Hemolysis of autologous erythrocytes modified with common enterobacterial antigen in immune and non-immune rabbits

Antigen for immunization	Mean antibody titers (reciprocal) against common antigen	Antigen for challenge	Hemoglobinemia		Hemoglobinuria		Deaths		Total
			Number and percentage of animals						
Common antigen	320	Common antigen[a]	25 (100%)		18 (72%)		9 (36%)		25
None	10	Common antigen	1 (11%)		1 (11%)		1 (11%)		9
Common antigen	400	Staphylococcus	0 (0%)	1 (4%)	1 (13%)	2 (8%)	0 (0%)	1 (4%)	8
None	10	None	0 (0%)		0 (0%)		0 (0%)		8

[a] Obtained from microorganism with O antigen unrelated to that of strain used for immunization.

or *vice versa*. In contrast, hemolysis occurred only in a small percentage of rabbits when similarly treated erythrocytes were injected into non-immune animals or when red blood cells modified with a completely unrelated antigen (staphylococcus) were administered into animals previously immunized with common antigen.

This *in vivo* hemolytic reaction, then, is effected by antibodies which neither cause precipitation of the corresponding antigen nor agglutination of many enteric bacteria possessing this antigen on the surface[5]. These antibodies readily cause hemagglutination and hemolysis *in vitro*[4]. It is of interest to note that antibodies against the common antigen have been detected in the serum of healthy individuals and in human γ-globulin preparations from various countries as well[6]. It has been shown also that one-third of children with enterobacterial infection respond with an increase in the titer of this antibody[6]. It remains to be determined whether hemolytic anemia in man accompanying or following enterobacterial infections may have, in part at least, a similar mechanism. From the present investigation and from previous studies, then, it is evident that autologous red blood cells modified with either O, Vi, or common enterobacterial antigen undergo rapid lysis in rabbits having the corresponding antibody in substantial titer[7].

Zusammenfassung. In Kaninchen, die mit gemeinsamem Antigen der intestinalen Bakterien immunisiert waren, sowie in nichtimmunisierte Kaninchen wurden Eigenerythrocyten, die mit dem Antigen vorbehandelt waren, intravenös injiziert. *In vivo*-Hämolyse-wurde nur bei den immunisiertem Tieren beobachtet. Erythrocyten, die mit heterologem Staphylokokken-Antigen vorbehandelt waren, wurden nicht hämolysiert.

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The Interaction of Free Radicals in Protein and Melanin

Introduction. Melanin is a comparatively inert substance and in biological systems it is assigned either a protective role (colour adaptation to the environment, absorption of light or heat) or else it is considered to be a metabolic by-product.

The experiments described in the present communication show that under experimental conditions, there can be an interaction between the free radicals in proteins (egg albumen) and those in melanin, and it is conceivable that melanin may play a more active role in cell metabolism than previously considered.

Methods. The melanin was supplied by L. Light and Co. (Colnbrook, Bucks, U.K.) and was synthesised by them from L-tyrosine by potassium persulphate oxidation.

25 mg of melanin was dissolved in 2.5 ml 0.1 *M* sodium phosphate buffer at pH 7.4. This was stock solution M, which was then diluted as shown in the Table.

A series of 'Vitreosil' tubes (internal diameter 3.0 mm) were filled with 5.0 ml of albumen and various concentrations of melanin as shown in the Table. The tubes were placed in an unsilvered 'Pyrex' vacuum flask containing liquid nitrogen and irradiated for 90 min with light at 366 mμ. A series of control tubes containing 5.0 ml of water

Test

Tube	a	b	c	d	e	f	g
1.0 ml melanin solution	M	M/2	M/4	M/5	M/10	M/50	M/100
5.0 ml. albumen solution	+	+	+	+	+	+	+
Irradiation time (min)	90	90	90	90	90	90	90

Control

Tube	a	b	c	d	e	f	g
1.0 ml melanin solution	M	M/2	M/4	M/5	M/10	M/50	M/100
5.0 ml water	+	+	+	+	+	+	+
Irradiation time (min and sec)	90	30	18	15	8	1 min 45 sec	54 sec

and 1.0 ml of melanin (concentration from M to M/100, as shown in the Table) were irradiated for a specified time and compared with those containing albumen. The times of irradiation were calculated from experimentally determined absorption coefficients of the melanin dilutions, so that an equivalent amount of energy would be absorbed by each melanin molecule.

