Two early post-operative arteriograms (Cases 6 and 21) and two re-explorations of the wound (Cases 5 and 7) have shown the vessel to be still patent. Six of these 11 patients have been either asymptomatic or objectively improved post-operatively. Two patients showed no change and the remaining two patients had temporary post-operative deterioration (Cases 5 and 9). One patient died at home four weeks post-operatively. Necropsy disclosed a cerebral haemorrhage on the side opposite the previous carotid occlusion. Both carotids were patent. The patient had spontaneously stopped oral anticoagulants three days before death, and it seems questionable whether or not anticoagulant treatment contributed in any way to the haemorrhage.

Of the 16 patients with complete occlusions, in only four were we able to restore the blood flow (Cases 16, 21, 23, and 27). None of the patients with complete occlusions demonstrated improvement which was not consistent with the normal progress of the disease. There were two deaths, necropsy showing a pulmonary embolus and a myocardial infarction as the presumptive causes of death (Cases 13 and 16).

It is not possible to analyse statistically such a small series of cases, particularly in a disease with as variable a course as carotid thrombosis. We believe, however, that there are theoretical considerations in favour of direct arterial surgery which are supported by a study of the patients in this series. The most appealing theoretical advantage of direct arterial surgery is the chance to interrupt a pathological chain of events which frequently ends in irreversible cerebral damage. There is no way to know if the surgically corrected incomplete occlusions in this series would otherwise have progressed to complete occlusions and severe loss of function. It is undeniable, however, that some of the cases of complete occlusion with hemiplegia had previous symptomatic partial occlusions which were operable before the occlusion became complete (Cases 12, 13, 23, and 26).

Another theoretical advantage of arterial surgery is the ability to relieve symptoms of cerebrovascular insufficiency by improving the cerebral blood flow. This has definitely been borne out in practice (Cases 1 and 2).

A further theoretical advantage of carotid arterial surgery is the ability to improve the cerebral blood flow and provide a better collateral circulation should future vascular occlusions occur elsewhere in the cerebral arterial system.

Patients with internal carotid thrombosis have been shown frequently to have atherosclerotic disease in the other carotid artery and in the vertebral arteries (Hutchinson and Yates, 1957).

Conclusions

Direct arterial surgery attempting to restore normal blood flow has been the primary method of treatment for 27 patients with symptomatic occlusions of the internal carotid artery. The ability to restore blood flow has been found to depend largely on whether the occlusion is partial or complete. In complete occlusions blood flow can be re-established only during the short time before the clot extends into the cranial cavity. Even then, irreversible cortical damage may have occurred (Cases 5, 21, and 23). In partial occlusions a good blood flow can nearly always be re-established. The risk of surgery is not great, only 2 out of 27 patients having any post-operative exacerbation of their neurological symptoms.

At present we feel that the patients most likely to benefit from surgery are those with incomplete occlusions who first come to a doctor because of symptoms of cerebrovascular insufficiency. Restoring flow in these patients not only frequently relieves the symptoms of cerebrovascular insufficiency but may prevent the later development of complete thrombosis and irreversible cerebral damage.

We wish to thank Sir Russell Brain, Dr. Denis Brinton, Dr. Macdonald Critchley, Dr. Colin Edwards, Dr. C. H. Edwards, Dr. C. Groves, Dr. Philip Harvey, Dr. R. A. Henson, Dr. Rowland Hill, Dr. Denis Leigh, Dr. John Marshall, Sir George Pickering, Dr. Blake Pritchard, Dr. Kenneth Rawlings, Dr. Kenneth Robertson, and Sir Charles Symonds for referring patients to us, and Dr. David Sutton and the other radiologists who have performed the arteriograms.

