proliferating. It is convenient to refer to such clones and the immunological patterns they carry as "forbidden" clones or "forbidden" patterns, and to regard the mechanism by which they are eliminated as part of the general homoeostatic control of body structure and function.

A wide variety of medical conditions in the body represent the partial failure of homoeostatic mechanisms —auto-immune disease can be interpreted best as another example of partial and often temporary failure of one such mechanism. Instead of being eliminated certain forbidden clones of cells find opportunity to proliferate and, in reacting with the antigen involved, inflict damage on the body.

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[*The second Croonian Lecture will appear in next week's issue.*]

EXPERIMENTAL STUDY OF AN ERYTHROPOIETIC PRINCIPLE PRODUCED IN THE KIDNEY

BY

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The present paper is concerned with problems related to erythropoiesis. An attempt is made to extend our knowledge of the mechanism regulating the level of erythrocytes in the body, and to throw light on the pathogenesis of some types of anaemia. Special attention is given to anaemia developing in the uraemic condition.

Anaemia occurs in most types of human renal insufficiency. This form of anaemia, like that of chronic infection, appears to be due both to a reduction of the life-span of the red cell and to deficient marrow response (Wintrobe, 1956). That author states: "It has been repeatedly noted, however, that the rate of corpuscular elimination has not been such that it could not be met by increased production, were erythropoiesis accelerated to the full capacity of the bone marrow. It is noteworthy that erythropoiesis in the bone marrow may not be reduced below the normal, and, although there is usually no increase in reticulocytes, an occasional normoblast may be found in the blood smear. Nevertheless, red cell production does not accelerate sufficiently to meet the demand." The cause of the anaemia, however, in renal disease, as well as in chronic infection, hepatic disorder, hormonal disturbance, neoplastic disease, etc., is still obscure.

In the experiments young male mice of the WLO (White Label, Oslo) strain were used. A great number of the animals were rendered nephritic by local *x*-ray irradiation of the kidneys, done under ether anaesthesia. During the irradiation the whole animal was shielded with lead, with the exception of the exteriorized kidney, which was hanging down in a plastic chamber through which Ringer's solution at 37° C. was flowing. The physical data were: 50 kV, 2 mA, hvl 0.3 mm. Al. The distance from focus to mid-axis of the kidney was 31 mm. The dose rate was about 1,700 r/min. An even dose distribution was obtained by irradiation from both sides.

Reticulocytes were counted in Nile-blue Giemsa-stained blood smears; 2,000 cells were counted on each slide. As an immediate reticulocyte response of short duration may be regarded as an outflow of reticulocytes not related to cell production (unpublished personal material), only protracted reticulocytosis of several days' duration has been used as an indicator of red-cell production. The haemoglobin value was measured by means of Ljungberg's colorimeter. Blood-urea values were determined in all nephritic and some of the normal animals by means of a gas volumetric method (Horn, 1951). In some of the animals, erythrocytes, haematocrit, and CO_2-combining power ("alkali reserve") (Roughton and Scholander, 1943; Scholander and Roughton, 1943; Scholander *et al.*, 1947) were determined. With exception of the reticulocyte values, only a few of the results of these laboratory investigations are reported here: they are presented in the accompanying charts. To indicate the variation between the observations, ± 1 standard deviation has been calculated, and plotted in the graphs.

Evidence that an Erythropoietic Principle is Produced in the Kidney

The anaemia developing in uraemic mice with irradiated kidneys was found to vary from hypochromic to normochromic. Generally the reticulocyte count was slightly lower in the uraemic than in the normal mice. With increasing blood nitrogen accumulation, the bone marrow showed a hypoplastic erythropoiesis.

During a study, begun in 1956, on functional and anatomical changes in mice produced by *x*-ray irradiation of the exteriorized kidneys, the following observations were made: (1) By irradiation of the kidneys in mice a condition resembling the essential features of glomerulonephritis in man was produced. (2) In mice with total irradiated kidneys a definite but slight decrease in the haemoglobin values was noted, with only slight rise of the blood urea. (3) After unilateral nephrectomy alone, the blood urea rose from the normal 20 mg. per 100 ml. to about 30, and on removing one kidney and half of the other the blood urea rose to about 50 mg. per 100 ml. These animals, however, had only a mild, transient anaemia in spite of their raised blood urea. (4) In mice in which a small amount of kidney tissue was shielded during the irradiation, it was possible to produce uraemia with a blood-urea value about 150 mg. per 100 ml. without anaemia (Osnes, 1958).

These observations led to the following conclusions: The failure of the kidney as an excretory organ and the development of anaemia are parallel phenomena and independent of each other. These findings also indicate that the kidney may produce a principle of importance in erythropoiesis, and lack of this principle

may be the cause of the anaemia developing in *x*-irradiated nephritic mice.

In the animals, mentioned above, with partial nephrectomy or partial irradiated kidneys, normal kidney tissue functioning as an excretory organ was present. It may be disputed whether a uraemic intoxication in such a condition is the same as when all the excretory kidney tissue is involved in disease.

As a further test to show that the kidney produces an erythropoietic principle the following experiments were done:

Three groups of mice were irradiated with 8,000 r on the *left* kidney. In group A the right kidney was removed (control group). In group B the ureter of the right kidney was ligated. In group C a peritoneal outlet from the ureter of the right kidney was made. It will be observed that the right kidney in groups B and C was no real excretory organ because of the ligation of the ureter (group B), or because the urine produced was reabsorbed from the peritoneal cavity (group C). Excretion of urine to the urinary bladder was possible only from the left irradiated kidney in all groups, but the excretory function was so impaired as to produce a marked uraemia in all of them. The average blood-urea value in groups A, B, and C seven weeks after irradiation was 156, 152, and 160 mg. per 100 ml. respectively. The average haemoglobin value seven weeks after irradiation was 46.5% in group A, 66.3% in group B, and 99% in group C (Fig. 1).

