AN "OUTBREAK" OF JUVENILE DIABETES MELLITUS: CONSIDERATION OF A VIRAL ETIOLOGY

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Huff, J. C., J. C. Hierholzer (Respiratory Virology Section, Center for Disease Control, Atlanta, Ga. 30333) and W. A. Farris. An "outbreak" of juvenile diabetes mellitus: consideration of a viral etiology. Am J Epidemiol 100:277-287, 1974.—Nine of twelve cases of juvenile diabetes mellitus, representing an unusual geographic and temporal cluster, were investigated for evidence that a specific viral infection might be etiologically related to their occurrence. Eight diabetics had experienced recent "viral-like" illnesses, predominantly respiratory, but these illnesses bore no uniform temporal relation to their onsets of diabetes. Diabetics demonstrated no serologic evidence of a recent viral illness common to all. Elevated titers to only one virus, coxsackie B-3, were more prevalent in diabetics than in controls (33% vs. 6%), but geometric mean titers of diabetics to a panel of 26 common viral antigens were similar to those of controls. These data neither support nor negate the hypothesis that infection with a specific virus precipitates juvenile diabetes mellitus.

diabetes mellitus, juvenile; respiratory tract infections

INTRODUCTION

Recent experimental and epidemiologic data have generated a growing interest in the possibility that viral infections may be involved in the pathogenesis of diabetes mellitus. Scattered case reports have suggested that human diabetes may follow such viral diseases as mumps (1-3) and congenital rubella (4, 5). Craighead and co-workers (6, 7) have provided experimental evidence that mice infected with a strain of encephalomyocarditis (EMC) virus may develop damage of the β-cells of the pancreatic islets and a resultant "diabetic-like" state. Furthermore, Gamble and co-workers (8) have reported that the Coxsackie B viruses, picornaviruses similar to EMC virus, may be associated with human diabetes. Gamble's data included higher prevalence of antibody to Coxsackie B viruses in individuals with juvenile diabetes of recent onset than in controls or long-term diabetics.

In early 1972, a group of pediatricians in Pinellas County, Florida, noted a large number of new cases of juvenile diabetes. This report describes the results of an epidemiologic and laboratory investigation of these cases.

BACKGROUND

Pinellas County lies along the Gulf Coast of Florida just north of the mouth of Tampa Bay. The flat lowlands of the
county are covered by contiguous urban and suburban areas, where 96 per cent of the county's half million residents live (1970 census). The largest cities are St. Petersburg (population 216,232) and Clearwater (population 52,074). Tourism is the main industry in the county. Socioeconomically, most residents are middle class. Because the county is situated on a peninsula and excellent medical care is available in the county, Pinellas County residents generally seek medical care within Pinellas County.

Methods

Epidemiologic. Medical records at the seven hospitals in Pinellas County where children are usually admitted were reviewed. Records of individuals up to age 14 who were admitted between January 1968 and March 1972 as cases of diabetes mellitus were examined. A new "case" of juvenile diabetes was defined as an illness 1) diagnosed as diabetes in a child not previously known to have the disease, 2) characterized by glycosuria and hyperglycemia (a blood glucose of a least 200 mg/100 ml), and 3) treated with insulin at the time of hospital discharge. The "incidence" of juvenile diabetes mellitus in the pediatric age group was estimated by plotting all new cases by month of hospitalization. No effort was made to identify new cases in older age groups or new cases in persons not hospitalized.

Investigation of cases. Diabetic children hospitalized early in 1972 were investigated in detail. With the consent of the child's parents, a detailed medical history was taken from each family member. Each family was questioned concerning associations between them and the families of other diabetic children.

Review of public health records. Physicians' reports of viral diseases to the county health department were examined for the time period November 1971 through March 1972. Records of positive viral cultures and positive viral serologic tests from Pinellas County were also reviewed for the same period.

Laboratory. In mid-March 1972, rectal and throat swabs were taken from newly diabetic children and other members of their households. Specimens placed in tryptose phosphate broth with 0.5 per cent gelatin were frozen in Dry Ice. In the laboratory, specimens were processed for inoculation onto two strains of human diploid lung fibroblast (RU-1 and WI-38), one line of human epithelium (HEp-2), and primary rhesus monkey kidney cell (MKC) cultures, as previously described (9). The specimens were also inoculated into 10-day embryonated eggs (10). Each specimen was passaged at least three times for a total incubation period of four weeks.

