TABLE I.
Effect of Isomeric Thiocyanobenzoic Acid and the Thiocyanate Ion on Cytochrome Oxidase.

Compound	Effective conc. for cytochrome oxidase inactivation, mg %
Sodium thiocyanate	5000
Ortho-thiocyanobenzoic acid	300
Meta- " "	6
Para- " "	0.18

zoic acid shared this activity. In concentrations of from 1-7000 to 1-1000 the decolorization time for methylene blue was approximately doubled. This property is not shared by the thiocyanate ion(1).

Effect on cytochrome oxidase. A suspension of rabbit's brain brei supplied the cytochrome oxidase. Its activity was measured by the Nadi reaction. The reaction was allowed to proceed for 5 minutes, at 25°C. At the end of this period the absence of color compared with a control was considered inactivation of the oxidase.

These studies show that the thiocyanate ion is ineffective in inactivating cytochrome oxidase. Ortho-thiocyanobenzoic acid exhibits little inhibition of cytochrome oxidase activity. The para compound, however, shows a powerful nullifying action on cytochrome oxidase. This compound was shown to be free from any contamination with cyanide by chemical means.

Discussion. The thiocyanate radical attached to a phenyl group is a potent depressor agent, if the radical is meta or para, to the carboxyl group. The ortho compound does not share this activity. The relaxant action of the three isomeric acids on smooth muscle appears to be of the same order of magnitude. Brain reductase systems are inactivated by small concentrations of each of the isomeric acids. However, cytochrome oxidase is inactivated by the meta and para compounds but is not strongly affected by the ortho acid. Indeed on the blood pressure and on cytochrome oxidase the action of the ortho acid is somewhat similar to the thiocyanate ion and markedly dissimilar to the meta- and para-thiocyanobenzoic acids.

Summary. 1. The pharmacologic responses to the three isomeric thiocyanobenzoic acids have been investigated. 2. The meta and para compounds are powerful depressors. This action is not shared by the ortho compound. 3. p-Thiocyanobenzoic acid inactivates cytochrome oxidase in concentrations similar to cyanide. The meta compound is less active and the ortho isomer exhibits little inhibition. 4. All three isomers are relaxants to smooth muscle.

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Increases in Serum Thyroxin during Uncomplicated Pregnancy.* (17892)

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It has been established that an increase in serum precipitable or protein-bound iodine to levels above the euthyroid range of 4 to 8 γ % frequently occurs during pregnancy in human subjects(1). The studies herein reported indicate that the serum thyroxin also rises.

Materials and methods. Sera have been obtained at random for analyses from 57 gravid women usually during the second or third trimester. With the exception of 4 under the care of private physicians all were regular visitants to the dispensary of the Elizabeth Steel Magee Hospital. In each

* Aided by a grant from the Committee on Growth, National Research Council.

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TABLE I.
Circulating Protein-bound Iodine (PBI) and Thyroxin Are Increased in Pregnancy.

Group	No. of subjects	No. of sera*	PBI (Mean ± S.D.) γ %	No. of subjects	No. of sera*	Thyroxin (Mean ± S.D.) γ %
Control†	25	89	5.4 ± 0.7	23	23	3.7 ± 0.8
Pregnant	57	81	7.8 ± 1.3‡	21	23	5.4 ± 1.0§

* Analyzed in duplicate or triplicate.

† Consisting of healthy adults, males and non-pregnant females, since no statistically significant difference could be demonstrated in their protein-bound iodine and thyroxin concentrations.

‡ Significantly different from control group since "t" was found to be 11.57 and "p" = <0.0001.

§ As in preceding footnote with "t" = 4.76 and "p" <0.0001.

instance pregnancy proved to be free from major complications and terminated in the delivery of a living child at term. A total of 81 sera have been analyzed in duplicate or triplicate for the concentration of protein-bound or serum precipitable iodine by the method of Barker as used in this laboratory(2,3). In 43 of the patients blood was withdrawn for analysis only once. In the remaining 14 repeat samples were obtained one or more times during gestation. In view of the variable relationships between the serum proteins and protein-bound iodine(4), albumin and globulin concentrations(5,6) have been measured in all but a few of the pregnant subjects. In 23 sera from pregnant women with protein-bound iodine concentrations within and above the euthyroid range thyroxin levels were also measured using the method of Taurog and Chaikoff(7). For purposes of comparison thyroxin and protein-bound iodine analyses have been conducted in healthy non-pregnant adult females and males. The results are presented in Table I.

Results. A. *Protein-bound iodine levels.* The average value for this fraction in pregnancy proved to be significantly higher, statistically, than the mean recorded in non-pregnant adults (Table I). These results agree closely with the findings of others and support the conclusion that uncomplicated pregnancy is usually associated with a rise in the serum precipitable or protein-bound iodine(1). In 28 of the patients in our series the increase was of sufficient magnitude to place the protein-bound iodine above the upper limit of the range characteristic of euthyroid non-pregnant adults(8), 8.0 γ %. The highest value observed was 11.3 γ %. There were no discernible clinical signs of thyrotoxicosis in this subject nor in those with lesser elevations in protein-bound iodine. The results of serial analyses indicate that this rise is not necessarily stable. At times slight but probably definite increases or decreases were subsequently observed, while in others previous values were reduplicated. This contrasts with the relative constancy of this serum iodine fraction in non-pregnant subjects(9). In the patients in whom longer periods of observation are available there was evident a distinct trend upward during successive analyses. The recorded rises were not always of sufficient magnitude however to place the protein-bound iodine value above euthyroid limits.

