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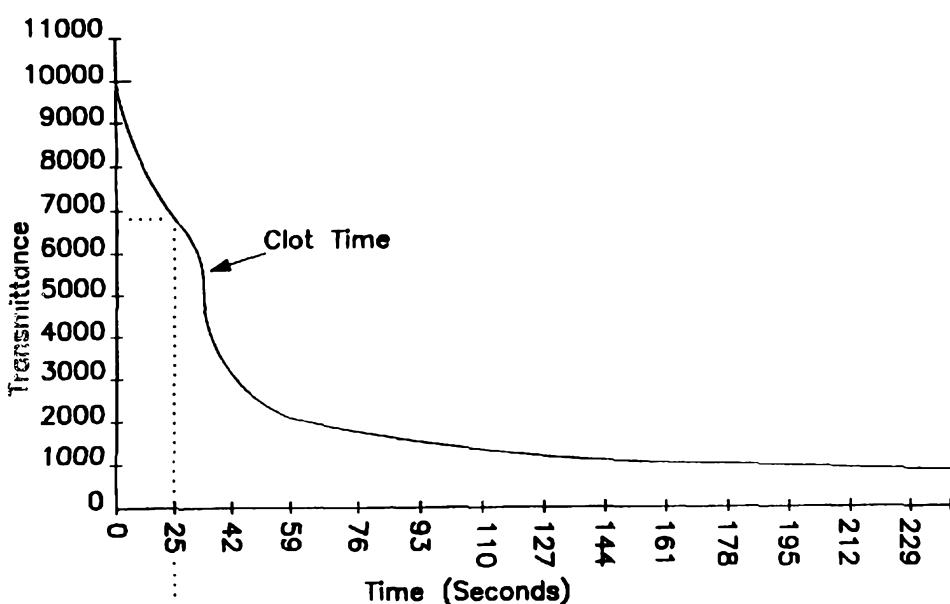
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(54) Title: A METHOD FOR PREDICTING THE PRESENCE OF HAEMOSTATIC DYSFUNCTION IN A PATIENT SAMPLE



(57) Abstract: Disclosed is a method for detecting a precipitate in a test sample in the absence of clot formation. The precipitate detection allows for the prediction haemostatic dysfunction in patients, which can lead to bleeding or thrombosis or particularly to Disseminated Intravascular Coagulation (DIC).

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**A Method for Predicting the Presence of Haemostatic Dysfunction in a Patient Sample**

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BACKGROUND OF THE INVENTION

This application is a continuation-in-part of U.S. patent application 09/244,340, filed February 4, 1999, the subject matter of which is incorporated 10 herein by reference. This application also relates to U.S. patent 5,646,046 to Fischer et al., the subject matter of which is incorporated herein by reference.

Blood clots are the end product of a complex 15 chain reaction where proteins form an enzyme cascade acting as a biologic amplification system. This system enables relatively few molecules of initiator products to induce sequential activation of a series of inactive proteins, known as factors, culminating in 20 the production of the fibrin clot. Mathematical models of the kinetics of the cascade's pathways have been previously proposed.

Thrombosis and hemostasis testing is the in vitro 25 study of the ability of blood to form clots and to break clots in vivo. Coagulation (hemostasis) assays began as manual methods where clot formation was observed in a test tube either by tilting the tube or

removing fibrin strands by a wire loop. The goal was to determine if a patient's blood sample would clot after certain materials were added. It was later determined that the amount of time from initiation of 5 the reaction to the point of clot formation in vitro is related to congenital disorders, acquired disorders, and therapeutic monitoring. In order to remove the inherent variability associated with the subjective endpoint determinations of manual 10 techniques, instrumentation has been developed to measure clot time, based on (1) electromechanical properties, (2) clot elasticity, (3) light scattering, (4) fibrin adhesion, and (5) impedance. For light scattering methods, data is gathered that represents 15 the transmission of light through the specimen as a function of time (an *optical time-dependent measurement profile*).

Two assays, the PT and APTT, are widely used to screen for abnormalities in the coagulation system, 20 although several other screening assays can be used, e.g. protein C, fibrinogen, protein S and/or thrombin time. If screening assays show an abnormal result, one or several additional tests are needed to isolate the exact source of the abnormality. The PT and APTT 25 assays rely primarily upon measurement of time required for clot time, although some variations of

the PT also use the amplitude of the change in optical signal in estimating fibrinogen concentration.

Blood coagulation is affected by administration 5 of drugs, in addition to the vast array of internal factors and proteins that normally influence clot formation. For example, heparin is a widely-used therapeutic drug that is used to prevent thrombosis following surgery or under other conditions, or is 10 used to combat existing thrombosis. The administration of heparin is typically monitored using the APTT assay, which gives a prolonged clot time in the presence of heparin. Clot times for PT assays are affected to a much smaller degree. Since a number of 15 other plasma abnormalities may also cause prolonged APTT results, the ability to discriminate between these effectors from screening assay results may be clinically significant.

20 The present invention was conceived of and developed for predicting haemostatic dysfunction in a sample based on one or more time-dependent measurement profiles, such as optical time-dependent measurement profiles. In addition, the present invention is 25 directed to predicting the presence of Disseminated Intravascular Coagulation in a patient based on a

time-dependent profile, such as an optical transmission profile, from an assay run on the patient's blood or plasma sample.

SUMMARY OF THE INVENTION

5 The present invention is directed to a method for detecting a precipitate in a test sample in the absence of clot formation. The method includes providing a test sample and adding thereto a reagent, the reagent alone or in combination with additional 10 reagents causing the formation of a precipitate. The reagent preferably comprises a metal divalent cation and optionally includes a clot inhibiting substance. The detection of the precipitate can be qualitative or quantitative, and the precipitate can be detected such 15 as by a clotting assay, a latex agglutination or gold sol assay, an immunoassay such as an ELISA, or other suitable method that would allow for detection and/or quantitation of the precipitate. The formation of the precipitate can be detected as an endpoint value, or 20 kinetically. This precipitate detection allows for predicting Haemostatic Dysfunction in patients. The present invention is useful for predicting Haemostatic Dysfunction that can lead to bleeding or thrombosis, or specifically to Disseminated Intravascular 25 Coagulation (DIC).

More particularly, the present invention is directed to a method comprising adding a reagent to a test sample having at least a component of a blood sample from a patient, measuring the formation of a precipitate due to the reaction of the test sample and the reagent, over time so as to derive a time-dependent measurement profile, the reagent capable of forming a precipitate in the test sample without causing substantial fibrin polymerization. The invention is 10 also directed to a method for determining whether or not a patient has haemostatic dysfunction, comprising obtaining a blood sample from a patient, obtaining plasma from said blood sample, adding a reagent capable of inducing the formation of a precipitate in patients 15 with haemostatic dysfunction without causing any substantial fibrin polymerization, taking one or more measurements of a parameter of the sample wherein changes in the sample parameter are capable of correlation to precipitate formation if present, and 20 determining that a patient has haemostatic dysfunction if precipitate formation is detected.

The present invention is also directed to a method for determining in a patient sample the presence of a 25 complex of proteins comprising at least one of a 300 kD protein, serum amyloid A and C-reactive protein,

comprising obtaining a test sample from a patient, adding an alcohol, a clot inhibitor, and a metal cation, wherein a precipitate is formed which comprises a complex of proteins including at least one of a 300 kD 5 protein, serum amyloid A and C-reactive protein.

The invention is also directed to a method comprising adding a coagulation reagent to an aliquot of a test sample from a patient, monitoring the formation 10 of fibrin over time in said test sample by measuring a parameter of the test sample which changes over time due to addition of the coagulation reagent, determine a rate of change, if any, of said parameter in a period of time prior to formation of fibrin in said test sample, if the 15 determined rate of change is beyond a predetermined threshold, then with a second aliquot of the patient test sample, add thereto a reagent that induces the formation of a precipitate in the absence of fibrin polymerization, measuring the formation of the 20 precipitate over time, and determining the possibility or probability of haemostatic dysfunction based on the measurement of the precipitate.

The invention is also directed to a method for 25 monitoring an inflammatory condition in a patient,

comprising adding a reagent to a patient test sample, the reagent capable of causing precipitate formation in some patient test samples without causing fibrin polymerization, measuring a parameter of the test sample 5 over time which is indicative of said precipitate formation, determining the slope of the changing parameter, repeating the above steps at a later date or time, wherein an increase or decrease in the slope at the later date or time is indicative of progression or 10 regression, respectively, of the inflammatory condition.

The invention is further directed to a method for diagnosing and treating patients with haemostatic dysfunction, comprising adding a reagent to a test 15 sample that causes precipitate formation without causing fibrin polymerization, taking measurements over time of a parameter of the test sample that changes due to the formation of the precipitate, determining the rate of change of said parameter, determining that a patient has 20 haemostatic dysfunction if said rate of change is beyond a predetermined limit; intervening with treatment for said haemostatic dysfunction if said rate of change is beyond the predetermined limit.

The invention also is directed to a method comprising adding a reagent to a patient sample capable of causing formation of a precipitate in said sample, monitoring a changing parameter of said sample over time, said parameter indicative of said precipitate formation, determining the rate of change of said parameter or whether said parameter exceeds a predetermined limit at a predetermined time, repeating the above steps at least once, each time at a different plasma/reagent ratios, measuring the maximum, average and/or standard deviation for the measurements; and determining haemostatic dysfunction based on the maximum, average and/or standard deviation measurements.

15 The present invention is further directed to an immunoassay comprising providing a ligand capable of binding to C-reactive protein or the 300 kD protein in lane 5 of Fig. 21, adding said ligand to a test sample from a patient and allowing binding of said ligand to C-  
20 reactive protein or said 300 kD protein in said test sample, detecting the presence and or amount of C-reactive protein or said 300 kD protein in said sample, and diagnosing haemostatic dysfunction in the patient due to the detection and/or amount of C-reactive protein  
25 or said 300 kD protein detected.

The invention further relates to a method for testing the efficacy of a new drug on a human or animal subject with an inflammatory condition and/or 5 haemostatic dysfunction, comprising adding a reagent to a patient test sample, said reagent capable of causing precipitate formation in some subject test samples without causing fibrin polymerization, measuring a parameter of said test sample over time which is 10 indicative of said precipitate formation, determining the slope of said changing parameter and/or the value of said parameter at a predetermined time, administering a drug to said animal or human subject, repeating the above steps at a later date or time, wherein an increase 15 or decrease in said slope or value at said later date or time is indicative of the efficacy of said drug.

BRIEF DESCRIPTION OF THE DRAWINGS

20 Figures 1a and 1b illustrate transmittance waveforms on the APTT assay with Fig. 1a showing a normal appearance, and 1b showing a biphasic appearance; Figure 2 illustrates transmittance levels at 25 seconds in relation to diagnosis in 54 patients with bi- 25 phasic waveform abnormalities. The horizontal dotted

line represents the normal transmittance level;

Figure 3 illustrates serial transmittance levels (upper panel) and waveforms (lower panel) on a patient who developed DIC following sepsis and recovered;

5 Figure 4 illustrates serial transmittance levels (upper panel) and waveforms (lower panel) on a patient who developed DIC following trauma and died;

Figure 5 illustrates ROC plots for the prediction of DIC transmittance at 25 seconds (TR25), APTT clot 10 time, and slope\_1 (the slope up to the initiation of clot formation);

Figures 6 and 7 show histograms for DIC, normal and abnormal/non-DIC populations for TR25 and slope\_1;

Figures 8 and 10 show group distributions for 15 slope\_1 and TR25 respectively;

Figures 9 and 11 show partial subpopulations of the data shown in Figures 8 and 10;

Figure 12 is an optical transmission profile for an APTT assay;

20 Figures 13 and 14 are optical transmission profiles for PT assays;

Figure 15 is an illustration of two waveforms where (x) is a test run on a sample using an APTT clotting reagent and resulting in a biphasic waveform, and (y) is 25 a test run where a clot inhibitor is used;

Figure 16 is a standard curve for ELISA of CRP;

Figure 17 shows an isolated precipitate after gel filtration;

Figure 18 is a graph showing the time course of 5 turbidity in a sample upon adding a precipitate inducing reagent;

Figure 19 is a graph showing the relationship between maximum turbidity change and amount of patient plasma in a sample;

10 Figure 20 shows the results of anion exchange chromatography of material recovery after fractionation of patient plasma;

Figures 21a and 21 show the non-reduced and reduced SDS page of various fractions of patient plasma;

15 Figure 22 shows immunoblots of CRP in normal and DIC plasma;

Figure 23 illustrates the turbidity change upon adding divalent calcium to materials obtained upon Q-sepharose chromatography in the absence of plasma  
20 (except top curve);

Figure 24 is a table showing CRP determined by ELISA;

Figure 25 shows the response to increasing calcium concentrations in optical transmission profiles;

25 Figure 26 shows a more pronounced slope without the

use of a clotting agent;

Figure 27 is a calibration curve with heparin;

Figure 28 shows CRP levels in 56 ITU patients plotted against transmittance at 18 seconds; and

5 Figure 29 shows more samples with CRP and decrease in transmittance at 18 seconds (10000- TR18).

#### DESCRIPTION OF THE PREFERRED EMBODIMENTS

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In the present invention, not only can a particular abnormality (Haemostatic Dysfunction) be detected, but in addition the progression of the disease can be monitored in a single patient. Haemostatic Dysfunction, 15 as used herein, is a condition evidenced by the formation of a precipitate prior to (or in the absence of clot formation, depending upon the reagent used).

Disseminated intravascular coagulation (DIC - a 20 type of Haemostatic Dysfunction) prognosis has been hampered by the lack of an early, useful and rapidly available diagnostic marker. The invention has been found to be not only useful as an early diagnostic and single monitoring marker of DIC, but in addition the

quantifiable and standardizable changes also allow for prognostic applicability in clinical management.

Disseminated intravascular coagulation (DIC) is a 5 secondary response to a pre-existing pathology whereby the haemostatic response becomes perturbed and disseminated as opposed to the focused events of normal haemostasis. Despite improvements both in the intensive care management of patients and in our basic knowledge 10 of haemostatic mechanisms in DIC, survival in this patient group is still very discouraging. Fundamental to the management of this complication is the implementation of aggressive therapy directed at forestalling or eradicating the primary pathology as the 15 source of the initiating stimulus. However, in practical terms, the problem remains one of early identification of DIC to facilitate immediate and appropriate intervention. Although the technological armory available to the clinical investigator has 20 expanded enormously, the pace of acute DIC precludes most of the more specific tests and reliance is still placed on traditional screening tests such as the prothrombin (PT), activated partial thromboplastin time (APTT) and platelet count. These tests lack specificity 25 on an individual basis and are only useful in DIC if they lead on to further determinations of fibrinogen and

fibrin breakdown products/D-dimers. However, changes in these parameters may not occur all at the same time and as such, serial testing is often needed which inevitably leads to a delay in diagnosis and clinically useful 5 intervention.

The normal sigmoidal appearance from an APTT transmittance waveform (TW) changes to a "bi-phasic" appearance in DIC patients. This represents a loss in the plateau of a normal APTT-TW, with development of an 10 initial low gradient slope followed by a much steeper slope (Figures 1a and b). In addition, this bi-phasic pattern can be seen even when the APTT clotting time result is normal.

Freshly collected blood samples that required a PT 15 or an APTT were analyzed prospectively over a two week working period. These were in 0.105M tri-sodium citrate in the ratio of 1 part anticoagulant to 9 parts whole blood and the platelet-poor plasma was analyzed on the MDA (Multichannel Discrete Analyzer) 180, an automated 20 analyzer for performing clinical laboratory coagulation assays using an optical detection system (Organon Teknika Corporation, Durham, NC, USA). In addition, to deriving the clot times for both PT (normal 11.2-15s) using MDA Simplastin LS and APTT (normal 23-35s) using 25 MDA Platelin LS with 0.025M calcium chloride (Organon Teknika Corporation, USA), an analysis of the TW for the

APTT was performed on each occasion at a wavelength of 580nm. To quantitate the visual profile, the amount of light transmittance at 25 seconds was recorded. A normal waveform has a light transmittance of 100% that is represented on the analyzer and in Figure 1a without the decimal point as 10000. As such, a bi-phasic change will have a reduced light transmittance of less than 10000. As can be seen in Fig. 1b, decreasing levels of light transmittance prior to clot formation correlate directly with increasing steepness of the bi-phasic slope. The recording of the light transmittance at 25 seconds also allows for standardization between patients and within the same patient with time. If the minimum level of light transmittance for each sample were to be used instead, this would be affected by variations in the clot time of the APTT and would therefore not be ideal for comparisons.

To ensure that no cases of DIC were overlooked, the following criteria was followed. If (a) an abnormal bi-phasic TW was encountered, or (b) a specific DIC screen was requested, or (c) if there was a prolongation in either the PT or APTT in the absence of obvious anticoagulant therapy, a full DIC screen was performed.

This would further include the thrombin time (TT) (normal 10.5-15.5 seconds), fibrinogen (Fgn) (normal 1.5-3.8 g/l) and estimation of D-dimer levels (normal < 0.5

mg/l) on the Nyocard D-Dimer (Nycomed Pharma AS, Oslo, Norway). Platelet counts (Plt) (normal 150-400 10<sup>9</sup>/l) performed on an EDTA sample at the same time were recorded. In addition, clinical details were fully 5 elucidated on any patient with a bi-phasic TW or coagulation abnormalities consistent with DIC.

The diagnosis of DIC was strictly defined in the context of both laboratory and clinical findings of at least 2 abnormalities in the screening tests (increased 10 PT, increased APTT, reduced Fgn, increased TT or reduced Plt) plus the finding of an elevated D-dimer level (>0.5 mg/l) in association with a primary condition recognized in the pathogenesis of DIC. Serial screening tests were also available on those patients to chart progression 15 and confirmation of the diagnosis of DIC as was direct clinical assessment and management. For statistical analysis, values for the sensitivity, specificity, positive and negative prediction of the APTT-TW for the diagnosis of DIC were calculated employing a two-by-two 20 table. 95% confidence intervals (CI) were calculated by the exact binomial method.

A total of 1,470 samples were analyzed. These were from 747 patients. 174 samples (11.9%) from 54 patients had the bi-phasic waveform change. 22 of these 54 25 patients had more than 3 sequential samples available for analysis. DIC was diagnosed in 41 patients with 30

of these requiring transfusion support with fresh frozen plasma, cryoprecipitate or platelets. The underlying clinical disorders as shown in Table 1

TABLE 1 GOES IN HERE

40 of the 41 patients with DIC had the bi-phasic TW. The one false negative result (DIC without a bi-phasic 15 TW) occurred in a patient with pre-eclampsia (PET) where the single sample available for analysis showed a prolonged PT of 21.0s, APTT of 44.0s and raised D-dimers of 1.5mg/l. 5 other patients were identified in this study with PET and none had either DIC or a bi-phasic 20 TW. Of the 14 patients with a bi-phasic TW which did not fulfil the criteria of DIC, all had some evidence of a coagulopathy with abnormalities in one or two of the screening tests. These abnormal results fell short of the criterion for DIC as defined above. 4 of these 14 25 patients had chronic liver disease with prolonged PT and

mild thrombocytopenia. A further 2 patients had atrial fibrillation with isolated elevation of D-dimer levels only. The remaining 8 patients were on the ICU with multiple organ dysfunction arising from trauma or 5 suspected infection but without the classical laboratory changes of DIC. These patient profiles were described in the ICU as consistent with the "systemic inflammatory response syndrome" (SIRS). Based on these figures, the bi-phasic TW has a 97.6% sensitivity for the diagnosis 10 of DIC with a specificity of 98%. Use of an optical transmittance waveform was found to be helpful in detecting the biphasic waveform.

TABLE 2 GOES HERE

The positive predictive value of the test was 74%, which

increased with increasing steepness of the bi-phasic slope and decreasing levels of light transmittance (Table 2 and Figure 2). In the first two days of the study, there were 12 patients who had an abnormality in 5 the clotting tests plus elevation of D-dimer levels. These were patients who were clinically recovering from DIC that occurred in the week preceding the study. This led to the impression that TW changes might correlate more closely with clinical events than the standard 10 markers of DIC.

TABLE 3 WILL GO HERE

The availability of more than 3 sequential samples 20 in 22 patients allowed for further assessment. Table 3 illustrates one such example with serial test results from a patient with *E. coli* septicaemia.

The appearance of a bi-phasic TW preceded changes in the standard tests for the diagnosis of DIC. It was 25 only later in the day that the PT, APTT, Plt and D-dimer

levels became abnormal and fulfilled the diagnostic criteria of DIC. Treatment with intravenous antibiotics led to clinical improvement by Day 2 with normalization of her TW in advance of the standard parameters of DIC.

