

being ventilated. These changes were prevented by bicarbonate therapy, since there was no appreciable difference in type of surgery, postoperative analgesia, intravenous fluid therapy, or anaesthetic technique between the two groups. The incidence of postoperative radiological changes in the chest, however, was significantly reduced in group 2. The improvement in acid-base balance in patients receiving bicarbonate therapy and the relative failure of these patients to increase both their alveolar ventilation and tidal and minute volumes, even in the presence of chest complications, indicated a considerable difference in gas exchange which cannot be fully explained without concomitant measurements of CO<sub>2</sub> production. Simultaneous measurements of oxygen transfer by arterial PO<sub>2</sub> and expired gas measurements may resolve the problem.

I would like to thank Mr. H. Shucksmith, Mr. L. Lawson-Light, Mr. G. Wilson, and Mr. D. Pratt, consultant surgeons, for permission to investigate patients under their care. I also thank Dr. Alan Ness, Mr. G. G. Bird, Dr. J. Glanville, Dr. H. Herlinger, consultant radiologists; Mr. A. Powell, and the pathology depart-

ment of St. James's Hospital, Leeds, Mr. M. P. Robinson, orthopaedic surgeon, Robert Jones and Agnes Hunt Hospital, Oswestry; and Dr. Susan L. Lewis for her help in preparing this paper.

## REFERENCES

- Astrup, P., Jørgensen, K., Andersen, O. S., and Engel, K. (1960). *Lancet*, 1, 1035.  
 Bendixen, H. H., Bullwinkel, B., Hedley-Whyte, J., and Laver, M. B. (1964). *Anesthesiology*, 25, 297.  
 Collins, C. D., Darke, C. S., and Knowelden, J. (1968). *British Medical Journal*, 1, 401.  
 Lewis, F. J., and Welch, J. A. (1965). *Surgery, Gynecology and Obstetrics*, 120, 305.  
 Mellemaard, K., and Astrup, P. (1960). *Scandinavian Journal of Clinical and Laboratory Investigation*, 12, 187.  
 Ngai, S. H. (1967). In *Modern Trends in Anaesthesia*, ed. F. T. Evans and T. C. Gray, vol. 3, p. 184. London, Butterworths.  
 Nunn, J. F., Coleman, A. J., Sachithanandan, T., Bergman, N. A., and Laws, J. W. (1965). *British Journal of Anaesthesia*, 37, 3.  
 Paine, E. G., Boutwell, J. H., and Soloff, L. A. (1961). *American Journal of the Medical Sciences*, 242, 431.  
 Palmer, K. N. V. (1955). *Postgraduate Medical Journal*, 31, 25.  
 Price, H. L., et al. (1966). *Anesthesiology*, 27, 24.  
 Prys-Roberts, C., et al. (1967). *Lancet*, 2, 399.  
 Stringer, P. (1947). *Lancet*, 1, 289.

## Rhinoviraemia

G. E. D. URQUHART,\* M.B., M.R.C.PATH. ; E. J. STOTT,† PH.D.

*British Medical Journal*, 1970, 4, 28-30

**Summary:** Rhinoviruses have been isolated from the serum of two infants at necropsy. Failure to isolate viruses from ten other sera from infants who yielded rhinoviruses from their respiratory tracts suggests that true rhinoviraemia occurs rarely, and is infrequently associated with rhinovirus infections, both clinical and subclinical, and death. It is suggested that this is the first report of isolations of human rhinoviruses from the blood.

### Introduction

A viraemic phase of infection has been shown or is presumed to occur in many virus infections. Rhinoviruses, though primary respiratory pathogens, are members of the picornavirus group, many members of which have been shown to have a viraemic phase. We report two cases of sudden unexpected death of infants from whose blood rhinovirus H strains were isolated.

### Case Reports

*Case 1.*—A girl aged 3 weeks died in January 1968. There was no significant clinical history. The baby was taken into bed with its parents because it was crying during the night. Next morning the mother awakened to find it lying upside down, face down under the blankets, and dead. At necropsy the lungs were grossly congested and oedematous, serous thoracic petechiae were numerous, and both adrenal medullae were haemorrhagic. The brain was macroscopically normal. Histological sections were not prepared. The cause of death was certified as asphyxia, but the findings were also consistent with acute anaphylaxis.

