mains constant after the first decrease. Arsenic trioxide and mercuric bichloride are poor preserving agents to employ as they immediately react with the iodine until one or the other is completely used up. Alcohol is the best preservant, with cavity fluid and the 10 per cent formaldehyde solution second and third, respectively. The tissue alone will return as much iodine as that preserved in either of the formaldehyde solutions and, as iodine inhibits putrefaction, if nothing else were available but these two agents it would probably be better to leave the material unpreserved.

It is evident that the toxicologist, by making the preliminary examination as proposed, would be able to tell immediately within 10–15 per cent the quantity of free iodine present in the body at the time of death.

REFERENCES.

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A COMPREHENSIVE STUDY OF THE PREPARATION OF COLLOIDAL SILVER IODIDE AND A REPORT OF ITS BACTERIOCIDAL AND BACTERIOSTATIC VALUE.*

BY W. A. LOTT AND W. G. CHRISTIANSEN.

It has long been felt desirable to obtain a non-irritating, non-staining germicidal colloidal silver compound to supplant the mild silver-protein compounds which have objectionable staining properties and to supplant the strong silver-protein compounds which produce irritation as well as stains. In fact, colloidal silver halides to which germicidal activity is ascribed have been marketed for some years as germicides. This laboratory has throughout several years conducted a study of colloidal silver iodide made by a great variety of methods. These methods can be indicated briefly as follows:

I Double decompositions.
  A. $AgCl + KI \rightleftharpoons AgI + KCl$
  B. Ag proteinate + KI $\rightleftharpoons$ AgI + K proteinate
  C. Ag soap + KI $\rightleftharpoons$ AgI + K soap
  D. $Ag_2O\text{-}(H_2O)_4 + KI \rightleftharpoons AgI + KOH$

II Oxidation and reduction.
  A. $Ag^\circ + I^\circ \rightleftharpoons AgI$

III Decomposition of double salt.
A. $KAgI_2 \rightleftharpoons KI - AgI$

With each method a very thorough study was made of the effect of varying the conditions during every step in the method on the colloidality and germicidal behavior of the silver iodide.

These conditions themselves seem endless in variability, and no attempt will be made to discuss them all, but it might be pointed out that significantly different effects are obtainable by altering the (1) kind of protective protein, (2) the degree of hydrolysis of this protein, (3) p_H of the protein during the several

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steps in preparation, (4) concentration of electrolytes during and after the formation of the AgI, (5) which of the two reactants in the double decompositions is present in excess, and in what concentration, (6) method and degree of removal of electrolytes after the formation of AgI, (7) temperature of drying.

On account of the difficulty it caused during the early part of the work, it seems desirable to discuss the question of discoloration of the AgI colloids by light, and the control of this phenomenon. The brown color developed in the light sensitive products was due, of course, to the reduction of silver to the metallic state. It was felt at the outset that this could be controlled by keeping the concentration of hydroxyl-ions (OH^-), and silver ions (Ag^+) low. This was confirmed by all the facts later developed. For example: The addition of a small amount of acetic acid to a light sensitive product made it stable to light because of the decrease of the hydroxyl-ion concentration. The addition of moderate amounts of ammonia stabilized a product, probably by the decrease of the silver-ion concentration by the formation of the $Ag(NH_3)^+$ complex ion, and possibly also by decreasing the hydroxyl-ion concentration. A product already turned brown could be made yellow or white by the addition of sufficient iodine to oxidize metallic silver to silver iodide. A product which has been precipitated out of water solution by ordinary denatured alcohol tended to be less sensitive to light than one which was precipitated by isopropyl alcohol. This is explained by the fact that potassium or sodium hydroxides are insoluble in isopropyl alcohol and therefore much less soluble in somewhat diluted isopropyl alcohol than in equally diluted denatured alcohol; therefore when isopropyl alcohol is used the fixed alkali will tend to remain in the gelatinous product. This, of course, would make the solution of the redissolved material higher in hydroxyl-ion concentration and more light sensitive.

Practically then, the conversion to AgI must be carried out completely and when this conversion is completed the hydroxyl ion must be low. In the double decomposition this is accomplished by using mixtures of KI and I_2, the quantity of I_2 being determined by the amount of fixed alkali in the solution which must be neutralized. In the oxidation-reduction method we must be sure that there is not too much fixed alkali present for the conversion to AgI and that the oxidation is really completed. This is determined by the golden-yellow color of the product at the end-point.

EXPERIMENTAL PART.

I. Preparation by Means of Decomposition of Potassium Argento Iodide.—(*a*) This was first accomplished by stirring 11.6 Gm. of Ag_2O with 12.7 Gm. I_2 and 18 Gm. of KI in a solution of 40 Gm. of hydrolyzed, dialyzed and desiccated gelatin and 15 cc. water until the mixture became clear. This preparation was dried to transparent flakes at room temperature in vacuum. The redispersion was not rapid, and gave rise to a milky, not entirely stable colloidal solution.