5 D-dimers and Plt were still respectively abnormal 24 and 48 hours later.

This correlation between clinical events and TW changes was seen in all the DIC patients where samples were available to chart the course of clinical events.

10 As the TW changes were quantifiable and standardizable through recording of the transmittance level at 25 seconds, this analysis provided a handle in assessing prognostic applicability. Figure 3 illustrates the results of a patient who initially presented with

15 peritonitis following bowel perforation. This was further complicated by gram negative septicaemia post-operatively with initial worsening of DIC followed by a gradual recovery after appropriate therapy. As DIC progressed initially, there was increasing steepness in

20 the bi-phasic slope of the TW and a fall in the light transmittance level. A reversal of this heralded clinical recovery. Figure 4 illustrates the results of a patient who sustained severe internal and external injuries following a jet-ski accident. Although

25 initially stabilized with blood product support, his condition deteriorated with continuing blood loss and

development of fulminant DIC. The bi-phasic slope became increasingly steep with falls in transmittance level as the consequences of his injuries proved fatal.

5 As DIC can arise from a variety of primary disorders, the clinical and laboratory manifestations can be extremely variable not only from patient to patient but also in the same patient with time. There is therefore, a need for systems that are not only 10 robust in their diagnosis but simple and rapid to perform. Although it has been shown that the bi-phasic TW appeared to be sensitive for Haemostatic Dysfunction (e.g. DIC) and was not seen in other selected patient groups with coagulation aberrations or influenced by 15 either (i) pre-analytical variables, (ii) different silica-based APTT reagents, (iii) the use of thrombin as the initiator of the coagulation reaction or (iv) treatment in the form of heparin or plasma expanders, the robustness of this assay for DIC could only be 20 addressed through a prospective study. This study has shown that the bi-phasic TW provides diagnostic accuracy in DIC with an overall sensitivity of 97.6% and specificity of 98%. In contrast, none of the standard parameters on an individual basis (i.e., PT, APTT, TT, 25 Fgn, Plt, D-dimers) or even in combination, has ever reached the degree of sensitivity or specificity. The

ready availability of TW data from the MDA-180 would also fulfil the criteria of simplicity and rapidity unlike the measurements of thrombin-antithrombin complexes or other markers that are dependent on ELISA 5 technology. In addition, the advantages of TW analysis are that: (a) the bi-phasic TW change appears to be the single most useful correlate within an isolated sample for DIC and as such, reliance need no longer be placed on serial estimations of a battery of tests, and (b) the 10 appearance or resolution of the bi-phasic TW can precede changes in the standard, traditional parameters monitored in DIC with strong, clear correlation to clinical events and outcome.

Although the bi-phasic TW was also seen in patients 15 who did not have DIC *per se* as defined by the above criteria, the clinical conditions were associated with Haemostatic Dysfunction - namely activated coagulation prior to initiation of clot formation resulting in a biphasic waveform (for example in chronic liver disease 20 or in the very ill patients on the Intensive Care Unit who had multiple organ dysfunction). It appears that bi-phasic TW is sensitive to non-overt or compensated DIC and that a transmittance level of less than 90% (Figure 2) or sequential falls in that level (Figure 4), 25 reflects decompensation towards a more overt manifestation and potentially fulminant form of DIC.

This line of explanation is supported by the observation of only a mild bi-phasic TW (transmittance level of about 95%) in 2 patients with atrial fibrillation; a condition that is associated with mild coagulation 5 activation and elevated D-dimer levels. As no follow-up samples were available on these 2 patients whose clinical details were otherwise unremarkable, their bi-phasic TW could well have been transient. Nonetheless, these cases illustrate that the lower the level of light 10 transmittance, the more likely the bi-phasic TW becomes predictive of Haemostatic Dysfunction, particularly DIC.

The observation of a normal TW in a patient with PET and DIC needs further exploration as the study did 15 not selectively aim to examine any particular patient groups and only had a total of 6 patients with PET; the remaining 5 of which did not have DIC. One explanation which would be supported by other findings in this study is that the patient could have been recovering from PET 20 and DIC at the time of the sample. There may already have been normalization in the bi-phasic TW in advance of the other parameters which were still abnormal and indicative of DIC. Another explanation is that the disturbed haemostatic process in PET is more localized 25 and different from the DIC that arises from other conditions. Such patients respond dramatically to

delivery of the fetus which suggests anatomical localization of the pathological process to the placenta despite standard laboratory clotting tests implying systemic evidence of the condition.

5 Example:

Though analysis of the transmittance at a time of 25 seconds is helpful in predicting DIC, a second embodiment of the invention has been found that greatly improves sensitivity and specificity. It has been found 10 that looking at transmittance at a particular time can result in detecting an artifact or other decrease in transmittance at that point, even though the waveform is not a bi-phasic waveform. For example, a temporary dip in transmittance at 25 seconds would cause such a 15 patient sample to be flagged as bi-phasic, even if the waveform was normal or at least not bi-phasic. Also, if a patient sample had a particularly short clotting time, then if clot formation begins e.g. prior to 25 seconds (or whatever time is preselected), then the waveform 20 could be flagged as biphasic, even though the real reason for decreased transmittance at 25 seconds is because clot formation has already begun/occurred.

For this reason, it has been found that rather than analysis of transmittance at a particular time, it is 25 desirable to calculate the slope of the waveform prior

to initiation of clot formation. This calculation can involve determination of clot time followed by determination of waveform slope prior to clot time. In an additional embodiment, the slope (not transmittance) 5 is determined prior to clot time or prior to a preselected time period, whichever is less. As can be seen in Figure 11, when transmittance is used for determining e.g. DIC, there is poor specificity and sensitivity. However, as can be seen in Figure 9, when 10 slope prior to initiation of clot formation is used, specificity and sensitivity are greatly improved, and are better than standard tests used in the diagnosis of Haemostatic Dysfunction, such as DIC.

Additional testing was performed on three sets of 15 patients. The first set consisted of 91 APTT assays run on samples from 51 different confirmed DIC patients. The second set of data consisted of 110 APTT assays run on samples from 81 different confirmed normal patients.

The third set of data included 37 APTT assays run on 22 20 abnormal, non-DIC samples. Fig. 5 illustrates ROC plots for the prediction of DIC for three different parameters derived from the APTT assay using the combined data sets described: (1) transmittance at 25 seconds (TR25), (2) APTT clot time, and (3) slope 1 (the slope up to 25 initiation of clot formation). Slope 1 exhibited the best predictive power, followed by TR25. It has also

been shown that transmittance at 18 seconds has predictive value, particularly when the APTT clot time is less than 25 seconds. The "cutoffs" associated with the highest efficiency for the three parameters are 5 listed in Table 4:

Parameter	Cutoff
TR25	< 9700
Clot Time	> 35
Slope 1	< -0.0003

Table 4

10

It should be noted that these cutoffs have shifted with the addition of the third set, and would likely shift again, depending on the sample populations. Figures 6 and 7 show the histograms for the DIC, normal and 15 abnormal/non-DIC populations for TR25 and slope 1 respectively. Tables 5 and 6 show the data for the histograms in Figures 6 and 7 respectively:

20

Bins	DIC	Normal	Abnormal/Non-DIC
-0.006	3	0	0
-0.005	2	0	0
-0.004	1	0	0
-0.003	10	0	0
-0.002	24	0	0
-0.001	33	0	0
-0.0005	12	0	0
-0.0002	5	5	2
-0.0001	1	37	13
More	0	68	22

Table 5

5

Bin	DIC	Normal	Abnormal/Non-DIC
7000	34	1	0
8000	18	2	0
9000	26	6	1
9500	8	3	0
9600	3	2	1
9700	1	0	0
9800	1	3	0
9900	0	21	4
10000	0	62	30
More	0	10	1

Table 6

Figures 8 and 10 show the group distributions for 10 Slope 1 and TR25 respectively; and Figures 9 and 11 show the group distributions for Slope 1 and TR25

respectively. Figures 9 and 11 show partial subpopulations of the data shown in Figures 8 and 10.

When the prediction of Haemostatic Dysfunction is performed on an automated or semi-automated analyzer, 5 the detected bi-phasic waveform can be flagged. In this way, the operator of the machine, or an individual interpreting the test results (e.g. a doctor or other medical practitioner) can be alerted to the existence of the biphasic waveform and the possibility/probability of 10 Haemostatic Dysfunction such as DIC. The flag can be displayed on a monitor or printed out. A slope of less than about -0.0003 or less than about -0.0005 is the preferred cutoff for indicating a bi-phasic waveform. An increasing steepness in slope prior to clot formation 15 correlates to disease progression.

The above examples show that waveform analysis on the APTT assay can identify characteristic bi-phasic patterns in patients with haemostatic dysfunction. In the majority of cases, this dysfunction could be 20 labelled as DIC. This diagnostic waveform profile was seen in all APTT reagents tested, which were either silica or ellagaic acid-based. It has also been surprisingly found that a bi-phasic waveform can also be seen on PT assays with particular reagents, and that the 25 bi-phasic waveform is likewise indicative of haemostatic dysfunction, primarily DIC.

Using samples that give bi-phasic APTT waveforms, the PT waveform profile was derived using PT reagents (thromboplastin), namely Recombiplast™ (Ortho), Thromborel™ (Dade-Behring) and Innovin™ (Dade-Behring).

5 Both Recombiplast and Thromborel were particularly good at showing bi-phasic responses. Innovin was intermediate in its sensitivity. Using the transmittance level at 10 seconds into the PT reaction as the quantitative index, Recombiplast and Thromborel 10 objectively showed lower levels of light transmittance than Innovin. Thromborel can show a slight increase in initial light transmittance before the subsequent fall. This may be, in part, related to the relative opaqueness of Thromborel.

15 Further studies were performed comparing APTT profiles using Platelin™ and PT waveform profiles using Recombiplast™. Consecutive samples over a four week period from the intensive care unit were assessed. Visually, and on objective scores (comparing TL18 for 20 APTT and TL10 for PT), the APTT profile was more sensitive to changes of haemostatic dysfunction and clinical progression than the PT profile. This relative sensitivity can be seen in the APTT profile of Figure 12 (Platelin) compared to the PT profiles of Figure 13 25 (Recombiplast) and Figure 14 (Thromborel S). Invariably, at smaller changes in light transmittance,

the APTT waveform detected abnormalites more easily than the PT waveform. Nonetheless, in severe degrees of haemostatic dysfunction, both bi-phasic profiles were concordant.

5        In a further embodiment of the invention, the time dependent measurement, such as an optical transmittance profile, can be performed substantially or entirely in the absence of clot formation. In this embodiment, a reagent is added which causes the formation of a  
10 precipitate, but in an environment where no fibrin is polymerized. The reagent can be any suitable reagent that will cause the formation of a precipitate in a sample from a patient with haemostatic dysfunction, such as DIC. As an example, divalent cations, preferably of  
15 the transition elements, and more preferably calcium, magnesium, manganese, iron or barium ions, can be added to a test sample. These ions cause activation of an atypical waveform that can serve as an indicator of haemostatic dysfunction. It is also possible to run  
20 this assay in the absence of a clotting reagent (APTT, PT, or otherwise). As part of the reagent that comprises the activator of the atypical waveform, or separately in another reagent, can also be provided a clot inhibitor. The clot inhibitor can be any suitable  
25 clot inhibitor such as hirudin, PPACK, heparin, antithrombin, I2581, etc. The formation of the atypical

waveform can be monitored and/or recorded on an automated analyzer capable of detecting such a waveform, such as one that monitors changes in turbidity (e.g. by monitoring changes in optical transmittance).

5

Fig. 15 is an illustration of two waveforms: waveform (x) is a test run on a sample using an APTT clotting reagent and resulting in an atypical (biphasic) waveform, whereas waveform (y) is a test run on a sample 10 where a clot inhibitor is used (along with a reagent, such as a metal divalent cation, which causes the formation of a precipitate in the sample). Waveform (y) is exemplary of a waveform that can result in patients with haemostatic dysfunction where no clotting reagent 15 is used and/or a clot inhibitor is added prior to deriving the time-dependent measurement profile. Generally speaking, the greater the slope of the waveform (the larger the drop in transmittance in the same period of time) due to the precipitate formation, 20 the greater severity of the patient's haemostatic dysfunction. Fig. 16 is a standard curve for ELISA of CRP (CRP isolated from a patient used as the standard).

The precipitate formed in the present invention was 25 isolated and characterized by means of chromatography

and purification. Gel Filtration was performed as follows: A column (Hiprep Sephacryl S-300 High resolution - e.g. resolution of 10 to 1500 kDa) was used. The volume was 320 ml (d=26mm, l=600mm), and the flow rate was 1.35 ml/min. Fig. 17 shows the isolated precipitate.

Fig. 18 is a graph showing the time course of turbidity in a sample upon adding a precipitate inducing agent (in this case divalent calcium) and a thrombin inhibitor (in this case PPACK) to mixtures of patient and normal plasmas. Fig. 19 is a graph showing the relationship between maximum turbidity change and amount of patient plasma in one sample. 0.05 units implies 100% patient plasma. (Data from Fig. 18).

The steps used in the purification of components involved in the turbidity change in a patient's plasma were as follows: PPACK (10  $\mu$ M) was added to patient plasma. Calcium chloride was added to 50mM, followed by 8 minutes of incubation, followed by the addition of EtOH to 5%. The sample was then centrifuged 10,500  $\times$  g for 15 minutes at 4 degrees Celsius. The pellet was then dissolved in HBS/1mM citrate/10  $\mu$ M PPACK, followed by 25 35-70%  $(\text{NH}_4)_2\text{SO}_4$  fractionation. Finally, sepharose

chromatography was performed using a 5ml bed, 0.02-0.5M NaCl gradient and 50ml/side, to collect 2ml fractions.

Fig. 20 shows the results of anion exchange chromatography (Q-sepharose) of material recovery after 5 the 35-70% ammonium sulfate fractionation of patient plasma.

Figs. 21a and 21b show the non-reduced and reduced, respectively, SDS PAGE of various fractions obtained 10 upon fractionation of patient plasma. The loading orientation (left to right): 5-15% gradient/Neville Gel. (approximately 10 $\mu$ g protein loaded per well). In lane 1 are molecular weight standard (94, 67, 45, 30, 20 and 14 kDa from top to bottom. In lane 2 is 35%  $(\text{NH}_4)_2\text{SO}_4$  15 pellet, whereas in lane 3 is 70%  $(\text{NH}_4)_2\text{SO}_4$  supernate. Lane 4 is Q-sepharose starting material. Also shown in Figs. 21a and b are (from Fig. 20) peaks 1, 2a, 2b and 3 in, respectively, lanes 5, 6, 7 and 8. Lane 9 is pellet 1, whereas in lane 10 are again, molecular weight 20 standards. Results of NH<sub>2</sub>-terminal sequencing showed peak 3, the 22 kD protein in lanes 8 and 9 to be C-reactive protein (CRP), and the 10 kD protein in lane 9 to be human serum amyloid A (SAA). Peak 1 in lane 5 is a 300 kD protein which, as can be seen in Fig. 23, is 25 part of the complex of proteins (along with CRP) in the precipitate formed due to the addition of a metal

divalent cation to a plasma sample.

Immunoblots of CRP were performed in normal and DIC plasma. Blot 1 (see Fig. 22): (used 0.2  $\mu$ l plasmas for 5 reducing SDS-PAGE/CRP Immunoblotting). Loading orientation (left to right): NHP; Pt 5; 3; 1; 2; 4; and 8. For Blot 2: Loading orientation (left to right): NHP; Pt 9; 10; 11; 7; 6; 12. For Blot 3: (CRP purified from DIC patient plasma) - Loading orientation (left to 10 right; ng CRP loaded): 3.91; 7.81; 15.625; 31.25; 62.5; 125; 250. The Blots were blocked with 2% (w/v) BSA in PBS, pH 7.4 and then sequentially probed with rabbit anti-human CRP-IgG (Sigma, Cat# C3527, dil 1:5000 in PBS/0.01% Tween 20) and then treated with the same 15 antibody conjugated to HRP (dil 1:25000 in PBS/0.01% Tween 20).

Fig. 23 illustrates the turbidity changes upon 20 adding divalent calcium to materials obtained upon Q- sepharose chromatography in the absence of plasma. No single peak gave a positive response, but a mixture of peak 1 and peak 3 materials did give a positive response indicating the involvement of CRP, a 300 kD protein, and 25 one or more other proteins in the precipitate (peak 3 +

plasma was the control). Fig. 24 is a table showing CRP,  $\mu\text{g}/\text{ml}$  determined by ELISA. Delta A405nm is the maximum turbidity change observed when patients' plasma was recalcified on the presence of the thrombin 5 inhibitor PPACK). Fig. 24, therefore, shows that patients with increased absorbance have varying elevated levels of CRP, once again indicating that more than one protein is involved in the precipitate formation.

10        In one embodiment of the invention, the reagent to plasma ratio is varied between multiple tests using a reagent that induces precipitate formation. This variance allows for amplifying the detection of the precipitate formation by optimization of reagent to 15 plasma ratio (e.g. varying plasma or reagent concentrations). In the alternative, the slope due to the precipitate formation can be averaged between the multiple tests. As can be seen in Fig. 25, the response to increasing calcium concentrations is shown in optical 20 transmission waveform profiles. The left panels show two normal patients where calcium concentrations were varied (no clotting agents used), whereas the right panels show two patients with haemostatic dysfuntion (DIC in these two cases) where the metal cation 25 (calcium) concentration was varied (the calcium alone being incapable of any substantial fibrin

polymerization).

Though precipitate formation is capable of being detected in patients with haemostatic dysfunction when a clotting agent is used, it is beneficial that the reagent used is capable of forming the precipitate without fibrin polymerization. As can be seen in Fig. 26, the slope is more pronounced and more easily detectable when a reagent such as calcium chloride is used (right panel) as compared to when a clotting reagent such as an APTT reagent (left panel) is used. As can be seen in Fig. 27, when a clot inhibitor was added (in this case heparin), all parameters including slope\_1 gave good results, and slope\_1 showed the best sensitivity. For the above reasons, a reagent capable of precipitate formation in the absence of fibrin polymerization and/or a clot inhibitor are preferred.

As can be seen in Fig. 28, CRP levels from 56 ITU patients were plotted against transmittance at 18 seconds. The dotted line is the cut-off for an abnormal transmittance at 18 seconds. Fig. 29 shows more samples with CRP and decrease in transmittance at 18 seconds (10000 - TR18). These figures indicate that 25 patients with abnormal transmittance levels due to

precipitate formation all have increased levels of CRP.

However, not all patients with increased levels of CRP have abnormal transmittance levels thus indicating that more than CRP is involved in the precipitate.

5

In a further embodiment of the invention, the formation of the precipitate comprising a complex of proteins including CRP is detected and/or quantitated, by the use of a latex agglutination assay. In this 10 method, antibodies are raised against either the 300 kD protein or CRP. Whether monoclonal or polyclonal antibodies are used, they are bound to suitable latex and reacted with a patient test sample or preferably with the precipitate itself having been separated from 15 the rest of the patient plasma, in accordance with known methods. The amount of agglutination of the latex is proportional to the amount of the CRP complex in the sample.

20 Alternatively, immunoassays can be performed, such as ELISA's, according to known methods (sandwich, competition or other ELISA) in which the existence and/or amount of the complex of proteins is determined.

For example, an antibody bound to solid phase binds to 25 CRP in the CRP protein complex. Then, a second labeled

antibody is added which also binds to CRP in the CRP protein complex, thus detecting the complex of proteins. In the alternative, the second labeled antibody can be specific for the 300 kD protein in the complex. Or, in 5 a different assay, the antibody bound to solid phase can bind to the 300 kD protein in the complex, with the second (labeled) antibody binding either to the 300 kD protein or to CRP. Such immunoassays could likewise be adapted to be specific for SAA, where antibodies, bound 10 and labeled, bind to SAA, or where one antibody binds to SAA and the other either to CRP or the 300 kD protein. The above techniques are well known to those of ordinary skill in the art and are outlined in Antibodies, A Laboratory Manual, Harlow, Ed and Lane, David, Cold 15 Spring Harbor Laboratory, 1988, the subject matter of which is incorporated herein by reference.

It is to be understood that the invention described and illustrated herein is to be taken as a 20 preferred example of the same, and that various changes in the methods of the invention may be resorted to, without departing from the spirit of the invention or scope of the claims.

