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THERAPEUTIC COLLOID

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Our invention relates to the art of the production of substances in colloidal state to be used therapeutically and more particularly to the production of fatty sols that adsorb the toxins that pervade the human blood stream when the body is in a state of unhealth.

The object of our invention is the preparation of stable fatty sols by a novel process which can be administered intravenously or subcutaneously for the purpose of adsorbing and fixing the toxins that are frequently the evidentiary manifestation of disease and for the ultimate purpose of restoring the health of an individual to an accepted norm.

The following description of our experiments and observations not only sets forth our corroboration of the early researches and developments in this particular field of fatty sols and their therapeutic application but also discloses our subsequent extension of these early principles into a novel and workable therapy.

Previously, only sporadic mention had been made by various observers of fine particles occurring normally in the blood serum and plasma to which the name "Blood dust" had been generally applied. One of the early observers had previously suggested the term "Chylomicron" for these fine particles, inasmuch as investigation had established their fatty character and approximate size, one-half to one micron; other facts established by the early workers were that chylomicrons had their origin from fat and not from protein or carbohydrates, that the number circulating on the blood stream has a constant relation to the food intake and the approximate numerical relationship to other formed elements were estimated, i. e. in a cubic millimeter of blood there are about eighty million chylomicrons to five thousand white and five million red. The last point seems to us specially important,—the tremendous quantity of these particles in relation to the blood cells. It is obvious that such a predominance of one subject in the blood serum should have more biological significance than simply the function of nutrition and hence might logically be affected by the various states of health and disease in the same manner that the red or white cells are.

Search of the literature for further knowledge of this important emulsion brought to light a series of papers by certain workers whose careful work concerned itself with the physical and chemical aspects of the chylomicron emulsion. They concluded that each fatty particle was coated with a protein film, presumably a mixture

of albumen and globulin and further that the number of the circulating particles had no effect on the specific gravity or the viscosity of the blood as shown by the methods they employed in their work.

Further research revealed that the extension ultramicroscopic investigation of the blood serum has been done by certain other individuals. These workers consider the electrophysical state of the particles to be the determining factor in health and disease and utilizing the modern conception of the structure of the atom they feel that an explanation of all diseased processes is possible by their hypothesis. The essential fatty character of these minute particles is not recognized by these last mentioned individuals but then, as has been shown previously, the surface film may well be protein, this being a major factor in the stabilization of such an emulsion.

From this work, there is no doubt that there is a very suggestive relationship between the state of health of the individual and the morphology and characteristics of the small particles.

We have examined over a thousand cases under the dark field covering a wide range of sickness and it is quite possible to describe an illness in terms familiar to the physical chemist by observing the ultra-microscopic picture of a blood serum.

Before discussing the common changes observed under the dark field, a few definitions should be in order:

Brownian movement.—This is a vibratory motion peculiar to small particles in a state of colloidal suspension. It is due apparently to the electronic bombardment of the particles by the external phase.

Hydration.—Where the field shows a number of variations under different heads, for example—a fewer number of particles—an increased size of the particle—a sluggish repulsion between particles—a sluggish Brownian movement—the presence of clumps.

Dispersion is the opposite of the above and is identified generally with the normal appearing field—that is a goodly number of particles, excellent motion, usually uniform in size, and no clumps.

After stating the possible biological variations in the characteristics of a fatty emulsion, we must note some of the more obvious changes, commonly observed by looking under the dark field. The commonest findings are a lessened activity and diminution in the number of these circulating Chylomicrons, especially in wasting diseases. In diseases of an endotoxic character, clumping and

agglutination under the microscope of these individual particles seem to be a sign of infection or of existing toxemia. This clumping and agglutination by the fat particles in the presence of a circulating toxin suggests that perhaps fat plays a part in detoxification. In other words, fats are toxiphilic and "fix" a toxin, the union being chemical or physical, and resulting in an innocuous substances.

The ability of a lipid and fat to fix toxic radicals is well known, and certainly the colloidal form circulating in the blood is, by virtue of its tremendous surface development, singularly well adapted to this role.

Some examples of this power of fat to absorb may be given: the affinity of diphtheria and tetanus for the lipoids of our body. If we delay in giving antitoxin for instance, there will be no unbound toxins circulating to meet it and, hence, the danger of delay in giving antitoxin is serious.

Again, we know that a prolonged toxemia of an organ leaves a deposition of fat in it. Fatty degeneration and infiltration are every day experiences to the pathologist. A kidney which has lost its function through inflammation is apt to be full of fat, a liver likewise reacts by having fatty replacements, especially in severe poisoning. It was formerly believed that this depended on a transformation of the proteins of the tissues into fat, under the influence of the poison. It is evident that if such a change were as wide-spread as the fat deposits would lead one to suppose, there would be a great loss of protein in the entire body and an increase of fat. Analysis of the entire body of such small animals as mice, frogs, etc., shows that no change occurs and that foreign fat such as beef suet or codliver oil given to mice is deposited in the liver cells. There is no longer any doubt that the fat in the liver, kidneys, and other paraneoplastic cells is not formed from their own protein, but is deposited after transportation from fat deposits already existing.

