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THE MUTAGENIC EFFECT OF LYSERGIC ACID DIETHYLAMIDE
III. EVALUATION OF THE GENETIC RISK OF LSD IN MAN

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SUMMARY

In view of the necessity to determine the mutagenic activity of chemical substances in man, the genetic risk of LSD is discussed on the basis of our own experiments in relation to the findings of other authors and the present way of interpretation. The genetic hazard of LSD is evaluated according to the Bridges scheme. After analysing the approaches to the evaluation of possible LSD hazard, we conclude that LSD studied on various organisms is a mutagenic substance inducing gene mutations, chromosome breaks and non-disjunction. The genetic risk of LSD to the human population is discussed. It is suggested how the risk might be diminished by using the drug only in specific psychiatric indications, and then contraception should be used by treated patients.

The current philosophy in interpreting the results of mutagenicity testing was fundamentally expressed in the form of a question by AUERBACH[1], "Is the medium of our test the message of our conclusions?", and by BRIDGES[2], "Environmental genetic hazards—the impossible problem?"

During the past 5 years many papers have been published on the mutagenic activity of various chemicals we meet in our environment. But there are only few published opinions on the extrapolation of experimental results to man[12,16].

Many methods for mutagenicity testing have been devised and recommended, and in many countries they have become part of general toxicological evaluations particularly of new drugs. If there is alarm about the genetic risk of chemicals and if there are methods to study this problem, then it should also be said what ought to be done with the results. If there is no agreement in the interpretation of results of mutagenicity testing, then no recommendation is justified for mutagenicity testing of groups of chemicals (drugs, pesticides, food additives) representing some hazard to the population.

A study on the mutagenic activity of LSD determining sex-linked recessive lethals in Drosophila, cytogenetic analysis of chromosomal aberrations in the meiosis

of male rats and mice, and the dominant lethal test in mice was completed in Czechoslovakia in 1971. The following quotation comes from the report[22].

"It was confirmed that sex-linked recessive lethals as well as delayed mutations were induced in Drosophila. The cytological analysis of spermatocytes in diakinesis–first metaphase showed irregularities manifesting themselves in the loss of pairing ability or in a premature disjunction of bivalents. The dominant lethal test made it evident that LSD is capable of inducing dominant lethals, primarily the preimplantation lethality. After a single dose of 10 μg LSD/kg body weight changes were induced just on the statistical significance level.

"Taking into account the results published so far, as well as our own experimental findings, as to the direct effect of LSD on humans we came to the conclusion that a single dose of 200–400 μg LSD has no adverse effect on progeny, especially if the recommendation is heeded to allow a time lapse of at least three months between application and conception.

"However, in the case of repeated doses, changes do arise owing to the summation of the applied doses. In a long-term LSD therapy in psychiatric indications we have recommended contraception being used by patients. In patients who had been treated after conception or shortly prior to conception with a high dose of LSD the possible interruption of pregnancy should be considered. The use of LSD during pregnancy, especially in the first three months, is, in our view, a clear indication for the interruption. Very questionable is the therapeutic application of LSD in child psychiatry, particularly from the viewpoint of later consequences.

"On the basis of a complex assessment of the genetic risk of LSD administration we thought it fit to advise the further use of this drug only in strictly specified psychiatric indications, provided that an efficient anticonception in the treated persons and their partners is ensured. In view of the possible late effects we do not regard it as medically ethical to conduct LSD experiments on volunteers at a reproductive age."

Despite no other substance having been so widely tested on humans, whereby the evaluation of the genetic hazard continues to be contradictory (DISHOTSKY *et al.*[5], LONG[14]), we regard it as suitable to re-assess the genetic hazard of LSD to man by answering the five questions posed by BRIDGES[2]. The difficult and important problem of estimating the risk to human populations is analysed in relation to one substance, in the hope that the type of analysis will be analogically suitable for similar tests of different substances. We assume that this procedure will call forth an extensive discussion on the correct evaluation of results obtained with various tests.

(1) Is LSD mutagenic?

For an estimation of mutagenic activity the results obtained in mammals are considered to be the most important. A rapid penetration of LSD into all tissues has been proved in mice, where its concentration exceeded the concentration in blood. For the estimation of LSD mutagenic activity the finding that this substance easily penetrates the cell membrane is significant.

