Placental Transfer of 2,3,5-Triiodobenzoic Acid in the Rat

RICHARD W. McDOWELL*, ROBERT R. LANDOLT, WAYNE V. KESSLER, and STANLEY M. SHAW

Abstract □ The placental transfer of 2,3,5-triiodobenzoic acid and/or metabolites was studied using ^{14}C-labeled 2,3,5-triiodobenzoic acid. Placental transfer of 2,3,5-triiodobenzoic acid and/or metabolites occurred in both cold-stressed and control rats. The maternal blood of cold-stressed and control rats contained significantly higher concentrations of 2,3,5-triiodobenzoic acid and/or metabolites than the blood of respective fetal rats. However, cold stress did not significantly alter either placental transfer or the level of 2,3,5-triiodobenzoic acid and/or metabolites in the maternal and fetal tissue. Cold stress had no apparent effect upon the plasma level of either 2,3,5-triiodobenzoic acid, 2,5-diiodobenzoic acid, or 3,5-diiodobenzoic acid in maternal or fetal animals. While nonpregnant rats acclimated to cold stress, pregnant rats did not, indicating cold stress and pregnancy may act synergistically.

Keyphrases □ 2,3,5-Triiodobenzoic acid-^{14}C—placental transfer □ Placental transfer, 2,3,5-triiodobenzoic acid-^{14}C—rats □ Fetal, maternal levels—2,3,5-triiodobenzoic acid-^{14}C, metabolites □ Cold-stress effect—urinary excretion, 2,3,5-triiodobenzoic acid-^{14}C □ Scintillometry—analysis

The presence of 2,3,5-triiodobenzoic acid in the environment has created concern regarding its toxicity. Results of various studies (1–3) show that the compound is tolerated in the adult rat, guinea pig, and man. Increased fetal malformations in mice and rats by environmental contaminants (4) strongly suggest that the degree of placental transfer is another parameter for the determination of the potential toxicity of an environmental contaminant.

The present study was conducted to determine the placental transfer, metabolism, and urinary excretion of 2,3,5-triiodobenzoic acid in rats. Cold-stressed as well as normal rats were studied since stress has been shown to alter the metabolism (5), the excretion (6), and the action (7) of various compounds. The influence of stress on placental transfer seems pertinent since many environmental stimuli have been found to cause a stressed condition in man and animals.

EXPERIMENTAL

Radiochemical—Carboxyl-^{14}C-labeled 2,3,5-triiodobenzoic acid (2,3,5-triiodobenzoic acid*) was available from the work reported by Spitznagle *et al.* (8) and was purified by thick-layer chromatography using the procedure of Jarboe *et al.* (9). Chemical purity was determined by spotting separately both 2,3,5-triiodobenzoic acid* and reference standard 2,3,5-triiodobenzoic acid[1] on thin-layer plates of purified silica gel[2]. Separate plates were developed in one of two solvent systems: petroleum ether–propionic acid (10:1 v/v) or propionic acid–methanol–benzene (1:2:10 v/v). UV light and a 0.1% bromcresol green solution were used to detect spots. No chemical impurities were found.

Radiochemical purity was determined by spotting 2,3,5-triiodobenzoic acid* on thin-layer plates of purified silica gel[3] and developing the plates in the solvent systems, already described. Radioactive spots were located with X-ray film[4]. The spot containing 2,3,5-triiodobenzoic acid* was scraped into a counting vial, and the radioactivity was determined. The radioactivity of the remaining sorbent on the plate was determined similarly. Of the total radioactivity recovered, 97.9 ± 0.3% was present as 2,3,5-triiodobenzoic acid*.

Radioactivity Measurements—Tissue samples (30–75 mg.) were placed in tared counting vials and weighed. Then 1 ml. of a tissue-digesting solution[5] was added to each vial, and the vials were heated for 2 hr. at 60° with occasional agitation. One-half milliliter of 30% hydrogen peroxide was added to decolorize the samples. The samples were heated at 60° for an additional hour and cooled. Glacial acetic acid, 0.5 ml., was added to each sample to reduce protein phosphorescence.

