$Ba(OH)_2$ is added until the suspension is pink to phenolphthalein. Whereas NaOH would dissolve all the dilution precipitate under these conditions, $Ba(OH)_2$ dissolves only a part of the protein but all the carboxypolypeptidase. After removal by centrifugation of the undissolved protein 1 N acetic acid is added to the supernatant solution until the solution is orange to phenol red. The globulin crystals thereupon appear, promptly if the solution is seeded, slowly if it is not. The protein can be dissolved with NaOH and recrystallized by neutralization.

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THE EFFECTS OF PITUITARY IMPLANTS AND EXTRACTS ON THE GENITAL SYSTEM OF THE LIZARD

OVULATION has been induced in a serpent, *Xenodon merrimi,* six days after five homoplastic whole pituitaries were implanted.[1] Hypertrophy of the genital system has been produced in Lacerta[2] and in young alligators[3] by means of mammalian pituitary extracts. Removal of the pituitary causes atresia in the testes of the garter snake (*Thamnophilis sirtalis* and *Thamnophilis radix*) followed by a partial restoration to normal when pituitaries are implanted.[4]

In a series of experiments carried on between October 30, 1933, and April 4, 1934, fifty-five females and seventy-nine males of *Anolis carolinensis* received injections of Antuitrin S (human pregnancy urine extract, Parke Davis), while twenty-five females and twenty-five males received injections of sheep pituitary (whole pituitary extract of Parke Davis). Approximately fifty animals were kept as controls. A single dose with either extract was not more than .02 cc diluted with two or three volumes of cold-blooded Ringer. This proved to be the maximum dose that was safe to use.

The males responded very completely to both extracts and could be very easily distinguished from controls in the following particulars: (1) The dorsal crest along the neck and back was raised, often to the height of an eighth of an inch. (2) The hemipenes could be everted. (3) The testes were often enlarged to two or three times the size of those of controls. (4) The epididymis and vas deferens were always greatly enlarged. In an extreme case of hypertrophy a single loop of the epididymis was found to be at least fourteen times the diameter of that of a control which was killed at the same time. (5) Spermatozoa were found in the epididymis after the fourth daily injection. (6) The vas eferens was slightly enlarged. (7) Courtship and fighting were a common activity on every sunny day during winter and early spring.

In regard to the females, hypertrophy of the ovaries and oviducts was produced with both Antuitrin S and sheep pituitary, but actual egg-laying resulted only with the latter extract. Some females which were injected with sheep pituitary retained mature ova within the ovaries. These eggs were slowly resorbed during the ensuing three months. Neither the ovaries nor oviducts enlarged as much with Antuitrin S as with the sheep extract. After twelve injections of sheep pituitary, two eggs were laid on March 23 and three more the next day. A sixth egg was laid on April 11. The first egg to be laid by any of the controls was on April 18 and a second egg on May 8. No more eggs were laid by controls until June, July and August.

The metabolism of injected animals was greater than that of controls as was shown by an increase both in appetite and in the amount of food eaten. Also, food was required oftener. General activity and speed of movement were undoubtedly greater. Moulting occurred more frequently.

The after-effects were noticeable. Four months after the last injection found many of the treated lizards to be persistently thin, although on an average they ate more than the controls. A few died apparently of starvation while controls, which had received approximately the same amount of food, lived in a perfectly healthy condition.

Complete details concerning these experiments with mammalian pituitary extracts are to be reported later.

It may be of further interest to state that in connection with some experiments with pituitary implantations in *Anolis carolinensis* (December 21, 1933, to April 14, 1934), one female which received four whole pituitaries (taken from males of the same species) failed to ovulate, but the genital system was approximately twice the size of that of controls. Out of three females which received five similar homoplastic implants, two died before ovulation (autopsy showed hypertrophy of the genital system) but the remaining female actually laid two eggs, one on March 24 and another four days later. Another female, which received three frog pituitaries, laid an egg on April 12.

When the two females last mentioned were killed (April 14) they showed mature ova just ready to leave the ovary. Only one ovary in each female, however, contained a mature ovum, although the oviducts

[1] B. A. Houssay, *Compt. Rend. Soc. Biol.*, 106: 377–378, 1931.
[2] M. Herlant, *Arch. de Biol.*, 44: 347, 1933.
[3] T. R. Forbes, *Proc. Soc. Exp. Biol. Med.*, 31: 1129, 1934.
[4] W. H. Schaefer, *Proc. Soc. Exp. Biol. Med.*, 30: 1363–1365, 1933.

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 ± 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.