anoxaemia are anaemia, present in Cases 1 and 3, and alcoholic intoxication. McCormick (1961) noted the association between sickling crises and alcohol and it may have been an important factor in the death in Case 6, although inhalation of vomit probably caused the extra hypoxia that is presumed to have precipitated a terminal sickling crisis.

One further feature common to all subjects developing splenic infarcts in the present series was their lack of mobility prior to the onset of symptoms. All had been sitting, either in an aeroplane or propped up in a hospital bed, for some time previously, and it may be that this tended to favour stasis of the splenic circulation, thus potentiating locally any anoxaemia consequent on hypoxia or infection. Once established, splenic infarcts appear to be highly susceptible to secondary infection, and prophylactic antibiotics are advocated to prevent abscess formation and its attendant complications (Conn, 1954).

In contrast to the sickle-cell trait clinical manifestations of the haemoglobin C trait are few. Two cases of idiopathic haematuria were reported by Myerson *et al.* (1959) and Smith and Krevans (1959), but otherwise the condition appears to be essentially benign.

In conclusion it seems that heterozygous carriers of haemoglobin S are better protected against the lethal effects of malaria than are haemoglobin C heterozygotes, but that possession of haemoglobin S in concentrations greater than 40% can give rise to undesirable side-effects. Conversely, the protective ability suggested for haemoglobin C is apparently unaccompanied by any comparable disadvantages, but, being of a lower order, it is less easily demonstrated convincingly in a small sample.

Summary

Previously quoted figures comparing malarial parasite densities in Accra children have been re-analysed. Results for carriers of haemoglobin C, although less conclusive

than originally claimed, do suggest some degree of protection against *P. falciparum* infections. The superior efficiency of haemoglobin S in this respect is confirmed.

Case reports illustrating the occurrence of splenic infarcts and a fatal crisis in persons with the sickle-cell trait are presented. The roles of infection and circulatory stasis, and of the proportion of haemoglobin S present in the blood, are discussed in relation to the development of sickling *in vivo* in such cases.

I wish to thank my wife once again for her great assistance in matters technical and typing. Dr. Hermann Lehmann kindly estimated the percentage of haemoglobin S in several blood samples. I am grateful to Dr. J. A. Fraser Roberts, Dr. C. A. B. Smith, and Dr. J. P. Garlick for their helpful criticisms.

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USE OF A PROTHROMBIN METER FOR QUICK'S ONE-STAGE TEST

BY

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In spite of the current controversy over the effectiveness of anticoagulant therapy, there is at the moment no sign of a reduction in the number of prothrombin estimations requested to be carried out in routine laboratories. Thus any mechanization that lessens the amount of labour involved in performing these estimations by Quick's method is to be welcomed. The standard technique involving holding the tubes containing the reagents, manipulating them in the water-bath, and working the stop-watches is both tiring and monotonous. Further, in order to obtain consistent results, a degree of skill has to be acquired to judge when clotting has occurred.

Toohey and Cook (1960) described a photoelectrical system for measuring prothrombin times, using the Quick technique, and this machine was later marketed by the Evans Electro-selenium Company as a "prothrombin meter" (Fig. 1). Five hundred comparative tests were carried out by Toohey and Cook, and they concluded that the machine produced more accurate and reproducible results than the manual method, but no statistical analysis was made of their results. Before introducing this machine into routine use in this laboratory it was decided to determine prothrombin times on a series of specimens with technicians

employing the orthodox method as well as the mechanical method, and then submit the results to statistical analysis.

Material and Methods

Venous blood, collected without stasis, was mixed with 3.8% sodium citrate in the proportion of 9:1. After mixing and centrifuging, the plasma was tested within two hours by a technician who determined the prothrombin time according to Quick's one-stage method (see Dacie, 1956), using 0.1-ml. volumes of citrated plasma, human brain thromboplastin, and M/40 calcium chloride. The thromboplastin used gave times of 11–12 seconds with normal plasma. Each specimen was tested at least twice by the manual method and using the prothrombin meter.

The meter as originally described by Toohey and Cook (1960) determines the time for clot formation of the plasma by shining a beam of light through the specimen under test on to a photosensitive cell. A clock which is incorporated in the machine is started as the reagents are mixed, and a change in the turbidity of the specimen takes place just before clotting occurs, which reduces the amount of

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light reaching the photocell. The fall in output from the cell consequent on this reduction allows a relay controlled cut-out system to operate which halts the timing mechanism.

