THE MECHANISM OF ARTHUS REACTIONS. I. THE RÔLE OF POLYMORPHONUCLEAR LEUCOCYTES AND OTHER FACTORS IN REVERSED PASSIVE ARTHUS REACTIONS IN RABBITS.

J. H. HUMPHREY.

From the National Institute for Medical Research, Mill Hill, London, N.W.7.

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THE Arthus reaction, one of the classical types of allergic reaction, is evoked by the introduction of antigen into the tissues (usually into the skin) of animals whose plasma contains precipitating antibodies. The experimental animals have usually been rabbits, because rabbits are excellent producers of precipitating antibodies, but characteristic reactions occur in other species, including man, dog, guinea-pig and rat. It has been abundantly shown that ability to produce an Arthus reaction can be passively transferred by injection of either homologous or heterologous precipitating antibodies, and there is good evidence that neither non-precipitating antibodies nor cell-borne antibodies (Culbertson, 1935 ; Cannon and Marshall, 1941 ; Fischel and Kabat, 1947) are essential, or are even involved in the reaction. The macroscopic appearance of an Arthus-type reaction depends on its severity, but there is characteristically an inflammatory reaction with erythema, oedema and a varying degree of haemorrhage and necrosis, apparent after 1–2 hr. and maximum after 8–24 hr. or more, after which it slowly subsides. In the early stages there is massive oedema and invasion of the tissues at the injection site with polymorphonuclear leucocytes (polymorphs), which are concentrated mainly in and around the small blood vessels but also in the connective tissue and other structures. There are many thrombi consisting of a mixture of platelets and polymorphs, and the vessel walls (particularly those of the venules) are necrotic, with varying degrees of haemorrhage. Mononuclear cells are not prominent during the early stages. After 12–24 hr. the number of polymorphs declines rapidly, and the polymorph invasion gives way to infiltration by mononuclear cells, which, according to Gell and Hinde (1954), arise partly by invasion from the blood stream and partly by proliferation of local histiocytes.

Thus the Arthus reaction, as normally elicited, contains at least two elements, namely the consequences of the local combination of antigen with antibody, and of the local introduction of a foreign protein. It was the aim of this work to study the first element only. By using reversed passive Arthus (R.P.A.) reactions the problem can be much simplified. In this variation of the reaction, antigen is given intravenously into normal animals, and antibody is injected soon afterwards into the skin (see Fischel and Kabat, 1947). There is no question of any pre-existing cellular hypersensitivity in the test animal, and, if homologous antibody is used for injection into the skin, there is no local foreign protein reaction at the injection site, since the antigen is distributed generally throughout the circulation. Furthermore (Humphrey, 1951) R.P.A. reactions can be repeatedly reproduced in different skin sites on the same animal for periods up to six days, and it is possible

to assess the effects of various treatments against control periods in the same animals. There is the added advantage that variations in antibody levels, which complicate experiments using active immunisation, are eliminated.

R.P.A. reactions in the rabbit are uninfluenced by antihistamine drugs, though they are somewhat diminished by large doses of sodium salicylate (Smith and Humphrey, 1949) and considerably diminished by single doses of cortisone or ACTH (Humphrey, 1951). These observations, together with the time relationships of the reactions and the observed histological changes, suggested that the cellular invasion might be an essential part of the reaction, and not merely a consequence of secondary tissue damage. A means of investigating this point was suggested by Thomas and Stetson's (1949) use of nitrogen mustard (HN2) temporarily to diminish circulating polymorphs, during their studies of the Shwartzman reaction in the rabbit. The effect of suitable doses of HN2 was striking—R.P.A. reactions in rabbits being virtually abolished so long as the polymorph count was low.

While this work was in progress, Stetson (1951) extended his study of the Shwartzman reaction to include Arthus reactions. He drew attention to the similarity of the two reactions, both in their behaviour towards HN2 treatment and in their early histological appearances and suggested that in both instances an abnormal metabolic process developed in the injected skin areas which increased the vulnerability of the local capillaries and venules to leucocyte-platelet thrombi. The circumstantial evidence that invasion by polymorphs is an important link in the chain of events which make up the Arthus reaction is quite good. Before coming to definite conclusions, however, it seemed wise to use other agents than HN2 for depleting the animals of polymorphs, and to investigate other species besides rabbits. It was also desirable to show that replacement of the polymorphs in a depleted animal would restore the capacity to produce a reaction, since such a demonstration would prove that they were essential.

The following papers describe a study of R.P.A. reactions in the rabbit and the guinea-pig, and of the effect upon them of agents which affected the numbers or the properties of the circulating polymorphs and platelets. Although attempts to replace polymorphs by transfusion to deficient animals failed, the remaining evidence suggests that polymorphs are essential for oedema formation and for vascular damage in the rabbit. The reaction in the guinea-pig, which is more complicated and in only part of which are polymorphs concerned, is described in a separate paper.

METHODS.

Animals.

Mature rabbits of either sex, from the Sandylop and albino strains maintained at the National Institute for Medical Research, London, were used throughout. They were fed on pelleted diet No. 81 of Bruce and Parkes (1946) supplemented with hay.

Antigens and antibodies.

Antigens used were crystallised bovine serum albumin (Armour), 5 × recrystallised hen ovalbumin, human γ-globulin and a preparation of the capsular polysaccharide of pneumococcus Type III. Antisera were prepared by immunisation of rabbits with the antigens, either incorporated in Freund's adjuvant mixture, or adsorbed on alum, or, in the case of anti-pneumococcus sera, by intravenous injection of formalin-killed whole organisms. The sera contained 4·5–15 mg. of specifically precipitable antibody per ml.