Single (convalescent) serum specimens were collected by venipuncture in mid-March from newly diabetic children, members of their households, and a control group of children from Pinellas County with no history of diabetes. Non-diabetic controls were matched with the newly diabetic children for age and sex. Serum samples were stored at −30°C until tested.

The sera were tested for antibodies to the prototype strains of a large number of respiratory viruses by complement-fixation (CF), hemagglutination-inhibition (HI), indirect hemagglutination (IHA), or serum neutralization (SN) tests, as appropriate for each particular virus. The sera were heat-inactivated (56°C, 30 minutes) for all serologic tests. Antibodies to influenza A and B, parainfluenza 1, 2, and 3, the "soluble" and "viral" antigens of mumps virus, the group-specific hexon antigen of adenovirus, respiratory syncytial virus (RSV), Mycoplasma pneumoniae, herpes simplex 1, and cytomegalovirus (CMV) were assayed by the standardized CF test with overnight fixation of 5 units of complement (11). Antibodies to influenza C, parainfluenza 4A and 4B, and coronavirus OC 43 were assayed by the standardized HI
test with 0.01 M phosphate-buffered saline diluent and spectrophotometrically standardized 0.4 per cent mammalian or 0.5 per cent avian red blood cells (12, 13). Hemagglutinating antigens of parainfluenza 4A and 4B were prepared for use in the HI test as described by Killgore and Dowdle (14). Antibodies to herpes simplex 1 and CMV were determined by the IHA procedures of Bernstein and Stewart (15, 16) and coronavirus 229E antibodies were measured by a similar IHA test (17). Neutralizing antibodies to the six Coxsackie B viruses were assayed by SN tests in MKC tissue culture (9). Neutralizing antibody titers to EMC virus (r+ variant), assayed by plaque neutralization tests in L-cell tissue culture, were generously provided by Dr. John E. Craighead, University of Vermont.

RESULTS

Epidemiologic. Thirty-five cases of juvenile diabetes diagnosed in children between January 1968 and March 1972 are presented by month of hospital admission in figure 1. In the 48 months prior to January 1972, 23 new diabetics were hospitalized, an average "incidence" of one new case every 2 months. The maximum number in a consecutive 2-month period was four in 1971. In February and March 1972, 12 cases occurred. Based on an "expected" rate of four new cases or fewer in a 2-month period, the occurrence of 12 cases was a very rare event (p < 0.001).

Investigation of cases. Nine of the 12 patients hospitalized in February and March and their families agreed to participate in the detailed case investigations. The nine children with newly diagnosed diabetes included five males and four females, ages 4 to 13 (mean age 8.3). Although all nine had been hospitalized at either of two hospitals in central Pinellas County, their places of residence were scattered throughout the county. No diabetic child and family was acquainted with any other child and family, and none were associated in educational, religious, occupational, or recreational activities. No medication or toxic exposure was common to the nine.

A summary of data from case investigations is shown in table 1. Onset of diabetic symptoms preceded hospitalization by 1 to 7 weeks (mean 3 weeks). Two factors com...
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**Table 1**

*Summary of epidemiologic data for nine new cases of juvenile diabetes*

| Patient | Age | Sex | Date of initial symptoms* | Date of hospitalization | Family history of diabetes | Viral illness in 3 months preceding hospitalization |
|---------|-----|-----|---------------------------|-------------------------|---------------------------|--------------------------------------------------|
| 1       | 6   | M   | 1/16*                     | 2/1                     | Maternal great-aunt       | 12/29 URI,† otitis media                          |
| 2       | 4   | F   | 1/29                      | 2/11                    | Paternal aunt, paternal great-grandmother | 12/25 "fluenza-like" illness                     |
| 3       | 11  | F   | 1/11                      | 2/11                    | Maternal grandfather      | 2/7 Pharyngitis                                   |
| 4       | 9   | F   | 2/7                       | 2/14                    | Maternal and paternal cousins | 1/15 Gastroenteritis                              |
| 5       | 6   | M   | 12/25                     | 2/16                    | Paternal grandmother      | 12/25 URI                                        |
| 6       | 10  | M   | 2/8                       | 2/22                    | None                      | 2/18 URI                                         |
| 7       | 12  | M   | 2/14                      | 3/4                     | Maternal grandmother     | 3/4 ? URI, otitis media                           |
| 8       | 13  | F   | 2/18                      | 3/9                     | Unknown (adopted)         | None ? URI, otitis media                          |
| 9       | 4   | M   | 2/24                      | 3/9                     | Maternal grandmother     | 2/15 "fluenza-like" illness                       |

