than with the latter. The dye is more toxic in light, even the light of the microscope condenser, than in darkness.

That the results described are due to reduction, not to diffusion outward of the dye is shown by the fact that after reduction the color returns within a few seconds with increase in oxygen content of the fluid. It may also be noted that in all cases the reduction gradient in animals not irreversibly injured is essentially identical with the gradient of susceptibility to a large number of chemical and physical agents in gradually lethal concentration or dosage.

The anteroposterior reduction gradient in *Paramecium* was noted in an earlier paper.[1] Recently Roskin and Semenoff,[2] using a leucobase only, have concluded from the observed course of reduction that oxidation occurs most rapidly in the posterior region of *Paramecium*. Their results can be duplicated by following their procedure but this procedure results in deeper staining of the anterior ectoplasm and less rapid or no reduction there while more posterior regions are still able to reduce, consequently they have failed to observe the normal anteroposterior reduction gradient.

7545 C

Effect of 1-2-4 Dinitrophenol on Cellular Respiration.

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Numerous investigators (Plantefol,[1] Field, Martin and Field,[2] Ehrenfest and Ronzoni[3]) have reported that 1-2-4 dinitrophenol added *in vitro* to plants, yeast cells and frog tissues increases their respiration. This increase seems to be associated with an increased aerobic fermentation in the case of yeast cells (Cutting and Tain-

[1] Child, C. M., and Deviney, E., *J. Exp. Zool.*, 1926, **43**, 257.

[2] Roskin, G., und Semenoff, W., *Z. f. Zellforschung u. mikr. Anat.*, 1933, **19**, 150.

* Rockefeller Foundation Fellow.

[1] Plantefol, L., *Ann. Physiol. Physicochim. Biol.*, 1933, **8**, 127.

[2] Field, J., 2nd, Martin, A. W., and Field, S. M., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 56.

[3] Ehrenfest, E., and Ronzoni, E., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 318.

[4] Cutting, W. C., and Tainter, M. L., *J. Pharm. and Exp. Ther.*, 1933, **48**, 410.

ter[4]) and an acceleration of anaerobic lactate production in the case of frog tissues (Ehrenfest and Ronzoni).

The experiments here reported were performed in an attempt to determine the mechanism of this rise in cellular respiration. The oxygen consumption was measured with the Warburg apparatus. The yeast (baker's) was suspended in 0.066 M phosphates; the frog tissues, in frog Ringer's, buffered with phosphates to pH 7.46; the goose red cells in 0.9% NaCl buffered with phosphates to pH 7.46. All these solutions contained 0.2% glucose. Gonococci were suspended in 0.9% NaCl and buffered with phosphates to pH 7.01 and pH 6.0.

Field, Martin and Field's contention that dinitrophenol is active only in its undissociated form is not supported by the findings of Ehrenfest and Ronzoni, nor by our own experiments. Dinitrophenol increased the respiration of yeast at pH 6.64 (where the concentration of the undissociated form was only 0.0158 mg. per liter), and the respiration of frog tissues and goose red cells at pH 7.46 (where dinitrophenol is practically wholly dissociated) (Table I).

TABLE I.
Effect of 1-2-4 Dinitrophenol on Respiration of Tissues and Cells.

Tissue	Concentration of Dinitrophenol mg./L.	O_2 Consumption		% Increase
		Before D.N.P. c.mm./hr.	After D.N.P. c.mm./hr.	
Yeast	50	121.8	151.6	20
pH = 6.64		136.5	156.3	13
T = 25°		140.5	155.1	9
		124.6	157.2	21
		137.3	184.7	26
		114.0	174.5	35
Frog Kidney	5	21.2	37.8	44
pH = 7.46		4.6	7.2	36
T = 30°		12.0	20.4	41
		22.6	34.2	34
Frog Liver	5	15.6	21.4	27
pH = 7.46		15.8	24.6	36
T = 30°		24.6	34.0	28
		11.0	12.8	14
Goose Red Cells	7	73.6	113.5	54
pH = 7.46		76.3	112.0	47
T = 37°		77.7	119.3	54
	13	75.6	140.0	85
		82.3	140.0	70

