THE DISTRIBUTION OF THE BLOOD GROUP A ANTIGEN IN HUMAN TISSUES

E. J. HOLBOROW, PATRICIA C. BROWN, L. E. GLYNN, MARY D. HAWES, G. A. GRESHAM, T. F. O'BRIEN AND R. R. A. COOMBS.

From the Medical Research Council Rheumatism Research Unit, Canadian Red Cross Memorial Hospital, Taplow, Maidenhead, Berks., and the Department of Pathology, University of Cambridge

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IT has been known for many years that blood group antigens are present in many human tissues and body fluids other than red cells and saliva. Their presence has usually been demonstrated by agglutination inhibition tests, using extracts of organs or tissues to inhibit specific agglutinating systems—for example, group A red cells and anti-A serum.

This approach has contributed little to the precise localization of these antigens within organs and tissues. More recently, however, several different methods have been used to study the distribution of blood group antigens at a cellular level.

Thus Coombs, Bedford and Rouillard (1956), have developed a specific mixed agglutination test in which tissue cell suspensions, sensitized with specific anti-A or anti-B agglutinin are mixed with human group A, B or O red cells respectively. Where the A or B antigen is present on both tissue cell and red cell, the presence of the specific agglutinin causes adherence of red cells to the tissue cell surface—the " mixed agglutination " effect. This method, therefore, allows observation of single cells, and has been used to reveal blood group antigens on epidermal cells, and on platelets (Coombs *et al.*, 1956; Coombs and Bedford, 1955).

Another recently introduced method is to absorb agglutinating antisera with a tissue cell suspension, and subsequently to elute specific anti-A or anti-B activity from the cells (Nelken, Nelken, Michaelson and Gurevitch, 1956; Berroche, Maupin, Hervier and Dausset, 1955).

A method which is particularly apt for locating tissue antigens *in situ* is that of Coons, in which a high-titre specific antiserum, conjugated with fluorescent dye, is used to " stain " fresh tissue sections. The sites of antigen-antibody reaction in the section then appear as fluorescent areas on ultra-violet microscopy. The use of this method to study blood group antigens in the epithelium of gastric mucosa and in some other situations had been described (Glynn, Holborow and Johnson, 1957; Glynn and Holborow, 1959).

The present work is an attempt to describe the distribution of the human blood group A antigen in human tissues, in terms of two of the methods outlined above—the mixed agglutination test and the fluorescent antibody technique. These two techniques, independently carried out, have revealed a remarkably close correspondence of results.

MATERIALS AND METHODS

Fluorescent Antibody Technique

Tissues.—At first, most of the tissues examined were obtained post-mortem, up to 24 hr. after death. Later, tissues were taken at autopsies on new-born infants, performed within a few hours of death, and these specimens usually yielded more satisfactory results. Tissue from 2 foetuses (about 16 and 20 weeks respectively) freshly obtained from abortions, also yielded very good results. Small blocks of unfixed tissue were placed in closed polythene containers and quickly frozen in dry-ice/alcohol mixture, and thereafter stored at $-20°$ until examined.

Sections.—Blocks of frozen, unfixed tissue were transferred to a cryostat at $-20°$, in which sections were cut with a microtome, according to the technique described by Coons and Kaplan (1950). Such frozen sections, placed on slides, and thawed, were subsequently treated with anti-A globulin conjugated with fluorescein isocyanate.

Conjugate.—Rabbit antisera were prepared against human blood-Group-A substance (batch 203/112/1, prepared from ovarian cyst material and kindly given by Professor W. T. J. Morgan, Lister Institute, London). The method of immunization, using coated streptococcal vaccine, has been previously described (Glynn, Holborow and Johnson, 1957). These antisera specifically precipitated human A-substance, and gave no cross-reaction with B, H or Le^a substances.

The preparation from these antisera of fluorescent conjugate (using florescein isocyanate), the method of absorbing the conjugates to increase specificity, and the method of staining sections have been previously described (Glynn, Holborow and Johnson, 1957).

Mixed Agglutination Technique

Glassware.—Siliconed glassware was used throughout.

Saline.—Normal NaCl solution buffered at pH 7·2 by adding 10 ml. Sørensen's phosphate buffer to each litre salt solution.

