OBSERVATIONS ON THE CHANGES PRODUCED IN THE BLOOD AND BONE MARROW BY HÆMOR- RHAGE AND BLOOD DESTRUCTION.¹ (PART II.)

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IN a previous paper² attention was directed to the changes seen in the blood and bone marrow of rabbits after hæmorrhage. The present research was undertaken in order to study these changes more fully, and to contrast them with the changes following blood destruction by phenylhydrazin, and to consider more especially whether the fact that the remnants of red cells are left in the body produces any variation in the type of red cell regeneration from that which is followed after hæmorrhage. The megaloblastic marrow and megalocytic blood which are the essential characteristics of pernicious anæmia are strongly contrasted with the low colour index of post-hæmorrhagic anæmia, and they have been looked upon by Hunter (1909¹) and others as the effect rather than the cause of the pronounced blood destruction which occurs in that disease. On this hypothesis regeneration in the marrow may be presumed to be stimulated by the products of red cell destruction.

Bunting (1906²) has noted the megaloblastic response to ricin, and Paton and Goodall (1903³) and Kamminer (1906⁴) to phenylhydrazin, in rabbits. References to the literature of the action of phenylhydrazin on blood will be found in Itami (1908⁵), Ritz (1909⁶), Lewin (1901⁷), Heinz (1901⁸), Morawitz and Pratt (1908⁹), and Rosenthal (1910¹⁰).

I. METHODS EMPLOYED.

Estimations of hæmoglobin were made with a Gowers-Haldane standardised hæmoglobinometer. Enumerations of the red and white blood cells were made with Bürker's modification of the Thoma-Zeiss hæmocytometer. The diluting solution used in the earlier observations was Toisson's fluid; it was,

¹ Towards the expenses of this research a grant was made by the Science Committee of the British Medical Association. [Received April 7, 1911.]

² *Journ. Path. and Bacteriol.*, Cambridge, 1911, vol. xv. p. 4.

however, found¹ in counting the red cells of rabbits which had been freely bled, and also of those which had received phenylhydrazin, that the counting field contained, besides the usual red cell appearances, a number of very pale red cells, many of which were very difficult to see and readily escaped being counted, the result being an erroneously low red cell count and a correspondingly raised colour index. It was found that the number of these pale cells was increased with the interval of time after the mixing of the blood and Toisson's fluid in the counting chamber, and many cells became quite invisible with any ordinary methods of illumination. To obviate this error the Toisson's fluid was replaced by Hayem's solution.

The diameters of the red cells were measured with the help of a camera obscura, the image of the microscope field being projected on to a table where the individual cells were outlined in pencil; the apparatus being adjusted for a magnification of 1000 diameters, the cells were measured in μ directly with a millimeter scale. The sizes of the red cells varied from $4\ \mu$ to $12\ \mu$, and were classed in groups progressing from $4\ \mu$ by $0.25\ \mu$ up to $12\ \mu$; it was found that in a series of successive 100 cells the distribution in these groups was not constant, but if the average diameter is calculated of each successive 100 the figures are in good agreement.

The appearances of the red cells and the differential counts of the white cells were observed on blood films stained with Jenner's stain. The marrows were examined by the glycerin film method, and the classification adopted was that which I have already described.² The bleedings were made on successive days from the auricular veins. The inoculation of phenylhydrazin was made under the skin in the groin, the dose, in all cases, being 10 c.c. of a 1 per cent. solution of phenylhydrazin in 0.8 per cent. salt solution.

II. THE EFFECTS OF HÆMORRHAGE ON THE BLOOD AND BONE MARROW.

Observations were made on eight different rabbits which were bled to amounts varying from 54 to 76 per cent. of the estimated total oxygen capacity of the animal. The details are given in Tables I. to VIII. It is convenient to follow the effects observed on one of these animals, and subsequently to note to what degrees the effects produced on the others agree or vary.

Rabbit BA (male, 2980 grms.).—On three successive days 43, 46, and 40 c.c. of blood respectively were removed, containing in all 73 per cent. of the estimated total oxygen capacity of the animal. For the three days previous to the first bleeding the average number of red cells was 6.5 millions per c.mm., and the colour index was 0.62 on the human scale; this was now considered as unity. On the fourth day, *i.e.*, three days after the first, and one day after the last bleeding, the red cells numbered 2.1 millions per c.mm., and the colour index was 1.1, rising on the two following days to 1.3. The average diameter of 100 red cells rose from $5.8\ \mu$ before bleeding to $6.7\ \mu$, $6.5\ \mu$, and $6.6\ \mu$ on the days succeeding the last bleeding, the range of diameter rising from $2.25\ \mu$ to $5.25\ \mu$, $5\ \mu$, and $5\ \mu$ respectively. The number of polychromatic red cells in 100 red cells rose from none before bleeding to 18, 11, and 15 on these days; and the number of nucleated red cells to every 100 of white cells counted in a stained film, rose from none to 5, 60, and 18 on these same days. The animal was then killed, *i.e.* on

¹ *British Medical Journal*, London, 1910, June 4.

² *Journ. Path. and Bacteriol.*, Cambridge, 1911, vol. xv. p. 5.

the fifth day after the first, or third day after the last bleeding. Marrow films were at once prepared, and on examination showed—

Small primitive cells and halo cells	20.4 per cent.
Large primitive lymphocytes	39.6 „
Other lymphoid cells	7.2 „
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Total lymphoid cells	67.2 per cent.
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Total leucoid cells	32.8 per cent.
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The large primitive lymphocytes (myeloblasts), which I regard as the progenitors of metrocytes (premegaloblasts), show a large increase over the normal percentage of about 15 per cent.

The number of nucleated red cells to every 100 white cells was 71 as against 15 per cent. for normal animals. These cells were grouped as—

Normoblasts	5.4 per cent.
Primitive erythroblasts	3.2 „
Metrocytes	16.2 „
Megaloblasts	13.0 „
Free nuclei	33.2 „

The marrow therefore shows great lympho-erythroblastic activity, which is especially marked by the great increase of large primitive lymphocytes and the presence of a large number of metrocytes; and is associated with raised colour index, polychromasia, increased diameter of red cells, and the presence of large numbers of nucleated red cells in the blood.

Of the other seven rabbits, four were not killed, and the marrow was therefore not examined. In these animals, for the three days previous to the first bleeding, the average number of red cells was 6.7, 6.5, 5.4, and 5.7 millions per c.mm. respectively, and the colour indices 0.64, 0.63, 0.61, and 0.64 respectively. As before, these last figures were then regarded as unity for each rabbit. On the fifth day after the first bleeding the red cells numbered 2.6, 2.4, 2.7, and 2.6 millions per c.mm., and the colour indices, were 1.1, 1.3, 1.1, and 1.3 respectively; the average diameter of 100 red cells was increased by about 1μ in each case; the proportion of polychromatic red cells was respectively 11, 16, 17, and 6; and the numbers of nucleated red cells for every 100 white cells counted were 6, 8, 4, and 3; so that apparently the changes in the blood of these animals were rather less than in the case of Ba, which is consonant with the fact that they were bled to less degree. Of the remaining three animals, Bb 2, which was bled to rather greater amount than Ba (76 per cent. of the estimated total oxygen capacity being removed), showed a markedly less degree of change in its blood than the others, although the marrow showed extreme erythroblastic activity. It is interesting to note in this connection, that when the animal was killed it was found to be pregnant, a condition in which it might be supposed that the blood was in training to accommodate itself to an increased marrow activity.

