THE HÆMATOLOGY OF CARBON-MONOXIDE POISONING.

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(*A contribution from the Laboratory of the Provincial Board of Health of Ontario.*)

That the poisonous nature of coal or illuminating gas[1] is due to the carbon monoxide which it contains has long been known. Haldane[2] has shown that the hæmoglobin of the blood has an affinity for carbon monoxide about 140 times as great as that which it has for oxygen. He has also placed beyond doubt the fact that carbon monoxide is not used up or oxidized in the body but is excreted by the lungs unchanged, and concludes that carbon monoxide acts as a poison purely in a negative way by uniting with the hæmoglobin of the blood, thus throwing it out of commission as an oxygen carrier and thereby preventing normal oxidation in the tissues.

It was with the object of ascertaining what the ultimate action of carbon monoxide was upon the body and whether it played any great part in producing anæmia, which has so often been associated with leaking gas pipes and sometimes found in persons breathing gas contaminated atmosphere, that this investigation was undertaken. Moreover, it was hoped that we might arrive at some conclusion as to why some individuals subjected to acute coal-gas poisoning recover rapidly and suffer little after-effects, while others linger for three or four days and finally succumb. Our experiments have been made throughout on guinea-pigs.

Chronic coal gas poisoning.

At the beginning of this work the animals were placed in a cage and a mixture of air and pure carbon monoxide passed through the cage until 50 % of the hæmoglobin of the guinea-pigs was saturated with gas. As guinea-pigs just retain consciousness with 50 % saturation they were kept at this point for one hour and then removed. Such doses having

no apparent effect when repeated daily for weeks either on the blood, weight, or health, another method was resorted to. The animals were placed in cages in an air-tight chamber continuously exhausted by means of an electric fan. The air drawn through the chamber entered at the bottom through a wide pipe. A gas-tube entered this pipe so that the outlet dipped into a cup of mercury on the end of a beam. This beam revolved on an axle in such a way that the current operating the electric fan ran through the coils of a temporary magnet, and thus drawing up the beam allowed the gas to pass. If, for any reason, the fan stopped running the gas was instantaneously cut off.

Twelve guinea-pigs were taken, six males and six females, and placed in two cages in the gas chamber after making careful estimations of the white and red blood corpuscles and hæmoglobin. Gas was then allowed to mix with the air drawn through the chamber until the mixture was of such a strength that 25 % of the hæmoglobin of the guinea-pigs was saturated with carbon monoxide. Haldane's method for the estimation of carboxy-hæmoglobin was used. As blood will take up only a definite quantity of carbon monoxide from any definite mixture of carbon monoxide and air the blood saturation for our purposes was a sufficient indication of the strength of the gas mixture. For the sake of brevity, we will term this saturation of the hæmoglobin of the blood with CO as "hæmoglobin saturation."

Erythrocyte and leucocyte counts were made as frequently as possible, together with hæmoglobin estimations and differential counts. For the total red counts Hayem's solution was used; for the total white counts dilute acetic acid. The hæmoglobin was estimated by Dare's hæmoglobinometer. The differential counts were made from smears stained by Wright's method, occasionally checked by Ehrlich's triacid and Jenner's stains.

After the first few weeks all counts and smears were taken after the guinea-pigs had been kept without food for at least fifteen hours, thus obviating the large error which we found due to the leucocytosis of feeding. The animals were usually transferred to cages without food at night and the estimations made before feeding them next day. All hæmoglobin estimations were made after the animal had been left in the fresh air for one and a half hours, since it was found by experiment that the blood with 25 % hæmoglobin saturation became completely free from CO in that time.

The weight of the animals was occasionally noted, and at intervals,

one of them was taken and killed by a blow on the head; the organs were hardened in alcohol, alcohol and formaldehyde or Zenker's fluid, to examine for possible pathological changes which might have taken place.

Of the twelve guinea-pigs originally placed in the gas chamber fifteen months ago, two are still apparently as healthy as ever, one died of old age after two months confinement, four died at one time through the carelessness of the attendant, one after seven months was transferred to fresh air again, and five have been killed for pathological study. Other guinea-pigs have been put in and taken out of the gas at various times for confirming certain points.

None of the animals decreased in weight: on the contrary most of them gained. After the first few days, those living with 25 % of their hæmoglobin saturated with CO appeared just as active and happy as those living in the air.

It was naturally expected that with only three-fourths of their hæmoglobin available for oxygen carrying purposes the animals would fail to resist and that symptoms of anæmia would follow. The reverse process actually took place and to our surprise the animals succeeded in resisting. The blood showed degeneration of the erythrocytes on the second day, followed on the third day by the appearance of normoblasts. A steady rise in the number of erythrocytes after a few days indicated clearly that the gas was causing increased proliferation of the erythroblasts and that compensation was taking place. After three to four weeks the animals attained their maximum of red blood corpuscles for that saturation and the counts then remained approximately constant. As compensation took place the degeneration of the erythrocytes gradually disappeared. The leucocytosis, present at first, also disappeared although the leucocyte count after compensation had taken place remained above that of guinea-pigs living in air. The normoblasts disappeared within a few days of their first appearance, as did poikilocytes which were sometimes present at the beginning. The animals in short became acclimatised.

