PAPERS AND SHORT REPORTS

Reversible inhibition of leucocyte sodium pumps by a circulating serum factor in essential hypertension

VERNON M S OH, ELIZABETH A TAYLOR

Abstract

To test whether leucocyte sodium pumps function abnormally in patients with essential hypertension specific tritium-ouabain binding (number of pumps) and ouabain sensitive uptake of rubidium-86 ($^{86}\text{Rb}^+$) (transport activity) were measured in mononuclear leucocytes from 37 untreated hypertensive patients and 85 normotensive subjects. Ouabain binding was lower and transport activity per binding site higher in the hypertensive patients before incubation ($p < 0.001$), but both variables were normal after incubation for 72 hours with fetal calf serum. To determine whether a circulating inhibitor of sodium pumps was present in patients with hypertension ouabain binding and $^{86}\text{Rb}^+$ uptake were measured in normal leucocytes before and after incubation for 72 hours with serum from 13 untreated hypertensive patients and 18 normotensive subjects. Ouabain binding was lower after incubation of cells with serum from hypertensive patients than after incubation with normal serum both before ($p < 0.01$) and after ($p < 0.001$) dialysis of the serum.

The results suggest that in hypertension a circulating serum inhibitor of the sodium pump causes a chronic but reversible reduction in the number of pumps.

Introduction

Defective transport of cations across plasma membranes in essential hypertension is associated with an increase in the sodium ion concentration in leucocytes.¹⁻³ This abnormally high concentration might be due to (a) intrinsically impaired function of Na^+/K^+ adenosinetriphosphatase (ATPase) (the sodium pump); (b) inhibition of functionally normal sodium pumps by a circulating serum factor^{4,5}; or (c) a combination of (a) and (b).

We have shown that incubation of human leucocytes with fetal

calf serum or human serum in vitro enhances both specific tritium (^3H)-ouabain binding and ouabain sensitive uptake of rubidium-86 ($^{86}\text{Rb}^+$) proportionately.⁷ Table I shows the values predicted by the three hypotheses for specific ^3H -ouabain binding and ouabain sensitive $^{86}\text{Rb}^+$ uptake in mononuclear leucocytes from hypertensive patients before and after incubation with serum. Moreover, if hypothesis (b) were true and the serum of hypertensive patients contained a sodium pump inhibitor specific ^3H -ouabain binding would be lower in normal leucocytes after incubation with serum from hypertensive patients than after incubation with serum from healthy normotensive subjects.

We therefore carried out a study in two parts. Firstly, we measured specific ^3H -ouabain binding and ouabain sensitive $^{86}\text{Rb}^+$ uptake in intact mononuclear leucocytes from patients with untreated essential hypertension and from healthy normotensive subjects before and after incubation with a standard fetal calf serum in vitro. Secondly, we measured specific ^3H -ouabain binding in normal mononuclear leucocytes before and after incubation with undialysed and dialysed serum from patients with untreated essential hypertension and from healthy normotensive subjects.

Subjects and methods

In experiment 1a we studied 37 consecutively recruited patients with untreated essential hypertension and 85 healthy normotensive volunteers. Whenever possible the normotensive subjects chosen were of the same sex and race as the hypertensive patients and were of similar age, height, and body weight (table II). In experiment 1b we studied the first 23 patients with essential hypertension and the first 18 normotensive subjects recruited for experiment 1a. In experiment 2 we studied 13 consecutively recruited hypertensive patients and 18 similarly matched normotensive subjects (table II). All subjects gave informed consent.

Blood pressure (Korotkoff phase V diastolic) was measured on three occasions seven days apart with a Hawksley random zero sphygmomanometer after subjects had been lying supine for five minutes. Mean arterial pressure was calculated as the sum of one third of the systolic pressure and two thirds of the diastolic pressure. All the volunteers and their first degree relatives had resting blood pressures $< 140/90$ mm Hg. All the patients had resting blood pressures $\geq 140/90$ mm Hg; none had any clinical or biochemical evidence of renal disease; and all had normal electrocardiograms, chest and abdominal x ray films, intravenous urograms, and 24 hour urinary excretion of vanillylmandelic acid. None of the subjects smoked; for two weeks before, and during, the study they refrained from taking caffeine, alcohol, and other drugs.

