COMMUNICATIONS TO THE EDITOR

METHIONINE AND ISOLEUCINE CONTENT OF MAMMALIAN HEMOGLOBINS. ITS SIGNIFICANCE FOR NUTRITIONAL AND METABOLIC STUDIES

Sirs:

Reports indicate that rats fail to grow when the only dietary protein is human or beef globin unless supplemented with isoleucine.[1,2,3]

Adult dogs receiving dog hemoglobin as virtually the sole source of protein maintain approximate nitrogen balance with slight loss of weight.[4,5] Dog hemoglobin supplemented with isoleucine is no better utilized.[5] However, supplementation with methionine results in positive nitrogen balance and maintenance of, or slight gain in, weight.[5] Further addition of isoleucine does not improve the effect of methionine.[4,5]

The nutritive value of "hemoglobin" seems, therefore, to depend on experimental conditions. It will be shown, however, that the results are exactly what could be expected from the difference in isoleucine and methionine content of hemoglobins.

From the data in the accompanying table,

ISOLEUCINE AND METHIONINE CONTENT OF MAMMALIAN HEMOGLOBINS

Assumed Molecular Weight = 66,700

	Isoleucine[6]			Methionine[7]		
	Protein, %	$M/10^3$ g.	M/m	Protein, %	$M/10^3$ g.	M/m
Human[8]	0	0	0	1.32	8.85	5.9
Dog[7,9]	1.36	10.4	6.9	0.42	2.81	1.9
Horse[10]	0[10]	0	0			4[12]
Bovine, adult[13]	0	0	0	1.76	11.8	7.9
Bovine, fetal[14]	0.63	4.80	3.2	0.97	6.50	4.3

it can be seen that the isoleucine content of dog hemoglobin is appreciable (1.36%) whereas human, horse and adult bovine hemoglobins lack this amino acid.[14]

Methionine is present in all hemoglobins studied, to the extent of 1% or higher, except in dog hemoglobin (only 0.4%).

The amino acid requirements for hemoglobin formation would seem to be different in different mammals. Whereas the human, the horse and cattle need for this purpose appreciable amounts of methionine but no isoleucine, the dog on the other hand needs isoleucine and only small amounts of methionine. The amino acid composition of hemoglobin is of metabolic significance since its breakdown contributes appreciably to the protein economy of an animal.

That fetal and adult bovine hemoglobins are two different proteins is again[13] illustrated by the difference in their methionine and isoleucine content.

This work was carried out in part under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Columbia University.

DEPARTMENT OF BIOCHEMISTRY
COLLEGE OF PHYSICIANS AND SURGEONS
COLUMBIA UNIVERSITY — ERWIN BRAND
NEW YORK, N. Y. — JEAN GRANTHAM

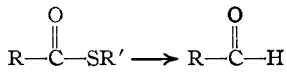
RECEIVED MARCH 16, 1946

(1) Devline and Zittle, *J. Biol. Chem.*, **156**, 393 (1944).
(2) Albanese, *ibid.*, **157**, 613 (1945).
(3) Orten, Bourque and Orten, *ibid.*, **160**, 435 (1945).
(4) Whipple, Robscheit-Robbins and Miller, *Ann. N. Y. Acad. Sci.*, in press.
(5) Miller, *Federation Proc.*, **5**, 73 (1946).
(6) Microbioassay with *L. arabinosus* 17-5 and with *L. mesenteroides* P-60, *cf.* Brand, *et al.*, THIS JOURNAL, **67**, 1524 (1945).
(7) Microbioassay with *L. mesenteroides*.
(8) Prepared by R. K. Cannan and obtained from H. B. Vickery.
(9) Prepared by L. L. Miller.
(10) Prepared by G. L. Foster (*J. Biol. Chem.*, **159**, 431 (1945)).
(11) Microbioassay with *L. arabinosus*.
(12) Brand and Kassell, Abstracts, Am. Chem. Soc., Boston meeting, Sept. 1939.
(13) Prepared by Wyman, *et al.*, *J. Biol. Chem.*, **153**, 275 (1944), and obtained from H. B. Vickery, *ibid.*, **156**, 283 (1944).
(14) Devline and Zittle[1] and Albanese[2] report an isoleucine content of 0.29 and 0.8%, respectively, in human hemoglobin.

A NEW ALDEHYDE SYNTHESIS

Sir:

In connection with our previous study on the hydrogenolysis of thioacetals,[1] it was apparent that the reductive desulfurization reaction therein described might be extended to compounds such as thiol esters in which case the corresponding aldehyde would be obtained.



This would be of particular interest in the sugar series since it would constitute a new method for synthesizing aldehydo derivatives. We have established the above reaction by the hydrogenolysis of the thiol esters of benzoic, propionic and tetraacetyl-D-ribonic acids which gave benzaldehyde, propionaldehyde and *aldehydo*-D-ribose tetraacetate, respectively.

The thiol esters were prepared by the reaction of the acid chloride with an excess of ethyl mercaptan in pyridine or by reaction of the acid chloride with lead mercaptide in dry ether (as for ethyl thiolpropionate, b. p. 133–135°). The hydrogenolyses were carried out with Raney nickel in 70–80% ethanol under reflux essentially as described[1] for the hydrogenolysis of thioacetals. After catalyst removal, benzaldehyde and propionaldehyde were isolated in the distillate as their bisulfite compounds in 60–70% yield and identified by definitive derivatives. The residual sirup obtained on hydrogenolysis of ethyl thiol-

(1) M. L. Wolfrom and J. V. Karabinos, THIS JOURNAL, **66**, 909 (1944).

