INCREASED "TAKE" RATE OF ORAL ROTAVIRUS VACCINE IN INFANTS AFTER MILK FEEDING

SIR,—A vaccine prepared from the attenuated bovine rotavirus strain RIT 4237 is immunogenic and safe in children[1,2] and the vaccine-induced immunity has protected children over at least one epidemic season of subgroup 2 rotavirus infection.[2] A single oral dose of $10^{8\cdot1}$ $TCID_{50}$ (median tissue culture infective dose) elicited an antibody response in 50–70% of seronegative vaccinees—a satisfactory seroconversion rate but one that could be better . In our previous trials we gave no specific instructions about feeding but in practice no food was given immediately before vaccination. We show here that the seroconversion rate can be increased by giving oral RIT 4237 vaccine after milk, to neutralise gastric acidity.

The vaccinees were healthy infants aged 5–6 months. Our intention to enrol only bottle-fed infants was frustrated by the high rate of breast-feeding in Finland and most of the babies studied were partly breast-fed. However, they received their breast milk early in the morning or late in the evening, and the vaccinations were done at mid-day. Those in the "milk" group (n = 19) were given at least 100 ml of infant formula or diluted cow's milk just before vaccination. Those in the "no milk" group (n = 24) were deprived of feeds for 2 h before and 2 h after the vaccinations. The RIT 4237 vaccine[3] (lot L 991) had a titre of $10^{8\cdot1}$ $TCID_{50}$ per dose. The reconstituted vaccine (0·5 ml) was placed in the back of the baby's mouth and those in the milk group were given some of their usual milk immediately after vaccination.

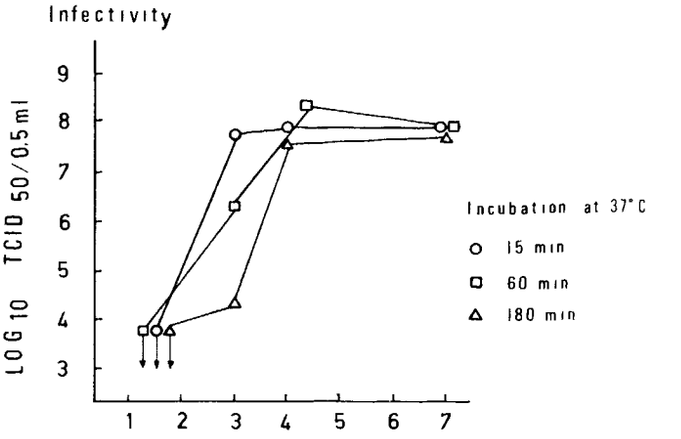
Sera were collected before and 4 weeks after vaccination, coded, and shipped frozen to the Smith-Kline RIT laboratories in Belgium, where a double sandwich enzyme immunoassay with hyperimmune rabbit antiserum against the RIT 4237 virus and, as antigen, a concentrated preparation of the RIT 4237 strain was used in serological studies. Rotavirus IgG was demonstrated with rabbit antihuman IgG conjugated to peroxidase (Miles Laboratories) and 'ABTS' (Boehringer Mannheim) as substrate. A control antigen, prepared from uninfected Ma 104 cells, was included. Absorbances were measured at 414 nm on a 'Titertek Multiskan' (Flow Laboratories) spectrophotometer. Seroconversion was defined as a change in titre from less than 200 to 400 or more.

In separate pH stability studies RIT 4237 virus was diluted 20-fold in buffered solutions to give stable pH of 1·5, 3, 4, and 7, and incubated at 37°C for 15, 60, or 180 min. Ten-fold dilutions were then made in minimum essential medium supplemented with 0·1% lactalbumin hydrolysate, 0·1 μg/ml of trypsin, and 0·5% sodium bicarbonate. The infectivity of the virus was assayed in Ma 104 cells grown on microtitre plates. The infectivity titre was read by cytopathic effect on day 7.

2 infants in the milk group and 4 of the controls were initially seropositive and none of them responded serologically to the vaccine. 1 child in the milk group who had a natural rotavirus diarrhoea 2 days after vaccination was also excluded from calculations of seroconversion rates. 14 of the remaining 16 infants (88%) in the milk group seroconverted compared with 9 out of 20 (45%) of the controls ($p<0\cdot01$). The magnitude of the antibody response in the seroconverters was similar, geometric mean titres being 971 and 800 in the milk group and no-milk group, respectively.

