glucagon and/or pancreatic polypeptide immunoreactivities, approximately 75% of the cells contain only glucagon immunoreactivity, 15% contain only pancreatic polypeptide immunoreactivity, whereas 10% of the cells contain both immunoreactivities. The detailed distribution of these three cell types in islets of different regions of the pancreas remains to be studied. Presence of multiple peptides in one cell type is not uncommon in nervous and endocrine systems[11], including the A cells in endocrine pancreas[12–14]. In some instances, such presence of multiple peptides can be attributed to the presence of a large precursor hormone[15,16]. However, presence of separate synthetic machineries in the same cells is also possible[17]. The possibility that the present observation represents the identification of a new peptide which contains the immunodeterminants of both glucagon and pancreatic polypeptide also exists. Relevant to the present observation are the reports of presence of bovine pancreatic polypeptide, glucagon and glicentin immunoreactivities in human colorectal mucosa[18] and in cat intestine[19]. Glicentin is known to contain the immunodeterminants of glucagon[20,21]. But, the relationship of glicentin to bovine pancreatic polypeptide is unknown.

Nevertheless, the presence of glucagon and pancreatic polypeptide immunoreactivities in the same cells in frog[4,5] and, to a lesser extent, in rat suggests a close relationship of the two peptides. It is tempting to speculate that, perhaps at some point in vertebrate evolution, these two peptides were handled simultaneously in their production, and that these glucagon-pancreatic polypeptide cells in rat represent a primitive cell type which handles glucagon and pancreatic polypeptide production in a similar manner as frog islet cells do.

In addition to immunohistochemical studies in other vertebrates, future biochemical and physiological studies are necessary to understand the exact nature of the relationship between glucagon and pancreatic polypeptide and the significance of the glucagon and pancreatic polypeptide containing cells in rat islets.

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Loss of antibody production accompanied by chromosome loss in a cloned hybrid line secreting antibodies to sheep red blood cells

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Summary. Somatic cell hybrids between Sp2/O-Ag14 mouse myeloma cells and lymphocytes derived from BALB/c mice hyperimmunized with sheep red blood cells (SRBC) were produced. One hybrid producing IgG_1 antibody to SRBC was selected, cloned twice and subsequently transferred to BALB/c mice. After a number of transfers it was found that the antibody titer in ascites fluid gradually decreased. Cytogenetic analysis revealed gradual chromosome loss in the hybrid clone, which produced progressively less antibody.

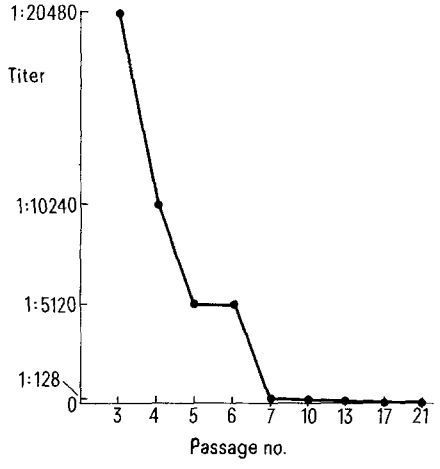
Key words. Myeloma cells, mouse; lymphocytes, mouse; cloned hybrid cell line; somatic cell hybrids; antibody production; chromosome loss.

The somatic cell hybridization technique developed by Kohler and Milstein[1,2] has made it possible to generate hybrid cell lines synthesizing monospecific antibodies. However, the production of antibody is often lost when the hybrids are grown in mass culture. This could probably be due to a number of factors. In the work described in this report it was found that the loss of specific antibody production is accompanied by, and is most likely due to chromosome loss.

Materials and methods. BALB/c mice were injected twice with 2×10^7 SRBC i.p. at two-week-intervals and boosted after three weeks with the same dose. Spleens were taken three days later. 4×10^6 pooled spleen cells from three mice were fused with 2×10^6 Sp2/O-Ag14 myeloma cells using polyethylene glycol 1500 (Merck), essentially as described previously by Galfre et al.[3]. Specific antibodies to SRBC were detected using a standard hemagglutination assay. Cloning and recloning of hybrid cultures was performed under limiting dilution conditions[4]. The karyotype of ascites fluid cells was analyzed according to the method of Ford[5].