With twenty-five per cent. of its blood rendered useless for oxygen carrying purposes by its union with CO the guinea-pig is capable of compensating and will manufacture new red corpuscles until it has reached a total of about 8,000,000 with a corresponding hæmoglobin content of 105 %. The normal number of erythrocytes in the peripheral circulation of guinea-pigs is about 6,000,000 with 88 % hæmoglobin. The animal with true compensation has three-fourths of 8,000,000 or

6,000,000 erythrocytes, and three-fourths of 105 % or 79 % of its hæmoglobin still available. Thus there is only a total loss for oxygen carrying purposes of 9 % of its hæmoglobin.

The guinea-pig responds very rapidly in its effort to counteract the effect of the CO and will manufacture 2,000,000 additional corpuscles per cubic millimetre in three or four weeks.

TABLE I. *To illustrate the increase in the number of erythrocytes in blood of guinea-pigs living in a gas atmosphere so that* 25 % *and* 35 % *of the hæmoglobin was saturated with carbon monoxide.*

(The numbers of the erythrocytes have in each case to be multiplied by 1000.)

FEMALES.

No. of guinea-pig	June 4—6. Saturation 0	June 9—12. Saturation 25 %	June 28—30. Saturation 25 %	June 7—10. Saturation 25 %	After compensation with 35 % sat.	Hæmoglobin
1	6,630	7,232	7,032	8,064	9,416	104 %
2	6,280	6,367	7,680	7,016	9,864	107
4	5,360	6,757	7,360	8,424	9,224	104
5	5,576	7,296	7,896	7,176	9,440	103
6	5,552	5,976	8,032	9,120	9,712	105
Average	5,879	6,726	7,600	7,960	9,531	104·6

MALES.

	June 7—9	June 12—15	June 23—27	July 4—6		
1	6,361	6,152	8,232	8,800	—	—
2	6,797	6,702	8,208	7,896	9,976	107 %
3	5,944	6,360	7,776	6,848	9,216	105
4	5,472	6,995	8,388	8,004	9,744	106
5	6,643	6,739	7,632	7,752	9,108	108
6	6,536	6,829	7,768	7,848	9,256	106
Average	6,292	6,629	8,001	7,858	9,544	106·4

When a guinea-pig has attained full compensation, however, and is brought into air the erythrocytes do not show an immediate diminution; the number may in fact remain oscillating for several weeks about its compensation point before coming back, perhaps quite rapidly, to its original normal number. The hæmoglobin on the contrary shows a steady decrease from the beginning as may be seen from the following example. No. 4, female, was taken from the gas in which it had been living for several months and put in the air of the laboratory on Nov. 27th, 1905.

TABLE II. *No. 4—Female.*

Date	Erythrocytes	Hæmoglobin
Nov. 27	7,832,000	106 %
Nov. 29	8,688,000	106
Dec. 9	9,072,000	103
Dec. 13	8,160,000	—
Dec. 18	7,992,000	98·5
Dec. 30	7,680,000	90·0
Jan. 8	6,184,000	89·0
Jan. 29	5,936,000	88

A second guinea-pig placed in the gas Nov. 27th, 1905 to Feb. 21st, 1906 with hæmoglobin saturation gave the following results:

TABLE III. *No. 7—Male.*

Date	Erythrocytes	Hæmoglobin
Nov. 27	5,912,000	88 %
Dec. 1	6,042,000	—
Dec. 4	6,440,000	—
Dec. 7	7,080,000	93
Dec. 13	7,440,000	95
Dec. 15	8,768,000	—
Dec. 22	7,920,000	100
Jan. 6	8,088,000	98·5
Jan. 8	7,648,000	100
Jan. 15	8,032,000	—
Jan. 26	7,968,000	105
Feb. 21	8,024,000	105

One of the animals which had been for a year in coal gas with 25 % hæmoglobin saturation, then for several weeks at 35 % saturation, and finally for three weeks at 45 % saturation, was put into the air. It then had 10,093,000 erythrocytes and 110 % hæmoglobin. One month later it had 10,720,000 erythrocytes and 109 % hæmoglobin. One month later still, no examination having meanwhile been made, the blood showed only 6,346,000 erythrocytes, 6,000 leucocytes and 89 % hæmoglobin.

The effect of depriving an animal of the use of part of its hæmoglobin by allowing it to unite with CO is also in many respects similar to depriving it of part of its blood by bleeding. In the latter case the blood is rapidly renewed by the activity of the hematopœitic organs, as evidenced by the appearance of normoblasts and the increasing quantity of erythrocytes and hæmoglobin.

Evidences of degeneration are to be seen in the erythrocytes within twenty-four hours after the guinea-pigs enter the gas-chamber at 25 %

saturation, followed by the appearance of erythroblasts between the sixtieth and seventieth hours. If the animal resists, these erythroblasts disappear in about a week, and as true compensation obtains the degenerated forms also disappear so that the blood becomes histologically normal again. These phenomena are again repeated if the hæmoglobin saturation is again increased.

In gas-poisoning at least the degeneration of the erythrocytes does not begin with an increase in the pale centre of the corpuscles as Maragliano[3] and Castellino have pointed out sometimes occurs, but rather in a lessening or disappearance of this normal biconcavity. The degenerating corpuscle in specimens stained with Wright's stain assumes a very finite, granular, homogeneous appearance of a pale, violet or greyish tinge. These changes are accompanied by an increase of about 1 m. in the diameter of the corpuscles.

In stained specimens as the amount of hæmoglobin decreases in the corpuscle this tint becomes more intense until we have present all stages from a pale purple, or violet, to an intense blue. This erythrocyte degeneration, as evidenced by a more or less nuclear staining of the discoplasm of the corpuscle, does not advance from the periphery to the centre, or *vice versa*, for we find islands of protoplasm both at the centre and the periphery still tinged with hæmoglobin. As degeneration advances we get a vesiculated appearance of the protoplasm, in many cases surrounding an island of hæmoglobin holding protoplasm. This vesiculated appearance is followed by vacuolation of the cells and in acute degeneration a mere shell-like form may persist, Hayem's achromocytes.

