of 25 mM Pi buffer (pH 7.5); 1.25 mM agmatine (Sigma), and enzyme (1 ml) in a total volume of 4 ml. After incubation at 37 °C for 1 h, the reaction was terminated by adding 0.5 ml of 10% TCA. In the blank, agmatine was added after incubation. The supernatant, after removal of proteins by centrifugation, was used for the estimation of N-carbamylputrescine by the method of Archibald[10]. The enzyme unit is defined as the amount of enzyme required to liberate 1 µmole of N-carbamylputrescine/h under the assay conditions. The protein content of the tissue was determined by the method of Lowry et al.[11] with bovine serum albumin as a standard. Dry weight of cotyledons and embryo was determined by drying fresh tissue at 60 °C to constant weight.

Results and discussion. Seeds were collected and grouped into different stages of development as described in the experimental section. The average fresh weight of the seed and dry weight of the cotyledons and embryo was determined. Stage 9 represents the fully mature seed, each weighing between 0.6 and 0.8 g. Storage of these seeds, over a period of 1 year, resulted in about 40% decrease in fresh weight (table). Dry weight of the cotyledons increased progressively up to stage 7 and was fairly constant after that. Storage resulted in a slight increase in dry weight. The dry weight of the embryo remained constant from stage 6 with some increase during storage. Protein content of the cotyledons and the embryo increased rapidly up to stage 7 and thereafter the increase was slow. It is suggested that during cotyledon development there are 2 phases of growth[12]; an initial one of cell division and a later one of cell expansion. Accumulation of storage material occurs mainly in the expansion phase. The final phase of seed development (maturation) is marked by a severe water loss, a gradual reduction of metabolic activity and a very small increase in the dry weight. The protein level of the kidney bean has been found to be relatively constant during development whereas it increases in the cases of pea, peanut and soybean[13]. The protein content of soybean increased throughout development, whereas the increase in peanut took place early, and that in pea took place later in development[13]. Our results are in agreement with the data reported for peanut[13]. During the germination of seeds, the protein content of both cotyledons and embryo decreased progressively over a period of 8 days. Agmatine iminohydrolase activity increased during development of the cotyledons but was constant in the embryo. In seeds stored for 1 year, decreased activity of the enzyme was found in both cotyledons and embryo. During germination, the enzyme activity increased further in the cotyledons. In the embryo, however, it increased up to day 3 but came back to its original level with further germination.

The studies reported above thus suggest that during the development of groundnut seeds, the reserves in the cotyledons are built up, as shown by an increase in dry weight and protein content associated with increased agmatine iminohydrolase activity. Germination of seeds represents a process where the reserve material of the cotyledons is being utilized, as shown by a decrease in protein content. The enzyme activity increased during the development of cotyledons, and also during germination, suggesting that a part of the arginine released from the reserve proteins of the cotyledons may be utilized through the agmatine iminohydrolase reaction.

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Solubilization of human red cell membranes by lysolecithins of various chain lengths

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Summary. Red cells and membranes prepared from them were treated with graded amounts of palmitoyl, myristoyl and lauroyl lysolecithins. There was no release of phospholipids from the intact red cells in the absence of hemolysis. The solubilization pattern of lipid and protein from the red cell membranes by lysolecithins varies with their chain length.

Lysolecithins are amphiphatic compounds which, like the anesthetics[3], interact with the red cell membrane inducing concentration-dependent effects such as red cell crenation, sphering, protection against hypotonic lysis and finally, hemolysis[4,5]. At much higher concentrations lysolecithins act as detergents and solubilize membranes. The present study reports on the solubilization of red cell membrane components by lysolecithins of various chain lengths. Red cells were separated from fresh human blood, washed in isotonic buffered saline (pH 7.4) and resuspended at about 40% hematocrit. Red cell membranes were prepared from recently outdated blood, essentially according to Dodge et al.[6] by hemolyzing washed red cells in 30 vol. of 10 mM Tris-HCl buffer (pH 7.4) supplemented with the proteinase inhibitor Trasylol (19 KIU/ml), repeatedly washed and resuspended in the hemolysis buffer to give 50 µg lipid phosphate/ml. Palmitoyl (C 16:0), myristoyl (C 14:0) and lauroyl (C 12:0) lysolecithins were 10 µmole/ml in either isotonic buffered saline or in hemolysis buffer. Since the lysolecithins presented residual venom phospholipase A activity, both the red cell and the membrane suspensions were supplemented with 1 mg/ml EDTA prior to addition