Technique of reversed passive Arthus reactions.

Normal rabbits, whose bellies had previously been depilated, were injected, intravenously with 20–30 mg. of the protein antigens, or 2–3 mg. pneumococcus polysaccharide. After ½–1 hr., 0·5 ml. of antiserum, diluted to contain 3–5 mg. antibody/ml., was injected intracutaneously on each side of the belly. The rabbits were examined at intervals, and the oedema volumes measured as described by Humphrey (1951). Four days later, except where otherwise stated, a further injection of antigen was given and the procedure was repeated at different, but corresponding sites on the belly wall.

When leakage of Pontamine Blue was to be studied, the rabbits were given 5 per cent Pontamine Sky Blue 6BX intravenously in a dose of 0·6 ml./kg. This was usually injected ¼ hr. before the test antibodies, but in certain experiments injection was delayed until after the Arthus reactions had begun to develop.

Collection of polymorphonuclear leucocyte exudates.

Exudates were induced in normal rabbits by intraperitoneal injection of 150 ml. 3 per cent peptone in 0·9 per cent NaCl, previously warmed to 37°. After 12 hr. a further 50 ml. was injected, and the exudates were aspirated 3 hr. later, using the technique described by Mackaness (1952). All glassware used was treated with silicone, but a variable amount of fibrin clot generally formed during the subsequent manipulations. When the cells were to be used for transfusion within an hour of collection, clot formation was prevented by addition of sufficient sodium citrate or sodium ethylenediamine tetra-acetate, and the exudate was cooled slowly by standing in a refrigerator. The cells were deposited by centrifugation at 800 g. and, after removal of most of the supernatant fluid, were re-suspended in approximately 10 ml. Differential counts showed that 85–95 per cent were neutrophil polymorphs. Nearly all were actively motile when examined on a warm stage microscope.

When it was desired to transfuse exudate cells produced in a particular animal back into the same animal after depletion of its circulating polymorphs by nitrogen mustard, it was necessary to maintain the cells viable for at least 8 days—allowing 4 days for the animal to recover from the peritoneal lavage and for the scar to unite, and 4 days for the HN2 to take effect. With the help of Dr. Audrey Smith, of this Institute, it was found that exudate cells would remain largely viable—as judged by ability to show amoeboid movements and to ingest staphylococci—if they were stored as a deposit in the original exudate fluid at the bottom of a centrifuge tube at 2°.

Fewer cells remained viable when anticoagulants were added, but without them there occurred extensive formation of fibrin threads and clumping of the leucocytes on the threads. In order to obtain suspensions of dispersed cells suitable for intravenous injection, the following procedure was adopted. The exudate was aspirated into sterile flasks, and the peritoneal cavity washed out with Ringer solution containing 0·1 per cent gelatin and 10 u./ml. penicillin. The combined exudate and washings were agitated gently at room temperature while a fine clot formed, and they were then poured through silicone-treated muslin which retained most of the fibrin. After cooling slowly to 2°, the cells were deposited by centrifuging at 800 g. and were stored at 2° for 8–14 days. After storage for this time, 70–80 per cent appeared to be fully viable, and about 5–10 per cent were dead, when examined by phase contrast illumination on a warm stage. The cells were in clumps, however, and although they could be partly dispersed by gentle agitation for ½ hr. at 37°, it was found more convenient to treat the suspension with 2000 units streptokinase + 500 units streptodornase (Burroughs Wellcome and Co.) for 5 min. at 37° immediately before intravenous injection into the rabbits. This treatment yielded a uniform suspension which caused no apparent ill effects on the recipients, although—as is described below—no rise in the systemic polymorph counts resulted.

Dosage of nitrogen mustard.

" Mustine " (Boots Pure Drug Co.) was injected intravenously immediately after solution, over a dose range 0·5–2·0 mg./kg. and polymorph counts were made daily for 6 days. The changes in white cell counts followed a constant pattern, which is reported in the text of the paper below. Although 1 mg./kg. was usually sufficient to lower the polymorph count below 50/c.mm. on the fourth day, 1·75 mg./kg. was chosen as the dose which caused a consistent fall without any obvious general ill effects on the animals.

RESULTS.

Experiments using Nitrogen Mustard.

Oedema in R.P.A. reactions.

The typical course of development of oedema at a reaction site is shown in Fig. 1. The volume of measurable oedema increases rapidly, reaching a maximum at 7–10 hr. This pattern is constant, especially when duplicate sites are injected, and repeatable so long as the amount and volume of antibody are standard. In all experiments oedema volumes were measured at regular intervals, but the 7 hr.

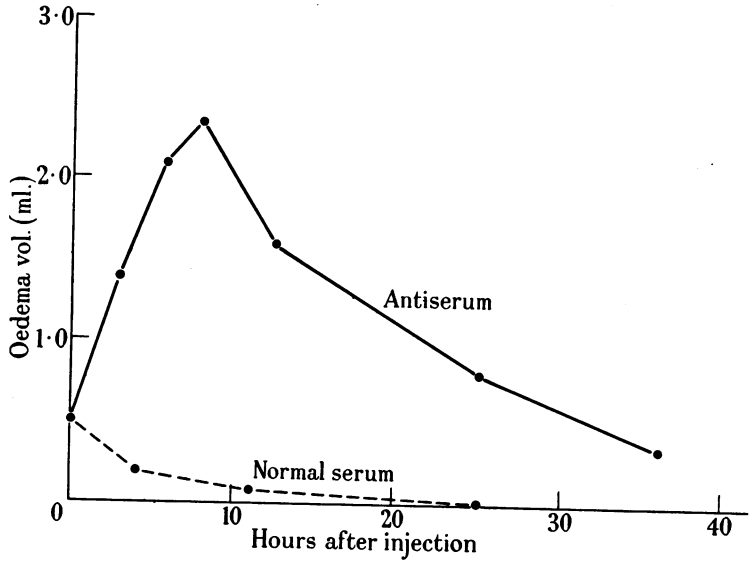

FIG. 1.—Time course of oedema at site of reversed passive Arthus reaction.

