Lehmann (1962) from Accra, while Went and MacIver (1958) described it from the West Indies. The differential diagnosis of adult sickle-cell disease reported " SS " is given in Table V. Clinically some cases of sickle-cell F-thalassaemia may be difficult to differentiate from $SF_{highgene}$ cases, but the simple Kleihauer and Betke staining technique (Lehmann and Huntsman, 1966) will distinguish between the two by their foetal haemoglobin distribution in the red cells. In F-thalassaemia the distribution of Hb F in the individual red cells is quite heterogeneous, while in $F_{highgene}$ it is relatively uniform. We have found this test useful in Accra, where the level of Hb F may be more than 10% in sickle-cell anaemia or sickle-cell thalassaemia.

The contribution that adult sickle-cell disease patients make towards the persistence of the S gene in the population is greater than is usually realized. It is no mere conjecture when we state that here in Ghana this contribution will soon outstrip that supposed to be made by balanced polymorphism through *falciparum* malaria. Widespread haemoglobin genotyping starting from schools and subsequent genetic counselling of young adults ought to be pursued relentlessly (Konotey-Ahulu, 1968) if the morbidity and mortality caused by sickle-cell disease are to be appreciably reduced on the African Continent.

We are grateful to Professor H. Lehmann, M.R.C. Abnormal Haemoglobin Unit, University Department of Clinical Biochemistry, Cambridge, for his advice ; to Miss P. Kynoch (Cambridge) for technical advice and help ; and to Professor S. R. A. Dodu, Head of Department of Medicine and Therapeutics, Ghana Medical School, for his encouragement.

REFERENCES

Allison, A. C. (1956a). *Ann. hum. Genet.*, **21**, 67.
Allison, A. C. (1956b). *Trans. roy. Soc. trop. Med. Hyg.*, **50**, 185.
Boi-Doku, F. S., and Ofori-Atta, Susan (1967). *Ghana med. J.*, **6**, 45.
Dunn, J. M., and Haynes, R. L. (1967). *Amer. J. Obstet. Gynec.*, **97**, 574.
Edington, G. M. (1963). *Ghana med. J.*, **2**, 83.
Edington, G. M., and Laing, W. N. (1957). *Brit. med. J.*, **2**, 143.
Edington, G. M., and Lehmann, H. (1954a). *Lancet*, **2**, 173.
Edington, G. M., and Lehmann, H. (1954b). *Trans. roy. Soc. trop. Med. Hyg.*, **48**, 332.
Edington, G. M., and Lehmann, H. (1955a). *Brit. med. J.*, **1**, 1308.
Edington, G. M., and Lehmann, H. (1955b). *Brit. med. J.*, **2**, 1328.
Edington, G. M., and Lehmann, H. (1956). *Man*, **56**, 34.
Ghana med. J., 1964, **3**, 1.
Harris, F. C., and Lomax, G. D. (1954). *W. Afr. med. J.*, **3**, 29.
Hendrickse, J. P. de V., and Watson-Williams, E. J. (1966). *Amer. J. Obstet. Gynec.*, **94**, 739.
Humble, J. G., Anderson, I., White, J. C., and Freeman, T. (1954). *J. clin. Path.* **7**, 201.
Jacob, G. F., and Raper, A. B. (1958). *Brit. J. Haemat.*, **4**, 138.
Konotey-Ahulu, F. I. D. (1968). *Ghana med. J.*, **7**, 118.
Konotey-Ahulu, F. I. D., Gallo, E., Lehmann, H., and Ringelhann, B. (1968). *J. med. Genet.*, **5**, 107.
Lehmann, H. (1954). *Eugen. Rev.*, **46**, 101.
Lehmann, H., Beale, D., and Boi-Doku, F. S. (1964). *Nature (Lond.)*, **203**, 363.
Lehmann, H., and Huntsman, R. G. (1966). *Man's Haemoglobins*, p. 293. Amsterdam.
Marengo-Rowe, A. J. (1965). *J. clin. Path.*, **18**, 790.
Neel, J. V. (1957). *New Engl. J. Med.*, **256**, 161.
Neel, J. V., Hiernaux, J., Linhard, J., Robinson, A., Zuelzer, W. W., and Livingstone, F. B. (1956). *Amer. J. hum. Genet.*, **8**, 138.
Poulik, M. D. (1957). *Nature (Lond.)*, **180**, 1477.
Ringelhann, B., Dodu, S. R. A., Konotey-Ahulu, F. I. D., and Lehmann, H. (1968). *Ghana med. J.*, **7**, 120.
Ringelhann, B., Lewis, R. A., Lorkin, P. A., Kynoch, P. A. M., and Lehmann, H. (1967). *Acta haemat. (Basel)*, **38**, 324.
Ringelhann, B., and Makunga, J. (1965). *Ghana med. J.*, **4**, 124.
Russo, G., and Mollica, F. (1965). *Pediatria (Napoli)*, **73**, 63.
Serjeant, G. R., Richards, R., Barbor, P. R. H., and Milner, P. F. (1968). *Brit. med. J.*, **3**, 86.
Thompson, G. R., and Lehmann, H. (1962). *Brit. med. J.*, **1**, 1521.
Went, L. N., and MacIver, J. E. (1958). *Blood*, **13**, 559.

