nisse erweitern die am Gesamtkollektiv erhobene Aussage dahingehend, dass nicht einmal in der Positivität des Korrelationskoeffizienten eine allgemeine Gesetzmässigkeit liegt.

Summary. 169 cats were immunized with heat-killed antigen of *Brucella abortus*. No correlation between the albumin-γ-globulin-quotient and the level of agglutinating antibodies could be demonstrated.

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Hemolysis in Immune Rabbits of Autologous Erythrocytes Modified with Common Enterobacterial Antigen

CEPPELLINI and DE GREGORIO[1] reported that red blood cells modified with Vi antigen undergo rapid lysis in immune, but not in non-immune, rabbits. Subsequent experiments with ^{51}Cr-labeled erythrocytes revealed that hemolysis also occurs when autologous cells modified with enterobacterial O antigen (*S. typhosa*) are injected into rabbits previously immunized with the corresponding O antigen[2]. The degree of hemolysis depends upon the amounts of antigen used for the treatment of erythrocytes and the O antibody titer of the serum. The present study was undertaken to determine whether erythrocytes modified with the recently discovered common enterobacterial antigen undergo lysis in immunized animals. This common antigen was found in numerous species of enteric bacteria, including *Escherichia coli*, *Aerobacter aerogenes*, *Salmonella*, *Shigella*, and *Proteus*[3,4]. This antigen-antibody system has several unusual features. The antibody is elicited by intravenous injection of *E. coli* 014 and a few other serogroups of *E. coli*, but not by numerous other enteric bacteria containing this antigen. For unexplained reasons, too, antibodies neither cause precipitation of soluble common antigen nor bacterial agglutination of many of the above species, but cause specific hemagglutination of erythrocytes modified with the antigen[3,4]. Finally, this antigen-antibody system is not operative in latex agglutination[4].

For immunization and challenge of rabbits (2 to 3 kg), common antigen was obtained from smooth strains of *E. coli* 014 and 0111 and from *S. typhimurium*. The strains were grown on brain veal agar in Kolle flasks for 18 h at 37°C. The organisms were suspended in 25 ml of phosphate hemagglutination buffer (pH 7.3; Difco). The suspension was heated for 1 h in boiling water and centrifuged at $23{,}500 \times g$ for 30 min; the resulting clear supernate was used as antigen. For immunization mixtures of equal volumes of antigen and complete Freund's adjuvant (0.2 ml; Difco) were injected into each of the footpads at weekly intervals for a total of 4 series of injections. This immunization procedure results in the formation of antibody against the common antigen[5].

Titration of antibodies against common antigen was carried out by means of the hemagglutination test, as described previously[4]. Briefly, erythrocytes (2.5% suspension) were washed 3 times with phosphate buffer and treated with a 1:10 dilution of the common antigen. The antigen was obtained from a strain whose O antigen is unrelated to that used for immunization. Serial two-fold dilutions of serum (0.2 ml) were mixed with equal volumes of modified erythrocytes. The mixtures were incubated at 37°C for 30 min, and hemagglutination was read grossly after centrifugation for 2 min at $1350 \times g$. Confirmation of the identity of the antibody was obtained by inhibition of the resulting hemagglutination by antigen from a third strain. For example, serum from rabbits immunized with *E. coli* 0111 were tested with red cells modified with *E. coli* 014 antigen, and the inhibitory antigen was obtained from *S. typhimurium*.

Challenge of immunized and non-immunized rabbits was carried out in the following manner. Heparinized arterial blood (5 ml) was obtained from the ear of the rabbit. The erythrocytes were washed 4 times with 30 ml of phosphate buffer. To the sediment was added common antigen (5 ml) from one of the above microorganisms, and the mixtures were incubated for 30 min at 37°C in a water bath. The red blood cells were again washed 4 times in 30 ml of buffer and resuspended in 5 ml of the same diluent. For control purposes, erythrocytes were treated under the above described conditions with staphylococcal antigen, prepared in the same manner as the enterobacterial antigens; this antigen is unrelated to the common antigen of gram-negative bacteria. Unmodified red blood cells, washed in parallel, were also used. The erythrocyte suspensions were injected into the marginal vein of the ear. Only autologous red blood cells were used in these experiments, and antigens for immunization and challenge were obtained from microorganisms with unrelated O antigens.

After the injection of modified autologous erythrocytes, samples of blood were obtained in capillary tubes 30 min, 1 h, $2^1/_2$ h, and 18 h after challenge. Hematocrit was determined, and the degree of hemolysis of the plasma noted by gross inspection. Urine was collected throughout the observation period on filter paper and tested for presence of hemoglobin with Hematest Reagent tablets (Ames Company, Elkhart, Indiana).

The results of the challenge with modified and non-modified autologous erythrocytes in immune and non-immune rabbits are recorded in the Table. It can be seen that red blood cells modified with common enterobacterial antigen undergo lysis in immune animals, as evidenced by the finding of hemoglobinemia in 100% and of hemoglobinuria in 72% of animals. Hemoglobinemia, at the times tested, was maximal 30 min after challenge and became progressively less during the ensuing 18 h. No difference was noted between animals immunized with common antigen from *E. coli* 014 and challenged with common antigen from either *E. coli* 0111 or *S. typhimurium*,

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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[3]. 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