TABLE II. Reactivity of Human Hemoglobin After Chemical Treatment.

Treatment	Ratio of reactivity with human serum* (%)
Hemoglobin + .02 M NaCN + .2 M potassium ferricyanide	0
" + .5 M potassium ferricyanide	77.5
" + .5 M NaCN	100
" + .5 M sodium azide	100
" + .1 M β-mercaptoethanol	100
" (2.0 cc) + carbon monoxide (500 cc, research grade, room temp & pressure) †	100
" + .15 M NaCl (control)	100

Adult hemoglobin, 25 mg/ml, (95% Hgb A) was used. Samples were reacted at room temperature for one hour, then dialyzed against 3 changes of 0.15 M NaCl, 18 hr at 4°C before testing. Tests were made in "buffered agar" containing 0.5 mg/ml of the treated hemoglobin, incubated 20 hr, 23-24°C.

* Ratio, in %, of precipitate ring sizes formed by several concentrations of human serum in agar containing each hemoglobin sample relative to ring sizes formed by the same concentrations of this serum with agar containing the control hemoglobin.

† This sample was not dialyzed before testing.

Thus far the precipitate reaction between albumin and hemoglobin has been noted only in agar medium, and several attempts to demonstrate it in liquid medium, or in gelatin gels have failed(6,12).

Summary. Human serum albumin precipitates in agar gels with hemoglobin. This reaction can be quantified by means of a diffusion ring test, described, and is maximal between pH 6 and 8 when the medium contains 0.01 to 0.1 M NaCl. Several human and animal hemoglobins display varying reactivities. Conversion of hemoglobin to cyanmethemoglobin abolishes reactivity. It is suggested that the specificity of the reaction depends on both the globin and heme moieties of hemoglobin.

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Release of Permeability Factors from the Blood Platelet.* (30356)

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Increased vascular permeability is a characteristic of intravascular immune reactions in which there are platelet and leukocyte aggregates in the lumen(1). Polymorphonuclear

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leukocytes are known to release a factor or factors which increase vessel permeability and there is evidence that this may be a substance released from the leukocyte granules (lysosomes)(2,3). The release of permeability factors from leukocytes has been found to occur during the phagocytosis of antigen-antibody (Ag-Ab) complexes(2). The part played by the platelet, however, in vessel permeability changes is not known.

Recent evidence indicates that the platelet granules have a composition compatible with that of lysosomes(4) and that the platelet itself is capable of phagocytosis(5,6). During phagocytosis of particulate matter the platelet granules disappear(7) and serotonin and histamine are released(8). Loss of platelet granules is also known to occur when platelets are exposed to collagen fibers(9) or to proteolytic enzymes such as thrombin(10). The purpose of the experiments reported here was to observe whether factors which induce platelet aggregation and degranulation release a substance or substances which can increase vessel permeability.

Materials and methods. Blood was withdrawn from humans or pigs and placed into silicone-coated glass tubes in the proportions of 9 parts blood to one part of a 2% EDTA anticoagulant solution. A once-washed Tyrode-gelatine platelet suspension was prepared by methods that have been described (6,7). The gelatine concentration in the Tyrode solution was 0.25%. The platelet suspensions contained fewer than one red cell per 70,000 platelets and fewer than one granulocyte per 100,000 platelets. Ten parts of the platelet suspension were added to one part of the test material in silicone-coated glass tubes. The resulting mixture was incubated at 37°C for 15 minutes with continuous gentle agitation. Following this the tubes were centrifuged at 3,600 r.p.m. (RCF 1,570) at 4°C and the supernatant from this centrifuged at 12,000 r.p.m. (RCF 17,300) at 4°C. The upper half of the supernatant was removed, transferred to silicone-coated glass tubes and kept at 4°C until used in experiments on the day of preparation. Control samples consisted of the platelet suspension to which Tyrode solution was added or Tyrode-gelatin solutions to which the test materials were added. The test materials used in these experiments consisted of Ag-Ab complexes (washed immune precipitates), collagen prepared from rabbit tendon by the method of Hovig(9), adenosine diphosphate and adenosine monophosphate (Sigma Chemical Co., St. Louis), thrombin (Parke Davis, bovine thrombin), latex particles (Hytex, Hyland Laboratories), heat aggregated human serum albumin (Connaught Medical Research Laboratories, Toronto), and non-heat aggregated human serum albumin.

The supernatants from these samples were injected intradermally (0.3 ml) into rabbits which had received an intravenous injection of Evans blue (1.0 ml/kg of a 0.5% solution). The resulting skin lesions were examined and graded 10, 30 and 60 minutes after injection. In some experiments the rabbits were injected intravenously with 35 μc of I^{131}-labelled human serum albumin (Abbott Laboratories) 10 minutes prior to the experiment. In these experiments, the animal was bled out, and the area around the site of injection excised and placed in formamide solution. The radioactivity was determined in a Nuclear Chicago Model 4204 Gamma Detector. In addition the Evans blue in the excised area was extracted by the formamide, and measured by reading the resulting color at 620 mμ in a colorimeter.

Results. The results for one of 6 experiments are shown in Table I. The supernatant from the platelet-Ag-Ab complex reaction produced an intense blueing response. There was no blueing response at site of injection of the supernatant from the Tyrode-Ag-Ab complex control mixture. The supernatant from the thrombin-platelet mixture also produced an intense blueing reaction. A moderately intense reaction was obtained with the supernatant from the latex particle-platelet or collagen fiber-platelet mixture. The supernatant from the aggregated albumin-platelet mixture also produced some blueing. Both the AMP- and ADP-platelet mixtures appeared to release factors which caused a slight blueing response (final concentration of ADP 100 or 10 μg/ml). When the extent of increased permeability was assessed using

TABLE I. Platelet Permeability Factors.

