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The Susceptibility to Autoxidation of Human Red Cell Lipids in Health and Disease

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SUMMARY. The susceptibility of human red cells to autoxidation was measured (i) in various groups of normal subjects, (ii) in haemolytic states, and (iii) in non-haemolytic disease. Many types of haemolytic disease—notably thalassaemia major and autoimmune haemolytic anaemia—were associated with a greatly and persistently increased susceptibility of the red cell lipids to autoxidation. In two patients with haemolytic disease this was the only biochemical abnormality found. Susceptibility to autoxidation is about three times higher in the newborn period than it is in adult life; and there is a comparatively wide scatter in old age.

Although it is at present impossible to demonstrate spontaneous autoxidation in human RBCs, the susceptibility of RBC lipids to autoxidation under oxidative stress can be accurately measured (Stocks & Dormandy, 1971). In a preliminary communication, moreover, Stocks *et al* (1971) reported that this susceptibility is greatly increased in certain haemolytic states as well as in the neonatal period. Further study was prompted by three considerations. First circumstantial evidence suggests that the autoxidative breakdown of polyunsaturated lipids may be a causative factor both in normal and in premature cell ageing. Second, it is possible that in certain states the abnormal susceptibility of the RBC lipids to autoxidation reflects a similar abnormality in other organs and tissues. Third, since susceptibility to autoxidation can be greatly reduced by antioxidants *in vitro* the abnormality might be amenable to correction *in vivo*.

METHODS

Lipid Autoxidation

Our method of measuring susceptibility to autoxidation is based on the generation under oxidative stress of malonyldialdehyde (MDA), a secondary breakdown product of lipid peroxides (Dahl *et al*, 1962). The principle and various experimental procedures have been described in previous publications (Stocks & Dormandy, 1970, 1971). Our standard assay is carried out as follows:

Reagents. (1) *Buffered saline*: 17.6 ml 0.5 M-KH_2PO_4 + 60.8 ml 0.5 M-K_2HPO_4 are made up to 1 litre with deionized water. An aliquot of this solution (100 ml) is added to 1 litre 0.15 M-NaCl, pH 7.4. (1) *Azide buffer*: 1 litre buffered saline + 10 ml 0.4 M-sodium azide. (3) *TCA–arsenite solution*: 280 g trichloracetic acid (TCA) are added to 500 ml deionized

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water followed by 13 g sodium arsenite. The mixture is dissolved by heating, then cooled. Finally, it is made up to 1 litre with deionized water and filtered. (4) *Hydrogen peroxide solution*: Immediately before use 5.5 ml standardized 9 M-H_2O_2 ('Aristar') is made up to 100 ml with deionized water. Two ml of this solution is made up with buffered saline to 100 ml. (5) *TBA solution*: 5 g thiobarbituric acid (TBA) is dissolved by heating in 200 ml deionized water together with 25 ml N-NaOH. The solution is cooled, made up to 500 ml with deionized water and filtered.

Procedure. Freshly withdrawn heparinized blood is spun. The plasma is aspirated and replaced by an approximately equal volume of azide buffer. The cells are resuspended. A 2 ml cell suspension is diluted with 8 ml azide buffer and after mixing the cell suspension is spun. The supernatant and the remainder of the buffy coat are removed. Five ml of azide buffer is added to the packed cells and the cell suspension is mixed. (3.5 or 4 ml azide buffer is added when the initial PCV is less than 25%.) The Hb concentration is measured by transferring 50 μl of the suspension to 5 ml of Drabkin's reagent. To 5 ml of the cell suspension buffered saline is added to bring the final Hb concentration to exactly 3 g/100 ml. Five ml of this suspension is transferred to a glass boiling-tube and equilibrated in a 37°C shaking water-bath for 10 min. Five ml of hydrogen peroxide solution is added by allowing the solution to run down the side of the tube (zero time). The mixture is then incubated at 37°C for 2 hr.

MDA estimations. Three ml of the cell suspension is added to 2 ml TCA–arsenite solution. The mixture is spun, 3 ml of the supernatant is transferred to a 15 ml centrifuge tube and 1 ml of TBA solution is added. An air condenser is fitted to the tube and the mixture is incubated for exactly 15 min in a boiling-water bath. The tube is cooled. The absorption spectrum of the mixture between 500 and 600 nm is plotted by a recording spectrophotometer. The formula $(OD_{532} - OD_{600}) \times 900$ gives the MDA concentration in n-mol/g Hb.

