(Table II). We cannot yet say whether it will prove possible experimentally to stimulate erythropoiesis to the point of actual polycythaemia. Dr. G. Hudson is at present reinvestigating both the short-term and long-term effects of A.C.T.H. and 11-oxysteroids on the haemopoietic system, with the object *inter alia* of trying to ascertain whether he can induce an experimental overproduction of red cells.

Anoxaemia

Finally I had hoped to be able to say something about our studies on the effects upon the bone marrow of anoxaemia. Last summer our group (Drs. E. H. Batten, W. J. Gall, G. Halley, R. S. Harris, A. F. Rogers, and myself) went to the Jungfraujochstation, at a height of over 13,000 ft. (3,960 metres), to investigate this problem. Unfortunately, we have not yet completed the statistical analysis of our extensive data, but it may perhaps be of interest to say that during the first five days of exposure to the lessened oxygen content of the air at the Jungfraujoch the two most significant changes in the bone marrow were an increase in its erythroid cells, as might be expected, but also a very definite decrease in the marrow lymphocytes. This response of the lymphocytes recalls observations such as those of Dameshek and Valentine (1937), who noted that in cases of pernicious anaemia the marrow lymphocytes were above the normal level, but fell markedly soon after treatment was begun. A fall in the marrow lymphocytes in circumstances in which there is a sudden increase in red-cell formation could be explained in several ways. It might be argued that lymphocytes were being rapidly discharged from the marrow to make room for the increase in red cells. But in our guinea-pig experiments a simple calculation indicates that this ought to lead to an appreciable lymphocytosis, which does not in fact occur.

Another possibility is that lymphocytes are becoming transformed into red-cell precursors, and the evidence of our Jungfraujoch material, which we hope to present in full before long, seems to point in this direction.

Summary

After a brief review of lymphocyte production, a quantitative technique is described for studying the nucleated cells of bone marrow. The three main cell groups in the marrow are (1) erythroid, (2) myeloid, (3) lymphocytes. In the marrow of 17 normal guinea-pigs the mean count of these cells per c.mm. was approximately: erythroid, 273,000; myeloid, 390,000; lymphocyte, 310,000.

In relation to the cell content of the blood, the marrow appears to possess a greater reserve of myeloid than of erythroid cells. Brief mention is made of some of the problems to which the quantitative study of the bone marrow is being applied.

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THE CONTROL OF ANTICOAGULANT THERAPY IN MYOCARDIAL INFARCTION

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Anticoagulant treatment with drugs of the coumarin type is now recognized to be a great advance in the management of acute myocardial infarction. In the published series which evaluate the effect of this treatment the evidence suggests that the mortality and complication rates are reduced for the first month after an infarction (Tulloch and Gilchrist, 1950, 1951; Irving Wright *et al.*, 1948; Irving Wright, 1952; Loudon *et al.*, 1953). The necessity for a control series in all such studies is generally agreed, but the validity of finer classification of cases into good or bad risks at the onset of the illness has been questioned, and some—for instance, Irving Wright (1953)—do not believe that such detailed classification is possible or that it is justifiable to withhold anticoagulant treatment even in what seems to be a "good risk" case at the outset. It appears, therefore, that all patients with acute myocardial infarction should receive a course of treatment in hospital with a coumarin anticoagulant unless there is a clear contraindication.

While the immediate benefit from such short-term treatment is not in doubt, there is as yet no information about its effect on the late mortality in survivors of a single attack, say at the end of a year. If it were shown that the benefit ceased with the anticoagulant course, there would be a strong case for prolonged out-patient treatment, provided this could be made safe and effective for general use. In any event, it is probable that a tendency to intravascular thrombosis persists in patients who have had one myocardial infarct, and the experimental work of Payling Wright *et al.* (1953) suggests that long-continued administration of coumarin anticoagulants does speed up recanalization of blocked arteries. For these reasons it is likely that large-scale out-patient treatment will have to be considered very

seriously, and not least from the point of view of safe and effective control. The standard method of treatment, based on frequent "prothrombin time" estimations and an empirical therapeutic range, is safe in hospital if scrupulously carried out, but its well-known risk of haemorrhage makes it more difficult to apply to outpatients.

