SINDBIS VIRUS INFECTION IN MAN

REPORT OF A CASE WITH RECOVERY OF VIRUS FROM SKIN LESIONS

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The clinical features of Sindbis virus infection in man have not previously been fully described. In the present study, the isolation of virus from skin lesions and the demonstration of antibody rise confirm the association of this virus with a clinical syndrome.

CASE REPORT

Clinical Findings

P.G., a White woman aged 45 years, was examined at her home 7 miles north of the centre of Johannesburg on 3 January 1963. Her illness had commenced 6 days previously with malaise and severe gripping frontal headache. The headache lasted for only half a day; but within 48 hours there was marked soreness of tendons and joints, particularly those of the hands and feet, the latter being more severely affected, and the patient complained of intermittent feelings of heat and cold. On the 3rd day a transient stiffness of the neck was noted, a few red macules appeared on her back, and 3 very painful vesicles, which later merged to form a single blister, developed on the medial aspect of the left great toe. During the next 3 days smaller vesicles appeared on and between the toes of both feet, the macular rash became more florid and general in distribution, pain and swelling of the hands and feet were severe, and the patient took to her bed with a fever varying between 99° and 100°F.

Respiratory symptoms were absent, and there was no sore throat. The patient had no abdominal pain, constipation or diarrhoea; but anorexia and nausea without vomiting persisted intermittently. There was no pain on swallowing. Bladder function was not impaired. The headache did not recur, there was no dizziness, and the patient slept well. Pains in the eyes were not felt. Paraesthesiae and skin tenderness were not experienced; the body aches seemed deep, but muscle tenderness was not noted. The large joints were not involved.

When examined on the 6th day of the disease, the patient appeared ill, with a temperature of 99·8°F. A profuse rash was present on the face, trunk and limbs, including the palms and soles. The lesions were discrete, being composed mainly of red macules, with some papules 3 - 4 mm. in diameter; these had the appearance of early vesiculation. A painful blister, measuring approximately 6 mm. × 12 mm., was present on the left great toe, with numerous smaller vesicles on and between the toes of both feet. The rash was not pruritic, except on the feet, where vesicles had appeared and itching was marked. A few red blotches were noted on the hard and soft areas of the palate, and 2 painful, small, shallow ulcers were present in the mouth on the inner aspect of each lip. There was mild photophobia, but the conjunctivae were not suffused. Lymphadenopathy was absent, except for a few small nodes which could be felt in the posterior triangles of the neck. The fingers and feet were swollen and painful. The central nervous system, heart and lungs appeared to be normal, and the blood pressure was 130/80 mm.Hg. Right subcostal tenderness was present, but neither liver nor spleen was palpable.

On the 8th day of illness the rash was still profuse over the trunk and limbs, but was less marked on the face. On the right great toe there were several recent vesicles measuring about 4 mm. in diameter, and crops of smaller vesicles were present on the dorsa of both feet and on the fingers of both hands.

The illness ran a protracted course, and the patient remained in bed for 2 weeks. Nausea became more pronounced, and on the 12th day culminated in a severe bout of vomiting, precipitating carpopedal spasm. The rash remained bright for 10 days, and then faded to leave brown stains that were still visible on the 28th day. An electrocardiogram taken on the 34th day was normal. Marked physical tiredness, pain in the hands and feet, slight swelling of the fingers, and a tender swelling in the left tendo achillis were still present after 10 weeks.

Differential Diagnosis

The patient lived in a well-wooded area, near to riding stables and a veterinary hospital. Mosquitoes were numerous, necessitating nightly spraying of the bedroom. She had not been away from Johannesburg recently, except that 5 weeks before her illness she had paid an evening visit lasting several hours to the Hartebeespoort Dam, where she had been exposed to the bites of mosquitoes.

The following possibilities were considered in the differential diagnosis of this very unusual rash:

(*a*) Epidermophytosis with dissemination.

(*b*) Rickettsial infections, including rickettsial pox, South African tickbite fever, and Q fever.

(*c*) Viral infections, including herpes, Coxsackie and arthropod-borne viruses.

