cortical potentials. Various controls (confirming Chang and Kaada's conclusion for the sensory response) indicate that these spike-like potentials are in both cases afferent fiber potentials, and thus represent presynaptic events for the cortical neurones. The significance of the formal analogy between the sensory and the callosal response of visual area is discussed. A callosal volley can exert on a subsequent sensory response of the visual area the same facilitatory effect which has been described by the author for auditory-callosal and -sensory impulses.

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Received July 1, 1955. P.S.E.B.M., 1955, v90.

Influence of Pyridoxine on Transaminase Activity of Human Placenta, Maternal and Fetal Blood.* (21929)

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The response of pregnant women to tryptophane(1,2) and pyridoxal loading tests(3) are characteristic of subjects with vit. B_6 deficiency(4,5). The abnormal excretion of xanthurenic acid after tryptophane loading can be abolished by daily administration of 10 mg of pyridoxine hydrochloride(6). This would imply that the altered tryptophane metabolism in normal pregnancy is the result of a deficiency in vit. B_6. Nevertheless, Vilter *et al.*(7) were "unable to demonstrate any other evidence of vit. B_6 deficiency by blood, urine, or tissue levels of this vitamin, or by measuring output of pyridoxic acid in urine." It would seem desirable, therefore, to search for some other evidence of vit. B_6 deficiency to justify large supplements of pyridoxine to diets of pregnant women. There is considerable evidence that pyridoxine derivatives, pyridoxal phosphate and pyridoxamine phosphate, serve as coenzymes of transamination reactions(8). Some tissues of pyridoxine deficient animals have a reduced transaminase activity.

Therefore, we investigated the effect of pyridoxine supplements on transaminase activity of certain fetal and maternal tissues. Blood levels were studied, inasmuch as Marsh, Greenberg and Rinehart(9) have demonstrated a correlation between pyridoxine intake and glutamic-aspartic transaminase activity of hemolyzed blood in monkeys and man. The placenta was also studied because it is the only other available fetal tissue.

Methods. Subjects were divided as follows: Group I: Ten non-pregnant healthy women who did not receive a pyridoxine supplement. Group II: Eighteen pregnant women without pyridoxine supplement.

* Supported by grants from the National Heart Institute of the National Institutes of Health, Public Health Service; and from the Christine Breon Fund.

Group III. Eight pregnant women who received 10 mg pyridoxine hydrochloride per day during the last 4-6 weeks of pregnancy.† Transaminase activity was measured on bloods of Groups I to III and on placentas of Groups II and III. Cord bloods as well as maternal bloods were collected in 10 subjects of Group II. Blood samples from pregnant women were collected at time of delivery, using potassium oxalate as the anticoagulant. After aliquots were removed for cell counts and hematocrit levels, the remainder was repeatedly frozen and thawed in an equal volume of distilled water. Hemolyzed blood was centrifuged lightly for one minute and the clear red supernate used for transaminase determinations. Freshly delivered placentas were drained of blood, placed in plastic bags and stored in frozen state. Breis were prepared by dissection and homogenization for 20 seconds in a cold Waring Blendor. Undiluted breis were routinely freed of connective tissue and frozen before further extraction. Frozen breis retained their original activity for several months despite repeated freezing and thawing. At the time of determination, 0.5 g of freshly thawed brei were ground in a cold mortar and transferred to a graduated centrifuge tube with small portions of ice cold 0.1 M phosphate buffer, pH 7.4. The final volume was adjusted to 10 ml. The homogenate was centrifuged lightly for one minute and 0.2 ml of supernate used in each tube for transaminase determinations. Glutamic-aspartic transaminase activity was determined by a modification of the method of Tonhazy, White and Umbreit(10). The reagents were the same as those of the original method except that trichloracetic acid was omitted because of high blank values in its presence. Instead, enzyme reaction was stopped with aniline citrate to which had been added hydrochloric acid equivalent to the trichloracetic acid omitted. Blanks and standards contained the same amount of homogenate and substrates as did the reaction mixtures. The substrates (and pyruvate in the standards) were added *after* the aniline citrate reagent in the blanks and standards. Inordinately high blanks were obtained if alpha-ketoglutaric acid was omitted. It was necessary to use the means of triplicate determinations for blanks, standards and reaction mixtures to obtain consistent results. Some of the difficulties encountered with this method have been discussed by Marsh, Greenberg and Rinehart(9). Preliminary investigation indicated that Q_T^{10}, as defined by Tonhazy, *et al.* (10), best expressed enzyme activity for comparison between placentas. The activity of blood was expressed as the number of μl of oxalacetate formed per hour per ml of blood, calculated from the amount of pyruvate obtained after incubation of 0.1 ml of blood for 10 minutes.‡