The increase in cellular respiration observed after the addition of dinitrophenol seems not to be due to a direct oxidizing action of this compound on the oxidizable substrates. Thus dinitrophenol did not oxidize lactate activated by α-hydroxyoxidase of gonococci, an oxida-

tion readily catalyzed by reversible dyes (Barron and Hastings[5]) nor did it oxidize linseed oil, an oxidation catalyzed by hemin (Robinson[6]). Furthermore, when cellular respiration was inhibited by the addition of specific inhibitors of the oxidizing enzymes, KCN and CO, there was no increase of respiration after addition of dinitrophenol. Indeed some decrease of respiration was observed upon the addition of dinitrophenol to cyanide-treated goose red cells. These experiments were performed with frog kidney and liver, and goose red cells in the case of cyanide (0.002 M) and frog kidney and yeast in the case of CO (Table II). We may add that the formation of the potassium salt of metapurpuric acid by the action of KCN on aqueous solutions of dinitrophenol, occurs only in alkaline reaction and at 60°. At 38° and pH 7.46 (the pH of our experiments) such a reaction does not occur.

Dinitrophenol showed no accelerating influence on oxidations produced by gonococci. As oxidizable substrates, glucose, lactate, and pyruvate were used (Table III).

TABLE II.
Effect of 1-2-4 Dinitrophenol on Cells and Tissues after Inhibition of Respiration by KCN and CO.

Tissue	Inhibitor	O_2 Consumption	
		Before D.N.P.	After D.N.P.
		c.mm./30 min.	c.mm./30 min.
Frog Kidney	KCN	2.5	1.0
		2.5	3.1
		5.0	4.9
		4.6	4.1
		3.3	2.6
Frog Liver	KCN	2.9	1.7
		7.3	2.5
		6.1	3.7
		6.4	6.0
		4.0	0.7
Goose Red Cells	KCN	17.9	10.8
		19.7	9.6
		31.1	6.8
		20.2	5.2
Frog Kidney	$CO:O_2$ (96.5:3.5)	1.1	0.4
		0.4	0.4
		1.4	1.1
		1.3	0.9
Yeast	$CO:O_2$ (90:10)	64.0	66.0
		66.2	64.6
		52.2	55.0
		64.2	65.2

[5] Barron, E. S. G., and Hastings, A. B., *J. Biol. Chem.*, 1933, **100**, 155.
[6] Robinson, M. E., *Biochem. J.*, 1924, **18**, 255.

TABLE III.
Dinitrophenol and Oxidations Produced by Gonococci.

Substrate	O_2 Consumption in 30 Minutes	
	Without D.N.P.	With D.N.P.
Glucose	234.8	234.8
Lactate	48.2	44.0
Pyruvate	130.0	91.9

Conclusion. Since dinitrophenol is unable to oxidize such a labile compound as lactate activated by α-hydroxyoxidase; is without effect when the respiration of cells and tissues has been inhibited by cyanide or carbon monoxide; and has no action on the respiration of certain bacteria, where the complicated controlling mechanisms present in highly organized cells are absent, it is concluded that the increase in respiration produced by dinitrophenol is not due to direct oxidation of the oxidizable substrates. It is suggested that dinitrophenol acts by combining with some of the substances acting as agents for the control of the speed of cellular oxidations, thus increasing the activity of the oxidizing enzymes.

7546 P

Effect of Histamine and Alcohol on Acid Secretion of Stomach of Postoperative Cases.

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In order to stimulate the secretion of acid by the stomach of postoperative surgical cases, the action of histamine and alcohol was tested. In 10 cases a Rehfuss tube was inserted through the nose

TABLE I.
Histamine Tests.

No.	Control		15 min.		30 min.		45 min.	
	Free	Total	Free	Total	Free	Total	Free	Total
1	0	11	12	36	82	102	73	95
2	0	—	30	48	68	85	55	75
3	0	—	27	51	90	112	80	105
4	0	—	27	51	90	112	80	105
5	30	55	32	54	72	89	65	87
6	0	—	0	—	0	—	0	—
7	0	—	0	—	—	—	60	81
8	0	—	20	38	89	104	119	129
9	0	—	0	—	0	-	0	—
10	0	—	112	134	124	142	135	155