Results and discussion. Spleen cells from BALB/c mice hyperimmunized with SRBC were fused with Sp2/O-Ag14 myeloma cells and plated in two 96-well tissue culture clusters at 0.2 ml

per culture. Out of 192 cultures 51 showed growth of hybrid cells. In 21 cultures hybrid cells secreted antibodies specific for SRBC. The culture with the highest titer of anti-SRBC antibody was selected and cloned twice under limiting dilution conditions. Then the clone was expanded to mass culture and injected i.p. to BALB/c mice. Testing ascites fluids two weeks later by Ouchterlony analysis showed that the clone was secreting IgG_1 antibody. Antibody titer was assessed after subsequent in vivo passages of hybridoma cells. After a number of passages it was found that the hybrid clone produced progressively less antibody (fig.). Ascites fluid from the third passage had the titer of 1:20,480 which dropped to 1:5120 after the next three passages. After seven passages, that is approximately 4.5 months after fusion, the antibody titer decreased gradually. Finally after the 13th passage, that is six months after fusion, the antibody titer was negligible. Eight months after fusion (21st passage) the antibodies to SRBC were not detectable in ascites fluids.



Karyotype of hybridoma cells producing antibody to SRBC, compared with the antibody titer

Number of metaphases analyzed	Chromosome number	Passage number	Antibody titer
50	104 ± 4*	3	1:20,480
47	104 ± 7	6	1:5120
50	94 ± 4**	13	1:4
49	96 ± 7**	21	0

* Mean value ± SD. ** $p < 0.001$ vs. the chromosome number in the passage No. 3.

It is of interest that the decrease of antibody titer was accompanied by a decrease in the chromosome number in the hybrid cells. Chromosomal analysis showed that the hybridomas secreting specific antibodies had a mean chromosome number of 104 ± 4 (table). Six months after fusion, the chromosome number dropped to 94 ± 4 and did not change significantly in the following two months (21st passage; table). Loss of antibody production by hybridoma cells is probably due to a number of factors. The simplest situation is when the antibody forming hybridoma is one among a number of non-antibody-secreting hybrids in a given culture. When the antibody producing hybridoma is growing more slowly than non-producing hybrids, then it may rapidly be overgrown by the other cells in the culture. This possibility was excluded in the present study by cloning twice the hybrid clone producing antibodies to SRBC. Loss of antibody production may also be due to segregation of the genes for antibody heavy and/or light chains[6,7]. On the basis of the data presented here it may be postulated that the observed loss of antibody production is most probably due to chromosome loss in the hybrid line studied.

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On the mechanism of killing of *Trypanosoma cruzi* by human polymorphonuclear leukocytes[1]

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Summary. The temperature-dependence of some processes involved in the killing of sensitized *T. cruzi* epimastigotes by human polymorphonuclear leukocytes (PMN) was determined. The rate of the reactions was related to the temperature of incubation according to the Arrhenius equation and the apparent energies of activation (Ea) were calculated. The Ea values separated these complex reactions into two groups: one with Ea of about 10 kcal/mol for the phagocytosis of the parasites and the release of lysosomal enzymes by PMN, and the other with Ea of about 22 kcal/mol for the cytotoxicity against sensitized *T. cruzi*, the rate of oxygen consumption by PMN, and the lysis of the parasites with added hydrogen peroxide.
Key words. Trypanosoma cruzi; polymorphonuclear leukocytes.

Peripheral human polymorphonuclear leukocytes (PMN) are able to kill in vitro the epimastigote and amastigote forms of *T. cruzi* in the presence of antibody directed against the parasite[3-6]. At present only the characteristics of the killing of the epimastigote forms of *T. cruzi* had been extensively documented[3,4,6-10]. It had been demonstrated that the interaction between the Fc receptor of PMN and the Fc from the antibody bound to *T. cruzi* epimastigotes leads to phagocytosis and par-