WE CLAIM:

1. A method comprising:
  - a) adding a reagent to a test sample comprising at least a component of a blood sample from a patient;
  - b) measuring the formation of a precipitate due to the reaction of the test sample and the reagent, over time so as to derive a time-dependent measurement profile, said reagent capable of forming a precipitate in the test sample without causing substantial fibrin polymerization.
- 15 2. The method according to claim 1, wherein said reagent comprises a metal ion.
3. The method according to claim 2, wherein said metal ion is a divalent metal ion.
- 20 4. The method according to claim 3, wherein said divalent metal ion is a metal ion from the transition elements.

5. The method according to claim 2, wherein said metal ion comprises one or more of calcium, magnesium, manganese, iron or barium.
- 5 6. The method according to claim 1, wherein a clot inhibitor is provided as part of said reagent or as part of an additional reagent added to said test sample.
- 10 7. The method according to claim 6, wherein said clot inhibitor comprises one or more of hirudin, heparin, PPACK, I2581, and antithrombin.
- 15 8. The method according to claim 1, wherein the formation of said precipitate is correlated to the existence of haemostatic dysfunction in the patient.
- 20 9. The method according to claim 8, wherein the greater the formation of said precipitate, the worse the existence of haemostatic dysfunction in the patient which can be quantified by constructing a reference curve to compare said patient test sample with previous patient
- 25

samples.

10. The method according to claim 1, wherein the time dependent measurement profile is an optical transmission profile, and wherein the greater the decrease in transmission in the test sample, the greater the formation of said precipitate, and the greater the haemostatic dysfunction in the patient.
- 10 11. The method according to claim 1, wherein said precipitate comprises a protein weighing approximately 20 kD.
- 15 12. The method according to claim 11, wherein said protein is insoluble in saline, EDTA and Imidazole, and soluble in 5 molar urea.
- 20 13. The method according to claim 1, wherein said reagent is added to said test sample in the absence of clot inducing reagents.
14. The method according to claim 1, wherein the formation of the precipitate is measured at least once after time\_0.

15. The method according to claim 14, wherein a single endpoint measurement is made of precipitate formation after time\_0.
- 5 16. The method according to claim 1, wherein said reagent is capable of causing precipitate formation completely in the absence of fibrin polymerization.
- 10 17. The method according to claim 10, wherein the amount of fibrin polymerization in the method, if any, causes no change in optical transmittance.
- 15 18. A method for determining whether or not a patient has haemostatic dysfunction, comprising:
  - a) obtaining a blood sample from a patient;
  - 20 b) obtaining plasma from said blood sample;
  - c) adding a reagent capable of inducing the formation of a precipitate in patients with haemostatic dysfunction without causing any substantial
- 25

fibrin polymerization;

- d) taking one or more measurements of a parameter of the sample wherein changes in the sample parameter are capable of correlation to precipitate formation if present;
- e) determining that a patient has haemostatic dysfunction if precipitate formation is detected.

10

19. The method according to claim 18, wherein a plurality of measurements are made after addition of said reagent.

15

20. The method according to claim 18, wherein a single reagent is used prior to taking said measurements.

20

21. The method according to claim 18, wherein said measurements are measurements of optical transmission or absorbance through said sample.

22. The method according to claim 21, wherein said reagent comprises a metal ion.
23. The method according to claim 22, wherein said metal ion comprises one or more of calcium, 5 magnesium, manganese, iron or barium.
24. The method according to claim 18, wherein a clot inhibitor is provided as part of said reagent or as part of an additional reagent 10 added to said test sample.
25. The method according to claim 24, wherein said clot inhibitor comprises one or more of hirudin, heparin, PPACK, I2581 or 15 antithrombin.
26. The method according to claim 18, wherein said one or more measurements are unaffected by 20 clot formation due to lack of fibrin polymerization.
27. The method according to claim 18, wherein the one or more measurements are a plurality of 25 measurements, and wherein a rate of change of

said plurality of measurement is determined, and wherein haemostatic dysfunction is determined based on the determined rate of change.

5

28. The method according to claim 18, wherein said haemostatic dysfunction is DIC.

10 29. A method for determining in a patient sample the presence of a complex of proteins comprising at least one of serum amyloid A and C-reactive protein, comprising:

15 a) obtaining a test sample from a patient;

b) adding an alcohol, a clot inhibitor, and a metal cation;

wherein a precipitate is formed which comprises a complex of proteins including at least one of serum amyloid A and C-reactive protein.

20

30. The method according to claim 29, wherein said patient test sample is a sample of whole blood or a portion thereof.

25

31. The method according to claim 30, wherein said alcohol is methanol or ethanol.

32. The method according to claim 30, wherein said metal ion is a divalent metal cation selected from the group consisting of calcium magnesium, manganese, iron and barium, and wherein said clot inhibitor is selected from the group consisting of hirudin, heparin, 10 PPACK, I2581 and antithrombin.

33. A method comprising:

- a) adding a coagulation reagent to an aliquot of a test sample from a patient;
- b) monitoring the formation of fibrin over time in said test sample by measuring a parameter of said test sample which changes over time due to addition of said coagulation reagent;
- c) determine a rate of change, if any, of said parameter in a period of time prior to formation of fibrin in said test sample;



37. The method according to claim 35, wherein said reagent that causes precipitate formation comprises a clot inhibitor.

5

38. The method according to claim 37, wherein said clot inhibitor is hirudin, heparin, PPACK, I2581 or antithrombin.

10 39. The method according to claim 33, wherein at least one measurement in the test of the second aliquot with the reagent capable of forming a precipitate without causing fibrin polymerization is at a time greater than the 15 clotting time of the first aliquot.

40. A method for monitoring an inflammatory condition in a patient, comprising:

20 a) adding a reagent to a patient test sample, said reagent capable of causing precipitate formation in some patient test samples without causing fibrin polymerization;

25 b) measuring a parameter of said test sample over time which is indicative

of said precipitate formation;

5 c) determining the slope of said changing parameter;

d) repeating steps a) to c) at a later date or time;

wherein an increase or decrease in said slope at said later date or time is indicative of progression or regression, respectively, of said inflammatory

10 condition.

41. The method according to claim 40, wherein a plurality of measurements are taken over time so as to provide the slope of said changing 15 parameter.

42. The method according to claim 40, wherein said reagent comprises a metal divalent cation.

20 43. The method according to claim 42, wherein said reagent comprises calcium, magnesium, manganese, iron or barium.

44. The method according to claim 42, wherein said

reagent further comprises a clot inhibitor.

45. The method according to claim 44, wherein said clot inhibitor is heparin, hirudin, PPACK, I2581 or antithrombin.

5 46. The method according to claim 40, wherein said changing parameter is optical transmission or absorbance.

10

47. The method according to claim 40, wherein said inflammatory condition is one or more of rheumatoid arthritis, sepsis, or a condition due to surgery or trauma.

15

48. A method for diagnosing and treating patients with haemostaic dysfunction, comprising:

a) adding a reagent to a test sample that causes precipitate formation without causing fibrin polymerization;

20 b) taking measurements over time of a parameter of the test sample that changes due to the formation of the precipitate;

25

- c) determining the rate of change of said parameter;
- d) determining that a patient has haemostatic dysfunction if said rate of change is beyond a predetermined limit;
- e) intervening with treatment for said haemostatic dysfunction if said rate of change is beyond the predetermined limit.

10

49. The method according to claim 48, wherein said treatment includes administration of antibiotics and/or clot inhibitors.

15

50. The method according to claim 48, wherein said treatment comprises identifying and correcting the underlying cause of said haemostatic dysfunction.

20

51. The method according to claim 50, wherein said treatment comprises the administration of a broad spectrum antibiotic, evacuation of the uterus in abruptio placentae, blood replacement therapy, administration of

25

platelet concentrates to correct thrombocytopenia, administration of fresh plasma, administration of one or more blood factors, and/or treatment with interleukin-1.

5

52. The method according to claim 48, wherein said haemostatic dysfunction is DIC or haemostatic dysfunction with the potential to lead to DIC.

10

53. The method according to claim 48, further comprising repeating steps a) to d) at one or more later times or dates, comparing the later one or more rates of change of the parameter with the earlier, and optimizing treatment based on increases or decreases in the rates of change of the parameter.

15

54. The method according to claim 53, wherein said changing parameter is changing optical transmittance through the test sample.

20

55. The method according to claim 54, wherein the test sample is whole blood or a portion thereof.

25

56. The method according to claim 55, wherein said test sample is a plasma sample.
57. The method according to claim 48, wherein said reagent that causes the precipitate formation in the absence of fibrin polymerization comprises a divalent metal cation.
- 10 58. The method according to claim 57, wherein said reagent comprises barium, iron, manganese, magnesium and/or calcium.
- 15 59. The method according to claim 57, wherein said reagent further comprises a clot inhibitor.
60. The method according to claim 59, wherein said clot inhibitor is antithrombin, I2581, PPACK, heparin and/or hirudin.
- 20 61. A method comprising:
  - a) adding a reagent to a patient sample capable of causing formation of a precipitate in said sample;
  - b) monitoring a changing parameter of

said sample over time, said parameter  
    indicative of said precipitate  
    formation;

5           c) determining the rate of change of

    said parameter or whether said  
    parameter exceeds a predetermined  
    limit at a predetermined time;

10           d) repeating steps a) to c) at least  
    once, each time at a different  
    plasma/reagent ratios;

15           e) measuring the maximum, average and/or  
    standard deviation for the  
    measurements in step c); and

          f) determining haemostatic dysfunction  
    based on measurements of step e).

62. The method according to claim 61, further  
    comprising repeating steps a) to e) at a later  
    time or date to monitor disease progression or  
    regression.

63. The method according to claim 61, wherein said  
    haemostatic dysfunction is DIC or haemostatic  
    dysfunction with the potential to lead to DIC.

64. The method according to claim 61, wherein said reagent further comprises a clot inhibitor.

5 65. The method according to claim 64, wherein said reagent comprises a metal cation capable of inducing precipitate formation.

10 66. The method according to claim 65, wherein said reagent comprises calcium, magnesium, manganese, iron or barium as divalent cations.

15 67. The method according to claim 61, wherein said different plasma/reagent ratios are a result of altering the concentration of said reagent from test to test.

20 68. The method according to claim 61, wherein said different plasma/reagent ratios are a result of altering the concentration of said test sample from test to test.

69. The method according to claim 68, wherein said test sample is a plasma sample that is diluted at different ratios from test to test.

70. An immunoassay comprising:

- a) providing a ligand capable of binding to C-reactive protein or the 300 kD protein in lane 5 of Fig. 21;
- b) adding said ligand to a test sample from a patient and allowing binding of said ligand to C-reactive protein or said 300 kD protein in said test sample;
- c) detecting the presence and or amount of C-reactive protein or said 300 kD protein in said sample; and
- d) diagnosing haemostatic dysfunction in the patient due to the detection and/or amount of C-reactive protein or said 300 kD protein detected.

71. The immunoassay of claim 70, wherein said C-

20 reactive protein or said 300 kD protein detected is part of a complex of proteins formed upon addition of a metal divalent cation to the test sample.

25 72. The immunoassay of claim 71, wherein said

complex of proteins comprises both CRP and said 300 kD protein and further comprises serum amyloid A.

5        73. The immunoassay of claim 70, wherein said haemostatic dysfunction diagnosed is DIC or haemostatic dysfunction with the potential to lead to DIC.

10       74. The immunoassay according to claim 71, wherein the diagnosed haemostatic dysfunction has the potential to lead to bleeding or thrombosis.

15       75. The immunoassay of claim 71, wherein said C-reactive protein is modified, cleaved, mutated or whole C-reactive protein.

20       76. A method for testing the efficacy of a new drug on a human or animal subject with an inflammatory condition and/or haemostatic dysfunction, comprising:

25               a) adding a reagent to a patient test sample, said reagent capable of causing precipitate formation in some subject test samples without causing

fibrin polymerization;

b) measuring a parameter of said test sample over time which is indicative of said precipitate formation;

5 c) determining the slope of said changing parameter and/or the value of said parameter at a predetermined time;

d) administering a drug to said animal or human subject;

10 e) repeating steps a) to c) at a later date or time;

wherein an increase or decrease in said slope or value at said later date or time is indicative of the efficacy of said drug.

15

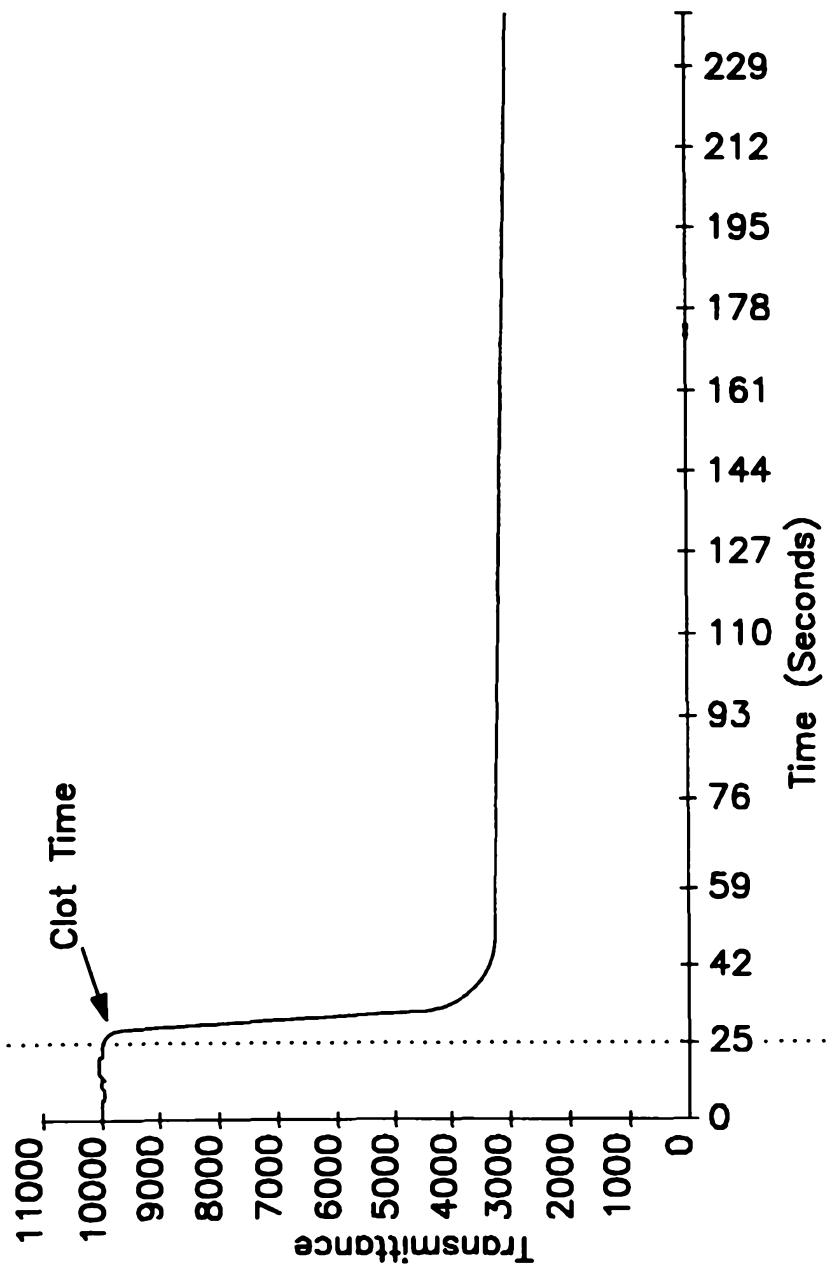
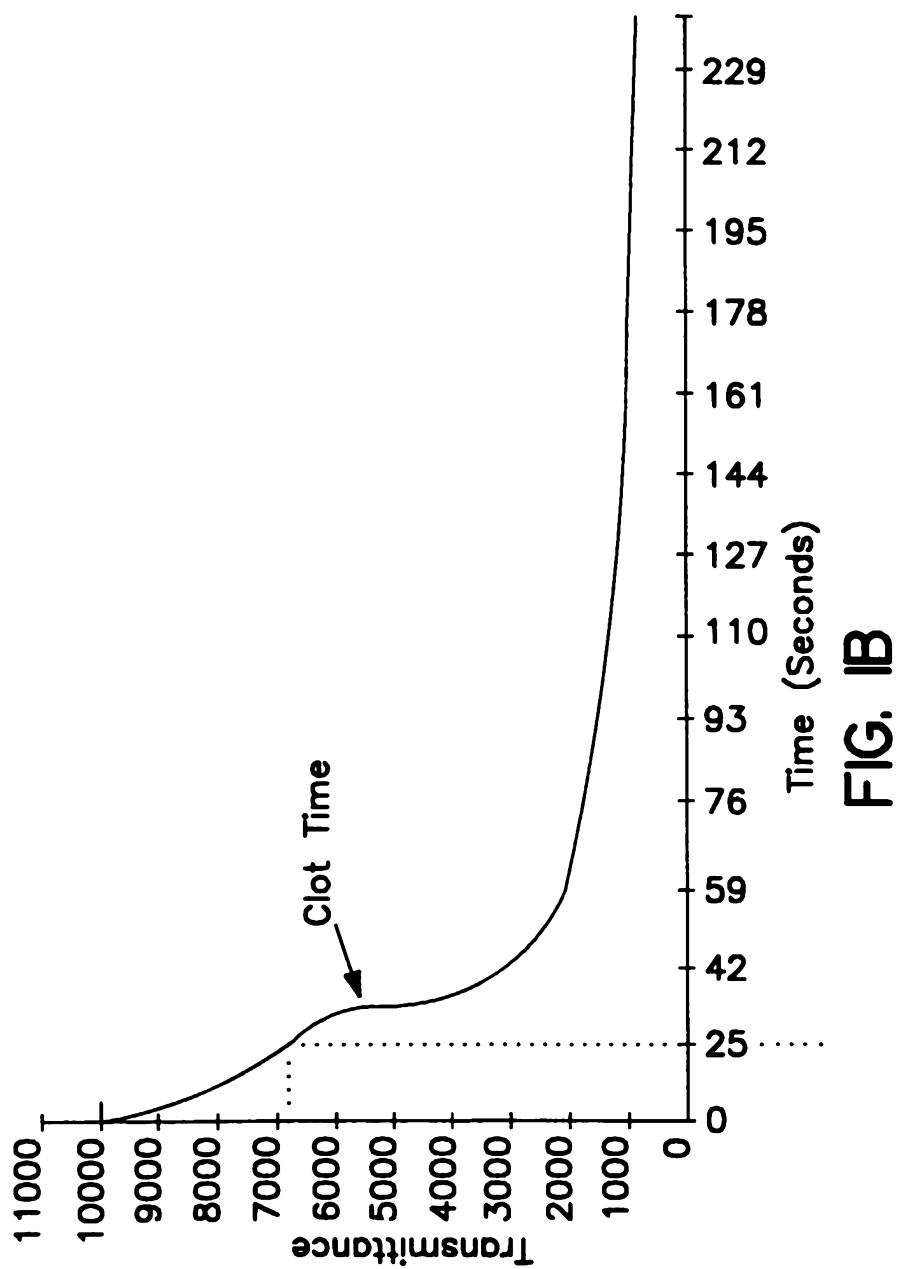


