THE BLOOD IN THE SPLEEN PULP.

By J. BARCROFT, F.R.S. and L. T. POOLE, *Major* R.A.M.C.

(*From the Physiological Laboratory, Cambridge.*)

The investigations described below were undertaken in order to supply, if possible, independent evidence of the observations made by Cruickshank(1) and Scheunert and Krzywanek(2) that the blood which comes from the spleen during its contraction contains a greater quantity of hæmoglobin than that in the general circulation. The extra-cutaneous(3) spleen preparation described in the previous paper serves excellently for this purpose. If the spleen be stabbed with a needle, or better with a hypodermic needle, blood as a rule issues. This blood is not necessarily pure blood from the spleen pulp; the needle of course penetrates the capsule and may enter trabeculæ, or pierce vessels of considerable size. Nevertheless in so far as it differs from the blood of the general circulation, it will probably reflect the composition of the contents of the spleen pulp. Two methods have been adopted for testing the blood, the hæmatocrit and the Haldane-Gowers hæmoglobinometer. Controls were taken from the ear vein, and, as is emphasised later, from the capsule of the spleen.

Experiments were made (1) with the hæmatocrit, (2) with the hæmoglobinometer.

(1) For the determination of the corpuscle volume blood was collected in the following way. From a piece of sheet rubber about 1 mm. thick a portion was cut about 2 cm. by 1. In the middle of this a hole was punched of about 3 mm. diameter. A little vaseline was placed on the bottom of this small piece of rubber and it was laid on the spleen. A stab was made in the middle of the hole with a needle; the rubber formed a small cell which collected the blood, a pair of hæmatocrit tubes was filled with it and was rotated for ten minutes, after which time the centrifugalisation reached a state of finality. A trace of dry heparine was put on the needle with quite satisfactory results. The spleen is quite insensitive. The dog apparently was usually not conscious of the prick.

The first pair of readings was taken from the ear vein. Readings from there always agree well with one another and with readings taken from an artery. Thus, taking duplicates on different occasions:

Hæmatocrit readings ("Nigger").

Ear vein	(1)	36	37	33	38	34
	(2)	37	35	33	36	34
Artery	(1)	—	—	—	—	33
	(2)	—	—	—	—	33

It may be taken, therefore, that a disparity of more than 3 p.c. in the readings either of duplicates, or as between one vessel and another, has some real meaning.

To turn to the readings from the spleen; the first pair was taken usually immediately after exercise; the spleen was pale and shrunken; the duplicate readings agreed well; it is usual for the blood from the first puncture to have approximately the same hæmoglobin content as that of the ear vein. Once they were markedly below those of the ear vein. This proved a very unusual occurrence; it may be that we went into a lymphatic or some serous cavity. And after all in view of the fact, which will be shown, that the corpuscles become more concentrated in the spleen than in the blood, there must be some situation in which the reverse is true, *i.e.* in which the fluid draining away from the corpuscles is capable of being tapped.

We pass now to the subsequent three punctures. In these readings from the spleen the duplicates differed greatly. In each pair one reading was much above that of the ear vein, the other was either approximately the same or a few per cent. higher. Of such a pair of readings the first taken was always the highest. It occurred to us that the cause of the great disparity might be as follows: that both samples of blood were a mixture of fluid coming from the pulp and from the capsule, that in the first sample the preponderance of blood might have been from the pulp, but that this source quickly dried up, leaving the later hæmorrhage from the superficial area. Or it might have been the other way round, the more concentrated blood from the capsule and the less concentrated from the pulp. It is quite easy to obtain a sample of blood from the capsule by making a puncture which does not go below that investiture. The result is always a sample not richer in hæmoglobin than the blood from the ear vein.

Thus

Hæmoglobin value (H.-G. hæmoglobinometer).

