

of yeasts by washed cultures of *Acetobacter* under conditions in which no bacterial growth took place.

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## GENETICS

### Frequency of Glucose-6-phosphate Dehydrogenase Deficiency, Red-Green Colour Blindness and Xg<sup>a</sup> Blood-group among Chamorros

PROGRESSIVE fatal neurological diseases, such as amyotrophic lateral sclerosis, occur with exceptionally high incidence in the indigenous population of Guam and the other Mariana Islands in the Western Pacific. Recent investigations indicate that these neurological problems are not limited to the Chamorros, the indigenous population of the Marianas<sup>1,2</sup>. Since extensive pedigree and epidemiological data revealed no single genetic defect which might reasonably account for such abnormalities, ancestral relationships among different aboriginal populations of the Western Pacific are being examined. If the ancestry of these groups can be defined in terms of several common genetic markers, then a comparison of the incidence of amyotrophic lateral sclerosis, Parkinsonism-dementia or other neurological diseases occurring within these groups might facilitate the separation of genetic and non-genetic disease-producing factors. This communication reports the results of a preliminary survey for three sex-linked traits: glucose-6-phosphate dehydrogenase (G-6-PD) deficiency, red-green colour blindness and Xg<sup>a</sup> blood-group in Chamorro males. The results are summarized in Table 1.

The sample tested were males attending the only two high schools on the Island. Compulsory education of all Chamorros ensured random representation of natives from all parts of Guam by such a sampling technique. No sibship in the sample was represented by more than one sibling.

The G-6-PD determinations were carried out by the method of Motulsky<sup>3</sup>. Of 246 students tested, the blood from one (0.4 per cent) had a decolorization time of greater than 3 h on duplicate determinations. This frequency is the same which Blumberg *et al.* found in other population isolates in Micronesia<sup>4</sup>. These results should also be compared to the frequency of G-6-PD deficiency in Carolinians from four islands in Micronesia: Angour, 9 per cent; Koror, 8 per cent; Ifalik, 6 per cent; Ulithi, 0 per cent<sup>5</sup>. There is no malaria in Guam.

Table 1. FREQUENCY OF G-6-PD DEFICIENCY, RED-GREEN COLOUR BLINDNESS AND Xg<sup>a</sup> BLOOD-GROUP IN CHAMORROS

	No. of males tested	No. positive	Percentage positive
G-6-PD deficiency	246	1	0.4
Red-green colour blindness	246	8	3.3
Xg <sup>a</sup> blood-group	109	71	65

246 students were tested for red-green colour blindness with American Optical Pseudo-isochromatic Plates. 8 (3.3 per cent) were colour-blind. About 4 per cent of Filipinos<sup>6</sup> and Japanese<sup>7</sup> are colour-blind, whereas in the United States and in Europe about 8 per cent of the male population have some type of colour-blindness.

109 Chamorro boys were tested for Xg<sup>a</sup> blood-group by the technique of Mann *et al.*<sup>8</sup>. 71 (65 per cent) were positive. This result does not differ from the frequency of the Xg<sup>a</sup> gene in Caucasians (62 per cent) and in Negroes (59 per cent).

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## VIROLOGY

### Relationship between Adenoviruses and Canine Hepatitis Virus

IT was shown by Kapsenberg<sup>1</sup> and Heller and Salenstedt<sup>2</sup> that infectious canine hepatitis virus (ICH) and adenoviruses possessed common complement fixing antigens. Heller and Salenstedt<sup>2</sup> demonstrated five precipitin lines in Ouchterlony plates in the reaction between ICH and convalescent serum from a dog infected with ICH. Three of the antigens forming these precipitin lines were common to adenovirus type 7 which itself gave five precipitin lines with human anti-adenovirus type 7 convalescent serum. The immunological relationship between the two viruses was one-sided, as anti-adenovirus type 7 serum would react in Ouchterlony plates with ICH, but anti-ICH would not react with adenovirus type 7. Recently, Darbyshire and Pereira<sup>3</sup> have demonstrated two antigens in the livers of ICH infected dogs which can be precipitated with dog anti-ICH. One of these antigens is precipitated by rabbit anti-adenovirus type 5 serum. In the experiments reported here it was found that ICH infected dog kidney cultures produced only two soluble viral antigens, one of which is closely similar to the A (group specific) antigen of adenoviruses types 5 and 7 and another which is specific for ICH.