REFERENCES

Adams, R. D., and Vander Eecken, H. M. (1953). *Ann. Rev. Med.*, **4**, 213.
Behrman, S. (1954). *Postgrad. med. J.*, **30**, 570.
Bering, E. A., Taren, J. A., McMurrey, J. D., and Bernhard, W. F. (1956). *Surg. Gynec. Obstet.*, **102**, 134.
Denny-Brown, D. (1951). *Med. Clin. N. Amer.*, **35**, 1457.
—— (1952). *New Engl. J. Med.*, **246**, 839.
Eastcott, H. H. G., Pickering, G. W., and Rob, C. G. (1954). *Lancet*, **2**, 994.
Edwards, C., and Rob, C. G. (1956). *British Medical Journal*, **2**, 1265.
Fisher, M. (1954). *A.M.A. Arch. Neurol. Psychiat.*, **72**, 187.
Hunt, J. R. (1914). *Amer. J. med. Sci.*, **147**, 704.
Hutchinson, E. C., and Yates, P. O. (1957). *Lancet*, **1**, 2.
Johnson, H. C., and Walker, A. E. (1951). *J. Neurosurg.*, **8**, 631.
Millikan, C. H., and Siekert, R. G. (1955). *Proc. Mayo Clin.*, **30**, 186.
Shapiro, S. K., and Peyton, W. T. (1954). *Neurology*, **4**, 83.
Stone, H. H., Donnelly, C., and Frobese, A. S. (1956). *Surg. Gynec. Obstet.*, **103**, 313.
Thomas, M. H., and Petrohelos, M. A. (1953). *Amer. J. Ophthal.*, **36**, 335.

SICKLE-CELL DISEASE: NEW METHOD OF TREATMENT

PRELIMINARY REPORT

BY

G. HILKOVITZ, M.B., B.Ch.

From the Medical Unit, Hospital for Tropical Diseases, London

Sickle-cell disease is an inherited malady with a poor prognosis. The misshapen erythrocytes are destroyed in the body, resulting in a haemolytic anaemia, and they adhere to each other to form intravascular thrombi with infarctions of various tissues.

The defect resides in the haemoglobin, which, when deprived of its oxygen by the respiring tissues, undergoes molecular rearrangement to form long rod-like particles with resulting alteration of the erythrocyte contour. The only treatment of value is blood transfusion, which may be life-saving during a haemolytic crisis. Anticoagulants do not prevent the thrombotic phenomena.

Sickled cells are not usually seen in blood obtained from a patient by venepuncture, because in the process of examination the blood becomes re-oxygenated and the erythrocytes are restored to their normal shape (Hahn, 1928). The usual method by which sickled cells are demonstrated is that of sealing a drop of blood under a coverslip on a slide. The consumption of oxygen in such a preparation by the respiring erythrocytes, leucocytes, and contaminating bacteria results in the reduction of the haemoglobin and consequent alteration in the shape of the red cells.

Tomlinson and Jacob (1945) washed the red cells from a patient with sickle-cell disease repeatedly in normal saline and then resuspended them in the patient's plasma. They made sealed preparations and demonstrated that the red cells failed to sickle. They showed also that the addition of zinc acetate or sodium cyanide solutions to blood from a patient prevented the occurrence of sickling. These substances are known inhibitors of the enzyme carbonic anhydrase. These workers concluded that they had removed a factor necessary for sickling from the erythrocytes by washing, and suggested that this factor might be carbonic anhydrase.

New Approach to Treatment

In cases of cyanide poisoning the venous blood remains fully oxygenated. Cyanide is a known inhibitor of carbonic anhydrase. It seemed reasonable to assume that use might be made of a carbonic anhydrase inhibitor, less toxic than cyanide, to control the reduction of haemoglobin in sickle-cell disease to a point where the occurrence of sickling is suppressed.

A powerful yet relatively non-toxic carbonic anhydrase inhibitor is available in acetazolamide ("diamox"). This drug is a valuable diuretic, and its action as such is dependent upon its property of suppressing carbonic anhydrase activity in the kidney. It was decided to test the drug on blood from a patient with sickle-cell disease.

A negro baby aged 8 months came under the observation of the Medical Unit at the Hospital for Tropical Diseases, London, on April 1, 1957, because of proved sickle-cell disease. Electrophoretic studies had shown the presence only of haemoglobin S in the child's blood, while both parents were trait-bearers, as shown by the presence of haemoglobins A and S in their blood. The baby was anaemic (haemoglobin of 9.1 g. per 100 ml. of blood) and febrile, and the liver was palpable two fingerbreadths below the right costal margin. The dorsum of the right hand, the proximal phalanx of the right ring-finger, and the dorsum of the left foot were swollen. *X*-ray films demonstrated bony changes, characteristic of sickle-cell disease, underlying the swollen parts.

