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Test for Genetic Recombination in Kappa Particles of Paramecium aurelia, Variety 4

Kappa particles are microscopically visible structures, about a micron in length, which are distributed at random in the endoplasm of certain strains of paramecia. Furthermore, these particles, numbering several hundred per paramecium, are self-reproducing and mutable, and they contain deoxyribonucleic acid and are capable of infecting other paramecia under certain conditions (*1*). It is clear that the kappa particle shows many properties of a microorganism; hence, another property, genetic recombination, was also looked for.

At least two pairs of characters which do not lie at the same genetic locus must be studied to detect recombination. With kappa particles two such pairs are (i) ability, or lack of it, to produce the poison paramecin and (ii) ability, or lack of it, to persist in paramecia under certain culture conditions. The reason for assuming that these characters are not at the same locus is that they mutate independently of one another. Specifically, *slow hump kappa* particles (*2*) produce paramecin but are lost from animals grown at maximal fission rate at 31°C; *pi* particles (*3*) do not produce paramecin and are not lost under the growth conditions just described. Both types are mutants of the *normal hump kappa* particles found in stock 51 of *Paramecium aurelia,* variety 4. Normal hump kappa particles, like slow hump kappa, produce paramecin, but, like pi are not lost from well-fed paramecia grown at 31°C.

The test for recombination is to determine whether, following placement of slow hump kappa and pi particles in a common cytoplasm, there will appear particles capable both of producing paramecin and of persisting in host organisms grown at maximal rate at 31°C. That is, will normal hump kappa-like particles appear? This test was made as follows. Three types of paramecia, containing pi and slow hump kappa particles, containing only slow hump kappa particles, and containing only normal hump kappa particles, were all grown under conditions which would result in the marked decrease or loss of slow hump kappa particles, as indicated by a marked decrease or loss of poison production. Then the progeny of all three sets were tested for production of paramecin. The animals containing pi and slow hump kappa particles gave results comparable to those given by animals containing only slow hump kappa, both groups showing little or no paramecin production, whereas the normal hump kappa animals produced large amounts of the poison.

If the animals containing both pi and slow hump kappa particles had shown paramecin production similar to that of the normal hump kappa animals, then appearance of a recombinant type of particle in the former would be a possible explanation. In the absence of such a finding, the simplest conclusion is that under the conditions of this experiment no recombination occurs. This experiment assumed that the recombinant would behave as normal hump kappa do in the presence of pi—that is it would become abundant in each organism (*4*). However, it is possible that the recombinant might be of a new type which would exist only in a very low frequency. The experiment, as executed, does not test this possibility (*5*).

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Acetylcholine Hydrolysis in Psychiatric Patients

In a recent report (*1*) I suggested the possibility that the mechanism underlying the relationship between scotopic threshold changes and psychiatric disorders may be a deficiency in the acetylcholine-cholinesterase balance.

Granger (*2*) reported that schizophrenics manifested a significant elevation of the dark-adaptation curve along the log-luminance axis, without alteration of shape, as compared with normals. Rubin and Goldberg (*3*) employed Sarin (isopropyl methylphosphonofluoridate), an anticholinesterase, in experiments designed to determine the effect on scotopic visibility of measured quantities of inhaled material and found significant elevations of the absolute visual threshold. A second study (*4*) indicated that conjunctival instillation had no significant effect on dark adaption, while significant elevation of the dark-adaption threshold did occur when both eyes were protected from the atmosphere containing Sarin and in both eyes although only one had been protected. Rubin *et al.* (*5*) then performed another test of the hypothesis that the eventuation of the threshold response was localized in the central nervous system. Following exposure to Sarin, it was found that the tertiary atropine salt (atropine sulfate) reduced the elevated threshold to the preexposure level while the quaternary methosalt of atropine (atropine methyl nitrate) had no effect on the Sarin-produced elevated threshold. The ability of the tertiary salt to pass the blood-brain barrier while the quaternary salt does not suggested that the cholinergic blocking effect observed was acting centrally. It was further suggested that acetylcholine-cholinesterase concentrations are important determinants of the dark-adaption process. These results suggested that the elevated dark-adaptation thresholds observed in schizophrenia could be attributed to some biochemical imbalance between the enzyme and its substrate.

With this paradigm in mind, it was posited that the hydrolysis rate of acetylcholine by erythrocyte cholinesterase should be different for normals and for patients admitted to a psychiatric institute (*6*). Blood samples (5 ml) were obtained from 10 volunteers among the hospital staff and from 23 patients within 24 hours after admission and prior to the administration of any kind of therapy. Michel's electrometric method (*7*) was employed to determine the activity of the cholinesterase (*8*). The rate of hydrolysis of acetylcholine by erythrocyte cholinesterase for the normals and institutionalized patients is presented in Table 1. The standard error of regression for the normals was found to be $s_b = \pm 0.0003$, and the fiducial limits of the slope at the 5-percent level of confidence for $(n\text{-}2)df$ was found to be $l_1 = -0.0106$ and $l_2 = -0.0088$. The slope for each patient was calculated and then evaluated relative to the fiducial limits calculated for the normal sample. Six out of 23 patients had slopes that fell within the fiducial limits of the sample, while 17 distributed themselves into two groups that differed significantly from the linear regression that characterized the sample of normals. Ten patients showed significantly slower, and seven patients showed significantly faster, hydrolysis of acetylcholine by erythrocyte cholinesterase. Of the six patients whose slopes were in the normal hy-

Table 1. Linear regression equations and ΔpH/min. for the normal subjects and the two groups of patients who differed significantly from the normals as well as from each other.

