These are possibly incomplete forms of Behcet's syndrome, but it is not known at present how many cases of recurrent orogenital ulceration ultimately develop iritis, the third symptom of Behcet's triad.

Laboratory tests in two patients showed evidence of a systemic disturbance, while the third was found to have discoid lupus erythematosus.

The herpes neutralization tests were carried out by Mr. Brian Moore, of the Institute of Medical and Veterinary Science, Adelaide.

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BACTERAEMIA IN HUMAN BRUCELLOSIS

BY

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The studies presented in this paper were undertaken to investigate the incidence of bacteraemia in human brucellosis at Malta, and to find out the relationships between recovery of brucella from blood and from marrow as well as between bacteraemia and pyrexia.

Methods and Results

A study was carried out on the result of blood cultures drawn from 93 cases of brucellosis that were admitted to medical wards of St. Luke's Hospital, Malta, without having yet received antibiotic treatment. In one of these cases (Case 1) blood was drawn and cultured three times. In 11 cases blood was drawn and cultured twice, first for diagnostic purposes, and then to correlate the results of cultures of blood and marrow drawn simultaneously as described below. In no instance was antibiotic treatment started before these samples had been collected. The total number of blood cultures was 106. At least 5 ml. of blood was collected by sterile syringe from an antecubital vein and cultivated in tryptose broth and agar, using Castaneda's combined method of culture. No sample was stated to be sterile until 21 days had elapsed. Where culture was negative the diagnosis was based on serum agglutination of *Brucella melitensis* at a titre of at least 1 in 160 at some period of the disease. *Br. melitensis* was recovered from 69.8% of instances (Table I).

The results of 45 bone-marrow cultures drawn from the sternum of 45 out of the 93 patients were also studied. The samples were obtained by aspirating at least 2 ml. of bone-marrow into sterile syringes fitted with Salah needles, and were cultivated by the same

TABLE I.—Result of 106 Blood Cultures from 93 Cases of Brucellosis

	No.	%
Blood culture positive	74	69.8
" " negative	32	30.2

method as described for blood. The presence of marrow in the drawn samples was confirmed in all instances by demonstrating marrow cells in slides that were prepared from samples. *Br. melitensis* was recovered from 66.7% of samples (Table II). When the results of cultures of blood and of marrow samples, collected simultaneously from 35 of the above cases, were compared with each other, it was found that bone-marrow samples could be sterile when bacteraemia was present, and vice versa (Table III).

TABLE II.—Result of Bone-marrow Cultures from 45 Cases of Brucellosis

	No.	%
Marrow culture positive	30	66.7
" " negative	15	33.3

TABLE III.—Result of Simultaneous Blood and Marrow Cultures from 35 Cases of Brucellosis

Marrow Culture	Blood Culture	No.	%
Positive	Positive	16	45.7
"	Negative	7	20.0
Negative	Positive	7	20.0
"	Negative	5	14.3

Blood was collected for culture at random with no reference to the degree of the patient's temperature on the day when the blood was collected or on the days preceding the collection. Each blood sample was collected during the morning, one to two hours after the patient's temperature had been measured and recorded. The relationship between the result of blood culture and the patient's temperature on the day of collection was studied by comparing the result of the blood culture with the morning temperature as well as with the higher of the morning or the evening temperature. When blood was collected for culture more than once from the same patient, only the result of the sample collected on the first occasion was considered.

The results observed in the 93 cases are presented in Tables IV and V, which also show estimates of the number of positive and negative cultures that would

TABLE IV.—Number of Positive and Negative Blood Cultures Observed in 93 Cases of Brucellosis in Relation to the Morning Temperature on the Day When the Blood was Collected and the Numbers Expected if There Was No Association Between Pyrexia and Bacteraemia