Effect of Acetazolamide in Vitro

First Experiment

Method.—Acetazolamide was dissolved in sterile normal saline solution to give a concentration of 2.5 mg. per 100 ml. Blood was obtained from the patient by venepuncture. It was mixed with 2 mg. of heparin and was allowed to become oxygenated by standing in contact with air. A portion of this blood was used to obtain red cells, which were washed five times in a sterile normal saline solution by making repeated suspensions of the red cells. Five sealed slide preparations were made from each of the following: (*a*) heparinized and oxygenated whole blood; (*b*) washed red cells suspended in normal saline; (*c*) heparinized oxygenated blood mixed with sterile normal saline (one drop of each); and (*d*) a mixture of one drop of heparinized oxygenated blood and one drop of acetazolamide solution. The sealed preparations were incubated for 24 hours at 37° C. and were then examined, using an oil-immersion lens. The sickled cells in 32 fields were counted on each slide and selection of fields was avoided by using the corresponding portions of each slide.

TABLE I.—*Percentages of Sickled Cells in Various Preparations of Patient's Blood*

Preparation	Total No. of Cells Counted	Percentage of Sickled Cells
Patient's whole blood	2,378	18·7
Washed cells	2,306	5·7
Blood with normal saline	2,039	4·3
Patient's whole blood with acetazolamide	3,067	2·4

The results are shown in Table I. The addition of acetazolamide to whole blood had achieved a statistically significant reduction in the number of sickled cells. Washing the erythrocytes had had the expected effect of reducing the number of sickled cells. A considerable reduction was also achieved by mixing blood with normal saline.

Second Experiment

Another experiment was therefore designed to observe the effect upon sickling of acetazolamide without using normal saline either as a control or as a solvent for the drug and to eliminate the factor of dilution.

Method.—First 500 mg. of acetazolamide was dissolved in 100 ml. of sterile distilled water. Then 0.01 ml. of this solution was added to 1 ml. of heparinized oxygenated blood from the patient, giving a concentration of 5 mg. of acetazolamide per 100 ml. of blood. One drop of this blood was used for making a sealed-slide preparation. A sealed-slide preparation was also made using one drop of heparinized oxygenated blood. The two preparations were incubated at 37° C. for 24 hours and examined under high-power magnification.

In the preparation containing acetazolamide an occasional sickled cell was seen, whilst the majority of the erythrocytes were sickled in the other preparation. It was concluded that a concentration of 5 mg. of acetazolamide per 100 ml. of blood is sufficient to inhibit almost entirely the phenomenon of sickle-cell formation *in vitro*.

Effect of Acetazolamide on Sickling Phenomenon in Vitro in Deoxygenated Blood

If acetazolamide is effective in suppressing oxygen loss from haemoglobin, thereby inhibiting sickle-cell formation, it should have no observable effect when mixed with deoxygenated blood. The following experiment was carried out.

Method.—Blood was obtained from the patient by venepuncture. Sealed preparations were made in triplicate from this blood, with and without the addition of acetazolamide, before sufficient time had elapsed for the blood to become oxygenated. The concentration of acetazolamide used was 5 mg. per 100 ml. of blood. The preparations were incubated at 37° C. for 12 hours and then examined under a high-power lens.

Sickle-cell formation was pronounced in all the preparations. It is concluded that acetazolamide inhibits sickling by preventing oxygen loss from the haemoglobin of the erythrocytes.

Action of Acetazolamide on Sickling Phenomenon in Vitro when Administered Orally

It remained to be observed whether the oral administration of the drug had any effect on the sickle-cell test *in vitro*.

Method.—A drop of blood was obtained from the patient by pricking the skin before the drug was administered. The drop of blood was mixed on a slide with 0.06 mg. of heparin and allowed to stand in contact with moist air for 15 minutes. A sealed preparation was then made. A single dose of 7 mg. of acetazolamide per kg. body weight was administered to the patient orally. Blood was obtained by pricking the skin one, three, five, and six hours after administration of the drug. Sealed preparations were made from these samples of blood by the same techniques as that described for the pre-administration sample. All preparations were placed in an incubator at 37° C. as soon as they had been made, and each slide was examined at half-hourly intervals to observe the time of onset of sickling and the time taken for sickling to be completed.