Our point about this whole matter is the assumption that a toxic organ has fat brought to it by nature in an effort to detoxicate the existing condition and is not simply a phenomenon per se of the action of toxin on cells.

It may not be an exogenous type like diphtheria or tetanus. It may be endogenous or intracellular, exerting its effects only upon cell destruction. It may be on the other hand an untoward by-product of deranged metabolism, or finally and most probably, it may be a so-called change of physical or chemical equilibrium and not a separate substance at all.

We have prepared lecithin sols but consider lanolin superior, which is a tri-cholesterol-ester of the three fatty acids palmitic, stearic and oleic. It is produced by condensation methods using alcohol and water, and when finished is extraordinarily stable, but extremely sensitive to a toxic radical. The actual method of procedure is indicated as follows:

Anhydrous lanolin is used because it is slightly soluble in alcohol and partly mixes with water without separating.

Quantitative measures are not necessary in the preparation of this sol because there is only a certain amount absorbed by the alcohol, the rapidity and quantity varying with temperature of the alcohol. For this reason warm alcohol is used, to save time and conserve alcohol, though it may be made with cold. A sufficient amount,

usually about fifty grams of lanolin is stirred in a large beaker by means of a mechanical stirrer for one and a half to two hours. Samples are taken after the first hour and mixed with an equal quantity of distilled water. If a prompt, white precipitate forms, which after several samples does not increase in density, this is taken as evidence of sufficient saturation.

The volume of lanolin-alcohol mixture is then mixed with three times its quantity of distilled water and an immediate white emulsion is formed.

The next step is the driving off of the alcohol over a steam bath at a temperature varying from eighty to ninety degrees. The volume at the end of this process is roughly half that of the original mixture and alcohol should not be detected by smell although there is a small quantity always present by conventional tests. If warm alcohol is used there is usually a creamy scum of undispersible fat floating on top of the dispersed fat in a state of emulsion. After cooling to room temperature, it is filtered through paper.

The preparation is now ready for use subcutaneously but if intravenous use is contemplated it is wise to add powdered glucose, making a 5 per cent solution such as is commonly used for intravenous medication.

The sol as completed is extremely stable. Tests have shown it to be sterile from all types of organism and spore formers. Freezing promptly breaks the emulsion, and agitation fails to restore it.

It may be stored in a cool place with a cork or cotton plug. Before clinical use, it is brought to a boil or is autoclaved, and injected slowly at body temperature.

Its physical properties are: a milky-appearing aqueous liquid, consisting of particles about one micron in size showing active Brownian movements. It carries a negative charge obeying the general rule that the phase having the lower dielectric constant carries a negative charge, the positive phase in this case being water. Its pH varies slightly, but may be adjusted easily to 5.5. There is undoubtedly an orientation of the fatty molecules with the polar group turned toward the water. Hence, any toxin with which it united must present a similar active or polar group as recognized by colloid chemistry.

We contend that our emulsion is not stabilized by any film, dispersion being quite permanent even after three years observation. Presumably, its stability depends upon two factors: one being the electric charge and the other a "hydration factor" spoken of by Freundlich.

The sol is thermo-stable even at boiling temperature and ultra-violet light rather increases the energy of the Brownian movement.

In a general way, dispersion is enhanced with an increase of the pH or a shift to the basic side of the solution, while hydration is brought about by the addition of acids. Thus, precipitation of 100 cc. of colloid by 30 minimum lethal doses of diphtheria toxin will occur in lanolin sol at a pH value of 4.5, but will not occur at a pH value of 5.0. Most diseases of a toxic nature are associated generally with acidosis which is compensated for by the buffer salts. In these diseases, we note routinely a state of marked hydration of the chylomicrons in the blood serum. Conversely in diseases characterized by alkalosis, the chylomicrons in the serum are routinely highly dispersed.

The effect of a number of substances upon this colloid was investigated next. From test-tube experiments, we demonstrate that a drop of "toxic" blood will precipitate approximately 3 cc. of this lanolin. "Toxic blood" is meant simply that the blood comes from a case showing ordinary symptoms of toxemia, such as fever, leucocytosis, malaise, sweat, etc.

