univoltine species. These in turn are at the threshold of social behavior which, in at least one species, has already been crossed.

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Wheat Gluten–Schizophrenia Findings

Singh and Kay (*1*) reported that 30 out of 39 measures of psychopathology and social participation reflected non-therapeutic changes during the period of gluten challenge. In fact, only 5 of the 14 reported measures yielded changes sufficiently large to reach the commonly accepted .05 level of statistical significance. Moreover, symptoms characteristic of schizophrenia, such as poor impulse control and thought disorder, failed to attain this criterion. Indeed, 9 out of 13 measures yielded two-tailed probability values of .2 or less, indicating trends in the data, yet not direct confirmation of their hypotheses. Even the one measure on which the authors reported significant improvement, passive or apathetic withdrawal, represents only a weak trend at the .2 level.

The authors stated that group changes during gluten challenge "occurred against the expected course of improvement with neuroleptic treatment." However, deterioration during gluten treatment occurred only in the five most seriously ill patients with a less favorable therapeutic outcome, while the other nine patients with more favorable outcomes were not adversely affected. These figures indicate that the sample was not homogeneous with regard to premorbid history, number of prior hospitalizations, or severity of illness, all of which covary with prognosis.

Perusal of Singh and Kay's graphs does not convincingly indicate pathologic increases during the period of gluten challenge; indeed, decreasing trends in the pathology ratings occurred concurrently with gluten challenge. Moreover, the fact that overall therapeutic outcome and gluten response were not independent of each other suggests that a different type of analysis would have been more suitable in handling the data. The basic methodological question is whether pathologic increase during gluten challenge retains significance after the overall decrease in symptomatology covering 12 weeks is statistically controlled.

Analysis of the data would further require separating subgroups with differing premorbid histories and paranoid-nonparanoid status. In addition, the heterogeneity of the sample indicates that this group of schizophrenics includes both acute and chronic subjects. Moreover, the mean IQ of this sample was 78.57; borderline intelligence is not representative of hospitalized schizophrenics, particularly at the age of this sample, and this points to another source of confounding in the data. Such factors as these may significantly affect drug responsivity and prognosis independently of gluten treatment.

We doubt that the reported findings would persist if the analyses recommended above were carried out. If gluten impairs the psychological status of schizophrenics, it is important to provide sound data to demonstrate that effect. Appropriate statistical tests of the data and replication of the results of the study are called for.

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The recent report by Singh and Kay (*1*) appears to support the hypothesis that wheat gluten is a pathogenic factor in schizophrenia. However, it suffers from several serious methodological flaws.

The chi-square analysis they present utilizes the averages of 33 dimensions of psychopathology obtained with 14 patients as if they were independent observations, a basic requirement in any application of the chi-square test (*2*). However, since these dimensions of psychopathology are correlated, the chi-square analysis is not valid.

The correlated t-test comparing group changes across all 33 dimensions of the psychopathology rating schedule is also inappropriate since the sampling distribution for the correlated t-test is predicated on the fact that the rows are independent, a condition which again does not exist. The usual approach to answering the question the investigators appear to be asking in the analysis would be to compute a correlated t-test (or nonparametric Wilcoxon matched-pairs signed-ranks test) between the gluten and nongluten mean psychopathology scores with the data of the 14 subjects entered as rows.

Since no control groups were studied which received the placebo drink for all 12 weeks, it is impossible to determine how much of the attenuation of clinical effect after 6 weeks of neuroleptic treatment is due to the gluten intervention and how much is due to the normal process of recovery from a schizophrenic episode (that is, rapid neuroleptic effect followed by a more gradual and fluctuating recovery).

While the authors note that "each patient was interviewed for 90 minutes and independently rated by a specially trained psychiatrist and a psychologist," it is unclear whether the nonblind principal investigator (M.M.S.) conducted or participated in these clinical interviews. Since the interviewer is an important factor in any clinical interview situation, participation of the nonblind principal investigator in these interviews would make the ratings based on the information obtained during these interviews less independent of the experimental conditions than one would be led to believe.

