

## EXPERIMENTAL PURPURA.

(Second Communication.)

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IN a preliminary communication by one of us (Ledingham<sup>1</sup>) to this journal on June 13th, 1914, the production of purpura hæmorrhagica in guinea-pigs, after inoculation with an antiserum prepared by immunising rabbits with guinea-pig blood plates, was placed on record. The present paper deals mainly with conjoint work carried out in June and July of last year, together with additional experimental data since obtained by one of us (L.) in the absence of the other (B.) on military service. The blood changes in guinea-pigs inoculated with anti-plate serum have been followed out in detail, and we propose in the first place to submit data on this point. A description will follow of experimental purpura in the rabbit and rat induced by inoculation with their respective anti-plate sera.

*Blood Changes in Guinea-pigs after Inoculation with Anti-guinea-pig-plate Serum.*

*Notes on technique.*—The blood plates were counted in a Thoma-Zeiss chamber, the blood from the ear being received into citrate solution containing brilliant cresyl blue which showed up the blood plates very clearly. The figures for the plate content per c.mm. obtained by this method in a series of normal guinea-pigs were as follows: 856,000, 752,000, 340,000, 640,000, 672,000, 688,000, 590,000, 696,000, 664,000, 592,000 (the latter four obtained from the same animal on four successive days), 592,000, and 584,000. The average of nine animals works out about 638,000 per c.mm. The hæmoglobin was estimated by Haldane's instrument. Attempts were also made to obtain coagulation times, but as we have not satisfied ourselves that the methods employed yield reliable results, at least so far as the guinea-pig is concerned, we refrain at present from alluding to them. The statement in the preliminary communication that the clotting is delayed rested entirely on observations of the bleeding time after puncture. Prolongation of the bleeding-time after puncture of the ear vein is a very marked feature in experimental guinea-pig purpura (as Duke asserts to be the case in human purpura), but this function may prove to have no direct relation to the coagulation time as ordinarily understood and calculated.

The three animals, B 3, B 4, and B 5, whose blood changes, &c., have been followed daily, received each one dose of anti-plate serum (1.5 c.c.) subcutaneously immediately after the control normal samples had been taken. The animals were purposely chosen of large size (over 400 gm.), so that a lengthy observation period would be available before the fatal conclusion (or possibly recovery) resulted. It had previously been determined that in animals of 250 gm. a dose of 1 c.c. of a particular batch of anti-plate serum had produced death in three, four, or five days with typical purpuric lesions. The data obtained from these three large animals, together with notes of the general condition, are found in Table I. (see p. 312).

<sup>1</sup> Ledingham (1914): The Experimental Production of Purpura in Animals, &c., THE LANCET, vol. i., 1914, p. 1673.

*Remarks on Table I.*—It will be observed from the table that two of the animals died in 4 and 8 days respectively, while the third animal (B 3), whose initial weight was 450 gm., made a complete recovery after exhibition of purpuric skin lesions. On July 14th—i.e., 36 days after the first dose—it received a second dose of 2 c.c. of the serum. The reaction to this second dose was very slight, the blood plates only, showing a marked fall, while the purpuric skin spots were few. It is impossible here to analyse the blood changes in detail, but certain points deserve notice. 1. The total leucocyte count is not appreciably affected, though there is a distinct tendency to relative lymphocytosis (see the column of differential counts). 2. The blood plates are the elements which suffer the earliest and promptest reduction. Except in the case of B 5, which showed an abnormally low initial plate content, the plates fell to one-sixth to one-tenth of their initial figure. In animal B 3, which recovered, the rapid regeneration of red cells and hæmoglobin is well seen. In the last two columns data are appended with reference to the presence of hæmagglutination in the shed blood, the minute changes in the red cells, and the general condition of the animals.

*Post-mortem appearances of the above three animals. B 3.*—Killed for examination on July 18th. A few skin hæmorrhages present at site of last inoculation. A few groups of faded punctiform hæmorrhages in stomach wall. No petechiæ on heart or lungs. Lymph glands not of hæmolymph character. Urine not stained with hæmoglobin.

*B 4.*—Dead June 16th. This animal had shown numerous skin hæmorrhages during the early days following inoculation of the serum. At necropsy these had faded and skin hæmorrhages were found only in the neighbourhood of the inoculation site. The red colour of the lymph glands was disappearing. Several hæmorrhages in the lungs and very numerous petechiæ in intestine. Stomach mucosa showed two round patches—one about the size of a sixpence, the other half that size—studded with small punctiform hæmorrhages. There was no ulceration. Liver yellowish. Spleen large, dark red, and friable. Kidneys and adrenals apparently normal.

*B 5.*—Dead June 12th. Numerous hæmorrhages at site of inoculation and a few scattered about elsewhere. Only a few lymph glands show the hæmolymph change. No hæmorrhages in intestine. Liver pale and yellowish. Spleen greatly enlarged and friable. Urine blood-stained. A few small hæmorrhages in testicles. *Both eyes show cataract.*

*Blood Changes in Smaller Animals.*

To complete this part of the subject tables are also appended showing the variations in the red cells, blood plates, hæmoglobin, &c., in three guinea-pigs of less weight. Each was inoculated on June 30th, 1914, with 1 c.c. of anti-plate serum subcutaneously. (See Tables II. A, II. B, and II. C).

