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Use of the protamine titration and antiheparin compounds in certain hemorrhagic conditions

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THE USE OF THE PROTAMINE TITRATION AND ANTIHEPARIN COMPOUNDS IN CERTAIN HEMORRHAGIC CONDITIONS

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TABLE OF CONTENTS

The protamine titration is one of the more recent approaches to the study of abnormal bleeding. It is a test performed on venous blood which indicates the presence of an excess of circulating heparin or heparinoid anticoagulants. In certain hemorrhagic conditions it may also be considered a basis for effective therapy, for if the test results indicate an excess of heparinoid substances, the antiheparins, protamine sulfate or toluidine blue, may be used in treating the condition.

In 1948, Allen and his co-workers (1) first reported the presence of a heparin-like anticoagulant in dogs which had been exposed to total body irradiation. It was found that the plasma from the irradiated animals caused a delay in clotting times when mixed with normal dog plasma whereas saline mixed with normal plasma did not cause this delay. Therefore, the investigators reasoned that an anticoagulant was present. Since heparin was the only known naturally occurring endogenous mammalian anticoagulant, they tried the antiheparins and found that either toluidine blue or protamine sulfate restored the clotting time to normal in vivo and in vitro. Their conclusion, therefore, was that the anticoagulant present was either heparin or a heparin-like substance.

Following their initial article, Allen and his associates reported (2) that a means had been found to determine this clotting inhibitor titrametrically. It was noted that normal dog blood would tolerate far greater amounts of heparin before the clotting time was increased than would the blood from irradiated dogs. Once the amount of added heparin began to increase the clotting time, very small amounts then greatly increased it. Therefore, to make a more practical and sensitive test, the blood specimen was made incoagulable by the addition of a standard amount of heparin and then back titrated with a standard heparin inactivator (protamine) to a clotting end point. Theoretically, the amount of protamine required to re-establish coagulation would vary in accordance with the concentration of endogenous heparin or heparinoid substances. Using this test, the protamine requirement under standard conditions was found to be remarkably constant in dog and man. The investigation then continued to determine whether the test had any clinical value.

Subsequent study revealed that the protamine titration was elevated in many hemorrhagic conditions indicating an increase in circulating anticoagulants and suggesting that the antiheparins, protamine

sulfate and toluidine blue, might be beneficial. Therefore, attempts were carried out to employ these agents and although results were inconclusive in some instances, in others the response was quite gratifying.

Before considering the clinical aspects of the test, a brief review of the agents used and of the theories on which the test is based may be of some aid in understanding and evaluating the problem.

Toluidine Blue

According to Jorpes (3), heparin is produced by the mast cells in the connective tissues around the blood vessels. The cell contains many closely packed metachromatic granules which have a special affinity for the basic aniline dyes. It is this affinity, supposedly, which provides a means of neutralizing heparin by inhibiting its production. Previous to the advent of the protamine titration, however, the dye had not been used clinically as an antihemorrhagic agent to any appreciable degree.

One report was made in 1947 by Allen (4) on the use of intra-venous toluidine blue in six cases of thrombocytopenic purpura. All six patients were benefited to the extent that petechiae were controlled.

Since the platelet counts showed no response and remained at a low level after the use of the dye, Allen felt that the toluidine blue must have had some affect on the capillary permeability. It must be remembered, however, that this report preceded the advent of the protamine titration and it is now believed that some cases of thrombocytopenic purpura show an increased level of circulating anticoagulants which are neutralized by toluidine blue.

The dye is now available for clinical trial (Abbott Laboratories) in two forms: 1. oral, in 100 mg. capsules and 2. intravenous, in 0.1% solution. There appears to be no marked side effects from the use of the drug although too rapid intravenous administration or too large a dose have been observed by various investigators to cause nausea and vomiting. The drug is excreted in the urine and feces so the patient should be told that his urine will turn blue. Dysuria or burning on urination have also been reported, but subside upon increasing the fluid intake.

