CYTOGENETICAL AND IMMUNOMORPHOLOGICAL STUDY OF CHLORPROMAZINE EFFECT ON HUMAN LYMPHOCYTE CULTURES IN VITRO

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Phenothiazine neuroleptics are the subject of a great number of studies aimed at estimating their mutagenic threat for man. For the purpose outlined a variety of test-systems are employed, such as genic mutations of microorganisms with or without beforehand metabolite (or with hepatic homogenate) activation of the drug under test within the organism of mammals; genetic (point) mutations in Drosophila melanogaster; dominant lethal mutations in mammals; cytogenetic investigation of bone marrow, tissue and cell cultures in mammals; mutagenesis of cultures from specially treated cell strains; micronuclear test in mammals.

In the treatment of humans it is possible to assay both mutagenic action of the medicament, through cytogenetic study of lymphocyte cultures, and its teratogenic action in case of eventually observed monstruosities in the offspring. Under experimental conditions, the cytogenetic investigation of lymphocyte cultures treated in vitro is very extensively used.

According to survey data, submitted ty Y. L. Shapiro et al (1972 — 31), a teratogenic effect was recorded twice in the course of stelazine (trifluoperazine) treatment of pregnant women (B. Horner and G. Hall — 1973). Also, reports are available on chromosome aberrations among individuals undergoing stelazine treatment (E. Jenkins, 1970 — 45). However, on drosophila (I. A. Rappoport et al, 1971 — 21) and on lymphocyte cultures (V. S. Zhurkov, 1975 — 12) no mutagenic activity of the preparation has been found.

In the opinion of some authors (K. Petersen et al, 1973 — 53) triflupromazine causes dominant lethal mutations in mice, whereas according to others (V. Ray et al, 1973 — 54) on the basis of complex investigation of the preparation, mutagenic activity is detectable neither in mice insofar as dominant lethal mutations and chromosome aberrations in bone marrow are concerned, nor in salmonella with metabolite activation and in human lymphocyte cultures.

Reports on mazheptil (thio- /pro/ perazine) claim an insignificant increase in chromosome breaks in the course of treatment (M. Krachunova, 1975 — 16), whilst in lymphocyte cultures, in vitro, it exerts a mild cytogenetical effect, manifested at concentrations exceeding the therapeutical dose 50—100 times (12). On drosphila no mutagenicity is recorded (21), while in mice bone marrow it is not cytogenetically active (L. Chioreame et al, 1967 — cited by 31; Y. A. Revazova et al, 1975—22). Also, no point mutations on salmonella are found, although dominant lethal mutations in mice, as well as certain increase in chromosome breaks in lymphocyte cultures, treated in vitro, are established, warranting its classification as a weak mutagen (22)1

Perphenazine, according to some authors (J. Nielsen et al, 1969 — 51) augments chromosome deletions among treated individuals, which is denied

by others (M. Cohen et al, 1972 — 38). On drosophila etaperazine (perphenazine) is manifested as a medium strong mutagen (I. A. Rappoport et al, 1965—20)

Phrenolon (metaphenazine) and propazine (promazine) do not prove mutagenically active on drosophila and lymphocyte cultures (21, 12).

Pipolfen (promethazine) which has a marked mutagenic activity for drosophila, failed to manifest a similar activity on lymphocyte cultures (12).

Treatment with thioridazine and fluphenazine does not account for increase in chromosome aberrations in the lymphocytes (M. Cohen et al, 1969 — 36). Fluphenazine (liogen) does not produce mutations on drosophila (21).

Similarly, no mutations on drosophila are observed in the course of experiments with neuleptil (propericyasine), randolectil (butylperazine) and teralen (methylpromazine) (L. M. Filipova et al, 1975 — 26).

Chlorcisine (diethylaminopropyl-chlor-phenothiazine) exerts a strong mutagenic effect on drosophila (20).

Chlorpromazine (CP) — dimethylaminopropyl-chlor-phenothiazine — known also under the trademark names largactil, plegomazine, chlorazine, megafen, thorazine, aminazine etc — is a neuroleptic most frequently and widely used in the medical practice, and also, the subject of numerous researches.

As regards megaphen (49) and aminazine (21), no mutagenic activity on drosophila whatever is established.

CP administration to pregnant rats produces abortions and underdevelopment of the fetus (1. Chambon, 1955); administered to female and male mates it reduces fertility while the progeny shows poor development (B. and L. Doty, 1963). In mice, however, CP does not account for lethal mutations (S. Epstein et al, 1968 — 40). According to more recent data, in mice it inhibits the fertilized ovum implantation and methionine transport towards blastocyte (R. Borland et al, 1975 — 33).

CP at dose 1—20 mcg/ml, administered over a period of 24 hrs on cell cultures from Chinese hamster, doesn't cause chromosome aberrations (F. Kelly—Garvet et al, 1973 — 48).

