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The Toxicity and Bactericidal Power of the Bile Acids and Derivatives

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Introduction

Pneumonia is generally recognized as an acute infection of the lungs characterized by a massive inflammatory exudate in one or more lobes.It is one of the more serious infectious diseases afflicting man,ranking next to tuberculosis as a cause of death.Of 2000 cases collected at the Bellevue Hospital in New York City, 95.6 % were caused by the Diplococcus pneumoniae(5).Of 529 cases at the Hospital of the Rockefeller Institute 86 % were caused by this organism(1).

At present there is no specific treatment of definite value for pneumococcus pneumonia except for those cases infected with type 1.For these homologous anti-pneumococcus serum has proven of value in reducing the death rate(1,50).After the work of Neufeld(39),and of Dochez and Gillespie,and others at the Rockefeller Institute(8,11,13,1),in which the differentiation into the various types and its importance was shown,it was found that type 1 antiserum,used early in the course of the infection and in large amounts,gave the patients having this type of pneumococcus a certain degree of immunity and kept the blood cultures negative(1,5,9, 50).In the large,unsellected series of cases studied at the big hospitals the death rate has been reduced about one third,while under more

favorable conditions, such as in private practice, or if the cases are sellected, the decrease is much more.

An extract of immune serum(~~30~~), essentially a serum free solution of the antibodies, has been tried with equably favorable results. It was prepared in a polyvalent form, but was of questionable value in type 2 cases and was definitely without any in cases due to type 3 and 4(6). As with the whole antiserum, only in infections of type 1 pneumococcus was there any action. Its advantage lies in the fact that it does not contain the blood proteins which often cause unfavorable reactions.

The antibodies have been concentrated as much as five to fifteen times(20,11) using a method of precipitation and resolution. With this preparation much more of the effective antibodies can be given in a smaller volume of solution and without many of the unfavorable reactions(7,6). It has given results comparable to those obtained with whole antiserum and with extract.

Vaccines have been tried rather extensively, but have proven useful only as a prophylactic measure. Their therapeutic value is very doubtful(5,47,50). Immune responses can not be elicited rapidly enough to influence the course of the disease.

The first compound tried extensively as a therapeutic agent in pneumonia was ethyl-hydrocuprein(Optochin)

Morgenroth and Levy,who introduced it(36),showed that it killed the pneumococcus in very high dilutions,and that it had some protective and curative action when administered to infected animals.Two years careful trial of Optochin at the Rockefeller Institute(35) has proven that its use does not reduce the death rate. By careful regulation of the amounts given to the patients,it was possible to keep the blood cultures negative in many cases.However its action was confined to the blood stream;the course of the pneumonic lesion was uninfluenced.In addition some developed symptoms of poisoning.Cecil(5) likewise has found it of no value

Other cinchona derivatives have been tried experimentally without promise(18).

A number of commercial quinine preparations have been examined for their ability to kill the pneumococcus in vitro(21,22,32),and have been suggested for clinical trial.Quinine in various forms has been examined experimentally(32,46,47,52),and has been used clinically,especially by Solis-Cohen.He claims as good results for his "definite" treatment of all pneumonias with quinine as have been obtained with antiserum in the treatment of type 1 infections(48).However most clinicians do not consider it favorably(5).

Many dye substances and coal tar products have been examined with respect to toxicity and bactericidal

properties.Felton and Dougherly (17) examined derivatives of triphenyl methane,of acridine ,quinolin,safrinin,phenazin,quinon,and quinine; only in the latter case were any compounds found with a very great action on the pneumococcus.in vitro.A number of mercurials were found to have comparatively little action on the pneumococcus in vitro(51).Metaphen has been shown to have some protective and curative action in experimental infections(12).No reports of the clinical trial of these substances was found.

Micro-organisms have been isolated which decompose the specific polysaccharide of types 1,2,and 3 pneumococci(14,43).The enzyme that acts on the specific polysaccharide of type 3 pneumococcus was isolated from the organism,and was found to cure rabbits infected intradermally with a type 3 pneumococcus.(2,23,24). Apparently this enzyme has not been tried clinically.

Comparatively little appears to have been done in regard to the use of the bile salts in pneumonia.Castellanos has used bile salts for treating empyema.And Barjot has tried a solution of sodium taurocholate on negroes suffering from pneumonia(57).The latter had but four treated cases so that his favorable results can not be accepted as conclusive. Elton(17) appears to have

found some correlation between the development of jaundice and recovery in pneumonia patients.Ziegler(56,57,58) has tried sodium taurocholate and sodium dehydrocholate clinically;he feels that the bile salts have a specific action in terminating the pneumonia.Experimentally sodium dehydrocholate merely prolonged the lives of infected rabbits.

By using small amounts of bile or of sodium taurocholate Hilgermann(23) showed a curative action in mice infected peritoneally or subcutaneously with pneumococcus.Larger anmounts of the bile salts,themselves nonfatal,killed the animals in a very short time.Eddy(15) found that mice infected peritoneally with a pneumococcus died when bile salts were given intraperitoneally.Often the peritoneum was sterile,but blood cultures were positive.Hilgermann feels that the bile salts act to dissolve enough of the infecting pneumococci so that endotoxins are formed in sufficient amounts to produce an immune response,while not in large enough amounts to be very toxic.Ziegler(57) has shown that pneumococci treated with sodium dehydrocholate immunized rabbits very rapidly.It would appear then,if the bile salts do have any curative action,that it is a secondary one in that they merely aid in the production of an immunity.

Bile salts injected intravenously have been shown

to disappear from the blood very rapidly(25).It is doubtful whether appreciable amounts of the known bile salts,on intravenous injection,would reach the pneumococci in an actual pneumonic lesion.This together with the fact that only under special experimental conditions did they show a protective action makes it improbable that any of the bile salts investigated to-date will actually prove useful in the treatment of pneumonia.Pneumocholin(pneumococci treated with bile salts) may find a use in rapidly inducing an immune reaction,but its value is necessarily limited in that it must be used very early in an infection.

The therapeutic agent desired is one that will arrest the development of the pneumonic lesion and effectively prevent it spreading.This is borne out by a new treatment which seems very promising.If the infection is confined to one side,the lung on that side may be collapsed,localizing the infection by cutting out the large peripheral circulation.The course of the infection then resembles that of a common cold.The chemotherapeutic agent must be relatively inert in the animal body,being neither rapidly removed nor toxic,and it must act upon the pneumococcus in comparatively high dilutions.If auch a satisfactory therapeutic agent derived from or related to the bile acids is to be ~~ex-~~

obtained,we must know just what chemical structures of the bile acids are responsible for their properties.