Rabbits Bc and Bd were only bled on two successive days, and were killed on the eighth day after the last bleeding. They showed the same series of changes in the blood, but to a much less degree, and the marrows though still actively erythroblastic were much less so than in the case of Ba, the numbers of metrocytes per 100 white cells being only 1.4 and 5 respectively. The total number of nucleated red cells, in the case of Bd, was still very high (89), but this included 40 of free nuclei, an indication that the most active

phase of forming red cells, which is associated with an urgent need for hæmoglobin in the circulation, had now passed.

A number of white cell enumerations were made, and also day by day estimations of the differential percentages of the various white cells, but no special effect was noticeable as the result of the bleeding. Generally speaking, rabbits have two types of white cell percentages: (*a*) those with excess of non-granular cells, (*b*) those with excess of granular cells; as a rule, the type of blood remains fairly constant after becoming more lymphoid or more leucoid, as the case may be, on the first few days that follow the bleeding.

III. THE EFFECTS OF SUBCUTANEOUS INJECTION OF PHENYLHYDRAZIN.

Nineteen rabbits were employed for the purpose of observing the effects of phenylhydrazin on the blood and bone marrow. In each case 10 c.c. of a 1 per cent. solution in 0·8 per cent. salt solution was injected subcutaneously, and in no case did the animal appear to suffer any pain or discomfort. Twenty-four hours after the injection the blood was found to have undergone very marked changes. When examined in the hæmoglobinometer tube it appeared brown and cloudy, so that accurate estimation of the amount of hæmoglobin present could not be made. The number of red cells was diminished usually to a considerable degree. The stained films showed many polychromatic red cells, and occasional basophilia, normoblasts, and free nuclei, also poikilocytes and degenerated shadow cells.

This cloudy brown condition lasted for about a week, so that all the hæmoglobin estimations made before the eighth day were unreliable and were disregarded.

The nature of this brown coloration requires further investigation. It resembles no known derivative of hæmoglobin; it gives no obvious absorption band in the spectrum; it is not produced by the same substance that gives the brown colour to the blood in experimental anilin poisoning;¹ it is not methæmoglobin.

It is still an open question whether blood cell destruction continues during the whole time that this brown colour is present, or whether it ceases soon after the inoculation of the phenylhydrazin; and in comparing this series of experiments with those described above, it is difficult to be certain whether the differences in the effects observed are due to the more prolonged destruction, or to the influence of the products of blood cell destruction, which in the second series are retained in the circulation. This question is, however, in part answered by the results of these experiments.

For the sake of convenience, the days after the day of inoculation are considered as comparable with the days after the first bleeding. As in the case of the rabbits that were bled, it is useful to follow the effects observed in the blood and bone marrow of one representative animal, and subsequently to note in what degree the effects produced in the others agree or vary. The details are given in Tables IX. to XVI.

Rabbit 14 (2646 grms.).—On the three days previous to inoculation the colour index averaged 0·69, and this was then considered as unity. The

¹ *Guy's Hosp. Reports*, London, 1909, vol. lxiii. p. 313.

blood was clear, and the films were healthy, excepting an occasional polychromatic cell. When examined on the day after the inoculation the blood was cloudy and brown, so that a reliable colour index could not be determined. The red cells had fallen in number from 4.98 to 2.88 millions per c.mm.; there was no increase in the number of white cells; stained films showed some polychromasia, many poikilocytes, and in a count of 300 white cells there were 12 nucleated red cells, of which 9 were normoblasts and 3 were free nuclei.

The average diameter of 100 red cells, which for three days previous to the inoculation had been $6.3\ \mu$, with a range of $2.75\ \mu$, was now only $5.4\ \mu$, with a range of $3.75\ \mu$; and the number of polychromatic red cells was 5 per cent.

On the sixth day after inoculation the blood was clear but still slightly brown; the red cells had steadily fallen in number day by day to 1.58 millions per c.mm.; the stained films showed a severe condition of polychromasia (28 per cent.), there was much poikilocytosis and basophilia, and in a count of 300 white cells there were 105 nucleated red cells, composed of 84 normoblasts, 6 megaloblasts, 13 free nuclei, and 2 microblasts. As the white cell count was 14,000 per c.mm., there were nearly 5000 nucleated red cells per c.mm. The average diameter of 100 red cells measured $7.9\ \mu$, with a range of $5.5\ \mu$.

On the following day (seventh day after inoculation), the blood was clear and free from all trace of brown coloration. The red cells had risen in number to 2.38 millions per c.mm., and the colour index was 1.3. Stained films showed similar appearances to those of the previous day, with 21 per cent. of polychromatic red cells, much basophilia, and in a count of 300 white cells there were 44 nucleated red cells, of which 27 were normoblasts, 2 megaloblasts, and 15 free nuclei; the white cells numbered 7700, so that there were over 1100 nucleated red cells per c.mm. The average diameter of 100 red cells was $8.5\ \mu$, with a range of $3.7\ \mu$.

On the next day (eighth day after inoculation), the number of red cells was 2.5 millions per c.mm., and the colour index was 1.3; the stained films showed the same appearances with 22 per cent. of polychromatic red cells, and 50 nucleated red cells in a count of 300 white cells, made up of 25 normoblasts, 24 free nuclei, and 1 megaloblast. The average diameter of 100 red cells measured $8.0\ \mu$, with a range of $6.0\ \mu$. The animal was then killed. Marrow films were at once prepared, and on examination showed—

Small primitive cells and halo cells	38.8 per cent.
Large primitive lymphocytes	28.8 „
Other lymphoid cells	11.2 „
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Total lymphoid cells	78.8 per cent.
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Total leucoid cells	<u>21.2 per cent.</u>

Both the small primitive cells and the large primitive lymphocytes were thus increased to nearly twice the normal number, and the nucleated red cells to about four times; these cells were grouped as—

Normoblasts	4.4 per cent.
Primitive erythroblasts	11.4 „
Metrocytes	0.2 „
Megaloblasts	8.0 „
Free nuclei	43.8 „

The marrow therefore shows much lympho-erythroblastic activity of an exaggerated normal type, as evidenced by the excess of small primitive cells and primitive erythroblasts; the large number of free nuclei suggesting a

recent rapid production of red cells. This marrow contrasts with the marrows of rabbits 8 and 10 and 11 and 12, which were killed on the third and fifth days after inoculation, in which erythroblastic activity was much greater and associated with the presence of a large number of metrocytes, as was also the case with the bled rabbit BA described above, which also may be regarded as a fifth-day marrow.

There does not seem to be so marked a connection between the presence of increased numbers of large primitive lymphocytes and metrocytes, or between the very much increased cell diameter and raised colour index and the number of metrocytes, but I think it must be assumed that the appearances in the blood are the result of an earlier condition of marrow.

Rabbit 15 (2400 grms.) was treated on the same day with the same dose of phenylhydrazin as rabbit 14. Previous to inoculation the colour index of this animal was slightly lower than rabbit 14, being only 0.60; there was marked polychromasia amounting to 1 per cent., also some basophilia, and 2 normoblasts in a count of 300 white cells, equivalent to 48 nucleated red cells per c.mm.; the average diameter of 100 red cells was 6.7μ , with a range of 2.75μ . It would seem, therefore, that this was not a perfectly normal blood, and that some blood cell regeneration was in progress.

On the sixth day after inoculation the blood was clear but still brown, and the number of red cells had fallen day by day from 5.81 to 1.13 millions per c.mm. The stained films showed severe polychromasia of 14 per cent., much poikilocytosis and basophilia, and in a count of 300 white cells there were 119 nucleated red cells, composed of 62 normoblasts, 38 free nuclei, 18 megaloblasts, and 1 metrocyte, and equivalent to 12,000 nucleated red cells per c.mm., there being a lympho-leucocytosis 31,000 per c.mm. The average diameter of 100 red cells measured 8.1μ , with a range of 6.25μ .

