fact that the oxidation is inhibited by the presence of iodoacetate, especially after prolonged incubation, either aerobic or anaerobic. Iodoacetate is believed to exert its inhibitory effects by combination with active thiol groups.[6-10]

Summary. It has been shown that although minced Flexner-Jobling rat carcinoma has an aerobic respiration equal to that of rat skeletal muscle mince, the oxygen uptake in the presence of glutathione of thermostable powders (essentially denatured proteins) prepared from the tumor tissue is considerably lower than the oxygen uptake of similar powders prepared from muscle. This seems to parallel the fact that although the tumor powder and the muscle powder have approximately the same total sulfur content, the percent of the total sulfur which is present as sulfhydryl sulfur is much lower in the tumor powder than in the muscle powder.

11740 P

Oxidation of Native Protein by Glutathione-Copper Catalysis.

ROBERT N. FEINSTEIN* AND F. J. STARE.† (Introduced by H. C. Bradley.)

From the Department of Physiological Chemistry and the Cancer Research Laboratory, University of Wisconsin, Madison, Wis.

That protein can, under certain conditions, consume oxygen has been known for some time. In explanation of this phenomenon 3 factors have been stressed: (1) heavy metals, (2) a low molecular weight source of reversibly oxidizable sulfur such as cysteine or glutathione, and (3) the content and chemical state of the sulfur of the protein substrate.

Our work has been entirely with native egg albumin and gelatin, as examples of proteins of high and low sulfur content respectively. We were interested in studying the effects of variations in the three

6 Dickens, F., *Biochem. J.*, 1933, **27**, 1141.

7 Dickens, F., *Nature*, 1933, **131**, 130.

8 Rapkine, L., *Compt. rend.*, 1933, **112**, 790.

9 Rapkine, L., *Compt. rend.*, 1933, **112**, 1294.

10 Rapkine, L., *J. chim. phys.*, 1936, **33**, 493.

* This report is from a thesis submitted by Robert N. Feinstein to the Graduate School of the University of Wisconsin in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

† Now at the School of Medicine, University of Chicago, Chicago, Illinois.

TABLE I.
Effect of Cu, Fe, and Glutathione (GSH) on Oxygen Consumption of Native Egg Albumin.

g of albumin	Molarity of GSH	Molarity of $FeCl_3$	Molarity of $CuSO_4$	cu mm O_2 used, 6 hr
.15	M/200	M/1000	0	30
.15	M/200	0	M/1000	62
.15	M/200	M/1000	M/1000	57

factors listed above. Measurements of oxygen uptake were made in the Warburg manometric apparatus at a temperature of 38.2°C. The protein was weighed directly into the flasks, and the other materials were dissolved in unbuffered Ringer's solution brought to pH 7.4 and pipetted in. The final volume of the flask contents was 2.2 cc.

Results. In Table I are listed the results of variations in heavy metal. If either glutathione (GSH) or all heavy metals are absent, no oxygen is consumed. If the protein is omitted, the oxidation of the GSH is so rapid as not to be measured by the technic employed. It is of particular interest that gelatin, under the same conditions, consumes approximately the same quantity of oxygen, despite the wide disparity in sulfur content of the two proteins.

Another point of interest is the difference in behavior of egg albumin and gelatin to dilute and concentrated solutions of $CuSO_4$. The data are presented in Table II, which shows that gelatin is inhibited in its oxygen consumption about 20% by concentrated solutions of $CuSO_4$, while the egg albumin is not inhibited but somewhat increased. Not only is the rate of oxygen uptake increased by the M/10 $CuSO_4$ in the latter case, but also the albumin consumes considerably more oxygen before the reaction ceases.

Part of this excess oxygen uptake is independent of the presence of the GSH. In one experiment, 0.15 g of egg albumin in the presence of M/200 glutathione and M/10 $CuSO_4$ consumed 77 cu mm of oxygen in 6 hours, while 0.15 g of the protein in M/10 $CuSO_4$ with no added glutathione still used 56 cu mm (both reactions still well in

TABLE II.
Difference in Effect of Concentrated and Dilute $CuSO_4$ on Gelatin and Egg Albumin.

Protein	Molarity of $CuSO_4$	cu mm O_2 used, 4 hr
Gelatin	.002 M	81
"	.1 M	64
Egg Albumin	.002 M	76
" "	.1 M	85

GSH = 3 mg (M/200); Protein = .15 g.

progress). This GSH-independent oxygen consumption is possibly due to denaturation of the protein by the copper, with consequent exposure and oxidation of latent -SH groups. Gelatin, with its low sulfur content, is not similarly affected.

Summary. Native egg albumin and gelatin, in the presence of M/1000 $CuSO_4$ and M/200 glutathione, consume significant and approximately equal quantities of oxygen. When the $CuSO_4$ is replaced by M/1000 $FeCl_3$, the oxygen uptake is diminished by about 50%. If the M/1000 $CuSO_4$ is replaced by M/10 $CuSO_4$, the gelatin consumes less oxygen than before, and the egg albumin more.

11741

Calcification of Trichina Cysts *in vitro*.*

BERNARD D. ROSS. (Introduced by Franklin C. McLean.)

From the Department of Physiology, University of Chicago, Chicago.

Previous attempts to produce pathological calcification *in vitro*[1, 2] have failed to give consistent results, presumably owing to failure to employ a readily calcifiable tissue. Because trichina cysts become calcified in a uniform manner *in vivo,* we attempted to reproduce this phenomenon *in vitro,* employing the technics used for rachitic cartilage.[3] Preliminary experiments failed to produce calcification even when serum of high Ca^{++} x P product was used. In view of the findings of von Brand, Holtz and Vogel[4] and of Wantland[5] in which it was demonstrated that large doses of viosterol greatly accelerate the calcification of the cysts *in vivo,* calcification *in vitro* was attempted in infected muscle from rats which had received one or more toxic doses of viosterol. The experiments demonstrated that following this preliminary treatment, calcification of the cysts *in vitro* could be readily obtained.

Methods. Adult rats of varying strains, ages and weights, which

* This work was aided by a grant from the Josiah Macy, Jr., Foundation. We are indebted to Mead Johnson and Co. for supplies of viosterol, 1,000,000 units per gram.

1 Rosenheim, A. H., and Robison, R., *Biochem. J.,* 1934, **28**, 712.

2 Law, K. A. O., and Robison, R., *Biochem. J.,* 1936, **30**, 69.

3 Shipley, P. G., Kramer, B., and Howland, J., *Biochem. J.,* 1926, **20**, 379.

4 v. Brand, T., Holtz, F., and Vogel, H., *Z. f. Parasitenk.,* 1933, **6**, 308.

5 Wantland, W. W., *J. Parasitol.,* 1938, **24**, 167.