Clinical Pathological Investigations

A blood count taken on the 7th day of illness showed the haemoglobin level to be 15 G. per 100 ml. There were 5,100 leucocytes per c.mm., comprising neutrophils 57·5%, monocytes 10·0%, eosinophils 5·5%, and lymphocytes 27·0%, of which some had atypical features, including vacuolation of the cytoplasm and fenestration of the nucleus. The erythrocytes were normal in appearance, but the sedimentation rate (Westergren) was 24 mm. in the 1st hour, the upper limit of normal being 7 mm. A second blood count on the 28th day gave essentially similar values. On the 46th day the blood count was also within normal limits, but occasional atypical lymphocytes were still present, and the sedimentation rate was 29 mm. On the 57th day the sedimentation rate was still raised, being 28 mm. in the 1st hour.

Liver-function tests on the 46th day did not show marked changes except for a +++ colloidal-red reaction. The protein values were: total protein 7 G., albumin 3·3 G., globulin 3·7 G., and gamma globulin 1·39 G. per 100 ml. On the 57th day the colloidal-red tests gave a ++ reaction.

Bacteriological cultures of blood taken on the 7th day remained sterile, and no fungi were recovered from material taken on the 8th day of illness from the skin lesions and cultured on actidione agar. Rickettsial complement-fixation tests were negative on the 7th and 57th days.

Virological Investigations

Complement-fixation tests for herpes simplex virus were negative on the 7th and 30th days.

Specimens for virus isolation were taken on the 8th day, transported to the laboratory at approximately 4°C., and inoculated into tissue cultures or suckling mice within 3 hours.

Primary monolayer tissue cultures of kidney epithelium from the vervet monkey *Cercopithecus aethiops pygerythrus* were used, roller tubes with or without free coverslips being maintained up to 21 days at 37°C. in a medium consisting of balanced salt solution with 0·5% lactalbumin hydrolysate and antibiotics. The antibiotics were: penicillin, streptomycin, neomycin and nystatin. Litters of six 24-hour-old white mice were inoculated by the combined subcutaneous and intracerebral route, each mouse receiving approximately 0·01 ml. intracerebrally and 0·04 ml. subcutaneously. The mice were observed for 24 days.

The following virus studies were undertaken:

(*a*) *Blood.* 20 ml. of heparinized blood were centrifuged, and the buffy layer, together with some red cells, was resuspended in a portion of the patient's plasma. Two tissue-culture tubes were inoculated with 0·2 ml. of the suspension, and were maintained for 21 days. No cytopathic changes were observed. Mice were not inoculated with blood.

(*b*) *Throat swab.* A throat swab was agitated in 2 ml. of buffered gelatin saline containing antibiotics, and after centrifugation 0·2 ml. of the fluid was introduced into each of 2 tissue-culture tubes, and 2 litters were inoculated. No virus was recovered.

(*c*) *Rectal swab.* A rectal swab was agitated in 2 ml. of buffered gelatin saline containing antibiotics. After centrifugation, 0·2 ml. of the fluid was put into each of 2 tissue-culture tubes, and 2 litters were inoculated. Virus was not isolated.

(*d*) *Skin lesions.* A number of vesicles on the hands and feet were wiped with swabs which were moistened with alcohol, before being opened. Vesicle fluid was withdrawn on swabs which were then agitated in a tube with 2 ml. of buffered gelatin saline containing antibiotics. To the contents of this tube were added small portions of the skin overlying some of the vesicles. After centrifugation, 0·2 ml. amounts of the fluid were inoculated into each of 2 tissue-culture tubes. One tissue-culture tube showed no changes over 21 days. In the other culture, cytopathic changes developed after the second day, being well advanced by the 5th day, when the fluid was harvested. Sub-inoculation from this tube into 4 tissue cultures resulted in cytopathic changes within 48 hours. A coverslip culture included in this passage was fixed with Bouin's solution and alcohol, and was stained with haematoxylin and eosin. Focal degeneration was seen, and many of the nuclei contained multiple small eosinophilic inclusions. Virus was not recovered when 2 litters were inoculated with the original suspension of material from the skin lesions. Only a small quantity of this suspension was left for an attempt at re-isolation in tissue culture, which was negative. It is probable that the amount of virus present was small, since only 1 of 2 cultures became infected in the original isolation. The possibility that this agent may have been a laboratory contaminant can be excluded, because the unit in which the isolation was made is concerned chiefly with the study of enteroviruses, and a similar agent had not previously been handled there.

