HERPES GENITALIS* CLINICAL AND VIROLOGICAL STUDIES

BY

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This paper presents the results of clinical and virological investigations on patients with genital herpes attending the venereal disease department at St. Thomas' Hospital between February and December, 1966. The results of some studies on cases of extra-genital herpes are also included for comparison.

Primary herpes simplex virus (HSV) infection is usually subclinical (Scott, 1957), but may occasionally, especially in childhood, produce severe infection with marked constitutional symptoms, *e.g.* disseminated visceral herpes (McKenzie, Hansen, and Becker, 1959), gingivostomatitis (Dodd, Johnston, and Buddingh, 1938), follicular conjunctivitis (Granström, 1937), meningoencephalitis (Whitman, Wall, and Warren, 1946) and vulvovaginitis (Slavin and Gavett, 1946). Primary herpes genitalis appears to be a rarity. One well-documented case in a man aged 23 has been reported, this being associated with aseptic meningitis and a 22-fold rise in neutralizing antibody titre (Duxbury and Lawrence, 1959). After primary infection, subsequent attacks may present as localized lesions, common sites being the lips, cornea, buttocks, nipples, and genitalia. Such lesions may be recurrent and are often provoked by such excitants as fever, sunshine, trauma, and stress (Scott and Tokumaru, 1965). When herpes infection recurs there is a strong tendency for the same site to be involved (Burnet and Williams, 1939; Scott, 1957). The presence of neutralizing antibody does not necessarily protect against recurrent infection (Andrewes and Carmichael, 1930; Scott, 1957).

Genital lesions appear initially as grouped vesicles 2 to 3 mm. in diameter, often with some surrounding erythema. Regional nodes are not usually enlarged unless secondary bacterial infection is present. The vesicles often persist for 1 to 4 days and then either become pustular and dry, or rupture leaving superficial ulcers.

Herpes genitalis is usually considered to be venereally acquired (Unna, 1883; Fournier, 1896; Tyler, 1957). It is a condition almost exclusively of young adults (*i.e.* of the age of maximum sexual

activity) and an initial attack, but not necessarily subsequent attacks, usually follows a few days after intercourse. In men, lesions may occur on the prepuce, glans penis, or penile shaft; in women, lesions may occur on the vulva, vagina, or cervix. The diagnosis is simple enough when typical vesicles are present but, when ulceration occurs, other conditions have to be differentiated. The most important of these is syphilitic chancre, but such diseases as chancroid, lymphogranuloma venereum, candidosis, erythema multiforme, and Behçet's syndrome may also have to be considered.

Material and Methods

A consecutive series of sixty patients, thought on clinical grounds (*vide supra*) to have genital herpes, were investigated. The patients were asked the duration of their lesions and whether they had previously experienced either a similar genital eruption or herpetic lesions elsewhere.

Syphilis was excluded by serological tests (Wassermann reaction and Reiter protein complement-fixation test). Where appropriate, dark-ground studies on material taken from genital ulcers were also performed.

Vesicular fluid or scrapings from the base of ruptured vesicles were obtained from genital lesions. The material was immediately immersed in 3 to 4 ml. of virus transport medium (Hanks's BSS; bovine plasma albumen 0.4 per cent.; and antibiotics), and within 2 hours of the specimen's arrival at the laboratory was inoculated into HeLa cell cultures (0.2 ml.) and whenever possible onto the chorioallantoic membrane (CAM) of 11-day-old fertile hens eggs (0.05 ml.). The presence of HSV in cultures exhibiting cytopathic effect (CPE) was confirmed by neutralization tests employing virus-specific rabbit antiserum and by inoculation of culture fluid into eggs and inspecting the CAMs after incubation at 36°C. for the characteristic "pin-point" HSV lesions. The CAMs from specimens initially inoculated into eggs were likewise examined and, if negative, the CAMs were ground and re-passed into fresh eggs before the specimens were declared negative.

