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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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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was determined. Shrinking was measured under a load of 50 mg, which does not significantly affect the value of the half-time. Our measurements were performed in different media, namely in $6M$ urea, $0.1N$ NaOH, $0.1N$ CH_3COOH and $2.5M$ KI. For each of these solutions the parameters of formation of an activated complex of denaturation were evaluated. To enable the determination of the enthalpy change of activated complex formation, the measurements were performed at different temperatures: 28°, 32°, 37°, 42°, and 47°C. For determination of free energy and enthalpy change in distilled water, the measurement was performed at 58° and 62°C. For comparing values obtained in distilled water with those obtained in different media at much lower temperature, the values obtained in distilled water were extrapolated to 37°C.

Calculation of the thermodynamic parameters of activated complex formation was as follows:

Assuming that activated complex formation is a first order reaction, the velocity constant at any temperature will be

$$K = \frac{0.6932}{t_{1/2}}$$

and the equilibrium constant of the complex formation is then

$$K' = \frac{3.31 \cdot 10^{-11}}{T\, t_{1/2}},$$

where $3.31 \cdot 10^{-11} = 0.6932 \cdot h/k$, h and k designate Planck's and Boltzmann's constant respectively, T is absolute temperature and $t_{1/2}$ is the half-time of the reaction.

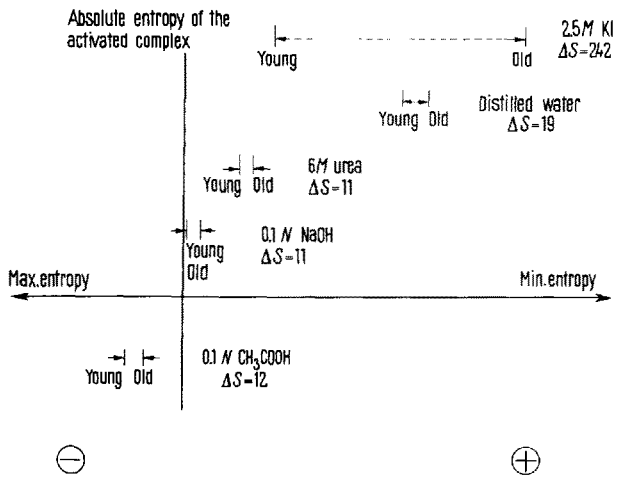
From the value of equilibrium constant the free energy change is computed:

$$\Delta F^{\ddagger} = -2.303\, R\, T \log K'.$$

The temperature dependence of the velocity constant K enables one to determine the enthalpy change: plotting $\log K'$ against $1/T$ we obtain a straight line, the slope of which is equal to $\Delta H^{\ddagger}/2.303R$.

The entropic effect is evaluated from the formula:

$$\Delta F^{\ddagger} = \Delta H^{\ddagger} - T\,\Delta S^{\ddagger}.$$



Comparison of entropic changes accompanied with activated complex formation supposing that the absolute entropy of activated complex at both young and old collagens is the same.

In our results summarized in Table II it is shown that all changes in the structure take place on account of entropy change, and that the free energy change for formation of the activated complex of denaturation is the same in old and young rats. This can be illustrated in the following way: Let us consider an axis of absolute entropy values as shown in the Figure. The absolute entropy of the activated complex of denaturation is always the same independent of the age and the mode of denaturation. Plotting the entropy changes combined with the formation of the complex for old and young animals in different media, we see that in spite of the different numerical values, there

Table II. Numerical values of thermodynamic magnitudes of activated complex formation in different media for old and young individuals. Each value is an average from ten measurements. Standard deviation in $\Delta F^{+}_{+} = \pm 2.5$ kcal per mole, in $\Delta S^{+}_{+} = \pm 4.0$ cal per grad mole

