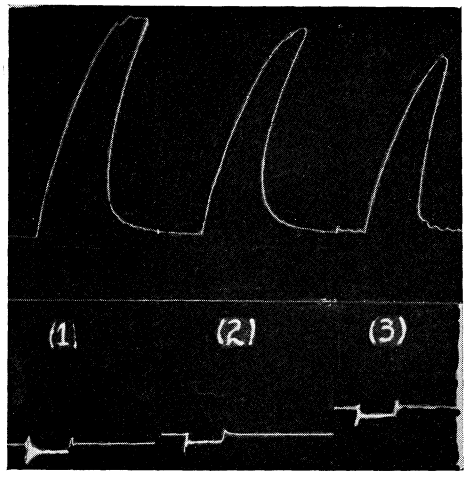

FIG. 1.
Kymograms showing the effect of stimulation of the contra-lateral sciatic nerve in causing reflex contraction of (1) the normal (left) soleus, (2) the transplanted (right) soleus, (3) the normal (left) tibialis posterior.

minimal degree of after-discharge. It is apparent, nevertheless, that the characteristic of the contraction is more nearly that of pale muscle than of red.

In Table I are compared the analyses for iron and myohemoglobin in normal soleus, transplanted soleus, and normal tibialis posterior muscles. It is evident from these results that the alteration in function of the red muscle has resulted in an iron and myohemoglobin content very similar to that of normal pale muscle and far below that of normal red muscle.

TABLE I.
Comparison of Myohemoglobin and Iron Contents in Normal Red, Transplanted Red, and Normal White Muscles.
All muscle weights corrected to 5 g. All values represent averages in mg per 5 g muscle of the number of determinations indicated in parentheses.

Muscle	Iron content	Myohemoglobin content
Tibialis posterior (2)	32.50	10.00
Transplanted soleus (3)	34.00	10.29
Normal soleus (3)	53.49	15.74

Summary. Conversion of red muscle to pale muscle can be accomplished by changing the position of the tendon of insertion to that of a white muscle. In thus assuming the function of a pale synergistic muscle, the red muscle in turn assumes the properties of pale muscle as evidenced by similar types of reflexly induced contractions and by similar myohemoglobin and iron contents. Acknowledgment is due Mrs. G. W. Wyatt for aid in the chemical determinations.

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Sulfhydryl and Disulfide Content of Normal and Arsenic-Resistant Trypanosomes.*

STEWART C. HARVEY.† (Introduced by E. M. K. Geiling.)

The sulfhydryl group has been regarded as a more or less specific receptor for tervalent arsenicals since it was postulated by Voegtlin[1] that arsenicals are bound through the formation of dithioarsenite complexes. It was further suggested by him[2] that differences in the equilibrium position of the glutathione system or in the absolute quantity of glutathione in sub-strains of a given strain of trypanosomes were responsible for the phenomenon of arsenic-resistance, the resistant strain presumably having a greater sulfhydryl reserve with which to detoxify the arsenical. Since then, the concept of the arsenic receptor has been broadened to include the fixed sulfhydryl groups of proteins,[3,4] the importance of which has been emphasized by the work of Barron

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† John Jacob Abel Fellow in Pharmacology.

1 Voegtlin, C., Dyer, H. A., and Leonard, C. S., *U. S. Pub. Health Rep.*, 1923, **38**, 1882.

2 Vogetlin, C., Dyer, H. A., and Miller, D. W., *J. Pharm. and Exp. Therap.*, 1924, **23**, 55.

3 Voegtlin, C., and Rosenthal, S. M., *J. Pharm. and Exp. Therap.*, 1930, **39**, 347.

and Singer.[5] The presence of the sulfhydryl group has been demonstrated in *T. equiperdum* by Voegtlin,[1] but no quantitative estimations have hitherto been reported. Reiner *et al.*[6,7] report that under comparable conditions of exposure, normal and resistant strains of *T. equiperdum* bind essentially the same amount of arsenic, irrespective of the drug used, which offers indirect evidence that the sulfhydryl group is not involved in the phenomenon of arsenic-resistance. In a more direct attempt to elucidate the role of the sulfhydryl group in arsenic resistance, a quantitative study of the sulfhydryl and disulfide contents of normal and oxophenarsine-resistant strains of *T. equiperdum* and *T. hippicum* was made.

Materials and Methods. The normal strain of *T. equiperdum* was one obtained from the laboratory of Dr. A. L. Tatum, and the oxophenarsine-resistant strain was a substrain produced in this laboratory.[8] The normal strain of *T. hippicum* was obtained from Dr. M. H. Soule and the resistant strain from Dr. S. H. Sandground. The trypanosomes were propagated in a Sprague-Dawley strain of albino rats.

