Studies in the Pathogenesis of Autoerythrocyte Sensitization Syndrome

By GEORGE S. CROCH, STUART C. FINCH, WILLIAM ROgowAY and DAVID S. FISCHER

The AUTOERYTHROCYTE SENSITIZATION SYNDROME was first described as a distinct clinical entity by Gardner and Diamond in 1955.1 They studied four women who developed purpura, erythema, edema and painful ecchymoses either spontaneously or at sites of minimal trauma on the trunk and extremities. Extensive studies of standard hematologic and coagulation parameters failed to reveal any characteristic abnormality. Skin tests showed that the factor responsible was present in the red cell stroma but not in the hemoglobin, serum or plasma. Since then, several additional cases have been reported,2-4 but there has been no further characterization of the factor in the erythrocyte stroma which is responsible for the cutaneous reactions. All of the patients with this disorder have been women and most of them have had some rather profound emotional problems. This has led to the suggestion that these patients express their emotional problems in a physical form through both hysterical mechanisms and psychophysiological reactions5,6 or as factitious purpura.7

Recently two patients with the characteristic clinical picture of the autoerythrocyte sensitization syndrome were referred for evaluation. We had the opportunity to study one of them in considerable detail. Our primary interest was the possible identification of a biochemical factor in the erythrocyte stroma responsible for development of the cutaneous lesions. Subsequent to the identification of phosphatidyl serine as a provocative factor in the second patient, a third patient was seen for brief evaluation.

Patient #1

E. K., a 41 year-old female, was admitted to the Yale-New Haven Medical Center in October 1962 for investigation of an increased bruising tendency of 9 months duration. In December 1962 she developed a painful nodule in the abdominal wall at the site of a
previous benign ovarian implant. This was surgically incised and only a small amount of hemorrhage was noted. In mid-January she developed some painful hemorrhagic lesions along the course of the veins in both lower extremities and occasionally in the deltoid regions. This was thought to represent thrombophlebitis. She was treated for several weeks with anticoagulants and bed-rest, without improvement. During the next several months she intermittently developed crops of spontaneous painful purpuric spots. These occurred mostly on the legs but not infrequently on any portion of the trunk, arms, face or scalp. She stated that each new lesion was red, swollen and painful during the first 6–12 hours. During the next 12–24 hours the pain and swelling continued as each lesion developed a “black and blue” discoloration. After the first 24–48 hours the pain generally subsided but the discoloration lasted for 7–10 days. In July 1962 she was skin-tested with her own red cells and saline. There was no local response to the saline but at the blood injection site the complete cycle of one of her purpuric lesions was reproduced. Several weeks of therapy with 40–60 mg. prednisone daily did not alter her clinical course.

At the age of 23 the patient was divorced from her first husband. She remarried and subsequently had two children. Her 11-year-old son was premature and suffered severe visual impairment due to retrolental fibroplasia. She had 3 miscarriages. There was a total of 9 hospital admissions between the years 1951 and 1962. The first 4 admissions were for various gynecologic problems and each resulted in some form of gynecologic surgery. Two admissions in 1961 were for gastroenteritis and another in 1961 was for laryngitis and some symptoms suggestive of Sjögren’s Disease. Later in 1961 she developed some rather bizarre neurologic symptoms. Several neurologists felt that this represented multiple sclerosis while several others believed that the entire picture was factitious in origin.

The patient was a thin, tense female with gross tremor of her arms and a slow scanning type of speech. She was alert and well oriented. Several moderately tender, slightly raised purpuric spots were present on the arms and thighs. Several similar lesions in the scalp were exquisitely tender. The conjunctivae, ocular fundi and mucous membranes were negative for evidence of increased bleeding. The remainder of the physical examination was unremarkable. There was no nystagmus and the deep tendon reflexes were equal and active. No pathologic reflexes were elicited.

Routine laboratory studies were unremarkable. Bleeding time, clotting time, platelet count, prothrombin time and partial thromboplastin times all were within normal limits. A PPD skin test was strongly positive. A characteristic purpuric lesion developed on the forearm following the intradermal injection of 0.1 ml. of a 60 per cent concentration of her own erythrocytes. The saline control was negative.

It was possible only to complete limited skin testing on patient E. K. Shortly after discharge from the hospital she moved to another city. Subsequently she was divorced and has been lost to follow-up.

Patient #2

J. S., a 46-year-old, unmarried white female secondary school teacher, was first seen in July 1963 for determination of the cause of her skin hemorrhages.

