Papers and Originals

THE SPLEEN AND ANAEMIA*

BY

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The spleen has not been a popular subject for Goulstonian lectures, and a perusal of the records reveals that one of the last times in which it was taken as a subject was in 1722. In that year William Stukely gave his lecture entitled "Of the spleen, its description and history, uses and diseases, particularly the vapours with their remedies." It is an interesting lecture for several reasons. It starts off with a sonnet by the Duchess of Winchelsea and ends with a description of the dissection of an elephant, but the main part of the lecture contains a good review of what was thought about the spleen in the eighteenth century, and from this we can see by how much our own knowledge has progressed.

At that time the spleen was customarily thought of as the seat of emotions. It was thought to have a glandular function and to supply heat and blood to the stomach during digestion. Discussing these functions, Stukely (1722) states that "laughter is a passion proper to the human race . . .," that "diaphragmatic concussions reciprocally assist the spleen, whence mirth at meals must be very useful towards a good digestion"; he goes on to say, "Wisely, therefore, did our ancestors keep their jesters to entertain them at dinner, whence from having a good digestion they begat an athletic and hardy race." He then concludes as follows: "Quite contrary is the practice in religious houses and colleges, where the Scripture is preposterously read at mealtimes and a superfluous demureness of countenance prepares them all for the diseases of an unactive spleen. . . . The remedy, therefore, is obvious, and without the concurrence of cheerfulness, exercise, open air and conversation, all medicine is impotent." Despite this rather tautologous reasoning and the fanciful eighteenth-century physiology, Stukely later draws on his vivid imagination and reaches an inspired conclusion from his rather limited macroscopic observations on the appearance of the spleen. He states: "This contrivance in Nature can serve no other purpose than most effectually to form a sponge which when filled by the arterial blood can at pleasure, upon its contraction, throw it all out again into the great channel of the splenic vein." This conclusion forestalled that of Joseph Barcroft by two hundred years, while in the interval Hodgkin (1822) demonstrated the contraction of the spleen of cats after immersion of their limbs in cold water and discussed the discharge of blood from the spleen under stress.

Our knowledge of splenic function has increased by some extent since those days. We have discarded some of the more fanciful presumptions of our predecessors, but, while in general terms we know more of what functions the spleen accomplishes in the body, we are ignorant of many of the details by which these activities are achieved. It is my purpose in this lecture to consider in some detail the relationship of this organ to the red cell, and I shall approach the problem initially from the viewpoint of the physiologist and later see how this view accords with clinical phenomena. Most of the work which I shall

describe would not have been possible without the help of many colleagues, and I would like particularly to record my thanks to Miss D. Nightingale, Miss J. M. MacAlister, Drs. A. J. Bowdler, J. Richards, and P. J. Toghill, and to a number of our students who have so willingly submitted to our investigations.

Destruction of Red Cells in the Spleen

There is no very conclusive proof that the spleen destroys red cells in health. However, red-cell fragments may be found in the phagocytic cells of the spleen (Doan, 1926) and to a small extent there is an accumulation of radioactivity over the spleen if radioactively labelled cells are injected into a healthy subject and the fate and site of accumulation of radioactivity followed (Schloesser, Korst, Clatanoff, and Schilling, 1957). If cells are similarly labelled and injected, but prior to injection treated so as to render them metabolically inactive, then they disappear from the circulation rapidly with a half-time of 12 hours and radioactivity accumulates in the spleen, liver, and bone-marrow (Harris, MacAlister, and Prankerd, 1957). Normal red-cell destruction has now been clearly demonstrated to be a function of cell age (Callender, Powell, and Witts, 1945), and since many chemical studies indicate that ageing of red cells is accompanied by their diminishing metabolic activity (Prankerd, 1961) it is likely that the injection of non-metabolizing cells simulates over a short time-scale the normal destruction of ageing cells. This suggests that the role of the spleen in normal cell destruction is only a passive one along with the other component parts of the reticulo-endothelial system, and the fact that splenectomy does not influence normal cell survival would support this conclusion.

In disease, however, this state of affairs is often reversed, and we are all familiar with instances in which the spleen has become the principal destructive site of red cells. An invariable feature that seems to accompany this change in splenic activity is an increase in the total red-cell content of the spleen. This can be estimated from the haemoglobin content of a portion of spleen removed at operation. The meagre amount of blood present in the normal spleen (that is, one usually removed for rupture or during gastrectomy), which consists of about 40–60 ml., is shown in Table I together with the remarkable increase in red-cell content which may occur in haemolytic states in which there is

TABLE I.—*Total Red-cell Contents of Various Spleens Removed at Operation*

	mg. Hb/g. Spleen	Spleen Wt. (kg.)	Total R.B.C./ml.
Normal (total gastrectomy) ..	5.9	0.145	26
Normal (ITP)† ..	6.7	0.170	35
Hereditary spherocytosis {	180	0.645	350
	210	0.466	295
	175	1.950	970
Lymphoma {	67	0.490	100
	142	0.824	360
	179	1.76	960
Primary hypersplenism ..	182	3.1	1,700
Autoimmune haemolytic anaemia ..	155	0.770	380

†ITP = Idiopathic thrombocytopenic purpura.

