A case of vaccinia necrosum (or progressive vaccinia), with severe hypogammaglobulinaemia, treated with n-methyl isatin beta-thiosemicarbazone (33T57)

T. H. FLEWETT AND F. L. KER

From Little Bromwich General Hospital, Birmingham

SYNOPSIS A fatal case of vaccinia necrosum treated with antivaccinal γ globulin and N-methyl isatin β -thiosemicarbazone (33T57) is described. Histological abnormalities found at necropsy included intranuclear as well as cytoplasmic inclusion bodies, absence of lymphoid germinal centres, grey hepatization, and bronchiolar epithelial hyperplasia. Virus titres were highest in the original skin lesion; virus was also found in the lymph node draining it and in the kidney and the brain. No toxic effects could be clearly attributed to the drug used in treatment.

This case is reported because, like that recently reported by Connolly, Dick, and Field (1962), passively transferred immunity and 33T57 failed to influence significantly the course of the disease, and also because of some unusual histological features.

HISTORY

A West Indian negro boy was born on 23 April 1961 and was vaccinated with Lister Institute lymph on 31 August 1961. The batch of lymph used gave normal results when used for very many other vaccinations. The vaccination 'took', the lesion grew larger and 'became infected'. The infant was admitted to a residential nursery by the local health authorities because of this, and was transferred to the Children's Hospital, Birmingham, on 18 December because the lesion was becoming larger.

On admission there was a large sloughing ulcer on the left deltoid area, with no inflammation at the margin. Around the margin (at distances up to 2 cm.) there were crops of vesicles of various sizes (Fig. 1). Isolated vaccinal lesions were also present on the buttocks, scrotum, and left scapular area. The liver was large and firm. Spleen and lymph nodes were not palpable. The haemoglobin level was 9 g./100 ml.; W.B.C. 2,700 per c.mm. (polymorphs 72%, eosinophils 1%, lymphocytes 23%, and monocytes 4%); E.S.R. 2 mm. in the first hour. No sickle cells were found. The blood contained 5 mg./100 ml. of γ globulin but no γ macroglobulin (syn. β_{2m} , 19S component) was detectable. The γ globulin and γ macroglobulin were determined by an agar-diffusion method (Gell, 1957; Soothill, 1962b). The β_{21} component was 3% of that of a standard 'normal' serum. Total serum

protein was 4.2 g./100 ml. The chest radiograph showed large areas of infective opacity. Vaccinia virus was isolated in eggs and tissue cultures from the principal lesions. *Proteus vulgaris*, a faecal type of *Strep. viridans*, and *Bact. coli* were isolated from the skin lesions, and coagulase-positive staphylococci from the nose.

TREATMENT

For fear of infecting eczematous children in adjacent cubicles, the child was transferred to the infectious diseases unit of Little Bromwich General Hospital.

A transfusion of 110 ml. of blood was given, and penbritin, tetracycline, nystatin, colomycin, novobiocin cream, and polybactin spray were all used to control the secondary infection, as different organisms succeeded each other locally.

Five hundred milligrams immune antivaccinal γ globulin and 500 mg. pooled γ globulin were given.

Within the next two days antivaccinal γ globulin and pooled human γ globulin, 1,000 mg. each, were given in divided doses. Afterwards 500 mg. of antivaccinal γ globulin was given weekly.

As the γ globulin was having no apparent effect upon the inexorable progress of the lesions (Figs. 2 and 3), N-methyl isatin β -thiosemicarbazone (33T57) was given six hourly in a dose of 250 mg.

Further vesicles did not appear, although fresh vesicles had up to this time been appearing regularly at the spreading margins of the main lesion. It was our impression, though it cannot be put more strongly, that some of the lesions were tending to dry up (Fig. 4). However, four days after administration of the drug began, the child developed diarrhoea and died three days later. No symptoms or signs other than the diarrhoea were observed which could have been attributed to the 33T57.

Throughout the period spent in hospital, the child's

FIG. 1. *The lesion on the left arm the day after admission.* FIG. 2. *The same six days later* FIG. 3. *The same nine days after Fig. 2.* FIG. 4. *The same eight days after Fig. 3.*

pulse rate varied between 120 and 140; temperature was normal on admission, but rose to 101°F. (38.3°C.) for two days on the eighth and twelfth days after admission. These rises in temperature were not accompanied by corresponding changes in pulse and respiration rate, and did not appear to be related to any change in the state of the lesions.

POST-MORTEM FINDINGS

The heart showed no gross abnormality.