Temperature Class Intervals		Blood Cultures			
		Positive		Negative	
(°F.)	(°C.)	Observed	Expected	Observed	Expected
97.0-97.8	36.1-36.6	4	5.59	4	2.41
98.0-98.8	36.7-37.1	16	20.27	13	8.73
99.0-99.8	37.2-37.7	20	16.80	4	7.20
100.0-100.8	37.8-38.2	12	11.18	4	4.82
101.0-101.8	38.3-38.8	5	4.19	1	1.81
102.0-102.8	38.9-39.3	3	2.10	0	0.90
103.0-103.8	39.4-39.9	5	4.89	2	2.11
104.0-104.8	40.0-40.5	0	0	0	0
105.0-105.8	40.6-41.0	0	0	0	0
Total		65		28	

Test of significance: $\chi^2 = 7.60$ and $0.05 < P < 0.10$. The first two and the last five classes have been combined.

have been expected to occur if there were no causal relationship between pyrexia and bacteraemia. No significant difference between the observed and the expected numbers has been obtained. Furthermore, cases have occurred where *Br. melitensis* has been recovered from the blood of patients who had been running normal or subnormal temperatures for periods of days or weeks, as in Case 1. It appears that the comparatively large incidence of positive blood cultures at

TABLE V.—Number of Positive and Negative Blood Cultures Observed in 93 Cases of Brucellosis in Relation to the Higher of the Morning or Evening Temperatures on the Day when Blood was Collected, and the Numbers Expected if There Were No Association Between Pyrexia and Bacteraemia

Temperature Class Intervals		Blood Cultures			
(°F.)	(°C.)	Positive		Negative	
		Observed	Expected	Observed	Expected
97.0-97.8	36.1-36.6	1	1.40	1	0.60
98.0-98.8	36.7-37.1	9	12.58	9	5.42
99.0-99.8	37.2-37.7	17	18.17	9	7.83
100.0-100.8	37.8-38.2	15	12.58	3	5.42
101.0-101.8	38.3-38.8	9	8.40	3	3.60
102.0-102.8	38.9-39.3	5	3.49	0	1.51
103.0-103.8	39.4-39.9	6	5.59	2	2.41
104.0-104.8	40.0-40.5	1	1.40	1	0.60
105.0-105.8	40.6-41.0	2	1.40	0	0.60
Total		65		28	

Test of significance: $\chi^2 = 6.765$ and $0.05 < P < 0.10$. The first two and the last five classes have been combined.

TABLE VI.—Result of Cultures from 11 Patients Whose Blood was Drawn and Cultured Twice

Patient	Days from Date of Admission	Temperature of Patient		Result of Culture
		(°F.)	(°C.)	
1	3	100	37.8	+
	6	100	37.8	+
2	3	103	39.4	+
	7	100	37.8	+
3	2	103.4	39.7	+
	3	105	40.6	+
4	3	98.5	36.9	+
	13	98	36.7	+
5	6	99.6	37.6	+
	52	101	38.3	+
6	3	99.4	37.4	—
	11	99.6	37.6	—
7	1	98.5	36.9	—
	7	102.0	38.9	—
8	1	98.5	36.9	—
	4	99.4	37.4	—
9	1	99	37.2	—
	12	98	36.7	+
10	7	104	40.0	—
	3	101	38.3	+
11	2	98.2	36.8	+
	9	100.2	37.9	—

temperatures ranging from 98 to 101.8° F. (36.7 to 38.8° C.) is due to the higher incidence of brucellosis patients presenting at hospital with this range of temperature, be they culturally positive or negative. When blood was collected twice from the same patient the result of the second culture was unpredictable. Table VI shows the results of these cultures with reference to the patients' highest temperature on the day when blood was collected, as well as to the time interval in days from the day of admission to hospital.

The following case is selected for publication because of the prolonged period of bacteraemia without pyrexia.

Case Report

A 9-year-old boy attended the out-patient clinic on September 11, 1958, with a history of three episodes of fever to 103° F. (39.4° C.), each lasting about three weeks. The first began in April. After a remission he had developed

low-grade pyrexia on September 4, and this had persisted. Serum agglutination with *Br. melitensis* had given negative results on several occasions. There was no history of exposure to infection with brucella. The boy was pale but did not look ill; temperature 99.5° F. (37.5° C.). The spleen was about 3 cm. below the costal arch, firm, not tender; the liver was not appreciably enlarged; lymph nodes were enlarged at the anterior and posterior triangles of the neck, at the axilla, and at the groins; they were discrete, mobile, rather soft, not tender. The largest of these nodes was at the neck. When removed (October 21) it measured 1.7 by 1.8 by 1.2 cm. Histological examination showed only follicular hyperplasia and sinus catarrh. Physical examination was otherwise essentially negative. Chest x-ray films showed clear lungs; Mantoux intradermal test was negative at 1 in 1,000 dilution, Paul-Bunnell test, Wassermann reaction, and Kahn test of the blood were negative. Serum agglutination with *Br. melitensis* was positive only at a titre of 1 in 40. This test was repeated after five days with the same result.