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intrasplicenic red-cell destruction. Such a change in red-cell content implies the possibility of a change in the circulation through the spleen in order to bring it about, and I wish in this lecture to devote the first part to a consideration of certain aspects of the splenic circulation.

Dynamics of Splenic Circulation

The vascular anatomical arrangements of the spleen have been the centre of a long argument regarding the possibility of closed and open types of circulation (Knisely, 1936; Björkman, 1947). I do not intend to enter this conflict, but I wish to consider first some new facts concerning the dynamic state of the splenic circulation. This is most readily studied by injecting red cells labelled with radioactive chromium into the peripheral circulation and recording their entry and flow in the splenic circulation by means of a continuous recording external counter (Harris *et al.*, 1957). At the same time rates of flow in other organs can be monitored and the disappearance of the cells from the circulation can be followed. The studies which I shall be describing have been made either in our own students, who have been fully aware of the objects of the experiments and have volunteered for these studies, or they have been made in patients as part of what are now routine investigations into the advisability of splenectomy, the necessity and object of the investigations having been explained to them. With such a monitoring system a rather striking feature about the normal splenic circulation emerges (Fig. 1). This feature is the rapidity in which equilibrium

FIG. 1.—Uptake of radioactivity recorded by an external counter over the spleen and heart after injection of ^{51}Cr -labelled normal red cells into a normal subject.

is attained over the spleen compared with the same phenomenon over the heart.

By plotting the differences between the counts at given times over the spleen and that at equilibrium on semi-logarithmic paper one finds that the mixing curve is a single linear function (Fig. 2). Although the processes resulting in this logarithmic function are certainly complex and include extrasplenic phenomena, such as peripheral circulatory mixing and recirculation, it is probably fair to conclude that these apply equally to the precordial curve and that the rate of establishment of equilibrium is of the same order over the splenic as over the precordial site. Thus the circulation through the spleen appears to be rapid and undelayed. Additional evidence that there is no delay in transfer of normal cells through the spleen or elsewhere can be obtained from the peripheral blood counts, which reach an equilibrium in some two minutes after the injection of labelled cells and thereafter remain constant (Fig. 3). Furthermore, after the injection of normal labelled plasma the curve obtained from counting

over the spleen can be superimposed over that obtained with labelled red cells, indicating that there is unlikely to be any separation of these components within the spleen (Fig. 4).

The two principal observations which I have mentioned so far—firstly, that the red-cell content of the normal spleen is only some 20–30 ml., and, secondly, that the blood flow has features of a fast-flowing communication between artery and vein—make it very unlikely that the human spleen has any function as a storage organ of blood in man. If there were a reserve of red cells in the spleen attaining its own equilibrium with labelled cells in the rest of the circulation, then one would expect the mixing

FIG. 2.—Semilogarithmic plot against time of the differences between successive points in the splenic radioactivity curve and its equilibrium level, obtained in man and cat.

FIG. 3.—Levels of radioactivity in the peripheral blood of man and cat after injection of ^{51}Cr -labelled normal red cells. In the top instance a cat was infused with noradrenaline.

curve recorded by external counting to show two phases of radioactive accumulation, and the peripheral blood counts to continue to fall during the period of mixing. It is, however, still often quoted that the spleen acts as a storage organ for blood in man, and this belief appears to emanate from the dangerous practice of arguing physiological phenomena from animals to man. Barcroft and Barcroft (1923), in their well-known studies on the spleens of rats, rabbits, and cats, showed that the splenic blood became more slowly saturated than the peripheral blood on exposing these animals to carbon monoxide, and that the reverse held on re-exposing the animals to air. They

FIG. 4.—Uptake of radioactivity by the spleen of a normal subject after injection of ^{51}Cr -labelled compatible red cells from a patient with hereditary spherocytosis (H.S.) and ^{51}Cr -labelled plasma on separate occasions. Plasma radioactivity reduced by one-half.

concluded that these experiments showed a slowly exchanging pool of blood in the spleen, confirming incidentally Stukely's original suggestion. In further experiments they showed that under conditions of excitement the animals no longer had a slower uptake of carbon monoxide in the splenic blood—evidence that this pool of blood was under some sort of nervous control—and later radiographic studies (Barcroft *et al.*, 1925) revealed that there was a contraction of the spleens of these animals during excitement, although it was not possible to show this in the spleens of monkeys.

If we apply our same radioactive experimental technique to animals and study the rate of equilibration of radioactively labelled cells in the cat's spleen, then we find quite a different type of equilibration curve from that which I have described in man (Fig. 5, B). In this animal no quick equilibrium is achieved, and the curve, after an initial rapid increase, continues to rise more slowly for many

FIG. 5.—Uptake of radioactivity by the spleens of two cats (one given noradrenaline) after injection of ^{51}Cr -labelled cat red cells. A=After 2 μg . noradrenaline. B=Control.