TABLE I. Transaminase Levels in Blood.

Group	No. in group	Transaminase activity (μl oxalacetate)
I. Non-pregnant women	10	734 ± 129*
II. Pregnant women	18	748 ± 173
III. *Idem* + 10 mg pyridoxine HCl daily	6	984 ± 129
IV. Cord bloods†	10	1467 ± 194

* Means and stand. dev. of observations.
† From babies of 10 of the women of Group II.

Results. Levels of transaminase in blood samples are shown in Table I. Pregnancy appeared to have no effect. Addition of 10 mg of pyridoxine hydrochloride to the daily diet of pregnant women for several weeks resulted in a significant increase in transaminase activity ($P<0.01$). In contrast to effect on blood activity, supplements of pyridoxine had no effect on the transaminase activities of the placentas. The mean Q_T^{10} values with standard deviations were 37.9 ± 6.2 and 35.3 ± 6.0 for the supplemented and unsupplemented groups respectively. Cord bloods all contained high concentrations of transaminase activity. There was no correlation between blood levels of mothers and those of their infants. Although transaminase activity does exist in plasma(11), the method used is not sensitive enough for its measurement. Therefore, the

† We are indebted to the Stuart Co. for a generous supply of their Prenatal Capsules, some of which were specially prepared free of pyridoxine.

‡ Units, expressed as μg of sodium pyruvate/10 min./ml of blood(9), multiplied by the factor 1.22 correspond to the units used here.

TABLE II. Red and White Blood Cell Counts. Ten patients in each group.

Group	R. B. C. (millions)	W. B. C. (thousands)
I. Non-pregnant women	4.44 ± .8*	8.2 ± 1.8
II. Pregnant women (during delivery)	4.74 ±1.1	16.0 ± 3.4
IV. Cord bloods	4.76 ± .4	16.4 ± 3.7

* Means and stand. dev.

variations noted must have occurred in activities of individual cells, or in the number of cells, or both. The red cell counts of cord bloods did not differ significantly from those of any other group, nor did the white cell counts of cord bloods differ from those of mothers, although both were twice those of the non-pregnant controls (Table II). It would, therefore, appear that the activity of fetal blood cells was exceptionally high, when compared to maternal or non-pregnant subjects.

Transaminase activity of blood could be influenced very rapidly by raising the level of pyridoxine ingestion. This was demonstrated in the case of a patient hospitalized for mild toxemia (Table III). After transaminase activity had been determined on a control blood sample, 150 mg of pyridoxine hydrochloride per day were given by mouth. On the third day of supplementation, the transaminase activity showed an increase of 81%, and 32 hours later, an increase of 120%. Transaminase activity of cord blood fell within the range of those from unsupplemented mothers.

Discussion. The average American daily diet contains from 1.4 to 1.7 mg of pyridoxine (12). On the basis of observations of Marsh, Greenberg and Rinehart(9), it would be expected that a true deficiency of vit. B_6 should result in blood transaminase levels significantly lower than those found in healthy non-pregnant subjects. No significant difference was found as the result of normal pregnancy. Pregnant women responded to B_6 supplements with increased levels of blood transaminase, but this is equally true in non-pregnant subjects(9).

TABLE III. Effect of Pyridoxine Supplements on Blood Transaminase in the Same Individual.

Time of sample	Transaminase (μl oxalacetate)	R.B.C. (mill.)	W.B.C. (thous.)
8/10/54 (control level)	390	4.54	9.4
8/13/54	661	4.46	9.4
8/14 *	855	4.88	18.4
Cord blood	1660	5.10	20.8

* Immediately after delivery of baby.