reading was arbitrarily chosen as the standard for convenience. Fig. 2 and 3 summarise the results of many experiments carried out with HN2, and show the relationship between the oedema at 7 hr. and the numbers of circulating neutrophil polymorphs and of mononuclear cells (lymphocytes + monocytes) respectively. Each point is the mean of two reaction sites, and every animal tested is represented twice—once during the test and once during the control period. It is evident that there is no correlation between oedema and monocuclear cells, but a definite correlation with polymorphs.

When the polymorph count approached zero, the visible reaction was abolished; it increased with increasing numbers of circulating polymorphs until the count was about 1500/c.mm. Above this level the response, though variable, was probably maximal. (The variability shown is not solely due to variation from animal to animal, but is enhanced by the inclusion in these scatter diagrams of experiments using several different antigen-antibody systems and with somewhat varying doses of antibody.)

After an injection of HN2 (1·5–1·75 mg./kg.) the number of circulating polymorphs does not drop greatly during the first two days, but falls precipitately

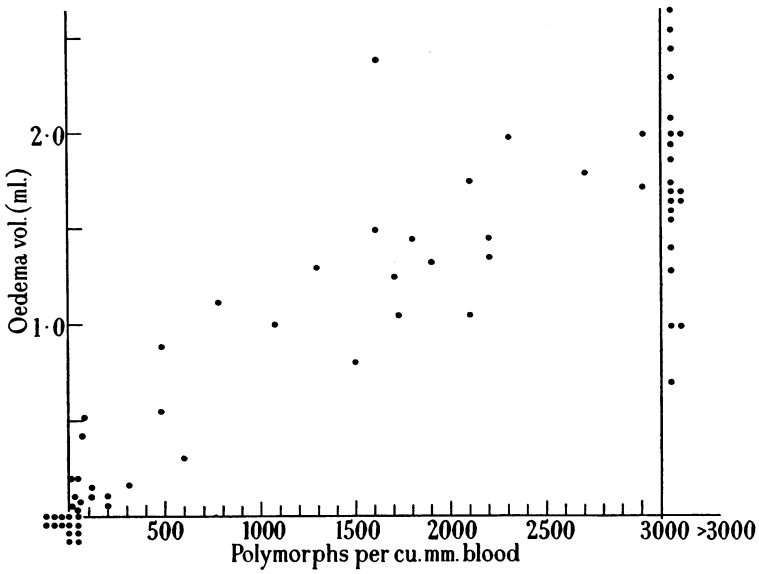

FIG. 2.—Correlation between oedema volume after 7 hr. at site of R.P.A. reaction and blood neutrophil count.

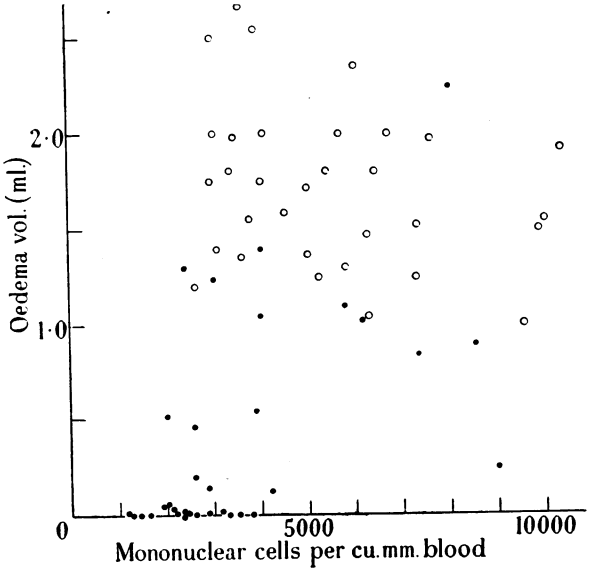

FIG. 3.—Correlation between oedema volume after 7 hr. at site of R.P.A. reaction and blood lymphocyte + monocyte count.

● 4 days after HN2.

○ 8 „ „ „

thereafter. By the 5th to the 6th day regeneration is occurring, and the blood contains many primitive as well as normal cells. After 8 days, the blood picture is restored to approximately normal. This sequence of events is probably brought about by an immediate toxic effect of the HN2, which prevents any new production of granulocytes by the bone marrow for about 5 days, although it does not impair the function of mature cells, which continue in the circulation and disappear with a normal half life. Repeated duplicate tests were made in two rabbits at intervals after HN2 administration, and Fig. 4 shows that the size of the reaction closely