Outbreak of Brucella melitensis Type 2 Infection in London

N. S. GALBRAITH,* M.B., D.P.H. ; M. S. ROSS,† M.R.C.S., L.R.C.P., F.C.PATH.
R. R. DE MOWBRAY,‡ M.D., M.R.C.P. ; D. J. H. PAYNE,§ M.B., F.C.PATH.

Brit. med. J., 1969, **1**, 612–614

Summary: An outbreak of seven cases of Brucella melitensis infection in London was traced to Italian pecorino cheese (cheese made from unpasteurized sheep's milk) which had been obtained from village markets in central Italy, brought back to England, and distributed to the affected persons.

It is emphasized that pecorino cheese made from unpasteurized milk should not be eaten unless it is known to have been stored for at least 90 days, the period during which these cheeses have been shown to become free from viable brucella organisms.

Introduction

An outbreak of seven cases of *Brucella melitensis* type 2 infection took place in November and December 1965 in persons of Italian origin resident in West Ham, London. The only food common to all the affected persons was pecorino cheese which had been obtained in August 1965 from village markets in central Italy.

* Deputy Medical Officer of Health, Newham, London E.15.
† Pathologist, Queen Mary's Hospital, London E.15.
‡ Physician, Queen Mary's Hospital, London E.15.
§ Director, Public Health Laboratory, Portsmouth.

We report this outbreak because *Br. melitensis* is rare in the United Kingdom (Dalrymple-Champneys, 1960) and because it appears that this is the first recorded outbreak where the patients have acquired their infection in this country.

The Outbreak

The seven patients, four adults and three children (see Table), belonged to two Italian families who had lived in West Ham for many years ; five persons in these two families were not affected. Three of the patients and one of the unaffected persons visited relatives in central Italy in August 1965, and brought home with them two pecorino cheeses. All the affected persons consumed the cheese. Two other Italian households consisting of four adults and three children were also given some of the cheese ; all were symptom-free and none had serological evidence of brucella infection.

The two pecorino cheeses were purchased from two village markets in the last few days of August 1965 and were brought back to England on 2 September. They were eaten from the latter part of September onwards, being served grated on spaghetti. A small sample of one of the cheeses, which was remaining in January 1966, was examined but no brucella organisms were isolated. Serum samples from two relatives in central Italy, who regularly purchased pecorino cheese from

the village markets concerned, showed evidence of brucella infection ; one had an agglutination titre of 1 : 80 and the other a titre of 1 : 640 against *Br. abortus*.

Clinical Features

The onset of symptoms was insidious (see Table). Mr. A and Mr. C dated their illnesses from the day they stopped work and J. C. from the day he was off school. The main features were fever, general malaise, anorexia, and pains in the limbs and back. In five of the patients symptoms persisted for several months. Mr. C was off work for a year and Mr. A for four months.