*Case 2.*—A girl aged 11 weeks died in November 1968 after a four-day history of "cold" with cough and diarrhoea. She was put to bed after feeding, being found dead in the cot next morning.

At necropsy patchy lung congestion and scanty serous thoracic petechiae were found, the bowel contents were yellow and fluid, and the brain was congested. Some milky material—possibly aspirated stomach contents—was found in the trachea. The cause of death was certified as acute tracheobronchitis. As in Case 1, no histological sections were available for examination.

### Investigations

Blood was collected post mortem by cardiac puncture in situ and multiple other specimens were taken with precautions to avoid cross-tissue contamination so far as possible. Specimen extracts, swabs, and blood fractions were stored at -35° C. between test procedures in the laboratory.

Rhinovirus isolation procedures were carried out in WI 38 and secondary monkey kidney tissue cultures incubated in conditions of reduced pH and rolled at 33° C. At least two tissue culture passages were made before a negative result was accepted. All cytopathic agents isolated were shown to be acid-labile and chloroform-resistant; they failed to grow in WI 38 or monkey kidney tissue cultures incubated at 37° and 33° C respectively. Acid lability, chloroform stability, and other laboratory procedures were performed as described by Grist *et al.* (1966).

Guinea-pig antisera were prepared by inoculating 1 ml. of virus (grown in WI 38 tissue culture to a titre of 10<sup>3.5</sup> TCD<sub>50</sub>/0.1 ml.) intraperitoneally, plus 2 ml. intramuscularly, with an equal volume of Freund's complete adjuvant, and bleeding out one week later.

### Virus isolations

Rhinovirus H strains were isolated initially from a tracheal swab taken from Case 1 and a nasal swab taken from Case 2. During a routine homologous neutralization test with serum from Case 2 it was noted that the tissue culture tubes inoculated with inactivated serum diluted 1/8 as a toxicity control developed cytopathic effects similar to those of a rhinovirus. Tissue culture passage and physicochemical tests confirmed that these effects were due to a rhinovirus H strain. Reisolation procedures on a fresh but uninactivated portion of this serum confirmed that a rhinovirus was present in the original specimen.

\* Lecturer in Clinical Virology, Glasgow University Department of Infectious Diseases, Ruchill Hospital, Glasgow N.W.

† M.R.C. Common Cold Research Unit, Harvard Hospital, Salisbury, Wilts.

Uninactivated serum from Case 1 that had not undergone homologous neutralization testing because of the small quantity available was inoculated into tissue cultures; as in Case 2, cytopathic effects developed after three days and proved to be due to a rhinovirus H strain. The unwashed blood-cell deposit from Case 1, containing both white and red blood cells, also yielded a rhinovirus.

Reisolation and titration procedures carried out on the five virus-positive specimens yielded rhinoviruses from all except the tracheal swab from Case 1. The highest concentration of virus, about 8 TCD<sub>50</sub>/0.1 ml., was in the serum specimens (Table I).

TABLE I.—*Rhinovirus Isolations*

Case No.	Code No.	Specimen	Virus Content TCD <sub>50</sub> /0.1 ml.	Reisolation
1	646-68	Tracheal swab	1	Negative
1	652-68	Blood cell deposit	1	Positive
1	653-68	Serum	8	Positive
2	7,479-68	Nasal swab	1	Positive
2	7,488-68	Serum	8	Positive

Portions of sera which had been sent to a separate laboratory for immunoglobulin estimations immediately after separation were retrieved, and virus isolation was attempted on three separate occasions with negative results. These latter specimens had been stored at 4° C. for 2 to 11 months, were bacteriologically contaminated, and required chloroform treatment before tissue culture inoculation.