B

minutes. Over the same period of time the peripheral blood counts persistently fall. Analysis of this splenic curve shows that it consists of two logarithmic components with considerably different rate constants (see Fig. 2). One can show mathematically that the curves in the cat are consistent with the expected pattern of a two-compartment system in which one compartment is exchanging slowly with the other, the labelled red cells behaving no differently from those of the rest of the population, as has been shown to be the case in certain abnormal states in man (Bowdler, 1962). Eventually, after about 20 minutes, a final equilibrium is achieved, and from the fall in the peripheral blood count one can calculate the amount of blood in the slowly exchanging compartment; it amounts to about 6–10% of this animal's total red-cell mass.

There is therefore evidence of a slowly exchanging red-cell pool in the spleen of cats but not in man. In the cat this pool can be eliminated by the injection of noradrenaline (Fig. 5), and no fall in peripheral blood counts occurs (see Fig. 3). On the other hand, if the injection of noradrenaline is delayed until after radioactive equilibrium has been reached then the radioactivity recorded over the spleen diminishes while simultaneously that in the animal's peripheral blood increases (Fig. 6), indicating the discharge

FIG. 6.—Record of splenic radioactivity in the cat after injection of noradrenaline. Radioactive equilibrium following the injection of ^{51}Cr -labelled normal red cells had previously been reached.

of a concentrated pool of labelled cells into the circulation. Since, in man, there is no evidence of a pool, one would not expect any response to the administration of noradrenaline. However, the spleen counts do drop after the injection of noradrenaline into man, but there is no rise in the peripheral blood counts (Fig. 7). The drop in the spleen counts

FIG. 7.—Record of splenic radioactivity in man after infusion of noradrenaline. Radioactive equilibrium following the injection of ^{51}Cr -labelled normal red cells had previously been reached.

most probably represents a generalized vasoconstriction in the spleen with a reduction in its vascular bed, and the only red cells lost from the spleen have the same specific activity as those in the peripheral blood.

Production of Red-cell Pooling in Man

Although the normal spleen of man perfused by normal red cells shows no evidence of a red-cell pool, it is possible to produce red-cell pooling by altering the conditions of examination (Harris *et al.*, 1957). This can be done most readily by facilitating the introduction of abnormal cells into the spleen. Those that produce the greatest degree of pooling are spherocytes. The injection of naturally occurring spherocytes from a patient with hereditary spherocytosis into the peripheral circulation of a compatible recipient or the injection of artificial ones, prepared by heating cells to 50° C. for 5 to 10 minutes (Harris *et al.*, 1957), will produce varying degrees of pooling in the normal spleen (see Fig. 4). In fact, the injection of almost any naturally occurring cell of abnormal shape will produce some degree of pooling, and this can be shown with thalassaemic cells, sickled cells, cells from patients with pernicious anaemia in relapse, and with elliptocytes, but I have not been able to demonstrate pooling with the small cells of patients with iron deficiency.

Although spherocytic cells are removed from the circulation permanently at a more rapid rate than normal, with some of the other abnormal cells the rate of permanent removal is only slightly increased, or even normal. In other words, some types of cell can survive the effects produced by pooling in the spleen reasonably well, while others cannot. Apart from altering the shape of cells, other changes in the physical characters of the cells may also produce splenic pooling. It seems that an alteration in the surface charge, which may be produced by coating the cells with antibody (Harris *et al.*, 1957), by removing some of the surface lipid components (Harris *et al.*, 1957), or by blocking the surface sulphhydryl groups (Jacobs and Jandl, 1962), will also produce the same effect. The effect of antibodies on the removal of red cells from the circulation is a phenomenon very dependent on the type of antibody used, and, while some lead to removal of coated cells by the spleen, others lead to removal by the liver (Mollison, 1959).

It is interesting that a red-cell pool produced in the normal spleen in this manner behaves like the red-cell pool in the cat's spleen after an injection of noradrenaline. In Fig. 8 the pooling was again produced by the injection of cells from a patient with hereditary spherocytosis, and after this had reached a reasonable degree an infusion of noradrenaline at 16 $\mu\text{g./min.}$ was started. A prompt reduction in splenic surface counts was observed which is similar to that observed with the normal spleen infused with normal cells. However, in this instance there was

FIG. 8.—Interrupted record of splenic radioactivity in a normal subject after injection of ^{51}Cr -labelled compatible red cells from a patient with hereditary spherocytosis, before, during, and after an infusion of noradrenaline.

a marked rise in radioactivity in the peripheral blood (Fig. 9), indicating that the trapped cells had been discharged into the circulation. After cessation of the infusion an equivalent amount of radioactivity appears to return to the spleen quite rapidly.

FIG. 9.—Peripheral blood count from the subject in the experiment shown in Fig. 8.