The object of this paper is to discuss, in the light of recently acquired knowledge and techniques, some fundamental problems in regard to the mode of action of coumarin drugs and, consequently, the safest effective method of controlling their dosage; and to suggest a possible approach to a solution of these problems.

Action of Coumarin Anticoagulants

When the one-stage test of Quick was introduced it was believed that it measured prothrombin (Quick *et al.*, 1935). In recent years it has become evident that this is not so, and that severe lack of prothrombin (below 10%) (Biggs and Douglas, 1953a) will prolong the clotting-time in the one-stage Quick test by only 5–10 seconds, depending on the activity of the tissue thromboplastin used—a prolongation below the conventional therapeutic range for anticoagulant therapy. Prothrombin, in fact, is affected to a very variable degree by coumarin drugs, rarely, with therapeutic doses, falling below 30% as measured by the two-stage area method of Biggs and Douglas (1953a), and may be within normal limits while the one-stage test is adequately prolonged (see Table). Severe depression of prothrombin can, however, result from overdosage.

A normal clotting-time in the one-stage test depends on at least two factors other than prothrombin, lack of either of which will produce a prolongation of the clotting-time far in excess of that produced by prothrombin deficiency. The best-known of these is factor V or ac-globulin (Quick, 1953; Owren, 1947). Fortunately it is unaffected by coumarin-type anticoagulants, and therefore need not be considered further. The other factor—factor VII (Koller *et al.*, 1951), cothromboplastin (Mann, 1949), SPCA (Alexander *et al.*, 1949), convertin (Owren, 1951)—is the one principally affected by those drugs (Douglas, 1952; Tudhope and Hunter, 1953). It is reduction in the concentration of factor VII which produces the prolonged clotting-time in the Quick test when coumarin anticoagulants are given, since the one-stage clotting-time is corrected by the addition of this factor (see Table).

Drug	One-stage (Quick) Test			Two-stage Prothrombin % of Normal
	Normal Plasma (secs.)	Patients' Plasma (secs.)	Patient's Plasma + 10% factor VII (secs.)	
Ethyl biscoumacetate ..	12	24	13	84
" ..	12	45	16	42
" ..	11	36	17	21
" ..	11	26	14	33
" ..	11	27	13	56
Phenylindanedione ..	11	26	13	83
" ..	11	22	13	73
" ..	13	28	14	85
Naphthylhydroxy-coumarin	12	24	14	72

The precise means whereby lack of factor VII affects the coagulation is by impairment of intrinsic blood thromboplastin generation (Biggs and Douglas, 1953b; Walker and Hunter, 1954) as studied by the blood thromboplastin generation test (Biggs *et al.*, 1953; Biggs and Douglas, 1953b), and it is probably on this that the therapeutic action depends. The situation, however, is complicated by the observation, made by Koller (1953) and ourselves (Walker and Hunter, 1953, unpublished observations), that in patients given coumarin drugs the generation of intrinsic blood thromboplastin may be impaired at a time when the one-stage test is normal or only slightly prolonged. We have noted this impairment both before the one-stage test has been prolonged (Fig. 1) and on cessation of treatment shortly after it has returned to normal (Fig. 2), either under the influence of vitamin K_1 (Koller) or spontaneously (Walker and Hunter, unpublished observations).

Impaired blood thromboplastin generation has also been found in patients under treatment whose one-stage clotting-times had temporarily fallen below 20 seconds, and in patients in whom the conventional therapeutic level was not achieved at all. To date, 37 out of 46 patients with one-stage clotting-times of 20 seconds or under have shown impaired blood thromboplastin generation when their serum was used in the generating mixture. This phenomenon is not due to lack of factor VII, unless we assume that the thromboplastin generation test is a much more sensitive measure of factor VII deficiency than the one-stage test. It may equally be due to lack of the Christmas factor, which cannot be measured by the one-stage test, since the levels of this factor in coumarin-treated patients have not been studied in an adequate number of cases (Biggs, 1953, personal communication). We do not think that the evidence at present known to us justifies the postulation of an additional serum factor (factor X, Koller, 1953), though we have not yet had the opportunity of studying Koller's data or of ascertaining that we are observing the same phenomenon.

