(70°, 31°), respectively. The angle between the path region A–42SEV and the direction of σ_3 is 38°, and that between the path region A–58K and the direction of σ_3 is 20°. Thus, if cracks formed in response to the tectonic stress field, the experiment reported here was appropriate for detecting their effect on V_P.

As a further check of the possibility that the ray paths considered here might not have been suitable for detecting precursive changes in seismic velocities, V_P for paths from region A to geophones 66H and 68H were measured (in Fig. 1 geophone 68H would plot slightly north of the upper left corner); both paths are also within the source dimension of the tremor of 27 March. Between 8 February and 30 March, V_P for the path to 66H was 6.04 km/sec, within 4 percent, and V_P for the path to 68H was 6.00 km/sec, within 2 percent. The scatter in V_P about the mean values for these two paths appeared random and did not suggest any precursive changes (*14*).

In most respects tremors induced by mining at depth appear to be identical to natural crustal earthquakes (*7, 15*), so it is somewhat disturbing to find that a prediction scheme reported for at least a certain class of earthquakes (*16*) is not of any use for mine tremors. There are, however, some differences between the situation of the event reported here and those of the three regions where anomalies in V_P/V_S have been documented. First, all shocks for which precursive changes in V_P/V_S have been reported have had focal mechanisms of the thrust-fault type. At ERPM the predominant mode of failure is normal faulting (*17*). Initial motions of *P* waves for the event of 27 March are appropriate for normal faulting.

Second, strain rates associated with the seismicity at ERPM are about 10,000 times greater than geological strain rates. Typical strain rates in the seismic regions of the mine are 10^{-5} day^{-1}, whereas normal geological rates are 10^{-6} year^{-1} or less. At present, the effect of strain rate on precursive changes in V_P/V_S is not known.

Third, the rock at ERPM is largely devoid of groundwater at depths greater than 1 km; there are a few fissures in the mine where groundwater percolates deeper, but these are rare. Explanations that have been presented for the precursive changes in V_P/V_S are based on the effect of dilatancy in the presence of groundwater; V_P is reduced at the onset of dilatancy and then resumes its normal value before the earthquake when groundwater flows into the hypocentral region to fill the voids. Although there is no appreciable groundwater at ERPM, one might expect to observe a reduction in V_P due to large-scale dilatancy.

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8. The values of V_P/V_S in Fig. 2C are considerably lower than the normal values of 1.75 found in New York State and southern California. The reason for these unusually low values is that Poisson's ratio, γ, for the quartzite at ERPM is very low. Laboratory measurements of γ for quartzite similar to that along the ray paths to 42SEV and 58K typically give a value of 0.16 [E. R. Leeman, *J. S. Afr. Inst. Min. Met.* **65**, 254 (1964); in *Rock Mechanics and Strata Control in Mines* (South African Institute of Mining and Metallurgy, Johannesburg, 1965)]. Values of γ inferred from V_S/V_P for ray paths to 42SEV and 58K are 0.20 and 0.12, respectively.
9. Seismic locations are calculated primarily from *P*-wave arrival times at the four geophones closest to a particular hypocenter. Arrival times at the further geophones, including 42SEV and 58K, are used mostly to check the precision of a location. The distances to the four geophones nearest to region A (Fig. 1) are so small that changes in compressional velocity of 10 percent or less would have very little effect on the seismic locations. Thus, the hypocentral coordinates in region A are, to a large extent, independent of seismic velocities along the ray paths region A–42SEV or region A–58K.
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Reoviruslike Agent in Stools: Association with Infantile Diarrhea and Development of Serologic Tests

Abstract. *Reoviruslike particles were visualized by electron microscopy in stool filtrates prepared from stools of infants and young children with severe acute gastroenteritis. Patients who had such particles in their stools and whose paired acute and convalescent serums were tested developed an antibody response to the reoviruslike agent, which was measured by immune electron microscopy and by complement fixation. The reoviruslike agent was antigenically related to the epizootic diarrhea of infant mice virus and the Nebraska calf diarrhea virus.*