Special diluent for cell suspensions.—To prevent red cells and tissue cells sticking to the glass, the cells were suspended in 1 : 200 dilution of normal rabbit serum in saline. The normal rabbit serum had previously been heated at 56° for 30min. and absorbed free of antibodies to human A, B and O red cells.

Anti-A sera.—Human anti-A serum of the so-called " immune " type (from a Group O person) was used. This serum had an agglutinin titre of 2000 for human A red cells. The rabbit anti-human-A-substance serum used in the fluorescent antibody study (absorbed with human O red cells) also gave satisfactory results. Ordinary human anti-A grouping sera was used, but gave generally weaker results. The sera were heat inactivated before use.

Preparation of tissue cell suspensions

Human tissues were obtained from surgical and post-mortem material. The post-mortem samples were generally procured within 24–36 hr. of death.

Cell suspensions were prepared either by gently scraping the tissue surface with a cataract knife or a brush, or by mincing the tissue with scissors in a small volume of the normal rabbit serum diluent. If cellular degeneration was evident, the suspension was discarded. A fine suspension could be obtained by selective use of the pipette. The cells were deposited by gentle centrifugation (1000–1500 r.p.m.) and washed $\times 3$ with diluent. The cells could be used at this stage or after storage in diluent containing 20 per cent glycerol at $-30°$ or $-70°$.

Mixed agglutination reaction

The method of testing for mixed agglutinatiou has been described previously (Coombs, Bedford and Rouillard, 1956). Suspensions of cells were obtained from various human tissues and treated as described above. The blood group of the patients was established by standard methods of red cell typing. Tissues from 9 group A patients and 5 group O patients were examined.

The mixed agglutination reaction was performed using the human " immune " anti-A serum at a dilution of 1 in 5.

RESULTS

Mixed Agglutination Technique

Cells from most of the tissues from Group A patients showed strong mixed agglutination with human A red cells—there being no mixed clumping with human O red cells. No mixed agglutination with human A red cells was obtained with cell suspensions from Group O tissues. The result of testing cell suspensions from specific tissues are given in the Table, and the typical appearances of positive and negative tests shown in Fig. 1 and 2.

TABLE.—*Distribution of A Antigen in Human Tissues*

Tissue	Mixed-agglutination method	Fluorescent anti-A antibody
Mouth epithelium	+	+
Tongue epithelium	+	+
Pharynx epithelium	+	+
Oesophagus epithelium	+	+
Stomach epithelium	n.t.	+*
Duodenum epithelium	n.t.	+
Small intestine epithelium	n.t.	+ (jejunum, ileum).
Large intestine epithelium	+ (anal canal and rectum)	+ (appendix, colon).
Salivary glands	n.t.	+ (mucous cells).
Pancreas	n.t.	+ (foetal ? islets).
Lachrymal gland	n.t.	0
Thyroid epithelium	+	0
Pituitary (pars intermedia)	n.t.	+
Adrenal (parenchyma)	n.t.	0
Kidney (collecting tubules)	+	+
(glomeruli)	n.t.	+
Ureter epithelium	+	+
Bladder epithelium	+	+
Urethra epithelium	+	n.t.
Trachea epithelium	+	n.t.
Bronchus epithelium	n.t.	+
Fallopian tubes	+	+
Uterus (endometrium)	+	+
Vas deferens	+	n.t.
Testis	n.t.	0
Spermatozoa	n.t.	0
Skin (epidermis)	+†	+
Liver (parenchyma)	0	0
(Kupffer cells)	+	0
Skeletal muscle	0	0
Cardiac muscle	+ (weak)	0
Parietal pleura	+ (weak)	n.t.
Parietal pericardium	+	n.t.
Vascular endothelium	+	+

n.t. = not tested.
* Glynn, Holborow and Johnson (1957).
† Coombs, Bedford and Rouillard (1956).

Fluorescent Antibody Technique

Results with this method are also given in the Table, for comparison with those obtained by the mixed agglutination technique. A brief descriptive account of the findings in the various body tissues follows.

The distribution of the A antigen in tissues may be described under 2 headings (i) its occurrence in epithelial cells, or tissues arising wholly or partly from epithelial origins, and (ii) its distribution in non-epithelial tissues.