TABLE II.A.—Guinea-pig 8.

Date.	Red cells.	Plates.	Hb.	Weight.
June 30th*	5,744,000	640,000	96%	240 gm.
July 1st	5,944,000	512,000	94%	220 "
" 2nd	5,130,000	320,000	80%	210 "
" 3rd	664,000	98,000	—	200 "

Death between 1 and 2 P.M. on July 3rd.

\* Received 1 c.c. anti-plate serum subcutaneously.

*Notes on blood picture and general condition.*—July 1st: Blood picture normal except for one or two red cells showing polychromatophilia. Well-marked hæmagglutination. Pig lively. Distinct infiltrate over abdomen. July 2nd: Some irregularity in shape of red cells. Megalocytes of medium size, fairly numerous, mostly showing polychromatophilia. Some red cell shadows. Pig seems ill. Infiltrate softening. Some skin hæmorrhages. Gums of fairly good colour. July 3rd: Hæmagglutination of shed blood most pronounced. Great irregularity in size of red cells. Megalocytes showing

TABLE I.  
Guinea-pig B 3.

Date.	Red cells.	Leucocytes.	Platelets.	Differential leucocyte count.				Hb.	Temperature.	Weight.	Colour index.	Appearance of blood on slide.	Remarks.
				Poly-nuclears.	Eosino-philic.	Lymphocytes.	Mast.						
June 8*	7,520,000	17,400	856,000	77.3	1.8	18.8	1.4	96	98.0°	450	1	—	—
9	7,808,000	17,400	680,000	76.4	0.4	22.6	0.4	92	97.0°	410	0.92	—	Pig lively. Firm infiltrate over abdomen.
10	7,024,000	16,400	250,000	64.0	2.0	33.6	0.6	90	98.4°	395	1.0	Hæmagglutination commencing	Infiltrate more marked.
11	6,256,000	14,800	148,000	56.0	1.5	42.0	0.5	76	99.0°	375	0.98	Hæmagglutination marked.	Animal ill; numerous skin hæmorrhages present.
12	3,968,000	14,400	180,000	—	—	—	—	62	99.0°	380	1.21	—	Animal very ill; infiltrate softening.
13	1,952,000	16,400	80,000	40.0	3.5	50.5	1.0	36	99.4°	400	1.4	Poikilocytosis; micro- and macrocytes; polychromatophilia; normoblasts and megaloblasts.	Condition unchanged.
14	2,924,000	16,200	398,000	39.0	4.5	56.0	0.5	30	99.8°	390	0.9	—	Much livelier; infiltrate disappearing.
15	2,064,000	20,400	368,000	38.0	2.3	61.6	1.0	50	98.6°	380	1.8	Hæmagglutination no longer present.	Condition good.
16	2,960,000	25,000	472,000	55.6	3.2	40.5	0.5	66	98.4°	—	1.7	Nucleated red cells, 3.0%.	„
17	4,356,000	24,200	428,000	42.0	1.0	57.0	—	80	99.8°	360	1.4	„ „ 2.3%	„
18	4,400,000	16,200	592,000	32.5	0.5	66.0	1.0	86	98.0°	370	1.4	„ „ 1.0%	„
19	4,544,000	18,400	436,000	42.0	—	57.0	1.0	90	100.0°	355	1.5	„ „ Nil.	„
20	4,240,000	14,200	624,000	32.6	0.3	67.3	—	94	98.4°	360	1.7	—	„
22	4,920,000	16,400	720,000	51.0	0.3	47.6	0.6	100	98.4°	360	1.5	—	„
29	5,520,000	8,800	896,000	45.0	0.5	54.5	—	100	99.8°	350	1.5	Except for some polychromatophilia blood picture is normal.	„
Interval of 3 weeks.													
July 14†	5,952,000	—	712,000	—	—	—	—	104	—	320	1.3	Blood picture normal.	Animal in good health.
15	6,312,000	—	708,000	—	—	—	—	104	—	300	1.2	—	Small infiltrate at site of inoculation.
16	5,324,000	—	304,000	—	—	—	—	98	—	320	1.4	Hæmagglutination; some irregularity in size of red cells.	Animal ill; infiltrate softening; one or two skin hæmorrhages seen.
17	5,104,000	—	240,000	—	—	—	—	98	—	300	1.5	Some polychromatophilia; no nucleated red cells seen.	—
18	5,248,000	—	608,000	—	—	—	—	96	—	320	1.4	—	Killed for examination.

Guinea-pig B 4.

