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Coagulation Factor Analysis in Patients On Long-Term Anticoagulation

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■ *Ninety studies on 58 patients undergoing chronic warfarin therapy included Quick prothrombin times, partial thromboplastin times, thromboplastin generation tests and assays for clotting factors II, V, VII, VIII, IX, X, XI and XII. The results indicate no benefit from supplementation of the Quick tests by any of these other procedures. It is suggested that the Quick test uniformly performed, using a standard uniform thromboplastin, would be the procedure of choice.*

COUMARIN DERIVATIVES for oral administration are the most practical agents for long-term anticoagulation. Careful screening of patients, with emphasis on alcoholism and hypertension and on detecting lesions which may bleed, helps to avoid complications. The attending physician must be aware of other drugs which affect the absorption, binding, and utilization of the anticoagulant as well as the balance with vitamin K.[1] In spite of all caution there is still a disturbing frequency of bleeding. Purpura, ecchymosis, menorrhagia and epistaxis may occur in 40 to 50 percent of patients but are usually minor. The incidence of serious or life-threatening bleeding varies in reported series. In 2,189 cases collected from the literature,[2] 5.6 percent of patients had serious bleeds, mostly gastrointestinal, urinary and intracerebral.

The correct dose of coumarin anticoagulant is commonly determined by its effect on the prothrombin time as determined by the method of Quick. The Quick prothrombin time is affected by depression of those coagulation factors in-

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fluenced by coumarins (Factors II, VII, and X) as well as by two others (Factor V and fibrinogen). It is not influenced by Factor IX, which is lowered by the anticoagulant. It has been suggested by some authorities that bleeding which occurs in the presence of Quick prothrombin time within the usual therapeutic range may be explained by undetected disproportionate depression of Factor II,[3] Factor X[4] or Factor IX.[5] This naturally leads to the conclusion that alternative or supplementary procedures to the Quick prothrombin time determination are necessary. In contrast to these reports, other publications have indicated that clotting factors depressed by long-term coumarin therapy are affected to a consistent extent, which correlates with the results of the Quick test.[6-8]

Differences in the procedures used to assay coagulation factors could explain the conflicting results. Tissue thromboplastins are known to vary in their relative sensitivity in detecting deficiency of different clotting factors. A further report on the relation between results with the Quick prothrombin time and specific coagulation factor assays thus seems warranted. The data to be presented do not support the notion that disproportionate depression of an individual clotting factor undetected by the Quick test is the explanation for spontaneous bleeding when the prothrombin time is in the usual therapeutic range.

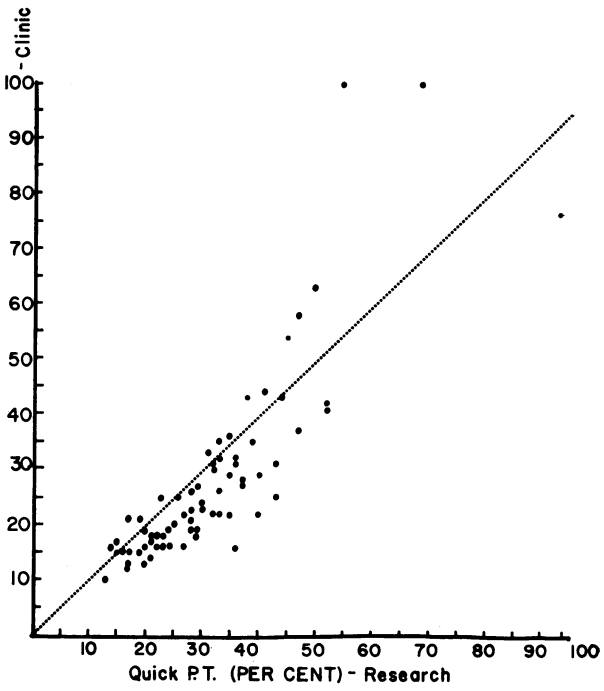

Chart 1.—Each dot compares the results of the one-stage Quick prothrombin time test on a single specimen as obtained in the research laboratory and in the hospital clinical laboratory. In this and subsequent charts the higher values were obtained on patients after warfarin therapy had been discontinued.