Epithelial Tissues

Buccal epithelium, tongue, pharynx and oesophagus (Fig. 3).—These tissues have been examined only in a 20-week foetus. The stratified squamous epithelium in all these sites was positive for the A antigen in all except the deepest epithelial layer. The mucous glands in the region of the pharynx were also strongly positive.

Salivary and lachrymal glands.—The A antigen is present in considerable quantities in the mucus-secreting cells of the mixed salivary glands but absent from the serous cells (7 specimens examined). Thus, the submaxillary gland gives positive staining in regions corresponding to the mucous cells, but parotid gland is uniformly negative. Some submaxillary tissue examined has been negative, but this may be associated with the non-secretor trait. In general, the secretor status has not so far been taken into account in these studies (with the exception of stomach, see below). One specimen of lachrymal gland was negative.

Thyroid gland.—The epithelial cells of the thyroid acini have been uniformly negative in 7 specimens examined.

Pituitary gland.—Positive staining in the pituitary (2 adult specimens) is confined to the colloid and lining cells of the vesicles of the pars intermedia. Other parts of the pituitary have shown no staining.

Thymus.—In foetal tissue and in the new-born, the outer concentric cells of Hassall's corpuscles stain specifically for A substance (4 specimens). The inner degenerate cells do not appear to stain but have a pearly blue auto-fluorescence. Thymuses from older subjects have not been examined.

Adrenal.—The cells of the cortex and medulla were uniformly negative, but the lining cells of the sinusoids, and occasional isolated unidentified cells in the medulla gave specific positive staining.

Liver.—No specific staining has been found in a number of specimens of liver nor has the A antigen been detected in the epithelial lining of bile ducts or gall-bladder. The Kupffer cells appear negative.

Pancreas.—Groups of cells which appear to be developing islets of Langerhans stain specifically for A substance in tissue from foetuses and from premature or new-born infants (4 specimens). The pancreatic acinar cells themselves, and the ducts, are negative. In adult pancreatic tissue, all obtained at routine post-mortem, tissue was unsatisfactory owing to autolysis.

Stomach and first part of duodenum.—The findings here have been fully described elsewhere (Glynn, Holborow and Johnson, 1957).

Small and large intestine.—In both adult and foetal tissue strong positive staining for the A antigen is present in the goblet cells of the mucosa of the jejunum, ileum, appendix, caecum, colon and rectum.

Respiratory tract.—In the lungs the most striking positive staining occurs in the peribronchial mucous glands which lie in relation to the bronchi and bronchioles. In these glands the mucous cells (as in the salivary glands) stain brightly, while their duct linings and the lining epithelium of the bronchi themselves also stain, though rather faintly. The remainder of the lung tissue is negative, including the bronchial cartilage, except for capillaries (see below) in the vicinity

of the peribronchial glands. The alveolar capillary network appears consistently negative.

Kidney (Fig. 6).—The glomerular tufts and peritubular capillaries in infants and foetuses (4 specimens) show positive staining of the "capillary" type (see below). This has not been seen in the few adult kidneys examined, possibly due to post-mortem change. Strong positive staining is seen in the cells lining the collecting tubules throughout the kidney, and in the cells lining the calyces and pelvis. The convoluted tubules are negative.

Ureter and bladder (Fig. 4).—The transitional epithelium of ureter and bladder is positive throughout its thickness, except in the deepest cell layer.

Uterus, Fallopian tubes and ovary.—These have been examined only in foetal material (1 specimen). The endometrium and the lining epithelium of the Fallopian tubes were both positive throughout their thickness. A line of stronger staining was noted at the endometrial surface. In the ovary no definite staining was seen except of the vascular tissue.

Spermatozoa and testes.—No specific staining of sperms from 4 group A adults was found, nor in sections of 2 infant testes.

Skin and its appendages (Fig. 5).—Sections of skin (20-week foetus, 3 infants and 1 child, aged 8 yr.) show specific staining at well-defined sites. These are the ducts of the sweat glands, but not the sweat glands themselves, the ducts of the sebaceous glands, but not the sebaceous glands themselves and the stratum granulosum of the epidermis. The other layers of the epidermis (stratum Malpighii and stratum corneum) and the hairs are negative. In the hair follicles a layer of cells continuous with the stratum granulosum is positive, and in one instance positive granules were seen in the external root sheath.