As already stated erythroblasts appear after about 60 hours with 25 % hæmoglobin saturation, and after remaining from four to seven days again disappear. In the course of a 400 leucocyte count probably only four or five will be found. In the case of the guinea-pig put directly into gas producing 45 % hæmoglobin saturation they numbered 400 to a 400 leucocyte count after a few days, and were present in the blood for some weeks. These forms appear in the blood to a large extent not as normal erythroblasts but as degenerated forms whose protoplasm shows nuclear staining. A few, however, show no degeneration, the number of these normal forms increasing as compensation takes place.

As the erythrocytes of normal blood are being used up and replaced continually, and even under ordinary circumstances show these various stages in their life history, so this process is constantly taking place only more rapidly in the gas. The life history of the cells in the latter

case is shorter because they are living under conditions where the normal metabolism of the cells is interfered with; even where true compensation has taken place the blood corpuscles show greater evidence of breaking down, the degenerating corpuscles being regarded as older forms having less hæmoglobin. In the case of the nucleated red corpuscles we find degeneration of the corpuscles in acute stages of poisoning; we must regard these also as being deficient in hæmoglobin. The nucleated forms not showing degeneration are probably young cells which have just recently entered the blood stream from the marrow.

In the stages of degeneration in cases where erythroblasts persist until death, erythrocytes were observed with basophile granulations, the granules studding the protoplasm of the cell. The cells in stained specimens showing this granulation were of a greyish tint and did not show advanced signs of degeneration. We regard these as degeneration products rather than the results of nuclear disintegration. When erythroblasts are numerous in the blood stream many free nuclei are found, while some normoblasts with two nuclei and others with the nucleus actually undergoing the process of division were occasionally observed.

Nucleated red corpuscles taken from the bone marrow of a guinea-pig with 10,000,000 erythrocytes when well stained with Wright's stain show a peculiar brownish red colouration of the hæmoglobin holding protoplasm unlike the ordinary erythrocytes which are coloured more of a pinkish red.

From what has been said above it is clear that the effects of chronic CO poisoning in the blood is similar to that which occurs at high altitudes. It is true that in the latter case nucleated red blood corpuscles have not been found, but we think the only reason for this is that the ascent has not been made rapidly or high enough. The similarity is obviously explained by the view that a lack of oxygen is the chief cause in both cases of the changes in the blood.

The effects of a high hæmoglobin saturation on the red blood corpuscles of guinea-pigs. As it was of physiological interest to determine how high a percentage of saturation guinea-pigs can stand continuously, the gas was increased so that the blood attained a saturation of 35 %. After a couple of weeks when the erythrocytes had increased to 9,000,000 the saturation was raised to 45 %. The animals looked ill for a few days and a collapse was feared, more especially as they seemed to lose their appetites. In four or five days they again began to eat heartily and gradually regained their normal appearance; examination of the

blood showed a vast amount of degeneration of the erythrocytes and large numbers of normoblasts appeared. The reds rapidly increased, however, and in three weeks the blood of the four guinea-pigs at this saturation showed 10,500,000 erythrocytes per c.mm. The corresponding hæmoglobin content was then 110 %. The blood was so thick that it became increasingly difficult to obtain good smears owing to the fact that the blood would not spread on the glass slides.

To show what the effect would be on a normal animal when put in an atmosphere which would cause 45 % hæmoglobin saturation, one was placed in a cage with the other four acclimatized guinea-pigs. It died in four days. As the animal constantly shivered and showed every indication of being cold, another guinea-pig was put in a cage with a current of warm water circulating below the pan of sawdust in the bottom. This kept the temperature quite warm, but the animal died in three days with exactly the same symptoms, except that it did not appear to be cold. A third guinea-pig was put in with the other four and showed all the typical symptoms up to the fourth day. When removed to the air it could not move and lay on its side for a couple of hours apparently dying. It then began to improve, ate a little food and after 7—8 hours was put back in the gas box. For a few days it appeared ill and became very thin but eventually recovered. In 4 to 7 days from its entry into the gas the red blood corpuscles showed such an amount of degeneration that it was difficult to understand how the animal could possibly maintain life. Many of the corpuscles were mere husks or shells with the ragged remnants of an interior; normoblasts were extremely numerous and their free nuclei appeared quite frequently. Many of the normoblasts showed degeneration of their protoplasm which sometimes consisted of a mere ragged remnant stained blue. If, therefore, a guinea-pig were taken into an atmosphere containing 7 % oxygen, or elevated 28,000 feet above the sea level we should expect it to die in about 3 or 4 days, since this height corresponds to a hæmoglobin saturation of 50 %. As a matter of fact it would probably die long before this time on account of the intense cold, dilation of blood vessels, and other effects resulting from rarefied atmospheres.

THE LEUCOCYTES OF GUINEA-PIGS, DURING HUNGER, AFTER FEEDING AND DURING CARBON MONOXIDE POISONING.

The total number of leucocytes per cubic millimetre in the guinea-pig when taken carefully so as to exclude the effects of food and excitement is about 6,000. For instance, the blood of ten guinea-pigs which had been kept in the laboratory for some time in order to allow them to become used to their surroundings, when kept without food over night and taken early next morning gave the following numbers.