Methods

Ninety studies were performed on 58 outpatients who had been taking warfarin for months or years. Four patients who completed a course of treatment were restudied after the warfarin had been discontinued. The blood was drawn during a routine clinic visit, and the patients were questioned and examined for bleeding problems at the same time.

For Quick prothrombin time determination in the hospital clinical laboratory, 4.5 ml of blood in 0.5 ml of 3.8 sodium citrate solution was used. The thromboplastin was Simplastin® (Warner-Chilcott). Percent activity was calculated from saline solution dilutions of pooled normal plasma.

For tests in the research laboratory, a second syringe (plastic) was filled with blood through the same needle and mixed with one-ninth volume of acid citrate anticoagulant[9] in a graduated polycarbonate centrifuge tube. The blood was centrifuged at once, the plasma transferred in portions to siliconed capped tubes and frozen at —30° for

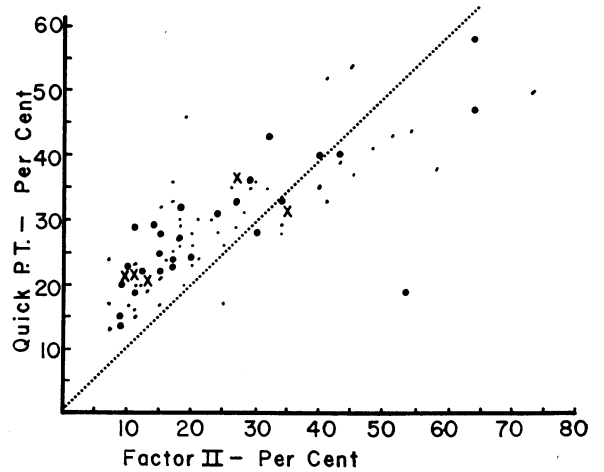

Chart 2.—Each dot compares the results of the Quick test with the Factor II assay on a single patient specimen. In this and in Charts 3, 4, and 5, the X's refer to values obtained on patients who were actively bleeding. The large dots refer to values obtained on patients who bled before or after the sample for testing was obtained. The small dots refer to values obtained on patients who did not bleed.

TABLE 1.—*Quick Prothrombin Times—Effect of Different Commercial Thromboplastins*

Patient No.	Rabbit Brain		Rabbit Brain and Lung	
	Seconds*	% Activity	Seconds*	% Activity
1	15	56	17	31
2	20	36	22	21
3	20	36	23	20
4	22	32	29	14
5	26	24	32	11.5
6	26	24	30	13
7	36	15	43	<10

*Rounded off to the nearest whole second.

a maximum of two weeks before assay. Once thawed, the plasma was used within several hours or discarded. Serum for the thromboplastin generation test was obtained from 2 ml blood incubated in a new 12 x 75 mm glass tube for three hours at 37°C. It was also stored in the frozen state before testing.

For Quick prothrombin time determination in the research laboratory a saline solution extract of acetone-dehydrated human brain was used.

Factor II was assayed in a one-stage procedure,[10] using human brain thromboplastin and, as substrate, a mixture of equal parts of barium sulfate absorbed oxalated beef plasma and human serum completely deprived of prothrombin by clotting whole blood in the presence of silica powder (Celite-Analytical Filter-Aid, Johns Manville).

Factor VII was measured along with Factor X in a similar system, replacing the human serum with bentonite-absorbed human plasma.[11]

Assay of Factor X differed from assay of Factors VII and X by the use of Russell's Viper Venom in the place of brain thromboplastin.

Factor V was evaluated by the technique of Borchgrevink, Pool and Stormorken.[12]

Factor VIII was measured by a thromboplastin generation technique as previously described.[13]

Factors IX, XI and XII were assayed in a partial thromboplastin time system. For Factors IX and XII, the substrates were plasmas of congenitally deficient patients. An artificial substrate was used for the Factor XI assay.[14] Plasmas were activated with Celite for one hour.

For the partial thromboplastin time, cephalin prepared by the Bell and Alton method[15] was used. Plasma was activated with Celite for six minutes. Our normal range is 35 to 50 seconds.

The thromboplastin generation test employed cephalin, aluminum hydroxide-absorbed citrated plasma and serum in the incubation mixture. Outdated blood bank plasma (in acid-citrate-dextrose solution) was the substrate for the second stage. Normal plasma and serum reagents were tested each day, and always had a substrate clotting time less than 12 seconds after six minutes of generation.