The results are given in Table II. It is shown that the administration of acetazolamide by mouth has a pronounced inhibiting effect upon sickle-cell formation *in vitro*. This effect was greatest in blood taken one hour and five hours

TABLE II.—*Delaying and Inhibiting Effect of Orally Administered Acetazolamide Upon Sickling in Vitro*

Time when Blood was Taken	Interval between Removal of Blood from Patient and Onset of Sickling	Time taken for Sickling to Become Complete
Before administration of drug	½ hour	1 hour
1 hour after administration of drug		Very few sickled cells after 48 hours' incubation
3 hours " " "	2½ hours	3½ hours
5 " " " "		Very few sickled cells after 48 hours' incubation
6 " " " "	½ hour	1 hour

after the drug was given, while six hours after administration sickle-cell formation proceeded to completion at the pre-administration rate. The sample of blood taken three hours after the drug was given sickled more slowly than a pre-administration sample.

The absence of any demonstrable inhibiting effect upon sickle-cell formation six hours after administration corresponded to the known rate of excretion of the drug (50% of the dose is excreted in six hours) (manufacturers' data). The diminished activity of the drug three hours after administration is interesting because it occurs between two peaks of activity at one hour and five hours after administration. It confirms, however, the fact observed by the manufacturers that a single dose administered to a dog by mouth results in two peaks of concentration within the erythrocytes. This is described in the section dealing with the pharmacology of acetazolamide.

These results were confirmed by a second experiment, using the same technique but with a reduction in the dose to 2 mg. per kg. of body weight. With this small dose there was noticeable delay in the onset of sickle-cell formation *in vitro* in blood taken one hour, two hours, four hours, and five hours after the drug was administered, and once again three hours after administration the inhibiting effect was less marked.

Effect of Orally Administered Acetazolamide on Sickling Phenomenon in Vivo

The occurrence of sickling in a sealed-slide preparation is an indirect demonstration of the phenomenon *in vivo*. With a view to discovering what proportion of cells were sickled in the venous circulation a further experiment was designed.

Method.—The patient was given by mouth a dose of 2 mg. of acetazolamide per kg. body weight. One hour later 1 ml. of blood was taken by venepuncture from the jugular vein into a syringe containing sterile liquid paraffin to prevent contact of the blood with air. The blood was then immediately injected into 2 ml. of 10% formol-saline solution under a layer of oil, the object being to fix the red cells in their circulating shape. Thin films were made and stained with eosin, and the percentage of sickled cells was counted under an oil-immersion lens. The patient was then given a dose of 7 mg. of acetazolamide per kg. body weight and one hour later 1 ml. of venous blood was obtained anaerobically and injected into 10% formol-saline solution under oil. Films were made and stained with eosin.

TABLE III.—*Decrease in Number of Sickled Cells Following an Increased Dose of Acetazolamide*

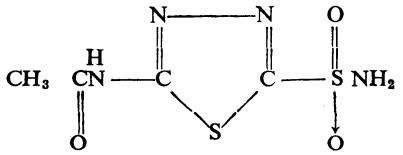
Preparation	No. of Cells Counted	Percentage of Sickled Cells
Films made from blood taken one hour after 2 mg. of acetazolamide per kg. body weight had been given orally	3,000	11·8
Films made from blood taken one hour after 7 mg. of acetazolamide per kg. body weight had been given orally	3,000	7·5

Standard error of difference, 0·75.

The results are shown in Table III. The number of sickled cells present in venous blood after a dose of 7 mg. of acetazolamide per kg. body weight was significantly smaller than after a dose of 2 mg. per kg. body weight.

Pharmacology of Acetazolamide

The structure of the drug is as follows (manufacturers' data)



The drug inhibits the activity of carbonic anhydrase. Its continued administration to man in oral doses of 8 to 16 mg. per kg. body weight per day results in lowering of the carbonic anhydrase activity of the whole blood to one-third of normal value. The drug is remarkably non-toxic and has been given to children for periods up to seven months in oral doses of 25 mg. per kg. body weight per day in the treatment of epilepsy. No toxic effects have been observed on the bone marrow, the liver, kidney, or the central nervous system. The limiting factor to dosage has usually been drowsiness and a feeling of fatigue on the larger doses.