Recent studies by Bishop *et al.* (*1*) in Australia, Flewett *et al.* (*2*) in England, and Bortolussi *et al.* (*3*) in Canada have suggested that an orbiviruslike or a reoviruslike particle might be an important etiologic agent of acute nonbacterial gastroenteritis of infants and young children. The group of investigators in Australia demonstrated the presence of the agent by electron microscopy in epithelial cells of duodenal mucosa, and all three groups observed it by electron microscopy in fecal extracts. In addition, the investigators in Canada also found the agent in duodenal fluids. Utilizing the technique of immune electron microscopy (IEM) in which convalescent

serum was employed as the source of antibody in order to enable the recognition of a viral agent, we previously described the visualization of a parvoviruslike agent in feces of individuals infected with a filtrate derived from a community outbreak of acute nonbacterial gastroenteritis in Norwalk, Ohio; we were also able to demonstrate serologic evidence of infection with this agent by IEM (*4, 5*). In addition, with this same technique, it was possible to visualize another parvoviruslike agent in stools from patients with hepatitis A and to demonstrate serologic evidence of infection with it (*6*). We recently expanded our gastroenteritis studies, which had been limited to outbreaks in schools, institutions, the military, and families, to include infants and young children admitted to the Children's Hospital of the District of Columbia with acute gastroenteritis in an attempt to elucidate by IEM and other techniques as well the etiologic agent or agents of this disease in this young age group. Prior to studies by Bishop *et al.*, Flewett *et al.*, and Bortolussi *et al.*, attempts to find such viral agents in this age group had been almost completely unsuccessful (*7*).

Stool specimens were obtained during the first 4 days of illness. After appropriate tests for pathogenic bacteria were carried out, specimens which did not yield salmonella or shigella were processed to yield 2 percent (approximately) stool filtrates by a method similar to that described (*4, 8*). Serum was obtained during the acute phase of illness and again approximately 3 to 6 weeks later (paired serums). The stool filtrates were examined by IEM, with the convalescent serum from a patient as the source of specific antibody. We took this approach in order to ascertain whether virus particles, if present, would be easily recognizable

