It will be seen that 16 of the 28 died of injuries to the chest and upper abdomen, and that, of these, eight had died of a most unusual injury—rupture of the aorta in its descending thoracic part. I have seen only three other cases of death from traumatic rupture of the aorta in 25,000 necropsies, and, of these three, one was the radio-operator of the Dakota which crashed at Mill Hill a fortnight earlier; the other two resulted from crushing injuries to the chest in traffic accidents. The radio-operator of the Viking was among those dying of rupture of the aorta.

The ruptures of the aorta were associated with fractures of the ribs, disruption of the intercostal muscles, fractures of the spine, or laceration and bruising of the anterior surface of the liver. These injuries were, in my opinion, caused by acute flexion of the body over the safety-belt. A similar mechanism could well have accounted for the findings in the eight who died from laceration of the heart, lungs, or liver and spleen. It would also appear that had these travellers been seated with their backs "to the engine" and supported by cushioned upholstery these particular types of injury might have been avoided.

It is clearly of little consolation to escape a sudden death only to die in a conflagration, but not all crashed aircraft catch fire, and presumably with advances in foam-protected petrol tanks the danger of fire will diminish.

Witnesses were not available to tell how the passengers in the aircraft were seated, but a study of the injuries revealed a remarkable similarity between the injuries of seven pairs of victims who might reasonably be expected to be sitting together. Thus two married couples all had ruptures of the aorta, another couple both had ruptures of the liver and spleen, while two men who had good reason to be seated together were the least severely injured—both sustained head injuries only, of a severity which suggested that had the speed of impact been a little less they might well have escaped. This is pure hypothesis, but suggests that certain parts of an aircraft are more dangerous than others in the event of a crash—a fact which is possibly obvious, and well supported by the escape of the steward of the Dakota at Mill Hill and the stewardess of the Viking, who were both in their correct positions in the tail of the aircraft.

Conclusions

Such a disaster as the crash of a passenger-carrying aircraft presents special problems to a pathologist called on to perform necropsies.

(1) The post-mortem examination of air-crash victims requires a small but efficient organization to facilitate identification and burial.

(2) Blood-urea estimations are of no value in estimating the time of death.

(3) The problem of identification should be approached by dealing with those easily identifiable first, and so narrowing the field of the more difficult cases.

(4) Identification by means of dental records is the most certain method in this class of accident.

(5) The immediate cause of death in more than half of the victims was acute flexion of the body over the safety-belt.

(6) This hazard might be lessened by air passengers travelling "back to the engine."

THE PROZONE PHENOMENON IN HAEMAGGLUTINATION REACTIONS

WITH SPECIAL REFERENCE TO
THE QUANTITATIVE ANTIGLOBULIN TEST*

BY

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Quantitative studies of precipitin and flocculation reactions have shown that both require optimal proportions of antigen and antibody. It is well known, for instance, that a series of test-tubes in which decreasing amounts of diphtheria antitoxin are mixed with a constant amount of toxin often shows a "prozone" of antibody excess where the antigen-antibody complex remains soluble (Ramon, 1922). Similarly, a flocculating system of decreasing amounts of horse serum and a constant amount of rabbit antiserum shows a prozone where the antigen-antibody complex is soluble in an excess of antigen (Dean and Webb, 1926). Many other examples have been published since these original reports.

It is well known, of course, that the optimal ratio differs for each antigen-antibody complex if either the constant antigen or the constant antibody technique is alternately used. But this has no bearing on the fact that prozones are in both cases due to an excess of antigen or antibody where the complex is "protected" against the precipitating effect of environmental factors, especially of salts.

Prozones have also been met with in the agglutination reactions. In this particular case of the antigen-antibody reaction, however, it is noteworthy that each bacterial or red cell has a fixed "load" of antigen. As the agglutination can be easily read only in a very narrow range of strength in the cell suspension, it is very difficult to vary the antigen concentration in the system. The prozones observed in agglutination reactions are then always situated in the antibody-excess range and could be interpreted as a purely quantitative phenomenon.

