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1 Jasmin, C., Chermann, J. C., Piton, C., Mathé, G., and Raynaud, M., *CR Acad. Sci.*, **268**, 876 (1969).
2 Jasmin, C., Chermann, J. C., Raynaud, M., and Mathé, G., *CR Deuxième Coll. Intern. sur les Virus Oncogènes* (CNRS, 1969, in the press).
3 Rauscher, F. J., and Allen, B. V., *J. Nat. Cancer Inst.*, **32**, 269 (1964).
4 Bernard, C., Silvestre, D., Tanzer, J., Peries, J., and Boiron, M., *Exp. Cell. Res.*, **40**, 513 (1965).
5 Chirigos, M. A., *Cancer Res.*, **24**, 1035 (1964).
6 Hirsch, M. S., and Harvey, J. J., *Intern. J. Cancer*, **4**, 440 (1969).
7 Lindberg, O., and Ernster, L., in *Methods of Biochemical Analysis* (edit. by Glick, D.), III, 1 (Interscience, New York, 1956).
8 Jasmin, C., Chermann, J. C., Mathé, G., and Raynaud, M., *Europ. J. Clin. Biol. Res.*, **15**, 56 (1970).
9 Raynaud, M., Chermann, J. C., Jasmin, C., and Mathé, G., *CR Acad. Sci.*, **270**, 578 (1970).
10 Wright, B. S., and Lasfargues, J. C., *Proc. Amer. Ass. Cancer Res.*, **5**, 70 (1964).
11 Wright, B. S., and Lasfargues, J. C., *J. Nat. Cancer Inst.*, **35**, 319 (1965).
12 Bernard, C., Boiron, M., and Lasneret, J., *CR Acad. Sci.*, **264**, 2170 (1967).
13 Bernard, C., Guillemain, B., Peries, J., and Boiron, M., *Intern. J. Cancer*, **3**, 558 (1968).
14 Bather, R., Leonard, A., and Yang, J., *J. Nat. Cancer Inst.*, **40**, 551 (1968).
15 Levy, J. P., Silvestre, D., Leclerc, J. C., and Boiron, M., *Virology*, **29**, 681 (1966).
16 Sinkovic, J. G., Howe, C. D., and Shullenberger, C. C., *Proc. Third Intern. Symp. Comp. Leukaemia Res., Paris 1967, Bibl. Haemat.*, No. 31, 73 (1968).
17 Boiron, M., Lévy, J. P., and Peries, J., *Prog. Med. Virol.*, **9**, 341 (1967).
18 Wright, B. S., and Lasfargues, J. S., *Nat. Cancer Inst., Monog.*, **22**, 685 (1966).

Isolation and Antigenic Characterization of Lassa Virus

LASSA fever as it was first noticed early in 1969 in three missionary nurses in Nigeria will be described by Frame *et al.*[1]. The disease later affected two laboratory workers at the Yale Arbovirus Research Unit (YARU) in New Haven. Two of the nurses and one of the laboratory workers died.

Studies at YARU have been concerned with attempts to isolate and establish the aetiological agent in experimental hosts—cell cultures and mice—and with its antigenic characterization. A detailed account of this work will be published elsewhere[2], but the current interest in this virus seems to warrant an advance summary.

Sixteen virus isolates, subsequently proved to be alike, were obtained from the following materials: serum taken between days 6 and 12 after onset from the two nurses and laboratory worker who died; serum and pleural fluid taken between days 5 and 14 from the surviving nurse; and serum (days 7 and 9), throat washings (days 9 and 14) and urine (days 9, 17 and 32) of the surviving laboratory worker. (The infection in this last patient will be described elsewhere[3].)

The isolations of Lassa virus were done in Vero cells. A cytopathic effect appeared as early as 4 days after inoculation, and in some instances progressed to complete destruction of the cell sheet within 5–8 days. In addition, distinct plaques measuring 2 mm in diameter were observed 5–8 days after inoculation. Both the cytopathic effect and the production of plaques were specifically inhibited by convalescent serum from the two patients who survived.

