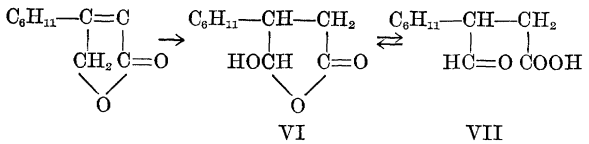

VI VII

or VII was shown by the preparation of the semicarbazone of the methyl ester. (Calculated for $C_{12}H_{21}O_3N_3$: C, 56.4; H, 8.3; found: C, 56.5; H, 8.5.)

The possible bearing of these observations on the structure of the cardiac aglycones and on the transformation of the aglycones into the iso-aglycones will be discussed in detail in a forthcoming communication.

JOSEF FRIED
MARTIN RUBIN
WALTER D. PAIST
ROBERT C. ELDERFIELD

DEPARTMENT OF CHEMISTRY,
COLUMBIA UNIVERSITY

NATURE OF THE PASTEUR ENZYME[1]

WHEN fermenting cells are brought in contact with oxygen, normally less carbohydrate is broken down and less fission products are formed. This phenomenon was discovered by Pasteur in 1861 and is to-day known as the Pasteur reaction. This effect has been interpreted in terms of an oxidative resynthesis of carbohydrate from the end products of fermentation (Meyerhof) of a suppression of fermentation by respiration (Warburg), and of an inhibition of fermentation dependent on the oxygen tension (Lipmann, Kempner, Laser). The selective inhibition of the Pasteur reaction by ethyl isocyanide,[2] by a lowering of the oxygen tension[3] and by suitable concentrations of carbon monoxide[4] indicates that a catalyst distinct from the respiratory enzyme is involved and that this agent contains heavy metal. The name *Pasteur enzyme* appears to be appropriate for this thermolabile catalyst.

The reversal of the carbon monoxide inhibition of the Pasteur effect by white light[4] has enabled us to apply the classical photochemical technique of Warburg to the study of the spectrum of the Pasteur enzyme. Rat retina was chosen for these experiments because its respiration remains unaffected by carbon monoxide at concentrations sufficient to inhibit the Pasteur enzyme.[4] The photochemical effect of monochromatic light of three different wave-lengths on the glycolysis in this system has been attributed by Warburg and Negelein[5] to the action of carbon monoxide and light on the respiratory enzyme causing a secondary effect on glycolysis. This view is no longer tenable in the light of Laser's observations,[4] which we have been able to confirm.

In the present work sixteen different wave-lengths of monochromatic light of high intensity have thus far been used for the charting of the relative photochemical absorption spectrum of the Pasteur enzyme in the visible region. The pattern obtained is that of an iron-porphyrin-protein, showing a high γ- or Soret band at 440–455 mμ, and lower β- and α-bands at 500–520 mμ and 570–590 mμ, respectively. The position of the maxima of these bands is indicated in the diagram (Fig. 1) together with the corresponding maxima of the bands of carbon monoxide hemoglobin and the carbon monoxide complex of the respiratory enzyme.

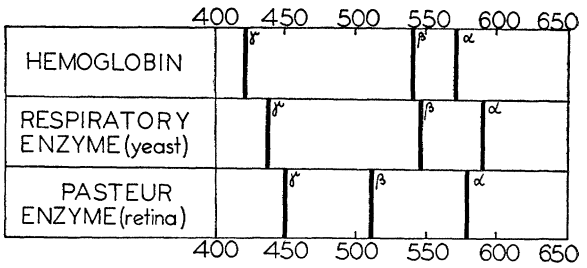

FIG. 1. Diagram showing the position of the absorption maxima of the carbon monoxide compounds of hemoglobin, the respiratory enzyme in yeast (Warburg) and the Pasteur enzyme in retina (present work) in the visible region of the spectrum.

A well-defined band at 450 mμ has also been recorded by direct spectrography of carbon monoxide-treated rat retinas. This band coincides in position with the main or γ-band of the Pasteur enzyme as revealed by the photochemical experiments.

The Pasteur enzyme in retina appears to be a pheohemin protein like the respiratory enzyme in yeast and in Acetobacter. It differs from them with regard to its affinity for carbon monoxide and oxygen as well as the position of the absorption bands of the carbon monoxide compound.

KURT G. STERN
JOSEPH L. MELNICK[6]
DELAFIELD DUBOIS

YALE UNIVERSITY SCHOOL OF MEDICINE

PROLIFICACY OF RATS TREATED WITH MARE GONADOTROPIC HORMONE

As an earlier paper[1] has demonstrated, adequate amounts of mare gonadotropic hormone will produce superovulation and estrus in immature rats. Mating of these rats[2] resulted in the implantation of as many

[1] This work was aided by a grant from the Jane Coffin Childs Memorial Fund for Medical Research.
[2] O. Warburg, *Biochem. Zeitschr.*, 172: 432, 1926.
[3] H. Laser, *Biochem. Jour.*, 31: 1671, 1937.
[4] *Ibid.*, 31: 1677, 1937.
[5] O. Warburg and E. Negelein, *Biochem. Zeitschr.*, 214: 101, 1929.
[6] Finney-Howell Research Foundation fellow, 1939–40.
[1] H. H. Cole, *Amer. Jour. Anat.*, 59: 299–331, 1936.
[2] H. H. Cole, *Am. Jour. Physiol.*, 119: 704–712, 1937.

as 28 normal-appearing fetuses. Further, 2 of 14 rats which were allowed to bear their young had 17 living young each. Apparently Engle[3] misinterpreted this report, for he states regarding it, "Immature animals, however, showed superfecundity, but these immature animals were not permitted to carry their young to term so that the number of viable young is unknown."

