Dissertation & Research on
The Bactericidal action of some
Compounds of Silver -

being
Thesis submitted for degree of M.D

by
Edward. James. Tyrrell M.B. Ch.B
(Edin 1901)

"Abertay"
Maydulen Yard 1a
Dundee

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— Views regarding Bactericidal Action —

The group of pathogenic & putrefactive micro-organisms constitute one of the most important & interesting class of morbific agents, which we now recognise as efficient causes of disease & death. The extrinsic means which we adopt for combating such agents & their effects appear crude & trifling when compared with what we already know of the natural powers of resistance and adaptation possessed by the living tissues themselves; yet, with the dawn of reason & inductive power during the process of organic evolution, a new factor in combating disease appeared, and we trust we are not unduly arrogant in believing that we can aid the defensive processes of the silent tissues by applying the analytic & reflective powers of the human mind to the study of disease, its prevention & cure.

The most obvious & direct method of dealing with micro-organisms when they occur in places where they may be injurious to man or animals, is to destroy them, or render them harmless. The various methods of disinfection have this for their object, & the principle has been fully justified

by the splendid results obtained by the methods of Antiseptic Surgery, and public & private Sanitation.

The usual method of dealing with micro-organisms when we wish to destroy them, is to subject them, for a more or less lengthened period of time, to the action of heat or certain chemical agents, when such agents can be applied without injury to the object to be disinfected. These Antibacterial agents have been variously termed Antiseptics, Disinfectants, Bactericides &c and there appears to be some want of agreement as to which of these terms is most suitable and what is definitely implied by the terms themselves.

For Clinical purposes an Antiseptic may be defined as a substance or agent which acts on pathogenic as well as putrefactive bacteria in such a manner that they are prevented from producing their specific effects & so rendered harmless.

In the laboratory, "the death of bacteria is usually judged by the fact, that when they are transferred to a fresh Quantity of an artificial medium in which

they previously grew, no growth takes place"
(manual of Bacteriology – Muir & Ritchie – p. 32)
In accordance with this, any agent is a Bactericide if it is capable of affecting the vitality of bacteria to such an extent that they no longer grow on artificial media. It is conceivable, however, that bacteria so enfeebled that they fail to grow on an artificial basis, may, under more favorable conditions – as for instance, the animal body – still arrive at development.
It is probable, however, that all or nearly all forms of pathogenic & putrefactive bacteria may be totally destroyed when directly submitted, ~~for~~ during a suitable period of time, to the action of the more potent antibacterial agents, so that most of these agents may truly be termed Bactericides.
During the plague of Athens, Acron, according to Plutarch, stayed the spread of epidemics by lighting fires in the public places, & in the streets where death occured; And at the present time it is recognized that heat is the most effectual bactericide

and when it is possible to apply it, the exposure of objects to be disinfected to a temperature of 180°C. for one or two hours is sufficient to destroy the most resistent forms of bacterial spores. In other cases chemical agents alone, are applicable. Aromatic substances were used in primitive times to disguise or counteract noxious odours, and in the time of Hippocrates, Sulphur was regarded as an antidote against the plague. According to Ovid, Sulphur was also employed by shepherds for bleaching & purifying wool from contagious diseases. About the middle of the 18th century, Pringle in his "Memoire sur les substances Septiques et Antiseptiques", describes experiments with regard to the anti-putrefactive action of such substances as Sodium Chloride, Camphor, Borax &c. In 1800 Guyton de Morveau in France & Cruikshank in England recommended Chlorine as a disinfectant; & in 1847 Semmelweiss prescribed washing of the hands with Chlorine water in Gynecological practice. The rational use of antiseptiques, however, was not understood until Lister – inspired by the work of Pasteur – revolutionised general & operative surgery in

the 6th decade of the 19th century, by the introduction of the antiseptic method of dealing with wounds by means of solution of Carbolic Acid. In 1878 Koch & others recommended Solutions of Mercuric Chloride (1:1000-2000) for similar purposes. Since then, numerous antibacterial agents - more or less efficient - have been recommended for surgical & other purposes.

The potency of chemical antibacterial agents has been investigated by numerous observers - Miquel, J. de la Croix, Duclaux, Koch, &c. Until recently, however, there has been no unobjectionable & trustworthy method that could be applied for ascertaining the capacity of different disinfecting agents for effecting the purpose for which they are used.

With regard to Chemical agents in general, it is probable that there is some connection between their chemical nature and their effects as disinfectants. Such probability has been increased by the theoretical views put forward by van t'Hoff, Arrhenius & others as to the condition of substances in solution, for it is in that state they are chiefly employed as disinfectants.

As is well known, according to the Ionic Theory all salts, acids, & bases in aqueous solution undergo electrolytic dissociation to a greater or less extent, the molecules of these substances in solution being split into electrically charged parts called ions, thus conferring on the solution the property of conducting electricity. For example the molecule $AgNO_3$ separates into the positive Ag ion (Cation) + the negative NO_3 ion (Anion) In like manner HCl in solution furnishes the Cation H and the Anion Cl, while NaOH in sol^{n} furnishes the Cation Na and the Anion OH. In this condition of electrolytic dissociation these substances possess properties very different from the ordinary condition. Chemical interaction of inorganic substances in solution is explained by dissociation + regrouping of ions. In the case of dilute solutions of neutral salts, strong acids and bases, the dissociated part is much greater than that not dissociated. In consequence of the partial dissociation into ions, the character of watery solutions of salts, acids, + bases is influenced by the ions as well as by the unaltered molecules. According to

Behring the disinfecting value of mercuric compounds is essentially dependent upon the amount of Hg capable of solution whatever the state of combination may be. The investigations of Krönig & Paul (Zeitschr. f. Hygien. u. Infectionskr. XXV 1897) appear to prove, however, that although the disinfecting power of mercuric & other salts is in a great degree dependent upon the extent of their dissociation, the concentration of metallic ions in a solution plays a prominent part in the effect produced. Similarly the disinfectant power of solutions of acids and bases was found to be proportional to the extent of dissociation. These observers (Krönig & Paul) further showed the connection apparently existing between antibacterial action & the concentration of metallic ions, by mixing, in aqueous solution the well dissociated electrolyte NaCl with the less freely dissociated electrolyte $HgCl_2$. In such a solution according to the laws of chemical mass action the amount of dissociation of the inferior electrolyte is reduced and the result of the experiment showed that the disinfecting action of the mercuric chloride was also reduced.