Urine samples were prepared by adding 50–100-μl. aliquots of urine to the counting vials.

Fifteen milliliters of scintillator[6] was added to each urine or tissue sample. The samples were counted in a liquid scintillation spectrometer[7] using the internal standard method (11) to determine the absolute disintegration rates. The maximum counting error was 1.2%.

Animal Care and Mating—Sprague-Dawley[8] female rats, weighing 200–250 g., were housed in individual screen-bottom hanging cages and allowed free access to food[9] and water. The animals were maintained in a lighted environment from 6 a.m. to 8 p.m. and were handled for 5–10 min. daily during pregnancy to reduce stress (12). They were mated during the period from October to April using the procedure described by Mork *et al.* (13). They were assigned to treatment groups by using a table of random numbers.

Placental Transfer of 2,3,5-Triiodobenzoic Acid*—Forty pregnant rats, weighing 290–350 g., were injected intraperitoneally with 15 μc. (1.77 mg.) of 2,3,5-triiodobenzoic acid* per 300 g. of body weight on Day 19 of gestation. Four days prior to injection, 20 pregnant rats were placed in individual metabolism cages in an environmental chamber[10] maintained at 4° and illuminated for 14 hr. daily. The remaining animals were placed in individual metabolism cages at room temperature with the same light conditions.

Five animals from each treatment group were sacrificed 2 hr. after injection. Additional groups of five animals from each treatment group were sacrificed at 4, 6, and 8 hr. after injection. Urine was collected at 2, 4, 6, and 8 hr. after injection.

Maternal animals were sacrificed with a small guillotine and exsanguinated. The blood was collected in a heparinized beaker. Samples were removed for measurement of radioactivity of whole blood. The remainder was transferred to a heparinized 15-ml. test tube and centrifuged. The plasma was decanted and stored frozen for metabolite studies. The maternal adrenals were removed, trimmed to remove connective tissue, weighed, frozen in liquid nitrogen, and stored frozen until assayed for ascorbic acid by the procedure of Maickel (14). Samples of maternal liver were obtained.

The fetuses were removed from the uterus of each maternal animal. Samples of whole fetal blood were collected in heparinized capillary tubes[11], centrifuged, and stored frozen for metabolite studies. Samples of fetal whole blood, fetal liver, and entire fetus were also obtained for each maternal animal. To obtain a homogeneous sample of the entire fetus, two fetuses per maternal rat were frozen in liquid nitrogen, placed between sheets of plastic, and pulverized. A small precooled spatula was used to mix the fragments thoroughly. Different fetuses were used for the different tissue samples.

[1] International Minerals and Chemical Corp., Skokie, Ill.
[2] A *mixture of Adsorbosil-1* and Adsorbosil P-1 (2:1), Applied Science Laboratories, State College, Pa.
[3] Adsorbosil-1.
[4] No-Screen X-Ray Film, Eastman Kodak Co., Rochester, N. Y.
[5] A methanolic solution of hyamine hydroxide prepared according to Bruno and Christian (10) from hyamine 10-X crystals, Rohm & Haas, Philadelphia, Pa.
[6] Contained 1 part *p*-xylene, 3 parts *p*-dioxane, 3 parts 2-ethoxyethanol, 8% naphthalene, and 1.0% 2,5-diphenyloxazole.
[7] Tri-Carb, Packard Instrument Co., Inc., Downers Grove, Ill.
[8] Sprague-Dawley, Inc., Madison, Wis.
[9] Wayne Lab-Blox, Allied Mills, Inc., Chicago, Ill.
[10] Model 683, Hotpack Corp., Philadelphia, Pa.
[11] The method of Mork *et al.* (13) was used.