At the age of 20 (1937) the patient experienced an ice skating injury to her left knee which bled profusely. The following year a semilunar cartilage was removed from the left knee. At the age of 22 (1939) she suffered a fall and the apparently healed surgical site opened. Hospital records substantiate the fact that this continued to bleed intermittently for the next 8 years despite 3 separate surgical procedures designed to close the wound, including autologous skin grafts. There was never any evidence of underlying osteomyelitis. An admission note at Memorial Hospital (N. Y.) in 1945 (6 years later) described “a 2-inch ulceration draining bright red blood over the left patellar region.” During this period the patient was also under the care of a hematologist who felt that she had a defect in her clotting mechanism of unknown type. Multiple hematologic studies failed to reveal any abnormalities. The wound gradually improved and was healed by 1947. In 1956 a hysterectomy was performed for myomata of the uterus.
THE AUTOERYTHROCYTE SENSITIZATION SYNDROME

She remained well until approximately 2 years prior to admission, when she awakened one night with a large painful ecchymosis on one of her arms. Subsequently she experienced multiple painful ecchymoses limited to the upper and lower extremities and rarely the face. A stinging or burning sensation often was felt at the site of a skin hemorrhage from 12 to 24 hours prior to the development of any visible skin involvement. Usually some pain persisted as the lesion evolved. The ecchymoses recurred in crops at 2–3 week intervals without relation to trauma, activity, diet or previous menstrual pattern. Each skin lesion began as a 1–2 cm. area of erythema which rapidly became indurated and slightly swollen. Within 18–24 hours a hemorrhagic border developed. This spread both outward and toward the center of the lesion so as to produce an ecchymosis, usually from 4 to 6 cm. in diameter, which gradually underwent resolution in 5 to 7 days. Occasionally, individually ecchymotic areas were from 10–15 cm. in diameter. There was no history of other systemic signs or symptoms except for a feeling of malaise for 1–2 days at the peak of the ecchymotic phase.

Shortly after the onset of the disorder the patient underwent extensive studies of her coagulation mechanism. There was no evidence of any clotting abnormality. Studies also were negative for increased fibrinolytic activity, for the presence of a circulating anticoagulant, for lupus erythematosus or rheumatoid arthritis; all other laboratory studies were unremarkable, including a serum electrophoresis.

Physical examination revealed a moderately obese, pleasant-appearing white female in neither acute nor chronic distress. There were eight discrete reddish blue areas on the forearms and legs. Some of these lesions were slightly tender to palpation. Except for the old scars about her left knee the remainder of the examination was within normal limits.

The subsequent clinical course was characterized by the intermittent appearance of spontaneous, painful purpuric skin lesions on the forearms, legs, anterior chest and rarely the face. The lesions never appeared on her back. They generally occurred in crops over a period of several days, often not developing again for several months. During 1963 and 1964 the patient was admitted several times to the Clinical Research Center for skin testing and other studies. The clinical course was not altered with the daily administration of 40 mg. of prednisone for several weeks. Autologous erythrocyte desensitization over a period of several months did not alter her course. Chloroquine therapy was attempted but the patient was intolerant of this drug. Considerable improvement has been experienced, however, following administration of trepelenamine (25 mg. q.i.d.). At the present time she is having minimal difficulty with purpura while taking this antihistamine.

Patient #3

A. W. was a 58-year-old white female with a history of spontaneous, recurrent painful purpuric skin lesions of 7 years duration. Onset followed injury to her right leg with subsequent venous thrombosis. The patient stated that most, but not all, skin lesions were preceded by a local burning or tingling sensation. Each local lesion initially was red, swollen and painful and most, but not all, then became purpuric. Skin lesions frequently occurred on the arms and legs, rarely on the abdomen and never on the face, scalp, chest or back.

Physical findings were unremarkable except for the presence of well-circumscribed, red, swollen and tender areas on the left forearm, right patella, calf and popliteal areas. There was slight left upper quadrant abdominal tenderness. Moderate osteoarthritic changes of the terminal interphalangeal joints were present.

All routine laboratory studies, as well as the platelet count, prothrombin time and partial thromboplastin time, were within normal limits.

MATERIALS AND METHODS

Patient #1, E. K., was available for limited testing of her skin reactivity. Intradermal skin tests at 6 separate sites on the posterior thorax were performed with 0.1 ml. of either 60 per cent or 80 per cent autologous erythrocytes in saline, mixed with 1.2 mg. of
hydrocortisone, or mixed with 1.2 mg. of diphenhydramine hydrochloride. Each injected area was examined at intervals of several hours during the next 24 hours.

