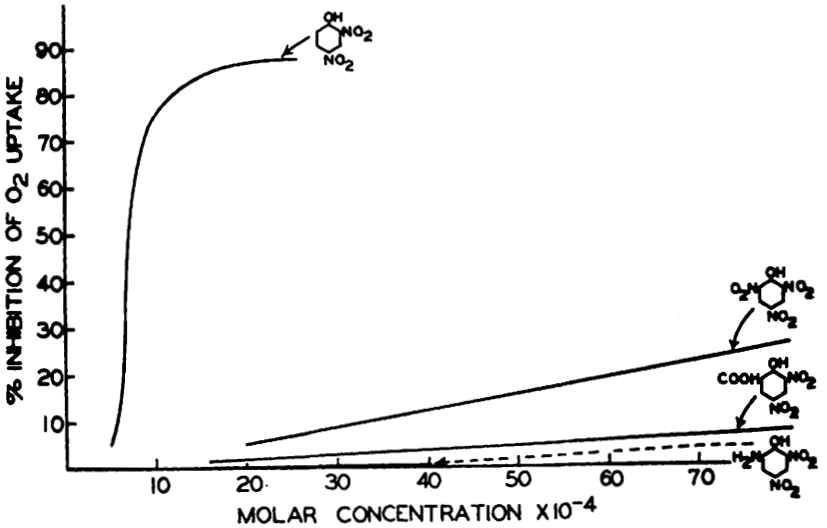

FIG. 4. Relative inhibitory effect on oxygen consumption of *M. verrucaria* spores of 2,4-dinitrophenol and compounds substituted in the 6-position with the nitro, carboxyl or amino group.

Summary. (1) The relative effects of 16 dinitrophenols in inhibiting the oxygen uptake mechanism of spores of *Myrothecium verrucaria* were studied. (2) The alkyl-, cycloalkyl-, or aryl- groups substituted ortho to the hydroxyl group in the 2,4-DNP molecule significantly increased the poisoning effect on this oxidative mechanism as compared to 2,4-DNP. (3) Effective concentrations of all active compounds manifested their full effect within an hour following contact with the spores. (4) The inhibitory effect as caused by all the alkyl substituted 2,4- and 2,6-DNPs averaged 85-90%. (5) Concentrationwise, the 6-*n*-hexyl-2,4-DNP compound proved the most potent, while dinitrosalicylic, picramic, and picric acids were significantly ineffective.

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Primary Amide Groups of Human Hemoglobin.* (18872)

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Pauling *et al.*(1) observed that the hemoglobin isolated from patients with sickle cell anemia possesses a higher isoelectric point (IEP) than that from normal individuals. Schroeder *et al.*(2) have shown that the amino acid composition of the two proteins is almost identical, and postulated that differences in the folding of the peptide chains were responsible for the electrophoretic results.

An increase in the number of primary amide groups of the sickle cell hemoglobin with a consequent decrease in free carboxyl groups might also be responsible for the higher IEP of this protein. The data of Table I, however, indicate that the number of primary amide groups in normal hemoglobin and in hemoglobins isolated from patients with sickle

* Aided by a research grant from Armour and Co.

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TABLE I. Number of Primary Amide Groups per Mole of Human Hemoglobin.

Non-sickle cell anemia hemoglobin source	Primary amide N, residues/mole	Sickle cell anemia hemoglobin source	Primary amide N, residues/mole
Mr. L. (polycythemia vera)	26	Miss S.	28.7
	22.9		25.9
	26		24.4
" M. (normal)	26.1		26.3
	25.8		26.7
	26.4	" P.	26.8
	23.5		26.8
	23.9		25.8
" C. (normal)	25.9	" B.	26.1
	25.9		26.5
Mrs. R. (acute rheumatic fever)	26.8		25.4
	26.8		
Miss T. (subacute bacterial endocarditis)	26.3		
	27.3		
Mrs. Q. (pulmonary infarction)	26.1		
	26.1		
Avg	25.7	Avg	26.3
Standard deviation = 1.22		Standard deviation = 1.15	

cell anemia, polycythemia vera, acute rheumatic fever, subacute bacterial endocarditis, and pulmonary infarction is essentially constant. An average of 26 primary amide groups per mole was obtained for all the hemoglobin samples analyzed.