June 8*	7,280,000	18,600	752,000	75.7	2.6	21.0	0.4	92	98.8°	400	1.0	—	—
9	7,808,000	15,800	752,000	71.6	1.5	25.9	0.8	92	99.6°	360	0.98	—	Well marked firm infiltrate over abdomen.
10	4,704,000	18,200	182,000	66.2	1.2	28.2	—	80	100.0°	380	1.3	Hæmagglutination commencing; some poikilocytosis.	Animal ill; numerous skin hæmorrhages.
11	3,952,000	14,800	190,000	56.0	1.5	42.0	—	66	100.0°	365	1.3	A few microcytes and megalocytes.	Animal very ill; infiltrate softening.
12	2,896,000	8,800	122,000	35.0	3.0	62.0	—	40	101.2°	380	1.1	Hæmagglutination marked; polychromatophilia; many nucleated red cells, 3%.	Condition unchanged.
13	1,232,000	18,800	144,000	29.0	2.0	68.0	1.0	30	99.8°	390	1.8	Normoblasts and megaloblasts very numerous, 8%.	„ „
14	1,296,000	8,400	248,000	36.0	2.3	61.0	0.6	30	98.4°	310	1.8	Some punctate basophilia noted; phagocytosis of red cells by large mononuclears.	„ „
15	?	8,600	286,000	34.8	1.3	61.1	2.3	24	96.0°	290	—	Nucleated red cells, 3%.	„ „
16	1,952,000	15,200	248,000	50.2	—	47.7	1.7	32	94.0°	250	1.4	„ „ 2.5%.	Animal cold and collapsed; died during night.

Guinea-pig B 5.

June 8*	5,840,000	12,800	340,000	78.2	0.4	20.3	—	94	98.6°	420	1	—	—
9	7,728,000	17,800	1,064,000	80.0	—	20.0	—	90	99.8°	385	0.74	—	Fairly well marked infiltrate over abdomen.
10	7,312,000	11,600	284,000	75.6	—	23.0	1.0	80	99.0°	380	0.61	Agglutination of red cells commencing. A few microcytes and poikilocytes.	Animal ill; no skin hæmorrhages seen.
11	2,980,000	9,400	298,000	58.0	4.0	35.0	0.5	66	99.0°	375	1.3	Hæmagglutination well marked; numerous polychromatophilic megalocytes.	Infiltrate softening; a few skin hæmorrhages to be made out.
12	1,200,000	10,800	330,000	61.0	6.0	32.0	1.0	—	94.0°	350	1.3	Fair number of normoblasts and megaloblasts, 3%.	Animal <i>in extremis</i> , cold and collapsed; died between 9 and 10 P.M.

\* Subcutaneous injection of 1.5 c.c. anti-guinea-pig platelet serum.

† Received second dose of 2 c.c.

polychromatophilia, very numerous; poikilocytes; no punctate basophilia. A few normoblasts, some showing pyknosis and karyorrhexis of nucleus. A few red cells with "Jolly" bodies. Pig cold and collapsed; blood thin and watery; no eye changes observed. Post mortem: Gelatinous blood-stained infiltrate over abdomen and thorax. A fair number of small punctiform hæmorrhages in skin. Numerous hæmorrhages in subcutaneous tissue. No eye changes. Spleen dark red and friable. Bladder filled with dark, blood-stained urine. Testicles showed several large hæmorrhages. No hæmorrhages in stomach or intestines. Lymph glands of red hæmolymp character. Bone marrow dark red.

TABLE II.B.—Guinea-pig 9.

Date.	Red cells.	Plates.	Hb.	Weight.
June 30th*	6,620,000	672,000	98%	260 gm.
July 1st	5,952,000	480,000	96%	260 "
" 2nd	5,616,000	272,000	84%	240 "
" 3rd	720,000	392,000	16%	230 "

Death between 1 and 2 P.M. on July 3rd.

\* At 5 P.M. received 1 c.c. anti-plate serum subcutaneously.

*Notes on blood picture and general condition.*—July 1st: Pig lively. Well-marked firm infiltrate over abdomen and extending forward to thorax. Blood picture normal except for one or two red cells showing polychromatophilia. July 2nd: Hæmagglutination well marked. Some red-cell shadows. Irregularity in size of red cells commencing. Megalocytes of medium size showing polychromatophilia, but not numerous. Animal ill. Infiltrate softening; some skin hæmorrhages; gums of good colour. July 3rd: Hæmagglutination extreme. Blood very watery; oozing after vein puncture. Irregularity in size of red cells. Megalocytes showing polychromatophilia; a few deeply basophile red cells; some poikilocytes. A few normoblasts, some showing punctate basophilia of cytoplasm. Pig collapsed and cold. No eye changes visible. Post mortem: A few small punctiform hæmorrhages in skin. Glands do not show hæmolymp change. No eye changes. Bladder contained dark blood-stained urine. No hæmorrhages in internal organs.

TABLE II.C.—Guinea-pig 10.

Date.	Red cells.	Plates.	Hb.	Weight.
June 30th*	6,630,000	688,000	102%	360 gm.
July 1st	6,170,000	384,000	100%	320 "
" 2nd	5,504,000	328,000	84%	300 "

Death during night of July 2nd-3rd.

\* At 5 P.M. received 1 c.c. anti-plate serum subcutaneously.

*Notes on blood picture and general condition.*—July 1st: Blood picture normal. Hæmagglutination well marked. Pig lively. Infiltrate not very well marked. July 2nd: Irregularity in size of red cells. Fair number of megalocytes, mostly showing polychromatophilia. Some red-cell shadows. Pig not well. Infiltrate more marked, but softer. Some skin hæmorrhages, one about the size of a threepenny-piece. Post mortem: Well-marked gelatinous blood-stained infiltrate. Numerous small punctiform hæmorrhages in skin. Glands of hæmolymp character. A few small hæmorrhages in lungs and in wall of left ventricle. Spleen much enlarged and friable. One or two minute petechiæ in pancreas. Bladder full of blood-stained urine. Numerous hæmorrhages in wall of large gut and in testicles.