Antisera were prepared in guinea-pigs against both serum rhinoviruses and used in cross-neutralization tests. These showed that the viruses were similar within each case but that each infant had been infected with a different rhinovirus (Table II). Four

TABLE II.—*Cross-neutralization Tests with Antisera to Serum Viruses*

Virus	Virus Dose TCD <sub>50</sub>	Antiserum Titres‡	
		653-68	7488-68
646-68	32;100†	128	< 8
652-68	32	16	< 8
653-68	1000;32†	> 8	> 8
7,479-68	100	—	32
7,488-68	320	—	32
*6,043-68	1000	—	< 8
*7,742-68	1000	—	< 8
*8,360-68	32	—	< 8
*8,710-68	320	—	< 8

— = Not tested.

\*Concurrent rhinovirus H strains.

†The 2 doses given refer to amounts of virus used in tests with 653-68 and 7488-68 antisera respectively.

‡Expressed as reciprocals.

No neutralization occurred with preimmune sera.

other rhinoviruses, handled in the laboratory over the same period as the serum isolations were made, were not neutralized by antiserum prepared with the virus isolated from the serum of Case 2.

Attempts to isolate rhinovirus from extracts of lung, brain, suprarenal fat, bowel, and myocardium from both cases gave negative results. In addition a tracheal swab and a thymus extract from Case 2 were also negative for rhinovirus; blood-cell deposit was not available from this case for rhinovirus isolation procedures. The brain extracts were also inoculated into rhinovirus-sensitive Manchester HeLa tissue cultures with negative results. All of these specimens underwent initial virus isolation procedures in WI 38, secondary monkey kidney, and Bristol HeLa tissue cultures incubated in non-rhinovirus isolation conditions with negative results. Attempts to isolate rhinoviruses were unsuccessful with uninactivated sera from eight surviving infants and post-mortem sera from two other "sudden death" infants.

### Typing of viruses

Before typing tests were carried out it was confirmed (E.J.S.) that the viruses isolated had the physicochemical properties of rhinoviruses. Cross-neutralization tests with standard rhinoviruses and antisera showed that the tracheal isolation from Case 1 was similar to rhinovirus type 15 and the nasal isolation from Case 2 was similar to a Glasgow strain 1321-62 which itself is related to

TABLE III.—*Typing of Rhinovirus Isolates*

Virus	Virus Dose TCD <sub>50</sub>	Titres to Antisera				
		653-68	RV15	7488-68	RV22	1321-62
Case 1	646-68	320	20	1280	—	—
	RV15	680	20	≥ 1280	—	—
Case 2	7479-68	680	—	—	20	40
	RV22	1800	—	—	10	≥ 10,240
	1321-62	68	—	—	80	2560

— = Not tested. RV = Rhinovirus.

rhinovirus type 22 (Table III). Antiserum to the virus 653-68 did not show neutralizing activity against the viruses used in this test, probably owing to the high dose of viruses used. No reactions were detected with antisera to remaining virus types 1A to 5S.

### Serum immunoglobulins

Serum levels of IgA, IgG, and IgM were estimated by a radial diffusion technique and found to be normal in both cases except for a raised level of 93 mg. of IgM per 100 ml. in Case 2.

### Comment

Many viruses have been isolated from blood, but we believe that we are the first to report isolations of human rhinoviruses from this site. That rhinoviruses can be isolated outside the respiratory tract is shown by studies such as those described by Cate *et al.* (1964, 1967), who isolated rhinoviruses from the lower gastrointestinal tract of a small proportion of volunteers inoculated intranasally.

The amount of virus in untreated serum from both infants was about 8 TCD<sub>50</sub>/0.1 ml., and the initial finding that heat-inactivated serum from Case 2 was positive for rhinovirus when diluted 1/8 suggests that the serum protected virus from thermal inactivation, as a loss of at least tenfold in infectivity would be expected by inactivation procedures (Dimmock and Tyrrell, 1964).

