injection of the mixture of *C. albicans* and L1210 cells. Histopathological study by a pathologist without the knowledge of the experimental design confirmed this finding.

Discussion. This study fails to demonstrate immunodepression in mice bearing L1210 leukemia until the terminal stage of the disease. Our data support the observation of STUTMAN[11] who indicated that immunosuppression had minimal effects on tumor development induced by chemical carcinogens. Using methylcholanthrene or urethan, STUTMAN did not detect significant differences in tumor incidence or latent period of tumor development between the immunologically deficient nude mice and their normal immunologically competent siblings. Recently, GILLETTE and COX[12] showed that T-cell deficiency did not affect the tumor incidence and latency of mice with methylcholanthrene induced primary tumor.

FAUVE et al.[6] reported that the development of murine teratocarcinoma did not impair the cellular immunity of the host against *Listeria monocytogenes*. Their data actually showed an increased resistance against *L. monocytogenes* by the tumor-bearing animals. They further demonstrated that several malignant cells elicited an antiinflammatory response and suggested that this antiinflammatory property might have allowed the tumor to develop in the susceptible host. We show in this study that L1210 cells are also capable of suppressing the inflammatory response. This antiinflammatory reaction may possibly facilitate the rapid growth of the tumor in the absence of immunodepression.

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[12] R. W. GILLETTE and A. COX, Cell. Immun. *19*, 328 (1975).

Staphylococcus epidermidis in the Circulating Blood of Normal and Thrombocytopenic Human Subjects: Immunological Data

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Summary. Immunological studies, performed with human sera and rabbit antisera upon some strains of *Staph. epidermidis* carried in the circulating blood of normal and thrombocytopenic subjects, indicate that the reactivity is very low and almost completely related to antigenic properties common to *Staph. aureus*.

The presence of cell wall deficient (CWD) forms of Gram positive Cocci has been recognized within the platelets of normal human subjects[1]; foetal blood drawn at birth from the umbilical cord showed a comparable situation[2]; patients affected by thrombocytopenia from autoimmune disease carried in addition a relevant number of conventional forms free in the circulation and phagocytized by leucocytes[3]. Following BERGEY[4], such microorganisms may be recognized as belonging to various strains of *Staphylococcus epidermidis*. Here we describe the results of experiments carried out in order to compare some antigenic properties of our isolates with those of a strain of *Staphylococcus aureus*, and also to evaluate the immunological situation of normal and thrombocytopenic human subjects with regard to such microorganisms.

Methods. The sera of 2 autoimmune thrombocytopenic patients and sera from normal subjects were used. Their reactivity was tested against some strains of *Staph. epidermidis* cultured from the blood of normal and thrombocytopenic subjects and against *Staph. aureus* Rose ATCC 14154.

[1] G. G. TEDESCHI, D. AMICI, I. SANTARELLI, M. PAPARELLI and C. VITALI, in *Microbial Ultrastructure* (Technical Series of the Society for Applied Bacteriology), No. 10, p. 325.
[2] G. G. TEDESCHI, D. AMICI and I. SANTARELLI, Experientia *32*, 925 (1976).
[3] G. G. TEDESCHI, D. AMICI and I. SANTARELLI, Experientia *31*, 1088 (1975).
[4] BERGEY'S *Manual of Determinative Bacteriology*, 8th ed. (Williams & Wilkins, Baltimore 1974).

Table I. Human sera: each specimen tested against *Staph. aureus* Rose and some strains of *Staph. epidermidis*

Immunological tests	No. and origin of serum specimens	*Staph. aureus* Rose		*Staph. epidermidis*				
		n of positivities/ *n* of proofs	End point of positive sera	*n* of strains	*n* of positivies/ *n* of proofs		End point of positive sera	
Slide agglutination	2 thrombocytopenic	2/2	160	6	7/12[a]	1/12[b]	5–20[a]	10[b]
	4 normal	4/4	320–1280	6	15/24[a]	5/24[b]	5–20[a]	5–10[b]
Complement fixation	2 thrombocytopenic	none	–	4	none	–	–	–
	32 normal	8/32	50	4	3/128[a]	none[b]	10[a]	none[b]
Indirect agglutination	2 thrombocytopenic	none	–	4	none	–	–	–
	3 normal	3/12[c]	512[c]	4	3/12[c]		3–32[c]	
Indirect immuno fluorescence	2 thrombocytopenic	2/2	5	4	8/8[a]	1/8[b]	5[a]	5[b]
	3 normal	3/3	10[d]	4	12/12[a]	3/12[b, c]	5[a]	5[b]