FIG. IA



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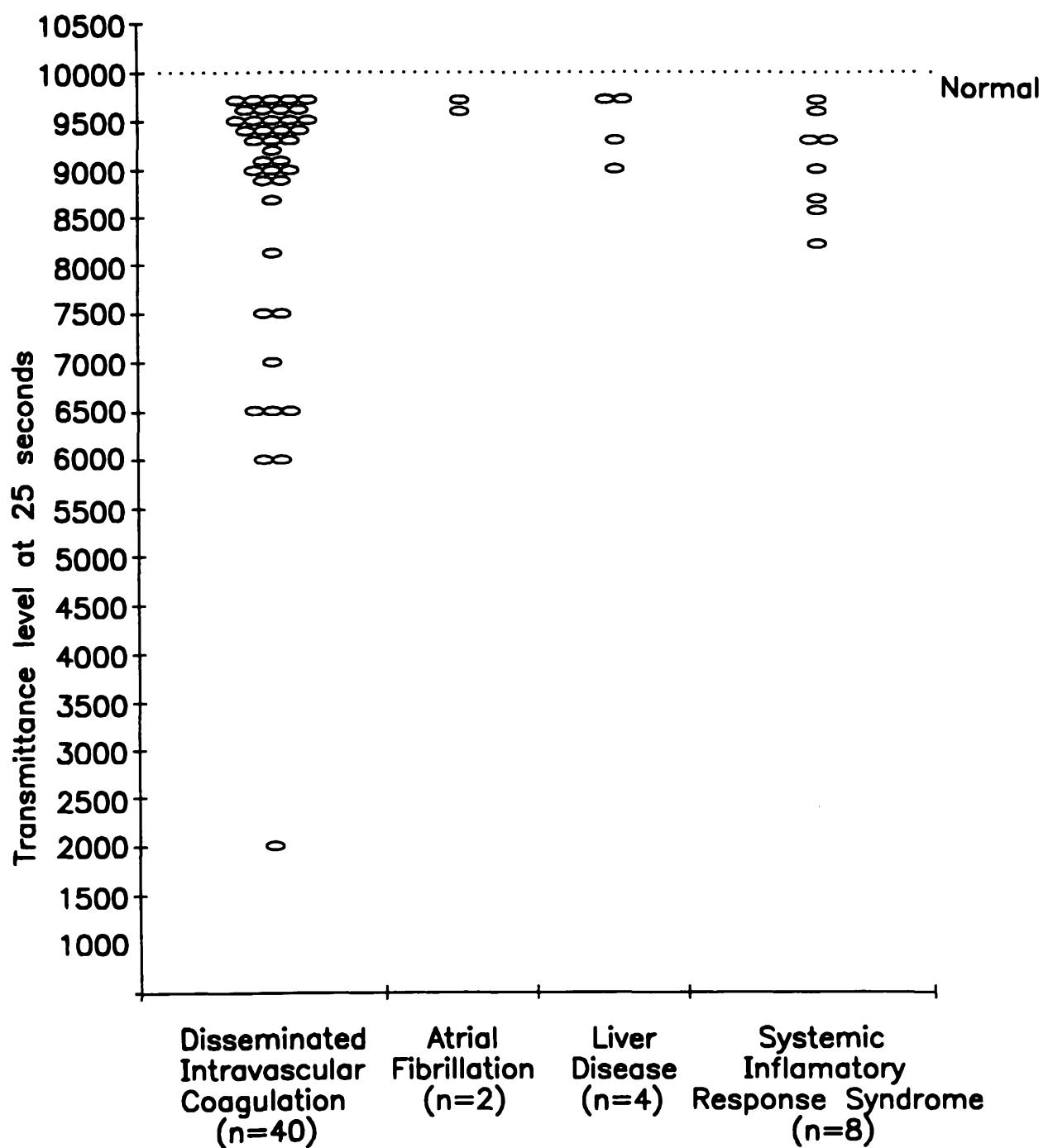
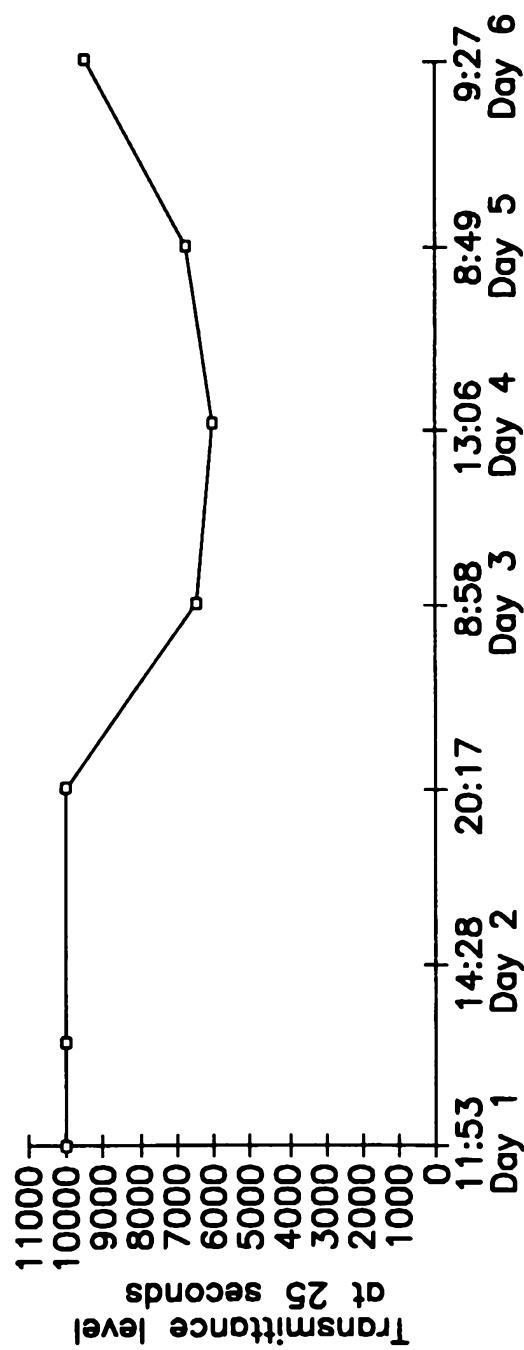


FIG. 2



### FIG. 3A

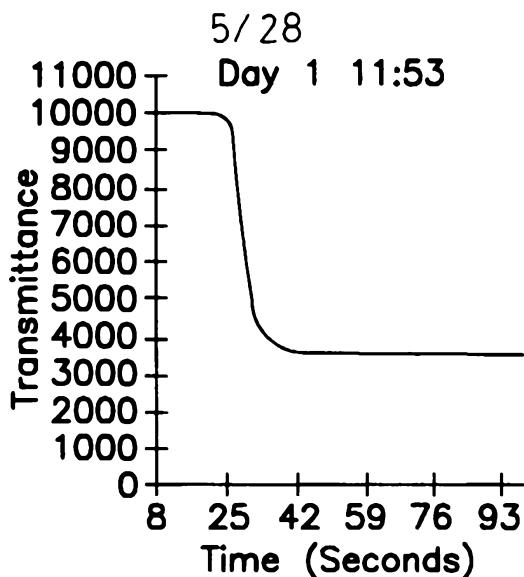


FIG. 3B

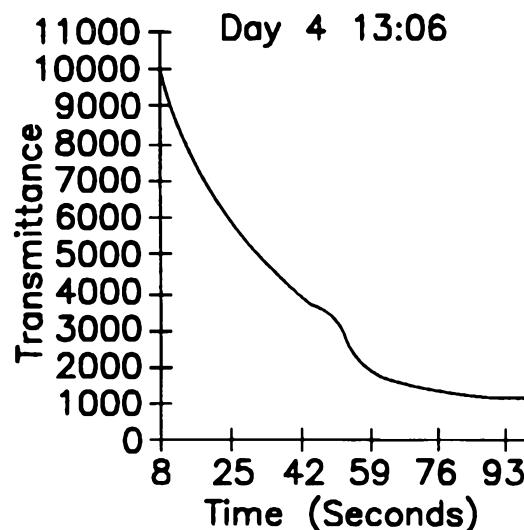


FIG. 3C

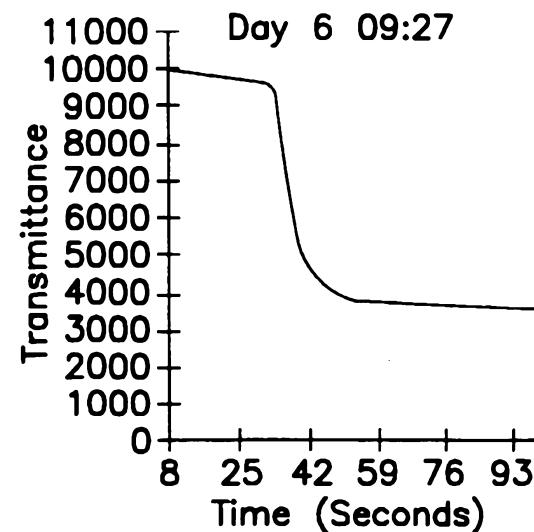


FIG. 3D

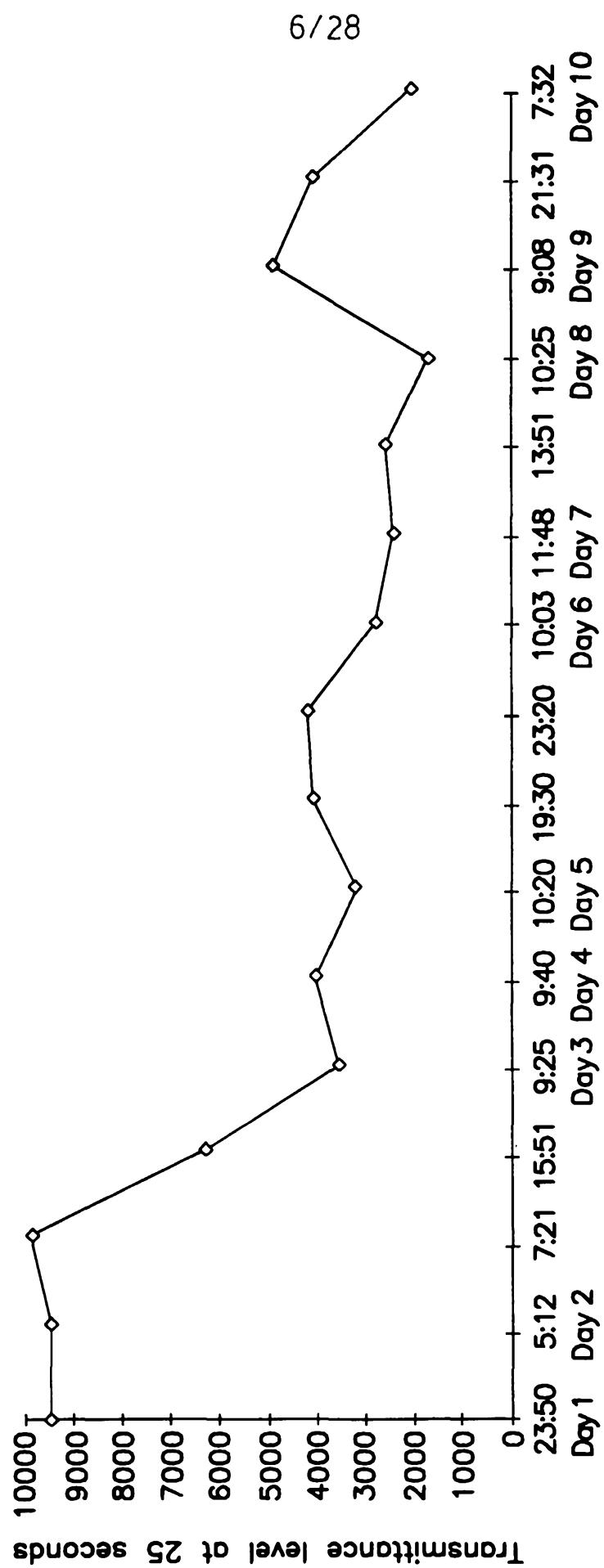


FIG. 4A

FIG. 4B

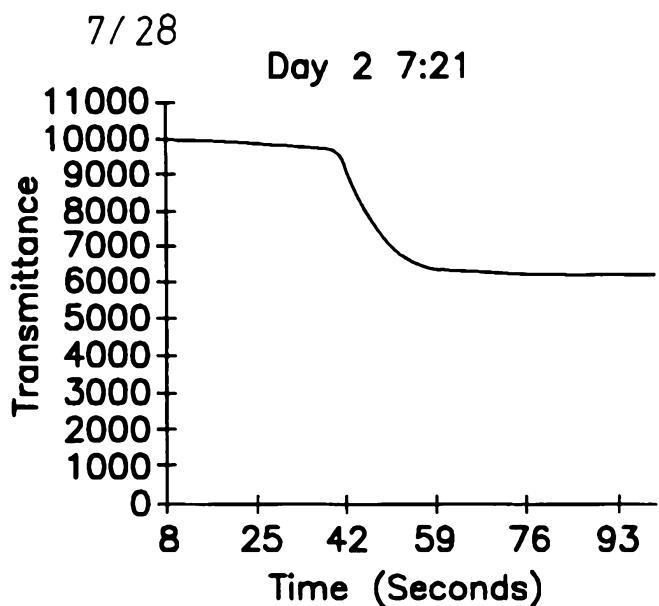


FIG. 4C

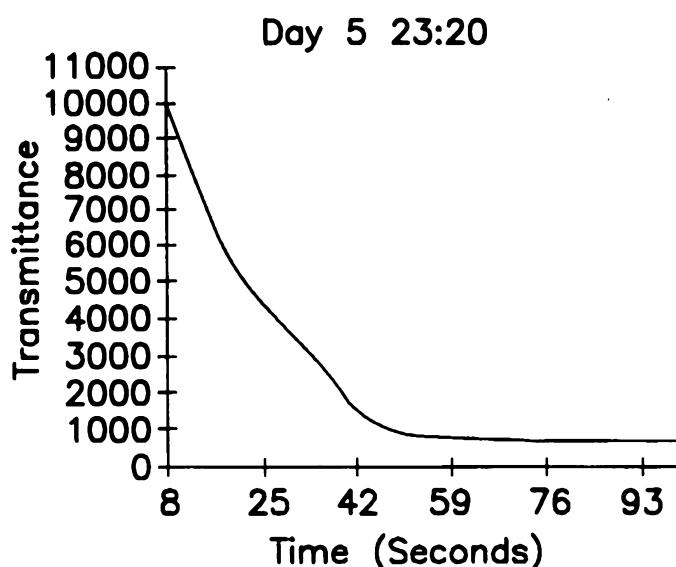
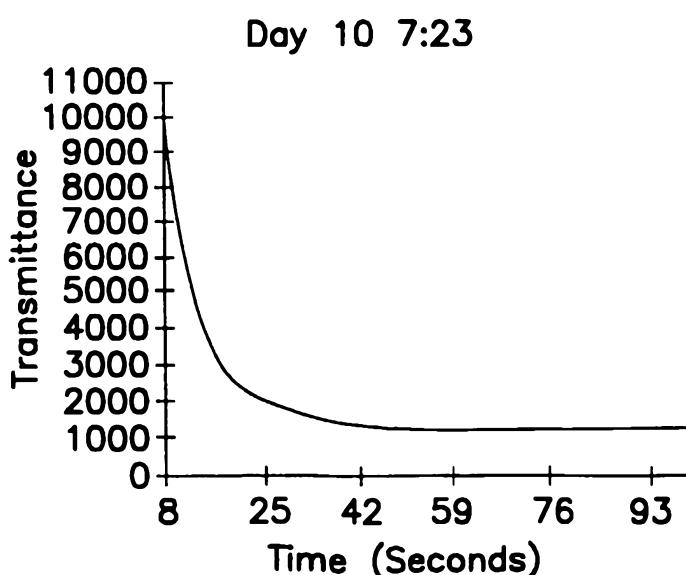


FIG. 4D



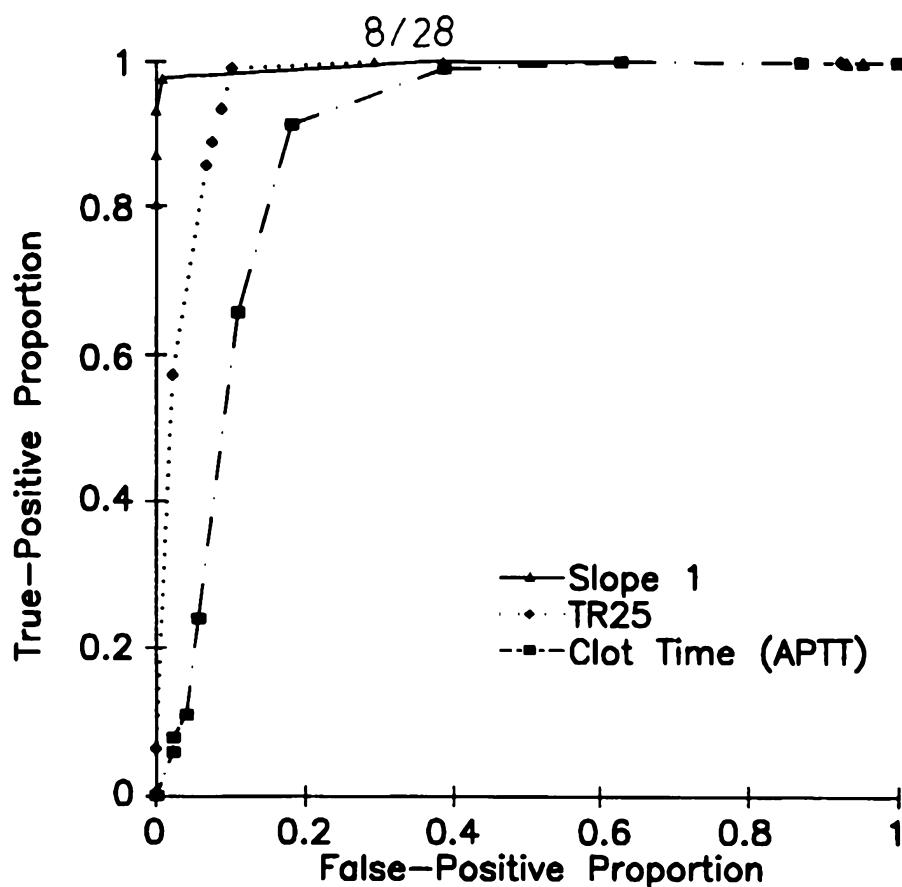


FIG. 5

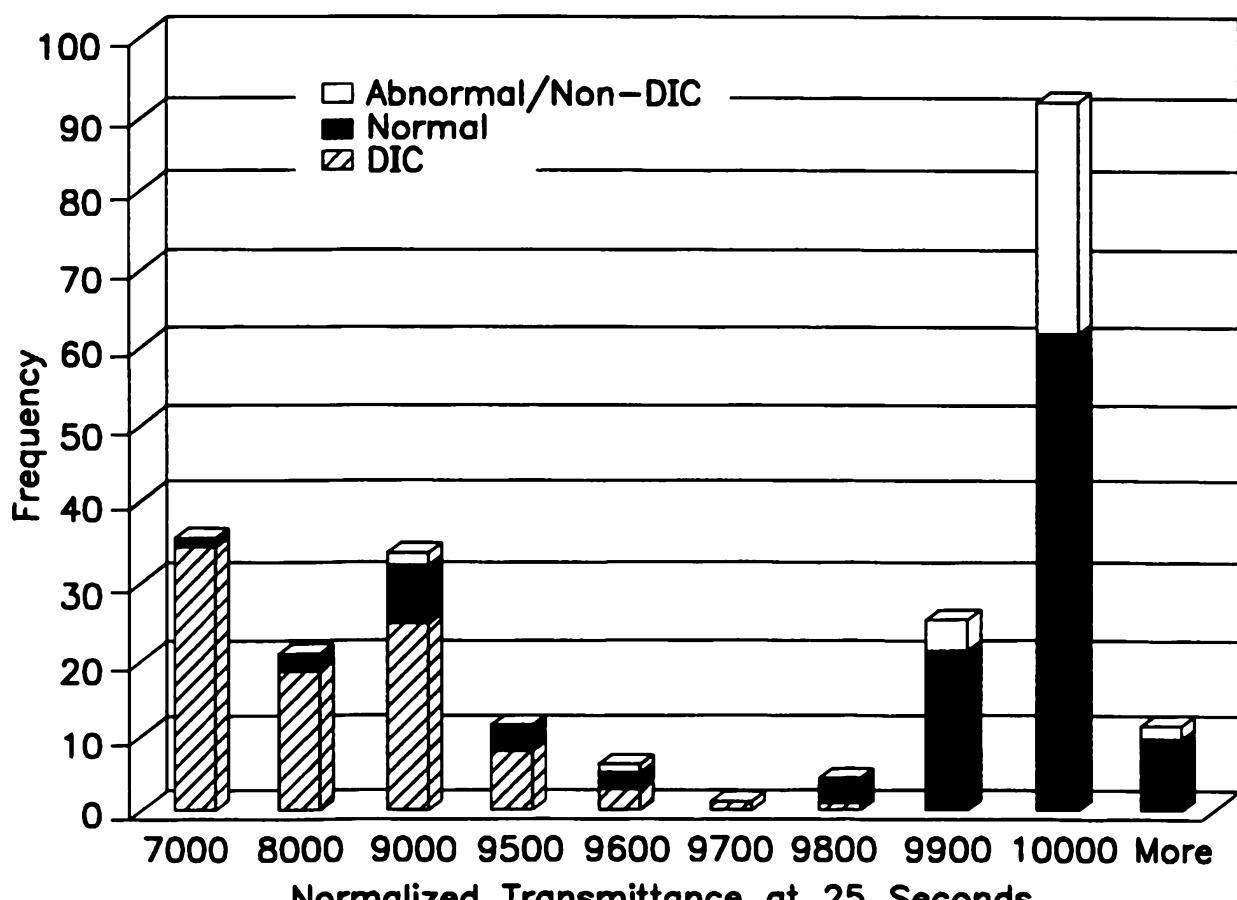


FIG. 6

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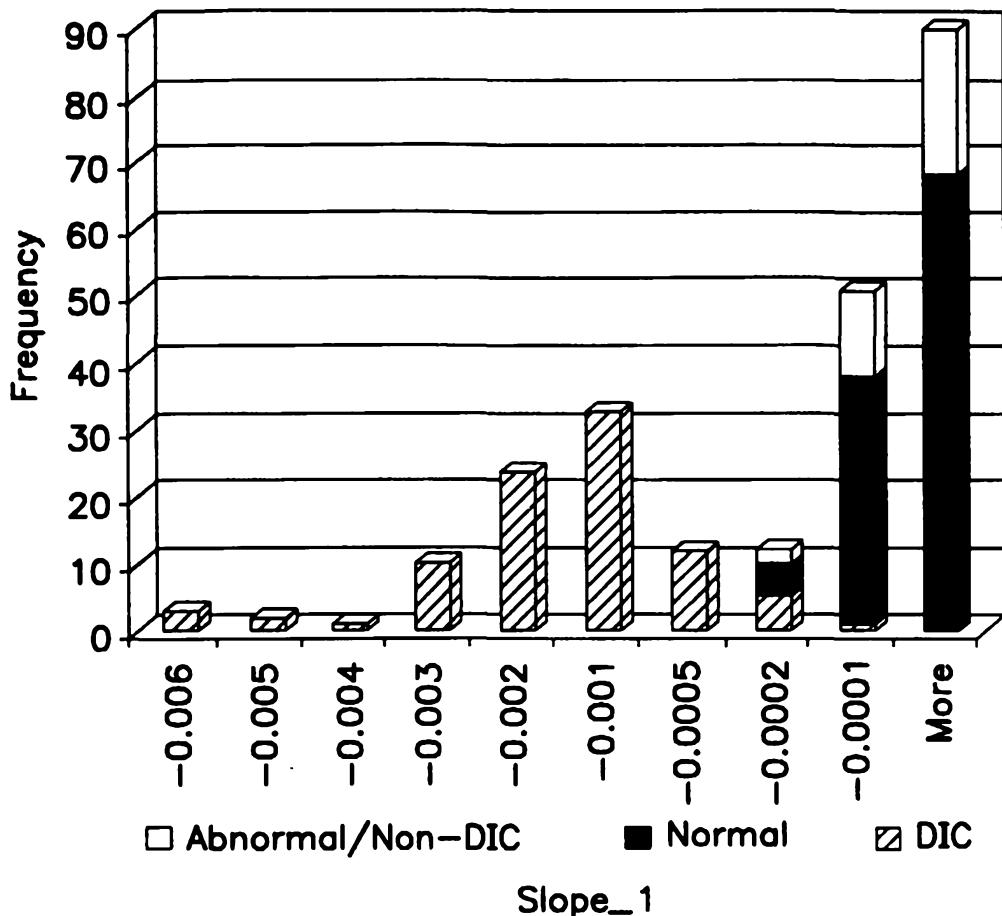


