4. Fitzgerald, L., Physiol. Rev. 34, 325 (1957).
5. Kihn, L., Arch. Physik. Therapie 15, 1 (1963).
6. Malkinson, F. and Rothman, S., in "Handbuch der Hautund Geschlechtskrankheiten," Jadassohn, J., ed., Springer, Berlin, p. 90, 1964.
7. Kligman, A. and Christophers, E., Arch. Dermatol. 88, 702 (1963).
8. Christophers, E. and Kligman, A., Advan. Biol. Skin 6, 163 (1964).
9. Tuwiner, S., "Diffusion and membrane technology," Reinhold, New York, 1963.

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Relation of Pock Size on Chorioallantoic Membrane to Antigenic Type of Herpesvirus Hominis* (32861)

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Recently we have observed that strains of herpesvirus hominis (HVH) could be differentiated antigenically into two types. Type 1 was found to be primarily associated with nongenital infection and transmission, whereas type 2 was primarily associated with genital infection and transmission (1). In order to further characterize the two antigenic types, biological markers were sought. Other investigators (2–4) found that the pock size on embryonated egg chorioallantoic membrane (CAM) with certain HVH strains were larger. In addition, various types of histological changes have been observed (5–8) in the CAM after infection with different HVH strains. This report presents studies on the relation of pock size on CAM to antigenic type of 79 strains of HVH which were isolated from genital and nongenital sites, and suggests the use of this technique as a presumptive test for the differentiation of HVH strains. Histological studies of the CAM infected with serotypes 1 and 2 also are included.

Materials and Methods. Virus strains. The source and passage history of the majority of the strains used for this study have been described (1). In addition, 4 strains isolated from eye infections, 2 from infections of newborns and a vaginal isolate from an 8-year-old girl are included. The majority of strains were primary isolates with 1 or 2 tissue culture passages, mainly in primary rabbit kidney cells. Only 2 strains had prior egg passage.

Egg inoculation. Embryonated eggs 10–12 days old were inoculated with 0.1 ml of varying dilutions of virus on the CAM using the false air sac technique (9). At least 2 eggs were inoculated with each virus dilution and the eggs were incubated at 34–35°C for 3–4 days. To avoid the possible effect of population density on pock size, measurements were made, whenever feasible, when the number of pocks was between 20 and 100. The membrane was removed onto a plate containing saline. The morphology of the pocks was examined and the diameter of the pocks was measured with the aid of an ocular micrometer mounted to the eyepiece of a binocular dissection microscope. Strains which did not produce pocks with undiluted virus were tested at least twice.

Criteria for "large" and "small" pocks. Up to 20 pocks were measured for each strain. Virus strains were divided into those producing "small" or "large" pocks on the basis of the following criteria: Small—average diameter of pocks counted was less than 0.5 mm and diameter of any one pock did not exceed 1.0 mm (Fig. 1). Large—Average diameter of pocks counted was greater than 0.5 mm and many pocks exceeded 1 mm in diameter (Fig. 2). Pock size measurements were made on

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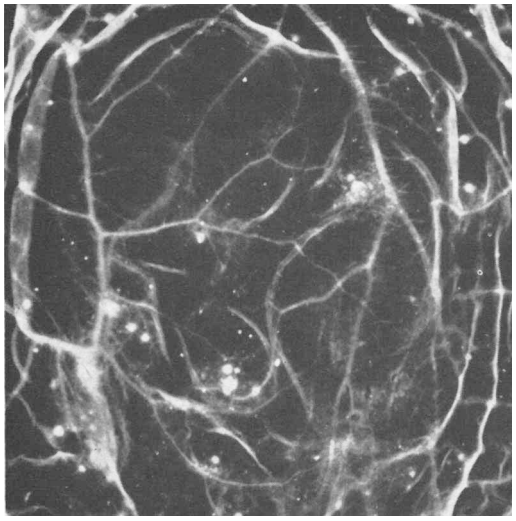

FIG. 1. Pocks produced by a type 1 herpesvirus hominis strain.

strains without prior knowledege of their antigenic type.

Antigenic typing. Methods for antigenic typing of HVH strains using a microneutralization technique and the pN method of analysis have been described previously (1).

