Influence of Diet on Stability of Rat Erythrocytes. (19358)

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The structural stability of the mammalian erythrocyte is in part dependent upon the state of order among the components which constitute its surface(1). This stability resides in the quantitative and spatial relationships of lipids and proteins forming the cell membrane, which is built up as a tricomplex system due to the ionic polarity of the specific lipids and proteins. It has been shown by hemolytic experiments that calcium may enter the membrane structure and considerably enhance its stability through more powerful ionogenic forces(2). Variations in other components of the complex may theoretically alter the stability, although such changes have not been accomplished experimentally.

Alterations in the lipids and proteins of erythrocytes in anemias were observed by Erickson and her colleagues(3). It is perhaps significant that in hemolytic and hypochromic anemias the quantity of total lipid and especially the proportions of the phospholipids, acid soluble lipid and cholesterol were decreased. The deviations in these two diseases suggest that it may be possible to initiate similar changes experimentally and alter the stability of the cell. In the present studies dietetic conditions which affect the osmotic stability of the rat erythrocyte have been examined. The effect of alloxan, in view of its *in vivo* hemolytic action, was also investigated.

Materials and methods. Male Sprague-Dawley albino rats, weighing 200 g, were placed without restriction for a period of 3 months on the diets indicated in Table I. One hundred rats were maintained on the pellets, and 2 groups of 48 rats on each of the synthetic diets. Samples of blood, collected from the tails into dried tubes containing 0.2 mg of sodium oxalate, were suspended as 0.01 ml aliquots (by Breeder-Brew pipette) in 2.0 ml volumes of various concentrations either of saline or of a solution which contained 9 mg % of alloxan monohydrate (Eastman Kodak No. 1722) dissolved in either 0.5% or 1.0% sodium chloride. The extent of hemolysis was estimated spectrophotometrically with a Coleman Spectrophotometer No. 14 by the technic of Hunter(4), and the percentage hemolysis was so calculated as to determine the cumulative curve or ogive by Bolton's procedure(5). Two or more analyses were made on each rat so that each point of the curve represented the averages of between 96 and 200 separate analyses.

TABLE I. Composition of Diets.

Synthetic	
Sucrose	710 g
Casein (vit. test)	210
Salt mixture 4(6)	40
Cystine	2
Choline	1
Ca. pentothenate	20 mg
Niacin	10
Riboflavin	3
Pyridoxin	2.5
Inositol	100
Biotin	.1
Folic acid	.2
Thiamin	2
Halibut liver oil	2 drops/wk
Fat*	—
Pellet (master mix rabbit pellets)†	
	%
Ground wheat	.5
Irradiated yeast	.9
Ground limestone	.9
Defluorinated phosphate	.3
Salt content:	
Manganese sulfate	.003
Potassium iodide	.0028
Iron oxide	.00032
Copper sulfate	.00026
Cobalt sulfate	.00026
Alfalfa meal	Amt unspecified
Ground barley	" "
Beet pulp	" "
Linseed oil meal	" "
Ground corn meal	" "
Molasses	" "
Soybean oil meal	" "
Pulverized oats	" "

* To constitute the final high fat diet either lard or "spry" was melted and mixed with above mixture, so that each 100 g of final diet contained 20 g of fat.

† This pellet diet, as obtained from McMillen Feed Mills, Fort Wayne, Ind., is specified to contain 2% crude fat and 18% protein.

Results. The behavior of the erythrocytes

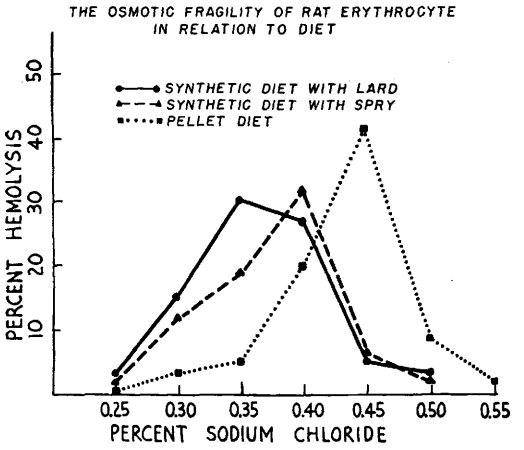

FIG. 1. The red cells were suspended in sodium chloride solutions of varying tonicity. Extent of hemolysis is estimated by determination of hemoglobin released after constant time of 30 min.