*Remarks on Tables II.A, II.B, and II.C.*—These three animals, whose initial weights were 240 gm., 260 gm., and 360 gm., did not survive beyond the third day. All showed pronounced purpura. The effect of the serum on the blood elements is essentially similar to that obtained in the case of the larger animals of Table I. Notes regarding the minute changes in the red cells, the general condition of the animals during life, and the post-mortem appearances are appended to the tables.

#### Control Experiments with other Sera: Action of Anti-leucocytic Serum.

This serum was prepared by immunising rabbits with leucocytes obtained from the peritoneal exudates of guinea-pigs which were killed 4 hours after an intraperitoneal inoculation of 5-10 c.c. of sterile broth. Such exudates, as is

well known, consist almost entirely of polymorphonuclear leucocytes, and if great care is taken in manipulation of the animals after death the risk of admixture with red cells can be very largely avoided. The exudates are taken up in citrated saline and thereafter washed in normal saline. The serum of the rabbits so immunised proved to be quite as fatal to guinea-pigs as the anti-plate serum, but did not produce purpuric phenomena. We append two tables (Nos. III.A and III.B) showing the action of the serum on the blood elements throughout the course of the experimentally induced disease.

TABLE III.A.—Guinea-pig 15.

Date.	Red cells.	Plates.	Leucocytes.	Differential count.†				Hb.	Weight.
				P.	E.	L.	Ma.		
July 8th*	6,128,000	592,000	10,200	40.5%	0.5%	58.5%	0.5%	100%	290
" 9th	4,440,000	456,000	2,900	3.0%	0.5%	96.0%	0.5%	80%	250
" 10th	2,080,000	640,000	10,800	10.0%	1.0%	88.0%	1.0%	40%	230
" 11th	?	?	?	25.0%	2.0%	72.5%	1.5%	?	220

Death at 3 P.M. on July 11th.

\* At 5.30 P.M. received 1 c.c. anti-leucocyte serum subcutaneously.

† P = Polymorphonuclears. E = Eosinophiles. L = Lymphocytes. Ma = Mast cells.

*Blood picture and general condition.*—July 9th: Red cells irregular in size; numerous polychromatophilic megalocytes; a few red cells showing "Jolly" bodies; no nucleated red cells. The polymorphonuclear leucocytes are degenerated, their protoplasm being vacuolated and the granules appearing as indistinct basophile knots in cytoplasm. Pig ill. Small infiltrate under skin; no skin hæmorrhages. July 10th: Large numbers of megalocytes; some microcytes; well-marked polychromatophilia; nucleated red cells (normoblasts) 1.5 per cent. of white cells. Some phagocytosis of red cells by mononuclears. General condition unchanged. No skin hæmorrhages. July 11th: Some red cells show punctate basophilia; nucleated red cells (normoblasts and megaloblasts) 21 per cent. of white cells. Neutrophile myelocytes present. Pig ill and collapsed; hæmoglobinuria; no eye changes. Post mortem: No skin hæmorrhages. Lymph glands appear normal. No hæmorrhages in internal organs. Spleen greatly enlarged and friable. Bladder full of blood-stained urine.

TABLE III.B.—Guinea-pig 16.

Date.	Red cells.	Plates.	Leucocytes.	P.	L.	Ma.	Hb.	Weight.
July 8th*	6,368,000	584,000	14,400	51.0%	49.0%	—	98.0%	270
" 9th	5,560,000	648,000	2,600	3.0%	97.0%	—	90.0%	240
" 10th	2,680,000	640,000	8,000	6.6%	88.6%	3.3%	52.0%	220
" 11th	1,608,000	776,000	13,200	34.0%	67.5%	0.5%	22.0%	200

Pig dead at 6 P.M. on July 12th.

\* At 5.30 P.M. received 1 c.c. anti-leucocyte serum subcutaneously.

*Blood picture and general condition.*—July 9th: Slight hæmagglutination. Irregularity in size of red cells; poikilocytes; polychromatophilic megalocytes; one nucleated red cell seen. Degeneration of polymorphonuclears as in No. 15. Pig seems ill. Very slight infiltrate; no skin hæmorrhages. July 10th: Marked irregularity in size of red cells; no nucleated red cells. Infiltrate soft; no skin hæmorrhages. July 11th: Many megalocytes showing polychromatophilia; some red cells with punctate basophilia; nucleated red cells (normoblasts) 3.5 per cent. of white cells. Phagocytosis of red cells observed. Myelocytes 4 per cent. (total neutrophiles, 34 per cent.). Pig very ill. No eye changes. Post mortem: No skin hæmorrhages. No eye changes. No hæmorrhages in internal organs. Spleen slightly enlarged. Hæmoglobinuria. Liver yellowish.

It will be seen from the tables that while the red cells were very considerably affected, the blood plates were not appreciably altered in number. The most striking phenomenon in connexion with this serum is the great but temporary fall in the absolute number of leucocytes. In this fall, as the differential counts show, the polymorphonuclear cells are chiefly involved. No hæmorrhages were seen on the skin during life or in the organs after death. In both cases hæmoglobinuria was a well-marked feature.