According to data published by R. Shram in 1974, the mutagenic activity of aminazine is manifested on cell cultures from mammals and man, and on microorganisms upon metabolic activation, whereas insofar as dominant lethal mutations in mammals are concerned, the results are rather controversial (N. P. Bochkov, R. Y. Shram et al, 1975 — 5).

Regarding the effect of chlorpromazine on the fetus during treatment of pregnant women, reports are available (cited by 31) both on hyperbilirubinemia in the newborns (P. Scokel et al, 1962 and V. Apgar 1964), and absence of relationship whatever between icterus neonatorum and CP (A. Belmont et al 1963). Out of a total of 52 women receiving more than 500 mg CP daily during the last months of pregnancy, stillbirths are recorded in two cases, abortion — in one, and convulsive seizures — in one (D. Sobel, 1960). Also, a child with congenital monstruosity is observed (J. L. and J. A. O'Leary, 1964).

In the first cytogenetic investigation of schizophrenic patients in this country, M. Tzoneva, L. Ivanov and B. Petrov (1965 — 29) establish a predisposition to chromosome deletions, thereafter assuming (1967 — 59) that the changes recorded in the karyotype maybe due to secondary factors, the application of simultaneous neuroleptic treatment inclusive. In a subsequent study of schizophrenic patients, L. Ivanova and D. Boneva (1967 — 13) found that patients undergoing CP treatment have a greater number of chromosome aber-

rations thereby presuming that chlorpromazine could be accepted as the causing factor of their occurrence. During the same year, M. Cohen et al repeatedly reported on an increased number of chromosome deletions in the course of CP treatment (1967 — 34, 35). K. Hirschhorn and M. Cohen (1968 — 42), J. Nielsen et al (1969 — 52), E. Jenkins (1970 — 45) described analogical findings. M. Cohen et al also published in 1969 and 1972 (36, 38) their own, and those of A. Sandberg (cited by 36), negative cytogenetic results among patients undergoing CP treatment. In 15 schizophrenic patients treated with phenothiazines (without specification of the preparations) and other neuroleptics, V. Ionchev, M. Tzoneva, M. Krachunova and G. Valkova (1975 — 15) found unreliable increase in structural aberrations, as compared to a group of untreated patients. Using the method of Moorhead et al, as modified by M. Tzoneva 1967 — 28), L. Ivanova and D. Boneva (1973 — 43) studied lymphocyte cultures from 21 patients with schizophrenia and other affections prior to and after CP treatment for one month. Their findings point to a dose-related increase in chromosome breaks.

In lymphocyte cultures treated in vitro with CP, M. Cohen et al (1969 — 36) and N. Kamada et al (1971 — 46) failed to observe chromosome aberrations. L. Ivanova and D. Boneva (1973 — 43) established a significant increase in structural aberrations upon administration of 5 mcg/ml CP for 72 hours to cultures from 21 healthy individuals and patients with psychogenic and schizophrenic conditions. In compliance with data published by R. Shram (1974 — cited by 5), the results of aminazine treated lymphocyte cultures are conflicting. Reports by O. Rendon (1974—55) on the effect of chlorpromazine in vitro, and by V. S. Zhurkov (1975—12) in terms of aminazine are negative.

The brief survey of literature data on the issue shows that there are a great number of dissenting results concerning the mutagenic action of phenothiazines, chlorpromazine in particular, and in most instances it is a matter of uncomparable findings because of differences in the methods employed. In lymphocyte cultures treated in vitro, the differences involve mainly the method and length of cultivation, as well as the concentration and duration of CP treatment. Experience had to date shows that colchicine is toxic for cells whenever administered at dose exceeding 0.5 mcg/ml (after the method of P. Moorhead et al, 1960 — 51); in 72-hour cultures, most of the cells are already in the second phase of mitosis, and the number of aberrations which occurred during the first mitosis (by the 54th hour) is altered (K. Bakton, G. Evans, 1975 — 1). It is pointed out that prolonged mutagen exposure is inexpedient, on the one hand, owing to the fact that its concentration is altered as the result of splitting, and on the other, because it acts in different stages of the mitotic cycle (K. N. Yakovenko et al, 1974 — 32) at appreciably modified sensitivity (N. P. Bochkov, 1975 — 4).

The facts outlined above led us to undertake a comparative study on the effect of chlorpromazine on lymphocyte cultures, using different methods and time of cultivation, as well as different treatment periods and concentrations of the medicament.

Material and method

Lymphocyte cultures from the venous blood of fourteen healthy individuals (5 women and 9 men) with average age 29 years, were investigated. Part of each culture was set apart as a control (K). The remainder was distributed into experimental setups with addition of sterile solution of CP substance in physiological saline. Each setup was processed in 2—3 parallel repeats. Nutrient medium 199 "Pasteur" without additives and aeration was used. Colchicine treatment lasted for three hours. The preparations were stained after Giemsa.