At present we know that the most active bactericidal bile salts are these containing two hydroxyl groups in positions 3 and 12, the salts of deoxycholic acid,of apocholic,and of the various choleic acids being about equal in their power to kill the pneumococcus(18,30).These salts are also the most toxic(18,30 48).The salt of cholic acid,which contains three hydroxyl groups in positions 3,7,and 12,is a much weaker bactericidal agent than the salt of the dihydroxy acid, and is much less toxic.The salt of the triketo acid is a less active bactericidal agent than the salt of the diketo acid.Further it is known that the salt of the triketo acid has a lower bactericidal action and toxicity than the salt of the corresponding trihydroxy acid,and that the salt of the diketo acid is a weaker bactericidal agent than the salt of the dihydroxy acid. (18,30).However no very complete study of these substances appears to have been made.

It has been found that the absence of hydroxyl and (or) keto substitutents on the cholane nucleus,as in cholanic and cholatrienic acid,and that rupture of the ring structure,as in bilianic and cilianic acids,destroys cholagogue action(38).

The following investigation is an attempt to elucidate more fully upon what chemical structure of the bile acids and derivatives the well known toxic and bactericidal powers of these substances depend.

It has been shown that lecithin will antagonize the toxicity of the bile salts for guinea pigs(48),and that lecithin does not antagonize the action of the bile salts in inhibiting the growth of the pneumococcus in vitro(15).However we have found that a solution containing equal amounts of the conjugated bile salts and of lecithin had no favorable influence upon rabbits infected intradermally with a type 1 pneumococcus.It was thought advisable,then,to study the effect of lecithin upon the toxicity and antipneumococcal activity of the substances being examined.

Preparations

Pure cholic and deoxycholic acids were prepared from beef bile according to White(52).The cholic acid after removal of the alcohol of crystallixation,melted at the correct temperature,197 degrees centigrade. All the melting points were obtained in an apparatus such that the whole of the mercury column of the thermometer was heated to the same temperature.They were not corrected.The acetic-choleic acid(the molecular compound of acetic and deoxycholic acids)was recrystallixed twice from ethanol and heated at 110-120 degrees until the melting point came up to 170-171 degrees.The temperature reported is 172(54).

The sodium salts of the two acids were prepared by neutralization of the acids with sodium hydroxide in absolute ethanol and recrystallizing once.The preparation of sodium deoxycholate was attended with considerable loss since it crystallized poorly.The final product was really the salt of ethanol-choleic acid.The sodium deoxycholate was found to crystallize very well from butanol,but the presence of butanol in the product was considered less desirable than that of ethanol.

Dehydrocholic acid was prepared by the oxidation of cholic acid according to Hammarsten(27).Recrystallized

from acetone a pure product was obtained with the reported melting point(60),long,flat plates,almost needles,m.p.237 degrees centigrade.Since no solvent was found from which the salt could be crystallized,the free acid was used in the experiments and neutralized with the equivalent amount of sodium hydroxide in the solution to be employed.

Dehydro-deoxycholic acid was prepared by the oxidation of deoxycholic acid with chromic acid(54).If the product obtained from the reaction mixture was too impure,it would not crystallize from ether as described (54),being too soluble.However it was observed that an excess of ether precipitated out amorphous material,and almost pure dehydro-deoxycholic acid crystallized out when the supernatant solution was concentrated.Recrystallized twice from 70 % acetone it melted at 186 degrees,2 degrees below that reported.The sodium salt was prepared by neutralization of the acid with sodium hydroxide in methanol,evaporation to dryness,and crystallization from 91 % isopropanol.

The oximes of the two keto acids were prepared by treating their alkalin solutions with hydroxylamine(38) Hydroxylamine hydrochloride was added to the solution of the salt and then sodium hydroxide to redissolve the precipitated acid.After about eight hours on the steam

bath the reaction is completed.The oximes were precipitated with acid and recrystallized from 95 % ethanol. The trioxime of dehydrocholic acid melted,with decomposition,at 270-272 degrees.Mylius gives the melting point as 270 degrees with decomposition(30).The dioxime of dehydro-deoxycholic acid did not crystallize well from 95 % ethanol since it was rather insoluble. But by prolonged treatments with large volumes of alcohol it was possible to effect purification.The crystals were small and grew very slowly on digestion,but finally were obtained some large enough that their form could be made out.They were composed of two six sided pyramides attached at their bases,one being about 3-4 times as tall as the other.They sintered at 169-170 degrees and melted at 183.5-184 with decomposition as described(41).

Dehydrocholic acid was condensed with isatin by heating an alkalin solution of the two.The product was precipitated with acid and recrystallized from absolute ethanol(4).Purification was rather difficult so that but a small amount of the pure product was obtained.The flat needles decomposed at 296-297.The product used for the determination of toxicity and bactericidal power was not this pure;it had a slight brown color and decomposed about 290 degrees.

Bilianic acid was prepared by the oxidation of cholic acid at room temperature with potassium permanganate.

It was precipitated with acid,after decolorization with bisulfite as described(38),and crystallized from hot, diluted ethanol.It melted at 264 degrees.The reported melting point is 263.My product was a mixture of bilianic and isobilianic acids,no attempt being made to separate the two because of the small amount obtained.

Methyl deoxycholate was prepared by the usual method of refluxing the acid with methanol and sulfuric acid.The ester was separated from the alcohol by an excess of water.This ester was very difficult to purify since no means of getting it to crystallize out free of the impurities could be found.A fair degree of purity was obtained in the following manner.The hot solution of the ester in methanol was diluted with water just to slight opacity and cooled.Amorphous material separated out which contained most of the coloration.If cooled in the ice box for a sufficient time,some of the ester would crystallize.After separation of the colored material,the rest of the ester was precipitated with more water.Repetition of this process several times removed all the coloration.The final colorless product melted at 65-66 degrees on two successive purifications and was composed of long needles.The reported melting point of methyl deoxycholate is 71-73 degrees(56).

Methyl cholate was made by heating the acid and the alcohol with sulfuric acid on the steam bath and

cooling the concentrated solution in the ice box.The ester crystallized out in clusters of needles.Recrystallized several times from methanol it melted at 147 degrees(42).According to Z. Uraki[49], after repeated crystallizations from alcohol,the ester melts at 155 degrees.

The methyl ester of dehydro-deoxycholic acid was prepared as was the ester of deoxycholic acid.It crystallized well from ether;the final product melted at 131.5-132 degrees,which is the reported melting point.

Methyl dehydrocholate was prepared,m.p.242 degrees, but was not used.

