carbon atom of the testosterone molecule, which is the only manner in which pregneninolone differs from testosterone. Second, the striking difference in the biological properties displayed by the same compound when administered to experimental animals and to humans.

The results of this study draw attention to the inadvisability of applying to humans, observations concerning the biological properties of hormones as determined in animal experiments and emphasizes the importance of determining (by means of objective studies) the biological properties of these compounds in humans before using them as therapeutic agents.

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Thermostable Oxidations in Tumor and Muscle Tissue.

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Hopkins and Dixon[1] showed that if a tissue was minced, thoroughly extracted with boiling water, dehydrated with alcohol, dried and finely ground, the resulting "thermostable powder" would not take up oxygen by itself, but in the presence of a little glutathione would take up considerable amounts of oxygen. Further work by Hopkins[2] indicated that the oxygen uptake of these thermostable powders was due to the oxidation of fat at an acid pH and the oxidation of protein at neutral or slightly alkaline pH.

We were interested in determining whether tumor tissue possessed any thermostable oxidative properties similar to those Hopkins found for muscle tissue. For our experiments we have used Flexner-Jobling rat carcinoma and have compared all results to those ob-

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A preliminary report of this work was presented before the thirty-third annual meeting of the Federation of American Societies for Experimental Biology in Toronto, April, 1939.

1 Hopkins, F. G., and Dixon, M., *J. Biol. Chem.*, 1922, **54**, 527.

2 Hopkins, F. G., *Biochem. J.*, 1925, **19**, 787.

tained with corresponding thermostable powders prepared from normal rat muscle. Muscle was selected as the comparison tissue principally because the aerobic respiration of fresh minced tissue of the Flexner-Jobling rat carcinoma is of the same order of magnitude as the aerobic respiration of fresh rat skeletal muscle mince.

Methods. The powders were prepared by mincing the tissue in a Latapie mincer, suspending them in 3 volumes of distilled water, boiling for 10 minutes, decanting the supernatant fluid, and resuspending in boiling water. The powders were then washed with hot water by decantation 9 times. From the time the tissue was first suspended in water, anaerobic conditions were maintained by bubbling a stream of nitrogen through the solution. After the last decantation, the tissue was washed twice with 95% alcohol and finally with absolute alcohol. The resulting material was dried *in vacuo* at a temperature of 60°C. When dry, it was thoroughly ground and stored in an evacuated Thunberg tube in the ice box. For some experiments, the powders were extracted with chloroform for 24 hours in Soxhlet extractors, and the resulting fat-free powders were used. When the powders were thus extracted, it was found most important to remove all traces of the chloroform, for the barest trace of any volatile, water-insoluble solvent can introduce considerable error in manometric experiments.

The glutathione used was a crystalline preparation of reduced glutathione. In the presence of a little copper, it took up oxygen equivalent to the theoretical amount as calculated from the equation:

$$4\ GSH + O_2 \rightarrow 2\ GSSG + 2\ H_2O$$

The measurements of oxygen uptake were made in the Warburg manometric apparatus at a temperature of 38.2°C, and oxygen uptakes were calculated on the basis of cu mm of oxygen per gram of powder. 0.15 g of the thermostable powder was weighed into the flask; then the desired amount of glutathione, usually 3 mg, was added. As the flasks were calibrated to contain 2.2 cc, the concentration of glutathione was M/200. The medium used was an unbuffered Ringer's solution brought to the desired pH with HCl or NaOH. Such a Ringer's solution is satisfactory for the respiration medium, since no acid is produced. CO_2 production was found to be negligible, so no KOH was used in the flasks. When any substance, such as $CuSO_4$, iodoacetate, etc., was to be added, it was dissolved in Ringer's solution and brought to the proper pH.

Chemical determinations were run on the fat-free powders. Total nitrogen was determined by Kjeldahl digestion with a $CuSO_4$-K_2SO_4-H_2SO_4 mixture. Total sulfur and total phosphorus were determined on a single sample, by sodium peroxide oxidation in a Parr sulfur

bomb. The sulfur was precipitated as $BaSO_4$ and determined gravimetrically, while the phosphorus was precipitated from the filtrate as the strychnine phosphomolybdate.[3] This determination of phosphorus compared satisfactorily with the H_2SO_4-HNO_3-$HClO_4$ digestion method of Gerritz.[4] The sulfhydryl content of the powders was determined by the method of Todrick and Walker,[5] measuring the reduction of 2,6-dichlorophenolindophenol in a boiling water bath. To prevent recoloration of the dye the determinations were carried out in test tubes bearing one hole rubber stoppers and filled with nitrogen before placing in the bath. Since the dye was not calibrated against pure cysteine, but was simply weighed out and made to volume, no accuracy is claimed as to the *absolute* figures for -SH content, but the striking *differences* in -SH content observed are significant. Determinations of -SH content of the tumor and the muscle powder were run alternately, during the course of a single hour, and with the same solution of the dye, so that exactly the same titer of the dye was used for both.

