Biochemistry. — *On saponin-hemolysis.* By E. GORTER, F. GRENDEL and W. A. SEEDER. (Communicated by Prof. P. EHRENFEST.)

(Communicated at the meeting of March 28, 1931.)

We have studied saponin-hemolysis by determining the surface occupied by the saponin in a monomolecular layer at a water-air interphase, and the minimum amount necessary to produce hemolysis of a certain number of red blood cells, of which the surface has been measured.

In a previous publication [1]) we have been able to show that the surface of red blood cells is formed by a double layer of lipoid molecules. It is highly probable, that the molecules (lecithin and cholesterol) are orientated in such a way, that one molecule is fixed with its polar groups to the interior of the red blood cell (formed by a concentrated hemoglobin solution) and the other stretches its polar groups to the exterior (formed by the plasma).

The surface of the saponin.

Through the courtesy of the late Dr. A. W. VAN DER HAAR we were able to study *parillin,* a saponin having a molecular weight of 872, containing three molecules of hexose in its molecule.

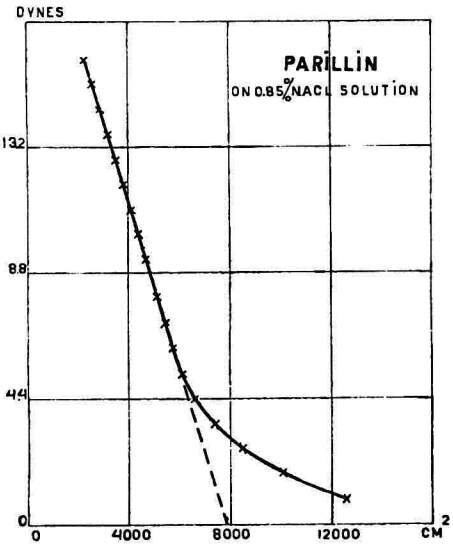

Fig. 1.

When it is spread in a monomolecular layer on the water of a Langmuir-Adam apparatus, the surface per milligram is *0.80 square meter.* This number is very little influenced by temperature, acidity of the water in the tray, etc.

It is very remarkable that one can spread from a alcoholic solution as well as from a water-colloidal solution, and that the spreading in both cases is the same.

The surface of the red blood cells.

The red blood cells from 1 cc. of blood were careful washed with 0.185 %

[1]) GORTER and GRENDEL, J. of exper. med. **41**, 1925, p. 439; F. GRENDEL, Biochem. Zeitschr. **214**, 1929, p. 231.

NaCl-solution and their number determined in the usual way. The diameter (D) was measured with the aid of a Zeiss drawing apparatus, and from this number the surface was evaluated using the formule $2D^2$ [1]).

The amount of saponin necessary to produce complete hemolysis.

We have determined the minimum amount of saponin necessary to produce complete hemolysis in the following way.

In a series of ten testtubes we added to a constant number of red blood cells an increasing amount of saponin in watery suspension and have examined 5—10 series of the same range of saponin, each series containing an increasing amount of red blood cells.

It was easy to show in the case of parillin that the surface of saponin is equal to the surface of the red blood cells in that tube, where all the red blood cells are hemolysed, and no excess of saponin is present.

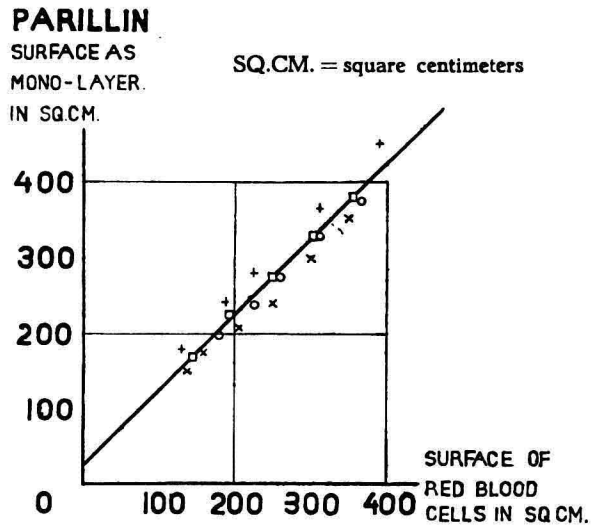

Fig. 2.

The points in this figure indicate several experiments that show conclusively the relation 1 : 1.

For other saponins: we tried digitonin (MERCK) and Achras-saponin (VAN DER HAAR); it was difficult to obtain reliable results.

First of all the surface occupied in a monomolecular layer on water was difficult to determine with accuracy, because of the form of the compression—surface-area curve.

Moreover the amount necessary to produce a complete hemolysis was sometimes exactly that, demanded by the above theory, but in modifying the concentration of blood and saponin, irregularities were observed.

We believe that the discrepancies are due in both experiments to a too

[1]) KNOLL, Arch. f. d. ges. Physiol. **198**. 1923, p. 367.

small tendency towards spreading of these saponins, that are somewhat too soluble in water, or to impurities of the products examined.

If one adds too much blood, there remains a certain number of red blood cells un-hemolysed.

Summary. Complete hemolysis occurs at the moment that there is sufficient saponin available to cover the surface of the cells with a layer. one molecule thick.
