A fuller account of this work will be published elsewhere. We are much indebted to Dr. J. G. Humble, M.V.O., for advice and criticism. The work was supported by generous grants from the Endowment Funds of Westminster Hospital.

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Dr. B. W. Lacey asked if colostrum fat had been tried as a source of clotting factor and if the action of the clotting factor was influenced by the level of cholesterol.

Professor N. F. Maclagan in reply: We have not tried colostrum fat. So far as we know, the plasma cholesterol does not affect clotting systems directly.

Staphylococcal Antibody in Osteomyelitis and Suppurative Arthritis

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SEROLOGICAL tests for the diagnosis of staphylococcal infection are of poor repute. Nevertheless recent experience has led me to the conviction that an examination of the blood for staphylococcal antibody should be carried out more frequently for the diagnosis of unexplained pyrexias and for bone and joint disease.

Table I lists the commoner antigens of pathogenic staphylococci which have been characterized; the percentages are approximations and are based on published figures.

TABLE I.—DISTRIBUTION OF STAPHYLOCOCCAL ANTIGENS

Antigen	Produced by approx. %
Coagulase	100
Alpha-hæmolysin	80–100
Delta-hæmolysin	97
Panton-Valentine leucocidin ..	50
Hyaluronidase	95
Staphylokinase	90

If coagulase is produced by all pathogenic strains then the titration of anti-coagulase would appear to be the obvious choice, but this is impracticable at present. There appear to be at least three antigenically-distinct coagulases, and we wait on the provision of purer coagulase preparations as well as on purer fibrinogen substrate. Alpha-hæmolysin is the only staphylococcal antigen available as a standardized commercial preparation; its antibody is the one I have been measuring. Though delta-hæmolysin and hyaluronidase have almost as universal a distribution as coagulase, they are poor antigens. Interest in the Panton-Valentine leucocidin has recently been revived by Gladstone and van Heyningen (1957). I am not aware of any reports on anti-staphylokinase.

To return to the measurement of anti-alpha-hæmolysin, this is one of the simplest of serological tests and can be performed by a technician in an hour and a half. The patient's serum is inactivated by holding at 56° C. for 30 minutes. Dilutions of serum are made in 0·5 ml. volumes of saline. To each dilution of serum is added one unit of standard toxin in 0·5 ml. of saline +0·1% gelatin. The toxin is prepared from the Lyophilized Staphylococcal Alpha Hæmolysin of the Wellcome Research Laboratories. After 30 minutes standing at room temperature, 0·1 ml. of a 10% suspension of fresh rabbit cells is added to all tubes, including two controls—one serum alone and one toxin alone. All tubes are incubated for an hour and the end-point of inhibition by anti-toxin is determined by the tube showing 50% hæmolysis. As one unit of toxin has been placed in each tube, the titre of

antihæmolysin is the denominator of the dilution fraction. Most workers agree that the majority of healthy people have titres of less than 2 units.

Table II shows the distribution of antibody levels among patients at the Royal National Orthopædic Hospital; and Table III the totals of patients with raised titres expressed as

TABLE II.—ANTI-ALPHA-HÆMOLYSIN TITRES OF HOSPITAL PATIENTS

	Osteomyelitis	Secondary staph. infection	Presumed staph. infection	No staph. infection
Less than 2 units/ml. ..	33	20	—	231
2–4 units/ml.	21	11	—	13
4–8 units/ml.	10	10	1	1
8–16 units/ml.	5	2	1	—
16–32 units/ml.	5	3	2	—
Total 369	74	46	4	245

TABLE III.—PERCENTAGE OF PATIENTS WITH TITRES OF 2 UNITS OR OVER OF ANTI-ALPHA-HÆMOLYSIN

	%
No staph. infection	5·7
Staph. osteomyelitis and suppurative arthritis ..	55·4
Staph. infection elsewhere	56·5

percentages. It may be true that higher levels of antibody are encountered more frequently in patients with osteomyelitis, but in this series a patient with staphylococcal pyelonephritis had as high a titre as any patient with osteomyelitis, and the highest titre I have seen recorded (Bergquist, 1950) was that of a patient who developed staphylococcal empyema and whose titre rose from a pre-infection level of 1 unit to 440 units within a month.

The fact that about 45% of patients known to have staphylococcal infection, whether osteomyelitis or otherwise, have titres of less than 2 units would seem at first to make this test of very little value. On the other hand, we have found that a high titre, and more especially a progressively rising titre, is of the greatest help on occasions, as in the following cases.