Certain other experiments of Kronig & Paul are worthy of note as bearing upon the mode of action of Bactericidal solutions. In their experiments with organic compounds they were able to confirm an observation by Scheurlen, that the disinfecting action of aqueous solution of Phenol is very considerably augmented by the addition of salts such as Sodium Chloride, Lithium Chloride &c. Liquefied Carbolic acid (90% pure Phenol) proved to be somewhat less effective as a bactericide than 5% aqueous solution. Solutions of Phenol in Alcohol or Caustic Soda Solution proved almost entirely destitute of disfecting power. Solutol, Lysol, Creolin proved to be incapable — either in concentrated state or when diluted with water — of destroying the spores of Bacillus Anthracis of average resistance within a period of 4 days. After 10 minutes exposure to 35% solution of Formic Aldehyde several hundred spores of Anthrax remained capable of development. Mercuric Chloride or Silver Nitrate dissolved in strong Ethyl Alcohol proved to have very little action on the spores of Anthrax, but when dissolved in dilute alcohol — $HgCl_2$ in 25% & $AgNO_3$ in 50% Alcohol — the action was considerably stronger

than that of a pure water solution. The addition of Ethyl Alcohol to the aqueous solution of Carbolic acid or Formic Aldehyde considerably reduced the disinfecting action of these substances.

For the comparative investigation of different chemical agents & for establishing their relative value as disinfectants (Krönig & Paul have concluded that the following conditions must be fulfilled: — (1) Equimolecular quantities of the substances to be investigated must be compared. (2) The bacteria ~~testing~~ serving as test objects must possess the same powers of resistance. (3) The number of Bacteria made use of in any comparative experiment must be the same. (4) The bacteria must be introduced into the disinfecting solution without any of the nutritive basis upon which they have been cultivated. This condition is necessary because most Metallic Salts are precipitated by organic substances and are thus rendered less effective as disinfectants. (5) The disinfecting solutions must always have the same temperature since their disinfecting power is not inconsiderably augmented with increase of temperature. (6) After the action of a disinfecting material the bacteria must be separated from it as completely as possible.

(7) After having been submitted to the action of the disinfectant, the bacteria must be sown in equal quantity upon the same nutritive bases and at the same temperature for growth.
(8) The number of bacteria remaining capable of reproduction, and that have formed colonies upon the nutritive basis must be ascertained after the lapse of the same period of time.
All these conditions were observed in their experiments, and I have endeavoured to fulfil them in my research with regard to the bactericidal action of Silver compounds on Staphylococcus pyogenes aureus.

— Bactericidal action of Silver & its Compounds —

The object of the present research was to investigate the bactericidal action of certain compounds of Silver. Within the last few years a large number of these have been placed upon the market, and at present there is no satisfactory experimental evidence as to their individual value. This, it has been my endeavour to supply. As substances for comparison I have employed $AgNO_3$ and $Ag(NH_2)Cl$. But before dealing with the experiments in detail it is necessary to

refer to the history of the compounds.

In 1828, Higginbottom as a result of his own practical experience, recommended the application of dilute solutions of $AgNO_3$ for the treatment of various septic affections e.g. purulent conjunctivitis erysipelas, foul wounds &c; and it is now recognised and sufficiently proved by experimental research and clinical experience that, like the metal salts in general, the Silver salts in particular, possess high antiseptic and disinfectant properties. Miquel in his investigations with regard to the antiputrefactive power of various chemical substances found that Silver Nitrate even excelled Mercuric Chloride (Organismes de l'atmosphère vivant. 1883). Martens in his investigation found 1 in 1000 to be the "Antiseptic equivalent" of $AgNO_3$ & of $HgCl_2$ i.e he fid that solution of strength indicated prevented growth of Staphylococcus pyo. aureus. Krönig & Paul (1897) concluded from their experiments that exposure of Staphylococcus pyo. aureus for 3 minutes to .42% $HgCl_2$ or .08% $AgNO_3$ completely destroyed the organisms, but that the action of $HgCl_2$ on Anthrax spores

was superior to that of $AgNO_3$, which however, in the strength of 4·25% Sol^{n} completely destroyed them in $8\frac{3}{4}$ hours. Although we know that Silver Nitrate, to which until recently the therapeutic employment of Silver was exclusively confined, possesses several deficiencies, its sphere of employment was nevertheless fairly large & firmly established, which proves that we are satisfied with its curative effect & that the results obtained with it were favourable. Silver Nitrate, however, forms insoluble compounds with albumin & Chlorides of the tissues thus creating a barrier which interferes with its penetration into the tissues & acting as a strong irritant & Caustic. These objectionable qualities of Silver Nitrate — its causticity & lack lack of penetrating power — detract from its utility as a desinfectant and particularly so in the treatment of various affections of the mucous membranes especially those due to micro-organisms, in which it is necessary to exert a deep effect without producing irritation. It is known that Silver Chloride is readily soluble in solution of ammonia & Sodium Thiosulphate but this knowledge does not seem to have been utilised for desinfectant purposes, although it had been

used to determine the general pharmacological effect of the metal.

To preserve the antibacterial action of Silver and at the same time avoid irritating action various other salts and organic compounds of silver have recently been introduced & used with satisfactory results. In 1894 a substance known as Argentamine was placed upon the market. It was first described as a solution of Silver phosphate + Ethylene diamine but at present Silver lactate is used instead of the phosphate. Ethylene diamine being a base resembling Ammonia doubtless acts in a similar manner, and Argentamine like ammoniacal solution of AgCl prevents precipitation of albumin + Chlorides. Schäffer who investigated this substance found it a potent bactericide with regard to Gonococci and states that its penetrating power is 5 time that of Silver lactate solutions.

The next step was the introduction in 1895 of a Silver-casein compound known as Argonin. The bactericidal action of this substance has been investigated by Rudolph Meyer who compared it ē $AgNO_3$ + Argentamine. Meyer found that Solution of Argonin 1 in 750 has same bactericidal action on micro-organisms suspended in water as

Argentamin 1 in 4000 or Silver Iodide 1 in 3000.
On micro-organisms in albuminous fluids he found that 1 in 750 sol. of Argonin was equivalent to 1 in 4000 Argentamin or 1 in 1000 $AgNO_3$. The addition of a little ammonia to the argonin solution increased its bactericidal power – exposure of gonococci to 1 in 30,000 ammoniacal solution of Argonin completely stopped all growth. He also found that it does not penetrate very deeply into the tissues. Ammoniacal solution increased penetrating power but deprives the substance of its bland & non-irritating qualities.
In 1895 Crédé of Dresden introduced his Silver treatment of wounds &c by means of Itrol (Citrate of Silver) & Actol (Lactate of Silver). Numerous clinical reports &c by various authors testify to value of these two silver salts as reliable antiseptics. Crede's experiments to produce general disinfection of the body in septic conditions by means of Itrol & Actol, failed, & in 1897 he introduced a Soluble form of Silver known as Collargol or Colloid Silver hoping that it might prove of value as a general disinfectant in septic conditions. He recommends it to be used for this purpose, either

