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Plasma vitamin A and beta-carotene in retinitis pigmentosa

WAGIHA H. MASSOUD,* A. C. BIRD, AND E. S. PERKINS
From the Departments of Experimental Ophthalmology and Clinical Ophthalmology, Institute of Ophthalmology, University of London

The pathogenesis of retinitis pigmentosa as an isolated genetically determined disorder is still obscure. Whether the retinopathy is due to a systemic disorder or to an abnormality restricted to the retina is uncertain. Whilst pigmentary retinopathy has been described with multiple system diseases (Laurence and Moon, 1866; Flynn and Aird, 1965; Kearns, 1965) and progressive neurological disease (Winkelman, 1932; Jampel, Okazaki, and Bernstein, 1961; Weiner, Konismark, Stoll, and Magladery, 1967), systemic biochemical disorders have been identified in only two diseases in which pigmentary retinopathy occurs: Refsum's syndrome (Refsum, Salmonsen, and Skatvedt, 1949; Billings, O'Callaghan, and O'Day 1957; Levy, 1970) and a-betalipoproteinaemia (Bassen and Kornzweig, 1950).

Particular attention has been paid to the possible role of abnormal vitamin A metabolism because of its importance in the formation of rhodopsin. Deficiency of this vitamin in man causes retinal degeneration which can be reversed by vitamin A administration (McLaren, 1963; Norden and Stigmar, 1969; Fells and Bors, 1969, 1971), and which histologically resembles genetically determined retinitis pigmentosa (Cogan, 1950). Similarly, experimental vitamin A deprivation of rats causes degeneration of rod outer segments and finally receptor cell death. Furthermore, the pigmentary retinopathy of a-betalipoproteinaemia appears to be due to abnormalities of vitamin A metabolism (Lloyd, 1968; Sperling, Hiles, and Kennerdell, 1972).

However, therapeutic trials with vitamin A in patients with genetically determined isolated retinitis pigmentosa have given no clear indication of the role of abnormal vitamin A metabolism in the pathogenesis of this condition. The results of treatment with vitamin A appear to have impressed some investigators favourably (Town, 1951; Friede, 1952) while others have reported little therapeutic success (Levine, 1933). In 1962, an uncontrolled therapeutic trial suggested that patients with retinitis pigmentosa benefited from intramuscular and oral administration of 11-cis vitamin A, but a rigidly controlled trial by the same investigators failed to confirm this (Chatzinoff, Nelson, Stahl, and Clahane, 1968). The experience of this group illustrates the difficulties in assessing the results of uncontrolled therapeutic trials, and puts into perspective the results of previous studies on the therapeutic value of vitamin A in retinitis pigmentosa.

The results of metabolic studies have been similarly inconsistent. Campbell (1962), Campbell and Tonks (1962), and Campbell, Harrison, and Tonks (1964) reported reduced plasma levels of vitamin A in patients with retinitis pigmentosa when compared with levels in the general population, and concluded that vitamin A deficiency played a part in the pathogenesis of retinitis pigmentosa. They received support from some investigators (Shearer, 1964; Rogers, 1966; Soliman, Abboud, Osman, and Massoud, 1970), though others could not confirm the original findings (Mehra and Khare, 1965).

It should be emphasized that the workers who studied vitamin A metabolism and conducted therapeutic trials took no account of the mode of inheritance of retinitis pigmentosa in the patients studied. Since each form of retinitis pigmentosa is a distinct disease likely to have a different pathogenesis from other forms, mixed populations were studied. For this reason it is difficult to draw any definite conclusions from the data obtained by these workers.

It was the purpose of the present study to measure the plasma levels of vitamin A and of its precursor, beta-carotene, in patients with genetically determined retinitis pigmentosa.

Material

39 patients with genetically determined isolated retinitis pigmentosa were included in the study and the clinical details are shown in Table I.

