was a slight fall in the whole blood chloride, however no hypochloremia as in the water experiments. The plasma chloride was high. There was no tetany although the blood calcium fell to 3.8 mg. per 100 cc.

When the combined intravenous infusion and transperitoneal perfusion was similarly maintained for 12 hours no symptoms developed. At the end of that time the intravenous injection of sodium chloride was stopped, but the perfusion of distilled water with glucose continued. Muscular twitchings then developed, became more severe and finally convulsions occurred and death followed after 5 hours of perfusion alone. In another experiment the simultaneous intravenous administration of Ringer's solution *without the sodium chloride* did not prevent the development of symptoms nor the lethal effect, and this animal died 5½ hours after the perfusion began.

These experiments represent a study of certain effects of peritoneal dialysis. It is difficult properly to evaluate, with the data at hand, the complex physico-chemical changes which result in the blood and tissues of the dialyzed animals. However, the experiments demonstrate the importance of sodium chloride to life and a state of well being.

4568

## Phagocytosis of Red Blood-Cells Treated by Tannin.

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Northrop and Freund[1] found that red blood-cells of sheep suspended in dextrose solution are agglutinated by electrolysis whenever the potential is depressed to 6 millivolts or less, except in the case of $MgCl_2$ and $CaCl_2$. When, however, cells are sensitized by specific antibody, ricin,[2] collodial stannic hydroxide,[3] an emulsion of paraffin oil,[1] they are agglutinated below about 12 millivolts, that is, at a potential higher than the critical potential for agglutination of unsensitized cells. This observation constitutes evidence that from the physical-chemical point of view specific antibody mediates agglutination by the same mechanism as ricin, collodial stannic hydroxide solution, and paraffin oil.

[1] Northrop, J. H., and Freund, J., *J. Gen. Physiol.*, 1924, vi, 603.

[2] Stillmark, H., *Arb. pharmacol. Inst. zu Dorpat*, 1889, iii, 59.

[3] Landsteiner, K., and Jagic, N., *Wien. Klin. Woch.*, 1904, xvii, 63.

Recently Reiner and Fischer[4] have been able to show that another substance, tannin (carefully neutralized), causes agglutination of red blood-cells of sheep, ox, and guinea pig and that red blood-cells treated with tannin become hemolyzed in the presence of guinea pig complement.

Repeating and extending Reiner's and Fischer's work I have found that erythrocytes of sheep exposed to 0.4 and 0.1 pro mille tannin solution, after they have been washed by centrifugalisation, are hemolyzed by the addition of guinea pig complement. If the mechanism of the action of tannin upon erythrocytes is similar to the action of immune serum, in which the electrical charge of the cells plays a rôle, agglutination and hemolysis should not occur when the suspending medium is free from electrolytes. When the experiment was repeated using isotonic dextrose instead of saline solution, neither agglutination nor lysis occurred. When tannin was added to red blood cells suspended in salt solution, the cells became agglutinated, but when the tubes were centrifugalized and the supernatant fluid replaced with dextrose solution, the cells could be suspended evenly and the addition of complement failed to cause lysis.

It was further observed that when from 0.4 to 0.1 pro mille tannin is added to red blood-cells suspended in either salt or dextrose solution a large number of the cells lose their original disc shape and become more or less angular. A similar change in shape takes place to a less extent when specific serum is added to red blood-cells. The final concentration of immune serum that caused such change ranged from 1:5 to 1:80.

Since immune serums prepare red cells for phagocytosis as well as for agglutination and lysis by complement, the question arises whether tannin prepares them for phagocytosis also. To test this possibility the following experiment was made. To 1 cc. of carefully neutralized tannin salt solution was added 0.2 cc. of a 1% suspension of washed erythrocytes of sheep. The mixture was kept at room temperature for 15 minutes and washed twice by centrifugalization. The red blood-cells were resuspended in 0.2 cc. of salt solution and mixed with 3 drops of a dense suspension of washed leucocytes of rabbit.

Both in the hanging drops and in the fixed preparations made from tubes containing red blood-cells treated with tannin and suspended in salt solution one could observe the following. A great number of red blood-cells were in clumps, some of which had surrounded leucocytes. Red cells were attached to many (often 60%) of the

[4] Reiner, L., and Fischer, O., *Z. f. Immunitatsf.*, 1929, lxi, 317.

white cells, but only very exceptionally were they actually found within them. In preparations made from tubes containing red blood-cells treated with tannin and suspended in dextrose solution, or red blood-cells not treated with tannin and suspended in saline solution, neither agglutination nor attachment of red blood-cells to more than a very few leucocytes was found. Furthermore, in hanging drops from these tubes we could observe that moving red blood-cells would come into contact with leucocytes, slide on their periphery and then move away.

On completing this work I was of the opinion that the attachment of the red blood-cells to the leucocytes marked the beginning of phagocytosis, and that phagocytosis could be studied in the action of tannin upon erythrocytes. After the above paragraphs were written, a paper by Reiner and Kopp[5] appeared describing similar experiments (with guinea pig leucocytes) and arriving at similar conclusions, which they carried a step further. By using a longer period of incubation they observed complete phagocytosis of practically all the red blood-cells. This observation I have since confirmed.

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## Correlation of Ultra-Violet Absorption to the Development of Immunity.

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It has been observed that the absorption spectrum for blood serum in the ultra-violet has a characteristic absorption band at 2800 Ä. Judd Lewis[1] was the first to observe variations from the normal in the absorption curve of various pathological sera. In a second publication Lewis[2] shows that the protein content of serum is the absorbing constituent and gives curves for the absorption of the 3 protein fractions, pscudoglobulin, euglobulin, and albumin. Stenström and Reinhard[3] showed that a synthesis of the amino acids in the proportion in which they occur in blood serum gave a similar

[5] Reiner, L., and Kopp, H., *Z. f. Immunitatsf.*, 1929, lxi, 397.

[1] Lewis, S. Judd, *Proc. Royal Soc. London*, 1915, lxxxix, 327.

[2] Lewis, S. Judd, *Proc. Royal Soc. London*, 1922, xciii, 178.

[3] Stenström, W., and Reinhard, M. C., *J. Biol. Chem.*, 1925, lxvi, 819.