by means of Inunction or by intravenous injection & particularly upholds its use in General infection due to Staphylococcus or Streptococcus. Extensive literature has been published with regard to use of Collargol and many Clinicians have obtained favorable results with it. Kurz-Krause and Lange investigated action of Collargol by means of intravenous injection in Rabbits & Dogs — Silver was not deposited at place of introduction, it was absorbed by the blood & distributed Amongst the body. If animal was killed 3 hrs after injection the silver could be detected in blood, heart, lungs, Spleen, liver, Kidneys & intestine. Lange summarises as follows "as a direct consequence of the introduction of Silver in form of Colloidal Silver, a general distribution throughout the whole organism may be assumed to occur, this distribution however is not of a permanent character & even from the places where the silver is principally deposited, it is secreted again in a comparatively short space of time. Ernst Cohn's investigations (1902) have shown that Aqueous Solution of Collargol 1 in 30 killed Staphylococcus Pyo. Aureus in 10 hours Streptococci in 8 hours, Diphtheria Bacilli in 6 hours & B. Anthracis in 4 hours. Cohn found

that Collargol is soluble in blood-serum to the extent of 5-10% and the solution in serum proved to be as active as an antiseptic as the watery sol: Intra-venous injection was followed by no toxic symptoms in rabbits + examination showed wide distribution throughout the body. Forty-five minutes after injection into the blood the silver was found deposited in all the organs + was no longer present in the blood. Further experiments with rabbits injected i Staphylococci, Streptococci, B. cholerae, B. Anthracis led Colin to conclude that intravenous injection of Collargol cannot be accepted as an efficient means of producing general disinfection.

Protargol was introduced in 1897 + very favourably reported upon by Neisser + others as an efficient Antagonist to gonorrhoeal infection of Genito-urinary tract + Conjunctivis. Caccianiga has used an hypodermic injection of dilute sol^{n} of Protargol as a means of treatment in Croupous Pneumonia. Largin is another albuminous compound of Silver introduced in 1897 + the substance was investigated by Pezzoli + its bactericidal action compared

with Protargol, Argentamin & Silver Nitrate. His results will be referred to later.

Nargol introduced about 1899 is a compound of Silver with nucleinic acid. Recommended for use in Gonorrhoeal urethritis &c.

Ichthargan (1900) is combination of Ichthyol & Silver. It has been investigated by Aufrecht who showed it to be far less toxic & more strongly bactericidal than Silver Nitrate. According to Ehrmann Ichthargan penetrates tissues much better than Silver Nitrate.

Among other compounds less widely used & some of them difficult to obtain in this country are Albargin (Gelatose Silver) Argyrol (Silver Vitellin) Argentol, & Silbernol. These will be referred to shortly in the next section. Silver Acetate has been used & recommended by Zweifel for the prophylaxis of Ophthalmia Neonatorum.

Silver Fluoride (AgFl) has been recently recommended as an antiseptic in Surgical practice (1:1000 to 1:100)

– Chemical Composition & Properties of Compounds of Ag –

(1) Silver Iodide – Composition & properties well known.

(2) Argentamine – This is a 10% solution of Silver Iodide in 100 parts of 10% Aqueous Solution of Ethylene-diamine. It is a colorless, transparent, odorless, limpid fluid. The presence of Ethylene-diamine prevents precipitation of Chlorides & albumin. Its action is therefore more penetrating than that of Silver Iodide solution. Contains 6·3% of Silver.

(3) Argonin. – This is a compound of Silver (4·2%) Casein and Alkali prepared by adding a solution of the Sodium compound of Casein to a solution of Silver Iodide and precipitating the newly formed substance by the addition of alcohol. The resulting white powder must be free from HNO_3 & Alkali. The powder tends to darken & becomes yellow brown in Colour. It is odourless. Insoluble in cold & imperfectly so in hot water. Solution darkens when exposed to light

(4) Thiol – This is described as pure Silver Citrate. It is a fine, light, odourless, tasteless powder. Color is white but darkens to grey when kept for some time. Said to be soluble in water 1:3800 Solutions darken when heated & on exposure to light. My specimen contained 60·36% of Silver.

(5) Actol — Lactate of Silver. A white, odorless, tasteless powder. Soluble in cold water (1 in 20). Precipitates albumin & chlorides. Solution darkens on exposure to light. Contains 53.39% Silver.

(6) Collargol — This is soluble form of Silver also known as Colloid Silver. A similar substance was prepared by Careylea in 1891. Collargol is a modification of ordinary Silver which renders it soluble in water and albuminous fluids. It is produced from a solution of Silver Nitrate in a supersaturated solution of Ammonium Citrate by reduction with sulphate of Iron, the Collargol being the fine dark precipitate thrown down. Collargol is put on the market in the form of small, hard, green-brown lumps which have a metallic lustre. The lumps are soluble in cold distilled water (1 in 25) & also in blood-serum to the extent of 5-10%. A 1% solution in water is dark brown in color, opaque & slightly viscid. According to Klimmer, Solution of Collargol with albumin & gum-arabic added till, is not precipitated in the Stomach by Hydrochloric & Lactic acid. My specimen contained 82% Ag. Solution darkens when exposed to light. Solutions are weakened by precipitation of ordinary metallic Silver, so that after few days Strength of Silver in Solution = ½ or ⅓ original strength.

Quite recently Harriot has stated that Collargol is a compound of Ammonia & an acid which he terms Collargolic acid.

(7) Protargol — This preparation is a Proteid compound of Silver said to contain 8% of Silver. It is a pale yellow powder, odorless, & tasteless. Readily soluble in water. The solutions are not affected by heat, albumin, HCl, or weak solutions of NaCl or NaOH. Darkens by light.

(8) Largin — This is a Silver albumen compound An albumenoid constituent of which is Protalbin - a new spirit-soluble derivative of the para-nucleo-proteids. It is a cobalt grey a yellow powder, Soluble in water, glycerin & blood-serum. The 5% solution is orange-brown in color, slightly darkened by light. Largin is said to contain 11-10% Ag — my specimen contained 9.2% Ag.

(9) Nargol — This is stated to be a Compound of Silver with Nucleinic acid containing 10% Silver. Specimen estimated by me contained 9.5% Ag. Nargol is a yellow-brown powder easily Soluble in water — 2% Sol^n is orange-brown in color. Solutions are not precipitated by Albumen or Chlorides.

(10) Ichthargan — This is a compound of Ichthyol-sulphonic acid & Silver, described as Argentum thiohydro-carbuno-sulphatum solubile. It is a green-brown, faintly aromatic powder, readily & completely soluble in water, Glycerin, & dilute alcohol. It is insoluble in strong alcohol Ether & Chloroform. It is said to contain 30% of Silver — My Specimen contained 29.02%. Solution of Ichthargan 1:100 is a rich pale brown color, slightly darkened by light. Concentrated solutions are precipitated by Sod. Chloride & by Albumen, the precipitate being redissolved on adding excess of the reagent.