Other Measurements

All other haematological and biochemical investigations were performed by established methods (Dacie & Lewis, 1968). Serum tocopherol was measured by the method of Hashim & Schuttringer (1966). Our normal range with this method is 0.5–2.0 mg/100 ml.

MATERIAL

The present paper gives our findings in over 500 assays. The majority of these were performed on staff and patients of the Whittington Hospital, London.

RESULTS

1. *Normal Variations*

Adult range. As reported previously, the mean 2 hr MDA in healthy adults was 138 (±71) nmol/g Hb. For routine clinical purposes 250 nmol can be taken as the upper limit of normal. In no subject so far tested was the 2 hr MDA less than 80 nmol. In only one normal subject was the 2 hr MDA significantly and persistently above the normal (varying between 400 and 650 nmol over a 2 yr period). In any one person the 2 hr MDA tends to remain fairly

constant from day to day and from season to season. Diurnal variations, if any, are small; and normal meals and slight variations in diet have no noticeable effect. There is no significant sex difference; and between 17 and 52 yr (the age limit of the series) there is no discernible upward trend.

Pregnancy. The mean 2 hr MDA in pregnant women in the second and third trimesters of pregnancy was 121 (± 39) nmol/g Hb. Only two subjects in our series of 62 had a 2 hr MDA above the normal range (Fig 1).

Newborn infants. The mean 2 hr MDA in umbilical cord bloods from normal full-term infants was 768 (± 63) nmol/g Hb, i.e. approximately three times higher than the adult

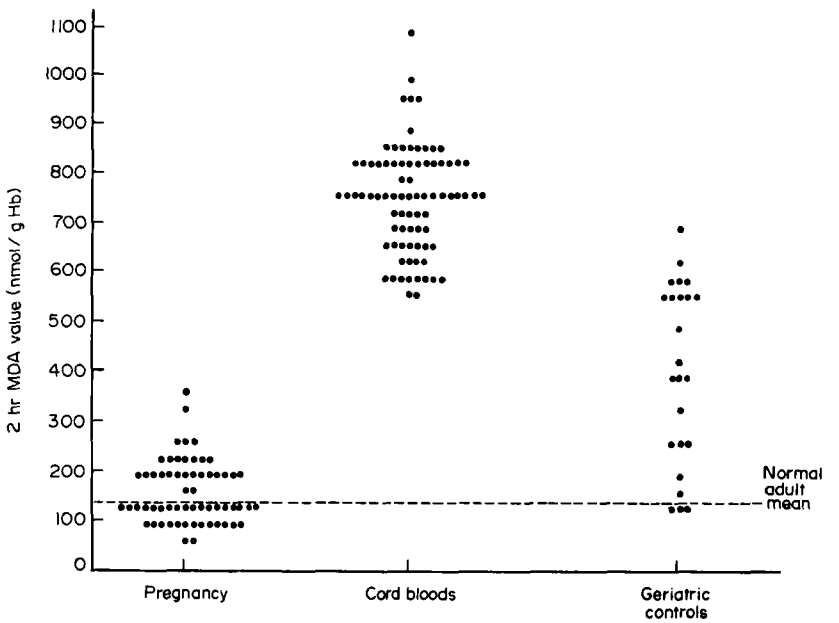

FIG 1. The 2 hr MDA results in normal pregnant women, in normal cord bloods and in a normal geriatric control series.

range and mean. The extreme limits in our series of 88 blood samples were 1080 and 580 nmol (Fig 1). All but two of the serum tocopherol levels were low by adult standards (mean 0.28 ± 0.11 mg/100 ml). The latter finding is in agreement with several previous reports (Oski & Barness, 1967; Hashim & Schuttringer, 1966).

Old age. The mean 2 hr MDA in a geriatric control series of 24 people over 74 yr was 486 (± 122) nmol/g Hb. All subjects were as active and fit physically and mentally as compatible with advanced age in our local population. As shown in Fig 1, the range of results was much wider than in the normal adult control series. There was no correlation between 2 hr MDA and Hb, and most serum-tocopherol levels were within the normal range.

