COENZYMES I AND II IN THE HUMAN EPIDERMIS*

CHANGES IN NUTRITIONAL SUN-SENSITIVITY (PELLAGRA) AND DURING CYTOSTATIC DRUG THERAPY

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The commonest sun eruption in South Africa is pellagra. Although a niacin deficiency in pellagra is clearly not the whole story, it suggests a means by which nutritional and photodynamic influences may reinforce one another.

Nicotinamide is known to be built by cell nuclei into the pyridine nucleotides, coenzymes I (DPN) and II (TPN), which are essential in the cellular respiratory chain. These coenzymes are not only altered by ultraviolet light, but are influenced by free radicals resulting from the action of light. Other parts of the respiratory chain are also influenced by diet, light and sun-sensitizing drugs. It therefore seems that altered intracellular respiration may offer a pathway for the action of light in vitamin-deficiency states, thus bringing about abnormal changes in epidermal cells.

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Since nucleic acid synthesis is affected by cytostatic drugs, a few observations were made to test their action on pyridine nucleotide synthesis in the epidermis. These are also briefly reported, although the changes are obscure.

METHODS

Only the oxidized phosphopyridine nucleotides were assayed. These represent DPN and TPN, coenzyme III, and such precursor substances as also yield fluorescent products in the technique used. No enzymatic separation of these components was attempted.

Skin assays were made from samples of whole-thickness epidermis removed with a 3 mm. Volkmann spoon under procaine anaesthesia. These epidermal samples were blotted gently in the spoon, removed as a 'plug', weighed wet without mincing, and homogenized at once. Roughly 10 mg. epidermis could be obtained from 1 square cm. of

skin. Oxalated venous blood was used for the assay on whole blood. All assays were performed promptly on collecting the specimens.

The method used was the methyl ethyl ketone fluorimetric assay of Kaplan and Ciotti,[1] adapted to the Eel fluorimeter. The fluorimeter was calibrated over the range 0 - 4 μg. DPN, using DPN (Sigma Chemical Co.) of strength assayed independently by the cyanide addition procedure[1] on the ultraviolet spectrophotometer. Quinine sulphate in 0·1 N. H_2SO_4 was then used as a stable substitute for the DPN standard in concentrations yielding equal fluorescence. Duplicate assays were made throughout and were usually within 0·6 μg. per g. or 0·6 μg. per ml. or less, of one another.

RESULTS

Assays of oxidized pyridine nucleotides in skin and/or blood were performed on 48 patients, the results of which appear in the tables. All blood values are expressed as equivalents of DPN in μg. per ml., corrected to a hematocrit of 45%. Skin values are likewise equivalents of DPN in μg. per g. moist weight.

Blood Values

In White and Bantu patients without pellagra (cases 1 - 23), the blood values of pyridine nucleotides in the Bantu patients were on a rough average 25% lower than those in White patients, though still within the accepted normal range. The lowest values were found in 3 Bantu patients (cases 21 - 23) who were sick but showed no pellagra. The arithmetical average of the Bantu values (cases 7 - 23) was about 30 μg. per ml., while the average in a few White patients (cases 1 - 6) was 40 μg. per ml. In 13 Bantu patients with pellagra (cases 24 - 28, 33, 35 - 41) who were not critically ill and who are presumed to have had no vitamin treatment, the average value was 34 μg. per ml. Making allowances for the sampling errors in both groups, one may say that the blood values in the Bantu subjects are not only roughly normal, but are also roughly the same whether they suffer from pellagra or not.

Skin Values

The skin values of pyridine nucleotide showed a greater range of variation. In 7 White patients who had had no vitamins (cases 1 - 6, 47) the values lay between 36 and 120 μg. per g. In one non-pellagrous Bantu subject studied (case 48) the values also fell largely within this range. No other non-pellagrous Bantu cases were studied, if we exclude the values obtained on the unaffected skin

TABLE I. NORMAL AND ABNORMAL SKIN AND BLOOD VALUES OF PYRIDINE NUCLEOTIDES FROM WHITE PATIENTS

Case	*Test*	*Sex*	*Age*	*Blood (μg./ml.)*	*Skin (μg./g.)*	*Remarks*
1	a	F	47	40·9	36·1	Atopic eczematous skin, back.
	b				36·0	Normal skin, back.
2		F	18	54·9	110	Normal skin, shoulder.
3		M	29	41·7	67	Normal skin, back.
4		F	52	37·6	70·5	Normal skin, arm.
5		F	51	37·4	64·0	Normal skin, shoulder.
6	a	M	77	34·6	61·0	Non-scaly psoriatic skin.
	b				14·5	Dry, exfoliated, psoriatic scalp scales.

