Leukopoietic Activity in Human Urine Following Operative Procedures[1] (35186)

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Previous studies from this and other laboratories have shown the presence of a factor in the serum and urine of animals and man which will stimulate the formation of maturing granulocytic colonies from normal bone marrow cells *in vitro* (1–5). This factor can be detected and quantitated in the urine of all normal human subjects (3–5). Alterations have been found in the levels of this material in the serum and urine of patients with various disease states, particularly the granulocytic leukemias (4). Although it has been suggested that this factor may represent a true leukopoietic material stimulating granulocyte formation *in vivo*, this suggestion awaits confirmation. The present studies were undertaken to determine the levels of this factor in the urine of hematologically normal human subjects undergoing elective operative procedures. A study has been made correlating the rises and falls in total white blood cell and granulocyte levels in the peripheral blood of these individuals and their urinary leukopoietic activity. These studies have shown that levels of urinary leukopoietic activity rise during periods of granulocytosis and return to normal with the granulocyte count.

Methods and Materials. Subjects for study were patients undergoing elective operative procedures on the gynecological and surgical services of the University of Colorado Medical Center. Urinary collections were made in sterile glass bottles during the time intervals noted (4 to 24 hr). Urine samples were kept refrigerated, but not frozen, during collection. 50-ml aliquots were removed and dialyzed for 72 hr against three changes of distilled water. Following dialysis, samples were centrifuged at 9500 rpm for 15 min and the sediment was discarded. The supernatant portion to be used for testing was sterilized by filtration through 0.45 mμ Millipore membranes and stored at 4° prior to testing. Complete blood counts were done by finger stick at the times noted.

The method of determining leukopoietic activity in human urine has been previously recorded (3, 4). 0.15 ml of urine to be tested is placed in 5 replicate 35-mm plastic petri dishes. To each plate is added 1 ml of a 9:1 mixture of McCoy's 5A medium (supplemented by 15% fetal calf serum) and boiled 3% agar (Bacto-agar, Difco). Each milliliter of medium contains a single cell suspension of 75,000 nucleated bone marrow cells obtained from the femurs of C57/Bl mice, 2 to 3 months old. After mixture of medium and urine the plates are allowed to gel at room temperature and are then incubated at 37° under humidified conditions with a constant flow of 7.5% carbon dioxide in air. Microscopic colonies are visible at the second to the third day of incubation. Colony counts are done at the seventh to the tenth days of incubation with a dissecting microscope at magnification of 10 times. Previous studies have shown a linear correlation between the amount of active test material used (serum or urine) and the number of colonies detected (1, 5). Thus by counting the number of colonies, a quantitative estimate of the leukopoietic activity of various samples is obtained. Variation in urinary output with consequent concentration or dilution of the active factor is taken into account by expressing activity as the number of colonies stimulated by 0.15 ml of a standardized 24-hr

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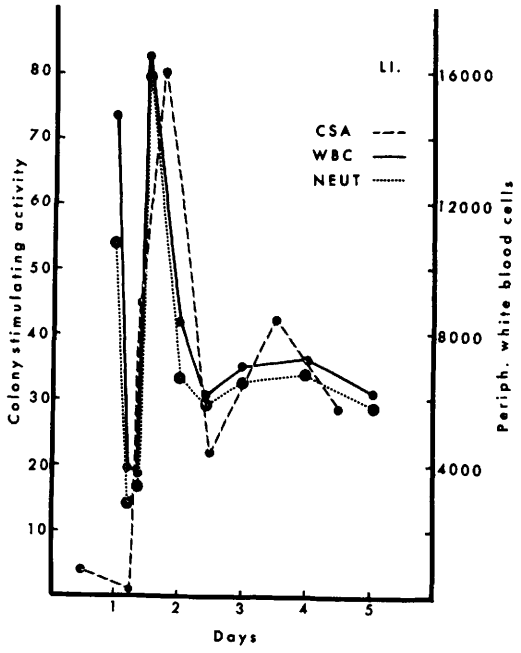

FIG. 1. Urinary CSA correlated with total WBC and absolute neutrophil counts in a 38-year-old female undergoing a vaginal hysterectomy. The time the operation was begun is indicated as day 1. The first points on all curves represent preoperation values. Points for urinary CSA are plotted at the midpoint of the time of collection. Each point on the CSA curve represents the mean colony count of 5 plates.

urine output of 1000 ml.