Considering these methodological problems, I am reluctant to conclude that the hypothesis that wheat gluten is pathogenic in schizophrenia was confirmed.

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A brief consideration of our research design, schematized in Fig. 1, may clear up some of the objections raised in the two comments. The therapeutic efficacy of neuroleptics in schizophrenia is established, so a therapeutic course would be expected with adequate medication. Two changes will then have to happen for any intervention made along the course of treatment to be considered countertherapeutic: a therapeutic arrest or reversal during the period of intervention, followed by a therapeutic enhancement or return to the previous therapeutic course after the intervention is ended. In other words, both periods A and A' (Fig. 1) have to be considered at once in relation to the test period. Figure 1 in our report (*1*) illustrates that, on the average, both these criteria were met. Levy and Weinreb may have mistakenly considered the last pregluten rating (week 6) as being in the gluten challenge period to conclude that "decreasing trends in the pathology ratings occurred concurrently with wheat gluten challenge."

For statistical analyses, we calculated the gluten effects by subtracting $(A + A')/2$ from B, using the final ratings in each period (indicated by arrows in Fig. 1). If B exceeded $(A + A')/2$ in terms of pathology, it was counted as an hypothesized or countertherapeutic effect. This procedure not only accounted for the medication effect, but controlled for the order effects that would obtain if B were compared separately with A and A' because of the expected course of therapeutic changes and would thus introduce both errors of commission (type 1) and errors of omission (type 2). Furthermore, by calculating $B - (A + A')/2$ separately for each patient and then applying a test such as the correlated t-test to the difference values obtained, we were able to use each patient as his own control and thus obviate the problem of errors due to variability that stems from the multifarious heterogeneity of schizophrenia.

Our design and statistical approach, therefore, took into account both the heterogeneity of schizophrenia and the therapeutic activity of the medication, which Levy and Weinreb as well as Smith commented on. Their criticisms are derived from the perspective of the usual cross-sectional types of comparisons of a test group with a control group. We rejected that for a longitudinal method, in which each patient was his own control, so as to get around the problem of the premorbid, phenomenologic, nosologic, prognostic, therapeutic, and possibly etiologic diversity of schizophrenia, and focus only on the changes produced by

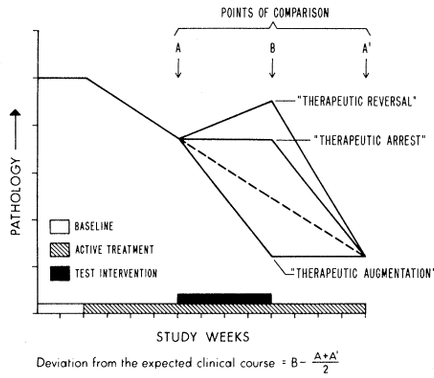

Fig. 1. Research design. Downward curve indicates decreasing severity of pathology.

the test intervention. Heterogeneity not only poses a problem of matching controls with test subjects, especially since the relevant matching criteria are uncertain, but makes a cross-sectional approach less than ideal because meaningful differences may be lost in the variability of the samples, while the so-called significant differences may be no more than a nonspecific lowest common denominator in different subgroups after the fashion of IQ in the many etiologically distinct types of mental retardation.

An example of cross-sectional logic applied to a longitudinal design is Smith's comment about the lack of a control group to establish gluten effects in relation to the course of treatment. Contrary to his opinion, with the formula we used to determine gluten effects, a natural plateau in the therapeutic course would yield B equal to or less than $(A + A')/2$ and would not appear as adverse gluten effect. We believe that this also answers Levy and Weinreb's concern about considering gluten effects against the expected therapeutic effects of the medication. (Smith incorrectly refers to six neuroleptic treatment weeks before the gluten challenge; there were only four. Levy and Weinreb are also inaccurate in stating that only five patients were adversely affected by wheat gluten; ten patients were described by us as having shown varying degrees of countertherapeutic gluten effect.)

