Antiserum raised in guinea-pigs immunized with HAA (purified by zonal centrifugation), and shown to be both sensitive and specific for the detection of HAA by gel diffusion, counter current immunoelectrophoresis, and complement fixation, was used to coat 0.81- μ m polystyrene latex spheres in a 0.5% suspension. The latex was stabilized with bovine serum albumin and preserved with 0.1% sodium azide. The reagent is stable for at least three months at 4°C.

During preliminary work on the test it was found that about 3% of 200 normal human sera caused non-specific agglutination of the latex particles. This false-positive reaction could be inhibited readily by the addition of diluted normal guinea-pig serum to the serum under test before the addition of the latex reagent. This non-specific reaction seems to be peculiar to the latex test as no serological reaction is demonstrable by the gel diffusion, counter current electrophoresis, or complement fixation test.

To carry out the test, a drop (about 25 μ l) of the test sample is placed on a microscope slide and a similar drop of 1:20 normal guinea-pig serum is added. This is then stirred gently with a disposable wooden applicator stick for a few moments. A drop of the latex reagent is added and the mixture is stirred until the diameter of the drop is about 2 cm. The slide, placed on a black tile, is then gently rocked to and fro for two minutes. By this time an HAA-positive serum will show a definite agglutination, though a strongly positive sample will agglutinate the latex within 15-30 seconds. HAA-negative sera produce no visible agglutination within two minutes. The distinction between a positive and negative result becomes more difficult when weak antigens are tested, but it has been our experience that blind trials with different observers who have some experience of the test show that variations in interpretation are minimal.

The latex test works well with serum or plasma that has been stored at 4°C for 24 hours, but very fresh plasma may cause non-specific agglutination. No agglutination could be seen when whole blood or whole lysed blood was used. Serum or plasma containing much visible particulate matter was filtered before testing.

Results

The latex test was evaluated on a panel of 60 sera provided by the National Institutes of Health, Washington, D.C., on a panel of 28 HAA-positive sera collected for antigen purification, and on a total of 551 sera collected from presumed

normal volunteers. The results of this evaluation are shown in the Table.

Results of Evaluation

Source of Sera	No. of Sera Tested	No. of Sera Positive by HAA Tests			
		Gel Diffusion	Counter Current Immuno-electrophoresis	Complement Fixation	Latex Agglutination
N.I.H. panel†	60	38	38	42	41
Pfizer HAA-positive panel	28	12	26	28	25
Pfizer HAA control panel 1	51	0	N.T.	0	0
Pfizer HAA control panel 2	500	N.T.	N.T.	N.T.*	3†

N.T. = Not tested.

*Not tested except as confirmation of latex-positive sera.

†One HAA-positive, two false positives (see text).

‡The N.I.H. panel consists of 60 sera, 47 of which are stated to be HAA-positive. The complement fixation titre ranges from 1:4 to >1:256.

The latex test has a sensitivity comparable to the counter current immunoelectrophoresis technique and nearly equal to the complement fixation test. The value of the test for routine use was shown during examination of 500 sera, as a result of which one positive serum from a symptomless carrier was found. This was confirmed by gel diffusion and complement fixation tests, and serum from the same donor remained positive when test bleeds were taken on two subsequent occasions at monthly intervals. Agglutination of both the remaining positive sera was abolished when undiluted normal guinea-pig serum was used as the inhibitor.

These results indicate that the latex agglutination test is similar in sensitivity to the routine tests in current use, while the advantage of extreme rapidity will make it of value in screening donors before bleeding. The very occasional false positive is unlikely to be of significance in practice as it would be the normal procedure to confirm a suspected positive serum by one of the other tests available.

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MEDICAL MEMORANDA

Occult Carcinoma of the Breast

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Occult carcinoma of the breast may be defined as a carcinoma of the breast in which metastases are clinically evident before the primary tumour in the breast is detectable. Usually metastasis to the axillary lymph nodes is the presenting feature,

but cases with metastases to other sites have been described. The present case is remarkable in that a period of 17 years elapsed between the excision of an axillary lymph node containing metastatic breast carcinoma and the subsequent clinical manifestation of the primary tumour in the breast.

Case Report

In 1953 a woman aged 33 noticed a painless swelling in the right axilla. On examination the swelling was found to be an enlarged lymph node. No other lymph nodes were palpable. The upper outer quadrant of the right breast was noted to be slightly nodular but this was thought to be of no clinical significance. No discrete masses were palpable in either breast. Examination of the chest and abdomen showed nothing abnormal.

In May 1953 the axillary lymph node was excised. Histological examination showed that it contained irregular masses of large

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spheroidal and polyhedral malignant cells displaying pronounced pleomorphism and numerous mitotic figures. Initially a diagnosis of Hodgkin's disease was considered, but on review it was decided that the appearances indicated an anaplastic secondary carcinoma compatible with a metastasis from a primary breast neoplasm.

Because of the absence of a palpable mass in the breast and in view of the patient's age it was decided that immediate mastectomy was not indicated. The right axilla and supraclavicular region were treated by radiotherapy (1,400 rads) but the breast itself was not irradiated. The patient was kept under regular observation.

