The relatively specific effect of alcohol on magnesium excretion, together with the observation that low serum-magnesium levels are more frequent and last longer than other electrolyte changes in patients with acute alcoholism (Martin et al. 1959), provide strong circumstantial evidence that the hypomagnesemia develops in these patients principally because an increased excretion of magnesium results from the intake of alcohol. The observation that administration of alcohol to rats increased their requirement for dietary magnesium (Gottlieb et al. 1959) is also explicable by this hypothesis.

Summary

The incidence of hypomagnesemia among fifty chronic alcoholics was 25%. This depletion was not considered important in the clinical condition of these patients, and it was usually corrected within a week of a return to normal diet.

Administration of alcohol to normal subjects caused a large rise in urinary magnesium excretion without producing a comparable effect on other cations. This was due, predominantly, to variation in the activity of the renal tubules. An increased magnesium excretion after consuming alcohol is believed to be the principal cause of hypomagnesemia among patients with chronic alcoholism.

We wish to thank Miss M. Nicholson, Miss J. Carter, Mr. W. B. Simpson, and Mr. K. Taylor for technical assistance.

REFERENCES


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that the linear relationship between the distance of the precipitin line from the antiserum cup and the concentration (Le Bouvier 1959, Beale and Mason 1962) holds over only a limited range (fig. 1). D-antigen assay is therefore only reliable when the D-antigen preparations are diluted or concentrated so that their precipitin lines fall on the linear part of the curve.

Standard preparations of all three types of poliovirus have been prepared and assigned a unitage such that a "D line" of 24 mm. in the test described by Beale and Mason (1962) corresponds to 600 units of D antigen for each type. The results of ten replicate titrations and the unitage assigned for 1 ml. of the standard preparations are shown in table I. These preparations have been dispensed in 0.5 ml. amounts in ampoules. An ampoule contains in 0.5 ml. the following amounts of D antigen: type 1, 440; type 2, 195; type 3, 235. The standard preparations deposited with the Division of Immunological Products Control Laboratory, Medical Research Council Laboratories, Hampstead, are used to control the potency of the poliovaccine components of the new quadruple vaccine. The poliovaccine components are assayed against these standards, and they are included at such levels that one dose of the vaccine contains not less than 75 D-antigen units of type 1 and not less than 1 D-antigen unit of each of type 2 and type 3. It should be noted that the D units defined here are not the same as the arbitrary units referred to by Beale (1961).

The D-antigen content of several commercial batches of killed poliovaccines have been tested by our method (Beale and Mason 1962) and found to contain 1 to 8 units of type-1 D antigen per dose. Such vaccines have been obtained both from this country and from several countries abroad. Fewer observations have been made on types 2 and 3, but routine batches of inactivated poliovaccine made by us have contained about 0.5 unit of D antigen per dose. Clinical trials have demonstrated that when they are incorporated in quadruple vaccine little if any increases are required for types 2 and 3 for satisfactory results; by contrast a substantial increase in type-1 component was required to give a satisfactory response to this antigen. The vaccine (8A) used by Dane et al. (1962), for example, contained 200 D units for type 1 and 1 unit each for types 2 and 3. In unpublished trials Dick and Dane showed that a batch of quadruple vaccine (7C) containing 15 units of type-1 D antigen was inadequate for producing a satisfactory immune response in children.

Gaisford and Perkins have carried out trials with four batches of quadruple vaccine. The poliovaccine and the freeze-dried triple-antigen components of these quadruple vaccines was stored separately, because of doubts expressed in the U.S.A. about the stability of the individual components. They were mixed immediately before inoculation. Gaisford and Perkins used a batch of quadruple vaccine 7A which contained 150 units of type-1 D antigen.

Preliminary results on children who have had a primary course of immunisation with these vaccines, beginning at one week old, suggest that batch 7A provides an adequate antigenic stimulus. This would be expected, since Gaisford and Perkins (see Perkins 1962) have shown that the response of one-week-old children to a concentrated purified poliovaccine (Merck, Sharpe & Dohme) was satisfactory. This vaccine has been found to contain 150 D-antigen units per ml. for type 1; the recommended dose is 0.5 ml., but Gaisford and Perkins used 1 ml.