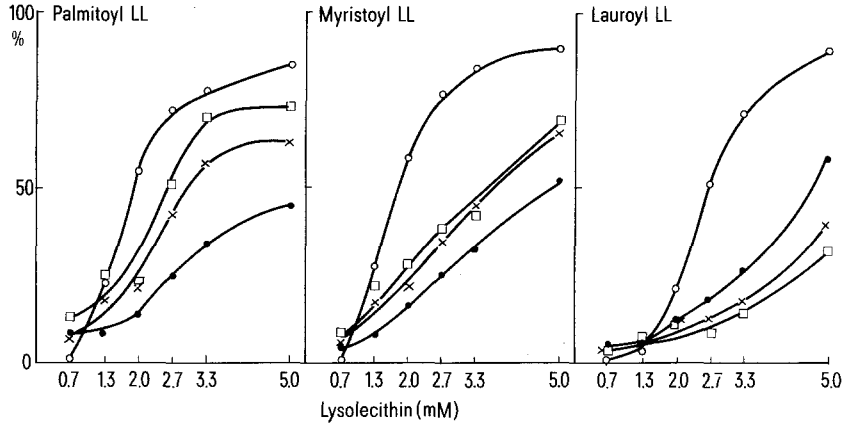

Fig. 1. Solubilization of red cell membrane components by lysolecithins. The figure is based on a representative experiment in which the lysolecithins were tested on the same membrane preparation. The incubation system contained, in a 3 ml volume, lysolecithin as indicated and an amount of membrane having an A_{450} of 1.8, 86 µg lipid phosphorous, 1.9 mg cholesterol and 4.6 mg protein. Following sedimentation at $6\times10^6\times$ g min both pellets and supernatants were analyzed. % solubilization was calculated, for each membrane component, as [(supernatant)/(supernatant + pellet]) × 100. A_{450} is given in % decrease. In addition to the experiment illustrated above, each lysolecithin was tested 2–3 times on separate membrane preparations with essentially similar results. ○: A_{450}; □: cholesterol; X: phospholipid; ●: protein.

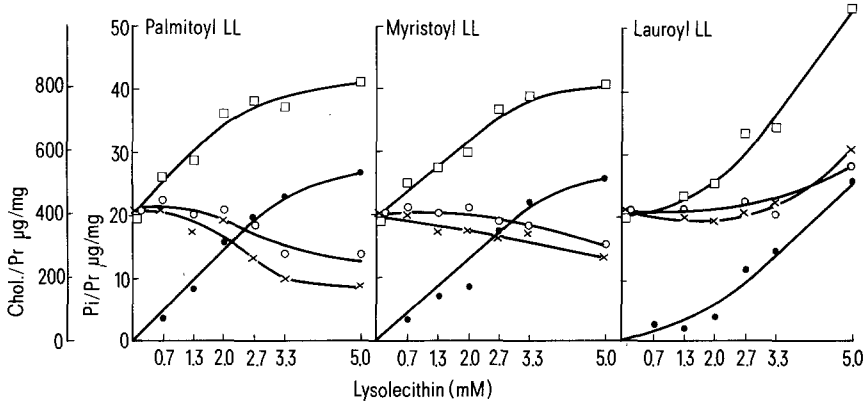

Fig. 2. Composition of membrane pellets following extraction by lysolecithins. Pellets from the experiment illustrated in figure 1. Lysolecithin and phospholipids are given in µg phosphorus, cholesterol in µg, all values being expressed per mg protein. ○: membrane phospholipids, i.e., sum of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol and sphingomyelin; ●: lysolecithin; X, cholesterol; □, total phospholipids i.e., sum of membrane plus lysolecithin.

of known amounts of lysolecithin solutions. Red cell suspensions were incubated 60 min. with lysolecithins at 37 °C, aliquots were removed for hematocrit determinations and the suspensions sedimented at 50,000 × g for 20 min. Supernatants were assayed for hemoglobin at 540 nm and analyzed for lipids. Membrane suspensions containing about 80 µg lipid phosphate were incubated with lysolecithins for 30 min at room temperature and optical densities were read in a Gilford spectrophotometer at 450 nm. The OD 450 of controls varied between 1.7 and 1.8. Insoluble material was pelleted at 100,000 × g for 60 min and resuspended in Tris buffer. From aliquots of both pellets and supernatants lipids were extracted[7], washed[8], and phospholipids chromatographed on thin-layers[9]. We also assayed lipid phosphorous[10], cholesterol[11], protein[12] and performed polyacrylamide gel electrophoresis in the presence of SDS[13,14].

Results and discussion. Lysolecithins hemolyze red cells in the sequence palmitoyl > myristoyl > lauroyl. Coleman and Holdsworth[15] and Billington et al.[16] found that taurocholate and glicocolate release from erythrocytes, prior to hemolysis, phospholipids which are located in the outer half of the membrane. Lysolecithins however, did not solubilize lipid in the pre-lytic phase, thus resembling the effects of Triton X-100[15]. When red cell membranes were incubated with graded amounts of C 16:0, C 14:0, and C 12:0 lysolecithins, there was no difference in protein solubilization, whereas their lipid-solubilizing ability (i.e., phospholipid and cholesterol) decreased with decreasing chain length (figure 1). The decrease in turbidity caused by palmitoyl and myristoyl lysolecithins was similar, whereas with lauroyl lysolecithin the curve was shifted towards higher concentrations (figure 1). The clearing effect does not seem to correlate with either lipid or protein solubilization. As seen in figure 2, all lysolecithins bind to the membranes

Selective solubilization of sphingomyelin and phosphatidylserine from red cell membranes by lauroyl lysolecithin