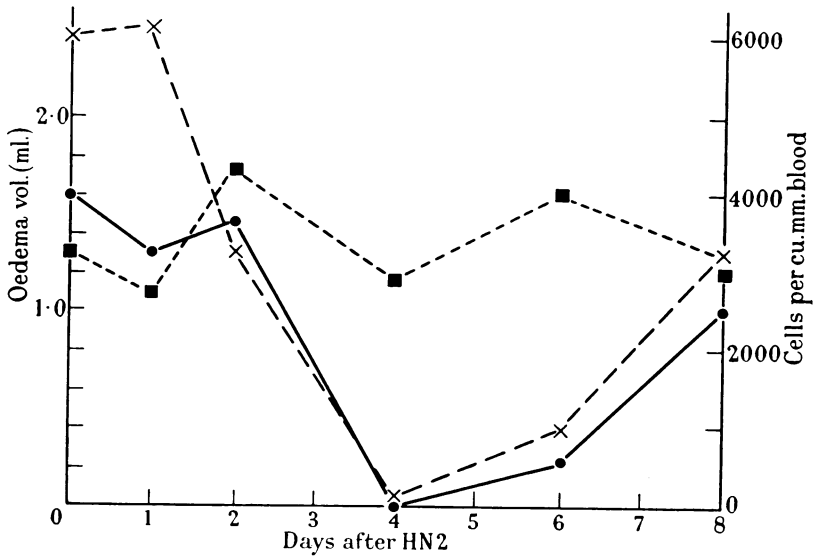

FIG. 4.—Correlation between blood white cell count and oedema volume after 7 hr. at site of R.P.A. reaction performed in same rabbit at varying times after HN2.

● oedema volume.
× neutrophil polymorph count.
■ mononuclear cell count.

followed the polymorph count. Thus the effect of HN2 on the reaction shows the same latency, and disappears at the same rate as its effect on polymorphs.

The correlation in time between the number of circulating polymorphs and ability to elicit an Arthus reaction was well demonstrated in another experiment in which the direct passive Arthus reaction was being studied. Two rabbits which had received HN2 4 days previously were injected intravenously with 150 mg. homologous antibodies against Type III pneumococcus polysaccharide. Intracutaneous injections of 0·5 mg. antigen 2 hr. later produced no reaction, although in control rabbits, immunised in the same way, vigorous haemorrhagic Arthus reactions appeared. Two days later, however, when polymorphs began to return to the circulation in the treated animals, the injected sites showed typical moderately severe reactions. Presumably the reaction of antigen with antibody had occurred in treated and controls alike, but any changes produced were harmless

until polymorphs arrived on the scene. Similar observations were made by Stetson (1951) in actively immunised rabbits.

Control experiments with HN2.

Nitrogen mustard, in doses sufficient to eliminate neutrophil polymorphs from the circulation, is likely to affect other tissues besides the bone marrow—*e.g.*, liver, spleen, adrenals, gastro-intestinal tract, as well as the skin in which the reactions are elicited. As was shown by Becker (1948) and by Stetson and Good (1951) in their studies of the Shwartzman phenomenon, the neutrophil depletion after intravenous HN2 administration can to a large extent be prevented if the

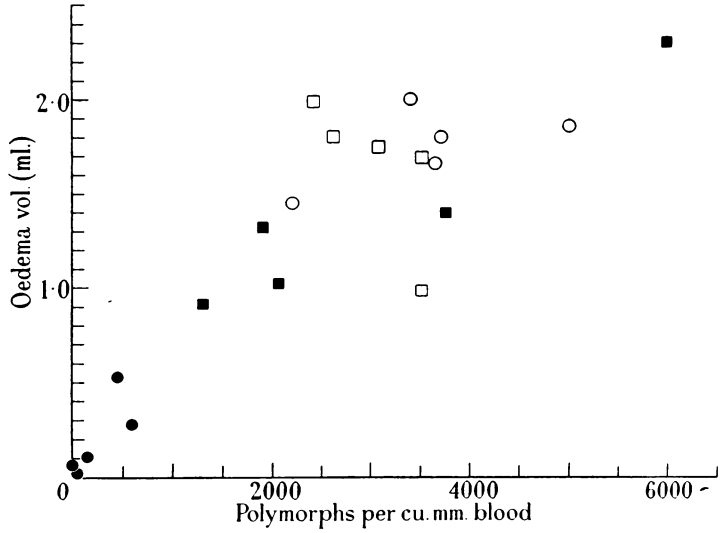

FIG. 5.—Correlation between oedema volume after 7 hr. at site of R.P.A. reaction and blood neutrophil count in rabbits treated with HN2 with and without clamping of the aorta.

○ Aorta not clamped 8 days after HN2.
● ,, ,, ,, 4 ,, ,, ,,
□ ,, clamped 8 days after HN2.
■ ,, ,, 4 ,, ,, ,,

abdominal aorta of the rabbit is clamped just above the bifurcation for 5 min. during and after the injection of HN2. By this means the legs, including their femoral bone marrow, escape the toxic effects of HN2, although the rest of the body is subjected to the drug. Animals which were injected while their aorta was clamped showed a reduction in neutrophil leucocyte count to about half the on groups of 5 animals subjected to the following treatments : HN2 injection with laparotomy, but without clamping the aorta ; HN2 injection with clamping of the pre-operation levels. R.P.A. reactions were performed 4 and 8 days after injection aorta ; saline injection with clamping of the aorta. This last group showed no difference from unoperated normal controls, and are not included. The results obtained in the first two groups are shown in Fig. 5.

It is evident that, so far as ability to elicit an Arthus reaction is concerned, HN2 had no effect when the aorta was clamped, even though the skin sites to be tested had been subjected to its influence. Furthermore, histological examination of biopsies of the reaction sites revealed no difference between the reactions in control animals and in those which had received HN2 with the aorta clamped, although the reaction was virtually abolished in the animals which received HN2 under laparotomy only (Fig. 6). These observations provide strong evidence, although not complete proof, that HN2 abolishes the reaction solely by virtue of its effect on the polymorphs.

Effect of HN2 on platelets and on complement.