Histological sections. Chorioallantoic membranes inoculated with 5 different type 1 HVH strains and 10 different type 2 strains were cut and fixed with Bouin's fluid. Sections were made and examined after staining with hematoxylin–eosin.

Results. Relation of pock size to antigenic type. In Table I, the type of clinical involvement, site of HVH isolation, antigenic type and pock size of 79 HVH strains are presented. The 9 strains which did not produce pocks after at least 2 attempts also are listed. Almost all the genital strains from males or females were type 2 and produced large pocks on the CAM. Two exceptions, commented on in a previous report (1), were in children—a 3-year-old boy with penile lesions and a 6-year-old girl with vulvar lesions, both having HVH type 1 also recovered from their throats.

All nongenital strains were type 1, except for 3 strains recovered from thigh lesions in women who also had type 2 virus recovered from genital sites.

Histopathology. As summarized in Table II, there are definite differences between the histological features of the pocks of type 1 and 2 HVH strains. In the case of type 1 infection, an ectodermal basal cell hyperplasia predominates (Fig. 3). Eight to 12 layers of round basal cells are squeezed together showing little variation in size or shape. The nuclei of these cells are round or oval with slightly irregular nuclear membrane, granular chromatin, and prominent round nucleoli. Their cytoplasm is adequate and vacuolated without inclusions. No necrosis and only rare hemorrhages are seen. Mild congestion, fibroblastic hyperplasia, and erosion are occasionally present and the few inflammatory cells found are mainly monocytic in type. The infected cells have single, enlarged, but bland nuclei, with thick nuclear membranes. The occasional intranuclear inclusions are poorly defined and often difficult to recognize. The multinucleated giant cells are scarce and limited to the margin of the lesion.

After infection with type 2 HVH, the entire thickness of the CAM is often involved (Fig. 4). Large areas of the ectoderm are ulcerated with marked necrosis, acute mixed inflammation and congestion of the subectodermic tissue. Extensive hemorrhages are present, even at some distance from the pock formation. In the depth of the lesion, abundant hyperplastic fibroblasts are mixed with numerous

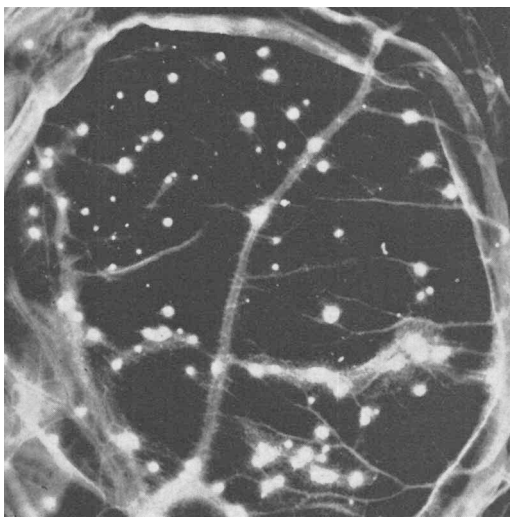

FIG. 2. Pocks produced by a type 2 herpesvirus hominis strain.

TABLE I. Pock Size on CAM, Type of Clinical Involvement and Site of Isolation of 79 Herpesvirus Hominis Strain by Antigenic Type.

		Type 1				Type 2				
			Pocks				Pocks			
Type of involvement	Site of isolation	Total	Large	Small	None	Total	Large	Small	None	Total
Genital tract										
male	penis	1		1		5	5			6
female	cervix					12	10		2	12
	endocervix					4	3		1	4
	vagina	1		1		11	9		2	12
	vulva					6	6			6
Neonatal herpes	skin					4	4			4
Meningoencephalitis	spinal fluid	2		1	1					2
	brain	8		6	2					8
Eczema herpeticum	skin	1		1						1
	lung	1		1						1
Dermatitis	face or trunk	4		4						4
	eyelid	1		1						1
	thigh					3	3			3
Keratitis and/or conjunctivitis	eye	4		4						4
Herpes labialis	lip	5		4	1					5
Gingivostomatitis	pharynx	2		2						2
Asymptomatic	pharynx	4		4						4
	Total	34	0	30	4	45	40	0	5	79

TABLE II. Histologic Differentiation of the Pocks on the Chorioallantoic Membrane of Embryonated Eggs after Infection with Herpesvirus Hominis (HVH) Type 1 and Type 2.