Case I, a girl, and *Case II*, a boy, both aged 14. Each first complained of pain in the right side, the girl for eleven weeks and the boy for eight weeks. Both were diagnosed as having appendicitis, but appendicectomy did not cure the pain. In both cases the diagnosis was then changed to tuberculosis of the spine on radiological grounds. The girl was put on a course of anti-tuberculosis therapy. Both were eventually transferred to our hospital. Fig. 1 shows our findings with respect to the girl.

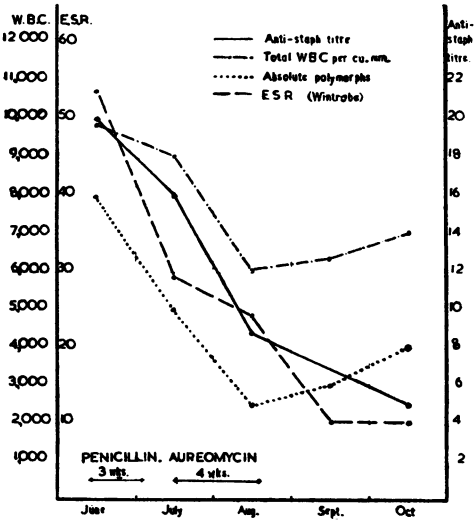

FIG. 1 (*Case I*).

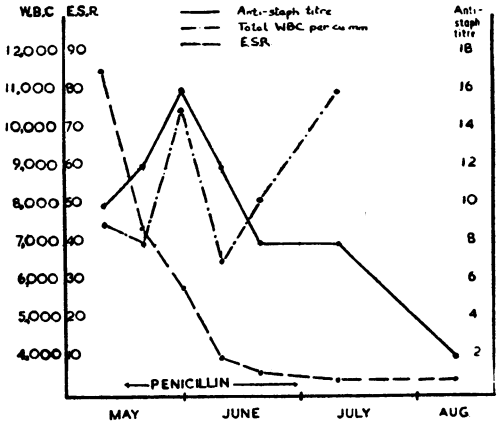

FIG. 2 (*Case II*).

On admission she was found to have 20 units of staphylococcal antibody and straight away was given penicillin. After three weeks this was changed to Aureomycin, which was continued for four weeks. By the time she was discharged her titre had fallen to 4 units.

The boy, who came to us twelve weeks after the onset of his pain and two weeks after his appendicectomy, had a titre of 10 units on admission (Fig. 2), which continued to rise for a time, while receiving penicillin, and then came down to 2 units. His total leucocyte count was even lower than the girl's, being only 7,500 per c.mm. and the absence of leucocytosis made some clinicians doubt whether this could be a staphylococcal infection.

Case III.—Woman, aged 58, developed pain radiating down the back of her right leg following a fall in December 1954. In March 1955 there was a sudden exacerbation of pain and she was admitted with a diagnosis of chronic back strain. Her antibody titre on admission was found to be 20 units, sedimentation rate 50, and leucocyte count 6,050 (Fig. 3). X-ray showed gross narrowing

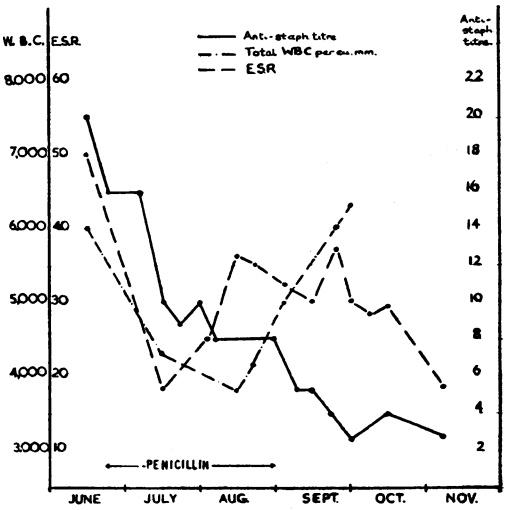

FIG. 3 (*Case III*).

of the I.V. space 4/5 and erosion of the L.V.4 anteriorly. Her antibody titre fell steadily on penicillin until, at her discharge on October 18, it was 4 units and her sedimentation rate 27 mm. in one hour (Westergren). She was then free from pain and X-ray showed that her lumbar vertebræ were fusing. A month later her antibody titre was 3 units, sedimentation rate 18 mm. and she was in excellent health. There was satisfactory consolidation of her lumbar vertebræ with no deformity.

Case IV.—A woman of 49 developed bronchitis and pleurisy at the end of October 1954. She spent fifteen days in bed and was given sulphonamide tablets. She then developed pain in the middle of her back at the level of the tip of her scapula, particularly noticeable at night. X-ray showed a destructive lesion of D.11, with loss of disc space, and the patient was admitted with a provisional diagnosis of spinal tuberculosis. On admission her only abnormal sign was tenderness over L.1 and 2, her temperature was normal and her white cell count was 17,800. Sedimentation rate 13 mm. in one hour (Westergren). Mantoux positive. Her anti-alpha-hæmolysin titre was 4–5 units. She was given penicillin and her titre fell to 3 units and her total white count to 8,000. She was discharged in good health.