No side-effects were seen and no other child had diarrhoea before post-vaccination sera were collected at 4 weeks. The vaccine was given in November, 1983, and the infants were followed up from December to April, when rotavirus was prevalent in the community. 4 further cases of rotavirus diarrhoea were recorded—1 mild case in the milk group (in a child who had been successfully vaccinated) and 3 cases in the no-milk group, all in children who had not seroconverted.

In acid conditions RIT 4237 virus remained stable at pH 4 but was labile at a lower pH (see figure).



Infectivity of RIT 4237 virus for bovine kidney cells after exposure to acid pH and pH 7.

The major problem with RIT 4237 vaccine has been the incomplete seroconversion rate after one oral dose. Although the rate can be improved by giving several doses (Just M, personal communication), this is impracticable—and, in view of our results, perhaps unnecessary. A seroconversion rate of almost 90%, achieved by simple milk feeding before vaccination, brings RIT 4237 rotavirus vaccine into the same category as many other human vaccines and makes routine rotavirus vaccination of infants more feasible. The inferior rates in our previous trials probably resulted from exposure of the virus to the acid conditions in an empty stomach. Other rotaviruses are known to be labile at pH less than 3,[5,6] and tissue-culture-passaged human rotavirus (Wa strain) in adults achieved a serological response of only 50%,[7] suggesting that Wa is as acid-sensitive as RIT 4237. Gastric acidity could be neutralised by, for example, sodium bicarbonate but this seems much less predictable than milk feeding in developing countries.

We need to know the effect of breast milk on the serological response to rotavirus vaccine since breastfeeding is recommended in the target age group for rotavirus vaccination. Pooled human breast milk protects against rotavirus diarrhoea,[8] so breastfeeding might interfere with the response to rotavirus vaccine. Maternal antibody in breast-milk may adversely affect oral poliovirus vaccination only in the first days of life.[9] If oral rotavirus vaccine can be effectively protected by milk in the stomach it might be possible to reduce the titre of the vaccine virus and so reduce the unit price.

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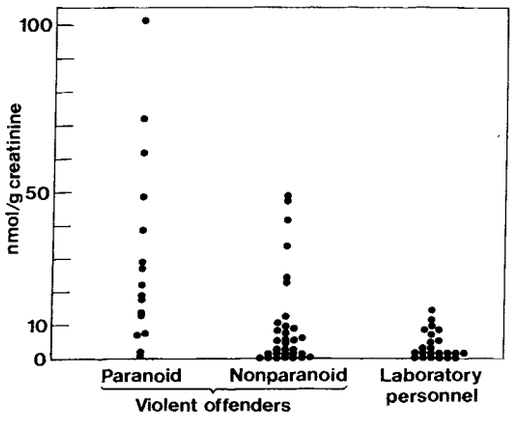
INCREASED URINARY EXCRETION OF BUFOTENIN BY VIOLENT OFFENDERS WITH PARANOID SYMPTOMS AND FAMILY VIOLENCE

SIR,—The excretion of bufotenin and other N,N-dimethylated indoleamines, dimethyltryptamine (DMT) and 5-methoxy-DMT, in psychiatric patients has been the subject of interest for almost two decades.[1] We have described an isotope dilution assay for bufotenin by mass fragmentography,[2] and found that this dimethylated



Urinary excretion of bufotenin among paranoid and non-paranoid violent offenders.

hallucinogenic[3] derivative of serotonin is a normal constituent of human urine. The presence of bufotenin in human urine had earlier been confirmed, by mass spectrometry, only in psychiatric patients.[4]

The urinary excretion of bufotenin was examined in 48 male violent offenders undergoing a forensic investigation on a closed ward. All the men had committed murder or attempted murder or other grave assault. In 11 cases the violence had been inflicted on near relatives only. The psychiatric diagnoses (DSM-III) were made on the basis of written records without knowledge of the results of bufotenin analyses. The records were based on a careful psychiatric interview, behaviour on the ward, psychological tests, and detailed life history.