Levy and Weinreb confuse the issue of the determinants of prognosis in schizophrenia and the presence or absence of gluten effects. The therapeutic outcomes were assessed after the end of the study, many weeks after the wheat gluten challenge. The poor outcomes were no more attributable to "gluten treatment" than were the good outcomes.

Our main purpose in discussing the greater conspicuousness of hypothesized gluten effects in the poor-outcome patients was to suggest that wheat gluten probably did not act by interfering with the absorption or pharmacological activity of the drugs—a conclusion also supported by the data on side effects. This does not mean that the therapeutic activity of neuroleptics did not oppose the manifestation of possible pathogenic effects of wheat gluten. Neuroleptics must be effective in the presence of wheat gluten as it is an abundant constituent of the ordinary diet, so that in the medicated patients any pathogenic influence of wheat gluten can be manifest only to the extent that the counteractive effect of neuroleptics is not successful. Such a possibility is also suggested by the fact that symptomatic improvement in schizophrenia requires continuous long-term treatment with neuroleptics, and withdrawal of the medications even after many years is frequently attended by an exacerbation of the psychosis. This would mean that, within the framework of our experiment, gluten effects would be less evident than, for example, if wheat gluten were given to unmedicated recovered schizophrenics.

Subgroup analyses would certainly be desirable, but would hardly be appropriate with a total sample of 14. Nevertheless, the absence of such analyses should not detract from the principal finding that we presented.

In view of the controls built into our study design, which we believe protect against type 1 error; the likely attenuation of the possible pathogenic effects of wheat gluten by the concurrently given therapeutic medication; and the serious consequences of a type 2 error, we consider that a somewhat less stringent than usual level of statistical significance is called for. The use of a one-tailed test is also appropriate because only one end of the probability curve is under evaluation.

Our report that a change in 30 of 39 measures was in the hypothesized direction was based on group means for $B - (A + A')/2$ for each parameter and was presented as descriptive statistics to indicate how extensively the gluten effect was reflected in the clinical picture. The nonparametric and parametric tests were used to further consider the consistency and extent of the gluten effect across parameters. This was important in that, with the heterogenous character of schizophrenia, which would be expected to lead to differential results in the individual parameter analyses, it served to show that the wheat gluten effect may have a generality that transcended the apparent diversity of schizophrenia. Smith has confused the two perspectives—consistency across parameters and consistency across subjects—within which we tried to consider the

gluten effects. It is debatable if within the former perspective, mean gluten effects on individual parameters could not be considered as "independent" observations, both because schizophrenia is clinically heterogenous and because in performing the ratings each dimension has to be regarded as separate and independent. It is certainly unlikely that the arguable relatedness of parameters could account for the probability of 1 in 10,000 obtained with the correlated t-test. The method Smith suggests deals with the second perspective. For that, the separate parameter-by-parameter analyses that we performed are to be preferred because the method of averaging across dimensions involves the unacceptable assumption of clinical equivalence of all parameters. He ignores the evidence from individual parameter analyses in concluding that the gluten hypothesis is not supported by our work.

The nonblind investigator (M.M.S.) interviewed patients only in the initial drug-free weeks to establish baseline pathology, but not in any of the periods relevant to the experiment.

The mean IQ of our sample (78.57) was not atypical when compared with the published figures from large samples indicating a mean IQ of 84.28 ± 16.6 for a diagnostic composition such as ours (*2*).

With the prevailing uncertainty about the syndrome called schizophrenia, our research decisions represented the best compromises we could devise between conflicting considerations, both practical and theoretical. After considering the criticisms, we remain convinced of the soundness of the decisions. Whether we are correct can only be determined by further research. One possibility is to test the wheat gluten effects in remitted, drug-free schizophrenics.

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Minimal Brain Dysfunction: Dopamine Depletion?