B. *Thyroxin concentrations.* From Table I it is apparent that the serum thyroxin level measured usually in triplicate during pregnancy ranged between 3.4 and 6.9 γ %,

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averaging 5.4 ± 1.0. This differs significantly from the mean of 3.7 ± 0.8 γ % recorded in non-pregnant control subjects. These values represent the actual results of analyses. They have not been corrected for the loss, approximating 10%, observed in thyroxin recovery studies in this and in other laboratories(7), because of the uncertainty of its magnitude in serum analyses. Such a correction however would only serve to emphasize the validity of our findings.

A good correlation is evident between the level of thyroxin and of protein-bound iodine in pregnancy, represented in the line of best fit $y = 1.0008 + 0.5741x$, where y indicates thyroxin and x the protein-bound iodine. The standard error of estimate of "y" proved to be 0.667, and the correlation coefficient, +0.734.

Discussion. The reality of the rise in circulating protein-bound iodine during uncomplicated pregnancy in human subjects has been confirmed in these studies. It has also been shown that this rise is associated with a significant increase in the serum thyroxin which is of course a subdivision of the protein-bound iodine. It seems reasonable to suggest furthermore that changes more marked than these may occur in pregnancy since in most of our studies serum specimens were obtained at random and hence probably do not represent the maximal values attained. These findings invite speculation concerning factors possibly operative in inducing these changes. Excluding artefacts such as those induced by administering organic iodine dyes (10) the concentrations of circulating thyroxin and protein-bound iodine can be affected by (a) the rates at which these compounds are synthesized, (b) the rates at which they are released from their sites of origin, (c) the characteristics of the plasma transport system, and (d) the rates at which they are metabolized or excreted. No data are available which conclusively implicate or exclude any of these factors in the changes which have been observed. Although it has been shown in animals that the thyroidal uptake of radioactive iodine is increased during pregnancy, no comparable studies in humans have been reported(11). Furthermore, though it is true that an increased intake of exogenous inorganic iodide raises the protein-bound and thyroxin iodine in the gland(12) as well as the circulating inorganic and protein-bound iodine(13), there is no evidence that pregnancy is necessarily associated with a higher ingestion of iodine or iodide. The persistence of the elevations in thyroxin and protein-bound iodine makes it quite improbable that the observed changes represent merely an increase in the rate of discharge of stored thyroid gland products. On the other hand it is possible that during gestation the plasma proteins bind thyroxin and organic iodine in larger quantities than usual. In our series of patients, the concentrations of serum albumin and globulin were 3.55 ± 0.42 and 3.20 ± 0.34 g %. In keeping with the findings of others these averages are lower and higher, respectively, than those characteristic of non-pregnant control subjects(14). If it is accepted that the organic iodine of serum is bound chiefly to albumin, one would then expect a fall rather than a rise in protein-bound and thyroxin iodine. It is by no means certain however that serum albumin and protein-bound iodine levels are so directly related(4,15). It is possible therefore that protein fractions do bind more organic iodine during pregnancy. There is no evidence for or against the possibility that in these patients the excretion of iodine compounds through the liver, gastro-intestinal tract or other routes(16,17) is diminished. Moreover, the known elevation in basal metabolic rate which usually occurs in the last

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trimester(18), does not resolve any of the uncertainties which have been cited, since (a) the rise in protein-bound iodine can be demonstrated in the first trimester(1) and (b) euthyroid protein-bound iodine values have been recorded in our series during the last trimester. It is obvious that these questions can probably be answered by studies which define the rates of production, release, and degradation of thyroxin and related compounds. In conducting such studies with radioactive tracers it must be kept in mind that the fetal thyroid in humans assimilates iodide from the 12 to 16 week point onward (19). Finally, though it is not apparent which of the above factors are present in the observed findings, it seems reasonable to suspect that they are mediated through hormonal changes occurring during pregnancy.

Summary and conclusions. The rises in protein-bound iodine which usually occur in uncomplicated pregnancy in human subjects are associated with an increase in the thyroxin fraction of the protein-bound iodine.

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Differentiation between Bacterial and Tryptic Gelatin Liquefaction as Aid to Diagnosis of Fibrosis of Pancreas. (17893)

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The failure of fecal specimens from patients with cystic fibrosis of the pancreas to cause liquefaction of the gelatin coating on x-ray film, in contrast to specimens from normal children or patients suffering from other maladies, has been shown by Schwachman(1,2) to be a useful aid to the diagnosis of this pancreatic disease. The test is based on the fact that in cystic fibrosis of the pancreas trypsin is absent or present in subnormal amounts in the feces. As shown by Johnstone(3), cultures of certain bacterial species, particularly members of the genera *Proteus* and *Pseudomonas*, may also cause a positive x-ray film test. These microorganisms are not rarely encountered in the feces of infants and young children(4) and their presence may be the cause of false positive x-ray film liquefaction tests. An attempt was made, therefore, to develop a test which may differentiate between gelatin liquefaction due to pancreatic trypsin and that due to bacterial enzyme.

Material and methods. The x-ray film liquefaction test as used for the diagnosis of cystic fibrosis of the pancreas has been previously described(2,3). Since Eastman Kodak "Blue Brand" x-ray film proved to be more sensitive than either Dupont "Xtra Fast Medical" x-ray film or Hammer panchromatic portrait photographic film for this test, the former was used throughout this investigation. Soybean trypsin inhibitor was procured from Worthington Biochemical Laboratory (Freehold, N. J.) and was dis-

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