Characterization of the Virus

A known strain of Sindbis virus (AR 86) and antisera prepared against Sindbis, Chikungunya and Middelburg viruses were made available to us through the courtesy of Dr. B. M. McIntosh of the Arthropod-borne Virus Research Unit of the Poliomyelitis Research Foundation.

The following was the characterization of the virus of our patient (P.G.):

(*a*) *Cytopathic effect.* The small nuclear inclusions resembled those known to be produced in tissue culture by some arthropod-borne viruses (Fig. 1).

(*b*) *Histopathology.* The inoculation of suckling mice resulted in lesions, described more fully later, involving a number of tissues including the heart, fat, thymus and striated muscle. However, the very extensive destruction of connective tissue indicated that this was not a Coxsackie virus.

(*c*) *Haemagglutination and haemagglutination-inhibition.* Using tissue-culture fluid centrifuged at 2,500 r.p.m. for 30 minutes, optimal haemagglutination with 0·5% goose erythrocytes was obtained at pH 6·0. Since several group-A arthropod-borne viruses are known to be present in South Africa, haemagglutination-inhibition was tested, using an antiserum prepared in mice against a known strain of Sindbis virus and an antiserum prepared in guinea-pigs against Chikungunya virus. Sera taken from the patient P.G. on the 8th day (acute) and on the 28th day (convalescent) were included in the test. All sera were treated with kaolin to remove non-specific inhibitors. The results are given in Table I, where it can be seen that haemagglutination-inhibiting antibody was present in the patient's convalescent serum, but not in the acute-phase serum. Complete inhibition of haemagglutination by the patient's virus was obtained with Sindbis antiserum. The slight inhibition observed with Chikungunya antiserum indicates a group reaction only.

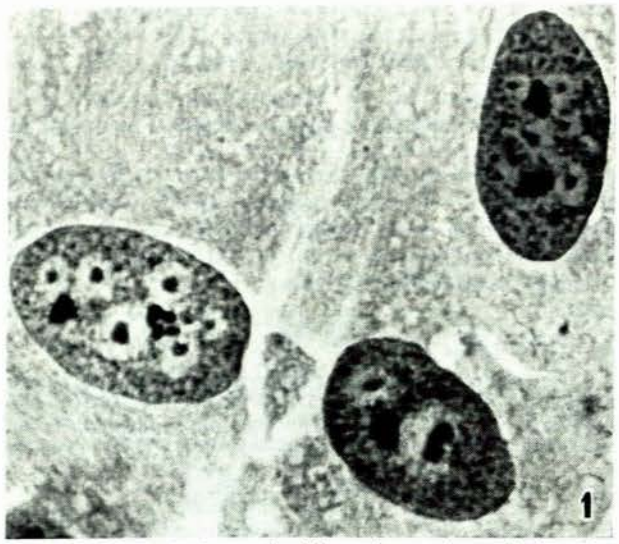


Fig. 1. P.G. strain in monkey-kidney tissue culture. Nuclear inclusions.

TABLE I. INHIBITION OF P.G. VIRUS HAEMAGGLUTINATION

	P.G. virus dilutions							
	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
P.G. virus only	+	+	+	+	+	±	—	—
P.G. virus with P.G. acute serum 1:80	+	+	+	+	+	—	—	—
P.G. virus with P.G. convalescent serum 1:80	+	—	—	—	—	—	—	—
P.G. virus with Sindbis antiserum 1:80	—	—	—	—	—	—	—	—
P.G. virus with Chikungunya antiserum 1:80	+	+	+	+	—	—	—	—

+=haemagglutination.
−=no haemagglutination.

