

SOME OBSERVATIONS ON THE CONDITION OF THE
BLOOD IN MEN ENGAGED IN ANILIN DYEING AND
THE MANUFACTURE OF NITRO-BENZINE AND ITS
COMPOUNDS.

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I. *Preliminary.*

It has long been known that men engaged in Anilin dyeing and in the manufacture of Nitro-benzine frequently suffer from various symptoms of poisoning. In the majority of cases the symptoms are slight and are not sufficiently severe to prevent the men from working; in some cases men are obliged to leave their work for longer or shorter periods. In a few cases death has occurred from the severity of the poisoning.

At the request of the Home Office Committee on Industrial Diseases, the towns of Bradford and Huddersfield were visited in January 1907 in order that information might be obtained as to the condition of the blood of the men engaged in Anilin black dyeing in the former and the manufacture of Nitro-benzine and its derivatives in the latter.

The following points were observed in all the cases examined:

The man's age, length of employment, number of times off work from illness due to trade; general appearance as indicated by cyanosis, and

conjunctival pallor. Examination of the blood included spectrum analysis, estimation of specific gravity, counts of both red and white corpuscles per c.mm., differential percentage count of the different kinds of leucocytes by means of stained films, and examination of the physical condition of the red corpuscles by the same means.

II. *Anilin Dyers.*

The men examined were selected on account of their being engaged in those processes which seemed most likely to lead to poisoning; in fact two of the cases were men who were off work in consequence of having been recently poisoned (Nos. 1 and 11). It will be noticed that of the 13 men examined none had been employed for less than one year, the longest, being 15 years. Of these thirteen men, 8 had never been off work at any time, but all had suffered at some time or other from symptoms of anilin poisoning; those most frequently complained of being headache, drowsiness, nausea, want of appetite, shortness of breath, palpitation, and tingling sensations in the feet and legs. In both the factories from which the cases were taken there was evidence that anilin vapour was present in the atmosphere. All the unpainted deal woodwork was stained a bright yellow colour which was said to be due to the anilin fumes in the air. In order to verify this statement strips of unpainted deal were exposed to anilin vapour in the laboratory when it was found that they quickly became stained yellow in the presence of even very small amounts of anilin vapour, thus giving a very delicate test for detecting its presence in the air. In every case the blood was examined spectroscopically but in no case was the spectrum of met-haemoglobin found to be present nor were any of the men cyanosed. Subsequent experiments proved however that the met-haemoglobin band could not be detected unless that substance was present in at least the proportion of 1 to 10 of oxy-haemoglobin.

The specific gravity of the blood was taken in every case and the percentage of haemoglobin calculated from it by means of Hammer-schlag's table, this being the only available method of estimating it and one which gives fairly accurate results; from this the colour index was determined.

The red corpuscles and leucocytes were counted with a Thoma-Zeiss haemacytometer, and the differential percentage count of the leucocytes was made from films stained by Jenner's method.

Among the anilin dyers the evidence of blood destruction was not very apparent from the blood counts. It will be seen from Chart No. I

TABLE I. *Anilin dyers.*

Case	Age	Length of employment		Off work	Cyanosis	Pallor	Specific gravity	Colour index	Red corpuscles	Leucocytes	Eosinophils	Polymorpho-nuclears	Lymphocytes	Large mono-nuclears	Mast	Basophil reas
	Years	1	2						5 4 4	15 10 5						
1	30	1	3	0	0	0	1056	1.0	4,448,000	7,280	.6	70	23	5	.3	+
2	34	1 1/2	0	+	+	+	56	.8	5,100,000	5,160	.6	66	30	5	.3	-
3	68	2	1	+	0	+	58	.8	5,400,000	10,000	1.6	63	30	6.3	1.0	-
4	26	2 1/2	1	+	0	+	52	.6	4,776,000	10,840	4.3	66	26	4.3	1.3	-
5	38	3	0	+	0	+	56	.8	4,840,000	9,960	4.3	70	22	3.3	.5	+
6	25	4	0	+	0	+	51	.8	4,456,000	12,440	5.3	43	48	3.6	2	+
7	46	4	0	0	0	0	54	.7	5,200,000	10,800	4	50	44	1.7	1	+
8	56	4	0	+	0	+	54	.7	4,464,000	4,240	0	50	47	2.5	2.5	-
9	27	4	0	+	0	+	48	.5	4,944,000	12,680	.6	80	17	3.6	.6	-
10	55	9	4	+	0	+	51	.6	5,280,000	6,640	1.6	69	22	5.3	1.3	-
11	28	9	1	0	0	0	56	.7	5,680,000	11,080	2.3	60	34	2.7	.3	-
12	46	12	2	+	0	+	51	.8	4,400,000	6,800	1.3	54	37	2.6	.9	+
13	35	15	1	0	0	0	58	.8	5,222,000	9,440	3.6	52	33	5.3	.3	+

