

Incubation of endogenous serum pyrogen with nitrofurazone did not alter its pyrogenicity.

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Adenovirus Neutralizing Antibodies in Persons on Taiwan.* (27366)

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"Serological epidemiology" used by Paul to study poliomyelitis in different areas of the world(1) has been followed by application of serological test surveys of different population groups to understand better the epidemiology of other viral diseases. Soon after discovering the adenovirus group, Huebner and co-workers studied the distribution of antibodies against 6 types in children and adults in Washington, D.C.(2). These studies showed that several of the types were very common and appeared to infect very early in life, while other types were less common and infected older children and adults. These observations have been confirmed in the United States(3-5). Recently reports have appeared from Taiwan(6) and Japan(7,8) concerning the presence of adenovirus antibodies. This study is an extension of the earlier one on Taiwan(6) to include 6-7-year-old children and more areas of the island.

Material and methods. Sera. Blood specimens were obtained from residents of 10 different counties of Taiwan, including the nearby Pescadore Islands. Recent primary school

entrants (6-7 years old) were bled as part of an islandwide study of eye infections(9). Adult sera (age 16-25) were provided by the laboratories of the Provincial Health Administration after testing for syphilis. The sera were frozen at -20°C prior to testing.

Test procedure. The "improved technic for the neutralization test"(10) was followed closely. The H. Ep. #2 tissue culture cell line (11) was substituted for HeLa cells and calf serum was used in place of horse serum. In this test the serum, virus and tissue culture cells are added to culture tubes at the same time and the test read in 48 hours (also at 72 hours). During this period the virus alone causes typical cytopathogenic effect (CPE), while specific antibody prevents these changes and allows normal growth of the H. Ep. #2 cells. Serum was heat inactivated (56°C for 30 minutes), diluted 2-fold from 1:5 to 1:40 and added in 0.1 ml amounts. Virus suspension was added in 0.2 ml amounts in a concentration in excess of that needed to cause +++ CPE (one-half $\log_{10}$ greater than the amount needed to damage 50% of the cells(10)). The H. Ep. #2 cell suspension consisted of 100,000 cells in 0.7 ml. Two tubes per serum dilution were employed in each titration.

Virus. Adenovirus prototype strains were used(10). Types 11-18 have recently been obtained from Dr. Wallace Rowe. Four strains isolated in Taiwan and called Taiwan A (#276), B (#1004), C (#6778) and D

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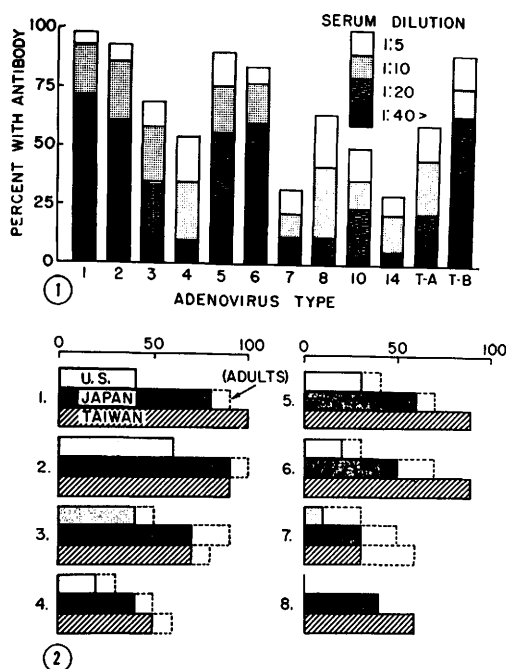


FIG. 1. Frequency of adenovirus antibody in 6-7-yr-old children on Taiwan by type. Children from 10 counties of Taiwan tested. One hundred sera (10 from each area) tested with adenovirus types 1-6 and 50 sera (5 from each area) with the other types.

FIG. 2. Comparison of percent with antibody against adenovirus types 1-8 in primary school aged children in the U. S., Japan and Taiwan. When adults had higher percent of antibody than children, it is shown by broken line extension of bar.

