lication it has been found that extraction of treponeme smears with acetone for 15 minutes prior to use of serum reduces background fluorescence so is a preferred method.

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Paroxysmal Nocturnal Hemoglobinuria. Evidence of Defect of Red Cell Stroma Manifested by Abnormalities of Lipids. (23513)

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Paroxysmal nocturnal hemoglobinuria (PNH) may be a manifestation or symptom of a chronic hemolytic anemia characterized by an abnormality of the red blood cell which renders it susceptible to hemolysis by the action of the "properdin system." The components of this system are normal constituents of the plasma namely properdin, Mg^{++} and cofactors resembling complement(1). The system apparently is not active at all against normal human red cells. A great amount of work has been done to study and define the plasma factors of the PNH hemolytic system, but the erythrocyte which carries the pathognomonic defect has received little attention except as an indicator of the reactions of the plasma factors. It has been accepted that the PNH erythrocyte is faultily constituted, but the nature of fault is unknown(2). The hemoglobin has a normal solubility and electrophoretic mobility. It has been suggested that the defect resides in the red cell stroma or in the metabolic machinery that maintains the stroma throughout the life span of the cell. The present study was undertaken to learn whether or not the stromal lipids of the PNH erythrocyte are different from those of normal red cells.

Materials and methods. The blood for these determinations was obtained from 2 patients with classical history and signs of PNH. Patient A has been known to have the disease since 1939. During the past 10 years he has gradually improved until at present he shows no evidence of hemolytic disease or anemia. His red cells, though giving the characteristic reactions *in vitro,* survive normally when transfused into the patient or a normal recipient. Patient B has a serious hemolytic disease, with anemia and hemoglobinuria, and 15 per cent of his red cells are reticulocytes. Control studies were done on the red cells of 14 normal adults. The lipid analyses were performed on the washed red cells. The details of the procedure are the subject of a separate report. In brief, the lipids were extracted by boiling ethanol-ether and the analysis was carried out under nitrogen. Total fatty acids were determined by weighing. Saturated fatty acids were precipitated as the lead salts and weighed (3,4). Polyethenoid fatty acids were measured by the alkali-isomerization technic of Herb and Riemenschneider(5,6). Oleic acid was computed to be the difference between the total fatty acids and the sum of the saturated and polyethenoid acids. Iodine number was determined by the Yasuda procedure(7). Cholesterol was determined by a standard method(8,9). Recovery experiments and parallel and segmental analyses on red blood cells from the same patient permit a high degree of confidence in the methods and results. The mean corpuscular area was determined by the photomicrography of fresh red cells in rouleaux by an original method which gives a high degree of accuracy. Mean corpuscular volume

TABLE I. Red Cell Lipids from 2 Patients with PNH Related to Cell Surface Area and Compared with Values from 14 Normal Patients.

	Normal ranges	Normal avg	PNH Case A	PNH Case B
Cell vol (μ^3)	74–92	82	105	80
Surface area (μ^2)	128–144	135	160	150
Fatty acids				
Iodine No.	99–126	112	175	121
Total fatty acids				
mg/ml cells	1.2–2.2	1.7	1.2	1.7
mg/$10^{-14}/\mu^2$	74–135	105	79	91
Unsaturated F.A.				
Linoleic				
(% total)	4.4–7.3	5.7	8.6	5.2
(mg $10^{-14}/\mu^2$)	4–9	6	7	5
Linolenic				
(% total)	0	0	0	0
(mg $10^{-14}/\mu^2$)	0	0	0	0
Arachidonic				
(% total)	14.8–19.4	17.1	22.9	22.2
(mg $10^{-14}/\mu^2$)	11–24	18	18	20
Pentaenoic				
(% total)	2.7–4.4	3.6	5.8	4.6
(mg $10^{-14}/\mu^2$)	2–5	4	4	4
Hexaenoic				
(% total)	2.1–6.1	3.8	5.2	5.4
(mg $10^{-14}/\mu^2$)	2–8	4	4	5
Total polyethenoid				
(% total)	24.0–37.2	30.2	42.5	37.4
(mg $10^{-14}/\mu^2$)	19–46	32	33	34
Oleic (% total)	41.8–57.5	48.2	39.9	29.8
(mg $10^{-14}/\mu^2$)	34–60	50	32	27
Saturated F.A.				
(% total)	13.5–28.1	22.5	17.6	32.8
(mg $10^{-14}/\mu^2$)	14–31	23	14	30
Free cholesterol				
(mg %)	117–158	132	143	152
(mg $10^{-14}/\mu^2$)	79–99	80	92	81

was computed from these same measurements. The mean corpuscular lipid was related to the surface area because most if not all of the lipids are found at the surface of the red cell (10).