The protein samples were prepared in the following manner: When the number of trypanosomes in the blood of the rat rose above 500,000 per cmm, the animal was decapitated and exsanguinated into a beaker which held 2 cc of heparinized Ringer-Locke solution (U.S.P.XIII) containing 0.75% glucose and 0.05% sodium bicarbonate. The volume of blood obtained in this way was usually 7-13 cc. The blood was centrifuged 8 minutes, the trypanosome layer was pipetted into 10 cc of the above Ringer-Locke solution, and then recentrifuged. This process was repeated until the trypanosomes were free

TABLE I. Sulfhydryl and Disulfide Content of Two Strains of *T. equiperdum* Expressed as the Cysteine Equivalents.

Normal strain					Arsenic-resistant strain				
Sulfhydryl mg/g		Combined mg/g		Disulfide mg/g	Sulfhydryl mg/g		Combined mg/g		Disulfide mg/g
Mean	Standard error of mean	Mean	Standard error of mean	Difference	Mean	Standard error of mean	Mean	Standard error of mean	Difference	Standard error of diff.
0.32	±0.05 (17 analyses)	6.00	±0.04 (28 analyses)	5.68	±0.06	0.40	±0.06 (10 analyses)	2.17	±0.14 (16 analyses)	1.77	±0.15

TABLE II. Sulfhydryl and Disulfide Contents of Two Strains of *T. hippicum* Expressed as the Cysteine Equivalents.

Normal strain					Arsenic-resistant strain				
Sulfhydryl mg/g		Combined mg/g		Disulfide mg/g	Sulfhydryl mg/g		Combined mg/g		Disulfide mg/g
Mean	Standard error of mean	Mean	Standard error of mean	Difference	Mean	Standard error of mean	Mean	Standard error of mean	Difference	Standard error of diff.
0.31	±0.01 (8 analyses)	1.49	±0.10 (22 analyses)	1.18	±0.10	0.27	±0.01 (8 analyses)	2.37	±0.04 (16 analyses)	2.10	±0.04

[4] Schmitt, F. O., and Skow, R. P., *Am. J. Physiol.*, 1935, **111**, 711.

[5] Barron, E. S. G., and Singer, T., *J. Biol. Chem.*, 1946, **157**, 221, 241.

[6] Reiner, L., Leonard, C. S., and Chao, S. S., *Arch. Intern. pharmacodynamie*, 1932, **43**, 199.

[7] Pedlow, J. T., and Reiner, L., *J. Pharm. and Exp. Therap.*, 1935, **55**, 179.

[8] Schueler, F. W., Chen, G., and Geiling, E. M. K., *J. Infect. Dis.*, 1947, **81**, 14.

from erythrocytes. The washed trypanosomes were next pipetted into 10 cc of 15% trichloroacetic acid, centrifuged, resuspended again in 10 cc of the trichloroacetic acid, and recentrifuged. To the protein residue were added 20 cc of anhydrous acetone, and the suspension was shaken for 5 minutes, whereupon 1 drop of concentrated hydrochloric acid was added with shaking and the mixture centrifuged. This was repeated, after which the acetone was drawn off under reduced pressure until the residue became pasty. It was then ground in a hot mortar until a fine powder was obtained. After drying 30 min. at 105°C. the sample was ready for analysis. These preparations were white or slightly buff colored. About 60 mg of dry protein were yielded by 10^{10} trypanosomes.

The analyses of the sulfhydryl and disulfide groups were carried out by the method of Mirsky and Anson,[9] with only such minor adaptations as are required for use with the spectrophotometer (Coleman Universal, Model 11).

Results. The values given in Tables I and II are expressed as the cysteine equivalents of sulfhydryl and disulfide in mg per g of dry protein. The values of the disulfide content reported are the differences between the two means of the original reduced sulfhydryl and the total (combined) sulfhydryl found after thioglycollate reduction of the protein.

Discussion. Although the mean of the sulfhydryl content of the arsenic-resistant strain of *T. equiperdum* is 25% greater than that of the normal strain, *the difference is not statistically significant.* On the other hand, the resistant strain of *T. hippicum* shows 13% less sulfhydryl than the normal strain, and this value has a moderate statistical significance (significant difference, $t=2.8$). More significant, however, are the differences in the disulfide contents of the normal and resistant strains. If it be assumed that the disulfide groups are sulfhydryl reserves which may be actively converted into sulfhydryl under the equilibrium displacements effected by the arsenical, the fact that the resistant strain contains only 31% as much disulfide as the normal strain, in the case of *T. equiperdum,* might seem to agree with reports that arsenic-fast strains of *T. equiperdum* bind less arsenic in the presence of glucose than normal strains.[7,10] Reiner *et al.*[6,7] warn that such differences in arsenic binding are probably the result of comparing two strains of trypanosomes not in the same degree of suppression. Furthermore, the differences shown in our results are too small to account for the high degree of resistance manifested toward certain arsenicals, the resistance factors of which may approach 200,[10] nor can any hypothesis based upon the sulfhydryl or disulfide content as yet explain the marked influence of the side chain upon the resistance factor. The fact that arsenic-resistant *T. hippicum* contains not less, but more, disulfide than the normal strain complicates even more the problem of establishing any direct relationship between the magnitude and state of the sulfhydryl system and arsenic-resistance. It can only be asserted that certain constitutional changes probably giving rise to arsenic-resistance also give rise to changes in the sulfhydryl system, but that the latter is not directly, at least, responsible for the drug fastness.