Consolidation, in the stage of grey hepatization, was seen in the whole of both lower lobes and much of the upper and right middle lobes of the lungs. Considering the duration of the extensive pneumonia, shown radiologically to be present on admission, histologically there was a rather scanty polymorphonuclear infiltration of the alveoli. There was

also some haemorrhage into alveoli locally. The bronchiolar epithelium in some areas showed marked proliferation, making the epithelial lining of the bronchioles several cell layers thick, and in others forming small collections of cells almost like small adenomata, as in Fig. 5, where the extension of the bronchiolar epithelium into the surrounding stroma is clearly shown.

RETICULO-ENDOTHELIAL SYSTEM The thymus was represented by two thin thread-like strands of ill-defined grey tissue. The spleen was small and no Malpighian follicles could be seen. Lymph nodes were smaller than usual in a child of this age. Histologically, the spleen, thymus, and lymph nodes showed a complete absence of germinal centres and plasma cells, and lymphocytes were very scanty (Figs. 6 and 7). The cells in the splenic and lymph

node pulp were mostly of the fixed tissue or 'littoral' variety. Hassall's corpuscles were not found in the thymus. The usual small aggregations of lymphocytes were not found in the lungs, and even in the appendix wall lymphocytes were almost completely absent, being found only in one small area.

The alimentary tract showed only mild patchy inflammation of the intestines, with very inconspicuous lymphoid tissue. The liver showed a moderate degree of uniform fatty change. Histologically, there were numerous bile thrombi, swelling of the Kupffer cells, and evidence of a moderate degree of fatty change in the liver cells. Apart from the absence of lymphoid tissue, no histological abnormality could be detected in the small bowel, as is often the case in gastroenteritis.

The skin lesion on the arm showed the kind of histological picture described in previous reports, namely, a central area of necrosis with a very scanty cellular infiltration with polymorphs and even fewer lymphocytes with no aggregations of plasma cells. Many epithelial cells at the edge of the zone of necrosis showed Guarnieri bodies in their cytoplasm (Fig. 8), but in addition eosinophilic intranuclear inclusions were found, some with a 'ring' structure (Figs. 9 to 11).

The kidneys showed no gross abnormality. Histologically, the mononuclear cell infiltration in the medulla described as occurring in fatal smallpox was not found.

Except for the skin, no inclusion bodies were found in the various organs.

VIROLOGY Some of the principal organs were titrated on the chorioallantoic membranes of chick embryos in the usual way to determine their content of viable vaccinia virus. The titres are given below and are expressed as pock-forming units/g. of tissue:

Edge of skin lesion.....	22,500
Brain	5
Kidney	8
Left axillary lymph node.....	25
Right axillary lymph node....	nil
Lungs (both).....	nil
Colon.....	nil
Jejunum.....	nil
Spleen	nil

DISCUSSION

Despite attempts at treatment the course of the infection was similar to those described by other authors; the evolution of the disease was much more rapid than in the case described by Connolly *et al.* (1962).

The histological picture seen in the organs was similar to that previously described in severe hypogammaglobulinaemia. However, the lesions in the lungs, with the proliferation of bronchiolar epithelium, are unusual and have not been described in this condition, though Lewis and Johnson (1957) described squamous metaplasia of the bronchioles in their case of progressive vaccinia; Kozinn, Sigel, and Gorrie (1955), whose patient had been treated with A.C.T.H. and cortisone, found coagulation necrosis in several organs, which was not present in this case. Whitwell (1955) described 24 cases showing 'tumourlets' in the lung; most of these were associated with chronic lung infection (bronchiectasis or chronic abscess) and took a benign course clinically. His youngest patient, however, was 18 years old. He was able to show by serially sectioning some of these tumourlets that each was a continuous multi-lobed structure, usually derived from the bronchiolar epithelium; the connexion with bronchiolar epithelium can be seen in Fig. 5 of this paper. Such tumourlets were quite numerous in our patient, four being found in one section about half an inch square. No inclusion bodies were observed in the cells, and there is no evidence to connect them with the vaccinal infection; furthermore no virus was isolated from the lungs after death. Inclusion bodies were found only in the skin lesion.

Although intranuclear inclusions have been reported in smallpox (Torres, 1936), vaccinia virus has not as far as we know been found to produce intranuclear changes. It is unlikely that the inclusions in our patient were due to herpes simplex virus, as there was no other evidence that it was present at the same time as the vaccinia; the inclusions are not like the classical Cowdry type A inclusions usually associated with herpes infection, and no herpetic pocks appeared among the vaccinal lesions in the eggs inoculated with skin material. It also seems unlikely that the 33T57 caused this; Sheffield, Bauer, and Stephenson (1960) did not note any nuclear changes in cells infected with rabbit pox virus and treated with a closely related thiosemicarbazone compound. There was no evidence of cytomegalic virus infection or of *Pneumocystis carinii* infection.