Condition	Type 1 HVH	Type 2 HVH
Site of involvement	Ectoderm mainly	Entire thickness (ectoderm, mesoderm, and endoderm)
Ectodermal hyperplasia	Marked	Moderate
Congestion	Mild	Marked
Hemorrhages	Rare	Common
Necrosis	Rare	Common
Erosion	Mild	Marked
Fibroblastic hyperplasia	Mild	Marked
Inflammatory cells	Mild (monocytes)	Marked (mixed: monocytes and polymorphonuclear leukocytes)
Multinucleated giant cells	Rare	Common
Intranuclear inclusions	Occasional	Common

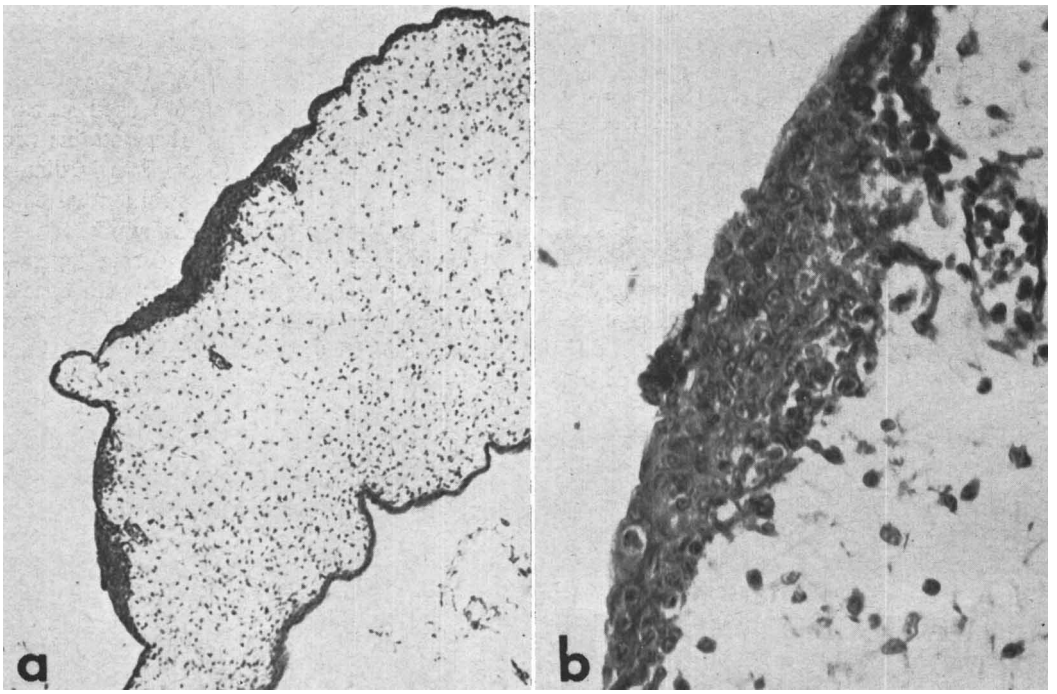

FIG. 3. Histological section of pock produced by a type 1 HVH strain; H and E. Note marked ectodermal hyperplasia with little subectodermal reaction. a. 68 × b. 400 ×