Protamine

The history of the use of protamine as an antiheparin agent is far more complex than that of toluidine blue. Its antiheparin properities were first discovered by Chargoff and Olson (5) in 1938. In a very

interesting study on the mode of action of various anticoagulants in dogs, these investigators found that the animal organisms seemed to be able to dispose of heparin in a comparatively short time. Therefore, an attempt was made to prolong the short lived activity of heparin by combination with protamine. This was done in view of the results which Hagedorn and Jensen had obtained with the combination of insulin and protamine. However, the result was far from that expected for the anticoagulant action of heparin in vivo was entirely stopped by protamine. Chargoff and Olson therefore suggested that protamines might be used to neutralize heparin in man. They also felt that this action of protamines might be useful in determining the presence of heparin in hemorrhagic diseases.

The next two articles which appear in the literature concerning the use of protamine clearly indicate the trends which research on this substance was to follow for approximately the next ten years. Jorpes and his workers (6) noted no toxic symptoms following the administration of protamine intravenously to rabbits or dogs either alone or after heparin. Therefore, they tried it on man and found no toxic symptoms when given intravenously. On the other hand, Mason (7) reported

that protamine was toxic if administered in any form intravenously.

Later reports by other authors showed large doses of protamine to be toxic to mice (8), rabbits (9) and rats (10). Hodgkins and associates (10) demonstrated that the addition of protamine to whole human blood perfusing isolated organs caused cessation of flow. It was felt, therefore, that the protamines exerted their toxic effects thru embolic vascular phenomena and that large doses of protamine were not without danger. Such reports naturally led to the assumption that protamine could not be administered safely to human beings although it had been carried out previously by Jorpes (6). It was not until 1947 that Parkin and Kvale (11) again demonstrated that protamine could safely be administered to man intravenously. They used 40-50 mg. of protamine with no toxic effect and later reports by Cowley and Lam (12) using 1 mg./Kg. and Portman and Holden (13) using 100 mg. demonstrated no untoward reactions. A subsequent article by Parkin & Kvale (14) revealed that the speed of injection of the protamine was an important factor in producing toxic reactions in animals and they therefore recommended slow intravenous injections in man.

The exact reaction in which protamine enters as an antihemorrhagic agent in the theory of coagulation is not known for although it does neutralize heparin and probably other natural anticoagulants, it may also act as an anticoagulant in large doses. Jaques (15) found that the protamine heparin complex behaves according to the law of mass action and he was able to demonstrate that either an excess of heparin or of protamine caused a prolongation of the clotting time. Chargoff (16) had previously reported that the protamines had anticoagulant properties which he described as being antithromboplastic. Tocantins (17) also believed the protamines to be antithromboplastic but a later report (13) failed to confirm this action. Other investigators have reported that protamine is antiprothrombic (18) and that it precipitates fibrinogen (19). Clinically, however, no anticoagulant actions have been reported. Everard and Fantl (20) present a possible explanation for this in that they believe that there is a protaminase in humans which breaks down protamine into products which conceivably may not enter into the reactions mentioned above.

The Nature of the Clotting Defect Measured by the Protamine Titration

The possibility exists that several types of naturally occurring anticoagulants are responsible for an

increase in the protamine titration and also that the anticoagulant present may differ in various hemorrhagic conditions. The response of patients with a clotting disturbance to the heparin inhibitors toluidine blue or protamine sulfate is not complete proof that the anticoagulant present is heparin. Unfortunately these agents can also react with other endogenous substances, some of which are mildly anticoagulant and not closely related to heparin (21). Nucleic acid is an example of such a substance. Another difficulty arises in the fact that the present means for heparin identification are not suitable for human use because of the large amounts of blood required to isolate a few micrograms of material (22). Therefore, it is impossible to state definitely that any one anticoagulant or clotting defect is present in a particular hemorrhagic condition.

Allen and his co-workers have performed the major part of the basic work in this field. Since their investigations originated with the study of the hemorrhagic syndrome associated with total body exposure to ionizing radiation, I shall first present their concept of the condition (23). It was found that the hemorrhagic picture present in experimental animals following acute whole-body exposure to radiation was very

similar to that seen in man following the bombing of Hiroshima and Nagasaki. The hemorrhagic disease was accompanied by a thrombocytopenia, poor clot retraction and an elevation in both bleeding and clotting times. Therefore, many considered the condition to result from the thrombocytopenia. The authors concluded, however, that the thrombocytopenia played only a secondary role in the production of hemorrhage and that the presence of an increased amount of free heparin in the blood was the agent of greatest significance. Their conclusion was based on the following facts: 1. If the clotting time of their experimental animals was sufficiently prolonged, the blood of an irradiated dog would delay the clotting time of normal blood indicating that some type of an anticoagulant was present. 2. Evidence that this anticoagulant was probably heparin was indicated since toluidine blue and protamine returned the clotting time to normal. 3. Evidence that thrombocytopenia was not the primary cause was based on the fact that the time of onset of thrombocytopenia and hemorrhage did not coincide plus the fact that the antiheparins controlled the bleeding though the platelet count was not raised.