Mr. A aged 57 was the first case to come to notice. He fell ill on 15 November. His sister-in-law Mrs. B aged 65, who lived in the same house, developed symptoms at the beginning of December. In January 1966 Mr. A reported that his relative Mr. C was suffering from backache and fever. On investigation Mr. C. aged 54, Mrs. C. aged 46, and three of their five children aged 15, 11, and 5 years were found to have *Br. melitensis* infection. Mr. C had been ill since 9 November 1965 ; Mrs. C recalled a mild febrile illness at about the same time as her husband fell ill ; one child had "flu" beginning on 12 November 1965, another had shoulder pains and was off school in the early part of November, and the youngest child had been unwell for some while but with no definite date of onset of symptoms.

All the patients were treated first with a month's course of tetracycline, two were also given streptomycin. There was a satisfactory response in five of the patients, symptoms being alleviated and fever disappearing. In the remaining two patients, Mr. C and Mrs. B, there was little improvement after the course of tetracycline, but they both subsequently responded satisfactorily to a month's course of ampicillin 500 mg. twice daily. In January 1967, about 14 months after the onset of symptoms, all the patients were symptom-free and they have since remained well.

Laboratory Methods

Blood Culture.—Venous blood 3–5 ml. was added to 100 ml. of 0·1% glucose broth and incubated at 37° C. in an atmosphere of 10% carbon dioxide. Subcultures were made on alternate days for the first week and then weekly for a further five weeks. All positive isolations were obtained from subcultures made during the first week. No growth was visible on 5% horse-blood agar after 24 hours' incubation, but at 48 hours minute colonies were observed which reached the size of 2 mm. after seven days. The organism isolated was recognized as belonging to the genus *Brucella* and was finally identified as a strain of *Br. melitensis* biotype 2, having the following characteristics: it was a small Gram-negative coccobacillus which grew aerobically ; growth was not enhanced by CO_2, nor did it produce H_2S ; it grew in the presence of thionin, basic fuchsin, methyl violet, and pyronin ; it was agglutinated by monospecific abortus antiserum and not by monospecific melitensis antiserum ; it was not lysed by abortus (Tbilisi) phage at routine test dilution or at 10,000 × routine test dilution.

Serological tests were carried out as described by Kerr *et al.* (1968).

Cheese Examination.—Samples were examined at Queen Mary's Hospital Pathological Laboratory and also at the Public Health Laboratory at Portsmouth and the Veterinary Investigation Laboratory at Leeds. About 2 g. of cheese was emulsified in 5 ml. of nutrient broth. Then 2-ml. portions were inoculated into the subcutis of each side of the back of a guinea-pig and 1 ml. into the peritoneal cavity. All the animals remained in good condition. Serum samples obtained from the guinea-pigs six weeks after inoculation contained no agglutinins for brucella. At necropsy there was no abnormality to be seen.

Discussion

Pecorino cheese is made from unpasteurized sheep's milk (Davis, 1965). Although the farms where the two cheeses were made could not be traced, it is known that *Br. melitensis* infection in sheep is widespread in central Italy, and cheese made from sheep's milk is recognized as an important source of human infection (Cariello and Tursi, 1964). Since this Italian cheese was apparently the only food common to the affected persons, it seems very probable that it was the source of infection.

Gargani (1952) studied the duration of infection with *Br. melitensis* in pecorino cheese by artificially inoculating milk before manufacture of the cheese. The cheese was self-sterilizing, but the artificially contaminated cheese was not free from viable brucella organisms until 90 days after manufacture. In the outbreak described the cheese was presumably infective in September 1965 but was free from brucella organisms when examined four months later in January 1966.