FIG. 7

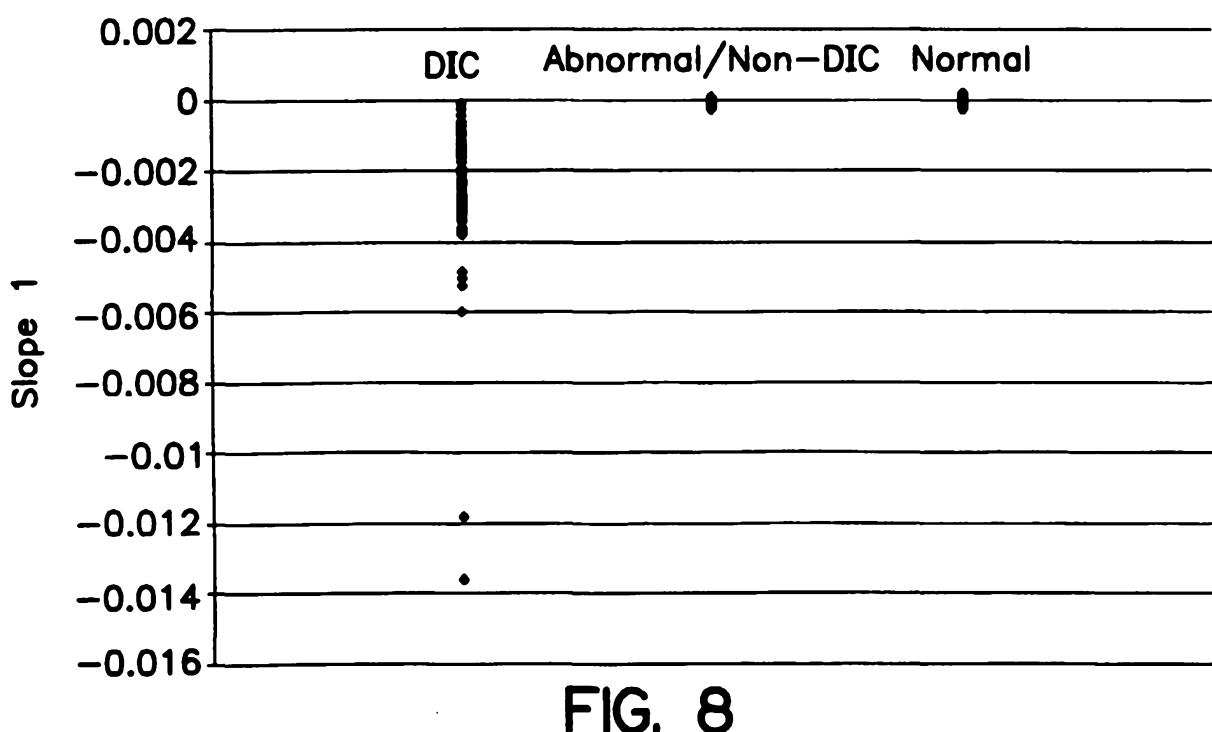


FIG. 8

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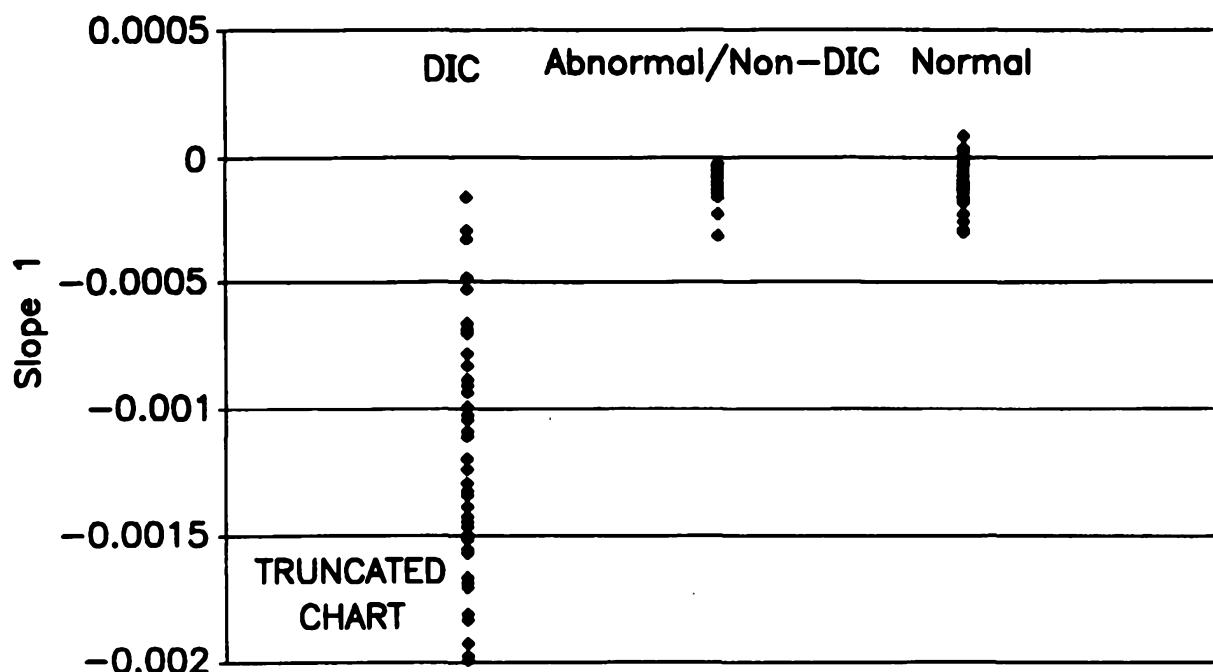


FIG. 9

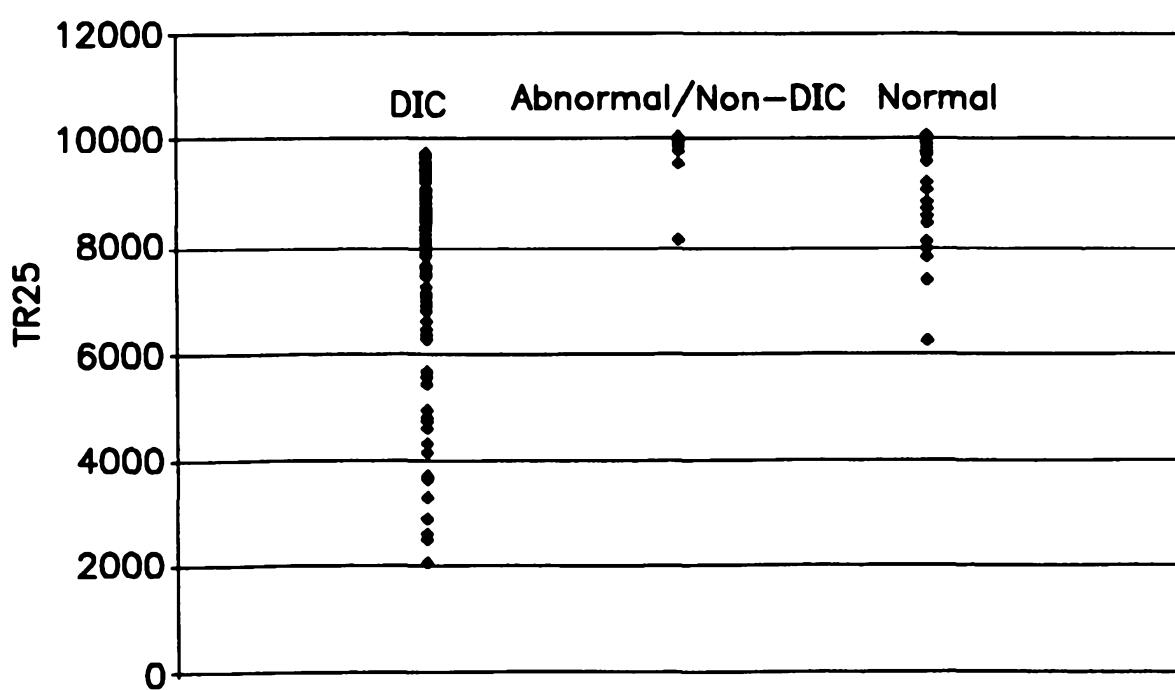


FIG. 10

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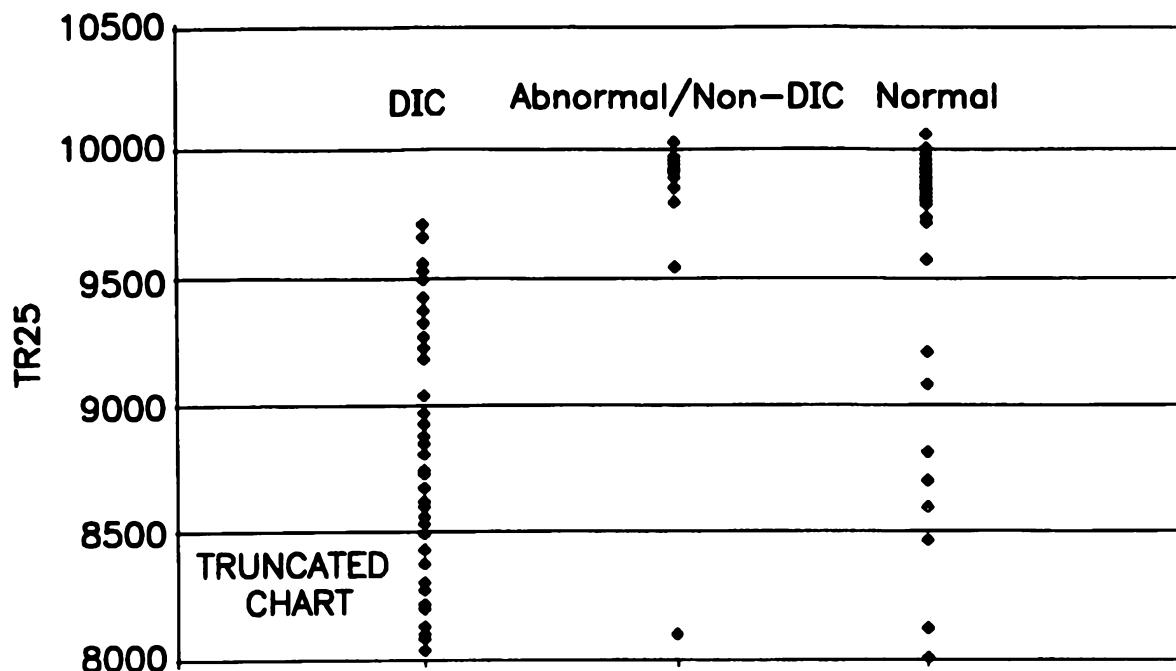


FIG. 11

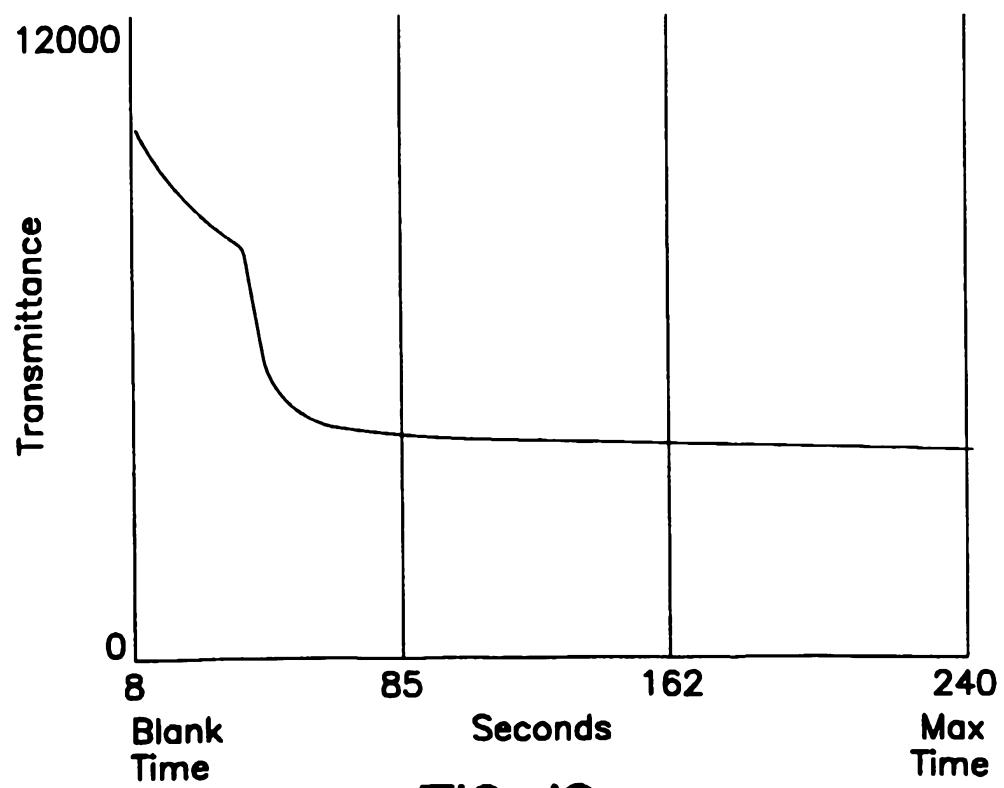


FIG. 12

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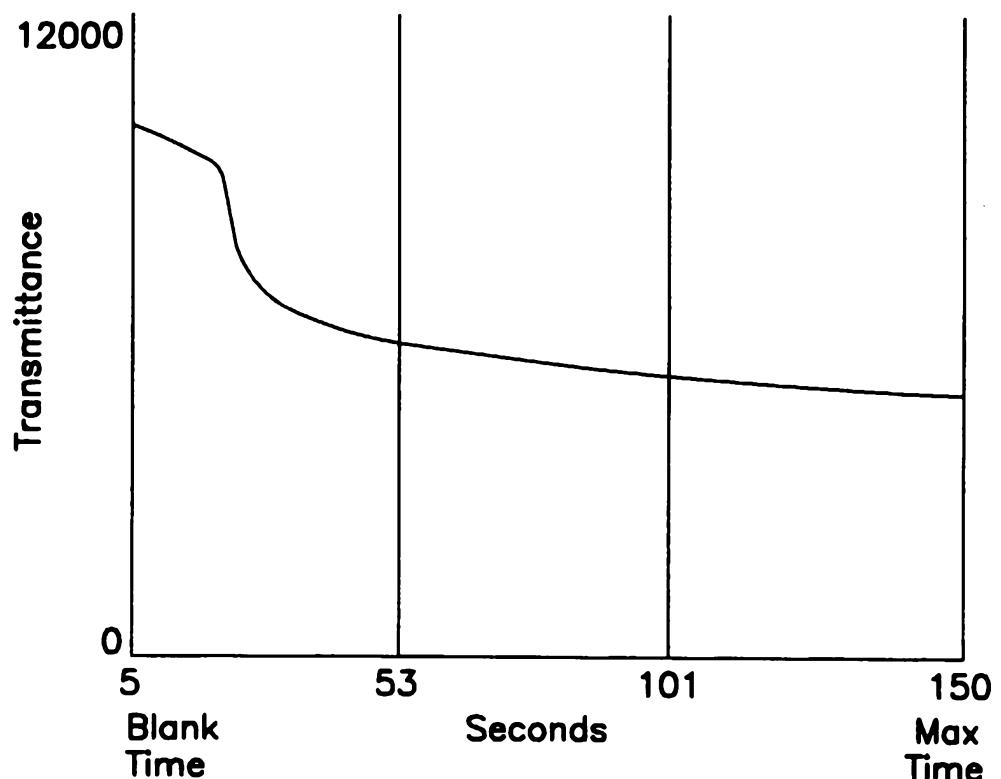