Table I—Concentration of 2,3,5-Triiodobenzoic Acid[a] in Various Rat Tissues

Treatment[b]	Hours[c] 2	4	6	8
		Maternal Blood		
Cold-stressed	21.76 ± 3.90	15.90 ± 3.40	21.05 ± 4.08	15.93 ± 5.64
Control	13.44 ± 6.06	15.52 ± 5.18	20.23 ± 3.31	18.73 ± 2.65
		Fetal Blood		
Cold-stressed	3.80 ± 1.91	4.40 ± 0.98	5.01 ± 0.96	8.03 ± 4.04
Control	3.42 ± 0.51	3.81 ± 1.32	6.49 ± 1.07	5.61 ± 0.42
		Maternal Liver		
Cold-stressed	6.71 ± 2.09	5.31 ± 0.99	6.69 ± 1.26	5.14 ± 1.55
Control	5.66 ± 1.36	5.45 ± 1.06	6.15 ± 1.30	5.82 ± 0.48
		Fetal Liver		
Cold-stressed	2.28 ± 0.39	3.12 ± 0.56	4.26 ± 0.59	4.75 ± 2.19
Control	2.44 ± 0.90	2.92 ± 0.89	4.85 ± 1.34	4.31 ± 0.56
		Fetal Tissue[d]		
Cold-stressed	1.57 ± 0.52	2.50 ± 0.67	3.76 ± 0.98	4.08 ± 1.50
Control	1.29 ± 0.23	2.58 ± 0.59	3.89 ± 0.64	4.74 ± 0.67

[a] Expressed as micrograms of 2,3,5-triiodobenzoic acid and/or metabolites per gram of tissue ± standard deviation. [b] Five maternal animals per treatment group per time interval. [c] Time elapsed between injection and sacrifice. [d] Homogeneous sample of entire fetus.

Plasma Metabolites—Maternal and fetal blood plasma was analyzed to determine the relative amounts of 2,3,5-triiodobenzoic acid and its metabolites that transferred to the fetus. The plasma was extracted with ethanol, which was found in preliminary studies to remove greater than 99% of the 2,3,5-triiodobenzoic acid and its metabolites. Fifteen-microliter aliquots of the ethanol extracts were spotted on TLC plates of purified silica gel[2]. Reference standard 2,3,5-triiodobenzoic acid and the two suspected metabolites, 2,5-diiodobenzoic acid and 3,5-diiodobenzoic acid, were spotted on each plate. The plates were developed in petroleum ether–propionic acid (10:1 v/v). Each spot was located with UV light and was then scraped quantitatively into counting vials containing liquid scintillator. The vials were counted and the results were expressed as the percent of total radioactivity recovered from each sample spotted on the thin-layer plate.

Effect of Cold Stress on Urinary Excretion of 2,3,5-Triiodobenzoic Acid—Twelve nonpregnant female rats were injected intraperitoneally with 5.06 μc. (0.52 mg.) of 2,3,5-triiodobenzoic acid*. Six were placed in metabolism cages and were cold stressed as already described for 4 days previous to the administration of 2,3,5-triiodobenzoic acid* and during the time of urine collection. The other six were kept in metabolism cages at room temperature. Urine from each animal was collected at 3-hr. intervals over a 24-hr. period. Aliquots were prepared for counting and the ^{14}C was determined. The results were expressed as the percent of the ^{14}C dose excreted. After 24 hr., the animals were sacrificed and the adrenals were removed, weighed, frozen, and stored for ascorbic acid determination.

Statistical Analysis—Analysis of variance (15) was run to test for significant differences, at the 0.05 confidence level, in the amount of 2,3,5-triiodobenzoic acid in each tissue sample. Variables tested included treatment, time, and interaction between treatment and time. If no interaction between treatment and time was found, stress and control data were pooled at each time interval and com-

Table II—Relative Amount 2,3,5-Triiodobenzoic Acid and Metabolites in Blood Plasma