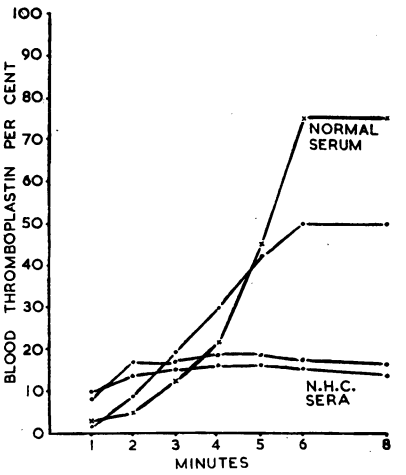

FIG. 1.—Thromboplastin generated from same mixture of normal platelets and normal Al(OH)$_3$-treated plasma using normal serum (upper curve) and serum from three patients 24 hours after 200 mg. of naphthylhydroxycoumarin (N.H.C.) by mouth, when one-stage time was still normal (lower three curves). Serum is source of factor VII and Christmas factor. 100% thromboplastin represents a clotting-time in the substrate plasma of 10 seconds.

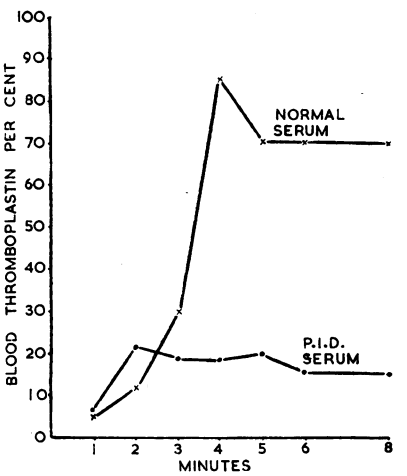

FIG. 2.—Blood thromboplastin generated from normal reagents (upper curve) and from same mixture using serum from patient receiving phenylindanedione (P.I.D.), when the one-stage time had returned to normal (lower curve).

These observations, if confirmed, may have an important bearing on the dosage necessary for therapeutic effect.

The Therapeutic Level

A one-stage clotting-time (Quick) of about 30 seconds with a control time of 12–14 seconds is considered to be the therapeutic level. The reason for this may simply be that it indicates the largest dose that can be given with reasonable safety, although an additional point is that the whole blood of such a patient will show a definite prolongation of its clotting-time in lustroid or silicone tubes. This level is now known to be associated with therapeutic benefit. In view of the actions described above, however,

it is worth while considering which effects are essential to therapeutic benefit and should therefore be aimed at with minimal dosage in the interests of safety.

Of the factors involved, prothrombin is the least likely to be concerned with the therapeutic action. Dicoumarol has a greater effect on prothrombin than ethyl biscoumacetate ("tromexan"), and Irving Wright (1953) found no significant difference in clinical results between series treated with these two drugs. This conforms with the variable and often high prothrombin levels found in patients with a one-stage test within the therapeutic range.

If reduction in factor VII is the important action, then the one-stage test should give a reliable measure of therapeutic level. But reduction in factor VII results in impaired blood thromboplastin generation, and this effect has been achieved while the one-stage clotting time is normal or nearly so. If it were found possible to bring about consistent impairment of blood thromboplastin and therapeutic benefit, with minimal prolongation of the one-stage clotting-time, then it might be possible to treat patients adequately with much smaller doses than hitherto believed necessary, thus eliminating the danger of bleeding—a most important consideration in out-patient treatment.