In view of the facts that leucocyte-platelet thrombi are a prominent feature of Arthus reactions, and that rabbit platelets contain large amounts of histamine and 5-hydroxytryptamine releasable by antigen-antibody reactions (Humphrey and Jaques, 1953), it was necessary to investigate the effect of HN2 on platelets as well as on polymorphonuclear cells. In the doses used the numbers of platelets were reduced from about 700,000/c.mm. to between about 250,000 and 500,000/c.mm. Their histamine content, estimated biologically on washed buffy layer of the blood, was always within normal range. It seems improbable that the small reduction in numbers could account for the observed changes.

Since complement fixation is a feature of antigen-antibody reactions in the rabbit (Stavitsky, Stavitsky and Ecker, 1949) the effect of HN2 administration on the level of haemolytic complement was examined. In four animals, which showed an almost complete absence of polymorphs 4 days after HN2, no significant changes in blood complement were found.

Effect of HN2 on reactions to histamine, leukotaxine and the histamine liberator 48–80.

It was possible that the effective action of HN2 might be not so much upon the polymorphs as upon the reactivity of the small blood vessels to some unknown product of antigen-antibody reactions. Miles and Miles (1952) showed that the susceptibility of the cutaneous vessels of guinea-pigs to various agents could be studied by measurement of the area of blueing produced by injections of graded quantities of these agents, under standardised conditions in the skin of animals which had previously received intravenous injections of Pontamine Blue. The substances studied by Miles and Miles, namely, histamine, leukotaxine and the histamine liberator 48–80, were chosen as representative capillary-damaging agents.

In the present experiments they were injected in graded doses over the range 0·05–12, 0·8–200, and 0·05–50 μg. respectively, distributed at random in the skins of 10 rabbits, 5 of which had received HN2 previously and 5 were controls. All the rabbits had received intravenous injections of 0·6 ml./kg. 5 per cent Pontamine Sky Blue 6BX $\frac{1}{2}$ hr. beforehand. Measurements of the area and intensity of blueing at the injected skin sites revealed considerable differences between animals of the same group, but no significant difference between the groups.

The results make it very improbable that changes in resistance to capillary damaging agents could explain the effects of HN2 on Arthus reactions.

Attempted Replacement of Polymorphs in Depleted Animals.

Systemic injections.

Numerous attempts were made to raise the number of circulating polymorphs in depleted animals by transfusion of washed or unwashed cells obtained from peritoneal exudates (see Methods). In 6 cases the exudates had been obtained from the recipient animals themselves before HN2 administration and had been preserved for 8–14 days at 2°, while in 4 the exudates were freshly obtained from normal rabbits of the same breed. Although the transfused cells were in good physiological condition (judged by motility, phagocytic activity, and failure to stain with methylene blue), and were not clumped, and although the number of cells injected was sufficient to raise the polymorph counts in the recipient animals to or above normal levels, in only one instance was the level raised significantly.

No difference was observed between the results of injection into the marginal vein of the ear or of injection into the left side of the heart. Presumably the cells from the peritoneal exudates were in some way altered so as to adhere to the walls of the blood vessels (see Heinle, Weisberger and Guyton, 1950). In the single animal which showed a rise after transfusion, intravenous injection of 2×10^9 fresh cells from another donor produced a sustained increase in the polymorph count from 20 to 480/c.mm. The mean volume of oedema after 7 hr. at the R.P.A. sites in this animal was 0·54 ml. compared with 2·0 ml. when the reaction was repeated 4 days later at a time when the polymorph count was 3400/c.mm. A volume of 0·5 ml. of oedema is about the amount expected at a polymorph level of 400/c.mm. rather than at a level of 20 c.mm., but a single partly successful experiment cannot be claimed as proof that polymorphs are essential for development of the reaction.

Local injection.

Although local injection of polymorphs mixed with the test sera does not achieve the same distribution of these cells as results from invasion from the bloodstream, some experiments were made to test their effect in restoring reversed passive Arthus reactions in depleted animals. When 10^8 fresh exudate cells (*i.e.*, the amount present in about 20 ml. blood) were added to antiserum or to normal serum and injected intracutaneously into depleted animals which had already received antigen, some increase in oedema was observed at 6–7 hr. Table I shows the effect in 3 rabbits of added polymorphs, the oedema volumes being expressed as a percentage of the full-size reactions obtained on re-testing the rabbits 4 days later when polymorphs had returned to the circulation. Addition

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EXPLANATION OF PLATES.

FIG. 6–11 show site of R.P.A. reaction.

FIG. 6.—Polymorph-depleted rabbit : 5 hr. (× 15).