(*b*) Better results were obtained by mixing 25.8 Gm. AgI and 18 Gm. KI in 129 Gm. of a solvent made by dissolving 48 Gm. of hydrolysed, dialysed and desiccated gelatin in 120 Gm. of diethylene glycol. This gave a clear liquid product which was a true solution of $KAgI_2$. When diluted with water it was rapidly redispersed to a fine, stable colloidal solution of AgI.

(*c*) By dissolving 10 Gm. of AgI and 7 Gm. of KI in 90 Gm. of diethylene glycol, a clear non-hygroscopic solution of $KAgI_2$ was obtained, which gave a stable colloidal solution of AgI when mixed with water.

II. Methods Depending upon Double Decompositions between KI and Various Colloidal Silver Salts.—It was found empirically that very highly dispersed AgI was obtained by the gradual addition of KI to colloidal solutions of only slight soluble silver salts. The explanation for this undoubtedly lies in the slight concentration of electrolytes present during the conversion to the AgI.

(*a*) *Double Decomposition between KI and Silver Proteinate*—360 Gm. of gelatin were hydrolysed in 825 cc. of water with 25 Gm. of 44% KOH by boiling one-half hour. Ten Gm. of phenol (which is later removed) was added, and the solution filtered with suction, a small quantity of Kieselguhr being added to assist filtration; 85 Gm. of $AgNO_3$ in 250 cc. water were added gradually, with efficient mechanical agitation. The silver proteinate was precipitated with two liters of denatured alcohol and dehydrated by disintegrating in two liters of a 1:1 mixture of acetone and methyl alcohol, whereby a light yellow powder was obtained. This was dried at room temperature *in vacuo.* The dry silver proteinate was not repeptized in 500 cc. water and agitated, while 80 Gm. of KI and 15 Gm. I_2 in 250 cc. water were added dropwise. A golden-yellow transparent solution of colloidal AgI resulted, which was precipitated with one gallon of No. 1 special denatured alcohol, and the sticky precipitate dried *in vacuo* at 80° C. A fine looking yellow flaky product resulted, which repeptized readily to a yellow transparent solution. The solid product contained about 26% AgI.

(*b*) *Double Decomposition between KI and Colloidal AgCl.*—One hundred thirteen Gm. of gelatin were hydrolysed in 200 cc. water with 45 cc. of aqueous caustic soda containing 0.1 Gm NaOH per cc., 4 Gm. of phenol were added. After boiling $^1/_2$ hour the preparation was filtered as previously described. A solution of 34 Gm. $AgNO_3$ in 40 cc. water and a solution of 13 Gm. NaCl in 40 cc. water were added dropwise, simultaneously to the agitated gelatin solution keeping the chloride slightly ahead.

Fifty-one Gm. of I_2 were now dissolved in 160 cc. aqueous sodium hydroxide containing 0.1 Gm. NaOH per cc. and 20 cc. water. This solution was now added dropwise to the above colloidal silver chloride solution, while agitating. A liter of 95% alcohol and 365 cc. ether was used to precipitate the colloid. After drying it was redispersed readily in water, giving a milky, stable colloidal solution.

Other modifications of this method wherein KI was used for the formation of the AgI and in which less excess iodide was used gave approximately the same result.

(*c*) *Double Decomposition between KI and Silver Soap.*—One hundred seventy Gm. of $AgNO_3$ were dissolved in 4 liters of water. To this was added, gradually, a solution made by neutralizing 150 Gm. of double distilled cocoanut oil fatty acids with 40 Gm. 33% NaOH in 4 liters of water. The silver soap, made in this manner, precipitates in a granular condition and can easily be washed free of electrolytes on a suction filter.

Four hundred Gm. gelatin were hydrolysed in 1500 cc. water with 50 Gm. of 44% KOH by boiling 35 minutes; 20 Gm. of phenol were added, and the solution filtered.

The silver soap was dispersed in the gelatin solution by a "lightnin" agitator with the beater attachment. The conversion to AgI was accomplished by adding a solution of 170 Gm. KI and 20 Gm. I_2 in 1250 cc. water, dropwise, while agitating. A slight excess of the iodine solution must be added after the golden-yellow end-point is reached in order to insure light stability.

The colloid was precipitated by 15 liters of No. 1 denatured alcohol. Thorough washing with alcohol or reprecipitating with alcohol was necessary to remove the soap formed as a by-product. After drying *in vacuo* at about 80° C. a fine looking yellow flaky product was obtained, which repeptized readily in water to give a translucent white stable solution. The solid material averaged 30% AgI.

A number of other silver soaps were used, but the cocoanut oil soap gave the best results, especially from the standpoint of color and general appearance of the final product.

