that the deforming stress was unidirectional. A consideration of the mechanics of this type of deformation shows that the cup was active and the cone passive, and that the shock was applied from the direction in which the apexes point. Therefore, the orientation of the shatter-cones suggests that, assuming that the beds were essentially horizontal prior to deformation, the shock force resulted from some type of explosion directly above the beds rather than from a cryptovolcanic explosion below the beds.

A probable interpretation for the observed orientation of these shatter-cones is that the Kentland disturbance is the "root" structure of a meteorite crater which was formed after late-Paleozoic time and deeply eroded prior to the Pleistocene. In fact, it is difficult to conceive of any other type of explosion than that of a large meteorite which would act from above the strata. Boon and Albritton (*1*) have developed evidence to show that structures of the Kentland type are the product of a meteorite impact. According to these writers, high-velocity impact, many times faster than the velocity of a shock wave in any type of rock, compresses the rocks elastically, rather than deforming them plastically, after which they are "backfired" into a damped-wave disturbance. Shatter-cones pointing toward the impinging body may be formed during the initial or compressional stage of such a meteoroid impact.

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Evidence That the Hemolytic Anemia Caused by Fat and Choline Is Not Due to Lipotropic Action[1]

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Johnson and co-workers (*6*, *7*) have shown that the feeding of fat to dogs increases the fragility of their red blood cells and causes an increased destruction of erythrocytes as judged by an increased output of bile pigment. The hemolytic agents from fat are presumably fatty acids and soaps which have escaped resynthesis into neutral fat during absorption (*4*, *5*). Although a high fat diet does not by itself produce anemia, Dupee, *et al.* (*3*) have shown that it can produce hyperplasia of the red bone marrow in dogs.

We have demonstrated (*1*, *2*) that the administration to dogs of choline chloride in addition to a high fat diet causes the rapid development of an acute hemolytic anemia, accompanied by a rise in the icterus index, which rapidly regresses upon cessation of administration of either the fat or the choline (if the latter has not been fed for more than 8 or 10 days). To explain the mechanism of the production of this anemia, we have postulated that the choline exerts a "holding" action upon the bone marrow to prevent any great increase in its rate of erythropoiesis, while the fat furnishes products which cause the actual hemolytic destruction of erythrocytes.

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Our experiments did not preclude the possibility that choline might exert its lipotropic action with the fat and thereby, in some manner, produce anemia. The present investigation was made to test this possibility, by the administration of atropine, which does not antagonize the lipotropic action of choline, but does block its pharmacologic vasodilator action.

Normal erythrocyte counts and hemoglobin percentages (Sahli) were determined, over a period of days, on one splenectomized and two normal dogs. The three animals were then given 60 grams of fat,[2] and 10 mg./kg. of choline chloride daily, in addition to their regular adequate diet. After the first day of the experiment, two of the dogs were given daily subcutaneous injections of atropine sulfate (0.5 mg./kg.) in addition to the orally administered choline and fat. Erythrocyte numbers and hemoglobin concentrations were determined daily on the blood of each dog during the experimental period.

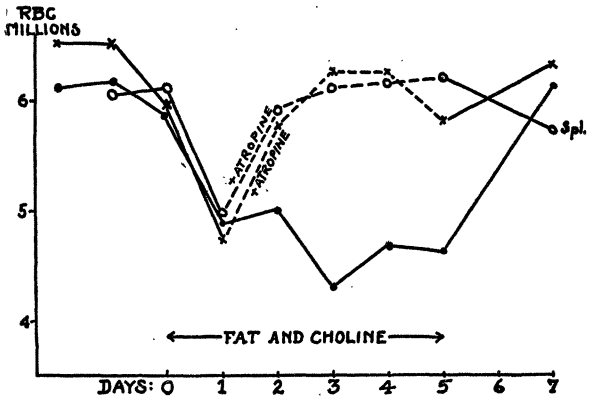

Fig. 1. The effect of atropine upon the erythrocyte counts of dogs rendered anemic by fat and choline. Dashed lines indicate periods during which atropine, in addition to fat and choline, was administered to two dogs. Open dot line (labeled Spl.) represents erythrocyte numbers of a dog which had been splenectomized about one year prior to this experiment.

It will be seen in Fig. 1 that the feeding of fat and choline caused reductions in the erythrocyte numbers of the three dogs, as observed about 24 hours after the first daily doses. At this time, two of the dogs were started on daily doses of atropine in addition to the choline and fat, and as a result, their erythrocyte numbers (dashed lines, Fig. 1) returned to normal within two days. The third dog, whose erythrocyte count is recorded as a solid line throughout Fig. 1, did not receive atropine and remained anemic during the period of fat and choline feeding. Hemoglobin percentages were observed to change proportionately to the erythrocyte counts.