The source of viruses in the blood is unlikely to have been contamination at the time of sampling, since no virus was isolated from other tissues in the thoracic cavities, but possibly post-mortem cell autolysis and blood fibrinolysis may have allowed rhinoviruses to pass into the circulation after death. Laboratory cross-contamination by aerosol spread from concentrated seed virus to the serum of Case 2 during the homologous neutralization test is a theoretical possibility without precedent in our laboratory. Neutralization tests ruled out cross-contamination with other rhinoviruses in the laboratory and confirmed that Case 1 viruses were similar to rhinovirus type 15 and that Case 2 viruses related to rhinovirus type 22. Failure to isolate viruses from 10 other sera from infants who yielded rhinoviruses from their respiratory tracts suggests that true rhinoviraemia occurs rarely. The first record of an equine rhinovirus, however, was an isolation from a batch of serum taken from horses (Sellers and Fitzpatrick, 1962), so that a precedent exists for rhinoviraemia.

The significance of rhinovirus in blood is difficult to evaluate, as the association of rhinoviruses with severe disease or death is unusual. Holzel *et al.* (1965) reported the association of two rhinovirus types (RV1B and 2) with four cases of meningoencephalitis in infants aged 2 to 29 months, but there was no suggestion of encephalitis in our cases. Circumstantial evidence supports the diagnosis of asphyxia in Case 1, but the bilateral adrenal haemorrhages are similar to those associated with overwhelming infection. Craighead *et al.* (1969) isolated rhinovirus type 13 from the lung of a fatal case of adult pneumonia whose defence mechanisms were depressed, but our cases did not show depressed immunoglobulin levels; other potentially protective factors such as interferon were not estimated. Despite the history of respiratory infection in Case 2, there was no evidence of extension of rhinovirus infection to the lungs. Since this case had rhinoviraemia

along with respiratory signs, it was unlike the influenza A viraemia reported by Khakpour *et al.* (1969) during the asymptomatic incubation phase of the infection but not during clinical illness.

We cannot exclude rhinoviraemia occurring as a post-mortem artefact, but true viraemia, if it exists, appears to be infrequently associated with rhinovirus infections, both clinical and subclinical, and death.

We wish to thank Dr. R. W. Logan for carrying out immunoglobulin estimations. This work was supported by M.R.C. Grant G967/102/C.

## Preliminary Communications

### Intestinal Absorption of Calcium-47 after Treatment with Oral Oestrogen-gestogens in Senile Osteoporosis

*British Medical Journal*, 1970, 4, 30-32

**S**ummary: Intestinal absorption of radiocalcium was measured in 15 postmenopausal women with osteoporosis before and after six months' treatment with an oral oestrogen-gestogen combination. Comparison with a control group indicated a significant improvement in intestinal absorption after treatment. Though there is no evidence that oestrogens have an anabolic effect on human bone, these results indicate that they affect the intestinal absorption of calcium directly.

#### INTRODUCTION

Intestinal calcium absorption can be measured by the oral administration of calcium-47 and by following the plasma concentration of the tracer at one and two hours (Blau *et al.*, 1954; Bhandarkar *et al.*, 1961; Cameron *et al.*, 1962; Caniggia *et al.*, 1963; Gennari, and Bianchi, 1963; Jaworski *et al.*, 1963; Caniggia and Gennari, 1964; Degrazia and Rich, 1964; Robinson *et al.*, 1964; Avioli *et al.*, 1965; Kinney *et al.*, 1965; Rose *et al.*, 1965).

In previous papers we showed that in patients affected by senile osteoporosis radiocalcium appeared in the plasma later than in normal subjects and that often the maximal peak of activity was lower (Caniggia *et al.*, 1963, 1965a). This has been confirmed by Parsons *et al.* (1968), who found that two hours' plasma activity corresponding to a given fractional absorption of calcium-47 was 1.8 times greater in the "remaining group" than in the "osteoporotic group" ( $P < 0.01$ ).

This paper presents data on the intestinal absorption of radiocalcium before and after six months' treatment with an oral oestrogen-gestogen combination in 30 women suffering from senile osteoporosis.

