Suzman (1956) and Suzman, Ruskin, and Goldberg (1955) have confirmed that the incidence of repeated attacks of coronary thrombosis is less in patients undergoing prolonged anticoagulant therapy than in untreated cases. The main objection to anticoagulant therapy, however, is the necessity for strict laboratory control to avoid therapeutically induced haemorrhage. In an editorial in the *Lancet* (1956) it is stated that in "good risk" cases "the relatively small advantage to be obtained from anticoagulants must be set against the risk of transport to hospital; if the assessment is 'poor risk,' then the patient should be got to hospital for anticoagulant treatment as soon as possible." It appears from this statement that adequate control of anticoagulant therapy necessitates admission to hospital. We cannot agree that this is necessary or, in fact, advisable, our view being based on an experience extending over several years during which over 1,000 patients have been under laboratory control either domiciliary or domiciliary. Haemorrhage in adequately controlled cases has been rare. It has, however, hitherto apparently not been customary in England to undertake laboratory control in domiciliary practice. The major objections have been (a) the fear of haemorrhage, which certainly occurred more frequently (Biggs and Macfarlane, 1949; James, 1949) when prothrombin estimations were performed with Russell's viper venom as the source of thromboplastin (Fullerton, 1940), and (b) the necessity of using freshly drawn blood for Quick, Stanley-Brown, and Bancroft's (1935) one-stage prothrombin test. The latter has apparently been based on Quick's (1951) statement that for research studies blood should be used within 30 minutes of collection, whereas up to four hours' storage was permissible for routine clinical purposes.

Since specimens submitted by distant medical practitioners could not reach our laboratories within four hours after collection, an investigation was undertaken in 1953 similar to that reported by Watson (1956), but no report was made at the time. However, in view of the increasing use of anticoagulants and of favourable recent reports (Suzman et al., 1955; Suzman, 1956; Wright, Marple, and Beck, 1948; Gilchrist and Tulloch, 1956), it was thought of interest to record a confirmation of Watson's findings. The main purpose of the study was to determine the effect of a 24-hour delay on the estimation of the prothrombin index and to assess the significance of the possible loss of labile factor (Factor V).

**Methods**

**Determination of Prothrombin Index.**—This follows its main essentials Stein's (1941) modification of the method of Quick et al. (1935). To duplicate samples of 0.1 ml. of plasma and 0.1 ml. of an approximately 6% solution of thromboplastin (prepared by incubating acetone-dried human brain with saline at 37°C. for 15 minutes), 0.1 ml. of M/40 calcium chloride solution is added, a stop-watch started immediately, and the end-point determined as described by Stein (1941). We have preferred in routine practice to express our results as the "prothrombin index" (P.I.) which is obtained by dividing the prothrombin time of normal plasma by that of the patient's plasma and expressing the result as a percentage. The effect of adding labile factor or Factor V was studied by adding 0.1 ml. of barium-sulphate-absorbed, i.e., prothrombin-free plasma prepared according to the directions of Rosenfield and Tuft (1947) to 0.9 ml. of test plasma. Samples each of 0.1 ml. of the mixture were then tested as described above.

**Discussion**

Like Watson (1956), little difference was found whether the sample of blood used for the estimation was fresh or 24 hours old. Stein (1941) had already suggested that plasma, if separated and kept in a cool place, may be used 24 hours later without showing much change in the prothrombin index, but this report has apparently
been overlooked by later workers. Although Stein
does not specifically state that the plasma remained
corked after separation, he has confirmed (per-
sonal communication) that this was in fact done.
This is of some significance as our experiments
have demonstrated.