Of the three tests recommended for determining mutagenic activity (dominant lethal test, cytogenetic analysis of bone marrow and host-mediated assay)[27], positive results were achieved with the dominant lethal test after single and repeated administration of LSD[23], positive as well as negative results in the cytogenetic analysis of bone marrow, and negative results in the host-mediated-assay[13] were achieved.

A cytogenetic analysis of mouse bone marrow demonstrated the ability of LSD to induce chromosomal aberrations[4]. EGOZCUE AND IRWIN[6], however, obtained negative results. They report in the same paper an increased frequency of chromosomal aberrations in the peripheral lymphocytes of *Macaca mulatta* in two out of three animals. Similar findings were obtained by KATO *et al.*[11].

Analysing the effect of LSD on chromosomes of germinal cells, COHEN AND MUKHERJEE[4], SKAKKEBAEK *et al.*[19] and SKAKKEBAEK AND BEATTY[18] described positive results in mice, whereas negative findings in mice and Macacus were described by JAGIELLO AND POLLANI[10] and EGOZCUE AND IRWIN[6].

The results of cytogenetic analysis of chromosomal aberrations in bone marrow and meiosis must be viewed as contradictory. An explanation was suggested by KATO *et al.*[11] who pointed to the possibly different individual reaction in the induction of chromosomal breaks.

From a number of papers published it is not clear whether or not LSD is a substance capable of inducing structural chromosomal changes in mammals. In our experiments we did not find any structural aberrations in the chromosomes of germinal cells in male rats and mice. An increased frequency of univalents, however, signalizes a possible irregularity in the actual meiotic division, though the mechanism is not quite clear[7].

Another possible genetic hazard from LSD may result from non-disjunction, which was proved on *Drosophila melanogaster*[3,20]. It may be that non-disjunction is the cause of aneuploidy in meiosis and of preimplantation losses of non-viable zygotes in the dominant lethal test.

Many authors regard an evaluation of the number of chromosomal aberrations in the mitotic as well as meiotic cell as problematical, especially the evaluation of hypodiploidia. If, however, there is a distinct difference in the spectrum of individually analysed categories of cells with a deviation in the number of chromosomes between the control and treated groups, then presumably such changes could be attributed to the drug tested. We do not think it is justified to declare, on the basis of such considerations, that the substance is not mutagenic because it does not induce distinct structural chromosomal aberrations. In such cases attention must also be given to other manifestations of the drug effect on the cell and its division; specifically, it is important to assess the significance of negative findings of structural chromosomal aberrations from the aspect of other test results, by examining the mutagenic effect of the given drug.

In a host-mediated assay, negative results on the mutagenic activity of LSD were obtained with the use of *Salmonella typhimurium* st. G 46 (nonsense mutant)[13].

In view of the contradictory results obtained in mammals it appears to be useful to analyse the observations obtained at a submammalian level. Mutagenic activity of LSD was proved in *E. coli*[26], in barley[17] and in *Drosophila melanogaster*[3,20,21]. Results in *Drosophila melanogaster* were negative after lower doses[3,25]; and in *Vicia faba* the lack of results was attributed to the reduced penetration of LSD to the analysed roots[14,24].

On the basis of all these results it can be assumed that LSD induces, on the submammalian level, primarily gene mutations. From this aspect the hypothesis that LSD acts on DNA by intercalation to the DNA molecule[28] appears to be acceptable. Our findings in the dominant lethal test and in the cytogenetic analysis of male meiotic

cells may be explained by non-disjunction, lack of rearrangement formation from broken chromosomes, or questionably they may be induced by gene mutations.

Though we are aware of the difficulties in assessing the positive and negative results in the literature as to the mutagenic LSD activity, we believe, in view of our own experimental findings, that LSD is a mutagenic substance.

(2) Is LSD likely to be mutagenic to man

The possibility of inducing genetic damage in man with LSD has been studied only with a cytogenetic analysis of chromosomal aberrations in lymphocytes *in vitro* and *in vivo*.

In experiments *in vitro*, positive results predominate. However, when data from the literature are compared, and in view of the non-homogeneity of the control samples, it is not possible to determine the correlation between the frequency of aberrations and the LSD dose used[5,11]. In addition, the difference between experiments *in vitro* and *in vivo* represents another principal obstacle in the extrapolation of results. Another question is whether the phytohaemagglutinin-activated lymphocyte is a suitable cell for testing mutagenic activity.

