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MUTATION ACTIVITY OF CERTAIN CHOLINESTERASE INHIBITORS

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-USSR-

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MUTATION ACTIVITY OF CERTAIN CHOLINESTERASE INHIBITORS

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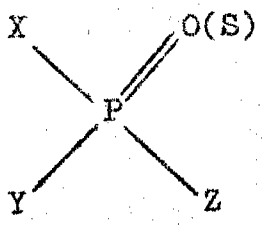
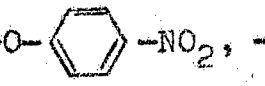
[Following is a translation of the article entitled "Mutatsionnaya aktivnost' nekotorykh ingibitrov kholinesterazy" (English version above) by I. A. Rapoport and R. G. Kostyanovskiy in Doklady Akademii Nauk SSSR (Reports of Academy of Sciences USSR), Vol 131, No 1, Moscow, March/April 1960, pages 191-194.]

(Presented by Academician N. N. Semenov on 28 September 1958.]

It has been established by now that a large number of chemically active organic compounds have mutation-causing properties. The more effective mutation-causing compounds were found among the alkylating agents (1) (yperites (2), ethylenimine, ethylene oxide (3) et al.) and the acylating agents agents (ketones (4), lactones (5) et al.) -- a fact which testifies to the nucleophilic character of the chromosome substratum which is the seat of the mutational process. The majority of the above-mentioned mutation-causing agents (chloroethylamines, chloroethyl sulfides, ethylenimines, epoxides) display a sharply defined anticholinesterase action both in vitro (6) and in vivo (7). In this connection it is interesting to study the mutation-causing activity of the cholinesterase inhibitors belonging to the group of phosphorylating compounds:



where X and Y could be -R, -OR, or $-N(R)_2$, while Z is a halide or a pseudohaloid group

(-CN, -CNS, ).

The first members of compounds of this type (the dialkylfluorophosphates) were synthesized by Lange and Kruger (8) in 1932, and were found to be extremely active physiologically. The numerous studies on this series which followed led to the synthesis of a group of parasympathomimetic medicinal preparations, as well as to the discovery of some effective insecticides which found extensive application in agriculture.

Our investigations were concerned with the genetic effects of a series of phosphoro-organic cholinesterase inhibitors which were found to induce some increase in mutational incidence. The present brief report gives the results of experiments employing the fluoric anhydride of methylphosphinic isopropyl ether (MFF -- ftorangidrid izopropilovogo efira metilfosfinovoy kisloty) -- a compound found to bring about frequent genetic alterations.

The studies were carried out on larvae (24-48 hours old) and imagoes of the fruit fly Drosophila melanogaster. Chemically pure IMFF (boiling point 55° at 15 millimeters pressure, $d_4^{20.5} = 1.10$) (9).

The materials being processed were placed in desiccators when the vapor concentration for the substance under study in the apparatus reached the equilibrium point. The larvae and imagoes were contained in test tubes with an even coating of agar-agar nutrient. Test tubes with imagoes were covered with a double layer of gauze; those with larvae were fitted with cotton plugs which were removed during exposure. Two series of experiments were performed: in Series I, the IMFF concentration was 12 milligrams/liter; in Series II, the concentration used was 8.5 milligrams/liter. Processing time ranged from 3 to 25 minutes. In Series I the specimens were exposed to a single treatment; a five-minute treatment for the images, moreover, constitutes approximately an LD80 reaction, while according to the number of insects that hatch, it is an LD70 reaction. In Series II, a 3-5 minute IMFF treatment was followed by a second toward the end of the pupation process for the young groups. In this case the emergence of imagoes from the pupae was totally unaffected in terms of numbers, while the toxicity for the imagoes amounted to LD50-LD80. The indicated disparity between the physiological reactions for specimens in all three metamorphic stages is explained, first of all, by the specific character of

pupal metabolism. It may be mentioned that an analogous relationship was found to hold in experiments using other mutation-causing agents.