* December 1971 to March 1972.
† URI, upper respiratory infection.

Common to the majority of the diabetics were a family history of diabetes and an illness in the 3 months preceding hospitalization considered by the patient's physician to be of possible viral etiology. Seven of the nine families knew of relatives with diabetes, but further information concerning the diabetic relatives was unavailable. Two of the diabetic relatives were reported to have had onset of disease in young adulthood; the others apparently were adult onset, insulin-independent diabetics.

Eight of the diabetics had histories of a preceding illness of possible viral etiology. The etiology of the illnesses was not determined. Seven of the illnesses were respiratory, ranging from simple upper respiratory to febrile lower respiratory illnesses. In only four of the eight cases, however, did the illness precede the onset of diabetic symptoms.

**Review of public health records.** In a search for viral illnesses prevalent in Pinellas County prior to the cluster of cases of juvenile diabetes, reports to the county health department of viral diseases were reviewed. An outbreak of measles occurred in Clearwater in December 1971, and influenza was recognized throughout the county in January 1972. Outbreaks of other reportable viral illnesses, including mumps and rubella, had not occurred. Influenza was confirmed by isolation of A/Hong Kong/68 (H3N2) strains and by positive serologic studies. Positive serologic tests were also obtained for parainfluenza and adenovirus. ECHO-11 and Coxsackie A-12 were isolated from individual patients, and in addition, Coxsackie B-2 and ECHO-8 were isolated from sewage effluents. With the exception of influenza, however, none of these agents was known to be associated with significant morbidity.

**Laboratory.** Cultures of rectal and throat swabs taken from eight newly diabetic children and nine of their family members yielded no virus isolates. At the time of attempted isolation in mid-March, there was no respiratory illness in any of the individuals tested; isolation attempts were therefore made only on the chance that there might still be virus excretion from a preceding illness.

Single serum samples were taken from
nine diabetic patients within 6 weeks after hospitalization and within 12 weeks after onset of symptoms. Unfortunately, earlier sera for either the respiratory or diabetes illnesses were not available. Single serum specimens were also collected from 21 family members (contacts) and from 36 matched and 20 unmatched control children at this time (table 2). All of these sera were tested for antibody to 15 common respiratory viruses and to M. pneumoniae. The antibody levels of the case, contact, and matched control groups were tabulated both as numbers of persons with "significantly high" antibody titers (table 3) and as geometric mean titers (GMT) (table 4).

"Significant" antibody titers were defined as those levels of antibody (serum endpoint dilutions) which we considered, through diagnostic experience with the test procedures used, to be suggestive of recent infection. The prevalence of significantly high titers of antibody to 15 viruses and to M. pneumoniae were quite similar in diabetics and controls for all agents except Coxsackie B-3 (table 3). Three of nine diabetics (33 per cent) had significant titers ($\geq 1:80$) to this virus, whereas only 2 of 36 matched, non-diabetic controls (6 per cent) had similar titers. This difference

| Table 2 |
|---------|
| **Age and sex characteristics of study population** |
| Sex | Age |
| Total | Male | Female | Median | Range |
| Diabetics | 9 | 5 | 4 | 9 | 4-13 |
| Family contacts | 21 | 10 | 11 | 26 | 3-46 |
| Controls, matched | 36 | 20 | 16 | 9* | 4-13* |
| Controls, total | 56 | 23 | 33 | 8 | 4-13 |

* Diabetics and controls matched for age (within 1 year), sex, locality, and time of serum collection.