For microscopic study, colonies were removed from the agar with a fine drawn Pasteur pipette, flattened on glass slides with coverslips and stained by floating aceto-orcein stain into the wet mount preparation as previously described (1).

Results. Colonies stimulated by urine in the present studies were similar in character and cellular morphology to those previously described (1, 4). They were composed almost exclusively of granulocytes at day 7 of culture regardless of the time at which urines were collected.

In general colony stimulating activity in the urine of these subjects followed closely rises and falls in the peripheral granulocyte count. Figure 1 shows the rise and fall of urinary colony stimulating activity (CSA) correlated with the peripheral granulocyte and WBC counts in a 38-year-old mother of seven (LI), undergoing an elective vaginal hysterectomy. Initial urinary CSA was normal with a value of 4. Mean values for normals in our laboratory are 0 to 40 with a mean of 20 (4). The initial granulocyte count prior to operation was slightly elevated at 10,500. During the first 4 hr while the patient was in the operating room, the granulocyte level fell markedly. During this time urinary CSA also declined to undetectible levels. Subsequently there was a rise in granulocyte count with a concomitant rise in urinary CSA. On the second postoperative day the granulocyte count had returned to normal as did urinary CSA. During the subsequent 3 days until the time of discharge from the hospital, the patient's total white blood count

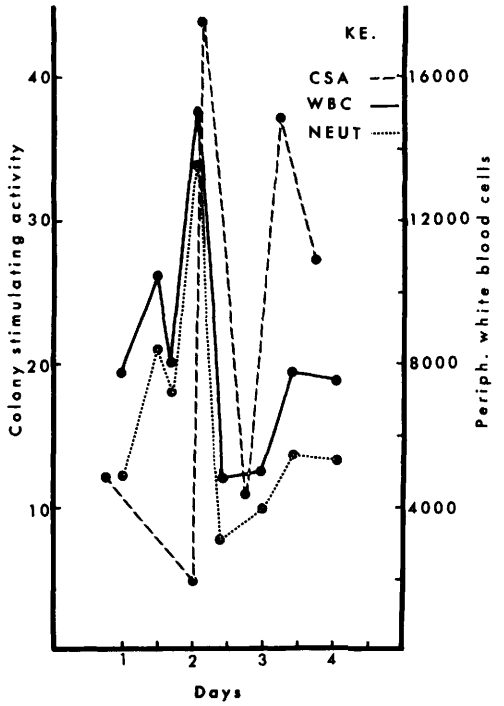

FIG. 2. Urinary CSA correlated with total WBC and absolute neutrophil counts in a 70-year-old man undergoing an elective herniorrhaphy. The time the operation was begun is indicated as day 1. The first points on all curves represent preoperation values. Points for urinary CSA are plotted at the midpoint of the time of collection. Each point on the CSA curve represents the mean colony count of 5 plates. Note the initial fall and late rise in CSA.

and granulocyte counts remained normal; and urinary CSA, while still mildly elevated, was in a normal range.

Figure 2 shows the course of patient KE, a 70-year-old man undergoing an elective herniorrhaphy. Initial granulocyte counts and urinary CSA were within normal range. CSA could not be measured during the first 18 hr postoperatively because the patient was unable to void. At 18 hr urinary CSA had declined; but the following day, with a concomitant rise in granulocyte count, CSA increased. This was followed by a subsequent fall in both granulocyte counts and CSA levels. A final spike of CSA activity was noted prior to discharge and this was correlated with a slight rise in the patient's peripheral granulocyte count.