Connolly *et al.* (1962) had noted the occurrence of a blotchy erythematous rash on the hands, feet, and back and abdomen occurring 10 days after they started treatment; when they stopped the drug the rash faded. We found no such rash, although of course toxic symptoms were being looked for; if present, it could perhaps have been masked by the brown skin. But Hitzig and Willi (1961) reported a morbilliform rash in some of their cases of severe hypogammaglobulinaemia treated with γ globulin.

FIG. 5. An area of bronchiolar epithelial hyperplasia; epithelium can be seen spreading from the bronchiolar lining $\times 88$. Haematoxylin and eosin.

FIG. 7. Spleen showing absence of germinal centres and Malpighian bodies $\times 55$. Haematoxylin and eosin.

FIG. 6. Thymus (the photograph includes almost the entire width of the lobe) $\times 55$. Haematoxylin and eosin.

FIG. 8. Cytoplasmic inclusion bodies in skin epithelium; they appear to lie within a space surrounded by a membrane. One nucleus (arrow) was full of eosinophilic material $\times 1,200$. Haematoxylin and eosin.

FIG. 9

FIG. 10

FIG. 11

FIGS. 9, 10, and 11. Intranuclear inclusions in skin epithelial cells. These were eosinophilic, sometimes ring-shaped, with small vacuoles, sometimes filling the whole nucleus, or in aggregations of globules of different sizes $\times 2,200$. Haematoxylin and eosin.

However, the course of events in this patient was more rapid than in theirs; he did not live 10 days after treatment was started. Turner, Bauer, and Nimmo-Smith (1962) noted severe and persistent diarrhoea and vomiting in their patient aged 5 months treated with this compound for eczema vaccinatum. Although, unlike Turner *et al.*, we had no reason to relate the diarrhoea in this patient to possible cross-infection, as the child was being barrier-nursed in a cubicle, the possibility cannot be excluded but one cannot assert that the diarrhoea was due to the drug. According to Bauer (personal communication) and Bauer and Goodwin (1962) and from our experience in treating one adult with smallpox (Ker, 1962), no subjective or objective toxic effects occur in adults treated with it.

The titres of virus in the tissues were not as high as those found by Keidan, McCarthy, and Haworth (1953), who recorded a titre of 10^5 pock-forming units/g. of tissue in lymph node. However, a certain amount of blood in all these organs contains antibody injected during life, and this may possibly have had some effect on virus titre; it is possible that the drug given also reduced the titre by preventing fresh virus growth in the last few days before death. As the patient reported by Keidan *et al.* (1953) was also treated with hyperimmune γ globulin, the latter explanation may be the better one.

Failure of treatment was not due to absence of antibodies. The serum γ globulin on 10 January was 240 mg./100 ml. following γ globulin injections, in contrast to the pre-treatment level of 5 mg./100 ml. Although no circulating antibodies for vaccinia virus were detectable in the serum taken on admission, after administration of the hyperimmune vaccinal γ globulin 100 pock-forming units of virus were neutralized by a 1:8 dilution of serum. Such a titre is comparable with that found after vaccination, though lower than the very high level achieved by Connolly *et al.* (1962) and it should have been enough to cure if antibodies were all that were needed for this. We were reluctant to take numerous blood samples; a fresh spreading pock rapidly developed at the point where the transfusion was given (into the temporal vein) soon after admission to hospital.

Most children with hypogammaglobulinaemia react normally to vaccination and other virus infections (Apt, 1953-4). Hypogammaglobulinaemia may sometimes, but not always, be associated with a deficiency of the two other immunoglobulins, β_2 and β_2 (or macroglobulin) (Gitlin, Hitzig, and Janeway, 1956). Deficiency of the latter is associated with a more permanent deficiency of γ globulin, and presumably represents a more severe form of the disease (Soothill, 1962a). This

child had the more severe form but such children have reacted normally to vaccination (Soothill, personal communication), so the serum protein deficiencies do not alone account for the occurrence of progressive vaccinia, and other coexistent deficiencies (perhaps of interferon production or cellular immunity) must be sought.