Supernatant platelet suspension plus	Evans Blue Visual extraction§ Diameter, cm	Intensity	% Transmission 620 mμ	I^{131} albumin, cpm/mg tissue
Ag-Ab complex*	1.2 × 1.2	++	58.5	22,603
Thrombin (100 units/ml)	1.1 × 1.6	+++	62.2	21,095
Collagen (undil.)	1.1 × 1.3	+	85.1	8,724
Latex (5% suspension)	1.0 × 1.0	+/—	96.2	13,477
ADP (1 mg/ml)	1.0 × .9	+/—	95.7	7,802
ADP (100 μg/ml)	.9 × .8	+/—	93.1	6,500
AMP (1 mg/ml)	Diffuse†	+/—	96.7	5,136
Aggregated HSA‡	Diffuse†	+/—	94.3	8,939
HSA‡	—	—	100.0	1,142
Tyrode solution	—	—/+	99.0	718
Control—Supernatant tyrode solution plus				
Ag-Ab complex	—	—	100.0	705
Thrombin (100 μ/ml)	.6 × .15	—/+	98.5	1,362
Collagen (undil.)	—	—	100.1	507
ADP (1 mg/ml)	—	—	99.0	810
Normal skin (control)	—	—	100.0	610

* BSA—Anti BSA (1.8 μg N and 13.7 μg N).
† No sharp demarcation and therefore not possible to measure extent accurately.
‡ Human serum albumin (1.5 μg N)—the aggregated material was prepared by heating albumin at 70°C for 30 min.
§ Not corrected for weight of extracted tissue.

I^{131}-labelled human albumin, immune complexes and thrombin induced the greatest release of permeability factors from the platelets (Table I). Both AMP and ADP produced a moderate release of permeability factors as did collagen, latex and aggregated protein. These results were similar to those obtained by assessing the degree of permeability change by quantitating the amount of Evans blue released into the tissues at the injected sites (Table I).

Discussion. These studies indicate that a substance or substances are released when platelets come in contact with certain surfaces or proteolytic enzymes such as thrombin. These factors are known to induce membrane changes in the platelet, degranulation, release of serotonin and histamine and release of ADP, which causes platelet aggregation. The action of ADP and AMP is more difficult to understand. These compounds have not been considered to produce significant morphological changes in the platelets or to release substances such as serotonin. However, in some of our investigations we have found that ADP causes some platelet degranulation around the periphery of aggregates(11). In addition, we have found some evidence that ADP induces or makes available platelet factor III activity(12). It should be pointed out that the minimum concentration of ADP used in these experiments was between 10 to 100 times that required to induce platelet clumping in citrated platelet-rich plasma. However, we have found a final concentration of between 1 to 10 μg of ADP/ml suspension necessary to induce platelet aggregation in the washed suspensions that were used.

It would appear that the platelet permeability factor or factors may contain serotonin, histamine and lysosomal enzymes, the latter derived from the platelet granules. Preliminary experiments carried out using platelet fractions indicate that the granule fraction which is rich in lysosomes can increase vessel permeability, as can lysed PMN-leukocyte granules(2,3). The platelet granules(13), like PMN-leukocyte granules(14) have proteolytic properties with peak activity at low pH values, indicating that they contain acid cathepsins. Serotonin and histamine are known to increase vascular permeability(15).

The observations show that a variety of stimuli which produce platelet aggregation cause release of permeability factors. This

may be important in the injury of both large and small vessels, *e.g.*, in immune reaction and large vessel disease such as atherosclerosis.

Summary. Incubation of platelets with antigen-antibody complexes, thrombin, collagen, latex particles, ADP, AMP or aggregated human serum albumin is associated with aggregation of platelets and release of permeability factors into the ambient fluid. The permeability response was most intense with the platelet-antigen-antibody complex and platelet-thrombin mixtures. It is postulated that the increased permeability is due to release of histamine, serotonin and lysosomal enzymes from the aggregated platelets.

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Influence of Oligodeoxyribonucleotides on the Immune Response of Newborn AKR Mice.* (30357)

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Adult AKR mice immunized with sheep red blood cells and treated with oligodeoxyribonucleotides display, 48 hours after antigen administration, a significantly greater number of hemolysin-producing cells in the spleen than animals that received antigen only(1,2,3). These differences become less pronounced as time between immunization and assay increases(1) and they are not detectable in the early responses of mesenteric and pancreatic lymph nodes(2). This paper reports additional results of similar studies carried out in newborn AKR mice.

Methods. Five- or seven-day-old AKR littermate mice were injected intraperitoneally with 10^8 sheep red cells in the presence or absence of oligodeoxyribonucleotides (= oligonucleotides), which were prepared by enzymatic digestion of calf thymus DNA as described previously(4). Treatment with oligonucleotides, or injection of corresponding amounts of biological saline into controls, was started with 0.05 ml on the day of immunization, was continued on the next 2 days, and increased to 0.1 ml on the fourth day after immunization. All injections were intraperitoneal and were continued daily until sacrifice. Animals were sacrificed at various times after immunization. Their blood was collected for determination of circulating antibody, and cells from individual spleens, as well as from pools of mesenteric and pancreatic lymph nodes, respectively, were as-

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