60 (2583)

A method for counting blood platelets in the rat.

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Methods for counting platelets are either direct or indirect. As noted in the preceding paper we have used both. In our hands an indirect method, based on that described by Cramer, Drew and Mottram[1] using the diluting fluid recommended by Rees and Ecker[2] has yielded the most reliable results. The fluid is 3.8 per cent sodium citrate, to which 0.2 percent formaldehyde and 0.1 percent brilliant cresyl blue are added. It is filtered clear every three days. The same pipettes and counting chambers have been used throughout.

The tip of the tail is shaved, then scrubbed clean and coated with vaseline. With vaselined scissors about 1 cm. of the tail is clipped off, the skin on the stump pushed forward and the bared tip quickly immersed in the solution and a single drop of blood allowed to flow into the fluid. Immediately thereafter, with the red pipette, filled to the 0.5 mark, blood is sucked up from the tail stump to the 1.0 mark and then again with diluting fluid to the 1.01 mark. This gives the sample for the direct red and platelet count.

The solution containing the first drop of blood is mixed thoroughly for a few moments, a drop withdrawn, placed upon a thoroughly cleaned slide, covered with a clean cover glass and sealed with paraffin. After standing, in order that the reds and platelets may settle out, a proportional count is made. Usually three hundred red cells are counted.

By the use of this solution the platelets take on a light blue stain and their structure is more distinctly made out. This, coupled with their examination by means of an oil immersion objective, minimizes the possibility of mistaking other refractile bodies for platelets. We find that in the direct method there is a considerable clumping of platelets as the table in the preceding

[1] Cramer, Drew and Mottram, *Proc. Roy. Soc.*, London, 1922, B xciii, 449.

[2] Rees and Ecker, *J. Am. Med. Assn.*, 1923, lxxx, 621.

paper shows by comparison with the counts made by the indirect method.

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61 (2584)

Observations on the theory of tetany.

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It has been found that in the sea urchin egg within certain time limits isotonic solutions of sodium phosphate at various $[H^+]$, inhibit cell division only by the limiting effect of the hydrogen ion concentration of the external solution. By direct analysis of the eggs it has been shown that within limits the $PO_4 =$ anion does not penetrate.

Eggs treated with isotonic phosphate over a wide hydrogen ion range, subsequently placed in isotonic electrolytes, and finally placed in sea water to determine their viability, show that (1) KCl has practically no injurious effect, (2) NaCl is most toxic with the peak of minimum toxicity at 7.4-7.8, (3) $CaCl_2$ is toxic with the minimum peak at 7.8, (4) $MgCl_2$ has relatively little toxicity but markedly shifts the peak towards the alkaline side.

$CaCl_2$ and $MgCl_2$ are remarkable in that eggs removed from the ovaries and placed directly into isotonic solutions of these salts even on the most vigorous washing and centrifuging do not cytolyse while the same eggs if allowed to stand in sea water for ½ hour or over, agglutinate and cytolyse immediately. Dr. Heilbrunn[1] has independently confirmed this point. This very extraordinary change is of interest from the point of view of the theory of agglutination and ageing.

Our experiments are directed towards the investigation of the mechanism of tetany. With such simple protoplasmic systems as the sea urchin egg many more environmental factors are under our control than in cells in organoid form. Inasmuch as phos-

[1] Heilbrunn, L. V., Personal Communication.