D-ribonate tetraacetate (m. p. 87–87.5°, $[\alpha]^{27}D$ +17° in chloroform), after removal of catalyst and solvent, was crystallized from acetone–methanol–(petroleum ether) and yielded *aldehydo*-D-ribose tetraacetate[2] in rather low yield.

Full details will be reported in a latter communication.

(2) R. Pasternack and E. V. Brown, U. S. Patent 2,237,263 (1941).

CHEMICAL LABORATORY
THE OHIO STATE UNIVERSITY M. L. WOLFROM
COLUMBUS, OHIO J. V. KARABINOS

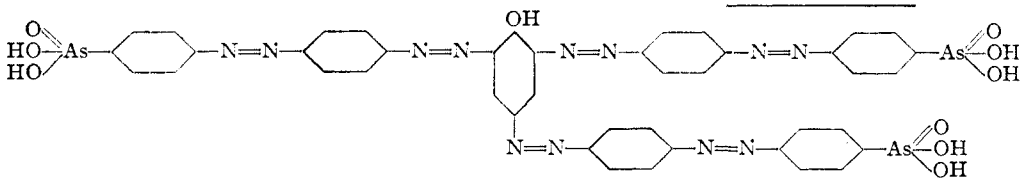
RECEIVED FEBRUARY 25, 1946

PRESSURE AND SPECIFIC PRECIPITATION

Sir:

Although the reactions that lead to specific precipitation evidently involve a combination between a di- or polyvalent antigen or hapten and specific sites on the antibody protein, probably resulting in the formation of a framework comprising large aggregates of the two species of molecules,[1] convincing evidence is lacking as to whether the protein itself becomes significantly altered in the process. The following observations bear on this question and suggest an approach for further study.

The precipitation system consisted of serum from rabbits immunized against arsanilic-azo-sheep serum and a simple trihaptenic dye antigen, *viz.*



To slow the precipitation for practical purposes, the serum was diluted 1:2, and the antigen 1:160,000. Visible precipitation occurred within a few minutes, and went to about three-fourths completion in an hour at room temperature. Small test-tubes were filled with corresponding specimens, stoppered with a rubber stopper, then placed in a water-filled steel pressure chamber, and hydrostatic pressure applied from a hydraulic pump within one to two minutes after mixing. At 10,000 pounds per square inch a scarcely visible precipitate formed during slightly more than an hour. After the pressure was released, precipitation continued in apparently the normal manner. Lower pressures were less effective, but quantitative analyses of protein nitrogen in precipitates centrifuged within less than five minutes after releasing pressure, showed decreases ranging from 55 to 77% under pressures between 1,500 and 8,000 pounds, in comparison with the amount under normal pressure. These data indicate a molecular volume increase on the order of 50 cc. or more per mole, although an accurate value requires detailed data on the rate of the reaction at different pressures.

Pressures of this magnitude oppose the reversible and irreversible denaturations of certain enzyme systems[2] and greatly retard the denaturation of purified human serum globulin at 65°.[3] These pressure effects are considerably greater than those encountered with reactions of small molecules and indicate that a number of groups are involved, or perhaps the protein molecule as a whole. They suggest that changes as extensive as those which take place in denaturation occur also in specific precipitation. An extensive quantitative study of pressure effects on the rate of specific precipitation will perhaps yield cogent data regarding the mechanism of the reaction.

THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY DAN H. CAMPBELL
CALIFORNIA INSTITUTE OF TECHNOLOGY FRANK H. JOHNSON[4]
PASADENA, CALIF.

RECEIVED FEBRUARY 6, 1946

(1) Pauling, Campbell and Pressman, *Physiol. Rev.*, **23**, 203 (1943).

(2) Johnson, Eyring, Steblay, Chaplin, Huber and Gherardi, *J. Gen. Physiol.*, **28**, 463 (1945).

(3) Johnson and Campbell, *J. Cell. Comp. Physiol.*, **26**, 43 (1945).

(4) Guggenheim Fellow.

METABOLIC PRODUCTS OF *ASPERGILLUS USTUS*

Sir:

Publication of preliminary results by Hogeboom and Craig[1] on the application of the latter's countercurrent distribution method to the problem of isolating bacteriologically active components from the culture filtrate of *Aspergillus Ustus*[2] prompts us to report briefly the progress of our work carried out in collaboration with Mr. Joseph Kurung and Dr. Harry Bray, Superintendent of the New York State Hospital for Incipient Tuberculosis.

The crude active material,[3] obtained by ether extraction of the culture filtrate or mycelium, has been divided into a bicarbonate soluble, a carbonate soluble and a neutral fraction. By fractional crystallization three substances have been isolated from the neutral fraction in small, variable amounts, and never all three from a single sample of crude material: A.U.N.-1, m. p. 155–6°, (C, 38.24; H, 4.10; Cl, 23.87); A.U.N.-2, m. p. about 270° with severe decomposition, inadequately characterized at present; A.U.N.-3,

(1) Hogeboom and Craig, *J. Biol. Chem.*, **162**, 363 (1946).

(2) Kurung, *Science*, **102**, 11 (1945).

(3) Generously supplied to us by Mr. Joseph Kurung and the Wallerstein Company, Inc.