Although the above mentioned evidence is quite convincing that thrombocytopenia is not the cause of

an increased protamine titration, some investigators failed to agree. Conley, Hartman and Lalley (24) observed that normal human plasma could be made more sensitive to the effects of added heparin by removing most of the platelets by centrifugation. They concluded that this plasma data was applicable to the protamine titration and stated that the test could be explained by the variation in platelet concentration alone. However, as Allen points out (25) the protamine titration is carried out on whole blood and is not dependent on the platelet level. To prove his point he presented evidence from his work on various hemorrhagic conditions. In 21 patients with a variety of disorders showing hemorrhagic tendencies, the platelet counts were normal, but all had a mild to marked increase in the protamine titration. On the other hand, 18 patients with similar bleeding disorders had moderate to marked thrombocytopenia but in these the protamine titration was normal. In those who responded to therapy with toluidine blue, partial or complete control of bleeding occurred but the platelet count was not appreciably altered. This evidence indicates that the protamine titration may be elevated in the presence of normal platelet counts as well as

severe thrombocytopenia and also that thrombocytopenia may occur without an increased titration.

Except for the experimental study on post irradiation hemorrhage, none of Allen's work has shown that the clotting defect demonstrated by the protamine test is due to heparin per se. In other studies he has shown (25) that the principle difference between the titratable defect and that due to heparin injections appeared to be in anticoagulant potence. The blood of patients with bleeding disorders rarely inhibited or delayed the coagulation of normal blood. Also the protamine titration was increased in many patients to such an extent that had the increased titration resulted from heparinemia post injection, the blood would have been incoagulable. Incoagulable blood was found in only a few instances. Generally the clotting time was increased only 2 to 4 times the normal values.

The possibility of a decrease of heparin co-factor has also been explored (26). (The heparin co-factor is believed to be closely associated with the albumin fraction of the plasma protein and its presence is necessary for the action of heparin.) The possibility was suggested by the fact that the amount of heparin required to alter the protamine titration in normal patients or dogs

renders the Lee-White clotting time incoagulable. This, as has been mentioned, is in contrast to the defect seen in patients with this bleeding syndrome whose clotting time may be only moderately prolonged but whose protamine titration is markedly altered. However, since it was found that in vivo the administration of rapid intravenous heparin, 0.25-0.50 mg./Kg. to such patients or to irradiated dogs had a much greater effect upon the whole blood clotting time than usually occurs, it was believed that it was unlikely that the heparin co-factor was abnormal.

Before proceeding to the clinical reports, it should be noted that the protamine titration may be influenced by factors other than anticoagulants; in fact, it is probably elevated by any disturbance which interferes with fibrin formation and is capable of prolonging the whole blood clotting time (21). A prothrombin deficiency may increase the titration, however it must be of such a degree that it will prolong the whole blood clotting time -- under 40% of normal activity. Hemophilia is another factor, and although all hemophiliacs do not show an increased protamine titration, it is usually present in severe exacerbations (2). Any impairment of the conversion of

prothrombin to thrombin or the absence of fibrinogen would also affect the test (25). Therefore, adequate blood studies should be performed to evaluate a patient with an increased protamine titration.

After considering the preceding reports, it is apparent that the etiology of the clotting defect measured by the protamine titration is not known. Allen and his associates (26) have summarized the present knowledge as follows: "Since many of the plasma and tissue proteins are antiheparins, it may be that the associated protein changes in some of these disorders in part account for the disturbance through the release of heparin or heparin-like substances which may then affect the system. Present studies suggest that a decrease in the antiheparins of the plasma and/or an increase, relative or actual, of the plasma heparinoid substances are the systems most likely affected."

The Clinical Aspect

Following Allen's initial work, a number of articles have appeared in the literature concerning the clinical application of the protamine titration and the use of protamine sulfate and toluidine blue. The results in some conditions are inconclusive while in others they are quite impressive and seem to warrant more widespread clinical trial.