FIG. 13

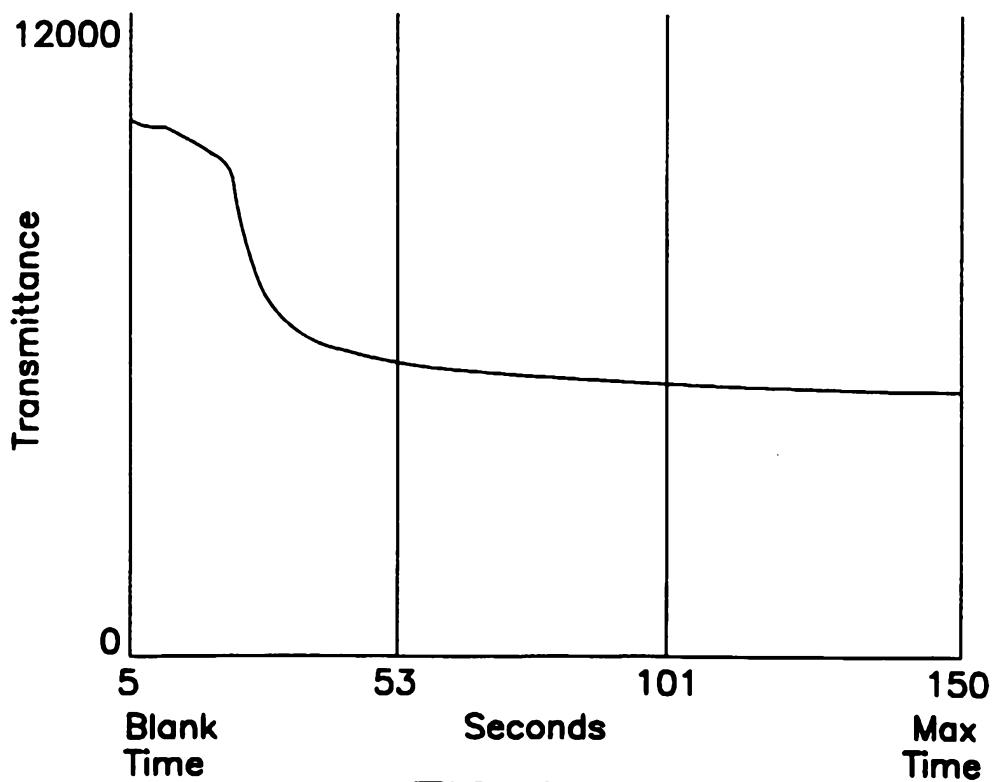


FIG. 14

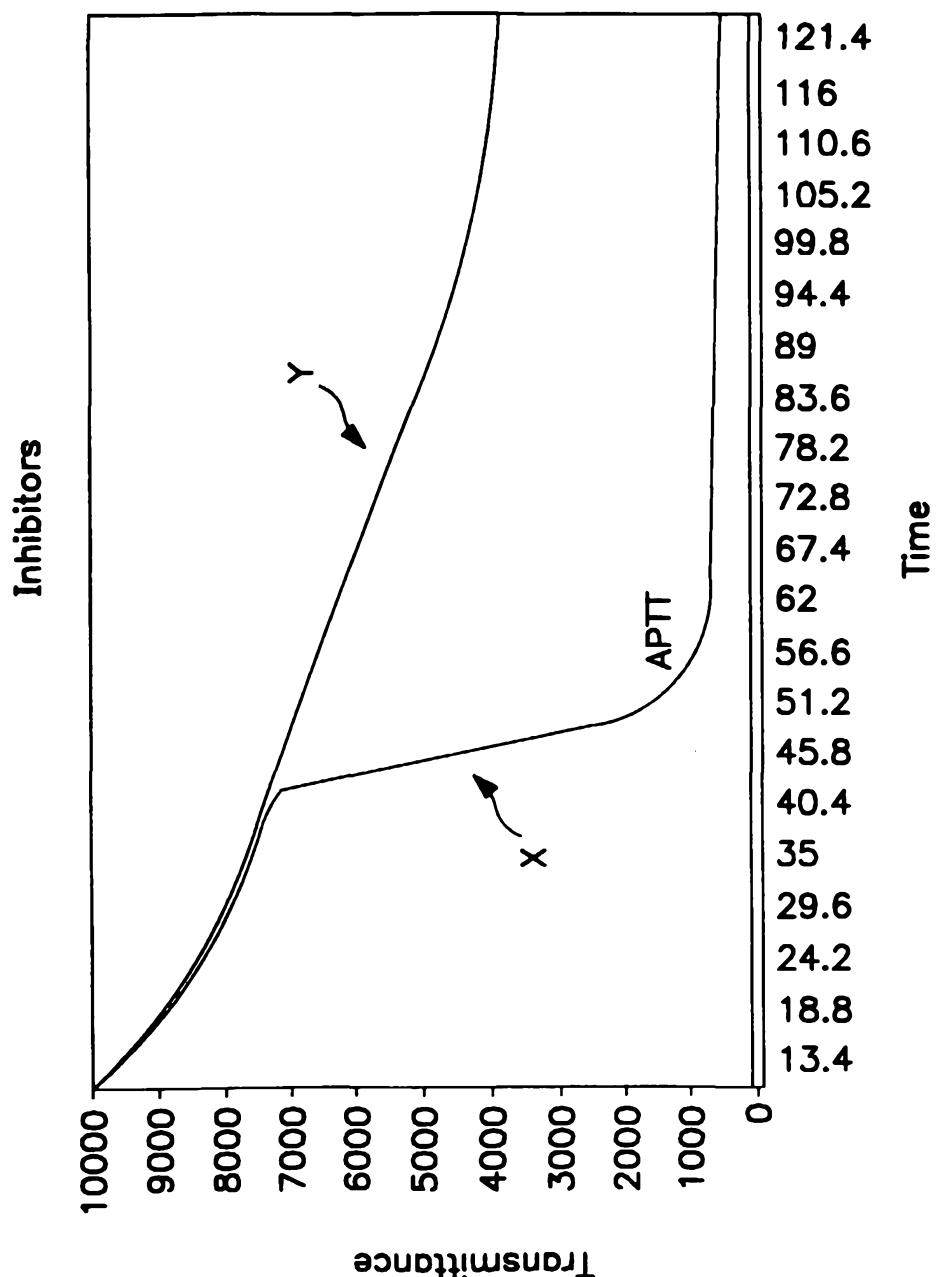


FIG. 15

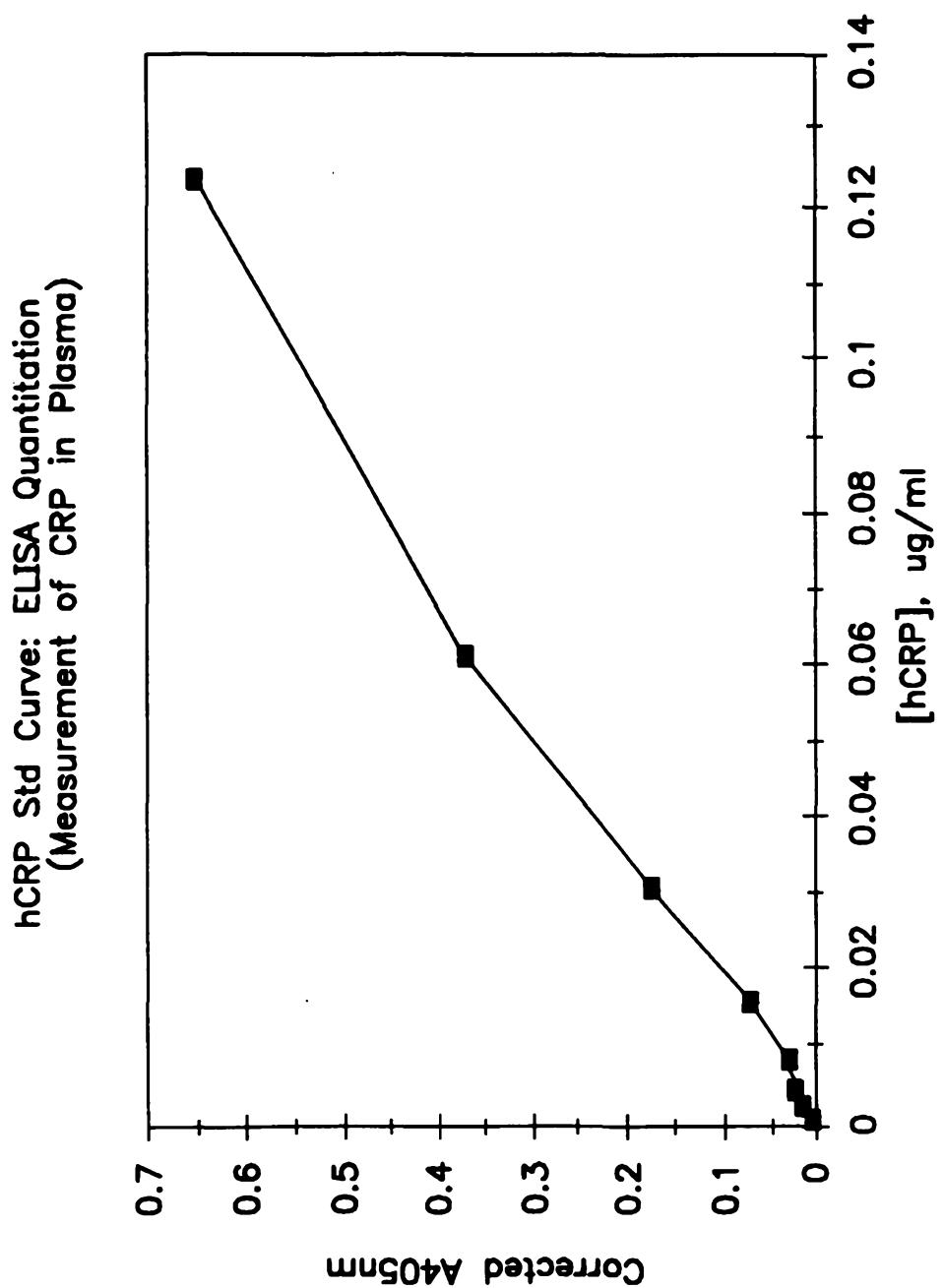


FIG. 16

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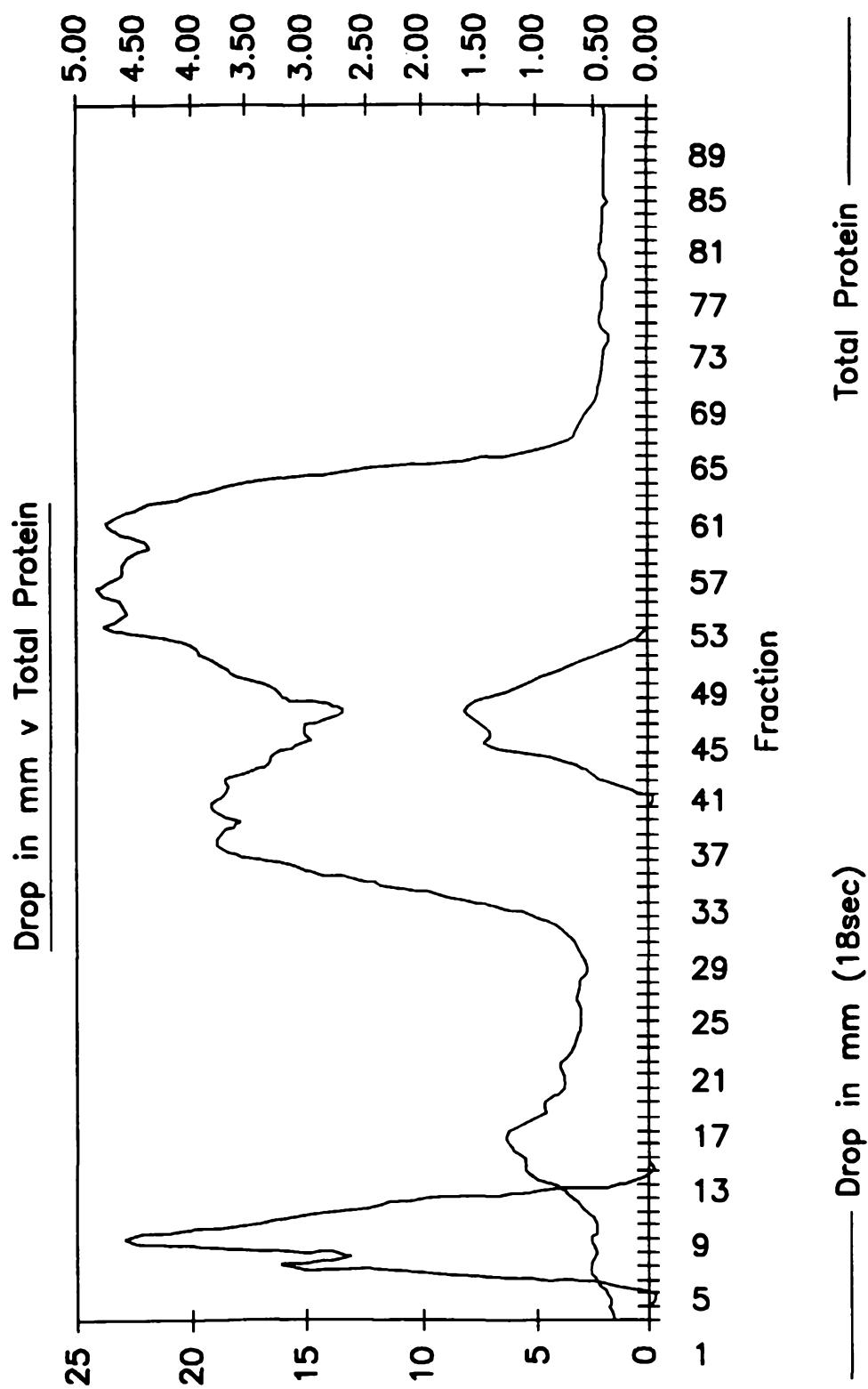


FIG. 17

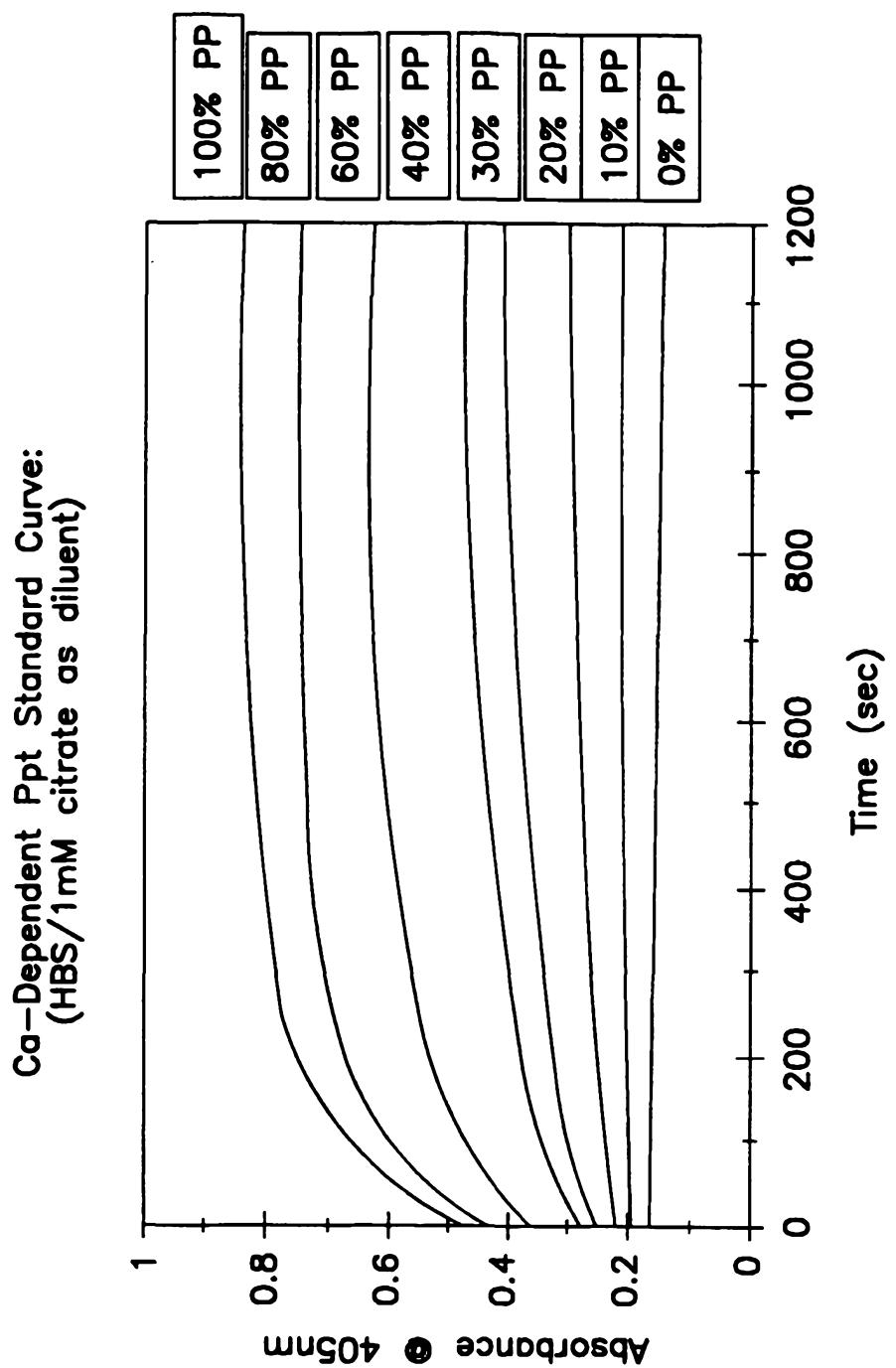


FIG. 18

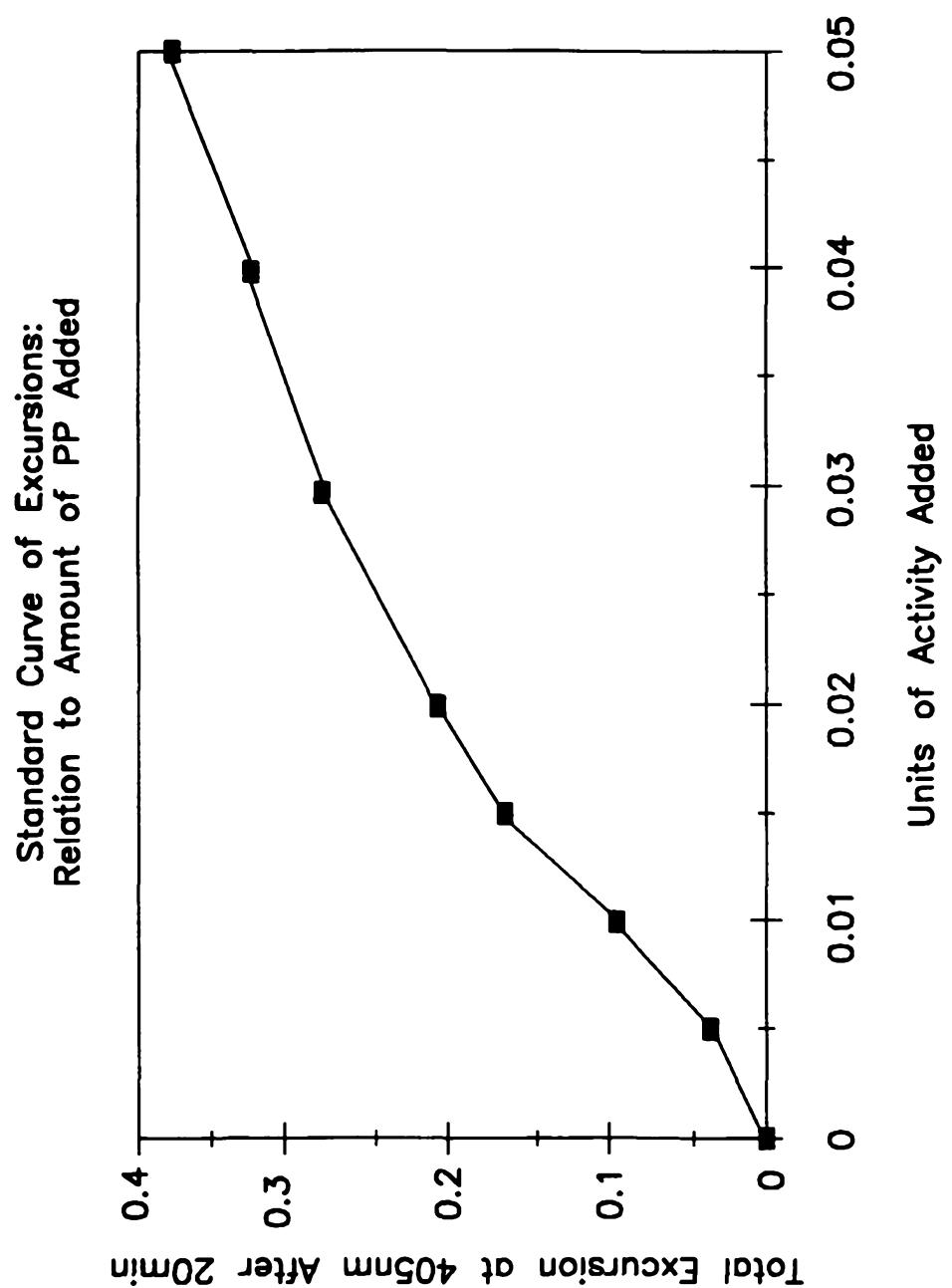


FIG. 19

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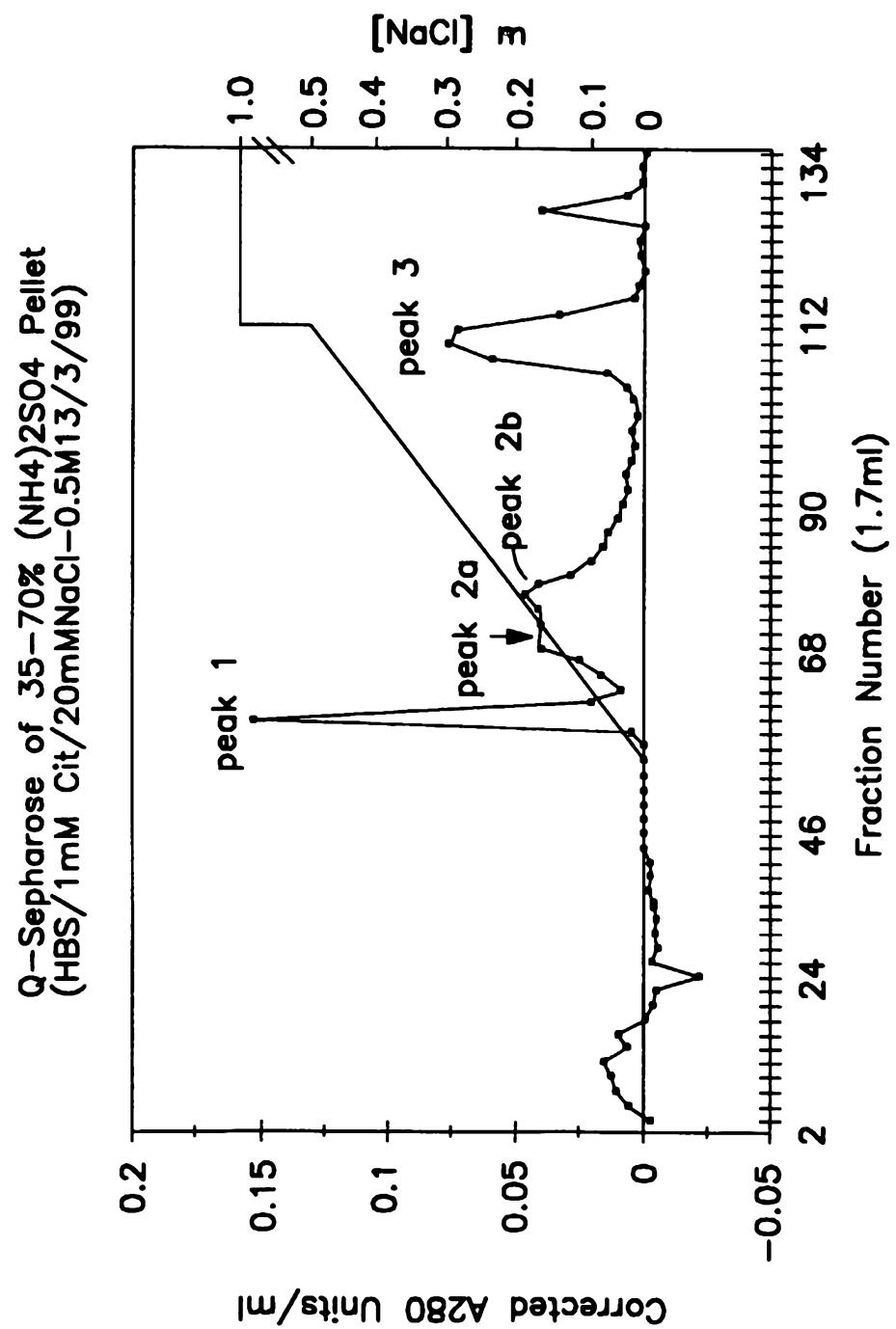