| Table 3 |
|---------|
| **Prevalence of elevated antibody titers in diabetic patients, family members, and matched controls** |
| Virus | Test | Elevated (significant) titer* | % with elevated titer |
| | | | 9 patients | 21 family members | 36 matched controls† |
| Influenza A | CF | 1:32 | 11 | 14 | 19 |
| Influenza B | CF | 1:32 | 0 | 0 | 0 |
| Parainfluenza 1 | CF | 1:32 | 0 | 0 | 0 |
| Parainfluenza 2 | CF | 1:32 | 0 | 0 | 0 |
| Parainfluenza 3 | CF | 1:32 | 11 | 0 | 8 |
| Mumps (soluble) | CF | 1:16 | 0 | 5 | 5 |
| Mumps (viral) | CF | 1:32 | 0 | 0 | 8 |
| Adenovirus | CF | 1:16 | 0 | 14 | 6 |
| RSV | CF | 1:32 | 11 | 14 | 14 |
| Herpes 1 | CF | 1:32 | 0 | 10 | 7 |
| M. pneumoniae | CF | 1:32 | 0 | 0 | 16 |
| Coxsackie B-1 | SN | 1:80 | 0 | 10 | 4 |
| Coxsackie B-2 | SN | 1:80 | 0 | 14 | 13 |
| Coxsackie B-3 | SN | 1:80 | 33 | 10 | 6 |
| Coxsackie B-4 | SN | 1:80 | 11 | 38 | 17 |
| Coxsackie B-5 | SN | 1:80 | 0 | 0 | 4 |
| Coxsackie B-6 | SN | 1:80 | 0 | 0 | 0 |

* A titer of this level or higher is considered elevated and therefore may be an indication of recent infection (within approximately 5 months). See text for complete definition.

† The frequency of elevated titers in the diabetic and control groups was not significantly different at the .05 level by Fisher's Exact Test for any virus except Coxsackie B-3 ($P = .047$).
was of borderline statistical significance ($p = .047$) by Fisher's Exact Test.

Family members of the diabetics were a diverse group of 12 adults and nine children and therefore were not compared with the control group except in general terms. Elevated titers to adenovirus, herpesvirus, and Coxsackie virus B-4 were most prevalent among family members.

The serologic data expressed as GMT’s to 17 viral antigens did not reveal any single agent to which diabetics possessed a significantly higher mean titer (table 4). The GMT of diabetics to Coxsackie B-3 was almost two dilutions higher than controls, but statistically, the difference was not significant by the $t$-test ($p = .083$). The GMT’s of family members, despite their age and sex diversity, were similar to those of diabetics and controls for all agents except Coxsackie B-4; the GMT of family members to Coxsackie B-4 was approximately one dilution greater than the GMT of diabetics and controls.

Results of GMT’s to nine additional viral antigens for all diabetics, selected family members, and an unmatched group of non-diabetic children are shown in table 5. As in table 4, antibody levels to most viruses were typical of pediatric groups. The GMT to CMV, which was somewhat high in diabetics, was nonetheless quite similar to that of the non-diabetic group and was not in an unusual range. The CF and IHA tests for herpes and for CMV measure different aspects of infection, and neither test indicated that these viruses were associated with the respiratory illnesses of the diabetics before they were hospitalized.

Antibody levels to the six Coxsackie B viruses are presented for each of the nine cases, together with the serologic identification of other possible recent respiratory illnesses, to provide a summary of the most salient serologic data (table 6). The pattern seen in this summary is one of total randomness. Virtually each case had serologic

