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On the rôle played by antagonistic ions in the process of blood coagulation.

By G. H. A. CLOWES and F. WEST.

[*From the Biological Chemical Department of the State Institute for the Study of Malignant Diseases, Buffalo, N. Y.*]

Citrated plasma prepared by admixing three parts of blood with one of 2.38 per cent. sodium citrate and centrifuging, coagulates almost immediately when admixed with an amount of $CaCl_2$ chemically equivalent to 4/10 to ½ of the citrate present. If additional citrate is added to the plasma, it is necessary to proportionately increase the amount of calcium added to induce coagulation. With thrombin, however, coagulation of plasma takes an entirely different course, the velocity of coagulation is considerably slower, but is apparently entirely uninfluenced by an excess of citrate. It may be concluded, therefore, that calcium induces coagulation by liberating thrombin from the blood platelets or other cells present in suspension in the plasma. To throw further light on this point, equal volumes of the same citrated plasma were precipitated by means of a considerable excess of a mixture of acetone and ether (an agent which has proved of considerable value in the preparation of thrombin) an excess of calcium chloride being added in one case immediately before and in the second case immediately after precipitation. The mixtures containing acetone ether were evaporated in vacuo at room temperature, the residues taken up with water and added to further amounts of citrated plasma to which an excess of citrate had previously been added. In the first case in which the calcium chloride was added before precipitation, coagulation took place rapidly, indicating the presence of free thrombin. In the second case in which the calcium was added after precipitation, no coagulation took place on addition of plasma, from which it must be concluded that by the process of precipitation the cells containing the thrombin have been rendered resistant to the action of calcium. Since the acetone-ether mixture removes not only water but fats, and since the precipitated material re-suspended in

water with the addition of $CaCl_2$ no longer produces thrombin, it must be concluded that the removal of the fatty portion of the cell membranes has rendered them non-sensitive to calcium, a point of view which harmonizes with the experiments on aqueous oil systems reported in the previous papers. As a further proof that thrombin is derived from cells suspended in the plasma which remain intact so long as the proportion of citrate to calcium exceeds 2.5 : 1, we followed the procedure recently adopted by Cramer [1] in studying oxalate plasmas, and filtered the citrated plasma through a bougie before the addition of calcium. It was found that the filtered citrated plasma was easily coagulated by thrombin but could not be precipitated by an excess of calcium even after a period of 24 hours. The residue on the filter was washed with salt solution containing a small amount of citrate, was then washed back by means of water pressure, and the aqueous suspension thus obtained divided into two parts. One part was treated with a slight excess of calcium, and when added to filtered plasma containing a large excess of citrate immediately caused coagulation from which it must be concluded that thrombin had been liberated by the calcium from cells incapable of passing through the bougie. The second portion was divided into two parts both of which were precipitated by means of acetone-ether following the procedure outlined above, the calcium chlorid being added in one case before and in the other case immediately after the addition of the acetone ether. The same results were obtained as in the previous case. The portion treated with calcium before precipitation rapidly coagulated filtered citrate plasma containing an excess of citrate. The second portion precipitated after the addition of calcium, exerted absolutely no effect upon the citrated plasma. From these experiments we are justified in concluding that thrombin is contained in blood platelets and possibly leukocytes suspended in the plasma, that the addition of a sufficient proportion of calcium to disturb the colloidal equilibrium of the lipoids in the cell membranes brings about the liberation of the thrombin, which in its turn causes precipitation of the fibrinogen, this later process being presumably entirely independent of calcium since it takes place rapidly in the presence of a large excess of

[1] *Quarterly of Exper. Physiology*, Vol. VI, p. 1.

citrate. It should be particularly noted that the ratio between calcium and citrate is approximately the same as that observed in the previous experiments on coagulation of oleate, toxicity to mice and interference with complement hemolysis, a fact which lends further support to the theory that the liberation of thrombin is associated with a disturbance in the colloidal equilibrium of fatty substances present in the cell membrane.

These experiments prove, furthermore, that the membrane contains substances other than fats and lipoids and, in the absence of the latter, is apparently uninfluenced by the addition of an excess of calcium to the system. In the light of these experiments, it should be possible to greatly simplify the existing theories regarding blood coagulation and to reduce the whole question to one of the liberation of thrombin as a result of a disturbance in the colloidal equilibrium of the platelet membrane under the influence of electrolytes and the subsequent precipitation of fibrinogen by adsorption of thrombin.

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On analogous effects exerted by anesthetics in physical and biological systems.

By G. H. A. CLOWES.

[*From the Biological Chemical Department of the State Institute for the Study of Malignant Diseases, Buffalo, N. Y.*]

Lillie has recently demonstrated that anesthetics used in certain concentrations are capable of functioning in a manner similar to calcium salts, protecting *Arenicola* larvæ from the destructive effect of pure salt solutions. Since calcium, on the one hand, and anesthetics, on the other, are capable of rendering a concentration film of fatty acid salts or lipoids relatively more soluble in oil and less soluble in water, it appeared possible that the agents in question protect the cell protoplasm by counteracting the destructive effect of negative ions on similar surface films formed between an external lipoid phase of protoplasm, and adjacent aqueous phases. If this theory were correct it should be possible to counteract the effect of negative ions in purely physical