to understand the true aetiology of menopausal flushes. It has been realized for some time that there is no clear connexion between the hormone levels in blood and urine and severity of flushes. The events at vascular level have never been fully investigated—in fact, the recent bibliography on the menopause is remarkably short. We hope that the present report will stimulate further investigation of menopausal vasomotor symptoms and of factors that precipitate them, so that they may be related more closely to other vasomotor disorders.

We may state in conclusion that clonidine, in doses similar to those used for migraine prophylaxis, seems a useful and safe alternative or even addition to other treatments for hot flushes and that it has no important side effects.

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Specific Laboratory Test for Diagnosis of Multiple Sclerosis

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Summary

Lymphocytes from patients with multiple sclerosis are much more susceptible to the inhibitory activity of linoleic acid (0·08 mg/ml) when tested for sensitization to thyroid by the macrophage electrophoretic mobility test (91% inhibition) than are those from normal subjects (57% inhibition). Cells from patients with a variety of other neurological diseases give 47% inhibition with linoleic acid. These differences are specific for multiple sclerosis and can be used as an in-vitro diagnostic test for the disease. Nearly 43% of clinically normal near relatives of patients with multiple sclerosis show an "anomalous" figure of about 77%; in the remainder the figure is the same as in the general population (57%). An anomalous result is compatible with lifelong freedom from M.S. Possibly a congenital anomalous handling of unsaturated fatty acids is a constant feature of the disease.

Introduction

While most cases of multiple sclerosis (M.S.) present little difficulty in diagnosis there are instances in which it must be left tentative, and the lack of some specific diagnostic in-vitro test is acutely felt. Lymphocytes from patients with M.S. are much more severely depressed by linoleic acid in their recognition of antigen than are those from patients with other neurological diseases or from normal subjects (Mertin *et al.*, 1973). This difference is sufficiently consistent to constitute a specific diagnostic test for M.S.

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Subjects and Methods

Four groups of subjects were studied—(1) 33 patients with M.S., of whom all except one were in a quiescent phase of the disease; (2) 27 patients with other neurological diseases; (3) 46 normal subjects aged 22 to 59 years drawn from the general population (not matched for age and sex); and (4) 96 relatives of M.S. patients, all of whom were clinically without neurological disease. This group comprised fathers, mothers, sons, daughters, brothers, sisters, nephews, and nieces of patients. In addition a few grandparents and grandchildren were studied.

Lymphocytes were prepared from 10-15 ml venous blood by the methylcellulose and carbonyl iron method of Coulson and Chalmers (1967) as modified by Hughes and Caspary (1970). Lymphocytes from normal subjects (Field *et al.*, 1970) as well as from those with a variety of diseases—for example, cancer, sarcoidosis, M.S., and other neurological diseases—interact with thyroglobulin (F1 fraction of thyroid). Indeed no subject has been seen (save with early measles infection) who did not respond fully to thryoglobulin, so that this may be used as a universal test antigen for measuring lymphocyte-antigen interaction. (Cells from chimpanzees, rhesus monkeys, sheep, and guinea-pigs also react with human thyroglobulin.) There is also almost universal sensitization of human lymphocytes (in Britain) to P.P.D. (purified protein derivative of tuberculin)—again with the exception of measles—only three out of several hundred tests giving negative results. Lymphocytes with M.S. or other neurological diseases react with encephalitogenic factor (Caspary and Field, 1970). In all these studies the method used was the macrophage electrophoretic mobility test (Field and Caspary, 1970), a detailed account of which, together with a protocol in extenso, has been published (Caspary and Field, 1971).

In principle the method depends on the observation that when lymphocytes are brought into contact with an antigen to which they are sensitized they elaborate a material that causes normal guinea-pig peritoneal macrophages to travel more slowly in an electric field. Normal guinea-pig macrophages are thus used as an indicator system for lymphocyte-antigen interaction. The substance which produces the slowing we have called macrophage slowing factor, and it may well be the same as the well known macrophage inhibitory factor. If t_c represents the time of electrophoretic migration of guinea-pig macrophages in the presence of human lymphocytes alone and t_e the migration time when antigen is also present then in general $t_e > t_c$ and $((t_e - t_c)/t_c) \times 100$ is a measure of lymphocyte sensitization. All measurements are ordinarily carried out in a balanced salt solution medium (Parker 199) and in the absence of serum. Recently Mertin *et al.*, (1973) found that physiological levels of linoleic acid incorporated into the test system interfere with the recognition of antigen by lymphocytes, so that the percentage slowing is less. This reduction was systematically studied when 0·08 mg linoleic acid per ml was used with lymphocytes from groups 1, 2, and 3. If a% be the percentage macrophage slowing in any particular instance and b% the slowing when 0·08 mg linoleic acid per ml is also present in the system then $a > b$ and $((a - b)/a) \times 100$ is the percentage change brought about by linoleic acid.

