lay. 83% (10 of 12) of Strain B birds, and 56% (23 of 41) Strain C birds had parthenogenetic development in newly laid eggs. Likewise 63.6% (7 of 11) Strain A birds, 33.3% (4 of 12) Strain B birds, and 7.3% (3 of 41) of Strain C birds produced eggs showing parthenogenetic development as observed after 9-10 days incubation. On an egg basis the microscopically observed incidence of parthenogenesis (57%) establishes the Dark Cornish breed as the highest producer of eggs showing parthenogenetic development of all domestic breeds of chickens reported on to date.

These data indicate definite strain differences within the Dark Cornish breed with regard to incidence of parthenogenesis. While Olsen(8) suggests that heredity is not exclusively in control, there is strong indication here that genetic factors are significant. Genetic differences are quite evident at the breed level since the incidence in these stocks is four times that reported by Kosin(6) in 2 different breeds of chickens.

It is apparent from the method employed that Olsen and Marsden(5) did not count those eggs which may have had parthenogenetic cells in the blastodisc at laying but did not develop further when incubated. It would be interesting to know whether some of the eggs considered non-parthenogenetic by Olsen and Marsden would show parthenogenetic cells if examined at the time of lay.

Summary. Infertile eggs from 3 strains of virgin Dark Cornish pullets were examined either microscopically at laying or macroscopically after 9-10 days incubation to determine relative incidence of parthenogenetic development. The data indicate the incidence of parthenogenesis may vary not only in different breeds or strains of birds, but also the observed incidence may vary with different technics of observation and different times at which these observations are made.

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Some Sources of Error in the Akerfeldt Test for Serum Oxidative Activity.* (23780)

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The procedure recently introduced by Akerfeldt(1) for estimating serum oxidative activity is based on the rate at which color develops after equal parts of serum and of a 0.1% aqueous solution of N, N'-dimethyl-p-phenylenediamine (DPP • 2HCl) are mixed in the absence of added buffer. According to Akerfeldt, normal sera caused little color change in the first 3 to 5 minutes whereas sera from patients with psychoses (and with many other diseases, as well as normal pregnancy) caused rapid development of color. However, observations made in these laboratories demonstrated that Akerfeldt's method does not accurately measure oxidative activity and cannot be used as a test for psychosis.

Observations. 1) *Temperature*: We found that small variations in initial temperature of the reaction mixture, or merely absorption by the mixture of heat generated in the spectrophotometer, significantly altered the

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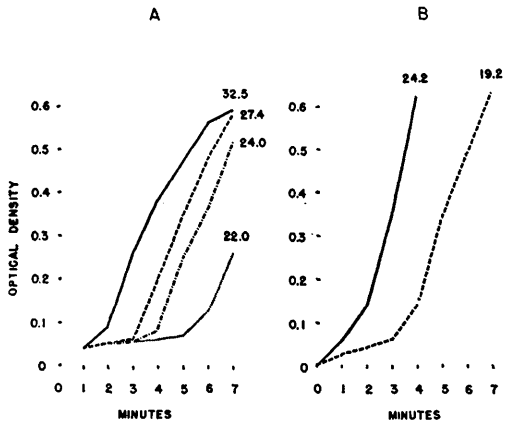

FIG. 1. Effect of temperature on Akerfeldt reaction. A—normal subject. B—psychotic patient.

velocity constant of the color-producing reaction and hence also the length of lag period which preceded it. Fig. 1A and 1B show the curves obtained with Akerfeldt's technic on sera drawn from 1 normal and 1 psychotic subject. The temperatures given are initial temperatures of the reaction mixture. Some curves also were obtained at temperatures other than 23°C. It is clear that at temperatures above 27°C all sera tested gave "psychotic" curves (*i.e.*, caused rapid onset of color) and that at temperatures below 17°C all sera tested were "normal" (*i.e.*, showed slow onset of color).

2) *pH*: The color-producing reaction is strongly inhibited at pH's above 7 and below 6. Variations in serum pH and buffer capacity have caused "psychotic" reactors to appear "normal" when an external buffer was not added to the reaction mixture (Fig. 2).

3) *Diet of the subjects*: Akerfeldt suggested that 3 distinct steps are involved in this reaction: 1. The DPP is oxidized linearly by blood ceruloplasmin, to a colored semi-quinone, DPPox. 2. This DPPox is immediately reduced to DPP by ascorbic acid of the serum. 3. After the ascorbic acid is used up, DPPox can re-form, imparting color to the reaction mixture. Later the DPPox reacts with other endogenous inhibitors in the serum—which perhaps includes a protein-containing sulfhydryl group—and thus the linearity of the first-order reaction is no longer apparent.