Total Leucocytes per c.mm.

1.	6,800	6.	6,000
2.	5,900	7.	6,500
3.	6,100	8.	5,900
4.	6,700	9.	5,500
5.	5,700	10.	6,000
		Average	6,110

When these precautions are not taken, however, the number may and does vary widely from this normal count, commonly ranging between eight and thirteen or fourteen thousand. Our counts and smears were therefore always taken with these facts in mind in order to obviate the errors which others have committed in this regard.

In our differential classification we have divided the leucocytes into:

1. Eosinophils—corresponding to the coarsely granular oxyphil cells.
2. Pseudo-eosinophils (Kurloff)—corresponding to the finely granular oxyphil cells or polymorphonuclear cells or polynuclears.
3. Mast cells—of Ehrlich—with coarse basophil granulation.
4. Small lymphocytes.
5. Large lymphocytes.
6. Vacuole forms—of Kurloff.
7. Transitional forms.
8. Large mononuclears.

In the earlier differential counts we placed the transitionals and large mononuclears in one class, but later sometimes separated them to see whether we could discover variations in either class during prolonged or acute leucocytosis of the oxyphil forms.

Of these classes perhaps nothing need be said, except with regard to the vacuole forms of Prof. Kurloff who worked in Ehrlich's laboratory

on the blood of the guinea-pig[4]. He states that they are characteristic of the blood of the guinea-pig, are without granulation and show transitions from large mononuclear to polynuclear forms. They appear to contain vacuoles filled with substance secreted by the cell and constitute 15—20 % of the colourless blood corpuscles.

To show the relative percentage of the various forms we have taken the counts of ten guinea-pigs which showed a total average of about 7,000 leucocytes. The table will serve also to illustrate the variations in the differential counts; the eosinophil cells, particularly even in the same guinea-pig under the same conditions, vary considerably from day to day.

TABLE IV. *Differential counts of* 10 *guinea-pigs taken before feeding.*

Eosinophils	Pseudo-eosinophils	Small lymphocytes	Large lymphocytes	Mast cells	Vacuole forms	Transitionals and large mononuclears
4·50	29·00	10·25	52·75	0	2·00	1·50
10·00	28·25	2·50	52·50	·50	4·75	1·50
3·00	28·75	1·50	61·50	·25	4·50	·50
·00	21·75	1·00	73·75	·25	2·25	1·00
12·25	17·25	·75	54·50	·50	7·25	7·50
5·25	27·50	1·00	57·25	·25	5·50	3·25
10·75	22·00	0	60·00	0	3·00	4·25
7·25	16·25	7·25	63·00	0	1·75	4·50
4·25	31·50	13·00	46·75	0	3·50	1·00
8·25	15·50	11·75	59·25	0	4·00	1·25
Average:—						
6·55	23·78	4·90	58·12	·18	3·85[1]	2·73

We have considered the blood of the guinea-pig after at least twelve to fifteen hours hunger to be the normal blood. When digestion is going on there is very marked hyper-leucocytosis. For instance, one hour after feeding, the blood of guinea-pigs shows an increase in the total number of leucocytes amounting to almost 100 %, while the character of the increase is quite different to that of digestion leucocytosis in man. A series of five guinea-pigs taken one hour after feeding shows the following total and differential counts.

[1] Ledingham in the *Lancet*, June 1906, since the above was written, says that the description of the minute structure of the body given by Kurloff and Burnett holds good. On an average they form about 8 % of the total leucocytes with a variation of from 4 to 11 %. None are found in animals under 3 weeks old. He thinks the peculiar structure suggests the idea of its being a parasite or leucocytozoon of some sort.

TABLE V. *Total and differential counts of 5 guinea-pigs taken 1 hour after feeding.*

Total	Eosinophils	Pseudo-eosinophils	Small lymphocytes	Large lymphocytes	Mast cells	Vacuole forms	Transitionals and large mononuclears
12,500	4·50	39·50	5·25	48·75	0	1·25	·75
10,400	5·00	54·25	1·00	36·75	·25	1·75	1·00
12,600	19·00	41·00	·50	26·00	1·00	5·00	6·50
15,300	7·25	32·75	14·00	40·00	1·25	3·75	1·00
15,400	7·75	30·75	2·75	52·25	·50	4·00	2·00
12,780	8·70	39·65	4·70	40·75	·60	3·15	2·25

A glance at this table will serve to show that the total count has increased from 6,000 leucocytes to 12,000 in one hour, that the eosinophils have undergone little change, that the pseudo-eosinophils have increased by about 15 %, that the lymphocytes have decreased by about 15 % and that the other forms have shown hardly any variation. The absolute number of all forms, however, has increased.

This average for the eosinophils is not a fair one since, as we have stated before, the number varies greatly in individuals. When each

TABLE VI. *Differential counts to show the eosinophilia of digestion in guinea-pigs.*