On the seventh day the blood was clear and free from brown coloration; the red cells had risen in number to 1.9 millions per c.mm., and the colour index was 1.3. The appearances of the stained films were similar to those of the previous day; the polychromasia amounted to 19 per cent.; in a count of 300 white cells there were 76 nucleated red cells, including 61 normoblasts, 7 free nuclei, and 8 megaloblasts, and corresponding to 4375 per c.mm., there being a lympho-leucocytosis of 17,500 per c.mm. The average diameter of 100 red cells measured 8.3μ , with a range of 6.75μ . The animal was then killed, and marrow films were at once prepared, which on examination showed—

Small primitive cells and halo cells	33.0 per cent.
Large primitive lymphocytes	25.2 „
Other lymphoid cells	5.6 „
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Total lymphoid cells	63.8 per cent.
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Total leucoid cells	36.2 per cent.

The differential count thus closely resembles that obtained from the marrow of rabbit 14. The number of nucleated red cells to every 100 white cells was 37.6, more than twice the normal number: they were grouped as—

Normoblasts	2.0 per cent.
Primitive erythroblasts	3.6 „
Metrocytes	0.6 „
Megaloblasts	2.2 „
Free nuclei	29.2 „

which also resembles in type the nucleated red counts in the marrow of rabbit 14, but suggests, in comparison, that the marrow had been depleted and that many nucleated red cells had been thrown out into the blood stream, as was indeed found to be the case.

Rabbit 6 (2660 grms.) and rabbit 7 (2450 grms.) were killed on the day following inoculation. In the case of rabbit 6, the red cells were reduced in number by 500,000 per c.mm.; the average diameter of 100 red cells which before inoculation was $6.5\ \mu$, with a range of $2.5\ \mu$, and only slight, less than 1 per cent. polychromasia, was reduced to $5.8\ \mu$, with a range of $2.75\ \mu$, and a polychromasia amounting to 2 per cent. In the case of rabbit 7, the red cells were reduced in number by 1.4 millions per c.mm.; the average diameter of 100 red cells measured before inoculation $6.37\ \mu$, with a range of $2.5\ \mu$, was reduced to $5.8\ \mu$, with a range of $2.75\ \mu$, and there was less than 1 per cent. of polychromasia. In both animals 3 nucleated red cells were noted in a count of 300 white cells. The white cells showed but slight change, but in each case there was a relatively increased percentage of polymorpho-nuclear leucocytes. So that in the blood of these animals there was but little to notice beyond the cloudiness and brown coloration, and diminished red cell diameter.

The marrows of these animals agreed very closely in type, both showing marked erythroblastic activity, and only slight increase of lymphoid cells, though in both cases this is marked by the increased percentage of polymorpho-nuclear leucocytes (also in evidence in the blood films), so that if these cells were excluded the lymphoid character of the marrow would be more striking. In both marrows the red cells numbered over 40 per 100 white cells; they are grouped as:—

	Rabbit 6.	Rabbit 7.
Normoblasts	11.4 per cent.	11.6 per cent.
Primitive erythroblasts . .	5.8 "	1.6 "
Metrocytes	0.4 "	0.0 "
Megaloblasts	11.4 "	7.2 "
Free nuclei	12.2 "	23.2 "
Total	<u>41.2 per cent.</u>	<u>43.6 per cent.</u>

Rabbits 8 and 10 were killed on the third day after inoculation. The number of red cells diminished day by day to 1.4 and 1.3 millions per c.mm.

In the case of rabbit 8 the average diameter of 100 red cells on the four days of observation were respectively $6.5\ \mu$, $5.7\ \mu$, $5.2\ \mu$, and $6.3\ \mu$, with ranges of $2.25\ \mu$, $2.25\ \mu$, $3.0\ \mu$, and $4.5\ \mu$; the polychromasia being less than 1 per cent. on the first day was increased to 20 per cent. on the last day. With rabbit 10 the diameters were $6.2\ \mu$, $5.7\ \mu$, $6.2\ \mu$, and $6.5\ \mu$, with ranges of $2.5\ \mu$, $3.25\ \mu$, $4.75\ \mu$, and $4.5\ \mu$; and the polychromasia increased from less than 1 per cent. to 13 per cent. on the last day.

Films prepared from the marrows of these animals showed marked erythroblastic activity, but in both, as in the marrows of the preceding rabbits (rabbit 6 and rabbit 7), there was no evidence of an increased number of lymphoid cells, in fact, the marrows had a leucoid type; this was chiefly due to the presence of an increased proportion of polymorpho-nuclear leucocytes, which was not in evidence in the blood. This condition seems to indicate an attraction to the marrow for these cells on account, possibly, of some toxic irritation.

The small primitive lymphoid cells were proportionally diminished in number. In this connection it may be noted that the giant cells, which I regard as the source of these cells, were comparatively few in number, and of these none showed typical distinct multinucleated masses, and in many

instances the nuclear mass was free, unsurrounded by any granulous cytoplasm. Is it possible that the poison was acting directly upon these cells?

The large primitive lymphocytes also showed no increase in percentage number; many of these cells were undersized, as if due to multiplication by division, many show changes towards the formation of fine oxyphil granular myelocytes, and various transitional forms to metrocytes; of these last there is a marked increase in number compared with the marrows of rabbit 6 and rabbit 7 killed on an earlier day, and most of them show active nuclear division and formation of megaloblasts. The nucleated red cells of these two marrows closely agree:—

	Rabbit 8.	Rabbit 10.
Normoblasts	2.4 per cent.	0.8 per cent.
Primitive erythroblasts	6.8 „	4.4 „
Metrocytes	5.6 „	4.2 „
Megaloblasts	2.0 „	2.0 „
Free nuclei	14.8 „	19.4 „
Total	<u>31.6 per cent.</u>	<u>30.8 per cent.</u>

Passing now to rabbit 11 and rabbit 12, which were similarly treated but killed on the fifth day after inoculation, it is found that the marrows in both cases present a very marked lympho-erythroblastic activity, the increased percentage of the lymphoid cells being chiefly accounted for by the large primitive lymphocytes; these marrows correspond in type to that of rabbit B_A; see Tables VIII. and XVI. The blood of these rabbits showed similar changes to those of the other inoculated rabbits, but no measurements were made of the red cell diameters.

Rabbit 25 was killed on the eleventh day after inoculation. The blood showed appearances similar to those seen in the other animals (Tables IX. to XIII.), but no measurements were made of the red cell diameters, nor was the amount of polychromasia estimated. The marrow showed a most intense lympho-erythroblastic activity, and contrasts most markedly with the marrows of those rabbits which were killed at earlier dates after inoculation, especially with regard to the number and differential grouping of the nucleated red cells

Small primitive cells and halo cells	30.0 per cent.
Large primitive lymphocytes	23.4 „
Other lymphoid cells	9.6 „
Total lymphoid cells	<u>63.0 per cent.</u>
Fine oxyphil myelocytes	13.4 per cent.
Coarse oxyphil myelocytes	1.0 „
Basophil granular myelocytes	1.6 „
Polymorpho-nuclear leucocytes	21.0 „
Total leucoid cells	<u>37.0 per cent.</u>

The lymphoid character was probably masked by the large proportion of polymorpho-nuclear leucocytes. The number of nucleated red cells enumerated in a count of 500 white cells was 745, or 149 per cent.; they comprised—

Normoblasts	35.8 per cent.
Primitive erythroblasts	6.8 „
Metrocytes	1.4 „
Megaloblasts	21.2 „
Free nuclei	83.8 „

A large number of the free nuclei were megaloblastic.