Sample	T (°K)	$t_{1/2}$ (sec)	K'	ΔF^{+}_{+} (kcal per mole)	ΔH^{+}_{+} (kcal per mole)	ΔS^{+}_{+} (cal per grad mole)
			Heat only			
Young rats	331	352.0	$2.84 \cdot 10^{-16}$	23.35	91.5	206
(10 weeks)	335	52.0	$1.90 \cdot 10^{-15}$	22.70	91.5	206
Old rats	331	732.0	$1.37 \cdot 10^{-16}$	24.00	100.0	229
(10 months)	335	96.0	$7.50 \cdot 10^{-16}$	23.10	100.0	229
			$6M$ urea			
Young rats	301	330.0	$3.32 \cdot 10^{-16}$	21.35	37.3	53.0
(10 weeks)	305	132.0	$8.23 \cdot 10^{-16}$	21.00	37.3	53.4
	310	72.0	$3.24 \cdot 10^{-15}$	20.95	37.3	53.0
	315	27.5	$3.83 \cdot 10^{-15}$	20.70	37.3	52.6
	320	16.5	$6.27 \cdot 10^{-15}$	20.65	37.3	52.2
Old rats	301	840.0	$1.31 \cdot 10^{-16}$	21.80	41.3	64.7
(10 months)	305	194.0	$5.15 \cdot 10^{-16}$	21.30	41.3	65.5
	310	112.0	$9.50 \cdot 10^{-16}$	21.25	41.3	64.7
	315	33.0	$3.18 \cdot 10^{-15}$	20.80	41.3	65.0
	320	22.0	$4.70 \cdot 10^{-15}$	20.90	41.3	63.7
			$0.1N$ acetic acid			
Young rats	301	36.0	$3.32 \cdot 10^{-15}$	19.80	5.4	−47.7
(10 weeks)	305	30.0	$3.62 \cdot 10^{-15}$	20.10	5.4	−48.0
	310	24.0	$4.45 \cdot 10^{-15}$	20.30	5.4	−47.8
	320	16.0	$6.45 \cdot 10^{-15}$	20.7	5.47	−47.5
Old rats	301	42.0	$2.63 \cdot 10^{-15}$	19.9	9.60	−34.4
(10 months)	305	36.0	$3.01 \cdot 10^{-15}$	20.2	9.60	−34.7
	310	30.0	$3.56 \cdot 10^{-15}$	20.5	9.60	−34.6
	320	18.0	$5.75 \cdot 10^{-15}$	20.8	9.60	−34.9
			$0.1N$ natrium hydroxide			
Young rats	305	144.0	$7.52 \cdot 10^{-16}$	21.0	21.45	1.5
(10 weeks)	315	40.0	$2.50 \cdot 10^{-15}$	21.0	21.45	1.4
	320	36.0	$2.87 \cdot 10^{-15}$	21.3	21.45	0.5
Old rats	305	300.0	$3.21 \cdot 10^{-16}$	21.6	25.50	12.8
(10 months)	315	101.0	$1.04 \cdot 10^{-15}$	21.6	25.50	12.4
	320	48.0	$2.16 \cdot 10^{-15}$	21.3	25.50	13.1
			$2.5M$ KI			
Young rats	305	20	$5.43 \cdot 10^{-15}$	19.9	45.40	83.5
(10 weeks)	310	5	$2.14 \cdot 10^{-14}$	19.2	45.40	84.5
Old rats	305	18	$6.04 \cdot 10^{-15}$	19.8	119.00	325.0
(10 months)	310	1	$1.07 \cdot 10^{-13}$	18.4	119.00	342.0

is a shift in the values corresponding to old animals in the sense of lower values of absolute entropy. This means simultaneously an increase in absolute negative entropy, which is in good agreement with the idea that living organisms as open systems are fed by negative entropy. Older structures have therefore less freedom, are more ordered, and have a lower value of absolute entropy.

Zusammenfassung. Bei der Prüfung der thermodynamischen Denaturationsparameter der Kollagenstruktur wurde festgestellt, dass die Erhöhung der strukturellen Stabilität der Erniedrigung der Entropie entspricht. Entropieänderung während der Denaturation der Struktur stimmt mit den Änderungen der Enthalpie völlig überein, so dass die freie Energie im Tierexperiment andauernd konstant bleibt.