By mode of work, the investigations performed were divided up into four groups. In the first the cultivation was done after the method of Moorhead et al, as modified by M. Tzoneva (28), using the phytohemagglutinin of Tzoneva. Colchicine at dose 10 mcg/ml was administered, and hypotonic processing was made with distilled water. In the other three groups 0.5 mcg/ml colchicine, 0.555 per cent potassium chloride as hypertonic solution (1) and phytohemagglutinin "NIEM" were utilized.

Thus the following groups were established:

Group I — four individuals, cultivation term — 72 hrs. Experiment with 5 mcg/ml CP for 72 hrs;

Group II — four individuals, cultivation term 72 hrs. Experiments with 5 mcg/ml CP for 72, 48,24 and 4 hours, administered accordingly at 0, 24, 48 and 68 hours;

Group III —two individuals, cultivation term 72 hrs. Experiments with 50 mcg/ml CP for 4, 24 and 72 hrs;

Group IV — four individuals, cultivation term 56 hrs. Experiments with 50 mcg/ml CP for 4, 32 and 56 hrs, administered at 0,24 and 52 hrs.

Metaphase plates with adequately scattered and clearcut chromosomes, displaying moderate spiralization, diploid (44—47 chromosomes) or tetraploid, one hundred per setup, were subjected to cytogenetical study. Metaphases not exceeding 25 from two coded preparations each were picked out from each duplication. Analysis of a total of 5000 metaphases was made under microscope, photographing only those with aberrations, except for group I where all metaphases were photographically recorded. Cells without karyotyping and chromosomes with breaks and reorganizations, as well as cells with associations of acrocentric chromosomes, and chromosomes participating in the latter were recorded by number and type. Achromatic disorders without dislocation of chromatids were not included.

To perform the immunomorphologic study, 5000 in group I, and 2000 cells each per experimental setup in the other groups were differentially counted from the same preparations, totalling 124 000 cells. Apart from blast and mitotic index (BI, MI), tetraploid mitoses and amitoses were also recorded.

The results from the cultures experimented upon were subjected to intragroup comparison with those from the control cultures to assay the effect of CP. Intergroup comparison of control cultures was made with due consideration to the different conditions of cultivation.

Statistical elaboration was performed using the methods of variation and alternative analysis (D. Sepetliev, 1972—25).

Results

Aneuploidy does not show significant changes in any of the experimental setups with CP treatment.

Structural aberrations (Fig. 1) in group I show a reliable increase, with the percentage of damaged cells rising from 3.50 to 19.50, and the average aberrations per 100 cells — from 4.00 to 28.25 ($p < 0.001$).

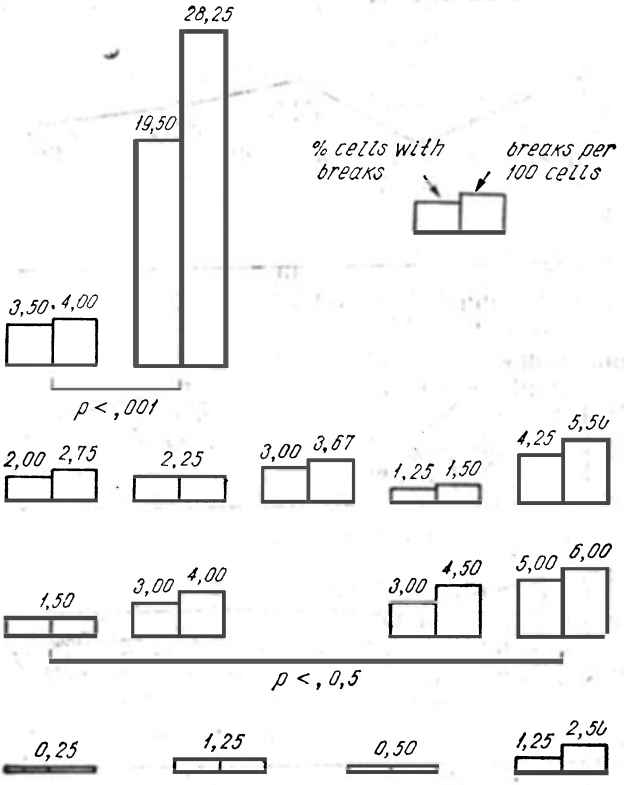

Fig. 1: Chromosome aberrations.

In group II and III, the 72-hour-long incubation does not account for noteworthy differences both upon treatment with 5 and 50 mcg/ml CP. The percentage of aberrant cells alters accordingly from 2.00 (1.50) to 2.25 (3.00). Insignficant are likewise the differences in the mean number of breaks per 100 cells. The same holds true for the experiments with 4-, 24- and 48-hour-long CP treatment at dose 5 mcg/ml, as well as with 50 mcg/ml CP for 24 hours. Only upon 4-hour treatment with 50 mcg/ml CP (group III), the percentage of cells with breaks, and the average number of breaks per 100 cells augment reliably from 1.50 to 5.00 and 6.00 respectively ($p < 0.005$).