2. *Haemolytic States*

The series excludes neonatal haemolysis and illnesses in which the haemolytic element is generally regarded as a secondary feature (e.g. vitamin B_{12} deficiency). The apparent cause

or mechanism of the haemolysis was investigated in all patients; but normal or negative results of RBC enzyme assays, osmotic fragility, Coombs test, Hb electrophoresis, and acid-serum test are omitted from the tables.

Autoimmune haemolytic anaemia. The findings in seven patients are summarized in Table I. The main criterion for inclusion was past or present clinical haemolysis associated with a positive direct Coombs reaction (transient in Case 6 and intermittent in Case 7). Case 2 and 3 appear to be instances of the rare familial form of the disease (Kissmeyer-Nielsen *et al*, 1952; Richmond, 1965); unfortunately, after the initial testing it was not possible to investi-

TABLE I. Susceptibility of RBC lipids to autoxidation in autiommune haemolytic disease

Case No.	*Age and Sex*	*Main clinical features*	*Main laboratory findings**		*2 hr MDA (nmol/g Hb)*
			Hb (g/100 ml)		
1	25 F	Crisis when 34 weeks pregnant. Steroids: blood transfusions.			
		Delivery by Caesarean section	4.8	Retics 6%; Se.br. 4 mg/100 ml	590
		2 days later. Further transfusions	7.0	Retics 8%; Se.br. 6 mg/100 ml	630
		1 week later. On steroids.	8.2	Retics 9%; Se.br. 2 mg/100 ml	700
		3 mth later. Post-splenectomy	12.5	Retics 4%	770
		6 weeks later. On steroids	12.5	Retics 4%	780
2	32 F	Long-standing anaemia: crises 2 weeks ago	8.4	Retics 6%	765
3	64 F	Mother of Case 2. Fluctuating anaemia	7.8	Retics 8%	570
4	23 F	Anaemia for years. Crisis with 'flu 3 weeks ago. Responding to steroids.	8.6	Retics 18%	985
		Much improved. Coombs −ve	11	Coombs−ve	380
5	8 M	Crisis 2 yr ago: no known precipitating cause. On steroids. Resistant anaemia	8.8	Retics 12%	1055
6	5 M	Crisis complicating pneumonia	5.8	Retics 19%; Se.br. 3 mg/100 ml	990
		4 day later. Much improved	10.2	Retics 10%;100 ml Coombs−ve	440
7	78 M	Crisis (?) precipitated by urinary infection. Jaundice; circulatory collapse	5.4	Retics 20%; IgG +++ in serum: lysin at 37°C	640
		2 days later. Steroids: blood transfusions	7.0	Retics 18%	600
		1 week later. Started on azathioprine	6.8		580
		1 mth later. Symptom free	13.0	Coombs −ve	240
		6 weeks later	14.4	Coombs −ve	200
		4 mth later: emergency admission	8.4	Retics 4%. Acidosis.	
		Pneumonia, septicaemia, anaemia Died 2 days later.		Coombs +ve	470

* Direct Coombs test +ve unless indicated.

TABLE II. Susceptibility of RBC lipids to autoxidation in thalassaemia major: untransfused patients

No.	*Age and Sex*	*Splenectomy*	*Hb (g/100 ml)*	*Serum tocopherol (mg/100 ml)*	*2 hr MDA (nmol/g Hb)*
1	17 M	Yes	10.0, 10.5	0.22	1070, 1150
2	13 M	Yes	9.0, 9.6	0.42	1025, 1200
3	11 F	Yes	8.8, 9.4	0.80	815, 920
4	5 F	No	8.2	0.72	555
5	1 M	No	8.5, 9.4		650, 730
6*	5 M	No	7.5, 8.2	0.50	1050, 1080
7	9 M	Yes	7.8, 6.8, 6.8	0.44	800, 1150, 1350

* Sickle-cell thalassaemia.

gate the patients further. It may be noted that, although the MDA results were abnormal in all cases at all times when the direct Coombs test was positive, the clinical course and severity of the illnesses did not always parallel the increase in the 2 hr MDA value. Case 1 was fairly well controlled on steroids when last seen; but at that time she had a higher 2 hr MDA value than immediately after the onset of her haemolytic crisis. (In this respect the biochemical pattern was similar to that seen in RBC enzyme deficiencies which tend to appear more

TABLE III. Susceptibility of RBC lipids to autoxidation in thalassaemia major: transfused patients