The patient was kept in hospital from September 18 to November 10, 1958. From September 21 there was apyrexia, yet *Br. melitensis* was recovered three times from the blood and once from the bone-marrow. The agglutination titre rose, glandular enlargement regressed, and splenic enlargement remained. The child's colour returned soon after admission; his appetite was good and his general condition excellent.

Investigations.—Haematological examination on October 8 showed: haemoglobin, 11.4 g./100 ml.; erythrocytes, 5,110,000/c.mm.; packed cell volume, 38.5%; mean corpuscular volume, 75.5 cubic microns; mean corpuscular haemoglobin, 22.3 μ g.; mean corpuscular haemoglobin concentration, 29.6%. Blood smear showed a moderate increase in central achromia of erythrocytes; polychromasia, stippling, and normoblasts were absent; there was anisocytosis, with a tendency to microcytosis but also with rare macrocytes; poikilocytes rare; reticulocytes 1%. White blood cells numbered 6,650/c.mm. (36% polymorph neutrophils, 6% eosinophils, 1% basophils, 49% lymphocytes, 8% monocytes). No immature leucocytes were seen. A myelogram showed: myeloid, 55.9% (leucoid, 42.2%); and erythroid 7.6%; the myeloid:erythroid ratio being 7.3:1 and the leucoid:erythroid ratio 5.5:1. Total serum proteins were 9 g./100 ml., and the albumin:globulin ratio 2:1. Serum electrophoresis showed an essentially normal pattern. Serum glutamic oxalacetic transaminase was 19 units/ml. (control: 11 units/ml.) and serum glutamic pyruvic transaminase 10 units/ml. (control: 13 units/ml.). Plasma bilirubin, thymol turbidity, and zinc sulphate turbidity were normal.

"Achromycin V" (1 g. daily in four equal doses by mouth from October 22 to November 2) caused bacteraemia to disappear, but splenic enlargement remained. The spleen was still 1 cm. below the costal arch when the patient was seen at the out-patient clinic on December 1, 1958, and January 12 and 26, 1959. After leaving hospital he went to school and played games; yet he remained apyrexial except for an evening temperature of 99° F. (37.2° C.) from December 9 to 12, 1958, and of 100° F. (37.8° C.) on January 1 and 2, 1959. Samples of blood cultured on November 17 and December 1, 1958, were sterile.

Comments

Human brucellosis has protean manifestations. Its onset may be acute or insidious; fever is highly irregular in degree, duration, and course; long remissions may occur; and its symptoms are exceedingly variable in nature and in severity. Diagnosis is derived from agglutination tests with brucella and is confirmed by culture of brucella from the blood-stream, bone-marrow, or other sources. It appears, however, that the occurrence of bacteraemia is as unpredictable as the clinical findings.

The predilection of brucella for the reticulo-endothelial system has been proved by clinical, post-mortem, and experimental studies (Sharp, 1934; Huddleson, 1943; Spink, 1956). Meyer (1943) suggested that continuous bacteraemia might be maintained by focal lesions in bone-marrow. The importance of bone-marrow as a reservoir of brucella can be seen from the fact that brucella can be recovered from bone-marrow with the same facility as blood, as suggested by Tables I and III as well as by the findings of Hamilton (1954), who obtained the same recovery rate of *Br. melitensis* from samples of venous blood as from samples of marrow. Simultaneous blood and marrow samples, drawn by Spink (1956) in the U.S.A. from 59 cases of brucellosis, yielded brucella from the marrow in five cases when bacteraemia was absent, but in no instance was brucella recovered from blood when the marrow sample was sterile. These findings differ from what is reported in Table IV, where blood culture was positive in seven cases from which sterile bone-marrow was drawn. It is therefore suggested that brucella may not be uniformly distributed in bone-marrow and that bacteraemia might be also maintained from other sources as spleen or liver.