In none of the experiments with 56-hour cultivation (Group IV) are reliable differences established in terms of aberrations in comparison with the controls.

Structural aberrations in the control cultures present deletions of one chromatid and four isochromatid fragments altogether in group I and II. In part of the experiments in group II (24 hrs) and group IV (56 hrs) only monochromatid breaks are present, whereas in the other groups they predominate. By fre-

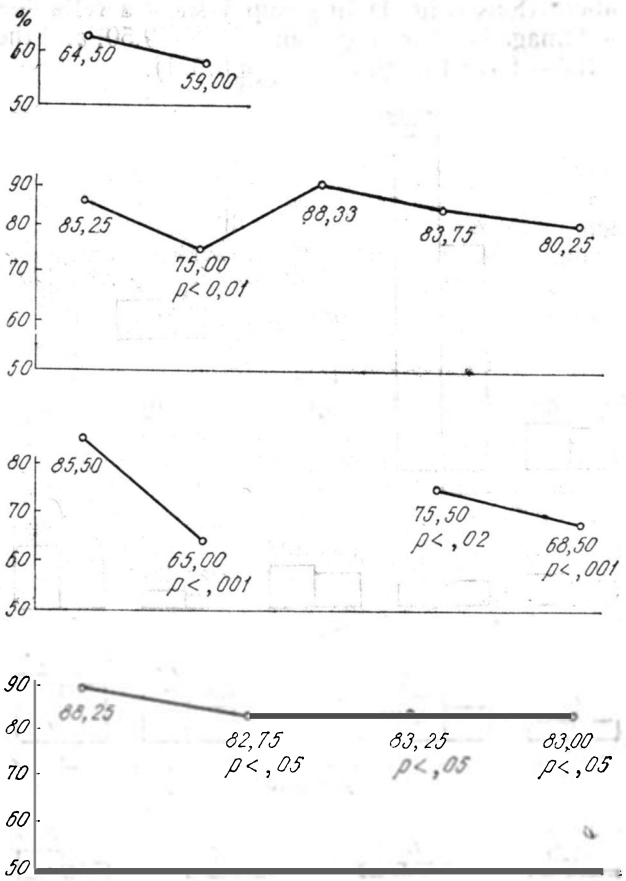

Fig. 2: Percentage of cells with associations.

quency next rank paired acentric fragments and seven centromere divisions in all. The least are the reorganizations yielded by a total of four dicentric chromosomes in 3 cells for all experimental setups.

The associative capacity of acrocentric chromosomes estimated by the percentage of cells with associations, average number of associations per cell, average number of associated chromosomes per cell, and the percentage of associated chromosomes D and G (relative to their total number) in treated cultures is inhibited. An exception in this respect is the 48-hour experiment in group II where the values of the indicators augment, rather considerably for the average associations and associated chromosomes per cell. In the other experiments the values of the five indicators listed above decrease, although not always reliably. This is most strongly pronounced in group III, where only

the percentage of associated D chromosomes shows an insignificant difference at 24 hours. Upon 72-hour treatment in group II, similarly only the percentage of D chromosomes is not significantly diminished. In group IV the percentage of cells with associations shows a significant decrease in all groups, whereas

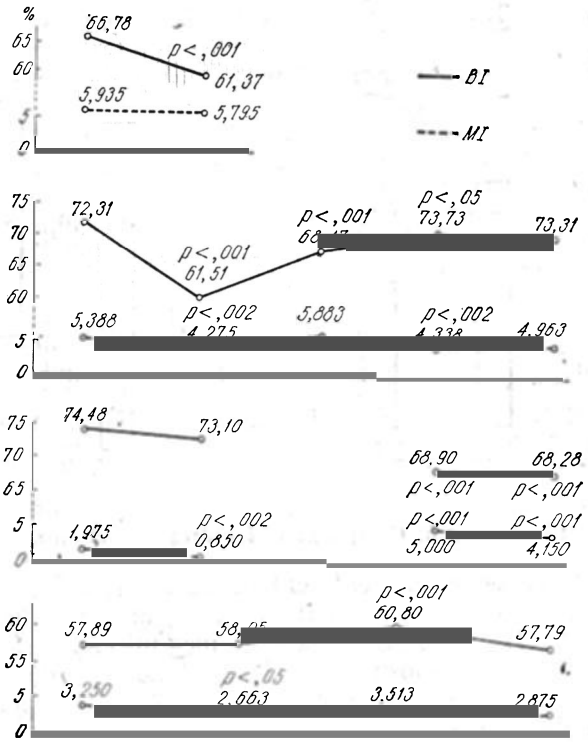

Fig. 3: Immunoproliferative activity.

associations and associated chromosomes in the average per cell — upon 56 and 32-hour treatment. In group I, all the values decrease insignificantly.