The question is, what is the reason for the difference in the haemoglobin values in these cases? If we postulate production of an erythropoietic principle by the kidney tissue, it may be supposed that the left

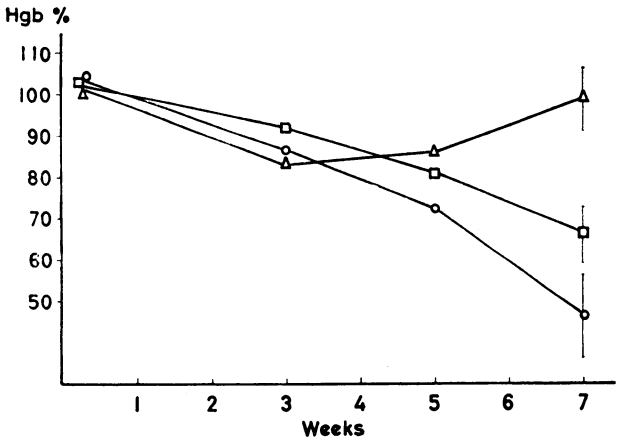

FIG. 1.—Haemoglobin values in mice with *x*-irradiated left kidney (8,000 r). A, Right kidney removed (17): O——O. B, Right ureter ligated (14): □——□. C, Right ureter peritoneal outlet (6): △——△.

irradiated kidney produces the same amount of this principle in all groups. In group A, with the right kidney removed, only the left irradiated kidney could produce this postulated erythropoietic principle. In group B hydronephrosis of the right kidney developed, with partial destruction of the kidney tissue, and a postulated endocrine function might be impaired in this kidney. In group C, however, there was no destruction of the right kidney at all; on the contrary, a marked hypertrophy was observed. If the kidney produces (or activates) any principle of importance in erythropoiesis it may be expected that this function would be intact in this non-irradiated kidney.

In accordance with this, a progressive anaemia developed in group A. In group B an anaemia also developed, but to a lesser degree. In group C an initial anaemia was observed, but later the haemoglobin value increased to nearly normal. The initial anaemia observed in the latter group might be caused by a transitory non-bacterial infection (ureo-peritonitis).

The results of this experiment clearly indicate that the kidney produces (or activates) a principle of importance for the erythropoiesis, and that *x*-ray irradiation of the kidney impairs the production of this principle. Beside

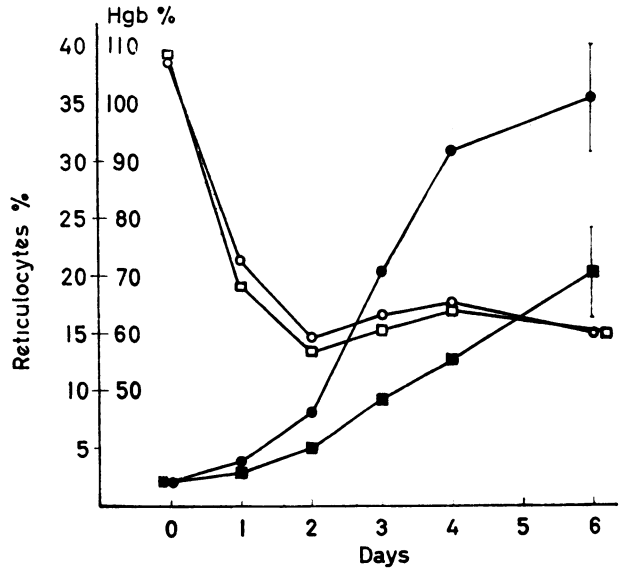

FIG. 2.—Haemoglobin values and reticulocyte response. Normal bled mice (6): O——O, Hb; ●——●, reticulocytes. Partially nephrectomized bled mice (6): □——□, Hb; ■——■, reticulocytes.

this, the experiment shows that retention of toxic products does not necessarily impair erythropoiesis.

These findings are consistent with the following experiments. Fig. 2 shows the haemoglobin value and the reticulocyte response after daily bleeding in mice with intact kidneys, and in mice with partial nephrectomy, only half a kidney being left intact. With the same degree of anaemia in the two groups, the reticulocyte response in animals which had one kidney and half of the other removed was only about one-half of that in the group with intact kidneys. This result is in accordance with the interpretation that a reduction in the mass of kidney tissue implies a reduction in the amount of kidney factor available.

Fig. 3 shows the reticulocyte response in three groups of mice bled to the same degree (0.5 ml.). In group A both ureters were ligated. In group B both kidneys

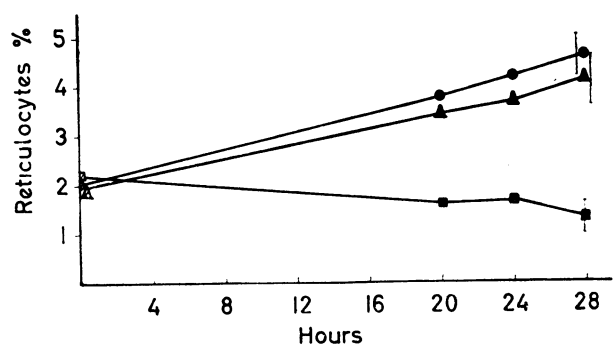

FIG. 3.—Reticulocyte response in 3 groups of mice bled 0.5 ml. A, Ureter-ligated bled mice (6): ●——●. B, Nephrectomized bled mice (6): ■——■. C, Nephrectomized bled mice treated with activated serum (6): ▲——▲.

were removed. In group C both kidneys were removed and the animals were treated with a single injection (2% of body weight) of serum from bled mice ("activated" serum). A good reticulocyte response is seen in animals with ligated ureters (group A), but no reticulocyte response in animals with kidneys removed (group B), in agreement with the findings of Jacobson *et al.* (1957). Group C, however, with kidneys removed, followed by injection of activated serum, shows good reticulocyte response. The average blood-urea levels in these groups 28 hours after intervention were 335, 339, and 332 mg. per 100 ml. respectively. The average haemoglobin values were 70, 71, and 73.3% respectively. The life span after intervention varied from 28 to 40 hours in all groups.

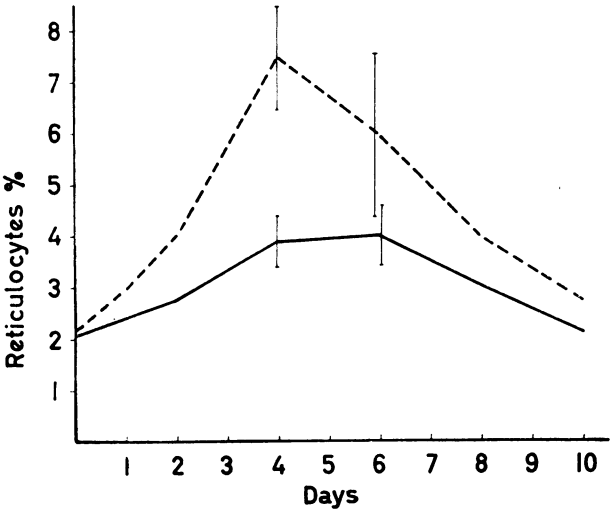

FIG. 4.—Reticulocyte response of normal mice and of nephritic mice treated with one injection of activated serum. Donors: Bled mice. Recipients: ——, normal mice (5); - - - -, nephritic mice (6).

