level falls with advancing pregnancy allowance must be made for the stage of gestation at which the test is carried out, and a bilirubin value at 34 weeks that might indicate the need for active intervention would not necessarily have the same import at 24 weeks. Technical errors—for example, owing to exposure of liquor to daylight, to contamination with maternal or foetal blood, to interference by haemoglobin absorption, etc.—can be minimized by careful technique, but each laboratory needs to standardize and evaluate the method it uses.

Little is known of how bilirubin enters liquor or of its rate of turnover, but the water content changes rapidly, and if this were associated with variation in the total liquor volume, changes in the bilirubin value might be secondary to this.

Bilirubin is insoluble in water, and hence in liquor, as in plasma, it is attached to protein. Protein turnover in liquor amnii is slow in comparison with the water component, so theoretically could give an indirect measurement of liquor volume. Thus by relating bilirubin to protein as the bilirubin/protein ratio errors secondary to changes in liquor volume would be eliminated. Dunstan (1968), however, did not find any relation between protein content and liquor volume.

The present study of two series of cases of Rh isoimmunization comprising 477 cases in all and using two different methods of protein estimation confirms that the bilirubin and protein values do correlate with severity of haemolytic disease, but that

bilirubin is superior to protein and is not improved, indeed is impaired, by relating to protein values.

Because these findings are in direct conflict with those of the other two groups of workers cited, we have considered whether technical differences in protein estimation between the three laboratories could be the explanation. A direct comparison of the protein values in these series is summarized in Table VI.

For the three laboratories the mean values are not different, though the ranges differ. This is probably because the published figures are based on extremely small series, but the method of selecting patients for amniocentesis may also have played a part.

We therefore conclude that the estimation of protein in liquor amnii does not give as good a forecast of severity of haemolytic disease as is possible by bilirubin estimation and that the value of bilirubin is not improved by relating it to the protein value.

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TABLE VI.—Protein Levels in Liquor Amnii—Summary of Results in Present and Two Relevant Published Series

	Method	No. of Cases	Gestation	Protein Level (g./100 ml.)	
				Range	Mean
Present series 1 ..	F & C	298	All	0.1–1.39	0.213
Present series 2 ..	Biuret	179	All	0.05–1.1	0.318
Morris <i>et al.</i> (1967)	Biuret	25	30–32 weeks	0.236–0.587	0.374
Cherry <i>et al.</i> (1965)	Biuret	39	All	0.155–1.520	0.463

F & C = Folin and Ciocalteu technique.

Sickle-cell Anaemia, Sickle-cell Thalassaemia, Sickle-cell Haemoglobin C Disease, and Asymptomatic Haemoglobin C Thalassaemia in one Ghanaian Family

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Summary: A Ghanaian family is described in which a sickle-cell haemoglobin C man married to a sickle-cell thalassaemia woman produced 12 children (eight alive). Four children have sickle-cell anaemia, two sickle-cell haemoglobin C disease, one has sickle-cell thalassaemia, and one is asymptomatic haemoglobin C thalassaemia.

It is emphasized that the contribution that adult sickle-cell disease patients make, through procreation, to the persistence of the S gene may be greater than is normally supposed, and that this contribution may soon outstrip that made by balanced polymorphism through falciparum malaria. Widespread haemoglobin genotyping in schools leading to genetic counselling is advocated to decrease the incidence of sickle-cell disease.

Introduction

Ghana abounds in abnormal haemoglobins. One person in four or five has either the sickle-cell trait (Hb AS) or the haemoglobin C trait (Hb AC). For West Africa as a whole Lehmann (1954) described the incidence of the sickle-cell trait as between 20 and 30%. Edington and Laing (1957) made the incidence of the sickle-cell trait in Southern and Northern Ghana to be 19% and 7% respectively. Edington and Lehmann (1954a, 1954b) first described Hb C outside the U.S.A. The main focus for Hb C is in West Africa (Allison, 1956a, 1956b; Neel *et al.*, 1956), with the highest frequency in Northern Ghana of 20–21% (Edington and Lehmann, 1956; Edington and Laing, 1957; Neel, 1957).