Normal blood will not do this. None of the four blood types taken from a well person has any precipitating effect on the colloid. We have tried many other substances to discover whether they can precipitate the colloid; but with one or two exceptions they do not. This is most important, because it is the presumed affinity of the fat for toxin which causes a precipitation; and if many other substances did this, it would be of no particular value. Other common chemical substances do not do this. Thus methylene blue (10%) and phenol (97%), arsenic (1-100), mercurochrome (2%), bichloride of mercury (10%) S. T. 37, ceanothyn (tincture), iodine (sat. sol.), sodium hydroxide (10%) have no precipitating effect according to our experiments.

Other observations follow: Precipitation is caused regularly by a pH more acid than 1.2; but acidity is not the only factor as we tested many sera which were definitely on the alkaline side, and a drop of which promptly precipitated the colloid. The promptness with which coagulation or precipitation takes place in the test tube after the addition of a drop of toxic blood from a sick patient is roughly parallel to the severity of the illness. Thus in a critical pneumonia case in the first stage, precipitation was prompt in 4 minutes and when the test was repeated 1 week later, with the patient considerably better, the test took 20 minutes before precipitation was noticeable. This phenomenon observed in vitro may be interpreted much as the blood sedimentation test for red cells.

Of the various salts, the Cl ion seems to be particular in causing precipitation in a lower concentration than other salts.

Regarding this precipitation or agglutination phenomenon we assume that the fat "fixes" a toxic radical; and in our test-tube experiments this precipitating effect is considered evidence of toxicity. When acid is the precipitating agent the result is due to the H charge neutralizing the negative charge on the particles.

Analyzing this phenomenon, it is clear that if the toxin in a toxic blood has not been "fixed" by the emulsion in the test-tube, the precipitate if used again against another specimen of colloid in a test tube would drop it. But this will not be so unless the number of drops originally added were too great for the 3 cc. of colloid.

Having made these test-tube experiments and assuming that a coagulated fatty emulsion is evidence of its innocuous state, we tried some biological experiments using guinea pigs and rabbits, which were kept under similar conditions. We found first that the colloid in 5 to 10 cc. doses was not harmful when injected into the earveins of a rabbit and doses of 250 cc. are not harmful to humans. The next step, we felt, was then to obtain a definite toxin of known strength instead of simply an unknown toxic serum. We obtained from a local laboratory diphtheria toxin of an M. L. D. sufficient to kill a 250 gram guinea pig in four days and was made up so that 1 cc. represented this dose. Adjusting the amount of toxin to the weight of a guinea pig, we made an-

other series of experiments, the conclusions of which follow:

The fatal dose when mixed with 3 cc. of colloid and injected together was without effect. The control animal died on the fourth day.

Three guinea pigs were given a lethal dose of toxin subcutaneously and then each was given respectively 1, 2, 3, cc. of colloid in divided doses starting 15 minutes after the original toxin injection and continuing every 2 hours for 4 days. The results were that the guinea pigs treated by the colloids showed no effects so far as we could judge by their appearance and behavior. The control animal died as before.

Further experiments brought out the fact that it takes more colloid to neutralize the toxin in the body than in the test-tube and also that if given all in one dose it is not as effective as the same amount divided over a period.

To test out the detoxicating power of fat in vivo, 3 minimum lethal doses of toxin were put into 10 cc. of colloid and allowed to precipitate. At the end of 24 hours, the clear portion was injected into a guinea pig and the precipitated portion was injected into another guinea pig. In both pigs no untoward effects were noted, which would seem to indicate that the toxin had been adsorbed by the precipitate rendering both clear portions and precipitate innocuous. Naturally, many experiments have been made with the end in view of shaking loose the toxic portion after its union with the fat, all being unsuccessful to date.

This however opens up a most fascinating investigation because, were it accomplished, one would be in a position of being able to isolate an endogenous toxin, such as that of pneumonia, typhoid, grippe, etc., and study it in its pure state as we do now diphtheria, tetanus, and botulinus.

In the preparation of this colloid for human injection, it was necessary to find out whether or not the buffer salts have a precipitating effect.

The addition of phosphates, sulphates and calcium carbonate to the lanolin sol does not influence the state of dispersion, the Brownian motion, the size of the particles, or its therapeutic efficacy in any way until a certain concentration is reached. Since this concentration is well above that found physiologically, it is possible to employ a sol to which has been added one or more of these salts in physiological proportions. The addition of sugar in the form of dextrose up to a 10 per cent solution with or without the presence of a salt, such as sodium carbonate, in no way militates against the stability of the sol. This finding is of value since it permits the use of a physiologic glucose solution (5 per cent).

Unadjusted, when freshly prepared, a colloidal sol of fat will be of a certain pH value. For instance, lanolin will have a pH value of about 4.5 or a little higher. This is not the pH of the body, but from our experience gives excellent therapeutic results and even when used in as much as a 500 cc. dose has no deleterious effects. It is wise, however, and it is our custom to adjust our sols by the use of small quantities of a very dilute sodium hydroxide to a pH of 7.5 or 7.6 before we use them. It has been found that, if a sol of lanolin has a pH of 4.5, the addition of sugar to make a 5 per cent solution will raise the pH value to almost a physiological normal due to apparent depression of ionization. For this reason, it is hardly necessary to adjust a sol to which glucose has been added. Somewhat the same effect, although not to the same degree, follows the intro-

duction of salt or sodium phosphate in a physiological percentage.