Lecithin was obtained from hog brain.After passing through a meat grinder the brain was thoroughly dried with acetone,and extracted with ether.The concentrated ether extracts were precipitated with an excess of acetone.The acetone insoluble fraction was dissolved in ether and precipitated with absolute ethanol.Precipitation of the concentrated alcoholic solution with acetone yielded crude lecithin.This product was largely freed of the ether-insoluble lipins by allowing the ether solution to stand in the ice box until all the precipitate had settled out.The clear supernatant solution was precipitated with acetone,and the precipitate dried in vacuo until free of the odor of acetone.

Methods

The M L D* for white mice was used as the measure of toxicity of the preparations. The animals were all of the same stock, and many were raised in the laboratory. For a time the diet was Pratts Rabbit Pellets, but later this was changed to Purina Dog Chow; this change did not affect the resistance of the animals. The mice appeared to do quite well on this feed, and breed readily.

The animals all were used approximately 24 hours after the last feeding, having been about twelve hours without food. In the morning they were isolated, and that evening weighed and injected intraperitoneally with a sterile solution of the calculated amount of the material being tested. Those that survived and apparently recovered fully from one experiment were used for a second. The M L D was taken as the smallest amount of substance per unit of weight that killed at least 90 % of the injected animals.

Solutions of the water soluble salts were made up in saline for the M L D 's and in broth for the experiments with pneumococci.

* In brevity the minimum lethal dose is here expressed by the letters M L D.

The esters were dissolved in olive oil that had been treated with sodium hydroxide to remove the free fatty acids.The salts were weighed out into graduated test tubes,dissolved in saline, and diluted to volume.The tubes were closed with cotton plugs and sterilized in live steam.Where the salt was not prepared,that amount of the free acid necessary to give the desired concentration of the salt was weighed out and sodium hydroxide added until neutral to phenolphthalein.Only in the case of the trioxime was the salt too insoluble to give a solution concentrated enough for use.However in this case the salt separated out,after neutralization of the acid with heating,in what appeared to be a very hydrated form so that a stable suspension was formed.This could be injected and was used as such.The solutions of the esters were made as were the salts except that olive oil was used as the solvent.Methyl cholate was too insoluble in oil but formed in it a supersaturated solution that was stable for as long as 12 hours.Methyl dehydrocholate could not be used as no suitable inert solvent could be found as a carrier.

To make the solutions containing the lecithin as well as the bile compound,an amount of it equal to the weight of the bile acid compound was weighed out in the test tube and the solution prepared as above.Usually

it was necessary to break up the lecithin particles mechanically to effect solution at all rapidly.

The solutions prepared in this manner and sterilized remained stable at least two months and probably longer. To the solutions of sodium deoxycholate and its derivatives in broth and to their solutions containing Lecithin ~~broth~~ it was necessary to add more alkali to redissolve free acid that precipitated. In the case of sodium dehydrodeoxycholate, when the solution in broth was sterilized, a crystalline precipitate formed which would not dissolve with excess alkali. But if the concentrated stock solution were made up in water or saline no precipitate formed on sterilization, and the sterile solution could be added to broth and incubated without its formation.

For the experiments on pneumococci a type 1 strain obtained from the Rockefeller Institute was used. The stock culture was kept in the ice box in serum semi-solid agar. The broth used was a beef heart infusion with 2 % peptone and 0.5 % sodium chloride added. It was adjusted to a final pH of 7.6-7.8. This plain broth was used for the concentrated stock solutions of the bile salts and for making the various dilutions in the bactericidal tests. It is necessary to use an enriched media for culturing the pneumococcus; this enriched

media was formed by adding to each 800 cc lot of the plain broth,at the time of dispensing,20 cc of sterile 20 % glucose and 25 cc of sterile rabbit blood.The broth was then dispensed into sterile test tubes,each containing at least 10 cc.This enriched broth was used for all culturing.

The action of the water soluble preparations upon pneumococci was tested in two ways.First the bactericidal concentration was determined,being taken as that concentration in plain broth which would kill enough of the organisms under the conditions of the experiment so that no growth would occur in a subculture made at a given time(15 minutes unless specified otherwise).The method more in detail is as follows:by dilution of the stock solutions of the bile salts with sterile plain broth 4.9 cc lots were made up containing varying amounts of the bile salt preparation being tested.Each was then inoculated with 0.1 cc of a 6-8 hour culture of pneumococcus and put in the incubator at 32-34 degrees.At different time intervals after the inoculation (15 min.,30 min.,1hr.,2hrs.,etc.)subcultures were made, 0.1cc being transfered into 10 cc or more of the enriched broth.Since there was at least a hundred fold dilution in the subculture of the bile salt of which the bactericidal action was being determined,and this amount was later shown not to be able to inhibit growth

it may safely be concluded that when no growth occured in the subculture, the bacteria had been killed by their exposure to the more concentrated agent.

The second action measured was the ability to inhibit growth. The clear supernatant broth after the red corpuscles had settled out was found suitable for the inhibition tests. It contained the added glucose and the serum, and supported a good growth. As before, 4.9 cc lots of broth (enriched) were made up containing varying amounts of the substances being tested. These were inoculated with 0.1 cc of a 6 - 8 hour pneumococcus culture and incubated 14 16 hours. The smallest concentration that consistently prevented visible growth in this time was taken as the inhibitive concentration.

The viability of the cultures used in each experiment was controlled by a test tube of broth without added bile salt. Sterility of the solutions and broth was controlled by tubes containing these without inoculation of pneumococci.

Experimental

TOXICITY OF SODIUM DEOXYCHOLATE: In table 1 are summarized the results of the determination of the M L D of sodium deoxycholate for male and female mice.

Table 1

Sodium Deoxycholate
(3,12-dihydroxycholanate)

Males

Dose mg/gm	Moles x 10^{-7}/gm	Total No.	No. Died	% Died
0.14	3.24	3	3	100
0.12	2.78	4	4	100
0.10	2.32	5	5	100
0.08	1.86	7	3	43
0.06	1.40	3	0	0
		Females		
0.16	3.70	4	4	100
0.14	3.24	4	2	50
0.12	2.78	3	2	66
0.10	2.32	3	1	33
0.08	1.86	4	0	0

In column 1 are the doses in terms of millegrams per gram of body weight, in the second column are the doses in terms of moles x 10^{-7} per gram of body weight, in the third column is the total number of animals injected, in the fourth column is the number of animals that died and in the fifth is the percentage of deaths.

The M L D for the male mouse is 0.10 mg per gm of body weight and for the female 0.16 mg per gm.

One cc of the solutions injected in these experiments contained 0.01 gm of the salt.

DETOXIFICATION OF SODIUM DEOXYCHOLATE BY LECITHIN: The effect of the addition of lecithin upon the toxicity of sodium deoxycholate is to decrease it, more perhaps in the case of the female.than of the male.This detoxifying action is shown in Table 2.