The most recent surveys in Ghana involved young adults from the north and south of the country. In the south frequency of the sickle-cell trait (AS) is 20% and the Hb C trait (AC) is 9%, while in the north (among 143 persons) Hb AS is 7%, Hb AC is 18%, β -thalassaemia is 4–5%, with one case of α -thalassaemia (Ringelmann *et al.*, 1968). Other

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qualitative or quantitative haemoglobinopathic genes that have been described as being found in Ghana include High F gene (Edington and Lehmann, 1955b), Hbs D, G, and K (Edington, 1963), Hb G_{Accra} (Lehmann *et al.*, 1964), thalassaemia (Harris and Lomax, 1954; Boi-Doku and Ofori-Atta, 1967), and recently a new haemoglobin variant—haemoglobin Korle Bu, $\alpha_2\beta_2$ 73Asp→Asn (Konotey-Ahulu *et al.*, 1968). With at least nine abnormal haemoglobin genes in Accra it is not surprising that various genotype combinations have been seen and described—namely, SS, SC, S-thalassaemia, S+F (in children), SF_{highgene} (in adults), CC, CF_{highgene}, and, recently, thalassaemia major (Boi-Doku and Ofori-Atta, 1967), GG_{Accra} (Lehmann *et al.*, 1964), SD_{Punjab} (Ringelhann *et al.*, 1967), and Hb S Korle Bu in two successive generations (Konotey-Ahulu *et al.*, 1968). If one includes the G-6-PD enzyme defect the incidence of hereditary qualitative and quantitative erythrocyte defects rises to one in three persons in Ghana.

This paper describes yet another gene interaction in Ghana, Hb C thalassaemia occurring in one member of a remarkable family of 14 (10 alive), none of whom has a normal haemoglobin genotype.

The Family

The family (Figs. 1 and 2), who now live 15 miles (24 km.) from Accra, belong to the Fante tribe. They were investigated because one of the children presented at the sickle-cell clinic of Korle Bu Hospital on 17 March 1967 with symptoms and

signs of sickle-cell anaemia. Table I summarizes the clinical features of the family. Table II gives the mother's obstetric history.

FIG. 2.—39-year-old sickle-cell thalassaemia mother with 10-year-old haemoglobin C thalassaemia daughter, both healthy looking.

TABLE II.—Mother's Obstetric History

Pregnancy	Delivery	Subsequent History of Child
1st	Normal. Female	1. Died suddenly aged 1 month
2nd	Normal. Female	2. Alive. 19 yrs. Sickle-cell anaemia (SS)
3rd	Normal. Male	3. Alive. 17 yrs. Sickle-cell anaemia (SS)
4th	Normal. Female	4. Died at 6½ yrs. after brief febrile illness
5th	Normal. Twin females	5. One died at 3½ yrs. Convulsions and fever
		6. Other twin alive. 12 yrs. old. Sickle-cell anaemia (SS)
		7. Alive. 10 yrs. Haemoglobin C thalassaemia
6th	Normal. Female	8. Alive. 8 yrs. Sickle-cell Hb C disease (SC)
7th	Normal. Female	9. Alive. 7 yrs. S-thalassaemia
8th	Normal. Female	10. Born at 8 months' cyesis after mother had severe rheumatic crisis. Died after 3 days
9th	Premature. Male	11. Alive and well. 4 yrs. Sickle-cell Hb C disease (SC)
10th	Normal. Female	12. Alive. 1 yr. old. Sickle-cell anaemia (SS)
11th	Normal. Male	

Note: 12 children, 4 dead, 8 alive with 4 Hb SS, 1 Hb S-thal., 2 Hb SC, and 1 Hb C-thal.

Investigations and Results

The haematological profiles are given in Table III. All 10 peripheral blood films showed target cells (between 1 and 20%); the least number was found in sickle-cell anaemia and sickle-cell thalassaemia, and the largest number where haemoglobin C occurs—namely, SC disease and C-thalassaemia (compare red cell fragility results below). Anisocytosis, poikilocytosis, and

FIG. 1.—The family. Back row, left to right: II 8, Hb SC; II 7 Hb C-thal; II 3, Hb SS; II 2, Hb SS; II 6, Hb SS. Front row, left to right: II 11, Hb SC; I 1, Hb SC; I 2, Hb S-thal; II 12, Hb SS; and extreme right II 9, Hb S-thal.