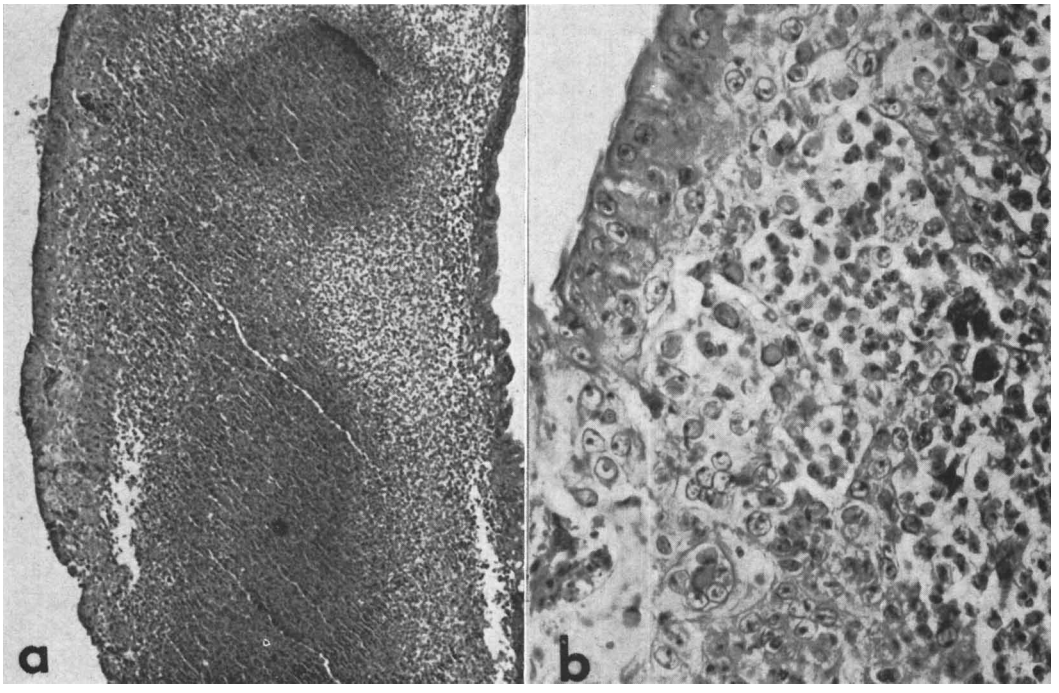

FIG. 4. Histological section of pock produced by a type 2 HVH strain; H and E. Note involvement of the 3 layers with necrosis, intense inflammatory response in mesoderm, and ulceration of ectodermal layer. a. 68 × b. 400 ×; also note multinucleated giant cells.

giant syncytial cells containing distinct, intranuclear inclusions. The nuclei of these cells are characteristically molded against each other and have prominent thickened, regular nuclear membrane. These histological changes were noted not only in large pocks with a diameter over 1.0 mm but also in the occasional pock which measured less than 1 mm in diameter.

Discussion. These observations provide further support that certain biological characteristics are associated with the antigenic differences previously reported among herpesvirus hominis (HVH) strains (10,14). Thus, strains belonging to type 2 and noted to be most frequently related to genital sites of isolation and mode of transmission (1) have been found to produce large pocks and characteristic histological features on the chorioallantoic membrane of embryonated eggs. We have also demonstrated a higher mortality in adult female mice inoculated via the genital tract with type 2 strains (11). In a recent publication (12), Hutfield cites unpublished observations of B. Barton of the Public Health Laboratory Service, Colindale, England, which are similar to our findings: the pocks produced when genital strains are freshly isolated on CAM are larger than those with nongenital strains. Schneweis (3) also noted that two type 2 strains produced larger pocks (0.8–1 mm) than strains belonging to type 1 (0.2–0.5 mm), although this investigator had to adapt his strains by egg passage first. Hamar (4) found 2 strains producing macropocks (3 mm in diameter) but failed to describe the origin of the strains. In addition, Jawetz *et al.* (13) in 1955 reported that their MAX strain produced large pocks on the CAM; this strain was later typed by Schneweis in 1962 and found to belong to type 2 (14).

Great caution must be placed on results of pock size obtained with HVH strains which have had several passages on eggs. Wildy (2), for instance, reported 6 strains of HVH of unknown type having multiple egg passages, 4 producing "large" pocks (1–1.5 mm in diameter) and 2 producing "small" pocks never exceeding 0.5 mm. Similar concern about the effect of egg passage or prior selection for a particular characteristic, such as cytopathic effect, might explain the conflicting reports of histological findings on CAM inoculated with HVH strains (5-8).