Case No.	*Age and Sex*	*T.R.**	*Splenectomy*	*Mean Hb† (g/100 ml)*	*Fall in Hb (%/day)*	*Relation to transfusion*	*Hb (g/100 ml)*	*Serum tocopherol (mg %)*	*2 hr MDA (nmol/g Hb)*
8	14 M	Im	Yes	10.2	0.56	Pre	7.0, 7.1	0.69	510, 540
9	13 M	Im	Yes	11.5	0.9	Pre	6.0, 6.2	0.30	600, 450
						Post	12.2	0.48	340
10	10 M	Im	Yes	11.0	0.9	Pre	7.1	—	480
11	9 M	Im	No	10.8	1.0	Pre	8.9	—	640
12	1 F	Im	No	9.9	1.2	Pre	5.8, 7.2	0.87	385, 405
13	20 F	Im	No	?	?	Pre	7.1, 9.1	0.29	600, 450
14	3 F	Im	No	11.6	0.9	Pre	7.2, 10.1	0.86	365, 105
15	4 M	Im	No	10.8	1.0	Pre	8.6, 7.5	0.78	135, 252
16‡	10 M	High	Yes	13.2	0.8	Pre	9.4, 10.7	0.67	450, 480
						Post	14.0	—	280
17‡	3 F	High	No	12.3	0.7	Pre	9.8	0.62	290
						Post	15.2	—	130
18	11 M	High	Yes	12.0	1.1	Pre	9.4, 10.7	0.69	270, 165
19	9 F	High	Yes	12.0	0.9	Pre	10.5	0.65	205
20	12 F	High	No	12.0	1.1	Pre	9.6, 10.5	0.69	120, 260
21	7 F	High	No	12.5	1.3	Pre	7.2, 9.4	0.62	270, 130
22	4 M	High	No	12.5	1.3	Pre	8.6	0.64	280
23	3 F	High	No	12.0	0.9	Pre	6.1, 10.0	0.48	85, 80

* Transfusion regime. Im = intermediate. See text and Fig 2.

† See Fig 2.

‡ Evidence of relatively brisk autologous red-cell production despite high transfusion regime.

severe as the anaemia improves.) Whereas in this patient the direct Coombs test remained positive, in Case 7 the reaction became negative on treatment with azathioprine. At that stage his 2 hr MDA value was also normal. During his terminal illness the direct Coombs test reverted to strongly positive and the 2 hr MDA value again became abnormal.

In view of the 'autoimmune' type of haemolytic anaemia reported in some patients on long-term treatment with methyldopa (Carstairs *et al*, 1966), a series of 16 patients on this drug were investigated. None had clinical evidence of haemolysis but two had a weakly positive direct Coombs test. The 2 hr MDA value in all 16 was normal.

Thalassaemia major. Our series of patients are listed in Tables II and III. They fall into three groups. Cases 1–7 do not receive regular transfusions and none had been transfused within 6 mth of the assay. Cases 8–15 are on a regular 'intermediate' transfusion regime. Cases 16–23 are on a regular 'high' transfusion regime. Fig 2 illustrates the pattern of change in Hb and

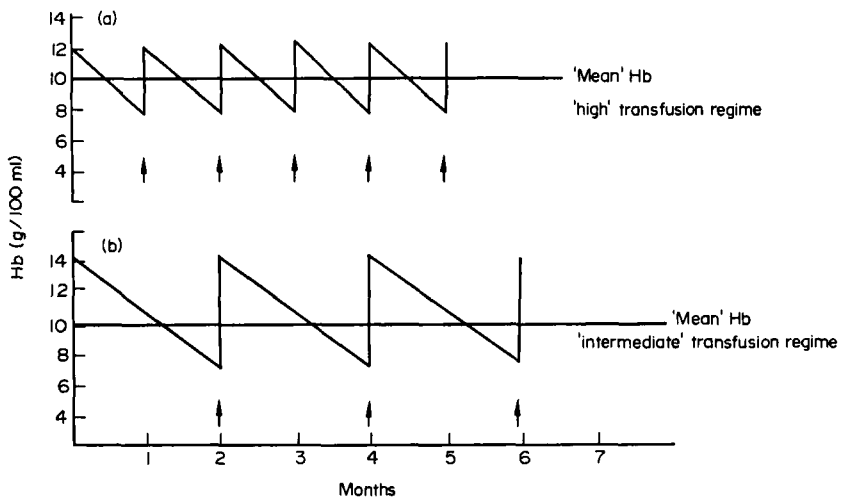

FIG 2. The pattern of change in Hb concentration in patients with thalassaemia major on (a) high transfusion regime and (b) intermediate transfusion regime. The arrows indicate times of transfusions.