The Danger of Haemorrhage

For many years a constant source of anxiety to the clinician has been unexpected and dangerous bleeding in patients under treatment, and one of the big problems of anticoagulant therapy is to determine the factor or factors principally concerned with the onset of haemorrhage. As these factors need not necessarily be the same as those principally concerned with the therapeutic effects, it may become possible to eliminate the danger of bleeding without lessening therapeutic efficiency. One such possibility has been mentioned in the preceding section.

It is generally agreed that bleeding is rare when the one-stage test is within the "therapeutic limits." Even within these limits, however, it was noted (Stirling and Hunter, 1951) that bleeding might occur, in the absence of obvious predisposing conditions such as peptic ulcer, whenever the one-stage time was more than 30 seconds, and it was also noted then that the patients in whom the time was above 40 seconds did not bleed more frequently than those in whom it was between 30 and 40 seconds. There is, therefore, beyond certain limits, no hard-and-fast correlation between the results of the Quick test and the tendency to bleed—suggesting that haemorrhage may not be primarily due to factor VII deficiency. The precise cause of haemorrhage is in fact obscure, but we suggest that one possible reason why most patients given coumarin anticoagulants never bleed is that their prothrombin concentration is adequate (see Table).

Three cases have recently been encountered (two out-patients and one in hospital) in which the continuation of bleeding was correlated with very low prothrombin levels —below 10% of normal—rather than with the one-stage test, and are to be described in detail elsewhere. Both out-patients had stopped their drug at least 24 hours before admission, and had therefore entered the recovery phase: their one-stage clotting-times were probably much longer at the onset of haemorrhage than they were found to be on admission. However, the fact remains that both were bleeding, one seriously, at a time when their one-stage times were within the accepted therapeutic level. In the third case the one-stage test was within therapeutic limits when bleeding started, and prothrombin was found to be almost absent. On the other hand, we have had cases in which the prothrombin fell to very low levels and no bleeding occurred.

The findings suggest that prothrombin deficiency may be related to the cause of haemorrhage and show that, whatever the one-stage time may be at the onset of bleeding, severe prothrombin deficiency and haemorrhage can exist when the one-stage time is only moderately prolonged. While it is admitted that with all the well-known anticoagulants prothrombin is not greatly reduced until very low levels of factor VII are reached and that scrupulous maintenance of the one-stage time in the region of 30 seconds is a reasonable safeguard, it is clear that with the doses at present regarded as necessary this safeguard, especially in the case of out-patients, is by no means absolute, and that any possibility of establishing a therapeutic range by impairing blood thromboplastin with minimal prolongation of the one-stage time, and hence with smaller doses and a greater margin of safety, is at least worthy of investigation. The possibility exists that the required dosage might be small enough to avoid prothrombin deficiency and also haemorrhage.

Comment

Although long-term anticoagulant therapy is probably very important in the treatment of coronary thrombosis, it is possible in the light of present knowledge, as we have indicated, that a period of more strict laboratory control and study of the different factors may further increase the benefits and lessen the dangers of this treatment. It is necessary to determine the minimal prolongation of the one-stage clotting-time which is associated consistently with impaired blood thromboplastin generation and to seek confirmation by clinical studies that the therapeutic results in these circumstances compare favourably with the present regime. For these reasons it is desirable that a standardized trial of out-patient treatment in specialized units should be made. For the trial period the laboratories concerned should carry out agreed standard techniques in order that their results may be comparable.

In the meantime, no revolutionary change in the method of management in hospital is proposed. Routine laboratories should continue to use the one-stage test and to accept its usual interpretation in terms of therapeutic and haemorrhagic levels. The other tests suggested above for research purposes are too complicated for routine use.

Summary

Some of the unsolved problems in the mode of action and control of treatment with anticoagulant drugs in myocardial infarction are described.

In view of the varying effect of coumarin anticoagulants on the known clotting factors, and our present lack of knowledge concerning which factor or factors are important, it is suggested that treatment might be made more effective and less dangerous, and eventually more simple and capable of application in domiciliary practice, if for a trial period clinical investigations were correlated with detailed laboratory studies to determine the safest and most effective method of control.

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