FIG. 20

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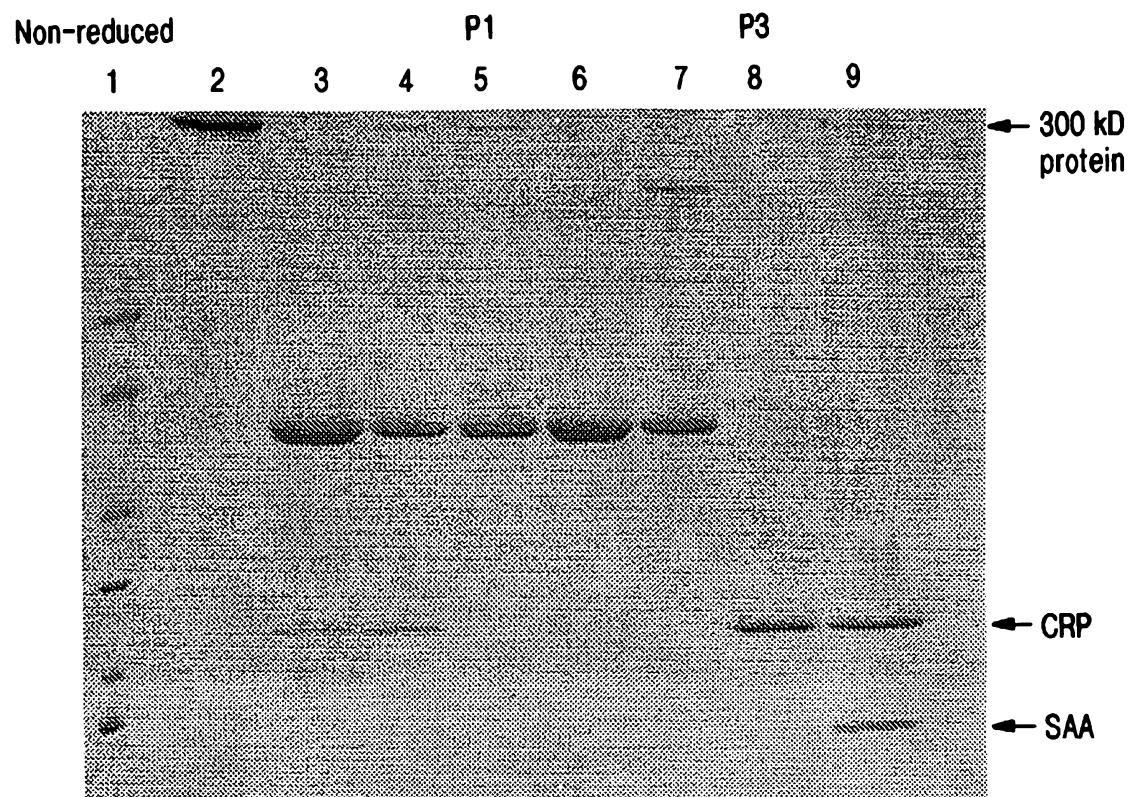


FIG. 2IA

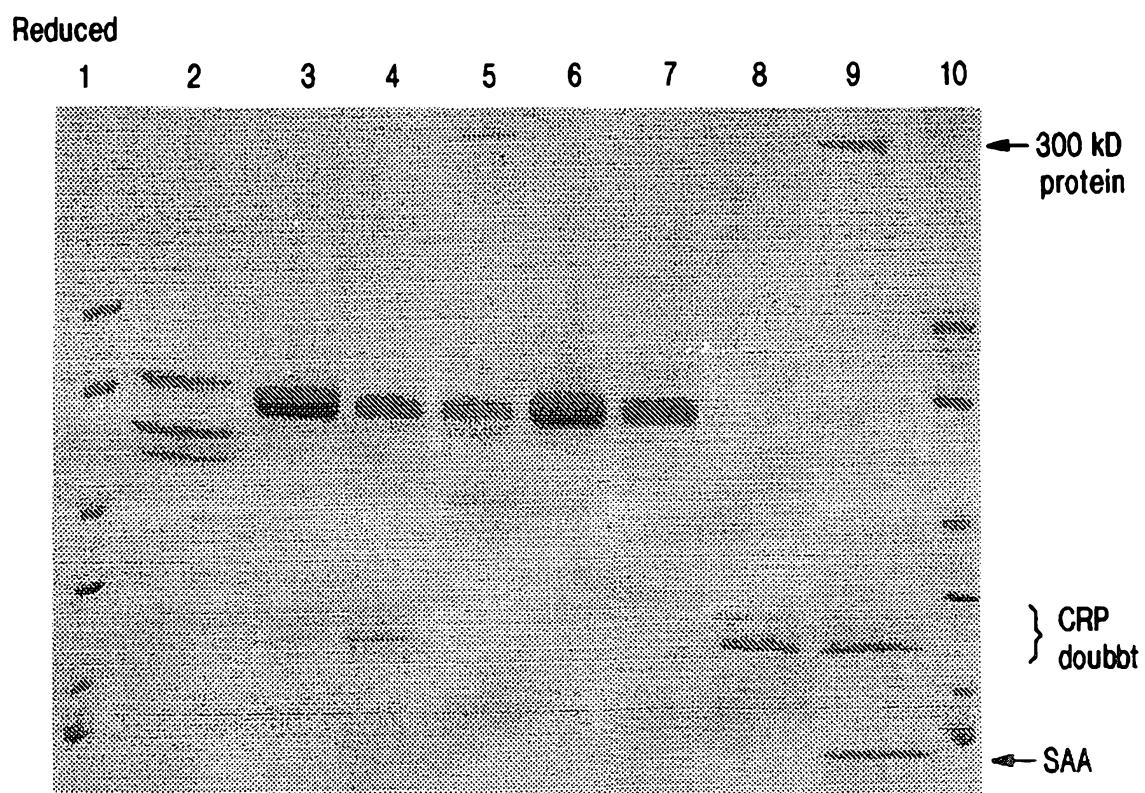


FIG. 2IB

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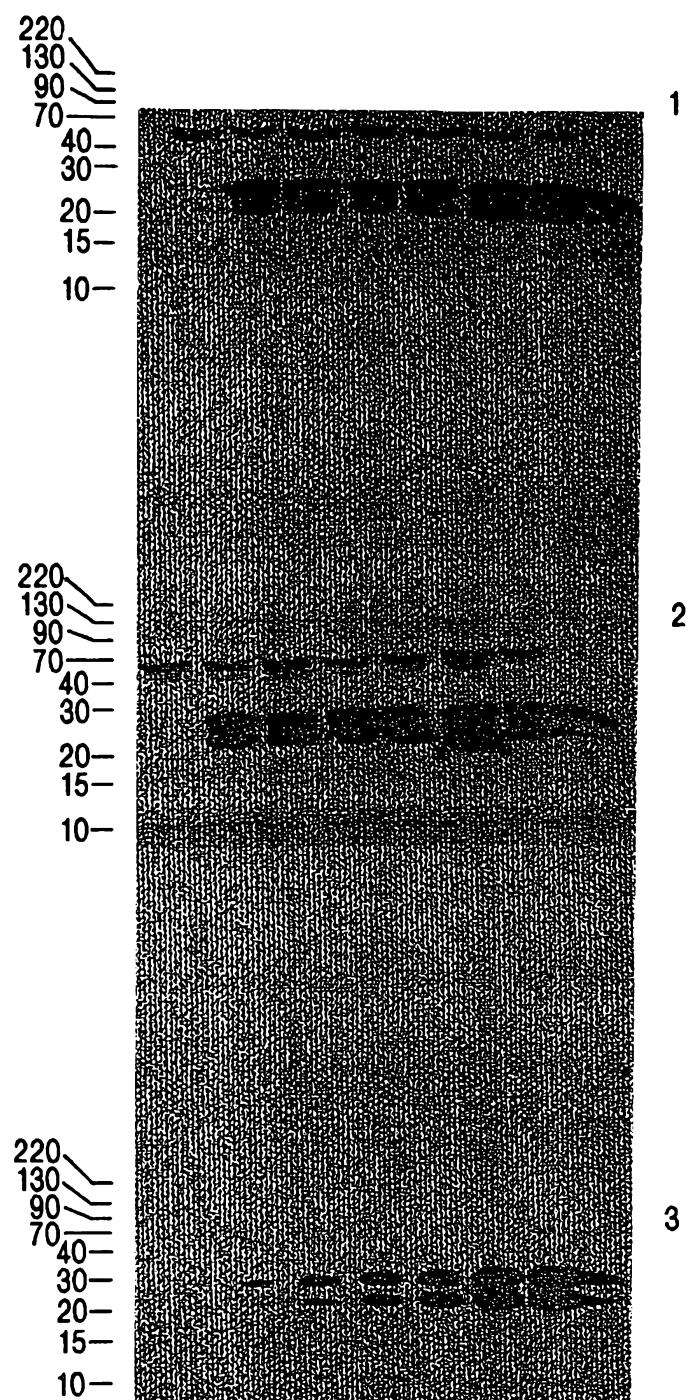


FIG. 22

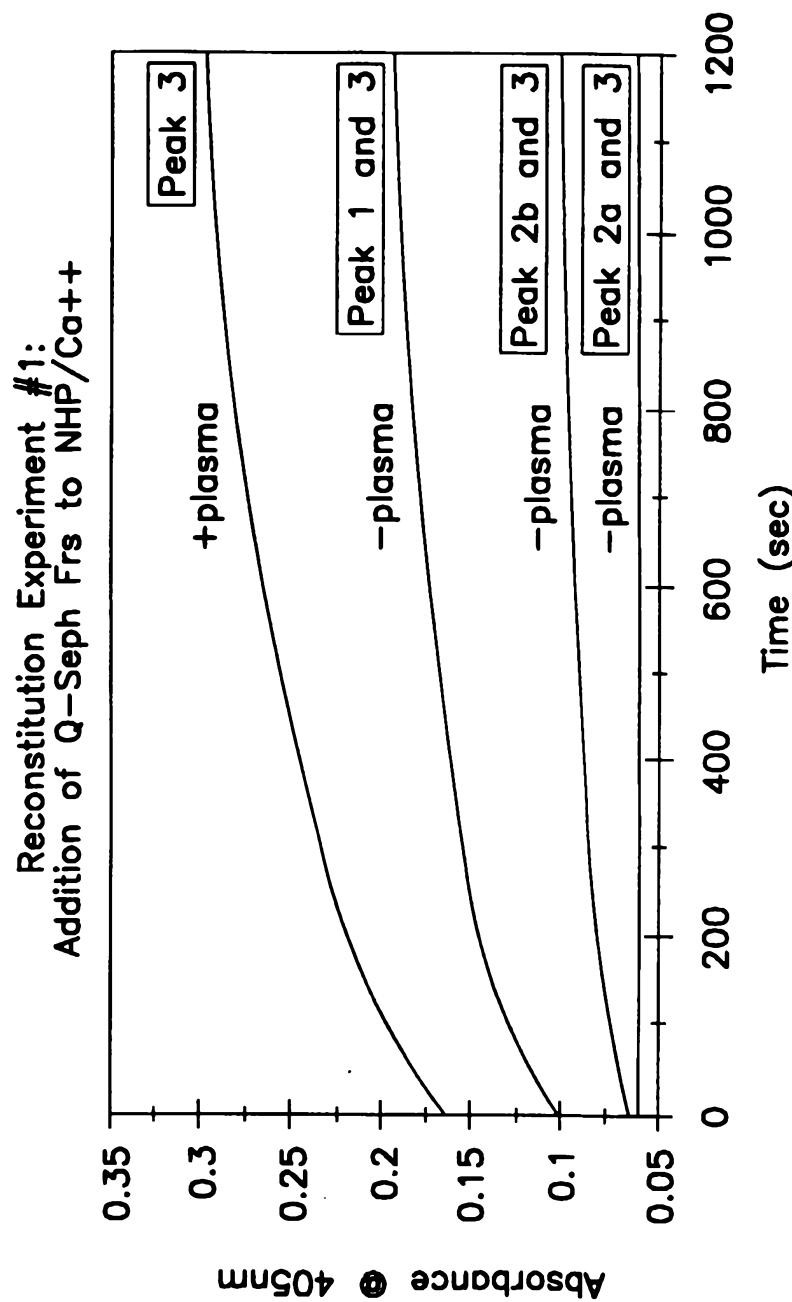


FIG. 23

**Plasms CRP Levels and Calcium-Induced Thurbidity Changes.**

Plasma Sample	[CRP], ug/ml	delta A405nm
Normal Human Pool	0.73	0
Pt#1	248	0.329
Pt#2	277	0.235
Pt#3	319	0.345
Pt#4	443	0.170
Pt#5	478	0.640
Pt#6	492	0.230
Pt#7	528	0.140
Pt#8	576	0.640
Pt#9	600	0.390
Pt#10	639	0.160

**FIG. 24**

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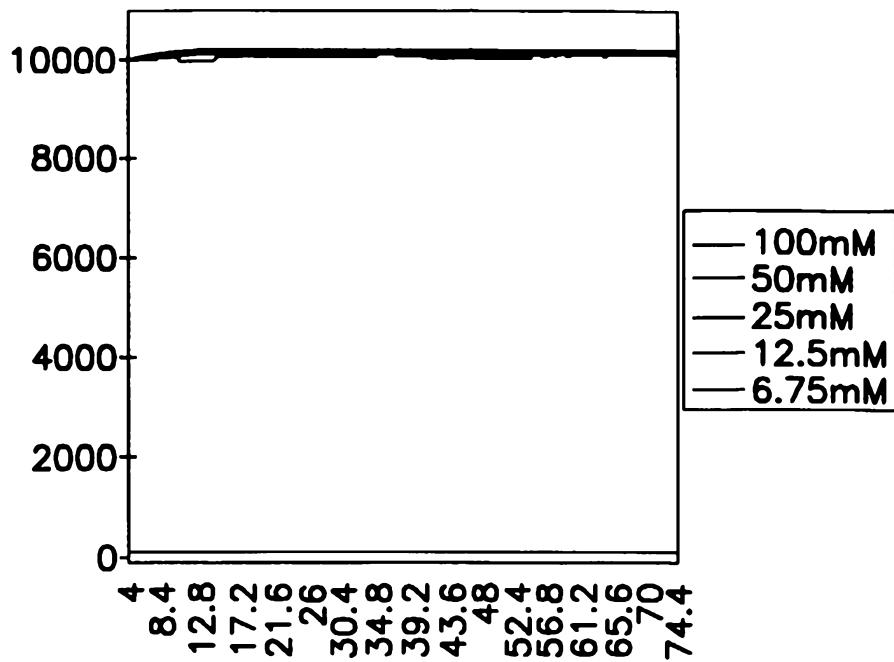


FIG. 25A

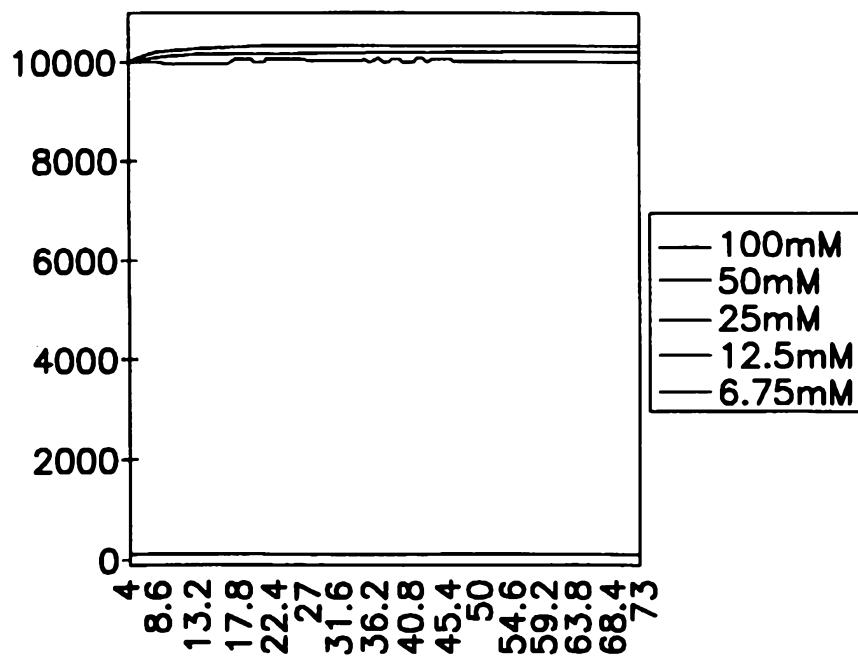


FIG. 25B

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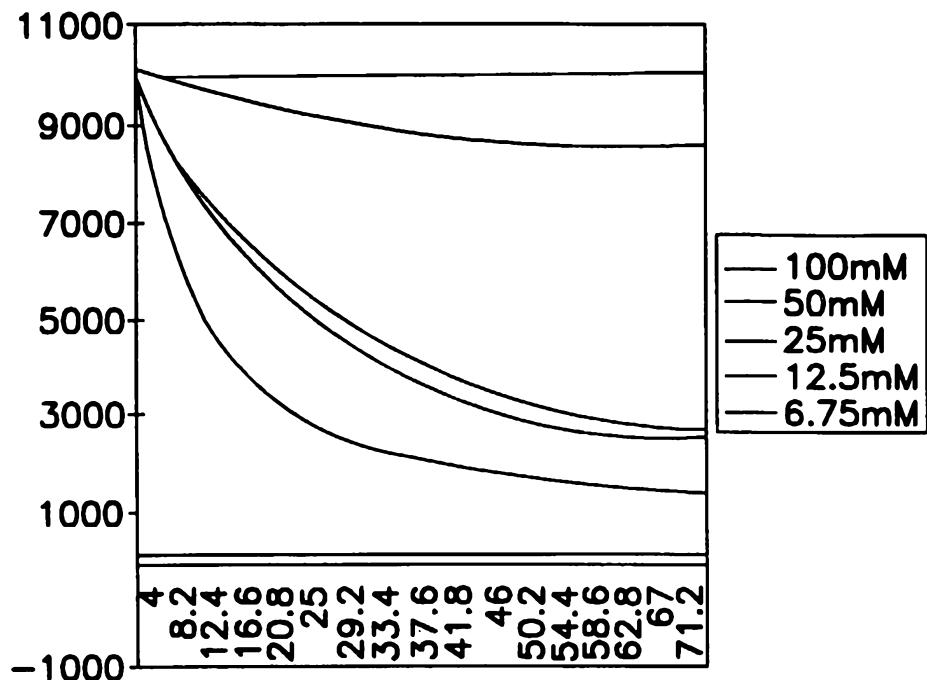