Thus, when Coriell *et al.* (7) used primary isolates of throat and skin, these strains produced only small pocks (0.1–1 mm in diameter). Histological findings—proliferated ectodermal cells with minimal inflammatory reaction in mesodermal and endodermal layers—are similar to those noted by us with type 1. Anderson (6) found that first CAM generations of the HF strain produced superficial and proliferative lesions (similar to our type 1 findings). By the thirtieth passage in eggs there were signs of extensive involvement of the mesoderm with hemorrhages (similar to our type 2 findings). Dawson (5), also using the HF strain passed 6 times in eggs, found both types of lesions to be produced by this virus: some with marked hyperplasia of ectoderm with a minimal amount of necrosis, and others with necrosis and little or no ectodermal hyperplasia. On the other hand, Wheeler's HF strain (15) (typed by us as type 2 in contrast to 3 other HF strains we have tested to be type 1) was more virulent for the chick embryo than his HPF strain (type 1), a variant producing syncytial giant cells in tissue culture. Similarly, Nii and Kamahora (8) distinguished 3 substrains of HVH by their cytopathic effect on FL monolayers. Their +GC strain, which produced syncytial formation, caused histological changes on CAM suggestive of our type 2 findings, with necrosis and inflammatory reaction in the mesodermal layer. They could not find any giant cells in the CAM inoculated with either their —GCr or —GCf strain.

Recent observations by Taniguchi (16) suggest that the greater destructive effect of type 2 strains on the CAM may be related to continued viral growth. This worker, using the SK strain isolated from a patient with eczema herpeticum, usually associated with type 1, found that the growth of virus on the CAM was interrupted 2 days after infection. Thus the CAM may provide a good model for the study of host factors responsible in either curtailing infection with type 1 strains to the ectodermal layer or allowing rampant in-

volvement of subectodermal layers with type 2 strains.

An analogy between the pock size and histological findings with the two antigenic types of HVH may be made with those noted with variola and vaccinia viruses. The pock size of variola virus on CAM is 0.5–1 mm (17) (slightly larger than type 1 HVH), whereas the pock size of several strains of vaccinia virus ranges from 1.3 to 3 mm (17,18) (similar to type 2 HVH). Histologically, variola lesions on CAM show primarily ectodermal proliferation, whereas vaccinial lesions are more extensive and more destructive, without significant proliferation of the ectodermal cells (17). Although the pocks of HVH (type 2) may be confused macroscopically with those of vaccinia, histological sections would readily differentiate HVH with its intranuclear inclusions from vaccinia with its intracytoplasmic inclusions.

At present, antigenic typing of HVH strains by either the plaque neutralization method (14) or microneutralization technique (1) is a specialized procedure and requires prior virus titrations. The determination of pock size on CAM is an easier test, although it should still be considered a presumptive test. Recently we have used it as a rapid screening test in the ongoing study of genital herpetic infections. Thus, when a strain producing small pocks was observed among 30 genital strains, which would have been expected on the basis of the findings noted here to produce large pocks, the virus was immediately submitted to antigenic typing. This small pock strain was recovered from an adult female with recurrent vulvar lesions and was found to belong to type 1. Similarly, an HVH strain recovered from the pharynx of a 9-year-old girl with herpetic vulvar lesions was noted to produce large pocks resembling the isolate from the vulvar lesions. On antigenic testing, both isolates were found to belong to type 2. In addition, strains recovered from the throat and eye of a newborn infant which produced large pocks were given priority antigenic testing and found to belong to type 2. Such results also point to the occasional association of type 1 with genital sites and type 2 strains with nongenital sites.

Summary. Strains of herpesvirus hominis (HVH) which were recovered primarily from genital sites and which belonged to antigenic type 2 were found to produce large pocks (av diameter $>$ 0.5 mm) on the chorioallantoic membrane (CAM) of embryonated eggs. Type 1 HVH strains isolated primarily from nongenital sites produced small pocks (av diameter $<$ 0.5 mm) on the CAM. Histological examinations of the infected CAM have revealed that type 1 HVH strains produce ectodermal proliferation with little subectodermal involvement. Type 2 HVH strains involve ectodermal, mesodermal and endodermal layers with marked necrosis, hemorrhages and inflammatory cells. Multinucleated giant cells are more readily found in CAM infected with type 2 strains. The conflicting reports of other workers regarding pock size and histological findings after infection with various HVH strains may be explained by their multiple passages in eggs and other selective factors. Also included is a comparison of CAM infection with type 1 and 2 HVH and variola and vaccinia viruses. Evaluation of the pock size of freshly isolated strains of HVH which have had no prior egg passage could serve as a rapid presumptive test for ascertaining the antigenic type of HVH strains.