The antigens used in this study were the F1 fraction of thyroid and P.P.D. In the case of groups 1 and 2 encephalitogenic factor was also used.

Linoleic acid (C 18:2—that is, 18 carbon acid with two double bonds) was obtained from Sigma Chemicals and used at a concentration of 0·08 mg/ml, corresponding with twice the normal level of unesterified linoleic acid in serum. The acid was dissolved in ethanol, but preliminary experiments showed that the concentration used had no effect on the results.

Results

As expected, lymphocytes from groups 1, 2, and 3 were sensitized to thyroid and to P.P.D. but cells sensitized to encephalitogenic factor were found in only groups 1 and 2 (Caspary and Field, 1970). In all three groups linoleic acid caused a significant inhibition of lymphocytic response to all antigens tested. Consistently this was much greater with M.S. lymphocytes (group 1) than with those from normal subjects (group 3) or patients with other neurological diseases (group 2) (table 1). Student's *t* test showed the mean reductions with thyroid to be all highly significantly different ($P \ll 0.001$). With encephalitogenic factor as antigen measurements were made in only groups 1 and 2, since it is known that in normal subjects sensitization is low (Field and Caspary, 1970). With all the test antigens studied reduction was much greater with M.S. lymphocytes than with those from groups 2 and 3. A curious feature was the small reduction seen with lymphocytes from patients with other neurological diseases as against the much greater reduction with M.S. lymphocytes when compared with normal. For practical reasons further extensive family tests were undertaken with thyroid antigen alone.

When the relatives of M.S. patients (group 4) were tested it quickly became apparent that they fell into two clearly defined groups (table 1). Of the 96 tested 55 gave the same result as that shown by the normal subjects and 41 showed a mean reduction of about 77%—approximately mid-way between the normal value of 57% and the 91% seen in patients with M.S. These subjects were perfectly normal and had never shown any form of nervous disease. Among them were several parents of over 70 years of age, the oldest being a mother aged 88. Clearly an "anomalous" figure of 77% is not incompatible with life long freedom from M.S. Every mother of an M.S. patient gave the anomalous result but no father did so, though only a few were available (table II). In general the anomalous figure was more common in female relatives than in males. Overall 42·7% of the near relatives tested gave the anomalous result.

TABLE I—*Percentage Inhibition of Lymphocytic Response to Antigens in Presence of Linoleic Acid. Numbers of Subjects are given in Parentheses*

	Group 1	Group 2	Group 3	Group 4	
				Normal Results	Anomalous Results
	Thyroid Antigen (F1)				
	(26)	(8)	(27)	(55)	(41)
Mean ± S.D.	91·03 ± 3·00	46·25 ± 2·48	56·76 ± 2·32	56·98 ± 1·60	76·81 ± 1·45
Range	85·3 – 95·9	42·6 – 47·7	50·0 – 60·6	52·8 – 59·2	72·9 – 80·0
	P.P.D. Antigen				
	(6)	(8)	(19)		
Mean ± S.D.	87·12 ± 2·11	46·64 ± 0·86	61·20 ± 2·20		
Range	84·0 – 89·7	45·4 – 47·7	56·3 – 66·5		
	Encephalitogenic Factors as Antigen				
	(11)	(17)			
Mean ± S.D.	89·15 ± 1·33	46·12 ± 3·22			
Range	87·7 – 91·9	42·0 – 47·6			

Tests of significance of differences with thyroid antigen.—Group 1 *v.* group 3: $P \ll 0.001$; group 1 *v.* group 2: $P \ll 0.001$; group 2 *v.* group 3: $P \ll 0.001$; group 4 anomalous *v.* group 3; $P \ll 0.001$; group 4 normal *v.* group 3: N.S.

TABLE II—*Percentage Reduction of Lymphocyte Thyroid Interaction Produced by Linoleic Acid in 96 Relatives of M.S. Patients (Group 4)*

	77% Anomalous)	57% (Normal)	No. of Subjects	Proportion of Subjects with Anomalous Result
Fathers ..	0	6	6	0
Mothers ..	11	0	11	100%
Brothers ..	1	15	16	6·3%
Sisters ..	13	5	18	72·2%
Son	2	11	13	15·4%
Daughters ..	4	1	5	80·0%
Nephews ..	3*	9‡	12	25·0%
Nieces ..	7†	8§	15	46·7%
Total ..	41	55	96	42·7%

*Children of a sister of a propositus.
†Four of these were children of a sister of a propositus and three belonged to a brother of a propositus.
‡Six of these were children of a sister of a propositus and three belonged to a brother of a propositus.
§Four of these belonged to a sister and four to a brother of a propositus.