(11) Albargin — This is a compound of Silver & Gelatose which latter is a transformation product of Glue. It is described as a bulky powder of pale yellow color, freely soluble, without decomposing, in cold & warm water. The solutions are stated to be neutral & dialyse through animal membranes. It is said to contain 15% Silver — (I failed to obtain a specimen)

(12) Argentol = oxy-chinolin-sulphonate of Silver ($C_9 H_5 N . OH . SO_3 Ag$). This is a compound of Silver & Quinosulphol (ortho-oxychinolin-meta-sulphonic acid). In presence of alkalic chlorides

it is stated to split up into oxy-quinoline & metallic Silver. (Not used in my research)

(13) Silberol = Silver Sulpho-carbolate contain 28% Ag. Solutions in water (2%) are used in ophthalmic operation for disinfecting Conjunctiva & Cornea (not used in my research)

(14) Silver Acetate — lately recommended as a substitute for $AgNO_3$ in ophthalmic practice.

(15) Silver Fluorate — Recently recommended as a reliable antiseptic (not envelopes)

— Previous researches concerning the bactericidal power & penetration of Silver Compounds —

The bactericidal action and penetrating power of Silver Compounds has received much attention during the past few years & some of the more important results are summarized in the following tables —

I. Bactericidal action – Results of previous investigations. Gonococci.

Substance	Author	Concentration	Duration of action 3′	5′	6′	10′	15′
Silver Nitrate	Schäffer	1 : 4000	—	Abundant			
	Steinschneider + Schäffer	1 : 2000	—	abundant		abundant	
		1 : 3000	—	"do"		"do"	
		1 : 4000	—	"do"		"do"	
	Meyer	1 : 4000					
		1 : 6000					
Argentamin	Schäffer	1 : 4000		very scanty			
	Steinschneider + Schäffer	1 : 2000	—	—			
		1 : 3000	—	one colony			
		1 : 4000	—	one colony			
	Kamen	1 : 2000	—	—	—	—	
		1 : 3000	—	scanty	—	—	
		1 : 4000	—	moderate growth	—	scanty	
Argonin	Meyer	1 : 750	—		—		
		1 : 4000	one colony		—		
		1 : 6000	—		one colony		
	Kamen	1 : 2000		—		—	
		1 : 3000		scanty		—	
		1 : 4000		abundant		moderate	
Protargol	Kamen	1 : 2000		—		—	
		1 : 3000		moderate		—	
		1 : 4000		"do"		scanty	
	Pezzoli	1 : 2000		moderate		very scanty	
		1 : 3000		abundant		"do"	
		1 : 4000		"do"		abundant	
Largin	Kamen	1 : 2000		—			
		1 : 3000		scanty			
		1 : 4000		moderate			
	Pezzoli	1 : 2000		scanty			
		1 : 3000		"do"			
		1 : 4000		"do"			

II. { Previous researches on the inhibitory action of Silver Salts on Culture Media.

Substances	Author	Concentration	Duration of action 5'	Duration of action 10'
Silver Nitrate	Steinschneider & Schäffer	1 : 1000	—	—
		1 : 2000	—	—
		1 : 5000	2-3 Colonies	1 Colony
		1 : 10,000	many colonies	2-3 Colonies
	Meyer	1 : 2000	—	—
		1 : 6000	Scanty	Isolated colonies
Argentamin	Meyer	1 : 2000	Mod. abundant	—
		1 : 6000	Abundant	Moderate
	Steinschneider & Schäffer	1 : 1000	abundant	Moderate
		1 : 2000	do	Mod. abundant
		1 : 4000	do	abundant
		1 : 8000	do	do
Argonin	Meyer	1 : 2000	Moderate	
		1 : 6000	abundant	Moderate
Protargol	Pezzoli	1 : 1000	Scanty	Scanty
		1 : 2000	abundant	—
		1 : 4000	do	Moderate
Largin	Pezzoli	1 : 1000	Moderate Slimy	Scanty
		1 : 2000	Fairly abundant	do
		1 : 4000	abundant	Moderate.

III

Pezzoli tested penetrating power of Silver Solutions (1) into pieces of liver. He measured Zone of penetration indicated by dark color produced on treating it Amm. Sulphide Sol. (2) He tested penetration into Gelatine culture by pouring Test Sols upon Gelatin slope cultures of B. coli & measured Zone of inhibition of growth.

Results :— Relative

(1) = Penetration into liver

Argentamin	= 100
$AgNO_3$	= 66
Largin	= 58
Protargol	= 38

(2) Penetration into Gelatin

Argentamin	= 16
Largin	= 10
Protargol	= 5
$AgNO_3$	= 4

- Research by the present writer -

Reasons for my own research are as follows:-
(1) Previous results contradictory & only extended over part of the field.
(2) Methods in many cases were not scientific
(3) I wished to determine the influence of ionised & non-ionised silver.
(4) The importance & extensive therapeutic use of the substances investigated.
& investigations regarding the bactericidal power of these silver compounds the following considerations may be noted. (1) The anti-bacterial action is not necessarily simply proportionate to the percentage of silver contained in it. The condition of the silver in the compound as regards its capability of acting as a bactericide in solution is of first importance:
(2) With regard to albuminous & similar compounds of silver it is probable that electrolytic dissociation in solution is a small & negligible quantity (probably not more than 1 mg per litre); (3) With the exception of Argentamin + Isthargan it is probable that the substances in combination ē silver do not in

themselves possess appreciable antibacterial action (4) The penetrating power of solutions of the substances into organic matter is of importance.

In view of these considerations the methods adopted were as follows:

(1) The percentage of Silver in each substance was estimated.

(2) Equal - Silver - Content Solutions (usually 1% Aq Sols) were prepared & comparative investigations carried out with these. (Herein my investigation differs from that of all others — The solutions previously adopted compared being prepared in terms of the amount of the Compound in Solution)

(3) The comparative action of the Test solutions on Saprophytic bacteria in a fluid containing Chlorides (slight albumin was investigated — here the solutions were proved in fluid to some extent analogous to tissue fluid.

(4) The action of the Test solutions was tried on dried films of Staphylococci pyo. aurei. The films were prepared by submerging small glass balls in a watery emulsion of the cocci.

(5) Rate of diffusion thro' agar medium was tested.

