N,N-Dimethyl-p-phenylenediamine Oxidation by Serum from Schizophrenic Children

Interest in the oxidation of aromatic amines has been renewed by the work of Akerfeldt (*1, 2*) with the in vitro oxidation of N,N-dimethyl-*p*-phenylenediamine dihydrochloride (DPP) by the serum of normal and schizophrenic patients. Since a survey of the literature failed to reveal any published data concerning this oxidation reaction in the case of children, we have examined the sera from 23 children hospitalized because of psychiatric illness. The group ranged in age from 6 to 13 years. There were 5 female and 18 male children.

The Akerfeldt test (*1*) was modified slightly to yield a final solution of serum and DPP whose *p*H was between 7.00 and 7.15 (*3*). Three parameters were used in the analysis of the biochemical data. The first was the value of the slope when the optical density at 552 mμ was plotted against time. The second was the length of lag period which resulted in the majority of cases prior to the oxidation of DPP. The optical density at 552 mμ, determined 5 minutes after the addition of DPP to the serum, served as the final parameter. The length of the lag period has been shown to be a function of the amount of ascorbic acid in the serum (*1, 3*). The rate of change of optical density is the result of the influence of ceruloplasmin and the apposing activity of sulfhydryl groups (*2*) as well as other metabolites (*4*).

Observations in ten cases in which the psychiatric diagnosis (*5*) was not schizophrenia (group A) are given in Table 1. Seven cases specifically designated as schizophrenia (group B) by the attending psychiatrist, as well as an additional group of six cases in which the diagnosis of schizophrenic reaction is likely but not satisfactorily documented beyond reasonable doubt (group C), are also presented.

The biochemical criteria used to determine an abnormal response were as follows: (i) a lag period in the 0- to-1.6-minute range *and* (ii) an optical density reading of 0.39 or higher at 552 mμ after 5 minutes. Both criteria had to be met; if only one was satisfied the test was considered to be borderline.

The lag periods and optical density readings of the sera from the children in group A indicate that eight were "biochemically normal," one (No. 22) was borderline, and one (No. 21) showed an abnormal response. On the basis of these biochemical measurements, child No. 21 was thought to be schizophrenic; this interpretation was not in agreement with the psychiatric diagnosis. In the remaining nine subjects the absence of an abnormal response bore out the psychiatric diagnosis.

Of the children in group B, only in cases 5 and 14 did the results permit a prediction of schizophrenia on the basis of the biochemical data. In group C, only cases 11 and 15 met both biochemical criteria for such a prediction. The results in the cases of the remaining children in groups B and C were typical of those obtained from normal adults.

Statistical analysis of the data in groups A, B, and C failed to reveal any significant differences between the values for optical density, slope, or lag period obtained from biochemical measurement of the sera of schizophrenic children and those obtained from measurement of the sera of nonschizophrenic children. These results, therefore, offer little or no support for the suggestion that the Akerfeldt-type reaction can be used to distinguish between schizophrenic and nonschizophrenic children. Similarly, Horwitt *et al.* (*6*) were unable to distinguish, on the basis of the Akerfeldt test, between normal and schizophrenic adults.

Table 1. Biochemical parameters and psychiatric diagnosis of 23 children hospitalized because of psychiatric illness.

