Serum Leucine Aminopeptidase in Twin Pregnancy.*† (25962)

JOHN J. SCIARRA AND DONALD A. BURRESS (Introduced by J. W. Jailer)
Columbia University College of Physicians and Surgeons, Dept. of Obstetrics and Gynecology, N. Y.

Since the original report(1) describing colorimetric determination of leucine aminopeptidase activity (LAP) in human sera using L-leucyl-β-naphthylamide hydrochloride as a synthetic substrate, many observers(2,3,4,5,) have recorded elevated levels of this enzyme in the sera of pregnant human females. Published results are in general agreement in showing a gradual rise in serum levels of LAP values throughout the second and third trimesters of pregnancy, with the amount of this enzyme at term being from 2 to several times the amount present in the normal non-pregnant female or in the pregnant female during the first 12 weeks of pregnancy. It has been reported(3) that in 3 cases of hydatidiform mole and one case of choriocarcinoma LAP activity of the serum was normal, and it has been stated(4) that serum levels of this enzyme in patients with pre-eclampsia or chronic hypertensive disease were found to be the same as in normal pregnancy. No data are yet available on serum LAP activity in multiple gestation, therefore we report the following series of patients with documented twin pregnancies.

Material and methods. Sera were obtained, during the third trimester, from 11 patients with radiographically proven twin pregnancies, and from 25 patients during the same stage of gestation with single fetuses. Serum LAP activity was determined by hydrolysis of L-leucyl-β-naphthylamide hydrochloride, with a colorimetric assay of the β-naphthylamine liberated. A .2 ml sample of serum is diluted with 9.8 ml of distilled water and a 1 ml aliquot of this mixture is incubated with 0.18 mg of L-leucyl-β-naphthylamide hydrochloride dissolved in 2 ml of a 0.18 M phosphate buffer at pH 7.2. Incubation is for 2 hours at 37°C. Hydrolysis is then stopped with 1 ml of 40% trichloracetic acid and 1 ml of 0.1% sodium nitrite is added to a 1 ml sample of the mixture in a colorimetric tube. Three minutes later, after diazotization is complete, excess sodium nitrite is decomposed with 1 ml of 0.5% ammonium sulfamate. A blue color is then produced by addition of 2 ml of N-(1-naphthyl) ethylenediamine dihydrochloride in 95% ethanol (0.5 mg per ml) and this is read against a blank on the spectrophotometer at 560 mμ. Values are recorded as optical density x 10^3.

TABLE I. Comparison of Serum Leucine Aminopeptidase (LAP) Activity* in Single and Twin Pregnancy in the Third Trimester.

Pregnancy	No. of cases	Wk gestation	Mean values and S.D.	Range
Twin	11	31-43	1072 ± 84	920-1230
Single	25	31-41	618 ±128	347- 860

* Values recorded as optical density × 10^3.

Results are presented in Table I. Comparison of the 11 patients with proven twin pregnancies with the control group representing the same stage of gestation (third trimester) reveals a significant difference. Using the t test of significance for non-paired samples, t = 12.7***, which exceeds the 0.1% level of significance.

LAP activity of amniotic fluid was found to be 50 units on 2 determinations.

Discussion. The etiology of the increase of serum LAP activity in single and twin pregnancy as well as in cancer and other diseases is unknown. Umbilical cord blood shows low levels of activity as do sera from patients with trophoblastic tumors(3). Tissue analyses of this enzyme in human placentas reveal no significant increase in LAP activity per unit of weight of placenta as gestation progresses(4).

Since the placental mass in twin pregnancy is usually greater than the placenta of a single fetus, our results would suggest that the amount of normally functioning placental tissue is related to LAP activity in the maternal serum.

Summary. Serum leucine aminopeptidase

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(LAP) activity during third trimester of pregnancy was significantly increased in 11 patients with documented twin pregnancy, when compared with 25 patients with a single fetus of same gestational age.

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Biological Assay of Human Urinary Erythropoietic Stimulating Factor (ESF)* (25963)

JOHN W. WINKERT† AND ALBERT S. GORDON

Department of Biology, Graduate School of Arts and Science, New York University, N. Y. City

Previous studies have demonstrated the presence of ESF in urine of patients with a variety of anemias(1-4). Because of the ready availability of Thalassemia patients with predictably low levels of hemoglobin our efforts have been restricted to these subjects. An attempt has been made to correlate concentration of ESF in the urine with hemoglobin level in the blood of donor subjects at the time urine was collected. Although the data obtained previously were insufficient to indicate any association between these factors(4), the evidence now available is sufficient to suggest that concentration of ESF in urine does relate to severity of anemia. This information is useful as a guide in selection of patients as donors of ESF. Based upon the hematocrit and reticulocyte responses to ESF, a bioassay has been developed which is probably more reliable than existing ones which depend upon the use of only one parameter in evaluating erythropoietic responses.

Materials and methods. Thirty-four samples of urine were collected from 13 patients with Thalassemia major at hemoglobin levels ranging from 2.5% to 8.0%. The samples were frozen immediately and subsequently thawed, filtered, and frozen in sterile injection bottles until assayed. Mature 170 to 230 g Long-Evans female rats, maintained upon a definite surplus supply of water and Purina Laboratory Chow for at least 2 weeks after removal from the stock cages, were used for assessment of erythropoietic effects. The size of the groups used varied from 3 to 5 animals depending upon the precision required and availability of material. Each rat received 5 daily 3 ml subcutaneous injections of urine or extract. Microcapillary hematocrit and reticulocyte values were determined prior to assay, and upon the day after last injection. Reticulocytes were stained with new methylene blue according to the procedure of Brecher *et al.*(5). With the aid of an ocular counting disk, one-fourth of the erythrocytes in a microscopic field were counted while all reticulocytes in the field were enumerated and divided by 4. The percent reticulocytes was calculated after counting 1000 cells.

Results. In Fig. 1 and 2, reticulocyte and hematocrit responses are plotted separately as functions of hemoglobin levels of donor patients. Two equations were derived from the analysis of these relations by the method of least squares(6). The first equation describes the regression of reticulocyte responses in recipient rats (y) upon hemoglobin levels of donor patients (x).

$$(a)\quad y = -2.1x + 13.2$$

The second equation represents the regression of hematocrit responses in recipients (z) upon donor hemoglobin levels (x).

$$(b)\quad z = -1.8x + 11.1$$

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