Patient #2, J. S., was studied intensively with a great variety of substances during 4 separate periods of hospitalization. The substances injected and their amounts are shown in Table 1.

The initial testing procedure for patient J. S. involved an evaluation of her skin reactivity to various components of her own red cells. Later red cells from a patient with polycythemia vera who was known to be free of viral hepatitis were used. The total lipids were extracted from thrice saline-washed erythrocytes with diethyl ether or by the chloroform-methanol technic of Farquhar. An erythrocyte stromal fraction was prepared from thrice saline-washed red cells lysed osmotically with 4 volumes of distilled water and separated by centrifugation. The supernatant was dialyzed, concentrated and used as a "hemoglobin solution." The stromal fraction was extracted with lipid solvents as detailed above, and the insoluble residue was used as the stromal protein fraction (elmin).

The phosphatide and neutral lipid fractions were prepared from the erythrocyte total lipid fractions by the silicic acid-silicate-water column chromatograph technic of Rouser et al. Separation of phosphatidyl serine and phosphatidyl ethanolamine was accomplished on silicic acid columns with chloroform-methanol mixtures. Purity of the phosphatide fractions was established by thin-layer chromatography on silicic acid with the chloroform-methanol-ammonia solvent of Skipski et al. and verified by the chloroform-methanol-glacial acetic acid-water technic of Skipski et al.

Lysophosphatidyl serine and lysophosphatidyl ethanolamine were prepared by the method of Rouser et al., using crotalus adamanteus venom. Purity of the lysophosphatides was verified by thin-layer chromatography on sodium acetate-silicic acid plates.

Platelets from a patient with polycythemia vera were prepared by differential centrifugation. They were either resuspended directly or their total lipids were extracted with diethyl ether or chloroform-methanol (4:1). Both preparations were used for intradermal skin testing.

The following materials were obtained from outside sources: phosphatidyl ethanolamine, lot #2845, and lecithin (vegetable), lot #1501, from Nutritional Biochemical Corp.; phosphatidyl serine, lot #G1838, K1614 and L1332, stearic acid, lot #G2423, and palmitic acid, lot #3050, from Mann Research Laboratories; L-α-cerophalin (β, γ dipalmitoyl-L-phosphatidyl ethanolamine), lot #5313-1330, from Sigma Chemical Company; L-serine, Lot #5650. and α-phospho-L-serine, lot #5253. and deoxyribonucleic acid (highly polymerized. salmon sperm origin), lot #30193. from California Corp. for Biochemical Research; inositol phosphatide. lot #P16-3. from Corn Products Refining Company; phosphatidyl-L-serine (extract of beef brain, Folch Fraction III), lot #9296. from Light and Company, Ltd.; oleic acid. lot #1-A, arachidonic acid. lot #4-A, methyl oleate. lot #1-M. and methyl arachidonate. lot #4-M. from the Hormel Institute; ribonucleic acid (yeast), lot #6111. from Worthington Biochemical Corp.; crotalus adamanteus venom from Ross Allen's Reptile Institute; and PPD, intermediate strength. lot #095152 D, from Parke. Davis and Company.

Synthetic γ-oleic, β-palmitic, L-α-phosphatidyl-D, L-serine was prepared by Prof. L. L. M. van Deenen, University of Utrecht. The Netherlands; calf thymus deoxyribonucleic acid was prepared by Dr. Charles E. Carter, Western Reserve University School of Medicine; the phosphatide fraction of PPD of human tuberculosis bacilli came from the laboratory of Drs. D. S. P. Patterson and M. I. W. Lesslie, Central Veterinary Laboratory, New Haw, Weybridge, Surrey, England.

All test substances for J. S. were prepared by aseptic techniques when possible and all but the phosphatidyl serine were suspended in 0.15 M sterile saline. Serial dilutions of the phosphatidyl serine were made in distilled water. The phosphatidyl serine was dispersed with a Bronson Instruments sonifier, model LS-75-5. Hypertonic saline then was added to isotonicity with minimal clouding. Chemicals in aqueous (saline) solution were sterilized by
Table 1.—Results of Skin Test in Patient #2

