Table I. Rat strains and source

Agus PVG/c	MRC Laboratory Animals Centre Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey, Great-Britain.
August Long-Evans Sherman	Centre de Sélection et d'Elevage des Animaux Laboratoire, C.N.R.S., F-54 Orléans-la-Source, France.
B.N. Gunn	Proefdierencentrum K.U.L., De Croylaan 34, B-3030 Heverlee, Belgique.
C.D.	Charles River France S.A. B.P. 29, F-76 St-Aubin-les-Elbœuf, France.
LOU	Animalerie de l'Ecole de Santé Publique, U.C.L., Avenue Chapelle-aux-Champs 4, B-1200 Bruxelles, Belgique.
OFA Fischer	Centre de Recherche et d'Elevage des Oncins, IFFA-Credo, F-69 St-Germain-sur-l'Arbresle, France.
OKAMOTO	Continental Pharma, Stw. Haacht 30, B-1830 Machelen, Belgique.
Wistar R	Département de Radiobiologie, Centre d'Etude et de l'Energie Nucléaire, CEN-SCK, B-2400 Mol, Belgique.

Table II. Geometrical mean (×2) dilution titres of sera giving complete and incomplete haemolysis

Strains	No Rats	Complete haemolysis	Incomplete haemolysis
Agus	5	< 1 [a]	3.2 (3–4) [b]
August	5	1.2 (0–2)	5.6 (5–6)
BN	5	< 1	5.8 (0–8)
CD	5	< 1	2.8 (2–3)
Fischer	5	< 1	1.6 (0–3)
Gunn	5	< 1	6.4 (1–10)
Long-Evans	5	< 1	5.2 (0–9)
LOU	5	< 1	9 (7–10)
OFA	5	3.6 (2–5)	8 (6–10)
PVG/c	5	< 1	4.6 (1–8)
Sherman	5	< 1	2.8 (1–4)
Wistar R	5	2.2 (1–2)	6 (4–7)
F1 (August × LOU)	5	< 1	10.6 (9–11)
F1 (CD × LOU)	5	< 1	8.8 (5–11)
F1 Okamoto × LOU)	5	< 1	6.6 (2–10)

[a] Haemolysis still incomplete with 1:2 diluted rat serum. [b] Range.

The highest dilution of serum producing complete haemolysis was read as the end point, incomplete haemolysis also being taken into account. The titres were recorded as the reciprocals of these serum dilutions.

Results. The highest mean serum dilution giving complete or incomplete haemolyse are listed in Table II. The results represent geometrical mean (×2) dilutions titres; the range is also indicated. With the test conditions employed, complete haemolysis was observed only with 3 of the rat strains, viz. OFA, Wistar R and August. The highest antidody response was observed with OFA rats. All strains responded with humoral antibodies to SRBC but important strain differences were evident.

Discussion. The discrepancies observed between the mean antidoby responses of different rat strains to the same immunogenic challenge with sheep erythrocytes strongly suggest that a genetic factor is involved in this type of immune response. This hypothesis is also consistent with the observation that hybrids between a good responder strain (August) and a moderate responder strain (LOU), or between a moderate (LOU) and a poor responder strain (CD) performed in a manner rather similar to that of the better of the two parent strains.

In this respect our finding with sheep erythrocytes given to rats is reminiscent of those that have been observed with other antigens in various species, viz. the mouse, rat and guinea-pig[2–6]. In the present case the response was not an all-or-not matter, but this may have been due to the fact that the sheep erythrocyte antigen was not administered in a limiting dose.

Résumé. Des rats appartenant à plusieurs souches ont été immunisés par injection i.p. de 2×10^8 globules rouges de mouton. La mesure du titre des haemolysines réalisée 6 jours plus tard montre des différences quantitatives selon les souches. Une réponse importante n'a pu être décelée que pour 3 d'entre elles (August, OFA et Wistar R).