The irradiated tubes were placed in an electron spin resonance spectrometer at 9000 Mc/s (X-band) with a magnetic field of 3000 gauss. The pen recorder traced either the derivatives or the integral of the absorption curves.

Results. Figure 1 shows the results of these experiments. Unless otherwise stated, all results refer to irradiated solutions. The figures a, b, c etc. are of the differential of the absorption curves, whilst a′, b′, c′ etc. are of their corresponding integrals.

Figure 1a shows the signals from the concentrated melanin solution (M), the albumen (A), and the mixture (M+A). The shape and line width of the two signals was quite different; melanin gives a sharp signal, whilst that from albumen is much broader. Nevertheless, the albumen has a considerable number of free radicals, as shown by the integral curve a′. The initial concentrations of melanin and albumen were chosen so that each, when separately irradiated for 90 min, would have the same concentration of free radicals. In Figure 1a, M has 61.8×10^{14} free radicals/*g*, whilst A has 63.5×10^{14} free radicals/*g*. The mixture M+A has fewer free radicals (35.2×10^{14} free radicals/*g*). The rest of the figure shows the signals obtained from the various dilutions of melanin, and the mixture of melanin and albumen.

Figure 1b shows the signal from half the melanin concentration (M/2) which had been irradiated for 30 min, compared with albumen plus melanin (A+M/2), which had been irradiated for 90 min. The mixture had a smaller signal even though it had a longer irradiation time.

Figure 1c shows M/4, which had been irradiated for 18 min, compared with the mixture irradiated for 90 min.

Figure 1d shows M/5 irradiated for 15 min compared with the mixture irradiated for 90 min. The integral curves 1a to 1d show that the area under the curves in the case of the mixture is smaller than that for the melanin alone, hence the free radical concentration in the mixture is less than that present in the melanin alone. At great melanin dilutions (1e to 1f and 1g) the shape and line width of the curves indicate that there is a gradual return of the albumen signal, until 1d′ is much the same as 1a′ (A).

The results are summarized graphically in Figure 2.

Discussion. The results indicate that there is an interaction between melanin and the albumen so that there is a diminution in the concentration of free radicals in the mixture after irradiation. This diminution is not due to the difference in the time for which the specimens were irradiated.

All the tubes containing the mixture of albumen and melanin were irradiated for 90 min. The control tubes, containing melanin and water, were irradiated for a proportionately smaller time. Thus the control tube containing half the concentration of melanin (M/2) was irradiated for 30 min, whilst that containing one fifth the quantity of melanin (M/5) was irradiated for 15 min. In all these cases down to concentrations M/5, the control, which had the shorter irradiation time, had more free radicals than the mixture which had been irradiated for 90 min. Our control experiments have shown that the number of free radicals produced on irradiation of either melanin or albumen is directly proportional to the duration of the irradiation time, up to 90 min, i.e. the longer the irradiation time, the greater the concentration of free radicals. The free radical concentration is also proportional to the concentration of melanin or albumen. Thus one would expect that the concentration of free radicals in the control would be equal to, or even smaller than the free radical concentration in the mixture. Yet, in all cases down to