Table 2

Sodium Deoxycholate plus Lecithin

Dose mg/gm	Moles x 10^{-7}/gm	Total No.	No. Died	% Died
	Males			
0.18	4.16	1	1	100
0.16	3.70	1	1	100
0.14	3.24	3	3	100
0.12	2.78	2	2	100
0.10	2.32	2	0	0
0.08	1.86	3	0	0
	Females			
0.18	4.16	3	2	66
0.16	3.70	4	1	25
0.14	3.24	1	0	0

The M L D for the male was increased to 0.12 mg per gm of body weight.The number of females used is small, but it is seen that the M L D for them is raised to at least o.18 mg per gm.

The concentration of the solutions used was such that 1cc contained 0.01 gm of the bile salt and 0.01 gm of lecithin.The volumes injected ranged from 0.15 to 0.32 cc.

THE ACTION OF SODIUM DEOXYCHOLATE ON PNEUMOCOCCUS: In four experiments the bactericidal concentration of sodium deoxycholate was found to be 0.001 gm per cc.

The inhibitive concentration was found to be 0.0006 gm per cc in three experiments.

THE ACTION OF SODIUM DEOXYCHOLATE PLUS LECITHIN UPON PNEUMOCOCCUS: In the presence of an equal amount of lecithin by weight, the bactericidal concentration is increased to 0.004 gm per cc. The inhibitive action of this bile salt, however, is increased, the inhibitive concentration was 0.0004 gm per cc in three experiments.

TOXICITY OF SODIUM CHOLATE: Sodium cholate is much less toxic than sodium deoxycholate, and like the latter would appear to be a little more toxic for the male than for the female mouse. Table 3;

Table 3

Sodium Cholate
(3,7,12-trihydroxycholanate)

mg/gm	Moles x 10^{-7}/gm	Total No.	No. Died	% Died
	Males			
0.32	7.14	2	2	100
0.30	6.70	2	1	50
0.28	6.25	2	2	100
0.26	5.80	6	6	100
0.24	5.35	8	3	37
0.20	4.25	9	3	33
0.18	3.80	4	0	0
	Females			
0.30	6.70	3	3	100
0.28	6.25	4	4	100
0.26	5.80	8	5	62
0.24	5.35	5	2	40
0.22	4.70	4	0	0
0.20	4.25	1	0	0

The M L D for the male was 0.26 mg per gm of body weight,and for the female 0.28 mg per gm.

DETOXIFICATION OF SODIUM CHOLATE BY LECITHIN:Both sexes are protected somewhat by lecithin and to about the same extent.See Table 4.

Table 4

Sodium Cholate plus Lecithin

Dose mg/gm	Moles x 10^{-7}/gm	Total No.	No. Died	% Died
	Males			
0.40	8.93	1	1	100
0.38	8.49	3	3	100
0.36	8.04	2	2	100
0.34	7.59	3	1	33
0.30	6.70	3	1	33
0.26	5.80	1	0	0
	Females			
0.44	9.91	1	1	100
0.40	8.93	4	4	100
0.36	8.04	3	2	66
0.32	7.14	3	2	66
0.28	6.25	1	0	0

The M L D for the male mouse is increased from 0.26 to 0.36 mg per gm of body weight,and for the female from 0.28 to 0.40 mg per gm of body weight.

In most of the experiments on the M L D of sodium cholate a 2.5 % solution was used,and the volumes injected varied from 0.20 to 0.62 cc.In some of the tests a 1 % solution was used,apparently with identical results.

THE ACTION OF SODIUM CHOLATE ON PNEUMOCOCCUS:The bactericidal concentration of sodium cholate was 0.012

gm per cc.This value was repeatedly confirmed since sodium cholate was used as a control for the other bile salts tested.The inhibitive concentration was 0.0010 gm per cc in three experiments.

THE ACTION OF SODIUM CHOLATE PLUS LECITHIN ON PNEUMOCOCCUS:Lecithin decreased the bactericidal activity of sodium cholate,but much less than that of sodium deoxycholate.The bactericidal concentration was 0.018 gm per cc in four experiments.Lecithin had no effect upon the inhibitive action of sodium cholate.In three experiments the inhibitive concentration of sodium cholate ,in the presence of an equal amount of lecithin was 0.0010 gm per cc,the same as of the bile salt alone.

TOXICITY OF SODIUM DEHYDRO-DEOXYCHOLATE.Oxidation of the hydroxyl groups to carbonyl greatly lowers the toxicity.The M L D of the sodium salt of dehydro-deoxycholic acid was found to be 0.44 mg per gm of body weight for the male mouse and 0.48 mg per gm for the female.Table 5.

Table 5

Sodium Dehydro-deoxycholate
(3,12-diketo-cholanate)

Dose mg/gm	Moles x 10^{-7}/gm	Total No.	No. Died	% Died
		Males		
0.44	10.33	4	4	100
0.40	9.39	6	5	80
0.38	8.45	9	6	66
0.32	7.51	10	2	20
0.28	6.57	2	0	0
		Females		
0.48	11.27	3	3	100
0.44	10.30	7	6	80
0.40	9.39	6	3	50
0.36	8.45	3	0	0

DETOXIFICATION OF SODIUM DEHYDRO-DEOXYCHOLATE BY LECITHIN: Lecithin did not protect male mice, the M L D being the same with and without it, while for the female the M L D was raised to 0.56 mg per gm of body weight. Table 6.

Table 6

Sodium Dehydro-deoxycholate plus Lecithin

Dose mg/gm	Moles x 10^{-7}/gm	Total No.	No. Died	% Died
		Males		
0.50	11.73	1	1	100
0.44	10.33	3	3	100
0.40	9.39	3	2	66
0.36	8.45	3	0	0
		Females		
0.56	13.12	2	2	100
0.52	12.20	2	1	50
0.48	11.27	3	0	0

Although the number of female mice receiving this bile salt plus lecithin is small, it is enough to indicate that there is a fair amount of protection.

In the experiments on the determination of the M L D of sodium dehydro-deoxycholate, 1 cc of the solution contained 0.025 gm; the volumes of solution injected ranged from 0.15 to 0.46 cc.

The reactions of the animals to sodium dehydro-deoxycholate were rather spectactular. With all the other preparations there was at the most an irritation immediately after the injection; the animals would become quiet, apparently comatose, and die with occasional mild convulsions. Death was rather slow and appeared to be due to respiratory failure. Only very large doses, several times the M L D, killed in a short time. With sodium dehydro-deoxycholate there was slight irritation immediately after injection, but within a very short time (2 - 10 minutes) the animals receiving a sufficient amount went into convulsions. The convulsive movements were confined to the hind quarters, as nearly as could be observed, and became quite severe. A large amount of fluid, colorless, and probably from the salivary glands collected around the mouth and caused choking. Those that died did so in less than 60 minutes, the breathing

gradually becoming slower,convulsive,and finally stopping.There was a marked cyanosis and many showed hemorrhage at the nose and mouth(those with hemorrhage invariably died).In those that recovered,the breathing began to improve in a short time,and would reach a remarkably rapid rate.By means of the presence or absence of hemorrhage and of the condition of the breathing it was possible to predict quite accurately whether or not a given animal would survive.Lecithin had no apparent effect upon the reactions.