Non-epithelial Tissues

Vascular endothelium.—Specific staining of capillaries has frequently been noted in a variety of organs obtained post-mortem—namely thyroid, thymus, heart muscle, pancreas, salivary and lachrymal glands, skin and subcutaneous

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EXPLANATION OF PLATE

FIG. 1*a*.—Mixed agglutination reaction between human Group O oesophageal epithelial cells and human group A red cells. Negative result—red cells have no affinity for serum-treated epithelial cells. (Phase contrast microscopy : ×407.)

FIG. 1*b*.—Mixed agglutination reaction between human Group A oesophageal epithelial cells and human group A red cells. Positive result—red cells adhering specifically to serum-treated epithelial cells. (Phase contrast microscopy : ×407.)

FIG. 2*a*.—Mixed agglutination reaction between Group O endothelial cells from vena cava and human group A red cells. Negative result. (Phase contrast microscopy : ×407.)

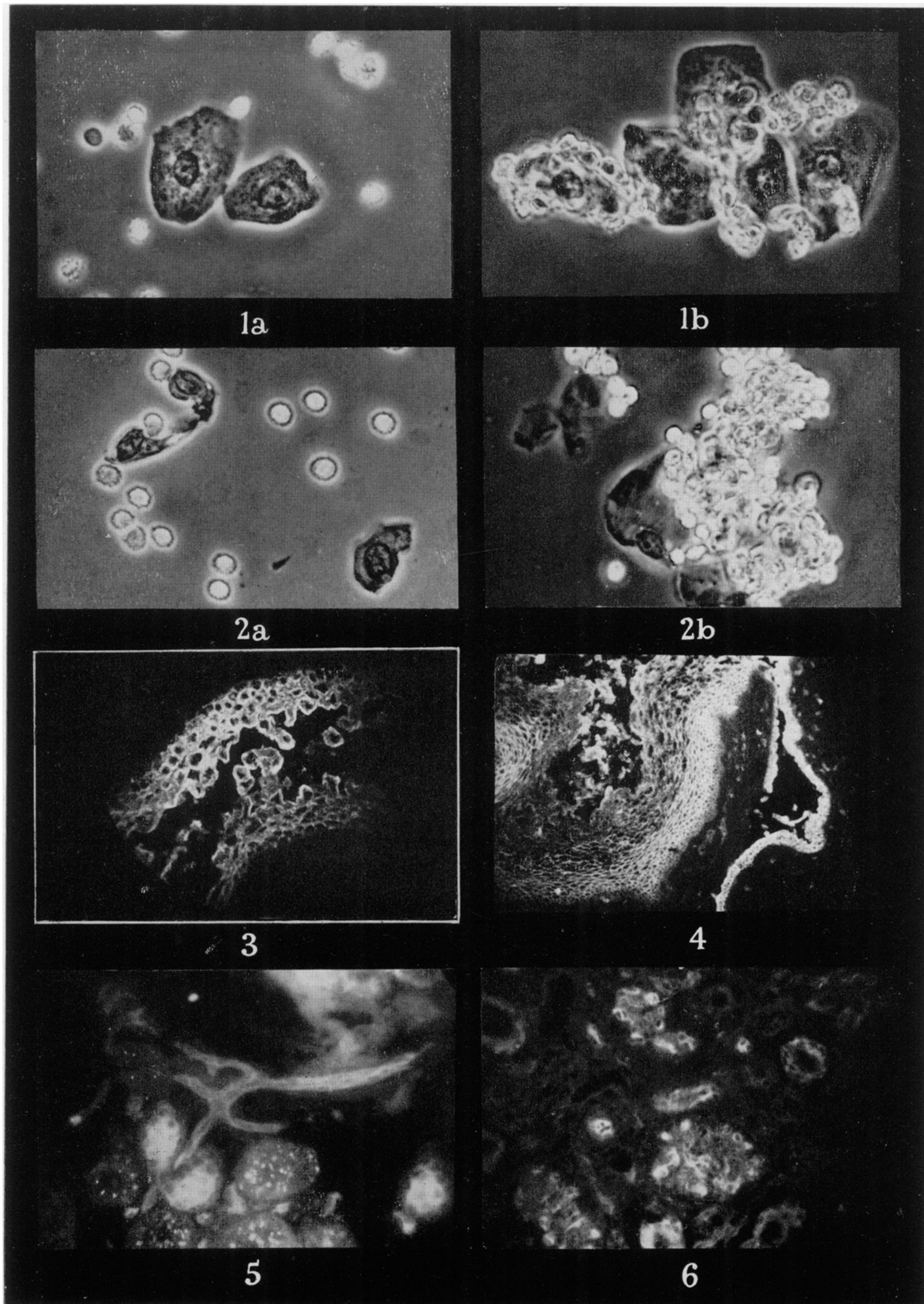
FIG. 2*b*.—Mixed agglutination reaction between group A endothelial cells from vena cava and human group A red cells. Positive result. (Phase contrast microscopy : ×407.)