Hours[a]	Percent of Total Radioactivity[b] Origin	2,3,5-Triiodobenzoic Acid	2,5-Diiodobenzoic Acid	3,5-Diiodobenzoic Acid	Solvent Front
			Stress Maternal[c]		
2	8.7 ± 4.1	73.8 ± 5.3	12.1 ± 1.3	2.0 ± 0.6	1.2 ± 0.4
4	7.8 ± 3.9	71.8 ± 2.6	16.0 ± 4.2	2.6 ± 1.2	1.5 ± 1.4
6	10.6 ± 4.7	67.7 ± 8.6	21.0 ± 4.4	2.3 ± 0.7	0.5 ± 0.6
8	5.7 ± 0.1	64.6 ± 9.5	26.6 ± 9.3	2.4 ± 1.1	0.5 ± 0.5
			Stress Fetal		
2	7.4 ± 4.7	62.5 ± 10.8	18.0 ± 1.6	6.9 ± 3.5	5.2 ± 3.4
4	5.6 ± 2.5	65.5 ± 2.4	18.6 ± 3.7	7.0 ± 2.9	3.3 ± 1.5
6	5.2 ± 2.6	65.7 ± 7.1	22.3 ± 5.0	4.2 ± 3.5	2.4 ± 3.1
8	8.4 ± 3.9	57.1 ± 9.9	22.1 ± 5.6	6.6 ± 3.1	5.7 ± 3.7
			Control Maternal[d]		
2	15.5 ± 7.4	68.2 ± 8.7	14.4 ± 2.9	1.5 ± 0.4	0.4 ± 0.2
4	15.5 ± 7.5	63.9 ± 2.9	18.2 ± 7.0	1.9 ± 0.6	0.5 ± 0.4
6	11.3 ± 8.0	67.0 ± 8.0	17.6 ± 1.6	2.3 ± 1.2	1.4 ± 1.2
8	9.6 ± 2.6	63.4 ± 9.9	21.9 ± 11.4	1.7 ± 0.1	0.9 ± 0.9
			Control Fetal		
2	4.1 ± 2.9	61.5 ± 13.7	20.1 ± 8.6	7.6 ± 3.4	6.1 ± 4.8
4	10.4 ± 4.1	53.1 ± 8.4	23.7 ± 6.5	7.3 ± 3.6	5.5 ± 2.1
6	3.8 ± 1.9	56.5 ± 16.0	18.0 ± 4.2	7.8 ± 5.2	13.8 ± 10.7
8	8.6 ± 5.8	60.7 ± 8.2	20.9 ± 7.5	4.4 ± 2.3	5.4 ± 4.2

[a] Time elapsed between injection and sacrifice. [b] Percent of total radioactivity recovered from a sample spotted on a thin-layer plate expressed as mean value ± standard deviation. [c] Four maternal animals per time period. [d] Five maternal animals 2 hr. postinjection, six maternal animals 4 hr. postinjection, and four maternal animals each at 6 and 8 hr. postinjection.

Table III—Fetal/Maternal Blood Plasma Ratios of 2,3,5-Triiodobenzoic Acid[a]

Treatment	Hours[b] 2	4	6	8
Cold-stressed	0.85 ± 1.15	0.91 ± 0.04	0.98 ± 0.12	0.87 ± 0.15
Control	0.94 ± 0.14	0.84 ± 0.16	0.84 ± 0.20	0.97 ± 0.20

[a] Ratios calculated from data in Table II. [b] Time elapsed between injection and sacrifice.

pared to pooled data at other time intervals. The Student *t* test (16) was used to determine significant differences at the 0.05 confidence level for urine samples as well as for 2,3,5-triiodobenzoic acid and its metabolites in blood plasma.

RESULTS AND DISCUSSION

Placental Transfer of 2,3,5-Triiodobenzoic Acid—The ^{14}C activity detected in tissue was expressed as 2,3,5-triiodobenzoic acid even though metabolites may have been present. Data in Table I show that placental transfer of 2,3,5-triiodobenzoic acid occurred at each time interval studied. The 2,3,5-triiodobenzoic acid levels in the tissue of cold-stressed maternal and fetal rats in relation to 2,3,5-triiodobenzoic acid in respective control tissue showed no significant differences. Since no interaction between treatment and time was found, stress and control data were pooled at each time interval and compared statistically. Although occasional statistically significant differences were observed, the pooled data did not consistently indicate an influence of time on placental transfer or the level of 2,3,5-triiodobenzoic acid in maternal or fetal tissue.

