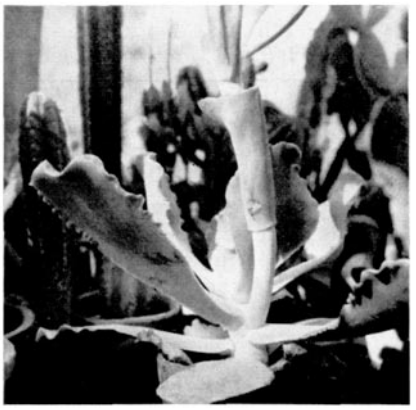
A Rare Type of Ascidium

Ascidia (scyphia) are of very rare occurrence in succulent plants. WOLTHUYS[1] reviews the literature and records his own observations. He quotes slightly more than a dozen examples recorded in almost a hundred years (the first by MORREN in 1852 and the last by himself in 1948). Seven of his examples are confined to the Crassulaceae whilst the remainder were noted in Agavacea and Liliaceae. The latter are recorded in HARRIS' *Missouri Botanical Garden Bulletin of 1906*. It is thus worth noting an ascidium on *Echeveria gibbosa*, var. *crispa*, Hort., grown in a private collection of succulent plants in Barry, Glamorganshire, in 1963. The connation has, once more, appeared in a member of the Crassulaceae. The ascidium, as shown in the photograph, is of the diphyllous type, goblet in form and open at the top as described by WOLTHUYS for other ascidia in the family. Here, however, the similarities end. The diphyllous ascidia described by WOLTHUYS terminated the shoot, the normal growing points aborted and thus further growth was impossible from within the goblet. Nearby dormant buds gave rise to side branches but nothing ever grew within the ascidium. The connation now under consideration, as can be clearly seen in the photograph, is borne on a side shoot which continues to grow from within the ascidium. Thus the growing points within the ascidium are not aborted. The shoot continues to grow from within the ascidium to end in a floral head. The flowers in this head were self-pollinated, and the seeds planted in 1964. Up to date, all the seedlings obtained are normal in every respect.



Résumé. Un type d'ascidie peu commun et jusqu'ici non enregistré dans les plantes charnues est décrit. Le point de croissance à l'intérieur du gobelet n'avorta pas, de sorte que le rejeton continua à croître à partir de l'intérieur de l'ascidie pour aboutir à une tête florale.

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Acid Phosphatases and Hemoglobin in Normal and G-6-PD Deficient Erythrocytes Incubated with Acetylphenylhydrazine under Various Conditions

One of the features of the G-6-PD[1] deficient red cells is a low GSH content; moreover, when these cells are treated in vitro or in vivo with particular substances (primaquine, acetylphenylhydrazine, etc.) a further drastic decrease, indeed almost the disappearance, of the GSH occurs. This is followed in vivo by a hemolytic crisis.

The connection, if any, between the hemolytic crisis and the GSH disappearance has not yet been elucidated. The GSH is supposed to protect the cells mainly through a specific protection of the sulphydrilic groups of their proteins. The GSH decrease could then result in the inactivation of a number of enzymes and in some modification of other proteins essential for the erythrocytes.

In fact, mixed disulphides of glutathione with hemoglobin appear when GSH is oxidized to GSSG; the resulting pigment (which shows a faster anodic mobility than the normal Hb at pH 8.6) is especially susceptible to oxidation to methemoglobin followed by denaturation and precipitation[2,3].

On the other hand, a decrease of activities in a number of enzymes, such as hexokinase[4-6], pyrophosphatase[7,8], glyoxalase[9], catalase[10], and acid phosphatases[11], has been observed in erythrocytes obtained from G-6-PD deficient individuals during the hemolytic crisis induced in vivo by various drugs (primaquine) or foodstuff (fava

[1] The following abbreviations have been used: glucose-6-phosphate dehydrogenase, G-6-PD; reduced glutathione, GSH; oxidized glutathione, GSSG; hemoglobin, Hb; acetylphenylhydrazine, APH.

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[4] G. J. BREWER et al., report in a recent paper[5] that the hexokinase activity did not appear to be reduced during the primaquine-induced hemolytic crisis in the G-6-PD deficient individuals.

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[10] A. R. TARLOV and R. W. KELLERMEYER, Fed. Proc. *18*, 156 (1959).