Adenovirus type 5 and anti-adenovirus type 5, which was produced by infecting rabbits with adenovirus type 5 (H.G.P. strain) and used as the  $\gamma$ -globulin, were obtained from Dr. H. G. Pereira (National Institute for Medical Research, London, N.W.7). Anti-adenovirus type 7, which was hyperimmune serum produced in rabbits, and adenovirus type 7 were obtained from Dr. M. S. Pereira. Virus Reference Laboratory, Central Public Health Laboratory, London, N.W.9.

The adenovirus type 7 was grown on HeLa cells in Parker's Medium No. 199 containing 0.5 per cent lactalbumin hydrolysate. The HeLa cells were propagated in Parker's Medium No. 199 supplemented with 10 per cent fresh calf serum. The adenovirus type 7 used in the experiments contained  $10^{7.5}$  TCD<sub>50</sub> per ml. ICH was grown on primary dog kidney cells. These cells were grown in Hanks's balanced salt solution containing 0.5 per cent lactalbumin hydrolysate, 0.05 per cent bicarbonate and 5 per cent horse serum, and were infected with virus in Parker's Medium No. 199. The virus collected was concentrated by ultrafiltration until it contained  $10^8$  TCD<sub>50</sub> per ml. Anti-ICH was obtained both from a dog that had been naturally infected with ICH and from one

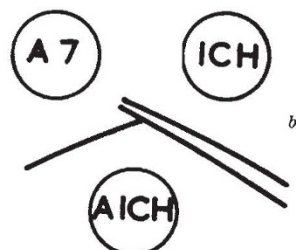
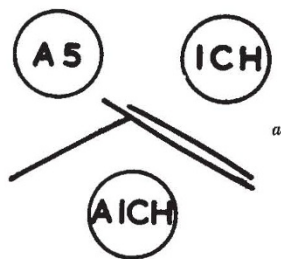


Fig. 1. Double-diffusion Ouchterlony plates. *a*, A5, adenovirus type 5; ICH, canine hepatitis virus; AICH, anti-canine hepatitis virus. *b*, A7, adenovirus type 7; ICH, canine hepatitis virus; AICH, anti-canine hepatitis virus

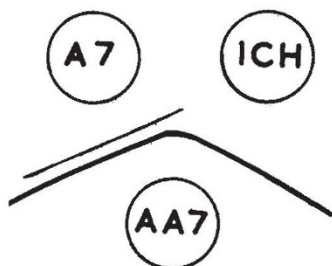


Fig. 2. Double-diffusion Ouchterlony plate. A7, adenovirus type 7; ICH, canine hepatitis virus; AA7, anti-adenovirus type 7

that had been immunized with formalin inactivated ICH. The cross-reactions between the antigens of ICH and adenoviruses types 5 and 7 with anti-ICH, and anti-adenovirus types 5 and 7, were analysed by double diffusion in agar with the technique described by Ouchterlony<sup>4</sup>. The plates were dried and stained by the method of Grabar and Burtin<sup>5</sup> after developing for 3 days at 20° C.