In the accompanying figures results are
shown on 55 specimens, 49 of which were from
patients on anticoagulant therapy. After estimat-
ing the prothrombin index on each specimen of
blood, other samples of the same specimens were
stored, corked, in a drawer which was opened and
closed several times during the day to simulate
conditions that might occur during the transporting
of the specimens to the laboratory. After 24
hours under these conditions the mean difference
in prothrombin times was only $0.7 \pm 1.4$ seconds.
Fig. 1 shows that in only three specimens was
an increase greater than 3 seconds obtained. When
the actual prothrombin index is 50%, which is the
level aimed at in therapy, an increase of 3 seconds
in the prothrombin time would mean that a pro-
thrombin index of 45% is reported. At lower
prothrombin indices, to produce an error of 5% would require even greater differences in pro-
thrombin times, e.g., at a prothrombin index of
40%, 4 seconds’ difference is required, and at 30%
8 seconds’ difference.

This error may be due either to inherent tech-
nical defects or to disappearance of labile clotting
factors, e.g., Factor V, from the oxalated blood
on standing. Either of these possibilities would
fit the observed facts that the variations shown
bear no constant relationship to the actual pro-
thrombin times recorded from 12 seconds (pro-
thrombin index 100%) to 30 seconds (prothrombin
index 40%). Since anticoagulant therapy is known
not to affect Factor V (Stefanini, 1950; Biggs and
Macfarlane, 1953), it might be expected that
destruction of the labile factor would be fairly
uniform in all specimens and independent of the
initial value of the prothrombin index. Since this
could account for changes in the prothrombin
times on standing over 24 hours, the effect of adding labile factor was determined. Fig. 2
shows that there is no decrease in the degree of
scatter, suggesting that there has been no signifi-
cant loss of the labile factor during storage of the
blood under the conditions specified. The prob-
ability, therefore, remains that technical errors are
responsible for the degree of scatter found.

When blood specimens are centrifuged soon
after collection and the centrifuged blood allowed
to stand in uncorked tubes at room temperature,
considerable differences in prothrombin times
(mean $6.5 \pm 7.4$ seconds) compared with whole
blood are found after 24 hours. These are only
partially corrected by adding labile factor (mean
$3.0 \pm 5.5$ seconds). However, when the separated
plasma is kept in corked tubes under similar con-
ditions to whole blood there is much less change
in the prothrombin times (mean $3.0 \pm 3.7$ seconds)
in most of the specimens, but prothrombin time
increases of over 5 seconds were observed in six
specimens and over 10 seconds in two specimens.
LABORATORY CONTROL OF ANTICOAGULANT THERAPY

The addition of labile factor produced no significant change in the prothrombin times (mean 1.9 ± 3.9 seconds). Chak and Giri (1948) showed that the loss in prothrombin activity of plasma on storage could be restored by passing carbon dioxide through the plasma, and claimed that the carbon dioxide in some way neutralized the action of inhibitors which are formed during the period of storage. As, however, the prothrombin times after 24 hours of the separated plasma are slightly longer than those of plasma left in contact with their erythrocytes, both being kept corked under similar conditions, Stein and Abrahams (personal communication) have suggested that the red cells exert a buffering action and thus retard the escape of carbon dioxide.

Summary

A report is given of the laboratory control of domiciliary and ambulatory cases on prolonged anticoagulant therapy. Several of these patients have been under treatment for over five years. In the authors' experience, which extends to over 1,000 patients on prolonged anticoagulant therapy, haemorrhage in adequately controlled cases is rare. The prothrombin index, performed on samples of oxalated venous blood, does not appreciably vary for 24 hours after withdrawal, provided the specimens are maintained corked. No relationship could be established between the variations found and possible deterioration of labile factor, and such variations are put down to experimental error.

REFERENCES

Laboratory Control of Anticoagulant Therapy: The Effect of Storage on Blood Used for Prothrombin Estimations

W. Lewin, B. M. Bloomberg and Irene Mrost

*J Clin Pathol* 1957 10: 351-353
doi: 10.1136/jcp.10.4.351

Updated information and services can be found at:
http://jcp.bmj.com/content/10/4/351.citation

**Email alerting service**

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Notes**

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/