Discussion

Clearly while the lymphocytes from all the subjects studied showed a reduction in their ability to react to thyroid, P.P.D., or encephalitogenic factor this reduction was much greater with lymphocytes from patients with M.S. than with lymphocytes from normal subjects or those suffering from some other neurological disease, including neurosyphilis. This latter is important, since in several immunological respects neurosyphilis presents findings similar to those in M.S.—for example, a raised gammaglobulin level in the spinal fluid; the myelinolytic factor in serum. There were no exceptions in the 33 M.S. patients studied and moderate doses of corticosteroids did not affect the results. The degree of interference with the response to antigen may thus be used as a specific test for M.S. If independent study confirms these findings (and they have been greatly extended in our hands in a familial study of the disease) then a procedure of much practical value in the occasionally difficult case will be available. Indeed experienced neurologists admit that as many as 20% of their primary diagnoses of M.S. have later been amended. A "blind" trial of neurological diseases, including M.S., is in progress.

There are certain possible applications of the test. (*a*) While all are agreed that a high (though variable) proportion of cases of retrobulbar neuritis turn out to be M.S., it should be possible to determine whether the residue are formes frustes or fundamentally different in their nature. Already it is clear that not all cases of retrobulbar neuritis give the M.S.-type result. (*b*) Controversy has long existed about the nature of Devic's disease. One classical example of this disease gave a result typical of that

expected with "other neurological diseases"—clearly different from M.S. (*c*) In Schilder's disease it should be possible to obtain evidence whether there is the lymphocytic anomaly characteristic of M.S.

The present findings also offer a rational basis for the claim by Millar *et al.* (1973) that supplementation of the diet by sunflower seed oil (rich in linoleic acid) had a beneficial effect on the course of M.S. over a two-year period. If lymphocyte interaction with encephalitogenic factor (the putative antigen in M.S. if it is to be regarded as an autoimmune or autoaggressive disease) is important at any stage in the disease (either in its initiation or in its periodic exacerbation) then linoleic acid would be expected to damp down its evolution. With such a rational basis it becomes of the greatest importance that the therapeutic findings of Millar *et al.* be confirmed. Almost a decade ago Thompson and his group (Thompson, 1973) first drew attention to the anomaly in handling of unsaturated fatty acids associated with M.S., an anomaly present not only in brain but also in erythrocytes (Gul *et al.*, 1970) and leucocytes (Mahler, 1971). Sinclair (1956) was one of the first to stress the possible importance in M.S. of a deficiency in unsaturated fatty acids, and Thompson, in his Jephcott lecture of 1966, suggested that some anomaly in the handling of unsaturated fatty acids might be "possibly inborn in nature."

The present family study indicates that unusually high (anomalous) lymphocyte susceptibility to linoleic acid is genitically determined. The presence of the anomaly itself is compatible with lifelong freedom from M.S. If M.S. occurs in any subject, however, the anomaly is found to be severe—91%, against 77% in the clinically normal subject. It is not known whether the development of M.S. converts a 77% type of result into the 91% type. Results greater than 90% in some very early and mild cases of M.S. (Field and Shenton, 1973) suggest that the high figure predates the development of the disease. "Anomalous" lymphocyte susceptibility to linoleic acid is clearly sex-associated in strong degree, but much more extensive studies are needed to clarify and quantify the familial pattern. The youngest relative found with the defect was an 18-month-old girl. This speaks for Thompson's suggestion mentioned above.

The deficiency in linoleic acid reported by the above workers might clearly work in several ways (Thompson, 1966)—(*a*) the abnormal constitution of myelin might make it susceptible to a patchy and premature abiotrophy; (*b*) it may be more susceptible as a target for some immunological or autoaggressive disease; (*c*) it may be more susceptible to viral infection (with measles, for example) either as a left-over from an acute infection or as a "slow" infection ab initio. In any case, it appears that such myelin is a prepared ground for the development of the disease. In a condition such as M.S., with a century of bleak history behind it, it is perhaps permissible to indulge in a little optimistic speculation. The test described above has already picked out "anomalous" relatives of M.S. propositi, and while the familial pattern has not been fully worked out it is already clear that an anomalous response to linoleic acid may be picked up long before the central nervous system is fully myelinated. Such children might well be given unsaturated fatty acids in the hope that they will develop properly constituted myelin before the virtual completion of the process round about the age of 16 and so acquire a nervous system unsuitable for the development of M.S. whatever its causal agent may be. Experimental support for an approach of this type comes from the work of Clausen and Møller (1967), who showed that feeding immature rats with polyunsaturated fatty acids diminished their susceptibility to experimental allergic encephalomyelitis.

"The final aim of the epidemiologist is to prevent the disease by removing one or more influences in the chain of causation. History shows that diseases have been prevented by such means when knowledge of their pathogenesis has been no less rudimentary than is that of multiple sclerosis" (Acheson. 1972).

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