The genetic action of IMFF was analyzed according to the incidence rate of sex-linked mutations. A y^{3P} line which had been used in a number of previous experiments was used for this purpose. After receiving treatment,

TABLE 1

	Concentration in mg/liter	Stage of Development	Exposure in Minutes	Number of Chromosomes	Number of Lethal Mutations	Percentage of Lethal Mutations
Experiment I	12	Larvae	3-5			
Experiment II	8.5	Larvae, pupae	15-25	445	15	3.4
Experiment III	12	Adult insects	3-5	229	27	11.7
Experiment IV	8.5	"	6-7			
Control	--	"	--	812	--	--

y^{3P} males were crossed with Bcl/white females. Lethal mutations, as well as mutations sufficiently clear to be observed without the aid of a microscope were in future hybridization identified by the absence of males in the offspring of Bcl females and y^{3P} males in the second X-chromosome of the diploid set, or by a similar apparent morphological change in all males bearing a sex chromosome originating from an X-chromosome which had been in contact with the chemical being studied. The experimental results are given in TABLE 1.

Four apparent autosomic mutations which had arisen in the experiments must also be mentioned. Three of these were observed in the offspring of a single male; it is highly probable that they represent the reduplication of a single change which arose at an early stage of embryonic cellular development. Thirty-four cases displayed the occurrence of manifestly independent sex-linked mutations -- these were observed in the offspring of various males. Paired mutations were twice observed under conditions

wherein it would have been impossible to guarantee independent occurrence were it not for the great morphological differences in the lethal mutations (mortality stages). There was but a single case of paired sex-linked lethal mutations arising as a result of a single spermatogonial mutation.

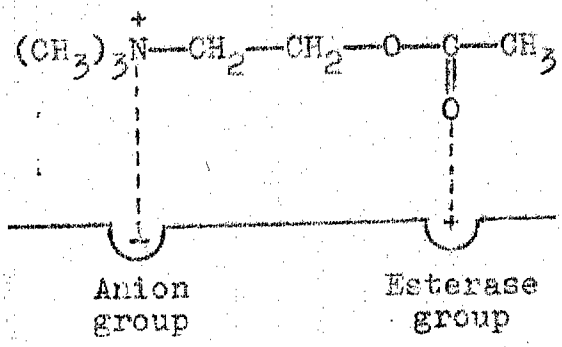
Three mutations of the number indicated in TABLE 1 were of the semi-lethal type with marked morphological characteristics. Of an especially remarkable character in these specimens was the simultaneous appearance of two discrete changes -- the distinctive fused-wing mutation (fused longitudinal nervures) and a markedly decreased eye size. This simultaneous mutation of several genes is linked very probably with an alteration of the chromosome structure, possibly of the inversive type.

Another of the lethal mutations consisted of a fractional alteration in connection with a reversion for a small portion of the spermatogonia, most probably for one cell in eight, judging by the relationship between the lethal and non-lethal mutational groups. The new mutational state turned out to be an unstable one for one of the cells, which then reverted to the norm.

The comparison between the incidence rate for chemically induced mutations in the sex chromosome and that for spontaneous mutations (1:700 ÷ 1:1000) testifies to the capability of the compound being studied to increase mutational incidence by approximately 100 times -- a factor which corresponds to, and in the long run may exceed the effectiveness of high-frequency radiation. Thus, the new supragenetic factor is of some interest in connection with pharmaceutical (antibiotic sources) and agricultural selection.

The data on the biochemical action of phosphoro-organic compounds given below afford some conception of several aspects of the mechanism involved in the interference of a new mutation-causing agent with the autocatalytic process. These data acquire an added interest in connection with the discovery of a significant cholinesterase content in cell nuclei (10).

