(27.3%) unsuccessful. The total successes from both vaccinations numbered 920 (92.6%).

Multiple pressure gave a higher success rate than the scratch technique in persons previously vaccinated, and the difference between the success rates for the two methods is due to the greater failure of the scratch method in previously vaccinated males. No significant difference for either sex or with either technique was noted among primary vaccinations.

Some possible explanations for the difference between the two techniques in previously vaccinated males are discussed.

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BONE INVOLVEMENT AFTER VACCINATION AGAINST SMALLPOX

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Involvement of bone is one of the rarest complications after vaccination against smallpox. The following case is presented as the first one in the British Isles confirmed by virus isolation, and also because the condition may be difficult to distinguish from the other hyperostoses of infancy.

Case History

The patient was the second male child of healthy parents. Neither the parents nor the elder brother had reacted abnormally to smallpox vaccination. At the age of 3½ weeks the child was vaccinated by scarification high over the left deltoid. The reaction was "severe"; and there was massive but intact vesiculation set in an area of intense erythema 1 in. (2.5 cm.) in diameter, which subsided completely in three weeks.

When 7½ weeks old and 28 days after vaccination, a diffuse, firm, and slightly tender swelling was found over the left scapula without evidence of superficial inflammation or of skin involvement and without much systemic upset. Eight days after this there was a loss of function of the left arm and the swelling over the scapula appeared larger. On admission to hospital the child was pale and looked ill, his Hb was 9.5 g./100 ml., the W.C.C. was 17,000/c.mm. (neutrophils 47%, lymphocytes 48%, eosinophils 2%), and the blood group was A, Rh+. An x-ray film of the left shoulder (Fig. 1) showed considerable deposition of periosteal new bone on both costal and dorsal surfaces of the body of the scapula, extending on to the lower surface of the spine. The scapular lesion was unilateral and the ipsilateral clavicle and humerus were not involved. Excessive periscapular soft-tissue swelling could be seen on x-ray examination at this stage.

Biopsies of the left scapula taken at 37 and 46 days after vaccination were not informative, being bacteriologically sterile and showing a non-specific histological picture of oedematous reactive changes in the periosteum and muscle fibrosis with a notable lack of inflammatory cell infiltration.

X-ray examination 56 days after vaccination showed a significant increase in the periosteal new bone deposited on the scapula. A poorly demarcated, translucent area was seen in the body of the left scapula in the region of the glenoid margin. This later spread in the body of the scapula and still later in the recovery stage a peripheral zone of sclerosis appeared, suggesting an attempt at repair. We feel it unlikely that these

changes are the result of a limited bone biopsy. They probably represent an area of osteitis. X-ray examination of the mandible, then, showed no abnormality.

Sixty-two days after vaccination his condition deteriorated and he was transferred to the surgical unit of the Royal Belfast Hospital for Sick Children. At this time he was pale and was running a low-grade pyrexia, though he was active and (at 12 weeks) weighed 14 lb. 14 oz. (6,750 g.). The scapular mass was now 4 cm. in diameter but no longer tender, and full passive movement was present at the shoulder-joint.

A third biopsy of the left scapula consisting of a piece of the body of the scapula, new bone, greatly thickened periosteum, and contiguous muscle was removed 67 days after vaccination. At operation the skin and subcutaneous fat looked normal, but the muscle was swollen, pale, unduly firm, and much less vascular than normal. Both bone and muscle

FIG. 1.—X-ray film of left shoulder region showing, in profile view of the scapula, considerable deposition of new bone. This was 40 days after vaccination and 12 days after the first local signs being noted.

fragments, when inoculated as a 20% suspension on to the chorio-allantoic membrane of embryonated eggs, produced typical vaccinia virus pocks. A 20% suspension of these infected membranes had a titre of 1/64 with vaccinia antiserum in the complement-fixation test.

Aerobic and anaerobic bacteriological cultures were sterile. The histology showed an irregular formation of osteoid with fibrosis of the marrow cavity. Mild, chronic, non-specific inflammatory changes were also evident. Virus inclusions were not seen, but these are difficult to demonstrate in mesodermal tissues (A. W. Downie, personal communication).

The day after the third biopsy the total plasma proteins were 5.1 g./100 ml. (albumin 2.52 g.; globulin α_1 , 0.39 g.; α_2 , 0.72 g.; β , 0.72 g.; and γ , 0.75 g.). The E.S.R. was 60 mm./hr., the Hb 7.6 g./100 ml., and the W.C.C. 15,000/c.mm. (neutrophils 74%, lymphocytes 20%, monocytes 6%). The white cells phagocytosed staphylococci normally. The child's serum vaccinal antibody titre measured with the haemagglutination inhibition (H.I.) test (Kempe, 1956) was 1:16, and serum from the child's mother had a titre of <1:2. (The serum titre was the dilution of serum before the addition of haemagglutinin.)