The maternal blood of cold-stressed and control rats contained significantly higher concentrations of 2,3,5-triiodobenzoic acid than did the blood of respective fetal rats at each time interval. However, cold stress did not significantly affect the levels of 2,3,5-triiodobenzoic acid in either the maternal blood or the fetal blood; it would thus appear that exposure to cold did not affect the placental transfer of 2,3,5-triiodobenzoic acid.

Plasma Metabolites—Actual 2,3,5-triiodobenzoic acid, exclusive of metabolites, was determined in this phase of the investigation. The relative amount of 2,3,5-triiodobenzoic acid in the maternal blood plasma of cold-stressed and control rats (Table II) did not vary significantly over the 2–8-hr. postinjection period. Except for the 4-hr. postinjection interval, the blood plasma level of 2,3,5-triiodobenzoic acid in maternal cold-stressed rats was not significantly different from that present in respective control animals. No significant differences between cold-stressed and control rats were found at any time interval for fetal blood plasma levels of 2,3,5-triidobenzoic acid. No significant differences in ratios (Table III) of 2,3,5-triiodobenzoic acid in fetal blood plasma to maternal blood plasma due to treatment or time within a single treatment were found. Apparently a constant percentage of the 2,3,5-triiodobenzoic acid in maternal blood plasma was present in fetal blood plasma at each time interval. The data suggest that exposure to cold did not affect the placental transfer of 2,3,5-triiodobenzoic acid.

Comparison of the concentration of 2,5-diiodobenzoic acid in the fetal blood plasma with 2,5-diiodobenzoic acid in respective maternal blood plasma showed no significant difference in the 2,5-diiodobenzoic acid level for stressed or control animals, with the exception of the cold-stressed group at the 2-hr. interval. The concentration of 3,5-diiodobenzoic acid was significantly higher in fetal blood plasma than in respective maternal blood plasma for both stressed and control rats at 2 and 4 hr. postinjection and at 8 hr. postinjection for the control animals. The data suggest a selective transport mechanism for 3,5-diiodobenzoic acid which was not affected by cold stress. The results of this phase of the investigation indicate that exposure to cold did not affect the placental transfer of either 2,3,5-triiodobenzoic acid or its metabolites.

Effect of Cold Stress on Urinary Excretion of 2,3,5-Triiodobenzoic Acid—Nonpregnant cold-stressed rats excreted a significantly higher