THE ACTION OF SODIUM DEHYDRO-DEOXYCHOLATE ON PNEUMOCOCCUS:The bactericidal concentration of sodium dehydro-deoxycholate was found to be nearly the same as that of sodium cholate,0.014 gm per cc.This concentration was effective in each of 4 experiments.The inhibitive concentrationwas 0.006 gm per cc,and was confirmin three experiments.

The effect of lecithin upon the action of sodium dehydro-deoxycholate on pneumococci was not determined.

TOXICITY OF SODIUM DEHYDROCHOLATE:The M L D of sodium dehydrocholate can not be defined as certainly for the female as for the male mouse.It is practically the same in each case,2.0 mg per gm of body weight. Table 7.A larger number of animals might possibly show a lesser toxicity for the female,as with sodium dehydro-deoxycholate.

Table 7

Sodium Dehydrocholate
(3,7,12-triketo-cholanate)

Dose mg/gm	Moles x 10^{-7}/gm	Total No.	No. Died	% Died
	Males			
2.0	47.6	3	3	100
1.8	42.9	5	3	60
1.6	38.2	5	2	40
1.4	33.5	6	0	0
1.25	28.3	3	0	0
	Females			
2.0	47.6	2	2	100
1.9	45.2	2	1	50
1.8	42.9	4	3	75
1.7	40.5	2	0	0
1.6	38.2	3	1	33
1.4	33.5	1	0	0

10 % solutions were used for the determination of the M L D of sodium dehydrocholate;the volumes injected varied from 0.24 to 0.70 cc.

The detoxification of sodium dehydrocholate by lecithin was not examined.

THE ACTION OF SODIUM DEHYDROCHOLATE ON PNEUMOCOCCUS

The bactericidal concentration of sodium dehydrocholate was found to be 0.1 gm per cc.It has been reported that at least a 2% solution of this bile salt is required to inhibit the growth of,pneumococci in ascitis broth(31). In the bactericidal test using a 10 % concentration, the concentration of the bile salt in the subculture will be one one hundredth of 10 % or 0.1 %,so that when no growth occured in the subculture it may be concluded that the organisms had been killed.A concentration of

0.06 gm per cc did not kill the pneumococcus in one hour, but 0.08 gm per cc did in this time. Table 8. These figures were checked but once.

Table 8

Sodium Dehydrocholate

Concn. gm/cc	Moles x 10^{-6}/cc	Time required to kill.
0.1	226	15 minutes
0.08	181	60 minutes
0.06	136	not in 60 minutes

No inhibitive tests were done with sodium dehydrocholate, nor was the effect of lecithin studied because of the small amount of the salt available for use.

TOXICITY OF THE SALT OF 7,12-DIKETO-5-CARBOXYLIC-3,4(α,β)-QUINO-CHOLANIC ACID: When a mol of isatin is condensed onto ring A of dehydrocholic there occurs a further decrease in toxicity. The M L D of the salt of the "quino" compound for both male and female is about 3.2 mg per gm of body weight. Since different doses were used in the experiments with male mice than in those with female, it appears that the preparation is slightly more toxic for the female. The difference is small, and probably apparent only. See Table 9.

Table 9

Salt of "Quino" Compound

Dose mg/gm	Moles x 10^{-7}/gm	Total No.	No. Died	% Died
	Males			
4.0	71.4	2	2	100
3.6	64.3	1	1	100
3.4	60.7	4	4	100
3.0	53.7	4	2	50
2.6	46.4	1	1	100
2.0	35.7	2	0	0
	Females			
4.0	71.4	2	2	100
3.6	64.3	1	1	100
3.2	58.9	4	4	100
2.8	50.0	3	0	0
2.4	42.8	4	0	0

THE ACTION OF THE SALT OF THE "QUINO" COMPOUND ON PNEUMOCOCCI: In Table 10 are the results of the bactericidal tests with this preparation.

Table 10

Salt of the "Quino" Compound

Concn. gm/cc	Moles x 10^{-6}/cc	Time required to kill
0.1	178	30 and 60 minutes
0.08	143	60 minutes
0.06	107	not in 60 minutes
0.05		not in 4 hours

By comparison with Table 8, it is seen that the salt of the "quino" compound is possibly a little more active in killing the pneumococcus than is sodium dehydrocholate. While it required 181 moles x 10^{-6} per cc of the latter salt to kill in 60 minutes, 143 x 10^{-6} moles ~~killed~~ per cc of the "quino" compound killed the pneumococcus

in 30 minutes in one experiment and in 60 minutes in a second. If it requires 178 moles x 10^{-6} per cc of the "quino" compound to be effective consistently, this salt is about equal to sodium dehydrocholate in bactericidal activity.

This condensation compound of isatin and dehydrocholic acid, first prepared by Borsche and Frank(3), and described by them as 7,12-diketo-δ-carboxylic-3,4(α,β)-quino-cholanic acid, was tested for its "physiological action". Because of its ring structure resembling the cinchona alkaloids it was thought that it might have some peculiar actions. However the above authors describe it as being "physiologically inactive".

TOXICITY OF SODIUM BILIANATE: If ring A of cholic acid is broken, forming bilianic acid, there is a further fall in toxicity. Table 11.

Table 11

Sodium Bilianate

Dose mg/gm	Moles x 10^{-7}/gm	Males Total No.	No. Died	% Died
10.0	193	2	2	100
8.0	155	1	1	100
6.5	126	2	2	100
6.0	116	2	1	50
5.5	106	2	0	0
2.0	36	2	0	0

The M L D for the male mouse is above 6.0 mg per gm of body weight, and is probably about 6.5 mg per gm.

The M L D for the female mouse was not determined, nor was the effect of lecithin studied because of the small amount of the preparation obtained.

THE ACTION OF SODIUM BILIANATE ON PNEUMOCOCCUS: In Table 12 are the results of the bactericidal tests. It is seen that sodium bilianate is a little less potent than the salts of the "quino" compound and of dehydrocholic acid.

Table 12

Concn. gm/cc	Moles x 10^{-6}/cc	Time required to kill
0.1	193	60 minutes
0.08	155	not in 3 hours
0.06	97	not in 4 hours

While an approximately 180 moles x 10^{-6} per cc solution of the salts of dehydrocholic acid and of the "quino" compound killed the pneumococcus in 60 minutes, it required a more concentrated solution of sodium bilianate to kill in this time, 193 moles x 10^{-6} per cc.