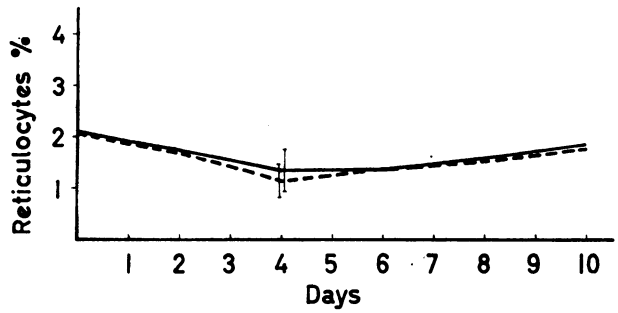

FIG. 5.—Reticulocyte response of normal mice and nephritic mice given one injection of normal serum. Donors: normal mice. Recipients: ——, normal mice (6); - - - -, nephritic mice (6).

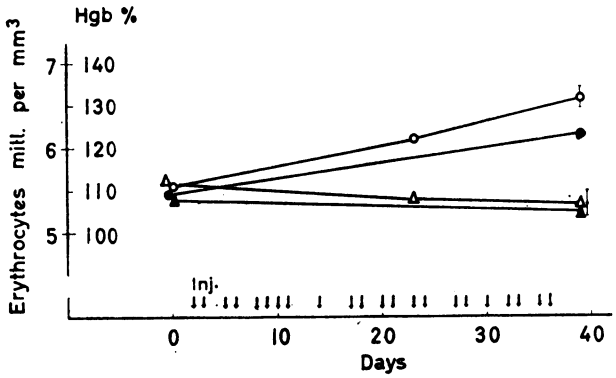

FIG. 6.—Haemoglobin values and red-cell counts. A, Normal mice treated with activated serum (6): O——O, Hb; ●——●, erythrocytes. B, Normal mice treated with normal serum (6): △——△, Hb; ▲——▲, erythrocytes.

The fact that ureter-ligated animals react with reticulocytosis after bleeding, whereas the nephrectomized animals do not, might indicate that the latter were in a condition unable to react with reticulocytosis. The fact, however, that the nephrectomized animals were able to respond to a substance present in serum from bled mice shows the opposite to be true.

These latter experiments agree well with the findings demonstrated in Fig. 1, and corroborate the conclusion that the kidneys produce a principle of importance in erythropoiesis.

Studies on Erythropoiesis-stimulating Substances in Serum from Mice

Carnot and Deflandre (1906) have discussed a factor in plasma capable of stimulating erythropoiesis. Since this observation was made a considerable literature, presenting rather divergent views on the subject, has grown up. Problems related to erythropoiesis have been reviewed by Grant and Root (1952), Erslev (1955), and Jacobs (1958).

The fact that anaemic serum injected into an animal may cause marked erythropoiesis points to a regulating factor in the blood. Many authors have dealt with the isolation of erythropoiesis-stimulating factors. No one, however, has been able to determine the chemical nature of the erythropoiesis-stimulating substances. The nature of the stimulus for secretion of the erythropoietic principle is uncertain, and opinions about which is the tissue of origin are controversial and without firm foundations. Villa and Sala (1937) suggested that a reticulocytosic factor was formed in the liver. These authors were able to demonstrate such a factor in blood from the liver vein in rats, but not in blood from the liver artery or the portal vein. Jacobson *et al.* (1958) found that cobaltous chloride administration, acute haemorrhage, and hypoxic hypoxia after bilateral nephrectomy fail to elevate plasma erythropoietin, whereas bilateral ureter ligation reduces but does not eliminate the rise of plasma erythropoietin. They concluded that the kidney may be the site of erythropoietin production. These authors, however, point out that they cannot state with certainty that a negative response obtained by use of their methods of assay indicates the absence of erythropoietin(s) from the plasma or plasma extracts investigated; and they add that perhaps the kidney may not be involved in erythropoietin(s) production at all.

The term *erythropoietin* is used in this paper to describe a principle found in serum capable of stimulating erythropoiesis when injected into a *normal* organism.

Fig. 4 shows the reticulocyte response in a group of normal mice and in a group of nephritic mice treated with a single injection (2% of body weight) of activated serum. The donor mice were rendered anaemic by bleeding two days in succession (to a haemoglobin value about 40%), and on the third day serum from these animals was given to the two recipient groups. A very good reticulocyte response was observed in normal as well as in nephritic recipients, reaching a maximum about the fourth day, and with a duration of about 10 days.

Fig. 5 shows a parallel experiment where normal and nephritic mice were injected with serum from normal unbled mice. This serum caused no reticulocytosis in the recipient groups; on the contrary, a depression of the reticulocyte values was observed in normal as well as in nephritic mice.

A rise of the reticulocyte values in the blood does not necessarily mean an increased production of red blood cells. In long-term experiments, however, repeated injections (each 0.3 to 0.4 ml.) of activated serum into normal mice (Fig. 6) were followed by a rise in haemoglobin values from 111 to 131.7% and a rise in red-cell count from 5.47 to 6.16 millions/c.mm., whereas the administration of normal serum in the same amounts caused a decrease in haemoglobin values as well as in red-cell counts. In accordance with these findings, the reticulocytosis observed in normal mice after injection of activated serum (Fig. 4) may be regarded as an indication of increased red-cell production.

Fig. 7 shows the haemoglobin values and the body weights in two groups of nephritic mice in which each kidney had been exposed to 4,000 r. At 75 days after *x*-ray irradiation the haemoglobin value, the body weight, and the condition of the animals were about the same in both groups. In group A repeated injections of activated serum (each 0.35 ml.) were started 79 days after irradiation. Group B (control group) was not treated with serum at all, because administration of protein to mice in a nephritic state of this degree had been found to be badly tolerated generally, whereas activated serum could be injected with caution. It is seen that progressive anaemia could be prevented by injections of activated serum, and that with closer-spaced injections the haemoglobin value finally increased. During the experiment the serum-treated animals (group A) showed only a slight decrease of the body weight, and by the end of the experiment they were in a much better condition than the animals in the untreated group. The average blood-urea value was 62 mg./100 ml. in group A, against 114.5 mg. in group B. The average kidney weight was 249 mg. in group A, against 190 mg. in group B. The average heart weight was 140.6 mg. in group A, against 149.2 mg. in group B.

These experiments are promising also with regard to the treatment of human nephritis. As mentioned above, an increased protein load in a nephritic animal usually lowers its condition. We observed that the animals receiving activated serum not only became less anaemic but their general condition, including the uraemia, improved.

