karyotype from that already described. In the spermatogonial cell at metaphase there are 17 chromosomes (Figure 5). 12 large autosomes and 2 small *m*-chromosomes are clearly distinguishable. The remaining 3 elements are probably the sex-chromosomes. The primary spermatocyte nuclei are seen to possess 3 distinct heteropycnotic bodies which represent the sex-chromosomes observed at first as 3 heteropycnotic masses (Figures 6 and 7) and at the later stages as deeply stained bipartite elements easily detectable from the autosomal bivalents (Figures 9 and 10). At the second spermatocyte metaphase, 2 different sets of cells, one set containing sex-pseudotrivalent in the order '*X* 1 supernumerary *Y*' or '*X Y* 1 supernumerary' (Figures 11 and 12) and the other containing the sex-pseudotrivalent in the order '*Y X* 1 supernumerary' (Figures 13 and 14), are observed.

The pairing of *m*-chromosomes occurs later than the association of the homologous autosomes and therefore the primary spermatocyte plates contained, independently of a different sex-chromosome number, an unequal number of the remaining elements (Figures 2, 3 and 9,10). The *m*-chromosomes, separated or paired, are easily detectable from the autosomal bivalents by their different size and from the sex-chromosomes, which are deeply stained and have bipartite structure, too. It is impossible not to distinguish the 2 sets of cells, although they have the same number of chromosomal elements.

The number, $2n = 16$ in *Trapezonotus arenarius* L. described by PFALER-COLLANDER[3] and by the author, seems to be a modal number of this species and *XX:XY* sex-mechanism is characteristic. On the other hand, however, the diploid number of chromosomes determined as 17 (based on the author's observations) clearly indicates the existence of a multiple sex-chromosome mechanism.

Zusammenfassung. Die charakteristische Grundzahl der Chromosomengarnitur bei *Trapezonotus arenarius* L. wurde mit $12A + 2m + X + Y$ gefunden, wobei ein Individuum mit überzähligem Geschlechtschromosom festgestellt werden konnte.

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Studies on Human Lymphocytes Stimulated in vitro with Anti-γ and Anti-μ Antibodies

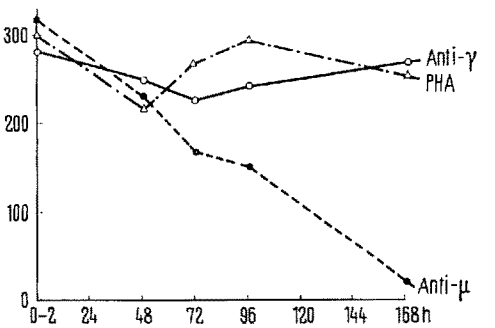
Peripheral lymphocytes of rabbits may be stimulated in vitro to transform into blast cells and to synthesize DNA if cultured in the presence of antisera to rabbit γG-globulin or specific antiallotype sera (SELL and GELL[1], SELL et al.[2]).

The present report describes experiments designed to see if human lymphocytes would transform into blast cells and synthesize DNA after being stimulated with antibodies against the heavy chains of γG-globulin (γ-chain) and of γM-globulin (μ-chain).

Materials and methods. Horse anti-γG-globulin serum (containing 9.2 mg of anti-γG/ml) was made specific to γG heavy chain (anti-γ) by inhibition with light chains prepared as described by FLEISCHMAN et al.[3]; the anti-μ serum was prepared by injecting γM-globulin into a rabbit and by inhibiting the antiserum with cord serum (ADINOLFI et al.[4]).

Before being used, the antisera were heated at 56 °C for 20 min and absorbed 3 times with a mixture of red and white cells.

Samples of blood were collected by venipuncture from a healthy donor (B.G.); 10 ml of blood were mixed with 0.1 ml of heparin (5000 IU/ml). After centrifugation at 2000 rpm the plasma was discarded; the buffy coat was recovered and the cells were washed 4 times in Hank's B.S.S. (Difco) and finally suspended in 5 ml of the same solution. Aliquots of 1 vol. of the cell suspension were mixed with 2 vol. of the solution containing each stimulating factor under test, i.e. phytohemagglutinin (Burroughs Wellcome), anti-γ and anti-μ. 0.6 ml of each mixture was transferred to culture bottles, each containing 2 ml of foetal calf serum (Grand Island Biological Co., USA) and 6 ml of T.C. 199 Difco. The cultures were gassed with 5% CO_2 and incubated at 37 °C; 1 culture from each group was terminated at intervals of 24 h; 2 h before



Number of cells in cultures stimulated with PHA, anti-γ or anti-μ (see text).