– Estimation of Silver –

Each of the following compounds were carefully ashed in a platinum crucible. The ash was then treated with Strong HNO_3 or $(NH_4)NO_3$ crystals & reheated until all organic matter was driven off. The resulting $AgNO_3$ was then dissolved in distilled water & the amount of Silver estimated by means of $\frac{N}{10}$ Ammonium thio-cyanate solution using Ferric Sulphate as indicator. The process was in each case carried out with minute precautions & reactions, & in some cases repeated. Results as follows

	% Ag		% Substance
Argonin =	4·2	∴ 1% Ag. Sol = 1 in 4·2 =	23·81%
Argentamin =	6·24	" 1 in 6·24 =	16·02
Protargol =	7·72	" 1 in 7·72 =	12·95
Largin =	9·20	" 1 in 9·20 =	10·87
Nargol =	9·46	" 1 in 9·46 =	10·57
Actol =	53·39	" 1 in 53·39 =	1·87
Itiol =	60·36	" 1 in 60·36 =	1·65
Collargol =	81·77	" 1 in 81·77 =	1·22
Ichthargan =	29·02	" 1 in 29·02 =	3·45

Largin was decomposed ē great difficulty.

– Preparation of percentage Silver – solutions used in this research –

(1) Largin dissolved by aid of heat in proportion of 1 in 9.2 (1% Ag) but on cooling the largin was precipitated & instead of a solution a grey greasy-looking paste resulted. 1 part of largin, however, dissolved in 18.4 parts of cold water forming a brown, clear, solution containing 0.5% Ag.

(2) Argonin even when well diluted failed to form a true solution: 1 part in 12.6 of water formed a turbid, opalescent pinkish fluid. I failed to produce a trustworthy solution & therefore did not use it in further experiments. The so-called Soluble Argonin (Argonin. L.) which has been more recently put upon the market, I was unable to obtain.

(3) Ithol is stated to be soluble 1 in 3,800 of water. The most concentrated solution which I coned obtain after 8 hours agitation in by means of Engine Stirrer was found to be equal to 1 in 4.300 & contained .014% Ag. The solution was clear & transparent.

(4) Collargol dissolved easily to form a 1% solution (i.e 1 in 82 appx). This solution was

dark brown – almost black – opaque & very slightly viscid. It is particularly rapidly darkened by light. An important fact with regard to Collargol Solutions, is that they are very unstable – after a few days the strength of Solution is reduced to $\frac{1}{3}$ by ~~pre~~precipitation of Silver (ordinary metallic) from the Solution. This precipitated Silver forms a grey black deposit at bottom of bottle in which the Solution is contained

(5) The other substances dissolved with comparative ease.

As heat & light appeared to decompose the Solutions, the Solutions were prepared with Cold distilled water & in a darkened room. They were kept in bottles completely covered by opaque black paper.

Portions of the Solutions were exposed for 3 hours to direct Sunlight. All of them were darkened by light, especially Solutions of Actol, Thiol & Collargol.

— General Bactericidal Effect on Saprophytic Bacteria in a Slightly albuminous fluid containing Chlorides —

Water which had been used for macerating pathological specimens was used — it contained slight amount of albumen + chloride + had a most revolting odour.

Method — Small quantities of macerating fluid were intimately mixed ē equal quantities of the various Test Solutions + after 5, 10, or 15 minutes a loopful of the mixture was stroked on agar slopes + incubated at 37.5 C. "Control" Experiment accompanied each series — "Control" = Equal parts of sterile distilled water + macerating fluid — loopful stroked on agar + incubated at same time as the others. Temperature practically constant during each series of Experiments. Same stock of Culture Medium was used: The agar-Medium Composition is as follows 10 grams Peptone + 5 grams Nacl + 2 grams Liebig's Extract + 1 Egg + Distilled water to 1000 C.C. Glycerin-Agar = Agar-Medium + 7% Glycerin. All thoroughly Sterilized before use.

This method has some practical value

because the conditions – albuminous fluid containing chlorides – closely simulates those of the body. It is, at least a test of the comparative potency of the solutions used. It is, however, by no means a scientific method for it involves (1) various kinds of bacteria possibly of varying resistance (2) variable number of Bacteria (3) Bacteria were not freed from small amount of Test solution adhering to them before they were sown on the nutritive medium.

The following tables include only a small part of the Experiments performed. As many tests with solutions of intermediate strengths were made which yielded no further notable result. They have been excluded

The Controls in each series of experiments showed copious growth within 36 hours.

– Bactericidal Action on Putrefactive Bacteria –

Table I.

I

Test Solution	Percentage of Ag in Solution	Percent. of Substance in Sol^{n}.	Duration of action 5′ 48 hrs	5′ 5th day	15′ 48 hrs	15′ 5th day	Remarks.
$AgNO_3$	$\frac{1}{8}$ = ·125	0·197	o	o	o	o	o = no growth + = growth ++ = much growth + = abundant.
$Ag(NH_2)Cl$	$\frac{1}{8}$ = "	as NO_3 = ·197	o	o	o	o	
Collargol	$\frac{1}{6}$ = ·170	0·20	+	++	+	+	growth retarded not prevented
'do'	$\frac{1}{3}$ = ·34	0·40	+	++	+	+	
'do'	$\frac{1}{2}$ = ·50	0·61	+	++	●	●	= no growth after 15′ exp^{n}.
'do'	$\frac{2}{3}$ = ·66	0·81	o	o	o	o	no growth after 7 days.
Protargol	$\frac{1}{8}$ = ·125	1·62	o	o	o	o	
Nargol	$\frac{1}{8}$ = "	1·33	o	o	o	o	
Largin	$\frac{1}{8}$ = "	1·36	o	o	o	o	
Argentamin	$\frac{1}{8}$ = "	2·00	o	o	o	o	
Actol	$\frac{1}{8}$ = "	0·23	o	o	o	o	

Table II

$AgNO_3$	$\frac{1}{10}$ = ·1	·157	o	+	o	o	growth retarded by 5′ Prevented by 15′
$Ag(NH_2)Cl$	$\frac{1}{10}$ = ·1	·157	o	+	o	o	
Protargol	$\frac{1}{16}$ = ·063	·81	o	o	o	o	no growth at end of 7 days.
Nargol	$\frac{1}{16}$ = "	·66	o	o	o	o	
Largin	$\frac{1}{16}$ = "	·68	o	o	o	o	
Argentamin	$\frac{1}{16}$ = "	1·00	o	o	o	o	
Actol	$\frac{1}{16}$ = "	·12	o	o	o	o	

– Bactericidal action on pathogenic Bacteria –

Table III

Test Soln	% Ag. in Soln	% Subst. in Soln	Duration of Action 5' 5th day	Duration of Action 15' 5th day	Remarks
Protargol	1/32 = ·03	0.41	0	0	
Nargol	1/32 = "	0.33	+	0	growth retarded but not prevented by 5' exposure.
Largin	1/32 = "	0.34	0	0	
Argentamin	1/32 = "	0.50	0	0	
Actol	1/32 = "	0.06	0	0	