that 6 out of the 13 cases examined had more than the normal number of red corpuscles per c.mm., the highest being 5,600,000 and the lowest 4,400,000. In all probability the effect of absorbing small doses of anilin daily, is to stimulate the production of red corpuscles by the bone-marrow, so that destruction is counter-balanced by renovation. Evidence of this was supplied by the low colour index and imperfect development of the corpuscles. None of the men showed signs of cyanosis at the time of examination, but nine of them showed conjunctival pallor. The specific gravity of their blood showed that there was a decrease in haemoglobin of from 5% to 50%. The colour index in the cases examined was with one exception below unity.

The number of leucocytes per c.mm. did not show any great departure from the normal; eight were above the average and five below. The highest was 12,000 and the lowest 4,000 per c.mm.

Examination of the stained blood films gave the most important indications of degeneration in the red corpuscles.

In most of the cases the variations in size of the corpuscles were considerable, ranging from 5 μ to 11 μ , the larger sizes being the most numerous. None showed any poikilocytosis but 6 out of the 13 showed basophil granulations in the red corpuscles. The number of cells affected varied from two or three in the whole film in the slight cases to 10 or 12 in every field of the microscope in the more pronounced ones. This reaction is of great significance, as it appears to be in anilin poisoning, as it is in lead poisoning, the earliest noticeable sign in the blood. No nucleated red corpuscles were found in any case, nor any pathological leucocytes.

The differential percentage leucocyte count showed a departure from the normal in several cases.

The number of polymorphonuclear cells showed a decrease in 9 out of the 13 cases; the decrease being considerable in cases 6, 7, 8, 12 and 13. There was a corresponding increase in the lymphocytes in these cases.

The mast cells were present in higher numbers than is normal in 6 of the 13 cases, but there does not appear to be any relation between these cases and those in which the polymorphonuclears were diminished. The eosinophils showed a percentage above the normal in three cases. Case No. 6, which showed the smallest percentage of polymorphonuclears, had the largest number of eosinophils, lymphocytes and mast cells.

III. *Conclusions concerning anilin.*

In anilin workers the following appear to be the most important points which are shown by a blood examination:

(1) An increase in the number of red corpuscles when the amount of poison absorbed is small and constant.

(2) A decrease in the specific gravity and haemoglobin of from 5% to 50%.

(3) A low colour index, showing that renovation of the corpuscles is proceeding more rapidly than the manufacture of haemoglobin.

(4) Degeneration or imperfect development of the red corpuscles as shown by the variations in size and the presence of corpuscles containing basophil granulations.

(5) Abnormal leucocytic percentages, consisting principally in a diminution in the polymorphonuclears and an increase in the other cells, particularly the lymphocytes, eosinophils and mast cells.

IV. *Nitro-benzine Workers.*

The 21 men examined were employed in the manufacture of di-nitro-benzol, as this substance is generally believed to be the most toxic of all the nitro-benzine series.

The men employed in the works visited are constantly shifted so that they do not work in the di-nitro-benzol department for more than a week or two at a time in consequence of the number of cases of poisoning which have occurred.

The examination was conducted on the same lines as that of the anilin dyers and the same facts noted. It will be noticed that the average length of employment is considerably shorter in the nitro-benzine workers than in the anilin dyers. Five of the 21 cases examined had been employed for one week or less, while only four had been employed for more than one year. This is no doubt owing to the dangerous nature of the trade. Very few workers escape poisoning at some time or other and so men do not care to remain long at it.