She remained well until 1966, when a small mobile swelling was noted in the left breast. Mammography showed no abnormality in either breast. The lump was excised and on histological examination it was seen to be an area of fibroadenosis with no evidence of malignancy. She continued to be examined regularly in the breast unit.

No masses were palpable in either breast until December 1970, when a discrete hard nodule 1.5 cm in diameter was noted in the upper outer quadrant of the right breast. This was tethered slightly to skin but not to muscle. Axillary lymph nodes were not enlarged and there were no other abnormalities on examination. Chest x-ray film and skeletal survey showed no metastases. A carcinoma, with microcalcification, however, was found in the right breast on mammography. The mass was excised. Frozen-section histological examination showed a carcinoma 2 cm in diameter. A radical mastectomy was then done. Histological examination showed a poorly differentiated intraduct and infiltrating carcinoma. Several axillary lymph nodes contained metastatic tumour deposits.

Comment

Occult carcinoma of the breast is rare. Owen *et al.* (1954), in a study of the clinical and pathological material at the Mayo Clinic from 1907 to 1950, collected 5,451 cases of carcinoma of the breast in which there was axillary lymph node involvement. In 27 of these cases no tumour was palpable in the breast, giving an incidence of 0.5%.

In 1909 Cameron advocated early mastectomy for occult carcinoma of the breast. Since then most cases with axillary lymph node metastases have been treated by radical mastec-

tomy after all other possible primary sites have been excluded by thorough investigation.

In cases not treated by immediate mastectomy the occult tumour has in most instances soon become palpable. Occasionally, however, a period of several months or even years has elapsed between the development of an axillary lymph node metastasis and the subsequent detection of the breast tumour. Klopp (1950) reported a latent period of four years, and Roux-Berger (1951) and Haagensen (1956) described cases where five years passed before the breast neoplasm became clinically evident. The latent period of 17 years in the present case appears to be unique in its length.

Bond (1968) postulated that metastasis formation in carcinoma of the breast may occur very early in the natural history of the condition. The present case shows that this can take place long before the tumour is clinically detectable. It also emphasizes that carcinoma of the breast may occasionally be very slow growing. Collins *et al.* (1956) suggested that at least half the duration of development of any cancer may occur before it enters the period of clinical observation. With breast tumours as slow growing as the tumour in the present case this interval can evidently be considerable.

I wish to thank Mr. W. P. Greening, director of the breast unit, for permission to report this case. Thanks are also due to Dr. N. F. C. Gowing, consultant pathologist, Royal Marsden Hospital, for the histological review.

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Pulmonary Aspergillomata in a Child Treated with Clotrimazole

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Pulmonary aspergillosis, most frequently caused by *Aspergillus fumigatus*, has been classified into five clinical types (Pepys, 1966). One of the commonest is the aspergilloma (Hinson, 1958), where the fungus forms a discrete, often solitary mycetoma (fungal ball) in the lung tissue, usually in cavitating or necrotic areas in the upper lobes.

Generally speaking the treatment of aspergillomata has been unsatisfactory (*British Medical Journal*, 1971). An orally administered synthetic antifungal agent, BAYb 5097 (bis-phenyl-(2-chlorophenyl)-1-imidazolyl-methane), later named clotrimazole, has been described (Plempel *et al.*, 1969) and its possible value in treating fungal infections in man discussed (*British*

Medical Journal, 1969). Clotrimazole has been approved by the Committee on Safety of Drugs for use in clinical trials under controlled conditions. It inhibits in vitro a large variety of yeasts and mycelial fungi at concentrations below 10 µg/ml and usually at concentrations of 1-2 µg/ml (Plempel *et al.*, 1969; Holt, 1970; Waitz *et al.*, 1970). The LD₅₀ for animals is about 1,000 mg/kg body weight, and doses of 150 and 60 mg/kg/day given to animals and man respectively are well tolerated. A single oral dose of 40 mg/kg in man produces a blood level of 2-4 µg/ml after four hours. Oberste-Lehn *et al.* (1969) treated a patient with sycosis barbae from which *Candida albicans* had been isolated, a patient with pulmonary aspergilloma, and a patient with a pulmonary infection due to *C. krusei* with clotrimazole (60 mg/kg body weight) without serious toxic effects and with encouraging therapeutic results.

We report a case of multiple aspergillomata treated with clotrimazole and, later, surgical excision of the cavities.

Case History

The patient, an 8-year-old boy, had had skin infections due to *Staphylococcus aureus* since the age of 2 weeks, and from the age of 4 months numerous chest infections and staphylococcal tension cysts in both lungs. When he was 6 years 5 months a further large tension cyst formed in the right upper lobe. After six months it was clear that the right upper lobe was destroyed and that lobectomy was necessary. There was no evidence of an immunological deficiency. X-ray examination showed what appeared to be a mycetoma in a cavity in the left lung (Fig. 1). The eosinophil count was greatly raised. There were numerous aggregations of fungal mycelium in the sputum and *A. fumigatus* was consistently isolated

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