[11] F. A. OSKI, N. T. SHAHIDI, and L. K. DIAMOND, Science *139*, 409 (1963).

beans) or in enzyme-deficient (for G-6-PD) red cells incubated in vitro with APH. Some of these enzymes (pyrophosphatase[7], catalase[10,12], acid phosphatases[11]) show low activities in the G-6-PD deficient red cells even independently from these clinical or experimental conditions.

The relationship, however, is not clear between the GSH disappearance and the decrease of some enzymatic activities, or between this last phenomenon and the hemolysis.

We have recently observed that a decrease of red cell acid phosphatase activity accompanied by a peculiar modification of their electrophoretic pattern (appearance of fast anodic components and reduction of the staining intensity of the bands) occurs when a hemolysate is incubated with GSSG[13].

The following results show that the same modifications can be induced in intact red cells when a decrease of their GSH content is induced with acetylphenylhydrazine (APH). Electrophoretic analysis was carried out according to HOPKINSON et al.[14] for acid phosphatases, and according to POULIK[15] for Hb. The acid phosphatase activity was determined with the method of TORRIANI[16],

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[15] M. D. POULIK, Nature *180*, 1477 (1957).
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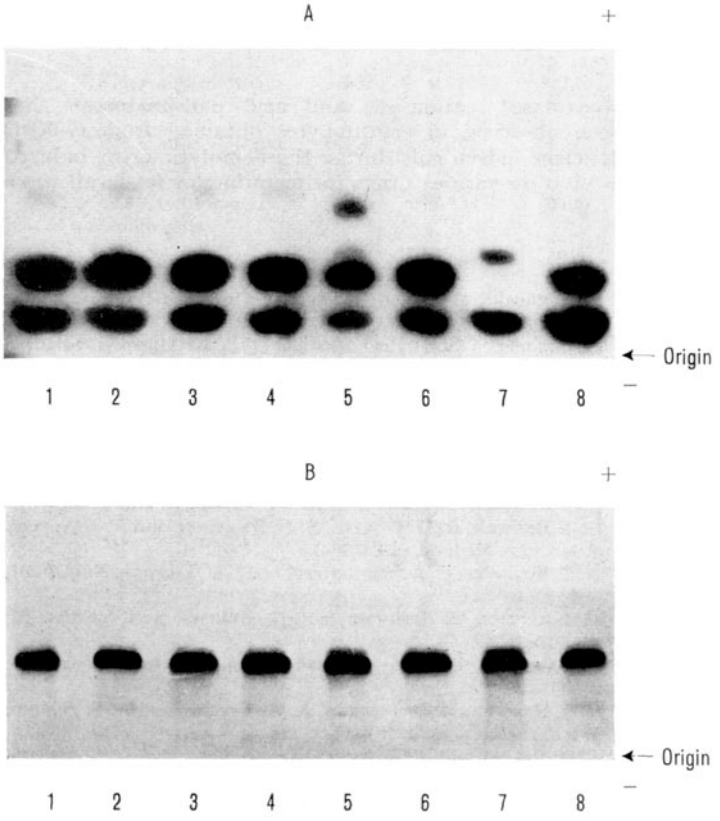
Effect of APH on normal and G-6-PD deficient human red cells

Mixtures incubated at 37°C		GSH mg%	Acid phosphatases		Hb fast component
			μM p-NP produced in 30 min per g of Hb	Electrophoretic pattern	
Whole normal blood	+ APH	30.7	138.4	normal	+
	control	32.5	185.1	normal	+
Normal erythrocytes in saline + glucose 2%	+ APH	29.3	160.6	normal	+
	control	28.9	176.2	normal	+
Normal erythrocytes in saline	+ APH	3.9	115.9	modified	+++
	control	32.8	181.4	normal	+
Whole G-6-PD deficient blood	+ APH	4.9	100.1	modified	++++
	control	23.3	195.8	normal	+



Acid phosphatases (A) and hemoglobin (B) starch gel electrophoretic patterns under various conditions. 1, Whole normal blood (B genotype) incubated with APH (acetylphenylhydrazine). 2, Whole normal blood (B genotype) control. 3, Normal erythrocytes (B genotype) in saline + glucose 2% incubated with APH. 4, Normal erythrocytes (B genotype) in saline + glucose 2% control. 5, Normal erythrocytes (B genotype) in saline incubated with APH. 6, Normal erythrocytes (B genotype) in saline control. 7, Whole G-6-PD deficient blood (CB genotype) incubated with APH. 8, Whole G-6-PD deficient blood (CB genotype) control. In A5 a new B pattern displaced towards the anode is evident; in A7 the fast component of the normal CB pattern is no longer visible and the slow component shows a decreased intensity; a new band appears, displaced towards the anode with respect to the slow component by the same amount that the new B pattern (in A5) is displaced with respect to the normal B pattern. In B5 and in B7 a fast (anodic) Hb band (Hb A_3) is clearly visible.

slightly modified to make it suitable for hemolysates. The GSH content of red cells was estimated according to BEUTLER et al.[17].

