5037

Changes Produced in Certain Constituents of the Blood by Dilutions of Sodium Citrate.

JOHN W. WILLIAMS. (Introduced by W. H. Harris.)

From the Department of Pathology, College of Medicine, Tulane University.

While working upon the relationship of sodium citrate to bile salt hemolysis, interest was aroused in the microscopic picture produced in blood by addition of this sodium salt. As far as has been ascertained the changes observed have not been heretofore described, nor has it been explained in what manner a 30% sodium citrate solution stops hemorrhage when injected intravenously or intramuscularly, whereas the less concentrated solutions (4-10%) act as anti-coagulants when added to blood for transfusion.

In our observations fresh defibrinated blood, serum from clotted blood, citrated blood and blood added to the dilutant from the finger tips were studied. In each case the findings corresponded. The solutions of sodium citrate used varied in dilutions from 0.2 to 50% by weight and in all cases distilled water was used as vehicle. Saline (0.2 to 30%) was run in comparison but findings with this salt were limited to crenation in the hyper and swelling with chromolysis in the hypotonic dilutions.

When blood serum is added to a 20-50% solution of sodium citrate a flocculent, milky precipitate is formed. This redissolves macroscopically on adding more serum. This precipitate also forms in 20-50% sodium citrate solution when serum which has been passed through a Berkefeld filter or has been kept at 55°C. for an hour is added. Positive tests for globulin are obtained from the precipitate, which microscopically appears as a coarse granular material with a tendency to aid in the clumping and enmeshing of red and white blood cells and which when observed under the microscope does not disappear *in toto* on the addition of more serum. Fragmenting leucocytes are seen in 20-50% dilutions of sodium citrate with thread-like filaments projecting from apparently injured portions of their surface membrane. These filaments appear to join the granular meshwork of the serum produced by these concentrations of the sodium citrate. In the 20-50% dilutions of the salt it is noted that the red cells are flat, irregular and crumpled (more marked in the higher concentrations) and appear in shape like paper plates, first knotted up and then let resume their original form as best they can. This flattening appears to aid in the clumping (30%

dilution especially) of the cells which clumping is irregular in outline. As the concentrations of sodium citrate decrease the cells tend to resume their normal size. In the 10% sodium citrate concentration there is considerable difference in size of the reds, less irregularity in their shape, and many of them appear to have a narrow outer ring of hemoglobin and a clear center. In the 5% solution of sodium citrate the red cells are smaller and the centers appear as clear spaces shaped like crosses, stars and round discs, while in the 1% dilution these clear centers become much smaller and disappear in the next larger dilutions. In the 1 and 5% sodium citrate dilutions white cells are sometimes seen 2 to 5 times their normal size with nuclei which have lost their polymorphous character (in the case of the polymorphonuclear leucocyte) and appear as grayish blue balls set in a clear outer ring (ring and nucleus occupy each about ½ the diameter of cell) containing many small red granules, some of which loosen themselves from the cell and float off in the solution. The outline of these white cells is slightly jagged and we have designated them asters because of their resemblance to the flower of that name. Another type of altered white cell occurs in like concentrations resembling in appearance a cross section of a pearl with a darker round nucleus set into a non-transparent, non-granular ring. These latter cells are regular in outline and many of them are larger than normal leucocytes. In the 0.5 and 0.2% dilutions swelling of the red cells, especially marked in the 0.2% dilution of sodium citrate, with resultant chromolysis and shadow (ghost) cell formation is seen.

In our studies we have noted 2 forms of crenated cells and feel they should be differentiated. The flat, misshapen paper plate type seen in the 20-50% sodium citrate dilutions and the smaller hand grenade shaped type with projecting knobs, present in all blood which has stood a short time, have nothing in common either as regards outer appearance or physico-chemical forces which cause their formation. The hand grenade shaped type we have designated as cockleburrs because of their resemblance to the seed of that name. In the latter are small conglomerate granules of hemoglobin often adjacent to or in the knobs, which granules on lowering the tonicity of the solution, detach themselves and leave empty teats projecting. The cockleburr form was present in all dilutions of sodium citrate examined, with the exception of the 0.2% dilution in which remnants of the cockleburr in the form of swollen, round, colorless cells with projecting teats were seen.

In an attempt to ascertain whether the granular material which

precipitated on the addition of serum to 20-50% sodium citrate would form when serum was not present, blood cells were washed with normal saline (20-50% sodium citrate dissolved in normal saline on the addition of serum produces this precipitate) and added to 20-50% dilutions of sodium citrate, but there was no precipitate or granular deposits and in addition a less marked clumping of the cells and a lesser flattening and irregularity of the red cells. When serum is allowed to stand a considerable time over a clot, less precipitate forms on addition to 20-50% sodium citrate and there is a slight variation in the amount of precipitate with the serum of different individuals. There was no typical rouleux formation as seen in normal blood in any of the dilutions (50, 40, 30, 20, 10, 5, 1, 0.5, and 0.2%) of sodium citrate to which red cells had been added.

In summary, it may be said that we believe there is a change produced in the serum, red and white cells and platelets by the 20-50% dilutions of sodium citrate which change is not entirely reversible on the addition of more serum and the resultant macroscopic clearing of the solution which takes place. The above changes would be more apparent in the muscle tissues after intramuscular injection than in the blood after intravenous injection because of remote possibility of great dilution in the former case. We believe the changes effected by 20-50% sodium citrate and described herein, namely, globulin precipitation and change in the cellular elements of the blood, fix the sodium citrate and stimulate the activity of coagulation on the part of the body. It is further thought that the calcium is not fixed (as in the case of lesser concentration of sodium citrate) in the 20-50% concentrations of sodium citrate; it is therefore available to aid in coagulation. Temporary decrease in coagulability of blood in intramuscular injections of 30% sodium citrate might be explained by the fact that there is an early small absorption of less concentrated sodium citrate into the blood stream and a resultant temporary fixation of the calcium in small part before reactions promoting increase in coagulability are effective.