#### *Action of Anti-red-cell Serum (Ordinary Lytic Serum).*

The effects of injecting lytic serum into the animal whose red cells have been used for immunisation have been studied by several workers, and notably by Muir and McNee (1912),<sup>2</sup> who have experimented with anti-rabbit red-cell serum prepared by immunisation of the goat with rabbit red cells. No record has been found of the production of purpuric lesions by such sera. It was, however, considered advisable to prepare a lytic serum for comparative tests, and for this purpose rabbits were immunised with washed guinea-pig red cells in the usual way. This serum has been tested on guinea-pigs only in a few cases so far. It is very lethal for guinea-pigs, but no evidence has been obtained that it can give rise to generalised purpura. As we shall see later, an anti-rat-red-cell serum proved to be equally devoid of purpura-producing effects when injected into rats, whereas the anti-rat-plate serum consistently produced purpura.

#### *The Question of Dosage of Anti-plate Serum.*

To test this point four guinea-pigs, each exactly 250 gm. weight, received the following quantities of anti-plate serum subcutaneously in the dorsal region on June 2nd, 1914: 2 c.c., 1 c.c., 0.5 c.c., and 0.1 c.c. (volume in all cases made up to 2 c.c. with saline). The animal which received 2 c.c. died on June 6th, showing an enormous number of hæmorrhages, with petechiæ on the heart and lungs and double cataract. The animal which received 1 c.c. died on June 4th (i.e., after 42 hours). There was beautiful miliary purpura over the whole skin, with petechiæ on the heart and lungs. Cataract had not developed. The animal which received 0.5 c.c. developed, like the others, a local swelling which had practically disappeared on June 6th. It died ten days later, but showed no purpuric lesions either internally or externally. Very probably any such lesions that may have occurred and escaped detection in the early days after inoculation had faded. The animal which received 0.1 c.c. showed only very slight local swelling for a few days after inoculation, and remained well.

#### *Experimental Purpura in the Rabbit.*

An anti-rabbit-plate serum was prepared by immunising the goat with rabbit blood plates recovered from rabbit blood by a method similar to that employed in the case of guinea-pig blood. Each dose given to the goat consisted of the blood plates recovered from the mixed blood of 12 rabbits. About nine such injections were given. The serum of the goat was tested at various times during the course of immunisation, and it will be convenient to detail briefly the results obtained with this serum on various rabbits, as in certain cases highly interesting pathological lesions were

found at necropsy. In the earlier cases of the series purpuric lesions in the skin were unfortunately not searched for.

*Rabbit 1.*—Received on June 20th 4 c.c. anti-plate serum subcutaneously (from test bleeding of goat after three doses). June 21st: Considerable soft local swelling. June 23rd: Swelling very slight. Rabbit remained well till July 15th, when sudden death took place. Post mortem: Animal well nourished. Peritoneal cavity filled with clotted blood and extravasated fæces. *Two large fairly clean-cut perforations of the bowel just below cæcum. A few purpuric spots in small intestine. Some faded purpuric spot in skin, especially that of hind limbs. Large hæmorrhages in the psoas muscle and in the muscles over tibia and fibula. Effusions of blood in the periosteal sheaths of these bones. Stomach mucosa showed a cluster of minute hæmorrhages, but there was no general purpura of the organ.* Liver and lungs healthy.

*Rabbit 2.*—Received on June 20th 1.5 c.c. intravenously (test bleeding after four doses). Remained well.

*Rabbit 3.*—Received on June 30th 2 c.c. intravenously (test bleeding after four doses). Remained well till July 3rd, when it was given a further dose of 3 c.c. intravenously. Death occurred two hours thereafter. Post mortem: *Perforated ulcer of colon adherent by film of lymph to abdominal wall. Purpuric patches round the ulcer.* The colon contained another large ragged ulcer with greyish scurf on it. It was not perforated. *In the stomach were two similar large non-perforated ulcers.* Right lung collapsed and congested. Some free blood in right pleural cavity. The liver contained a few nodules of pseudotuberculosis, so that some doubt remains whether the late purpuric lesions met with may have been of bacterial origin. This is, however, unlikely.

*Rabbit 4.*—Small Russian. Received on July 6th 5 c.c. intraperitoneally (test bleeding after four doses). July 7th: Three or four purpuric spots in skin over sternum. July 8th: Very large number of purpuric spots on skin of abdomen and back, and especially of extremities. Petechiæ on ear. On the same day it received a further dose of 3 c.c. intraperitoneally. July 9th: Rabbit looks well. Received a third dose of 3 c.c. intravenously and died in convulsions five minutes afterwards. Post mortem: Enormous number of skin hæmorrhages. Miliary purpura of stomach. One or two petechiæ on epicardium, and a good number on large-bowel and under kidney capsule. Very large number in skin covering fore and hind extremities and tail. No cataract. Large amount of free blood in peritoneal cavity. One or two reddish lymph glands in axilla and groin. Urine quite clear.

*Rabbit 5.*—Very small Russian weighing 440 gm. Received on August 27th 4 c.c. intraperitoneally (test bleeding after seven doses). August 28th: Animal looks fairly well. Numerous purpuric spots on skin of abdomen and extremities. Weight 431 gm. August 29th: Animal dead. Post mortem: Beautiful purpura of skin. *Extensive purpura of cæcum and large bowel.* A few spots in small intestine and in stomach. Numerous petechiæ in diaphragm.