It has been stated by Henschen and Howald (1929) that denervation of the cat's spleen produces a considerable increase in engorgement of that organ, and we have been able to show that autonomic blockade in the cat with trimetaphan is followed by an increase in red-cell pooling in the normal spleen (Fig. 10). In man, however, we have

FIG. 10.—Record of splenic radioactivity in a cat following the injection of ^{51}Cr -labelled red cells and 0.25 mg. trimetaphan after radioactive equilibrium had been obtained.

not found that autonomic blockade by continuous infusion of trimetaphan, sufficient to lead to a fall in blood-pressure of 70/50 with the subject at 45 degrees, would lead to pooling of normal red cells in the normal spleen.

Natural Occurrence of Red-cell Pooling in Man

The methods of producing splenic pooling which I have described so far all involve the introduction of abnormal cells to the normal spleen, and the phenomenon of pooling may or may not be associated with increased red-cell destruction. A splenic pool may also be found in other circumstances, such as when the red cells are normal but the spleen is abnormal as a result of disease (Harris, McAlister, and Pranker, 1958; Bowdler, 1962). In such instances there is always splenomegaly which is associated with a large increase in splenic red-cell content. I have observed such pooling of apparently normal red cells in

a pathologically enlarged spleen in the leukaemias, Hodgkin's disease and other lymphomata, Gaucher's disease, and idiopathic splenomegaly.

It seems also that a spleen already enlarged as a result of the continual circulation of abnormal red cells may show the characteristic of pooling as if it were a result of the consequent splenomegaly itself. This can often be demonstrated, for example, in the enlarged spleen of hereditary spherocytosis, over which pooling may also be sometimes found after the injection of labelled compatible normal cells. In congestive splenomegaly it is unusual to find evidence of a pool in spite of the intense splenic congestion, and the reason for this is apparent from the histology.

We may summarize the problem of splenic pooling as so far discussed in the following way. Firstly, normal splenic blood flow in man appears to be entirely a rapid affair, in contrast to that in certain other mammals, such as the cat and goat, in which a compartment of slow pooling exists; this second compartment can be expelled into the general circulation under the influence of noradrenaline. Secondly, a similar slowly exchanging pool of cells can be produced in the spleen of normal man by the injection of certain abnormal types of cells; they can also be expelled by adrenaline. Pooling is also found to occur in certain instances of splenomegaly; and, as might be expected, these spleens are especially vascular.

Anatomical Site of Pooling

At this point it is probably as well to consider what anatomical evidence there is of the whereabouts in the spleen of the pooling which I have described. The human spleen is usually regarded as consisting of white and red pulp, but for the sake of clarity I shall refer to red cells in the sinusoids as intrasinusoidal and those outside the sinusoids as extrasinusoidal. In those animals which show pooling red cells can readily be demonstrated lying among the Malpighian corpuscles and the lymphatic cords, and the spleens of these animals have many muscular septa traversing their substance.

It does not appear to be possible to demonstrate sinusoids in the cat and goat spleen (Richards, unpublished observations), two animals that show marked pooling, so the arterioles would appear to end blindly in the splenic substance, the cells being collected from there into veins. In the human spleen sinusoids are abundant and red cells appear to be almost entirely within them, the lymphatic cords being predominantly empty and the substance of the spleen mainly devoid of muscular septa.

After the injection of noradrenaline into the cat the spleen appears to contract and to discharge almost completely the red cells which have accumulated in it. The spleen in this state no longer shows any isotope evidence of its containing a red-cell pool (see Fig. 5, A).

It would thus appear that in these animals intrasplenic spaces are the sites of red-cell pooling. It is perhaps worth mentioning here a fallacy in examining post-mortem spleens which may or may not apply to the pathological spleens of man. In the case of the goat, for instance, the mode of death produces gross changes in the distribution of red cells within the spleen. In a section of the spleen of a goat dying under pentobarbitone anaesthesia the spleen appeared full of red cells packed in the splenic substance. By way of contrast was the appearance of the spleen of a goat which suffered an agonal death as the result of an accident. This goat, which ended its peaceful existence after winding its tether around its stake until it asphyxiated,

had a spleen which appeared contracted and virtually devoid of red cells, reminiscent of that of the cat after noradrenaline.

Since pooling on a similar time-scale occurs under appropriate conditions in both cat and man it would seem unlikely that the sinusoids represent the site of pooling in man. Furthermore, in congestive splenomegaly, a condition showing marked sinus congestion and little if any extrasinusoidal congestion, it is most unusual to find evidence of pooling, while in the disease associated particularly with pooling—namely, hereditary spherocytosis—the spleen shows extrasinusoidal congestion most conspicuously, with no evidence of sinus congestion. In other patients who have shown pronounced evidence of pooling during isotope studies a marked degree of extrasinusoidal congestion was evident on microscopy of the removed spleens. Very occasionally, in congestive splenomegaly in which there is gross splenic congestion, extrasinusoidal extravasations of cells occur in the spleen, and in one patient whose spleen showed these changes pooling had been demonstrated.

It would appear very probable, then, that the site of pooling in the human spleen is extrasinusoidal amongst the lymphatic cords, a part of the spleen showing minimal evidence of red-cell circulation in the normal state.