### Table I—Distance of D Precipitin Line from Antiserum Cup for Standard D-Antigen Preparations

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Distance in mm.</th>
<th>Mean</th>
<th>Units per ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td>21.9, 22.9, 23.5, 23.6, 23.6, 23.0, 23.5, 22.8, 22.6, 21.7</td>
<td>22.9</td>
<td>880</td>
</tr>
<tr>
<td>Type 2</td>
<td>25.2, 24.6, 25.0, 24.8, 25.9, 25.4, 25.7, 25.5, 25.0</td>
<td>25.3</td>
<td>390</td>
</tr>
<tr>
<td>Type 3</td>
<td>24.6, 25.4, 24.6, 24.1, 24.8, 25.8, 24.4, 24.7, 24.0, 24.8</td>
<td>24.7</td>
<td>470</td>
</tr>
</tbody>
</table>
Fig. 3—Stability of diphtheria and tetanus toxoids in quadruple vaccine at 4°C.

The results for a batch of quadruple vaccine 7D containing thiomersal (100 parts per million) and sodium edetate (700 p.p.m.) are shown in fig. 2a and b and compared with those for the same vaccine (7c) without thiomersal but with half the amount of sodium edetate. It will be seen that there is a rapid fall in potency of the polio antigen, as measured by the antigen-extinction technique in chicks (Beale 1961) for batch 7D, whereas batch 7c is stable for at least one year. This rapid fall in poliovaccine potency when quadruple vaccine contains thiomersal and sodium edetate as bacteriostat has been found also in children (Perkins and Gaisford, Butler et al., personal communications). It has, it will be recalled, been established that plain killed poliovaccine is stable at 4°C in the presence of thiomersal and sodium edetate for at least 21 months (Davisson et al. 1956, Perkins and Yetts 1959).

The stability of the diphtheria and tetanus toxoids in the vaccine has proved satisfactory on storage at +4°C for one year, whether thiomersal was present or not (fig. 3). The results of tests for stability of the pertussis component (fig. 4) are given for five vaccines containing pertussis (quadruple vaccines batches 7c and 7D, the pertussis component made up as fluid triple antigen, the same material freeze-dried, and the British Standard). Table II shows the detailed results after one year's storage at 4°C.

The pertussis component of quadruple vaccine has recently been shown by tests done in the United States (Massachusetts Department of Health 1960, Pittman 1962) to lack stability. Various possible reasons for this lack of stability in presence of poliovaccine, despite its proven stability in triple antigen, have been advanced. Thiomersal, which has been used as the bacteriostat for triple antigen, has had to be replaced by other bacteriostats because of its deleterious effect on the poliovaccine component (fig. 2). Benzethonium chloride, which is commonly preferred in quadruple vaccine, has been found by Corkill (1962) to destroy the potency of pertussis vaccine when storage is prolonged or when the concentration exceeds 1/20,000. Corkill (1962) has also found in monkey-kidney tissue culture fluid two other factors that affect the pertussis vaccine. The first, which is destroyed by autoclaving, reduces the opacity of pertussis preparations. The second, which in fact destroys the pertussis antigen, is heat stable and may be dialysable because after osmoconcentration with polyethylene glycol it disappears from the vaccine. This heat-stable factor is neutralised by three treatments:

(a) addition of thiomersal (this cannot be used in quadruple vaccine because of its effect on the poliovaccine component);
(b) addition of sodium edetate;
(c) purification of the poliovaccine component by methanol precipitation.

These findings may well be the explanation for the difficulties with quadruple vaccine, particularly the observation of Pittman (1962) that the vaccine deteriorates more quickly under market conditions than during storage strictly at +4°C.

There is one other suggested cause of lack of stability of the pertussis component in quadruple vaccine. This is the protease found by Baron and Barnett (1960) in monkey-kidney tissue-culture fluid and in some samples of poliovaccine. The relationship of this factor to those described by Corkill is unknown, but his heat-sensitive factor might well be protease. It has been found during manufacture of inactivated poliovaccine in these laboratories that the protease present in the tissue-culture fluid is almost always removed by the Seitz filtration pads. In the few samples in which protease has been detected after filtration, only minute traces remained, requiring more sensitive techniques for their detection than those described by Baron and Barnett (1960). Subsequently our vaccine is purified by adsorption on and elution from aluminium phosphate (Fantes 1962). None of such purified samples has been found to contain protease. It is
probable that the aluminum-phosphate purification method would remove Corkill's heat-stable factor, but no work has been done on this question. Finally sodium edetate (350 p.p.m., but not thiomersal, is added to our quadruple vaccine. It seems that these procedures adopted in our laboratories remove factors responsible for the degradation of the pertussis potency and are the reason for the striking stability of the pertussis component of the particular quadruple vaccine we are describing (fig. 4 and table 11).