<table>
<thead>
<tr>
<th>Material</th>
<th>Amount*</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>0.1 ml.</td>
<td>0</td>
</tr>
<tr>
<td>Salmon sperm DNA</td>
<td>4.0 μg.</td>
<td>0</td>
</tr>
<tr>
<td>Calf thymus DNA</td>
<td>40.0 μg.</td>
<td>0</td>
</tr>
<tr>
<td>Inositol phosphatide</td>
<td>500.0 μg.</td>
<td>0</td>
</tr>
<tr>
<td>RNA (yeast)</td>
<td>5.0 mg.</td>
<td>0</td>
</tr>
<tr>
<td>Procain</td>
<td>0.2 mg.</td>
<td>0</td>
</tr>
<tr>
<td>Histamine</td>
<td>0.27 mg.</td>
<td>0</td>
</tr>
<tr>
<td>Isologous platelets</td>
<td>0.1 ml.</td>
<td>+</td>
</tr>
<tr>
<td>Isologous platelet lipid</td>
<td>0.1 ml.</td>
<td>+</td>
</tr>
<tr>
<td>PPD (intermediate)</td>
<td>0.1 ml.</td>
<td>+</td>
</tr>
<tr>
<td>Phosphatidyl ethanolamine</td>
<td>200 μg.</td>
<td>0</td>
</tr>
<tr>
<td>Phosphatidyl choline</td>
<td>200 μg.</td>
<td>0</td>
</tr>
<tr>
<td>Phosphatidyl serine</td>
<td>200 μg.</td>
<td>+</td>
</tr>
<tr>
<td>Phosphatidyl serine</td>
<td>25 μg.</td>
<td>+</td>
</tr>
<tr>
<td>Phosphatidyl serine</td>
<td>12.5 μg.</td>
<td>0</td>
</tr>
<tr>
<td>L-serine</td>
<td>500 μg.</td>
<td>0</td>
</tr>
<tr>
<td>O-phosphoserine</td>
<td>500 μg.</td>
<td>0</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>200 μg.</td>
<td>?</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>200 μg.</td>
<td>?</td>
</tr>
<tr>
<td>Methyl oleate</td>
<td>200 μg.</td>
<td>0</td>
</tr>
<tr>
<td>Methyl arachidonate</td>
<td>200 μg.</td>
<td>0</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>200 μg.</td>
<td>0</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>200 μg.</td>
<td>0</td>
</tr>
<tr>
<td>Lysophosphatidyl serine</td>
<td>200 μg.</td>
<td>0</td>
</tr>
<tr>
<td>Glycerylphosphoryl serine</td>
<td>200 μg.</td>
<td>0</td>
</tr>
<tr>
<td>Autologous white cell suspension</td>
<td>0.1 ml.</td>
<td>0</td>
</tr>
</tbody>
</table>

*In all instances the volume used for skin testing was 0.1 ml.

All test materials were prepared in the laboratory and placed in sterile, disposable, 1 ml coded syringes. The arms and forearms were used for intradermal skin testing except where otherwise noted. All injections in patient J. S. were performed by a physician who was not aware of the code until the reactions were evaluated and recorded.

Patient #3, A. W., was skin-tested on her forearms with autologous erythrocytes, saline, α-cephalin, DNA, RNA, phosphatidyl serine, phosphatidyl ethanolamine and PPD. The amounts injected were the same as were injected into patient J. S. A double-blind procedure also was used.

RESULTS

Patient #1, E. K., developed the characteristic lesions of autoerythrocyte purpura at all injection sites on her extremities and posterior thorax. Several hours after initiation of the skin test she noted pain and swelling at each injection site. The lesions were quite painful during the night and she slept with considerable discomfort. By morning each lesion was very swollen and was surrounded by purpuric discoloration. There was no evidence that either the antihistamine or hydrocortisone was effective in modifying the extent of the lesions. The lesions were swollen and painful for several days. The purpuric discoloration persisted for about one week.

Table 1 summarizes the reactions of patient J. S. to the test substances. It
was demonstrated repeatedly that her skin reacted locally to the intradermal injection of either autologous thrice-washed red blood cells in concentrations of 30 per cent or greater or washed red cell stroma. No reaction was elicited by the intradermal injection of serum, plasma, washed white cells, a hemoglobin solution, normal saline, histamine or procaine.

Following the fractionation procedure outlined in Figure 1, it was discovered that patient J. S. consistently reacted to a lipid extract of her own red cell stroma or those of 2 different donors. The "lipid free" stromal residue (elinin) either failed to produce a reaction or gave a much slower abortive type of reaction which did not progress to an ecchymotic stage. The "neutral fat" fraction derived from human stromal lipid gave a negative reaction. The residual cephalin fraction was separated into phosphatidyl ethanolamine and phosphatidyl L-serine. The former gave no reaction while the latter, phosphatidyl L-serine, reproduced the patient’s own lesions and those induced with either whole red cells or red cell stroma. Extensive double-blind testing with 4 different lots of the potassium salt of phosphatidyl L-serine from 2 different commercial sources always resulted in the development of a typical lesion. The skin tests with commercial phosphatidyl ethanolamine and phosphatidyl choline (lecithin) were consistently negative. Although the commercial phospholipids were crude by comparison with the chromatographically prepared materials, there were no ambiguous reactions. Skin reactions to injections of some of these test substances are shown in Figure 2. In the range of 25 µg. to 500 µg, a crude dose response relationship was obtained with phosphatidyl L-serine.