In experiments relating to the effect of pregnancy on growth we have bred about 100 more rats after the induction of precocious sexual maturity with mare gonadotropic hormone. These rats were given 8 rat units in a single injection at ages ranging from 30 to 33 days and were bred within 72 hours after the injection. In one instance 30, in another 32, and in still another 33 fetuses were found at midpregnancy, though some were in advanced stages of resorption.

The question arises, therefore, as to how many remain viable throughout pregnancy. We previously reported[2] finding 23 living young *in utero* on the 21st day of pregnancy. In the present series the uterus of one rat contained 20 on the 21st day of pregnancy; that of another, 23 living young on the 20th day. One rat (G7474), given 8 rat units on the 33rd day and bred 3 days later, gave birth to 23 living young on the 60th day of age.[4] The young, aside from being small, were normal. Six of these were raised by the mother (we destroyed the others at birth) and averaged 42 gm on the 21st day of age. As previously shown,[5] breeding of these precociously matured rats has no apparent deleterious effect. This particular rat subsequently raised four other litters; the number of young born at each succeeding parturition was 13, 10, 10 and 14, respectively. This rat, therefore, as the result of treatment with gonadotropic hormone while still immature as regards body size, gave birth to approximately twice the average number born under normal conditions. In our colony, the average litter size of untreated rats is 11.5, and 2 litters of 19 among several thousand have been encountered.

On the basis that 33 represents the maximum number implanted and 23 the largest number of young born, it appears that considerable resorption occurs. Evidently, however, not only can superfecundity be produced, but also the number of viable young carried to term can be increased above normal by treating immature rats with gonadotropic hormone. Our results indicate that the maximum prolificacy is obtained by treating rats just before the time of normal maturity.

H. H. COLE

UNIVERSITY OF CALIFORNIA,
DAVIS

SCIENTIFIC APPARATUS AND LABORATORY METHODS

AN ADAPTABLE ROTATING UNIT FOR ROLLER TUBE TISSUE CULTURES[1]

To meet the problem of rotating roller tubes in a water-jacketed incubator an adaptable unit was devised. This unit can support either a simple test-tube holder or a Novy jar. By means of the latter a controlled atmosphere can be provided for the cultures.

The rotator is supported by a heavy steel base (A) 18 cm wide and 27 cm long. The inclination of the whole unit can be regulated by means of the screws (B) at the front edge of the base plate. The upright plate (C) supports the rotator shaft (D) by means of the bearings (E). The adaptable holder consists of a circular rear plate (F) joined to an anterior ring (G) by three sturdy supports (H). The three adjustable clamps (L) in the anterior ring hold the test-tube support or Novy jar in place (diagram 2). It is better for the anterior ring to be of a size snugly to fit the anterior lip of the Novy jar rather than for the latter to project, as in the diagram. By means of a standard double stopcock the jar can be exhausted or various gases can be introduced. The same holder can also be employed for an aluminum test-tube rack whose front disc fits a recess in the anterior ring of the adaptable holder (diagrams 1, 3, 4). A much larger test-tube support could be used with a unit having a shorter base plate (A). The rotator is driven by a small electrical motor[2] outside of the incubator

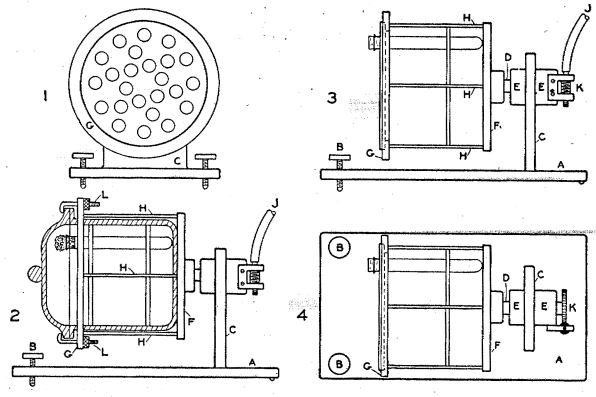

FIG. 1

[3] E. T. Engle, in "Sex and Internal Secretions," Williams and Wilkins Company, Baltimore, 1027, 1939.

[4] A prolonged gestation is frequently encountered in these precociously matured rats.

[1] This investigation was aided by a grant from the Jane Coffin Childs Memorial Fund for Medical Research.

[5] H. H. Cole and G. H. Hart, *Am. Jour. Physiol.*, 123: 589–597, 1938.

[2] Signal Electric Manufacturing Company, Menominee, Michigan. Type C2A 115 AC–DC Amp: 18 W15 Gear ratio 900–1.