Induction of an Auto-Haemoantibody Response in Rats Injected with a Rabbit's RNA-Immuno-Carrier

Evidence that an immuno-carrier RNA (RNA-I-C) is present in the serum of immunized rabbits has been reported in a previous paper[1]. RNA-I-C extracted from serum of an immune animal injected into another animal of the same species determines antibody production against the same antigen used for immunizing the first animal producer of RNA-I-C[2]. According to these previous results it was argued that the immune response might be possible, using the same RNA-I-C also in animals of another species. If this hypothesis is correct the injection in the rat of RNA-I-C from serum of a rabbit immunized against rat red blood cells may induce in the same rat auto-haemoantibody production. The present work describes results showing that, in the serum of rats injected with RNA-I-C extracted from an anti-rat erythrocytes rabbit serum, there is a production of auto-haemoantibodies.

Female rabbits weighing 1.5–1.8 kg, were immunized by 6–8 intravenous injections of rat or guinea-pig red blood cells (RBC). The RNA was extracted as described in previous papers[1,2] from rabbit hyperimmune sera, and then introduced by one intracardiac injection into normal rats, in the amount of 0.28 mg/100 g body weight, suspended in 1 ml of 0.154 *M* NaCl. Male and female Wistar strain rats, weighing 180–200 g, were used. Animals of equal age were selected for each experiment. A first group of rats was used as a control; a second group of rats was injected with RNA-I-C extracted from anti-rat RBC rabbit sera as indicated above, a third group of rats received only 0.07 mg/100 g body weight of RNA-I-C from anti-rat RBC rabbit sera; finally a fourth group of rats was treated with RNA-I-C from anti-guinea-pig RBC rabbit sera in the amount of 0.28 mg/100 g body weight. The following determinations were made on the rats of the four groups in order to ascertain an eventual haemolytic immune response *in vivo*: (i) the red blood cell count was repeated 12, 24, 36, 48, 72, 96, 120, 144 and 168 h after challenge; a drop of blood was collected from the tails of rats with the method suggested by CUSHNIE[3] and the count made by a Thoma counting chamber; (ii) haemoglobin estimation was made by Bausch-Lomb Spectronic 20 procedure and haemoglobinuria detected by the usual benzidine reaction on urine samples collected in special cages; (iii) the reticulocyte count was made by the brilliant cresyl blue dry method; (iv) at the end of the experiments, the spleens of the rats were removed, fixed in 95° alcohol, and haemosiderin was demonstrated in the sections by the Berlin blue and by Turnbull's methods.

The results of RBC counts are reported in Figure 1: a strong decrease in RBC number was observed only in the rats injected with 0.28 mg/100 g body weight of RNA-I-C from anti-rat RBC immune rabbit sera; a moderate decrease was obtained in the rats which received 0.07 mg/100 g body weight of RNA-I-C; no significative changes were observed in the rats injected with RNA extracted from rabbit anti-guinea-pig RBC sera and in the normal controls. Haemoglobin concentrations of red blood cells decreased only in the rats treated with RNA-I-C from anti-rat sera. Colour index remained approximately unchanged. Reticulocytosis was found 96 and 120 h after the injection only in animals which received the RNA from anti-rat RBC sera (Figure 2). The haemoglobinuria was detected only in the case of rats treated with RNA from anti-rat RBC sera: the benzidine test was strongly positive 48 h after the RNA injection. No red cells were detected in the sediment. Haemosiderin was found in more considerable amounts in the sections of spleen of rat injected with RNA-I-C from rabbits immunized with rat RBC; small amounts of haemosiderin were occasionally demonstrated also in the spleen of rats treated with RNA from rabbit anti-guinea-pig sera RBC. In both