the meaning of the term 'mean Hb' in the second and third groups. Patients in these two groups were also receiving the iron-chelating agent desferrioxamine. The whole series is part of a detailed long-term study conducted by one of us (C.B.M.) and all but one have been observed regularly since birth or for at least 7 yr. Assessment of autologous marrow activity was based on the rate of fall in Hb following transfusion (taken in conjunction with other clinical and laboratory findings). The 2 hr MDA in all the untransfused patients was high. In patients receiving regular transfusions the results showed close correlation with the rate of autologous RBC production. The 2 hr MDA value was lowest in patients in whom bone marrow activity was almost totally suppressed by the transfusion regime and highest in those whose bone-marrow was relatively active.

Other types of haemolytic disease. The results are set out in Tables IV–VI. Only one out of four patients with congenital spherocytic anaemia had an abnormal 2 hr MDA value, but none were in an acute haemolytic crisis at the time of testing. In the enzyme-deficiency group four out of five were abnormal; and four clinically-affected patients with haemoglobino-

TABLE IV. Susceptibility of RBC lipids to autoxidation in congenital spherocytosis

Case No.	*Age and Sex*	*Main clinical findings*	*Main laboratory findings**			*2 hr MDA (nmol/g Hb)*
			Hb (g/100 ml)	*Reticulocytes (%)*		
1	32 M	First crisis complicating glandular fever: improving	9.5	4	Se.br. 4 mg/100 ml	110, 140
2	4 F	Daughter of Case 1: symptom free	12.5	2		115
3	24 F	8 mth pregnant: diagnosis on routine film	10.8	2.5		370
4	50 F	Fluctuating jaundice for 3 mth: anaemia	11	8		195
5	32 F	Splenectomy 5 yr ago for anaemia: symptom-free since	13.4	1.8		220, 280

* Osmotic fragility of RBCs increased in all cases.

pathies had a raised 2 hr MDA. Several patients heterozygous for Hb S in whom the diagnosis was made on routine blood examination (during pregnancy or preoperatively) and in whom the abnormality was not clinically manifest gave normal results. Cases 10 and 11 in Table VI showed no biochemical abnormality other than the marked and persistent increase in the 2 hr MDA value. Although in Case 11 the acute haemolytic episode may have been precipitated by an overdose of tabs. codein co. the susceptibility of his RBC to autoxidation increased after the drug had been withdrawn and after clinical and laboratory evidence of haemolysis had disappeared.

3. *Non-Haemolytic Disease*

Blood dyscrasias and reticuloses. The 2 hr MDA results in a series of patients are set out in

TABLE V. Susceptibility of RBC lipids to autoxidation associated with enzyme defects

Case No.	*Age and Sex*	*Main clinical findings*	*Main laboratory findings*			*2 hr MDA (nmol/g Hb)*
			Hb (g/100 ml)	*Reticulocytes (%)*		
1	23 F	Family history of favism. Crisis 2 mth ago: now symptom free	12.4	1.5	Pre-transfusion G6PD < 10% normal	380, 350
2	18 F	Attempted termination of pregnancy with quinine. Anuria for 2 days. Now improving	10.0	6	Pre-transfusion < 5% normal G6PD	310 310
3	26 F	Crisis 3 yr ago following sulphonamides. Now symptom free	12.8	3	G6PD < 10% normal	630
4	8 M	Brother of Case 3. Mild anaemia	11.6	2	G6PD < 10% normal	500
5	23 M	Crisis precipitated by alcohol. Jaundice: anuria	8.0	3	Se.br. 5 mg/100 ml G6PD < 10% normal	215
		1 mth later: much improved	12.0			420