Changes in the percentage of cells with associations, and its values are illustrated in Fig. 2. In group II, it decreases significantly only in the 72-hour experiment, while in group III and IV — in all experimental setups.

The average associations per cell which in the controls are 0.925 (group I), 1.440 (II), 1.455 (III) and 1483 (IV) do not decrease significantly in group I, as well as in group II — on 48- and 24-hour treatment, and in group IV — on 4-hour treatment.

The number of associated chromosomes in the average per cell — 2050 (group I), 3.305 (II), 3.320 (III) and 3.520 (IV) — displays a modification parallel to the average associations per cell.

The percentage of associated chromosomes D — 18.17 (I), 28.04 (II), 30.67 (III) and 35.21 (IV) — decreases reliably only upon 72- and 4-hour treatment with 50 mcg/ml CP (group III).

The percentage of associated chromosomes G — 24.00 (I), 40.56 (II), 37.00 (III) and 35.19 (IV) — shows a stronger reduction than the percentage

of D, significant in the 72-hour setup of group II, and in all experimental setups in group III.

Blast activity is significantly modified in part of the experiments and the alteration is by no means simple. In 72-hour cultures with application of

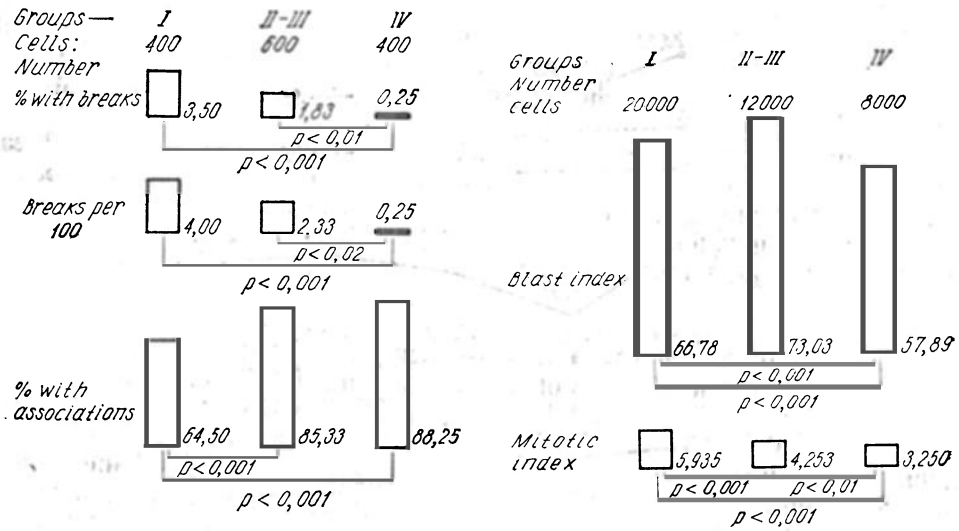

Fig. 4 and 5: Comparison of untreated cultures.

5 mcg/ml CP, it decreases after treatment for 72 hrs (gr. I and II) and 48 hrs (II), whereas upon 24-hour treatment it increases, and on 4-hour treatment it remains unchanged (II). Treatment with 50 mcg/ml causes blast activity reduction only after shorter exposures — 24 and 4 hours (III). In 56-hour cultures there is no reduction whatsoever, while the 32-hour experiment displays a significantly higher BI.

Mitotic activity (Fig. 3) does not follow the course of changes in blast activity. In the longest exposures MI decreases singnificantly in all groups except for group I, the latter corresponding to a change in blast index in group II alone. In the 24-hour experiment MI in group II is lowered at parallel increase in BI, while in 24- and 4-hour treatment (III), it augments contrary to BI reduction.

Tetraploid mitoses exhibit a significant change only in group I where they are substantially increased — from 0.00 to 0.51 per cent ($p < 0.001$).

Inasmuch as the percentage of cells in amitotic division shows significant changes (only in groups III and IV), it should be pointed out that they are opposing and do not correlate with the changes in BI and MI.

A comparison between the groups is made for some of the indicators of the control setups (Figs. 4 and 5). In the comparative study, the results for group II and III are summed up (group II—III) since CP concentration is the only difference between the 72-hour cultures, whereas group IV is with 56-hour cultivation, and group I differs from the remainder by the way of treatment.

Group I is distinguished by the highest number of chromosome breaks, least associations, average blast activity and highest mitotic activity. It should

be streassed that the greater MI in group I is largely attributed to mitoses hardly lending themselves to cytogenetic analysis, whilst in the other groups metaphases with a perfect morphology predominate. The percentage of cells with aberrations and aberrations per one hundred cells exceed those in group II—III, but differ reliably from group IV alone. The percentage of cells with associations is reliably lower than that of group II—III and IV. BI in group I differs essentially both from the highest one in group II—III, and from the lowest one in group IV. The mitotic index in group I exceeds reliably MI in the other groups.