Time	Total count	Eosino-phils	Pseudo-eosinophils	Small lympho-cytes	Large lympho-cytes	Mast cells	Vacuole forms	Transitionals and large mononuclears
10.30 a.m.	8,200	4·25	31·50	13·00	46·75	0	3·50	2·00
11.40 ,,	15,400	7·75	30·75	2·74	52·25	·50	4·00	2·00
12.40 p.m.	16,400	3·50	53·75	4·25	34·25	·25	3·00	1·00
3.40 ,,	11,400	1·75	56·50	25·50	13·00	0	2·00	1·00
11 a.m.	6,500	0	21·75	1·00	73·75	·25	2·75	1·00
12 ,,	12,500	4·50	39·50	5·25	48·75	0	1·25	·75
1 p.m.	10,900	3·00	49·00	0	43·00	0	3·75	1·25
3.15 ,,	6,700	1·50	39·00	·25	54·00	0	4·00	1·25
11 a.m.	7,300	3·00	28·75	1·50	61·50	·25	4·50	·50
12 ,,	10,400	5·00	54·25	1·00	36·75	·25	1·75	1·00
1 p.m.	8,600	3·00	48·25	9·75	37·50	·50	1·25	·75
3 ,,	6,200	2·00	37·25	6·75	48·50	·75	2·50	1·25
10.45 a.m.	9,400	4·50	29·00	10·25	52·75	0	1·00	1·50
12 ,,	15,300	7·25	32·75	14·00	40·00	1·25	3·75	1·00
12.45 p.m.	9,300	7·75	38·75	1·75	46·75	·75	2·00	2·25
3.15 ,,	9,900	4·25	29·75	6·00	54·50	·25	3·75	1·00
10.30 a.m.	8,900	8·25	15·50	11·75	59·25	0	4·00	1·25
11.45 ,,	11,300	8·75	20·00	·75	66·50	·75	2·75	·50
12.45 p.m.	7,400	10·00	46·50	·75	36·00	·75	4·50	1·50
3.45 ,,	5,700	·25	28·50	5·75	60·75	0	4·00	·75

daily differential count is considered by itself it is found that the eosinophils at first always show an increase, followed by a diminution so that a new total number is finally attained for that animal.

A few counts taken at random will serve to illustrate this point. A preliminary rise in the eosinophils is almost invariable; in about two hours they again reach approximately the original number, while a few hours later they are quite below it. Next day the number may be higher again. There is no constancy in the number of eosinophils present in blood of even the same animal from day to day.

The second hour after feeding, although the total count has undergone but little alteration, the change in the differential count becomes even more marked.

TABLE VII. *Average of total and differential counts 2 hours after feeding.*

Total	Eosinophils	Pseudo-eosinophils	Small lympho-cytes	Large lympho-cytes	Mast cells	Vacuole forms	Transitionals and large mononuclears
10,900	2·00	49·00	0	43·00	0	3·75	1·25
8,600	3·00	48·25	9·75	37·50	·50	1·25	·75
7,400	10·00	46·50	·75	36·00	·75	4·50	1·50
9,300	7·75	38·75	1·75	46·75	·75	2·00	2·25
16,400	3·50	53·75	4·25	34·25	·25	3·00	1·00
10,520	5·25	47·25	3·30	39·50	·45	2·90	1·35

On the whole the relative increase of pseudo-eosinophil cells seems to attain its maximum about two hours after feeding and still continues high several hours afterwards, with a corresponding diminution in the lymphocytes. The chemiotactic action due to digesting food therefore is to cause a large increase in the relative proportion of the pseudo-eosinophils, although the absolute number of all the other forms is raised.

According to Rieder[5] in the digestion leucocytosis of man the proportion of lymphocytes to polymorphonuclears practically undergoes no alteration, indeed the lymphocytes may be in excess. The eosinophils on the other hand show a marked relative reduction.

Ward[6] claims that there is a daily increase of about 20% in the number of leucocytes, the count being lowest in the morning and highest about 5 p.m., and that there is a diminution in the proportion of polymorphonuclear and a corresponding increase of lymphocytes, the eosinophils showing but little change.

This difference between the leucocytosis of digestion in man and guinea-pig seems to indicate a difference in the role of the polymorphonuclear in these two cases. It will be noted that in normal guinea-pig

blood the granular forms amount to about 30 % and the lymphocytes to 63 % of the total, which numbers are almost reversed in the human blood where the oxyphil granular forms are in excess. In both cases the leucocyte normally present in greatest number in the blood shows the greatest increase during the leucocytosis of digestion.

Prof. Kurloff working on the guinea-pig in Ehrlich's laboratory has obtained results markedly different from those found by us. For instance, in three examples quoted of normal guinea-pigs' blood he obtained 10,700, 12,000 and 15,000 leucocytes, or an average of 12,600 per cubic millimetre. As the result of very numerous counts taken when the guinea-pigs had been kept without food for about fifteen hours, we find that the guinea-pig has normally only 6,000 leucocytes per c.mm. Evidently Kurloff did not, as we at first did not, take this digestion leucocytosis into account and his differential counts are therefore not those of normal blood any more than the blood of an animal suffering from a toxæmia is normal blood.

Though we have not separated the vacuole forms from the lymphocytes as Kurloff has, still if these two classes be grouped together we find that Kurloff's normal count corresponds quite closely to that of our digestion leucocytosis. The average of his pseudo-eosinophils, 45 %, corresponds to our 47 %; his average of lymphocytes and vacuole forms, 50 % in all, corresponds to our 46 %. Kurloff's normal counts therefore are evidently made on guinea-pigs undergoing pseudo-eosinophil leucocytosis. The results which he obtained after two years on the blood of splenectomised guinea-pigs are the differential counts of unfed animals; those which he started out with are the differential counts of animals undergoing digestion (pseudo-eosinophil) leucocytosis.

So too the counts given by Kanthack and Hardy[7], were evidently made on the blood of guinea-pigs undergoing digestion leucocytosis.