Excluding the five men who had been employed one week or less, 11 out of the remaining 16 had been off work at least once in consequence of being poisoned. All the men, including those who had not left their work, had suffered from some symptoms of poisoning, such as headache, drowsiness, loss of appetite, nausea, shortness of breath,

palpitation, cyanosis and pains in the legs. Of the cases recorded in Table II, No. 1 had only been employed for one day and therefore serves as a control case for purposes of comparison with the others, his blood being quite normal. Of the other 20 cases, 13 showed some degree of cyanosis; in most of them it was slight and confined to the lips, but in cases 6 and 8 it was well-marked. In these two cases spectroscopic examination showed the band of met-haemoglobin: in none of the others was it visible.

Most of the men had conjunctival pallor, the exceptions being those employed for one week or less (Nos. 1, 2, 3, 4, and 5). The specific gravity of the men's blood was below normal except in the five previously mentioned cases.

The colour index was in most cases nearly normal, this being probably due to the fact that blood destruction was unaccompanied by regeneration.

The number of red corpuscles per c.mm. was normal in only five cases, these again being the men who had only been employed for a very short time. Most cases had from 4 to $4\frac{1}{2}$ million per c.mm., whilst three were below four million, the lowest being 3,600,000.

The leucocyte count varied from 4,000 to 21,000 per c.mm., eight being above 10,000, five below 7,000, the rest between these figures.

The differential percentage leucocyte count showed the same features as in the anilin dyers.

Thirteen of the 21 cases showed a decrease in the number of polymorphonuclears and a corresponding increase in the lymphocytes. It will be observed that in cases 2, 5 and 6 (men who had only been employed for a very short time) there was already a considerable decrease in the polymorphonuclears. In four cases (Nos. 7, 8, 13 and 21) there was a marked increase in the number of eosinophils, but these cases do not seem to bear any relation to those in which there was a decrease in polymorphonuclears.

In ten cases (Nos. 2, 4, 6, 7, 8, 10, 13, 14, 17 and 21) there was a considerable increase in the number of mast cells. No myelocytes were seen in any case. Only in three were any nucleated red cells found and these in very scanty numbers.

The red corpuscles varied very much in size in nearly all cases (from $3\ \mu$ to $12\ \mu$), the larger sizes being the most common. In cases 4 and 15 some poikilocytes were seen. In 16 of the 21 cases basophil granulations were present in the red corpuscles, three exceptions being men who had been employed for less than a week. In some cases they

TABLE II. *Di-nitro-benzol workers.*

Case	Age	Length of employment		Off work	Cyanosis	Pallor	Specific gravity	Colour index	Leucocytes		Postinphils	Polymorpho-nuclears	Lymphocytes	Large mono-nuclears	Mast	Basophil reds
		Days	Months						Red corpuscles							
1	30	1		0	0	0	1060	1.0	5,120,000	8,640	2.3	71	26	1.3	.3	-
2	42	4		0	0	0	58	1.0	4,840,000	7,200	1.6	55	37	4	1.3	-
3	36	4		0	0	0	60	1.0	4,976,000	5,240	1.6	71	25	2	.3	-
4	27	7		0	0	+	60	1.0	5,120,000	8,840	2.3	66	26	3.6	1.0	+
5	22	7		0	0	0	58	.9	5,168,000	10,160	1	58	36	5	0	+
6	30	21		0	+	+	52	1.0	3,712,000	15,640	2.3	56	42	2.6	1	+
7	45	1		0	+	+	54	.9	3,792,000	14,640	8.3	63	24	5	1.6	+
8	23	1½		1	0	+	52	1.0	3,864,000	14,480	10	63	26	1	1.6	+
9	30	2		1	+	+	55	.9	4,264,000	21,160	.6	60	37	1	0	+
10	24	3		0	+	+	52	.8	4,032,000	14,360	2.6	74	22	2.6	1.3	-
11	32	3½		1	+	+	55	.9	4,336,000	7,960	2.3	63	31	2.6	0	+
12	26	4		1	0	+	57	1.0	4,280,000	16,920	0	68	28	4	0	+
13	51	4		1	+	+	55	.8	4,784,000	12,320	9	54	31	3	1.3	+
14	53	5		0	0	+	55	.9	4,040,000	6,520	.3	55	40	4.6	2	+
15	29	9		1	+	+	56	.8	4,240,000	10,280	1	42	56	3	.3	+
16	49	10		1	+	0	54	.8	4,880,000	9,360	2.3	63	31	2.6	0	+
17	34	1½		1	+	+	57	1.0	4,448,000	9,320	.3	66	31	1	1.3	+
18	47	1½		1	+	+	54	.9	4,168,000	4,400	2.3	47	47	3	0	-
19	41	2		0	0	+	54	.9	4,256,000	6,240	1.2	60	30	4	0	+
20	35	8		2	0	+	57	.9	4,576,000	8,760	.5	72	25	2.5	0	+
21	42	10		1	+	+	52	1.0	3,880,000	5,960	7	50	40	2.6	1.5	+