Table IV

Protargol	1/34 = ·029	0.38	+		growth retarded but not prevented by 5' exposure
Nargol	1/25 = ·04	0.31	+		
Largin	1/34 = ·029	0.32	+		
Argentamin	1/34 = "	0.47	+		
Actol	1/34 = "	0.055	+		
Ithol	= ·011	0·02	+		

Table V

Protargol	1/64 = ·015	0.37	+	Slight	growth retarded not prevented by 5' or 15' exposure
Nargol	1/64 = "	0.11	+	Moderate	
Largin	1/64 = "	0.17	+	Very Slight	
Argentamin	1/64 = "	0.25	+	0	growth retarded by 5'
Actol	1/64 =	0.03	+	0	Prevented by 15' exposure.
Ithol	= ·009	0.015	+	0	

Conclusions drawn from Tables

I. II. III. IV. and V.

$AgNO_3$	$\frac{1}{8}$ % aq.	5′	–	Prevented growth
$Ag(NH_2)Cl$	$\frac{1}{10}$ % aq.	5′	–	Retarded "
		15′	–	Prevented "
Collargol	$\frac{1}{2}$ %	5′	–	Retarded "
		15′	–	Prevented "
	$\frac{2}{3}$ %	5′	–	Prevented. "
Nargol	$\frac{1}{16}$ %	5′	–	" "
	$\frac{1}{25}$ %	5′	–	Retarded "
	$\frac{1}{32}$ %	5′	–	" "
		15′	–	Prevented "
Protargol Largin	$\frac{1}{32}$ %	5′	–	Prevented "
Argentamin Actol	$\frac{1}{34}$ %	5′	–	Retarded "
Protargol Largin	$\frac{1}{64}$ %	5′	–	"
		15′	–	"
Argentamin Actol	$\frac{1}{64}$ %	5′	–	Retarded
		15′	–	Prevented
Ichol	.011	5′	–	Retarded
	.009	15′	–	Prevented.

— Bactericidal Action of Silver Compounds on Pyogenic Organisms —

Staphylococcus pyogenes aureus employed.

An important part of this section of my investigations was to ~~compare~~ determine the activity of Ionised as compared with non-ionised Ag. Silver Nitrate, Argentamin, Ihal + Actol together with the albuminous and other compounds are very suitable for investigation from point of view of the relation shown to exist between amount of electrolytic dissociation + bactericidal power. Inorganic Salts as a rule are less dissociated in Solution than Organic Salts; as for the albuminous Compounds it has been already mentioned that amount of dissociation is practically nil.

For this part of the research it was necessary to have a thin uniform film of bacteria in a non-albuminous solution containing no Chlorides, and it was necessary that the the Silver Solution should be capable of removal otherwise it would exert a retarding influence on growth. For a similar purpose

Krönig & Paul used purified garnets &c
As the garnet crystals obtainable by me
were too small & irregular for this purpose
I employed ground glass balls of uniform
size which were purified & sterilised before
each experiment, as follows:- (1) Shaking HNO_3
then washed with distilled water (2) Caustic Soda
Solution & Heat then washed in distilled water
(3) Alcohol 95% (4) Gently heated the balls in
Platinum Capsule.
Same stock of Bacteria (Staph. Pyo. aur) & Culture
medium (Glycerin agar) were used throughout.
The bacteria were mixed & sterile distilled
water, the glass balls (5 m.m diameter) were
submerged in the Bacterial mixture. The
balls were removed from Bacterial mixture
& dried in desiccator. After subjection to the
action of the Test Solution the bacteria were
freed from adherent-disinfectant by immersion
for ½' in sterile distilled water. The balls -
usually 3-4 in each case - with adherent
bacterial film were shaken up with melted
glycerin agar (40°C) by means of vigorous shaking
for 3'. The infected melted agar was then poured
into Petri's dish, incubated at 37.5°C & colonies
counted after lapse of 3-4 days.

Method –

(1) 6 loopfuls of Staph. pyo. aur. culture inoculated in 10 c.c sterile distilled water in test-tube – Shaken & inoculated: (2) This Bacterial mixture was then poured into sterile glass beaker, submerging sterile glass balls arranged in single layer on bottom of beaker (3) Fine sterile forceps were used to transfer the glass balls & adherent bacterial film to glass racks in sterile desiccator (4) 3–4 balls (same no in each series of Expts) were then submerged in Test-solution, then transferred to sterile distilled water for ½' (5) the ground glass balls with the bacterial film were then shaken up in 5 c.c sterile boiled Glycerin Agar (40°C) which was then transferred to Petri-dish, incubated, & colonies counted

The method is very tiring & requires much patience. The following tables are the result of my experiments.

Control Expt accompanied each series – the balls being submerged in sterile distilled water for a period corresponding to submersion in Test-sols

– Bactericidal action on Films of Staph. Pyo-aur –

Solution	Table I: % Ag	I: % Subst.	I: Exposure 4' Colonies	II: % Ag	II: % Subst.	II: Expos. 5'	III: % Ag	III: % Subst.	III: Expo 5'
"Control"			69			75	1/100		13
$AgNO_3$	1/40	·039	0	1/60	·03	0	1/100	·016	0
$Ag\,NH_2Cl$	"	·039	0	"	·03	61	1/50		0
Protargol	"	·324	0	"	·21	0	1/100	·130	0
Argyrol	"	·265	4	"	·17	34	1/50	·106	0
Largin	"	·272	0	"	·18	0	1/100	·109	0
Argentamin	"	·401	0	"	·27	0	1/100	·160	0
actol	"	·047	0	"	·03	0	1/100	·019	1
Thiol	·014	·024	1	·014	·014	0	·014	·024	3
Collargol	1/3	·40	0	1/3	·40	2	1/3	·40	2
Ichthargan							1/100		0
$Hg(NO_3)_2$							1/100 Hg		0
$HgNO_3$							1/100 Hg		0

It will be seen that the control in each case showed a number of colonies. The tables however are not conclusive as regards comparative potency of substances tested. $Hg(NO_3)_2$ & $HgNO_3$ were introduced by way of comparison with Ag compounds & to test the relative power of Mercuric & Mercurous Salt. Ichthargan was procured towards end of experiments.

(I hope to carry on these experiments)

– Investigation with regard to diffusion of Compounds of Silver –

In order that my research might be of greater practical value I endeavoured to find out the comparative diffusibility of the substances already tested. The method I employed for this purpose is a special application of the results of the diffusion experiments of Graham & of Voigtlander. Graham (1862) found that diffusion of Salt solutions through gelatinous substances e.g Starch mucilage, Agar-Agar &c obeyed the same laws as diffusion through pure water. De Vries, however, found that K_2CrO_4 diffused more slowly through gelatinous bodies than through pure water – but in this case a chemical reaction probably occurred. Voigtlander's researches (Ztschr. f. phys. chem. III. 316 (1889)) showed that the diffusion of substances having no chemical action on the agar follows the same laws as diffusion through water. He thus confirmed Graham's work.