The interpretation of cytogenetic results in the lymphocytes of LSD-treated humans is even more difficult. From the viewpoint of an exact analysis some studies are not easy to evaluate on the ground that they provide no clear-cut information about the quality of the LSD used (illicit LSD) or *e.g.* about the possible effect of other factors on the positivity or negativity of the findings (the simultaneous use of other drugs, interval between treatment and examination, individual reactivity, difference in dosage, size of sample, *etc*).

Only in peripheral lymphocytes can the ability of the drug to induce chromosomal aberrations be analysed. From this aspect some negative findings obtained in humans are no proof of the negative mutagenic activity of LSD. The results obtained in model organisms indicate an ability of LSD to induce not only structural chromosomal aberrations but also aberrations in the number of chromosomes as well as gene mutations which cannot yet be analysed in man. On the assumption that an identical mechanism gives rise to gene mutations and chromosome aberrations in humans and mammals, an analogous action of LSD on human genetic material could also be assumed leading to the conclusion that LSD is probably also mutagenic for man.

(3) What dose of LSD is being, or is likely to be, received by the population or individuals at risk?

A single dose of 100–150 μg LSD is used for inducing a psychedelic state which is then repeated individually in different ways. In psychiatry the therapeutic dose ranges from 100 to 750 μg in a single administration up to a total of 1–3 mg at repeated doses. Recently, the administration of high single, as well as repeated, LSD doses has been on the decline. Users of illicit LSD give the single dose as being 100–1000 μg, but can this information be accepted as true?

(4) What is the risk from being exposed to LSD?

From the viewpoint of the population the risk is small, and in psychiatric indications it can be controlled. The situation is different with regard to illicit use where there is no control. The danger of genetic damage in illicit users is more pronounced because of their fertile age.

The dose, which distinctly induces an increased frequency of linked recessive lethals in *Drosophila melanogaster*, is about 200 times higher than the average dose applied to man. An analysis of the dominant lethal test in mice showed, after a single application of 10 μg LSD/kg body weight, differences just on the level of statistical significance. This dose corresponds to 800 μg LSD when calculated for an adult man without using the safety cofficient (which is usual in pharmacological practice). It is impossible to prove at the present stage of knowledge whether the routinely used single dose of 200–400 μg LSD (2.5–5.0 μg LSD/kg body weight) is or is not mutagenic for man. This dose, calculated on the immediate concentration of LSD in human blood, may reach maximally 0.04–0.08 μg LSD/ml. It seems to us that the single dose of 200–400 μg LSD represents only a minute population hazard.

(5) What is the acceptable risk of LSD?

From the above proposition it follows that the genetic hazard of LSD to the human population is not very significant. With adequate preventive measures it can be reduced, even more by limiting the therapeutic application of LSD to strictly indicated cases. Particularly, its use in child psychiatry is extremely difficult to recommend. In the treatment of patients at a fertile age the genetic risk can be reduced by anticonception which should be applied during treatment and for a certain period after it. We have adopted an interval of 3 months following the termination of therapy. We are aware that this interval is justified to a certain degree only for men where the elimination of some directly affected stages of spermatogenesis can be assumed. If, however, gene mutations affect DNA in the stem cells then the possible continual transfer of the damage to the next cell generations must be expected and a 3-month safety interval (2 cycles of spermatogenesis) between the last administration and conception loses its significance. Anticonception in women during LSD administration is essential in order to avoid genetical damage to the foetus. As it cannot be predicted in which cycle the ovulation of a mutated oocyte will occur, the permanent contraception of women treated by LSD should be recommended.

Though the genetic risk of LSD does not appear to be a vital problem from the viewpoint of the population, the more so that in recent years the therapeutic use has been largely reduced in medical practice, the primary objective of this paper is to discuss openly the procedures for evaluating the mutagenic activity of any substance tested.

The importance of studying the genetic risk of chemicals has largely increased the range of tests whose merits are discussed and recommended by various authors. In our view an extensive approach to the use of various tests on a mammalian and a submammalian level, as well as a wide range of substances that are tested only with certain tests, is a limiting factor for getting a clear answer as to the possible genetic risk of individual substances for man.

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