were few in number, while in others, noticeably in Nos. 6 and 8, the affected cells were very numerous, as many as 20 being present in every field of the microscope. It will be noticed that in cases 4 and 5 men who had been working for only one week, and whose red corpuscles were normal in number, yet showed a distinct basophil reaction, emphasising the fact that this is the earliest detectable blood sign in di-nitro-benzol as in anilin poisoning.

Spectroscopic examination showed only the oxy-haemoglobin bands in 19 cases, while in two (Nos. 6 and 8) a faint band in the red indicated the presence of met-haemoglobin in a proportion of at least 1 to 10 of the oxy-haemoglobin.

V. *Conclusions concerning di-nitro-benzol workers.*

(1) Di-nitro-benzol is more toxic than anilin, and causes more cases of acute poisoning than any other of the nitro-benzine series.

(2) It quickly affects the men who work in it, even within one week.

(3) The occurrence of red corpuscles showing basophil granulations is the first recognisable sign in the blood of poisoning by this substance.

(4) The number of red corpuscles is reduced after a short time by 1 to $1\frac{1}{2}$ million per c.mm.

(5) The specific gravity and haemoglobin content are reduced in about the same ratio as the red corpuscles; therefore the colour index is not far removed from unity.

(6) Some leucocytosis, principally lymphocytosis, occurs at some stage in chronic poisoning.

(7) Even when there is considerable cyanosis the presence of met-haemoglobin cannot be detected spectroscopically except in the more severe cases.