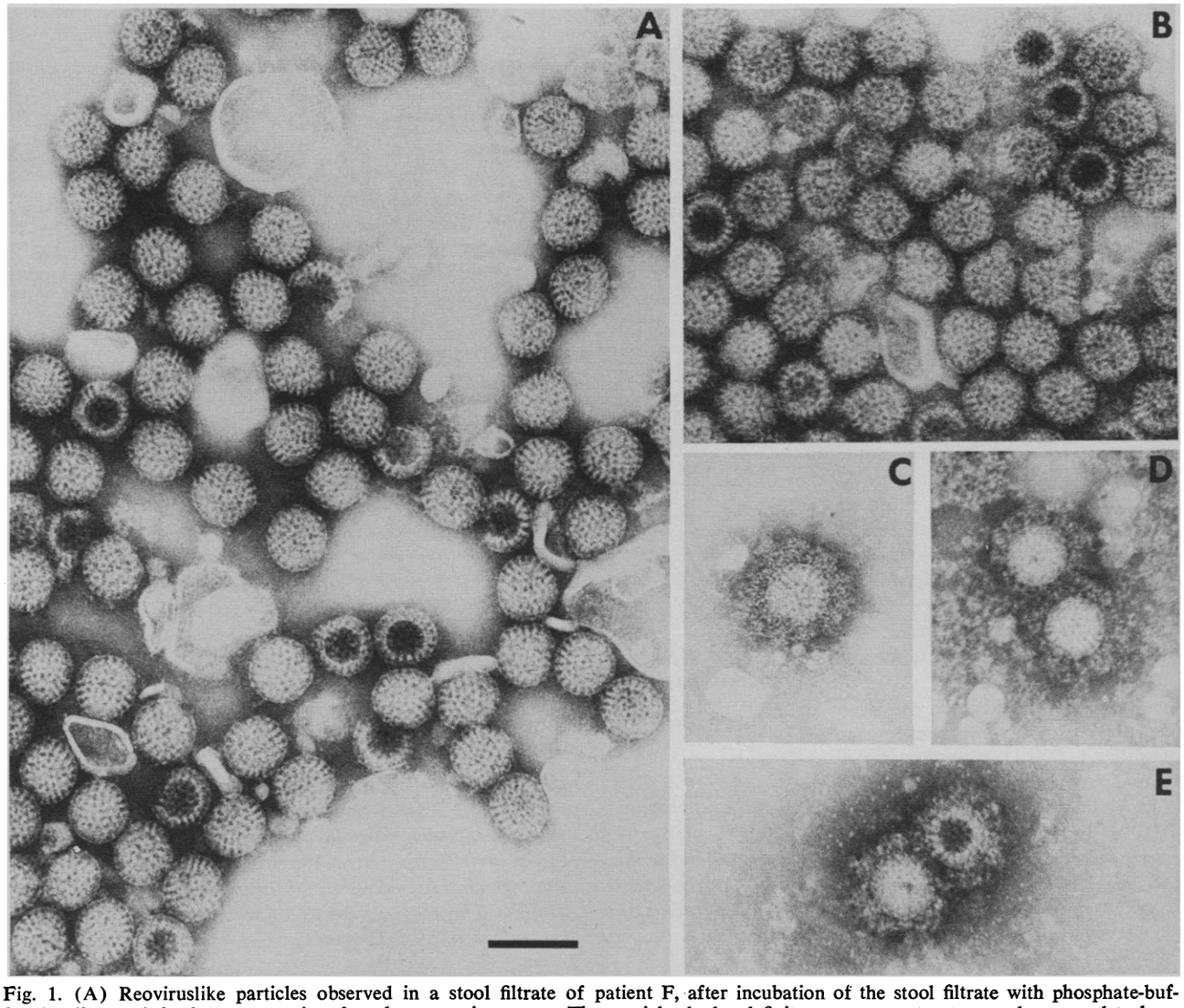

Fig. 1. (A) Reoviruslike particles observed in a stool filtrate of patient F, after incubation of the stool filtrate with phosphate-buffered saline and further preparation for electron microscopy. The particles had a definite capsomere structure and appeared to have a double-shelled capsid. Occasional "empty" particles were seen. (B) Reoviruslike particles observed after the stool filtrate of patient Ru was incubated with a 1 : 5 dilution of acute phase serum from this same patient, and then prepared for electron microscopy. No definite antibody was seen on these particles. This serum was given an overall rating of 1+ for antibody to the reoviruslike agent (Table 1). (C, D, and E) Reoviruslike particles observed after incubation of the stool filtrate of patient Ru with a 1 : 5 dilution of convalescent serum from this same patient, and further preparation for electron microscopy from the same experiment as (B) above. The particles appear to be heavily coated with antibody. The quantity of antibody on these particles was scored as 4+. This convalescent serum was given an overall rating of 4+ for antibody to the reoviruslike agent (Table 1). Bar represents 100 nm.

when coated with specific antibody present in a patient's convalescent serum (*4, 6, 9, 10*). A sample (0.8 ml) of stool filtrate was incubated with 0.2 ml of a 1 : 5 dilution of serum for 1 hour at room temperature prior to centrifugation and further preparation for electron microscopy (*4*). Some filtrates were also examined after incubation with phosphate-buffered saline (PBS) as a control to the IEM procedure (*4*).

Filtrates of stools collected during January, February, and March 1974 from 21 infants and children 2 to 20 months of age who had acute gastroenteritis, with diarrhea (with or without vomiting), severe enough to warrant hospitalization were studied by IEM for the presence of viral agents and, in some instances, by conventional electron microscopy as well. Stool filtrates from 14 of the patients were tested with their convalescent serums. In seven other patients, convalescent serums were not available for use in the IEM test and hence their stool filtrates were examined with convalescent serums obtained from either patients Ru or B (Table 1). Virus particles with characteristic reoviruslike morphology were visualized in stool filtrates from 13 (62 percent) of the 21 patients (Fig. 1A). A total of 44 stools was collected from the 21 children, and 26 were positive for the reoviruslike agent. The particles were so distinct in morphology and in most instances so numerous that of 19 virus-containing filtrates examined by both conventional electron microscopy and IEM each was positive by both methods. The particles in the 26 positive stools appeared similar morphologically and had a definite capsomere structure. They resembled the reoviruses or orbiviruses morphologically. The diameter varied from approximately 67 to 73 nm, with an average of approximately 70 nm. Many of the particles appeared to have a definite double-shelled capsid; "empty" particles were seen occasionally. In filtrates from 2 of the 13 individuals positive for the reoviruslike agent and in certain filtrates from 4 of the 8 who were negative for the reoviruslike agent, small enteroviruslike or parvoviruslike particles were also seen, but their significance is not yet known. In this latter group of 8, 1 of the 4 individuals positive and 1 of 4 individuals negative for the small particle were found to be shedding poliovirus type 3 in anal swab specimens, which were tested in cell cultures. Attempts to propagate the reoviruslike agent in cell cultures have thus far not been successful.