Fresh whole blood 100 ml. was transfused and oral iron and vitamin C were given. One week later intramuscular injections of human antivaccinal gamma-globulin batch EPG 101 (H.I. titre 1:640 and neutralizing titre 1:1,600) were started. The total gamma-globulin given in five courses spread over 103 days was 5.75 g. The child's vaccinal H.I. antibody fluctuated during gamma-globulin therapy between 1:16 on nine occasions, 1:8 on three occasions, and 1:4 on two occasions.

Seventy days after vaccination the child developed an illness like the prodromal illness of measles, with snuffles, conjunctivitis, and great pallor, but without rash or buccal lesions. Within 12 hours the lower jaw was swollen and slightly tender, mainly on the left side (Fig. 2). This swelling remained for the next three or four days, then the baby's general state returned to normal. Six days later a trace of periosteal new bone was discernible on the base of the left side of the body of the mandible, and 14 days later this deposition was quite considerable and extending round towards the opposite side. At no stage was there any evidence of bone destruction in the mandible.

FIG. 2.—Facial appearance one day after the appearance of the soft-tissue swelling over the left mandible, 71 days after vaccination.

side of the mandible. Normal health returned in a few days and the infant remained well with a normal E.S.R. and W.C.C. Three hundred and forty-five days after vaccination he was using the left arm freely with negligible passive restriction at the scapulo-humeral joint. Clinically no bone deformity or local swelling remained anywhere.

Serial x-ray examination during the recovery phase suggested that the scapular new bone was reamed out from within, giving the body a blown-up appearance similar to that described in infantile cortical hyperostosis (Caffey, 1961). A striking feature has been the progressive shrinkage and remodelling of the

scapula. On the latest films it is approximating to a normal shape, although somewhat globular on the A.P. view and a little widened in profile. This is the only deformity remaining, the mandibular new bone having completely disappeared.

Discussion

Virus involvement of bone is rare, and only smallpox virus (Cockshott and MacGregor, 1958) and vaccinia virus are known to affect bone tissue in man. The scapula and humerus were the bones most frequently affected in 10 probable cases of vaccinal osteomyelitis reviewed by Elliott (1959); but in only three cases was virus isolated from biopsy material (Barbero, Gray, Scott, and Kempe, 1955; Sewall, 1949). Mandibular involvement has not been described before.

Although this child was vaccinated at an early age, he reacted normally with a wide zone of erythema, followed by uncomplicated healing of the primary lesion. There is nothing in the family history, the normal γ -globulin level, or the normal vaccinal H.I. antibody level after vaccination (McCarthy, Downie, and Bradley, 1958) to suggest that antibody production was defective.

Apparently vaccinia virus localized intracellularly in bone during the viraemia following primary vaccination, and in this child set up a recurrent acute infection lasting about five months. It is of interest that although virus-infected biopsy material was removed on three occasions the skin wounds healed normally.

Certain features of this child resemble infantile cortical hyperostosis. The prodromal illness followed by acute episodes occurring 28, 70, 95, and 115 days after vaccination in which periosteal new bone was deposited as early as six days after the soft-tissue swelling, and reached a peak in three weeks' time, was clinically similar to infantile cortical hyperostosis observed in a non-vaccinated child (Cochran, 1962). The distinctive facies due to bilateral involvement of the mandible, the other gross lesions seen radiologically (Caffey, 1961), and the microscopical picture were also similar to infantile cortical hyperostosis. This similarity may occasionally demand biopsy for differentiation.

In the present case we believe there has been destruction in the subglenoid region of the scapula, but healing had taken over before any appreciable disability resulted. Elliott (1959) has, however, emphasized that in vaccinal osteitis, as in the variolar form, the process can progress to destroy the epiphysis and involve the joint, with later deformity and disability.

Summary

An example of vaccinal osteitis is described. This is one of the most infrequent complications of vaccination against smallpox, and this case is believed to be the first recorded in the British Isles in which the diagnosis was confirmed by isolation of the virus. From observation of the course of the illness, for almost a year, the most distinctive feature appeared to be the periodic deposition of periosteal new bone, preceded each time by a prodromal illness and transient soft-tissue swelling in relation to the bone to be affected. The close similarity of all features of the disease to infantile cortical hyperostosis is noted, and it is suggested that it may be difficult, on occasion, to differentiate the two conditions without culture of biopsy material.