35 patients had a history of alcohol abuse but they had been in prison and thus without alcohol for several months before entering the department. 17 patients were diagnosed as having an intermittent explosive disorder. In 10 patients paranoid personality traits were recorded on axis II. These patients, together with those with disorders with paranoid symptoms (2 with chronic paranoid schizophrenia, 1 with paranoia, and 3 with paranoid personality disorder) constituted 33% of the violent offenders. The mean age of the patients was 37 years (range 16–75 years). Most patients (35) were not given any psychiatric drugs during their stay on the forensic ward. 11 patients had received neuroleptics before the urine sample for bufotenin analysis was taken.

Urinary bufotenin excretion, measured by mass fragmentography,[2] in all 48 violent offenders ranged from 0·15 to 103 nmol/g creatinine (median 7·1) and levels were higher ($p=0·011$, Mann-Whitney U-test, two-tailed) than those in the reference group of laboratory personnel (n=23, range 1·23–14·1 nmol/g cr, median 1·76). Bufotenin excretion in the 16 patients with paranoid symptoms (figure) ranged from 0·44 to 103 nmol/g cr (median 19·9) and was clearly higher than that of the other violent offenders (range 0·15–48·6 nmol/g cr, median 4·83; $p=0·0026$). Among 11 patients who had been violent against near relatives only, bufotenin excretion was especially high (6·7–103 nmol/g cr, median 26·9, $p=0·0026$). Bufotenin excretion values did not correlate significantly with the use of psychiatric drugs, the diagnosis of alcohol abuse, or the diagnosis of intermittent explosive disorder.

The association of bufotenin excretion with paranoid symptoms was unexpected, since metabolic abnormalities are seldom thought to be linked with these personality traits or even paranoid psychoses. However, in mass spectrometric studies on blood levels of DMT an increase (not significant) was found in schizophrenic patients with high suspiciousness scores.[5] In that study suspiciousness was specifically noted because DMT was known to cause paranoid states.[6] There is also another link between paranoid symptoms and biogenic amines: amphetamine psychosis is often clinically indistinguishable from paranoid schizophrenia.[7]

Paranoid factors play a central role in family violence, and the highest bufotenin excretion values we found were in patients who had been violent against near relatives only. Bufotenin excretion was earlier thought to be associated with the acute phase of schizophrenia or psychosis in general. However, most of the high bufotenin excretors we found, including the 3 patients with the highest values, did not have a psychotic disorder. The bufotenin excretion of patients with intermittent explosive disorder did not differ significantly from that in the other violent offenders. Among these individuals there is evidence for low brain serotonin metabolism[8,9] and a reactive hypoglycaemic tendency in glucose tolerance tests.[10]

The significance of our finding is unclear. Violent offenders form a very selected group of individuals in very abnormal conditions. The prevalence of paranoid symptoms is high and the conditions themselves may, in part, contribute to the presence of paranoid thinking. In addition, according to our preliminary findings, high bufotenin excretion values are common also in other psychiatric patients without clear association with paranoid behaviour. Our study suggests that high bufotenin excretion is not related to psychosis but rather to paranoid thinking and family violence. Furthermore, our results seem to provide some evidence for a biochemical factor in the aetiology of various psychiatric disorders with paranoid symptoms.

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ANENCEPHALY: DISAPPEARING IN KUWAIT?

SIR,—The incidence of anencephaly has a striking geographical variation, probably dependent on genetic, maternal environmental, and dietary factors. In 1966 Stevenson et al[1] found the incidence of anencephaly to be highest in Belfast and lowest in Bogota (Colombia) and Lublijana (Yugoslavia). In a classification[2] based on epidemiological data Kuwait was put in the high-incidence group on the basis of a 1968 study showing an incidence of 3·2 per 1000. Incidences above 3 per 1000 were also reported for Egypt and the Lebanon.[5]

During 1983 all live births and stillbirths at the main maternity hospital and three regional hospitals in Kuwait were studied. Among 36 138 births there were only 48 cases of anencephaly (1·33 per 1000 or less than half the previous figure[3]). Within Kuwait there was an apparent geographical variation in the three regional hospitals, the incidence being highest in the Jahra area with its large Bedouin population who stick to their own traditions, including dietary habits, and lowest at Farwania Hospital, serving a mixed