Group IV (56 hrs) is characterized by the smallest mumber of breaks, lowest blast and mitotic activity, and most strongly pronounced associative capacity of acrocentricytes. By the signs outlined above it differs essentially not only from group I, but also from group II—III, except for the percentage of cells with associations.

Discussion

The research shows that upon treatment of cultures with 0.5 mcg/ml colchicine for 3 hrs, the addition of CP at dose 5 mcg/ml for 72, 48, 24 and 4 hrs (group II), and for 56, 32 and 4 hrs (group IV) does not produce an increase in chromosome aberrations. The great number of chromosome breaks at analogical CP concentrations in cultures treated with 10 mcg/ml colchicine (group I), which concentration is toxic for the cells (1), in our opinion, should be attributed to its combined effect with colchicine. The nearly twice as great number of breaks in the control cultures at high colchicine concentration — 4 per 100 cells for group I against 2.33 for group II-III — is accepted as an argument in favour of the above statement. The increase in structural aberrations among CP treated cases, observed with this particular method of cultivation (43), is most likely due to the combined action already referred to. However, for the time being, we are not in a position to confirm the hypothesis set forth with comparative data.

The authors claiming negative results in studies, in vitro, use 72-hour cultures, treated with different CP concentrations: 1 and 10 (12); 1, 10 and 100 (36); 0.7, 3.5, 7.1, 17.7 and 35 mcg/ml (46), for periods ranging from 6 to 72 hrs. In two of the reports there is evidence of 2-hour treatment with 0.2 mcg/ml colchicine (46) or 0.05 mcg/ml colcemide (36) corresponding to about 0.5 mcg colchicine. The CP concentrations under test, those used by us inclusive, cover also the theoretical therapeutical concentration. At dose 100 mg four times daily, received by an individual weighing 80 kg and blood totalling six liters, a concentration up to 16.6 mcg/ml blood may be attained, at 5 mcg CP per gram body weight daily. However, chlorpromazine splitting occurs very rapidly, and within 2 hrs of oral receipt, up to twenty CP metabolites are found in the blood. At 400 mg daily amount received, eleven of the metabolites after summation display values ranging from 0.340 to 0.450 mcg/ml (P. Kaul et al, 1972 — 47), while CP itself — from 0.105 to 0.336 mcg/ml (S. Curry, 1970—39). On the other hand, on prolonged use, chlorpromazine deposits unevenly within the tissues, and a concentration of 17.5 mcg/g has been established in the gonads of a deceased patient (E. M. Solomatin, 1966 — 23). According to the cited data, administration in vitro of doses substantially exceeding the realizable therapeutical concentration in the blood or gonads failed to produce chromosome disorders.

No data concerning the administration of 50 mcg/ml CP are available. As in treatment with 100 mcg/ml (36), the 50 mcg dose too yields a marked cytotoxic effect. One of the experimental setups (48-hour treatment), and part of the repeats were discarded because of the absence of mitoses suitable for cytogenetic analysis. Wherever such an effect was possible, the unfit mitoses compact or crushed and slovened chromosomes prevailed unlike control cultures where practically all mitoses were adequately scattered, with a clear-cut chromosome pattern. In all treatment terms there were cells with partial or complete "pulverization" of chromosomes. Hence it is believed that the increase in structural aberrations at four-hour exposure is an expression of a toxic effect. On longer exposure, it is hardly detectable, probably owing to the heavier lesion of cells, rendering them unfit for cytogenetical analysis. The significant rise of breaks recorded only after 4-hour treatment — in the G_2 period — might be related by analogy to the data concerning radiation mutagenesis (Z. A. Dzhemilev, 1967 — 9; A. V. Sevankaev et al, 1973 — 24), as well as to the greater vulnerability of chromosomes at this particular period of the mitotic cycle. Although the former explanation seems more verisimilar, the assumption is warranted that in the G_2-period the chromosomes are more vulnerable by chlorpromazine since among cultures treated with 5 mcg/ml CP too, the breaks are more numerous in the 4-hour exposure setup ($p > 0.05$).

We recorded changes in associations of chromosomal satellites as an additional cytological indicator in assaying the drug effect (M. Cohen et al, 1971 — 37). It is believed that associations are a very fine indicator for the general condition of the chromosome set (A. A. Profjeva-Belgovskaya, 1966 — 18), and may reflect the immunologic readiness of cells (V. M. Gindilis et al, 1967 —8). Presumably, in lymphocyte cultures all acrocentrics merge in associations (N. S. Zhdanova et al, 1975 — 11) which, larger ones in particular, undergo partial destruction or fragmentation depending on the methods used of cultivation and working out of preparations (N. S. Zhdanova, 1972 — 10).