In twelve patients the retinopathy was transmitted as an autosomal recessive characteristic (Cases 1–12), in

Address for reprints: A. C. Bird, F.R.C.S., Department of Clinical Ophthalmology, Moorfields Eye Hospital, City Road, London EC1V 2PD
* Present address: 10, Dr. Handoussa Street, Garden City, Cairo, Egypt

Table I *Results in 45 patients*

Case no.	Age (yrs)	Sex	Type of inheritance	Visual acuity R	Visual acuity L	ERG R	ERG L	Dark adaptation (Log. units above threshold) R	Dark adaptation (Log. units above threshold) L	Vit. A. (μg. per cent.)	beta-carotene (μg. per cent.)
1	25	F	AR	HM	6/36	–	–	–	–	73	118
2	10	M	AR	6/18	6/18	Absent	Absent	(1969) 2·5 (1970) 3	3 3	46	75
3	11	M	AR	6/60	3/60	Absent	Absent	–	–	61	77
4	9	M	AR	1/60	CF	Absent	Absent	–	–	64	65
5	44	M	AR	PL	PL	Absent	Absent	–	–	60	41
6	42	M	AR	6/12	6/12	a—20 μv b—30 μv No flicker	a—40 μv b—60 μv	3	1	54	162
7	26	M	AR	1/60	1/60	–	–	4	4	75	101
8	44	F	AR	6/12	6/9	a—40 μv b—80 μv	a—40 μv b—80 μv	N	N	61	142
9	38	F	AR	6/60	6/60	Absent	Absent	3	3	75	107
10	59	F	AR	PL	PL	–	–	–	–	66	197
11	40	F	AR	6/12	6/24	Absent	Absent	2	2	59	60
12	9	M	AR	6/60	6/60	Absent	Absent	–	–	56	129
13	38	M	AD	6/5	6/5	a—20 μv b—24 μv	a—16 μv b—24 μv	1	1	100	86
14	41	M	AD	6/24 pt	6/60	Absent	Absent	4	3·5	64	132
15	21	F	AD	3/60	6/9	a—40 μv b—60 μv	a—20 μv b—20 μv	4	3	66	113
16	24	F	AD	6/6 pt	6/9	a—10 μv b—20 μv	a—10 μv b—25 μv	1	1	64	169
17	40	F	AD	6/18	6/18	Absent	Absent	3	3	71	77
18	11	F	AD	6/9	6/9	–	Absent	2·5	2	51	92
19	6	F	AD	6/9	6/9	–	Absent	2	1·5	36	77
20	20	F	AD	6/18	6/18	Absent	Absent	3	3	56	110
21	23	F	AD	6/9	6/12	–	–	–	–	64	109
22	8	F	AD	–	–	–	–	2	1·5	51	71
23	6	F	AD	–	–	–	–	0·5 (40 min.)	0·75	56	86
24	28	M	AD	6/5	6/5	–	–	1	1·25	84	38
25	27	F	AD	6/36	6/60	Absent	Absent	4	3	69	52
26	38	F	AD	–	–	a—20 μv b—30 μv	a—40 μv b—100 μv	N	N	53	108
27	32	M	AD	2/60	6/24	Absent	Absent	3	3	48	175
28	33	F	AD	6/24	6/12					61	58
29	15	M	S	6/12	6/12	Absent	Absent	2	2	48	98
30	15	F	S	6/6	6/9	Absent	Absent	–	–	50	57
31	19	F	S	6/60	3/60	Absent	Absent	N	0·5	59	56
32	25	M	X-L	6/36	3/36	a—3·3 μv b—3·3 μv averaged	Absent	4	4	47	113
33	21	F	X-L	6/12	6/12	*1st visit* a—15 μv b—60 μv averaged *2nd visit* Absent	Absent	*1st visit* 0·75 *2nd visit* 4	0·75 4	45	160
34	19	M	X-L	6/24	3/60	Absent	Absent	4	4	69	113
35	16 17	M	X-L	6/18	6/12 pt	averaged 5 μv (2nd visit)		*1st visit* 1 *2nd visit* 2·5	0·75 2·5	74	81
36	13	F	Unknown	5/60	6/24	Absent	Absent	3	2·5	52	64
37	16	M	Unknown	6/12	6/12	Absent	Absent	4	4	79	192
38	12	M	LMBB	6/60	6/36	Absent	Absent	N	N	43	79
39	10	F	LMBB	6/18 pt	6/24	Absent	Absent	–	–	24	79
40	22	F	X-L (Heterozygote)	1/60	6/6	a—80 μv b—160 μv	a—80 μv b—200 μv	Normal		43	83
41	8	F	X-L (Heterozygote)	2/60 myope c̄ own gls.	2/60	a—60 b—120	–	2	1·75	43	77
42	21	F	X-L (Heterozygote)	6/4	6/4	N	N	N	N	69	202
43	32	F	AR (Heterozygote)	6/5	6/5	N	N	N	N	65	90
44	14	F	AR	6/4	6/4	a—160 μv b—28 μv	a—160 μv b—240 μv	N	N	53	136
45	35	F	AR (Heterozygote)	–	–	–	–	–	–	48	127

