on both sides were equal in size and enlarged to the maximum condition necessary for ovulation. None of the twenty-five controls ovulated during the period of this investigation.

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## DISCREPANCIES IN THE VALUE OF THE AEROBIC REDUCING INTENSITY OF THE YEAST CELL AND STARFISH EGG[1]

THE recent appearance of a paper by Green[2] on the oxidation-reduction potentials of cytochrome c has brought to light a discrepancy in the value of the aerobic reducing intensity of Fleischmann's yeast cells, as estimated (a) from the reactions of penetrating oxidation-reduction indicators of the Clark series, (b) from the reaction of the naturally occurring oxidation-reduction system cytochrome c.

As Keilin[3] has reported, well-aerated yeast shows none of the bands of reduced cytochrome. For cytochrome c Green[4] has reported $E_0'$ values of about + 0.125 v. for pH values between 4.59 and 7.14. Since in aerated yeast all components of cytochrome are so far oxidized that the bands of the reduced form are not detectable, we may safely assume that cytochrome c is at least 90 per cent. oxidized. Assuming for the yeast cell a pH value between 6.0 and 7.0 and taking for cytochrome c within the yeast cell an $E_0'$ value of + 0.125 v., we get for the aerobic reducing intensity of aerated yeast cells a value equal to or greater than + 0.18 v. (0.125 plus 0.058 $\log_{10}$ 9).

The stated value of the aerobic reducing intensity of these cells, as estimated from the reaction of penetrating oxidation reduction indicators, will depend upon the intracellular pH which we assign to the yeast cell. For a very large number of diverse cells Chambers and his collaborators[5] have found a cytoplasmic pH of $6.8 \pm 0.2$. Fleischmann's yeast cells stained with methyl red or propyl red take on the alkaline coloration of these dyes, indicating a pH value equal to or greater than 5.8 with methyl red, and equal to or greater than 6.2 with propyl red. It would certainly seem safe, therefore, to assume for the cytoplasm of the yeast cell a pH equal to or greater than 6.0.

In Table I is shown in tabular form the values of the aerobic reducing intensity of Fleischmann's yeast cells, as estimated from the reaction of penetrating oxidation-reduction indicators (previously reported by Beck and Robin[6]) if we assign to the cytoplasm of the yeast cell pH values of 6.0 and 7.0, respectively.

TABLE I

| Indicator | $E_0'$ values pH 6.0 | pH 7.0 | Condition in aerated yeast cells | Estimated value for aerobic reducing intensity pH 6.0 | pH 7.0 |
|---|---|---|---|---|---|
| Toluylene blue | 0.162 | 0.115 | Reduced | 0.124 or less(1) | 0.077 or less(1) |
| Thionin | 0.092 | 0.062 | Partially reduced at | 0.092(2) at | 0.062(2) |
| Cresyl blue | 0.089 | 0.047 | Largely oxidized | 0.103 or more(3) | 0.061 or more(3) |
| Methylene blue | 0.047 | 0.011 | Largely oxidized | 0.061 or more(3) | 0.025 or more(3) |

(1) Potential values estimated on assumption that toluylene blue is at least 95 per cent. reduced.
(2) Potential values estimated on assumption that thionin is 50 per cent. reduced.
(3) Potential values estimated on assumption that cresyl blue and methylene blue are at least 75 per cent. oxidized.

It is quite evident that whether we assume a cytoplasmic pH value of 6.0 or the much more probable value of 7.0 the aerobic reducing intensity of the yeast cell, as estimated with the penetrating indicators, is decidedly more negative than the value which we estimate from the reaction of cytochrome.

Chambers, Pollack and Cohen[7] had noted a similar though smaller discrepancy in their microinjection experiments on starfish and sand-dollar eggs. $K_4$ indigo tetrasulphonate, $E_0'$ value at pH 7.0 of $-0.047$ v., is not perceptibly reduced aerobically; ethyl Capri blue, $E_0'$ value at pH 7.0 of $-0.072$ v., is definitely partially reduced. This discrepancy is in all probability due to the fact that sulfonated dyes are reduced by the cellular dehydrogenase systems much more slowly than are basic dyes (as ethyl Capri blue) having $E_0'$ values of the same order.