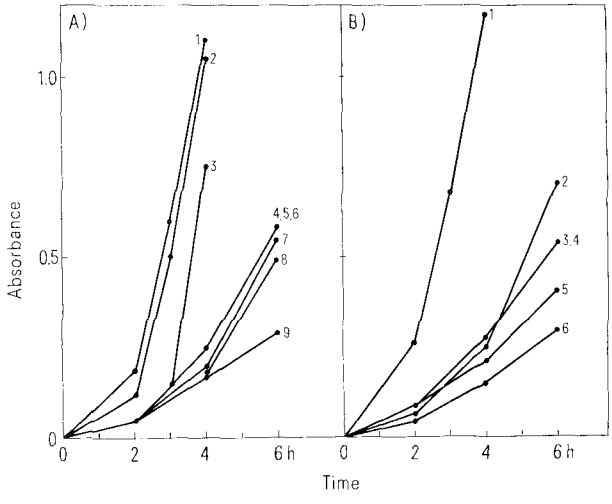
[a, b] Before and after absorption of the sera with *Staph. aureus* Rose. [c] The positive data refer to a single serum specimen showing a strong agglutinating activity towards *Staph. aureus* Rose (titre 1280). [d] Strong positivity, further dilutions not tested.

Table II. Rabbit sera; each specimen tested against *Staph. aureus* Rose and 10 strains of *Staph. epidermidis*

Immunological tests	Number and origin of serum specimens	*Staph. aureus* Rose		*Staph. epidermidis*			
		n of positivites/*n* of proofs	End point of positive sera	*n* of positivites/*n* of proofs		End point of positive sera	
Slide agglutination	4 normal	4/4	320	16/40[a]	none[b]	40[a]	–
	10 immune sera[c]	10/10	160–1280	40/100[a]	8/100[b]	40–640[a]	160–320[b]
Complement fixation	2 normal	2/2	160	not tested[a]	none[b]	–	–
	10 immune sera[c]	10/10	50–160	not tested[a]	12/100[b]	–	50[b]

[a, b] Before and after absorption of the sera with *Staph. aureus* Rose. [c] Against *Staph. epidermidis*.

For the preparation of the antisera, 10 strains of our isolates and *Staph. aureus* Rose ATCC 14154 were employed. These were grown for 18 h on nutrient agar, washed and resuspended in saline. On days 1, 3, 6, 9 and 20 rabbits received i.m. injections at the dose of half the growth of one tube per kg body weight. In some cases the rabbits were simultaneously treated with immunodepressant drugs: Flecocortid Richter (12 mg as hydrocortisone per kg) or Imuran Wellcome (12 mg Azathioprine per kg). Blood was withdrawn on day 30.



Staph. aureus Rose and a strain of *Staph. epidermidis* isolated from the circulating blood of a thrombocytopenic patient. The graphs show the turbidimetric values from the starting point to the end of the logarithmic phase of growth in DIFCO PPLO broth as such or added 1:10 with rabbit and human sera.
A) Rabbit sera. *Staph. aureus* Rose: 1, normal rabbit; 2, rabbit immunized against *Staph. epidermidis* and treated with immunodepressant drugs; 3, rabbit immunized against *Staph. epidermidis*. *Staph. epidermidis:* 4, normal rabbit; 5, as 4 after absorption with *Staph. aureus* Rose; 6, after immunization against *Staph. epidermidis*, administration of immunodepressant drugs and absorption with *Staph. aureus* Rose; 7, after immunization against *Staph. epidermidis* and absorption with *Staph. aureus* Rose; 8, after immunization against *Staph. epidermidis* and treatment with immunodepressant drugs; 9, after immunization against *Staph. epidermidis*. Immune sera against *Staph. epidermidis* impair the growth of the same microorganism as well as of *Staph. aureus* Rose; absorption of the sera with *Staph. aureus* Rose, and/or treatment of the rabbits with immunodepressant drugs, inhibit such activity.
B) Human sera. *Staph. aureus* Rose: 1, broth without serum. *Staph. epidermidis:* 2, broth without serum; 3, with serum from a thrombocytopenic patient; 4, as 3 after absorption with *Staph. aureus* Rose; 5, with normal human serum absorbed with *Staph. aureus* Rose; 6, with normal human serum. Normal human serum impairs the growth of *Staph. epidermidis;* such activity is not completely suppressed following absorption with *Staph. aureus* Rose. The serum from the thrombocytopenic patient does not affect the growth of *Staph. epidermidis* isolated from the blood of the same patient.