| Virus          | Test | 9 patients | 21 family members | 36 controls (matched only) | 56 controls (total) |
|----------------|------|------------|-------------------|---------------------------|---------------------|
| Influenza A    | CF   | 6.86       | 8.84              | 6.92                      | 7.38                |
| Influenza B    | CF   | 4.00       | 4.56              | 4.16                      | 4.16                |
| Parainfluenza 1| CF   | 4.32       | 4.72              | 4.58                      | 4.88                |
| Parainfluenza 2| CF   | 5.44       | 5.21              | 5.14                      | 5.32                |
| Parainfluenza 3| CF   | 8.64       | 5.75              | 8.00                      | 7.90                |
| Mumps (soluble)| CF   | 5.88       | 4.93              | 5.83                      | 5.80                |
| Mumps (viral)  | CF   | 5.88       | 4.56              | 8.32                      | 8.31                |
| Adenovirus     | CF   | 5.04       | 5.94              | 5.99                      | 6.09                |
| RSV            | CF   | 12.70      | 9.76              | 9.33                      | 9.83                |
| Herpes 1       | CF   | 4.00       | 7.74              | 5.24                      | 5.52                |
| M. pneumoniae  | CF   | 4.67       | 5.75              | 8.64                      | 8.83                |
| Coxsackie B-1  | SN   | 5.00       | 7.70              | 5.52                      | 5.96                |
| Coxsackie B-2  | SN   | 6.15       | 8.94              | 13.53                     | 13.75               |
| Coxsackie B-3  | SN   | 23.04      | 9.61              | 8.70                      | 8.92                |
| Coxsackie B-4  | SN   | 14.70      | 25.05             | 10.40                     | 11.56               |
| Coxsackie B-5  | SN   | 6.30       | 5.63              | 5.79                      | 5.74                |
| Coxsackie B-6  | SN   | 5.00       | 5.61              | 5.44                      | 5.14                |

* A geometric mean titer (GMT) of 4.00 $= <1:8$ for CF tests and of 5.00 $= <1:10$ for SN tests. GMT’s are listed as dilution factor of endpoint serum dilutions.

† The GMT in the diabetic and matched control groups was not significantly different ($P > .05$) for any virus by the $t$-test.
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TABLE 5

| Virus               | Test | Significant titer | Prevalence of significant titers in 9 diabetics (%) | Geometric mean titer* |
|---------------------|------|-------------------|-----------------------------------------------------|-----------------------|
| Influenza C         | HI   | 1:128             | 0                                                   | 13.72                 |
| Parainfluenza 4A    | HI   | 1:128             | 0                                                   | 13.39                 |
| Parainfluenza 4B    | HI   | 1:128             | 11                                                  | 9.69                  |
| Herpes 1            | IHA  | 1:2048            | 0                                                   | 6.35                  |
| Cytomegalovirus     | CF   | 1:256             | 22                                                  | 46.31                 |
| Coronavirus OC43    | HI   | 1:2048            | 11                                                  | 21.82                 |
| Coronavirus 229E    | IHA  | 1:64              | 11                                                  | 9.83                  |
| Encephalomyocarditis| PN‡  | 1:4               | 0                                                   | 1.00†                 |

* GMT of 4.00 = <1:8. GMT's are listed as dilution factor of endpoint serum dilution.
† Not done.
‡ GMT of 1.00 = <1:2.
§ PN = plaque neutralization.

TABLE 6

Summary of Coxsackie B antibody titer and significant titers to other viruses for 9 new cases of juvenile diabetes mellitus

| Case | B-1 | B-2 | B-3† | B-4† | B-5 | B-6 | Significant antibody to other viruses |
|------|-----|-----|------|------|-----|-----|---------------------------------------|
| 1    | 0‡  | 0   | 160  | 0    | 0   | 0   | None                                  |
| 2    | 0   | 10  | 0    | 40   | 0   | 0   | CMV                                   |
| 3    | 0   | 0   | 20   | 160  | 0   | 0   | None                                  |
| 4    | 0   | 0   | 0    | 0    | 10  | 0   | RSV, parainfluenza 4B                 |
| 5    | 0   | 0   | 40   | 0    | 0   | 0   | Coronavirus 229E                      |
| 6    | 0   | 0   | 160  | 40   | 0   | 0   | CMV                                   |
| 7    | 0   | 20  | 80   | 0    | 0   | 0   | Influenza A                           |
| 8    | 0   | 0   | 20   | 40   | 20  | 0   | Parainfluenza 3                       |
| 9    | 0   | 0   | 0    | 0    | 0   | 0   | None                                  |

* Serum neutralization (SN) titers listed as dilution factor of endpoint serum dilutions.
† Among the 56 controls, 37 had SN titers to B-3 of <1:10, 9 had titers of 1:10-1:20, 7 had 1:40-1:80, and 3 were ≥1:160; 35 had SN titers to B-4 of <1:10, 5 had 1:10-1:20, 12 had 1:40-1:80, and 4 were ≥1:160.
‡ 0 = <1:10.