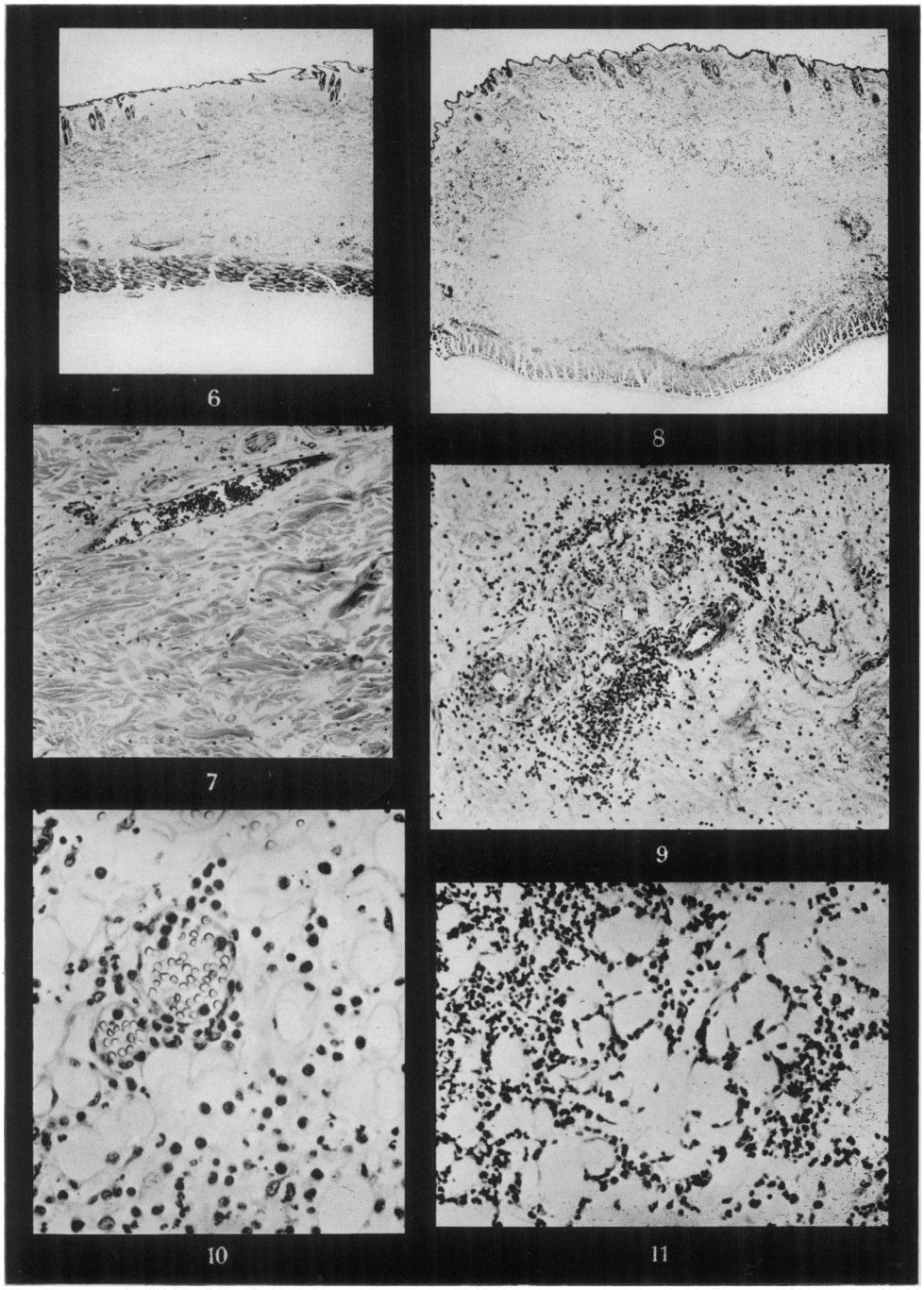
FIG. 7.—High power of Fig. 6 showing absence of perivascular infiltration (× 130).

FIG. 8.—Undepleted rabbit 6 hr. (× 15).

FIG. 9.—High power of Fig. 8 showing polymorph infiltration and early vascular necrosis (× 130).

FIG. 10.—Polymorph-depleted rabbit ; 24 hr.; large dose of antibodies. Shows pure mononuclear cell infiltration (× 130).

FIG. 11.—Undepleted rabbit 24 hr. ; large dose of antibodies. Shows mixed mononuclear cell infiltration and pyknotic polymorphs (× 130).


6
8
7
9
10
11

of polymorphs at the site of injection partly, but never completely, restored the reaction.

TABLE I.—*Mean Oedema Volumes after 6–7 hr. at Sites of R.P.A. Reactions in 3 Depleted Rabbits, and 2 Controls Expressed as Percentage of Mean Volume of Repeat Reactions 4 Days Later.*

	Depleted.	Controls.
Antiserum alone	4	81
Normal serum alone	1	—
10^8 polymorphs in saline	6	8
Normal serum and 10^8 polymorphs	24	—
Antiserum and 10^8 polymorphs	42	96

The mean polymorph count in the depleted rabbits was 26 per c.mm. The mean oedema volume in repeat reactions was 2·2 ml.

Capillary Permeability Changes at R.P.A. Sites.

The changes in capillary permeability at the sites of R.P.A. reactions can be studied in rabbits which have been given intravenous injections of Pontamine Blue (30 mg./kg.) ½ hr. beforehand. The sequence of events was fairly constant. For the first ½ hr. little or no blueing was observed at the reaction sites, but by 1 hr. a definite blue area appeared at the centre, and this area became more deeply stained during the next 3–4 hr. The peripheral area around the site meanwhile also became stained blue, although never so intensely as the central area. After 5 hr. no further changes occurred and the appearance (with the doses used) resembled a target, with a dark blue centre 10–15 mm. across, and a paler blue periphery 20–25 mm. in diameter. The area of visible oedema extended beyond the blue area. In the absence of antigen most whole sera caused little or no blueing, although normal or antibody-rich gamma globulin fractions (prepared by precipitation with Na_2SO_4) always caused deep central blueing, even when no antigen was administered and no oedema developed. In rabbits treated with HN2, the blueing at reaction sites due to the specific antigen-antibody reactions was almost completely abolished—*i.e.*, it was reduced almost to that produced by injection of the serum alone into normal animals. This degree of reduction was greater than that previously observed after single doses of cortisone (Humphrey, 1951).

Histological Changes.

The histological changes are described only in so far as they shed light on the pathogenesis of the reaction. Plates I–IV Fig. 6–9 show the striking difference after 7 hr. between the almost normal appearance of the skin reaction sites in depleted animals and the intense inflammatory changes in the untreated controls. The changes in the latter are essentially similar to those described in the review by Gell and Hinde (1954), except that the mononuclear cell reaction was quite inconspicuous up to 8 hr. Such small perivascular mononuclear cell collections as did occur were observed equally in both treated and control groups.