Mention has been made previously of Allen's use of toluidine blue in thrombocytopenic purpura (4). Two of the patients reported had primary thrombocytopenic purpura and it will be recalled they were benefited to the extent that petechiae were controlled. In a later article (25) six additional patients with this disease were considered. Three had normal protamine titrations and did not respond to toluidine blue, whereas three other patients had increased titrations and responded to therapy with toluidine blue to the extent that there was some decrease in bleeding. All required splenectomy, however. In this respect, Holoubek and his associates (27) suggested that toluidine blue might be used preparatory to splenectomy in thrombocytopenic purpura although their report on one patient showed no relief gained with the use of the dye. Another group, Hall, et al. (28) felt that the test and therapy were not indicated in this disease. They performed protamine titrations on 5 normal persons and 3 persons with essential thrombocytopenic purpura. Their results of the titration, even with the normal, were so varied that they were unable to draw any conclusions. Protamine and toluidine blue were injected intravenously in one

patient with purpura but the hemorrhagic phenomena continued. It was their conclusion that the test was unreliable because it involves the use of protamine which has so many effects on different phases of the blood clotting mechanism (antiprothrombin, etc.).

It is obvious that the evidence is inconclusive to date. In view of this, Yates (29) suggested that the antiheparins were probably of more value in secondary purpuras. He also reported one patient with primary purpura who did not respond to treatment but he noted that the literature reported better response to therapy if the thrombocytopenia was secondary. In this respect, small groups of cases have been reported where hemorrhagic states have responded to antiheparin therapy with primary diseases as leukemia, Hodgkins disease, aplastic anemia, etc.

Following the work of Grossman and his associates (30) another field was opened where the antiheparin agents might be of benefit. It was noted that the incidence of hemorrhage in patients with acute leukemia appeared to be increased during the early period of aminopterin therapy. Further study showed that toluidine blue was effective in controlling the hemorrhagic manifestations due to aminopterin. Now this type of

therapy is being tried clinically for the control of bleeding secondary to the use of such agents as radio-active phosphorus, urethane, nitrogen mustard, etc.

By far the most proliferative field as far as the number of cases reported concerning the use of the antiheparins has been in obstetrics and gynecology. Here the complaint of abnormal bleeding is frequently seen and therefore has lent itself most readily for evaluation by the protamine titration. A more thorough presentation of the literature on this aspect of the subject will be undertaken for it is in this field that my own observations, on which I shall report, have been made.

Allen and his associates in two articles (21 & 22) presented a total of 40 patients with postpartum hemorrhage or menorrhagia. Of the 8 patients with postpartum hemorrhage, all had increased protamine titrations before treatment while only 3 had increased clotting times. Bleeding stopped in all 8 patients treated with either protamine sulfate or toluidine blue. Of the 32 patients with menorrhagia, all had increased protamine titrations before treatment while only 24 had increased clotting times. Bleeding was controlled in 18 patients; decreased to some degree

in 7, while 7 patients had no response. It was felt that the intramuscular administration of protamine sulfate gave results equal to or better than those of the dye. Since it had a rapid action, Allen believed it best suited for the types of disorders mentioned above whereas toluidine blue with its slower but more prolonged action was suggested for patients with hemorrhagic diseases complicated by other disorders. It must be remembered, however, that Allen's series was on patients receiving the antiheparins parenterally whereas now the oral administration of toluidine blue lends itself readily to the treatment of such disorders as menorrhagia.

Following this initial work, Elghammer and his co-workers (31) made a study on 43 patients with menorrhagia and 23 normal females employing the protamine titration. No gross pathology was found nor did endometrial biopsies reveal any characteristic pattern in the patients with menorrhagia. The titrations were carried out at different intervals during the menstrual cycle and on the day of heaviest flow. During menstruation a slight increase in whole blood clotting time and a mild to moderate thrombocytopenia was seen in some patients, but the authors felt that the pro-

tamine titration was a better index of any hematologic disturbance. The titration was found to be increased in 80 of 106 determinations during menorrhagic periods but was increased in only 5 of 23 determinations made during menstruation in females whose menses was considered normal. When an increase did occur in the latter group, it was less pronounced than in those with menorrhagia. Intermenstrually, 82 titrations on the menorrhagic patients revealed 39 increased and 43 normal determinations while 35 titrations on the normal controls revealed 11 increased and 24 normal determinations. The majority of patients were treated with protamine sulfate -- 50 mg. intramuscularly on the day of heaviest flow. Of these, 30 patients had good results whereas temporary or no effects were obtained in 12 patients, of which 6 had increased protamine titrations. The response of many of the patients with menorrhagia to a single intramuscular injection of protamine could not be explained in that the duration of intramuscular protamine was considered to be less than four hours. The best results were obtained when the protamine was given on the second or third day of flow which was usually coincident with an increase in the protamine titration and the onset of profuse bleeding.