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In experiment 1a we measured specific ^3H -ouabain binding in freshly isolated mononuclear leucocytes from the 37 patients and 85 normotensive subjects. In experiment 1b we incubated cells from the first 23 patients and the first 18 matched normotensive subjects for 72 hours in RPMI 1640 culture medium (Gibco) containing 10% vol/vol fetal calf serum. We measured specific ^3H -ouabain binding and ouabain sensitive $^{86}\text{Rb}^+$ uptake before and after incubation without knowledge of the blood pressure. We wished to allow enough time for a putative inhibitor to dissociate extensively from the sodium pumps but to maintain cell numbers and viability. As the half life of dissociation of ouabain from leucocyte binding sites is about seven hours,⁶ we chose a period of more than eight half lives—namely, 72 hours. Because fetal calf serum stimulates specific ^3H -ouabain binding and ouabain sensitive $^{86}\text{Rb}^+$ uptake in a manner dependent on concentration we chose 10% vol/vol fetal calf serum to produce only a moderate increase in specific ^3H -ouabain binding and ouabain sensitive $^{86}\text{Rb}^+$ uptake.

In experiment 2 we incubated seven batches of cells from four normotensive volunteers for 72 hours in media containing 90% vol/vol RPMI and 10% vol/vol serum from 13 patients with untreated essential hypertension and 18 normotensive subjects. We measured specific ^3H -ouabain binding before and after incubation. In three studies we also measured specific ^3H -ouabain binding before and after incubation with dialysed serum from six patients with untreated essential hypertension and 10 normotensive subjects. We dialysed serum from both hypertensive patients and normotensive subjects to determine whether a difference in specific ^3H -ouabain binding found in experiment 1 might be due to serum from normotensive subjects containing more of the dialysable factor that enhances specific ^3H -ouabain binding during culture.⁷ We incubated cells for 72 hours to allow equilibration in the binding of a slowly associating inhibitor, if present, to the leucocyte sodium pumps. Specific ^3H -ouabain binding was measured without knowledge of the subjects' blood pressures. We also measured the potassium ion concentration in the serum of the 13 patients and 18 normotensive subjects.

Preparation, culture, and counting of leucocytes—Mononuclear leucocytes were isolated from 40–80 ml venous blood by centrifugation on Ficoll and sodium diatrizoate as described elsewhere.⁸ Sterile materials and aseptic technique were used throughout. The final cell suspension contained 99% mononuclear leucocytes, of which 87% were lymphocytes. The leucocytes were incubated in RPMI containing 10% vol/vol human serum or fetal calf serum for 72 hours at 37°C in a humidified atmosphere of 95% air and 5% carbon dioxide. The final cell density was about 10^6 leucocytes/ml culture medium. Cell viability was assessed by the trypan blue exclusion test and was always >97% after isolation and >92% after incubation of the cells. After culture cells were washed once with potassium free Ringer or RPMI and specific ^3H -ouabain binding and ouabain sensitive $^{86}\text{Rb}^+$ uptake were measured again. The leucocytes were then counted with a Coulter counter (model ID).

Serum—Human serum, obtained from 18 normotensive subjects and 13 patients with untreated essential hypertension, was stored at -70°C for up to 14 days before use. We used only one batch of fetal calf serum which had

been inactivated by heat for 30 minutes at 56°C by the manufacturer (Gibco). We dialysed serum across a Visking membrane with a molecular weight threshold of about 10 000 for 48 hours at 4°C against four changes of 5 litres each of distilled and deionised water. Before use human serum was passed through a Millex-GS filter (Millipore) of $0.22\text{ }\mu\text{m}$ pore size.

Measurement of specific ^3H -ouabain binding—We measured specific ^3H -ouabain binding by incubating leucocyte suspensions with 25 nmol specific ^3H -ouabain/l (14.6 ng/ml) (Amersham, UK) in potassium free Ringer for two hours at 37°C as described elsewhere.⁸ Specific ^3H -ouabain binding was defined as the total ^3H -ouabain bound minus the amount bound in the presence of digoxin $5\text{ }\mu\text{mol/l}$ ($3.9\text{ }\mu\text{g/ml}$).