TABLE II. BLOOD LEVELS OF PYRIDINE NUCLEOTIDES IN NON-PELLAGROUS BANTU SUBJECTS

Case	*Sex*	*Age*	*Blood (μg./ml.)*	*Diagnosis and remarks*
7	F	25	36·0	Pityriasis rubra pilaris.
8	M	8	36·2	Pityriasis rubra pilaris.
9	M	31	30·9	Stomatitis — ? type.
10	M	16	28·1	Lichen planus.
11	M	10	25·0	Eczema.
12	F	50	33·5	Lues III.
13	F	31	35·4	Thyrotoxicosis.
14	F	40	33·8	'Lodger'.
15	M	45	36·3	Seborrhoeic eczema.
16	F	14	28·2	Otitis externa.
17	F	40	30·9	Cardiac failure.
18	F	28	43·2	Not pellagra.
19	M	16	29·9	Not pellagra.
20	M	24	25·4	Colles fracture.
21	F	31	15·2	Gastro-enteritis — ? type.
22	M	45	17·4	Pneumonia — convalescent.
23	F	21	21·8	Acute salpingitis.

TABLE III. BLOOD LEVELS OF PYRIDINE NUCLEOTIDES IN PELLAGROUS BANTU SUBJECTS

Case	*Sex*	*Age*	*Blood (μg./ml.)*	*Remarks*
24	M	40	28·3	Untreated pellagra with dementia.
25	M	26	32·1	Untreated pellagra with dementia.
26	F	45	31·5	Pellagra with Riehl-like picture. No vitamins given for past 3 months.
27	M	19	26·4	Untreated pellagra. Peri-oral 'mudpack'.
28	F	30	28·8	Untreated pellagra.
29	M	8	38·2	Active pellagra, possibly treated.
30	F	27	49·3	Active pellagra with dementia, under treatment.
31	F	30	55·5	Active pellagra, under treatment.
32	M	40	53·7	Subsided pellagra, under treatment.

TABLE IV. PYRIDINE NUCLEOTIDE LEVELS IN THE BLOOD, AND THE NORMAL AND ABNORMAL SKIN FROM PELLAGROUS BANTU SUBJECTS

Case	*Test*	*Sex*	*Age*	*Blood (μg./ml.)*	*Pellagrous skin (μg./g.)*	*Adjoining non-pellagrous skin (μg./g.)*	*Remarks*
33		M	43	33·8	63	68	Non - keratotic pellagrous skin from extensor surface, forearm.
34		F	42		42	49	Forearm skin.
35		F	20	41·9	32·5	53·4	Recent case. Forearm skin.
36		M	16	32·0	27·5	28	Forearm skin. Not keratotic.
37		F	10	35·9	27·9	31·1	Forearm. 1 month duration. Non-keratotic skin.
38		M	22	28·3	24	30	Pellagra with dementia. Forearm skin.
39		M	60	33·4	36	49	Ordinary pellagra.
40		F	39	50·7	34·8	40·9	Pellagra. 3 months' duration.
41	a	M	42	48·5		29·8	Extensor surface forearm.
	b					30	Flexor surface forearm.
42		M	45	47·6	36·1	37·9	Pellagra dementia.
43		M	35		191	219	Several weeks' vitamin therapy. Skin lesions very evident.
44	a	M	?35	39·4	168	169·4	Several hours' intravenous vitamin-B complex administration. Pellagra with encephalopathy.
	b			45·3	133·2	121·3	5 days later. No further intravenous vitamin therapy. Skin lesions still evident.

