RELATIONSHIP BETWEEN PLASMA CHOLESTEROL LEVEL AND ERYTHROCYTES SHAPE IN RABBITS ON ATHEROGENIC DIET AND ONION EXTRACTS

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ABSTRACT

The relationship between plasma cholesterol level and erythrocytes shape in albino rabbits was studied. In animals maintained on cholesterol diet alone, erythrocytes began to show slightly crenated shape after four weeks of administration of cholesterol and by the end of three months, the cells showed marked crenation and aggregation. This abnormality was completely absent in the animals fed with cholesterol plus onion extract diets.

INTRODUCTION

IT has been observed that the erythrocytes in hypercholesterolemic rabbits begin to show crenation after 5–7 weeks of cholesterol feeding[1–3]. The precise level of the serum cholesterol with respect to the occurrence of crenation is not well known. In this report, we present the levels of plasma cholesterol of rabbits fed with atherogenic diet which influence the erythrocyte shape. A comparison has also been made with rabbits, at comparable levels of plasma cholesterol fed with the same atherogenic diet plus extract of onion and with normal rabbits.

MATERIALS AND METHODS

White albino rabbits of same age (2–3 months), sex and body weight were fed with normal diet (carrot, cabbage and greens). A set of animals was divided into four groups (10 animals in each group). Group I served as normal controls. Group II animals were fed with the normal diet plus extract of 20 g fresh onion. Group III animals were fed with atherogenic diet (normal diet + 0·5% cholesterol) and Group IV animals with atherogenic diet (as above) plus the extract of 20 g fresh onion. The administration of cholesterol was adjusted in such a way that the plasma cholesterol levels in Group IV animals were comparable to those in Group III, as shown in Table I. The onion extract, which is water soluble, was prepared by the method of Stoll and Seeback[4] as follows :

The onion bulbs after removing the outermost layers were frozen and crushed into small pieces and were treated with ethanol. The ethanol extract was evaporated at low temperature and reduced pressure. This extract, free from alcohol, was used as onion extract.

Blood samples were collected with ammonium oxalate as anticoagulant, every week by ear vein puncture. A minor prick was made in the side of the ear for the preparation of microscopic slides.

Plasma was separated by centrifugation at 3000 × g for 10 min and was used for cholesterol estimation by the method of Abell *et al.*[5]. The plasma fibrinogen was determined by the method of Goodwin[6]. The erythrocyte membrane was isolated by the method of Dodge *et al.*[7]. The total lipids were extracted with choloroform-methanol (2 : 1) mixture by the method of Folch *et al.*[8]. Total phospholipids were estimated by determination of phosphorus[9] and multiplying by a factor of 25. The microscopic slides were viewed under Leitz microscope and a magnification of 1000. The membrane total protein was determined by the method of Lowry *et al.*[10]

RESULTS

Table I shows the relationship between cholesterol levels and the occurrence of crenation and aggregation of erthrocytes of various groups. The cholesterol levels in Groups I and II were normal and low compared to Groups III and IV. Crenation and aggregation tendencies of the erythrocytes were absent in these groups. The crenation of erythrocytes in Group III started at the plasma choolesterol level of 170 mg%. The crenation and aggregation increased with the increase in cholesterol levels. The severity of crenation and aggregation was more at 600 mg% and above. The crenation and aggregation were completely absent in Group IV rabbits at the comparable cholesterol levels.

The total lipids, cholesterol and phospholipids levels of erythrocytes and erythrocytes membrane are given in Table II. The plasma cholesterol and fibrinogen levels in Groups III and IV were elevated and were at comparable levels. The erythrocytes total lipids, cholesterol and phospholipids were also elevated in these groups, whereas the erythrocyte membrane total lipids, cholesterol and phospholipids of Group IV were normal and at comparable levels of Groups I and II. The membrane lipids showed three fold increase in Group III.

TABLE I

A comparison of plasma cholesterol levels and erythrocyte shape in rabbits of various groups

Duration in weeks	Groups I and II			Group III			Group IV		
	Cholesterol mg %	Erythrocytes		Cholesterol mg %	Erythrocytes		Cholesterol mg %	Erythrocytes	
		b	*c*		*b*	*c*		*b*	*c*
I	100±10[a]	..	..	100±8	—	—	110±10	—	—
II	,,	—	—	140±10	—	—	135±10	—	—
III	,,	—	—	150±15	—	—	155±15	—	—
IV	,,	—	—	170±8	+	—	180±10	—	—
V	,,	—	—	220±15	++	—	220±15	—	—
VI	105±15	—	—	270±15	++	—	280±20	—	—
VII	,,	—	—	280±20	++	—	290±15	—	—
VIII	,,	—	—	300±10	+++	+	310±20	—	—
IX	,,	—	—	350±15	+++	+	370±30	—	—
X	,,	—	—	400±25	+++	++	410±20	—	—
XI	,,	—	—	530±30	++++	++	510±40	—	—
XII	,,	—	—	600±25	++++	++	610±30	—	—
XIII	142±23·5	—	—	1200±267·5	++++	+++	1142±150·4	—	—

a—mean ± S.D., *b*—crenation, *c*—aggregation

Following abbreviations are given to show the degree of changes :

—, absence: +, slight: ++, mild; +++, medium; ++++, severe.