The basic tissue reaction to invasion by brucella is granulomatous. Nodular foci have been reported in animals and man; they have been found in spleen, liver, lymph nodes, bone-marrow, etc. The reaction involves macrophage cells with or without the formation of giant cells, as well as plasma cells and lymphocytes. Necrosis is slight or absent, but abscess formation and even caseation may occur (Sundberg and Spink, 1947; Hamilton, 1954; Spink, 1956). Brucella organisms are located intracellularly and may result in a persistent infection for a long time (Spink, 1956). Whatever the factors may be that break the cellular barrier, it would not appear, from what has been reported above, that pyrexia is a sign that the cellular barrier has been broken and that brucella organisms are allowed to roam in the blood.

The pyrexia of brucellosis does not appear to be induced by bacteraemia but by some chemical change connected with the infected tissue cells and influencing the thermo-regulatory centre of the brain. This is supported by the fact that the clinical course of brucellosis is rapidly influenced by treatment with corticotrophin or cortisone, showing an abrupt drop in temperature, though bacteraemia may persist (Spink and Hall, 1952).

Investigations into the possible mechanisms of pyrexia have shown that many bacteria have in their structure lipopolysaccharides of high molecular weight, which, when injected intravenously, cause febrile responses that are preceded by prompt leucopenia (Wood, 1958). With injections that are repeated at intervals of a day or less, a state of tolerance is induced; this state, however, ceases to exist within two weeks after discontinuance of the injections. Further investigations suggest that bacterial pyrogens act upon polymorphonuclear leucocytes, with release of endogenous pyrogen from leucocytes or with alteration of chemical structure of bacterial pyrogen by some action of leucocytes (Snell, 1958). Polymorphonuclear leucocytes appear to have an insignificant role in brucellosis, but it would appear also that the presence of only a minimal number of leucocytes in the blood is necessary for the pyrexial reaction to take place (Cranston *et al.*, 1956). Brucellosis infection which is characterized by leucopenia, and

which may present bacteraemia without pyrexia, should offer a fruitful field for the elucidation of the mechanism of pyrexia in man. The significance of leucopenia in brucellosis also merits a careful and detailed study.

Summary

The results of blood and of marrow cultures from cases of brucellosis have been studied. *Brucella melitensis* was recovered from 69.8% of blood cultures and from 66.7% of bone-marrow cultures. Brucellosis bacteraemia is apparently unrelated to pyrexia. Brucellosis should be a fruitful field for the elucidation of the mechanism of pyrexia in man.

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ASCORBIC ACID INHIBITION OF THE GLUCOSE-OXIDASE TEST FOR GLYCOSURIA

BY

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A simple test for the presence of glucose in urine, using the enzyme glucose oxidase, was introduced about three years ago and marketed under the names "clinitix" and "tes-tape." Hunt *et al.* (1956) compared these two products with each other, and with two methods of glucose detection employing reduction of copper sulphate—namely, Benedict's reaction and "clinitest" tablets. They thought both new methods were equally satisfactory as qualitative tests, being as reliable as the copper sulphate reactions and perhaps more sensitive, but they considered tes-tape to be unreliable quantitatively. Like Tunbridge *et al.* (1956), they expressed a preference for clinitix. Luntz (1957), comparing clinitix and clinitest for clinic use, also was impressed favourably with the former.

Clinistix has now come to be used widely as a simple, quick test for glycosuria. According to the manufacturers the test is carried out by dipping the impregnated end of a strip of thick blotting-paper into the urine and noting the presence or absence of a blue colour after an interval of one minute. The end of the paper is impregnated with glucose oxidase, orthotolidine, and vegetable peroxidase. In the presence of atmospheric oxygen, glucose oxidase oxidizes glucose to glucuronic acid and hydrogen peroxide. The latter, in the presence of the peroxidase, gives a blue colour