Carbon monoxide calls forth a leucocytosis in the guinea-pig which varies in intensity with the strength of the saturation of the blood with this gas. For convenience we may designate this a carboxyphilic leucocytosis as indicating the cause producing it and the cells chiefly concerned in it, namely those with oxyphilic granulation. The guinea-pigs experimented upon were kept over night without food and after the first days during which several were made daily, the estimations were always made in the morning before feeding. When placed in an atmosphere of coal gas so that 45 % of their hæmoglobin was saturated with CO and the specimens of blood taken at hourly intervals an immediate change may be observed. The total count may or may not increase for

TABLE VIII. *Blood of guinea-pig A, male, placed in gas so that 45 % of its hæmoglobin was saturated with carbon monoxide. April 4, 1906, 11.45 a.m.*

Date	Eosin.	Pseudo-eosinophils	Small L.	Large L.	Mast	Vac. f.	Trans. f.	Large mono.	Erythrocytes	Leucocytes	Hæmo-globin	Eosin. myelocytes
Apr. 3, 1906	3·00	14·50	14·25	57·25	·50	3·75	3·50	3·25	6,420,000	7,200	88 %	
4, 12.45 p.m.	7·25	16·25	11·50	53·25	0	4·25	3·75	1·75	—	8,700	—	0
1.45 ,,	14·25	32·25	10·00	34·50	0	4·50	2·75	1·75	—	8,200	—	0
2.45 ,,	10·75	31·50	14·00	30·50	0	2·75	7·00	3·50	—	7,700	—	0
3.45 ,,	7.20	44·80	1·60	30·40	0	4·00	8·00	4·00	—	9,500	—	0
4.45 ,,	8·50	55·75	1·25	22·50	·50	4·75	4·25	2·50	—	10,600	—	0
8 ,,	6·00	50·75	5·00	26·25	0	3·25	5·75	3·00	—	11,100	—	0
9 ,,	4·20	45·80	14·80	29·80	·80	3·20	1·00	·40	—	10,000	—	0
11 ,,	8·00	54·00	13·50	17·50	·25	2·00	3·00	1·75	—	10,300	—	0
Apr. 5 a.m.	Smears could not be counted.			—	—	—	—	—	6,272,000	12,000	—	0
5 p.m.	,,	,,	,,	—	—	—	—	—	—	18,600	—	·46
6 ,,	18·40	49·20	2·06	22·52	0	2·76	3·22	1·38	—	20,600	—	1·75
7 ,,	11·00	45·40	6·25	27·75	·75	2·75	3·25	1·00	—	25,400	—	0
8	Died.											

TABLE IX. *Guinea-pig C, male, placed in gas with 45 % hæmoglobin saturation. April 16, 1906.*

Date	Eosin.	Pseudo-eosinophils	Small L.	Large L.	Mast	Vac. f.	Trans. f.	Large mono.	Erythrocytes	Total leucocytes	Hæmo-globin	Saturation
Apr. 16	10·00	14·00	12·75	43·75	·50	2·00	11·75	5·25	6,200,000	8,200	86·5 %	45 %
17	Smears could not be counted.			—	—	—	—	—	5,456,009	14,300	—	45
18	23·25	31·25	6·25	29·75	·75	·50	5·00	3·25	5,928,000	18,700	—	45
19	15·25	49·50	·50	27·00	1·00	·75	3·75	1·00	6,064,000	17,700	—	45

Eosinophilic myelocytes 1·25.

three or four hours but after that it steadily rises; the differential count alters from the beginning. The increase in the oxyphil granular cells is particularly noteworthy. The lymphocyte decrease is inversely as the increase of the granular forms. None of the other forms seem to show any constant variation. Table VIII illustrates the change.

It will be noticed that in this case there is an oxyphil leucocytosis. The rise in the total number of white cells, however, is not so rapid, while the differential change takes place just as quickly. In 12 hours the granular forms have increased from 18 % to 62 % while the lymphocytes have decreased from 71 % to 31 %. There seems to be little change in the other forms excepting a perceptible rise in the transitionals during the third and fourth hours.

In Table IX is given the blood history of the second guinea-pig placed in the gas chamber at 45 % saturation, and kept warm by means of a current of warm water constantly circulating beneath the floor of the cage. The animal died in three days and appeared from the first to be more prostrated than guinea-pigs newly introduced into the gas at ordinary room temperature.

The nature of the change in this guinea-pig is essentially similar to that of the previous one. Eosinophilic myelocytes appeared in both within three days, showing that there had been a great call on the source of supply in the bone marrow.

A very interesting case for comparison is that of a third guinea-pig which was placed in the same atmosphere and showed all the typical symptoms up to the fourth day, when it appeared to be dying. (See page 39.)

It is again to be observed that the same features are shown up to the fourth day at least; that on the third day eosinophilic myelocytes are present, that on the fourth day nucleated reds appear and increase in number from day to day, and that the leucocytosis reaches its maximum on the fifth day after which it begins to decrease. The numbers of nucleated reds given are those found during the differential countings of 400 leucocytes.

The cause of death of *A* and *C* was not due to starvation, for after observing that they refused to eat in the gas, they were taken out and left in the air for a while. They would then begin to eat in a short time and after a couple of hours would be returned to the gas. Of course normal assimilation would probably not take place under these conditions but they should obtain at least enough nourishment from the food eaten to support life.