Addendum: Parker and Banatvela [Brit. J. Venereal Diseases **43**, 212 (1967)] and Hutfield [Arch. Derm. Venereol. **47**, 118 (1967)] have recently reported that genital HVH strains produced large pocks and nongenital strains small pocks on the CAM.

1. Dowdle, W., Nahmias, A., Harwell, R., and Pauls, F., J. Immunol. **99**, 974 (1967).

2. Wildy, P., J. Gen. Microbio. **13**, 346 (1955).

3. Schneweis, K., Zbl. Bakter. I. Orig. **186**, 467 (1962).

4. Hamar, M., Arch. Klin. Exptl. Dermatol. **220**, 283 (1964).

5. Dawson, J., Am. J. Pathol. **9**, 1 (1933).

6. Anderson, K., Am. J. Pathol. **16**, 137 (1940).

7. Coriell, L., Blank, H., and Scott, T. McN., J. Lab. Clin. Med. **34**, 402 (1949).

8. Nii, S. and Kamahora, J., Biken's J. **6**, 205 (1963).

9. Tokumaru, T. and Scott, F. NcN., in "Diagnostic Procedures for Virus and Rickettsial Diseases," p. 388. Am. Public Health, New York, 1964.

10. Nahmias, A. and Dowdle, W. R., Progr. med. virol., in press (1968).

11. Nahmias, A., Naib, Z., Highsmith, A., and Josey, W., Pediat. Res. **1**, 209 (1967).

12. Hutfield, D., Wasley, G., and Gray, E., Brit. J. Venereal Diseases **43**, 48 (1957).

13. Jawetz, E., Coleman, V., and Merrill, E., J. Immunol. **75**, 28 (1955).

14. Schneweis, K., Z. Immunitaets forsch. **124**, 24 (1962).

15. Wheeler, C., J. Immunol. **193**, 749 (1964).

16. Taniguchi, S., Virology **30**, 333 (1966).

17. Downie, A. and Dumbell, K., J. Pathol. Bacteriol. **59**, 189 (1947).

18. Fenner, F., Virology **5**, 50 (1958).

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Studies of Adenovirus Type III Infection Treated with Methisazone and Trifluorothymidine* (32862)

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Adenovirus infection of man is common and some adenoviruses have been implicated as causative factors of cancer in animals. Some types of adenovirus produce sufficiently mild infections, however, so that volunteer studies with these agents appear relatively simple and risk free (1,2).

The testing of drugs on randomly occurring diseases is difficult, since a definite clinical diagnosis may not be possible until the disease is past. In addition, the variability of randomly occurring cases, except in very large epidemics, would require that very large numbers of people be tested with any new drug which is to be evaluated in order for such studies to be meaningful. Volunteer studies of induced infection appeared safer and more practical than the study of random cases as a method of drug evaluation, and adenovirus type III, passed only in human amnion cells, and isolated from a very mild local infection seemed an ideal agent to provide this test system.

Two drugs possess *in vitro* activity against adenovirus and seem reasonably safe for use in man. Methisazone (*N*-methylisatin-*B*-thiosemicarbazone) is of proven value in a variety of pox virus infections of man, clearly preventing smallpox in contacts and probably providing therapeutic benefit in the treatment of vaccinia gangrenosa (3–13). Recent studies of this drug in tissue culture indicate that it has some *in vitro* effect in suppressing adenovirus multiplication (14), and a broad experience with thousands of subjects provides detailed information as to its safety and toxicity (3–6). Trifluorothymidine (F_3TdR) is a potent and nontoxic antimetabolite of proven benefit in the experimental and clinical treatment of herpes simplex infection in the eye when administered locally as drops (15,16). Since it suppresses and interferes with virus DNA synthesis, and drugs of this class have been shown to have activity against adenovirus in tissue culture (14) the topical administration of trifluorothymidine also seemed of possible clinical value. It was considered unlikely that this drug could prevent systemic infection, and it seemed unlikely that it could penetrate into the conjunctiva and remain in vascularized tissues sufficiently long to prevent conjunctivitis, but it seemed possible that it might decrease the severity of the conjunctivitis, and more important, prevent the corneal opacities which are frequently associated with adenovirus infection.

Materials and Methods. One hundred and sixty male subjects at the Florida Division of Correction, Raiford, Florida, were tested for specific neutralizing antibody to adenovirus

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