Other explanations have arisen from the study of these prozones. Shibley (1929), for instance, observed prozones with moderately heated agglutinating antisera, and postulated that heat had modified the agglutinin into an agglutinoid which combined with the antigen without bringing an agglutination. He showed, indeed, that the cells within the prozone were subsequently unagglutinated by an agglutinating dose of fresh antiserum. The work by Bawden and Kleczkowski (1942) suggests that heat would combine some serum albumin with the antibody molecules and induce the blocking property.

On the other hand, with the discovery of the blocking effect in anti-Rh sera, Wiener (1944) suggested that blocking antibodies could be held responsible for the prozone phenomena met with in titrations of anti-Rh sera. This interpretation was supported by a blocking effect within the prozone.

While studying prozone-giving anti-Rh sera Levine and Waller (1946) observed that suitable absorption

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removed the blocking antibody, thus suppressing the prozone without any appreciable effect on the final titre of the agglutination.

Present Investigations

In various experiments in which blood from different species was injected into rabbits we have seen that after an inversion of the optimal temperature for activity (Millet and Hubinont, 1946) there is a further change in the agglutination pattern—namely, the development of a prozone (Hubinont, 1948) which gives a blocking effect—thus substantiating the opinion that it is due to the presence of blocking antibodies. It is noteworthy that these experimental prozones were found as the result of prolonged immunization, and also that they appeared quite regularly after a time, varying probably with the antigenic strength of the material injected and the individual response of the rabbit.

Quite similarly we found that the preparation of rabbits by injecting human globulins—alum-precipitated,

Thus the meaning of the prozone remains open to discussion. Either it is due to an excess of a unique antibody or it results from a blocking effect by a blocking antibody which should then coexist with an agglutinating antibody.

We have thought that the anti-human-globulin reaction could shed some light on the problem, for, unlike the haemagglutination reactions ordinarily met with, the amount of antigen could be varied in a very large range and, consequently, quantitative aspects of the antigen-antibody reaction could be studied also with the constant antibody technique. We have therefore carried out investigations with prozone-giving anti-human-globulin sera in the following way.

Experiment I

An equal quantity of O M CDe/cDE 1/5 suspension in saline was mixed in tubes with 128 volumes of two-fold dilutions in AB human serum of a strong blocking anti-D serum (titre 2⁻¹⁴ when 30% bovine albumin

TABLE I.—Sensitization of CDe/cDE cells^a by anti-D Sera of Increasing Potency (as Judged by the Titre in Albumin) (Hubinont and Massart-Guiot, 1950)

Name of Anti-D Sera	Titre in Albumin with CDe/cDE Cells	Increasing Dilutions of the Anti-globulin Serum (PR ₃ b)								
		1/2	1/5	1/10	1/20	1/50	1/100	1/200	1/500	1/1000
Dew	2-4	—	—	—	—	—	—	—	—	—
Mer	2-3	—	—	—	±	—	—	±	+	++
For.	2-5	—	—	—	±	+	+	++	+++	++++
Laur.	2-7	—	±	++	+++	++++	++++	++++	++++	++++
Berg.	2-7	+	++	+++	+++	++++	++++	++++	++++	++++
Beekm.	2-8	+	++	+++	+++	++++	++++	++++	++++	++++
Fr.	2-14	++	++	+++	+++	++++	++++	++++	++++	++++
Free.	2-18	++	+++	++++	++++	++++	++++	++++	++++	++++

following Proom (1943)—could lead to a definite prozone phenomenon when the anti-human-globulin serum so obtained was titrated with sensitized Rh-positive cells. In a certain number of cases we observed that the indirect Coombs test was positive in a very small range of dilutions of the anti-human-globulin serum, and we recommended that several dilutions of the reagent should be used before deciding that there was no sensitization (Hubinont and Massart-Guiot, 1950). Further, we were able to show that the prozone given by these particular anti-human-globulin sera was inversely proportional to the titre in albumin of the sensitizing anti-Rh serum. Thus we concluded that the range of the prozone was dependent on the degree of sensitization of the cells (Table I).

