

ABSORPTION OF OXYGEN BY BLOOD PLASMA FRACTIONS IN ALKALINE MEDIA

LEON L. GERSHBEIN AND BOGUSLAW K. KROTOSZYNSKI
Illinois Institute of Technology, Chicago

When a variety of plant and animal proteins is subjected to oxygen in alkaline media, absorption of gas ensues. The uptake of oxygen is due primarily to the cysteine or cystine contents of the proteins and, of the other amino acid residues investigated, only tyrosine, tryptophan and histidine contribute to a very small (each under $10 \mu\text{l.O}_2/\text{mg.}$) but definite degree (Gershbein, *et al.*, 1953).

The present study was undertaken with the view of ascertaining variations in oxygen uptake among blood plasma fractions isolated by way of the well-known procedure of Cohn and associates.

EXPERIMENTAL

Proteins. — The human plasma fractions were obtained from the Laboratory of Physical Chemistry and Public Health of Harvard University. Armour and Company was the source of porcine, ovine, canine, and equine fractions.

General Procedure. — The Warburg apparatus was employed in the determination of oxygen absorption (Umbreit, *et al.*, 1949). A weighed sample of protein together with 2 mls. of distilled water was introduced into the main chamber of the flask and 1 ml. of 3.0 N NaOH was placed

in the side-arm. The flask was attached to the manometer and the system gassed with pure oxygen prior to insertion into the bath maintained at 37.5°C . When constant conditions obtained, the alkali was tipped into the main chamber and readings were taken over a period of 20 hrs. Stirring was continued without interruption. Generally, up to ten determinations were carried out for each protein fraction.

RESULTS

Oxygen uptake values expressed as microliters of oxygen absorbed by one milligram of the human plasma fractions together with the respective average deviations from the means appear in Table 1. Findings for select hog, sheep, dog and horse fractions are shown in Table 2. The latter also contains oxygen uptake values for porcine and ovine plasma samples employed in the fractionation.

DISCUSSION

Each of the plasma fractions displayed oxygen uptakes in sodium hydroxide (final concentration: 1.0 N), the rate of absorption being greatest during the first two hours of reaction. The respective values are essentially "saturation" figures

TABLE 1.—Oxygen Uptake Values for Human Blood Plasma Protein Fractions Subjected to Treatment with Alkali (Time: 20 Hr.).

Fraction	Prominent components	Average microliters oxygen/mg. \pm average deviation from mean
I	fibrinogen	2.48 ± 0.31
II	γ -globulin	3.53 ± 0.57
III	β_1 -lipoproteins	
IV + V	β_2 -lipid-poor euglobulins and isoagglutinins	4.17 ± 0.18
	albumin, β -metal-containing proteins, α_2 -glycoproteins, α_2 -muco-proteins, α_1 -lipoproteins and iodo-proteins	
V	albumin	5.53 ± 0.80 6.12 ± 0.81

TABLE 2.—Uptake of Oxygen by Plasma Protein Fractions of Various Species in Aqueous Sodium Hydroxide (Time: 20 Hr.).

Species	Fraction	Average uptake, μ l. O ₂ /mg. \pm average deviation from mean
hog	plasma	5.03 ± 0.97
	I	3.50 ± 0.17
	II	4.29 ± 0.35
	IV-1	4.09 ± 0.40
	V	6.84 ± 0.75
sheep	plasma	5.93 ± 0.11
	II	4.88 ± 0.17
	IV	5.36 ± 0.78
dog	I	3.89 ± 0.46
	V	6.21 ± 0.10
horse	I	3.43 ± 0.21
	IV	3.61 ± 0.39
	V	5.71 ± 0.75

under these conditions, and increases in alkali concentration did not markedly alter the present uptakes.

For the human plasma fractions (Table 1), oxygen absorption was lowest with fibrinogen or Fraction I and increased progressively to the albumins (Fraction V) which displayed a maximal uptake value ($6.12 \pm 0.81 \mu\text{l.}/\text{O}_2/\text{mg.}$). These findings are consistent with the amino acid analyses, although the pertinent published data are sparse. Thus, the cystine content of blood albumin ranges two to three times higher than that of fibrinogen or the globulins; the levels of tyrosine, tryptophan and histidine, each of which figures to only a minor extent in the absorption of oxygen by proteins, occur in much narrower limits (Block and Weiss, 1956: 254-255, 341).

Except for minor discrepancies, the trend noted with the human proteins is also reflected in the findings with fractions from the four animal species listed in Table 2. As would be expected, plasma samples as such gave rise to uptake values which were higher than those of the corresponding Cohn fractions except for the albumins (Fraction V). This is exemplified in the last table by the porcine series.

SUMMARY

Protein fractions obtained from human plasma by the Cohn procedure absorb oxygen when exposed to the gas at 37.5°C in 1.0 N NaOH. The uptake values ($\mu\text{l.}/\text{O}_2/\text{mg.}$) were determined in the Warburg apparatus and found to be least for fibrinogen (Fraction I) and highest for the albumins (Fraction V),

thereby paralleling the respective cystine contents. Similar trends were also noted for several hog, sheep, dog and horse plasma fractions.

ACKNOWLEDGMENT

This study was aided by a grant from The Toni Division of the Gillette Company, Chicago, Illinois.

LITERATURE CITED

- BLOCK, R. J., and K. W. WEISS. 1956. Amino acid handbook. Springfield, Charles C. Thomas Publ., xiii + 386 pp.
- GERSHBEIN, L. L., B. KROTOSZYNSKI, J. A. Labow, and M. J. Brunner. 1953. Oxygen uptake by proteins during alkaline denaturation. *Fed. Proc.*, 12: 470-471.
- UMBREIT, W. W., R. H. BURRIS, and J. F. STAUFFER. 1949. Manometric techniques and tissue metabolism. Minneapolis, Burgess Publ. Co., iii + 227 pp.