Case No.	Biochemical parameters: Slope	Lag period (min)	Optical density (552 mμ) at 5 minutes	Psychiatric diagnosis
		Group A		
12	0.168	3.2	0.32	Passive-aggressive
22	0.162	1.8	0.48	Chronic brain syndrome
6	0.132	3.4	0.22	Anorexia nervosa
7	0.126	4.1	0.17	Passive-aggressive
21	0.120	0.0	0.56	Passive-aggressive
1	0.120	4.0	0.17	Psychoneurosis
20	0.120	4.2	0.13	Mental retardation
17	0.120	2.3	0.32	Maladjustment, childhood
2	0.108	6.0	0.02	Emotional instability
4	0.108	3.8	0.16	Psychoneurosis
		Group B		
5	0.300	< 0.5	0.80*	Schizophrenia, autism
18	0.138	3.2	0.30	Childhood schizophrenia
8	0.138	5.5	0.03	Childhood schizophrenia
14	0.120	0.0	0.45	Childhood schizophrenia
9	0.120	4.3	0.13	Childhood schizophrenia
3	0.114	4.1	0.12	Schizophrenic reaction
13	0.084	3.8	0.13	Schizophrenic reaction
		Group C		
15	0.138	< 0.4	0.60	Schizoid personality
10	0.138	3.4	0.25	Childhood schizophrenia
11	0.170	< 0.4	0.39	Childhood schizophrenia
19	0.120	4.0	0.15	Schizophrenia (questioned)
23	0.114	5.0	0.03	Schizophrenia (questioned)
16	0.090	3.7	0.11	Schizophrenic reaction

* At 3.8 minutes.

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References and Notes

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Absorption of C^{14}-Labeled Sucrose by Alfalfa Nectaries

The physiological significance of nectar in the life of the plant has not been adequately determined. Bonnier, in 1878 (*1*), observed that reabsorption of nectar took place if it were not removed from the flower before pollination. Pankratova reviewed this subject in 1950 (*2*).

In the studies reported here, C^{14}-labeled sucrose was used to demonstrate the reabsorption of nectar and its distribution in the plant. A special capillary pipette was used to place 0.001 ml of a 40-percent solution of C^{14}-labeled sucrose on the nectary of an alfalfa flower. All

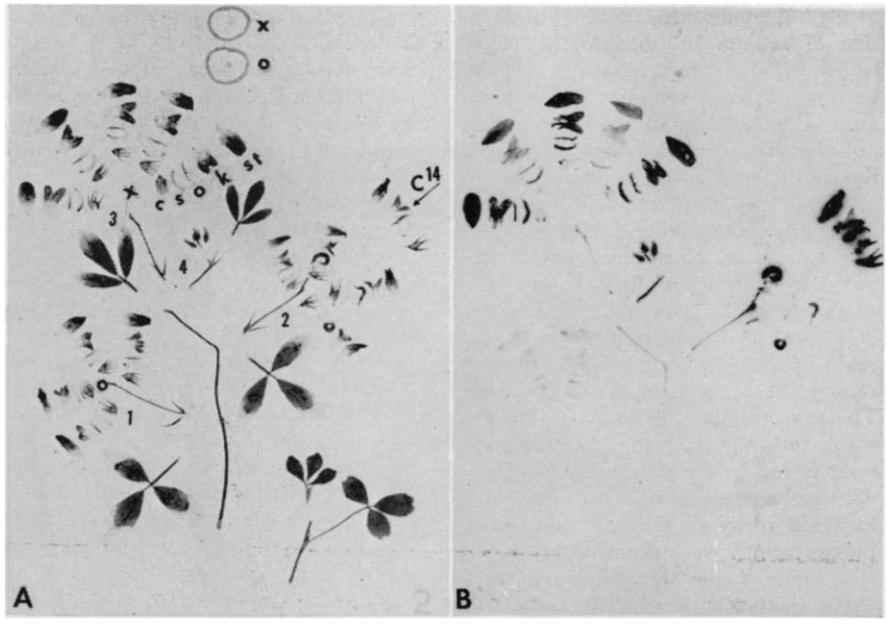

Fig. 1. Results of the experiments described in text, shown in photograph (*A*) and in autoradiogram (*B*). The point of application is indicated by the arrow. The flower parts are arranged from the outside in the following order: standard petal (*st*), wings and keel (*k*), ovary (*o*), staminal column (*s*), and calyx (*c*). On the raceme (2) with the treated flower, the pods from three of the flowers in addition to that from the treated one became radioactive, but the floral parts which had developed before the treatment did not react. One pod failed to grow. All parts of the five flowers of the upper raceme (3) which opened after the treatment became radioactive. Nectar from this raceme (3) and from the one below (1) was placed in the circles labeled *x* and *o* at the top of *A*. This nectar was also radioactive. The raceme at the lower left (1), which was in full bloom at the time of treatment, was not pollinated and became only partially radioactive. The terminal bud (4) located approximately in the center of *A* and the leaf at the lower right that developed after treatment were radioactive. Older leaves that had completed growth before treatment did not create an image on the negative. [Photographic work by W. P. Nye]