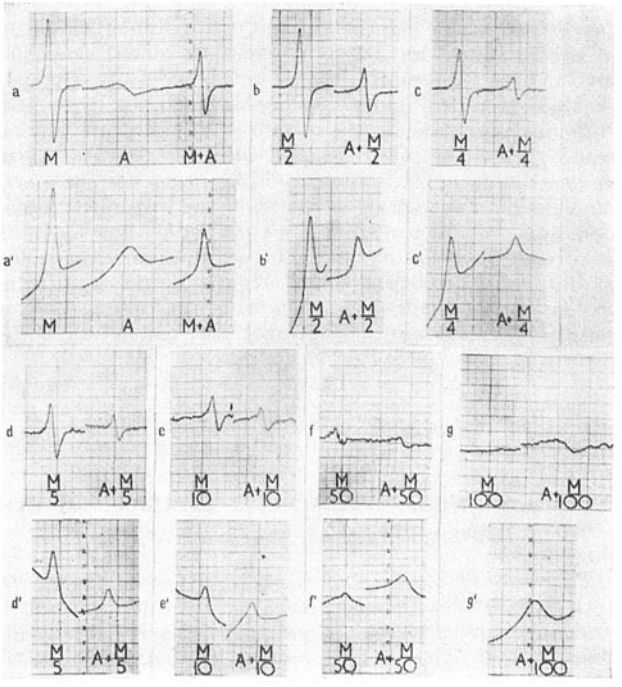

Fig. 1. Free radicals in melanin (M), albumen (A), and melanin albumen mixture (A+M). The concentrations of melanin are indicated on the Figure. Thus half the initial concentration of melanin is shown as M/2. The figures a, b, c, d, etc. are of the differential of the electron spin resonance signal whilst figures a′, b′, c′, d′, etc. are of the corresponding integrals. Full details are given in the text.

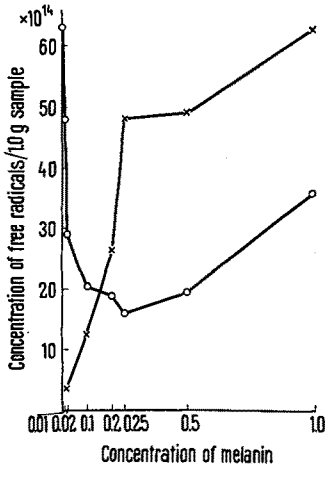

Fig. 2. The effect of melanin on the free radical concentration present in irradiated albumen. The free radical concentration of the melanin controls —x— remains greater than the free radical concentration present in the melanin albumen mixtures —o— down the dilution M/10.

concentrations of M/5, the opposite is true, the concentration in the mixture is smaller. This can only mean that there is some interaction between the melanin and the albumen. The effect was not due to mutual screening since the absorption of the mixture at 366 mμ was equal to the sum of the individual absorptions.

Very dilute concentrations of melanin (M/10–M/100) are unable to restrain the albumen free radical concentration and the free radical concentration increases as the melanin concentration decreases.

It has been suggested by PULLMAN and PULLMAN[1], and also by LONGUET HIGGINS[2], that a copolymer chain of quinonoid units can act as a one-dimensional semiconductor, with the bound protons acting as electron traps. The non-localized empty molecular orbitals that the Pullmans postulate in association with the whole chain, would give exceptional electron acceptor abilities. Such an electron acceptor system could be present in melanin, allowing it to accept electrons from the irradiated albumen. It is possible that such a system could allow melanin to protect proteins against damage due to ultra-violet irradiation. The experimental results described in the present paper suggest that the melanins might play a more active role than that of direct heat and light absorbing agents in biological systems. The melanins could possibly act as electron acceptors and so help control the free radical concentration in the cell. Another possible role of melanins could be the maintenance of a constant rH in the cell[3].

Résumé. L'addition de mélanine au blanc d'œuf frais diminue la concentration des radicaux libres due à la lumière de 366 mμ. Cela permet de supposer que la mélanine agit comme un capteur d'électrons.

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[2] H. C. LONGUET HIGGINS, Arch. Biochem. Biophys. *86*, 231 (1960).
[3] We are indebted to the Medical Research Council for financial aid during these experiments.