TABLE X. *Guinea-pig D, male, placed in gas so that 45 % of its hæmoglobin was saturated with carbon monoxide. April 16, 1906.*

Date	Eosin.	Pseudo-eosinophils	Small L.	Large L.	Mast	Vac. f.	Trans. f.	Large mono.	Total erythrocytes	Total leucocytes	Hæmo-globin	Satura-tion	Total nuc'd reds	Eosin. myelocytes
Apr. 3	—	—	—	—	—	—	—	—	6,480,000	7,500	88 %	0	0	0
16	2.75	48·75	5·50	27·25	0	1·75	10·00	4·00	—	9,300	—	45 %	0	0
17	24·00	40·00	·50	18·75	·50	2·00	9·00	5·25	6,136,000	12,700	—	45	0	0
18	13·25	42·00	3·50	26·25	·75	·50	10·75	3·00	5,808,000	12,400	—	45	0	0
19	23·50	23·75	2·25	33·75	1·00	1·75	8·75	3·00	5,752,000	18,300	—	45	0	2·25
20	22·00	37·75	8·75	21·75	·50	0	7·00	1·75	6,200,000	32,900	—	45	6	·50
21	16·25	45·75	7·00	16·75	·50	·75	8·75	3·75	5,680,000	37,700	—	45	90	·50
23	20·75	54·75	3·00	12·75	·25	1·25	3·75	3·00	5,968,000	17,200	—	45	130	·50
24	23·00	49·50	1·75	12·25	1·50	1·75	6·00	4·25	6,240,000	11,200	—	45	93	0
25	18·00	48·25	5·00	19·50	1·25	2·25	4·75	3·50	5,896,000	7,600	—	45	125	0
May 1	1·50	44·00	3·50	40·00	·25	1·00	5·25	4·25	7,464,000	10,300	93	45	298	·25

TABLE XI. *Showing effect of 54 % hæmoglobin saturation for three hours.*

Date and Time	Eosin.	Pseudo-eosinophils	Small lymph.	Large lymph.	Mast.	Vac. f.	Trans. f. and large mono.	Total erythrocytes	Total leucocytes	Hæmo-globin	Saturation
9/1/1906											
11 a.m. (before gas)	10·75	22·00	0	60·0	0	3·0	4·25	6,108,000	7,500	88·5 %	0
5 p.m. (after removal)	3·50	58·75	0	28·5	·75	2·0	6·50	6,736,000	23,100	—	54 %
8.15 p.m.	·50	81·00	0	14·5	·25	1·75	2·00	—	14,500	—	10
10.15 ,,	1·00	63·50	0	30·0	·25	2·00	3·25	—	12,200	—	0
10/1/06											
12.15 a.m.	2·00	69·00	1·25	22·75	·50	2·75	1·75	—	14,000	—	0
2.15 ,,	4·50	75·50	·50	13·75	0	3·50	2·00	—	10,200	—	0
4.15 ,,	3·50	51·00	1·00	38·75	·5	1·75	3·50	—	6,400	—	0
6.15 ,,	2·25	38·00	·50	50·75	0	3·50	5·00	—	8,300	—	0
8.15 ,,	4·30	34·20	4·30	43·55	0	4·30	8·50	—	6,800	—	0

TABLE XII. *Showing effect of 70 % hæmoglobin saturation for two hours.*

17/1/06											
11.15 a.m. (before gas)	7·25	16·25	7·25	63·00	0	1·75	4·5	6,072,000	7,500	87 %	0
1.15 p.m. (on removal)	7·50	33·50	2·25	46·50	·75	2·50	7·00	6,088,000	8,800	—	70 %
3.15 ,,	7·75	43·25	2·75	40·75	1·75	2·00	1·75	—	20,700	—	28
4.15 ,,	2·00	65·00	1·00	24·00	·75	2·25	5·00	—	23,100	—	0
5.45 ,,	·50	88·50	3·25	3·75	·50	1·00	2·50	—	17,100	—	0
8.15 ,,	0	85·75	4·50	7·25	·50	·75	1·25	—	14,000	—	0
10.15 ,,	0	87·75	·75	8·25	0	1·25	2·00	—	19,700	—	0
18/1/06											
12.15 a.m.	0	84·00	1·25	8·75	0	3·50	2·50	—	10,700	—	0
2.15 ,,	1·00	80·50	3·25	12·25	·25	2·00	·75	—	14,600	—	0
4.15 ,,	·75	69·25	5·25	22·25	0	1·50	1·00	—	18,600	—	0
6.15 ,,	·75	64·75	·75	27·50	·25	3·00	3·00	—	10,600	—	0
8.15 ,,	·75	25·75	10·50	58·75	·25	1·75	2·25	—	9,700	—	0
10.15 ,,	1·50	27·00	10·75	55·00	0	1·50	4·00	—	8,600	—	0
12.15 p.m.	(Could not be counted.)			—	—	—	—	—	8,300	—	0
2.15 ,,	,,	,,	,,	—	—	—	—	—	6,900	—	0
4.15 ,,	1·25	28·00	10·00	53·00	0	4·0	3·75	—	5,400	—	0
6.15 ,,	2·25	30·50	3·50	56·00	0	5·5	2·25	—	7,400	—	0

To complete this series blood counts are given (Tables XI and XII) of two guinea-pigs placed in gas of such strength that they were rendered unconscious. After being kept in that state for some time they were removed and the blood examined from time to time.

In these cases of acute poisoning, that is, when the animals are rendered unconscious and kept so for two or three hours, the blood picture differs from that of the chronic gas poisoning in two particulars. On the one hand the oxyphil leucocytosis is much greater in the acute cases, and on the other this oxyphil leucocytosis is in itself of a different character. The oxyphil granular forms increase from 23 % to 89 % in the second example, while the lymphocytes decrease from 70 % to 7 %. The coarsely granular form or eosinophil, about five hours after the removal of the guinea-pig from the gas, totally disappears for 6 or 7 hours and then gradually regains its former number.