Results

The Quick prothrombin time was tested on the same plasma specimens in the hospital clinical laboratory and in the research laboratory. Despite the use of rabbit brain and lung thromboplastin in the former laboratory and human brain thromboplastin in the latter, results showed a significant agreement (Chart 1). There was a tendency for higher percent activities with the human thromboplastin; and, in individual cases, the decision as to the requirement for increased warfarin or for its antidote would have been radically different if the human brain data had been used rather than that from the clinical laboratory. Discrepancies in percent activity in the Quick time due to different thromboplastins have long been recognized.[16] Table 1 demonstrates this in an experiment performed a number of years ago but not previously published. The two commercial thromboplastins had identical clotting times when tested against saline dilutions of normal pooled plasma, but obviously could lead to quite different interpretations when tested on the plasma of anticoagulated patients, whether the results were reported in percent activity or seconds.

Factor II, or prothrombin concentration, was generally lower than the one stage prothrombin time test (Chart 2). In the therapeutic range of 20 to 30 percent for the prothrombin time, the Factor II concentration varied from 7 to 34 percent with 90 percent of the values in the 10 to 30 percent range.

Factor VII (SPCA) was even more depressed than Factor II (Chart 3). When the prothrombin

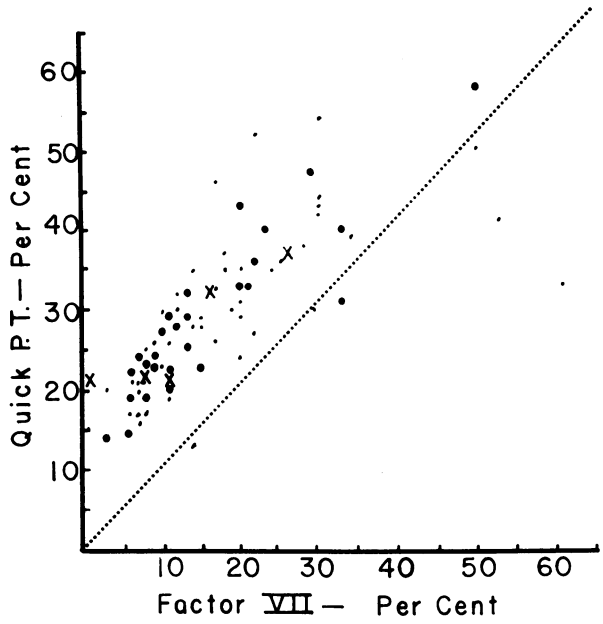

Chart 3.—Comparison of the Quick test with the Factor VII and X assay.

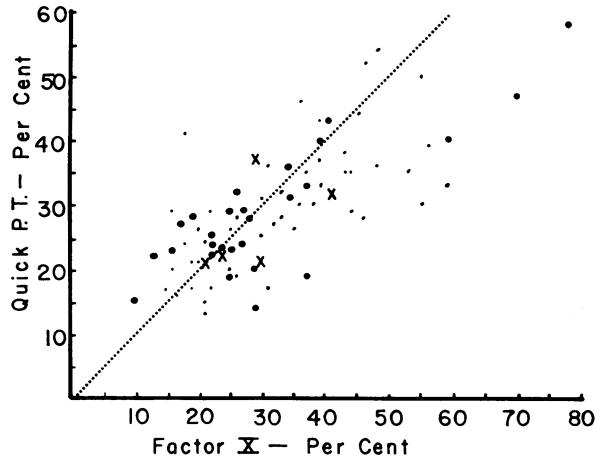

Chart 4.—Comparison of the Quick test with the Factor X assay.

time was in the therapeutic range Factor VII varied from 1 to 29 percent, and 90 percent of the values were 5 to 20 percent.