Rabbits 20 and 21 were killed fourteen days after inoculation. In both animals the colour index on the day of death was 1·2, and the number of red cells was still more than 2 millions per c.mm. less than on the day of inoculation; measurements of red cell diameter were not made; blood films taken day by day showed much anisocytosis, polychromasia, and basophilia, but the number of nucleated red cells was not so great as in the blood of the other animals. In both cases the marrow showed large excess of nucleated red cells over the assumed normal, the increase being relatively greatest among the normoblasts. In both there is a large percentage of polymorpho-nuclear leucocytes which is masking the number of the lymphoid cells, especially in the case of 20, where the marrow has a leucoid type.

The marrow of rabbits 10, 27, and 29 were not examined. Rabbit 10 is chiefly noticeable for the enormous number of nucleated red cells present in the blood on the seventh day after inoculation, 339 were noted in a count of 300 white cells. Rabbits 27 and 29 show very well the variations in diameter of the red cells over a long period.

Rabbits 3 and 4 were killed after an interval of two months; the marrow films then made still showed great excess of nucleated red cells due chiefly to free nuclei, many of which stained faintly and were less pycnotic and disposed to be diffuse, indicating probably a degenerated condition.

Rabbits 17 and 19 both received a second dose of 10 c.c. of the phenylhydrazin solution six weeks after the first dose. Rabbit 19, beyond a fall in hæmoglobin percentage and in the number of red cells, showed practically no changes in its blood, so much so that it seemed probable that the full dose had not been received; but, after the second dose, the changes, though more marked, were not so great as with rabbit 17, and the animal became ill and died eleven days after the second dose; the marrow was composed chiefly of granular myelocytes and leucocytes, and it was assumed that this rabbit had received some infection.

Rabbit 17 was killed on the fourteenth day after the second dose; the marrow showed a large excess of nucleated red cells, due chiefly to a relative increase in the number of normoblasts and free nuclei; the large primitive lymphocytes (myeloblasts) were also in excess of normal, but the lymphoid character of the marrow was masked by the large number (31·4 per cent.) of polymorpho-nuclear leucocytes (*vide* Tables IX. to XVI.).

The effects on the blood and bone marrow of a subcutaneous injection of 10 c.c. of a 1 per cent. solution of phenylhydrazin may now be summed up as follows:—

1. The production of cloudiness and a brown colour in the blood, which remain for a week after inoculation; the cloudiness apparently disappears before all trace of brown has gone, though possibly more delicate methods of observing slight degrees of opacity may demonstrate that these two appearances really disappear simultaneously.

2. The hæmoglobin percentage is lowered, falling apparently day by day, as is indicated, though probably not in an exact manner, by estimations made while the blood is still brown, to about 40 per cent. from the original percentage before receiving the drug, the lowest measurement being probably reached by the fourth day; by the fourteenth day the percentage figure has returned to within the limit of normal variation. This lowering of the hæmoglobin percentage is not, however, proportional to the lowering of the number of the red cells per c.mm., so that the colour index is raised, in all

cases reaching 1·3 to 1·6; this raised colour index still persists on the fourteenth day, and in several cases after an interval of three weeks.

3. The number of red cells per c.mm. is diminished; roughly, a fall of 1 to 1·5 millions occurs on each day succeeding the inoculation until the sixth day; a rise commences on the seventh day, and continues very gradually to approximate the original number by the end of three weeks (Table X.).

4. For normal rabbits the average diameter of 100 red cells was found to be about $6\cdot2\ \mu$; on the day after inoculation this was reduced by $0\cdot5\ \mu$ to $1\ \mu$, or to $5\cdot6\ \mu$; this diminution in size of the red cells continues up to the third day; I have no observations of measurements on the fourth and fifth days, but on the sixth day in the four rabbits that were examined the diameter was increased by $1\ \mu$ to $2\ \mu$, the highest average diameter recorded being $8\cdot4\ \mu$. This raised diameter persists up to three weeks after inoculation, and slowly returns to the normal in about six weeks. If the range of diameter of 100 red cells, which usually extends from $5\ \mu$ to $7\cdot5\ \mu$, is considered, it is found that the range increases on the first day to $3\cdot5\ \mu$ and continues to extend up to the eighth day, when in rabbit 15 it was as much as $7\ \mu$; about this time the range begins to diminish, and may return to the normal in about three weeks from the day of inoculation (Table XIV.).

5. Poikilocytes, schizocytes, and degenerated shadow cells were always seen in the stained films on the day following inoculation, usually increasing in number up to the eighth day, and then gradually disappearing by the fourteenth day. Polychromasia, amounting to over 1 per cent. of all the red cells, was also observed on the first day, and increased up to the eighth day, the highest record being 28 per cent. in rabbit 14 on the sixth day. Basophilia was usually first noted on the second day, increasing up to the eighth day, then diminishing, and being only occasionally met with on the fourteenth day (Table XV.).

6. Nucleated red cells usually appeared on the second or third day; in the case of rabbit 15 there were 14 per 100 white cells on the first day; the date on which the maximum is reached was variable, but usually this occurred six to eight days after inoculation. The number varies very considerably in different rabbits. Rabbit 10 showed as many as 113 per cent. on the seventh day, and 80 per cent. on the eighth day. Rabbit 15, 60 per cent. on the eighth day; after this date they rapidly diminish in number, and none were recorded after the thirteenth day in any animal (Table XIII.).

7. The number of white cells is usually slightly raised, but with the exception of rabbit 15 there was never any real leucocytosis; this raised white cells count, so far as was recorded, had ceased by the eighth day. The differential white cell counts, as mentioned above, showed no specific changes of type (Table XII.).

8. Analyses of the cellular elements of the bone marrow at different dates after inoculation show very marked changes from the normal. The most constant effect, which is especially apparent in the marrows of the first and third days after inoculation, is the increased percentage of polymorpho-nuclear leucocytes, which, as mentioned above, has a tendency to mask the true proportion of lymphoid cells, and is probably an indication that the marrow tissue is a seat of toxic irritation. In all the marrows there is, as might be expected, an increased number of nucleated red cells to every 100 white cells, but the proportions taken by the different kinds of nucleated red cells vary at different dates after inoculation. Table XVI. shows that on the first day the normal red cell regeneration is exaggerated, there being large increases in the number of normoblasts and megaloblasts, and the very large increase of free nuclei indicating that a number of new-made red cells have passed into the circulation. On the third day it seems as if the normal red cell regeneration cannot keep up with the blood demand, so that the normoblasts appear to be fewer, and the primitive erythroblasts are increased, the free nuclei, though still in excess, being also fewer, because many immature nucleated red cells have passed into the circulation; the supply of megaloblasts has been largely used up, so that these cells appear fewer, their place being taken by metrocytes. A similar process is noted on the fifth day. On the eighth day this is apparently still in progress, but the metrocytes are markedly less, and the free nuclei are increased in percentage, which is reflected in the raised number of red cells per c.mm., and by the diminished number of nucleated red cells in the circulating blood. The marrow films of the eighth day are also marked by a great increase in the number of small lymphoid cells and halo cells, a condition which I regard as preceding normoblast formation, and it is remarkable that in the marrow films of the eleventh and fourteenth days the number of normoblasts is enormously increased. Synchronously with this increase of small lymphoid cells, the number of metrocytes has diminished, or, in other words, the regeneration of red cells is leaving its unusual metrocytic type and returning to the normal type. It is also noticeable that in the marrows of the eleventh and subsequent days, the number of free nuclei show very high figures, which might be expected with an increased output of mature red cells, and the accumulation of their nuclei in the marrow. This condition persists in the marrow films prepared two months after inoculation.

It would therefore seem that after the subcutaneous injection of phenylhydrazin there is great red blood cell destruction, which continues for six to eight days; that regeneration commences on the first day after inoculation, but its effect is negatived by some direct toxic action in the marrow, which probably influences, firstly, the giant cells and their derivatives, the small lymphoid cells; and secondly, by increasing

the demand for polymorpho-nuclear leucocytes, and therefore for fine granular myelocytes, causes the large primitive lymphocytes—myeloblasts—to develop into granular myelocytes, so limiting their supply for the production of metocytes.