It has been observed (McDonald, 1966) that if CAMs are inspected at 7 rather than at 2 days, large necrotic lesions 3 to 5 mm. in diameter may occur with strains of virus isolated from patients with genital herpes, whereas lesions from strains from patients with extra-genital herpes remain typically small. Accordingly all isolates, including those originally identified in HeLa cell

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cultures, together with ten strains of HSV derived from extra-genital herpes infections from patients attending other departments of St. Thomas' Hospital, were inoculated into eggs which were harvested both at 2 and at 7 days.

Results

Herpes simplex virus was isolated from 23 (38 per cent.) of the sixty patients, 21 males and two females (Table I), whose ages ranged from 20 to 42 years. All but three men (Cases 4, 11, and 20) were uncircumcised (approximately 60 per cent. of all patients attending the clinic were found to be uncircumcised). Eight (34 per cent.) of the 23 patients from whom HSV was isolated gave a history of recurrent genital lesions, although only Case 11 gave a classical history of herpes labialis. It will be noted that some of the patients had classical grouped vesicles while others exhibited either pustules or ulcers which were presumably ruptured vesicles. The patient with pubic vesicles (Case 4), although not strictly a case of genital herpes according to the above definition, was a promiscuous

male whose lesions appeared during the course of treatment for gonorrhoea.

The likelihood of virus isolation appeared to depend more upon the duration of the lesion than its macroscopic appearance, for most lesions from which HSV were isolated, whether vesicles or ulcers, had been present for one week or less. Of 23 isolates made in HeLa cell cultures, CPE was usually evident by the third day, but on three occasions (Cases 6, 20, and 22) it was detected as early as 24 hours after inoculation. Of the fourteen isolates initially detected in eggs, only one required additional passage.

Although CAM lesions from genital and extra-genital strains appeared typical and identical at 2 days, by 7 days all the genital strains had produced large necrotic lesions, whereas nine of ten of the extra-genital strains showed little change from the small lesions seen at 2 days (Fig. 1, overleaf).

Table II (overleaf) lists the clinical diagnoses and size of lesions on CAMs from ten patients with extra-genital herpes (Cases A to J).

TABLE I
PARTICULARS OF 23 CASES OF HERPES GENITALIS (21 MALE, 2 FEMALE)

Case No.	Age (yrs)	Sex	Nationality	Marital Status	Lesions	Duration (days)	Consort	Lesions on CAM
1	27	M	British	M	Indurated coronal ulcers	5	Casual female	Large
2	25	M	B.W.I.	M	Shallow preputial ulcers	3	Wife	Large
3	30	M	British	M	Genital vesicles (recurrent)	7	Female friend	Large
4	20	M	British	S	Pubic vesicles	7	Female friend	Large
5	30	M	British	M	Preputial ulcers and oedema	10	Wife	Large
6	24	M	British	S	Subpreputial ulcers	7	?Homosexual	Large
7	26	M	British	S	Coronal ulcers (recurrent)	3	Female friend	Large
8	33	M	Asian	S	Healing penile ulcers (recurrent)	Not certain	Female friend	Large
9	31	M	B.W.I.	M	Penile vesicles (recurrent)	Not certain	Casual female	Large
10	39	M	B.W.I.	S	Coronal vesicles	7	Female friend	Large
11	42	M	British	M	Penile vesicles	Not certain	Wife	Large
12	25	F	Australian	S	Ulcer labium major	2	Male friend	Large
13	29	M	British	S	Coronal ulcers (recurrent)	3	Homosexual	Large
14	38	M	Polish	M	Preputial vesicles	1	Wife	Large
15	21	M	B.W.I.	S	Coronal vesicles and ulcers	4	Female friend	Large
16	23	M	B.W.I.	S	Coronal ulcers	7		Large
17	24	M	British	M	Preputial ulcers (recurrent)	Not certain	Wife	Large
18	28	M	British	M	Preputial vesicles and papules	7	Female friend	Large
19	25	M	Asian	S	Preputial pustules	1	Female friend	Large
20	41	M	British	S	Healing penile vesicles (recurrent)	Not certain	Prostitute	Large
21	32	M	B.W.I.	S	Penile ulcers	14	Homosexual	Large
22	22	F	B.W.I.	S	Vulval and vaginal ulcers	3	Male friend	Large
23	40	M	British	S	Penile vesicles (recurrent)	5-7	Female friend	Large

FIG. 1a.—Lesions on CAM from strain of HSV derived from genital lesions (7 days after inoculation).