The various component parts and hydrolysis products of phosphatidyl L-serine such as lysophosphatidyl L-serine, glycerolphosphorylserine, phosphoserine, L-serine, palmitic and stearic acids all gave a negative response when injected intradermally in patient J. S. (Fig. 3). Oleic and arachidonic acids, as well as their sodium salts, produced a local inflammatory reaction (present also in control subjects) which later became moderately ecchymotic. The methyl esters of oleic and arachidonic acids produced no reactions. The specificity of the reaction for the potassium salt of phosphatidyl L-serine was further borne out by the fact that the patient failed to react on 2 separate occasions to a synthetically produced phosphatidyl L-serine which differed from the natural variety only in that the unsaturated fatty acids were in the gamma rather than beta positions.

Thirteen additional female subjects ranging in age from 17 to 60 have been injected with the same preparations of phosphatidyl serine. Six of the 13 had been referred for investigation of purpura. None, however, reacted to local injections of autologous erythrocytes and therefore could not be characterized as having the autoerythrocyte purpura of Gardner and Diamond. The other 7 women were healthy young female subjects. None of the 13 women developed any objective local reaction following intradermal injection of amounts of the same phosphatidyl serine which consistently produced local reactions in patient J. S. Apart from the pain of the needle, none complained of burning or any other unusual subjective sensation at the sites of injection.

Following the intradermal administration of phosphatidyl serine at several separate sites in one forearm of patient J. S., the developing lesions were
Fig. 1.—Schematic representation of the erythrocyte fractionation scheme and the cutaneous purpuric response of patient #2, J. S., to injection of the fractions.

Fig. 2.—Local cutaneous response of forearm of patient #2, J. S., after intracutaneous injections of various test substances. Picture on left was taken at 18 hours showing reaction at F (autologous erythrocyte lipid fraction) and H (commercial phosphatidyl serine). Injections of phosphatidyl ethanolamine and DNA on the same forearm were negative. Picture on right shows positive reactions to autologous erythrocyte lipids and phosphatidyl serine at 72 hours.
Fig. 3.—Schematic structural formula for phosphatidyl serine. Each of its major components tested in patient #2, J. S., is underlined and the amount administered in micrograms is shown on the right.

Fig. 4a.—See figure legend on facing page.
biopsied 3, 12, and 18 hours later. The 3-hour biopsy section showed a moderate number of perivascular polymorphonuclear leukocytes in the upper corium. There was some associated "nuclear dust" but no evidence of necrosis. Endothelial cell swelling was questionable. At 12 hours, there was a massive increase in the number of perivascular polymorphonuclear cells and, in addition, these cells diffusely infiltrated the upper dermis, giving the appearance of a cellulitis. Endothelial cells and the surrounding connective tissue were considerably swollen. PAS stain showed focal areas of capillary wall fibrinoid necrosis along with some extravasation of fibrin-like material. The overall appearance was somewhat reminiscent of that of an acute allergic vascular purpura (Fig. 4). At 18 hours some capillary endothelial cell swelling persisted but the overall changes were much less impressive. The inflammatory reaction was most marked about 12 hours postinjection. Biopsies of the injection site in 3 control female subjects 12 hours after intradermal injection of the same lot of phosphatidyl serine showed only slight perivascular cuffing.

Fig. 4b

Fig. 4.—Biopsy of skin lesion from patient #2, J. S., at 12 hours following the intradermal injection of 100 μg. of phosphatidyl serine. (a) Low-power H & E stain showing acute inflammatory reaction, and (b) high-power PAS stain showing perivascular polymorphonuclear infiltration and fibrinoid necrosis of blood vessel walls.
Patient J. S. showed no local response to injection of calf thymus DNA, salmon sperm DNA, yeast RNA, or 100 μg of the phosphatide fraction of Weybridge PPD. However, she had an ecchymotic reaction to intermediate strength PPD on the forearm and back.