Fig. 8 shows the reticulocyte response in a group of normal mice and in a group of nephritic mice treated with a single injection (2% of body weight) of serum from nephritic mice. The serum donors with irradiated kidneys had haemoglobin values ranging from 30 to 68%. Fig. 8 shows that these nephritic donors also possessed substances in their serum able to produce reticulocytosis in normal mice (erythropoietin), but no demonstrable amounts of substances producing reticulocytosis in nephritic recipients. This experiment clearly demonstrates that the substance(s) producing reticulocytosis in normal mice and the substance(s) producing reticulocytosis in nephritic mice must be different.

Fig. 9 shows a similar experiment, but here the serum donors were nephrectomized and bled mice kept alive by means of intermittent peritoneal lavage (Grollman *et al.*, 1951). The serum transfusion was done 50 hours after nephrectomy and bleeding. The serum from these nephrectomized bled mice contained substances producing reticulocytosis in normal mice (erythropoietin) but no traceable amounts of substances producing reticulocytosis in nephritic mice. This observation does not agree with the findings of Jacobson *et al.* (1958) that there is no rise in the erythropoietin titre after bleeding in nephrectomized animals. The present experiment shows that the erythropoietin production can go on in the absence of the kidneys, and it must be concluded that the erythropoietin *per se*, as defined above, is produced outside the kidney.

Fig. 10 shows the reticulocyte response in nephritic mice treated with a single injection (2% of body weight) of serum from bilateral ureter-ligated and bled mice.

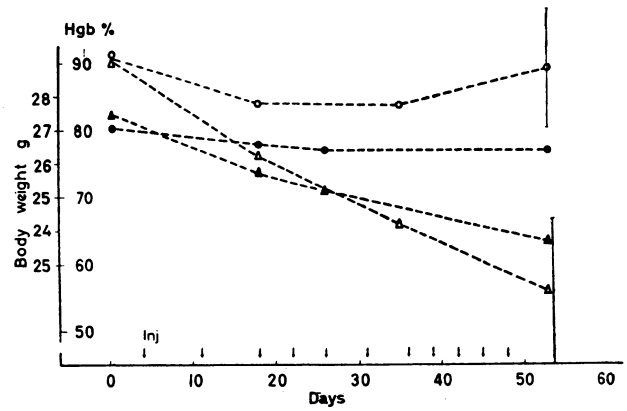

FIG. 7.—Haemoglobin values and body weights in mice exposed to 4,000 r. A, Nephritic mice treated with activated serum (7): ○---○, Hb; ●---●, body weight. B, Nephritic mice not treated (6): △---△, Hb; ▲---▲, body weight.

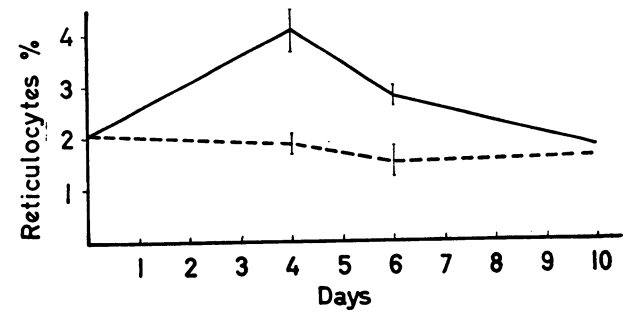

FIG. 8.—Reticulocyte response to a single injection of serum from nephritic mice. Recipients: ——, normal mice (6); ----, nephritic mice (4).

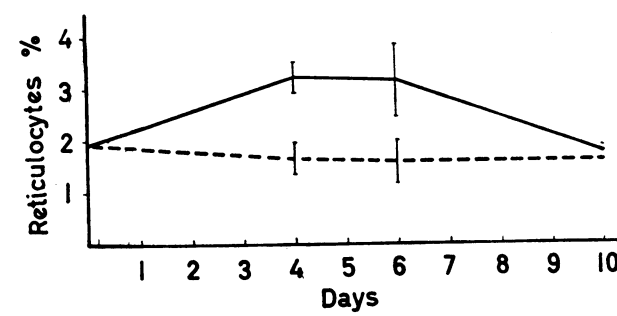

FIG. 9.—Reticulocyte response to a single injection of serum from nephrectomized bled mice. Donors: Nephrectomized bled mice. Recipients: ——, normal mice (6); ----, nephritic mice (4).

As in the former experiment, the donors were kept alive by means of intermittent peritoneal lavage. The serum transfusion was done 46 hours after ligation of the ureters and bleeding. This serum was able to produce reticulocytosis in nephritic mice, and thus the serum from ureter-ligated bled mice (as well as the serum from normal bled mice, Fig. 4) contained an erythropoietic principle which is not found either in nephritic anaemic mice (as shown in Fig. 8) or in nephrectomized bled mice (Fig. 10). In accordance with the findings already demonstrated it may be concluded that this erythropoietic principle, which is different from erythropoietin, is produced (or activated) in the kidney, and the lack of this principle may be the cause of the anaemia developing in *x*-rayed nephritic mice.

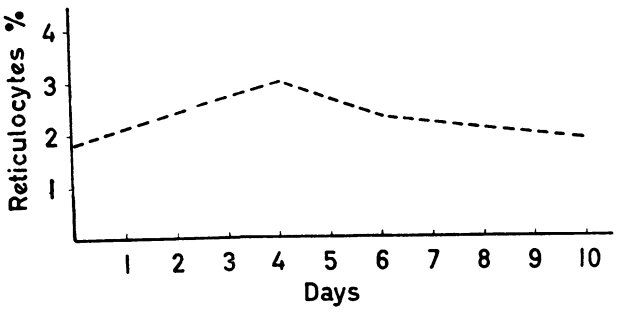

FIG. 10.—Reticulocyte response of nephritic mice to one injection of serum from bilateral ureter-ligated bled mice. Donors: Ureter-ligated bled mice. Recipients: ----, nephritic mice (3).

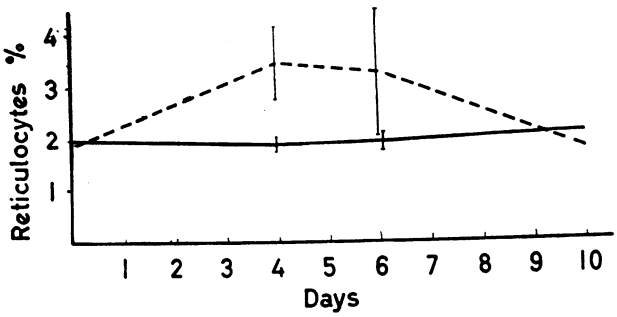

FIG. 11.—Mice treated with ultrafiltrate of activated serum. Recipients: ——, normal mice (6); ----, nephritic mice (6).

