3. Values for mannitol clearance determined by the slope method were consistently lower than simultaneously measured urine clearances; therefore, mannitol clearance cannot be determined by the slope method.

4. Clearance of venous plasma when plasma concentration is falling is lower than clearance of arterial plasma.

5. Neither mannitol nor PAH is metabolized, but they are excreted exclusively by the kidney, since a high urinary quantitative recovery of each was obtained in 24 hours, and neither disappeared from the plasma of two anuric patients.

6. Although the variation was not consistent, there was an average slightly greater urine clearance at lower plasma levels of PAH than at higher levels as the plasma concentration decreased.

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Pharmacology of Isomeric Thiocyanobenzoic Acids.* (17891)

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In a former communication(1) the authors and Tawab studied the pharmacologic responses to meta- and para-thiocyanobenzoic acids. In the main, the actions elicited by the two compounds were identical. Upon intravenous injection in the dog and rabbit they produced marked depressor responses. These were accompanied by electrocardiographic changes. The meta- and para-thiocyanobenzoic acids relaxed smooth muscle. In low concentrations they inactivated brain dehydrogenases and cytochrome oxidase. This effect was not shared by the thiocyanate ion. Through the kindness of Dr. G. F. D'Alelio† a sample of orthothiocyanobenzoic acid was provided. It is the most difficult of the three isomers to prepare. We decided to compare its pharmacologic activity with that of the meta and para compounds. Experimental studies were conducted with the soluble sodium salts of the 3 isomeric acids.

Blood pressure studies—dogs. The blood pressure studies were conducted on 6 dogs under ether anesthesia. Carotid artery blood pressure was recorded and injections of the various compounds were made into the saphenous vein. Solutions of 0.5% and 1.0% of the sodium salts were used. In our former studies it was demonstrated that the meta- and para-thiocyanobenzoic acids produced a marked depressor response when a dose of 0.5 cc/kg of a 0.5% solution was injected. The respiration was increased in rate and amplitude. This was followed by a depression of respiration. A constant finding was a marked accentuation of the T-wave of the E.C.G. in Lead II. A typical blood pressure tracing is shown in Fig. 1. The effect of the ortho compound illustrates that this isomer does not share with the meta and para compounds the capacity of eliciting a depressor response in non-toxic doses. Larger doses produce a progressive fall in blood pressure which is invariably fatal. In contrast to the constant E.C.G. finding of T-wave accentuation with the meta and para compounds, the ortho compound produced no changes in the E.C.G. in non-toxic doses. Toxic doses caused a depression of the Q R S complex and a flattening to an inversion of the T-wave.

Action on smooth muscle. The action of the meta- and para-thiocyanobenzoic acids on the smooth muscle preparations of the rabbit's intestine and the rat's uterus *in vitro* was markedly relaxant. A concentration of

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1. Tawab, S. S. A., Carr, C. J., and Krantz, J. C., Jr., *J. Pharm. and Exp. Therap.*, 1949, v96, 416.

† Director of Research, Koppers Company, Inc., Pittsburgh, Pa.

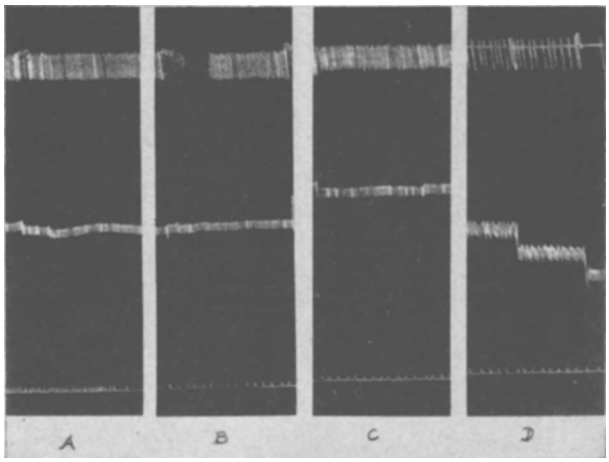

FIG. 1.
Effect of o-Thiocyanobenzoic acid on the blood pressure of the dog (male dog, 10.2 kg, ether anesthesia; normal blood pressure 114 mm Hg). A. Normal tracing. B. .5 cc/kg 1% sol. of o-thiocyanobenzoic acid. C. After 60 min. no appreciable change in blood pressure. D. 3 cc/kg additional in divided doses—progressive fall in blood pressure and followed by death.

6 mg of either isomer in a 30 cc bath caused a prompt depression of the frequency and amplitude of contraction. This action was not shared by the thiocyanate ion. Fig. 2 shows the action of ortho-thiocyanobenzoic acid on the rat's uterus; 12 mg of the ortho isomer elicited an activity comparable to 6 mg of the para compound.

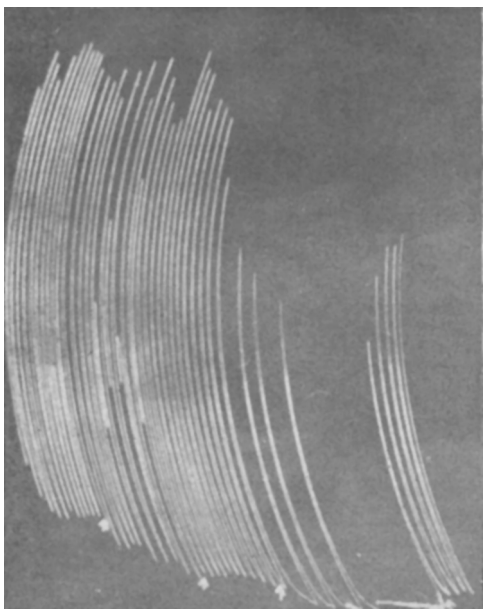

FIG. 2.
Effect of o-thiocyanobenzoic acid on the rat's uterus. At each arrow 3 mg was added to bath.

Acute toxicity—white rat. Acutely fatal intraperitoneal doses of the ortho-, meta- and para-thiocyanobenzoic acids elicited hyperirritability, hyperreflexia, tremors and transient convulsive seizures. This syndrome was followed rapidly by weakness of the extremities and flaccidity. The animals died of respiratory failure. The heart continued to beat for some time after respiratory collapse. Fatal doses usually killed within 30 minutes. The LD_{50} upon intraperitoneal injection into the white male rat (3 hours) for each of the 3 isomers is ortho 32 mg/kg, S.E. $= \pm 2.1$ mg; meta 17 mg/kg, S.E. $= \pm 1.3$ mg; para 22 mg/kg, S.E. $= \pm 1.9$ mg. For sodium thiocyanate the LD_{50}, under these conditions, is 540 mg/kg, S.E. $= \pm 42.5$ mg.

Effect on brain dehydrogenases. A suspension of rat's brain brei supplied the tissue dehydrogenase systems for these studies. The Thunberg methylene blue reduction technic was employed. The meta- and para-thiocyanobenzoic acids were shown to inhibit the dehydrogenase systems. Ortho-thiocyanoben-

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.

1. Heinemann, M., Johnson, C. E., and Man, E. B., *J. Clin. Invest.*, 1948, v27, 91.