When reaction sites were examined after 24 and 48 hr., however, there was much less to distinguish those in polymorph-depleted animals from undepleted controls, particularly when large amounts of antiserum were used. Control reaction sites at 24 hr. were still grossly oedematous, and contained many neutrophil polymorphs, most of which showed pyknotic degeneration ; after 48 hr. the

neutrophil polymorphs were all pyknotic, although intact eosinophils were fairly numerous, and the oedema was much less. In addition to these features, which were confined to the control animals, there was present in animals of both series a considerable mononuclear cell proliferation and invasion, similar to that described by Gell and Hinde at sites of direct Arthus reactions. This was diffuse throughout the dermis and the underlying *panniculus carnosus*, but particularly marked around small blood vessels (Fig. 10 and 11). By 48 hr. thrombosis of the venules, which was still evident at 24 hr., was relatively rare.

It appears, therefore, that the events at sites of reversed passive Arthus reactions take broadly the same course as at those of direct Arthus reactions although perhaps more slowly. Furthermore, suppression of the neutrophil polymorph invasion, though it greatly diminished thrombosis, oedema and vascular necrosis, did not prevent the succeeding phase of the reaction from following its usual course. The phase of mononuclear cell proliferation and invasion must therefore be directly related to the antigen-antibody reaction, and not secondary to the neutrophil polymorph phase.

The Effect of Other Treatments on R.P.A. Reactions.

Bacterial polysaccharide.

A potent pyrogenic polysaccharide, prepared from *Chromobacterium prodigiosum* by a modification of the method of Shear and Turner (1943), when injected intravenously in doses of 15 μg/kg., caused a fall in the polymorph counts to between 100 and 500/c.mm. and in lymphocyte counts to 1200–2100/c.mm. This fall occurred within $\frac{1}{2}$ hr. and lasted 4–5 hr., during which period neither oedema nor significant histological changes occurred at the sites of R.P.A. reactions. Interpretation of these results was complicated, however, by a prolonged fall in peripheral blood pressure, which accompanied the leucopenia and persisted for at least 12 hr.

Heparin.

Since Good and Thomas (1952) and Cluff and Berthrong (1953) claim that administration of heparin in large doses prevents local Shwartzman reactions in rabbits, it seemed worth while to test whether R.P.A. reactions would also be suppressed. Even very large amounts of heparin (British Drug Houses, Ltd.), however, reduced the oedema only by about one half, and did not alter their general appearance. The results of two cross-over experiments are given in Table II.

TABLE II.—*Effect of Heparin on Development of Oedema at Sites of R.P.A. Reactions.*

Total amount of heparin.	Dosage (I.V.).	Volume of oedema (ml.) at 6 hr.	
		Control period.	Heparin period
1,600 i.u.	2 × 800 i.u. ½ hourly	2·0	2·2
4,800 „	6 × 800 „ hourly	1·6	1·9
48,000 „	6 × 8000 „ „	2·25	1·45
48,000 „	6 × 8000 „ „	2·9	1·1

The rabbits were tested later with a different antigen-antibody system, 4 days after HN2 administration, and gave negligible reactions.

Denervation.

Since capillary tone, and to some extent permeability, are under nervous control, the effect of denervation on R.P.A. reactions was studied. The ears of three Sandylop rabbits were denervated on one side by removal of the stellate and superior cervical sympathetic ganglia, with and without section of the second cervical sensory nerve. R.P.A. reactions were induced in both ears by the usual technique and the results are shown in Table III. Although oedema was more rapid and severe on the denervated than on the control sides, the reactions appeared similar, and the more explosive development in the operated ears was probably due to the increased blood flow through them.

TABLE III.—*Development of Oedema at Sites of R.P.A. Reactions in the Ears of Rabbits which had been Denervated on One Side.*

Time.	Mean oedema vol. (ml.)	
	Unoperated side.	Denervated side.
4 hr. . .	1·0	1·7
6 hr. . .	1·4	2·7
23 hr. . .	1·4	0·6
32 hr. . .	1·2	0·2

DISCUSSION.

Large molecules, such as serum albumin, introduced directly into the circulation take 10–20 hours to come into complete equilibrium with the lymph protein in the tissue spaces (Wasserman and Mayerson, 1951). In the experiments described above, antigen was usually injected in amounts 200–300 times greater than those required to combine with the antibody, but at the actual site of injection antibody would certainly be initially present in excess. Thus there would be a pool of antibody, tending to diffuse slowly into the venous capillaries and lymphatics, meeting antigen diffusing steadily from the capillaries into the tissue spaces. Although we do not know the detailed mechanism by which proteins pass from plasma to lymph (see Pappenheimer, 1953) there is no doubt that the meeting place of antigen with antibody must be in or around the small blood vessels. The sequence of events following introduction of horse serum into sensitised rabbits, either locally into the tissues or into the systemic circulation, was observed by Abell and Schenck (1938) and by Ebert and Wissler (1951) using the ear chamber technique, Some 15–20 minutes after introduction of antigen into the moat of the chamber they observed intermittent and asynchronous contraction of arterioles, with complete obliteration of the lumina, which resulted in intermittent circulation of blood through the area. After 1 hour clumps of leucocytes began to appear, and this was rapidly followed by adherence of leucocytes to the walls of peripheral capillaries and venules. Both intermittent contraction of peripheral arterioles and increasing adherence of leucocytes to the walls of all the vessels continued for 2½ hours. About this time there began an emigration of leucocytes into the tissues, which continued until next day, by which time clumping in the vessels had ceased. When antigen was introduced repeatedly, at weekly intervals, the process became more marked during the later introductions, and intense leucocytic emigration was accompanied by extravasation of erythrocytes, and by injury to the endothelium and actual destruction of the vascular wall.