Because of the small amount of bilianic acid obtained no inhibitive tests were done, and the effect of lecithin was not examined.

TOXICITY OF THE SALT OF THE DIOXIME: Conversion of the keto acids to the oximino derivatives restores activity, but not equal to that of the hydroxy acids. The M L D for the sodium salt of the 3,12-dioximino acid was 0.22 mg per gm of bodyw weight for the male as compared to 0.10 mg per gm of the salt of the 3,12-

dihydroxy acid.The M L D of the salt of the dioxime for the female was 0.34 mg per gm of body weight.Table 13.

Table 13

Salt of 3,12-dioximino-cholanic acid

Dose mg/gm	Moles x 10^{-6}/gm	Total No.	No. Died	% Died
		Males		
0.22	4.73	4	4	100
0.20	4.32	4	3	75
0.18	3.93	4	3	75
0.16	3.49	2	1	50
0.14	3.06	3	0	0
		Females		
0.38	8.31	1	1	100
0.36	7.87	2	2	100
0.34	7.43	2	2	100
0.32	6.99	6	5	83
0.30	6.55	3	2	66
0.28	6.11	4	0	0
0.26	5.67	1	0	0

DETOXIFICATION OF THE SALT OF THE DIOXIME BY LECITHIN:The effect of lecithin upon the M L D of the oximino compounds,as regards the two sexes,is just the reverse of the effect of lecithin upon the M L D of sodium dehydro-deoxycholate.In the latter case it was the female that received protection;in the case of both the di- and tri-oximes the male receives protection. Table 14 shows the effect of lecithin on the M L D of the salt of the dioxime.

Table 14

Salt of the 3,12-dioximino-cholanic acid plus Lecithin

Dose mg/gm	Moles x 10^{-6}/cc	Total No.	No. Died	% Died
		Males		
0.36	7.87	2	2	100
0.34	7.43	3	3	100
0.32	6.99	2	1	50
0.30	6.55	3	1	33
0.26	5.67	4	3	75
0.22	4.73	1	0	0
0.20	4.32	1	0	0
		Females		
0.40	8.75	1	1	100
0.36	7.87	2	2	100
0.34	7.43	2	2	100
0.32	6.99	4	3	75
0.30	6.55	4	2	50
0.28	6.11	1	0	0

The M L D for the male mouse is increased from 0.22 mg per gm of body weight to 0.34 mg per gm by the addition of lecithin. The M L D for the female remains unchanged, 0.34 mg per gm of body weight.

In all the experiments on the M L D of the salt of the dioxime a 2.5 % solution was used; the volumes of solution injected ranged from 0.20 to 0.58 cc.

THE ACTION OF THE SALT OF THE DIOXIME ON PNEUMOCOCCUS: The bactericidal concentration of the salt of the dioxime is almost the same as that of sodium deoxycholate; in three experiments it was found to be 0.0012 gm per cc. A peculiar thing about this preparation, the inhibitive concentration is nearly equal to the bactericidal; 0.001 gm per cc was found necessary to inhibit

growth in two experiments.

THE ACTION OF THE SALT OF THE DIOXIME PLUS LECITHIN ON PNEUMOCOCCUS:Only one experiment was done on the effect of lecithin upon the bactericidal activity of the salt of the dioxime.It would appear to be decreased,how much can not be told.The inhibitive activity was found to be increased by the lecithin,0.0006 gm per cc of the salt in the presence of the lecithin prevented visible growth in each of two experiments, while it requires 0.001 gm per cc of the salt without lecithin.

TOXICITY OF THE SALT OF THE TRIOXIME:For the male mouse the M L D of the salt of the trioxime was 0.35 mg per gm of body weight.For the female the M L D was 0.65 mg per gm of body weight.Table 15.

Table 15

Salt of 3,7,12-trioximino-cholanic acid

Dose mg/gm	Moles x 10^{-7}/gm	Total No.	No. Died	% Died
		Males		
0.40	8.24	1	1	100
0.35	7.21	3	3	100
0.30	6.19	6	4	66
0.25	5.17	5	1	20
0.20	4.14	4	0	0
		Females		
0.70	14.37	4	4	100
0.65	13.35	4	4	100
0.60	12.33	4	2	50
0.55	11.31	6	0	0
0.50	10.28	5	0	0

DETOXIFICATION OF THE SALT OF THE TRIOXIME BY LECITHIN: The M L D for the male mouse is not raised to the same level as that for the female, but only to 0.55 mg per gm of body weight by the action of the lecithin. The results with the female are uncertain; the M L D might be lowered by the lecithin to 0.60 mg per gm, but this does not seem probable. At any rate the M L D is not increased for the female. Table 16.

Table 16

Salt of 3,7,12-trioximino-cholanic acid plus Lecithin

Dose mg/gm	Moles x 10^{-7}/gm	Total No.	No. Died	% Died
		Males		
0.55	11.31	6	6	100
0.50	10.28	3	0	0
0.45	9.26	7	2	28
0.40	8.24	5	2	40
0.35	7.21	3	0	0
		Females		
0.70	14.37	4	4	100
0.65	13.35	4	3	75
0.60	12.33	4	4	100
0.55	11.31	3	2	66
0.50	10.28	2	0	0

The concentration of the solutions injected for the determination of the M L D's of the trioxime was 2.5 %; the volumes ranged from 0.20 to 0.8 cc.

THE ACTION OF THE SALT OF THE TRIOXIME ON PNEUMOCOCCUS:No satisfactory results were obtained for the action of the trioxime on the pneumococcus,apparently because of the insolubility of the salt.The most concentrated solution obtained in broth without undissolved salt was 0.35 %.This concentration possibly acted upon the bacteria but very slowly.In the inhibition test this concentration did not prevent growth in as muchas a heavy flocculent precipitate formed on incubation(none in controls).This precipitate forms after a heavy growth.The tube appeared to be sterile immediately after the incubation period;nom growth occured in a subculture.This was not repeated.

TOXICITY OF METHYL CHOLATE:Methyl cholate was found to be approximately as toxic as sodium cholate, at least for the female.The results with the male mice are not as clear as those with the females.A peculiar thing about all three esters examined,there was ,comparatively,quite a wide range of doses over which male mice would be killed,making the determination of the M L D at first rather difficult.But with the female the break from fatal to nonfatal doses ~~ix~~ was much more abrupt.The M L D of methyl cholate for the male mouse is probably about 0.26 mg per gm of body weight;for the female it is 0.24 mg per gm.Table 17.