Placental transaminase activity was not influenced by the pyridoxine supplements. This could mean that glutamic-aspartic transaminase activity of the human placenta, like that of the adult rat liver(13), is relatively insensitive to profound changes in vit. B_6 concentration, or more likely, that it has accumulated from maternal sources an optimal supply of pyridoxine. Transaminase activity of term placenta, expressed as units/mg of solids, is considerably higher than fetal cord blood, which itself is much higher than maternal blood.

With respect to fetal blood, the data suggest that the infant's transaminase system is well supplied with pyridoxal or pyridoxamine cofactors, perhaps at the mother's expense. If this is a "desirable situation" for the fetus, it might be reasoned that it should be desirable for the adult. The pregnant woman may be able to maintain a "normal" blood transaminase level, by non-pregnant adult standards, and still have insufficient reserve on which to draw under such conditions of stress as tryptophane loading. If one assumes, however, that increasing the transaminase level of maternal blood by pyridoxine supplements is desirable, the same reasoning would hold true for the non-pregnant adult population.

Summary. 1. The glutamic-aspartic transaminase activity of whole blood from normal pregnant subjects is essentially the same as in non-pregnant subjects. Activity is significantly increased by supplementing the diet with 10 mg of pyridoxine daily. Transaminase activity of fetal blood is twice as great as in maternal blood in mothers not receiving additional vit. B_6. The transaminase activity of placental tissue is not altered by prior administration of pyridoxine for several weeks. 2. The results suggest that fetal tissues contain optimal quantities of B_6, whereas adults, both pregnant and non-pregnant, contain suboptimal concentrations for peak enzymatic

activity. The method employed is not sufficiently sensitive to demonstrate a reduced "reserve" of B_6 in normal gestation, but the data are compatible with the view that pyridoxine supplementation is desirable in human pregnancy.

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Received July 5, 1955. P.S.E.B.M., 1955, v90.

Effect of 2,4-Dinitrophenol on Sea-Urchin Sperm. (21930)

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2,4-Dinitrophenol (DNP) has a similar effect on many metabolic systems(1), stimulating respiration and inhibiting energy-requiring processes in low concentrations and inhibiting respiration as well as endergonic processes in high concentrations. Lardy and Phillips(2) showed that DNP stimulates respiration of bull sperm and reduces sperm motility. Since metabolism of sea-urchin sperm differs in several respects from that of mammalian sperm(3,4), it was of interest to examine the effect of DNP on sea-urchin sperm. Dinitrophenol stimulates respiratory rate and causes a reversible block to cleavage in sea-urchin eggs(5,6), but no investigations have been reported concerning the effect of DNP on sea-urchin sperm. Sperm of sea urchins and other animals exhibit "Dilution Effect" (4), characterized by rapid decrease in length of motile life and fertilizing capacity of sperm, and by temporary rise in respiratory rate, upon dilution of the suspension, the rate then falling off toward zero as sperm lose their motility. Metal-chelating agents, such as glycine and ethylenediamine tetracetic acid (Versene), can largely abolish the symptoms of the "Dilution Effect"(7-9), *i.e.* they extend the life span of sea-urchin sperm and suppress the temporary rise in respiratory rate, maintaining respiration at a low, relatively steady level.

It was, therefore, of interest to examine the effect of DNP on sea-urchin sperm in the presence of a metal-chelating agent such as Versene.

Materials and methods. Sperm from the sea urchin *Strongylocentrotus purpuratus* were used. Oxygen consumption was measured with the Warburg respirometer. Dry semen was diluted to a concentration of 4×10^8 to 6×10^8 cells per ml with sea water or with 10^{-3}M Versene† in sea water, and 3 ml of these suspensions were placed in the main compartments of 25 ml Warburg flasks; the gas space in the flasks contained air; CO_2 was absorbed with alkali. Experiments were done at 17.8°C. Dinitrophenol (0.3 ml) was added to sperm suspensions from magnetically control-

* Postdoctoral Fellow of the National Science Foundation.

† I am indebted to Versenes Inc., Framingham, Mass., for samples.