Whole blood from a normal subject, washed red cells from the same sample of blood suspended in saline + glucose 2%, the same red cells suspended in saline, and whole blood from a G-6-PD deficient individual were incubated for 2 h with and without APH. The tests listed above were then performed on hemolysates prepared from these different mixtures. Results are shown in the Table and the Figure.

It is clear that in the two mixtures in which a decrease of the GSH was obtained, the modifications of Hb and acid phosphatase referred to above also occurred.

As far as we know this is the first observation of a qualitative as well as a quantitative modification of an erythrocytic enzyme associated with the decrease of the red cell GSH.

The analogies between what has just been described and what happens to Hb in the same experimental conditions are striking: therefore the present results can be explained by assuming that the GSSG formed by the drug-induced oxidation of the GSH could combine with acid phosphatases producing molecules of the type 'protein S-SG' which would show an increased anodic mobility.

Experiments are being carried out in order to ascertain whether the activity of the molecules with higher anodic mobility is different from that of the native enzyme, as some of our data suggest, or whether the decrease of the activity is due only to the irreversible denaturation of the enzyme[18].

Riassunto. L'incubazione con GSSG determina nell'emolizzato una modificazione del quadro elettroforetico delle fosfatasi acide eritrocitarie ed una riduzione della loro attività.

Modificazioni identiche a carico dell'enzima possono venire indotte anche nel globulo rosso intero di soggetto normale o di enzimopenico per la G-6-PD, provocando una caduta del tasso di GSH mediante acetilfenilidrazina.

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[18] Acknowledgment: We wish to express our gratitude to Prof. D. CAVALLINI and to Prof. M. SINISCALCO for their helpful criticism and for having read the manuscript before publication. The skilful technical assistance of Mr. C. SANTOLAMAZZA has been very much appreciated.

Thermodynamics of Aging of the Collagenous Structure

In the recent past, several papers have appeared describing the aging of collagen as the manifestation of an increase in cross-linking density[1–3]. The nature of rising cross-links is however not clear enough. It seems that more factors cooperate in the resulting increase in structural stability of the aged collagen, as was shown recently by CHVAPIL and DEYL[4]. Excellent information about the changes that take place during the aging process could be obtained from the thermodynamic data. The great disadvantage of this method is based in the impossibility of direct enumeration of fundamental thermodynamic magnitudes like free energy, enthalpy and absolute entropy of the structure. This disadvantage could be abolished by taking into account the fact that the denaturation of collagen is a process consisting of at least two steps, the first one being the formation of an activated complex of denaturation. Assuming that the sterical configuration of the activated complex is always the same, independent of the nature of the denaturation, one can also consider that the thermodynamic parameters (in absolute measure) of activated complex of denaturation are independent of the nature of the denaturation process itself. This presumption could be easily verified by estimation of ΔF_{+}^{+} in the activated state, as will be shown below (see Table I).

In our work we tried to determine the changes in thermodynamic parameters on the aging of rat tail tendons. Male rats of Wistar strain aged 10 weeks and 10 months were used. The evaluation of free energy change in the formation of an activated complex of denaturation and enumeration of the enthalpic effect was performed in a similar way as described previously by WEIR[5]. From the contraction-relaxation curve of the tail tendon the half-time of shrinking (equal to half-time of denaturation)

Table I. Values of free energy of activated complex formation in different media for rat tail tendons from old and young individuals. Each value is an average from ten measurements; standard deviation is $\pm$ 2.5 kcal per mole. All measurements at 37°C, values for thermal denaturation are extrapolated

Sample	ΔF_{+}^{+} (kcal per mole)				
	Heat only	6 *M* urea	0.1 *N* CH_3COOH	0.1 *N* NaOH	2.5 *M* KI
Young rats (10 weeks)	26.5	20.9	20.3	21.0	19.2
Old rats (10 months)	28.7	21.2	20.5	21.6	19.5

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