What anatomical system of circulation is compatible with the findings which I have so far described? Firstly, I think that for the major part of the splenic circulation there can be no direct continuity between arteries and veins, for if there were it seems almost impossible to visualize a system which would selectively filter from the direct channels those abnormal cells which we know to be selectively retained in a splenic pool. A system which seems to me most probable is one in which the arterioles end amongst the lymphatic cords which form a loose filter and do not obstruct the flow of normal cells into the sinusoids, the bulk of the extrasinusoidal space being devoid of circulation. That some arteriovenous anastomoses may exist is quite possible, but dynamic studies will not demonstrate their existence. If there is any change in shape or surface charge of the red cells, then their permeation through the lymphatic filter becomes impeded and an obstructive vicious circle ensues, the cells being forced into the extrasinusoidal splenic tissue. In such a system any disease of the spleen may alter the filtering system by increasing the depth of the filtering cords or by diminishing their mesh, thus obstructing the flow of normal cells. The last situation may be expected to occur in diseases in which lymphatic proliferation occurs, while in Gaucher's disease, where the sinusoids become obstructed by the carbohydrate-loaded cells, congestion may become extrasinusoidal because of the more distal obstruction.

I want to consider next what is the significance of this pool of cells in the spleen in the production of anaemia. I think this question resolves itself into two parts. Perhaps the first is whether splenic pooling of red cells produces in them any particular deleterious effect, and the second what effect splenic pooling may have on the total circulation.

Significance of Red-cell Pooling

Effect of Pooling on Red Cells

To obtain information about the changes undergone by red cells in the splenic pulp is difficult, since it is not possible to sample this part of the spleen without contamination from cells in the rapidly flowing parts of the circulation such as the sinusoids. However, some information can be obtained from samples which are known to be

mixtures, if these show significant differences from the arterial blood. A number of analyses have been made by different investigators, and certain trends are clear. Usually the samples have been obtained at splenectomy from the splenic artery and vein, and from the splenic substance immediately after the spleen's removal by allowing the cut surface to drip; at any rate, this is so in our own studies. The clearest differences found have been in the osmotic fragility (Emmerson *et al.*, 1956; Motulsky *et al.*, 1958) and the mechanical fragility (Pranker, unpublished observations), which are increased, and the electrolyte content, which shows a fall in potassium-sodium ratio (Pranker, 1960). Metabolic changes such as rate of glucose utilization and phosphate ester composition have not shown such clear differences (Motulsky *et al.*, 1958; Pranker, 1960). In Table II it will be seen that cells in

TABLE II.—Osmotic Changes in Red Cells in the Spleen

Red Cells	Spleen	Pooling	% Lysis in Saline (0.5%)		
			Peri-pheral Blood	Splenic Pulp	Splenic Vein
Normal	{ Normal Gastrectomy	—	0	0	0
Normal	{ Enlarged ITP	Nil	0	0	0
Normal	{ Enlarged Hodgkin's	Nil	0	0	0
Normal	{ Enlarged Leukaemia	+	0	10	0
Coombs +ve .. .	Enlarged	+	20	70	40
Coombs +ve .. .	Enlarged	+	45	80	60
Hereditary spherocytosis	Enlarged	+	75	100	80
Hereditary spherocytosis	Enlarged	+	60	90	80
Thalassaemia .. .	Enlarged	+	0	5	0

or emerging from the spleens of patients with large pools show an increase in osmotic fragility which is not found in patients whose spleens do not show a pool.

Although it is difficult to demonstrate really impressive changes by these methods in red cells which have been through or are in the spleen, it is clear that certain changes are produced. It seems to amount to the fact that cells are conditioned by the spleen in certain ways. Conditioning can also be appreciated in its simplest form from the flattening of red cells observed after splenectomy, and in a more complicated form in an experiment of Motulsky *et al.* (1958). They studied twins with hereditary spherocytosis, one of whom had undergone splenectomy. The cells of the twin still retaining his spleen underwent 30% haemolysis in 0.5% saline, but the other's cells did not haemolyse at all at this saline concentration. After performing cross-transfusion experiments with labelled cells and estimating the osmotic fragility of the donated cells at various times in their new environment, they were able to show a progressive increase in fragility of the transfused cells in the recipient with the spleen, and the opposite effect in the cells transfused to the recipient without a spleen. This experiment demonstrated that the spleen produces an osmotic conditioning of red cells *in vivo*. From the observations I have already reported it would seem that the site of this conditioning effect must be in the extrasinusoidal spaces.

This brings us to a further point, which is whether pooling is synonymous with intrasplenic destruction of cells, perhaps brought about by some such changes as I have just been describing. To this question one can give a definite answer. In my experience, when significant intrasplenic destruction is occurring then red-cell pooling is always present, but the presence of pooling does not necessarily imply the occurrence of destruction.