C. André[7–8], H. Bazin[9], Andrée Beckers and J. F. Heremans

Faculté de Médecine,
Université Catholique de Louvain (Belgium),
4 August 1972.

[2] H. O. McDevitt and I. Green, in *Progress in Immunology* (Ed. B. Amos; Academic Press, New York 1971).
[3] H. O. McDevitt, K. B. Bechtol, F. C. Grumet, G. F. Mitchell and T. G. Wegmann, in *Progress in Immunology* (Ed. B. Amos; Academic Press, New York 1971).
[4] L. Ellman, I. Green and B. Benacerraf, J. Immun. *107*, 382 (1971).
[5] I. Green and B. Benacerraf, J. Immun. *107*, 374 (1971).
[6] B. Benacerraf and H. O. McDevitt, Science *175*, 273 (1972).
[7] Chargé de Recherche, Unité 45, Institut National de la Santé et de la Recherche Médicale, Lyon, France.
[8] Mailing address: Dr. C. André, Avenue Chapelle-aux-Champs 4, B-1200 Bruxelles (Belgium).
[9] Staff Member of the EURATOM Biology division, Publication No. 801.

Diffusible Factor of Thymus is Responsible for the Recovery From Some Effects of Heterologous Antilymphocyte Serum

The target of heterologous antilymphocyte serum (ALS) are thymus dependent lymphocytes[1] but the role of the thymus in the mechanism of action of ALS remains uncertain. Thymectomy potentiates the immunosuppressive effect of ALS[2], a fact not confirmed by others[3]. Jeejeebhoy and Singla[4] postulated that the delay in the recovery from ALS effects is due to the presence of free immunosuppressive antibodies in the blood of

experimental animals but the dependence of these antibodies on the thymus has not been demonstrated.

We designed several experiments to elucidate further the thymus dependence of the ALS level in the circulating blood of experimental animals.

Materials and methods. Two months old, male, highly inbred Balb/C mice were used throughout the experiments. Antimouse, rabbit ALS prepared as indicated in the legends to figures, was titrated by lymphoagglutination method of AMOS[5] and by the lymphocytotoxic test of DAUSSET[6]. Thymectomy of adult mice was performed according to MILLER[7]. In one group of thymectomized mice we installed i.p., one day after thymectomy, a millipore diffusion chamber (pore size 0.3 μm) with whole isologous 10-days-old thymus. We determined the titre of circulating free anti-lymphocyte antibodies (FALA) by sampling the serum from a single mouse at different time intervals after injection of ALS and checking for the presence of lymphoagglutinating and lymphocytotoxic activity by methods[5] and[6].

Results. A profound and prolonged lymphopenia is observed in the group of thymectomized mice as late as 96 h after treatment with a single dose of 0.5 ml of ALS (Figure 1). The recovery of lymphocytes was lower than that of leukocytes. Controls were performed by substituting ALS with normal rabbit serum (NRS) and isotonic saline for all groups of experimental animals. Figure 2 shows that, in nonthymectomized and in thymectomized mice with reconstituted thymus, the decay of the lymphoagglutinating activity of the serum is very fast. Similar decay was observed also for lymphocytotoxic activity. In thymectomized mice the decay is slow and even at 96 h the FALA are still detectable. After administration of NRS or isotonic saline no antisera could be detected. In experiments in which an empty, or an isologous spleen filled diffusion chamber was installed, the lymphocyte counts and decay kinetics of antibodies were similar to the values found in thymectomized mice.

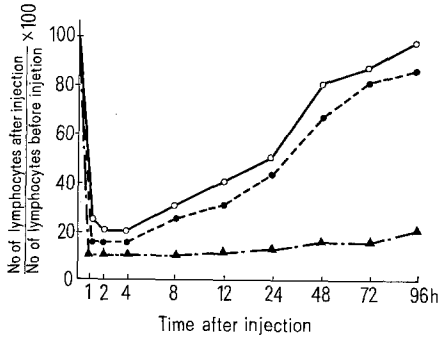

Fig. 1. New Zealand rabbits were given i.v. twice, 14 days apart, washed 10^9 thymus cells of Balb/C mice, then bled 7 days after the last injection. ALS from blood of 5 rabbits was pooled, decomplemented, liberated from hemoagglutinins and hemolysins against mouse erythrocytes by standard procedures. The figure shows the effects of the administration of 0.5 ml of ALS on the lymphocytes count in the peripheral blood of mice in various experimental conditions. Each point corresponds to the medium of 10 determinations.