Table 17

Methyl Cholate

Dose mg/gm	Moles x 10^{-7}/gm	Total No.	No. Died	% Died
0.30	6.81	2	2	100
0.26	5.91	2	2	100
0.22	5.00	2	1	50
0.20	4.55	2	1	50
0.18	4.09	2	1	50
0.16	3.64	4	3	75
0.14	3.18	1	1	100
0.12	2.73	1	0	0
	Females			
0.28	6.37	1	1	100
0.24	5.46	4	4	100
0.20	4.55	4	2	500
0.18	4.09	4	2	50
0.16	3.64	4	3	75
0.14	3.18	2	0	0

TOXICITY OF METHYL DEOXYCHOLATE:Methyl deoxycholate was found to be much less toxic than the salt of deoxycholic acid,especially for the female mouse.The M L D for the male is about 0.34 mg per gm of body weight,for the female probably 0.56 mg per gm,or even higher. Here again we see the comparatively wide range of doses over which the male is killed.Table 18.

Table 18

Methyl Deoxycholate

Dose mg/gm	Moles x 10^{-7} /gm	Total No.	No. Died	% Died
	Males			
0.34	7.98	5	5	100
0.32	7.51	1	0	0
0.30	7.03	6	5	83
0.28	6.56	1	1	100
0.26	6.08	9	6	66
0.24	5.61	1	1	100
0.22	5.13	1	1	100
0.20	4.66	3	1	33
0.16	3.71	1	0	0
	Females			
0.56	13.2	5	4	80
0.52	12.2	2	1	50
0.48	11.31	4	1	25
0.44	10.36	1	0	0
0.40	9.41	1	0	0

DETOXIFICATION OF METHYL DEOXYCHOLATE BY LECITHIN: The effect of lecithin upon the toxicity of methyl deoxycholate was tried only on male mice.Table 19.It is impossible to say what is the M L D from the results obtained.

Table 19

Methyl Deoxycholate plus Lecithin

Dose mg/gm	Dose Moles x 10^{-7}/gm	Total No.	No. Died	% Died
		Males		
0.38	8.93	1	1	100
0.34	7.98	3	2	66
0.30	7.03	3	2	66
0.28	6.56	1	1	100
0.26	6.08	2	1	50
0.24	5.61	3	1	33
0.22	5.13	1	1	100
0.20	4.66	3	2	66
0.18	4.18	1	1	100

TOXICITY OF METHYL DEHYDRO-DEOXYCHOLATE:The M L D of methyl dehydro-deoxycholate was 1.80 mg per gm of body weight for the male and 2.2 mg per gm for the female mouse.Table 20.

Table 20

Methyl Dehydro-deoxycholate

Dose mg/gm	Dose Moles x 10^{-7}/gm	Total No.	No. Died	% Died
		Males		
1.8	43.06	2	2	100
1.6	38.28	3	0	0
1.4	33.50	3	2	66
1.2	28.72	1	0	0
1.0	23.94	1	0	0
0.8	19.16	3	2	66
		Females		
2.2	52.63	3	3	100
2.0	47.84	2	1	50
1.8	43.06	4	2	50
1.6	38.28	2	1	50
1.4	33.50	5	1	20
1.2	28.72	1	0	0
1.0	23.94	2	0	0

Conclusions

Table 21 summarizes the results of the M L D experiments on all the preparations. In the first column are listed the substances tested; in the second the corresponding M L D'S for the male mouse; and in the third column the M L D's for the female mouse, in terms of moles x 10^{-7} per gm of body weight; In the fourth column are listed the corresponding comparative toxicities for the male, with sodium deoxycholate arbitrarily given the value of 100; and in the fifth column are the similar comparative toxicities for the female mouse.

In Table 22 are collected the results of the experiments on pneumococci. In the first column are the substances examined; in the second column the bactericidal concentrations in terms of moles x 10^{-6} per cc; in the third column are the comparative bactericidal activities in terms of sodium deoxycholate with an evaluation of 100; The fourth column contains the inhibitive concentrations in moles x 10^{-6} per cc, and the fifth contains the comparative inhibitive activities in terms of sodium deoxycholate with a value of 100.

Since sodium deoxycholate is the most toxic and also the most active against the pneumococcus, it has, as far as we know, the ideal structure for maximum

Table 21

Test Substance	M L D Moles x 10^{-7}/gm Male	Female	Comparative Toxicity Male	Female
Sodium Deoxycholate	2.32	3.24	100	71
plus lecithin	3.24	4.16	71	55
Sodium Cholate	5.80	6.25	40	37
plus lecithin	8.49	8.93	27	26
Sodium Dehydrodeoxycholate	10.33	11.27	22	20
plus lecithin	10.33	13.12	22	17
Sodium Dehydrocholate	47.6	47.6	4.9	4.9
"Quino" compound	60.7	58.9	3.7	3.9
Sodium Bilianate	126		1.8	
Sodium salt of the Dioxime	4.73	7.43	50	31
plus lecithin	7.43	7.43	31	31
Sodium salt of the Trioxime	7.21	13.35	32	17
plus lecithin	11.31	13.35	20	17
Methyl Cholate	5.91	5.46	40	42
Methyl Deoxycholate	7.03	13.2	33	17
Methyl Dehydrodeoxycholate	43.06	52.63	12	4.4

Table 22

Test Substance:	Bactericidal Action Concn. x 10^{-6}/cc.	Bactericidal Action Comparative Activity.	Inhibitive Action Concn. x 10^{-6}/cc.	Inhibitive Action Comparative Activity.
Sodium Deoxycholate	2.3	100	1.38	100
plus lecithin	9.24	25	0.92	150
Sodium Cholate	26.8	8.6	2.23	62
plus lecithin	40.1	5.7	2.23	62
Sodium Dehydrodeoxycholate	32.8	7.0	14	9.8
Sodium Dehydrocholate	226 (15 min.) 181 (60 min.)	0.010		
"Quino" compound	178 (60 min.)	0.010		
Sodium Bilianate	193 (60 min)	0.0092. By comparison with the "quino" compound.		
Sodium salt of the Dioxime	2.64	87	2.18	63
plus lecithin	<2.64	<87	1.31	101

activity both toward the animal organism and toward the pneumococcus.With the comparative activities of each preparation studied given in terms of it as 100,it is quite easy to follow the variations in each case.

The salts of 3,12-disubstituted acids are more toxic than the corresponding 3,7,12-trisubstituted ones, If the toxicity of the sodium salt of the dihydroxy acid for male mice is 100,the toxicity of the salt of the trihydroxy acid is 40,two fifths as toxic.On the same basis of comparison ,the toxicity of the salt of the diketo acid is 22,and of the triketo acid,5,about one fourth as toxic.The toxicity of the salt of the dioximé is 50 and of the trioxime 32.It is thus apparent that the substitution of hydrogen in position 7 has a definite effect in lowering the toxicity.