#### *Blood Changes in Experimental Purpura of the Rabbit.*

We append three tables (Nos. IV. A, IV. B, and IV. C) in which are recorded the blood changes, &c., in the case of three rabbits inoculated intraperitoneally with the same dose (3 c.c.) of anti-rabbit-plate serum.

TABLE IV. A.—*Rabbit 33.*

Date.	Red cells.	Plates.	Leucocytes.	P.	E.	L.	Hb.	Coag. time.
July 15th*	5,792,000	648,000	12,200	28.0 %	2.0 %	70.0 %	80.0 %	1' 47"
„ 16th	4,864,000	100,000	10,400	54.5 %	0.5 %	45.0 %	66.0 %	2' 55"
„ 17th†	4,872,000	496,000	9,800	27.5 %	1.0 %	71.5 %	64.0 %	1' 48"

\* At 4 P.M. received 3 c.c. anti-rabbit-plate serum intraperitoneally.

† Killed with ether to obtain specimen of skin.

<sup>2</sup> Muir, R., and McNee: The Anæmia Produced by a Hæmolytic Serum, *Journal of Pathology and Bacteriology*, vol. xvi., p. 410.

*Blood picture and general condition.*—July 16th: Hæmagglutination present. Some irregularity in size of red cells; a few red cells showing polychromatophilia; one or two nucleated red cells seen. Rabbit quite lively. Bleeds very readily when ear vein is punctured; marked purpura over entire skin. July 17th: No nucleated red cells seen; a few showing polychromatophilia. Rabbit quite lively. No fresh outburst of purpura so far as can be detected. Post mortem: Extreme purpura of skin. Lymph glands not of frankly hæmolymp character. A few punctiform hæmorrhages in stomach and large gut. Some extensive hæmorrhages in muscles of thigh. No hæmoglobinuria.

TABLE IV.B.—Rabbit 34, weight 920 gm.

Date.	Red cells.	Plates.	Leuco-cytes.	P.	E.	L.	Ma.	Hb.	Coag. time
July 20th*	5,424,000	672,000	12,400	15.0%	0.5%	83.5%	1.0%	80%	0' 58"
„ 21st	5,016,000	106,000	14,400	22.0%	—	77.5%	0.5%	70%	2' 0"
„ 22nd	3,824,000	232,000	12,100	30.5%	—	68.5%	1.0%	50%	1' 5"
„ 23rd	3,960,000	454,000	7,800	25.0%	0.5%	74.5%	—	56%	—
„ 24th	4,344,000	752,000	8,400	—	—	—	—	58%	—
„ 25th	4,184,000	760,000	18,000	—	—	—	—	58%	—
„ 29th	4,416,000	800,000	10,000	—	—	—	—	70%	—

\* At 4.15 P.M. received 3 c.c. anti-rabbit-plate serum intraperitoneally.

*Blood picture and general condition.*—July 20th: Before injection one or two nucleated red cells were seen in film. July 21st: Little change in red cells; one normoblast seen. Animal drowsy and unwell. Hæmorrhages in skin, and specially marked in skin of ears. The flicking or rubbing of the ears produces an immediate crop of small punctiform hæmorrhages. July 22nd: Many red cells showing polychromatophilia; marked irregularity in size of red cells; no nucleated red cells seen. Rabbit quite lively. Hæmorrhages still present in skin. July 23rd: Marked irregularity in size of red cells; some large megalocytes showing polychromatophilia; no nucleated red cells seen. Animal's condition good. July 29th: Animal's condition good. Purpuric spots in skin mostly faded. A few still present on ears.

TABLE IV.C.—Rabbit 35, weight 700 gm.

Date.	Red cells.	Plates.	Leuco-cytes.	P.	E.	L.	Ma.	Hb.	Coag. time.
July 20th*	4,240,000	640,000	9,600	25.5%	0.5%	72.5%	1.5%	62%	1' 2"
„ 21st	3,696,000	98,000	16,400	52.5%	—	47.0%	0.5%	50%	30"
„ 22nd	3,840,000	356,000	9,000	22.0%	2.0%	76.0%	—	44%	—
„ 23rd	3,856,000	472,000	6,600	50.0%	0.5%	49.5%	—	48%	—

Killed with ether at 2 P.M. on July 23rd.

\* At 4.45 P.M. received 3 c.c. anti-rabbit-plate serum intraperitoneally.

*Blood picture and general condition.*—July 20th: Before injection no nucleated red cells were seen. July 21st: Hæmagglutination present but not marked. Some irregularity in size of red cells; some poikilocytes; no nucleated red cells. Animal ill. Hæmorrhages in skin, and especially in skin of ears. July 22nd: No nucleated red cells. Animal's condition unchanged. July 23rd: No nucleated red cells. Condition unchanged. Post mortem: Skin shows numerous hæmorrhages. Lymph glands appear normal. Numerous intramuscular hæmorrhages in diaphragm. No hæmorrhages in stomach or intestine; no hæmoglobinuria. Some hæmorrhages of moderate size in thigh muscles.