Results. Although fresh minced tissue of the Flexner-Jobling rat carcinoma has an aerobic respiration of the same order of magnitude as fresh rat skeletal muscle mince, the thermostable oxidative properties are markedly lower in the tumor powder than in the muscle. A summary of some typical results is given in Table I.

From Table I it is observed that fresh minced tumor and muscle tissue consume approximately equal amounts of oxygen, namely 2600 and 2300 cu mm per hour per gram, respectively. Under optimal conditions, however, the thermostable powder prepared from tumor tissue consumes only 167 cu mm, while the muscle powder takes up 464 cu mm.

TABLE I.
Oxygen Uptake of Fresh Tissue and of Thermostable Powders Prepared from Fresh Tissues.

			Tumor	Muscle
			cu mm O_2 per g dry wt per hr	
Fresh Tissue (mince)			2600	2300
Thermostable Powder			32	104
,,	,,	+ M/1000 $CuSO_4$	60	176
,,	,,	+ M/200 glutathione	85	175
,,	,,	+ M/1000 $CuSO_4$ M/200 glutathione	167	464
,,	,,	+ M/100 NaCN	40	7
,,	,,	+ M/200 glutathione M/100 NaCN	36	7

[3] Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry*, Vol. II, p. 873, Williams and Wilkins, Baltimore, 1932.

[4] Gerritz, H. W., *Ind. Eng. Chem., Anal. Ed.*, 1935, **7**, 116.

[5] Todrick, A., and Walker, E., *Biochem. J.*, 1937, **31**, 292.

Table I shows also that both copper and glutathione are necessary for maximum oxygen uptake. For the oxidation 2 GSH—→GSSG, approximately 60 cu mm of oxygen are taken up by the 3 mg of glutathione used in this experiment. It is of interest that under the best conditions, the thermostable oxygen uptake of the muscle powder is equivalent to about 20% of the oxygen uptake of fresh muscle tissue; the thermostable oxygen uptake of the tumor powder is only 6% of the oxygen uptake of the fresh tissue. The cyanide inhibition of the thermostable oxygen uptake is markedly different; with the tumor powder an inhibition of 50% was observed, and with the muscle powder an inhibition of 95%.

In the thermostable powders, protein is probably the most important substrate. This view is suggested by several facts. First, the source and preparation of the powders are such that protein is the main constituent. Second, under similar conditions, pure native or denatured egg albumin, plus a little glutathione and copper, takes up oxygen. Third, if the thermostable powders, either normal or tumor, are extracted for 24 hours with chloroform, the oxygen uptake of the resulting fat-free powders is not decreased, but may actually be increased somewhat, as though the powders were losing so much inert material. Fourth, the powders, either fat-containing or fat-free, take up approximately the same amounts of oxygen at acid or neutral pH.

Table II gives the results of the chemical determinations. The total phosphorus is appreciably greater in the tumor powder than in the muscle powder. The nitrogen and the total sulfur content are not significantly different in the two powders. However, it appears of significance that the sulfhydryl content of the tumor powder is only 1/7 that of the muscle powder. Since the glutathione has been removed from these powders by the washing during their preparation, any sulfhydryl found is probably from the denatured protein.

The conclusion drawn by Hopkins[2] as to the importance of the protein -SH group in this oxidation seems to be confirmed by the

TABLE II.
Chemical Comparison of Thermostable Powders Prepared from Fresh Tumor and Muscle Tissue.

	Lipid-free tumor powder	Lipid-free muscle powder
Nitrogen	11.90%	13.80%
Total Phosphorus	0.74	0.11
" Sulfur (calculated as S)	1.16	1.14
" " (" " cysteine)	4.40	4.30
Sulfhydryl Sulfur (as cysteine)	0.07	0.51
% of total Sulfur present as Sulfhydryl	1.60	11.90

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.

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Oxidation of Native Protein by Glutathione-Copper Catalysis.

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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.

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