flowers on the raceme, including the treated one, were cross-pollinated by hand just before treatment. The plants were grown in a greenhouse in the absence of pollinating insects. After 6 days the plant was dissected, mounted on cardboard, pressed, and dried, and autoradiograms were prepared.

The results of the experiments, as shown by the autoradiogram (Fig. 1B) and picture (Fig. 1A), demonstrate that nectar is reabsorbed. The absorbed material is distributed primarily to growing parts of the plant, such as leaves, flowers, and pollen, but can also be found in the roots and in nectar of flowers that develop after the treatment.

Leaves which were completely developed before treatment did not become radioactive. On an adjacent raceme, flowers that had opened before treatment were much less radioactive than those that opened after treatment. However, nectar from flowers on both racemes showed images on the autoradiogram. In this case, nectar (as sucrose with C^{14}) was absorbed from a flower on one part of the plant and translocated and secreted in flowers some distance away (see Fig. 1). Similar results have been obtained with a number of other species.

In a time series test, parts of alfalfa plants adjacent to treated flowers were removed and checked for radioactivity at intervals of ½, 1, 3½, and 8 hours after treatment. These showed that reabsorption occurred within ½ hour after treatment and pollination, and that the amount of reabsorption was roughly proportional to the time interval after treatment.

On the assumption that sucrose is representative of nectar, it has been demonstrated that nectar is absorbed by nectaries as well as secreted by them. The fact that absorption occurred shortly after treatment suggests that nectar is not a static product (dissociated, so to speak, from the plant) but is in close contact with the plant system. Additional work will be needed to show whether or not nectar is also absorbed prior to pollination or senescence of the flower.

The question arises as to whether or not the nectar that is not removed by bees has any significance in the production of seed. If it does, the good production of alfalfa seed obtained by use of bees which collect mainly pollen may be partly a result of the fact that less nectar is collected. Obviously, nectar is a source of food adjacent to the developing embryos in a fertilized flower. Brink and Cooper (*3*) considered the nutrient supply to the seed to be a factor in seed failure.

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Conversion of Indirect- to Direct-Reacting Bilirubin in vivo

It is now well established that the difference between indirect- and direct-reacting bilirubin lies in the fact that the latter is conjugated with glucuronic acid (*1*). Considerable evidence indicates that indirect bilirubin in high concentrations is toxic to the nervous system (*2*), particularly in the neonatal period. Kernicterus, which is found in association with high levels of indirect bilirubin in the plasma, is regarded as bilirubin encephalopathy. If it were possible to control the high levels of indirect bilirubin in hemolytic disease of the newborn and in other hyperbilirubinemias seen at this time of life, the need for exchange transfusions would no longer exist.

It is known that an enzyme, glucuronyl transferase, involving uridine diphosphate glucuronic acid is concerned in the esterification of bilirubin with glucuronic acid (*3*), an enzyme which is deficient in the liver of the newborn (*4*). In addition, there appears to be an extrahepatic mechanism for the conversion of bilirubin to its glucuronide, although the

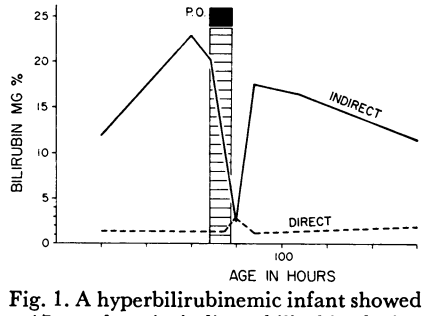

Fig. 1. A hyperbilirubinemic infant showed a 17-mg drop in indirect bilirubin during oral administration of 15 g of glucuronic acid.