γ globulin necessary to absorb completely anti-Vi activity. Typical results are shown in Fig. 2, in which Vi-specific activity remained after absorption. In addition, investigations utilizing inhibition of quantitative precipitation of human γ globulin at the equivalence point by the various primate γ globulins also failed to demonstrate Vi specific antigens in the non-human primates.

These studies would suggest that the Gm(b) factor is not only a mosaic of determinants varying between human races and in certain non-human primate families, but is also probably composed of more than a single determinant in the γG globulin of a single individual. Clearly, the Caucasian Gm(b) has one determinant which is not shared with non-human primates and which was detected in these investigations by an anti-Gm(b) raised in a rhesus monkey. One might anticipate that Gm(b) positive non-human primates may have a similar determinant in their Gm(b) 'area' which is specific for that particular primate family. In addition, at least one Gm(b) determinant found in Caucasian and Negro Gm(b) positive γ globulin occurs in the γ globulin of the *Pongidae* and *Cercopithecidae*, as is evident from their inhibition of the Gm(b) specific haemagglutination system utilizing an agglutinator from the non-rheumatoid human. This probably does not represent simple cross-reactivity in that the inhibition was quantitatively similar to that by human γ globulin. In addition, at present it is conceived that Gm specific agglutinators from non-rheumatoid donors are a uniquely specific form of anti-Gm agglutinator. It is impossible, however, to exclude cross-reactivity between similar but not identical Gm(b) determinants. There is no clear explanation of the variable degrees of inhibition seen in studies with the Gm(b) specific rheumatoid agglutinator. Such reagents may, in fact, be detecting several determinants of the Gm(b) mosaic, which may in turn appear in varying degrees in the non-human primates. Alternatively, cross-reactivity may have influenced results with this reagent to a significant degree.

The failure to demonstrate the Vi antigen to accompany Gm(b) determinants in the *Pongidae* and *Cercopithecidae* is of some interest. It is, of course, impossible to exclude totally the presence of this antigen. It is known that different anti-Vi-specific antisera may detect different areas of the γG globulin molecule[6]. If this indicates polymorphism of the Vi determinant, the use of the Vi specific monkey antiserum may have precluded demonstration of its presence. The most that can be said is that this determinant as it is recognized in man does not seem to occur in association with a Gm(b) determinant recognized by a human agglutinator in these primate γ globulins. This would support the original concept that the H-chain sub-group determinants are distinct from, but linked to, the Gm characters and occur in the same area of the H polypeptide chain, rather than the alternative suggestion that they are an epistatic configuration the structure of which is necessary for the appearance of the Gm(b) factor.

I thank Drs. E. Melby, H. Fudenberg and L. Martensson for certain primate sera and reagents used in this investigation, which was supported by grant *AI* 05780 from the National Institutes of Health, Bethesda, Maryland.

JAMES C. ALLEN

Department of Medicine,
The Johns Hopkins University School of Medicine,
Baltimore,
Maryland.

[1] Kunkel, H. G., Allen, J. C., Grey, H. M., Martensson, L., and Grubb, R., *Nature*, **203**, 413 (1964).
[2] Ropartz, C., Rivat, L., and Rousseau, P.-Y., *Vox Sang.*, **8**, 717 (1963).
[3] Podliachouk, L., *Ann. Inst. Pasteur*, **96**, 362 (1959).
[4] Boyer, S. H., and Young, W. J., *Science*, **133**, 583 (1961).
[5] Allen, J. C., Kabat, E. A., and Kunkel, H. G., *J. Exp. Med.*, **119**, 453 (1964).
[6] Grey, H. M., and Kunkel, H. G., *J. Exp. Med.*, **120**, 253 (1964).

HISTOCHEMISTRY

Ribonuclease and Deoxyribonuclease Substrate Film Methods: a Pitfall in the Interpretation of Results

DURING the course of histochemical investigations into various metabolic disorders, the substrate-film methods for the demonstration of ribonuclease and deoxyribonuclease[1,2] were examined. Satisfactory thin films were obtained by dipping chemically cleaned slides into the DNA or RNA gelatine mixtures at 60° C and then shaking off the excess liquid. The films were allowed to stand vertically at 60° C for 5 min before wiping clean the bottom quarter of the slide. This procedure gave stable films which did not lift or distort during the subsequent operations of fixation in formalin, incubation and staining. The gelatine was obtained from B.D.H. ('Gold Label' grade), and the DNA and RNA from L. Light.

The apparent distribution of DNase and RNase in tissue sections shown by the methods cited[1,2] led to the conclusion that not all the activity could be attributed to the respective enzyme. It was thought that quite a considerable proportion of the apparent activity was due to proteolytic enzymes dissolving the gelatine film, even though formalin fixed, thus releasing the substrate non-specifically. To test this hypothesis, substrate films of RNA and DNA were prepared in the normal manner and 0·1 ml. water, 0·1 ml. ribonuclease 0·01 per cent, and 0·1 ml. trypsin 0·01 per cent were placed separately on each film and the slides were incubated at room temperature for 1 h. The slides were then washed and stained for 10 min with 2 per cent toluidine blue.

The water controls left both films completely untouched and ribonuclease digested only the RNA film. Trypsin completely digested both films. From these results it is concluded that the present substrate film techniques for ribonuclease and deoxyribonuclease can lead to false results unless the gelatine used is found to be resistant to tryptic digestion after formalin fixation. Much of the apparent activity using B.D.H. 'Gold Label' gelatine is due to the action of proteolytic enzymes. Further work is in progress incorporating inhibitors in the gelatine substrate films and using agar as an alternative supporting medium.

I thank D. Barry for technical assistance. This work is supported by a grant from the National Society for Mentally Handicapped Children.

B. D. LAKE

Department of Morbid Anatomy,
Hospital for Sick Children,
Great Ormond Street, London, W.C.1.

[1] Daoust, R., *Exp. Cell. Res.*, **12**, 203 (1957).
[2] Daoust, R., and Amano, H., *J. Histochem. Cytochem.*, **8**, 131 (1960).

IMMUNOLOGY

Mouse Thymic Iso-antigens

Old, Boyse and Stockert reported the presence of a common iso-antigen (*LT*) in certain leukaemias of two strains of mice, and in thymus of only one of these strains and one unrelated strain[1]. Later, the *LT* iso-antigen was found in normal thymus of eight out of twenty-four strains[2]. The *LT* iso-antigen was not detected in the normal tissues examined other than thymus[1,2].

Following our initial report of a strong antigen associated with thymic lymphocytes of *AKR* mice[3], thymocytes from eighteen strains of mice were found to possess either the θ-*AKR* or the θ-*C3HeB/Fe* iso-antigens on their surface[4]. These thymic antigens were distinct from *LT*. In the four to six strains of mice that were tested further, neither splenic lymphocytes nor lymph node lymphocytes were cytolysed by iso-antisera that cytolysed thymocytes.

©1966 Nature Publishing Group