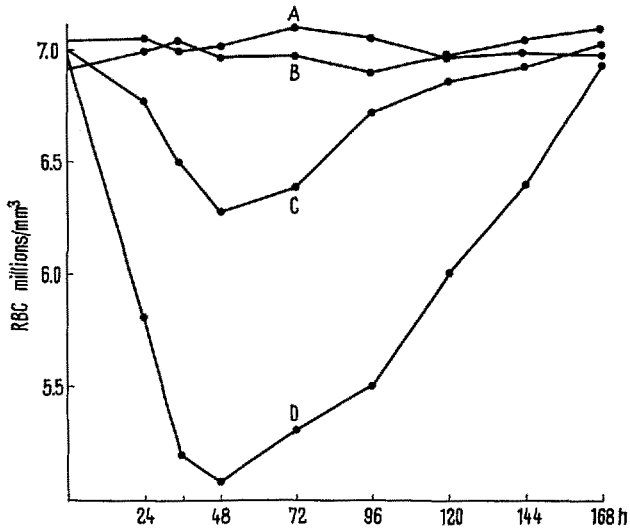

Fig. 1. RBC counts of A, normal rats; B, normal rats treated with anti-guinea-pig RBC RNA (0.28 mg/100 g body weight); C, normal rats treated with anti-rat RBC RNA (0.07 mg/100 g body weight); and D, with anti-rat RBC RNA (0.28 mg/100 g body weight).

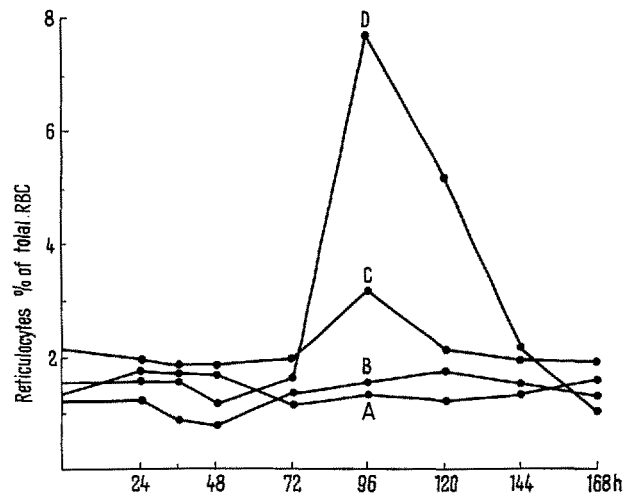

Fig. 2. Reticulocyte counts. Legend as for Fig. 1.

[1] L. MICHELAZZI, G. NANNI, and I. BALDINI, as reported to meeting Soc. Ital. Biol. sperim. (March 25, 1964).

[2] L. MICHELAZZI, G. NANNI, I. BALDINI, and A. NOVELLI, Exper. *20*, 447 (1964).

[3] G. H. CUSHNIE, in *The UFAW Handbook on the Care and Management of Laboratory Animals* (UFAW, London 1957), second ed., p. 368.

cases the lymphoid follicles were hypertrophics (Figure 3); the haemosiderin was scarce or absent and the lymphocytic centres were normal in the controls. Finally, controls of auto-haemagglutination and haemolysis *in vitro* on blood microsamples partially demonstrated a clumps formation only in rats treated with RNA from anti-rat RBC immune rabbit sera; a haemolytic response was observed, at titers of 1:20–1:40, only in the rats treated with 0.28 mg/100 g body weight of RNA from anti-rat RBC sera.

The presence of auto-haemoantibodies was also demonstrated by the usual immunofluorescence sandwich method: specimens of rat RBC treated with serum from rats injected with 0.28 mg/100 g body weight of RNA-I-C from anti-rat RBC immune rabbit sera, became fluorescent after incubation with fluorescein-conjugated anti-rat-globulin serum. The controls were always negative.

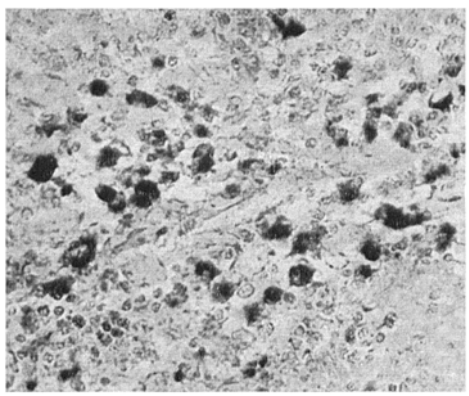

Fig. 3. Haemosiderin pigment in the spleen of a rat 7 days after injection of anti-rat RBC RNA (0.28 mg/100 g body weight).