The experiments reported above, in conjunction with Abell and Schenck's direct observations, suggest that the sequence of events is as follows.

Stage I.—A slowly developing, but relatively minor, damage to the local blood vessels, resulting from the union of antigen with antibody at the site and causing stickiness of the endothelium and arteriolar spasm. The mechanism of this damage is at present unknown, although it may perhaps be related to the changes occurring in immune plasma on addition of antigen, which cause release of 5-hydroxytryptamine and histamine from platelets (Humphrey and Jaques, 1953).

An increase in capillary permeability appears when the endothelial stickiness begins, but in the absence of Stage II it is transient and of limited extent. It is possible that the effect of cortisone administration (Humphrey, 1951) is to diminish the endothelial changes, and thereby to reduce the sequelae of the next stage.

Stage II.—Adherence of polymorphonuclear leucocytes to the walls, and intravascular clumping of leucocytes and platelets. Histamine and 5-hydroxytryptamine are probably released in significant quantities from the platelets, causing further increase in arteriolar spasm and in capillary permeability.

In the presence of sufficient antigen and antibody this process continues, and as the polymorphs accumulate the vessels become more or less plugged with cells and destruction of vessel walls occurs. This is particularly marked in venules and capillaries. At this stage there is extensive emigration of polymorphs into the surrounding tissues, and gross oedema becomes apparent.

Experiments not reported here indicated that oedema formation and emigration of polymorphs continue after leakage of Pontamine Blue from patent capillaries has ceased, the badly damaged vessels having meanwhile become completely plugged so that gross leakage does not occur fom them. The fluid which continues to accumulate in the neighbouring tissues (whose lymphatic channels are distended) presumably contains little plasma protein. Its accumulation implies that lymph drainage is obstructed, although the cause of this was not ascertained. Partial blocking by polymorphs is a possible cause.

All the changes of this stage were abolished or very greatly reduced by treatment with HN2 when, but only when, the circulating polymorph count was drastically lowered. This indicates that polymorph accumulation is essential for the vascular destruction and oedema, which are the main features of severe reactions. Adherence of platelets to the vascular endothelium could be seen in treated animals but was not extensive, and it is suggested that platelets are not essential for the destructive phase of the reaction, although their contained 5-hydroxytryptamine and histamine may well play a part by causing spasm and endothelial stickiness. Stage II is at its height 6–12 hours after the beginning of the reaction (depending on the severity) and it gradually gives place to Stage III.

Stage III.—This stage is characterised by invasion of the reaction site by macrophages, and by local proliferation of histiocytes. It is accompanied by degeneration and disappearance of the neutrophil polymorphs, by removal of leucocyte-platelet thrombi, and by repair of the vascular damage of Stage II by proliferation of endothelial cells. Since no histological observations were made beyond 48 hours, it is not possible to state whether the mononuclear cell collections develop into nests of plasma cells, as described by Gell and Hinde in direct Arthus reactions. Although the biochemical events which lead up to Stage III are unknown, it is interesting that the polymorph invasion of Stage II does not appear to be an essential factor.

The experimental work described above has largely been concerned with the rôle of neutrophil polymorphs, but it leaves the mechanism unexplained. Perhaps the most striking thing is the enormous number of these cells which are present at the reaction site. Of their known activities or properties any of the following might be important: proteolytic and other hydrolysing enzymes (Weiss, Kaplan and Larson, 1938; Barnes, 1940); very active anaerobic glycolysis with lactic acid production (Stetson, 1951); intense reducing activity (Rouser, 1951). Their concentration could well be sufficient locally to overwhelm any homoeostatic mechanism, and to cause a profound change in environmental conditions of the tissue cells.

SUMMARY.

A quantitative study was made of reversed passive Arthus reactions in rabbits, whose circulating neutrophil polymorphonuclear leucocytes had been reduced to very low levels by administration of nitrogen mustard.

In the absence of neutrophils, oedema formation was completely suppressed, and the early increase in local vascular permeability was much diminished.

Histological examination showed that, in the absence of circulating neutrophils, there was no local neutrophil infiltration of the reaction site, and very much less vascular necrosis. Mononuclear cell infiltration in the later stages was not affected compared with normal controls.

In rabbits which received nitrogen mustard, but whose neutrophil count was maintained by protection of the femoral bone marrow, Arthus reactions occurred normally.

The suppression of oedema did not depend on changes in other formed elements of the blood, or in haemolytic complement, or in skin reactivity to histamine 48–80 or leukotaxine.

Attempts to restore the level of circulating neutrophils in depleted rabbits, by transfusion of suspensions of fresh homologous cells or of stored autogenous cells, were largely unsuccessful.

The effect of other factors on the development of reversed passive Arthus reactions was studied, in an attempt to elucidate their mechanism. Heparin in very large doses caused only slight reduction in intensity. A bacterial polysaccharide which produced neutropenia very greatly reduced the reactions, but the observations were complicated by a prolonged fall in blood pressure. Arthus reactions in completely denervated ears differed from those in normal ears only by a more rapid course.

It is suggested that the reaction comprises three stages:

(I) Relatively rapid minor damage to the vascular endothelium accompanied by adherence of platelets and leucocytes, leading to—

(II) massive infiltration by neutrophil polymorphs which increases vascular damage and impedes lymphatic drainage, causing oedema formation;

(III) delayed infiltration and local proliferation of mononuclear cells.

Stages (II) and (III) overlap, but are independent. Only (II) depends on circulating neutrophil polymorphs.

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