		NO LL	8 µmoles LL		10 µmoles LL		15 µmoles LL	
		Pellet	Pellet	Supt.	Pellet	Supt.	Pellet	Supt.
I	SM%	28.1	33.6	23.1	31.6	21.5	42.6	17.8
	PS%	14.4	13.1	17.9	13.0	20.6	9.3	27.0
II	SM%	25.4	32.2	17.3	35.4	25.1	43.6	20.5
	PS%	15.6	10.1	19.7	9.6	16.4	3.9	17.3
III	SM%	25.9	28.0	17.7	28.0	20.5	37.3	24.3
	PS%	15.8	13.2	18.7	13.6	19.6	10.3	20.3

Membranes were incubated with lysolecithin and the pellets sedimented as described in 'Materials and Methods'. Phospholipid distribution in the pellets and supernatants was determined by thin layer chromatography. Results are from 3 separate experiments. There was no difference in the percentage distribution of phosphatidylcholine and phosphatidylethanolamine in sediments and supernatants, therefore it is not shown. LL: lysolecithin, SM: sphingomyelin, PS: phosphatidylserine, Supt: supernatant.

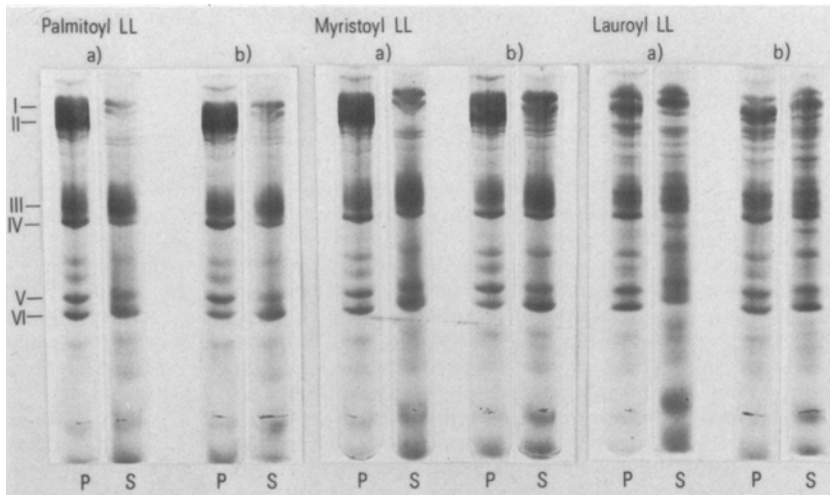

Fig. 3. Selective solubilization of red cell membrane polypeptides by lysolecithins. Acrylamide gel electrophoresis in presence of 1% SDS of pellets and supernatants, 35 µg protein per gel. a): 3.3 mM lysolecithin; b): 5 mM lysolecithin; P: pellet; S: supernatant. The polypeptide bands are designated according to Fairbanks et al.[17].

and, at low solubilization levels, elute in parallel both lipid and protein in a fairly constant ratio. At higher solubilization levels palmitoyl and myristoyl lysolecithins deplete the pellet of phospholipid and cholesterol whereas lauroyl lysolecithin produces a pellet slightly enriched in both lipids. Since lauroyl lysolecithin binds less to the membranes than its longer chain homologues, we compared the decrease in turbidity and solubilization of protein, phospholipid and cholesterol by the lysolecithins at equal binding levels (data derived from figures 1 and 2). Lauroyl lysolecithin appears to be as efficient as the other lysolecithins in decreasing turbidity and eluting total protein, but less efficient in solubilizing lipids, i.e., total phospholipid and cholesterol.

The distribution of phospholipid species in pellets and supernatants revealed that palmitoyl and myristoyl lysolecithins solubilized symmetrically, while lauroyl lysolecithins did so selectively, with sphingomyelin being mostly retained in the pellets and phosphatidylserine being preferentially extracted (table). Phosphatidylcholine and phosphatidylethanolamine were distributed similarly in pellets and supernatants. The polypeptide patterns in both pellets and supernatants were compared by acrylamide gel electrophoresis (figure 3). Lauroyl lysolecithin solubilized protein without discrimination whereas palmitoyl and, to a lesser extent, also myristoyl lysolecithin showed selectivity, the supernatants being enriched in protein bands 3 and 6, whereas bands 1, 2, 4, and 5 were poorly extracted (nomenclature according to Fairbanks et al.[17]).

Lysolecithins of various chain lengths differ in hemolytic ability[18] and characteristics of hemolysis such as temperature-dependence and effects on red cell species[19]. Also chain-dependent is their ability to solubilize microsomal membranes[20]. Our data indicate that the solubilization pattern of red cell membranes by lysolecithins is also a function of chain length. The longer chain lysolecithins, structurally similar to the endogenous phospholipids[21], delipidate the membrane probably by dissociation of hydrophobic lipid-protein bonds, exchange with the membrane lipid and solubilize mostly integral proteins. Thus, the overall effects of longer chain lysolecithins on red cell membranes resemble those elicited by Triton X-100[22–26]. Like Triton[26], palmitoyl lysolecithin might prove suitable for the isolation and study of red cell membrane proteins without denaturation, as previously achieved for the Ca^{2+}-ATPase of muscle micromes[27].

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