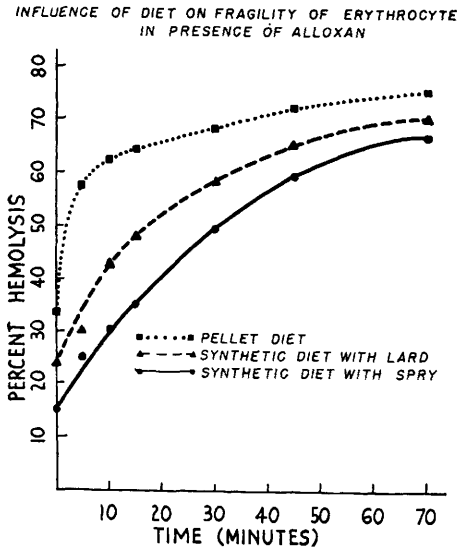

FIG. 2. Red cells are suspended in a solution of .5% NaCl containing 9 mg % alloxan. Extent of hemolysis is estimated by determination of hemoglobin released at staggered intervals up to 70 min.

from the 3 dietetic groups is depicted in Fig. 1, which demonstrates the considerable instability of the cells in animals on the pellet diet as compared with cells of rats on synthetic rations. The cells from animals on the two synthetic diets differ only slightly, with a slightly greater stability in those consuming lard. Microscopic examination of the cells substantiates the results observed in the fragility study. The cells from "pellet" fed rats pass rapidly through the stages of crenated disc, crenated sphere and prolytic sphere, whereas these changes are relatively slow in rats sustained on the synthetic diets. The red cell counts and the hemoglobin concentrations were not significantly altered in any of the groups.

In all groups the extent of hemolysis in 0.5% sodium chloride alone is insignificant. When alloxan is present in the suspending fluid (9.0 mg %) the cells of all groups are rapidly lysed (Fig. 2). The rate of hemolysis is most rapid in the cells from "pellet" fed animals and less in those of the other "synthetic" groups, which differ only slightly. When 1% sodium chloride is used no hemolysis occurs in the presence of alloxan, indicating a connection between pre-hemolytic swelling and the hemolytic action of alloxan on the cells.

Discussion. The stability of the erythrocyte of the albino rat is affected by alterations in diet. This is manifested by a change in osmotic fragility and an instability in the presence of alloxan. The relationship between the preconditioning of the cells by a non-hemolytic hypotonic level of saline prior to the action of alloxan is unique. It may be related to the conversion of alloxan into dialuric acid necessary for hemolysis by the compound(7); the prehemolytic swelling of the erythrocyte in the 0.5% sodium chloride allows the alloxan to penetrate the cell where, by interaction with glutathione(8), it is converted into the hemolytically active dialuric acid. This is further supported by the fact that the most easily lysed cells are those (pellet) which swell most readily in the hypo-osmotic solution.

Summary. The stability of the erythrocytes of albino rats is affected by alterations in diet. This change is reflected in both the osmotic fragility and is related to the rate of hemolysis in the presence of alloxan.

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Electron Microscope Studies on the Ciliary Apparatus of the Gill Cells of *Mya arenaria.** (19359)

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The nature of the ciliary apparatus has long been a subject of discussion. For reviews and literature pertaining to this subject consult Engelmann(1), Saguchi(2), Grave and Schmitt(3) and Worley(4). Morphology of the ciliary apparatus becomes especially significant in the formulation of theories concerning the physiology of ciliary movement (5). Clear pictures have been drawn of certain fixed ciliated cells showing the cilia, basal bodies and intracellular fibrils which often converge, forming a cone-like structure with its apex at one side of the nucleus(1). Because of the fact that the intracellular fibrils cannot be easily seen in the living cell with the light microscope, they have been thought of as artifacts by some (see discussion in 3).

The purpose of this report is to present electron micrographs of cells from the laterofrontal and frontal regions of the gill filaments of *Mya arenaria,* showing cilia, membranes, basal bodies, and intracellular fibrils.

Methods. Material used in this study was fixed in Regaud's solution and sectioned at 0.2 μ according to the method of Beams, *et al.* (6). The electron microscope used was an R. C. A. model EMU-2B, equipped with an unbiased gun. Magnification of the electron micrographs is indicated by the 1 μ scale drawn on Fig. 1.

Results. The cilia of laterofrontal cells (a, Fig. 1 and 3) appear to be compound *i.e.*, consisting of 3 to 4 filaments of approximately 250 Å in diameter. However, extensive fraying of them into the individual fibrils was not observed as has been demonstrated for the cilia of *Paramecium* and filaments of sperm tails (unpublished). At their bases the cilia are spaced only about 500 Å apart. This is close as compared to the distance between the cilia of the frontal cells (Fig. 2). The bodies of the cilia seem to be in many instances in contact with each other. However, no mucous-like substance which might function to hold them together as a unit was observed. This is mentioned in view of the findings of Carter (7) who observed that in the laterofrontal cells of *Mytilus* all the elements of the vibratory apparatus of a cell beat as a unit or "cilium", comparable to the cirri of certain protozoa(8).

Short extracellular filaments (Fig. 3) are thought to be broken cilia. Some evidence for a cuticular border consisting of short filamentous processes is shown at upper left in Fig. 1. However, it cannot be determined for certain whether or not these filaments are continuous all the way across the border. In the frontal cells, definite cuticular processes of about 400 Å in diameter and 8300 Å in length are sharply defined (g, Fig. 2). These are in many ways comparable to those seen in the kidney tubules and certain intestinal cells (9,10). Only the proximal portions of the cilia are shown in Fig. 2; they are more widely spaced than in the laterofrontal cells

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