FIG. 25C

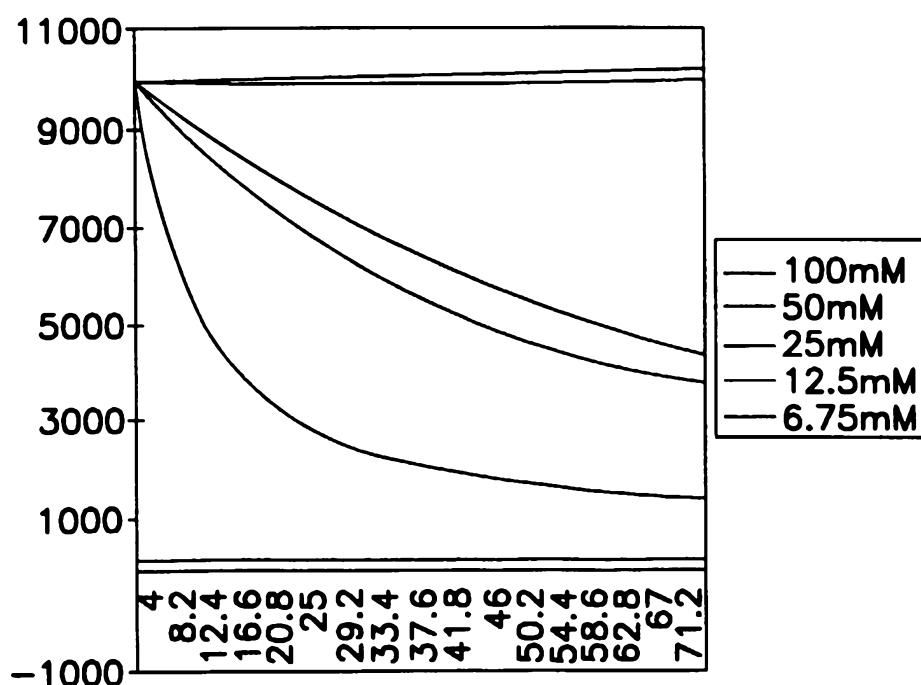


FIG. 25D

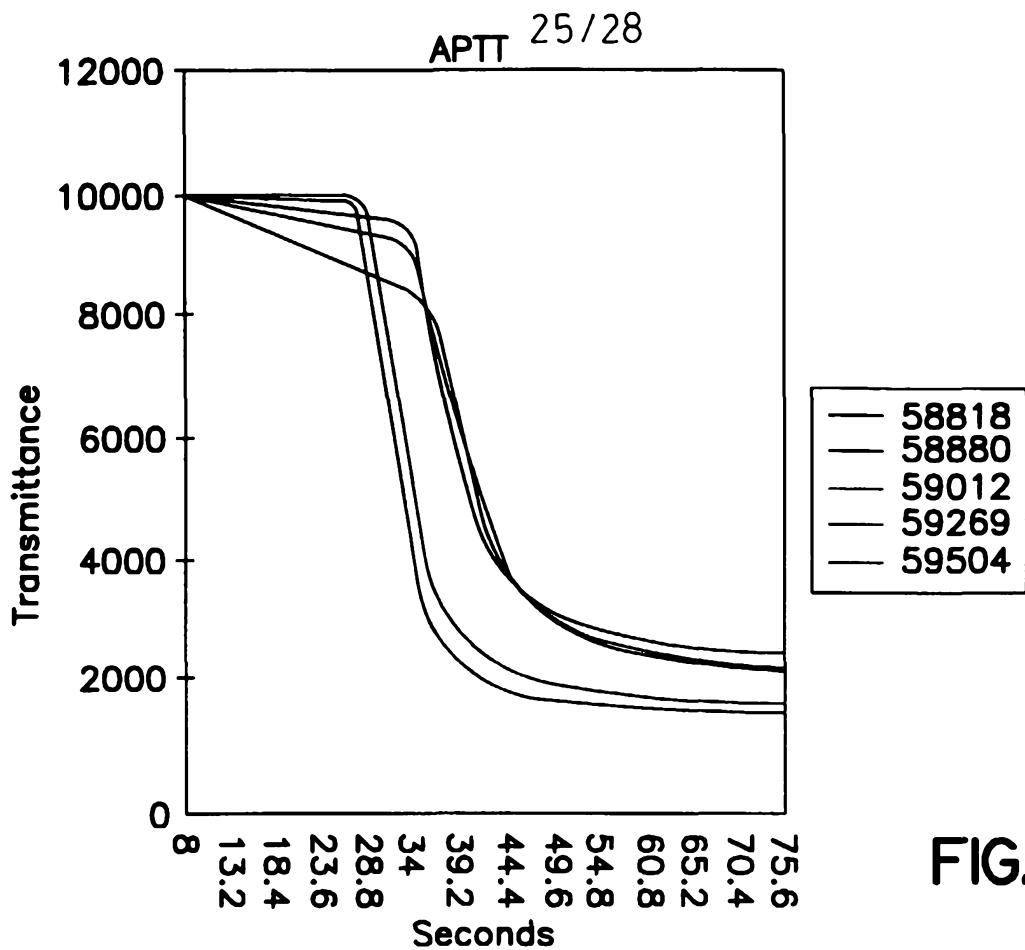


FIG. 26A

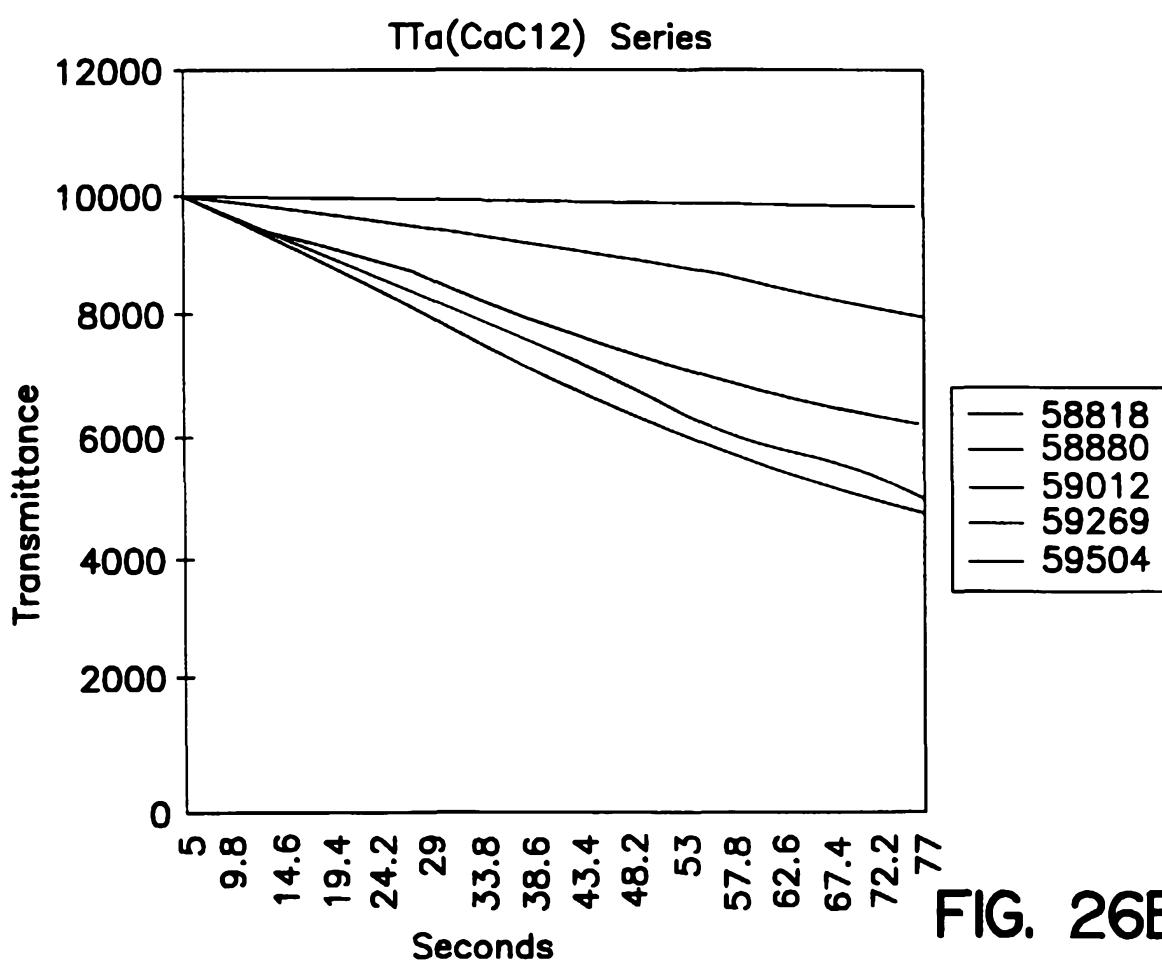
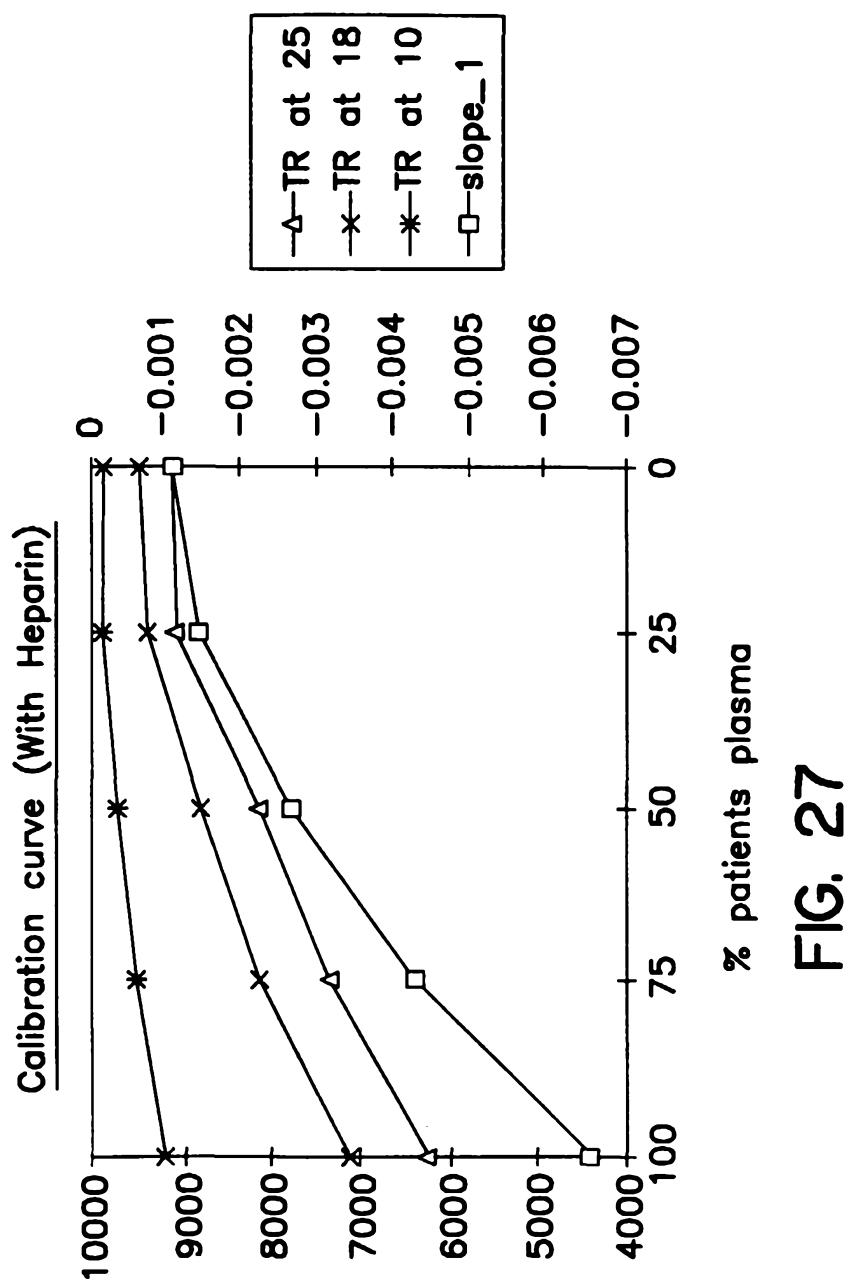


FIG. 26B

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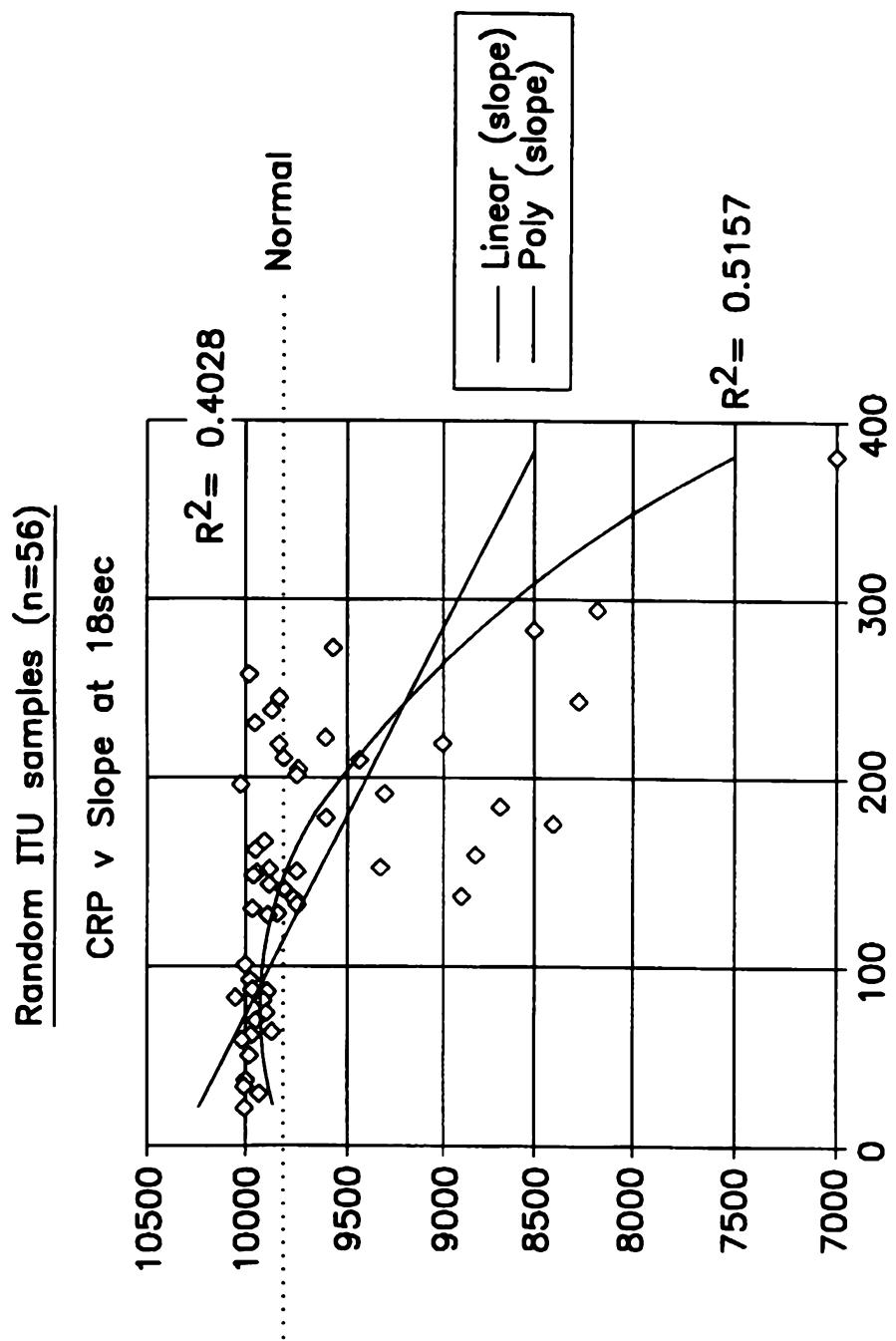


FIG. 28

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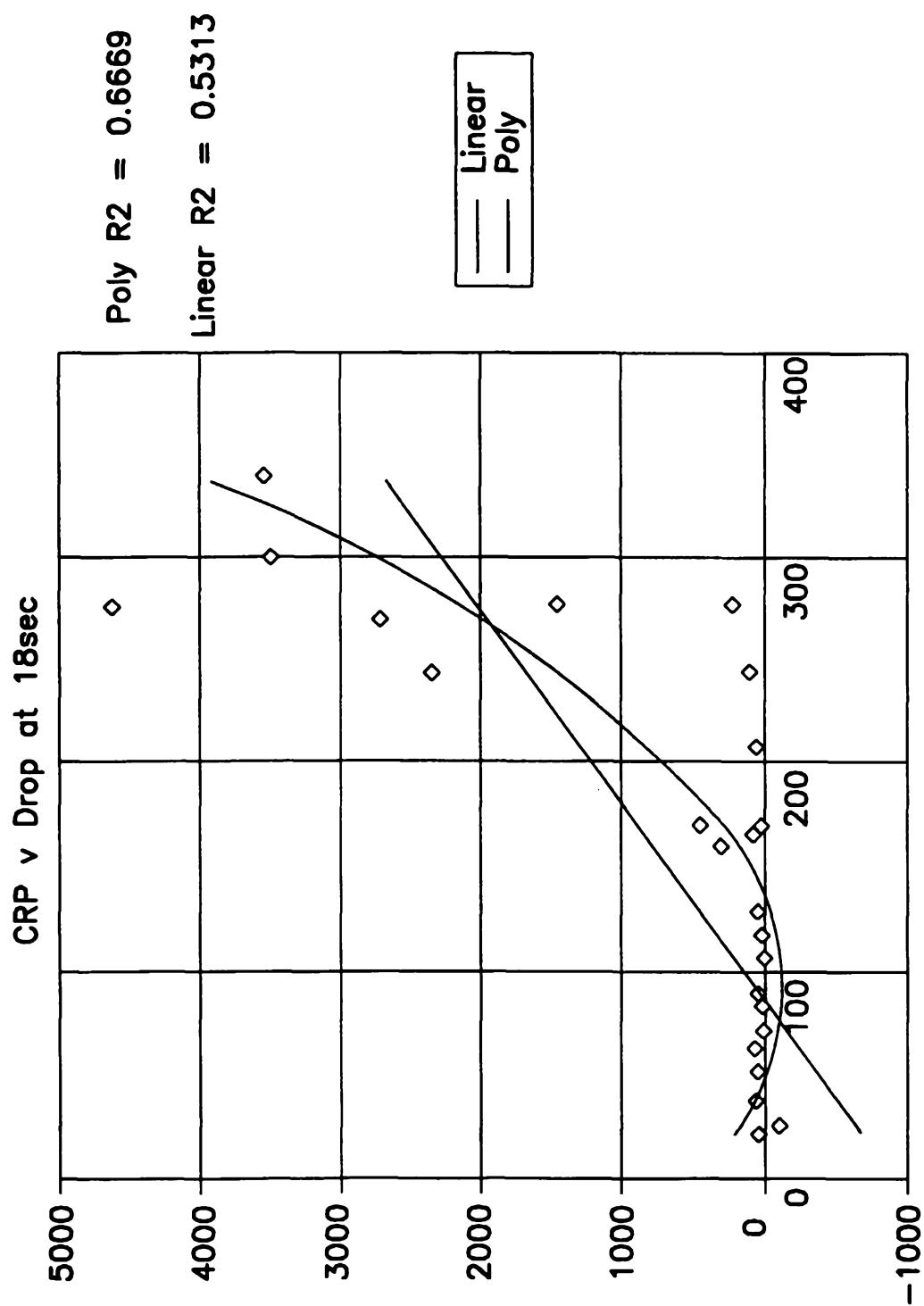


FIG. 29