Gilman and Marquardt (1951) in the United States recommended the pasteurization of milk used for making Italian cheese because they found that *Br. abortus* survived for long periods in the cheese. Some makers still feel that pasteurization of the milk affects adversely the flavour of the cheese, and for this reason prolonged storage has been recommended by the Italian Public Health Authorities. This outbreak shows that pecorino cheese made from unpasteurized milk should not be eaten unless it is known to have been stored for at least 90 days after manufacture.

The insidious onset of brucellosis and the vague symptomatology make diagnosis difficult ; the significance of illness in the C family was appreciated only when they were discovered to be relatives of the first two patients with brucellosis that came to notice. Blood culture may not be attempted in suspected cases of brucellosis ; furthermore, when it is undertaken and proves positive the culture may not be typed. It is possible, therefore, that *Br. melitensis* infection may escape recognition and that the mode of transmission of *Br. melitensis* from abroad, described in this paper, may not be as rare as this single report would suggest. Between 1957 and 1967 the Brucellosis

Clinical Features of Seven Cases of Brucella melitensis Infection

Patient	Age	Date of Onset	Duration of Illness	Clinical Features	*Br. melitensis* Blood Culture	Serum Agglutination Test*
Mr. A	57	15/11/65	4 months off work	Headache, sweating, shivering attacks, fever, anorexia	+ 2/12/65	1 : 800 2/12/65
Mrs. B	65	Early Dec. 1965	At least 8 months	Shoulder pain, fever	+ 14/5/66	1 : 400 4/1/66
Mr. C	54	9/11/65	12 months off work	Backache, pains in legs, fever, sweating	+ 13/1/66	1 : 800 11/1/66
Mrs. C ..	46	Early Nov. 1965	About two weeks	Mild febrile illness	–	1 : 800 11/1/66
A. C	15	Early Nov. 1965	About one week	Shoulder pains, general malaise, loss of energy, anorexia	+ 28/1/66	1 : 1,600 11/1/66
J. C	11	12/11/65	Several months	Influenza-like illness, loss of energy, anorexia	+ 14/1/66	1 : 1,600 14/1/66
L. C	5	Indefinite	Several months	"Off colour," poor appetite, pallor, pains in knees	–	1 : 3,200 14/1/66

* Standard agglutination test. Abortus antigen.

Reference Laboratory (now at the Public Health Laboratory, Portsmouth) received for identification 10 cultures of *Br. melitensis* that had been isolated in the United Kingdom. One of these cultures was biotype 2, and this was isolated on blood culture from an Italian aged 21 who had been resident in this country for two years; the source of infection was not discovered.

We wish to acknowledge the assistance in the clinical investigation of the following: Dr. D. K. Banerjee, Dr. P. D. Shanahan, Dr. J. C. Turner, general practitioners, and Dr. L. M. Webber, formerly medical registrar, Queen Mary's Hospital. Mr. A. J. Stevens, formerly of the Veterinary Investigation Laboratory, Leeds, kindly examined samples of the cheese.

REFERENCES

Cariello, M., and Tursi, L. (1964). *Ann. Sanità pubbl.*, **25**, 1041.
Dalrymple-Champneys, W. (1960). *Brucella Infection and Undulant Fever in Man.* London.
Davis, J. G. (1965). *Cheese*, vol. 1. London.
Gargani, G. (1952). *Clin. vet. (Milano)*, **75**, 257.
Gilman, H. L., and Marquardt, J. C. (1951). *J. Milk Fd Technol.*, **14**, 55.
Kerr, W. R., *et al.* (1968). *J. med. Microbiol.*, **1**, 181.

Controlled Trial of Dipyridamole in Cerebral Vascular Disease

JOAN ACHESON,* M.B., CH.B.; G. DANTA,† M.R.C.P., M.R.A.C.P.; E. C. HUTCHINSON,‡ M.D., F.R.C.P.

Brit. med. J., 1969, **1**, 614–615

Summary: A controlled double-blind study of the effect of dipyridamole was performed in 169 patients with established cerebral vascular disease. A dose of 400 mg. was used initially, given daily for an average of 14 months; the dose was then increased to 800 mg. daily for a further average period of 11 months. When the incidence of cerebral ischaemic episodes during treatment was compared in the drug-treated and placebo-treated groups no significant difference was found.