Since atropine was shown, previously, to prevent or abolish the hyperchromic anemia which was produced more slowly by the administration of choline alone (*1*), we rather expected that it would also antagonize the hemolytic anemia induced by fat and choline, as it actually did in this experiment.

We believe, therefore, that in these experiments, choline acts by the mechanism previously postulated (*1*), *i.e.* as a weak brake to inhibit any acceleration of erythropoiesis which may normally follow the hemolytic destruction of red blood cells. It probably does this by causing vasodilation and improved

[2] Wilson's "Advance," a shortening made from animal and vegetable fats.

blood and oxygen supply to the bone marrow, thus tending to depress erythropoiesis.

It is concluded that choline aids in the production of this anemia, *not* by virtue of a lipotropic action, but rather by its vasodilator or pharmacological action.

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A Nonrespiratory Variant of *Saccharomyces cerevisiae*[1]

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In studies of the toxic effect of ethylene oxide on a strain of *Saccharomyces cerevisiae*, it was noted that, at a time when almost all of the yeast cells in a suspension have been killed by the poison, some of the surviving cells give rise to colonies unlike those of the original organism. These colonies are smaller and rougher than the normal type. The organisms in them are usually more spherical, somewhat smaller, and in some cases occur in clusters. When restreaked on agar plates, they gave rise to colonies showing much variation in size. The ethylene oxide-induced variants can be obtained repeatedly from the original strain after repurification of the latter and thus do not represent a constant degree of contamination.

The occurrence of mutated colonies has been described before (*2, 3*) and interpreted as being due to the development of haploid yeast cells. However, such variation is usually observed only after sporulation of the yeast is followed by isolation on special media or by dissection and cultivation of individual ascospores.

In a few instances we have observed what appeared to be fusion between cells of the variant colonies to form a diploid yeast. Haploid yeasts are often unstable, not only because of possible conjugation but also because of the high rate of mutation to which they are subject. Instability has been noted in the variants obtained in this work and has led to difficulties in studying them. However, certain strains of the apparently haploid type have been sufficiently stable to be examined in some detail, and one, which did not undergo further variation over a period of several months, has shown very interesting characteristics.

Unlike suspensions of the parent strain, resting suspensions of this variant show no ability to oxidize either glucose or alcohol under aerobic conditions. In any case, the respiration is so slight that it is not measurable in Warburg respirometers. This is true even when the organisms have been grown in a shaking liquid medium containing 0.5 per cent glucose and 5 per cent yeast autolysate and have been harvested during the period of exponential growth. As will be seen in Table 1, the rate of fermentation is high, and no Pasteur effect can be observed.

Suspensions of the parent yeast examined spectroscopically show absorption bands corresponding to reduced cytochromes A, B, and C at 605, 552, and 563 A. Similar suspensions of the variant show a strong band at 563 A. (cytochrome C), but lack the other two even when reducing agent is added in excess.

TABLE 1

Rates of Respiration and Fermentation of Parent and Variant Strains at 30° C. With 2 Per Cent Glucose and 2 Per Cent KH_2PO_4

	Parent	Variant
Q_{O_2}	42.5	0
$Q^{air}_{CO_2}$*	62	274
$Q^{CO_2}_{CO_2}$	145	268

* Only aerobic fermentative CO_2 production included in $Q^{air}_{CO_2}$.

In addition, the variant gives no test for indophenol oxidase with Nadi reagents according to the method of Keilin (*1*). The parent strain gives a strong positive reaction under identical conditions. Spectroscopic examination for cytochrome oxidase was not made.

The variant can ferment the same sugars as the parent strain and use them as substrates for growth. As might be expected, however, it does not grow with alcohol as carbon source, although the parent strain is able to do so. Morphologically, it differs in size and in the shape of the cells (parent—(4.2–7.2) × (5.5–10.8)μ; average: 5.5 × 7.2μ; variant—(4.2–6.6) × (4.8–8.4)μ; average: 5.3 × 6.2μ). While the thallus of the parent strain occurs as single cells and pairs, that of the variant consists of clusters of from 3 to 20 cells. In old liquid wort cultures, the variant shows no surface growth or ring formation. There are also noticeable differences in gross morphology of streak and giant colonies. The variant has lost the ability to form ascospores.

The question as to why these haploid variants, if such they are, appear after ethylene oxide treatment has not been satisfactorily answered. It might be the result of a specific metabolic effect of the poison on the yeast cells, but it seems more likely that haploid cells or spores may be present in the normal cultures and survive the treatment with ethylene oxide better than the diploid cells because of their slightly greater resistance to the poison. Since the particular variant discussed occurs in clusters of various sizes, it is difficult to compare its death rate in ethylene oxide with that of the original strain by the use of viable counts. Since any difference in resistance cannot be great (all of the yeast cells are eventually killed by ethylene oxide), they are difficult to establish by ordinary statistical methods. However, in studies with another variant, which had similar physiological characteristics (*i.e.* absence of oxida-

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