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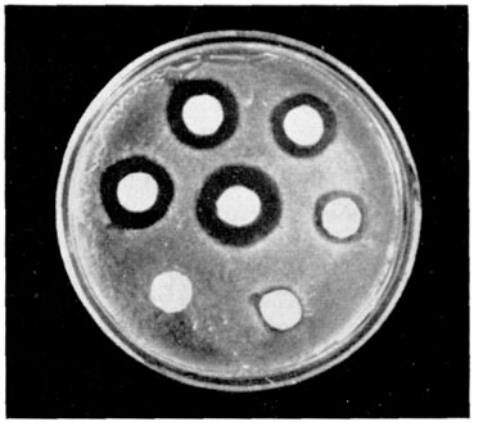
Neuere Angaben über Capsicidin

In einer früheren Mitteilung[1] wurde über die Isolierung von Capsicidin, eines neuen, besonders Hefen schädigenden Antibioticums aus Gewürzpaprika berichtet und der Wirkstoff als ein Saponin identifiziert. Weitere Versuche führten zu der Feststellung, dass Capsicidin in den Aleuronkörnchen der reifen Samen und in der Wurzelrinde von *Capsicum annuum* L. enthalten ist.

Ein Studium der *Aktivitätsbedingungen* des Wirkstoffes ergab Folgendes: (1) Aktive, wässerige Lösungen des Saponins jeglicher Herkunft lassen sich durch Cholesterin inaktivieren. Aus Samen extrahierte wässerige Aleuron*suspensionen* sind aktiv, jedoch durch Cholesterin nicht inaktivierbar, nur nach Desintegrierung der Körnchen durch Verreibung mit Sand. (2) Aktive, saponinhaltige Kaltwasserauszüge werden durch sofortiges Erhitzen, schwache Ansäuerung mit Mineralsäuren oder Alkalisierung vollständig inaktiviert. Erfolgt die Einwirkung nach einer gewissen Stehzeit der Extrakte, tritt keine Inaktivierung mehr ein. Mittlerweile spielt sich ein Stabilisationsvorgang ab (Figur). (3) In aktiven Wurzelextrakten bilden sich optisch anisotrope, in hitzeinaktivierten optisch isotrope Kristalle. (4) Durch Erhitzung in wässerigem Medium inaktivierte Substanz kann durch Kochen mit Xylol teilweise reaktiviert werden. (5) Bei trockener Erhitzung geht die Aktivität nur sehr langsam zugrunde und sie kann durch Xylol nicht wieder hergestellt werden. (6) Inaktive wässerige Extrakte hämolysieren auch nicht. Es ist daher nicht ausgeschlossen, dass allgemein bei nicht hämolysierenden Pflanzenextrakten durch sorgfältige Vermeidung inaktivierender Einflüsse noch neue Saponine entdeckt werden könnten. (7) Die Aktivität wird von der Zusammensetzung des Nährbodens wesentlich beeinflusst und zwar durch Beeinflussung der Lebensbedingungen und daher der Widerstandsfähigkeit des Teststammes: Hemmzonen von *Saccharomyces cerevisiae* var. *ellipsoideus* waren in Anwesenheit von 0,5% Glykose viel breiter als mit 10% des Zuckers. (8) Einfluss verschiedener zugefügter Stoffe auf die Aktivität: Methylalkohol, Äthylalkohol, Aceton, Glycerinmonostearat, -tristearat und -trioleat, Mannit, Glykose, Cystein, Pepton, Ovalbumin, Casein, raffiniertes Sonnenblumenöl, Stärke, Pektin, Bier, Apfelsaft verhielten sich indifferent. Lecithin inaktivierte vollständig, ebenso Milch, Weizenmehl, sterinhaltiges Sonnenblumenöl, mit Sand verriebener Hefebrei (weist auf die Oberflächenwirkung des Saponins hin).



Stabilisationsvorgang in einer Kaltwassersuspension 1:10 von Aleuron des *Capsicum annuum*-Samens. Mitte: Die Suspension ohne Erhitzung. Keine Hemmzone: Sofortige Erhitzung nach Extraktion. Ganz schmale Hemmzone: Erhitzung nach 5 min Stehzeit. Immer breitere Hemmzonen nach Stehzeiten von 10, 20, 30 und 50 min. Teststamm: *Sacch. cerevisiae* var. *ellipsoideus* T_{22}. Standard-Agar, pH 7,2.

Bei Fütterungsversuchen an 30 Ratten (1–4 Wochen lang) mit aktiven, stabilisierten Aleuronsuspensionen konnte, Mortalität und Blutbild betreffend, keine Giftwirkung nachgewiesen werden[2].

Summary. An account is given of the distribution of capsicidin – a saponin with antibiotic activity – in *Capsicum annuum* L., some conditions of its activity, and feeding experiments with rats.

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[1] I. GÁL, Z. Lebensmittelunters. *124*, 333 (1964).

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