XXXVIII. OBSERVATIONS ON THE VELOCITY OF THE PHOTO-OXIDATION OF PROTEINS AND AMINO-ACIDS.

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PHOTO-OXIDATION OF PURE PROTEINS.

IN earlier experiments [Harris, 1926, 1] it has been shown that, when blood is exposed to light, absorption of oxygen by the plasma occurs; further, that neither the saline constituents of plasma nor its glucose are responsible to any measurable extent for the uptake of oxygen. The proteins and lipins remain to be considered. As regards the proteins, since serum responds as effectively as plasma, attention may be devoted wholly to serum albumin and globulin. The only record of the effect of light on fibrinogen is that of Stalinger [1922], who found that when citrated plasma in the presence of eosin or ferrous sulphate is exposed to light the stability of the fibrinogen against a number of methods of precipitation is increased, but oxidation was not studied. In order to determine the locus of action of the photo-oxidation of plasma, the behaviour of other proteins was examined.

10 cc. of native egg-albumin suitably diluted were placed in the blood-tube [Harris, 1926, 1] in place of the usual 10 cc. of blood or plasma, and equilibrium established during agitation in the dark. On exposure to the radiations of the mercury vapour lamp after their passage through quartz and distilled water, the aqueous solution of egg-white took up oxygen at a steady rate of 17 mm³., or 0.17 volume % per minute. Plasma under identical conditions has an oxygen uptake of only 0.04 volume % per minute, the smaller figure here being no doubt due to the lower protein content. In the experiments on egg-white it became apparent that the size of the coagulum formed in the light (the bath being maintained at 37°) also depended on the length of exposure: whether the processes of coagulation and oxygen absorption are two independent or two related effects of ultra-violet light remains to be investigated. It is interesting to note in this connection that ultra-violet light is said to increase the egg globulins at the expense of the albumins [Chalupecky, 1917].

The photo-oxidation of egg-white, like that of plasma [Harris, 1926, 2], was found to be increased by haematoporphyrin (0.01 g.):

Vols. O₂ absorbed % per min. by egg-white in presence of haematoporphyrin when exposed to the radiations of Hg arc transmitted through colour filters:

Ruby	Canary yellow	Signal green	Blue	U-V
0.01	0.17	0.24	1.04	1.28

The spectral effect is identical with the regions of maximum absorption by the sensitiser subject to the reservations regarding these colour filters mentioned in the preceding paper [Harris, 1926, 2].

As a further step in the localisation of the oxygen absorption, pure proteins were examined. A crystalline preparation of ovalbumin subjected to examination responded as follows:

Vols. O₂ absorbed % per minute:

10 cc. 5 % ovalbumin	0.06
Ovalbumin + haematoporphyrin 0.01 g.	2.30

figures which are fairly close to those of goat's blood plasma for a like concentration of albumins + globulins.

A fat-free preparation of caseinogen (0.5 g.) yielded the following results on exposure to the radiations of mercury-vapour:

Vols. O₂ % per minute:

Caseinogen	0.12
Caseinogen + haematoporphyrin	1.67

The examination of a vegetable protein was undertaken with a view to determining whether proteins from plant sources behave like animal proteins and whether they respond alike to sensitisation by chlorophyll and haematoporphyrin. Crystallised edestin (0.5 g.) in aqueous solution was found to undergo photo-oxidation at a rate of 0.10 volume % per minute, which was increased to 1.54 on the addition of 0.01 g. haematoporphyrin. The effect of adding 0.1 cc. of the olive-oil emulsion of chlorophyll [Harris, 1926, 2] and exposing to various spectral regions is seen in the following figures, which give the rate of oxygen uptake in volumes % per minute:

Ruby	Canary	Green	Blue	U-V
0.06	0.18	0.24	0.66	0.85

It is seen that the effect of chlorophyll extends more powerfully into the red region of the spectrum. These figures must not be interpreted as representing true actinic values for reasons already given [Harris, 1926, 2]. Accurate actinometric measurements, while using specific chlorophylls, would be interesting in their possible relation to the rôle of this photo-sensitiser in photo-synthesis.

An outstanding exception to the photo-oxidation of proteins is that of gelatin. A highly purified sample in 2 % aqueous solution showed no absorption of oxygen in the light of the mercury arc; the figures were:

Vols. O₂ % per minute:

Gelatin 1 g.	0.00
Haematoporphyrin	0.03
Gelatin + haematoporphyrin	0.03

The transparency of gelatin solutions might be advanced from an optical standpoint as a partial explanation; even so, the addition of a highly absorptive sensitiser would be expected to activate it. It is more likely that the failure

of this protein to fall into line with those examined above is due to its exceptional constitution with respect to the particular amino-acids contained in it.

PHOTO-OXIDATION OF AMINO-ACIDS.

The amino-acids, when dissolved in distilled water and agitated with air, resolved themselves into two well-defined groups as regards the action of light. The following, in amounts of 0.2 g. each, effected no appreciable change in the volume of air in contact with their solutions: glycine, alanine, valine, leucine, asparagine, aspartic acid, histidine (HCl).