TABLE V. BLOOD AND SKIN PYRIDINE NUCLEOTIDE VALUES FROM PATIENTS UNDERGOING TREATMENT WITH CYTOSTATIC DRUGS

Case	Test	Sex	Age	Race	Blood (μg./ml.)	Skin (μg./g.)	Remarks
45	a	M	62	W	33·7	100	Cancer of stomach. Gastrectomy 10 days before with blood transfusion and vitamin-B complex. Shoulder skin. No cytostatics.
	b				33·8	89·6	After 1 week 5-fluorouracil. Treatment produced mouth ulcers. Shoulder skin.
46	a	M	50	W	31·3	178·3	Cancer of pancreas. Vitamins up to 1 week before. Shoulder skin.
	b				35·7	183·5	After 5 days' treatment with 5-fluorouracil. No side reactions. Shoulder skin.
47	a	F	72	W	29·2	120	Hodgkin's disease. Before endoxan treatment. No vitamins. Shoulder skin.
	b				35·6	79·3	After 5 days' endoxan treatment.
	c				36·5	33·5	After 9 days' endoxan treatment.
	d				40·1	58·4	After 11 days' endoxan treatment.
	e				38·3	62·1	After 16 days' endoxan treatment.
	f				31·1	35·7	After 20 days' endoxan treatment.
48	a	M	19	B	39·2	58·3	Hodgkin's disease. Before endoxan treatment. Shoulder skin. No vitamins. Ward diet, as given at non-European hospital.
	b				45·0	84·2	After 5 days' endoxan treatment.
	c				40·6	90·4	After 10 days' endoxan treatment.
	d				34·5	144·6	After 16 days' endoxan treatment.

of pellagrous Bantu subjects. The untreated pellagra patients (cases 33 - 42) showed generally low values in the skin by comparison, although values which did not differ greatly were found in non-pellagrous White subjects (cases 1a and b, 47c and f) as well. Comparing the levels in pellagrous skin with those of the unaffected skin nearby in the same subject, the pellagrous skin showed values about 15% less than the normal skin. This was probably not fortuitous, though one could ascribe it to dilution of the sample by scale or oedema, or variations according to site. The following observations suggest that the difference is important. Thus, case 1 (a and b) showed the same value for eczematous and non-eczematous skin; case 6 showed a normal value in abnormal skin; psoriatic scale (case 6b) despite dessication showed values of a quarter of the wet weight of whole epidermis with little scale (case 6a); case 41 (a and b) showed that the aspect of the forearm chosen for sampling did not influence the value obtained. Moreover, many of the pellagrous lesions were not notably scaly. From these considerations we may say that the skin value of pyridine nucleotides is low in pellagra, and lower in pellagrous skin as opposed to uninvolved skin in the same person, but that a low value as such is found in non-pellagrous subjects as well where there is no reason to suppose that they will develop pellagrous dermatitis.

The present study offers a few indications regarding the rate, extent and ability of the skin to synthesize pyridine nucleotides. Firstly it appears that severe illness does not interfere with the ability to use nicotinamide for nucleotide synthesis, and abnormal skin may do this without difficulty (cases 6 and 46). Secondly, case 46 shows that the levels readily rise above normal values on giving vitamin-B complex. Thirdly, cases 43 and 44 show that these high values are obtained in the skin in pellagra without any difficulty after nicotinamide administration, and that no obvious block in nicotinamide utilization exists. Fourthly, the same cases show that the skin can be loaded with more than sufficient vitamin within half a day if necessary, but that the lesions may still take a few weeks to heal.

Cytostatic Drugs

Four experiments were made to see if cytostatic drugs influenced the level of pyridine nucleotides in blood and skin. 5-fluorouracil in cases 45 and 46 appeared to have no influence despite toxic action on the oral mucosa in one (45b). Endoxan is known to affect the epidermis greatly in certain cases,[2] though it failed to do so visibly in cases 47 and 48 which were investigated in this study. The blood levels showed a slow rise and fall over a 3-week period in both cases but the skin changes could not be superimposed in the 2 cases, and the fluctuations observed are still obscure.

SUMMARY

Oxidized pyridine nucleotides which contain nicotinamide were assayed in the skin and blood. The values in the solar dermatosis of pellagra indicate that the level drops slightly in pellagrous skin and is generally lower than normal in this type of malnutrition. However, the ability to synthesize these coenzymes was evidently still normal, though the onset and cure of a pellagrous dermatosis cannot be clearly linked to the level of niacin-containing coenzymes in the skin. Cytostatic drugs influence the coenzyme levels in ways that cannot be interpreted at present.

REFERENCES

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