Voigtlander found that the rate of diffusion

through Agar was proportional to the square root of the time & the concentration, & varied also in a definite ratio with the temperature (Time, concentration, & Temperature remained relatively constant in my experiments)

In my experiments Equal-Silver-Content solutions were again used & compared with regard to their power of penetration into agar medium. (The composition of this medium has already been given & it will be noted that it contains albumen & chlorides). The agar medium was first inoculated with Staph. pyo. aur. which were diffused through it by shaking the medium melted at 40°C). The action of gravity was excluded by placing the tubes containing the agar in the horizontal position while submerged in the solution the diffusion of which was to be tested. Growth – or rather absence of growth, was the means by which I ascertained to what extent the solution had diffused. This might have been indicated by chemical means, but such a method is surely inferior to this biological test of diffusion & would not be a test of effective diffusion from point of view of antibacterial action, for it is

Conceivable that two different test solutions might diffuse at equal rate to same extent & yet the antibacterial action of the one might, during diffusion, diminish more rapidly than that of the other.

Preliminary investigation — I filled pieces of tubing (10 c.m long, 5 m.m bore) with melted agar. On cooling, I found that the agar could easily be shaken out of the tube as a cylindrical agar mould. Two such tubes were filled with agar, cooled, & placed in solution of Saffranin (1nd) & of Nitro-dimethylaniline (green). After ½ hr immersion in Saffranin solution the agar was found to be deeply stained for distance of 2 m.m. After 17 hours stained zone = 2.75 m.m that is diffusion had been stopped — the Saffranin acted as a dye (chemical inhibition) & prevented further diffusion. No capillarity

The agar tube in solution of Nitro-dimethyl amin showed a visible picture of diffusion. In 3 hrs green coloration of agar shading away from exposed end = 8 m.m, after 24 hrs the green zone = 22 m.m. No Capillarity.

Method used in Diffusion Experiments :-

1. Pieces of glass tubing 5 c.m long + 2 m.m bore were cleaned + sterilised, (2) 6 loopfuls of culture of Staphylococc. pyo. aur. was well shaken up in melted agar medium (40°C) (3) The pieces of tubing were filled with this infected agar; then cooled, + placed submerged horizontally in test solution for 20 - 22 hours: (4) The tubes were then removed, dipped in sterile water, + the infected agar cylinder was carefully shaken out of the tube into Petri's capsule containing 5 c.c of melted agar medium (The agar cylinders of each series of experiments were placed in parallel rows in the Petri dish). The dish was then placed in incubator at 37.5° + after 3 days the amount of penetration was measured as indicated by absence of growth towards end of cylinders. The extent of diffusion in millimetres was divided by the number of hours during which the cylinder had been subjected to the test-solution + the average hourly rate of diffusion was thus obtained. ("Control" experiment with each series)

Diffusion table indicating hourly-rate of diffusion thro Agar-medium.

	o/o of metal = 1/4 o/o	1/5 o/o	1/6 o/o	Average
$AgNO_3$	·65	·60	·60	= ·61
$Ag(NH_2)Cl$	·90	·80	·50	= ·73
Protargol	·85	·65	·65	= ·72
Nargol	·54	·51	·35	= ·47
Largin	·80	·65	·50	= ·65
Argentamin	·80	·80	·85	= ·82
Actol	1·00	·93	·85	= ·93
$Hg(NO_3)_2$	–	–	1·20	= 1·20
$Hg(NO_3)$	–	–	0·96	= ·96
Ihiol = (0·14 o/o)	·4	·43	0·40	= ·41
Collargol ($\frac{2}{3}$)	·75	·65	·35	= ·58
Ichthargan ($\frac{1}{6}$)	–	–	·45	= ·45

The percentages = per cent of metal. The mercury compounds introduced for comparison. Each of the mercury solutions contain ·364 o/o HNO_3 (required for solution of the mercurous salt.)

Silver salts arranged in order of diffusion is as follows on next page.

Ihiol was used in ·014 o/o Ag Sol. throughout.

Collargol was used in $\frac{2}{3}$ o/o Ag. Sol "

Ichthargan owing to lack of time was not used in earlier experiments.

Silver Salts arranged in order of diffusion-rate through Agar Medium —

(1) Actol = ·93
(2) Argentamin = ·82
(3) $Ag(NH_2)Cl$ = ·73
(4) Protargol = ·72
(5) Largin = ·65
(6) $AgNO_3$ = ·61
(7) Collargol = ·58
(8) Nargol = ·47
(9) Ichthargan = ·45 = Ichthargan.
(10) Ihrè = ·41

Except in the case of Ihrè & Collargol the Solutions contains equivalents of Silver.

After 24 hours immersion in the Solutions some of them (agar cylinders) showed precipitation at end abutting on the Solution :-

$AgNO_3$	½% Ag Sol	showed	4·5 m.m of white ppt.
$AgNH_2Cl$	"	"	none
Protargol	"	"	1 m.m of white ppt.
Nargol	"	"	" do "
Largin	"	"	" do .
Argentam	"	"	1 m.m grey ppt.
Actol	"	"	4 m.m white ppt.
Ihrè	·014%	"	none
Collargol	⅔%	"	6 m.m of dark colour ? no ppt.
Ichthargan	½%	"	1 m.m of white ppt.

– Therapeutics of Silver Salts –

The various silver salts have been chiefly used for treatment of gonorrhoeal affections of the genito-urinary tract. In this respect they have all proved useful and each has its supporters. In ophthalmic practice they have been used in gonorrhoeal & other ophthalmias. On the field of battle & in civil practice Actol & Ithol have been used as disinfectants in the treatment of wounds, ulcers, abscess cavities &c. Ithol has been much praised as an antiseptic dusting powder. Special interest is attached to Collargol because it has been asserted that it has produced general disinfection in general infection especially those due to Staphylococci & Streptococci. For this purpose it is used in the form of a 15% Ointment (Ung. Credé) rubbed into the skin until about one dram has been absorbed; it is also administered by intravenous injection & good results are reported in such varied conditions as puerperal septicaemia, Septic Endocarditis, Cerebro-spinal Meningitis, & even Tuberculous Meningitis (!) Protargol has been administered

subcutaneously in treatment of croupous Pneumonia & good results reported by Caccamiga. I think there is no doubt with regard to the assured position of some of the compounds in treatment of gonorrhoeal conditions;— results in treatment of the other conditions mentioned is chiefly if not wholly reported in foreign literature. It is possible that the use of Collargol for general disinfection in Staphylococcic & Streptococcic infections deserves further trial.

This Subject - Therapeutics - is scarcely within the scope of the present paper & I hope that the free bibliography which follows will indicate the immense interest which Silver salts have caused during the past few years.