What, then, is the critical factor in pooling which may produce red-cell destruction? I think this is the time factor; the duration of the pooling of each cell in the spleen. When long enough this might produce several changes in the cells, such as metabolic damage from glucose deprivation, as a result of close red-cell packing; cell-membrane damage, possibly from the liberation of lysolecithin, a mechanism not much in favour nowadays, or phagocytosis by cells of the reticulo-endothelial system. With regard to metabolic damage from glucose deprivation, swelling and gross metabolic damage occur *in vitro* in cells after four hours' existence devoid of glucose at 37° C. (Pranker, 1961), and this is well within the time for which cells may remain in the spleen in hereditary spherocytosis. We can calculate this time from a knowledge of the exchange rate of these cells passing into the spleen pool in steady state conditions, and the volume of red cells in the spleen. In one patient whose spleen was removed for this disease the total red-cell mass of the spleen was 250 ml. and the exchange rate into the pool 20 ml. per hour, giving a mean stay of cells in the spleen of 12½ hours. Although we do not know what concentration of glucose is available to red cells in the pulp of the spleen, other workers (Motulsky *et al.*, 1958) have shown that extracts of spleen tissue which include blood from the faster-flowing channels show a reduction of glucose concentration per gramme of haemoglobin in hereditary spherocytosis, compared with analyses on spleens in which pooling would not be expected.

Effect of Splenic Pooling on the Total Circulation

If, as I have suggested, the occurrence of pooling is not necessarily synonymous with intrasplenic red-cell destruction, what other role may pooling have in the production of anaemia? One other role is indicated by the following case history.

The patient, a young woman with Gaucher's disease, had a very large spleen. Her haemoglobin had remained at 9 g./100 ml. for 10 years, and, although reasonably well adjusted to her anaemia, her spleen had been progressively enlarging, and as she was unmarried it had become a source of social embarrassment. The survival of ⁵¹Cr-labelled cells was virtually normal, indicating no significant haemolysis, but there was considerable pooling of red cells in the spleen although there was no progressive rise of the splenic counts on subsequent daily counting. A spleen weighing 3.5 kg. was removed at operation, and after the operation the patient's haemoglobin rose to maintain a steady value of 15.8 g./100 ml. The explanation of this satisfactory rise in circulating haemoglobin after splenectomy could not have been that it was a result of an increase in red-cell survival, since this was unchanged after splenectomy. However, additional studies pointed to the cause. The total red-cell mass before splenectomy was 1,630 ml. and after splenectomy 1,234 ml. The plasma volume before splenectomy was grossly increased at 4,350 ml. and fell after splenectomy to 2,210 ml. Thus the whole-body haematocrit rose from 27.2% to 35.8% simply as a result of a contraction of plasma volume. These changes are summarized in Table III.

A response such as this is often taken as evidence that

TABLE III.—Special Investigations on a Patient With Gaucher's Disease

	Before Splenectomy	After Splenectomy	Normal
Body-weight .. .	60.8 kg.	56.3 kg.	—
Red-cell mass .. .	1,630 ml., or 26.8 ml. per kg.	1,234 ml., or 21.9 ml. per kg.	30.3 ± 5.6 ml./kg.
Plasma volume .. .	4,350 ml., or 71.5 ml. per kg.	2,210 ml., or 39.3 ml. per kg.	43.1 ± 5.6 ml./kg.
Blood volume .. .	5,980 ml., or 98.3 ml. per kg.	3,444 ml., or 61.2 ml. per kg.	70 ± 5.6 ml./kg.
Venous haematocrit ..	28.6%	41.4%	
Whole-body haematocrit	27.2%	35.8%	
Red-cell T½Cr survival	22 days	22 days	24–28

the removal of the spleen has removed an inhibitory factor acting on the bone-marrow, but in fact the fall in red-cell mass after splenectomy without change in red-cell survival must have indicated that the production of red cells by the marrow was less after splenectomy than before. A state of "splenic hydraemia" such as was shown in this patient will probably not produce anaemia unless the bone-marrow is also abnormal. In her instance the bone-marrow was widely infiltrated with Gaucher cells, and, although radioactive iron studies showed that marrow activity was increased even before splenectomy (Bowdler, 1963), this was insufficient to maintain a normal haematocrit, an accomplishment well within the range of the normal bone-marrow. Similar cases to this one have been well documented by McFadzean *et al.* (1958) in giant splenomegaly among the Chinese. The cause of the hypervolaemia is not clear, but in this instance a considerable part of the additional 1,800 ml. could easily have been accommodated in the very large spleen.

Although in this case the red-cell and plasma contents of the spleen at operation were not determined, in another patient showing a similar phenomenon the red-cell content of the spleen was 1,000 ml., and the plasma content 800 ml. as determined by isotope dilution at operation, so that part of the increased plasma volume could have been accommodated in the spleen, and was probably required there in order to fill the increased vascular space. This type of dilution anaemia may be found in chronic leukaemias, and a patient with this disease has survived three years after splenectomy, having been moribund from anaemia beforehand without any evidence of haemolysis. His response to splenectomy must also be attributed to the contraction in plasma volume which occurred.