Subjects	n	Hydrolysis rate	Regression equation	ΔpH/min
Patients	10	Slow	$Y = 7.917 - 0.0075X$	−0.0075
Normals	10	Normal	$Y = 7.906 - 0.0097X$	−0.0097
Patients	7	Fast	$Y = 7.922 - 0.0116X$	−0.0116

drolysis range, it is interesting to note in passing that one is presently considered by the clinical staff to have an organic lesion as indicated by electroencephalographic examination, and five are considered by the clinical staff to have neurotic depressions. Sixteen patients were evaluated clinically as schizophrenic.

These preliminary data do not contradict my general hypothesis that acetylcholine-chloinesterase imbalance may be a biochemical concomitant of the so-called "functional psychoses." A disturbance in enzymatic kinetics which may be reflected in excessive cholinesterase activity or deficient enzyme activity may be responsible for the maladaptive behavior which characterizes those that comprise the schizophrenic syndrome group. Since some pharmacological agents are now available whose effects on the acetylcholine-cholinesterase cycle are well known, experiments will be conducted to determine the behavioral changes that follow pharmacological manipulation of the kinetics of this enzyme system and its substrate. Specifically, it is predicted that marked behavioral changes should accompany the administration of an anticholinesterase—that is, diisopropyl fluorophosphate—to an individual whose hydrolysis rate for acetylcholine is too rapid, as compared with normal values; moreover, the administration of cholinergic blocking agents should produce marked behavioral changes in those individuals who manifest slow hydrolysis of acetylcholine by erythrocyte cholinesterase.

Although several investigators have previously focused attention on the role of acetylcholine-cholinesterase balance in schizophrenia and manic depressive psychosis, one may infer from their work that the hypothesis tested was limited to the role of deficient acetylcholine levels. Thus Fiamberti (*9*) has reported improvement in some schizophrenic subjects when intravenous injections of acetylcholine were administered. Rountree et al. (*10*) increased acetylcholine levels by administering diisopropyl fluorophosphate to schizophrenics and manic depressives without noting significant improvement in all subjects. The findings of the present study suggest that the administration of anticholinesterases or parasympathomimetic drugs could be effective only for those patients who manifested a rapid rate of hydrolysis of acetylcholine by the erythrocyte cholinesterase. If we can assume that the slopes obtained from the samples are reliable estimates of regression for populations, then we can deduce that, approximately, only half of a functionally psychotic population, selected at random, could be favorably affected by the pharmacological stimulation of a pronounced cholinergic effect. The remaining half, manifesting slow hydrolysis, would require cholinergic blocking agents.

It is not necessary, at this time, to consider whether or not this biochemical mechanism is the invariable concomitant of the functional psychoses. The next step requires that studies be undertaken to determine whether predictable behavioral changes result from systematic variations of enzymatic kinetics produced by appropriate pharmacological agents.

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Measurement of Observing Responses in Human Monitoring

The variables involved in visual monitoring and vigilance tasks need description and analysis, especially the observation, detection, and control response variables. In the usual study, control responses are measured, and then detection of a signal and observation of the display are inferred. Recently, however, Holland (*1*) has shown that direct measures of observing responses can be obtained. His subjects worked in a dark room and were required to detect and report deflections of a pointer on a dial. The dial could be seen only when the subject illuminated it for a short time by depressing a key. Each key depression was defined as an observing response. A second key was depressed by the subject to record signal detections and to reset the pointer. Holland found that, under fixed-interval schedules of pointer deflections, after each detection, observing responses ceased for a time and then resumed in an accelerated manner until the next detection. This resulted in scallop-shaped records of the subject's observing rate which were analogous to those obtained in other operant conditioning experiments with infrahuman subjects (*2*).

Generally speaking, observing responses refer to the relation, through time, between sense-organ orientation and displays. The visual sense is most commonly employed by humans in monitoring tasks. While Holland's technique insured that the observing response permitted observation of the display, the depression of a key may or may not be the same as actual head and eye movements involved in monitoring tasks. The experiment described in this report was designed to obtain a measure of observing responses which, while not eye movements, is assumed to be highly correlated with them. These measures were compared with those obtained by Holland.

Five male employees of the Research and Development Division of Electric Boat served as subjects, and each was given ten 30-minute sessions on a fixed, 1-minute interval schedule. As in Holland's study, the subjects worked in a dark room and had to detect deflections of a pointer from a null position. Detection was recorded and the pointer was reset when the subject pushed a button; the pointer remained deflected until the button was pushed. A continuous light source fixed atop elastic headstraps worn by the subject permitted him to observe the dial and pointer. The presence of the light on the dial was defined as an observing response. These observing responses can be thought of not as movements but as fixations of "holding responses" where the light is held on the dial. The light source was a 2.25-v flashlight bulb (lighted by a 1.5-v battery) fixed in the rear of a tube 10 in. long and ¾ in. in diameter. This tube was fixed to the elastic headstraps. At a normal viewing distance of 28 in., the circle of light was 3 in. in diameter and had an intensity of 3 ft-ca.

Before each session the subject sat in a comfortable position with his eyes on the dial. The tube was then adjusted by