Evidence of infection with a different virus in the preceding four to six months. It is therefore apparent that no one of the Coxsackie B viruses nor any one of the 23 viruses tested for could account for this "outbreak" of diabetes.

DISCUSSION

Heredity has been accepted as the major determinant of the occurrence of diabetes mellitus, but the exact mode of inheritance has not yet been clarified (18). Some investigators have concluded that both genetic and environmental determinants are involved (19). Among the characteristics suggestive of environmental determinants is a seasonality in the onset of juvenile diabetes. Both a 1926 study of patients from the north central United States (20) and a 1969 analysis of English diabetics (21) described a nadir in the incidence in late spring and early summer and a broad...
peak between late summer and early spring. Data collected in Pinellas County also hinted of a seasonal pattern: 11 of the 23 cases identified from 1968 through 1971 (figure 1) were hospitalized in the months January-April, approximately the time of the 1972 peak. Many viral respiratory infections have a similar seasonal pattern, and that characteristic has suggested a possible cause of the seasonality of juvenile diabetes.

Although case reports have indicated that a diabetic state may follow such diseases as mumps and congenital rubella (1-5), these reports have remained incidental observations. There is, at present, no firm evidence that one specific viral illness plays a significant part in the pathogenesis of human diabetes mellitus.

The most convincing data suggesting a viral etiology of diabetes come from the experiments carried out by Craighead and co-workers using a mouse model and the M strain of EMC virus (6, 7). In mice, this strain of virus caused necrosis of the $\beta$-cells of the islets, release of insulin, and, in some cases, a "diabetic-like state" characterized by hyperglycemia. Similar findings have recently been reported by other investigators (22, 23). Genetic factors also seem to be determinants in this model of "virus-induced" diabetes; only certain strains of mice, when infected, will develop the lesions suggestive of diabetes (24).

The fact that EMC virus is not a common human pathogen appears to preclude the possibility that EMC virus itself is involved in human diabetes. One study conducted by Craighead et al. showed a 20 per cent incidence of EMC antibody in a Panamanian population (25), and a later survey of a random population of hospitalized children in the New England states revealed a 15 per cent incidence of EMC antibody (26). Indeed, in the present study, only 10 per cent of the persons tested had measurable levels of EMC antibody (and none of these were diabetic children).

Attention has been focused therefore upon a similar group of picornaviruses, the Coxsackie B viruses, which are extremely frequent human pathogens. The Coxsackie B viruses, like EMC, may cause pancreatitis (27) and even diabetes (28) in experimental mice, and, in addition, they may attack the human pancreas in certain infant infections (29). Although there are no published case reports of diabetes in humans following proven Coxsackie B infections, the epidemiologic and serologic data of Gamble and co-workers (8) aroused considerable interest in a possible relation between these viruses and human diabetes. Gamble et al. reported that insulin-dependent diabetics within 3 months of onset had higher serum neutralization titers to Coxsackie B viruses, especially B-4, than did non-diabetic controls or longstanding diabetics (8). Controls in this study, however, were not closely matched for age, sex, residence, or time of collection of serum. Also, in their comparison of diabetics with controls, low complement-fixation titers ($\geq 1:5$) to Coxsackie B viruses were considered positive. Our experience with this test suggests that many sera at the 1:5 dilution fix complement with crude Coxsackie B antigens nonspecifically; hence, 1:5 "titers" might not be genuine indicators of past infection.

In a separate study, Gamble and Taylor showed a statistical correlation between the annual number of isolates of Coxsackie B-4 virus and the number of new cases of juvenile diabetes (21). The correlation was not seen for the other Coxsackie B viruses. However, attempts to show a temporal correlation between diabetes and other viral infections common during the same season were not made. Thus this temporal relationship remains open to question.

If diabetes mellitus is related etiologically to a specific viral illness, one might expect to see cases clustered in time following an outbreak of that viral illness in a population. One such study following a Coxsackie B-3 and B-4 mixed outbreak of
upper respiratory infection has recently been completed; no cases of diabetes were found among 109 children four years after the outbreak (30). Our purpose in the present investigation was to determine that the 12 cases of diabetes in Pinellas County represented an unusually high incidence and to look retrospectively for evidence that a single viral agent may have been associated with the cluster.

