

THE INFLUENCE OF THE SPLEEN IN CARBON MONOXIDE POISONING. BY J. BARCROFT, C. D. MURRAY, D. ORAHOVATS¹, J. SANDS¹ AND R. WEISS¹.

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THE frequency with which the operation of splenectomy has been performed, without apparently harmful results, has led to the conception on the part of many that the organism can get on as well without a spleen as with one. It was a matter of some curiosity to us to review this conception in the light of recent evidence which goes to show that the spleen is, among other things, a reservoir of red blood corpuscles held in reserve to meet such emergencies as demand an increase in the quantity of hæmoglobin present in circulation. On such a theory, were the organism presented with an issue which made its life depend on the amount of hæmoglobin it could produce, a splenectomised animal should die, when an animal possessed of a spleen should survive. Such an emergency would be a gradual hæmorrhage for it has been shown that if a cat be bled to the extent of 60 c.c. in an hour, the spleen will contribute 20 c.c. of fluid which is probably not less rich in red corpuscles than is the blood itself. The actual loss to the circulation would therefore only be 40 c.c.², which loss proved fatal. Had the cat had no spleen, the presumption is that it would have died when it had lost 40 c.c. Hæmorrhage is less susceptible of exact experiment than is carbon monoxide poisoning. We therefore determined to ascertain whether the animals possessed of a spleen survived those from which the spleen had been removed when both were exposed simultaneously to CO poisoning.

Two series of experiments were performed, in both cases on guinea-pigs. In series I the animals fell into three categories: (a) splenectomised; (b) operated controls, in which the abdomen had been opened and some omentum or pancreas removed in the vicinity of the spleen; (c) "normal controls" in which no operation was performed. The operations were carried out under ether four days before the animals were exposed to carbon monoxide, except in one experiment in which the time was three days. We found an ether-chloroform mixture unsuitable as an anæsthetic; the guinea-pigs developed lung trouble, in some cases fatal; with ether

¹ Travelling Fellows of the Rockefeller Foundation.

² This statement is based on radiographic work at present in progress.

the tissues remained normal. The general course of an experiment in series I may be followed from Fig. 1. In this case nine guinea-pigs were

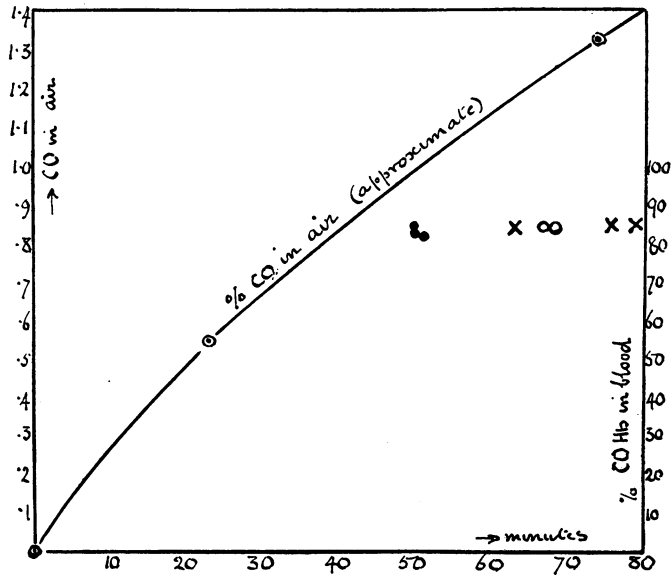


Fig. 1.

exposed, three of each category—one of the operated controls was found post-mortem to be pregnant and was discarded, as were other pregnant animals (except as stated on p. 84). Eight therefore are taken account of in the figure. The animals were more or less of the same size, as judged by the eye, no account was taken of sex. The guinea-pigs were placed in a chamber of 10 cubic metres capacity. Two gas taps in the chamber were turned on and a fan set going to keep the air thoroughly stirred. The CO in the air of the chamber was determined at intervals of about 20 minutes. The guinea-pigs were in wooden trays about 20 inches from the ground and were mixed together so that the observer who was outside the chamber was usually unaware into which category any particular guinea-pig fell. In some of the experiments we were able to secure an observer who was quite unaware of the object of the experiment. The duty of the "observer" was to note the time at which each guinea-pig died. Samples of the air of the chamber were taken for analysis, usually at intervals of about 20 minutes. These could be obtained without entering the chamber. In Fig. 1 the ordinates on the left give the percentage of CO in the chamber air and refers to the diagonal line. The abscissa is the time in minutes