TABLE I.—Clinical Features of Family

Subject	Age	Sex	Weight lb. (kg.)	Conjunctival Pallor	Jaundice	Lymphadenopathy	Palpable Liver	Palpable Spleen	Remarks
I 1	40	M	143 (65)	Nil	Nil	Nil	Nil	Nil	Healthy SC father. Joint pains rarely. Healthy looking S-thal mother. Late menarche. Joint pains in pregnancy
I 2	39	F	159 (72)	Yes	Yes	Nil	Yes	Nil	
II 2	19	F	101 (46)	Yes	Yes	Nil	Yes	Nil	Small size; SS. Late menarche. Joint pains only once
II 3	17	M	107 (48½)	Yes	Yes	Nil	Yes	Nil	Joint pains often. SS
II 6	12	F	56½ (25½)	Yes	Yes	Yes	Yes	Nil	Joint pains very often. Small size. SS
II 7	10	F	72½ (33)	Nil	Nil	Nil	Nil	Nil	Never complained. Healthiest of all. C-thal
II 8	8	F	50½ (23)	Nil	Nil	Yes	Nil	Nil	Joint pains rarely. Epistaxis. Dental caries. SC
II 9	7	F	51 (23)	Yes	Nil	Yes	Yes	Nil	Joint pains once when she had pneumonia. S-thal
II 11	4	F	31 (14)	Nil	Nil	Nil	Nil	Nil	Never complained. "Square-shaped" skull. SC
II 12	1	M	21 (10)	Nil	Nil	Nil	Yes	Yes	"No trouble." SS

TABLE III.—Haematological Profiles of D Family

Subject	Sickling Test	Hb Elect	Foetal (%)	Hb A ₂ (%)	Red Blood Cells							White Cells						E.S.R.	Genotype	
					Hb (g./100 ml.)	R.B.C. (mil.)	P.C.V. (%)	M.C.H.C. (%)	M.C.V. (cu.μ)	Retics.	Target Cells (%)	Blood Film	W.B.C.	P	L	M	Eo			Ba
I 1	Pos.	S + C. (C = 46%)	1.2	—	15.1	6.0	47	32	78	0.4	8	Anisocytosis, hypochromia, target cells	4.3	53	45	2	0	0	1	Hb SC
I 2	Pos.	S + A. (A = 21%)	2.3	6.1	11.0	4.7	35	31	74	0.2	1	Anisocytosis, hypochromia	4.6	56	41	1	0	2	18	S-thalassaemia
II 2	Pos.	S	10.2	3.1	8.0	2.8	26	30	92	3	1	Anisocytosis, polychromasia, 2 normoblasts per 100 W.B.C.	6.6	41	56	0	1	2	23	Hb SS
II 3	Pos.	S	2.4	—	7.0	3.0	23	30	77	4.5	3	Anisocytosis, poikilocytosis, 10 normoblasts per 100 W.B.C., sickled forms	9.2	60	35	2	2	1	33	Hb SS
II 6	Pos.	S	2.7	—	7.0	3.0	22	30	75	3.5	1	Anisocytosis, poikilocytosis, 1 normoblast per 100 W.B.C., sickled forms	7.2	45	45	1	9	0	26	Hb SS
II 7	Neg.	C + A. (A = 36%)	2.5	—	12.0	4.8	36	33	75	2	20	Anisocytosis, target cells	8.3	29	68	—	3	—	4	C-thalassaemia
II 8	Pos.	S + C. (C = 48%)	0	—	10.3	4.3	33	31	77	3	10	Anisocytosis, hypochromia, target cells	4.3	32	66	—	2	—	3	Hb SC
II 9	Pos.	S + A. (A = 29%)	4.1	—	10.0	3.6	32	31	89	0.8	5	Anisocytosis, poikilocytosis	3.6	50	48	1	1	0	15	S-thalassaemia
II 11	Pos.	S + C. (C = 48%)	1.8	—	10.5	4.0	33	32	83	1	9	Slight anisocytosis, hypochromia, target cells	6.6	27	72	—	—	1	15	Hb SC
II 12	Pos.	S	5.5	—	10.4	3.6	32	32	89	0.7	2	Slight anisocytosis	3.2	17	82	—	1	—	2	Hb SS

G-6-PD was normal in each case.

hypochromia were common. Sickled forms were seen in the plain film only in two Hb SS patients who also had the lowest haemoglobin and haematocrit values. Only the father (healthy SC) and one daughter (healthy C-thalassaemia) had haemoglobins of 12 g./100 ml. or more, with equivalently high haematocrits. There were no malaria parasites.

FIG. 3.—Osmotic fragility of red cells of four different haemoglobinopathies. Note fragility is least where haemoglobin C is present. Resistance to fragility progresses S-thal<SS<SC<C-thal.

Red cell fragility test was performed by the same technician on all the 10 samples on the same day. Osmotic fragility was not only decreased in all samples compared with normal—that is, the red cells were in all patients more resistant

to haemolysis than normal—but there seemed to be a definite pattern in the degree of resistance, thus $S\text{-thal} < SS < SC < C\text{-thal}$ (see Fig. 3).