The data obtained indicate that after injection into normal rats of a sufficient quantity of RNA extracted from rabbit anti-rat RBC immune sera a haemolytic immune reaction takes place in the rats with production of auto-haemoantibodies. This means that it is possible to obtain an immune response in an animal using an RNA-I-C produced by another species of animal. This immune response, according to previous results[1,2], is precocious but transitory: in fact it reaches the utmost degree 24–48 h after the RNA introduction and after 96 h it disappears[4].

Riassunto. Una produzione di auto-emoanticorpi è stata ottenuta in ratti normali iniettati con un RNA estratto dal siero di conigli immunizzati con globuli rossi di ratto.

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Der Sauerstoffumsatz der Leber von Ratten während der Höhenakklimatisation

Ausser den Untersuchungen von SUNDSTROEM und MICHAELS[1] gibt es keine Berichte über die Atmung von Organgewebe von Versuchstieren in der ersten Phase der Akklimatisation an Sauerstoffmangel. Die meisten Untersucher berichten, dass am Ende einer Höhenakklimatisationsperiode von mehreren Wochen und Monaten der O_2-Umsatz gleich ist wie der von Tieren die unter normalen Druck (760 mm Hg) leben. Da aus dem Abfall der Körpergewichtskurve, des Futterkonsums und der lokomotorischen Aktivität von Ratten während der ersten Tage in der Höhe (500 mm Hg) hervorgeht, dass die Tiere ihren Gesamtstoffwechsel reduzieren, ist anzunehmen, dass nicht nur der O_2-Umsatz der Muskeln, sondern auch der der inneren Organe herabgesetzt ist[2]. Um diese Hypothese zu prüfen, wurde der O_2-Umsatz der Leber, als das wichtigste Organ im intermediären Stoffwechsel, während der ersten 10 Tage der Akklimatisation an die Höhe von 3450 m untersucht.

Material und Methoden. Verwendet wurden junge und alte männliche Sprague-Dawley-Ratten, aus eigener Zucht und der Tierfarm Füllinsdorf (BL). Die Tiere wurden bei 22°C Raumtemperature und R.F. von 40–60% in geräumigen Käfigen auf Hobelspänen gehalten; Kunsttag von 07.00–19.00 h. Sie erhielten Wasser und Standardfutter (Altromin R) *ad libitum*. Die Tiere gleichen Alters wurden in Gruppen unterteilt, von denen ein Teil in den Tierraum der Hochalpinen Forschungsstation Jungfraujoch (3450 m) gebracht wurde und ein Teil als Kontrolle in dem Tierraum in Bern (540 m) blieb. Jedes Tier wurde täglich gewogen. Am Untersuchungstag wurden die Tiere morgens zwischen 10.00–11.00 h direkt oder nach Betäubung durch Schlag auf den Kopf guillotiniert, die Leber sofort herausgenommen, gewogen und bei 0–2°C gehalten. Die Lebern der Tiere aus der Höhenstation wurden einzeln in Gläser, die mit Krebs-Ringer-Lösung (0,6% $CaCl_2$) getränkte Watte enthielten, gefüllt und im Thermostopf mit Eis innerhalb 4 h zur Untersuchung nach Bern gebracht. Der O_2-Umsatz wurde nach Warburg gemessen. Schneiden der Leber nach DEUTSCH[3], Schnittdicke ca. 0,5 mm; 50–60 mg Feuchtgewebe pro Gefäss; Medium Krebs-Ringer-Phosphatlösung (0,61% $CaCl_2$); pH 7,3; Wasserbadtemperatur 37,5°C; Gasphase 100% O_2. Der Qo_2 wurde berechnet als mm^3 O_2/mg Trockengewicht/h. Von jeder Leber wurden Doppelbestimmungen vorgenommen.

[1] E. S. SUNDSTROEM und G. MICHAELS, *Memoirs University of California* (University of California Press, Berkeley and Los Angeles 1942), vol. 12.

[2] W. H. WEIHE, *Biometeorology II*, Proc. Third Int. Biometeor. Congr., Pau (S. W. TROMP and W. H. WEIHE, Ed.; Pergamon Press, Oxford), im Druck.

[3] W. DEUTSCH, J. Physiol. (Lond.) *87*, 56P (1936).