histologic studies of all types of such roots, the roots are fixed for at least 48 hours in a solution of 1.5 ml. of acetic acid, 8.5 ml. of formaldehyde, and 90 ml. of 50 per cent ethyl alcohol. Zirkle's (7) N-butyl alcohol procedure is used for imbedding in paraffin, and Land's gum arabic-potassium dichromate procedure, for attaching ribbons to the slides. The slides are (1) stained for approximately 30 minutes with dilute safranine prepared by adding 3 ml. of a 0.5 per cent solution of aqueous safranine to 70 ml. of water in a Coplin jar, (2) rinsed in water to remove excess stain, (3) stained for 5–10 minutes in a solution prepared by adding 3 ml. of Cartwright's picroaniline blue to 70 ml. of water in a Coplin jar, (4) rinsed again in water to remove excess stain, (5) dehydrated by carrying the slides through a series of alcohol dilutions to 95 per cent alcohol, (6) cleared in Diaphane solvent, and finally (7) mounted in Diaphane.

By this procedure the blue-staining hyphae of the Hymenomycetes associated with ectotrophic mycorrhizae are clearly contrasted with the red-staining elements of the short roots. The dark-colored hyphae of the pseudomycorrhizal fungi are not stained. Differentiation of the intracellular hyphae, which grow out from the Hartig net, was obtained by using Wratten filter B (No. 58) in the microscope lamp.

The results obtained by the use of the above technique suggest the possibility that the foliar decline diseases of pine may be related to a reversal of symbiosis, which causes the mycorrhizal fungi to become parasitic on the short roots when soil conditions become unfavorable.

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A Blood Test for Estimating the Week of Pregnancy[1]

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In a recent report (*1*) it was shown that the ability of human pregnancy plasma to inactivate pitocin increased over a thousandfold from the time of conception until term, and it was suggested that such a measurement might be useful in determining the week of pregnancy. The enzyme to which we refer as pitocinase is probably a peptidase and has not yet been fully characterized. In the present investigation it is shown that the concentration of this enzyme in the blood increases in a linear fashion for the first 16 weeks after conception, and that it is only during this period that the quantitative determination is reasonably accurate. Fortunately, it is during the first half of pregnancy that such information is usually desired, for beyond that time the activity, size, and osseous development of the baby are reasonably good criteria. The present report describes in detail the methods employed and the interpretation of the results.

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Method

Plasma from fresh, nonhemolyzed, oxalated blood, commercial pitocin, and saline are incubated at 37° C., and samples are removed at various times for assay of the residual pitocin. The plasma concentration and incubation times vary, of course, with the approximate duration of pregnancy. These are shown in Table 1. If the approximate duration is not known, more than one determination must be made. Immediately after mixing, one-quarter of the sample is withdrawn and diluted to 12.5 ml. with 0.85 per cent saline. One drop of 2 N acetic acid is added to bring the pH to about 5.5 (with chlorophenol red as an indicator), and the tube is placed in a boiling-water bath for 5 minutes. The sample is then filtered and neutralized (using bromthymol blue) with 1 drop of 2 N sodium hydroxide. This is the "zero time 100 per cent pitocin sample" with which the others are compared. The remaining portions, exactly equal in volume to the first, are withdrawn at the indicated times and treated in an identical manner. When incubations are prolonged for more than two hours, it is essential to incubate *in vacuo* in modified Thunberg tubes containing a few drops of toluene in order to prevent loss of enzyme activity through oxidation or bacterial action. Incubations of 24 hours are likely to give lower values because of shifting temperature optima.

TABLE 1

Wks. after conception	Range of units/ml. plasma	Incubation mixture (ml.)			Incubation times*	k′
		Pitocin	Plasma	Saline		
3–4	0.06–0.1	0.5	5.0	0	0, 18, 24 hrs.*	76
5–6	0.16–0.2	"	"	9.5	0, 18, 24 hrs.*	208
7–9	0.33–0.66	"	"	0	0, 1, 3, 5 hrs.*	76
10–12	1.0 –2.3	"	"	0	0, 20, 45, 90 min.	76
13–15	3.3 –6.0	"	"	4.5	0, 15, 30, 50 min.	139
16–18	10– 18	"	"	19.5	0, 15, 30, 45 min.	346
19–21	20– 30	"	"	Dilute to 50 ml.	"	693
22–24	35– 45	"	3.33		"	1,039
25–27	50– 65	"	2.0		"	1,732
28–30	60– 85	"	1.67		"	2,079
31–38	80–110	"	1.25		"	2,772

* When time exceeds 90 minutes, incubate anaerobically with toluene.

The relative amount of pitocin in each filtrate is then estimated by a standard U. S. P. oxytocic assay, using the isolated uterus of a rat or guinea pig. While details of the bio-assay cannot be presented in this brief communication, the technics and computations are well standardized (*2*). Obviously, the accuracy of the test hinges upon the care with which the bio-assay is made.

Using a sheet of semilog paper, the percentage of pitocin remaining in each sample is plotted (on the log scale) against time (on the arithmetic scale). Starting with 0 time and 100 per cent pitocin, a straight line is best fitted through the remaining points in order to obtain the average velocity of the reaction. The time in minutes where this line crosses the 50 per cent mark is called $t_{\frac{1}{2}}$, or the time for half destruction.

The value k′ (see Table 1) divided by the $t_{\frac{1}{2}}$ then gives the number of pitocinase units/ml. of plasma.