The technique when using the machine was the same as that for the Quick method, except that 0.2-ml. quantities of reagents were used instead of 0.1 ml., and that the tubes containing the final mixture had to be of specified dimensions so that they could fit into the tube-carrier of the machine. The required volumes of plasma and thromboplastin were pipetted into a pair of these tubes, which were then placed in a 37° C. water-bath. After approximately one minute the tubes were transferred to the machine, which carries its own thermostatically controlled heating system. As the machine contains two timing units, duplicate testing can be performed in rapid succession. With the tube in the carrier set in the light-path, M/40 calcium chloride was added, and the timing mechanism activated by moving the "Start-Read" switch to the "Start" position. The tube was then removed from the machine, its contents were mixed by inversion once, and it was replaced in position in the tube-carrier. The galvanometer needle was set to lie between the two black lines on its dial by adjusting the "sensitivity control," and finally the "Start-Read" switch was moved to the "Read" position.

The virtue of this system was that, having mixed the reagents, adjusted the galvanometer, and put the switch to the "Read" position, the operator can then carry on with the next estimation, for the timing mechanism is stopped by the action of the cut-out. The time in seconds for clot formation can be read off from the clock set alongside the galvanometer. A degree of dexterity is required to operate this machine, because once the calcium chloride has been added all the handling must be done within seven seconds, for if the clotting has begun before the tube has been returned to the machine the cut-out will not operate and no result can be obtained.

Results

Two hundred and fifty-nine specimens were tested in duplicate using the orthodox manual technique and the

machine. Technicians using either the manual method or the "meter" were allowed up to but not more than a two-seconds difference between the pair of times recorded for any individual specimen. If the difference exceeded two seconds both estimations were repeated, and only the set of times showing best agreement were recorded.

Fig. 2 shows the results of plotting the difference in seconds between the individual prothrombin times obtained on each specimen, grouped on the basis of those times under 20 seconds, 20-29 seconds, 30-39 seconds, 40-49 seconds, and 50 or more seconds. It shows that technicians, when using the orthodox manual method, are apparently able to obtain better agreement between their individual tests, whereas the machine has a wider "scatter" in its results, which increases as the prothrombin time becomes larger.

Fig. 3 shows the results of plotting the sum of the pair of the prothrombin times obtained by the manual method as the baseline, with the sum of the prothrombin times determined by the machine plotted above or below, depending on whether the machine's estimate was greater or less than the manual method's results. It can be seen that the machine tends to record a longer prothrombin time than that obtained by the manual method, and that this overestimate increases with the length of the prothrombin time.

The coefficient of correlation was determined for each of the groups (see Table). Good correlation was obtained between the machine and manual times at under 20 seconds and at 20-29 seconds. However, the correlation between the times obtained at 30-39 seconds was not so close. The number of tests in this group was, however, relatively small.

Coefficient of Correlation in 259 Specimens

Prothrombin Times	No. of Tests	Coefficient of Correlation between Manual and Machine
Under 20 secs	114	0.85
20-29 secs.	106	0.8
30-39 secs	26	0.48
40-49 secs	5	0.54
50 secs or more	8	0.87

FIG. 1

△ COINCIDENT VALUES FOR THE SUM OF 'MACHINE' AND THE SUM OF 'MANUAL' TIMES

FIG. 3

SEQUENTIAL PLOT OF PROTHROMBIN TIMES

Discussion

The benefits to be obtained from this prothrombin meter are its simplicity and relative ease of operation compared with the manual method. Further, inexperienced staff can rapidly be trained in its use. Against this must be weighed the wider scatter of results, and the decision on how many seconds' difference between the individual times obtained on a single specimen constitutes "good agreement." The tendency of the machine to record a longer prothrombin time, especially at and beyond the upper limit of the "therapeutic range," must also be considered when advising on dosage of anticoagulant drugs. Our range is taken as two to two and a half times the control time, which was usually 11 seconds. Since this trial was carried out the prothrombin meter has been put into routine use in this laboratory. No troubles (that could be attributed to the times determined on the prothrombin meter) have been experienced in controlling patients receiving anti-coagulant therapy. The machine can prove unreliable in determining the prothrombin time on specimens of plasma that are lipaemic or hyperbilirubinaemic, and particularly because of the significance of the prothrombin time in the

latter instance it is essential to test these using the orthodox manual method.