**Summary**

Methods used to measure and control the potency of the individual components in a quadruple vaccine are described, including the production of standard preparations of poliovirus D antigen. They make it possible to ensure that each batch of quadruple vaccine contains sufficient of all the antigenic components to give protective immunity to children.

The stability of all components of the vaccine after storage for one year at 4°C in the absence of a bacteriostat has been demonstrated. Reasons are given for believing that factors present in some poliovaccine preparations and destructive of pertussis vaccine have been removed from the quadruple vaccine described here.

It is concluded that an effective, stable, and safe quadruple vaccine has been developed.

Many colleagues have helped in the work on quadruple vaccine; in particular Dr. J. E. Crofts and Mr. I. E. Addison have prepared and carried out many tests on the triple antigen components. Dr. I. G. S. Furminger has allowed us to refer to his unpublished work on the protease present in monkey-kidney cultures. Dr. J. Corkill, of the Connaught Medical Research Laboratories, kindly allowed us to see the manuscript of his paper presented at Prague, which is being prepared for publication in a scientific journal. Dr. R. J. Wilson, also of the Connaught Medical Research Laboratories, closely concerned with the development of quadruple vaccine in Canada, has read our manuscript and made many helpful comments. We especially thank Dr. N. R. Butler, Dr. P. F. Benson, Dr. B. D. R. Wilson, Dr. J. A. Dudgeon, and Dr. J. Urquhart; Prof. W. F. Gaisford and Dr. F. T. Perkins; and Professor G. W. A. Dick and Dr. D. S. Dane for carrying out clinical trials on these newly developed vaccines and for many helpful discussions.

**REFERENCES**


**DIFFICULTIES encountered during and immediately after tracheotomy in infants**

**George Fennell**

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**DIFFICULTIES encountered during and immediately after tracheotomy in infants have prompted me to set forth the technique for the operation, and some suggestions on management immediately after operation.**

**Anaesthesia**

General anaesthesia is always advisable unless there is an obviously impassable obstruction, such as a foreign body, in the pharynx or larynx. If there is impassable obstruction, laryngotomy or tracheotomy without anaesthesia is probably needed immediately.

In the very young infant, perhaps only a few days old, the trachea should be intubated as the first step, and induction with nitrous oxide and oxygen can then be proceeded with. In older infants, induction should begin with nitrous oxide and oxygen given through a face mask, and after intubation anaesthesia should be continued with the addition of ether. As soon as possible after introduction of the endotracheal tube the anaesthetist should apply suction through a soft rubber catheter, to remove secretions from the bronchial tree.

**Position**

The infant is placed supine with a small sandbag beneath the shoulders, and the head is gently extended; hyperextension is undesirable as this interferes with the venous return from the head and neck and produces excessive bleeding. An assistant keeps the head in the extended position and is responsible for keeping chin, larynx, and suprasternal notch in a straight line throughout the operation. When the trachea has been exposed and all bleeding has been controlled, a little further extension of the head will bring the trachea nearer to the surface.

**Operation**

Skin preparation and the placing of towels must leave a narrow sterile area from the hyoid bone to the upper margin of the sternum. The cricoid cartilage is palpated and exposed. The isthmus is clamped on both sides of the larynx, and suprasternal notch in a straight line throughout the operation. When the trachea has been exposed and all bleeding has been controlled, a little further extension of the head will bring the trachea nearer to the surface.

The various tissue planes from skin to trachea should not be separated one from the other, but all these structures should be retracted laterally in one mass to reduce the risk of infection spreading into the fascial planes of the neck and the mediastinum. When the whole length of the incision is deepened, the trachea and thyroid isthmus lie exposed. The isthmus is clamped on both sides of the midline, divided, and tied. Division of the isthmus does much to overcome the difficulty of replacing a tracheotomy tube which has been coughed out, or is being changed, during the early postoperative period.

When the pretracheal fascia has been cleared from the third and fourth rings, a small transverse incision is made between them, and a semicircular piece of trachea is...