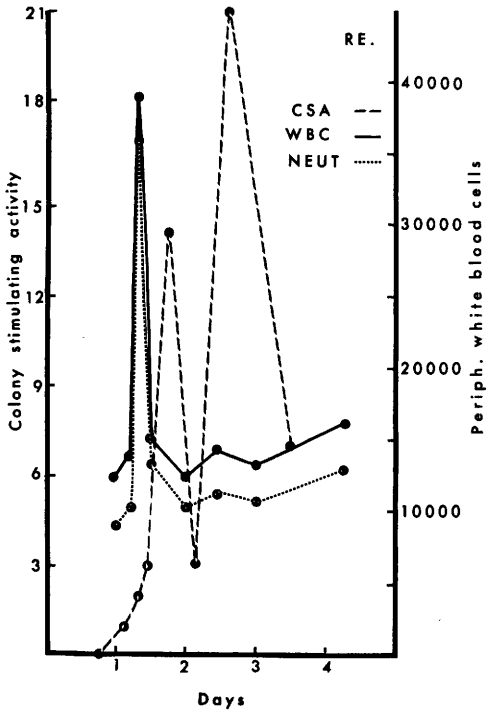

FIG. 3. Urinary CSA correlated with total WBC and absolute neutrophil counts in a 44-year-old male undergoing repair of an old hand injury. The time the operation was begun is indicated as day 1. The first points on all curves represent preoperation values. Point for urinary CSA are plotted at the midpoint of the time of collection. Each point on the CSA curve represents the mean colony count of 5 plates.

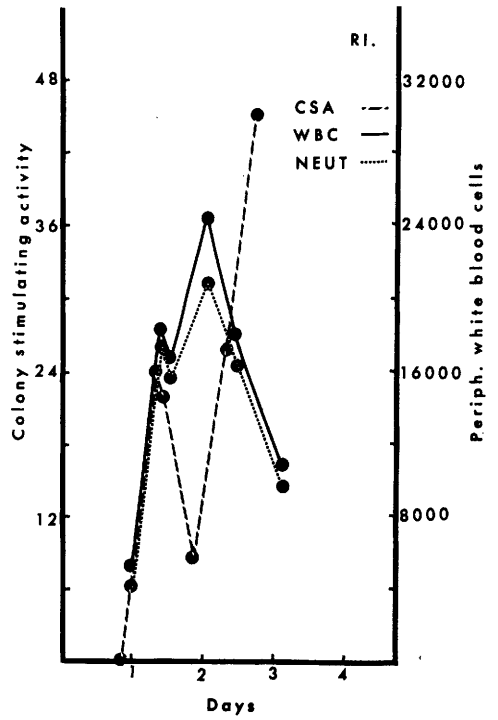

FIG. 4. Urinary CSA correlated with total WBC and absolute neutrophil counts in a 54-year-old woman undergoing an elective hysterectomy. The time the operation was begun is indicated as day 1. The first points on all curves represents preoperation values. Points for urinary CSA are plotted at the midpoint of the time of collection. Each point on the CSA curve represents the mean colony count of 5 plates.

Figure 3 shows the course of patient RE, a 44-year-old male undergoing an elective repair of an old hand injury. In this patient CSA levels were measured at 4-hr intervals during and immediately after operation. CSA during this period increased, correlated with a rise in granulocyte count; the rise in CSA peaked after the peak level of granulocytes in the peripheral blood. As in the last patient, a late peak in CSA was noted but in this patient was not correlated with a rise in peripheral neutrophil counts.

Figure 4 shows the course of patient RI, a 54-year-old woman undergoing an elective hysterectomy. In this patient the initial rise in urinary CSA levels during operation paralleled closely the rise in peripheral granulocyte count. Once again was noted a late rise

in CSA at 2 days postoperatively when the granulocyte count had returned to normal. This patient was not followed beyond this time.