	Exp.		
	(*a*)	(*b*)	(*c*)
Ear vein	67	73	71 p.c.
Capsule	64	75	71
	63	71	72

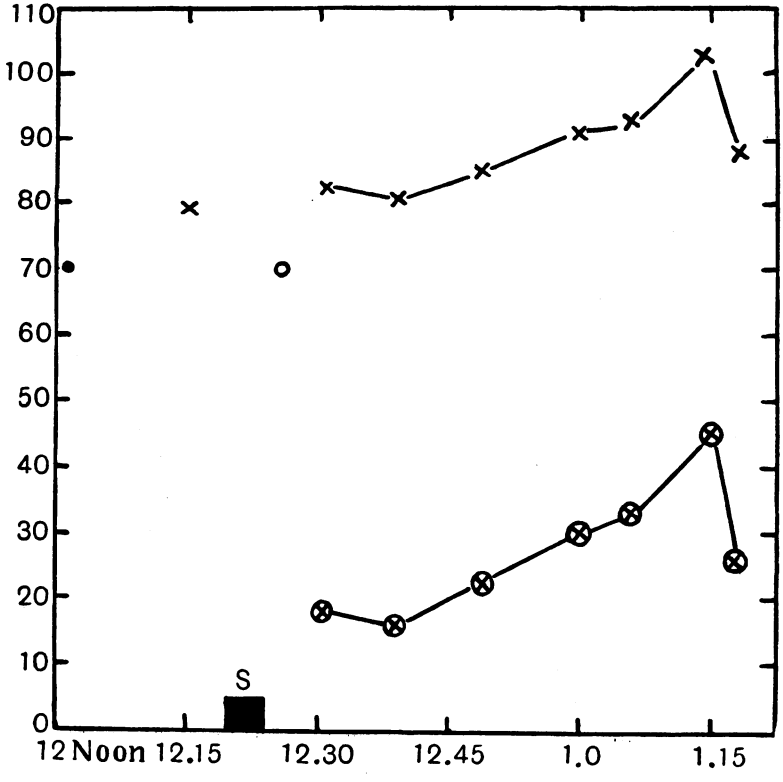
The above figures show that the blood from the ear vein and the capsule agree very well in hæmoglobin content and they cannot differ greatly in corpuscle content. Presumably then the high readings are those which come from the deeper regions of the spleen. In order to obtain a greater flow of pulp blood we gave up using an ordinary bayonet needle and resorted to a rather coarse hypodermic needle. This type of puncture gave much better duplicates; occasionally there would be a disparity and always the second sample collected was the lower one. It may be taken then that the topmost reading does not indicate a greater corpuscle content than the blood from the spleen pulp actually contains. Not that it is likely that the spleen pulp is uniform; a reading can only be referred to the position of the prick. Owing to unevenness in the composition of the pulp, in conjunction with the possibility of dilution of the pulp blood with capsule blood and blood from the trabecular muscle, etc., the readings must present a somewhat rugged appearance when plotted, but certain general features are clear of which the chief is that the blood from the pulp immediately after exercise is usually but little different from that of the general circulation, whilst the blood which subsequently comes from the pulp has a much greater corpuscle value and may have in extreme cases a corpuscle value greater in the ratio of 3 : 2 than that of the general circulation.

(2) Turning to the hæmoglobinometer experiments, of which we did three, the same general features may be seen, namely, a good concordance between the ear blood and the capsule blood and a greater amount of hæmoglobin in the blood of the spleen pulp than in that of the general circulation, with a tendency for the hæmoglobin to rise in the spleen after exercise. These features are shown in the figure which is a fair example of four carried out on the same dog, about six months after the spleen had been brought outside the body.

The fact having been established that the corpuscles become concentrated in the spleen pulp, the question must arise: By what mechanism is the concentration accomplished?

The concentration appears to depend upon the integrating of the nervous supply of the organ. The matter was tested in a dog, the upper portion (*i.e.* that nearest the backbone) of whose spleen was not denervated while the lower portion was. The method of denervation consisted in stripping the nervous elements from the vessels supplying the spleen and cutting away all adventitious material. The only connection between the lower two-thirds of the spleen and the rest of the body consisted in the denervated vessels. The upper portion of the spleen was innervated

normally. The denervation was effected at the same time as the spleen was made extra-cutaneous; that was about six weeks before the experiments here recounted took place.



Readings on hæmoglobinometer (ordinate).

Upper curve: blood from ● ear, ○ capsule, × spleen pulp.

Lower curve: percentage excess of hæmoglobin in spleen pulp as compared with general circulation.

Abscissa: time. Signal (S) = staircase exercise.

The innervated portion of the spleen reacted just as any normal spleen would have done. After exercise the haemoglobin value increased, and attained to a value, as a maximum, of about 50 p.c. higher than that of the blood in the normal circulation; thus on one occasion that of the general circulation gave a reading of 72 on the Haldane-Gowers hæmoglobinometer, whilst that of the spleen pulp was 106.

The denervated portion of the spleen never yielded blood which showed more than the most trifling sign of concentration. In these

experiments further precautions were observed in the collection of the blood from the spleen pulp. The denervated part was disinclined to bleed on an ordinary puncture. The blood was then collected, both from the innervated and denervated portions, in a glass pipette fitted at one end with a rubber teat and drawn at the other into what amounted to a hypodermic needle of glass, *i.e.* into a glass capillary stout enough to be thrust through the capsule. Heparine (powder) was used to prevent coagulation. Even after the above mode of collection was employed, however concentrated the blood found in the pipette, that which flowed from the site of the puncture subsequently was of the same hæmoglobin value as the blood of the general circulation.

Here also recurred a phenomenon to which attention has already been directed, namely, that the high hæmoglobin value is obtained only from the first blood withdrawn from a puncture. Blood which flows from it subsequently is of the hæmoglobin value of the general circulation. This seemed so definite as to make us doubt the adequacy of the explanation already given, namely that such blood merely comes from the capsule. It seemed possible that the damage to the finer structure of the spleen pulp caused by the puncture had been such as to impair the machinery necessary for concentration in the immediate vicinity. The efficiency of the mechanism would seem to depend upon its intactness.