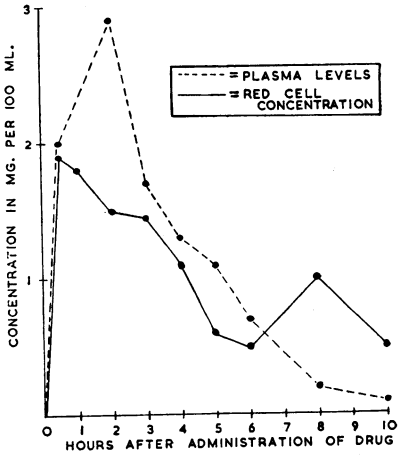


FIG. 1.—Chart showing the two peaks of red-cell concentration referred to in the text.

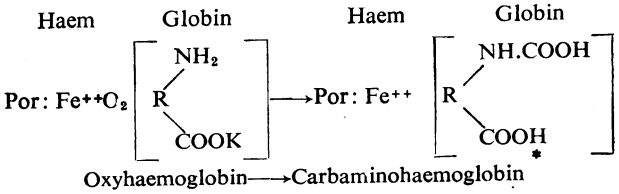
Absorption is complete after oral dosage, since the 24-hour urinary excretion is the same as that following intravenous administration. The drug is excreted unchanged by the kidney, 50% of the oral dose being lost via the urine in six hours. The plasma and red-cell levels in the dog after an oral dose of 20 mg. per kg. body weight are shown in the Chart (Fig. 1).

Theory of Action of Acetazolamide in Sickle-cell Disease

Carbonic anhydrase catalyses the reaction:

$$H_2CO_3 \rightleftharpoons CO_2 + H_2O$$

It is therefore required for the evolution of CO_2 from tissue cells. It is also necessary for the combination of CO_2 with H_2O within the red cell to form H_2CO_3. H_2CO_3 combines in the red cell with oxygenated potassium haemoglobin, with simultaneous release of oxygen. This reaction may be represented as follows (Wright, 1952):



In other words, when haemoglobin becomes reduced the globin molecule gains an H ion in the carboxyl group (marked *). This H ion is derived from the H_2CO_3 which carbonic anhydrase helps to form within the red cell. Therefore, interference with carbonic anhydrase activity retards the reduction of haemoglobin.

Following this line of argument, it becomes apparent that the bicarbonate shift mechanism is also retarded, thereby hindering the plasma buffering mechanism and inducing a mild acidosis.

The CO_2 tension in the plasma is dependent upon the amount of CO_2 in the plasma (in simple solution). The oxygen dissociation curve of haemoglobin is dependent upon the plasma CO_2 tension. If, therefore, the inhibition of carbonic anhydrase lowers the plasma CO_2 tension, it retards also the dissociation of O_2 from haemoglobin.

Since the occurrence of sickling depends upon the presence in the erythrocyte of reduced haemoglobin, a carbonic anhydrase inhibitor would tend to suppress sickle-cell formation.

Fig. 2 illustrates the chemical changes concerned.

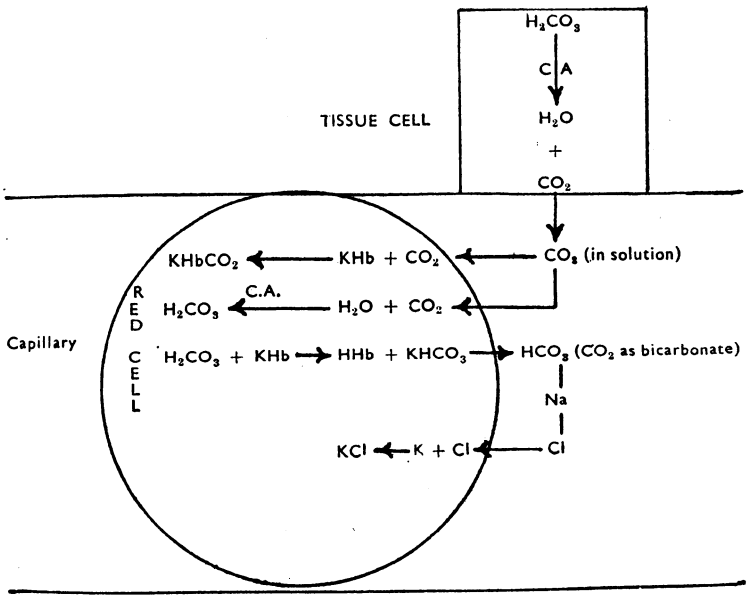


FIG. 2.—Chemical changes involved in CO_2 transport and the points of action of carbonic anhydrase.