All the human sera and the antisera were used as such or after absorption with *Staph. aureus* Rose. The antigens were prepared from bacteria cultured on agar, washed, resuspended in saline and utilized as such or, for complement fixation tests, also sonicated or treated with lysozyme.

The following immunological tests were carried out: slide agglutination; complement fixation: Kolmer's technique; and indirect agglutination for the detection of incomplete antibodies. Bacterial suspensions were sensitized by incubation in the presence of human sera diluted 1:80 with saline; the bacterial cells were washed 4 times with saline and the agglutination test was carried out with serial dilutions of an anti-human-globulin serum (Behringwerke AG) which had previously been incubated with suspensions of the same non-sensitized bacteria: this in order to avoid the possibility of a reactivity of the anti-globulin serum for the bacteria as such.

Indirect immunofluorescence. Bacterial smears were fixed with alcohol and incubated in the presence of serial dilutions of the sera to be tested; after washing, the smears were stained with rabbit anti IgG serum conjugated with FITC.

Bacterial growth in the presence of the sera. This was evaluated by means of turbidimetry (550 nm) in the course of the incubation at 37 °C in shaking flasks containing Difco PPLO broth added with the sera and with 12 h broth cultures of the bacteria to be tested at the concentration of 1:10 and 0.1:10 respectively.

Results and discussion. The results concerning slide agglutination, complement fixation, indirect agglutination and indirect immunofluorescence tests are summarized in Tables I and II. Considered as a whole, the results shown in Table I indicate that: a) in the sera from normal and thrombocytopenic subjects, antibodies reacting with *Staph. aureus* Rose and with our strains of *Staph. epidermidis* were present; b) the sera from thrombocytopenic patients showed a lower reactivity; c) the reactivity towards *Staph. epidermidis* was in every case of very low titre; d) following absorption with *Staph. aureus* Rose the reactivity towards *Staph. epidermidis* disappeared completely or almost completely.

The results shown in Table II indicate that the sera of normal rabbits reacted significantly with *Staph. aureus* Rose, and that, following absorption with this same microorganism, the reactivity towards our isolates disappeared. Immune sera demonstrated highly variable degrees of reactivity: 5 strains of our isolates which gave rise to a significant agglutinating activity (titre 160–320 following absorption with *Staph. aureus* Rose) gave origin

to antisera showing the highest complement fixation titre (50). Such proofs had been programmed in order to thy to serotype our isolates by means of cross reactions, but in view of the low titre remaining in only a few antisera following absorption with *Staph. aureus* Rose, no significant results were obtained.

The results concerning the inhibition of bacterial growth in the presence of rabbit antisera and of human sera are shown in Figure A and B. Microscopical examination demonstrated that the nephelometric variations were related to different rates of multiplication and not to processes of agglutination.

The data obtained indicate that the addition of rabbit immune sera and of human sera to the cultures interferred with the bacterial multiplication. The sera of the rabbits inoculated simultaneously with bacterial suspensions and immunodepressant drugs showed the same behaviour as the sera of normal rabbits. Antisera towards *Staph. epidermidis* inhibited significantly the multiplication of the same microorganism and of *Staph. aureus* Rose: the absorption on *Staph. aureus* Rose impaired the activity towards *Staph. epidermidis*. Such data are in full agreement with the results of the immunological assays previously described. The serum of the thrombocytopenic patient failed to interfere with the multiplication of the strain of *Staph. epidermidis* present within the same patient's blood; normal human serum significantly impaired the growth of this same microorganism: such activity was significantly but not completely depressed following absorption with *Staph. aureus* Rose.