In addition to the large increase in plasma volume found in these cases there is also the factor of the exclusion from the peripheral circulation of the large mass of red cells in the spleen; this must also be considered in relation to the development of anaemia. As I have indicated, this may in extreme instances amount to a litre or more of red cells trapped out of the circulation. In occasional instances such trapping can occur acutely and lead to rapidly progressive anaemia. This has been described in sickle-cell anaemia (Motulsky *et al.*, 1958), and in one such patient it was calculated that 40% of the red-cell mass became trapped in the spleen in 36 hours. Among the diseases in which I have found the phenomena of hypervolaemia being of critical importance in the development of anaemia in the presence of splenomegaly are the leukaemias, Gaucher's disease, and thalassaemia. It is probably also a contributory factor in many haemolytic anaemias associated with gross splenomegaly. The two indications that this state exists are the presence of splenic pooling, detected by surface counting, and an increase in the total plasma volume.

I have discounted the inhibitory effect of an enlarged spleen on the marrow because those case reports which show a normal red-cell survival before splenectomy with an increase in haematocrit afterwards have not been studied in enough detail to show that the changes in plasma volume which I have described could not have accounted for the post-operative improvement. In all our studies over the last 10 years I have not encountered any instance in which the occurrence of anaemia could not be accounted for on other grounds than the existence of a splenic inhibition of erythropoiesis.

Value of Splenectomy

I wish lastly to consider what the indications for splenectomy are in the treatment of anaemia. The problem may

be simple or extremely complex, but in either instance the value of isotopic studies is considerable, and it must be borne in mind that often much benefit may be achieved by splenectomy without a complete cure of the anaemia. The isotope studies include the estimation of red-cell survival, the determination of plasma and red-cell volumes, the detection of splenic pooling, and the determination of intrasplenic haemolysis. The last determination may prove difficult to assess (Hughes Jones and Szur, 1957).

From what has already been discussed it will be evident that splenectomy may benefit anaemia in one of two possible ways. Firstly, through the removal of a site of intrasplenic red-cell destruction or, secondly, through a decrease in plasma volume. The unquestionable example of the value of splenectomy acting through the first mechanism is hereditary spherocytosis (Young, 1955). Splenectomy should probably be undertaken in all affected individuals regardless of the presence or absence of anaemia, since it reduces the liability to crises and the development of gall-stones. This is probably the only disease in which the benefit of splenectomy on anaemia is predictable without recourse to the tests already outlined.

In the hereditary non-spherocytic haemolytic anaemias, such as those associated with glucose-6-phosphate dehydrogenase, pyruvic kinase, and diphosphoglyceromutase deficiency, the value of splenectomy is only slight (Dacie *et al.*, 1953). This contrast between the benefit of splenectomy in the spherocytic and non-spherocytic anaemias, in both of which there is defective red-cell metabolism, is probably based on the severity of the intracellular defect. In the last group the defect is so severe as to jeopardize survival anywhere in the circulation, whereas in spherocytosis the defect hazards the life of the cell only under conditions of stagnation in the spleen, the latter being brought about by the increase in the thickness of the cells. At most, splenectomy in hereditary non-spherocytic anaemias produces only a slight to moderate reduction in the rate of haemolysis and should be undertaken only if isotope studies demonstrate a marked degree of intrasplenic lysis.

Other conditions in which splenectomy is beneficial through a reduction in intrasplenic haemolysis are the autoimmune haemolytic states and cases of splenomegaly, either primary or secondary, to such diseases as the reticuloses. Splenectomy in these conditions is in my experience of benefit only if the ^{51}Cr red-cell survival is reduced below half its normal value and if in addition surface counting demonstrates a progressive rise in the ratio of splenic to liver counts during the four days after the injection of labelled cells. With less definite evidence of intrasplenic red-cell destruction the value of splenectomy in these conditions is doubtful. An example of these criteria is shown in Fig. 11, and the outcome of splenectomy was complete cessation of haemolysis.

The second mechanism through which splenectomy becomes beneficial, that of a reduction in total blood volume lessening the demand on red-cell production, may operate in a number of circumstances, but only when there is considerable splenomegaly. It would seem that an increase in plasma volume of about 50% is a likely indication of the benefit of splenectomy, especially if this is combined with evidence of red-cell pooling in the spleen and the peripheral blood counts show a reduction of more than 10% in the first hour. Following these criteria, I have seen successful rises in haemoglobin concentration after splenectomy in Gaucher's disease, two cases of leukaemia, and one patient with thalassaemia. It is probably through this mechanism rather than that of intrasplenic haemolysis

that benefit is derived after splenectomy in occasional patients with thalassaemia. The investigations of McFadzean *et al.* (1958) on Chinese with cryptogenic splenomegaly suggest that this anaemia responds to splenectomy through this mechanism also.