Introduction

Since the synthesis by Fischer and Roch (1951) of a new group of compounds containing two pyrimidine rings, there have appeared in the literature descriptions of the pharmacological properties and clinical effects of one of the derivatives, dipyridamole (Persantin). Experimentally this substance increases coronary blood flow and intercoronary anastomoses in dogs with surgically induced coronary artery constriction (Asada *et al.*, 1962; Vineberg *et al.*, 1962; Chiba, 1963; Fam *et al.*, 1964; Meesmann and Bachmann, 1966); it increases the concentration of adenosine triphosphate in heart muscle (Laudahn, 1961; Siess, 1962; DeGraff and Lyon, 1963), and a recent experimental study suggested that it enhances the vasodilatory effect of adenosine triphosphate (Afonso and O'Brien, 1967). Adenosine-diphosphate-induced platelet aggregation is inhibited by dipyridamole both in vitro and in vivo (Emmons *et al.*, 1965a, 1965b), and Sullivan *et al.* (1968b) showed that dipyridamole decreases platelet adhesiveness in patients with coronary artery disease.

Five double-blind studies in ischaemic heart disease have failed to show any beneficial effect of dipyridamole (Foulds and Mackinnon, 1960; Zion and Bradlow, 1961; Soloff *et al.*, 1962; Newhouse and McGregor, 1965; Sbar and Schlant, 1967). In one double-blind study, however, improvement was noted (Leiberman and Guglielmelli, 1964).

Sullivan *et al.* (1968a), in a preliminary report, found that dipyridamole in a dose of 400 mg. daily significantly reduced the incidence of emboli in patients who had undergone prosthetic cardiac valve replacement.

The modification of platelet aggregation by dipyridamole prompted us to examine the possibility that dipyridamole could influence the natural history of cerebral vascular disease. The daily dosage used in the cardiological studies has been of the order of 100 mg. Since this appears to be an ineffective dose we used a dose of 400 mg. daily in the first instance, and when no beneficial effect at this dose level was apparent we decided to double the dose.

* Research Assistant.
† Neurological Registrar.
‡ Consultant Neurologist.
North Staffordshire Royal Infirmary, Stoke on Trent, Staffs.

Method of Study

A total of 169 patients entered the trial. Each had partially or completely recovered from a clinical episode of cerebral ischaemia. In setting up the trial consideration was given to the known variations in the behaviour of cerebral vascular disease. It was therefore decided to match patients in pairs according to the following criteria: (1) sex; (2) age (within five years); (3) presence of hypertension (diastolic blood pressure of 100 mm. Hg or more); (4) clinical diagnosis at presentation according to the following subgroups: (*a*) transient ischaemic attacks (episodes of cerebral ischaemia lasting less than one hour), (*b*) single stroke, and (*c*) multiple strokes; and (5) length of history before beginning treatment.

Before treatment all patients underwent full clinical assessment, a full blood count was made, and an electrocardiogram taken. Liver and renal function tests were done before and six months after the beginning of treatment.

After the pairing, randomization was carried out by the statistical department of Boehringer Ingelheim Ltd., who supplied both the drug and the placebo. The active tablets contained 100 mg. of dipyridamole. The placebo tablets were of identical appearance. The information on the drug used in each patient was available in sealed envelopes throughout the trial. The patients were seen at monthly intervals for three months and then at three-monthly intervals by two observers. The clinical state was noted on each visit and any ischaemic episodes were recorded. Supplies of the drug were renewed at the time.

Results and Conclusions

Eighty-five patients received dipyridamole and 84 placebo. The relevant data pertaining to the two groups are set out in Table I. It will be seen that the treated and control groups are comparable with respect to the factors listed. Since the two groups were homogeneous, pairing was ignored at the final evaluation. The duration of disease before treatment ranged from three months to five years in both groups: 66% of the treated and 64% of the control group had a history of less than 12 months.