

quantitatively by the aspartase enzyme, and identified qualitatively in the form of the copper salt. Approximately the other half of the nitrogen (40–50 per cent) is precipitable by phosphotungstic acid. Since cystine, arginine, histidine and aromatic amino acids are absent, it is probable that this fraction consists of lycine. Further work is in progress to determine the nature of the compounds precipitated by phosphotungstic acid.

In an earlier paper<sup>1</sup>, it was mentioned that aspartic acid is not present in the amino acid mixture. This erroneous conclusion is ascribable to the fact that, owing to the small amount of material available, the determination was made from the solution from which ammonia had first been distilled off according to van Slyke, in which process aspartic acid seems to be precipitated as a calcium salt.

The composition of the excreted amino acid mixture is very interesting in several respects. In the first place, it confirms our earlier view that the excretion is not ascribable to decomposition of the nodules or the roots. According to our recent investigations the nodule protein contains a variety of different amino acids (tryptophane, tyrosine, arginine, etc.). Hence it follows that, should the excretion be due to decomposition of this protein, the amino acid mixture in the medium would also contain several different amino acids. In view of the fact that the rate of excretion is highest at an early stage of growth, it seems very likely that the excreted compounds represent the primary amino acids formed in the fixation of nitrogen. Since aspartic acid is formed from fumaric acid through a well-known enzymatic reaction, the excretion of large amounts of aspartic acid is a most interesting phenomenon. On the other hand, it is also interesting to note that, in sterile cultures, aspartic acid forms an excellent source of nitrogen for uninoculated leguminous plants, as we have earlier found.

ARTTURI I. VIRTANEN.  
T. LAINE.

Laboratory of the Foundation  
for Chemical Research,  
Helsingfors.  
Oct. 8.

<sup>1</sup> Virtanen, v. Hausen and Karström, *Biochem. Z.*, **258**, 106; 1933.

#### Quantitative Determination of Ascorbic Acid

At the sixth Caucasus Congress of Physiologists, Pharmacologists and Biochemists, held in Erivan, Armenian S.S.R., on October 11–17, 1934, a communication was made by A. G. Jonnissian under the title of "Über den Chemismus des Vitamin 'C'".<sup>1</sup>

Amongst other conclusions of the author occurs the statement "Fructose und Arabinose geben mit dem Reagens nach Tillman [sic] ein positives Resultat".

Tillmans recommended the use of 2:6 dichlorophenolindophenol, which has been found by many workers to be a quantitative reagent for ascorbic acid. It is known not to be strictly specific, but the number of naturally occurring substances that have so far been found to interfere with the determination of ascorbic acid by this dyestuff is not great, and methods have been evolved for eliminating their interference. If it were true that widely occurring sugars also interfered, the value of the reagent would be severely limited, and doubt might be cast on much published work.

Using the ordinary technique, we have examined specimens of purified *l*-fructose and *l*-arabinose, and in neither event have we been able to detect the slightest reducing action on Tillmans's reagent. We have no explanation to offer of Jonnissian's results, but we thought it might be of interest to publish the above facts, in case that author's statements should lead other workers to under-value what has come to be recognised as an extremely useful reagent.

A. L. BACHARACH.  
H. E. GLYNN.

Glaxo Laboratories, Ltd.,  
56 Osnaburgh Street,  
London, N.W.1.  
Oct. 18.

<sup>1</sup> *Proc. 8th Cauc. Cong. Phys. Pharm. Bio.*, Academy of Sciences Press, Moscow and Leningrad, 101; 1935.

#### Colloid Osmotic Pressure of the Body Fluids of Marine Animals

EXCHANGES of water between blood and tissues are generally believed, since Starling's work, to be regulated by the equilibrium of two forces, namely, hydrostatic capillary pressure which drives water out into the tissues, and blood colloid osmotic pressure which draws it into the vessels. Based on clinical and experimental investigations, I pointed out several years ago that this assumption leaves out of consideration the role of the tissues, which are, from a physico-chemical point of view, a much more complicated system than blood. In my opinion, the hydration of tissues determines the colloid osmotic pressure of blood, this pressure being but an intermediary between the different tissues<sup>1</sup>.

Comparative physiology has furnished a strong argument in favour of this view. According to Krogh<sup>2</sup>, "when the body is immersed in water the hydrostatic pressure is everywhere counterbalanced and all the capillary systems can be considered as being at the level of the heart. This is probably of some importance for large aquatic animals such as whales which should therefore, in spite of their enormous size, require only a low colloid osmotic pressure in the blood to prevent filtration". This presumption should, *a fortiori*, be valid also for animals of smaller size. I have therefore measured the colloid osmotic pressure of the body fluids of marine animals (serum of fishes, hæmolymph of Crustaceans, Molluscs and Tunicates, cœlomic fluid of *Sipunculus*). The following average values have been given by 140 measurements:

	Colloid osmotic pressure (cm. H <sub>2</sub> O)
<i>Sipunculus nudus</i>	0.95 (0.7–1.2)
Lamellibranch Molluscs	1.0 (0.8–1.2)
Gasteropod Molluscs	1.5 (1.2–1.9)
Cephalopod Molluscs	3.3 (2.8–3.8)
Decapod Crustaceans	3.6 (2.1–4.4)
Tunicates	1.7 (1.2–2.3)
Elasmobranchs	4.6 (3.1–6.4)
Teleosteans	19.1 (14.6–25.0)

These results show conclusively that neither the postulate of Krogh's theory, nor consequently its reasoning, can be correct: the blood of Teleosteans has a colloid osmotic pressure only a little inferior to that of the blood of the large terrestrial mammals (horse: 22.5–29.0 cm. H<sub>2</sub>O).

What is the explanation of the difference of colloid osmotic pressure of the different animals? An answer to this question is suggested by the striking differences between Elasmobranchs and