FIG. 3.—Unfixed frozen section of foetal human group A oesophagus stained with anti-A fluorescent conjugate. Stratified squamous epithelium positive for A antigen. ×130.)

FIG. 4.—Unfixed frozen section of foetal bladder and ureter stained with anti-A conjugate. Transitional epithelium positive for A antigen. (×32.)

FIG. 5.—Unfixed frozen section of child's skin (aged 12) stained with anti-A conjugate. Positive staining of A antigen in capillary endothelium, and in sweat duct epithelium. Sweat gland epithelium negative. (×130.)

FIG. 6.—Unfixed frozen section of foetal group A kidney stained with anti-A conjugate. Glomeruli and collecting tubules positive for A antigen. (×130.)



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tissue, stomach mucosa and kidney (both in glomeruli and in peritubular capillaries). In the foetal tissues examined the vascular endothelium of the larger vessels (including those in the umbilical cord), as well as that of the capillaries gave positive staining. In the 20-week foetus positive staining of the sinusoidal epithelium of the spleen and of the adrenal was clearly seen and also of the meningeal capillaries, the lining of the choroid plexus and of occasional blood vessels within the brain substance.

Effect of ethanol on sections

Pretreatment of sections with 90 per cent ethanol for 30 min. consistently prevented subsequent staining of vascular endothelium. Lower concentrations of alcohol (down to 45 per cent, 90 min. treatment) were also effective in abolishing staining of this type. Ethanol treatment removed all staining from spleen, adrenal, brain and thyroid, where all detectable A antigen is located in the endothelial cells lining capillaries or sinusoids. In contrast, epithelial staining is usually unaffected by ethanol, so that in the epithelium of the bladder, uterus and practically the whole lining of the alimentary tract, for example, the A antigen survives this treatment. In kidney, ethanol abolishes the glomerular staining, but leaves the collecting tubules positive, and in the thymus abolishes vascular staining, leaving the Hassall's corpuscles positive. The only exceptional epithelial tissue so far noted is the skin, where pretreatment with ethanol prevents positive staining in all the sites described.

Effect of other solvents on vascular endothelium

Chloroform treatment of sections completely prevented subsequent staining, and chlorobenzene almost completely prevented it. Acetone and ether were without effect.

Effect of ethanol on cells tested by the mixed agglutination method

Cells from the kidney pelvis, oral cavity, bladder, oesophagus, trachea and inferior vena cava endothelium were treated with 70 per cent alcohol for 40 min., and washed. All gave positive mixed agglutination tests with anti-A serum after this treatment.

DISCUSSION

Although both methods of detecting the A antigen have not been applied to all the tissues examined, both tests have been done on enough tissues to indicate close agreement between them. The results show that in group A individuals the A antigen is widely distributed in 2 principal sites—epithelial tissue and vascular endothelium.

The lining epithelium of the alimentary tract is strongly positive for the A antigen in all sites that have been examined (mouth, pharynx, oesophagus, stomach, duodenum, jejunum, ileum, appendix, colon, rectum and anal canal). The precise localization of the antigen is well shown by the fluorescent antibody method. In the columnar epithelium of the small and large intestine A substance is most conspicuous in the mucous droplets of goblet cells, although fainter

staining of indifferent epithelial cells may be seen, particularly at their luminal ends. The stomach and duodenum show special features in their distribution of blood group antigen (Glynn, Holborow and Johnson, 1957).