Patient #3, A. W., developed tender, swollen ecchymotic local areas on her forearms in response to the intracutaneous injections of autologous erythrocytes and intermediate strength PPD. Local pain and swelling occurred 4-6 hours postinjection. Maximum pain and swelling were noted at about 24 hours. In the course of the next few days all pain and swelling disappeared. Response was definitely negative to phosphatidyl serine, α-cephalin, phosphatidyl ethanolamine and DNA.

Discussion

The diagnosis of autoerythrocyte purpura in patient J. S. was well established. There were many features of her clinical history that were quite characteristic. Initial occurrence of purpura was preceded by several injuries and operations on the left knee associated with prolonged bleeding and poor wound healing. Her only other surgical operation was a hysterectomy. The purpuric lesions spontaneously occurred in crops on the face, trunk and extremities but not on the back. A local sensation of tingling or burning not infrequently preceded by several hours the actual development of a skin lesion. Virtually all of her lesions were unrelated to trauma. Each underwent a rather characteristic acute painful erythematous phase for 18-24 hours and then became purpuric and indolent for the next 5-7 days.

The diagnosis of autoerythrocyte purpura in the other two patients, E. K. and A. W., also was well documented. The clinical manifestations of each were somewhat different but both patients developed characteristic lesions in response to the intradermal injection of red cells but not saline. The syndrome developed in patient E. K. after 4 gynecologic operations, several episodes of functional illness and a long period of emotional distress. Her purpuric lesion frequently occurred in the scalp and on her back, in contrast to patients J. S. and A. W. who never had back or scalp lesions. It is of interest that A. W. first developed symptoms of autoerythrocyte purpura shortly after an injury to her leg and an episode of thrombophlebitis. Patient A. W. was a highly emotional woman with many somatic complaints. She had had two previous abdominal surgical operations.

Agle and Ratnoff have clearly emphasized many of the clinical features of the autoerythrocyte sensitization syndrome as demonstrated by their patients. All were women with onset between the ages of 19 and 51. Not infrequently the syndrome developed after operations, mostly related to the organs of reproduction. Often there was a history of rather prolonged bleeding episodes of one sort or another prior to the onset of purpura. The lesions usually occurred on the extremities, face and scalp and were much less likely to occur on the back. One of the most characteristic features emphasized by Agle and Ratnoff was the premonition that many patients have that they are about to develop a cutaneous purpuric lesion. Not infrequently, this was in the form of
a tingling, burning or stinging sensation at the precise spot where a lesion
developed several hours later.

McDuffie and McGuire\(^7\) evaluated the psychologic features by adminis-
tering the Minnesota Multiphasic Personality Inventory to 6 women aged 17 to 38
with characteristic purpuric skin lesions and abdominal pain. They found a
remarkably uniform personality pattern characterized by difficulty in handling
aggressive feelings, alteration between repression and hysterical acting out, a
superficial charm masking a demanding and selfish nature, lack of insight, and
resistance to psychotherapy.

The pathogenesis of the autoerythrocyte syndrome remains unclear. Gardner
and Diamond's patients developed the skin lesions only in response to injec-
tions of red cells or red cell stroma and not to a host of other substances.\(^1\) The
other materials injected included buffy coat, plasma and saline. Most other
authors have found similar results.\(^2,4\) Agle and Ratnoff have noted rather
nonspecific reactivity of the skin to injections of saline in some of their pa-
tients. The responses in some of their patients were modified by suggestion.
This peculiar pattern of reactivity occurring in women with a rather stereo-
typed altered emotional background has led them to feel that the emotional
factors are most important determinants in the development of symptoms. It is
their impression that the threshold of local reactivity is modified by the re-
sponse of the local tissue to autonomic stimuli. Other recurrent painful pur-
puric syndromes, possibly due to a variety of causes, have been described in
women. Lesions somewhat similar to those of autoerythrocyte purpura have
been reported in 3 women who have been found sensitive to DNA\(^18,20\) and in
1 woman who was sensitive to histamine.\(^21\)

We have found a negative skin test to red cells on a number of women with
quite characteristic histories for their disorders. Self-traumatization has been
the cause of the purpura in several other patients that we have observed. In
order to avoid confusion we have restricted our observations only to those
patients who react locally to autologous erythrocytes.