Independently, Van Loghem *et al.* (1950) had studied similar anti-human-globulin sera and, by sensitizing Rh-positive red cells with serial dilutions of anti-Rh sera, showed that the prozone phenomenon increased when the preliminary sensitization decreased. In explaining the phenomenon, they postulated (extending Wiener's views on prozones in agglutination by anti-Rh sera) that the antiglobulin serum should contain blocking antibodies (univalent) together with agglutinating antibodies (bivalent) at a higher titre, the former being responsible for the prozone when weakly sensitized cells are used.

Coombs (1950, personal communication) suggested that an excess of bivalent antibody by itself could interfere with the "lattice" formation, and eventually the addition of suitable amounts of gamma globulin (human) induced the agglutination within the prozone (see Van Loghem *et al.*, 1950).

(Armour) was used as diluent). The tubes were incubated at 37° C. for two hours. The cells were washed six times in physiological saline, and quantitative anti-globulin tests were performed on slides, the readings being recorded after ten minutes' gentle rocking.

We used AB serum as a diluent for the first stage (sensitization) in order to get a uniform sedimentation rate of the red cells, uniform environmental conditions, and also the same protein concentration when starting the washing. It was therefore believed that the chances that the antigen-antibody reaction would occur would be the same in all tubes, and, on the other hand, that there would be, if any, an equal aspecific absorption of the antiglobulin antibody by residual serum proteins after washing the cells in saline.

The result of this experiment is given in Table II. This can be read horizontally and vertically, giving the agglutination pattern either with constant antigen or with constant antibody titrations. It appears that both an excess of antigen and an excess of antibody correspond to a prozone where anti-human-globulin serum agglutinated sensitized Rh-positive cells, the result of the above-mentioned experiment being similar to observations in flocculation reactions.

The prozone met with in the constant antigen readings gave, of course, a blocking effect when an agglutinating dose of anti-human-globulin serum was added to the unagglutinated cells.

As there was a considerably lower titre of the anti-human-globulin serum when titrated with very strongly sensitized cells, we wondered if the very strong sensitization could not have brought down with the cells a greater quantity of aspecific serum proteins of which

TABLE II.—*Optimal Ratio Between the Degree of Sensitization of Rh-positive Cells and the Dilutions of Anti-human-globulin Serum*

Degree of Sensitization of CDe/cDE Cells (Washed) 6 Times	Increasing Dilutions of Anti-globulin Serum (PR ₂ b)										Saline
	1/1	1/5	1/10	1/50	1/100	1/500	1/1000	1/5000	1/10,000	1/50,000	
128/1 ..	+++	++	±	—	—	—	—	—	—	—	—
64/1 ..	++++	+++	+++	++	—	—	—	—	—	—	—
32/1 ..	++++	++++	+++	++	+	?	—	—	—	—	—
16/1 ..	++++	++++	++++	+++	+++	++	±	—	—	—	—
8/1 ..	±	+++	+++	+++	+++	+++	+++	+	±	—	—
4/1 ..	?	++	++	+++	+++	+++	+++	+++	+++	?	—
2/1 ..	—	±	+	+++	+++	+++	+++	+++	+++	+	—
1/2 ..	—	±	+	+++	+++	+++	+++	+++	+++	+	—
1/4 ..	—	—	±	++	+++	+++	+++	+++	+++	+	—
1/8 ..	—	—	?	+	+++	+++	+++	+++	+++	±	—
1/16 ..	—	—	—	±	++	+++	+++	+++	+++	±	—
1/32 ..	—	—	—	—	?	±	+++	+++	±	—	—
1/64 ..	—	—	—	—	—	—	++	+	—	—	—
1/128 ..	—	—	—	—	—	—	±	±	—	—	—
Unsensitized cells ..	—	—	—	—	—	—	—	—	—	—	—

the washings did not remove all traces in spite of the precautions described above. This was, however, very improbable, owing to the fact that supernatants gave a very slight but identical precipitate when tested with trichloracetic acid after the fifth washing.