It will be seen that all three animals developed marked generalised purpura from which they recovered. They were, however, killed for examination purposes at various dates, and the post-mortem appearances are appended to the tables. The marked and early fall in the plate content corresponding with the early onset of purpuric skin phenomena will be noted. In these rabbits coagulation times were taken by the Dale-Laidlaw method,<sup>3</sup> the blood being drawn into short capillary

<sup>3</sup> Dale, H. H., and Laidlaw, P. P.: A Simple Coagulometer, Journal of Pathology and Bacteriology, vol. xvi., p. 351.

tubes, each containing a small shot. The data so obtained are included, but we are not satisfied that they can be depended upon, in view of the variations we have obtained by this method in normal rabbits examined at short intervals. This question is reserved for further investigation.

#### Experimental Purpura in the Rat.

Rabbits were immunised with rat platelets recovered from fairly large quantities of rat blood. Each immunising dose consisted of the plates obtained from the mixed blood of 24 sewer rats. Three rabbits were immunised, two of which died during the course of immunisation. From the remaining rabbit a small test bleeding was taken a few days after it received its second dose. The serum so obtained was tested on a rat and found to be efficacious in producing purpura (see below). The rabbit received a third and a fourth dose of rat plates, but unfortunately a few minutes after the intravenous injection of the latter dose it developed marked anaphylactic symptoms. It was felt that the serum of such an animal would probably be largely deprived of its anti-plate elements owing to absorption by the large dose of fresh plates injected. The animal was, however, bled when moribund and the serum so obtained filtered. As we shall see, this serum possessed marked purpura-producing property.

#### Effect of Injecting Anti-rat-plate Serum into Rats.

*Rat 1.*—Received on Sept. 29th 2.5 c.c. subcutaneously in dorsal region near root of tail (from test bleeding after second dose). Sept. 30th: Numerous minute skin hæmorrhages round the area of inoculation and along the tail. None elsewhere. Oct. 1st: The animal now moribund was killed with ether. Post mortem: A large oval skin hæmorrhage was found at site of inoculation, with smaller petechiæ at the periphery. Numerous hæmorrhages in skin of tail. A few in pyloric portion of stomach. No generalised skin purpura. The result, however, showed that the serum from this early test bleeding possessed the purpura-producing property.

*Rat 2.*—Received on Oct. 13th, 1914, 3 c.c. subcutaneously (from moribund rabbit after its fourth dose). Oct. 14th: A few petechiæ noted on skin of hind limbs. Oct. 15th: Animal not examined. Oct. 16th: Animal very ill and drowsy; was accordingly killed with ether. Post mortem: Beautiful generalised skin purpura. Large hæmorrhage in muscles of one fore limb; marked purpura of tail. Several small hæmorrhages in pyloric portion of stomach. Lymph glands bright red in colour. No hæmoglobinuria. No cataract.

*Rat 3.*—Received on Oct. 14th 4 c.c. subcutaneously. Oct. 15th: Cluster of small hæmorrhages near site of inoculation. Oct. 16th: More hæmorrhages in the neighbourhood. Animal very ill and drowsy. Oct. 17th: Rat dead. Post mortem: Generalised skin purpura. Red lymph glands throughout. Petechiæ in pyloric portion of stomach and throughout a considerable part of the small intestine, also in sigmoid and rectum. Urine in bladder blood-stained.

On the whole, the lesions in rat purpura are essentially similar to those met with in experimental guinea-pig purpura except that cataract, so frequently found in guinea-pigs dying of the experimental disease, has not so far been noted in rats. As mentioned above, the effect of anti-rat red-cell serum was investigated on rats. No purpuric lesions resulted.

#### Action of Heterologous Anti-plate Sera.

The question of the specificity of these anti-plate sera is of importance, and at various times control experiments in settlement of this point have been made. Invariably the result has been that the animal does not respond to the heterologous anti-plate serum—e.g., only slight inconvenience and certainly no purpura follow the



injection of the guinea-pig with anti-rabbit-plate serum, and *vice versa*. Recently, being in possession of a supply of anti-guinea-pig-plate, anti-rabbit-plate, and anti-rat-plate sera, one of us has carried out the following experiment, which shows clearly that a heterologous anti-plate serum has no purpura-producing property.

TABLE V.

Rat A, 150 gm.,	received 7/12/1914,	3 c.c. anti-rat-plate serum.
" B, 90 "	" " "	3 " anti-guinea-pig-plate serum.
" C, 140 "	" " "	4 " anti-rabbit-plate serum.
(All inoculated subcutaneously.)		
Guinea-pig A, 230 gm.,	received 7/12/1914,	3 c.c. anti-guinea-pig-plate serum.
" B, 230 "	" " "	3 " anti-rat-plate serum.
" C, 230 "	" " "	4 " anti-rabbit-plate serum.
(All inoculated subcutaneously.)		
Rabbit A, 400 gm.,	received 7/12/1914,	4 c.c. anti-rabbit-plate serum.
" B, 380 "	" " "	4 " anti-guinea-pig-plate serum.
" C, 380 "	" " "	4 " anti-rat-plate serum.
All inoculated intraperitoneally.)		

On Dec. 8th Rat A showed several purpuric spots near tail and also on legs. Other two rats showed nothing and appeared quite lively. Guinea-pig A had an extensive hæmorrhagic infiltrate over the whole of the abdomen and did not appear well. The other two animals showed no purpura and were quite lively. Rabbit A had numerous purpuric spots all over its skin and was obviously ill. The other rabbits were quite lively and showed no purpura. On Dec. 9th the animals inoculated with the homologous sera were still more drowsy and listless. Other animals appeared quite well. On Dec. 10th Guinea-pig A and Rabbit A dead.