In this study cultures treated with 0.5 mcg/ml colchicine have associative indices reliably higher than in group one. Against the background outlined their reduction, dependent upon the time of CP treatment and concentration, stands out; in case of longer exposure and higher concentration, such a reduction involves mostly small acrocentrics.

Upon 6-hour cultivation, associations are the most numerous. This corroborates the report claiming that in the first metaphase associative indicators are higher than in the second one (10). Their lower values in group I are ascribed to the toxic colchicine effect.

L. Ivanova and D. Boneva (1972—14) found a lowered percentage of cells with associations following one-month CP treatment of schizophrenic patients, whilst in lymphocyte cultures treated with 10 mcg/ml CP, the average percentage of associations per cell were also lowered. In the course of psychogeny treatment (using rather low CP doses), and upon administration of 5 mcg ml CP in vitro to patients and healthy individuals, noteworthy differences in the associative capacity indicators are not manifested (the cultures are treated with 10 mcg/ml colchicine). No literature data concerning CP behaviour to associations are available. A significant reduction is observed 8—10 years after acute radiation disease (N. D. Okladnikova, 1970—17). Similar findings were described by L. Vasileva upon in vitro tetracycline treatment of lymphocyte cultures, as well as by I. V. Georgieva and M. Tzoneva (1967—7) — with diazinon and lindane.

In 72-hour cultures at 5 mcg/ml CP an inhibition of blast transformation is recorded provided treatment is begun within the first 24 hours. In 56-hour treatment no inhibition is noted. In 72-hour treatment with 50 mcg/ml CP the cytotoxic effect was so strong that one of the experimental setups had to be discarded. In the second experiment similarly a substantial number of degenerated cells were present, but in the preserved ones BI reduction was insignificant in contrast to shorter exposures. Mitotic activity was inhibited upon 5 and 50 mcg/ml CP addition after the start of cultivation (unreliable in group I only), regardless of the concentration used.

N. Kamade et al (46) established that 3.5 to 35.5 mcg/ml CP, added within the first 18 hrs of cultivation, inhibits the blast transformation of lymphocytes. According to M. Cohen et al (36), 100, 10 and 1 mcg/ml CP account for mitosis inhibition at linear dependence upon the duration of exposure. We observed (1973—2) a reliable reduction of blast and mitotic activity upon 72-hour treatment with 5, 10 and 15 mcg/ml CP; such a reduction was not manifested at 2 mcg. In patients undergoing CP treatment (1974—3), the effect was directly related to the dose and continuity of medication: in higher doses it resulted in a reduction, and in lower doses — in an increase in BI and MI. In either investigation the effect was rather pronounced in terms of the blast index, whereas in terms of mitotic index substantial individual fluctuations were noted. In the case material reported on the data concerning mitotic activity are in agreement with those published by M. Cohen et al (36) and by the authors (2), and concerning blast activity — with N. Kamada's and our own results (45,2).

The occurrence of polyploidy under CP effect, already observed by us earlier, under analogical conditions of cultivation, both in vitro with 5 mcg/ml CP, and in treatment using higher drug saturation (2, 3), was manifested in group I alone. This led us to the conclusion that cell division is impaired by the combined action of CP and high colchicine concentration. The cited authors who studied the CP effect in vivo and in vitro on lymphocyte cultures mentioned nothing about polyploidy. According to M. Tzoneva and M. Krachunova (1972—30), treatment with neuroleptics exerts a polyploidogenic effect.

It should be emphasized that between the individual groups differences exist not merely in terms of the treatment cultures, but also in terms of blanks with different way of cultivation. In group I a major part of the mitoses do not lend themselves to cytogenetic analysis. The fact of being compact or insufficiently scattered may be explained by the hypotonic processing with distilled water, whilst the short, thick and smeared chromosomes, in our opinion, should be ascribed to the higher colchicine concentrations used. To the latter we attribute also the enhanced breakability of chromosomes and the reduction of associations. Immunoproliferative indicators depend first and foremost on the qualities of the stimulator employed. In this group, treated with the phytohemagglutinin of prof. M. Tzoneva, the mitotic index is higher, and the blast one — lower than in II—III group, subjected to treatment with her preparation, produced by NIEM (batch one). In 56-hour cultures where only the first cell population is studied, the immunoproliferative indicators are the lowest, but chromosome integrity and associations are preserved ad maximum. Differences in blank cultures of the individual groups attest the great impact of the method and cultivation time on the results, and up to a certain extent — the quality of the materials employed.

The blast transformation of cultivated lymphocytes is an expression of their immunologic response to the stimulant. The established inhibitory effect of chlorpromazine on the latter response calls our attention to the problem of its therapeutical action . V. Ionchev, M. Tzoneva, M. Krachunova and G. Valkova found out that neuroleptics act immunosuppressively, and thus explained their therapeutical effect in schizophrenia. On the other hand, according to the cited authors, the continued treatment with high doses leading to cell destruction (confirmed also by H. Sommer et al, 1970—56), creates conditions for autoimmunization.