The next article to appear in this field was by Lathrop, Dieckman and Allen (32). Their report added a new approach in that they studied the protamine titration on 28 normal pregnant females. Titrations were performed on the day before delivery and again at irregular intervals throughout the postpartum period. Sixteen had increased protamine titrations pre-delivery and thirteen of this group returned to normal before the tenth postpartum day. Twelve patients had normal protamine titrations pre-delivery, however, 8 of these showed an increased titration at some time during the postpartum period. No abnormal bleeding or morbidity was noted in any of these patients regardless of the protamine titration. The authors also reported on 31 patients with hypermenorrhea. Nineteen of these were observed during the menstrual period, usually on the second day of flow, and 14 had an increased protamine titration. Of the 19 patients examined intermenstrually, 9 had an increased titration. Nine patients were treated with protamine and toluidine blue. Of these, 6 had an increased protamine titration and all reported good results. Of the 3 patients with a normal titration, 1 reported good; 1 fair, and 1 no results. Four of the patients with hypermenorrhea had hysterectomies. All

had increased protamine titrations preoperatively and all returned to normal 2-3 days postoperatively. Four patients with postpartum hemorrhage were also reported on. All of these had an increased protamine titration at the time of bleeding and were treated with toluidine blue and/or protamine sulfate. In each case bleeding stopped without the necessity of surgery.

The most recent work on abnormal uterine bleeding to appear in the literature which utilized the protamine titration is that of Rumbolz and his associates (33). Included in this report were studies on 8 normal females, 62 patients with menometrorrhagia and 8 patients with postpartum hemorrhage. Of the 8 normal women tested, 7 had a slight to moderate increase in their protamine titration with menstruation. Intermenstrually, however, they all had normal titrations. Of the 62 patients with menometrorrhagia, 47 had increased protamine titrations. None of these patients had any demonstrable pelvic pathology. Treatment by protamine sulfate or toluidine blue was used on 25 of the patients with menometrorrhagia and increased titrations. The majority of these showed a complete relief of symptoms and only 4 had no improvement. The group of untreated controls showed far fewer remissions and the majority required further procedure as

curettage, hormone therapy or hysterectomy. Eight patients with postpartum hemorrhage were also studied. Of these, 5 had increased titrations and were treated with one of the antiheparins. Bleeding stopped in 4 of these patients and in only 1 did the treatment have no effect. Two patients with menorrhagia but no increased protamine titration were given protamine sulfate empirically but no response was noted. The authors also noted, as did Allen (22), that the clinical response did not parallel the degree of elevation of the titration. It was also noted that longer courses of treatment were required in patients with prolonged bleeding histories.

The discussion following the article by Rumbolz (33) contained the following statement by Priddle: "If a patient is having abnormal bleeding and there is no gynecological reason for it, and even if she does not show an abnormal protamine titration, we feel that the patient should be given either protamine or toluidine blue empirically, for many times there will be a response." Lathrop and Carlisle (34) have carried this approach one step farther in that they have treated 63 patients with functional hypermenorrhea without making use of the protamine titration.

The patients were followed during an average of three menstrual periods while being treated with toluidine blue orally. Forty-three patients reported good results; 15 fair results, and 5 poor results. The authors felt that the good results reported were probably too high since the reports were obtained by phone and overzealous reporting, or the psycic effect of treatment coincidental with spontaneous improvement would both result in an increased figure.

Personal Investigation

This investigation has been undertaken with the following aim:

1. To determine the percentage of normal postpartum patients with elevated protamine titrations and by this means to evaluate the use of the test in patients with postpartum hemorrhage. If a significant number of normal patients could be suspected of having an elevated titration, then the value of this test in those patients with postpartum hemorrhage would be minimized.
2. To determine the percentage of patients with functional menometrorrhagia who

respond to antiheparin therapy but who have a normal protamine titration. If a significant number of such patients are found, the empirical use of the antiheparins would appear to be justified.