The following figures illustrate the point:

Hæmoglobin readings.

[Gowers hæmoglobinometer with Haldane's CO standard.]

Ear vein	72	72
Innervated portion of spleen	**87** 74	**105** 70 68
Denervated portion of spleen	71 69	72

The figures under the brackets are successive samples from the same puncture.

The fact of innervation seems most likely to influence the concentration of the blood through some muscular mechanism. Such a function might be connected with the rhythmic contractions of the organ; at least those contractions await some intelligible rôle. They are usually most considerable just after the spleen has been in contraction. They then gradually damp off. There is reason to believe that blood will not pass backwards from the spleen pulp into the artery, at all events it is said not to be possible to inject the artery from the vein, the pulp merely becomes distended. If then at each contraction lymph were squeezed out (as it is from a lymphatic gland (Florey(4)) when the muscle of the gland

contracts) and if at each relaxation some fresh blood entered the pulp from the artery, a mechanism would exist by which the corpuscles could be concentrated in the pulp. It is fair, however, to point out a criticism made to us by Dr Duncan Scott, namely, that we had probably destroyed the lymphatics as well as the nerves.

This is perhaps a suitable place to say a few words in discussion of some recent research. Scheunert and Binet and their respective co-workers have carried out researches in which they have found a considerable increase in the number of red blood corpuscles per cubic millimetre of circulating blood during conditions of exercise, asphyxia, etc., in the majority of which the polycythæmia has taken place in the normal but not in the splenectomised animals. Scheunert and Krzywanek(2), for example, in the horse found that after five minutes' work the red corpuscle count rose from 7·4 to 9·3 millions per cubic mm. and the corpuscle volume correspondingly rose from 29 p.c. to 36 p.c. (taking the average of the two jugular veins). This they attribute in part to the abstraction of water from the blood, but not entirely, for the changes in the refractive index of the plasma proteins, though they indicate a loss of water, do not indicate a sufficient loss of water to account for the whole increase in red cells. Nor is the increase in white cells commensurate with the increase in red cells. The question arises to what extent can the contraction of the spleen account for a change in the corpuscle volume of say from 29 to 39 p.c. (*i.e.* of approximately 25 p.c. of the final volume). The weight of the dead spleen was 2·6 kilos.—that after exercise on the analogy of dogs would be about 3 kilos. and before exercise would probably not be more than 9 kilos. on the same analogy. The corpuscles form 29 p.c. of the circulating blood and 61 p.c. of the blood added by the spleen (such is stated by Scheunert and Krzywanek). Let us further assume that the initial volume of blood in the animal was 40 litres.

	Corpuscle volume litres	Plasma volume litres	Total volume litres
Initial blood	11·6	28·4	40
Blood from spleen	3·7	2·3	6
	15·3	30·7	46

The corpuscle volume is now about 34 p.c. instead of 29 p.c.; so far no reabsorption of plasma by the tissues has been considered. The authors judge from the refractive index that such a reabsorption takes place and that the blood may be reduced to its original volume; if so there would be 15·3 litres of corpuscles in 40 litres of blood, which would be 38 p.c. That is something of the order which they noted. Whilst the

calculation is within the bounds of possibility it seems to us unlikely that the spleen has contributed the whole of the corpuscles which form the basis of the increased corpuscle volume; it appears more probable that the blood volume has increased and that some corpuscles have come from places other than the spleen. Any exact correlation between the nervous splenic mechanism which is responsible for the expulsion of the blood from the spleen pulp and the factors which regulate the imbibition of water by active muscle is not known to exist.

Somewhat similar experiments have been carried out by Binet(5) and his pupils, notably on asphyxia and hæmorrhage. Take one of their examples on the dog. The animal was made asphyxial, and with the pedicle of the spleen compressed the corpuscle count rose during asphyxia from 8·2 to 8·6 millions. The pedicle was released and there was a further rise from 8·6 to 9·3 millions. If these figures were applied to a dog with 400 c.c. of blood whose splenic blood contained 33 p.c. more corpuscles than the normal blood, the spleen would be expected to supply 100 c.c. of blood—that in our opinion is too much, at all events it is more than could be expected of an extra-cutaneous spleen. The principal difference between the basis of the above calculation and that made with regard to Scheunert and Krzywanek's experiments is that the latter authors definitely state that the blood in the splenic vein of the horse contains a corpuscle volume 100 p.c. greater than that for the general circulation and for Binet's asphyxial dog we have assumed 33 p.c. There is no evidence from the extra-cutaneous spleen suggesting that the venous blood from the dog's spleen contains anything like twice the volume of red cells found in the general circulation, but of course the extra-cutaneous spleen may not function so perfectly as the organ in its normal situation. Dr Feldberg of Berlin has informed us privately that he has obtained results which confirm the scale of splenic contraction involved in Binet's experiments.

There seems to be no doubt, however, that the spleen does play a considerable rôle in the increment of corpuscles both during asphyxia and during exercise, even though it may not be responsible for the whole phenomenon.

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