.

25 per cent Maximum Tolerated Dose.

Rat #31.

Killed seven days after injection.

The gross and minute ~~pathological~~ findings in the heart, liver, spleen, and kidneys are negative.

Rat #29.

Killed seven days after injection.

Gross and microscopic ~~pathologic~~ findings in the heart, liver, spleen, and kidneys are normal.

Rat #30.

Killed twenty-one days after injection.

Gross and histologic findings in the heart, liver, spleen, and kidneys are negative.

Rat #28.

Killed twenty-one days after injection.

Gross and histologic findings in the heart, liver, spleen, and kidneys are normal.

Discussion.

The administration of 150% of the Maximum Tolerated Doses of metaphen, mercurophen, and mercuric chloride, produced tissue injuries which were essentially identical in character and in degree. The changes produced by all three mercurials are therefore described in common as follows:

The heart generally showed no definite pathologic changes. In some animals there was transverse fragmentation of the muscle fibres, in a few, interstitial fibrosis and infiltration with lymphocytes.

The spleen was usually enlarged and engorged with blood. The Malphigian corpuscles were enlarged and sometimes contained plasma cells as well as lymphocytes. The pulp generally contained large amounts of hemosiderin and often many lymphocytes and a few plasma cells.

In most of the animals when any histologic change was noted in the liver, it comprised only cloudy swelling of the hepatic cells in the peri-portal areas. Relatively few of these cells contained small fat droplets. In one instance where the animal survived for a longer period of time, there was peri-portal infiltration with lymphocytes and plasma cells and some fibrosis.

The kidneys revealed the most constant and conspicuous changes. A typical toxic nephrosis was produced in each instance. The glomeruli generally were not involved, occasionally several

appeared hyperemic, others partially anemic, and infrequently a granular exudate was found in the capsular spaces of others. The convoluted tubules were altered most, the changes varying only in intensity and mostly governed by the length of time the animal survived. The lesions were characterized by cloudy swelling, casts, oxyphil, granular, and fatty degeneration, epithelial desquamation, coagulation necrosis, and calcification. Intracellular fibrin and small droplets of fat were found in the convoluted tubules in the outer and intermediate zones.

The straight collecting tubules, and the loops of Henle usually escaped severe injury. As a general rule the changes here were confined to moderate cloudy swelling, or a thinning of the walls. Animals surviving five days or more sometimes showed lymphocytic infiltration and the development of young fibrous tissue in the interstices of the tissue.

Mercuric chloride produced necrosis of the muscular tissue at the site of injection in two animals. This condition was not found in any of the metaphen and mercurophen rats.

A study of histological changes produced by the administration of 25% of the Maximum Tolerated Dose of metaphen, mercurophen, and mercuric chloride, showed tissue changes in only one animal --

a rat injected with mercurophen. The kidneys showed cloudy swelling of the convoluted tubules and lymphocytic infiltration and fibrosis. All other test animals showed no alterations of the tissues studied.

The results obtained in these experiments seem to justify the conclusion that 150% Maximum Tolerated Doses of metaphen, mercurophen, and mercuric chloride produced tissue changes which were essentially identical. Lesions of the heart muscle, spleen, and liver, were not constant and when demonstrated at all were of minor importance. The kidney showed the most characteristic changes; namely, a toxic nephrosis.

It was not possible to differentiate the lesions produced by the three mercurials studied from one another. The degree of tissue injury produced depended apparently on the amount of mercury administered and the length of survival. One fourth of the Maximum Tolerated Dose produced no evident histologic changes.

SUMMARY.

Yatren and hexylresorsinol (1-1000) do not have sufficient germicidal power to permit their use in small enough quantities for the preservation of biologics.

Colloidal chloro-cresol, 25%, and colloidal chloro-cresol, 50%, colloidal chloro-thymol, 25%, and colloidal chloro-thymol, 50%, because of their destructive action in precipitating the albuminous substances to such a marked degree are unsuited for biologic preservatives.

Intramuscular injections in rats of 1% solutions of metaphen and mercurophen were approximately 14 times more toxic than phenol and trikresol. In serum, mercurophen has an approximate germicidal potency 40 times greater, and metaphen 25 times greater than phenol and trikresol.

The increased germicidal power of the mercurials causes them to be less toxic than phenol and trikresol, when used as biologic preservatives.

Neither metaphen nor mercurophen precipitate proteins at their germicidal strength while both phenol and trikresol do.

The use of phenol and trikresol in the spinal canal is prohibited by ~~its~~ their extreme toxic action upon the respiratory and vasomotor centers.

Serums preserved with metaphen and mercurophen when injected into the spinal canal had no effect upon the blood pressure or respiratory movements.

It was demonstrated that the type of tissue injury produced by

metaphen and mercurophen is entirely dependent upon the quantity of ~~mercury~~ the drug administered. Twenty-five percent Maximum Tolerated Doses produce no histologic changes.

As far as could be determined neither phenol, trikresol, metaphen, nor mercurophen decreased the potency of toxins and antitoxins.

CONCLUSIONS.

1. Of the biologic preservatives studied the mercurial mercurophen appears to be the most satisfactory with metaphen as second choice.

2. Further studies on the adaptability of mercurials as biologic preservatives seem warranted.