Material and Method

The patients on whom I have performed protamine titrations have been from the hospital or dispensary services of the Department of Obstetrics and Gynecology of the University of Nebraska College of Medicine. Controls for the standardization of the protamine used have been obtained from the personnel of the University Hospital or medical students.

The procedure followed was that described by Allen and his associates (2). Venous blood is placed in a standard conical centrifuge tube containing 1.0 mg. (0.1 ml.) of commercial heparin, bringing the blood to the 11 ml. mark. The tube is then stoppered and inverted fifteen times to obtain thorough mixing. One ml. of the heparinized blood is pipetted into each of six serology tubes containing measured amounts of a 0.1% solution of protamine sulfate beginning with 0.1 mg. in the first tube and ending with 0.2 mg. in the sixth tube. Each tube is shaken briskly eight to

ten times to obtain reasonable mixing of the heparinized blood with the protamine solution. The tubes are then allowed to stand undisturbed for one hour before the end point is read. The end point is definied as the least amount of protamine required to form a solid clot. The normal end point varies depending on the stock of protamine used. Since the protamine I have used has been obtained from two different stocks, the initial normal end point was 0.14 mg. of protamine whereas that used at the time of this writing is 0.12 mg. as determined by normal controls.

To date, the only treatment employed has been oral toluidine blue. The drug is administered in 100 mg. capsules using one capsule twice daily for four days.

Results

A total of 41 protamine titrations have been performed on 39 patients. Eight of these patients had menometrorrhagia; 30 were postpartum patients, and 1 patient was receiving Roentgen ray therapy for a leiomyosarcoma of the uterus. The latter patient's protamine titration was normal on 2 occasions.

The majority of the protamine titrations on the postpartum patients were performed within 24 hours after delivery. All of the deliveries were normal

and uncomplicated except for 2 cesarean sections. All of the protamine titrations performed on this group were normal.

Of the 8 patients studied who had menometrorrhagia, 5 had normal protamine titrations. Three of the patients with normal titrations were treated with curettage, and all were relieved of their symptoms following the procedure. The pathological reports revealed that one patient had an endometrial polyp, another had an incomplete abortion, while the third demonstrated normal secretory endometrium.

The majority of the remaining 5 patients are still being followed. These cases are presented, however, to illustrate the comments to follow.

Two patients with normal protamine titrations were treated with toluidine blue.

Case 1. Mrs. D. E., aged 26 years, para iii, gravida iii, had a curettage and conization of the cervix on January 21, 1953 for acute chronic cervicitis and errosion. On January 27, 1953 examination revealed good healing of the cervix and no bleeding. On February 10, 1953, the patient entered with the complaint of bleeding of thirteen days duration. The bleeding had

started like a normal menstrual period but became progressively more severe. Previous menstrual periods had been normal. Bleeding appeared to be from the site of the conization. The protamine titration was 0.12 mgm. Toluidine blue, 100 mg. twice daily by mouth was administered. The patient returned on February 13, 1953 after taking 8 capsules of the dye. The bleeding had continued and the site of conization was packed with Gelfoam. Bleeding stopped within 48 hours.

Case 2. Mrs. D. S., aged 22 years para iii, gravida iv, was first seen on February 10, 1953 with a complaint of vaginal bleeding of one month's duration. The patient had no previous history of menometrorrhagia and her menstrual periods were entirely normal. Physical examination was negative except for a bloody discharge from the external os. Bleeding time and clotting time were within normal limits and the protamine titration was 0.12 mgm. Toluidine blue, 100 mg. by mouth, was administered. The vaginal bleeding stopped after the first day of treatment.

Three patients had elevated protamine titrations.

Case 3. Mrs. A. E., aged 54 years, para ii, gravida ii, was first seen on February 3, 1953 with the complaint of intermittent vaginal bleeding of six weeks duration. The patient gave a history of irregular and heavy menstrual flow of eighteen years duration. Pelvic examination revealed a polyp protruding from the cervical os and nodular enlargement of the uterus. Laboratory studies were: B.M.R. plus 40, bleeding time 2½ minutes, clotting time 9 minutes and prothrombin time 94 per cent. The protamine titration was 0.18 mg. A curettage was performed and the bleeding stopped without the use of toluidine blue.