From the results of the present researches the following considerations may be drawn: human subjects carry in the circulation strains of *Staph. epidermidis* with variable properties; the low immunological reactivity sometimes observed in assays carried out with human sera and rabbit antisera appears to be almost completely related to antigenic properties common to *Staph. aureus* (see [5], also for a review of the literature); the possibility may not be excluded that, within the circulating blood, a control of the multiplication of the bacterial forms under consideration derives from a non-specific immunological situation: the fact that the blood specimens from thrombocytopenic subjects showed a very high rate of growth of *Staph. epidermidis* together with a low reactivity towards *Staph. aureus* Rose might support this hypothesis. It appears that the microorganisms here considered, once they enter the host organism, become established in a state of equilibrium. The damage that can arise from the alteration of this equilibrium will be the object of a separate study.

[5] *The Staphylococci* (Ed. J. O. COHEN; John Wiley & Sons, New York 1972).

Lack of Platelet Factor-3 Activation After Incubation of Platelet-rich Plasma with Kaolin in the Rat[1]

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Summary. Stypven times, measured in rat platelet-rich plasma (P. R. P.) after incubation with kaolin, did not shorten as incubation proceeded, thus reflecting the lack of development of platelet factor-3 (PF_3) availability in this test system. Repeated freezing and thawing of P. R. P. or aggregation with collagen did result in PF-3 availability. Aggregation and PF-3 availability were inhibited by the compound VK774. These findings add another aspect to the list of species differences in platelet function.

Platelet factor-3 (PF_3) a lipoprotein fraction of platelets, contributes, by complex formation, to the activation of factor X and of prothrombin in the intrinsic pathway of coagulation.

For human cells, PF-3 can be made available by incubation of platelet-rich plasma (P. R. P.) with kaolin or collagen or by repeated freezing and thawing. PF-3 availability is then reflected by a shortening of the coagulation time of plasma mixed with Russel Viper Venom (Stypven time)[2]. The present paper reports upon aberrant behaviour of rat platelets in the determination of PF-3 availability after incubation with kaolin, as measured with the Stypven time[2].

Material and methods. 1. From rats (Wistar, male, 280–320 g), anaesthetized with pentobarbital 30 mg/kg intraperitoneally and Hypnorm® (Janssen Pharmaceutica) 1 ml/kg subcutaneously, blood was drawn by syringe from the surgically exposed inferior caval vein on citrate 3.8% (1 V/9 V of blood). The preparation of platelet-rich plasma (P. R. P), platelet-poor plasma (P. P. P.) and the counting of platelets in plasma was performed as previously described[3].

2. PF-3 availability after the activation of P. R. P. with kaolin was tested as follows[2]: 0.45 ml of plasma was incubated at 37 °C with 0.05 ml of kaolin suspension (final concentrations 20, 10, 5 mg/ml) or with buffer. Before and 20 min after the addition of kaolin or buffer, 0.1 ml of the mixture was coagulated with 0.2 ml of Stypven/$CaCl_2$. In order to check the reactivity of rat plasma in the Stypven time test, 50 µl of Thrombofax® (Ortho Pharmaceutica) was added to plasma-kaolin mixture in some experiments. Kaolin (Light, B. D. H.) was suspended in barbital-HCl buffer pH 7.4. Stypven (Burroughs, Wellcome and Co) was diluted 1/100,000 in buffer, mixed with an equal volume of $CaCl_2$ 0.05 M, and was kept on crushed ice.

3. PF-3 availability after aggregation induction with rat collagen in the presence or absence of an aggregation inhibitor was tested as follows: 0.7 ml of P. R. P. was incubated for 2 min at 37 °C with 0.1 ml of solvent or inhibitor solution and 0.1 ml of a mixture of $CaCl_2$ (final concentration 1×10^{-3} M) and $MgCl_2$ (final concentration 5×10^{-4} M) before the addition of collagen suspension or buffer. The mixture was continuously stirred in an ag-