The stratified squamous epithelium of mouth, pharynx and oesophagus differs in that the positive staining for the A antigen is not cytoplasmic, but appears to outline the cells, either in the cell membrane or in the intercellular substance. In contrast, in the mucus-secreting cells of the mucous glands of the mouth and pharynx, and of the sublingual and submaxillary salivary glands, the cytoplasm is strongly positive. Both methods show that the tracheal and bronchial epithelium possess the A antigen, but again the cells of the peritracheal and peribronchial mucus-secretory glands show the strongest cytoplasmic fluorescent staining.

The stratified squamous epithelium of the skin gives a somewhat different picture. Although the epidermal cells from the surface give a positive mixed agglutination test (Coombs, Bedford and Rouillard, 1956) the fluorescent method shows detectable antigen only in the granular-cell layer, in the sweat and sebaceous ducts, and in one cell layer of the hair follicles. This partial discrepancy between results by the two methods may be due to quantitative considerations discussed below.

In the urinary tract, A substance is detectable throughout the lining epithelium, extending from and including the collecting tubules in the kidney to the urethra. The transitional epithelium shows the non-cytoplasmic, intercellular type of staining seen in squamous epithelium, involving all layers except the Malpighian layer.

These results on epithelial tissue, particularly squamous and transitional epithelium, must be interpreted cautiously in view of the possibility that blood group substances may have been adsorbed by the cells from the secretions (saliva, urine, meconium) bathing them. When pharyngeal epithelial cells from a group O subject were incubated at 37° for 30 min. with boiled group A secretor saliva, and then washed twice in saline, they did not become positive in the mixed agglutination test with anti-A sera. Cells from group O bladder and ureter, however, did become positive on this treatment. Fluorescent anti-A serum stains the epithelium at these sites brightly and uniformly through several cell layers, and it is difficult to envisage how absorption of the very small amount of blood group substance in urine could give rise to such deposition of A antigen.

The presence of A antigen in vascular endothelium, which was previously reported (Glynn and Holborow, 1959) has been confirmed in a wide variety of tissues. The mixed agglutination method has been applied chiefly to endothelium scraped from the inferior vena cava, which has proved consistently positive in group A subjects. By the fluorescence method specific positive staining is seen chiefly in capillary endothelium, but also in the intima of the blood vessels of many tissues. The kidney glomeruli in new-born infants are conspicuously positive in this respect. The lung capillaries, however, have been negative in material strongly positive elsewhere. The ethanol solubility of this vascular antigen has been very strikingly consistent in the fluorescence study; that endothelium of the inferior vena cava remains positive in the mixed agglutination method after ethanol treatment suggests that the amount of ethanol-insoluble antigen present in endothelium may be too small for detection by fluorescent antibody. The same quantitative considerations may apply also to the A antigen in the cells of the epithelium.

Two discrepancies between the methods were found. Thyroid epithelium and Kupffer cells, both positive by the mixed agglutination method, were negative by the fluorescence method. It is possible that contamination of the cell suspensions with capillary endothelium contributed to this finding.

SUMMARY

The distribution of the blood-group A antigen in a variety of human tissues has been studied by 2 methods—the mixed agglutination method, using human immune anti-A serum, and Coons' fluorescence method, using a conjugate of rabbit antiserum to human A substance. Good agreement in results was found between the methods, which show that the A antigen is widely distributed in human tissues, principally in epithelial tissues and in vascular endothelium.

REFERENCES

Berroche, L., Maupin, B., Hervier, P. and Dausset, J.—(1955) *Vox Sang.*, **5**, 82.
Coombs, R. R. A. and Bedford, D.—(1955) *Ibid.*, **5**, 14.
Iidem and Rouillard, L. M.—(1956) *Lancet*, i, 461.
Coons, A. H. and Kaplan, M. H.—(1950) *J. exp. Med.*, **91**, 1.
Glynn, L. E. and Holborow, E. J.—(1959) *Brit. Med. Bull.*, **15**, 150.
Iidem and Johnson, G. D.—(1957) *Lancet*, ii, 1083.
Nelken, E., Nelken, D., Michaelson, I. C. and Gurevitch, J.—(1956) *Nature, Lond.*, **177**, 840.