Although the Factor VII assay used is also sensitive to Factor X, a specific assay for Factor X, or Stuart factor (Chart 4) seemed to agree better with the prothrombin time. Ninety percent of the values of Factor X were in the 13 to 37

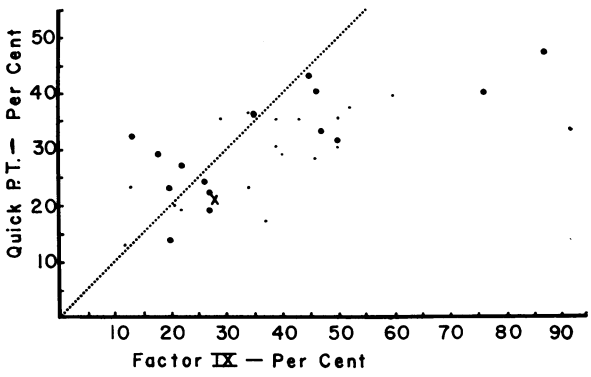

Chart 5.—Comparison of the Quick test with the Factor IX assay.

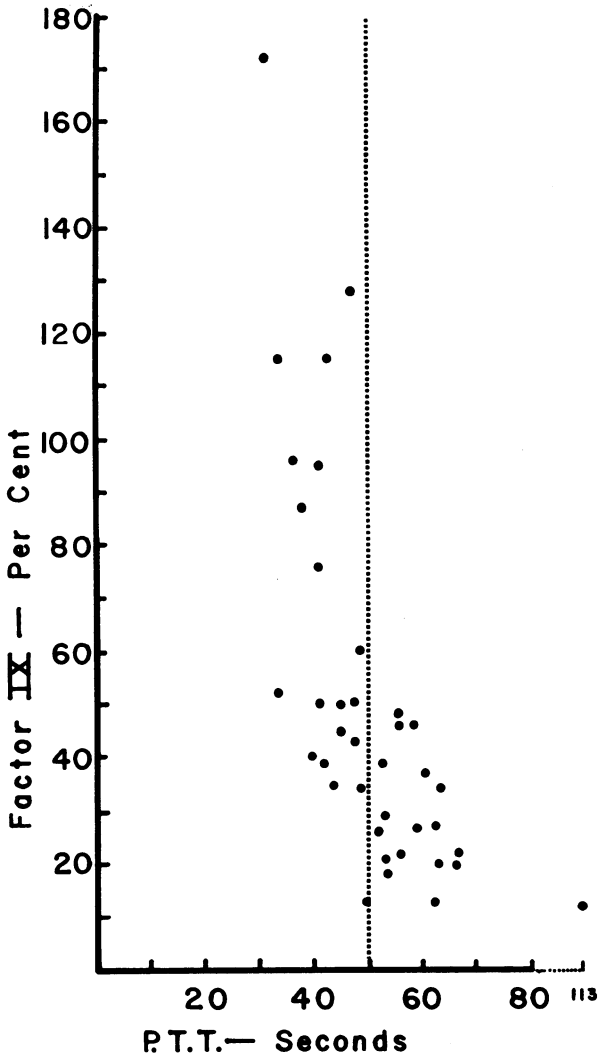

Chart 6. — Comparison of the partial thromboplastin time (PTT) with the Factor IX assay.

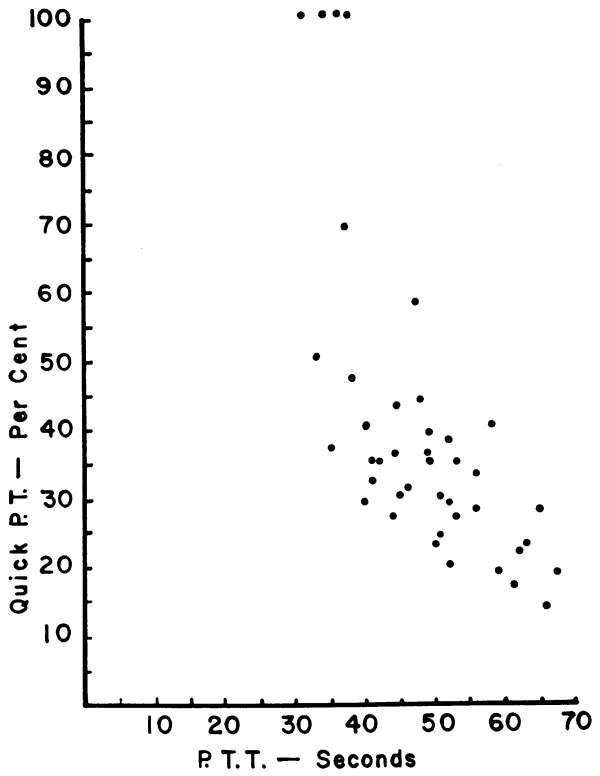

Chart 7.—Comparison of the PTT with the Quick test.