(*d*) *Double Decomposition between KI and Colloidal Silver Oxide.*—Fifty Gm. of gelatin were hydrolysed in 200 cc. water with 10 Gm. 40% KOH by boiling 30 minutes. After filtering the solution as usual, 17 Gm. of $AgNO_3$ in 50 cc. water were added dropwise, with agitation. The colloidal Ag_2O was formed by adding, dropwise, 5.6 Gm. of KOH in 50 cc. water. After the solution had been dialysed 24 hours at about 50° C., 12.7 Gm. of freshly precipitated iodine was now added and the mixture agitated until the solution became golden yellow in color, indicating that the conversion to AgI was complete. On some occasions, when an excess of I_2 was added, the solution became somewhat red, after the golden-yellow color had been reached. If this is allowed to go too far the solution becomes acid and the ultimate product will not be soluble in water.

After the characteristic yellow end-point was reached, the solution was immediately filtered to prevent further solution of iodine and the reaction mixture was evaporated *in vacuo* at about 80° C. Sometimes alcohol precipitation of the colloid was resorted to in order to get rid of most of the water.

The colloid repeptized to give a transparent solution which soon became opaque and some of the AgI settled out. If the colloidal silver iodide is not dialysed the repeptized product gives a somewhat milky solution, but shows no settling. No satisfactory explanation of the difference in stability has been worked out.

The solid product usually contained about 30% AgI.

III. Oxidation of Colloidal Silver by Elementary Iodine.—Seventeen hundred Gm. of gelatin in 5 liters of water were hydrolysed with 150 Gm. 43% KOH by boiling $37^1/_2$ minutes, bringing the finished solution back to its original weight with water. It was now filtered with the aid of Kieselguhr and centrifuged in a Sharples Supercentrifuge. Three hundred forty Gm. of $AgNO_3$ in 1500 cc. water were added dropwise (agitation) and converted to the oxide with 233 Gm. of 40% KOH. An additional 233 Gm. of 48% KOH were added and, while continuing the agitation, 250 Gm. of dextrose were dumped into the solution. In a few minutes the light colored solution suddenly became jet black, indicating that reduction to colloidal metallic silver had taken place. The colloidal silver solution was dialysed 24 hours at about 50° C. and sometimes, again centrifuged. The volume was decreased to six liters in a vacuum still and the colloidal Ag converted to AgI by agitating it with 300 Gm. of iodine. If a slight excess of iodine is dissolved the solution becomes somewhat red, but if the quantity of excess iodine is not more than slight, this was not detrimental. The colloid was now precipitated by 5 gallons of No. 1 denatured alcohol and dried at about 80° C. *in vacuo.*

The product was a fine looking yellow flaky solid which repeptized readily in water to give a transparent light golden-yellow solution, which was perfectly stable. The solution material usually contained about 30% AgI.

When the usual laboratory germicidal tests were made using both *B. Typhosus* and *Staph. Aureus,* variable results were obtained and in some instances the results appeared to indicate very high germicidal activity. However, a careful study of the ordinary germicidal test indicated that it is of very little value when applied to colloidal silver iodide, and an elaboration of the test showed that in those instances when the bacteria had apparently been killed, the bacteria had not been killed and that the original results were incorrect due to the limitations of the ordinary germicidal test. A careful study of the germicidal properties of all of our experimental samples of colloidal silver iodide of all purchased colloidal silver iodides proved that they are not germicidal and have little if any antiseptic activity when tested *in vitro* against *B. Typhosus* and *Staph. Aureus.*

Therefore, laboratory results fail to disclose any indications of useful antiseptic or germicidal properties in colloidal silver iodide.

The germicidal studies and tests were made by Dr. George F. Leonard in the Biological Research Laboratories of E. R. Squibb & Sons, and Dr. Leonard has discussed the limitations of the germicidal test as applied to silver preparations in a separate publication. (*Journal of Infectious Diseases,* 48 (1931), 358.)

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THE VITAMIN POTENCY OF VARIOUS GRADES OF COD LIVER OIL.*

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With the growing use of cod liver oil for animal husbandry purposes, knowledge of the vitamin activity of the various grades of cod liver oils commonly used for such purpose becomes important. These oils are from various grades of cod livers which become available in practice. Some oil is also obtained from pressing liver tissue or "chum," the residue left in steaming of fresh livers for the production of medicinal oil.

Five grades of cod liver oil seemed worthy of study in considering the above problem:

1. *No. 1 Medicinal Oil.*—Oil rendered from fresh cod livers by means of direct steam and skimmed off the settled mass within a short time after steaming (control oil to eliminate the natural variations in vitamin values existing in cod liver oil at different seasons and from different sources).
2. *Second Grade Oil.*—Oil skimmed off after the mass has stood twelve hours subsequent to the first skimming.

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