- Bibliography -

I. Silver Nitrate

Mays : Albany med. annual XXII Sept. 1901
Kerez : Centl. f. Bakter. u. s. w XXXII 8 u. 9

II Argonin

Meyer : Ungarische Beiträge z. Augenh. 1895 Bd XX
Jadassohn: Archiv. f. Dermatol. u. Syph. 1895
Kamen : Heilkunde 1899 No. 9 u 10
Jellinek : Wiener med. W'chschr. 1899 p. 209

III Argentamen

Bergel : Therap. monatsch. July 1900
Daxenbergen : Deutsche. med. Ztg 1901 No 4 p. 46
Imre : Ungarische Beiträge z. Augenheilk. Vol II 1900
Schäffer : Zeitschr. f. Hygiene 1894 Bd XVI

IV Actol

Schüle : Therap. monatsch. 1899 Nr. 3 u 4
Meyer : Deutsche Militärarzth. Ztsch. 1899 p 33
" " " 1900 No 11
Kornfeld : " " " 1900 No 12
Jasinski : Therap. Wochensch. 1897 No 3
Telge : Münchener med. Wchnschr 1897 No 6
Hindes u Balandehen : Archiv. russes de Pathol de Med. Clinq et de Bactériol 1901 Vol XI

- Bibliography -

V. Iris

Arlt: Wiener Klin W'chnschr. 1902. 1218

Bock: Wiener Med. Presse 1902 No 27

Bezer: Allg. Med. Cntr-Ztg 1896 No 97

Cewicek: Militärarzt XXXVI 19 u 20

Darier: Ophth. Klinik 1898 p. 91

Isaac: Ztschr f. prakt Aerzte Vol 8. 1899

Hengl: Aerztl. Rundschau 1897 p. 48

Nenadowitsch: Ophth. Klinik 1898 p. 91

Schlossberger: Deutsch. Archiv. f. Klin Med Vol 67. 5-6

Werler: Berlin. Klin W'chnschr. 1898 No 16

Weyer: Münchener Med. W'chnschr. 1901 N. 42

VI Collargol

Credé + Beyer: Collargol + Collargol Salbe als Antiseptica

Credé: Archiv. f. Klin Chirurg. Vol 56 No 4

Credé: Monatschr. f. Geburtsh. u Gynec. Vol 8 No 6

Liebreich: Therapeut. Monatshefte Dec 1897

Credé: Berlin Klin W'chnschr 1901 Sept 16th

Credé: Die Medicinische Woche 1901 May 28. June 3rd

Credé: Klinisch-Therapeut Wochenschr 1898. 14 u 15

Brown: Journ. of Nervous + Mental Disease 1900 June.

Wolfrom: Allg. Med. Centr-Ztg 1898 No 42

Werler: Deutsch. Med. Wchnschr. 1898 No 40

Collargol

Jacobi: Medical Record 1900 Feb 3rd
Kronfeld: Deutsch. med. Ztg Nov. 15. 1900
Kurz-Krause: Therap. Monatsh. 1900 Aug & Oct
Klimmer: Ztschr. f. Thiermedicin 1900 Vol 4
Klotz: Deutsch med. W'chnsch. XXVIII 29
Scheimer: New York med Monthly 1898 Nov.
Does: Ztsch f. Physiologie 1898 Vol 24 p 357
Cohn: Centr-bl. fur Bakteriologie, Parasitenk. u Infectionskrankh. XXXII 10 & 11 (1902)
Neissen: Wiener med. Wchnsch. 1898 No 44 & 45
Klien: Centrbl. f. Gynekologie 1898 No 10
Meier: Münchener med. W'chnsch. 1899 No 43
Dworetzky: Therap. Monatsh. 1900 No 3
Schlesmann: " " 1899 No 5 p. 278
Muller: Deutsche med. W'chnsch XXVIII No 11

VII Largin

Fürst: Dermatol. Ztsch. 1899 Vol. 9 p. 39
Fürst: Centralbl. f. Kinderheilk. 1901 No 3
Kornfeld: Wiener med. Presse Dec. 1898
Lassar: Dermatol. Ztsch. Bd VI 1899
Pezzoli: Wiener Klin. W'chnsch 1898 No 11 & 12
Porges: Wiener med. Presse 1899 No 44
Schuffan & Anfrecht: Allg. med. Ctr-Ztg 1898 No 84
Wellander: Archiv f. Dermatol u. Syph. XLVI 3

VIII Protargol

Davies: Die ophthmol. Klinik 1898 No 7

Cipriani: Annali di 1898 No 7

Fürst: Fortschritte der Medicin 1898 No 4

Donee: Clinique Ophthalmol 1898 No 14 p. 162

Pflueger: ophthalmol. Klinik 1898 No 11

Bistis: Clinique Ophthalm. 1898 N 20 p. 233

Walter: Ophthalmol. Klinik 1898 No 13

Welander: Deutsche Med. Ztg 1898 No 70

Rosenthal: Deutsche Med. Ztg 1898 41 p 407

Finger: Heilkunde 1898 March

Meyer: Die Ärztl Praktik 1898 No 4

Behrend: Berl Klin W'chrsch 1898 No 14

Alexander: Archiv f. Laryngol a Rhinolog vol 9

Caccianiga: Deutsche Med-Ztg 1900 No 35

Cohn H: Therap. d. Gegenw 1900 No 7

Koffs: Münchner Med W'chrsch 1899 No 50

Ruhemann: Deutsch Med W'chrsch 1899 No 40

Behrend: Berl Klin W'chrsch 1898 No 14 p 304

Stekel: Klin-Therap W'chrsch 1901 No 33

Zeisl: Wien Klin Med. Wochrsch 1901 No 8

Alexander: Wien. Med. Presse 1901 No 13 p 604

IX Nargol

Martin: Therap. Gazette 1.15.1900

Schwartz: Ophthalmic Record March 1902

X Ichthargan

Aufrecht: Deutsch. med. Wchnschr 1900 No 31

Bass: Deutsch Therapie Wchnschr 1901. No 14

Dubal: Ann. de la Policlin de Bruxelles Nov 1901

Falta: Archiv f. Augenheilk vol 43

Fürst: Deutsch. med. Wchnschr 1901 No 14

Leshkow: Monatsch. f. prakt Dermatolog. 1900 vol 31 p. 183

Lohnstein: Allg. med. Cent-Ztg 1900 No 80, 81

Saalfeld: Therap. Monatsch XVI 3 p 137

Schmidt: Dermatolog Centralbl. 1900 No 3

XI Albargin

Pfuhl: Skypiensche Rundschau 1902 No 3

Klotz: med. News 81-22. p 1019 (1902)

Baumann: Therap. d. Gegenwart März 1901

XII Argyrol

Barnes: Journ. of Eye Ear & Throat VII. S. p 11

Lederman: N. York med. Record LXII 21 p 810