TABLE II
PARTICULARS OF TEN CASES OF EXTRA-GENITAL HERPES (1 MALE, 9 FEMALE)

Patient No.	Sex	Age (yrs)	Clinical Diagnosis	Duration (days)	Lesions on CAM
A	F	18/12	Acute gingivostomatitis	5-7	Small
B	F	20	Keratoconjunctivitis pharyngitis	2	Small
C	F	23	Herpetic lesions on right middle finger	3 Recurrent	Large
D	F	26	Herpetic whitlow right index finger	10-14	Small
E	M	36	Conjunctivitis	Uncertain	Small
F	F	2	Acute gingivostomatitis	5-7	Small
G	F	19	Herpes labialis	2	Small
H	F	54	Acute febrile pharyngitis	4	Small
I	F	58	Acute stomatoglossitis	3	Small
J	F	22	Herpes labialis associated with Bornholm disease	4 Recurrent	Small

In view of these findings, the CPE of both genital and extra-genital strains was studied in HeLa, WI-38, and RK13 cultures. No difference in CPE between strains was found. Electron microscopy of three genital and three extra-genital strains revealed typical herpes-like virus particles (Fig. 2, opposite). All strains examined were morphologically identical.

Histological study of CAM lesions caused by strains of HSV from genital lesions revealed an ulcerated epithelium with marked round cell

infiltration, giant cell formation, and capillary proliferation. Examination of CAM lesions caused by extra-genital HSV revealed no ulceration, minimal round cell infiltration, and capillary proliferation, but no giant cells.

Discussion

This series shows that clinical cases of herpes genitalis are not uncommon. Records examined during the course of this investigation revealed that

FIG. 1b.—Lesions on CAM from strain of HSV derived from extra-genital lesions (7 days after inoculation).

FIG. 2.—Negatively-stained virus particle from genital lesions.

the incidence of virologically proven herpes genitalis approximated to that of primary syphilis. Barile, Blumberg, Kraul, and Yaguchi (1962), in an investigation of 35 patients with penile lesions in the U.S. Army in Japan, found that HSV was the most commonly encountered aetiological agent. As in our series, the majority of these patients (91 per cent.) were uncircumcised.

It is probable that many of the lesions in our series from which no virus was isolated were caused by HSV, but the long duration of many of them precluded virus isolation. This is broadly in agreement with the findings of Hutfield (1966).

There was a low incidence of herpes genitalis in females in this series. This may in part be due to the fact that they are less likely to notice lesions than men. Vulval lesions are more common than vaginal or cervical lesions (King and Nicol, 1964), but since HSV is rapidly inactivated below pH 7·0 (Munk and Ackermann, 1953; Farnham and Newton, 1959), the environment of the healthy vagina (pH 4·5) is unlikely to be suitable for virus replication. However, in a survey of genital herpes in 64 women,

Josey, Nahmias, Naib, Utley, McKenzie, and Coleman (1966) found that cervical lesions accounted for 46 per cent. of the lesions from which HSV was isolated. In these cases the cervix was often oedematous, bleeding on slight trauma. Less often, large punched-out or multiple superficial ulcers were also seen.

Only one extra-genital strain, from Patient C, a married woman aged 23 with recurrent herpetic lesions on the right middle finger, produced large necrotic "genital-type" lesions on the CAM. The possibility that this was derived from an inoculum from a genital lesion is, of course, speculative.

There have been no previous published reports of the marked morphological difference between the lesions which genital and extra-genital HSV strains produce on the CAM*. Although both genital and extra-genital lesions are clinically similar in appearance, and no difference in CPE or virus morphology could be demonstrated, it is possible that strains of HSV that cause genital lesions may differ antigenically from other strains.

Serological studies, notably kinetic neutralization tests, have revealed various antigenic types of HSV. (Schneweis, 1962; Ashe and Scherp, 1963). Similar studies on representative strains of HSV isolated in this study are in progress and will be reported later.