In order to avoid this bias up to a certain point we made the following experiment.

Experiment II

O M CDe/cDE packed cells were sensitized with a very powerful anti-D serum (titre 2^{-18} in 30% bovine albumin). After incubation for two hours at 37° C. they were washed six times in physiological saline. In another test-tube the same cells were mixed with equal proportions of human AB serum, incubated and washed in the same way as the sensitized cells. Sensitized and unsensitized cells were then mixed in various proportions in order to obtain identical volumes of suspensions of the same strength. These tubes were thoroughly shaken in order to ensure an even distribution of sensitized and unsensitized cells, and incubated for an extra hour. Quantitative antiglobulin tests were performed after resuspending the sediment in its own supernatant.

Table III shows that by mixing sensitized with unsensitized cells it is possible to reproduce a quantitative

Thus when mixing fresh to sensitized cells there seems to be a redistribution of the antibody amongst the cells, with the final result of a lessened statistical sensitization.

Arguments for a labile binding between the cell antigen and the Rh antibody could also be based on experiments, to be reported elsewhere, in which we were able, by washing, to "desensitize" Rh-positive sensitized cells up to a point where the antiglobulin reaction became negative, though the agglutinability of the cells was retained.

It should be remembered that Morgenroth produced a similar phenomenon by adding unsensitized to sensitized rabbit cells, all the cells undergoing haemolysis when complement was subsequently added.

Conclusion

Our data strongly suggest that the prozone phenomenon (at least in certain haemagglutination reactions) is more satisfactorily explained by a purely quantitative reaction between antigen and antibody in the antibody-excess ratio, when the constant antigen titration is used.

It is not claimed that there is absolute evidence that the prozone is not due to the presence of blocking antibody in the anti-human-globulin serum. But if we

TABLE III.—*Effect of Adding Fresh Cells in Various Proportions to Sensitized Cells*

Proportion between Sensitized Fresh Cells	Increasing Dilutions of Anti-globulin Serum (PR ₂ b)										Saline
	1/1	1/5	1/10	1/50	1/100	1/500	1/1000	1/5000	1/10,000	1/50,000	
10/0 ..	±	+	++	++++	++++	++++	+++	—	—	—	—
9/1 ..	—	±	++	++++	++++	++++	++++	±	—	—	—
7/3 ..	—	?	+	+++	+++	+++	+++	+++	++	+	—
4/6 ..	—	—	±	++	++	++	++	++	+	?	—
1/9 ..	—	—	—	+	+	+	+	±	—	—	—
0/10 ..	—	—	—	—	—	—	—	—	—	—	—

agglutination pattern which is quite similar to the one obtained in experiment I. When fresh cells are added to sensitized cells there is a remarkable decrease of the sensitization, for which a dilution effect cannot account, because (for instance in the 1/9 mixture) 1/100 and 1/500 dilutions of the antiglobulin serum gave a ++ reaction when most of the cells were involved. Examination under a low-power microscope showed that more than 90% of the cells were agglutinated, while in the original mixture only 10% were sensitized by the anti-D serum.

consider that the constant antibody technique gives also a prozone where the action of a "blocking antigen" is excluded up to a high probability, and that the reactions between sensitized cells and the dilutions of the anti-human-globulin serum suggest a linear relationship in the optimal proportions of antigen and antibody, the close analogy of the prozone phenomenon to precipitin or flocculation reactions is against any qualitative kind of explanation.

It would be of great interest to generalize our observations to other antigen-antibody reactions by varying the

amount of antigen on the cell's surface, or by the collodion-particle technique where this prospect could be more easily realized. We have carried out such an experiment with the agglutinating system "blocking" anti-D-trypsinized cells. A preliminary note on this subject will be published (Hubinont, 1951).