Although there was no family history of agammaglobulinaemia in our case, he would appear to come into the syndrome described by Hitzig and Willi (1961) as lympho-plasmocytic dysgenesis; their criteria fit quite well. It is interesting that they found that an intractable diarrhoea was a frequent feature of the clinical picture in their patients. They did not describe a case of progressive vaccinia (none of their case histories mentioned vaccination), although they discussed the possibility that some of those reported in the literature might have suffered from this kind of immunological defect. Barandun, Stampfli, Spengler, and Riva (1959), however, stated that their patients with primary hypogammaglobulinaemia when vaccinated reacted normally despite failure to form antibodies, and postulated that a cellular as well as an immunological deficiency must be present in cases of progressive vaccinia.

Connolly *et al.* (1962) found that locally injected interferon did not influence the spreading edge of lesions in their case. But perhaps they did not inject enough.

The drug 33T57 has produced no definitely beneficial effect in the cases in which it has been tried but in experimental animals the related compound has to be given early in the course of infection to prevent fatal rabbit pox.

Failure of inflammatory response in the skin lesions was striking and the phenomena of delayed hypersensitivity were all absent. It may well be that these phenomena are needed to contain the spread of the lesion. They are in part at any rate mediated through the activity of leucocytes, or at least of fragments of leucocytes. As the forms of treatment used on this child and on others have been uniformly unsuccessful, it might seem worth while to try repeated exchange transfusion with very fresh blood or to graft normal lymphoid tissue, perhaps into the bone marrow. This should be possible under the 'umbrella' of antivaccinal γ globulin treatment. Homografts occasionally 'take' readily in agammaglobulinaemia (Good and Varco, 1955a and b). The failure of thymic development in this child would suggest, following the views of Burnet (1962), that a homograft would have had a good chance of 'taking'. There might be a risk of producing runt disease by such a graft, but even to keep a child with vaccinia necrosum alive long enough for this to develop would be an achievement.

We should like to express our thanks to Dr. D. J. Bauer who supplied the 33T57 compound and for helpful discussions on treatment and toxicity; to Dr. J. F. Soothill who did the γ globulin estimations; to Dr. F. O. MacCallum for the serum neutralization titres; to Dr. R. G. F. Parker who performed the necropsy, made his histological preparations available to us, and drew our attention to Dr. Whitwell's paper; and to Professor J. R. Squire who read the draft and made some most useful comments and suggestions.

REFERENCES

- Apt, L. (1953-4). In *Year Book of Paediatrics*, pp. 67-69. The Year Book Publishers, Chicago.
- Barandun, S., Stampfli, K., Spengler, G. A., and Riva, G. (1959). *Helv. med. Acta*, **26**, 163.
- Bauer, D. J., and Goodwin, L. G. (1962). *Brit. med. J.*, **1**, 716.
- Burnet, Sir M. (1962). *Ibid.*, **2**, 807.
- Connolly, J. H., Dick, G. W. A., and Field, C. M. B. (1962). *Ibid.*, **1**, 1315.
- Gell, P. G. K. (1957). *J. clin. Path.*, **10**, 67.
- Gitlin, D., Hitzig, W. H., and Janeway, C. A. (1956). *J. clin. Invest.*, **35**, 1199.
- Good, R. A., and Varco, R. L. (1955a). *J. Amer. med. Ass.*, **157**, 713.
- , — (1955b). *J. clin. Invest.*, **34**, 910.
- Hitzig, W. H., and Willi, H. (1961). *Schweiz. med. Wschr.*, **91**, 1625.
- Keidan, S. E., McCarthy, K., and Haworth, J. C. (1953). *Arch. Dis. Child.*, **28**, 110.
- Ker, F. L. (1962). *Brit. med. J.*, **2**, 734.
- Kozinn, P. J., Sigel, M. M., and Gorrie, R. (1955). *Pediatrics*, **16**, 600.
- Lewis, H. M., and Johnson, F. C. (1957). *A.M.A. Arch. Derm.*, **75**, 837.
- Sheffield, F. W., Bauer, D. J., and Stephenson, Sylvia M. (1960). *Brit. J. exp. Path.*, **41**, 638.
- Soothill, J. F. (1962). Personal communication.
- (1962a). *Clin. Sci.*, **23**, 27.
- (1962b). *J. Lab. clin. Med.*, **59**, 859.
- Torres, C. M. (1936). *Proc. roy. Soc. Med.*, **29**, 1525.
- Turner, W., Bauer, D. J., and Nimmo-Smith, R. H. (1962). *Brit. med. J.*, **1**, 1317.
- Whitwell, F. (1955). *J. Path. Bact.*, **70**, 529.