Measurement of ouabain sensitive $^{86}\text{Rb}^+$ uptake—We estimated influx of potassium ions using $^{86}\text{Rb}^+$ as a tracer. $^{86}\text{RbCl}$ (Amersham, UK) was added to each Eppendorf tube (final concentration $8.3\text{ }\mu\text{mol/l}$ (709 ng/ml)), together with either RPMI alone or RPMI containing ouabain at a final concentration of $100\text{ }\mu\text{mol/l}$ ($58.5\text{ }\mu\text{g/ml}$), to identify total and ouabain sensitive $^{86}\text{Rb}^+$ uptake respectively. Incubation was started by adding cells in RPMI to each tube, which was then vortex mixed and placed in a water bath at 37°C . The final medium contained 4.48 mmol (mEq) potassium ions/l in each case. Incubation was stopped after one hour by centrifugation at 8800 g. The cells were quickly washed three times with potassium free Ringer at 4°C and lysed with distilled water. Finally, Cerénkov radiation was counted for 10 minutes in a liquid scintillation counter.⁷

Measurement of serum potassium ion concentration—Potassium ion concentration in the serum was measured in duplicate using an ion selective catharometer (Beckman Astra 8).

Statistical assessment—In experiment 1 we compared blood pressure, age, sex, weight, height, specific ^3H -ouabain binding, and ouabain sensitive $^{86}\text{Rb}^+$ uptake between groups using Student's *t* test for unpaired data. The relations between specific ^3H -ouabain binding and age, weight, mean arterial pressure, and ouabain sensitive $^{86}\text{Rb}^+$ uptake were tested by least squares regression. We analysed percentage changes in specific ^3H -ouabain binding and ouabain sensitive $^{86}\text{Rb}^+$ uptake to show changes in the proportions of binding sites occupied. In experiment 2 we used analysis of variance to compare both absolute and percentage changes in specific ^3H -ouabain binding after incubation. Percentage values were first subjected to angular transformation in both experiments.

Results

EXPERIMENT 1a

Mean (SD) specific ^3H -ouabain binding, representing the number of sodium pumps, was 49.7 (12.4) fmol (29.1 (7.25)pg)/ 10^6 cells in the patients with essential hypertension and 64.8 (13.3) fmol (37.9 (7.78)pg)/ 10^6 cells in the normotensive subjects ($p<0.001$). The differences between the groups in age, weight, and height were not significant (table II). Age and weight did not correlate with specific ^3H -ouabain binding in either group.

TABLE I—Values* of specific ^3H -ouabain binding (OB), ouabain sensitive $^{86}\text{Rb}^+$ uptake (RU), and RU/OB in mononuclear leucocytes from patients with essential hypertension predicted by three hypotheses

Hypothesis	Before incubation			After incubation		
	No of pumps (OB)	Transport activity (RU)	Transport activity/pump (RU/OB)	No of pumps (OB)	Transport activity (RU)	Transport activity/pump (RU/OB)
(a)	Normal or increased	Decreased	Decreased	Normal	Decreased	Normal
(b)	Decreased	Normal or increased	Increased	Normal	Normal	Normal
(c)	Decreased	Decreased	Decreased	Normal	Decreased	Decreased

*Expressed relative to values in healthy normotensive subjects.

TABLE II—Biological data on subjects. Values are means (SD)

Subjects	No	Age (years)	Sex	Body weight (kg)	Height (m)	Resting blood pressure (mm Hg)	Race
<i>Experiment 1a</i>							
Hypertensive	37	44.2 (15.0)	21M; 16F	65.4 (9.3)	1.66 (0.10)	166/106 (12.9)*	30 Chinese; 7 Indian
Normotensive	85	38.1 (13.5)	54M; 31F	63.6 (8.5)	1.70 (0.08)	113/73 (11.6)	74 Chinese; 11 Indian
<i>Experiment 1b</i>							
Hypertensive	23	45.5 (14.8)	13M; 10F	64.6 (9.6)	1.64 (0.05)	163/103 (14.10)*	19 Chinese; 4 Indian
Normotensive	18	40.0 (13.2)	12M; 6F	61.9 (8.2)	1.68 (0.09)	116/74 (10.7)	14 Chinese; 4 Indian
<i>Experiment 2</i>							
Hypertensive	13	44.8 (14.3)	9M; 4F	69.4 (10.7)	1.72 (0.12)	172/108 (14.8)*	7 Chinese; 6 British
Normotensive	18	39.1 (12.4)	13M; 5F	66.8 (8.6)	1.69 (0.07)	114/71 (10.7)	8 Chinese; 10 British

* $p<0.001$, Student's *t* test for unpaired data; group differences in all other variables were not significant.

EXPERIMENT 1b

Before incubation mean specific ³H-ouabain binding was significantly lower (*p*<0.02) in leucocytes from 23 patients with essential hypertension than in leucocytes from 18 normotensive subjects (fig 1, table III). After incubation with fetal calf serum mean specific ³H-ouabain binding was similar in the two groups. The mean percentage increase in specific ³H-ouabain binding was greater in cells from the patients with essential hypertension than from the normotensive subjects. The percentage increase in specific ³H-ouabain binding during incubation was directly proportional to mean arterial pressure in the patients with essential hypertension (*r*=0.63, *p*<0.002) but not in the normotensive subjects (*r*=0.16, NS) (fig 2). Similar results were obtained in both experiments 1a and 1b when matched Chinese and Indian groups were compared separately (not shown).