TABLE VI. Susceptibility of RBC lipids to autoxidation in haemolytic states

Case No.	*Age and Sex*	*Diagnosis*	*Main clinical and laboratory findings*	*2 hr MDA (nmol/g Hb)*
1	50 F	Bacteraemic haemolysis	Liver biopsy 2 days ago: collapse 2 hr later. Hb 6.7 g/100 ml. Se.br. 4.5 mg/100 ml. Blood culture *E. coli*	446
			Responding to treatment. Hb 8.8 g/100 ml	565
2	62 M	Uraemic haemolytic syndrome	Prostatic obstruction. Sudden onset of coma, haemolysis. Hb 7.8 g/100 ml. Retics 3%. 90% 'burr' cells. Blood urea 290 mg/100 ml	205
3	19 M	March haemoglobinuria	Symptom free between long-distance walks. Hb 14.2 g/100 ml	120
4	42 F	Incompatible transfusion	Exploratory craniotomy. Shock, anuria after incompatible transfusion. Hb 7.0 g/100 ml. Subsequent recovery	100
5	72 M	Elliptocytosis	Mild chronic anaemia. Hb 11.0 g/100 ml. Retics 4%. RBC survival 60% of normal	420
6	47 F	Paroxysmal nocturnal haemoglobinuria	Resistant iron-deficiency anaemia for 6 mth. Hb 9.8 g/100 ml. Retics 4%. Tests for PNH+ve	470
7	8 M	Hb SS	Abdominal crises; bone pains. Hb 10.0 g. Retics 5.5%	630
8	8 M	Hb SS	Twin of Case 7. Hb 9.4 g/100 ml. Retics 6%	800
9	41 M	Hb SC	Chronic anaemia: abdominal crises. Hb 12.0 g/100 ml. Retics 6%	410
10	2 M	Haemolysis: ? aetiology	Haemolytic crises complicating acute diarrhoea. Hb 10.2 g/100 ml. Retics 8%. Se.br. 4 mg/100 ml. Coombs −ve. RBC enzymes, fragility, electrophorosesis normal. Serum tocopherol 0.5 mg/100 ml. Parents normal	760
			2 weeks later. Well clinically. Hb 10.6 g/100 ml. Retics 4%	820
			1 mth later. Hb 12.2 g/100 ml. Retics 3%	760
			3 mth later. Symptom free. Hb 12.8 g/100 ml	280
11	31 M	Haemolysis: ? aetiology	Schizophrenic. No recent addition to battery of drugs. Crisis complicating cold. Hb 10.2 g/100 ml. Retics 20%. Coombs −ve. RBC enzymes, fragility, electrophoresis, O_2 dissociation curve normal. Serum tocopherol 1.3 mg/100 ml. Parents normal	720
			2 days later. Hb 9.8 g/100 ml. Retics 21%	800
			1 week later. Hb 11.5 g/100 ml. Retics 16%	940
			2 weeks later. Hb 11.1 g/100 ml. Retics 6%	900
			1 mth later. Hb 10.6 g/100 ml. Retics 2.5%	820
			3 mth later. Hb 12.8 g/100 ml. Retics 1%	860

Table VII. Interpreting the high values found in some cases, the wide 'normal' scatter in subjects over 70 should be recalled. (In an 82-yr-old woman, however, the 2 hr MDA fell from 500 to 210 nmol on successful treatment with vitamin B_{12} alone.) It may also be recalled that since MDA concentration is expressed in terms of g Hb, in large cells with a low MCHC the results could be regarded as 'spuriously' high.

Splenectomy. Because of the morphological (and perhaps biochemical) similarities between red cells from cord blood and from patients after splenectomy (Acevedo & Mauer,

1963; Holroyde *et al*, 1969; Nathan, 1969) the 2 hr MDA was measured in five subjects who had had their spleens removed some months or years earlier for non-haematological reasons. All five results were normal.

Malabsorption. Vitamin E deficiency has been recognized as an occasional feature of malabsorption states (Binder *et al*, 1965; Darby *et al*, 1946; Leonard *et al*, 1966; MacMahon & Neale, 1970). In a series of six patients whose faecal fat excretion exceeded 6 g/day the