IV. COMPARISON OF THE EFFECTS OF HÆMORRHAGE AND PHENYLHYDRAZIN.

Comparing with these results the effects that follow severe hæmorrhage, it is found that

1. There is never any production of cloudiness or brown coloration.

2. The hæmoglobin percentage is lowered; the lowest measurement is reached on the day following the last bleeding, after which it slowly returns to the original figure by the twelfth to fourteenth day. The colour index is usually raised, but not invariably, being about 1.1 on the day after the last bleeding, and has usually returned to the normal by the eighth day. The highest record was 1.3 (Tables II. and IV.).

3. The number of the red cells per c.mm. diminishes until the day after the last bleeding, and commences to rise on the following day, returning to the original number by the tenth to fourteenth day (Table III.).

4. The average diameter of 100 red cells shows no diminution in measurement; on the day after the last bleeding the diameter commences to increase, the highest record being $7.6\ \mu$ on the eighth day; a slightly raised measurement persists until the fourteenth day. The range of diameters increased up to the third day after the last bleeding, the highest record being $5.25\ \mu$. A high range, however, was noted in rabbit 7 as long as six weeks after the last bleeding (Table VI.).

5. Poikilocytes, schizocytes, and shadow cells were rarely met with in the stained films. Polychromasia was usually about 1 per cent. on the second day after the first bleeding, and was greatest on the third, fourth, and fifth days, the highest record being 18 per cent. (rabbit BA) on the third day after the first bleeding; the appearance soon decreased, and was only occasional by the eighth day (Table VII.).

6. Nucleated red cells appear in the circulation on the second or third day after the first bleeding; the highest record was 60 per 100 white cells (rabbit BA) on the fourth day; but this figure was quite exceptional, the next highest number being 18 per cent.; they were rarely met with after the sixth day (Table V.).

7. No marked leucocytosis was observed; day by day differential counts were made in all cases, but showed no specific change in type.

8. Analyses of the cellular elements of the bone marrow (Table

VII.) at different dates after bleeding show that, unlike the marrows of the rabbits that were treated with phenylhydrazin, there is no marked increase in the polymorpho-nuclear leucocytes, so that the lymphoid character of a red cell regenerating marrow is made prominent. In the films made on the day after one not very severe hæmorrhage there was no great erythroblastic activity noticeable beyond an increase of small lymphoid cells, normoblasts, and free nuclei; on the fourth day the large primitive lymphocytes (myeloblasts) are markedly increased, but the chief increase is seen in the nucleated red cells, due especially to the free nuclei, but also to the normoblasts, primitive erythroblasts, megaloblasts, and metrocytes. Similar type of marrow is seen in films of the fifth day, the metrocytes numbering 16.2 per 100 white cells, or more than three times greater than any metrocyte count in the phenylhydrazin marrows.

Marrows of the eighth day after the first bleeding show a persistence of this metrocytic type of red cell regeneration, and unlike the phenylhydrazin marrows of this date, as might be expected, the percentage number of the small lymphoid cells is diminished. Films made after three weeks, show that the marrow has returned to a normal lymphoid type, and the number of nucleated red cells is still high.

Therefore the marrow activity appears to be more intense and continuous in these bled rabbits than in those which received phenylhydrazin. Nevertheless it would seem from the preceding histological account, that with phenylhydrazin poisoning there is more evidence in the blood of red cell destruction and regeneration, and that these appearances persist for a longer period than in the blood of rabbits which have been bled severely on two or three successive days; so that it might be suggested either that the rate of regeneration after phenylhydrazin was slower than after hæmorrhage, or that the dose of phenylhydrazin was too great in comparison with the amount of bleeding.

Neither of these suggestions appears to be correct.

Itami (1908⁵) and Ritz (1909⁶) state that regeneration is quicker after phenylhydrazin than after bleeding, but their conclusions are based on observations of the hæmoglobin and red cells per unit volume of blood, and not on any estimation of the total quantity of hæmoglobin in the circulation. Hence there is no certainty that the two series of experiments are comparable to one another in respect of the quantity of red cells lost to the economy. I have a short series of determinations by Welcker's method of the total amount of hæmoglobin in rabbits at varying periods after receiving 10 c.c. of 1 per cent. phenylhydrazin. Assuming that each animal had 0.7 c.c. oxygen capacity per 100 grms. of body weight before treatment, the following figures show the amount present in percentages of the normal quantity.

Rabbits.	Days after Inoculation.	Percentage of Total Oxygen Capacity Present.
Normal	0	100
1 }	1 }	77 }
2 }	1 }	80 }
3 }	3 }	47 }
4 }	3 }	57 }
5 }	5 }	63 }
6 }	5 }	57 }
7 }	8 }	63 }
8 }	8 }	49 }
9 }	14 }	100 }
10 }	14 }	68 }

These figures appear to show, in the first place, that the amount of red cell destruction produced by the phenylhydrazin was at any rate not greater than the amount lost by bleeding. The dose of the phenylhydrazin given destroys 40 to 50 per cent. of all the red cells, whereas by bleeding from 54 to 76 per cent. were removed. They also show that the action of phenylhydrazin is not complete during the first twenty-four hours, that there is no gain in the total oxygen capacity during the first eight days¹ (presumably because the drug continues to act during that period), and that subsequently the animal regenerates about 4 per cent. of its total normal hæmoglobin per day. This is much faster than the figures hitherto found for regeneration after hæmorrhage, $1\frac{1}{2}$ to 2 per cent. per day,² though owing to the differences in the amounts of bleeding, it can at present only be concluded that the rate of regeneration after phenylhydrazin, once it has begun, is certainly not less than after bleeding.

Attention must now be directed to the further proposition that the megaloblastic marrow and megalocytic blood of pernicious anæmia, which contrast with the low colour index of post-hæmorrhagic anæmia, are the effect of the blood destruction occurring in that disease,—the marrow being stimulated by the products of red cell destruction. The blood condition in phenylhydrazin poisoning is analogous to that of pernicious anæmia; as contrasted with a normal condition, there is metromegaloblastic marrow, megalocytic blood, and raised colour index. In normal marrow the large primitive lymphocytes (myeloblasts) go on to produce granular myelocytes, whereas in conditions of anæmia they are in part deviated to the formation of metrocytes from which megaloblasts are derived.

The experiments show that after severe hæmorrhage there is also metromegaloblastic marrow, a slightly megalocytic blood, and also a slightly raised colour index. But they further show that, although

¹ For a similar delay after anilin poisoning, see *Journ. Path. and Bacteriol.*, Cambridge, 1909, vol. xiii. p. 279.

² *Ibid.*, 1909, vol. xiii. p. 274.

with phenylhydrazin poisoning the blood is megalocytic to a much greater degree, and the colour index is also much higher than in the case of the animals that were bled, yet it appears from the films that the marrow is metromegaloblastic to a much smaller degree at all dates; so that it seems at first sight that this high degree of megalocytosis in the blood is not dependent on a high grade metromegaloblastic marrow, or, in other words, megalocytes do not seem to arise directly from megaloblasts or metrocytes.

On further consideration, however, this fallacy is readily explained. A metromegaloblastic marrow from which large numbers of megalocytes are supplied to the blood, would show fewer metrocytes and megaloblasts than a marrow from which fewer megalocytes are passed into the blood. In the case of phenylhydrazin poisoning some influence is in progress whereby the normal blood cell formation from small primitive cells is partially inhibited, and has to be reinforced by the production of metrocytes, which, either directly or indirectly after forming megaloblasts, separate from their nuclei and pass into the blood as cells of large diameter; the marrow is thereby relatively depleted of metrocytes and megaloblasts; this depletion is especially marked because, in the earlier days of the poisoning, the production of metrocytes is interfered with by the demand on the large primitive lymphocytes (myeloblasts), to form granular myelocytes, so that the metrocyte production is relatively less, and owing to the urgent demand of the blood, the marrow depletion of these cells is relatively greater.