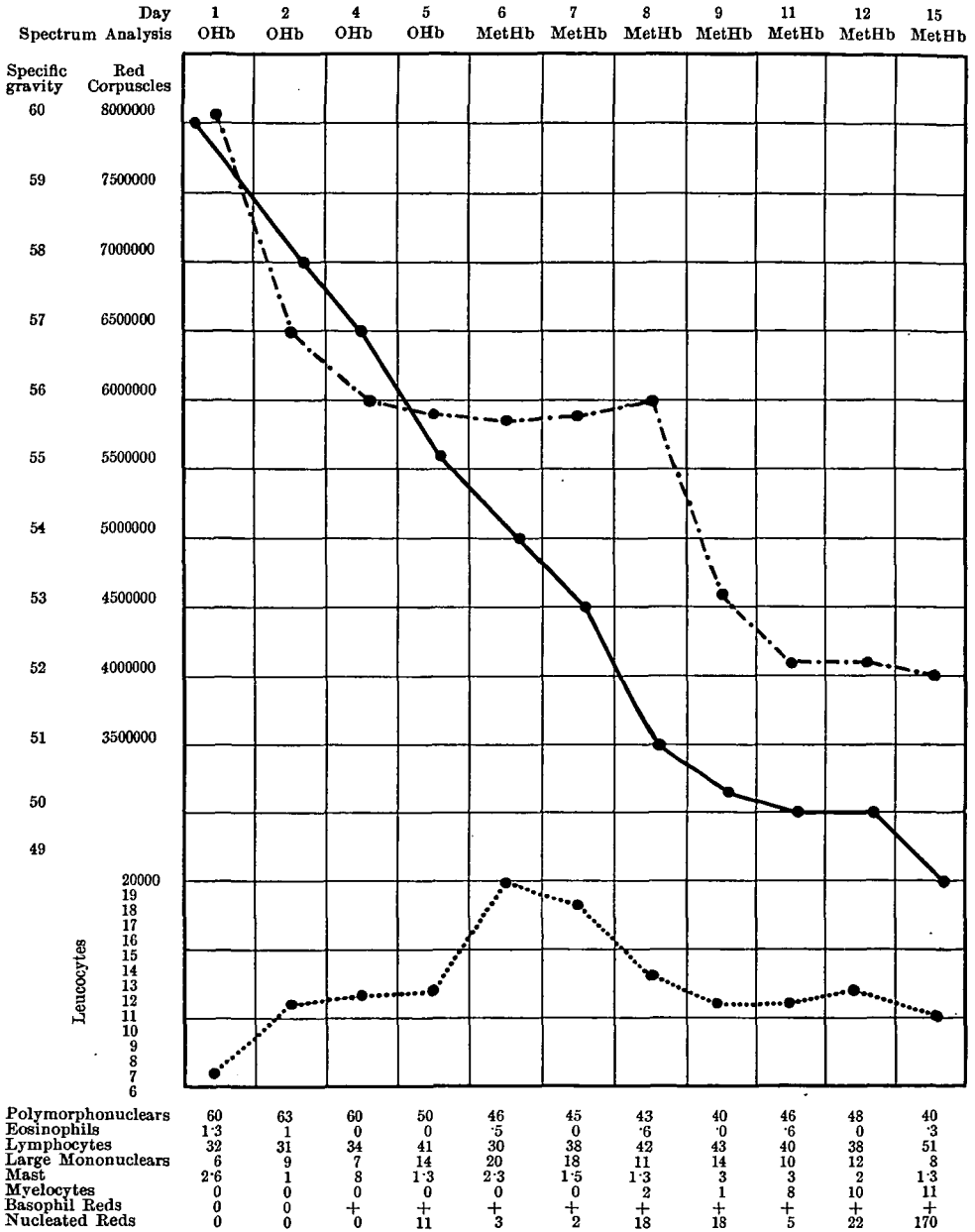
VI. *Animal experiments.*

In order to determine if possible the sequence of events in chronic anilin poisoning the following experiment was performed:

A rabbit weighing 2650 grammes was given subcutaneous injections of a 30% solution of anilin hydrochloride, and received 8 injections in 12 days; the initial dose was 0.1 c.c.: this was increased to .2 c.c. on the 5th day, and to .3 c.c. on the 6th and following days. On the last two days of the experiment the animal was placed in a chamber containing anilin vapour for half an hour.

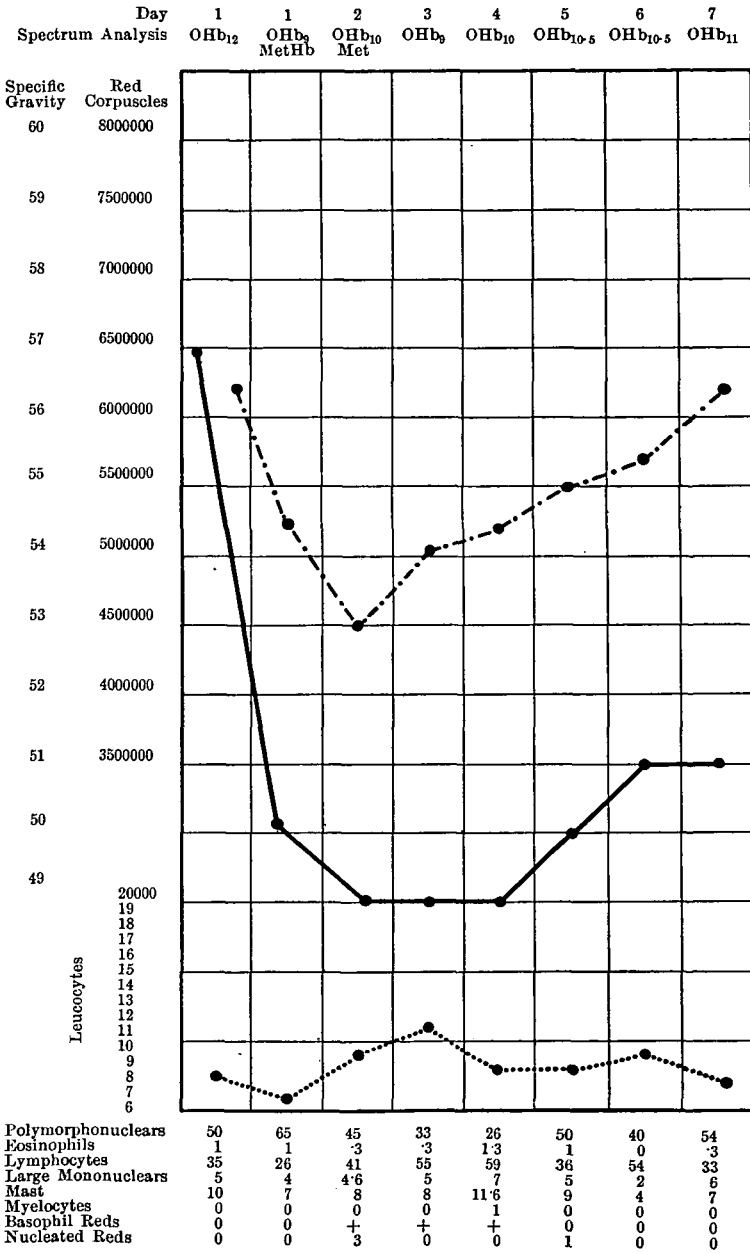
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CHART I.