from the turning on of the gas taps. In this particular experiment, which lasted 80 minutes, the final concentration of carbon monoxide in the chamber was 1.4 p.c. The ordinates on the right give the percentage saturation of the blood in each animal at death and refer to the dots, circles and crosses. These dots, circles and crosses refer to animals and are placed above the times at which they died. Reading from left to right, three splenectomised guinea-pigs (●) died 50, 50 and 51 minutes respectively after the gas was turned on. Their bloods contained 83.5, 85 and 83 p.c. of CO respectively. The next animal to die was a normal (×) after 63 minutes' exposure, the next an "operated control" (○) after the experiment had run for 67 minutes, and so on. Thus all the splenectomised animals died before any of the controls, and the operated controls and the normal controls were mixed up in the matter of their longevity. This experiment is typical save for the fact that the three splenectomised animals died within a minute of one another. Usually their deaths, as that of the other categories, were more spread out.

Six such experiments were performed, and for statistical purposes they are treated in the following way. In each experiment the average longevity of the normals in the chamber is calculated and called 100, the longevity of each animal is then calculated as a percentage of that. In the experiment under reserve, for instance, the longevities of the three normals were respectively 63, 76 and 79 minutes. The average longevity of the normals is therefore 72.7 minutes. The individuals are:

	Actual longevity (mins.)	Percentage longevity (mins.)	Mins.
Splenectomised	{ 50	{ 69	69
	{ 50	{ 99	
	{ 51	{ 70	
Operated controls	{ 67	{ 92	93
	{ 68	{ 93	
Normals	{ 63	{ 88	100
	{ 76	{ 104	
	{ 79	{ 108	

Treating each experiment in this way, the results of the whole six become comparable and may be summarised very shortly.

	Normals	Operated controls	Splenectomised
Number of observations	16	15	16
Mean longevity in chamber (<i>n</i>)	100	93.2	72.1
Standard deviation (σ)	8.14	13.02	17.86
Standard error of mean σ/\sqrt{n}	2.03	3.36	4.46

The question of course arises: are these differences between the categories significant? Taking the categories in pairs and comparing the normals with the operated controls, we find that difference of the means

is only 1.73 times the standard error of their difference $\left(\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}\right)$. No significance can therefore be attached to the difference between the normals and the operated controls; but as between the normals and the splenectomised animals the standard error of their difference is 5.68 times the difference of the means and 3.77 times as between the operated controls and the splenectomised; both of which are significant.

Working from the above data, it appears that the probability of the figures as between the normal and the operated animals, coming as they do by chance, is about 25 : 1 against and is of doubtful significance, but that corresponding probabilities as between the normal and the splenectomised and as between the operated controls and the splenectomised are respectively less than 1 in a 100,000,000 and 1 in 100,000, and may be regarded as of significance.

Although from the statistical point of view the above differences are satisfactory, we determined to carry out a control of a purely experimental kind. We therefore performed two experiments in which the gas used was not carbon monoxide, but one which kills the animal for a reason which is quite unconnected with the amount of hæmoglobin in its system. Such a substance is hydrocyanic acid. The animals normal and splenectomised were as in the experiment already described: the HCN was run slowly into the chamber from a burette situated outside. The concentration of gas was presumed to be that caused by the complete evaporation of the hydrocyanic acid measured as a liquid. The experiments lasted a somewhat shorter time than the carbon monoxide ones—about 40 minutes.

In one such experiment the percentage times were as follows:

Normals	69, 116, 116.	Mean 100
Splenectomised	85, 105, 131.	Mean 107

Taking the two experiments together, the mean percentage time both of the normals and the splenectomised animals came out at 100. There was no tendency, so far as could be observed, for the splenectomised animals to die before the normals.