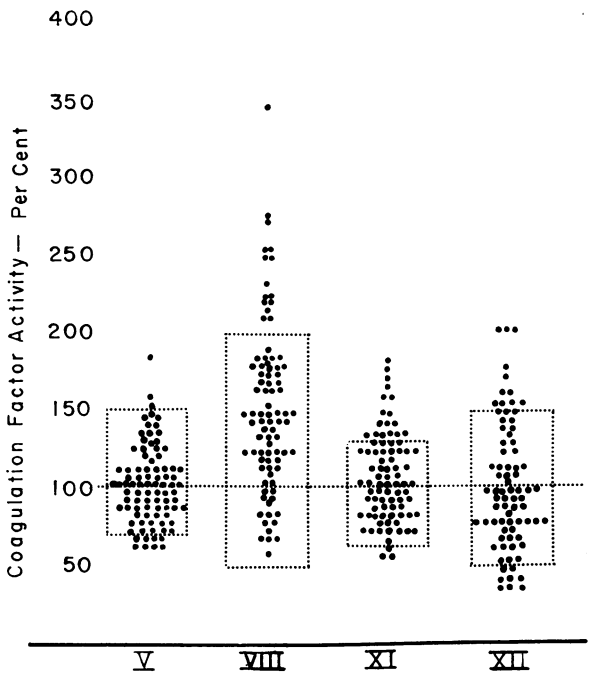

Chart 8.—Levels of Factors V, VIII, XI and XII. The rectangles outline the normal ranges.

percent range when the patient's prothrombin times were in the therapeutic range.

Factor IX (PTC) was not determined in all patients (Chart 5). It showed some correlation with the prothrombin time, but varied from it in a more unpredictable manner than the other factors. When the prothrombin time was in the therapeutic range, the Factor IX varied from 12 to 50 percent; and when the prothrombin time was in the 10 to 20 percent level, the Factor IX was 12 to 37 percent.

The partial thromboplastin time was investigated as a screening test for Factor IX depression (Chart 6). When Factor IX was under 30 percent the PTT varied from 50 to 113 seconds, but abnormal PTT's were seen with Factor IX levels as high as 47 percent. There seemed to be a better correlation of the PTT with the prothrombin time (Chart 7).

The thromboplastin generation test was abnormal in every patient under chronic warfarin therapy. The abnormality was confined to the patient's serum reagent. However, the defect in the thromboplastin generation test correlated very poorly with the Factor IX level, the Factor X level or the partial thromboplastin time.

Chart 8 shows the values obtained for levels of Factors V, VIII, XI and XII. In agreement with results reported by most previous investigators, these factors are not reduced by warfarin therapy, nor was there any evidence for a general compensating rise in these factors. Fifteen of the Factor VIII levels were above the upper limits of normal. Four of these patients were retested when anticoagulant therapy had been discontinued for more than a week, and two had returned to normal range.

Five patients were bleeding at the time blood was drawn for testing. The clotting values showed no tendency to be below the range obtained in the non-bleeding patients by any of the screening tests or factor assays (Charts 2 to 6). Twenty-nine studies performed on 13 patients who had bled before or after the withdrawal of test samples also showed no tendency to low values by any of the tests. Information on the patients who bled is outlined in Table 2.

Discussion

In more than 50 percent of patients in this study who bled abnormally during chronic anticoagulant therapy, a local cause for bleeding was