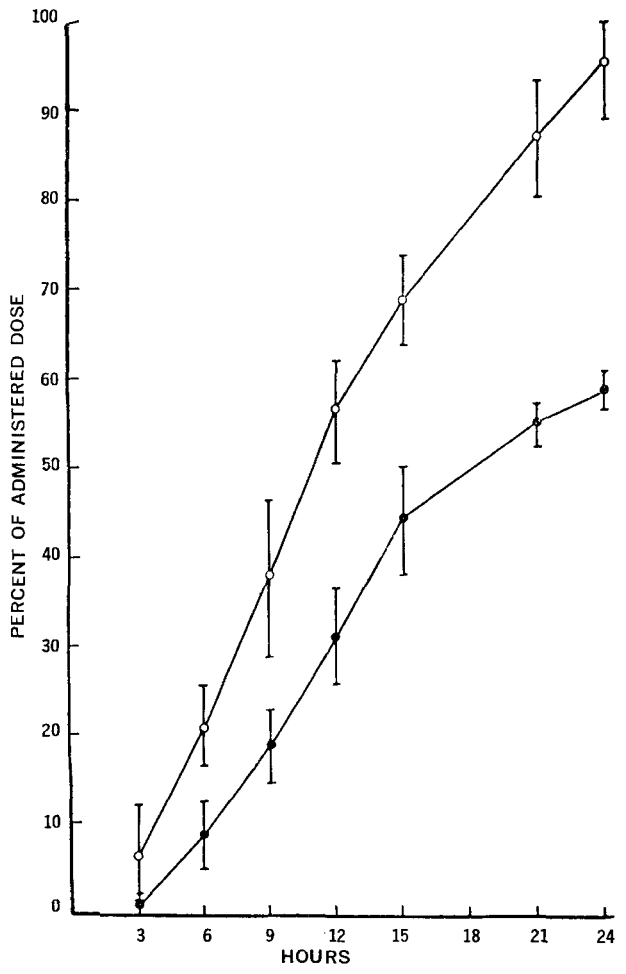

Figure 1—*Urinary excretion of 2,3,5-triiodobenzoic acid and/or metabolites by nonpregnant rats. Key: ○, cold-stressed; and ●, control.*

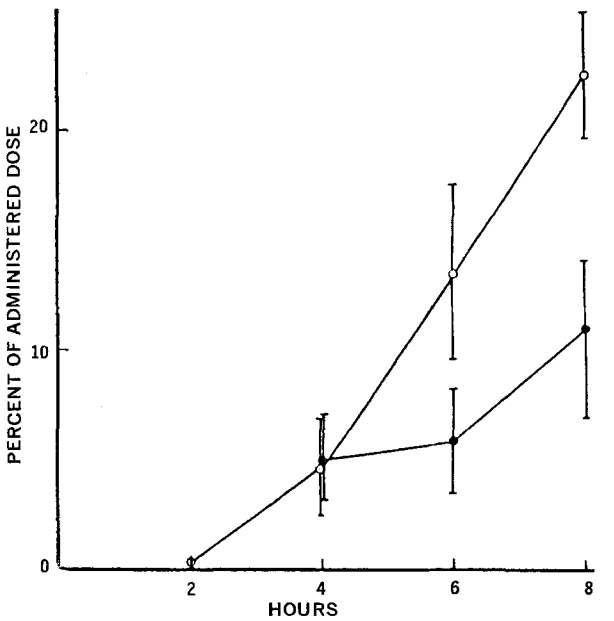

Figure 2—*Urinary excretion of 2,3,5-triiodobenzoic acid and/or metabolites by pregnant rats. Key: ○, cold-stressed; and ●, control.*

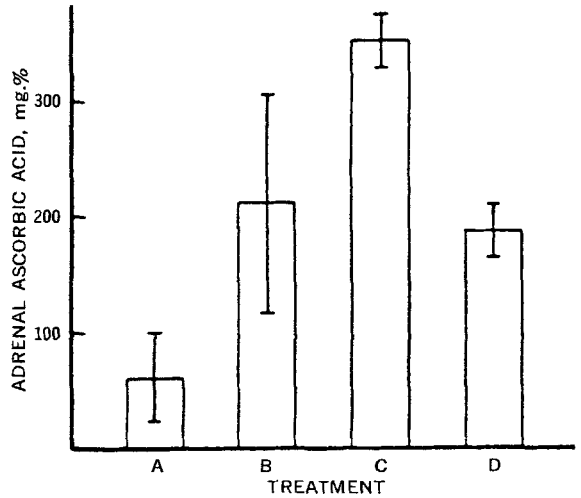

Figure 3—*Adrenal ascorbic acid. Key: A, cold-stressed pregnant; B, control pregnant; C, cold-stressed nonpregnant; and D, control nonpregnant.*