Our sols have routinely the appearance of milk and have a surface tension slightly above that of water. In each 1000 cc. there is approximately 4.5 to 5.0 grams of fat. It is practically odorless and produces nausea when an attempt is made to drink it.

Therapeutically, there is a choice among a number of preparations of fat. We have used lanolin almost exclusively, although kekuni nut sol is just as efficacious. However, almost any other fat can be used. The sol can be used in its freshly-prepared state or with the addition of dextrose. The method of administration depends upon the preparation to be used. With the freshly-prepared sol, hypodermoclysis is the method of choice and as much as 250 cc. will be absorbed readily in 15 minutes. If dextrose or the physiological salts or both are to be used, then the intravenous method is to be preferred, since the hypodermic method leads to discomfort because of the slow absorption. The intravenous use of the freshly-prepared specimen will at times cause severe lumbar pains after some 30 to 50 cc. have delivered. Since the material is kept as described, it is wise to boil the sol for 3 minutes before using, and to administer it at body temperature.

We have noted no sign nor symptoms of an allergic nature even of the most minute character in cases that we have treated. The initial dose is from 100 to 300 cc. depending upon the degree of toxicity. The subsequent doses, if necessary, are given at 2 to 5 day intervals, depending upon the progress of the case. Where there is marked dehydration, anorexia, or imperfect nutrition, we find it wise to use the sol containing sugar. Where the toxemic manifestations alone predominate, we merely use the adjusted colloid. The indications for its use are the infectious diseases, from the mild cases exhibiting a low-grade toxicity which show merely a generalized aching and soreness and tend to become chronic to the most virulent type of infections with temperatures ranging as high as 107 degrees. Among the cases treated successfully the streptococcic and staphylococcic infections, cellulitis, septic arthritis, puerperal sepsis. Also we have observed the toxic symptoms of tuberculosis improve after colloid injection (usually sub-cutaneous).

Clinical toxic symptoms of the case serve as an indication, rather than as a definite diagnosis. Also, since the affinity of the fat for the toxin is not bacteriologically specific, the type of infecting organism is not of very great importance, and the colloid may be indicated equally well in a case of typhoid, as in a case of pneumonia or meningitis. The best and most scientific indication for its use is to be had by a study of the serum

under the dark field; and if hydration and clumps are marked, it is indicated.

Following the use of the lanolin with dextrose intravenously, there is frequently met with a febrile reaction with a rise of temperature of 1 or 2 degrees and a concomitant increase of the pulse rate. This is followed usually by a decrease of the temperature to normal or thereabouts. There may be in 24 or 48 hours another increase of temperature, but it is wise to wait for at least 72 hours in a subacute case, longer in a chronic case, or 24 hours in an acute case. Before repeating the dose, the indications for repetition are found in the behavior of the temperature curve. The febrile reaction from a dose of 200 cc. intravenously is marked by chills, fever, and sweat which last from 15 to 20 minutes. This does not occur in all cases, however, and the febrile reaction is avoided by the hypodermoclysis method of administration. Contra-indications have as yet not been met with, although the use of the colloid is apparently contra-indicated in athermatous subjects and in those under heavy digitalis dosage.

We are convinced that the colloid has no effect directly on bacteria themselves, acting simply as a therapeutic "sponge" to absorb cellular and bacterial toxins and poisons and thereby give the patient a chance to capitalize on his natural resistance or the other treatment instituted.

We are aware that the invention may be embodied in other specific forms without departing from the spirit or essential attributes thereof, and we therefore desire the present embodiment to be considered in all respects as illustrative and nonrestrictive, reference being had to the appended claims rather than to the foregoing description to indicate the scope of our invention.

We claim:

1. A process for preparing lanolin sol which consists in agitating lanolin with ethyl alcohol, mixing said lanolin and said ethyl alcohol with approximately three times their volume of distilled water, expelling substantially all of said ethyl alcohol by heat, and filtering the hydro-alcoholic emulsion of said lanolin.

2. A detoxicant, therapeutic compound for parenteral use comprising lanolin sol of colloidal particles of substantially 1 micron in size and unstabilized by other substances.

3. A detoxicant, therapeutic compound for subcutaneous use comprising lanolin sol of colloidal particles of substantially 1 micron in size and unstabilized by other substances.

4. A detoxicant, therapeutic compound for intravenous administration comprising glucose and lanolin sol of colloidal particles of substantially 1 micron in size and unstabilized by other substances.

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