TABLE 2.—*Prothombin Time (Quick) in Patients Who Bled*

Actively Bleeding Patients	*Site of Bleeding*	*Quick PT (Percent)*	*Local Cause*
A	Dental	21	Diseased gums
B	GU	22	Stone
C	GYN	32	Endometrial hyperplasia
D	Epistaxis	21	None
E	Epistaxis	37	None
Patients Tested at a Time Remote from Bleeding	*Site of Bleeding*	*Lowest Recorded Quick PT*	*Local Cause*
F	Dental	14	Postoperative
G	Muscle	20	Scleroderma
H	GYN	22	Proliferative endometrium
I	GI and GU	23	Nephrotic syndrome (prednisone)
J	GYN	24	Enovid
K	Anal	28	Fissure
L	Dental	28	Diseased gums
M	GU and epistaxis	11	None
N	Ear and epistaxis	15	None
O	Epistaxis	19	None
P	GU	22	None
Q	Retroperitoneal	23	None
R	GU	25	None

evident. None of the five patients who were tested at a time when they were actively bleeding had a Quick prothrombin time value less than 20 percent. Although three of the remaining 13 patients who bled had prothrombin times below 20 percent, this proportion of low values was not greater than occurred among the non-bleeding patients. Moreover, none of the other tests performed provided results which would distinguish the bleeding patients.

The importance of local causes for bleeding in patients on long-term anticoagulant therapy has been stressed by other investigators. In Bjerkelund's[17] series, 18 of 52 patients who bled had some explanation other than the anticoagulant. Usually the lesions in such instances are evident, but occult tumors must be sought.[18]

The data obtained with the study indicate that, for all its defects,[19] the Quick prothrombin time method remains a satisfactory technique for control of anticoagulant therapy with medication which depresses the levels of vitamin K-dependent plasma coagulant factors. No additional information of benefit could be obtained by specific assays for coagulation factors nor by use of the partial thromboplastin time or thromboplastin generation test.

Within limits, the levels of Factors II, VII, IX and X could be predicted from the Quick prothrombin time. Levels of Factor VII would generally be expected to be significantly lower; levels of Factor II were somewhat lower; and levels of Factor X were approximately the same as the Quick values. Although Factor IX, as expected, correlated better with the partial thromboplastin time (which is influenced by Factor IX) than with the Quick test (which is not), there was no tendency for disproportionate depression of Factor IX or any other coagulation factor to correlate with bleeding. The relationship between coagulation factor levels and the Quick prothrombin time described here refers only to patients on chronic anticoagulant therapy. In the first few days of acute therapy, the Quick time primarily reflects Factor VII levels.

The thromboplastin generation test showed a poor correlation with all of the other tests. Most probably this reflects the varying states of activation and decay of Factor IX during the clotting of each specimen of blood[20] so that the Factor IX level resulting in the serum varied considerably.

There was a tendency for Factor VIII levels of these patients to be somewhat elevated. Although two of four patients returned to the normal range following discontinuation of therapy, it remains probable that Factor VIII elevations were a reflection of stress. Factor VIII rises with emotional disturbance and with tissue damage.

Voluminous data in the literature now indicate that the results of coagulation factor activity tests used to measure coumarin effect will vary widely with the test used and with the type of thromboplastin employed, whether results are reported in seconds or as percent activity. The therapeutic range desired can only be approximated by following published directions unless every aspect of the laboratory tests employed is identical. Each hospital has to establish the therapeutic range by a certain amount of trial and error. In the last analysis, each physician does this, unconsciously or otherwise, depending on the frequency of abnormal bleeding or thrombosis in patients under his care.

If agreement on a standard test and thromboplastin could be achieved,[21] patients would have far more uniform and successful care. Such agreement could be built around the Quick one-step prothrombin test, using acetone-dehydrated brain prepared according to the method of Quick.[22] The one advantage of thrombotest[23] is that it is a uniform reagent giving reproducible results from batch to batch supplied by a single manufacturer only. Similar results should be obtainable with a uniform Quick test and reagent.

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ORAL CONTRACEPTIVES AND MONILIA

"When you use a combination oral contraceptive agent, you're more inclined to have monilia in cultures. This distressing side-effect—and it is distressing because it interferes with the normal coital behavior of the patient and her husband—is very difficult to treat if the patient is maintained on the combination agent, just as it is almost impossible to clear up during pregnancy. In such a patient, if you'll stop the medication, you can put her on a local estrogenic cream; and it by itself, without any anti-monilia agent, will clear up the infection. Or if the patient wishes to stay on oral contraceptives, you can treat her with an estrogen or an oral monilia agent and switch her over to a sequential type of oral contraceptive. You're more apt to get a cure and keep the patient on oral contraception."

—HOWARD BALIN, M.D., Philadelphia

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