average increase was 0.058. In two experiments the same procedure was carried out after 3 days of fasting. The results did not differ from those obtained after food. In either case the presence of glucose caused an increase in the quotient, which may be attributed to the oxidation of sugar.

CONCLUSIONS.

1. It is shown by complete double respiratory quotients that in the case of renal tissue, the error of the Warburg-Barcroft manometers is, on the average, ±0.014.

2. The quotients were all between 0.7 and 1.0, and are fully accounted for by the oxidation of foodstuffs, without postulating the transformation of one into another.

3. As evidenced by the rise in the respiratory quotient, isolated tissue can oxidize glucose offered it in solution.

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Removal of Inhibiting Effects on Nitella of Certain Buffer Mixtures and Acids.

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Living cells of *Nitella* (collected in New York in autumn) were exposed[1] for 10 minutes to (1) M/150 phosphate buffer solution at pH 5.5, (2) 5×10^{-5} M phosphoric acid[2] (at about pH 4.3), and (3) 5×10^{-5} M hydrochloric acid (at about pH 4.2), and then placed in a solution of brilliant cresyl blue.[3] The rate of penetration of dye into the vacuole, as compared to that of the control (cells transferred from tap water into the dye solution), was found to decrease about 50 per cent in (1), 37 per cent in[4] (2), and 25 per cent[5] in (3).

This inhibiting effect was not removed when cells, previously exposed to the solutions above mentioned, were washed for ½ minute either in M/150 phosphate buffer solution at pH 7.7, or in M/150 borate buffer solution at pH 7.7 containing 0.02 M sodium chloride. In the latter case there was a greater inhibiting effect. The inhibiting effect of hydrochloric acid and phosphoric acid was very slightly reduced while that of the phosphate buffer mixture at pH 5.4 was

not changed when cells previously exposed to these solutions were washed in M/150 borate buffer solution at pH 7.7 for ½ minute.

The inhibiting effect, however, was completely removed when cells previously exposed to the solutions mentioned were treated in any of the following ways:

(a) By placing the cells in the dye solution made up with M/150 phosphate buffer mixture. The same result was obtained whether the dye solution was stirred or not.

(b) By placing the cells in the dye solution made up with M/150 borate buffer mixture, containing either 0.02 M sodium chloride or 0.01 M magnesium chloride.

(c) By washing the cells for ½ minute in 0.01 M magnesium chloride dissolved in M/150 borate buffer mixture at pH 7.7 and then placing them in the dye solution made up with borate buffer mixture.

Regarding the above experiments the following suggestions may be made:

(1) Magnesium chloride either displaces from the protoplasm the substances causing an inhibiting effect, or it acts on the protoplasm in such a way that it nullifies their effect (it may be that it decreases the viscosity of the protoplasm).

(2) The dye is affected by NaCl, and by phosphate buffer mixture, (in all probability chiefly by the base cations) in such a way that it is able to mask the effect of sodium chloride on the protoplasm.

(3) It is uncertain whether the dye is affected by magnesium chloride in the same manner as by sodium chloride as described in (2).

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[1] In all the experiments the solutions to which the cells were exposed before placing in the dye were not stirred.

[2] The pH value of phosphoric acid seemed a little higher than that of hydrochloric acid, but this difference is not sufficient to account for the difference in the behavior of the two acids toward protoplasm.

[3] The following holds for every experiment unless otherwise stated: The dye solution was 0.00014 M, made up with M/150 borate buffer mixture at pH 7.7. The dye solution was stirred at the rate of one revolution per second. The determination of the dye in the vacuole was made colorimetrically after the cells had been placed in the dye solution for ½ minute.

[4] The experiments with the same lot of *Nitella* showed that at pH 4.8 there is still 20 per cent decrease with phosphoric acid, while there is none with hydrochloric acid. (Irwin, M., *J. Gen. Physiol.*, 1926-27, x, 271.)

[5] This result is different from that in the writer's paper (PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 54.), where at pH 4 there was no inhibiting effect with hydrochloric acid while there was about 20 per cent decrease with phosphoric

acid. This difference is due to the fact that cells were collected in two different localities. In the former case the cells, which were collected in New York, were more sensitive than in the latter (cells collected in Cambridge). This is shown by the fact that when sufficiently low concentrations of these acids were used (0.000025 M at pH 4.8) with New York *Nitella* (see footnote 4) the same result was obtained as with Cambridge *Nitella* (using acids at pH 4.)

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Exit of Dye from Nitella with Different Initial Concentrations of Dye in the Vacuole.

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Living cells of *Nitella* were placed in brilliant cresyl blue solution for different lengths of time, so that the concentration of dye[1] (DB plus DS) in the vacuole varied from 0.000035 M to 0.0028 M (cell wall only very slightly stained). When such cells were placed in the M/150 phosphate buffer solution at pH 5.4 (solution changed and stirred), the rate of exit of dye from the vacuole was found to be about the same between 0.000035 M and 0.00014 M. Above the latter concentration it decreased as the concentration increased. There is also a decrease in the velocity constant, k, calculated from the equation $dx/dt = k(a-x)$, where a equals the initial concentration of dye in the vacuole, and x equals the amount of dye that has disappeared from the vacuole at time t. The pH value of the sap was about the same as that of the normal (determined after the dye has come out of the vacuole).

It may be assumed that the conditions, either in the vacuole or in the protoplasm or both, change as the concentration of dye increases. This change may be due to the production of a substance which (1) combines with part of DB to form a non-penetrating compound (other than DS), of the same color as DB and DS, which breaks down slowly to form DB during the exit of the dye, or (2) otherwise changes the concentration of DB.

[1] It is assumed that the dye exists in two forms, free base (for convenience called DB) and salt (for convenience called DS). DB penetrates the living cell very rapidly while DS penetrates so slowly that its penetration in this case is negligible. As DB enters the vacuole a certain proportion of it changes to DS. Though we measure the amount of DB plus DS in the vacuole, this method is justified because there is always a definite ratio of DB to DS, and because both DB and DS are of the same color. (*Cf.* Irwin, M., *J. Gen. Physiol.*, 1926-27, x, 75.)