Poterium spinosum und sein Einfluss auf den Blutzuckergehalt von Kaninchen

Poterium spinosum (im arabischen Sprachgebrauch auch Schich oder Belan genannt) wird in der arabischen Volksmedizin als Heilmittel gebraucht gegen Erkrankungen, die dem Symptomenkomplex eines *Diabetes mellitus* weitgehend ähneln. Diese Droge und die ihr zugeschriebene diabetesheilende Wirkung erregte in letzter Zeit in der deutschen Tagespresse viel Aufsehen, so dass wir uns auf Grund der zahllosen Anfragen sowie auch aus sozialen Gründen entschlossen, den Einfluss von *Poterium-spinosum*-Zubereitungen auf den Blutzuckergehalt näher zu untersuchen.

Die ersten Versuche wurden mit *Poterium-spinosum*-Dekokten – wie die Beduinen sie anwenden – an Kaninchen durchgeführt: Fein vermahlener Stamm mit Rinde und Wurzel *ana partes*. Die Zubereitungen wurden an nüchterne Kaninchen, die 24 Stunden vor dem Experiment ohne Nahrung belassen wurden, per Schlundsonde verabfolgt. Es wurden folgende Dosen appliziert: Leerwert, 0,33 g/kg, 0,62 g/kg, 5,0 g/kg. Die Blutentnahmen erfolgten in einstündigen Intervallen aus der Marginalvene. Die Bestimmung des Blutzuckergehaltes wurde nach Crecelius-Seifert durchgeführt. Jedes Experiment wurde an 8 Kaninchen durchgeführt.

Der Charakter der einzelnen Kurven im Durchschnitt geht aus Figur 1 hervor, wobei ersichtlich ist, dass zunächst ein Anstieg des Blutzuckergehaltes erfolgt, was auf einen bestimmten Kohlenhydratgehalt der Zubereitungen zurückzuführen ist. Der maximale Abfall der Blutzuckerkonzentration zeigt sich nach 4 h, um dann wieder allmählich anzusteigen, was mit einer spezifischen Wirkung der Droge zu erklären ist.

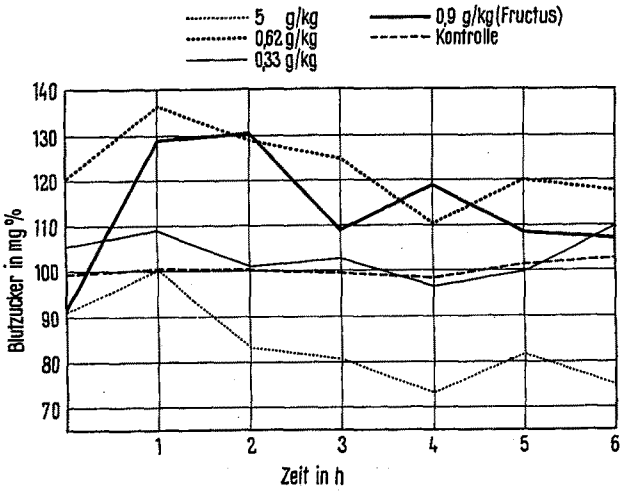

Fig. 1

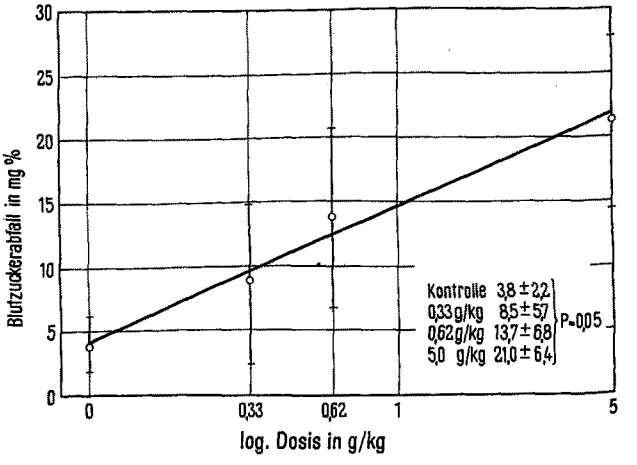

Fig. 2