Blastogenesis is provoked by one of two routes — through falling of the stimulant onto the cytoplasm, or through the effect it exerts on the cell surface (M. Radeva, Y. Stoichkov, 1971—19). Its action is instantaneous, but nevertheless requires a rather prolonged contact with the cell surface where it is retained (A. Y. Friedenstein et al, 1969—27). It is well known that CP inhibits the permeability of cell (A. Freeman et al, 1963—41) and mitochondrial (M. Spirites et al, 1963—57) membranes, decreases water permeability, and influences the passive transport of ions and some organic components. Methionine transport is likewise inhibited (33). The membranous action of chlorpromazine on lymphocytes may explain the inhibition of blast transformation upon treatment within the first 24 hrs of cultivation, i. e. at the time when the stimulant is supposed to trigger transformation.

Chromosome deletions under the simultaneous effect of CP and colchicine at toxic concentration are most likely due to the inhibition of cellular permeability by chlorpromazine. Presumably, part of the breaks taking place in the G_1-and S-period of the mitotic cycle, are repaired within 40 hours of cultivation, that is before the G_2- period (24) — a fact explaining among other things the highest number of aberrations upon treatment in the last few hours prior to mitosis. If the transport of constituents, indispensable for the restoration, across the cell membrane is hindered, this in turn would interfere with the reparative process itself. In the latter case the assumption is warranted that chlorpromazine potentiates the toxic action of colchicine on chromosomes by interfering with the transport of ingredients necessary for their integrity. It is furthermore possible that its action is added to that of colchicine. By the way, the work of L. Ivanova (1976—44) who, under equal conditions of cultivation, was successful in demonstrating a different mutagenic effect, dependent on the dynamics of schizophrenic disease, gives us sufficient reason to accept a potentiation of the colchicine effect by the action of other mutagenic factors.

Assessment of the mutagenic activity of drugs is an essential problem, especially when it is a matter of extensively and continuously applied preparations, such as chlorpromazine. Having in mind the impossibility to perform direct investigation on humans, it is recommended to extrapolate directly on man the positive effect established on any test-object, since underestimation of the mutagenic risk is more dangerous than its overrating (5).

Summarizing the data submitted concerning chlorpromazine effect on human lymphocyte cultures, in vitro, emphasis should be laid on the fact that its application at concentrations not exceeding the level required for the attainment of a therapeutical effect, by no means leads to chromosome aberrations. The latter are produced whenever it acts in combination with colchicine, at toxic concentration, where the presumption of a mutual drug poten-

tiation effect is by no means ruled out. Chlorpromazine impairs the association of acrocentrics. In 72-hour cultures it inhibits blast transformation provided it is administered within the first 24 hrs of cultivation, or else, at high concentration. Upon continued treatment it accounts for mitotic activity lowering.

The issue of the potentiation effect of colchicine in conjunction with other mutagenic factors gives us sufficient reason to pose the principally important question, both from theoretical and practical viewpoint, about the possibility of mutagenesis potentialization as a result of the interaction between various medicaments and chemical substances, demanding further researches along this line, and much greater caution on behalf of attending physicians confronting the so-called polypharmacy problem.

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ЦИТОГЕНЕТИЧЕСКИЕ И ИММУНОМОРФОЛОГИЧЕСКИЕ ИССЛЕДОВАНИЯ ВЛИЯНИЯ ХЛОРПРОМАЗИНА НА ЛИМФОЦИТНЫЕ КУЛЬТУРЫ В ПРОБИРКЕ ОТ ЧЕЛОВЕКА

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РЕЗЮМЕ

Проведено сравнительное исследование действия хлорпромазина (ХП) на лимфоцитные культуры от 14 здоровых лиц, распределенные на 4 экспериментальные группы. Исследованы цитогенетически 5000 метафаз, а иммуноморфологически — 124 000 клеток. Неодинаковые условия культивирования приводят, как в контрольных, так и в третированных ХП культурах к выраженным различиям. При применении ХП в дозах 5 мкг/мл, что превышает осуществимый терапевтически уровень в крови, повреждается хромосомная структура, только если в то же время имеется колхицин и в высокой концентрации, при которой увеличиваются хромосомные аберации и в контрольных культурах. Это может быть объяснено суммированием их действия, но более вероятным является, что ХП потенцирует мутагенное действие колхицина, так как подобный эффект на колхицин наблюдался и под влиянием другого фактора.

На основании экспериментальных данных обсуждается терапевтическое действие ХП на иммунный ответ и ставится вопрос о возможном риске потенцирования мутагенеза при медикаментозной полипрагмазии.