Results. (Table I). Abnormalities were found in the red cells of both patients: an increase in the concentration of arachidonic, pentaenoic and total polyethenoid fatty acids and a decrease in the concentration of oleic and total fatty acids. There were differences between cases A and B. In A the linoleic acid and the iodine number were high, in B they were normal. In A the saturated fatty acids were normal, in B they were high. In both cases the unesterified cholesterol was normal as was the concentration of the hexaenoic acids.

Both patients had macrocytosis and the surface area of the red cells was well beyond the normal range. When the abnormalities of the fatty acids are related to the surface area a different picture is obtained. The amount of fatty acid per μ^2 of surface is abnormally low because of the great surface area. Those highly unsaturated fatty acids which were found to be increased (arachidonic and pentaenoic) have normal values when computed in terms of mg per μ^2 of surface. The same is true for the saturated fatty acids in case B, but they are low in case A. Oleic acid which in both cases was low in terms of total amount is lower still when expressed as mg per μ^2 of surface.

Discussion. The fatty acids of normal red cells are concentrated at the surface in an amount sufficient to permit a bimolecular layer(10). The red cell fatty acids are structural in function and are probably present as components of the stromal lipoprotein. In PNH red cells the concentration of the total fatty acids is somewhat low but it lies within the normal range. However, the composition of the total is abnormal. The ratio of arachidonic and pentaenoic acids is high, and the total as well as the ratio of oleic acid is low. This means that the lipoproteins of the surface of the PNH red cell have different components from those of normal red cells. Whether or not this difference is related to the "PNH lesion" is not known.

The red cells of the 2 PNH patients differed from one another in at least 2 ways. The cells of patient B were more susceptible to the PNH hemolytic system, both *in vivo* and *in vitro*. Therefore the PNH lesion in case B may be said to have been more severe. The second difference is a consequence of the first. The red cells of patient B were much younger than those of patient A. Patient B had a severe hemolytic disease and his cells were rapidly turned over. They are estimated to have had an average age of less than 10 days. Patient A had cells that survived normally and their average age is estimated to have been 60 days. Lipid analyses of red cells in

other hemolytic anemias and in anemia due to hemorrhage have shown that young red cells are characteristically different from mature cells. The concentration of total fatty acids related to the surface of normal cells is the same in both, but the younger cells have a relatively high amount of saturated fatty acids. In comparing the 2 cases of PNH the outstanding difference is in the amount of saturated fatty acid. It is at the top of the normal range in case B, and at the bottom in case A. The difference is one that might be due to the ages of the cells rather than the severity of the PNH lesions.

Summary. The extracted lipids from the red cells of 2 patients were analyzed for cholesterol, unsaturated and saturated fatty acids. The ratios of the various lipids were established and the values were related to the total lipids and also to the surface area of the red cells. Compared with normal red cells the PNH cells showed an increased concentration of arachidonic and pentaenoic acids and a decreased concentration of oleic acid. These results suggest that the lipid pattern of the lipoproteins in the stroma of PNH cells is abnormal.

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Hypothermic Effect of Chlorpromazine, Histamine and Serotonin, and Acclimatization to Cold. (23514)

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Recent studies have shown serotonin and histamine to be some of the products of secretion of mast cells(1). We have reported definite changes in the appearance and abundance of these cells in animals exposed to low temperatures(2). Since chlorpromazine is believed to be a "serotonin liberator"(3), its effect was tested in animals which had been exposed to cold for varied periods of time. Similarly the effect of cold acclimatization on the body temperature response to serotonin and histamine was investigated.

Methods. Each group of animals was composed of 8 male rats weighing approximately 150 g. All injections were intraperitoneal in a 1% solution. In the first experiment 4 groups, after being exposed to cold (6°C) for 0, 1, 7 and 28 days respectively, were maintained at 21°C for 1 day and were then given an injection of 10 mg/kg chlorpromazine. In a second experiment 2 groups exposed to cold (6°C) for 0 and 28 days were injected simultaneously with 100 mg/kg of histamine phosphate and 2 mg/kg of serotonin. Immediately following these 2 injections, 4 doses of 2 mg/kg of serotonin were injected at 5 minute intervals. In a third experiment with 2 groups exposed to cold (6°C) for 0 and 24 days, 5 doses of 32.4 mg/rat of histamine phosphate, were injected at ½ hour intervals. The body temperature was measured with a thermocouple inserted at a depth of 40 mm.

Results. Fig. 1 shows that the intensity and duration of the hypothermic effect of chlorpromazine is greatly decreased by pre-exposing animals to cold for 7 and 28 days. No attempt was made to quantitate the chlorpromazine effect in terms of serotonin and histamine response. However, as shown in Fig. 1, the effect of acclimatization on the body tem-