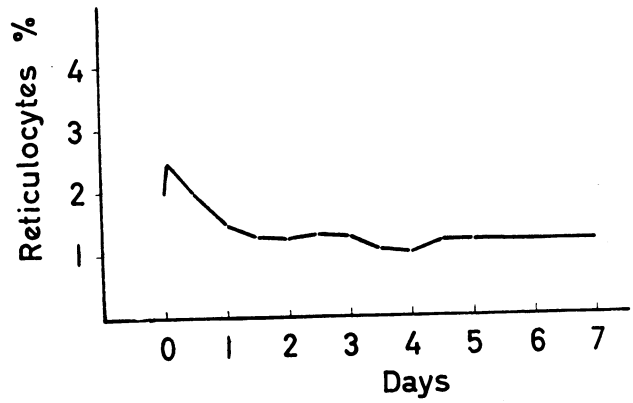

FIG. 12.—Reticulocyte response in six cordotomized bled mice.

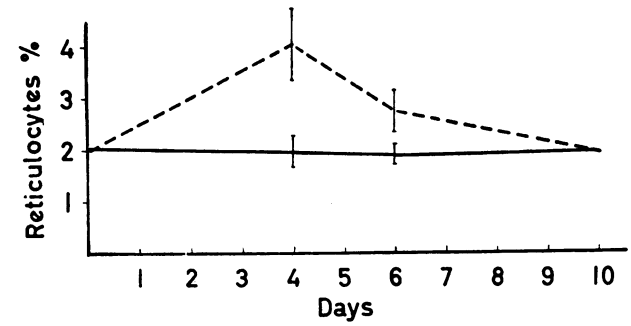

FIG. 13.—Donors: Cordotomized bled mice. Recipients: ——, normal mice (6); ----, nephritic mice (4).

In order to characterize the active principles discussed above, an ultrafiltrate of activated serum was injected into normal and nephritic mice (Fig. 11). The ultrafiltration was made at 2–4° C. in the course of about 28 hours. The recipients received a single injection (2% of body weight). This protein-free filtrate produced reticulocytosis in nephritic mice, but not in normal mice. This observation supports the idea that erythropoietin may be a protein or a protein-bound substance, whereas the principle producing reticulocytosis in nephritic mice is not a protein.

This active protein-free ultrafiltrate, treated in a boiling-water bath for three minutes, injected in the same dose as mentioned above, did not give rise to reticulocytosis in nephritic recipients. This shows that the erythropoietic principle produced in the kidney may be thermolabile.

In accordance with the experiments presented above, erythropoietin, as well as the erythropoietic principle produced in the kidney, may be regarded as necessary for the production of erythrocytes. As demonstrated in Figs. 8 and 9 the principle (erythropoietin) producing reticulocytosis in normal mice does not produce reticulocytosis in nephritic mice (nephritic mice themselves have raised erythropoietin titre—Fig. 8), and, vice versa, the principle producing reticulocytosis in nephritic mice (the erythropoietic principle formed in the kidney) does not produce reticulocytosis in normal mice (Fig. 11). By means of recipient groups of normal and nephritic animals we have a biological means of testing a serum with regard to these two different erythropoiesis-stimulating principles.

A very interesting problem is how the secretion of these active principles is regulated in the body. Hayashida and Ueno (1936, 1937) found no reticulocytosis after acute haemorrhage in rabbits with the spinal cord cut above Th. 2. Such animals, however, were able to respond to serum from normal bled rabbits with reticulocytosis. Serum from normal unbled rabbits, and serum from bled rabbits with cut spinal cord, produced no reticulocytosis when injected into rabbits with cut spinal cord. These findings indicate a nervous regulation of some erythropoiesis-stimulating principle. Hayashida (1936) tried to localize blood-regulating centres in the brain, and was of the opinion that such a centre existed in the area surrounding the tuber cinereum.

In accordance with the findings of Hayashida and Ueno, we obtained no reticulocyte response in a group of six mice which had the spinal cord cut at the level of C. 6 and were bled (Fig. 12). During the operation the animals were anaesthetized under ether for five minutes. Apart from a slight rise immediately after the operation, the reticulocyte values were found to be decreased.

Sera from cordotomized bled mice were tested in a group of normal and a group of nephritic mice (Fig. 13). The serum transfusion was performed 60 to 72

hours after cordotomy and bleeding. The recipients received a single injection of the serum (2% of body weight). No reticulocyte response was seen in the normal mice (no erythropoietin effect), whereas the response in the nephritic mice was pronounced (effect of the principle produced in the kidney). This indicates that the secretion of erythropoietin may be regulated by a central nervous mechanism through the spinal cord, whereas the secretion of the principle produced in the kidney is not. It is assumed that all nerve connexions from the brain and the brain stem to the kidneys are destroyed by cutting the spinal cord at the level of C. 6 (vagal nervous fibres to the kidney have never been demonstrated: Smith, 1951), and therefore the secretion of the erythropoietic principle produced in the kidney is probably not subject to nervous regulation at all. This is in close agreement with the successful homotransplantation of the human kidney between identical twins (Murray *et al.*, 1958). It is likely that the regulation of the erythropoietic principle produced in the kidney is humoral.

It has been suggested that the erythropoietic principle produced in the kidney is connected with the granules of the juxtaglomerular cells (Osnes, 1958). Further experiments (to be published) on the regulation of the kidney factor corroborate this view.

Studies on Erythropoiesis-stimulating Substances in Human Serum

Müller (1912) injected serum from anaemic guinea-pigs into bled mice. He found that the blood values of the mice did not fall at the rate shown in control experiments, and thus demonstrated that the species barriers could be crossed with respect to an erythropoietic principle. Loeschcke and Schwartzer (1939) and Schwartzer and Loeschcke (1940) injected serum into rabbits from newborn infants, and found a rise of the blood values of the animals. Since then serum from human beings with haematological disorders has been tested for erythropoietin by a number of investigators. In most of the anaemic patients investigated, as well as in cases of polycythaemia vera, a raised erythropoietin titre has been found. According to the majority of investigators, lack of erythropoietin does not seem to be a cause of anaemia in man.

It is of great interest to test human serum in *nephritic* mice in order to determine if lack of the erythropoietic principle produced in the kidney may be responsible for any type of human anaemia. Sera from 12 patients have been tested so far. Most of the sera were from patients with anaemia refractory to treatment. In each of the experiments one group of normal mice and one group of nephritic mice were given a single injection (2% of body weight) of human serum. The reticulocytes were counted before injection and 4, 6, and 10 days after.

Fig. 14 shows that injection of normal human serum caused a depression of the reticulocytes in normal as well as in nephritic mice, in accordance with the findings after injection of serum from normal mice (Fig. 5). This does not imply that erythropoietin and the erythropoietic principle produced in the kidney are not present in normal serum, but the amount of these substances may be too small to produce an effect discernible with the technique applied. Why the injection of normal serum causes a depression of the reticulocytes is uncertain. Steinberg *et al.* (1958) found that the maturation of bone marrow was suppressed by multiple injections of bovine and human serum into rabbits, and attributed this to the presence of specific factors in the serum inhibiting the erythropoiesis.