(*d*) *Neutralization tests.* Fresh passages of the virus from the patient P.G. and of the known Sindbis strain AR 86 were made in tissue cultures of vervet kidney. Harvests of both these agents titred $10^{5\cdot0}$ $TCID_{50}$ (doses infective for 50% of tissue cultures) per 0·1 ml., and approximately the same titre was obtained in suckling mice inoculated with these fluids. Equal volumes of undiluted serum, pre-treated with kaolin, and of tenfold dilutions of virus in buffered saline, were mixed and kept at 37°C. for 1 hour and 4°C. for 2 hours. 24-hour-old mice were then inoculated, each mouse receiving 0·01 ml. intracerebrally and 0·04 ml. subcutaneously. As controls, a limited number of mice were inoculated with Sindbis virus diluted 10^{-2} and P.G. virus diluted 10^{-3}, each mouse receiving 0·02 ml. In Table II it will be seen that, in the presence of serum from a pool of normal uninoculated guinea-pigs, the patient's virus titred approximately 10^{-5}. A similar titre was attained in the presence of an antiserum prepared in mice against Middelburg virus, another group-A arbor-virus isolated in South Africa. Virtually complete neutralization of P.G. virus in the dilutions used was observed in the presence of Sindbis antiserum. Table III gives the results of a test in which, under similar conditions, the patient's

acute and convalescent phase sera were challenged with Sindbis virus strain AR 86. Neutralizing antibody was present in

TABLE II. NEUTRALIZATION OF P.G. VIRUS IN 24-HOUR-OLD MICE

	P.G. virus dilutions				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
P.G. virus only	—	0/12	—	—	—
P.G. virus with normal guinea-pig serum	—	0/12	0/12	3/12	9/12
P.G. virus with Sindbis antiserum	11/12	8/12	11/12	11/12	10/12
P.G. virus with Middelburg antiserum	—	0/12	0/12	4/12	9/12

Numerator=survivors.
Denominator=mice alive 24 hours after inoculation.

TABLE III. NEUTRALIZATION OF SINDBIS VIRUS STRAIN AR 86 IN 24-HOUR-OLD MICE

	Sindbis virus dilutions				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Sindbis virus only	0/19	—	—	—	—
Sindbis virus with P.G. acute - phase serum	11/12	3/10*	12/12	9/12	12/12
Sindbis virus with P.G. convalescent serum	11/12	5/6	8/11	10/12	12/12

Numerator=survivors.
Denominator=mice alive 24 hours after inoculation.
* Histological examination of 2 sick mice showed very early lesions suggestive of Sindbis virus infection.

both sera. The acute-phase serum had been taken on the 8th day of illness, by which time neutralizing antibody could have been formed.

It is therefore evident that the virus recovered from the skin lesions was the cause of the patient's illness, and that it is virtually identical with the AR 86 strain of Sindbis virus.

SINDBIS VIRUS

Sindbis virus, which was named after the Egyptian village where it was first isolated by Taylor *et al.*[1] in 1952, is known to infect humans, domestic animals and birds, with the mosquito as vector. It is generally considered that birds probably form the chief reservoir, and that humans and other vertebrates are only occasional hosts not essential for the survival of the virus in nature. The agent has been placed in group A in the classification of arthropod-borne viruses.

Mosquitoes and Birds

Sindbis virus was originally isolated from Culex mosquitoes, which Taylor and his co-workers showed to be capable of transmitting the infection to animals. Strains of the virus have subsequently been recovered from Culex mosquitoes in India,[2] and from Culex and Mansonia mosquitoes in South Africa.[3,4]

Birds of various kinds can be infected. The virus was isolated from a crow in Egypt in 1953,[1] and from wagtails and mynas in India.[2] Serological evidence of infection in sparrows, pigeons and domestic fowls was obtained in Egypt,[1] and in South Africa antibodies have been found in the sera of domestic fowls and wild birds.[5]

Vertebrate Hosts

Antibodies against Sindbis virus were noted[1] in the sera of a number of domestic animals in Egypt, including the horse, donkey, cow, goat and sheep. In South Africa serological evidence of infection in sheep, goats and cattle has also been found.[6]

Taylor *et al.*[1] found antibodies in 122 (27%) of 445 human sera tested in Egypt. Human infection has undoubtedly occurred previously in South Africa, for the sera of persons in a number of places on the Highveld plateau have been shown to contain antibodies to Sindbis virus.[3,6] Unpublished findings have also been made available to us by Dr. J. H. S. Gear, concerning a patient seen in Johannesburg in 1958 suffering from headache, muscle and joint pains, and a papular rash extending over most of the body, including the palms and soles. Serological studies indicated infection with Sindbis virus, possibly contracted at the Hartebeespoort Dam one week before the onset of illness.