In the case of hæmorrhage, the normal red cell formation in the marrow is not interfered with; it is stimulated, but, to satisfy the demand of the blood, especially after severe hæmorrhage, this normal red cell production is not sufficient, and has to be supplemented by the formation of metrocytes. Owing to the continued and exaggerated normal red cell production, it is reasonable to suppose that by these combined forces the output of red cells is now able to cope with the demand of the blood, and the urgency of the call for red cells is not so acute, so that a relatively greater number of metrocytes remain in the marrow and have time to complete their transition into megaloblasts, and of these cells, again, some pass out into the blood as megalocytes, but a relatively larger number than in the case of phenylhydrazin poisoning remain in the marrow and give rise to normoblasts. The marrow after severe hæmorrhage is therefore not depleted to such an extent of its metrocytes and megaloblasts, and in marrow films these cells appear in greater numbers.

Hence it must be concluded that when the number of red cells in the blood is greatly diminished, either by severe hæmorrhage or by destruction inside the body, there results a metromegaloblastic marrow which produces megalocytes of high colour index, and that this effect is much greater when the red cells are destroyed inside the body.

The conclusions to be drawn from these observations are:

TABLE III.—*Showing Number of Red Cells in Millions per C.mm. in the Blood of Rabbits that were bled, at different Dates after last Bleeding.*

Rabbit	III. (T)	IV. (T)	VII. (H)	VIII. (H)	BA. (H)	Bb. ² † (H)	Bc.† (H)	Bd.† (H)
Average number of red cells for 3 days previous to 1st bleeding	6·7	6·5	5·4	5·7	6·5	6·4	5·5	6·4
Day of 2nd bleeding	4·8	4·5	4·3	3·3	—	3·6	3·8	3·8
" 3rd bleeding	3·0	3·1	2·3	2·3	..	3·2	2·1	2·2
1 day after last bleeding . .	2·5	2·4	2·3	1·9	2·1	3·6	2·8	2·3
2 days " " " " " " " "	—	—	2·9	2·3	2·2	3·5	3·1	2·5
3 " " " " " " " "	2·6	2·4	2·7	2·6	2·3	3·6	—	—
4 " " " " " " " "	3·0	3·0	—	—	..	..	3·7	3·6
5 " " " " " " " "	3·6	3·6	3·2	3·4	..	..	3·9	3·7
6 " " " " " " " "	3·6	3·9	3·5	3·5	..	..	4·1	3·4
7 " " " " " " " "	4·3	3·6	3·6	3·6	..	..	4·2	4·2
8 " " " " " " " "	5·4	6·6	4·1	3·8	..	..	..	..
9 " " " " " " " "	..	..	3·7	4·2	..	..	..	..
10 " " " " " " " "	..	..	—	—	..	..	..	..
11 " " " " " " " "	..	..	—	—	..	..	..	..
12 " " " " " " " "	..	..	4·2	5·0	..	..	..	..
13 " " " " " " " "	..	..	—	—	..	..	..	..
14 " " " " " " " "	..	..	4·0	4·8	..	..	..	..
15 " " " " " " " "	..	..	4·2	4·5	..	..	..	..
1 month later	..	..	4·2	5·1	..	..	..	..

† Only bled on two successive days. T = Toisson's fluid. H = Hayem's solution.

TABLE IV.—* *Colour Index at different Dates after Bleeding.*

Rabbit	III. (T)	IV. (T)	VII. (H)	VIII. (H)	BA. (H)	Bb. ² † (H)	Bc.† (H)	Bd.† (H)
Average colour index for 3 previous days.	0·64	0·63	0·61	0·64	0·62	0·63	0·70	0·63
Day of 1st bleeding	1·0	1·0	1·0	1·0	1·0	0·9	0·9	1·0
" 2nd bleeding	0·9	1·0	1·1	1·1	—	1·2	0·9	0·9
" 3rd bleeding	0·9	0·9	1·3	1·3	..	0·9	1·1	1·0
1st day after last bleeding .	0·9	1·0	0·9	1·2	1·1	1·2	1·0	1·2
2 days " " " " " " " "	—	—	0·9	1·2	1·3	1·2	1·1	1·1
3 " " " " " " " "	1·1	1·3	1·1	1·3	1·3	1·2	—	—
4 " " " " " " " "	1·0	1·2	—	—	..	..	1·1	1·0
5 " " " " " " " "	1·2	1·2	1·1	1·1	..	..	1·1	1·1
6 " " " " " " " "	1·1	1·1	1·0	1·3	..	..	1·1	1·2
7 " " " " " " " "	1·0	1·3	1·2	1·3	..	..	1·1	1·2
8 " " " " " " " "	0·9	0·7	1·0	1·3	..	..	..	..
9 " " " " " " " "	..	..	1·0	1·2	..	..	..	..
10 " " " " " " " "	..	..	—	—	..	..	..	..
11 " " " " " " " "	..	..	—	—	..	..	..	..
12 " " " " " " " "	..	..	1·0	1·1	..	..	..	..
13 " " " " " " " "	..	..	—	—	..	..	..	..
14 " " " " " " " "	..	..	1·0	1·1	..	..	..	..
15 " " " " " " " "	..	..	1·1	1·1	..	..	..	..
1 month later	..	..	1·2	1·2	..	..	..	..

* The index is calculated with the average of 2-3 previous days regarded as unity.
† Only bled on two successive days.
(T) = Toisson's fluid. (H) = Hayem's solution.

TABLE V.—*Showing Number of Nucleated Red Cells for each 100 White Cells counted on stained Films.*

Rabbit	III.	IV.	VII.	VIII.	BA.	Bb. ²	Bc.	Bd.
Day of 1st bleeding	0	0	0	0	0	0	0	0
" 2nd bleeding	0	0	0	1	0	0	0	1
" 3rd bleeding	13	5	0	1	2	0	0	1
1 day after last bleeding	7	8	1	0	5	0	2	2
2 days	—	1	2	60	0	0	—	1
3 " " " "	6	8	4	3	18	0	—	—
4 " " " "	15	8	—	—	..	..	0	0
5 " " " "	2	0.6	2	..	..	..	0	1
6 " " " "	5	0.6	3	0	..	..	0	0
7 " " " "	—	0	0	0	..	..	0	0
8 " " " "	1	0	0	0	..	..	..	..
9 " " " "	2	0	2	0	..	..	..	..
10 " " " "	..	..	—	—	..	..	..	..
11 " " " "	..	..	—	—	..	..	..	..
12 " " " "	..	..	3	0	..	..	..	..
13 " " " "	..	..	—	—	..	..	..	..
14 " " " "	..	..	0	0	..	..	..	..
15 " " " "	..	..	0	0	..	..	..	..
1 month later	..	..	1	0	..	..	..	..