(#7020), which have been tentatively classified as new strains (not types 1-18), were also employed as antigens in this study. All virus types were grown in H. Ep. #2 cell cultures and virus suspensions prepared by alternate freezing and thawing 3 times followed by low speed centrifugation.

Results. Fig. 1 shows the percentage of first grade school children with antibody and the serum titer against 12 adenovirus types. Almost all of the children had antibody against types 1,2,5,6 and Taiwan B and over half of the sera reacted at a 1:20 serum dilution or greater with these types. From one-half to three-quarters of the children had antibody against types 3,4,8,10 and Taiwan A. Only against types 7 and 14 were antibodies found in less than half of the children. High titer antibody was found infrequently with types 4,8 and 14. Twenty first-grade school

children's sera from 4 areas were tested with the Taiwan C and D strains. With the C strain 90% had antibody at the 1:5 serum dilution, 75% at 1:10, 65% at 1:20 and 30% at 1:40. The pattern with the D strain was 80, 70, 50 and 10% positive at the 4 dilutions.

Although there were differences in frequency of antibody and the types prevalent in the 10 counties of Taiwan, the small numbers tested from each area preclude attaching significance to these differences.

A total of 30 adult sera from 3 counties (Taipei, Taichung and Tainan) with large urban concentrations were tested against types 1-7 and 10. For types 1,2,5 and 6 the percentage positive was similar to that found in the children and shown in Fig. 1. There was an increase in frequency of antibody with types 3,4,7 and 10 to 83, 76, 70 and 60% respectively. The frequency of antibody in these adult sera from urban counties was somewhat higher than had been found in 2 rural Taiwan counties(6). One hundred adult sera from 10 counties were tested with the Taiwan A strain as antigen and 50 sera with Taiwan B. With Taiwan A, 67% were positive and with Taiwan B, 92% had antibody.

Fig. 2 shows a comparison of the frequency of adenovirus antibody in children of primary school age in the United States and in Japan with children on Taiwan. The studies used to compile these figures include reports from Cleveland(3), Washington(2) and Kansas City(5) in the United States and Okayama (7) and Tokyo(8) in Japan. When available, children in the 5- to 10-year age group are used. An original serum dilution of 1:4 was used in all studies. The results from Cleveland were reported as 1:8 final dilution which was equivalent to the 1:4 original dilution used in the other studies. The Taiwan results with an original serum titer of 1:5 are used for comparison. For comparison of antibody in adults, one additional study from Chicago is included for the United States(4) and one for Taiwan(6). For type 8, only the Chicago study has data from the United States and only the Tokyo study from Japan. Type 7 antibodies were not studied at Wash-

ington or in Okayama. Other published antibody studies of one or several adenovirus types agree in general with the figures presented(12,13). The percentages found in each study are averaged, irrespective of number of sera tested, and the resulting percentage is rounded off to the closest 10.

Fig. 2 shows that a considerably larger percentage of both children and adults in Japan and Taiwan had antibody against each of the 8 types of adenovirus than was found in the United States. The presence of type 8 antibody in the Orient and its rarity in the United States is well established(12). Taiwan children showed more antibody, particularly with types 1,5,6 and 8 than Japanese children.

In preschool children in Japan, the percentage with antibody was considerably less against many of the types than that shown in Fig. 2(7,8). Since the children on Taiwan tested had just begun primary school and are being compared with Japanese children in age groups of 6-15 and 5-8 years, it would appear that the age of occurrence of adenovirus infections on Taiwan compared to Japan is even earlier than the figure indicates.

In Japan there was a 20% increase in percentage of persons with antibody against types 3,6 and 7 between children and adults. In the United States and Taiwan only with type 7 was there more than a 10% increase from children to adults.