Summary

Herpes simplex virus was isolated from 23 (38 per cent.) of sixty patients with herpes genitalis. A history of recurrent lesions was common, and uncircumcised patients were more frequently involved. Genital herpes was detected in only two females in this series. Viruses isolated from patients with herpes were found to produce atypical large necrotic lesions when inoculated onto the chorio-allantoic membrane (CAM) of developing chick embryos. Viruses from extra-genital herpetic lesions, with one exception, produced classical small herpetic lesions on the CAM. No difference in cytopathic effect or virus morphology could be detected between genital and extra-genital strains.

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* Since this paper was submitted, D. C. Hutfield (*Acta dermatol. venerol.* (Stockh.), 1967, 47, 118) has also reported variations in size of CAM lesions produced by HSV derived from genital and extra-genital sources.

REFERENCES

- Andrewes, C. H., and Carmichael, E. A. (1930). *Lancet*, 1, 857.
 Ashe, W. R., and Scherp, H. W. (1963). *J. Immunol.*, 91, 658.
 Barile, M. F., Blumberg, J. M., Kraul, C. W., and Yaguchi, R. (1962). *Arch. Derm. (Chicago)*, 86, 273.
 Burnet, F. M., and Williams, S. W. (1939). *Med. J. Aust.*, 1, 637.
 Dodd, K., Johnston, L. M., and Buddingh, G. J. (1938). *J. Pediat.*, 12, 95.
 Duxbury, A. E., and Lawrence, J. R. (1959). *Med. J. Aust.*, 2, 250.
 Farnham, A. E., and Newton, A. A. (1959). *Virology*, 7, 449.
 Fournier, J. A. (1896). *Rev. gén. Clin. Thér. (Paris)*, 10, 177.
 Granston, K. O. (1937). *Acta ophthalm. (Kbh.)*, 15, 361.
 Hutfield, D. C. (1966). M.D. thesis, London.
 Josey, W. E., Nahmias, A. J., Naib, Z. M., Utley, P. M., McKenzie, W. J., and Coleman, M. T. (1966). *Amer. J. Obstet. Gynec.*, 96, 493.
 King, A. J., and Nicol, C. S. (1964). In "Venereal Diseases", pp. 25 and 243. Cassell, London.
 McDonald, J. R. (1966). Personal communication.
 McKenzie, D., Hansen, J. D. L., and Becker, W. (1959). *Arch. Dis. Childh.*, 34, 250.
 Munk, K., and Ackermann, W. W. (1953). *J. Immunol.*, 71, 426.
 Schneweis, K. E. (1962). *Z. Immun.-Forsch.*, 124, 24.
 Scott, T. F. McNair (1957). *Amer. J. Ophthalm.*, 43, no. 4, pt 2, p. 134.
 — and Tokumaru, T. (1965). In "Viral and Rickettsial Infections of Man", ed. F. L. Horsfall and I. Tamm, p. 892. Pitman, London.
 Slavin, H. B., and Gavett, E. (1946). *Proc. Soc. exp. Biol. (N.Y.)*, 63, 343.
 Tyler, D. E. (1957). *Brit. J. vener. Dis.*, 33, 228.
 Unna, P. G. (1883). *J. cutan. vener. Dis.*, 1, 321.
 Whitman, L., Wall, M. J., and Warren, J. (1946). *J. Amer. med. Ass.*, 131, 1408.

L'herpès génital. Étude clinique et virologique

RÉSUMÉ

Le virus de l'herpès simplex a été isolé de 23 des 60 malades (38 pour cent) atteints d'herpès génital. Un historique de lésions répétées avait été fréquent, et les malades non-circoncis avaient été atteints plus souvent. L'herpès génital a été découvert chez deux femmes seulement de cette série. On a trouvé que les virus isolés des malades atteints d'herpès avaient produit des grandes lésions nécrotiques atypiques quand ils avaient été inoculés à la membrane chorio-allantoïque des embryons du poulet en développement. Les virus provenant des lésions herpétiques extra-génitales avaient produit des petites lésions herpétiques classiques sur la membrane chorio-allantoïque sauf dans un cas seulement. Aucune différence dans l'effet cytopathique ou dans la morphologie des virus n'a pu être démontrée entre les souches génitales et extra-génitales.