Mean ouabain sensitive ⁸⁶Rb⁺ uptake (transport activity) was similar in the patients and normotensive subjects before and after incubation (table III), as was the mean percentage increase in ouabain sensitive ⁸⁶Rb⁺ uptake during incubation.

The mean ratio of ouabain sensitive ⁸⁶Rb⁺ uptake to specific ³H-ouabain binding, an estimate of transport activity/sodium pump, was significantly higher (*p*<0.05) before culture in cells from patients with essential hypertension than from normotensive subjects (table III); after culture it had decreased to a normal value in the patients with essential hypertension and was unchanged in the normotensive subjects.

Mean ouabain sensitive ⁸⁶Rb⁺ uptake was higher in women than men with

FIG 1—Experiment 1b: Mean specific ³H-ouabain binding to leucocytes from 23 patients with untreated essential hypertension and 18 healthy normotensive subjects before and after incubation with fetal calf serum.
Conversion: SI to traditional units—Ouabain 1 fmol≈0.59 pg.

TABLE III—Experiment 1b: specific ³H-ouabain binding (OB), ouabain sensitive ⁸⁶Rb⁺ uptake (RU), and RU/OB in leucocytes from untreated hypertensive patients and normotensive subjects before and after incubation. Values are means (SD)

	Before incubation			After incubation		
	Hypertensive patients (n=23)	Normotensive subjects (n=18)	Significance	Hypertensive patients (n=23)	Normotensive subjects (n=18)	Significance
OB (fmol/10 ⁶ cells)	51.0 (14.4)	61.9 (13.2)	<i>p</i> <0.02	75.0 (28.7)	74.3 (22.4)	NS
RU (pmol/h.10 ⁶ cells)	9.9 (2.0)	10.1 (3.4)	NS	11.3 (3.5)	11.0 (3.2)	NS
RU/OB (⁸⁶ Rb ions/pump h)	214.0 (66.3)	167.0 (48.5)	<i>p</i> <0.05	158.0 (47.8)	161.0 (68.3)	NS
% Change in OB				46.2 (27.8)	23.0 (26.3)	<i>p</i> <0.01
% Change in RU				17.1 (40.8)	15.0 (38.6)	NS
% Change in RU/% change in OB				0.03 (1.2)	2.68 (5.9)	<i>p</i> <0.01

Conversion: SI to traditional units—Ouabain: 1 fmol≈0.59 pg. Rubidium: 1 pmol≈85.5 pg.

essential hypertension (10.9 (SD 1.93) *v* 9.11 (1.81) pmol/h.10⁶ cells) (932 (165) *v* 779 (155) pg/h.10⁶ cells (*p*<0.05) but similar in normotensive men and women.

In both groups of subjects ouabain sensitive ⁸⁶Rb⁺ uptake was directly proportional to specific ³H-ouabain binding. The linear regression of ouabain sensitive ⁸⁶Rb⁺ uptake (*y*) on specific ³H-ouabain binding (*x*) was *y*=0.133*x*+4.43 (*r*=0.70, *p*<0.002) for cells from patients with essential hypertension, and *y*=0.090*x*+4.82 for cells from normotensive subjects (*r*=0.64, *p*<0.01).

FIG 2—Mean arterial pressure in hypertensive patients plotted against percentage increase in specific ³H-ouabain binding in their leucocytes after 72 hours' incubation. Least squares regression: *r*=0.63, *p*<0.002.

EXPERIMENT 2

Mean specific ³H-ouabain binding in normal leucocytes was lower after incubation with serum from patients with essential hypertension than with serum from normotensive subjects (table IV). The changes in specific ³H-ouabain binding during incubation were significantly smaller with serum from patients with essential hypertension than with serum from normotensive subjects, both when all hypertensive patients were compared with all normotensive subjects and when data on Chinese and British sera were assessed separately (not shown).

Both groups of serum were unable to stimulate specific ³H-ouabain binding after dialysis. None the less, mean specific ³H-ouabain binding was reduced significantly more by incubation with dialysed serum from patients with essential hypertension than with dialysed serum from normotensive subjects (*p*<0.001) (table IV).