sixteen it was an autosomal dominant (Cases 13–28), and in three it was sporadic (Cases 29–31). In four patients the disease was X-linked (Cases 32–35), and the inheritance was unknown in two (Cases 36–37).

In all these patients the fundus appearance was typical of genetically determined retinitis pigmentosa.

There were also two patients with Laurence–Moon–Bardet–Biedl syndrome (Cases 38–39) and six hetero-

zygotes from families with recessive disease (Cases 40–45).

Visual loss was due to retinal degeneration in all except Case 1, who had lens opacities, and Case 40, who had a right convergent squint with amblyopia. All were patients of Moorfields Eye Hospital.

84 normal volunteers (36 males and 48 females) acted as controls; nine were below the age of 20, 47 between 20 and 40, 25 between 40 and 60, and three over 60 years old (Table II).

Table II *Vitamin A and beta-carotene plasma level in 84 control subjects*

Males (36)			*Females* (48)		
Age (yrs)	*Vit. A (μg. per cent.)*	*beta-carotene (μg. per cent.)*	*Age (yrs)*	*Vit. A (μg. per cent.)*	*beta-carotene (μg. per cent.)*
7	41	65	18	57	112
14	46	81	18	74	240
19	60	179	18	27	58
21	66	152	18	71	63
22	51	92	18	61	121
22	64	223	19	56	221
22	51	54	19	50	75
23	60	82	20	66	66
23	69	117	20	50	68
24	46	79	20	46	63
25	108	136	21	64	85
26	71	197	21	65	144
26	61	137	22	38	115
27	117	67	23	65	134
27	89	208	23	102	81
28	59	180	24	50	119
29	78	131	24	46	118
30	74	105	25	56	160
30	64	83	25	107	190
31	56	109	25	41	163
31	78	187	25	89	92
31	66	89	26	93	216
33	76	152	28	71	102
34	71	148	29	60	85
35	73	212	29	64	187
39	80	104	33	56	137
40	87	32	34	70	103
42	74	167	38	31	132
42	69	137	39	61	142
43	51	68	39	92	317
48	57	97	43	65	94
51	59	63	43	84	165
54	60	55	44	41	77
54	92	193	45	46	37
63	57	57	47	93	130
65	89	117	47	43	88
			47	46	61
			47	64	133
			48	53	111
			49	69	73
			49	48	184
			50	66	144
			51	87	111
			52	46	138
			53	93	146
			54	38	95
			58	60	119
			65	65	248

Methods

Blood was taken after a 12-hr fast, and the method of Bessey, Lowry, Brock and Lopez (1946), with the modification of Abboud, Osman, and Massoud (1968) for the vitamin A and beta-carotene assay, was used for sample analysis.

A 4-ml. sample of blood in a lithium sequestrene tube was centrifuged at 3,000 r.p.m. for 15 min., and the plasma transferred into a clean dry plastic tube and frozen immediately for storage. Any sample with haemolysis was discarded.

0·5 ml. plasma was mixed with 0·5 ml. alcoholic KOH (10 per cent) in a 100 × 12 mm. Pyrex tube and placed in a water bath at 60°C for 20 min. After it had cooled to room temperature, 1 ml. kerosene/xylene mixture was added to each tube and the tube was covered with parafilm. (Kerosene white—Hopkin and Williams; Xylene—"M & B. Histologically pure"). Extraction of vitamin A into the kerosene/xylene layer was achieved by holding the tube at an angle of 45° against a whirlimixer for 45 sec. The tubes were cooled in iced water for 30 min., allowed to return to room temperature, and centrifuged at 3,000 r.p.m. for 15 min.