TABLE VI.—*Diameter of Red Cells at different Dates after Bleeding, representing the Average Diameter of 100 Red Cells in Terms of μ .*

Rabbit	III.	IV.	VII.	VIII.	Ba.	Be. ²	Range of Diameter.					
							III.	IV.	VII.	VIII.	Ba.	Be. ²
Average for 3 days before first bleeding	6.0	6.2	6.2	6.2	5.8	6.1	2.75	2.0	2.75	2.0	2.25	2.0
Day of 2nd bleeding	5.7	5.9	6.5	6.7	6.1	6.0	2.25	2.0	3.0	2.5	2.25	2.75
" 3rd bleeding	6.2	6.5	6.1	6.2	6.2	6.2	3.0	4.25	3.75	4.75	3.0	2.75
1 day after third bleeding	6.3	6.4	6.7	6.8	6.7	6.7	3.75	4.5	4.25	5.0	5.25	3.25
2 days			6.4	7.3	6.5	6.4			5.25	5.25	5.0	3.25
3 "	7.2	7.1	7.1	7.3	6.6	6.7	5.25	4.5	5.0	5.0	5.0	3.5
4 "	6.6	6.7					3.75	5.25				
5 "	7.1	6.7	7.1	7.6			5.25	4.0	3.25	4.0		
6 "	7.0	6.8	6.7	7.2			5.25	3.5	4.75	4.0		
7 "	6.5	7.0	6.6	7.3			4.0	4.5	4.25	3.5		
8 "	6.5	6.1	6.9	7.3			3.25	3.25	5.0	3.5		
9 "			6.7	6.8				4.5	4.0			
10 "												
11 "												
12 "			6.1	6.8					5.5	3.75		
13 "												
14 "			6.5	6.9					4.75	3.25		
1 month later			6.5	6.6					4.75	2.75		

TABLE VII.—*Showing the Number of Polychromatic Red Cells per 1000 Red Cells at different Dates after Bleeding.*

Rabbit	III.	IV.	VII.	VIII.	BA.	Bs. ²
1st day of bleeding	0	0	0	0	0	0
2nd " "	0	0	0	2	0	2
3rd " "	1	3	1	6	2	4
1 day after last bleeding	5	9	5	17	18	14
2 days	—	—	2	11	11	11
3 " " " "	11	16	17	6	15	3
4 " " " "	6	11	—	—	..	..
5 " " " "	5	10	2	7	..	..
6 " " " "	5	5	0	3	..	..
7 " " " "	1	0	0	0	..	..
8 " " " "	1	0	2	1	..	..
9 " " " "	..	..	5	2	..	..
10 " " " "	..	..	—	—	..	..
11 " " " "	..	..	—	—	..	..
12 " " " "	..	..	1	0	..	..
13 " " " "	..	..	—	—	..	..
14 " " " "	..	..	1	0	..	..
15 " " " "	..	..	..	..	..	..
1 month after	..	..	0	0	..	..

TABLE X.—*Showing Number of Red Cells in Millions per c.mm. in the Blood of Rabbits, at different Dates after receiving 10 c.c. 1 per Cent. Solution Phenylhydrazin subcutaneously.*

Rabbit . .	3 (T)	4 (T)	6 (T)	7 (T)	8 (T)	10 (T)	11 (T)	12 (T)	14 (T)	15 (T)	17 (T)	19 (T)	20 (T)	21 (T)	23 (T)	25 (H)	X (H)	27 (H)	29 (H)
Average of 3 days previous and on day of inoculation	5.1	5.6	5.5	5.8	5.4	4.4	4.3	5.3	4.8	6.0	5.7	4.5	5.2	6.5	6.5	5.0	5.3	6.2	7.0
1 day after	3.3	5.1	4.0	4.5	4.2	3.1	3.0	3.4	2.8	4.2	—	—	—	—	—	—	—	—	—
2 days after	2.5	3.0	—	—	3.1	1.6	—	—	2.2	2.8	—	—	—	—	—	—	—	—	—
3 " "	1.6	3.2	—	—	1.4	1.3	—	—	1.7	1.6	—	—	—	—	—	—	—	—	—
4 " "	1.4	2.7	—	—	—	—	1.6	1.9	—	—	—	—	—	—	—	—	—	—	—
5 " "	1.7	2.8	—	—	—	—	1.3	1.8	—	—	—	—	—	—	—	—	—	—	—
6 " "	—	—	—	—	—	—	—	—	1.5	1.1	—	—	—	—	—	—	—	1.9	1.4
7 " "	3.2	3.5	—	—	—	—	—	—	2.3	1.9	—	—	—	—	—	—	1.6	2.6	2.1
8 " "	—	—	—	—	—	—	—	—	2.5	2.0	1.9	3.5	2.5	2.5	1.7	2.2	2.0	2.6	2.4
9 " "	3.7	3.8	—	—	—	—	—	—	—	—	2.0	4.0	3.2	4.3	—	3.3	—	—	—
10 " "	—	—	—	—	—	—	—	—	—	—	2.4	4.4	—	—	—	—	3.5	3.3	2.9
11 " "	—	—	—	—	—	—	—	—	—	—	—	—	3.4	3.9	—	—	3.6	4.3	3.5
12 " "	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3.5	4.7	3.4
13 " "	—	—	—	—	—	—	—	—	—	—	3.4	4.5	3.6	3.9	—	—	4.0	4.7	3.6
14 " "	—	—	—	—	—	—	—	—	—	—	3.1	4.9	—	—	—	—	4.2	4.9	4.4
15 " "	—	—	—	—	—	—	—	—	—	—	3.4	4.7	—	—	—	—	—	—	—
16 " "	—	—	—	—	—	—	—	—	—	—	4.0	4.8	—	—	—	—	—	—	—
17 " "	—	—	—	—	—	—	—	—	—	—	3.3	4.6	—	—	—	—	4.9	4.9	4.3
18 " "	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
19 " "	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4.7	5.1	5.0
20 " "	—	—	—	—	—	—	—	—	—	—	4.5	4.7	—	—	—	—	—	5.2	4.6
21 " "	—	—	—	—	—	—	—	—	—	—	4.6	4.8	—	—	—	—	—	—	—
14 days later	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5.5	6.5	6.7

(T)=Toisson's fluid. (H)=Hayem's solution.

TABLE XI.—*Colour Indices at different Dates after receiving 10 c.c. 1 per Cent. Solution of Phenylhydrazin subcutaneously, the Average for Three Days previous to and including Day of Inoculation being regarded as Unity.*

Rabbit . .	3	4	6	7	8	10	11	12	14	15	17	19	20	21	23	25 (H)	X (H)	27 (H)	29 (H)
Day of inoculation	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
1 day after	1.22	0.92	1.0	1.0	1.12	0.82	0.92	1.12	1.32	0.92	—	—	—	—	—	—	—	—	—
2 days after	1.12	1.32	—	—	1.02	1.22	—	—	1.22	1.22	—	—	—	—	—	—	—	—	—
3 " "	1.22	1.12	—	—	1.52	1.32	—	—	1.22	1.52	—	—	—	—	—	—	—	—	—
4 " "	1.22	1.12	—	—	—	—	1.22	1.3	—	—	—	—	—	—	—	—	—	—	—
5 " "	1.12	1.12	—	—	—	—	1.22	1.3	—	—	—	—	—	—	—	—	—	—	—
6 " "	—	—	—	—	—	—	—	—	1.3	1.9	—	—	—	—	—	—	—	1.4	1.5
7 " "	—	—	—	—	—	—	—	—	1.3	1.3	—	—	—	—	—	—	1.5	1.3	1.4
8 " "	—	—	—	—	—	—	—	—	1.3	1.5	1.4	1.0	1.4	1.6	1.6	1.6	1.3	1.5	1.6
9 " "	1.0	1.2	—	—	—	—	—	—	—	—	1.7	0.87	1.1	1.0	—	1.1	—	—	—
10 " "	—	—	—	—	—	—	—	—	—	—	1.7	0.87	—	—	—	—	1.1	1.2	1.3
11 " "	—	—	—	—	—	—	—	—	—	—	—	—	1.2	1.1	—	—	—	1.1	1.3
12 " "	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.2	1.1	1.3
13 " "	—	—	—	—	—	—	—	—	—	—	1.4	0.9	1.2	1.2	—	—	1.0	1.2	1.4
14 " "	—	—	—	—	—	—	—	—	—	—	1.5	0.8	—	—	—	—	0.9	1.1	1.1
15 " "	—	—	—	—	—	—	—	—	—	—	1.3	0.7	—	—	—	—	—	—	—
16 " "	—	—	—	—	—	—	—	—	—	—	1.2	0.8	—	—	—	—	—	—	—
17 " "	—	—	—	—	—	—	—	—	—	—	1.3	0.8	—	—	—	—	0.9	1.2	1.2
18 " "	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
19 " "	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.0	1.2	1.0
20 " "	—	—	—	—	—	—	—	—	—	—	1.1	0.7	—	—	—	—	—	—	—
21 " "	—	—	—	—	—	—	—	—	—	—	1.1	0.7	—	—	—	—	—	1.0	1.1

(H)=Hayem's solution.