The mean (SD) potassium ion concentrations in the serum from the patients with essential hypertension and the normotensive subjects before incubation were similar (4.10 (0.36) and 4.19 (0.35) mmol/l respectively, NS).

Discussion

The results of experiment 1 show that the intact mononuclear leucocytes of patients with essential hypertension have on average fewer sodium pumps (lower mean specific ³H-ouabain binding) than

TABLE IV—Experiment 2: specific ³H-ouabain binding in normal mononuclear leucocytes before and after incubation with undialysed or dialysed serum from untreated hypertensive patients and normotensive subjects. Values are means (SD)

	Before incubation	After incubation			
		With undialysed serum		With dialysed serum	
		From hypertensive patients (n=13)	From normotensive subjects (n=18)	From hypertensive patients (n=6)	From normotensive subjects (n=10)
Specific ³ H-ouabain binding (fmol/10 ⁶ cells)	49.7 (4.0)	68.9 (15.0)	75.3 (11.3)	29.4 (16.7)	41.1 (17.4)
Mean % change from preincubation value		25.5 (32.5)	39.5 (34.7)	-38.6 (39.4)	-14.5 (42.4)
Analysis of variance:					
Absolute values			p<0.05		p<0.001
% Values after angular transformation			p<0.01		p<0.001

Conversion: SI to traditional units—Ouabain: 1 fmol=0.59 pg.

normal cells. Mean specific ³H-ouabain binding was similar in cells from patients with essential hypertension and normotensive subjects after 72 hours' culture in the presence of fetal calf serum, showing that the sodium pumps adapted by increasing in number during incubation (fig 1, table III). These data are consistent with hypothesis (b)—namely, that in essential hypertension a circulating substance competitively inhibits Na⁺/K⁺-ATPase. They are also consistent with the finding of fewer ouabain binding sites in the hearts and brains of spontaneously hypertensive rats.⁹

The greater increase in specific ³H-ouabain binding in leucocytes from patients with essential hypertension than in leucocytes from normotensive subjects during culture may have been due to more of the putative inhibitor dissociating from cells from the patients with essential hypertension (fig 3). Alternatively, the cells from patients with essential hypertension may have produced more new binding sites during culture. The percentage increase in specific ³H-ouabain binding during culture and, by extension, the proportion of inhibited sodium pumps before culture varied directly with the mean arterial pressure of the hypertensive patients. In another study the mean arterial pressure of patients with essential hypertension and normotensive subjects directly correlated with the plasma concentration of an inhibitor of Na⁺/K⁺-ATPase partially purified from canine kidney.⁶ Figure 3 shows the proposed mechanism that is supported by these data.

FIG 3—Schematic diagram showing most likely explanation for increase in mean specific ³H-ouabain binding to leucocytes from hypertensive patients and normotensive subjects during incubation with fetal calf serum for 72 hours.
Y = Sodium pump (specific ³H-ouabain binding site). Δ = Inhibitor molecule. Y = New sodium pump.

The mean transport activity of sodium pumps (ouabain sensitive ⁸⁶Rb⁺ uptake) was similar in leucocytes from patients with essential hypertension and normotensive subjects before culture (table III). After culture the enhanced activity in both groups was also similar. The mean activity per pump (ratio of ouabain sensitive ⁸⁶Rb⁺ uptake to specific ³H-ouabain binding) in leucocytes of patients with essential hypertension was increased before culture but normal afterwards, suggesting that transport activity per pump is chronically but reversibly up regulated in these patients. In the absence of a pump inhibitor the usual homeostatic response of the pump

to a raised intracellular sodium ion concentration is to increase transport.¹⁰ In the presence of the inhibitor, however, net ouabain sensitive ⁸⁶Rb⁺ uptake cannot increase because there are fewer free (unblocked) pumps. The results for ouabain sensitive ⁸⁶Rb⁺ uptake are therefore consistent with hypothesis (b). The finding that ouabain sensitive ⁸⁶Rb⁺ uptake varies directly with specific ³H-ouabain binding confirms that the two variables are stoichiometrically related.

The results of experiment 2 (table IV) suggest that the serum of patients with untreated essential hypertension contains a non-dialysable factor of molecular weight >10 000 that reduces the mean number of sodium pumps in normal leucocytes during culture for 72 hours. It might be argued that serum from patients with essential hypertension contains less of the dialysable factor in normal human serum that stimulates specific ³H-ouabain binding and ouabain sensitive ⁸⁶Rb⁺ uptake in leucocytes in culture.⁷ Though dialysis removed the ability of both sets of serum to stimulate specific ³H-ouabain binding, however, dialysed serum from patients with essential hypertension still reduced specific ³H-ouabain binding more than dialysed serum from normotensive subjects.