We also examined stool filtrates prepared from stools of 14 controls including infants and children admitted to the hospital during February and March 1974 for respiratory disease, and others returning for well-baby care, and we were unable to detect reoviruslike particles in 13 of these control stool filtrates. In these IEM tests a convalescent serum from patient Ru, which contained a high level of antibody, as judged by IEM, for the reoviruslike agent, was used for 13 of the 14 individuals since convalescent serum was available from only one of the controls. Reoviruslike particles were detected in a filtrate prepared from a stool from a 2-month-old infant admitted to the hospital with pneumonia. However, this infant had also developed diarrhea and vomiting shortly after admission to the hospital. This infant is omitted from tabulations of both the control and test groups. In certain filtrates from 4 of the 13 controls, small enteroviruslike or parvoviruslike agents were visualized. The controls were not matched precisely with respect to time with the diarrhea group; the ages of the controls ranged from 3 to 49 months with a mean of 15.8 months.

We used a stool filtrate that had readily detectable particles to examine paired serums from gastroenteritis patients for antibody to this reoviruslike agent by IEM. The uninactivated serum (0.2 ml of a 1 : 5 dilution) was mixed with 0.8 ml of the stool filtrate and incubated overnight (at least 17 hours) at room temperature and then prepared for electron microscopy in a manner similar to that previously described (*4*). The relative concentration of antibody in each serum was estimated by scoring the amount of antibody coating the particle on a 0 to 4+ scale as determined by electron microscopy. A 4+ rating indicated that single particles or particles in aggregates were generally heavily coated with antibody appearing prominently around the periphery of the particle. Ratings of 1+, 2+, and 3+ indicated the presence of antibody but in lesser amounts than a 4+ rating. A 1+ difference in antibody rating between paired serums was considered to be evidence of a serologic response. Each antibody evaluation of paired serums from patients by IEM was made by one observer on specimens that were coded in order to eliminate the possibility of biased interpretation.

Paired serums from four patients were examined by this technique for antibody to the homologous agent present in a stool filtrate; each individual demonstrated serologic evidence of infection to the reoviruslike agent (Table 1 and Fig. 1, B to E). We also tested paired serums from each of these patients and from an additional patient with gastroenteritis from whom a stool

Table 1. Serologic response of infants and children with gastroenteritis to 70-nm reoviruslike particle, as determined in tests with paired acute and convalescent (Conv.) serums.

Patient	Age (mo.)*	70-nm particle in stool	Antibody measured by immune electron microscopy †				Antibody titer measured by CF with 32 to 64 antigen units‡ §	
			Homologous stool particle		Particle from patient Ru			
			Acute	Conv.	Acute	Conv.	Acute	Conv.
B	8	+	0	2	0–1	4	< 4	≥ 64
Hi	8	+					< 4	32
Dt	9	+					< 4	32
Ru	10	+	1	4	0	3–4	< 4	32
F	11	+	0	3+	0	2–3	< 4	64
J	12	+					8	64
D	13	+	0	1	0	2–3	< 4	32
A	13	+					4	≥ 64
H	20	+					< 4	32
R	20	No stool			0	3–4	< 4	64
W	3	–					< 4	< 4
C	4	–					< 4	4
M	4	–					< 4	4
S	7	–					< 4	4

* Calculated to nearest month. † Quantity of antibody on virus particle was rated on a 0 to 4+ scale as described in text. ‡ The reciprocal is given. § Two percent stool filtrate from patient D used as antigen.

was not obtained, for antibody to virus particles present in the stool filtrate of patient Ru. Each of the five patients had serologic (IEM) evidence of infection with the reoviruslike agent indicating that the agents which infected these patients were either identical antigenically or very closely related (Table 1).