TABLE VII. Susceptibility of RBC lipids to autoxidation in blood dyscrasias

Case No.	*Age and Sex*	*Diagnosis*	*Main clinical and laboratory findings*	*2 hr MDA (nmol/g Hb)*
1	68 M	Multiple myeloma	6 mth history. Hb 12.2 g/100 ml	660, 640
2	82 F	Multiple myeloma	2 yr history. On cytotoxic therapy. Hb 11.0 g/100 ml	85, 150
3	87 F	Multiple myeloma	3 mth history. Pathological fractures. Hb 9.0 g/100 ml	200
4	72 F	Multiple myeloma	1 yr history. Renal failure. Hb 10.2 g/100 ml	440, 360
5	70 F	Chronic myeloid leukaemia	1 yr history. WBC 29 000/mm^3. Hb 13.0 g/100 ml	295, 240, 300
6	51 M	Chronic lymphatic leukaemia	2 yr history. WBC 18 000/mm^3. Hb 12.0 g/100 ml	200
7	48 F	Chronic lymphatic leukaemia	1 mth history. WBC 26 000/mm^3. Hb 11.0 g/100 ml	240
8	63 M	Acute monocytic leukaemia	1 mth history. WBC 43 000/mm^3. Hb 13.1 g/100 ml	110, 180
9	64 F	Megaloblastic anaemia	6 mth history. Serum-B_{12} <250 pg/ml. Hb 9.2/100 ml. Retics 3%	190
10	52 F	Megaloblastic anaemia	Rheumatoid arthritis: malabsorption. Serum folate <1 ng/ml. Hb 12.0 g/100 ml. Retics <1%	250
11	68 F	Megaloblastic anaemia	On chemotherapy for pulmonary tuberculosis. Serum folate <1 ng/ml. Hb 6.7 g/100 ml. Retics 1%	660
12	41 F	Megaloblastic anaemia	On anticonvulsant therapy. Serum folate 5 ng/ml. Serum-vit. B_{12} <250 pg/ml. Hb 8.0 g/100 ml. Retics <1%	550
13	85 F	Megaloblastic anaemia	6 mth history. Serum-B_{12} <100 pg/ml. Hb 5.0 g/100 ml	560
			2 mth later: on vitamin B_{12}. Hb 11.8 g/100 ml	300
			1 mth later. Hb 12.8 g/100 ml	180, 200
14	54 F	Megaloblastic anaemia	Carcinoma of stomach. Hb 8.6 g/100 ml	355
15	24 M	Aplastic anaemia	2 yr history. On testosterone. Hb 9.3 g/100 ml	125, 170
16	2 F	Erythrogenesis imperfecta	Presented at 2 mth. Abnormal tryptophane metabolism. On steroids. Hb 10.0 g/100 ml	400

plasma tocopherol and the 2 hr MDA concentration was normal in five (Although the plasma tocopherol was normal, the response to tocopherol by mouth was abnormal in four patients.)

Unselected in-patients under 60 yr. Patients known to belong to any of the groups already reviewed were excluded. The principal diagnoses were peptic ulcer, hypertension, diabetes, renal stone, chronic pancreatitis, nephrotic syndrome, salycilate overdose, myocardial infarction, virus hepatitis, rheumatoid arthritis, intracranial tumour and abdominal carcinoma.

Several patients had a mild or moderate iron-deficiency or normocytic anaemia; and two had had blood transfusions of more than 6 pints during the week preceding the assay. The essentially normal results are shown in Fig 3.

Unselected in-patients over 70 yr. The principal diagnoses in this series of consecutive admissions to the geriatric wards were: diabetes, senile dementia, carcinoma of breast, heart failure, chronic bronchitis, 'social admission', weight loss, cerebrovascular accident, arthritis, obstructive jaundice, carcinoma of the prostate and acute pulmonary infarction. The results showed a wide scatter (Fig 3).

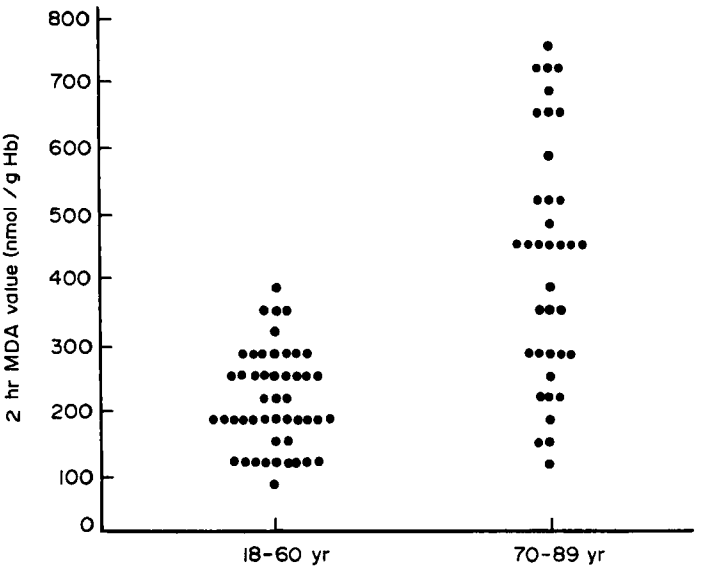

FIG 3. The 2 hr MDA results in unselected in-patients between 18 and 60 yr and above 70 yr. Known cases of haemolytic disease, severe anaemia and malabsorption were excluded.