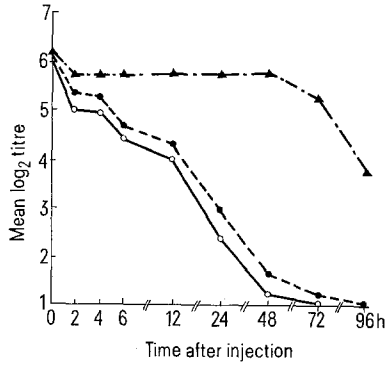

Fig. 2. Presence of free lymphoagglutinating antibodies in the sera of ALS treated mice. Each point corresponds to the medium of 10 determinations. 0.5 ml of ALS prepared as indicated above was given to experimental mice. ○—○, Nonthymectomized mice; ●---●, thymectomized mice with reconstituted thymus; ▲---▲, thymectomized mice.

Discussion. We have clearly shown that the recovery from lymphopenia induced by ALS, and the decay of FALA are strictly thymus dependent. Lymphopenia in ALS treated and thymectomized mice is associated with the high titre of FALA. In ALS treated thymectomized mice the repair of cell counts occurs after complete disappearance of FALA. It was suggested that the repair of immunological deficits in ALS treated animals is due to the migration of cells from bone marrow and thymus[8]. Our results indicate that the repair is caused by a diffusible humoral factor of the thymus. The absorption of FALA by the reconstituted thymus offers an alternative explanation of the lymphocyte count repair. However, the inability of the spleen put into the diffusion chamber to bind the antibodies does not seem to support such an alternative. The diffusible factor probably stimulates the recovery of leukocytes which consume the circulating FALA. In thymectomized ALS treated mice, the number of peripheral leukocytes is too low to cause any appreciable FALA consumption.

Riassunto. Nel sangue di topi timectomizzati trattati con ALS un alto e prolungato livello di FALA si accompagna ad una linfopenia mentre nei topi non-timectomizzati il pronto ripristino dei valori leucocitari si attua in concomitanza alla rapida scomparsa dei FALA dal siero. Viene messo in evidenza che responsabile del ripristino di questi effetti del siero antilinfocitario è un fattore umorale diffusibile del timo.

E. GARACI and W. DJACZENKO

Institutes of Microbiology, Chieti University, I-66100 Chieti, and Rome University, I-00100 Rome (Italy), 14 August 1972.

[1] W. J. MARTIN and J. F. A. P. MILLER, J. exp. Med. *128*, 855 (1968).
[2] A. P. MONACO, M. L. WOOD and P. S. RUSSEL, Ann. N.Y. Acad. Sci. *129*, 190 (1966).
[3] R. H. LEVEY and P. B. MEDAWAR, Proc. natn. Acad. Sci. USA *56*, 1130 (1966).
[4] H. F. JEEJEEBHOY and O. SINGLA, Immunol. *22*, 789 (1972).
[5] D. B. AMOS and N. PEACOCKE in, *Proc. 9th Congr. Europ. Soc. Haematol. Lisbon* (S. Karger, Basel, New York, 1963), p. 1132.
[6] J. DAUSSET in, *Histocompatibility testing*. Washington, D. C. Nat. Acad. Sci. (1965), p. 147.
[7] J. F. A. P. MILLER, Br. J. Cancer *14*, 93 (1960).
[8] H. F. JEEJEEBHOY and D. O. SINGLA, Immunol. *22*, 801 (1972).