We also were able to develop a complement-fixation (CF) test for the reoviruslike agent by utilizing as CF antigen a 2 percent (approximately) stool filtrate from patient D. In this test system, using 32 to 64 units of antigen we not only confirmed the above described serologic (IEM) evidence of infection in five patients but also found that the remaining five reoviruslike positive gastroenteritis patients with paired serums had serologic (as tested by CF) evidence of infection (Table 1). The differences in dates when the serums were acquired and in residences of the patients make it unlikely that a single adventitious agent was responsible for these infections. Paired serums were also available from four infants whose stool filtrates were negative for the reoviruslike agent, and each failed to give evidence of a significant response to the CF antigen (Table 1). In addition, we tested paired prechallenge and convalescent serums from four adult male volunteers who developed illness after challenge with the Norwalk agent and a significant increase in IEM antibody to the Norwalk agent; none of the four volunteers developed a serological response to the reoviruslike CF antigen. However, each of the four men had preexisting CF antibody. Failure of Norwalk-infected volunteers to develop a serological response to the reoviruslike antigen as well as the lack of a significant response of the four diarrhea patients who did not have reoviruslike particles in their stools suggests that the response of the particle-positive diarrhea patients were specific and not a manifestation of enteric disease in general. Further evidence of specificity was provided by our failure to detect a CF antibody response in paired (prior to challenge and convalescent) serums from volunteers infected with nonbacterial gastroenteritis agents derived from six separate and different family or institutional outbreaks. In addition, CF tests with paired (before illness or acute and convalescent) serums from ten other community-wide, institutional, or family outbreaks of gastroenteritis failed to demonstrate a relationship to the reoviruslike agent.

We also were able to demonstrate that the reoviruslike antigen from patient D was distinct from reovirus types 1, 2, and 3 since it failed to react with a 1:4 dilution of a polyvalent guinea pig antiserum to reovirus types 1, 2, and 3 which reacted to a 1:80 dilution with reovirus types 1, 2, and 3 antigens. In addition, paired serums from three patients who developed serological responses to the reoviruslike CF antigen were tested for a CF antibody response to reovirus types 1, 2, and 3 and no response to any of the reoviruses was observed. We also found that the reoviruslike CF antigen failed to react with a 1:4 dilution of three different polyvalent ascitic fluids from immunized mice, which contained CF antibody to 20 orbiviruses. The 20 orbivirus antigens used in preparation of these polyvalent grouping fluids are listed (*11*). From these data, it appears that the reoviruslike agent is distinct antigenically from the known reoviruses, and also from the 20 orbiviruses for which ascitic fluids were included in the CF test.

Since the reoviruslike agent resembled morphologically the epizootic diarrhea of infant mice (EDIM) virus (*12*) which causes a diarrheal disease in suckling mice, we utilized the CF test to determine if an antigenic relationship existed between these agents. Thirty-two units of the reoviruslike CF antigen reacted with serum from EDIM immune mice, which was diluted as much as 1:320 in the CF test; the EDIM serum exhibited the same titer with EDIM CF antigen. Serums from control mice failed to fix complement with the reoviruslike CF antigen as well as with EDIM virus. In addition, paired serums from nine of the infants and children who developed gastroenteritis and gave serologic evidence of infection with the reoviruslike CF antigen were tested for a serological response to the EDIM virus by CF. Two individuals developed a significant CF antibody response to the EDIM antigen. From these studies it appears that the reoviruslike agent is related antigenically to the EDIM virus.

Since the reoviruslike agent also resembled morphologically the Nebraska calf diarrhea virus (NCDV) (*13*), which causes a severe diarrheal disease in neonatal calves, we utilized both IEM and CF techniques to determine whether an antigenic relationship existed between these agents. In studies by IEM, we found that a 1:5 dilution (*4*) of rabbit antiserum to NCDV had significant antibody to the reoviruslike particle. In addition, 32 units of the reoviruslike CF antigen reacted with a 1:40 dilution of the rabbit antiserum to NCDV; NCDV CF antigen from a homologous system was not available for study. It appears from these studies that the reoviruslike agent is related to the NCDV also.

Our findings of a reoviruslike agent in stools of infants and children with gastroenteritis and of serologic evidence of infection with this agent (the latter accomplished by both IEM and CF) along with the absence of such particles in stools obtained from individuals without gastroenteritis suggests that this reoviruslike agent was the etiologic agent of the gastroenteritis observed in a majority of these hospitalized patients. We have also demonstrated that the reoviruslike agent is antigenically related to the EDIM virus and the NCDV. In addition, development of a CF test for antibody to this reoviruslike antigen should greatly facilitate laboratory and epidemiologic studies of the agent. Preliminary epidemiologic surveys in adults from many parts of the United States reveals that CF antibody to the reoviruslike antigen is quite common in serums from adults, an indication that this agent or a related agent is relatively ubiquitous. It is possible that this reoviruslike particle will emerge as a major etiologic agent of diarrhea of infants and young children.