Following the lead of Gardner and Diamond, our studies were concerned
with determining what portion of the red cell membrane was responsible for
eliciting the local purpuric reaction. The only component of the erythrocyte
membrane consistently capable of this reaction in the skin of patient J. S. was
phosphatidyl serine. The specificity of response to phosphatidyl serine was
most remarkable. Not only did she invariably respond to this material but in
no instance did she respond to other phospholipids, any of the individual
components of phosphatidyl serine or any of its hydrolysis products. The
response to PPD is not completely understood but it is possible that this also
represented a reaction to a phospholipid, possibly phosphatidyl serine, since
PPD has been shown to contain considerable phospholipid.\(^22,23\)

In these studies, particular effort was made to eliminate possibilities of
suggestion, trauma or bias in the development of lesions. In each instance the
occurrence of progressive induration and erythema culminating with a transit-
ion into a homogeneous, smoothly expanding area of purpura while under
close observation in a research unit would suggest that these lesions were not
self-induced. Furthermore, patient J. S. was subjected to repeated double-blind skin tests sometimes between 10 and 20 in number at any one session. Except for the color of the whole red cells, all other suspensions or solutions imparted no discoloration at the injection site. The red cell stroma and all of the extracts thereof were resuspended in physiologic saline, making any sensory identification of the test substances highly unlikely. In the late stages of the investigation when the patient was being tested with commercial chemical substances, a number of these did impart stinging or painful sensation, but they were not necessarily associated with a positive response. However, injections of oleic and arachidonic acids and their sodium salts, but not their methyl esters, caused burning and purpuric lesions without erythema or induration in patient J. S. The same offending materials caused burning and erythema, but no purpura, in control subjects. We believe that the lesions in J. S. were mediated by local bleeding, since similar atypical lesions were produced by injecting sterile water. The 13 control subjects did not report unusual or unpleasant sensation at the site of administration of the commercial phosphatidyl serine.

The possibility of self-trauma in the etiology of this disorder cannot be stressed too greatly. In a well-studied case by Gottleib et al., the patient was placed in a cast to preclude self-trauma, and it was demonstrated that she continued to develop lesions beneath the cast.4 In another patient who apparently reacted to one injection of autologous red cells but was suspected of self-traumatization, no lesions were found beneath the cast of two of her extremities after 10 days.7 Prausnitz-Küstner (P-K) tests were not carried out in our patients. In patient J. S., however, the skin of the posterior thorax was injected at one site with 0.5 ml. of autologous buffy coat and at another site with 0.5 ml. of autologous plasma. Both sites then were injected with 100 µg. of phosphatidyl serine. Shortly thereafter, erythema and pruritus developed, but no ecchymosis. Gottlieb et al. attempted two classic P-K tests and both were negative.4

The majority of reported patients with autoerythrocyte purpura have not developed lesions of the posterior thorax and the most prevalent site in all cases has been the extremities. One of our three patients was affected on the posterior thorax. In the patient reported by Gottlieb et al.,4 lesions could be induced on the back. In one of Gardner and Diamond's1 patients, after prolonged skin testing it became possible to induce lesions on the posterior thorax. This same type of distribution was noted in the DNA-sensitive patients. The work of Schwartz, Lewis and Dameshek19 clearly shows that the skin sensitivity is indeed generalized but that apparently localized factors, possibly vascular supply, have some bearing on the site of manifestation of the disorder.

Therapy of this disorder up to the present time has been most unsatisfactory. Administration of corticosteroids, antihistamines, antimalarials, and attempts at erythrocyte desensitization have been quite unsuccessful. One of our patients, J. S., has experienced some improvement on trepellenamine. The simultaneous test administration of red cells with either hydrocortisone or antihistamine did not prevent development of the lesions in patient E. K. Unfortunately, there was no opportunity to study the effects of therapy in this patient.
THE AUTOERYTHROCYTE SENSITIZATION SYNDROME

Table 2.—Summary of Previous Reports of Autosensitization Purpura

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Case No.</th>
<th>Age</th>
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<th>Reaction to Erythrocyte Stroma or Lysate</th>
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<td>Gardner and Diamond¹</td>
<td>1955</td>
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<td>4</td>
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<td>Gottlieb et al.⁴</td>
<td>1957</td>
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<td></td>
<td></td>
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<td>Lesions developed under plaster cast</td>
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<tr>
<td>Reed and Firkin²</td>
<td>1957</td>
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<td></td>
<td></td>
<td></td>
<td>Fibrinolysin demonstrated</td>
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<tr>
<td>Henstell and Kligerman¹</td>
<td>1957</td>
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<td></td>
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<tr>
<td>Shulman et al.²¹</td>
<td>1959</td>
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<td>Sensitive to histamine</td>
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<tr>
<td>Levin and Pincus¹⁸</td>
<td>1961</td>
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<td>DNA, buffy coat, O.T., lysed leukocytes all positive</td>
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<td>Schwartz, Lewis and Dameshek¹⁰</td>
<td>1962</td>
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<td>DNA, leukocytes, PPD all positive</td>
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<td>Agle and Ratnoff⁵,⁶</td>
<td>1962</td>
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<td>Little and Bell²⁰</td>
<td>1964</td>
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<td>DNA, lysed leukocytes positive, PPD negative</td>
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<td>McDuffie and McGuire¹⁷</td>
<td>1965</td>
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Compiled from published data: + = positive; 0 = negative; − = not tested; and ? = not stated in publication.