Post mortem: Guinea-pig A showed beautiful skin purpura, extensive œdematous and hæmorrhagic infiltrate spreading over abdomen from seat of injection and extending deeply into the thigh muscles, and miliary purpura of stomach. There was no cataract, and the urine was quite clear. Rabbit A showed a large œdematous and hæmorrhagic subcutaneous area over upper thorax, neck, and fore limbs, also a larger hæmorrhage under skin of left cheek. Miliary purpura of stomach and slight purpura of intestine. Liver yellow and mottled. No cataract. Urine quite clear.

The other animals remained well and put on weight.

Dec. 11th: Rat A dead. Post mortem: Purpura of skin particularly marked over skull and cheek. A few spots in pyloric portion of stomach and a few in small intestine. Urine was blood-stained (hæmoglobinuria).

The other animals, which received the heterologous plate sera, remained well and put on weight.

#### Summary and Conclusions.

1. Data have been recorded on the blood changes in guinea-pigs after inoculation with anti-guinea-pig-plate serum. The early fall in the number of platelets simultaneously with the outburst of skin purpura is found to be a marked feature.

2. Purpura has also been produced in rabbits and rats by inoculation with the respective anti-plate serum. The blood changes in rabbits have been followed with results similar to those obtained in guinea-pigs.

3. Anti-plate serum, in addition to its purpura-producing properties, possesses in common with other cytolytic sera considerable lytic powers, which in all probability contribute largely towards the fatal issue in small animals. Death, however, may occur as the result of extensive hæmorrhages alone without any obvious lytic changes as evidenced by hæmoglobinuria.

4. Sera obtained by immunisation with red cells or leucocytes do not produce purpura.

5. Purpura is not produced by inoculating animals with heterologous anti-plate sera.

The histology of the purpuric lesions will form the subject of a later communication.

## A CONTRIBUTION TO THE STUDY OF SHELL SHOCK.

BEING AN ACCOUNT OF THREE CASES OF LOSS OF MEMORY, VISION, SMELL, AND TASTE, ADMITTED INTO THE DUCHESS OF WESTMINSTER'S WAR HOSPITAL, LE TOUQUET.

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THE remarkably close similarity of the three cases which are described in this paper is shown in the following synopsis:—

—	Case 1.	Case 2.	Case 3.
Cause... ..	Shells bursting about him when hooked by barbed wire.	Shell blowing trench in.	Shell blew him off a wall.
	Preceding period of sleeplessness.	As in Case 1.	?
Vision ... ..	Amblyopia. Reduced visual fields.	"	As in Case 1.
Hearing ... ..	Slightly affected for a brief time.	Not affected.	As in Case 2.
Smell ... ..	Reduced acuity.	Total anosmia.	Unilateral anosmia and parosmia.
Taste ... ..	Almost absent.	Reduced acuity.	As in Case 2.
Other sensations ... ..	Not affected.	As in Case 1.	As in Case 1.
Volitional movements	"	"	"
Defæcation	Bowels not opened for five days following shock.	"	"
Micturition	Urine not passed for 48 hours.	Not affected.	As in Case 2.
Memory ... ..	Apparently slightly affected.	Very distinct amnesia.	"
Result of treatment	Gradual improvement by rest and suggestion.	As in Case 1, supplemented by hypnosis	As in Case 1.

CASE 1.—Private, aged 20. Admitted on Nov. 5th, 1914. On the nights of Oct. 28th and 29th he slept in the booking hall of X station; "not much sleep there." On the 30th he motored in a bus from X to Y, arriving there at 7.30 P.M.; billets found at 8 P.M.; on guard from 10–11.30 P.M., and from 1.45–3.45 A.M. At 11 A.M. on the 31st for the first time he went to the firing line. His platoon advanced to one set of trenches and then crossed the road to another, only to find it filled with cavalry and to be told that there was no room for them. During the retirement from this trench, at 1.30 P.M., they were "found" by the German artillery. Up to that time he had not been feeling afraid; he had "rather been enjoying it," and was in the best of spirits until the shells burst about him.

He was now retiring over open ground, kneeling on both knees and trying to creep under wire entanglements, when two or three shells burst near him. As he was struggling to disentangle himself from the wire three more shells burst behind and one in front of him. (An eye-witness in this hospital says that his escape was a sheer miracle.) After the shells had burst he succeeded in getting back under the wire entanglements; all his comrades had retired already. He managed to get into a trench, and as the firing slackened he rejoined his company. Immediately after the shell had burst in front of him his sight became blurred. It hurt him to open his eyes, and they "burned" when closed. The right eye seemed to have "caught it" more than the left. At the same moment he was seized with shivering, and cold sweat broke out especially round the loins. He thinks the shell behind him gave him the greater shock—"like a punch on the head, without any pain after it." The shell in front cut his haversack clean away and bruised his side, and apparently it burned his little finger. It was this shell, he says, which "caused his blindness."

Falling in with two comrades, he was led by them, one on either side, to the dressing station. He opened his eyes occasionally to see where he was going, but everything