As regards the bactericidal activity,it is possible to compare only the hydroxy and keto acids since no data wereobtained for the action of the trioxime on the pneumococcus.Again the salt of the dihydroxy acid is given the activity evaluation of 100.The bactericidal activity of the salt of the trihydroxy acid is but 8.6 The substitution of position 7 in the hydroxy acids reduces the bactericidal activity eleven times while the toxicity is reduced but about two and a half times.In the case of the keto acids,while substitution of

position 7 reduces the toxicity about four times, the bactericidal activity is reduced seven hundred times.

In comparing the hydroxy acids with the corresponding keto derivatives, we also find a greater fall in bactericidal activity than in toxicity, when the hydroxyl groups are replaced by keto. The toxicity of the salt of the disubstituted acid falls from 100 to 22 in this change of structure; the bactericidal activity falls from 100 to 7 (about 14 times). The conversion of the salt of the trihydroxy acid to the keto derivative lowers the toxicity about eight times, but the bactericidal activity is lowered 860 times.

It would then appear that conversion of the hydroxyl groups to keto, and that substitution of position 7 in these acids, are steps in the wrong direction in the search for a derivative with a high bactericidal action and a low toxicity. It has been reported(56) that sodium dehydrocholate was as active as sodium taurocholate in dissolving pneumococci in the peritoneal washings from an infected rabbit. In vitro, sodium dehydrocholate will not dissolve the pneumococcus in a concentration of 2 %. Quoting Ziegler,(30) "The chemistry of pneumococci grown on artificial media is apparently different from that of those grown in animals". If this is true, sodium dehydrocholate would be expected to be as effective in vivo as any of the known bile salts, even though its

action in vitro is very small.

If a molecule of isatin is condensed onto ring A of dehydrocholic acid, there occurs a further decrease in toxicity, with but a slight change in bactericidal activity. Conversion to the "quino" derivative lowers the toxicity of the salt from 4.9 to 3.7; the bactericidal activity may be increased. The change if any is small. If ring A is broken as in the formation of bilianic acid, there occurs a lowering of toxicity with a slight fall of bactericidal activity. The toxicity of sodium bilianate is but 1.8; the bactericidal activity (from comparison with the "quino" compound) is 0.0092. The bactericidal activity, once all the hydroxyl groups are removed, would then appear to be the property of that part of the molecule other than ring A, at least in the greatest part.

If the relationships found between the 3,7,12-trisubstituted and the 3,12-disubstituted acids holdx between the latter and the 3-monosubstituted, then we would expect to find that the principal activity was due to the four ring system having an hydroxyl in position 3. However, the salt of lithocholic acid is reported to be rather insoluble. This might affect its activity so that the dihydroxy acid would be the most active.

Very unfortunately,due to lack of material,it was impossible to prepare the 3-hydroxy-,7,12-diketo-cholanic acid and thus possibly find out how much the activity of the bile salts is due to the hydroxyl group in position 3 independent of hydroxyl groups in position 12 and (or) 7.

A further point in support of the importance of an hydroxyl group on the nucleus is found in the marked recovery of activity when the keto acids are converted to the oximes.The nitrogen present in the oximes exerts some influence,however,because recovery is not complete. A more important action of the nitrogen,possibly,is seen in the fact that the recovery of bactericidal activity in the disubstituted derivatives is greater than the recovery of toxicity.The toxicity of the salt of the dioxime was 50;the bactericidal activity was 37.While this separation of toxicity and bactericidal activity is not great enough that we may expect the dioxime to be of much value therapeutically,it does show that the toxicity and antipneumococcal activity can be influenced in the desired directions,and it indicates a possible way to compounds which may be valuable therapeutic agents.

The effects of lecithin are rather interesting.Due to incompleteness of the data obtained with lecithin we can point out some possible correlations only.It

would appear that the presence of hydroxyl groups favors the detoxifying action of lecithin. This action of lecithin was studied on only one derivative that did not contain hydroxyl groups, sodium dehydro-deoxycholate. Lecithin did not affect the toxicity for the male mouse and lowered the toxicity for the female but 15 %. The lowering of the toxicity by lecithin of the four hydroxyl-containing bile salts was greater in each case. The toxicity of sodium deoxycholate for the male was lowered 29 % and for the female 22 %; the toxicity of sodium cholate was decreased about 30 % for both male and female. While the toxicity of the salts of the oximino acids for the female mouse was unchanged by the lecithin, the toxicity for the male was lowered; in the case of the dioxime the fall in toxicity was 33 %, in the case of the trioxime, 40 %.

Lecithin lowered the bactericidal activity of those salts tested, and especially, the disubstituted ones of. The bactericidal activity of sodium deoxycholate is lowered 400 % while the activity of sodium cholate is lowered but a little over 30 %.

Lecithin had the opposite effect, if any, upon the inhibitive activity of the bile salts. In examining the amounts of the salts required to inhibit growth we see that the inhibitive concentration of sodium cholate is

only 8 % of the bactericidal concentration,but with the other salts the percentage is much higher,60 % for sodium deoxycholate,42 % for the salt of the diketo acid,and 32 % for the salt of the dioxime.Since in the inhibitive test serum was present while the pneumococci were exposed to the action of the bile salts,and since serum is known often to protect organisms in unfavorable environments,it seems probable that the serum is having such a protective action here.In the cases of the two disubstituted derivatives studied,deoxycholic acid and the dioxime,lecithin markedly increased the inhibitive action.The inhibitive action of the salt of the trihydroxy acid was unchanged by the lecithin.

It would appear,then,that the antipneumococcal activity of the disubstituted derivatives is peculiarly susceptible to the influence of other materials.This may be linked up with those factors which cause the dihydroxy acid to form the stable molecular compounds with other substances.The ability of the lecithin to promote the action of the disubstituted bile salts in the presence of serum in vitro indicates that its presence in actual therapeutic use might be desirable.

Two facts come out of the results of the M L D experiments with the three esters.One is the comparatively

wide range of doses over which male mice may be killed, while with the female the break from fatal to nonfatal doses is sharper. Tables 17, 18, 19, 20.

And while the esters of the 3,12-disubstituted acids are markedly less toxic than the salts of these acids, methyl cholate is as toxic as sodium cholate and possibly methyl cholate is as toxic as sodium cholate and possibly may mean that the ester itself exerts a toxic action. The much lower toxicity of the methyl esters of deoxycholic and of dehydro-deoxycholic acids compared to the salts of these acids indicates that the esters are probably inert, and that the salt of the acid, formed after the slow hydrolysis of the ester, is responsible for the toxic action.

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