Figs. 15 and 16 show the reticulocyte response in normal and nephritic mice after injection of human serum from one patient with severe haemorrhage and one with sideropenic anaemia respectively. These sera produced reticulocytosis in normal as well as in nephritic mice corresponding to the findings after injection of serum from bled mice (Fig. 4). From these observations it may be assumed that both the titre of erythropoietin and the titre of the erythropoietic principle produced in the kidney usually increase in the anaemic subject—that is, if the anaemia is not to be ascribed to lack of one of these principles.

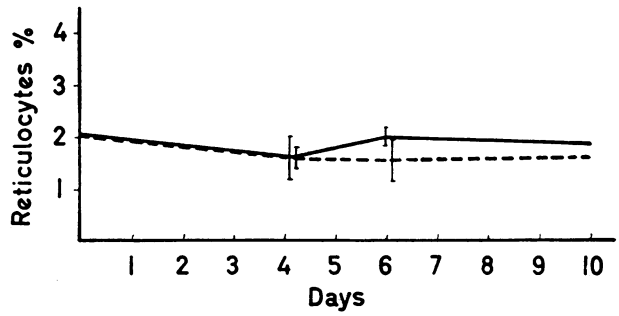

FIG. 14.—Findings after injection of normal human serum. Human donor (neurasthenia cordis; Hb 104%). Recipients: ——, normal mice (6); - - - -, nephritic mice (5).

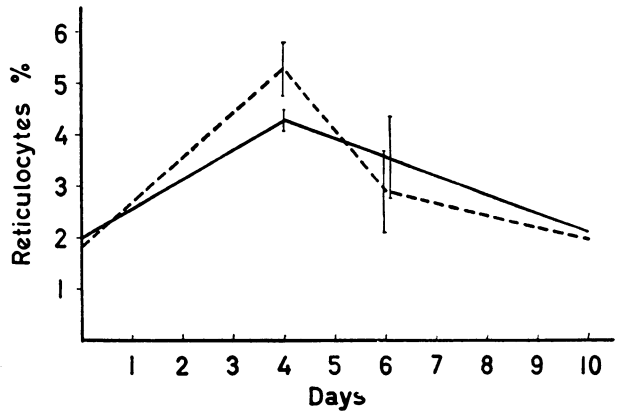

FIG. 15.—Human donor (anaemia posthaemorrhagica (Mb. Osler); Hb 27%). Recipients: ——, normal mice (6); - - - -, nephritic mice (6).

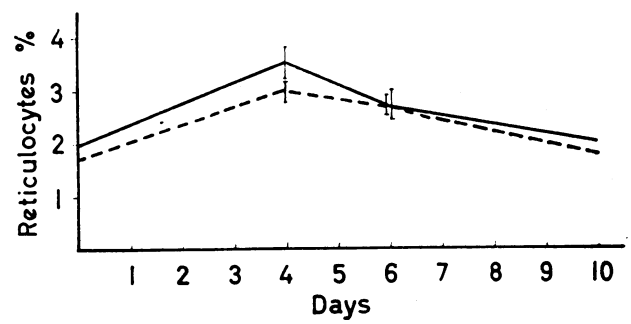

FIG. 16.—Human donor (sideropenic anaemia; Hb 53%). Recipients: ——, normal mice (6); - - - -, nephritic mice (6).

Figs. 17 and 18 show the reticulocyte response in normal and nephritic mice after injection of serum from anaemic patients suffering from chronic nephritis. These sera produced reticulocytosis in normal but not in nephritic mice, in accordance with the findings after injection of serum from *x*-irradiated nephritic mice (Fig. 8). These findings indicate that lack of the erythropoietic principle produced in the kidney may be the cause of the anaemia which accompanies chronic nephritis in man, and the pathogenesis of the anaemia may be the same in *x*-irradiated nephritic mice and in human nephritis.

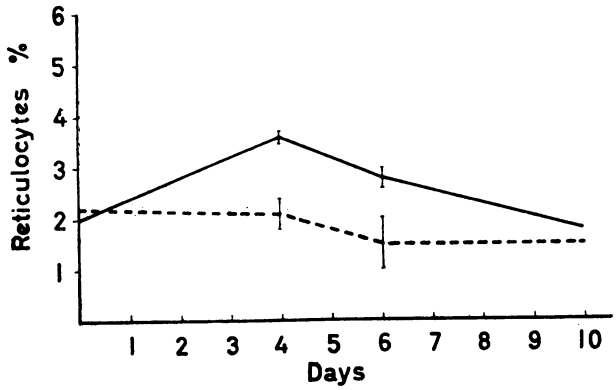

FIG. 17.—Human donor (chronic pyelonephritis; Hb 59%). Recipients: ——, normal mice (6); - - - -, nephritic mice (6).

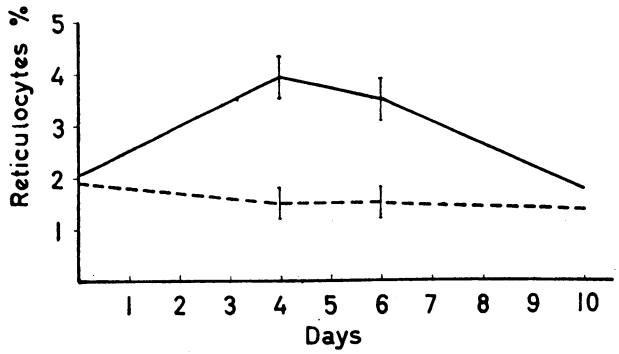

FIG. 18.—Human donor (chronic glomerulonephritis; Hb 59%). Recipients: ——, normal mice (6); - - - -, nephritic mice (6).

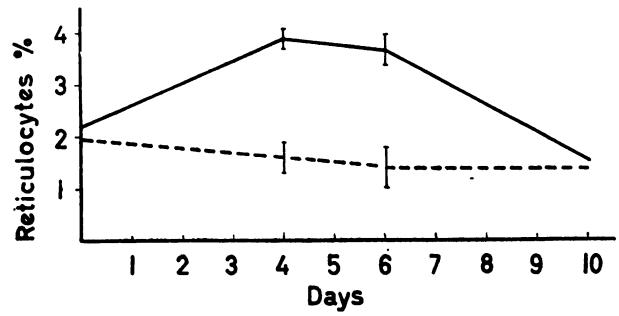

FIG. 19.—Human donor (ulcerative colitis; Hb 63%). Recipients: ——, normal mice (6); - - - -, nephritic mice (6).