The results for most adenovirus types in the individual studies within each country are quite similar despite the variation in test methods employed. One notable exception was type 4. In Japan no children in the Tokyo area were found to possess type 4 antibody while 73% of those in Okayama had antibody. Somewhat similar was the finding with adult women in Washington, D.C., where 71% had type 4 antibody while in the other studies in the United States the range in adults was 12 to 29%. In a previous study with adult sera on Taiwan, about half as much type 4 antibody was found in 2 rural counties (31%)(6) as was found in the 3 urban counties (76%) tested in this study.

Discussion. Demonstration of a higher prevalence of antibody against 8 adenovirus

types in children and adults on Taiwan and Japan than has been found in the United States suggests that these viruses spread at an increased rate in the Orient. A greater frequency of adenovirus eye infections has been recognized in the Orient. Eye infections would appear to account only in part for a higher percentage of antibody. The greater frequency of antibody in Chinese than in Japanese children would suggest that adenoviruses act similarly to certain other viruses (e.g., enteroviruses) in spreading more rapidly in more tropical countries and in those where sanitary and hygienic standards are not as highly developed.

The percentage of children with antibody against adenovirus types 4 and 8 was 40-60% for both Taiwan and Japan. This is of interest since type 4 has often caused eye infection on Taiwan(14) whereas it was not found to be a prominent cause of acute respiratory disease (ARD) in recruits(15). Presence of antibody in 40% of children may offer an explanation, on the basis of widespread immunity, for the failure of type 4 to cause ARD on Taiwan. The epidemiology of type 4 may have similarities with type 8 in the Orient. We have found type 8 apparently capable of causing respiratory disease(15).

The frequency with which persons on Taiwan possessed antibody to the 4 presumably new adenovirus strains suggests that they may be as important as the previously recognized types.

Summary. Children and adult sera from Taiwan were tested for neutralizing antibodies against a number of adenovirus types. Almost all 6-7-year-old children had antibodies against types 1,2,5 and 6. Over half of the children had antibody to types 3,4,8 and 10. Only for types 7 and 14 were less than half the children positive. Only for type 7 did adults show a considerably greater frequency of antibody than the children. Comparison of the frequency of adenovirus antibody on Taiwan with published data from Japan and the United States suggests that adenovirus infections are more common and occur at an earlier age on Taiwan. A high percentage of Chinese children showed neutralizing anti-

body against 4 apparently new types of adenovirus isolated on Taiwan.

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17 Hydroxycorticosteroids in the Stomach. (27367)

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One of the main functions of the stomach is the transport of materials from the vascular side of the gastric membrane to the lumen. The primary substances moved are water and ions. The analogy between the stomach and the kidney from this aspect is obvious and suggests that the stomach may also have an excretory function.

Recently, the presence of ketosteroids in gastric juice has been demonstrated(1). The largest fraction of adrenal steroid breakdown which appears in the urine is in the form of 17 hydroxycorticosteroid substances, specifically tetrahydrocortisol and tetrahydrocortisone(2). Not all of the steroid metabolites are excreted by way of the urine(3). It is not unlikely, therefore, that the stomach could be another avenue of steroid loss.

Results from an investigation of gastric juice 17 hydroxycorticosteroid content are presented. These include the nature of the steroid and its concentration compared with blood and urine values in the same animal in control and stimulated states.

Materials and methods. Nine unanesthetized mongrel female dogs weighing 9.5 to 17.5 kg were studied. Three of the animals (1013, 1128, and 1223) had recovered from partial hypophysectomy (anterior lobe) performed at least one month prior to this investigation. Twenty-four hours after the last feeding, urine, blood samples, and gastric juice aspirates were obtained. Histamine* (0.36 mg of base subcutaneously) was administered to insure an adequate stomach secretion. This constituted the control measurement for each animal. In 4 animals (1241, 1225, 1228, and 1208) after obtaining the 3 control body fluid samples, corticotropin** (90 units) was infused over a 3-hour period. Gastric juice stimulated by histamine was aspirated during the last hour of corticotropin administration; urine and blood samples were obtained at the end of infusion. In the remainder of the animals a week was allowed to

* Histamine phosphate, The Vitarine Co., Inc.

**Corticotropin, Parke, Davis and Co.