Reverting to the coal gas series, only in one experiment did a splenectomised animal survive animals with spleens. This animal was particularly large—a circumstance which first drew our attention to the influence of size. As all the animals had been weighed after death, we were able, on the assumption that the gas had run at the same rate in all experiments, an assumption which we know to be only very approximate, to plot the time of death against the weight of the animals for the whole six experiments. The result was so interesting that we determined a second series of experiments in which much greater care was taken to have the categories of approximately the same weight. Also in this series

we had operated controls of a different character. We used female guinea-pigs throughout, and in the case of the operated controls one horn of the uterus was removed, thus depriving the animal of a mass of tissue more comparable to the spleen in size and importance, than is a piece of omentum. Otherwise the experiments were carried out as in series I.

Three experiments in this series were performed. Of these Fig. 2 is typical. Here the longevity in the chamber (abscissa) is plotted against the weight of the guinea-pig.

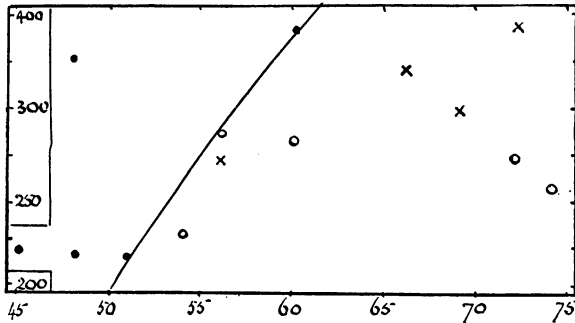


Fig. 2. Ordinate and weight of guinea-pig in grams: abscissa = longevity in chamber in minutes; ● = splenectomised; ○ = operated controls; × = normals.

It will be seen that the figure can be divided by a line into two areas: to the left are segregated all the splenectomised, to the right the normals and operated controls. The latter are scattered about their area quite indiscriminately.

Worked out statistically, the results of series II are as follows:

	Normals	Operated. One horn of uterus removed	Splenectomised
Number of observations	11	12	13
Mean longevity in chamber	100	99	75
Standard deviation (σ)	12.53	12.41	12.75
Standard error σ/\sqrt{n}	3.79	3.54	3.53

The probabilities against the differences observed occurring by chance are, as between the operated controls and the normals, 8 : 10; as between the normals and the splenectomised, less than 1 in 1,000,000; and as between splenectomised and the uterectomised, about 1 in 500,000. It would seem quite clear that the earlier deaths of the splenectomised animals as compared either with the operated controls or the normals is not a chance affair and that the mere fact of an operation does not influence the matter.

In the series very particular care was taken to avoid hæmorrhage, and it may be stated with great confidence that the splenectomised

animals had not lost more blood than the operated controls. In two cases¹ it was quite certain that the operated controls had lost more than the splenectomised, but their life in the chamber was not influenced by the loss. They lived as long as the normal animals.

The question naturally arises: How do the various categories compare in the matter of the saturation of the arterial blood with carbon monoxide? The following are the average figures for the percentage saturation of blood at death with CO:

	Splenectomised	Operated controls	Normals
Number of animals	28	26	28
Average percentage of CO in blood at death	81.9	83.5	83.9

In every experiment except one there was a slight, but very slight, tendency for the splenectomised animal to have a lower percentage saturation of CO than the normals, but on the whole it is remarkable how closely the figures agree. The suggestion is that there is a fatal percentage saturation which is approximately the same for all groups but that under the circumstances of the experiment it is reached sooner in the splenectomised than in the normal animals.

SUMMARY.

1. In an atmosphere which receives a continuous accession of coal gas, guinea-pigs from which the spleens have been removed die sooner than either normal guinea-pigs, those from which omentum has been removed, or those from which one horn of the uterus has been excised.

2. Excision of the spleen four days previously does not prejudice the length of life of guinea-pigs when exposed to an increasing concentration of hydrocyanic acid, a gas which kills by a means quite unconnected with hæmoglobin.

3. The effects of removal of the spleen are not due to hæmorrhage during the operation.

4. Having regard to the fact that in fatal pressure of carbon monoxide the spleen contracts, expelling its contents into the blood, it is suggested that the longer life of the normal animals is due to this accession of hæmoglobin to the blood.

5. The percentage saturation of the blood with CO is almost the same in all the categories of animals.

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¹ These were found to be pregnant.