Sources of hemoglobin. The group of 6 controls included 2 normal samples and one each from patients with the following diseases: Polycythemia vera, subacute bacterial endocarditis, pulmonary infarction, and acute rheumatic fever.† The experimental samples were obtained from 3 sickle cell anemic patients.‡

Isolation and analysis of hemoglobin. Solutions of carbon monoxide hemoglobin were prepared according to the method of Drabkin(3). They were stored under carbon monoxide at 4° until analysis. Hemoglobin concentration was determined with a Coleman Jr. spectrophotometer at 545 mμ(4).§

Determination of primary amide groups. A 1.00 ml aliquot containing approximately 8 mg of hemoglobin was pipetted into a test tube calibrated at 5.0 ml, then 4 ml of 3 N HCl was added. The tube was covered with a marble and placed in a boiling water bath for 3 hours. The ammonia liberated by this treatment was determined on an aliquot of this solution by microdiffusion in Conway vessels(5).

Results. The data, calculated as residues of primary amide groups per mole of hemoglobin, are set out in Table I. A value of 66,700 was used for the molecular weight of human hemoglobin(6).

Discussion. Schroeder *et al.*(2) found 36 residues of ammonia per mole in hemoglobin from normal individuals and 33 residues per mole in hemoglobin from patients with sickle cell anemia. These results are consistent with the values for the primary amide content reported here. Both groups of analyses indicate a constancy in the number of primary amide groups of the two proteins. The difference in the isoelectric points of the two proteins apparently is due to more subtle

† We wish to than Dr. George Cartwright, Department of Medicine, University of Utah, for obtaining these samples for us.

‡ We wish to thank Dr. Carl Moore, Department of Medicine, George Washington University School of Medicine, for sending these samples to us.

§ We wish to acknowledge the kindness of Dr. Joseph Kimmel for determining the oxygen capacity of a number of blood samples. These values were employed in the calibration of the colorimetric method.

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factors than the total number of amino acid residues or content of primary amide groups. Alteration in the sequence of the amino acids in the peptide chains is a possibility which should be considered in understanding this interesting problem.

Summary. The hemoglobin from sickle cell anemic individuals does not differ significantly in primary amide content from normal hemoglobin. Normal human hemoglobin was found to contain 26 primary amide groups per mole.

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Choline Requirement of Rats as Influenced by Age, Strain, Vitamin B_{12} and Folacin.* (18873)

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It was shown(1-3) that there was considerable variation in the minimum choline requirement of rats from different strains, sex, litter, and age. Copeland(3) noted that hooded rats of the Alabama Experiment Station strain (AES) had a much higher choline requirement for protection against renal hemorrhage than did albino rats of the Wistar strain. Since rats of the AES strain develop a high incidence of neoplasms(4-6) when subjected to chronic choline deficiency, it was of interest to investigate the choline requirement of the albino Sprague-Dawley (SD) strain as compared to the AES strain. The reports(7-9) that young rats of the Wistar strain are capable of synthesizing sufficient methyl and choline or of utilizing methanol for choline synthesis for growth and protection against renal damage prompted the investigation as to whether age and strain difference would influence these mechanisms under the experimental conditions employed in this laboratory.

Experimental. Weanling rats (21 days) of the SD and the AES strains weighing 40-45 g were placed in individual cages and uniformly grouped with respect to number, weight, sex, and litter. Feed and water were supplied *ad lib.* The basal diet contained extracted peanut meal 30 (50% protein)(10), sucrose, 39.5, extracted casein 6(11), salt mixture (undried) 4.4(11), L-cystine 0.1, cod liver oil 1, and lard 19. Vitamins were added (mg per kg of diet) as follows: Thiamine 2, pyridoxine 2, riboflavin 4, calcium pantothenate 10, niacin 20, i-inositol 200, alpha-tocopherol 25, alpha-tocopherol acetate 25, and 2-

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