Tryptophan and tyrosine, on the other hand, were extremely sensitive to light under similar conditions:

O₂ taken up per minute, mm.³:

{	Tryptophan 0.2 g.	24
	„ + haematoporphyrin 0.005 g.	96
{	Tyrosine: solution saturated at 37°	9
	„ : hot sat. soln. cooled to 37°	20
	„ + haematoporphyrin 0.005 g.	26

In view of their own photo-sensitivity, the acceleration caused by haematoporphyrin was necessarily much smaller than was the case with plasma. Since tryptophan and tyrosine constitute about 2.5 % of the amino-acids in serum globulin and albumin (Plimmer) an arithmetical computation will show that these two amino-acids are responsible for the bulk of the oxygen uptake by plasma. The presence of these two amino-acids in blood plasma and their absence from the insensitive gelatin invite very tempting deductions regarding the locus of the action of light on blood plasma, pending chemical analysis of the oxidation products formed under the action of light in all the above cases. It is interesting to note, however, on the physical side that tyrosine and tryptophan are known to be strong absorbers of ultra-violet radiation [Schmidt and Norman, 1920]. In the present experiments it was observed that what at first appeared to be a reversed phenomenon with the first group of amino-acids was due to the marked reflecting power of their solutions towards the incident radiations. This conformity of amino-acids to Draper's law of photo-chemical activity lends some support to the theory correlating light absorption with chemical constitution. It is interesting to recall the investigations of Witt [1876], who attributed colour to the existence of certain groups or radicles which he termed *chromophores*, a substance containing a chromophore being a *chromogen*; in the widest sense of the term a chromogen is a substance possessing a banded absorption in the ultra-violet or visible spectrum. A comparison of the behaviour of the amino-acids studied above seems to point to the benzene radical as the chromogen; benzene itself is photo-chemically very important, and in solution or as vapour exhibits seven absorption bands in the ultra-violet [Baly and Collie, 1905]. A sample of *l*-phenyl-alanine, isolated from silk, in a saturated solution in water, in which it is only sparingly soluble, showed a rate of absorption under the usual conditions of

these experiments of 7.5 mm.³ per minute in the light. An older sample, of American origin, which was a good deal more soluble, took up oxygen at a rate of 2.4 mm.³ per minute; this may have undergone oxidation during storage. Phenylalanine, however, was found in aqueous solution to be insensitive to light. The dihydroxy-derivative, like tyrosine, is very susceptible: thus 0.1 g. dihydroxyphenylalanine, on exposure to the radiations of mercury vapour, absorbed oxygen at a rate of 7.5 mm.³ per minute—this was not found to be accelerated by ferric chloride. It is a matter of interest that this substance is intimately related to melanin pigment. Groups other than phenyl therefore play an important part. Harden and Norris [1915] made the interesting observation that the reduction of methylene blue in the presence of lactic acid was greatly accelerated by light: they offered an explanation in terms of the methylene blue, but a consideration of the present experiments explains it as a photo-oxidation of lactic acid in the presence of an optically sensitising dye.

In view of the interesting findings of Hopkins [1925], cystine and cysteine were examined in some detail. They were, however, found to fall respectively into the afore-mentioned groups. Cystine, when suspended in distilled water or in buffered solutions of phosphates ($p_H = 7.4$), was found to be quite stable in the light and remained unaffected even with the addition of haemato-porphyrin; it is probable that its insolubility and light-reflecting power contribute largely to this inactivity. When, on the other hand, its reduced derivative was placed in distilled water at 37°, agitation in the dark caused an uptake of 0.1 cc. of oxygen per minute per g. cysteine. This rate was already so high in the dark that irradiation with the mercury lamp increased the velocity of the uptake by only 50 % and, further, the addition of haemato-porphyrin caused only a comparatively small increase. There must obviously be other limiting factors here affecting the rate of oxidation. It must be mentioned that Dixon and Tunncliffe [1923] found that direct sunlight had a strong accelerating influence on the reduction of methylene blue by glutathione. Examination of a substance related to cysteine which is stable in the dark showed definite photo-chemical sensitivity; thus, 0.1 g. sodium taurocholate absorbed oxygen at the same rate as 10 cc. of plasma on exposure to the radiations of the mercury vapour lamp.

SUMMARY.

A large number of proteins take up oxygen when exposed to the radiations of the mercury arc; gelatin is an exception.

Most amino-acids are insensitive to these radiations, but tryptophan and tyrosine absorb oxygen at a rapid rate in the light of the mercury vapour lamp. Cystine is quite stable (insoluble), but cysteine takes up oxygen so rapidly in the dark that the acceleration produced by light is small and negligible when compared with that of tryptophan and of tyrosine.

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REFERENCES.

- Baly and Collie (1905). *J. Chem. Soc.* **87**, 1332.
Chalupecky (1917). *Strahlenther.* **17**, 646
Dixon and Tunnicliffe (1923). *Proc. Roy. Soc. Lond. B.* **94**, 266.
Harden and Norris (1915). *Biochem. J.* **9**, 330.
Harris (1926, 1). *Biochem. J.* **20**, 271.
— (1926, 2). *Biochem. J.* **20**, 280.
Hopkins (1925). *Biochem. J.* **19**, 5, 787.
Schmidt and Norman (1920). *J. Inf. Dis.* **27**, 40.
Stalinger (1922). *Wien. klin. Woch.* **35**, 860.
Witt (1876). *Ber. deutsch. chem. Ges.* **9**, 552.