Thick line=specific gravity.
 Interrupted line=number of red corpuscles.

CHART II.



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Chart I gives the results of this experiment.

There was a constant and progressive fall in the specific gravity of the blood (thick line).

For the first ten days the oxy-haemoglobin bands were the only ones visible spectroscopically. On the 11th day the met-haemoglobin band was seen and remained visible until the animal was killed.

The red corpuscles showed an almost continuous decrease in number, rapid at first, but slower later on (interrupted line).

The number of leucocytes increased for the first 6 days from 7,000 to 19,000 per c.mm. and after that gradually fell.

The differential percentage leucocyte count showed a progressive decrease in the number of polymorphonuclears, with a corresponding increase in the number of lymphocytes.

The large mononuclears increased in number up to the 6th day and after that decreased.

On the 8th day of the experiment polymorphonuclear myelocytes were found to be present in the blood and continued in increasing numbers until the animal was killed.

At no time were many basophil red corpuscles found, but on the fourth day polychromasia was present and continued to increase, until on the last day of the experiment there were as many cells showing this change as there were normal corpuscles. Poikilocytes were also present during the last six days of the animal's life.

On the sixth day nucleated red cells were seen and continued in increasing numbers until the last day of the experiment, when they were present in very large numbers.

In order to determine the time taken to recover from severe anilin poisoning the following experiment was performed :

A rabbit weighing 2850 grammes was placed in a chamber of anilin vapour at a temperature of 30° C. for one hour. Before the experiment began the animal's respirations were 72 and its heart beats 128 per minute.

On removal from the anilin chamber the animal seemed much exhausted and was unable to stand up ; its respirations were 158 per minute and of the Cheyne Stokes type, the heart beats were too rapid to count accurately, but were over 200 per minute.

The veins in the ears were much dilated and looked blue, as did also the nose and lips.

The blood examined spectroscopically gave the met-haemoglobin band.

One hour after removal from the anilin chamber the animal had nearly recovered and was able to move about, and on the following day it appeared quite well and took its food well. The met-haemoglobin band was still visible spectroscopically.

On the third day the met-haemoglobin band had disappeared.

The next experiment was undertaken in order to determine the sequence of events which occur in the blood after a single exposure to anilin vapour. The results are indicated in Chart II.

A rabbit was placed in a chamber containing anilin vapour at a temperature of 32° C. for 1½ hours. Examination of the animal's blood before the experiment began, showed it to be normal in every respect. After removal from the anilin chamber the animal was much exhausted and was unable to stand or sit but lay in any position in which it was placed. Its respirations were very hurried and of the Cheyne Stokes type. The veins in its ears were much dilated and its nose and lips were blue. The animal remained in a semi-comatose state for about two hours and after that time gradually recovered. Its blood was examined two hours after removal from the anilin chamber; the results of this examination are shown in the second column of Chart II. The fall in specific gravity was very marked; that in the red corpuscles less so and in the leucocytes still less. The met-haemoglobin band was well marked when the blood was examined spectroscopically.

The differential leucocyte count showed a slight increase in the percentage of polymorphonuclears, but was otherwise not much changed.

On the following day the animal had quite recovered and took its food well.

It will be seen from the chart that the specific gravity had continued to fall. The number of red corpuscles per c.mm. had fallen considerably but the leucocytes had somewhat increased. The differential percentage leucocyte count showed a considerable increase in the lymphocytes and a corresponding decrease in the polymorphonuclears. The red corpuscles showed great variations in size with a moderate number of polychromatic and basophil cells. Three nucleated red cells were seen. The met-haemoglobin band had disappeared from the spectrum. On the succeeding days the specific gravity remained at 1049 for two days and then gradually rose.