By calculation from the mean value of the sulfhydryl content of *T. equiperdum* there are 2.7×10^{-3} milliequivalents of sulfhydryl per gram of protein or approximately 1.6×10^{-4} milliequivalents per 10^{10} trypanosomes, capable of binding 59μg of arsenic or 78μg of arsenic as As_2O_3 (assuming only 2 binding valences per atom). This amounts to 4.8×10^{6} molecules of arsenical per trypanosome. These calculations are not inconsistent with the results of Reiner *et al.*[11] who report an average binding of 0.1 microequivalent of arsenic per 10^{10} trypanosomes or 4×10^{6} molecules per trypanosome.‡ The maximum arsenic uptake (phenylarsenoxide) reported by Eagle and Magnuson[10] in a series of experiments is calculated to be 83μg of arsenic

[9] Mirsky, A. E., and Anson, M. L., *J. Gen. Physiol.*, 1935, **18**, 307.

‡ Their figure of 6×10^6 is too high by a factor of 3/2, since one equivalent is ½ atom in most organic arsenicals.

[10] Eagle, H., and Magnuson, H. J., *J. Pharm. and Exp. Therap.*, 1944, **82**, 137.

per 10^{10} trypanosomes. Even higher degrees of binding have been reported[6,12] which are not commensurate with the quantity of sulfhydryl found by us. This might be construed as evidence for binding as a monothioarsenite or by some other means not involving the sulfhydryl group. However, it is more likely that the discrepancy may be attributed to conversion of disulfide to sulfhydryl during exposure or to a sulfhydryl level in the living trypanosome higher than that found in this investigation. The presence of substrate is important to the degree of binding,[7] and it is not unlikely that during centrifugation the closely packed trypanosomes suffer a relative glucose deficiency, which would yield low values of reduced sulfhydryl. That oxidation during the preparation of the sample was not grossly responsible for low values was tested by comparison with samples of wet protein whose dry weight equivalent was reasonably established by count.

Summary. Normal and arsenic-resistant strains of *T. equiperdum* do not differ significantly with respect to their sulfhydryl content, but the former shows a considerable surplus of disulfide groups. The sulfhydryl content of normal *T. hippicum* is not appreciably different from that of an arsenic-resistant strain, but the disulfide content of the arsenic-resistant strain is higher than that of the normal strain. It is concluded that these differences are not the cause of the resistance but may be rather the result of some cytological reconstitution which probably also gives rise to resistance. The sulfhydryl content is found to be of the same order as the degree of arsenic binding.

11 Reiner, L., Leonard, C. S., and Chao, S. S., *Arch. intern. pharmacodynamie*, 1932, **43**, 186.

12 Eagle, H., *J. Pharm. and Exp. Therap.*, 1945, **85**, 265.

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Agents Influencing Experimental Radiation Injury. Effects of Folic Acid and Pyridoxine.

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In a previous communication by one of us,[1] it was noted that a high mortality rate occurred among tumor bearing mice which were treated with X-rays for therapeutic purposes. The survival time even of successfully treated animals was short. It has been assumed that the animals might have succumbed due to either the absorption of the disintegrated tumor, or to same toxic substances produced by secondary, or scattered, radiation. The latter assumption was based on observations made from earlier experiments using the tissue culture technique.[2] Thus it was noticed that tissue fragments remaining in the medium in which they were irradiated needed less dosage for preventing their growth *in vitro*, than those which were washed in Tyrode solution and transferred to a fresh non-irradiated medium. It was concluded that some toxic substances might have been produced in the culture medium by radiation. Similar observations were made by other investigators.[3,4] The so-called "radiation sickness" occurring among human patients treated with X-rays may also be the result

1 Goldfeder, Anna, *Radiology*, 1945, **44**, 283.

2 Goldfeder, Anna, *Radiology*, 1938, **31**, 73; 1940, **35**, 210.

3 Luria, S. E., and Exner, F. M., *Proc. Nat. Acad. Sci.*, 1941, **27**, 370.

4 Evans, T. C., Slaughter, J. C., Little, E. P., and Failla, G., *Radiology*, 1942, **39**, 663.