TABLE II

Lipid content of plasma, erythrocytes and erythrocyte membrane

Parameter analyzed	Group I	Group II	Group III	Group IV
Plasma cholesterol mg/100 ml	142·0 ± 23·5	135·00±18·5	1200 ±267·5	1142 ±150·4
Fibrinogen mg/100 ml	288 ± 15·0	278·0 ± 17·0	620 ± 24·0	600 ± 18·0
Erythrocyte lipids				
Total lipids mg/100 ml	425·48± 30·32	520·0 ±127·3	2519·0 ±128·5	2500 ±135·0
Cholesterol mg/100 ml	113·12± 18·17	142·0 ± 20·5	571·2 ± 20·8	571·2 ± 19·5
Phospholipids mg/100 ml	110 ± 10·25	134·0 ± 12·25	770·0 ± 35·5	340 ± 25·0
Erythrocyte membrane lipids (mg/g membrane protein)				
Total lipids	200·3 ± 17·2	217·2 ± 8·9	610·0 ± 45·70	203·4 ± 15·3
Cholesterol	123·4 ± 10·3	121·3 ± 9·7	450·0 ± 30·50	126·3 ± 10·7
Phospholipids	9·90± 1·25	10·10± 1·35	15·05± 1·5	10·5 ± 1·25

DISCUSSION

The elevated lipids level of the erythrocyte membrane leads to altered architecture of the membrane which finally leads to its crenation in Group III. Such crenation has also been observed in jaundice[11,12] in certain liver diseases[13–15] and in hereditary abetalipoproteinemia[16] where plasma cholesterol is elevated, which in turn elevates the erythrocyte cholesterol.

The normal levels of membrane lipids, cholesterol and total lipids in Group IV rabbits help to maintain

the normal shape of erthrocytes, even though the plasma cholesterol is elevated. This shows that the presence of onion extract helps to maintain the normal membrane lipid levels and thus in the maintenance of the shape of erythrocytes.

The altered appearance of erythrocytes and increased level of fibrinogen may be partly responsible for the increased aggregation tendency of erythrocytes of Group III. The absence of aggregation in Group IV indicates that the presence of some additional factors in the medium from onion extract which counterbalances the deleterious effect of cholesterol and fibrinogen on erythrocytes. The onion which is known for its fibrinolytic activity[17] may also explain the absence of aggregation in this group.

A comparison between Groups I and II shows that onion alone has no deleterious effect on the erythrocytes and the fibrinogen levels in these groups remain unaltered.

The presence of some additional factors of onion extract in Group IV plasma is well established by in *vitro* studies. When erythrocytes from Group III rabbits were incubated with the plasma from Groups II or IV, they regained their normal shape[1]. Identification of such factors in onion is in progress.

1. Vatsala, T. M. and Singh, Megha, *Curr. Sci.*, 1979, **48**, 797.
2. — and —, *Atherosclerosis*, 1980, **36**, 39.
3. Rogausch, M., *Pflugers Archiv.*, 1978, **373**, 39.
4. Stoll, A. and Seebeck, E., *Adv. Enzymol.*, 1951, **11**, 377.
5. Abell, L. L., Levy, B. B., Brodei, B. B. and Kendall, F. E., *J. Biol. Chem.*, 1952, **195**, 357.
6. Goodwin, J. F., *Am. J. Clin. Pathol.*, 1961, **35**, 227.
7. Dodge, G. T., Mitchell, C. D. and Hanahan, D. G., *Arch. Biochem. Biophys.*, 1963, **100**, 19.
8. Folch, J., Lees, M. and Stanley, G. H. S., *J. Biol. Chem.*, 1957, **226**, 375.
9. Fiske, C. H. and Subba Row, Y., *Ibid.*, 1925, **66**, 375.
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. T., *Ibid.*, 1951, **193**, 265.
11. Cooper, R. A. and Jandal, J. H., *J. Clin. Invest.*, 1968, **47**, 809.
12. —, *Ibid.*, 1969, **48**, 1820.
13. —, Kimbell, D. B. and Durocher, J. R., *New England J. Med.*, 1974, **280**, 1279.
14. Smith, J. A., Lonergon, E. T. and Starling, K., *Ibid.*, 1964, **271**, 396.
15. Powell, L. W., Halliday, J. W. and Cooksley, W. G. E., *Aust. New Z. J. Med.*, 1976, **6**, 3.
16. McBridge, J. A. and Jacob, H. S., *Br. J. Nemutol.*, 1970, **18**, 383.
17. Menon, I. S., Kendal, R. Y., Dewar, H. A. and Newell, D. J., *Br. Med. J.*, 1968, **3**, 351.

CONDUCTOMETRIC AND SPECTROSCOPIC STUDIES ON THE INTERACTION OF IMIDAZOLE WITH IODINE

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ABSTRACT

Interaction of imidazole with iodine has been examined by employing conductometric and spectroscopic techniques. Conductometric measurements indicate the formation of a 1 : 1 electron donor-acceptor (EDA) complex between imidazole and iodine. The σ_M and σ_P values for the complex are reported and the effect of solvent and temperature on σ_P has been studied. Spectral investigations show evidence for the transformation of the initially formed EDA complex into the inner complex thereby giving rise to the triiodide ion. Kinetics of the transformation reaction has been examined.

INTRODUCTION

MULLIKEN[1] pointed out that the interaction between an electron donor (D) and an electron acceptor (A) can give either the associative outer (EDA) complex or the dissociative inner complex depending on the distance of approach between D and A and the relative magnitude of the no-bond and dative wave functions. In many donor-acceptor systems with halogen acceptors[2–3], the formation of trihalide ion is often noticed which can only result through the formation of inner complexes from the initial EDA complexes. Electrical conductance of iodine in pyridine solution was one of the early evidences for