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On analogous effects exerted by antagonistic calcium and citrate ions in physical and biological systems.

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From the preceding paper it appears that antagonistic kations and anions exert their effect upon the equilibrium of aqueous oil systems by causing variations in the relative solubility of a concentration film of fatty acid salts in oil and water. The resemblance between the effects observed in purely physical systems of this type and in biological systems suggests the possibility that protoplasm may consist essentially of an aqueous lipoid system in which a film of lipoid material functions as the continuous phase. If this is the case, an excess of either positive or negative ions should exert a disturbing effect upon the physical equilibrium of the protoplasm corresponding with that observed above, and a critical point at which kations and anions exactly counterbalance one another should be found in biological systems corresponding with that observed in purely physical systems, the precipitation of lipoids and fatty acid salts, for example.

To test this question calcium was selected as a suitable kation, and citrate as a suitable anion on account of the facility with which the proportions in which they counterbalance one another in the process of blood coagulation may be estimated (see subsequent communication). A $m/5$ $CaCl_2$ and a chemically equivalent solution of sodium citrate were prepared and admixed in varying proportions ranging from 0 per cent. of Ca and 100 per cent. of citrate to 100 per cent. of Ca and 0 per cent. of citrate. On mixing these solutions with aqueous sodium oleate suspension, the following results were obtained.

Precipitation was complete from 100 per cent. to 40 per cent. of calcium, gradually diminishing from this point until virtually no precipitation was visible between 35 per cent. and 30 per cent. of calcium, once more rising to an almost complete precipitation between 25 per cent. and 5 per cent. and subsequently diminishing

until the solution containing no calcium and 100 per cent. citrate gave no precipitation. It will thus be seen that when approximately two equivalents of citrate were mixed with one of calcium no precipitation of the oleate solution occurred. The toxic effect of the same series of solutions when injected intravenously in mice showed a similar curve, the maximum dose of either the 100 per cent. calcium or the 100 per cent. citrate which could be administered to a mouse weighing 20 grammes being .25 c.c. As the point was approached at which no precipitation of oleate occurred, doses ranging from 2 c.c. to 4 c.c. could be injected intravenously, and at a critical point representing approximately 35 per cent. calcium it was not found possible to exert any appreciable effect upon the animals even by doubling the concentrations of the solutions employed. The effect exerted by the same mixtures of calcium and citrate upon the process of hemolysis of blood corpuscles by means of complement and amboceptor exhibited a similar curve. The solutions containing 100 per cent. calcium with no citrate and 100 per cent. citrate with no calcium entirely prevented hemolysis in the presence of an amount of amboceptor and complement four times that required to produce hemolysis in a normal system. With the decreasing proportion of calcium in the first case and citrate in the second, hemolysis took place more rapidly and at a critical point lying between 35 per cent. and 38 per cent. of calcium the interference with hemolysis was practically negligible. It will be seen, therefore, that the point at which oleate is not precipitated from its suspension in water corresponds almost exactly with that at which no disturbing effect is exerted on mice, or the process of hemolysis. At this critical point calcium and citrate are present in a ratio of approximately one chemical equivalent of calcium to two of citrate or three molecules of calcium chloride to four of trisodium citrate. This close coincidence lends support to the view that positive ions exert their protective effect in biological systems by counteracting the destructive effect of negative ions on a continuous or external lipoid phase of protoplasm in a manner similar to that outlined in a subsequent paper dealing with anesthetics.