The results of both experiments are therefore consistent with each other and with the lack of change in sodium ion efflux rate and decreased sodium ion efflux rate constant in leucocytes from hypertensive patients.^{1, 3, 6} It is generally agreed that sodium ion concentration is higher in leucocytes from patients with essential hypertension than from normotensive subjects.^{1, 2, 11-13} Because the sodium ion efflux rate is the product of the sodium ion efflux rate constant and intracellular sodium ion concentration our observations also fit in with this finding. All these findings may be explained by the inhibition of sodium ion extrusion from, and potassium ion uptake into, cells from patients with essential hypertension and they therefore support hypothesis (b) that in essential hypertension the increase in intraleucocyte sodium ion concentration is caused by a circulating serum inhibitor of functionally normal sodium pumps.

Moreover, in a study of the vasodilatory responses to an infusion of potassium ions at low concentration, which stimulates Na⁺/K⁺-ATPase activity, the increase in blood flow was smaller in the forearm resistance vessels of men with essential hypertension, compared with healthy normotensive controls.¹⁴ This result supports the concept that the Na⁺/K⁺-ATPase activity of vascular smooth muscle is chronically inhibited in essential hypertension.

The apparent discrepancy in cardiac glycoside binding in leucocytes of patients with essential hypertension between the present study and that of Boon *et al* is probably due to differences in technique and the selection of subjects.¹³ For instance, Boon *et al* incubated cells for 90 minutes in 100% vol/vol serum without keeping the pH of the serum normal. In our experiment 2 cells were exposed to serum for 72 hours and then washed with Ringer free of potassium so that specific ³H-ouabain binding was measured under the conditions that were used in experiment 1 to study the cells of hypertensive patients.

We conclude that the number of sodium pumps per cell is chronically but reversibly reduced in patients with untreated essential hypertension. Secondly, the serum of patients with untreated essential hypertension contains a competitive inhibitor of the sodium pumps of intact leucocytes. Thirdly, the apparent

ouabain binding capacity of cells from patients with essential hypertension can increase during incubation in the absence of the inhibitor. We do not suggest that the serum inhibitor is solely responsible for the disorder of leucocyte transport of cations in essential hypertension. Our study does not exclude a deficiency of other, ouabain resistant, cation transport pumps in the leucocyte. Our findings show, however, that the leucocyte is a useful model for studying in vitro the defects of transmembrane cation transport in essential hypertension.

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Benign melanocytic naevi as a risk factor for malignant melanoma

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Abstract

Examination of 180 patients with cutaneous malignant melanoma and 197 control patients in a case-control study showed that the risk of melanoma is strongly related to numbers of benign melanocytic naevi (moles). Some unusual features of naevi—a diameter exceeding 7 mm, colour variation, and irregular lateral outline—also showed a strong association with the risk of melanoma, but the relation of numbers of naevi to risk was present even in the group of patients whose naevi had none of these unusual features. Biopsy of clinically atypical naevi from several of the patients at highest risk generally did not show

dysplastic histology. Thus a group of people at high risk of melanoma may be identified by using simple clinical assessment of naevi.

Introduction

The incidence of and mortality from malignant melanoma of the skin have been rising rapidly in white populations around the world for many years.^{1,2} The search for high risk groups and preventive measures has, therefore, become particularly important. People with fair skin, blue eyes, and blond or red hair are at increased risk of melanoma,³ but the risks associated with these characteristics are moderate and because the characteristics are common among white populations it is not a useful way to define a high risk group who might benefit from preventive measures.

A high risk of melanoma is reported in patients with multiple histologically dysplastic naevi who come from families in which at least two members have had melanoma.⁴ Such families are, however, rare and account for only a small proportion of people with melanoma. The risks of melanoma in others with dysplastic naevi and with clinically atypical naevi have not yet been quantified. Furthermore, clinical criteria that reliably identify histologically dysplastic naevi have not been clearly determined: clinically atypical naevi do not always show⁵ the histological features described as characteristic of dysplastic naevi.⁶

Several recent case-control studies, whose cases were not selected because of familial melanoma, have found a raised risk of melanoma in people with increased numbers of naevi or large naevi.⁷⁻¹⁰ The number of clinically atypical naevi, however, was not determined, nor were sites other than the arms and legs examined for moles; no

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