The value k′ is derived from the formula for a first-order reaction and takes into account the plasma dilution. In the formula, $k = \frac{1}{t} \times \log_e \frac{C_o}{C_t}$, k represents the velocity, C_o the concentration of the substrate (pitocin) at 0 time, and C_t the concentration of pitocin at the time the sample is removed. When $t = t_{\frac{1}{2}}$, the fraction $\frac{C_o}{C_t}$ always equals 2, and the equation may be written: $k = \frac{0.693}{t_{\frac{1}{2}}}$. We have defined one unit of pitocinase activity as 100 × k at 37° C., since in this instance the concentration of enzyme is proportional to the velocity of the reaction. The number of units of pitocinase/ml. of plasma therefore equals $\frac{69.3}{t_{\frac{1}{2}}} \times$ the plasma dilution. The value k′ is simply the plasma dilution × 69.3.

Results

Plasma pitocinase determinations were made on 22 normal nonpregnant women, and the mean value was 0.023 units ± S.E. 0.0024, with a standard deviation of 0.011. Curiously enough, this value fluctuates in the normal menstrual cycle, becoming 0.018 during the two weeks centering about the onset of the menstrual period and 0.044 during the two weeks centering about the time of ovulation. The highest value found, 0.06 units/ml., is indicated by Line 0 in Fig. 1 and may be compared with the mean level for women, represented by Line M.

Determinations on 24 normal men gave a mean value of 0.01 ± 0.0014, S.D. = 0.007. This is significantly lower than the value for women, since the difference between the means is 4.8 times the standard error of the difference between the means. It was noted that in four men taking 5 mg. of stilbestrol daily (for carcinoma of the prostate), the mean value was 0.04, or about the level of ovulating women. The presence of jaundice likewise produces an elevated level. It is not known whether these low and fluctuating proteolytic activities are due to the same peptidase found in pregnancy or to some protease which may have some slight inactivating effect upon pitocin.

During the first 16 weeks of normal pregnancy, 37 determinations were made (Fig. 1). Eighteen of these were made on four individuals in order to establish the basic curve, and the remainder are unselected cases, excluding only those with abnormalities of pregnancy such as hydatid moles, ectopic pregnancies, and threatened or incomplete abortions—all of which are usually associated with lower values. When the pitocinase values are plotted on a logarithmic scale, they fall on a straight line with a standard deviation of ± 5 days. In each instance, conception was assumed to have occurred two weeks before the expected onset of the first missed period. During the latter half of pregnancy, the curve begins to flatten, and the scatter is greater. When pre-eclampsia supervenes, abnormal values are almost always noted (*1*).

The slope of the line illustrated may be represented by the expression 6 (log y + 1.7). Up to 16 weeks after conception, therefore, we may say that the week of pregnancy is equal to 6 × ($\log_{10}$ of the plasma pitocinase + 1.7) ± a standard deviation of 0.7 weeks.

It may be seen that values above 0.07 units/ml. of plasma, corresponding to four weeks after conception, are diagnostic of pregnancy in women who are not jaundiced. There is no known pregnancy test which may be considered accurate prior to the fourth week. Despite its economy, this method, when used as a purely qualitative indication of pregnancy, requires more time and skill in its present form than a Friedman or Aschheim-Zondek test. The present data suggest that the test measures the product of placental volume and some type of specific activity, and that the latter factor is relatively constant when pregnancy is normal. The method may find usefulness, therefore, when quantitative information is desired.

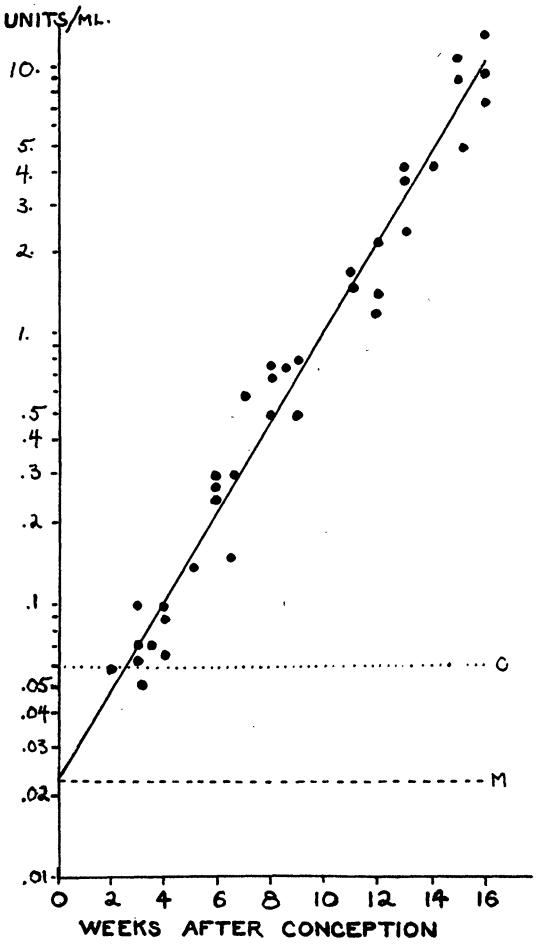

Fig. 1. Relation of plasma pitocinase concentration to the week of pregnancy. Ordinate represents the enzyme concentration in units/ml. plasma; Line M, the mean for nonpregnant women regardless of the phase of the menstrual cycle; Line O, highest level detected at the time of ovulation.

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