#### MATERIALS AND METHODS

Thirty women resident in hospital aged 50 to 66 were studied with the calcium-47 oral test before and after six months' treatment with an oral oestrogen-gestogen combination (ethyniloestradiol 0.1 mg. + vinylloestrenolone 2.5 mg.). These patients had senile osteoporosis, confirmed by clinical and laboratory features, x-ray examination, and needle biopsy of iliac crest.

#### REFERENCES

- Cate, T. R., Couch, R. B., and Johnson, K. M. (1964). *Journal of Clinical Investigation*, 43, 56.  
 Cate, T. R., Douglas, R. G., Johnson, K. M., Couch, R. B., and Knight, V. (1967). *Proceedings of the Society of Experimental Biology and Medicine* 124, 1290.  
 Craighead, J. E., Meier, M., and Cooley, M. H. (1969). *New England Journal of Medicine*, 281, 1403.  
 Dimmock, H. J., and Tyrrell, D. A. J. (1964). *British Journal of Experimental Pathology*, 45, 271.  
 Grist, N. R., Ross, C. A. C., Bell, E. J., and Stott, E. J. (1966). *Diagnostic Methods in Clinical Virology*, Oxford, Blackwell Scientific.  
 Holzel, A., Smith, P. A., and Tobin, J. O'H. (1965). *Acta Paediatrica Scandinavica*, 54, 168.  
 Khakpour, M., Saidi, A., and Naficy, K. (1969). *British Medical Journal*, 4, 208.  
 Sellers, R. F., and Fitzpatrick, M. (1962). *British Journal of Experimental Pathology*, 43, 674.

At 8 a.m. the fasting patients were given by mouth 40  $\mu$ Ci of calcium-47 dissolved in 10 ml. of a 10% calcium gluconate solution equivalent to 88 mg. of calcium carrier (according to our standard technique). Informed consent was obtained for the isotope studies from all patients. Samples of venous blood were drawn into heparinized syringes at one and two hours after administration of the dose: the radioactivity assays were carried out on 2-ml. plasma samples; counts were expressed as percentage of administered dose per litre of plasma. Calcium-47 as calcium chloride at raised specific activity was supplied by Sorin (Saluggia, Italy).

Radiochemical analysis was performed on plasma samples with a Tracerlab radiation analyser to screen out radiation from scandium-47; counts were corrected for decay.

The maximal peak of activity ranges normally from 2 to 2.5% of dose per litre of plasma (Gennari and Bianchi, 1963; Caniggia and Gennari, 1964; Caniggia *et al.*, 1965b). Cases below 2% of dose/l. can be considered as "hypoabsorbers" (Caniggia *et al.*, 1963, 1964). Cases above 2.5% of dose/l. can be considered as "hyperabsorbers" (Caniggia *et al.*, 1965a). According to the calcium-47 oral test 21 out of our 30 osteoporotic women could be regarded as hypoabsorbers and nine as normoabsorbers (Table I). These cases have been randomized with a double-blind technique so that after six months 15 patients had completed six cycles of the oestrogen-gestogen combination and 15 had been submitted to identical treatment with placebo.

The oestrogen-gestogen oral preparation (ethyniloestradiol 0.1 mg. + vinylloestrenolone 2.5 mg.) was given daily on a schedule of three weeks on and one week off. Of 15 women who took this preparation 14 had trivial vaginal bleeding when treatment was discontinued.

#### RESULTS

Statistically, no difference was found between the placebo group and the oestrogen-gestogen combination group before treatment as far as the maximal peak of activity after oral administration of calcium-47 was concerned. After six months' treatment with the oestrogen-gestogen combination there was a clear-cut improvement of intestinal absorption of calcium-47. The maximal peak of activity after the administration of calcium-47 rose in all but two cases, and often within the first hour (Table I). This effect was more evident in those who had been classified as hypoabsorbers; nevertheless, it was appreciable in the normoabsorbers as well. Statistically, there was a significant improvement in intestinal absorption of calcium-47 in the oestrogen-gestogen combination group compared with the placebo group (Table II).

Our plasma radioactivity values were corrected for body weight, and a mathematical analysis on a simple kinetic model was to evaluate the mean fraction absorbed and the