Case 4. Mrs. V. W., para ii, gravida ii, aged 23, was first seen on January 29, 1953 with the complaints of polymenorrhea and menorrhagia for the last seven years. Pelvic examination revealed retrodisplacement of the uterus. A protamine titration performed on January 29, 1953 was 0.12 mg. On February 6, 1953 the patient was seen again and was bleeding at

that time. A protamine titration was repeated and was 0.14 mg. The patient was placed on oral toluidine blue, 100 mg. twice a day for four days. The vaginal bleeding stopped after the first day of treatment.

Case 5. Mrs. L. C., aged 49 years, para ix, gravida x, was first seen on January 23, 1953 with the complaint of vaginal bleeding of five weeks duration. The patient stated that she had 2 menstrual periods in October of 1952 followed by 1 period in November of 1952. She then started bleeding in the middle of December of 1952 and had continued to bleed some every day up to the time of examination. Pelvic examination revealed a third degree rectocele and a second degree cystourethrocele. There was also an area of erosion on the lip of the cervix. The uterus showed some nodular enlargement. The protamine titration was 0.14 mg. On January 26, 1953 a curettage was performed and the pathological report revealed a proliferative endometrium with mild endometritis.

The patient was discharged on January 28,1953 -- asymptomatic with no bleeding. If menometrorrhagia again occurs, a trial of toluidine blue will probably be instituted.

Comment

The consistency with which normal protamine titrations were found in the postpartum patients studied suggests that the finding of an elevated titration in a patient with postpartum hemorrhage would be significant and would indicate the use of the antiheparins in treatment. The results obtained do not agree, however, with those of Lathrop, Dieckman and Allen (32) who found elevated protamine titrations in 24 of 28 postpartum patients studied. In their series, the titrations were performed at any time up to the tenth postpartum day, whereas in my series the majority of the titrations, 28 out of 30, were performed within the first 24 hours after delivery. The discrepancy in the two series, therefore, cannot be fully evaluated. The good results obtained by other investigators using the protamine titration and the antiheparins in the treatment of postpartum hemorrhage, suggests the value of the test and treatment in this hemorrhagic disorder.

The cases of menometrorrhagia are obviously too small a number to evaluate. However, if one considers the cases which are presented in conjunction with the results of other investigators, it is possible to arrive at certain conclusions.

It has been pointed out by others that a complete gynecologic study should be performed on all patients before accepting an elevated protamine titration as an indication for antiheparin therapy. Case 3 illustrates this point quite clearly, for not only did this patient have an endometrial polyp and probably fibromyomas of the uterus, but she also demonstrated a marked hyperthyroid state, all of which could account for her menometrorrhagia.

Case 4 illustrates the point that the protamine titration may be normal at one time and elevated at another in a patient who responds to therapy.

Case 1 and 2 illustrate that some patients with normal protamine titrations respond to antiheparin therapy whereas others do not.

Summary

1. The history of the development of the protamine titration and the use of protamine sulfate and toluidine is presented.

2. A review of the literature concerning the clinical use of the protamine titration and the antiheparins is made.

3. Results of an investigation of 30 normal postpartum patients using the protamine titration are presented.

4. Results of an investigation of 8 patients with menometrorrhagia using the protamine titration, and the results of treatment of three of these patients with toluidine blue is presented.

Conclusion

A. The protamine titration is probably of value as an indication for the use of protamine sulfate or toluidine blue in the treatment of postpartum hemorrhage.

B. After considering the results found in the literature and the points illustrated in the cases mentioned above, I feel that the empirical use of the antiheparins is justified in certain cases of menometrorrhagia. A complete gynecological work-up should be performed, of course, to rule out any other cause of the bleeding. My reasons for the above statement are:

1. Multiple titrations may be required to demonstrate one elevated protamine titration. Although the procedure is not difficult and

the cost is low, the inconvenience and time required both of the technician and the patient must be considered. Also the validity of only one elevated titration would certainly be questionable.

2. Certain patients can be expected to respond to antiheparin therapy al though their protamine titration is normal. Since other types of empirical therapy will then be tried, I feel that the trial of toluidine blue, to which no marked untoward effect has been attributed, is certainly justified.

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