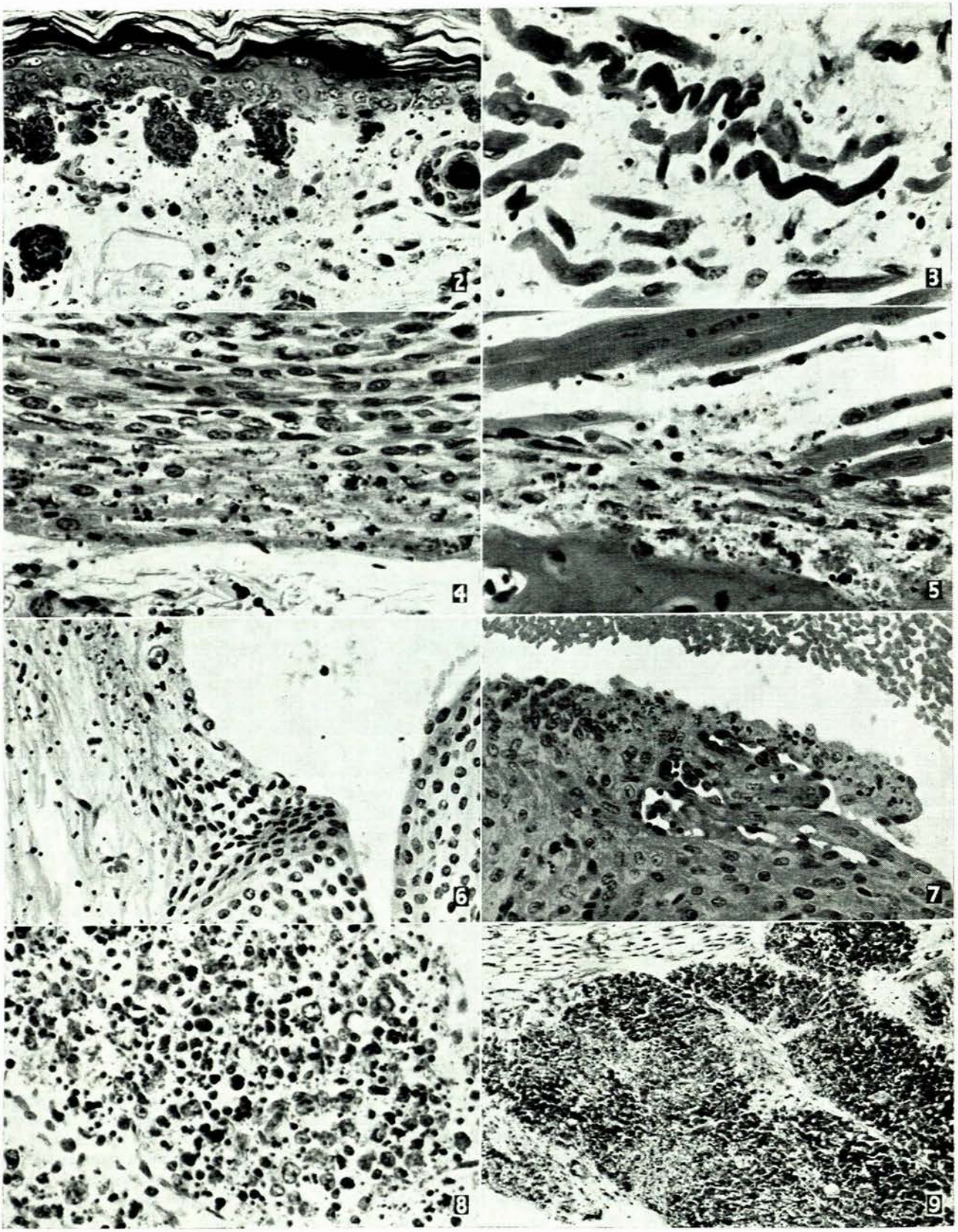
Sindbis Virus Isolations from Humans

The first isolations of Sindbis virus from humans were reported from Uganda by Haddow in 1961,[7] and further information concerning these was presented by staff members of the East African Virus Research Institute in 1962.[8] Virus was recovered from 5 persons by the inoculation of finger-prick blood into suckling mice. The patients were Africans aged between 25 and 38 years — they had been ill for 2 - 3 days, 4 had fever ranging between 99·2° and 102·6°F., all complained of headache, 2 had general malaise, and 1 complained of chest pain, 1 of widespread pains, and 1 of joint pains. No rashes were observed. Slight jaundice was noted in 2 patients, and 1 had an enlarged spleen. Neurological signs were absent. No further contributory clinical details could be ascertained.

The Pathology of Sindbis Virus Infection

The agent is pathogenic to chick embryos, and produces destruction of cells in chick-embryo tissue cultures.[9] Cytopathic effects have also been observed in other kinds of tissue cultures by a number of investigators. The virus is rapidly fatal to suckling mice in 2 - 3 days by any route of inoculation; but resistance to clinical disease markedly increases with age in mice. Taylor *et al.*[1] described the lesions in suckling mice inoculated by either the intracerebral or the intraperitoneal route: the central nervous system showed neurolysis without marked leucocyte infiltration, and the skeletal muscles underwent focal or diffuse degeneration with mononuclear infiltration. Myocarditis was rarely observed, and lesions of other organs were not seen. Weinbren *et al.*[3] noted similar changes in the brain and muscle of infected mice, and did not observe changes in other organs including heart, liver, pancreas, thymus, lung and fat-pad.