I have tried in this lecture to adopt a physiological approach to the pathological problem of the production of anaemia by the spleen by considering firstly our knowledge of the splenic circulation. I think this approach is

FIG. 11.—Splenic, liver, and blood counts in a man aged 20 with autoimmune haemolytic anaemia whose own red cells were labelled with ⁵¹Cr. Radioactivity in the portal-vein blood at splenectomy indicated that the rise in liver counts after the fourth day was due to the transfer of ⁵¹Cr liberated in the spleen from destroyed red cells.

leading, to a small extent, to an understanding of the mechanisms which may operate and to a clearer understanding of our approach to splenectomy. It is only a beginning, but future progress will come from further investigation of some of the clues which may have already appeared to us. We have advanced some way from the days

of Malpighi, when he said of the spleen, "What therefore may be thought of this artificial bowel? I am perfectly ignorant" (quoted by Stukely, 1722).

In conclusion may I quote again the words of William Stukely, who said: "Certainly if beauty be the first temptation to love we cannot but have a particular affection for the spleen. The delicacy of its construction will at first sight convey a strong desire to know what purpose thereby is aimed at in life."

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REFERENCES

- Barcroft, J., and Barcroft, H. (1923). *J. Physiol. (Lond.)*, **58**, 138.
 — Harris, H. A., Orahovats, D., and Weiss, R. (1925). *Ibid.*, **60**, 443.
 Björkman, S. E. (1947). *Acta med. scand.*, Suppl. No. 191, 1.
 Bowdler, A. J. (1962). *Clin. Sci.*, **23**, 181.
 — (1963). *Ann. intern. Med.*, **58**, 664.
 Callender, S. T., Powell, E. O., and Witts, L. J. (1945). *J. Path. Bact.*, **57**, 129.
 Dacie, J. V., Mollison, P. L., Richardson, N., Selwyn, J. G., and Shapiro, L. (1953). *Quart. J. Med.*, **22**, 79.
 Doan, C. A. (1926). *J. exp. Med.*, **43**, 289.
 Emmerson, C. P., Shen, S. C., Ham, T. H., Flemming, E. M., and Castle, W. B. (1956). *Arch. intern. Med.*, **97**, 1.
 Harris, I. M., McAlister, J. M., and Pranker, T. A. J. (1957). *Clin. Sci.*, **16**, 223.
 — (1958). *Brit. J. Haemat.*, **4**, 97.
 Henschen, C., and Howald, R. (1929). *Arch. klin. Chir.*, **157**, 667.
 Hodgkin, T. (1822). *Edinb. med. surg. J.*, **70**, 83.
 Hughes Jones, N. C., and Szur, L. (1957). *Brit. J. Haemat.*, **3**, 320.
 Jacobs, H. S., and Jandl, J. H. (1962). *J. clin. Invest.*, **41**, 1514.
 Knisely, M. H. (1936). *Anat. Rec.*, **65**, 23.
 McFadzean, A. J. S., Todd, D., and Tsang, K. C. (1958). *Blood*, **13**, 524.
 Mollison, P. L. (1959). *Brit. med. J.*, **2**, 1035.
 Motulsky, A. G., Cassard, F., Giblett, E. R., Broun, G. O., and Finch, C. A. (1958). *New Engl. J. Med.*, **259**, 1164.
 Pranker, T. A. J. (1960). *Quart. J. Med.*, **29**, 199.
 — (1961). *The Red Cell*. Blackwell, Oxford.
 Schlosser, L. L., Korst, D. R., Clatanoff, D. V., and Schilling, R. F. (1957). *J. clin. Invest.*, **36**, 1470.
 Stukely, W. (1722). Goulstonian Lectures, Royal College of Physicians, London.
 Young, L. E. (1955). *Amer. J. Med.*, **18**, 486.

STUDIES OF URINARY TRACT INFECTIONS IN INFANCY AND CHILDHOOD

I. ANTIBODY RESPONSE IN DIFFERENT TYPES OF URINARY TRACT INFECTIONS CAUSED BY COLIFORM BACTERIA

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A urinary tract infection caused by coliform organisms in man may elicit a measurable antibody response as shown as early as 1898 by Pfaundler, who employed a bacterial agglutination technique. Further studies by Siede and Luz (1941) using a similar technique indicated a certain difference in this antibody response in cases with pyelitis and those with cystitis. However, some workers (for example, Gillisen, 1954; Kaarsalo, Kasanen, Laurent, Piironen, and Raunio, 1962) have been critical of the possibilities of employing such a technique in the study of human urinary tract infections. Recent studies by Needell, Neter, Staubitz, and Bingham (1955) and by Slifkin, Ward, Fischer, and Cromartie (1959) using a haemagglutination method have given some evidence for the usefulness of such a method in the analysis of the antibody response in this type of coliform infection.

However, the characteristics of this antibody response are incompletely known. It seemed to us, therefore, that it would be interesting to pursue a systematic study of the antibody response, dealing particularly with its specificity and with the possibilities that antibody titration may offer in diagnosis, in following the course of infection, and in the anatomical classification of the site of the infection.

Patients and Controls

Twenty patients were studied, and there were 25 controls.

Sixteen of the patients had acute infections. They were hospitalized during the initial stage of the disease. Gross anatomical malformations were thought to be ruled out on the following grounds: in most patients by a rapid initial response to treatment and no recurrence during an observation period of at least six months; in those with a bad initial response to