Biochemistry. — *"On bimolecular layers of lipoids on the chromocytes of the blood."* By E. GORTER, M. D., and F. GRENDEL. (From the Laboratory of Pediatrics of the University of Leyden, Leyden, Holland.) (Communicated by Prof. P. EHRENFEST.)

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In a previous communication [1]) we have demonstrated that the quantity of lipoids contained in chromocytes of different animals is exactly sufficient to cover the surface of these chromocytes in a layer that is two molecules thick. In this paper we intend to discuss some technical details.

Before entering into this discussion we give some more results obtained with the technique described in our previous paper, making use of acetone as extractionfluid in large quantities.

TABLE I.

Acetone extraction, large quantities.

	Animal	Amount of blood used for the analysis.	No. of chromocytes per c.mm.	Surface of one chromocyte	Total surface of the chromocytes (*a*)	Surface occupied by all the lipoids of the chromocytes (*b*)	Factor $b:a$
		gm.		sq. μ	sq. m.	sq. m.	
20	Guinea-Pig B	10	5.630.000	100.8	5.7	11.6	2
21	„ C	1	5.800.000	92.5	0.53	1.02	2
22	Rabbit C	0.5	6.100.000	90	0.28	0.6	2.1
23	„ C	0.5	6.100.000	90	0.28	0.58	2.1
24	„ D	1	5.600.000	92.4	0.51	0.92	1.8
25	Goat 2	0.5	14.000.000	23	0.16	0.35	2.2
26	„ 2	5	14.000.000	23	1.6	3	1.9
27	„ 2	5	14.000.000	23	1.6	3.2	2.0
28	Man H G	2.5	5.200.000	123	1.6	3.0	1.9
29	„ J R	1	2.060.000	90	0.18	0.35	1.9
30	Dog B	5	9.700.000	101	4.9	9.8	2
31	„ C	1	4.340.000	124.8	0.54	1.08	2
32	„ C	1	4.340.000	124.8	0.54	1.08	2

[1]) *Journal of exper. medicine*, April 1, 1925, Vol. XLI, No. 4, 439—443.

We now want to emphasize the importance of using large quantities of acetone. When less than 3 × 100 cc. acetone per 1 cc. of blood are used, the extraction may be incomplete, so much so that in this case a second extraction may give an important part of the unextracted lipoids.

TABLE II.

Influence of quantity of acetone used.

	Animal	Amount of blood used for the analysis	Quantity of acetone used for the analysis	Total surface of the chromocytes	Surface occupied by the lipoids as determined	Bimolecular layer of lipoid. theoretical
		c.c.	c.c.	sq.m.	sq.m.	sq.m.
33	Man H.G.	1	2 × 30	0.64	0.67	1.28
34	„ „	1	3 × 30	0.64	0.84	1.28
35	„ „	2.5	3 × 125	1.6	2.7	3.2
36	„ „	2.5	3 × 150	1.6	3	3.2
37	Sheep 2	10	3 × 150	4.2	6.8	8.4
38	„ 2	10	3 × 150	4.2	15.1	8.4
				Second extraction	3 } 8.1	
39	Dog B	10	3 × 150	9.8	15	19.6
				Second extraction with alcohol	1.2 } 16.2	
40	„ „	10	3 × 150	9.8	14.4	19.6
				Second extraction with acetone	4 } 18.4	

Although in many of our experiments with acetone subsequent extraction with different solvents of lipoids had never yielded more than traces of spreading substances we have controlled our results by a different method.

We therefore made use of BLOOR's method of extracting lipoids.

In a glassbeaker of 100 cc. we filled 30—40 cc. of the alcohol-ether mixture (3 : 1) and under continuous shaking we added the washed chromocytes from 1 cc. of blood.

The mixture was then heated till boiling tempertature, cooled under the tap and made up to 50 cc. with alcohol-ether mixture and then filtered. The filtrate was evaporated to dryness on a waterbath and bumping of the fluid prevented by adding some platinum tetraeders. The residue was finally taken up in benzene and filtered into a measuring-flask of 10 cc. 0.1 cc. was pipetted on to the watersurface of the Langmuir-Adam apparatus.

The results obtained by this method were concordant with those already communicated, as is shown in table III.

TABLE III.

Results obtained by BLOOR's method.

	Animal	Amount of blood used for the analysis	No. of chromocytes per c.mm.	Surface of one chromocyte	Total surface of the chromocytes (*a*)	Surface occupied by all the lipoids of the chromocytes (*b*)	Factor *b* : *a*
		c.c.		sq. μ	sq. m.	sq. m.	
41	Man	1	5.200.000	123	0.64	1.29	2
42	„	1	5.200.000	123	0.64	1.26	2
43	„	2.5	5.200.000	123	1.6	3.1	2
44	Rabbit E	1	5.950.000	92.5	0.55	1.1	2
45	Goat 3	1	27.220.000	21.1	0.57	1.2	2.1
46	„ 3	1	27.220.000	21.1	0.57	1.15	2
47	„ 3	1	27.220.000	21.1	0.57	1.17	2

When, as we have tried to do, hoping to extract more lipoid matter, the extraction is made three times and the blood is heated for some time after the boiling temperature has been attained, the results are bad. It is highly

TABLE IV.

Results in using large quantities and prolonged heating.

	Animal	Amount of blood used for the analysis	No. of chromocytes per c.mm.	Surface of one chromocyte	Total surface of the chromocytes (*a*)	Surface occupied by all the lipoids of the chromocytes (*b*)	Factor *b* : *a*
		c.c.		sq. μ	sq. m.	sq. m.	
48	Sheep	10	11.500.000	36.5	4.2	9.9	2.3
49	„	10	11.500.000	36.5	4.2	10.4	2.5
50	Dog B	10	9.700.000	101	9.8	22.4	2.3
51	„ B	10	9.700.000	101	9.8	23.7	2.4
52	Rabbit C	0.5	6.100.000	90	0.28	0.7	2.5
53	„ C	0.5	6.100.000	90	0.28	0.65	2.3
54	„ F	5	5.550.000	87	2.4	5.7	2.4

probable that these results are due to other substances than lipoids because the extract is deep-brown.

Now blood-pigment and its derivatives spread on a watersurface. (Hemin in ether e.g. $\pm 70 \times 10^{-16}$ sq. cm.)

In our last experiments we modified the technique of determining the size of the bloodcells. Instead of drawing the cells on millimeterpaper we made use of PYPER's method [1]). In controlling determinations the numbers obtained by this method agreed fairly well with those from direct measurements.

It has struck us that direct measurement of the alcohol-ether mixture gave very variable results, mostly far too small. We were able to show that also by solving pure fatty acids in alcohol the results were often very bad, and in several instances one obtains half the right value. These results were partly due to the technical difficulty of bringing the alcohol exactly on the surface of the water in the tray, partly to the character of the substance, in so far as oleic acid and triolein were more apt to give good values than solid substances like palmitic acid or tripalmitin. Possibly the explanation of the half values lay in the association of the molecules of these fatty acids in alcohol [2]).

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[1]) BERGANSIUS, *Arch. ges. Physiol.*, 1921. CXCII, 118.

[2]) W. ROSS INNES, Journ. Chem. Soc. **113**, 1918, 410 found for hexadecylalcohol in alcohol 1.5 times the normal molecular weight.