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THE INJECTION TREATMENT OF HYDROCELES AND SPERMATOCELES

BY

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Despite the antiquity of injection treatment of hydroceles and spermatoceles there is still no general agreement regarding its value. The diversity of injection material is perhaps an indication that none is entirely successful, reflecting the multitude of operating techniques. The surgeon who promises a patient a brief stay in hospital, early return to work, and freedom from recurrence has either a short memory or little experience. It is felt that, if injection therapy has anything to offer, there is a place for it in the treatment of hydroceles and spermatoceles. It has been used sporadically in this hospital over a number of years, but unfortunately the records do not permit investigation of out-patients treated before 1948, when a new system of documentation was introduced. A fresh wave of enthusiasm for injection began in March, 1948, and it is the effect of this wave that I have attempted to discover.

Cases

The distribution of the cases is shown in Table I. The hydrocele clinic was a special clinic held on Saturday mornings for the repeated aspiration of hydroceles and spermatoceles. One patient had been attending for 40 years, several for more than 10 years; the average was about 5 years. The cases have been divided into frequent (average interval of three to four months between aspirations) and infrequent attenders (interval greater than a year). Each patient who attended the hydrocele clinic was injected at his first visit after March, 1948. Patients attending the hospital for the first time were seen by either a consultant surgeon or the R.S.O. acting as his deputy. All patients seen by the R.S.O. were referred to the hydrocele clinic for trial of injection

TABLE I.—Number of Lesions

	Total No.	Operation Advised	No Treatment	Aspiration	Aspiration + Injection
Hydrocele clinic	66	1	—	5	60
New patients:					
1948 { Hydrocele	61	23	4	2	32
{ Spermatocele	29	12	2	1	14
1949 { Hydrocele	53	21	1	8	23
{ Spermatocele	28	9	2	1	16

Eight patients had bilateral lesions. Many of the patients for whom operation was suggested had an associated lesion (e.g., hernia) for which operation was advisable.

before consideration of operative treatment. Of the patients seen by consultants, some were advised operation, some were reassured that no treatment was necessary, and others were referred to the hydrocele clinic; a few were treated by immediate aspiration (see Table I). To this extent the material is selected, probably accounting for the small proportion of young patients in the series. Many of these patients had been treated by their own doctors for some time, being referred to hospital either because of the increased pressure of work with the advent of the National Health Service or because they expressed a wish for operative treatment, being tired of repeated aspiration.

The distinction between a hydrocele and a spermatocele, while usually simple, is not always made, and in several cases it has been difficult to decide from the documents whether "hydrocele" meant in fact a hydrocele of the tunica vaginalis, or whether it was being used as a generic term for a fluid swelling in the scrotum. When the fluid has been aspirated several times it is often very difficult to be sure of the diagnosis; no attempt has therefore been made to classify the hydrocele cases attending the clinic as old patients.

Technique

A few patients were injected with "ethamolin"; this treatment was stopped when it was discovered that severe pain occurred, followed by collapse, the patient becoming cold, with a thready pulse. All cases recovered after resting for a few hours. I have not seen this collapse after injecting ethamolin into varicose veins, and do not think the reaction can be anaphylactic in nature. In one case procaine was injected after emptying the sac, five minutes before the ethamolin; the patient did not complain of pain and showed no untoward reaction—this supports the suggestion that the collapse is neurogenic—but he collapsed in the tram on his way home, and was confined to bed for two weeks with a red, painful, swollen scrotum, which subsided without discharge. This treatment seemed to be too drastic, and was abandoned, although the patient, who had been attending for aspiration of a spermatocele every two months, stated at a review two years later that "it was worth it," for there had been no return of fluid. It has not been possible to trace the records of the other patients who were treated in 1947; they have not attended hospital since, and so have not been included in the present series.

Quinine-urethane has been used since March, 1948. It has been prepared in the hospital dispensary, the following being the prescription:

Quinine hydrochloride	4 g.
Urethane	2 g.
Distilled water	to 30 ml.

The hydrocele sac is emptied as completely as possible, using a trocar and cannula, and 4 ml. of quinine-urethane is injected through the cannula. For a small spermatocele