FIG. 4.—Filter paper electrophoresis, tris-barbiturate buffer pH 8.6. The Hb A in the blood of I 2, II 7, and II 9 is present only in low concentration owing to the inhibitory action of the β -thalassaemia gene on the β -chain production of the normal A haemoglobin.

Haemoglobin electrophoresis was carried out on filter paper on a vertical Durrum type cell, with tris-barbiturate buffer at pH 8.6 (Fig. 4), and tris-borate buffer at pH 8.9 (Fig. 5). Where necessary starch-gel electrophoresis was carried out (Fig. 6) by the method of Poulik (1957), Hb A₂ determination by the method of Marengo-Rowe (1965), and quantitative determination of Hb A was made according to Ringelmann and Makunga (1965). The results show that not one member of the entire family of 10 has the normal genotype AA. The father (I 1) is SC and the mother (I 2) S-thalassaemia. The children come out SS (II 2, 3, 6, and 12), SC (II 8 and 11), S-thalassaemia (II 9), and C-thalassaemia (II 7) (Fig. 4).

Discussion

When an adult with sickle-cell haemoglobin C disease marries another with sickle-cell thalassaemia four different haemoglobinopathies may be, theoretically, expected in the offspring: SS, S-thalassaemia, SC, and C-thalassaemia. Provided the law of averages was obeyed and enough children resulted from the marriage one might expect these haemoglobinopathies to occur in roughly equal numbers. It is impossible to tell the genotypes of the four children who died out of 12 in this family, but it is not unexpected that of the eight alive all the four haemoglobinopathies are represented (Fig. 7). It is

FIG. 5.—Filter paper electrophoresis, tris-borate buffer pH 8.9. The Hb A₂ in the blood sample of I 2 is higher, 6.1%, than in the normal control, 2.1% (left).

FIG. 6.—Starch-gel electrophoresis pH 8.3. — The Hb A₂ is higher in the blood of I 2 (S-thalassaemia), compared with the control (AA) and II 2 (SS). In the latter there is a small F fraction between A and S. Hb C does not separate from A₂ at this pH in the blood of II 7 (C-thalassaemia).

not impossible to predict the haemoglobin genotype from the symptomatology in a place like Accra, where definite patterns of disease occur. Thus SC disease can often be distinguished clinically from adult cases of sickle-cell anaemia (Table IV),

FIG. 7.—Family tree.

TABLE IV.—Clinical Differentiation of Adult Hb SS and Hb SC Disease in Ghana

	Hb SC Disease	Sickle-cell Anaemia
Joint and bone pains ..	From late childhood	From early childhood. Getting scarce or ceased entirely
Growth ..	Normal	Stunted, normal, or very tall
Anaemia ..	Mild to moderate	Moderate to severe
Jaundice ..	Mild or nil in steady state	Always has a tinge even in steady state
Lymphadenopathy	Rare	Common
Splenomegaly ..	Common	Rare, but may be gross when seen
Hepatomegaly ..	Sometimes	Often
Leg ulcers ..	Rare	Common
Aseptic necrosis of femoral head ..	Common	Rare
Vitreous haemorrhage ..	Common	Rare
Precordial bruits ..	Rare	Common
Successful pregnancies ..	Multiple	One to three
Post-partum shock → death ..	Real danger	Real danger
Sickle-cell gnathopathy (front teeth and jaw protrusion) ..	Rare	Common
Longevity ..	Oldest alive to-day > 70 years	Oldest alive to-day 46 years

and the latter from sickle-cell thalassaemia and SF_{highgene} (Table V). The almost complete absence of joint pain history in the oldest girl (II 2), who otherwise has all the features of sickle-cell anaemia, can be explained by the high percentage (10.2) of foetal haemoglobin. The course of sickle-cell anaemia is milder when the proportion of Hb F is high.

We thought at first from the history that the mother was Hb SC, because of the sheer numbers of her children, but as it turned out she is the first sickle-cell thalassaemia patient on record with 11 pregnancies and 12 children (eight alive), none of the deliveries taking place in hospital. The combination of sickle-cell disease and thalassaemia has not only “seldom been reported in pregnancy but the condition of women with this combined haemoglobinopathy is believed to deteriorate during gestation” (Dunn and Haynes, 1967). However, Hendrickse and Watson-Williams (1966) state that “increasing anaemia near term, preventable by folic acid administration, is the only hazard.”