0·45 ml. of the kerosene/xylene mixture was transferred into quartz microcuvettes for spectrophotometer measurement. The Perkin–Elmer 402 spectrophotometer was used and transmission was measured at 328 mμ and 460 mμ.

The sample was compared with a blank which was prepared in the same way, except that 0·5 ml. distilled water was added in the place of plasma.

The mixture was transferred into silica tubes with Teflon stoppers and irradiated with an ultraviolet lamp (Mazda 125-watt high-pressure mercury lamp MBW/U) for 1 hr. The solution was returned to clean quartz microcuvettes and their optical density at 328 mμ re-measured.

Results

The levels of blood plasma vitamin A and beta-carotene in the patients are presented in Table I and those of the controls in Table II. On '*t*'-test no significant difference was found when the following groups were compared:

Patients and controls (Table III)
Patients and controls of similar ages (Table III)
Patients with autosomal dominant and autosomal recessive disease
Patients with autosomal dominant and X-linked disease
Patients with autosomal recessive and X-linked disease
Adult male controls and adult female controls

Comment

This work has failed to find any evidence that systemic vitamin A deficiency plays a role in the pathogenesis of genetically determined isolated retinitis pigmentosa. The failure to demonstrate any difference in plasma levels of vitamin A and beta-carotene between patients with retinitis pigmentosa and controls supports the observations of some workers (Hubbard, 1956; Chatzinoff and others, 1968; Mehra and Khore, 1965), but is at variance with others (Campbell, 1962; Campbell and Tonks, 1962; Campbell and others, 1964; Soliman and others, 1970).

Campbell and Tonks showed persistently low levels of vitamin A in patients with pigmentary retinal degeneration when compared with a control group. Their results for vitamin A levels in both patients (82 i.u./100 ml.) and controls (114 i.u./100 ml.)

Table III *Pathological "vitamin A"*

Comparisons	*Pathological v. normal*	*Patients*		*Controls*		*Standard error*	*'t' values*	*Significance*
		n_1	y_1	n_2	y_2		*Calculated*	
Males	Young (below 20 yrs)	7	61·143	3	49·000	7·680	1·581	$0{\cdot}05 < P < 0{\cdot}10$
	Adult (20 to 40 yrs)	5	67·800	23	70·783	8·550	0·349	$0{\cdot}35 < P < 0{\cdot}40$
	Adult (40 to 60 yrs)	3	59·333	8	68·625	8·500	1·093	$0{\cdot}15 < P < 0{\cdot}20$
Females	Young (below 20 yrs)	6	47·500	6	55·833	7·068	1·179	$0{\cdot}10 < P < 0{\cdot}15$
	Adult (20 to 40 yrs)	12	60·250	24	64·333	6·011	0·679	$0{\cdot}25 < P < 0{\cdot}30$
	Adult (40 to 60 yrs)	6	63·333	17	61·294	7·835	0·260	$0{\cdot}35 < P < 0{\cdot}40$
Total		39	59·821	84	64·952	3·182	1·613	$0{\cdot}05 < P < 0{\cdot}10$

were lower than those of other studies. This can probably be accounted for by their use of the relatively insensitive antimony trichloride method which has many disadvantages when compared with spectrophotometry (Abboud and others, 1968). In addition, their control values are those of the Medical Research Council and not their own. Soliman and others (1970) used the same techniques as in this study, and in patients with retinal degeneration they found reduced plasma levels of vitamin A at a low level of significance (1 per cent.) and a highly significant reduction in beta-carotene. It is possible that a significant number of their patients had liver disease and no account was taken of dietary factors. Their patients were not restricted to those with genetically determined disease, and some may indeed have had severe vitamin A deficiency and secondary retinal degeneration.

Summary

Plasma levels of vitamin A and beta-carotene were measured by spectrophotometry in 39 patients with genetically determined retinitis pigmentosa, No difference was found between the levels in these patients and the levels in a control group.

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