DISCUSSION

The autoxidation of polyunsaturated lipids is an irreversible destructive process; and in tissues it may be associated with accelerated cell ageing and premature cell death (Jensen, 1950; Harman, 1956; Dormandy, 1969; Barber & Bernheim, 1967). Since such biological autoxidation is an essentially slow process (corresponding to the latent phase rather than to the autocatalytic phase of non-biological rancidification), the quantitative measurement of susceptibility to autoxidation requires standard experimental 'stress' conditions. This in turn makes it necessary to identify as far as possible the critical variables which determine the results of the assay.

Theoretically three groups of factors could account for an abnormal increase in the 2 hr MDA concentration. Firstly, the increase might be the result of influences which promote autoxidation, e.g. exposure to high-energy radiation or a high oxygen pressure. Secondly, a high 2 hr MDA value could be the expression of a high initial substrate (polyunsaturated lipid) concentration. Thirdly, a true increase in susceptibility to autoxidation could reflect an impaired antioxidant protective mechanism. The autoxidation 'stress' created by peroxide in our assay is so much greater than other factors which might promote autoxidation that the

first group of variables can probably be ignored. Polyunsaturated lipid concentration does vary in RBCs both in health and in disease (Jacob, 1967; Farquhar & Ahrens, 1963); and the possibility that some abnormally high 2 hr MDA results might reflect an increased substrate concentration cannot be dismissed. On the other hand, variations in the polyunsaturated fat content of RBCs are probably not of the same order as variations in the 2 hr MDA value. Moreover, most conditions which lead to an increase in polyunsaturated fats in tissues tend to be accompanied by a parallel increase in antioxidant activity (Horwitt, 1962). Nevertheless, until variations in polyunsaturated fat and antioxidant concentrations in normal and in abnormal RBCs have been more fully correlated, the term 'increased susceptibility to autoxidation' could be qualified by 'relative to substrate concentration'.

With these reservations it seems reasonable to interpret the 2 hr MDA results in our assay as measuring the effectiveness of the antioxidant protective mechanism of the cells. This mechanism is itself complex and multifactorial (Dormandy, 1971). Circulating specific antioxidants—e.g. vitamin E—may be important, and much direct evidence suggests that in the neonatal period their role can be limiting (Garby *et al*, 1964; Oski & Barness, 1967; Ritchie *et al*, 1968). However, as our results show, vitamin E is by no means the only factor in antioxidant protection. The structural arrangement of the polyunsaturated lipids in the cells, especially in relation to stromal proteins and haemoglobin, may be equally critical; and structural integrity in turn is dependent on normal metabolic activity.

Whatever its precise mechanism, susceptibility to autoxidation is greatly increased in most, though not in all, haemolytic states. In at least one case, moreover, this increased susceptibility was the only biochemical abnormality detected; and the abnormality persisted beyond the phase of acute haemolysis. In assessing the significance of these findings the key question is to what extent the autoxidation of polyunsaturated lipids is a link in the chain between biochemical defect and physical cell breakdown, and how far it is an incidental effect (comparable perhaps to the change in the shape of cells). The weight of evidence at present favours the former possibility; and it can be said with certainty that whereas it is possible to accelerate cell ageing without inducing lipid autoxidation, lipid autoxidation inevitably leads to cell lysis. However, it would be important to establish whether or not in subjects showing no clinical evidence of haemolysis—e.g. in a comparatively large proportion of people over 70—the increased susceptibility to autoxidation is associated with a shortened red-cell life-span. If lipid autoxidation should prove to be an immediate aetiological factor in cell breakdown it would seem reasonable to try and prevent (or delay) it in patients with haemolytic disease even when the increased susceptibility to autoxidation is not the primary biochemical abnormality. We are at present exploring this possibility in thalassaemia major and in neonatal jaundice.

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