The lesions produced in suckling mice by the strain recovered from the patient P.G. appear to be more extensive than those noted by previous investigators. It is possible that the initial passage through monkey-kidney tissue culture may have selected a more virulent variant; but the severity of the illness in the patient suggests that this strain is *per se* particularly virulent. Most of the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infected mice died in 2 - 4 days, becoming progressively weaker without showing obvious paralysis. The lesions described below were found in the majority of mice, but occurred in varying degrees in individual animals. The rapid progression of the infection may account for the notable absence of inflammatory cellular reaction, although early polymorphonuclear infiltration was occasionally noted.

Lesions Produced by the P.G. Strain of Sindbis Virus in Suckling Mice

Skin. Necrosis of dermal connective tissue was usually marked (Fig. 2). Epidermal degeneration was rarely seen.

Skeletal muscle. Diffuse necrosis of interstitial connective tissue usually predominated, but patchy and occasionally widespread degeneration of muscle cells, with swelling of the fibres and loss of striation, also occurred (Fig. 3). Oedema was usually present, but inflammatory cellular infiltration was frequently absent. In many respects these lesions resembled those produced by the Coxsackie viruses, except when muscle fibres were unaffected and only the interstitial tissue showed degeneration. Patchy necrosis of tendons was seen (Fig. 4), and degeneration at muscle insertions was characteristic (Fig. 5).

Joints. The articular surfaces of bones appeared to be intact, but marked necrosis of peri-articular connective tissue and of capsular ligaments, with involvement of the synovial membrane, was frequently found (Fig. 6).

Bone marrow. Patchy necrosis of bone marrow was noted.

Smooth muscle. Necrosis of smooth muscle was seen around the oesophagus, gut and bladder, and in the walls of blood vessels, particularly in the lung.

Heart. Necrotic myocardial cells were often seen scattered throughout atria and ventricles, but degeneration was most marked at the atrio-ventricular junction, with occasional involvement of the valves (Fig. 7).

Lungs. Inflammatory lesions were not seen in the lungs, but necrosis was often marked in the walls of blood vessels, and scattered degenerated cells could be seen in the alveolar walls.