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processing, 0.4 ml of a solution of tritiated thymidine (5 μc/ml) (Radiochemical Centre, Amersham) was added. Immediately before processing, each culture was thoroughly mixed and 1 ml of the sample removed for white cell counting (Neubauer chamber) by 2 independent observers. The rest of the culture was centrifuged, the supernatant discarded and the cells washed in Hank's B.S.S. The cells were then processed as for chromosome studies (ROTHFELS and SIMONOVITCH[5], ARAKAKI and SPARKES[6]).

Autoradiographies were prepared by the stripping film technique, as described by LAJTHA[7]. The preparations were developed after 5 days and stained (GIANNELLI[8]). Only cells showing at least 5 autoradiographic grains were scored as labelled.

Results. In the first group of cultures the white cells were grown in the presence of phytohemagglutinin (PHA) anti-γ and anti-μ antibodies. In the experiment shown in the Figure, 0.4 ml of anti-γ, 0.4 ml of anti-μ or 0.4 ml of PHA diluted 1:8 were added to each culture. At time 0 each group of cultures contained about 300 white cells for 1 mm^3. The cultures were terminated at 2, 48, 72, 96 and 168 h. The number of the cells in each group of cultures dropped during the first 48 h; later the number of cells increased in the cultures stimulated with PHA and anti-γ antibody, but decreased rapidly in the cultures stimulated with anti-μ antibody. The transformation of lymphocytes to blast cells was observed in the cultures stimulated with PHA and with anti-antibodies.

The autoradiographic analysis on the DNA synthesis confirmed that PHA and anti-γ had a stimulating action on the cells; in fact, in the cultures stimulated by PHA, 4, 29, 47 and 16% of the cells were labelled at day 2, 3, 5 and 7. In the cultures stimulated with anti-γ, 3 and 6.6% of the cells were found to be labelled at day 4 and 7 (see Table I).

Table I

	0–2	24	48	96	168 h
PHA	0	4	29	47	16
Anti-γ	0	0	0	3	6.6
Anti-μ	0	0	0	0	0

Number of cells (expressed as %) incorporating tritiated thymidine in vitro. The cells were stimulated with phytohemagglutin (PHA), antibody against the heavy chain of γG-globulin (anti-γ) or antibody against the heavy chain of γM-globulin (anti-μ).

Table II

	0–2	72	96	120	144	168 h
(1) Anti-γ 0.4 ml	0	0.5	1.5	5	8	3
(2) Anti-γ 0.2 ml (then 0.2 ml after 72 h)	0	1.0	0	5.5	5.5	–
(3) Anti-γ 0.4 ml (for 30 min)	0	1.5	0	2	6	2.5

Number of cells (expressed as %) incorporating tritiated thymidine in vitro after stimulation with anti-γ serum.

The anti-μ antibody did not seem to induce transformation and synthesis of DNA; this negative result was confirmed in other series of experiments. Further investigations were carried out with anti-γ antibody. Table II shows the results obtained by treating the white cells under 3 conditions, in an attempt to investigate the dynamics of the transformation process. (1) In the first series of cultures 0.4 of anti-γ was added to each culture. (2) In the second series the cells were stimulated with 0.2 ml of anti-γ; after 72 h another 0.2 ml of anti-γ was added to each culture. (3) 1 vol. of the suspension of white cells was mixed with 2 vol. of anti-γ; the mixture was incubated at 37 °C for 30 min, then the cells were washed 3 times and resuspended in subcultures.

As shown in Table II, it appears that the % of cells which synthesized DNA was similar in each series of 3 cultures.

Discussion. As other investigators have reported, human peripheral lymphocytes treated in vitro with PHA are transformed to blast cells and synthesize DNA (MACKINNEN et al.[9], COOPER et al.[10], HAYHOE and QUAGLINO[11]). The present data demonstrate that a similar effect may be obtained by treating human lymphocytes in vitro with an antibody specific against the heavy chain of γG-globulin. The transformation and the synthesis were induced only when the anti-γ antibody was used in high concentration.

By comparison with PHA the induction, as judged by thymidine incorporation, was delayed. However, transformation and synthesis of DNA were noticed even when the lymphocytes were left in contact with the anti-γ antibody for 30 min only. The latter result is similar to that obtained by SELL et al.[2], using rabbit lymphocytes in the presence of antiallotype sera.

The failure to induce transformation and synthesis of DNA with an anti-μ antibody may be due either to the fact that peripheral lymphocytes do not release γM-globulin, or to the fact that the antibody used was not strong enough. Further investigations are being undertaken to clarify this problem.

Zusammenfassung. Es konnte gezeigt werden, dass sich menschliche periphere Lymphozyten, mit Antikörper gegen die H-Ketten des γ-Globulins behandelt, in Lymphoblasten umwandeln lassen und dass sie in vitro DNA synthetisieren.

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