Summary

A machine for determining the prothrombin time by the Quick one-stage method using a photoelectrical system is described. The results of a comparison between prothrombin times determined by technicians using the orthodox manual method and those measured by this machine are discussed. It is thought that the machine is efficient within the therapeutic range of prothrombin times but tends to overestimate prothrombin times over 30 seconds and is less consistent when compared with a trained technician using the orthodox manual method. The majority of technicians prefer the machine, which, for routine work, is most useful.

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Medical Memoranda

Anaphylaxis with Beeturia

This is a report of the case of a patient who presented with severe anaphylactic shock associated with beeturia.

CASE REPORT

A 44-year-old married woman was admitted to hospital at 2 p.m. on October 2, 1961, after the sudden onset of upper abdominal pain and vomiting. She was severely shocked, with a rapid pulse and blood-pressure of 60/0 mm. Hg. A slight generalized erythematous rash increased rapidly and became urticarial. Colicky abdominal pain with profuse vomiting and diarrhoea developed soon after admission. There was no blood in the vomit or stool. The vomitus contained clear fluid and food. The stool was of normal colour, but fluid.

The abdomen was soft, with neither tenderness nor guarding, but the bowel sounds were hyperactive. There were no other abnormal features.

The foot of the bed was raised, oxygen was given, and, on the presumptive diagnosis of an anaphylactoid state, intramuscular metaraminol, intravenous and intramuscular hydrocortisone, and intramuscular promethazine hydrochloride were administered. The blood-pressure responded rapidly to these measures and by that evening the vomiting and diarrhoea had ceased.

At 8 p.m. she passed urine of a bright-red colour. This persisted to a lessening degree for 12 hours, then the urine became normal in colour. The urine pigment was identified as betanin. There was no blood or other abnormal urinary pigment present. Questioning revealed that she had had a small amount of pickled beetroot with her evening meal on the previous day. Beetroot had been taken by her before without incident and without her noting a red urine after it. There was no history of previous skin rash, asthma, hay-fever, or any type of drug sensitivity.

Subsequent investigations showed normal blood values apart from an initial leucocytosis (13,000/c.mm.); the urine was normal chemically and microscopically; serial electrocardiograms were normal; serum amylase and serum glutamic oxaloacetic transaminase were normal. Chest x-ray examination showed slight ventricular enlargement. Barium series was negative. Faecal culture showed no pathogens.

A short course of oral steroid and antihistamines was given and convalescence was uneventful. At follow-up on November 8 she was feeling well but was determined not to eat beetroot again. She then told us that a cousin always passed

red urine after eating beetroot. Intradermal skin-testing with, firstly, a simple aqueous solution of beetroot pigment and, secondly, a prepared Bencard extract of beetroot gave a negative reaction in both cases. She refused to have even a small amount of beetroot orally.

COMMENT

There seems little doubt that this patient had an acute sensitivity reaction. Whether the beetroot contained the actual allergen, or whether the beeturia was the result of increased bowel permeability because of an allergic response to some other substance, is not clear. The negative intradermal tests with beetroot extract and the absence of previous upset after eating even larger amounts of beetroot seem to be against implicating beetroot as the source of the allergen. An association between allergy and beeturia was noted by Zindler and Colovos (1950). They described 26 cases of beeturia related to beet allergy and two cases in which beeturia occurred when beets were ingested with allergenic foods. Test-feeding beets to the 26 patients claimed to have beet allergy produced various responses, including urticaria, nausea, abdominal cramps, and one instance of severe diarrhoea. None of the patients apparently was as ill as ours.

Beeturia without related symptoms does occur (Ruh and Garvin, 1924; Cathala *et al.*, 1939). Elsewhere we have corroborated this and gone on to indicate some of the factors which may influence the absorption of betanin (Watson *et al.*, 1963). However, in more recent studies on the *in vivo* metabolism of purified betanin a 40-mg. dose of this compound given parenterally produced facial warmth and flushing in one subject, a symptom described by 3 of the 26 patients investigated by Zindler and Colovos. It is possible, therefore, that some or even all of the symptoms the patient reported here can be attributed to the amount of betanin absorbed.

We thank Professor E. M. McGirr for permission to report this case.

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