Discussion. It has previously been suggested that the bone marrow colony stimulating factor in human urine may represent a true leukopoietic substance stimulating granulocyte maturation and production *in vivo* (3, 4). This suggestion has been further strengthened by the present studies which have demonstrated a close correlation between rises and falls in the level of this factor in the urine of patients undergoing elective operative procedures and the peripheral granulocyte count.

Initial suggestions that bone marrow colony stimulating factor is a cell regulating substance came from biochemical studies which indicated a close similarity between this material and the red blood cell regulator, erythropoietin (6, 7). Both are glycoproteins with similar molecular weights and electrophoretic mobilities. Biological data, with the exception of the fact that this substance stimulates maturation and production of granulocytes *in vitro*, has however been largely lacking. No previous studies have appeared correlating rises and falls in the levels of this factor in humans with bone marrow activity or peripheral white blood cell levels.

The present work indicates a close relationship between levels of urinary CSA and peripheral granulocyte counts in hematologically normal humans with a changing leukodynamic status. The nature and meaning of this relationship must remain speculative, but some comments are in order. It is unlikely the rapid rise in peripheral granulocyte counts following operation is the result of the factor responsible for CSA. This rise is the result of the action of epinephrine, corticosteroids, endotoxin, and related factors leading to release of already mature granulocytes from the bone marrow and marginated sites (8, 9). Humoral factors regulating this arm of the granulocyte response have also been demonstrated by several authors (10, 11) and while these have not been well characterized, they appear to be biologically and chemically distinct from the urinary factor responsible for CSA. Releasing factors have been found to be at highest levels when the granulocyte count is low. This inverse relationship is the opposite of the results noted here where the two seem to parallel each other. Likewise biochemical data indicate that leukocyte releasing factors are heat labile substances of lower molecular weight and bear little resemblance to the factor in human urine under consideration.

Previous workers have speculated that the urinary CSA factor is involved in stimulation of maturation and production of new granulocytes in the bone marrow. This is particularly suggested by its action *in vitro*. In contrast to releasing factors it would be expected that maturation–production factors would be at highest levels when marrow reserves have been depleted and increased production is needed to meet demands and reconstitute marrow reserves. Such factors would thus be at highest levels at the same time, or shortly after, rises in peripheral granulocyte counts. The present data fit such a pattern and add further strength to the suggestion that CSA is related to increased production of granulocytes, *i.e.*, a granulopoietin.

The time points in the present studies do not allow us to determine accurately whether the rise in CSA follows or precedes the rise in peripheral granulocyte counts. In 3 of the 4 patients studied however, the peak rise in CSA followed the granulocyte peak in the peripheral blood. This also suggests that CSA is not responsible for the granulocyte rise seen and may be the result of it. While the source of urinary CSA has not been determined in humans, recent data from this laboratory indicate that mature circulating granulocytes themselves may be a major source exerting a positive feedback on the marrow in times of stress (12). This contention is also suggested by the present studies. Assays at closer time points are now under way.

In three patients in the present study, the late rise in CSA is unexplained. It may be that this represents the subsequent breakdown of granulocytes released in the preceding 48 hr immediately postoperatively. It would be anticipated that this might lead to a subsequent second rise in granulocyte counts at a time exceeding the limits of the present studies. Whether this is the case or

not, is also under further investigation.

The present studies have added further biologic information to the suggestion that the previously described bone marrow colony stimulating activity in human urine may represent a true leukopoietic material stimulating maturation and production of granulocytes *in vivo*.

Summary. Urinary bone marrow colony stimulating activity has been studied in four patients undergoing elective operative procedures. These studies have shown a close correlation between colony stimulating activity and rises and falls in the peripheral granulocyte count. These data add further biologic evidence to support the concept that urinary colony stimulating activity may represent a true leukopoietic substance.

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