TABLE V.—Differential Diagnosis of Adult Hb “SS” Patients in Ghana

True Genotype	History	Physical Examination	Special Investigations
SS	Joint and bone pains when young, with eye coloration. Joint pains less now, or ceased	Anaemia, jaundice, leg scars. May be stunted, normal, or very tall. Women may have borne 1 or 2 children. Gnathopathy is the rule. Typically no spleen	Sickling +. Electrophoresis S or S + F. Blood profile typical. (Family study)
S-β-thalassaemia ..	Joint pain history variable. Some often, others nil	May be like SS or may look quite normal and healthy. Spleen may be palpable. Women may have many children	Sickling +. Electrophoresis S + (A + F trace). A ₂ raised. Blood profile helpful. (Family study)
SF thalassaemia ..	Joint pains rare or never	May be anaemic or look normal. Rarely jaundiced. Spleen may be palpable. Women may have many children	Sickling +. Electrophoresis S + (A + F). A ₂ not raised. High % F. Kleihauer test—heterogeneous cell stain. (Family study)
SF _{highgene} ..	Joint pains rare or never	May be anaemic from other causes, or be quite normal. Not jaundiced. Women capable of having many children	Sickling +. Electrophoresis S + F. High % F. Kleihauer test—homogeneous staining of cells. Normal profile. (Family study)
SD Punjab ..	Joint pains as in SS when young	(Our 2 patients are still young, but behave like Hb SS)	Sickling +. Blood profile like SS. Paper and starch-gel electrophoresis. S. Agar-gel electrophoresis pH 6 “A + S.” Solubility tests. Fingerprinting of Hb. (Family study)
S Korle Bu ..	Never had joint pains	Normal. Women have several children	Fingerprinting. Normal blood profile. Sickling +. (Family study)

We have often seen Hb SC patients fare worse than Hb SS patients of the same age and sex. This is because of the varying standards of living in different families. An SC patient from a background with a bad social history (broken homes, poverty, unintelligent parents, etc.) has a poor medical history, while an SS patient from a good home may go from year to year without a single crisis. This family provides a remarkable built-in control: the children live under the same roof, are bitten by the same mosquitoes, and are given the same tender care by the same diligent, intelligent, Methodist parents. Given the same circumstances it can be seen from the clinical features and blood profiles that sickle-cell anaemia is the most severe disease, followed by SC and S-thalassaemia diseases, and the least severe is C-thalassaemia. Our particular C-thalassaemia girl is completely asymptomatic and would pass for a normal healthy Ghanaian schoolgirl; so is the SC father, whose Hb of 15.1 g./100 ml. is not unusual of Ghanaian SC haemoglobinopathy in the steady state.

Haemoglobin C thalassaemia is very rare; only 31 cases were recorded in the world press by 1965. In their detailed review of 24 cases from the literature Russo and Mollica (1965) observed that 17 were of the black race and seven were white, with the age range from 8 months to 68 years. Most of them were clinically anaemic, but at least three had haemoglobin values above 12 g./100 ml. In half of them the spleen was palpable. Red cell osmotic resistance was invariably increased, and target cells plus anisopoikilocytosis were constant features. The type of thalassaemia present in almost all cases was β-thalassaemia. This is the case in the present family where A₂ has been shown to be raised in the S-thalassaemia mother.

The comparatively excellent health of our C-thalassaemia patient and her S-thalassaemia mother, who was capable of passing through 11 pregnancies unscathed, may be due to the remarkable variability of the clinical results of the simultaneous inheritance of the traits of thalassaemia and a β-chain variant. Humble *et al.* (1954) found that, within the same family, S-thalassaemia could either produce an anaemia or be entirely symptomless. Almost asymptomatic adult sickle-cell disease patients are not uncommonly found in Ghana. Even with sickle-cell anaemia, which is reputed to kill patients before they are of childbearing age, our experience is similar to that of Serjeant *et al.* (1968), who reported “relatively benign sickle-cell anaemia in 60 patients aged over 30 in the West Indies.” We think this is because of the rising standard of living as emphasized by the *Ghana Medical Journal* (1964).

Frequency and severity of crises get less as sickle-cell anaemia (SS) patients get older, and this may cause confusion with other haemoglobinopathies that may also be returned “SS” on paper electrophoresis. Notable among these is the SF_{highgene} which was first described, also from Accra, by Edington and Lehmann (1955a, 1955b). This is a benign condition in which haemoglobin S together with a high level of foetal haemoglobin persists into adult life. Other reports from Africa were by Jacob and Raper (1958) in Uganda and Thompson and

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.

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Outbreak of *Brucella melitensis* Type 2 Infection in London

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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.

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

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