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Type 2 Hyperprolinemia: Absence of Δ^1-Pyrroline-5-Carboxylic Acid Dehydrogenase Activity

Abstract. *Δ^1-Pyrroline-5-carboxylic acid dehydrogenase activity was measured radioisotopically in normal and type 2 hyperprolinemia fibroblasts. The type 2 cells had no detectable activity over a range of reaction conditions whereas normal cells had easily measurable activity. This enzymatic defect accounts for the biochemical abnormalities in type 2 hyperprolinemia.*

The degradation of proline in man involves the conversion of proline to Δ^1-pyrroline-5-carboxylic acid (PC) by proline oxidase and the oxidation of PC to glutamic acid by PC dehydrogenase (*1*) (Fig. 1). Individuals with abnormalities in this pathway have been classified into two groups, on the basis of plasma and urinary findings, as type 1 and type 2 hyperprolinemia (*2*). Both types are characterized by elevated concentrations of proline in the plasma with resultant iminoglycinuria. Although plasma proline concentrations are of greater magnitude in type 2 hyperprolinemia, the distinguishing feature between the two types is that individuals with type 2 excrete large amounts of an *o*-aminobenzaldehyde reactive material, presumably PC, in their urines.

The metabolic defect in type 2 hyperprolinemia has been shown, in a single patient, to be a deficiency of proline oxidase (*3*). The enzymatic defect in type 2 hyperprolinemia, however, has not been defined. We now report that type 2 hyperprolinemia is due to an absence of PC dehydrogenase activity.

The patient is a 13-year-old asymptomatic Mexican-American female. She exhibits the characteristic biochemical abnormalities of type 2 hyperprolinemia, namely, a plasma proline concentration of 1.658 to 2.534 μmole/ml (normal, 0.07 to 0.15 μmole/ml), marked iminoglycinuria, and the presence of *o*-aminobenzaldehyde reactive material in her urine (*4*).

Using standard techniques, we obtained skin fibroblasts from the patient (HP type 2). Control cells included skin fibroblasts obtained from a 2-year-old white male with G_{M1} gangliosidosis (CJ) and normal fibroblasts from a human male (D550) and a human female (D551) from the American Type Culture Collection. All cells were grown in Eagle's minimal essential media with added nonessential amino acids, neomycin, glutamine, and 10 percent fetal calf serum. The concentration of proline in the medium was 0.1 m*M*. Cultures were routinely checked for bacterial and mycoplasmal contamination and were negative throughout the period of investigation.

To prepare enzyme extracts, we harvested the cells in the late log phase of growth; the cells were then washed and sonicated in a small volume of 50 m*M* tris buffer, *p*H 8.2, with 1 m*M* EDTA. The PC dehydrogenase activity of the extracts was determined by measuring the conversion of ^{14}C-labeled PC to ^{14}C-labeled glutamate (*5*). The reaction was stopped at 30 minutes by the addition of HCl, the final concentration being 1*N*. This acidified mixture was then mixed with an equal volume of a solution of *o*-aminobenzaldehyde (10 mg/ml in 10 percent ethanol and 1*N* HCl). The *o*-aminobenzaldehyde quantitatively combines with unreacted PC, forming a dihydroquinazolium compound which remains at the top of a Dowex column (150 by 5 mm), while the reaction product, ^{14}C-labeled glutamate, is eluted with 1*N* HCl. At reaction conditions of *p*H 8.2 and 30°C, this assay is linear up to 75 μg of added fibroblast protein and up to 30 minutes (for the reaction time). The recovery of glutamate is greater than 90 percent.

Ornithine δ-transaminase and PC reductase were assayed as described (*6*). Protein was determined by the method of Lowry *et al.* (*7*).

Table 1 shows the activity of PC dehydrogenase in control and type 2 hyperprolinemia fibroblasts. The mean activity of the control was 37.0 $\pm$ 3.6 nmole of glutamate produced per hour per milligram of protein, a value that represents about a tenfold increase in radioactivity as compared to either nicotinamide adenine dinucleotide (NAD) or enzyme blanks. In contrast, we were unable to detect any activity in HP type 2 extracts even with the addition of up to 180 μg of extract protein. Furthermore, we altered the reaction mixture by increasing PC concentrations 100-fold or by substituting nicotinamide adenine dinucleotide phosphate (NADP) for NAD, but we were still unable to detect PC dehydrogenase activity in the type 2 extracts. Thus over a range of reaction conditions, extracts of the type 2 hyperprolinemia cells have no detectable PC dehydrogenase activity.

The possibility of an inhibitor of PC