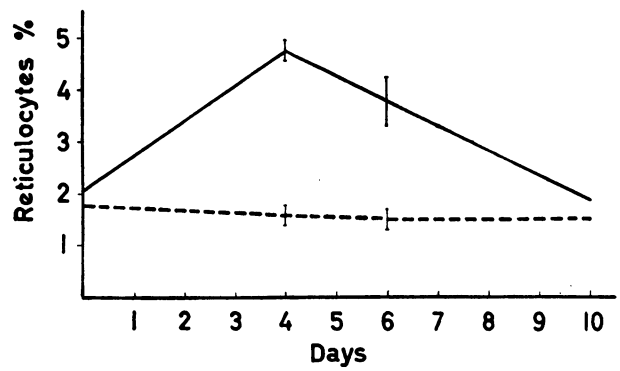

FIG. 20.—Human donor (chronic polyarthritis; Hb 63%). Recipients: ——, normal mice (6); - - - -, nephritic mice (5).

Figs. 19 and 20 show the reticulocyte response in normal and nephritic mice after injection of serum from anaemic patients suffering from chronic infection (ulcerative colitis, rheumatic arthritis). Normal mice responded with reticulocytosis, whereas nephritic mice did not. This is the same finding as after injection of serum from nephritic patients. Accordingly, it is suggested that some, if not all, the anaemias seen in chronic infection may be caused by lack of the erythropoietic principle produced in the kidney.

Figs. 21, 22, and 23 show the reticulocyte response after injection of serum from three patients with aplastic anaemia. Serum from two of these patients produced reticulocytosis in normal as well as in nephritic mice. Serum from the third patient, however, produced reticulocytosis in normal mice only. It is possible that the small amount of erythropoietic principle produced in the kidney was the cause of the anaemia in this patient. The apparent discrepancy underlines the possibility that these cases may differ with regard to pathogenesis.

Fig. 24 shows that injection of serum from a patient with polycythaemia caused reticulocytosis in normal but not in nephritic mice. This patient had also pyelo-ureteritis cystica and haematuria: the polycythaemia may be regarded as caused by the high erythropoietin titre.

In accordance with the results of the experiments with cordotomized mice (Fig. 13) the anaemia developing in man after lesions of the upper part of the spinal cord may be expected to be due to lack of erythropoietin. Such sera, however, have not yet been available for testing, but the results of testing the serum from a patient with chronic hepatitis are given in Fig. 25. No reticulocyte response was seen in the normal mice, whereas the response in the nephritic mice was marked. This indicates that the erythropoietin may be produced by the liver, and that the anaemia in hepatic disorders may be due to lack of erythropoietin.

The experiments presented in this section are pilot investigations of human sera in various haematological disorders. The number of investigations is too small to allow general conclusions. The results, however, are

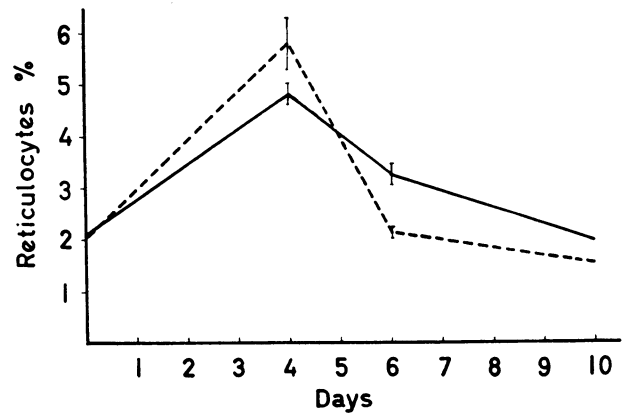

FIG. 21.—Human donor (pancytopenia; Hb 25%). Recipients: ——, normal mice (6); - - - -, nephritic mice (6).

in accord with those described in the previous two sections, and give some information about the mechanism which regulates the haemoglobin value in the body.

Discussion

The production of erythrocytes is a complex process, and a great number of different substances are necessary for erythropoiesis, such as proteins, minerals, vitamins, hormones, etc. With exception of erythropoietin, all these substances seem to have a common property in that an increased supply of one of them does not raise the haemoglobin value over the normal level. As already pointed out, the erythropoietic principle produced in the kidney has not caused reticulocytosis in normal mice with the technique used. It is assumed that an increased supply of this principle does not increase erythrocyte production in the normal subject. For this reason the erythropoietic principle produced in the kidney is *not* termed erythropoietin. Erythropoietin, however, is able to increase the haemoglobin value above the normal level, but it may be a condition that all other substances necessary for erythropoiesis are present in sufficient amounts. Erythropoietin therefore may be a key substance in regulation of the haemoglobin level.

As demonstrated by some of the above experiments, the erythropoietin titre is prone to rise in anaemic conditions. If anaemia is caused by lack of some substance necessary for erythropoiesis (as iron, erythropoietic kidney principle, etc.), then erythrocyte production is impaired because of this lack, and sufficient red blood cells cannot be produced in spite of the increase in erythropoietin titre. Such a serum, rich in erythropoietin, accelerates erythropoiesis when injected into a normal subject having all substances necessary for erythrocyte production, but having *a priori* an erythropoietin titre stimulating only to normal erythrocyte production. This explains why serum from an anaemic organism, unable to heal its own anaemia, may produce reticulocytosis when injected into a normal subject.

If, however, the production of erythropoietin is itself impaired, as demonstrated in cordotomized bled mice and in one anaemic patient suffering from chronic hepatitis, it is not to be expected that such a serum will produce reticulocytosis when injected into a normal subject.

Work is in progress to isolate the erythropoietic principle produced in the kidney. If this substance becomes available we may be able to treat anaemias caused by the lack of it. The experiments on mice illustrated in Fig. 7 give us reason to hope, too, that an increase of haemoglobin values in anaemic patients with renal disease may counteract progression of the disease itself.

Summary

By means of experiments in mice (partial *x*-ray irradiation of the kidneys, performing peritoneal outlet from the ureter, ureter ligation, partial and total nephrectomy, as well as injections into mice of serum from mice treated in different ways and from human beings with various types of anaemia) it has been possible to demonstrate that the kidney produces a principle of importance in erythropoiesis.

This erythropoietic principle differs from erythropoietin. Secretion of the former can take place also when the connexions from the brain and the brain stem to the kidneys have been destroyed. The principle is ultrafilterable and has been found thermolabile.

Lack of this erythropoietic principle produced in the kidney is found to be the cause of the anaemia developing in *x*-rayed nephritic mice. By treatment of such mice with serum from normal bled mice which is rich in this principle, it has been possible to prevent the anaemia, and atrophy of the kidneys and the degree of uraemia were less in the serum-treated animals than in the controls.