ICH formed two precipitin lines when reacted with anti-ICH in an Ouchterlony plate. This indicated the presence of at least two soluble antigens from ICH (Fig. 1*a* and *b*). Adenovirus type 5 (Fig. 1*a*) and type 7 (Fig. 1*b*) both formed a precipitin line with anti-ICH. This line formed a spur with the line furthest from the antigen well of ICH. The spur formation demonstrated a reaction of partial identity between the group specific antigens of ICH and adenoviruses types 5 and 7. Both the convalescent and artificially prepared dog anti-ICH sera gave the same result. Adenovirus type 7 gave two precipitin lines with its homologous antiserum, and the antigen forming the line nearest to the antiserum well gave a reaction of identity with the group specific antigen of ICH (Fig. 2). Klemperer and Pereira<sup>6</sup> showed that adenovirus type 5 gave three precipitin lines with its homologous antiserum. The line nearest to the antigen well was formed by the group-specific antigen, antigen A, and it was with this line that the line formed between ICH and anti-adenovirus type 5 formed a spur (Fig. 3).

These experiments show that ICH produces two viral antigens and not several. This is similar to other adenoviruses. Adenoviruses types 5 and 7, and ICH, all possess a common antigen, which can be precipitated in Ouchterlony plates by anti-ICH and anti-adenovirus types 5 and 7.

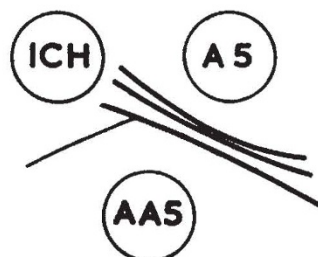


Fig. 3. Double-diffusion Ouchterlony plate. ICH, Canine hepatitis virus; A5, adenovirus type 5; AA5, anti-adenovirus type 5

The anti-adenovirus type 7 reacted equally well with adenovirus type 7 and ICH, and there was no spur formation between the precipitin lines of the common antigen. The other antisera, anti-adenovirus type 5 and anti-ICH, reacted more strongly with the homologous antigen, and the precipitin line from the latter formed a spur with that from the heterologous antigen. Thus ICH produces an antigen which is similar to, but not exactly the same as, the group specific antigen of adenoviruses and another antigen which is specific for ICH. The group specific antigens of adenovirus type 7 and ICH appear to be identical when reacted with anti-adenovirus type 7, but with anti-ICH and anti-adenovirus type 5 they are non-identical. This is probably because the anti-adenovirus type 7 was hyperimmune serum and presumably of lower specificity than the convalescent dog anti-ICH or the convalescent rabbit anti-adenovirus type 5 antiserum. These results do not agree entirely with the findings of Heller and Salenstedt<sup>2</sup>. It would seem possible that some of the antigens observed by these authors were of non-viral origin.

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### Effect of Actinomycin D and Ethylenediamine Tetraacetic Acid on the Multiplication of a Plant Virus in Etiolated Soybean Hypocotyls

THE recent demonstrations that actinomycin D which inhibits DNA dependent RNA synthesis<sup>1,2</sup> does not inhibit the multiplication of RNA containing animal<sup>2</sup> and bacterial viruses<sup>3</sup> led us to attempt to extend such observations to plant viruses.

The easiest, quickest and most economical way to introduce a chemical uniformly into plant material is to use small slices of tissue in submerged culture in shake flasks. Hypocotyls of etiolated soybean seedlings have been used previously in physiological studies<sup>4,5</sup> and were used here. *Glycine max* (Merr.) L. var. Hawkeye plants were grown at 30° C in the dark in 'Krum' moistened with water. 2.5-3 days after planting the emerging seedlings were inoculated by rubbing purified bean pod mottle virus (BPMV)<sup>6</sup> on the hypocotyls at a concentration of about 1 µg per plant. This would equal about 0.15 µg RNA of the infectious bottom component per plant. The virus was allowed to multiply for 2-3 days, depending on the experiment, and the hypocotyls were removed and sliced into sections about 2 cm long. The separately randomized virus-infected and control hypocotyl sections, usually 3 g per 10 ml. of solution, were then placed in the following basal medium: ATP-8-<sup>14</sup>C, 0.25 µc./ml.;