Treatment included five courses of human antivaccinal gamma-globulin spread over 103 days, and the outcome,

clinically and radiologically, was virtual complete resolution. It is, however, recognized that bone destruction and joint involvement can give rise to deformity and disability in the vaccinal as well as the variolar form of osteitis.

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CLINICAL EVALUATION OF CYTODIAGNOSIS IN BRONCHIAL CARCINOMA

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The first convincing account of the identification of malignant cells in sputum was given by Hampeln (1887), and by the end of the nineteenth century textbooks on laboratory techniques contained chapters on cytodiagnosis. At the beginning of this century sporadic papers appeared describing success in single cases, but Hampeln (1919) was the first to publish an objective assessment of the value of cytology in the diagnosis of bronchial carcinoma, recording 13 successes in a series of 25 cases.

Cytodiagnosis, however, did not become popular, the main disadvantages being loss of translucency and cellular distortion which occurred during the drying of smears rich in mucus. These difficulties were largely overcome by the introduction of wet fixation in alcoholic solutions (Dudgeon and Patrick, 1927). During the next 16 years important papers appeared in Britain stressing the value of sputum cytology (Dudgeon and Wrigley, 1935; Roodhouse Gloyne, 1937; Barrett, 1938; Gowar, 1943); and, in Denmark, Wandall (1944) published an authoritative monograph on neoplastic cells in sputum.

The vital phase of development occurred in America following the publication by Papanicolaou (1942) of his staining technique and in the following year of the classical monograph on the diagnosis of uterine cancer by vaginal smear (Papanicolaou and Traut, 1943). This stimulated extensive research in other fields of cytology, and during the 1940s many papers appeared confirming its value in the diagnosis of bronchial carcinoma; the percentage of positive findings varied from 55% (Farber *et al.*, 1950) to 80% (Herbut and Clerf, 1946).

In the United States, pulmonary cytodiagnosis soon became a routine procedure, largely because of the endorsement and support of the American Cancer Society and the United States Public Health Service (Papanicolaou, 1958).

Although so much of the pioneer work had been done in Britain, little attention was paid to the method until recent years, and, indeed, it is still regarded in some quarters as an expensive, time-consuming, and somewhat unreliable technique.

Material and Methods

The aim of the present study is to evaluate cytodiagnosis carried out as a routine procedure in a thoracic surgical unit served by a general laboratory.

During a two-year period cytodiagnosis was employed in 470 consecutive cases of suspected bronchial carcinoma.

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The diagnosis was confirmed in 240 cases, 202 (84%) of which were histologically proved; the remainder followed a typical malignant course to death.

We have analysed these 240 cases and have attempted to elucidate the following points: (1) comparison of cytodiagnosis with other diagnostic methods available; (2) the relative efficiency of the various methods of collecting specimens for cytological examination; (3) factors affecting the exfoliation of tumour cells; and (4) the causes of "false-positive" results.

Conventional Diagnostic Methods.—In addition to routine clinical and radiological examination, tomography was frequently employed. In practically every case bronchoscopy was performed, with biopsy of any abnormal tissue seen. When bronchoscopy was negative a bronchogram was done. Biopsy of scalene nodes, pleura, or distant metastases was carried out in a few cases.

Cytodiagnostic Methods

The following specimens were examined.

Sputum.—Three adequate samples were requested, but it was not always possible to obtain these, as treatment of the patient was never delayed in order to obtain a full cytological examination.

Bronchial Aspirate.—This was collected at bronchoscopy with the Mullard (1958) specimen-collector manufactured by Allen and Hanburys.

Bronchial Smears.—These were taken direct from suspicious areas seen at bronchoscopy.

From each specimen two slides were made and stained by the Papanicolaou method. The cells were graded according to Papanicolaou's criteria as follows:

Unsatisfactory Sample.—No report.

Negative.—Class 1 or class 2 cells present. Class 1 cells are normal sputum components. Atypical (class 2) cells are often found in inflammatory lung conditions.

Suspicious.—Class 3 cells present. In our experience 50% of the patients producing these cells eventually prove to have a carcinoma. This agrees with the findings of Rome and Olson (1957).

Positive.—Class 4 or class 5 cells present. Class 4 cells are highly suspicious of malignancy, but there is a slight element of doubt, especially in view of the bizarre appearances which may occur in squamous metaplasia. Class 5 cells are regarded as being as definite a proof of malignancy as a positive biopsy.