*Three cases had positive reaction to saline, but not individually identified. Presumably all the patients had a positive reaction to erythrocytes, but this is not explicitly stated.

In our opinion, the autoerythrocyte purpura syndrome probably represents only one aspect of a wide spectrum of disorders. These include factitious purpura, purpura possibly of purely psychic origin, DNA sensitivity purpura, and red cell membrane sensitivity purpura. There may be a continuous blending of these disorders. None, however, should be classified as truly autoerythrocyte purpura unless sensitivity to the red cell or red cell membrane is shown. Perhaps the entire group should be designated autosensitization purpura (Table 2). Our studies suggest that the phosphatidyl serine of the red cell...
membrane may play a role in the pathogenesis of the development of the skin lesions in at least one patient with autoerythrocyte purpura. It does seem possible that foci of increased vascular permeability to blood may develop for a variety of reasons. In response to the extravascular extravasation of red cells, a form of local allergic vasculitis may develop resulting in the development of a painful purpuric lesion. Vascular permeability factors and tissue enzymes may be of some importance.

At the present time one must consider the etiology of these disorders and their interrelationships to be in doubt. It is possible that skin testing is not specific and is not related to the pathogenesis of individual lesions. By analogy the relationship between the lupus phenomenon and the pathogenesis of disseminated lupus erythematosus or the presence of antithyroid antibodies in thyroid disease is not known. Although the local purpuric response to phosphatidyl serine is a highly reproducible phenomenon in our patient, it should be clear that we have no real understanding of its significance and can only speculate on its meaning.

**Summary**

1. Two of three female patients with the autoerythrocyte syndrome were tested for sensitivity to various components of the erythrocyte membrane. One was consistently sensitive to purified phosphatidyl serine.

2. It is suggested that in at least one patient with autoerythrocyte purpura the phosphatidyl serine of the red cell membrane may play a role in the pathogenesis of the purpuric lesions.

3. It is likely that a broad spectrum of autosensitivity-type purpuric disorders exists in women with complex emotional problems. The threshold of reactivity may be modified by the psyche.

**Summario in Interlingua**

1. Duo de tres patientes feminin con le syndrome de sensibilitate pro erythrocytos autologe esseva testate con respecto a lor sensibilitate pro varie componentes del membrana erythrocytic. Un del tres esseva uniformemente sensibile pro purificate serina phosphatidylic.

2. Es postulate que in al minus un del tres patientes, le serina phosphatidylic del membrana erythrocytic ha un rolo in le pathogenese del lesions purpuric.

3. Il es probable que un large spectro de disordines purpuric del typo autosensibile existe in feminas con complexe problemas emotional. Le limine del reactivitate es possibilemente modificate per le psyche.

**ACKNOWLEDGMENTS**

We would like to extend our thanks to Dr. Charles E. Carter, previously of the Department of Pharmacology, Yale University School of Medicine, and now at Western Reserve University School of Medicine, for the use of his laboratory and for the calf thymus deoxyribonucleic acid; to Dr. Peter Hukill for interpretation of the biopsies; to Drs. Irwin Braverman and Steven Malawista for performing the skin biopsies; to Dr. Frank Gardner of Harvard Medical School for referring patient A. W.; to Dr. Aaron Marcus of Cornell University Medical College for advice on lipid fractionation; to Drs. D. S. P. Patterson and M. I. W. Lesslie of Central Veterinary Laboratory, Weybridge, England, for the phosphatide fraction of P.F.D. of human tuberculosis bacilli; to Professor L. L. M. van...
Dennen of the University of Utrecht, The Netherlands, for synthetic γ-Oleic, β-palmitic, L-α-phosphatidyl D, L-serine; and to the patients themselves for their fortitude in permitting such extensive testing.

REFERENCES


Studies in the Pathogenesis of Autoerythrocyte Sensitization Syndrome

GEORGE S. GROCH, Captain, STUART C. FINCH, WILLIAM ROGOWAY and DAVID S. FISCHER

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