Shaywitz, Yager, and Klopper (*1*) propose that the hyperkinetic, or minimal-brain-dysfunction, syndrome in children may be due to a deficiency in the neural transmitter dopamine. As evidence, they demonstrate that rats selectively depleted of dopamine during infancy are significantly more active than normals during the 10- to 25-day range, and that they show learning deficits later in life. However, there are several difficulties with this analysis:

First, normal 10- to 25-day-old rats are "hyperactive" compared to older rats, apparently because certain adrenergic "activity" systems mature around age 10 days, while the cholinergic "inhibitory" systems, particularly the hippocampus and frontal cortex, approach maturity between ages 15 and 25 days (*2*). The hyperactivity produced by dopamine depletion was merely a moderate increase in the already-hyperactive behavior which is normal for this age. It disappeared at the same time that normal hyperactivity disappears, around age 25 days, presumably because of the maturation at this time of the cholinergic and perhaps also serotoninergic systems.

Second, Shaywitz *et al.* interpret the disappearance of hyperactivity in their rats at age 25 days as analogous to the amelioration of the hyperkinetic syndrome in children at age 10 to 12 years. However, age 25 days in a rat is merely the age of weaning. Puberty, corresponding to human age 10 to 12 years, occurs at about age 80 to 100 days in rats.

A more general hypothesis for hyperkinesis, which includes dopamine depletion as a special case, is that this syndrome is caused by a predominance of norepinephrine (NE) relative to other transmitters. Activity level and reward seem to depend on NE (*3*), and seem to be inhibited by acetylcholine (*4*), serotonin (*5*), and to a lesser extent dopamine (*6*). Rats with increased NE, or depletion of acetylcholine or serotonin, or damage to predominantly cholinergic structures, show high activity (*3–6*), deficits in habituation (*7*), difficulty with selective attention (*8*), and impaired punishment avoidance (*4, 9*). Adult human manics also show these four symptoms, plus euphoria (which is hard to demonstrate in rats); this parallel led to the now widely accepted theory that mania is due to an excessive level of NE relative to the other three transmitters (*8, 10*). The commonly reported characteristics of hyperkinetic children are, again, high activity, difficulty with selective attention, impaired punishment avoidance, and euphoria (*11*). One would suspect, therefore, that hyperkinetics, like manics, suffer from an excess of NE relative to the activity-inhibiting transmitters. (Curiously, mania is reportedly extremely rare in children, and hyperkinesis is unheard-of in adults.) Since the NE activating systems seem to mature before the cholinergic suppressive systems (*2*), any process which retarded the later periods of brain maturation would be expected to produce temporary dominance of the adrenergic systems, and therefore a hyperkinetic syndrome.

There are several other points about the hyperkinetic syndrome which make sense if we attribute it to delayed maturation of the later-maturing parts of the brain. First, the syndrome is three or four times more common in boys than in girls (*11*), which correlates with the fact that girls mature faster than boys. Second, the amelioration of the condition with age may be attributed to the continued, though belated, maturation.

Hyperkinesis, therefore, could be caused by anything which produced NE dominance with delayed maturation of cholinergic systems being perhaps the most common mechanism. One otherwise paradoxical phenomenon of hyperkinetic children makes sense on the basis of NE dominance: the activity-reducing effect of amphetamine. As Shaywitz *et al.* insightfully suggested, this result can best be explained by assuming that amphetamine stimulates the activity-suppressing transmitters dopamine and 5-hydroxytryptamine (5-HT) (*6, 12*) as well as NE. The question, of course, is why amphetamine effects on dopamine and 5-HT should dominate in hyperkinetics, although the effect on NE is greatly predominant in normals. An attractive possibility is that amphetamine's effects on dopamine and 5-HT become evident only when NE levels are so high that further increases would be ineffective. Indeed, analogous phenomena have already been demonstrated in rats: After NE has been highly potentiated by high doses of imipramine or amitriptyline, amphetamine has less activity-facilitating effect than usual. Also, when operant response rates are very high (analogous to hyperkinesis), amphetamine decreases the response rate (*13*).

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