Injection of serum from normal unbled mice into normal mice reduced the reticulocytes in the recipients;

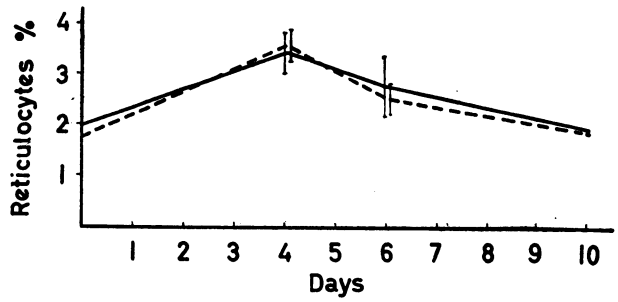

FIG. 22.—Human donor (pancytopenia; Hb 51%). Recipients: ——, normal mice (6); - - - -, nephritic mice (6).

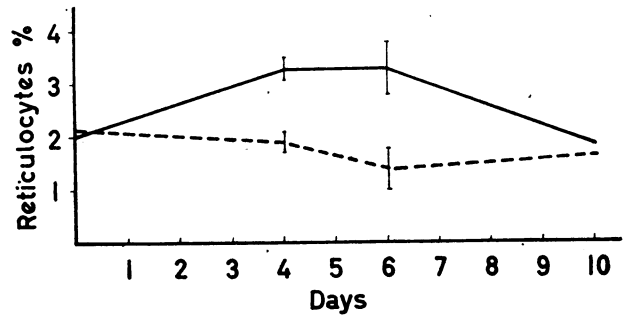

FIG. 23.—Human donor (aplastic anaemia; Hb 56%). Recipients: ——, normal mice (6); - - - -, nephritic mice (6).

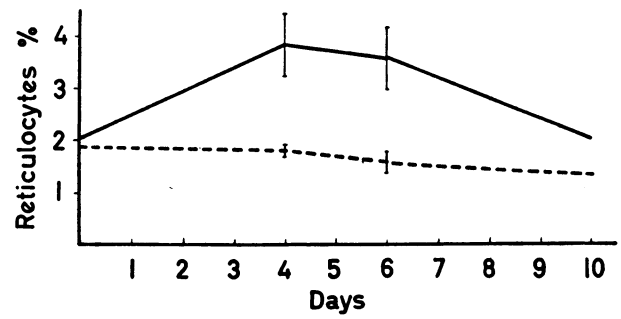

FIG. 24.—Human donor (polycythaemia; pyelo-urethritis cystica; Hb 98%). Recipients: ——, normal mice (6); - - - -, nephritic mice (6).

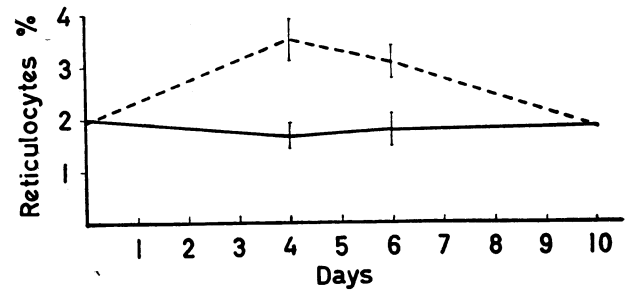

FIG. 25.—Human donor (chronic hepatitis; Hb 64%). Recipients: ——, normal mice (6); - - - -, nephritic mice (6).

continuance of injections caused a decrease in haemoglobin values as well as in red-cell counts.

Injection of serum from normal *bled* mice into normal mice caused reticulocytosis, and continued injections produced polycythaemia.

Erythropoietin produces reticulocytosis when injected into normal mice, but not in nephritic mice. The erythropoietic principle produced in the kidney, however, brings about reticulocytosis when injected into nephritic mice, but not in normal mice. From these findings it has been possible to test serum from mice as well as from human beings with regard to two different erythropoietic principles.

The titre of erythropoietin as well as that of the erythropoietic principle produced in the kidney generally increases in the anaemic patient, if the anaemia is not to be ascribed to lack of one of these principles.

No rise in the titre of the erythropoietic principle produced in the kidney has been found in anaemic patients suffering from chronic nephritis. This indicates that the lack of the erythropoietic principles produced in the kidney may be the cause of the anaemia accompanying nephritis in man.

No rise in the titre of the erythropoietic principle produced in the kidney has been found in anaemic patients suffering from chronic infection (ulcerative colitis, rheumatic arthritis). This finding has led to the conclusion that some, if not all, of the anaemias seen in chronic infections may be caused by the absence of the erythropoietic principle of the kidney.

Totally nephrectomized bled mice are able to increase their erythropoietin titre. This observation shows that erythropoietin is produced outside the kidney. No rise in the erythropoietin titre was found in a patient suffering from chronic hepatitis. This indicates that the erythropoietin is produced in the liver.

[Addendum.—Since this paper was written sera from another five anaemic patients have been tested.

Serum from one patient suffering from pernicious anaemia gave, as expected, good reticulocyte response in normal as well as in nephritic mice (no lack of the two erythropoietic principles discussed in this paper). Serum from one patient suffering from chronic glomerulonephritis and sera from two patients suffering from chronic rheumatic infection gave good reticulocyte response in normal but no response in nephritic mice (lack of erythropoietic factor produced in the kidney). Serum from one patient suffering from chronic hepatitis gave no reticulocyte response in normal but good reticulocyte response in nephritic mice (lack of erythropoietin).

These results show complete agreement with the tests described in the paper above.]

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NASAL STAPHYLOCOCCI AND SEPSIS IN HOSPITAL PATIENTS

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The noses of healthy individuals probably form by far the largest breeding-ground for the pathogenic staphylococci, and it is known that staphylococci from the nose can be responsible for septic lesions in the same individual. This has been shown for recurrent furunculosis (Valentine and Hall-Smith, 1952), for recurrent styes (Roodyn, 1954), and for minor industrial wounds (Williams and Miles, 1949). Studies of the incidence of cross-infection in one surgical ward at St. Bartholomew's Hospital have been in progress for the past three years, and a preliminary analysis (cited by Williams, 1958) suggested that nasal carriers of *Staphylococcus aureus* suffered wound infection more often than non-carriers. This analysis was therefore extended to the records from other wards.

Materials and Methods

The results described in this paper are derived from studies in three male surgical wards (A, B, and C) and one male medical ward (D). The analyses in the first half of the paper, on the nasal carrier state of 602 hospital patients, are based almost entirely on the records from ward A, while the analyses in the second half of the paper relating nasal carriage to sepsis are derived from the 1,358 patients in all four wards.