Previous reports suggesting a relationship between diabetes and viral infections have dealt primarily with the juvenile form of the disease. We, likewise, confined our investigation to cases of juvenile diabetes, specifically in the pediatric population.

For a number of reasons, we feel that our evidence supporting an unusual incidence of juvenile diabetes in early 1972 is valid. Firstly, our definition of a new case of juvenile diabetes was quite strict, and we included in our analysis only new cases in which there was little doubt of the diagnosis, cases which in a modern medical community would be hospitalized. Secondly, because the county where the investigation took place was somewhat isolated geographically and excellent medical facilities were available within the county, we feel it unlikely that cases would have been hospitalized elsewhere. Thirdly, our case finding techniques, viz., review of medical records, should have been equally sensitive throughout the 51-month period investigated. Finally, rather than comparing the incidence during early 1972 statistically with the “expected” incidence over the 4-year period, we compared it with the previous maximum incidence. Since the maximum occurred in 1971 and the Pinellas County population has been stable, growth in the pediatric population could not likely explain the peak of cases observed in 1972.

The detailed investigation of nine of the 12 cases which comprised the “outbreak” in early 1972 revealed no geographic clustering of cases within the county, no personal association between the families involved, and no single exposure common to the cases. Although seven diabetics had family histories of diabetes, there was no history of diabetes in their immediate families, and most of the relatives with diabetes apparently had adult onset disease. None of the diabetics had experienced a recent viral illness, such as measles or mumps, whose etiology could be identified. Furthermore, none had experienced any abdominal symptoms characteristic of pancreatitis. Illnesses of possible viral etiology were reported by eight of the diabetics. The illnesses, in general, were similar to common winter respiratory illnesses. But because these infections preceded symptoms of diabetes in only four cases, we could not relate these illnesses to the onset of diabetes.

The lack of viral isolates from the diabetic children and their family members was not unexpected; samples were taken weeks after the recent “viral-like” illnesses and after the onsets of diabetes, at a time when all individuals tested had recovered from their respiratory illnesses. It was therefore necessary to rely upon serologic testing of single “convalescent” serum specimens in a search for evidence of recent viral infection. Results on sera from diabetic children revealed no elevated viral antibody titers common to all. High titers in individual patients to a number of different viruses were observed: influenza A, parainfluenza 3, respiratory syncytial virus, Coxsackie B-3, Coxsackie B-4, parainfluenza 4B, cytomegalovirus, and coronavirus 229-E. Eight of the nine diabetics had at least one high titer suggestive of a recent viral infection. Whether these had been clinical infections or inapparent infections could not be determined. There was no evidence of recent mumps or EMC infection in any of the diabetics.

In comparison with non-diabetic controls, matched closely for time of serum collection, residence, age and sex, antibody to only one virus appeared more prevalent in diabetics. Three of nine diabetics had
high titers to Coxsackie B-3. Although the prevalence of high titers was significantly greater in the diabetics, the GMT of the group was not significantly different from matched controls. Family members of diabetics possessed high titers to a related virus, Coxsackie B-4. Since antibody to some of the Coxsackie B viruses can remain elevated for years following infection, the significance of the elevated B-3 and B-4 SN titers observed in our study is much more questionable than antibody levels to the other viruses. Perhaps Coxsackie B infections were related to some of the cases of diabetes, but we have no evidence that Coxsackie B viruses were responsible for the “outbreak” of diabetes. Furthermore, the cases of diabetes in this investigation occurred six months after the usual late summer-early fall peak of Coxsackie B infections (9).

Temporally, the cluster of cases of diabetes followed closely a community-wide influenza outbreak and the usual season of winter respiratory viral infections. A cluster of cases of diabetic ketoacidosis during an influenza epidemic in the winter of 1969–1970 has been previously reported (31). Six of 29 cases described in this report (31) were in individuals previously unknown to be diabetics. Despite the temporal association in our study, we were able to demonstrate an elevated titer to influenza in only one diabetic and in only three of the family members.

Our results, therefore, do not implicate any particular virus in this “outbreak” of diabetes. If viruses are indeed etiologically involved, the data at most suggest an effect of multiple viruses rather than any single entity upon genetically predisposed individuals.

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