Thymus. Extensive destruction of small thymocytes was common, while the reticular cells remained relatively unaffected (Fig. 8).

Brown fat. A characteristic degeneration of the brown fat was seen, areas of pale-staining cells with pyknotic nuclei contrasting with adjacent areas of unaffected cells (Fig. 9).

Liver and pancreas. Occasional degenerated parenchymal cells were seen in both these organs.

Kidney and spleen. No significant lesions were observed in the kidneys and spleens of infected mice.

Brain. Inflammatory reaction was usually absent, and mice inoculated by any route frequently showed only scattered degenerated cells in the brain. One animal, however, which had survived for 7 days after inoculation by the combined intracerebral-subcutaneous route with fluid diluted 10^{-5}, showed slight mononuclear infiltration of the meninges and cerebral vessels, while in the cortex there was an extensive area which stained pale and contained neurones showing marked vesication of the nucleus with margination of chromatin. Such nuclear vesication, often with a small red-staining body resembling a viral inclusion in the nucleus, was also observed in other affected organs in a number of mice.

While extrapolation from mouse to man is not always valid, some of the patient's signs and symptoms may be accounted for on the basis of the lesions observed in mice. In particular, the oedematous papules of the rash possibly resulted from damage to vessel walls and subsequent destruction of supporting connective tissue; while the pains in joints and tendons probably arose from a comparable pathological change. Although the patient P.G. did not suffer from pain about the eyes, this is a feature of some arthropod-borne virus infections, and sections through the orbits of infected mice showed extensive destruction of orbital muscles and of connective tissue in the eyelids. It may therefore be predicted that pain around the eyes will be observed in a proportion of human cases of Sindbis virus infection.

The regular occurrence of lesions in the heart, especially in the region of the valves, suggests that this organ may also be affected in humans, particularly in the infant. Similarly, destruction of the thymus, which we have also observed in suckling mice infected with certain Coxsackie and reovirus strains, may be of significance in the human infant, especially if the thymus plays a part in the development of immunological competence.

SUMMARY

A virus which is immunologically closely related to the known AR 86 strain of Sindbis virus, was isolated from the vesicular skin lesions of a patient suffering from low fever, malaise, pains in the joints and tendons, and a rash which was maculopapular over the trunk and limbs, but vesicular on the fingers and toes. A rise in haemagglutination-inhibiting antibody to this virus during the course of the illness was demonstrated.

The virus, which was recovered in tissue cultures of vervet-monkey kidney, is pathogenic for suckling mice, but produces lesions more extensive than those previously described for strains of Sindbis virus. Pathological changes in the suckling mouse involve connective tissue, muscle (including striated, smooth and myocardial fibres), central nervous tissue, brown fat, thymus, bone marrow and, to a lesser degree, liver and pancreas.

This paper presents the first association of a clinical syndrome with Sindbis virus infection in humans.

We are grateful to Dr. J. H. S. Gear, Director of the Poliomyelitis Research Foundation and of the South African

Figs. 2—9. P.G. strain in suckling mouse:
Fig. 2. Necrosis of dermal connective tissue.
Fig. 3. Destruction of skeletal muscle.
Fig. 4. Necrosis in tendon.
Fig. 5. Necrosis at muscle insertion.
Fig. 6. Necrosis of capsular ligament of joint.
Fig. 7. Degeneration of heart valve.
Fig. 8. Destruction of small thymocytes.
Fig. 9. Degeneration in fat-pad.

Institute for Medical Research, for his advice and encouragement in this study. We should also like to express our thanks to Dr. B. M. McIntosh, who is in charge of the Arthropod-borne Virus Research Unit of the Poliomyelitis Research Foundation, for his helpful advice and for providing antisera and the strain of Sindbis virus AR 86. Our thanks are also due to members of this Unit for their assistance in the haemagglutination tests; to the Histology Unit of the Poliomyelitis Research Foundation for the preparation of mouse sections; and to Mr. M. Ulrich, of the Photographic Unit of the South African Institute for Medical Research, for the photomicrographs.

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