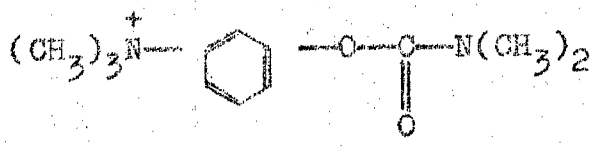
It was shown in 1947 (11) that the toxicity of phosphorus-containing compounds is tied in with a discrete cholinesterase-inhibiting action. The inhibition theory was worked out in detail by the American school of biochemists (12). In accordance with modern concepts, the active portion of the cholinesterase molecule contains an anion group and an esterase group at a distance of 4.5 Å (Angstrom units) away from it:



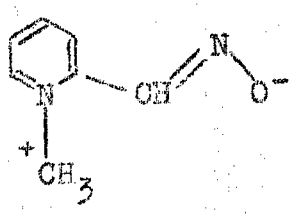
Under normal conditions and as a result of a precise geometric correspondence, the enzyme discretely adsorbs the acetylcholine and catalyzes its hydrolysis.

Upon completion of hydrolysis the active enzymatic surface is liberated and adsorbs a new molecule of the substratum.

There are two types of enzyme inhibitors. The first includes compounds which are not hydrolyzed at all or undergo only partial hydrolysis; these have coordinate groups equivalent to those of acetylcholine. One such compound is proserine:

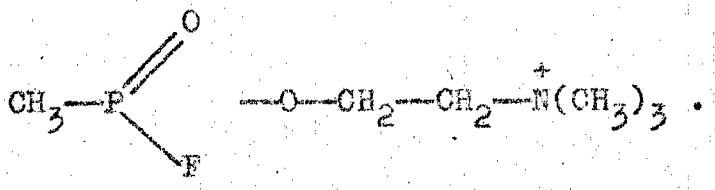


Phosphorylating compounds belong to the second type of inhibitors which block the esterase group in cholinesterase. Such hypotheses are supported by the existence of highly specific reactivator agents for phosphorylated cholinesterase. One of the strongest reactivators is pyridine-2-aldoximmethyliodide (PAM) (12,13), which has a nucleophilic oxime group at a distance of 4.5 Å

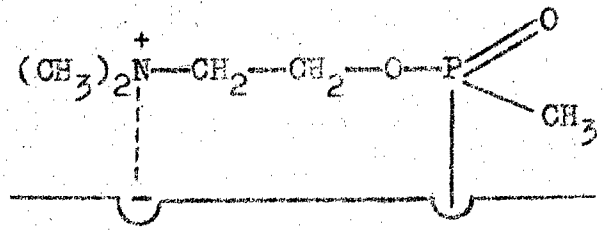


from the quaternary nitrogen. A shift of the aldoxime group into the β- or γ-position, moreover, considerably decreases activity. The reactivation obviously consists of the PAM coordination on the anion group of the phosphorylated enzyme and the subsequent rephosphorylation of the reactivating agent from an enzyme into a nucleophilic group. The possibility of such transformations is sufficiently supportable by the facts of chemistry (14).

In 1958 Enander showed that the action of a number of compounds including both essentially distinct types of inhibitors is not diminished by certain reactivators (15). These compounds, which were obtained by Tammelin (16) in accordance with the insecticides synthesized by Ghosh (17, 18), represent choline ethers of fluoromethylphosphinic acid:



The extremely high toxicity of these compounds (19) and their irreversible cholinesterase-inhibiting action are explained by the double blocking of the anion and esterase groups in the enzymatic molecule:



The part of the cholinesterase molecule which undergoes phosphorylation is the OH-group in serine. This has been demonstrated to hold in the case of cholinesterase (20) and also for chymotrypsin (21), which is also inhibited by phosphorylating agents. The phosphorylation of serine has been thoroughly investigated by chemists (22). The amino-acid sequence in blocking the enzyme (—Asp—Ser—Gly—) (23) common to many members of the esterase group has been precisely determined within recent times. In addition to the available facts on serine, there are also

data on the in vitro physiological phosphorylation of the OH-group in tyrosine (24).

The purport of the facts here presented is to admit of the possibility of direct phosphorylation within the chromosome substratum, which is the seat of the mutational process -- probably within the protein portion of the gene and most likely involving the aminoacids serine and tyrosine. The latter possibility may turn out to be a source of a certain degree of specificity in the mutational process; this supposition is apparently more amenable to experimental confirmation through the study of reverse mutation.

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