Therapeutic Trial with Acetazolamide in Sickle-cell Disease

At the time of writing the patient has been under observation for 70 days. In the period of 41 days preceding the administration of acetazolamide there was evidence of active haemolysis on two separate occasions, as shown by deterioration in the haemoglobin levels. The patient has so far been treated with acetazolamide for 29 days and there has been a steady rise in the haemoglobin levels during this period from 7.1 to 9.4 g. per 100 ml. of blood. The child has continued to gain weight and is more alert and active than she was before treatment.

A prolonged period of observation will be necessary, because natural remissions occur in this disease and no conclusions can be drawn from the present remission, which began with the beginning of treatment. A detailed account of the clinical and haematological assessment following treatment will form the subject of a further report.

Observations on the Numbers of Sickled Cells in the Venous Circulation

It has been shown that there was a significantly smaller percentage of sickled cells in venous blood after the administration of 7 mg. of acetazolamide per kg. body weight than there was after the administration of 2 mg. per kg. body weight. It was decided to count the number of sickled cells present in circulating blood while the patient was not having treatment and when she was taking the drug.

TABLE IV.—*Variations in Number of Sickled Cells in Venous Blood Before and During Cessation of Treatment and After Restarting Treatment*

Sample of Blood	No. of Cells Counted	Percentage of Sickled Cells
Taken 1 hour after last dose of acetazolamide ..	3,000	8·8
Taken 48 hours after last dose of acetazolamide ..	3,000	15·6
Taken 1 hour after administration of acetazolamide was restarted	3,000	8·9

Method.—Three 1-ml. samples of blood were obtained from the jugular vein. The first specimen was taken one hour after the last dose of acetazolamide and the second sample 48 hours after the last dose; the third specimen was drawn one hour after administration of the drug had been restarted. The three specimens were obtained by the same technique, as follows: 1 ml. of blood was drawn from the jugular vein into a syringe containing sterile liquid paraffin. The blood was immediately injected into 2 ml. of 10% formol-saline solution under a layer of liquid paraffin. Thin films were made from this blood and stained with eosin. The numbers of sickled cells were counted, using an oil-immersion lens.

The results are shown in Table IV. There was an increase in the number of sickled cells in the venous blood following withdrawal of the drug.

Summary

Evidence has been produced to show that acetazolamide inhibits the occurrence of sickling of red cells *in vitro* and *in vivo*. It is suggested that the drug exerts this effect by inhibiting the action of the enzyme carbonic anhydrase. The enzyme is concerned with the reduction of haemoglobin. The abnormal haemoglobin S in patients with sickle-cell disease undergoes molecular rearrangement during the process of reduction to form long slender rods which alter the shape of the red cell. The pathological manifestations of sickle-cell disease are determined by the alteration in the contour of the erythrocytes which adhere to one another to form intravascular thrombi and which are destroyed in the body, resulting in anaemia.

At the present time there is no effective treatment for sickle-cell disease, and it is suggested that acetazolamide may be employed to control the occurrence of sickling.

The preliminary experiments have shown the ability of acetazolamide to inhibit the sickling phenomenon *in vitro* when mixed with blood from a patient with sickle-cell disease. The effect was noticeable also when the drug was administered to the patient, it then being seen that the occurrence of sickling was inhibited *in vitro* as well as in circulating venous blood.

A therapeutic trial is in progress and the results so far are encouraging.

I would like to thank Professor A. W. Woodruff for his invaluable advice and encouragement, and for permission to publish. I am indebted to Drs. F. D. Schofield, S. Bell, and W. H. Jopling for helpful criticism; to Messrs. A. V. H. Allen and F. J. Barratt, of the pathological laboratory of the Hospital for Tropical Diseases, London, for much technical assistance; and to the nursing staff of Chamberlain Ward. My thanks are due to the manufacturers of acetazolamide (Lederle Laboratories Division, Cyanamid of Great Britain Ltd.) for supplies of the pure drug and permission to reproduce the pharmacological data. I am grateful to the parents of the patient for their co-operation.

REFERENCES

Hahn, E. V. (1928). *Amer. J. med. Sci.*, 175, 206.
Tomlinson, W. J., and Jacob, J. E. (1945). *J. Lab. clin. Med.*, 30, 107.
Wright, S. (1952). *Applied Physiology*, 9th ed., p. 424. Oxford Medical Publications.