TABLE XIV.—*Diameters of Red Cells at different Dates after receiving 10 c.c. Phenylhydrazin Solution subcutaneously. The Diameters represent the Average of 100 Cells expressed in Terms of μ .*

Rabbits . . .	Average Diameter of 100 Cells.								Range of Diameter.							
	6	7	8	10	14	15	27	29	6	7	8	10	14	15	27	29
Day of inoculation . . .	6.5	6.4	6.5	6.2	6.3	6.6	6.2	6.1	2.5	2.5	2.25	2.5	2.75	2.75	2.5	2.25
1 day after . . .	5.8	5.4	5.7	5.7	5.4	5.7	—	—	2.75	3.25	2.5	3.25	3.75	5.0	—	—
2 days " . . .	—	—	5.2	6.2	5.9	5.5	—	—	—	—	3.0	4.75	4.25	4.5	—	—
3 " " . . .	—	—	6.3	6.5	5.8	6.2	6.2	5.5	—	—	4.5	4.5	4.25	5.0	6.5	2.0
4 " " . . .	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5 " " . . .	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6 " " . . .	—	—	—	—	7.9	8.1	8.4	7.1	—	—	—	—	5.5	6.25	4.75	6.0
7 " " . . .	—	—	—	—	8.5	8.3	8.1	7.5	—	—	—	—	3.75	6.75	4.25	5.75
8 " " . . .	—	—	—	—	8.0	8.6	7.9	8.0	—	—	—	—	6.0	7.0	4.75	4.75
9 " " . . .	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10 " " . . .	—	—	—	—	—	8.1	7.8	—	—	—	—	—	—	—	4.5	3.25
11 " " . . .	—	—	—	—	—	7.6	7.2	—	—	—	—	—	—	—	4.0	4.25
12 " " . . .	—	—	—	—	—	7.5	7.3	—	—	—	—	—	—	—	5.0	3.75
13 " " . . .	—	—	—	—	—	7.4	7.6	—	—	—	—	—	—	—	3.5	3.75
14 " " . . .	—	—	—	—	—	7.4	7.6	—	—	—	—	—	—	—	3.0	3.0
15 " " . . .	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
16 " " . . .	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
17 " " . . .	—	—	—	—	—	7.1	7.3	—	—	—	—	—	—	—	3.5	2.75
18 " " . . .	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
19 " " . . .	—	—	—	—	—	7.2	6.5	—	—	—	—	—	—	—	3.0	2.75
20 " " . . .	—	—	—	—	—	6.9	6.8	—	—	—	—	—	—	—	3.0	2.5
21 " " . . .	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
22 " " . . .	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

TABLE XV.—*Showing Number of Polychromatic Cells per 100 Red Cells at different Dates after receiving Phenylhydrazin.*

Rabbits	6	7	8	10	14	15	27	29
Day of inoculation	0	1	0	0	0	1	1	0
1 day after	2	0	0	2	5	7	—	—
2 days "	—	—	9	11	9	5	—	—
3 " "	—	—	20	13	4	19	18	0
4 " "	—	—	—	—	—	—	—	—
5 " "	—	—	—	—	—	—	—	—
6 " "	—	—	—	—	28	14	17	14
7 " "	—	—	—	—	21	19	11	21
8 " "	—	—	—	—	22	18	3	12
9 " "	—	—	—	—	—	—	—	—
10 " "	—	—	—	—	—	—	4	13
11 " "	—	—	—	—	—	—	4	2
12 " "	—	—	—	—	—	—	3	2
13 " "	—	—	—	—	—	—	1	2
14 " "	—	—	—	—	—	—	0	0
15 " "	—	—	—	—	—	—	—	—
16 " "	—	—	—	—	—	—	—	—
17 " "	—	—	—	—	—	—	0	1
18 " "	—	—	—	—	—	—	—	—
19 " "	—	—	—	—	—	—	1	0
20 " "	—	—	—	—	—	—	3	1
21 " "	—	—	—	—	—	—	—	—
22 " "	—	—	—	—	—	—	—	—
1 month after	—	—	—	—	—	—	0	0

TABLE XVI.—*Differential Cellular Percentages of Bone Marrow at different Dates after receiving Phenylhydrazin.*

Days after Inoculation.	Rabbit.	Small Primitive and Halo Cells.	Large Primitive Lymphocytes (Megablasts.)	Other Lymphoid Cells.	Total Lymphoid Cells.	Fine Oxyphil Granular Myelocytes.	Coarse Oxyphil Granular Myelocytes.	Basophil Granular Myelocytes.	Polymorphonuclear Leucocytes.	Total Leucoid Cells.	Normoblasts.	Primitive Erythroblasts.	Metrocytes.	Megablasts.	Free Nuclei.	Total Nucleated Red Cells.
1	6	29.8	14.6	7.6	52.0	23.2	0.8	3.0	21.0	48.0	11.4	5.8	0.4	11.4	12.2	41.2
3	7	23.8	18.4	15.2	57.6	25.8	1.8	1.8	13.0	42.4	11.6	1.6	0	7.2	23.2	43.6
5	8	19.6	12.6	6.2	38.4	34.0	2.0	3.4	22.2	61.6	2.4	6.8	5.6	2.0	14.8	31.6
7	10	19.4	13.8	7.0	40.2	34.4	1.6	2.8	21.0	59.8	0.8	4.4	4.2	2.0	19.4	30.8
9	11	27.6	28.8	11.8	68.2	19.6	1.0	3.0	8.2	31.8	6.0	9.4	4.4	4.0	20.4	44.2
11	12	28.8	22.2	9.8	60.8	23.0	0.8	1.8	13.6	39.2	4.4	6.0	2.2	4.2	19.2	36.0
13	14	43.6	28.8	6.4	78.8	13.0	1.0	0.4	6.8	21.2	4.4	11.4	0.2	8.0	43.8	67.8
15	15	33.0	25.2	5.6	63.8	24.0	0.6	0.6	11.0	36.2	2.0	3.6	0.6	2.2	29.2	37.6
17	23	46.2	15.6	5.2	67.0	14.2	0.6	0.8	19.4	33.0	2.8	2.6	0.4	3.4	23.2	34.4
19	20	14.4	23.2	5.0	42.6	21.6	1.0	2.0	32.8	57.4	17.8	9.0	0	7.0	27.2	61.0
21	25	30.0	23.4	9.6	63.0	13.4	1.0	1.6	21.0	37.0	35.8	6.8	1.4	21.2	83.8	149.0
2 months after	3	25.8	16.8	7.6	50.2	20.0	0.6	2.2	27.0	49.8	11.0	5.6	0.2	3.6	57.0	77.4
Normal	4	28.0	15.8	9.0	52.8	21.4	1.6	3.6	20.6	47.2	8.8	1.0	0	3.2	64.8	77.8
Normal	..	24.9	15.5	9.1	49.6	42.2	1.5	2.1	4.5	50.4	4.8	1.6	0	3.0	6.2	15.6

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