The red corpuscles quickly increased in numbers and on the seventh day were as numerous as they were before the experiment. The leucocytes increased in number till the third day and then diminished.

The polymorphonuclears decreased till the fourth day and after that appeared in normal numbers. The lymphocytes on the other hand increased till the fourth day and then diminished. The other leucocytes did not vary in any marked degree. Basophil reds were present for eight days and after that ceased to appear. One myelocyte was seen on the fourth day and one nucleated red cell on the fifth day.

Conclusions from animal experiments.

These experiments show that anilin whether injected subcutaneously or inhaled as vapour very rapidly produces its destructive action on the blood.

This action is manifested by the following points in chronological sequence.

- (1) Production of met-haemoglobin in the blood.
- (2) Haemolysis and destruction of red corpuscles.
- (3) Rapid fall in the specific gravity and haemoglobin content of the blood, accompanied by a slight reduction in the number of leucocytes.

(4) A continued increase in the number of polymorphonuclears with a corresponding increase in the lymphocytes.

(5) The occurrence in the red corpuscles of basophil granules and polychromasia.

(6) The occurrence of nucleated red cells in severe cases.

(7) Recovery after severe poisoning is rapid and continuous and apparently no permanent disability is entailed.

Summary.

These animal experiments corroborate the findings in the blood of anilin and nitro-benzine workers and enable us to follow the sequence of events, which appear to be the same in cases of poisoning by either of these bodies, though they are much more marked in the case of the latter.

These bodies may gain access to the body either by (1) inhalation of their vapours; (2) absorption through the skin; (3) absorption from the alimentary canal after being swallowed.

Probably the first method occurs most commonly, since cases of poisoning are much more frequent in hot close weather than during the colder seasons of the year. No matter how they are absorbed these bodies appear to have the same action on the blood, which is as follows:

They first convert the oxy-haemoglobin of the red corpuscles into met-haemoglobin, this is followed by haemolysis, degeneration of cytoplasm and escape of haemoglobin into the plasma.

If a considerable amount of met-haemoglobin is present in the blood, the respiratory capacity of the red corpuscles is diminished, the patient becomes cyanosed and the colour of the blood is changed to a chocolate brown. This fact renders the estimation of haemoglobin by the ordinary colour standards impossible, as they are based on the colour of pure oxy-haemoglobin, which is quite different from that of met-haemoglobin.

In the less severe cases of poisoning in which met-haemoglobin is not present in sufficient amount to be detected spectroscopically, the changes which may be found in the blood are sufficiently characteristic to enable a diagnosis of anilin or nitro-benzine poisoning to be made.

These consist in:

(1) A decrease in the percentage of haemoglobin as estimated from the specific gravity of the blood of from 5% to 50%.

(2) A decrease in the number of red corpuscles if the amount of poison absorbed is considerable ; if the dose is very small this decrease is not found, blood formation apparently keeping pace with blood destruction.

(3) Degeneration and imperfect development of the red corpuscles as shown by the occurrence of basophil granulations, polychromasia, poikilocytosis, and variations in size.

(4) The presence of nucleated red corpuscles in severe cases.

(5) A decrease in leucocytes rapidly followed by an increase ; the increase being principally due to the number of lymphocytes.

The simultaneous occurrence of all or several of these signs in the blood enables a diagnosis of poisoning by anilin or nitro-benzine to be made in quite mild cases.

Cessation from work for a short time enables the blood to recover rapidly from the effects of poisoning.

The best treatment in any severe case of poisoning by these bodies appears to be inhalation of oxygen.

Haldane, Makgill, and Mavrogordato¹ found that mice could be kept alive in oxygen at a pressure of two atmospheres even when 90 % of their blood had been converted into met-haemoglobin by injection of sodium nitrite. They also showed that animals showing severe symptoms of di-nitro-benzol poisoning recovered rapidly when placed in oxygen, and that as other substances besides met-haemoglobin are produced by the decomposition of the haemoglobin, the absence of a met-haemoglobin band is no evidence that the symptoms are not due to oxygen asphyxia.

Permission to publish this paper has kindly been given by Mr H. L. Samuel, Chairman of the Home Office Committee on Industrial Diseases.

¹ *Journ. of Physiology*, Vol. *xxi*. p. 160, 1897.