This serological test has been of value in correcting the diagnosis in a variety of disorders, the most common being an early spinal lesion that has been labelled tuberculous. It has also given a clue to the cause of the fever of several patients who have been treated with cortisone for rheumatoid arthritis or lupus erythematosus and who have subsequently developed "silent" staphylococcal abscesses. In cases such as spinal disease, the results consequent on the use of this test have been highly gratifying—the patients have been cured much sooner than they would have been had they remained on their anti-tuberculous regimen. But why do only half of the patients with staphylococcal infection show this response. Is it due to lack of antigenic stimulus? By all accounts at least 80% of pathogenic strains of *Staph. pyogenes* produce alpha-hæmolysin *in vitro*. Incapacity to produce antibody is not likely to be an explanation, as the giving of toxoid to low-titre patients with staphylococcal infection has always produced a rise in antibody. The walling-off of the antigen may explain low titres in chronic cases, and we have examples of rise in antibody following operative interference, but this does not account for low antibody levels in early and progressive infections. Duration of infection cannot explain the discrepancy. We have the record of a patient whose titre rose to 8 units within three weeks of the onset of osteomyelitis. From Table IV it may be seen that the time factor does not appear to be very important.

TABLE IV.—ANTI-ALPHA-HÆMOLYSIN AND ANTI-LEUCOCIDIN TITRES OF PATIENTS WITH STAPHYLOCOCCAL OSTEOMYELITIS

Case	Duration		Anti-alpha	Anti-P.V.L.
1	3 weeks		8	80
2	1 month		10	200
3	1 month		2	12
			2–4	100
			2	40
4	6 weeks	Operation—	2	Not tested
			2	100
			4	80
5	7 months		16–20	100
			10–16	80
6	7 months		8–10	16
			4–8	16
			2–4	24
7	10 years	Operation—	2	16
			2–4	24
8	37 years		2	16–20

I shall refer briefly to another antibody, anti-leucocidin (Anti-P.V.L.). The normal level in serum seems to be up to 10 units. Gladstone has been measuring the anti-leucocidin titres in the sera of some of our patients and, so far, it appears that this may be a more sensitive indicator of staphylococcal infection in some cases. Table IV sets out for comparison the two levels in a few patients. In Cases III and IV the anti-leucocidin titre was a better guide, but in Case VI the anti-alpha-hæmolysin titres were more helpful. At present it seems worth while to carry out both tests.

I would like to thank Dr. Gladstone for allowing me to quote his findings.

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Dr. E. S. Duthie said that numerous measurements made by him on the antibody level against staphylococcal coagulase in the sera of normal individuals showed a wide variation. Similar results were found in 1950 by Rammelkamp, Hezebicks and Dingle in America and for this reason it was unlikely that the level of antibody against coagulase would be of value in the diagnosis of staphylococcal infections. On the other hand he had found high anti-alpha-lysin levels similar to those given by Dr. Lack in staphylococcal bone infections. Since the level of anti-alpha-lysin was relatively constant in normal cases he felt sure that this was a most valuable test and it would be interesting if at the same time anti-leucocidin values could be measured.

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Dr. Herta Schwabacher said that she had found the estimation of anti-alpha-hæmolysin to be of diagnostic value only when the focus of staphylococcal infection was in muscle or bone. She asked whether Dr. Lack had ever found a significant titre of staphylococcal antibody in staphylococcal skin infections? With the co-operation of Mr. H. F. Lunn, Casualty Officer, Peace Memorial Hospital, Watford, she had examined the sera in a small series of patients with furunculosis. On no occasion was antibody detected. These patients were given a course of staphylococcal toxoid and their sera when retested failed to show any rise in titre.

Dr. Lack in his reply stated that he too had never observed staphylococcal antibody in infections of the skin, but had done so when these patients had received toxoid therapy.

Dr. K. B. Rogers asked Dr. Lack whether his work would suggest that staphylococcal antitoxin would help in the treatment of extremely ill, toxic, cases of acute osteomyelitis as in the last few years two children with staphylococcal osteomyelitis had been lost, although the staphylococci from both cases were fully sensitive to penicillin.

Dr. Lack said that he had had no experience in the use of anti-toxin for the treatment of the toxæmia of acute osteomyelitis in children.

Dr. B. W. Lacey said that the cases mentioned by Dr. Rogers would seem to be ideal for the combined use of steroid and chemotherapy.