Infected Vero cells stained with Giemsa showed basophilic pleomorphic aggregates localized in the cytoplasm; the aggregates were similar in appearance to those described for Junin virus[4]. Electron microscopy revealed spheroid particles closely resembling those reported for lymphocytic choriomeningitis (LCM) virus[5]. Lassa virus failed to infect Singh's[6] *Aedes aegypti* and *A. albopictus* cell lines.

Lassa virus was propagated by intracerebral inoculation in 1 day old mice, which showed no signs of illness but later had antibody in their serum at the same time that virus was detected in their urine. In contrast, young adult mice similarly inoculated became ill, developed convulsions and died. This combined picture is reminiscent of LCM virus infection in mice.

Lassa virus is inactivated by sodium deoxycholate and by betapropiolactone. It appears to contain RNAd as determined by incorporation of 5-bromodeoxyuridine into the medium. As calculated by filtration, the virus has a diameter between 70 and 150 nm.

Complement-fixing antigens could be prepared from infected Vero cells and from infected newborn mouse brain tissue harvested 7 days after inoculation. In serological studies involving large-scale complement-fixing tests as well as haemagglutination-inhibition and plaque reduction neutralization tests, Lassa virus was found to be distinct from more than 200 viruses, including most known arboviruses. By complement-fixing test, a reproducible, low-level relationship was shown between Lassa and LCM viruses and also some members of the Tacaribe group[7].

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1 Frame, J. D., Baldwin, jun., J. M., Gocke, D. J., and Troup, J. M., *Amer. J. Trop. Med. and Hyg.*, **19** (4) (in the press).
2 Buckley, S. M., and Casals, J., *Amer. J. Trop. Med., and Hyg.*, **19** (4) (in the press).
3 Leifer, E., Gocke, D. J., and Bourne, H., *Amer. J. Trop. Med., and Hyg.*, **19** (4) (in the press).
4 Mettler, N., Buckley, S. M., and Casals, J., *Proc. Soc. Exp. Biol. Med.*, **107**, 684 (1961).
5 Dalton, A. J., Rowe, W. P., Smith, G. H., Wilsnack, R. E., and Pugh, W. E., *J. Virol.*, **2**, 1465 (1968).
6 Singh, K. R. P., *Curr. Sci.*, **36**, 506 (1967).
7 Downs, W. G., Anderson, C. R., Spence, L., Aitken, T. H. G., and Greenhall, A. H., *Amer. J. Trop. Med. and Hyg.*, **12**, 640 (1963).

Reactivity of anti-D Immunoglobulin G Subunits

IgG anti-A[1] and IgM anti-B[2] antibodies have been shown to bind by two binding sites to the surface of human group A and B cells respectively. I now report that IgG anti-D, on the other hand, only binds through one of the binding sites on the IgG molecule to Rh-positive red cells.

If IgG anti-D were to bind by both binding sites, the number of univalent Fab molecules with anti-D specificity capable of binding to Rh-positive red cells should be twice as great as the number of intact IgG molecules. Furthermore, the equilibrium constant of IgG anti-D binding by both sites to red cells would be much higher than that of the Fab piece binding univalently[1,3]. To investigate this, Fab pieces were obtained from an IgG preparation containing anti-D in high concentration (anti-D content approximately 1·6 per cent of the total IgG content) by both papain[4] and pepsin[5] digestion and subsequent alkylation with iodoacetamide; $F(ab')_2$ pieces were obtained by pepsin digestion alone (without added cysteine). Fab and $F(ab')_2$ pieces were separated from unsplit molecules and from peptides on a 'Sephadex G-100' column[6]. Intact IgG preparations and the subunits were trace-labelled[7] with ^{125}I. Protein concentrations for

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