It is felt that these discrepancies should be stressed, since they indicate that at least under aerobic conditions the underlying kinetic factors which determine whether a given oxidation-reduction indicator, or other reversible oxidation-reduction system, shall be present within a living cell chiefly in the oxidized or the reduced state, are affected not only by the oxidation-reduction potential of the indicator (or system) but also by its chemical nature. The failure of most workers to note similar discrepancies is probably due to the fact that most of the indicators having oxida-

[1] From the Lilly Research Laboratories, Marine Biological Laboratory, Woods Hole, Mass.
[2] D. E. Green, *Proc. Roy. Soc. B*, 114: 423, 1934.
[3] D. Keilin, "Ergebnisse der Enzymforschung," II. S. 239. Leipzig, Germany. Akademische Verlagsgesellschaft, 1933.
[4] *Loc. cit.*
[5] R. Chambers, *Bull. Nat. Research Council*, 69: 37, 1929.
[6] L. V. Beck and J. P. Robin, *Jour. Cell. and Comp. Physiol.*, 4: 527, 1934.
[7] R. Chambers, H. Pollack and B. Cohen, *Jour. Exp. Biol.*, 6: 229, 1929.

tion reduction potentials in the neighborhood of the ones showing partial reduction are closely related chemically to the latter.

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## ROLLER CANARY SONG PRODUCED WITHOUT LEARNING FROM EXTERNAL SOURCES

SINCE May 31, 1934, twelve roller canaries have been born and reared in soundproof cages, without hearing a song from any non-isolated bird. Eight are males, and four are females. Daily recordings of their vocal responses have been made on aluminum disks, motion picture films, or strobophotographic records.

The contest roller canary song consists of vocal effects known as rolls and tours. They are distinguished on the basis of sonance, or successive auditory fusion. In a roll, the successive pitch changes are perceived as unitary, whereas in the tour the patterns are perceived as discrete units. Physically, the distinction is one of rate of the successive patterns, the rolls having sufficient rapidity to be fused in auditory perception.

The basic song consists of a sequence of hollow roll, hollow bell, schockel, flutes and water roll. The first four, when graphed for rate of successive patterns, show a decreasing rate resembling a typical muscular fatigue curve. The rolls and tours of the main sequence are added to, substituted for or embellished by individual birds. The added effects are the bass roll, glucke, glucke roll, water glucke, schockel, deep bubbling water tour, bell roll, bell tour and bell glucke. It is rare that a single bird has all the effects in his song. The number generally varies from five to ten.

By January 7, 1935, the date of this writing, all the isolated males had produced recognizable effects of the roller canary song. These data have been checked by Mr. Frank H. Bires, of Whittier, California, an outstanding contest judge.

*Nest 1.* Males 51, 52 and 53, each aged 212 days, produced a hollow roll, schockel, flutes and water roll. Males 52 and 53 produced a hollow bell, and Male 51 a bass roll, bell roll and bell tour.

*Nest 2.* Male 24, aged 210 days, produced a schockel, flutes, water roll, hollow roll, deep bubbling water tour and water glucke.

*Nest 3.* Males 56, 57 and 58, each aged 163 days, sang a water roll and flutes. Males 56 and 57 developed a glucke and bell roll. Males 56 and 58 produced a hollow roll and schockel. Males 57 and 58 produced a water glucke, Male 56 the only bell tour in the nest, and Male 58 the only water glucke which has yet appeared in Nest 3.

*Nest 4.* Male 60, aged 224 days, produced a glucke, flutes, bass roll, hollow roll, hollow bell and bell glucke.

Taken together, the isolated birds produced all the effects. Four of them, Males 51, 52, 53 and 60, had from four to six effects when breaking into the mature roller song for the first time. They were subjected to inhibiting factors incidental to the original experiment, possibly the excessive heat, or, perhaps in the case of the first three, the fighting which often occurs when males are in the same cage. The other four, Males 24, 56, 57 and 58, developed one roll and tour after another from their baby song. The latter three were isolated at the first appearance of baby song, before any roll or tour appeared. The baby song is for the most part a nonsense melody of choppy notes covering a wide pitch range. The earliest baby song appeared at 60 days and the latest at 149 days.

Rolls appeared earliest in the cases of Males 51 and 52, specifically, at the age of 110 days. The slowest to develop a roll was from the same nest, Male 53, who was 179 days old at the time.

Males 24, 51, 56, 57, 58 and 60 heard no rolls or tours of any kind prior to producing them. Males 51 and 52 heard each other. Male 53 heard the song of Male 24.

The females have produced only a characteristic chirp and simple series of call notes. According to professional canary breeders, the female rarely has any of the rolls and tours. With this assurance, the mothers in this study were left with their young until weaned, the period varying from 25 to 40 days. The notes of the canary hens were observed and recorded, and no semblance of rolls or tours appeared. The males used in breeding were removed from the soundproof cages before the female was placed with the eggs. The eggs were removed from the breeding cage daily until all had been laid.

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