It will be remembered that in the tables of chronic poisoning shown, the eosinophils increased during the leucocytosis and remained abnormally high till death, or as in the case of the animal which survived until the time of writing, two months after it had been put in the gas chamber. In digestion leucocytosis there is invariably a rise of the eosinophils followed by a diminution so that the final number may be subnormal. The eosinophilic increase or decrease therefore seems to depend upon certain definite conditions. When there is unusual metabolic activity of the body cells, such as occurs after a meal, during which extraordinary anabolic and katabolic changes are taking place and extraordinary quantities of excretory products are forming, we have a slight but constant eosinophilia. When the body cells are deprived of their usual supply of oxygen and when therefore normal oxidation cannot take place we also have an eosinophilia, the extent of which varies directly with the percentage saturation of the hæmoglobin, or inversely as the supply of oxygen, up to a certain point at least. With a higher hæmoglobin saturation, or in what we may term acute gas-poisoning, we have a total disappearance of the eosinophils; that is, when oxidation of the tissues is so interfered with that really an acute toxic condition exists, in which we should expect to find an accumulation of the precursors of CO, possibly such products as amides and purin bases, and probably substances of the nature of toxins in the tissues we find a total disappearance of the eosinophils from the blood.

Fletcher[8] states that the hastening of rigor mortis and fatigue in muscle from which oxygen is withheld is due to an increased accumulation,

under circumstances of deficient oxidation, of the metabolic products within the muscles which are the potential precursors of CO.

Budgett[9] also says that the visible changes of structure shown by some protozoa when deprived of oxygen may be exactly reproduced by treatment with certain poisons: this indicates that either the poisons prevent oxidation or that lack of oxygen produces toxic substances.

From the results obtained by us it seems quite apparent that lack of oxygen does produce auto-intoxication, and that therefore gas-poisoning is really due to a toxæmia caused by lack of oxygen, the CO itself being merely the substance which prevents the oxygen from reaching the tissues.

Eosinophilia seems to be associated with moderate toxæmias, such as those caused by digestion, deranged metabolism, bacterial infections, or direct poisons. On the other hand a severe toxæmia of any description tends to cause a complete disappearance of the eosinophils.

Eosinophilia occurs in bronchial asthma, acute and chronic skin diseases, helminthiasis, malignant tumours and post-febrile conditions after infection. At the height of most acute infectious diseases the eosinophils disappear while abnormally high numbers appear during the post-febrile period.

Now in gas-poisoning none of the extraneous factors which cause leucocytosis are present. Food, bacterial toxins or other substances of that nature play no part; the CO itself is in every respect a neutral gas. We may safely claim, therefore, in such cases, having eliminated all other possibilities, that the lack of oxygen means deranged metabolic activity, and that excretory products usually eliminated as certain definite chemical compounds either must accumulate as some incompletely formed excretory product, or that abnormal constituents must be evolved, both of which act as poisons. Such extraordinary products may be nitrogenous and poisonous in nature (like the bacterial toxins) and prove positively or negatively chemiotactic to the oxyphil granular forms according to the quantity of the poison formed.

It is known that many, if not all, poisons cause a destruction of tissue, which destruction means production of poisons from the tissues themselves. All of these poisons affect the central nervous system and thus produce the abnormal symptoms of poisoning[10]. We have in gas-poisoning at any rate an indication, as evidenced by the severe leucocytosis and degeneration of the blood produced, that poisoning due to retarded metabolic activity has taken place. When this abnormal metabolism is continued for a length of time, especially when the

saturation of the blood with CO is high, the effect on the system is disastrous. The higher the saturation and the longer the time involved the greater will be the damage resulting to the body cells at large, and to those of the central nervous system in particular. It is probably for this reason that cases of poisoning produced by a rapid saturation of the hæmoglobin with CO and continued only for a short time recover rapidly when by fresh air and artificial respiration the gas is got rid of. When the inhalation of the gas has continued for a longer time the products of incomplete combustion in the body cells become so large and the consequent damage to the tissues so extensive that the disastrous effects upon the body prove irreparable, and perhaps several days after the gas has disappeared from the blood the patient will succumb. Taking the blood itself—a liquid tissue—as evidence, we see how extensive their degeneration may be.

CHIEF CONCLUSIONS.

1. Carbon monoxide acts as a poison solely by its ability to prevent the normal supply of oxygen from reaching the tissues and thereby deranging the normal metabolism of the body cells.

2. Guinea-pigs living continuously in a dilute carbon monoxide atmosphere so that the oxygen carrying capacity of the blood is reduced, are able, by increasing the quantity of hæmoglobin and number of erythrocytes to compensate for this loss and maintain an oxygen carrying capacity approximately equivalent to that of the original blood.

3. Carbon monoxide poisoning is followed by a leucocytosis of the eosinophil and pseudo-eosinophil forms. A moderate saturation produces a moderate toxæmia involving an eosinophilia like nearly all moderate toxæmias. A high saturation causes a severe toxæmia in the course of which the eosinophils disappear as in all severe toxæmia. A high prolonged saturation brings about the appearance of erythroblasts and myelocytes, indicating an hyperactivity on the part of the parent cells in the bone marrow.

4. The effect of carbon monoxide in increasing the number of erythrocytes in the blood is, in many respects, similar to those of high altitudes, in the peripheral circulation at least.

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