If the ascorbic acid is responsible for the lag period seen in this reaction, it would be reasonable to expect that differences in ascorbic-acid intake should cause variations in time that elapses before color-formation begins. It therefore becomes important to learn whether variation in ingested ascorbic acid might cause a positive test for psychosis in persons merely suffering from malnutrition. The method was first standardized with respect to temperature and pH. In this procedure 2 ml of fresh serum is mixed with 1 ml of 0.1 M phosphate buffer at pH 6.8 and ½ ml of distilled water, and the mixture cooled to 23°C. To this is added ½ ml of 0.4% DPP dissolved in 0.024 N HCl at 23°C (to yield DPP • 2HCl in solution). This mixture serves as its own blank in a Coleman spectrophotometer. The optical density of the mixture is measured at one-minute intervals at 552 μ; between readings it is kept at 23° in a water bath. Several minutes after the rate of the color-producing reaction has started to decrease, or about 10 to 15 minutes after the DPP is added, the readings are terminated and the pH of the mixture is determined. This should be in the range of 6.8 ± 0.2 and is generally over 6.8.

Serum-oxidase tests were carried out before and after ingestion of the ascorbic acid on 5 chronically psychotic patients at the McLean

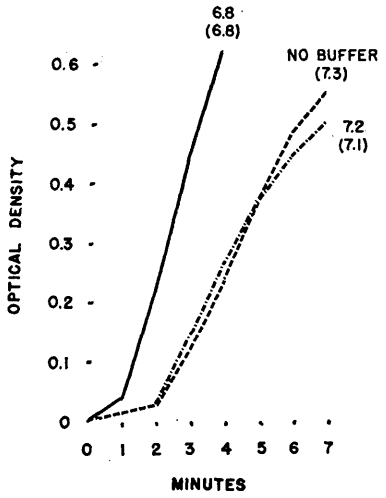

FIG. 2. Effect of pH on Akerfeldt reaction of psychotic patient. Figures indicate pH of buffer, where used; final pH of reaction mixture is in parentheses.

Hospital and on 9 healthy normal subjects. The first group, comprising 4 normal subjects, was given 48 oz. of orange juice (in addition to their normal diets), according to the following schedule:

1st test	10 A.M.
Orange juice, 12 oz	2 P.M.
Idem	8 P.M.
Orange juice, 24 oz	7 A.M.
2nd test	10 A.M.

A second group, consisting of 5 psychotic patients, was given 1000 mg of the ascorbic acid, 250 mg each at 2 p.m. and 8 p.m., and 500 mg at 7 a.m. the next day, to demonstrate that the ascorbic acid actually was the agent responsible for the change in lag-time. A third group of 5 normal subjects was similarly given 1000 mg of ascorbic acid over a 24-hour period.

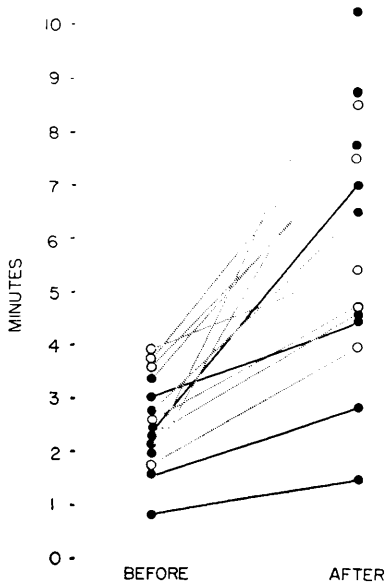

FIG. 3. Effect of ingestion of orange juice or of ascorbic acid on appearance time of color. Open circles indicate patients; solid circles indicate normal subjects. Solid lines indicate use of orange juice; dotted lines indicate use of ascorbic acid.

There was an increase in lag-time after ascorbic-acid ingestion in every case (Fig. 3). In the first group, the average increase was 1.9 minutes; in the second 2.7 minutes; and in the third group it was 4.9 minutes.

The findings are consistent with the reaction hypothesized by Akerfeldt. They also indicate that the amount of ascorbic acid ingested in the period preceding the test is probably the most important determinant of the lag-time. The difference in slope that was found after the color-forming reaction began did not show any consistent relation to ascorbic-acid ingestion. The increase in lag-time in normal subjects was found to be greater than that observed in psychotic patients in the small series reported here. It is evident that the test chiefly measures the state of nutrition with respect to vitamin C, and that the results should be abnormal in any condition in which vitamin C stores are depleted.

Summary and conclusions. The Akerfeldt test for serum-oxidase activity, originally proposed as a test for mental disease, should not be used for this purpose. Sera yield "normal" or "abnormal" results depending on temperature and pH at which the reaction is carried out. Even when the method is standardized to eliminate these two variables, the results obtained depend so greatly on the subjects' previous diet that they render the test of questionable value in the diagnosis of anything, except perhaps ascorbic-acid deficiency.

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