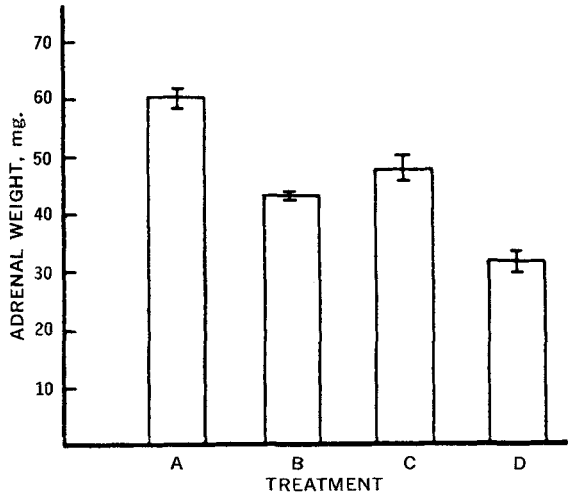

Figure 4—*Adrenal weight. Key: A, cold-stressed pregnant; B, control pregnant; C, cold-stressed nonpregnant; and D, control nonpregnant.*

percentage of injected 2,3,5-triiodobenzoic acid as 2,3,5-triiodobenzoic acid and/or metabolites than the nonpregnant controls for each time interval studied with the exception of the 3-hr. postinjection period (Fig. 1). Pregnant cold-stressed rats excreted a significantly higher percentage of injected 2,3,5-triiodobenzoic acid as 2,3,5-triiodobenzoic acid and/or metabolites than pregnant control rats at 6 and 8 hr. postinjection (Fig. 2). The nonpregnant cold-stressed rats excreted a higher percentage of administered 2,3,5-triiodobenzoic acid than the pregnant cold-stressed animals.

Adrenal Ascorbic Acid—The adrenal ascorbic acid level in the nonpregnant cold-stressed rats was significantly higher than in the nonpregnant controls (Fig. 3). The high levels of ascorbic acid indicate that the animals had acclimated to the stress (17). The pregnant cold-stressed rats had significantly lower levels of adrenal ascorbic acid than the pregnant and nonpregnant controls, which indicates that the pregnant cold-stressed rats did not acclimate to the cold. Pregnant control and nonpregnant control groups had similar levels of adrenal ascorbic acid, indicating no apparent stress from pregnancy on Day 19 of gestation. The data suggest that nonpregnant rats can acclimate to cold stress within 4 days while pregnant rats cannot. These data imply that cold stress and pregnancy act synergistically.

Cold stress has been shown to cause an increase in the size of the adrenal glands of rats (18). In the present study (Fig. 4), the pregnant cold-stressed rats had adrenal glands that were significantly larger than those of the other animals. The cold-stressed pregnant and cold-stressed nonpregnant rats had significantly larger adrenal glands than their respective controls; comparison between the two control groups revealed no significant difference.

SUMMARY

The results of the present investigation show that the placental transfer of 2,3,5-triiodobenzoic acid and two of its metabolites, 2,5-diiodobenzoic acid and 3,5-diiodobenzoic acid, occurred in cold-stressed and control rats on Day 19 of gestation.

Exposure to cold stress for a period of 4 days apparently did not influence the levels of 2,3,5-triiodobenzoic acid and/or its metabolites in the maternal and fetal tissue. The maternal blood of cold-stressed and control rats contained significantly higher concentrations of 2,3,5-triiodobenzoic acid and/or metabolites than the blood of respective fetal rats at each time interval studied. However, cold stress did not significantly affect the levels of 2,3,5-triiodobenzoic acid and/or metabolites in either the maternal blood or the fetal blood; it would thus appear that exposure to cold did not affect placental transfer. Cold stress had no apparent effect upon the plasma level of either 2,3,5-triiodobenzoic acid, 2,5-diiodobenzoic acid, or 3,5-diiodobenzoic acid in maternal or fetal animals. However, the data suggest a selective transport mechanism for 3,5-diiodobenzoic acid which was not affected by cold stress.

Pregnant cold-stressed rats excreted a significantly higher percentage of administered 2,3,5-triiodobenzoic acid as 2,3,5-triiodobenzoic acid and/or metabolites than the pregnant control rats. The same relationship was observed with nonpregnant cold-stressed animals when compared to nonpregnant control rats. Although an increased excretion of 2,3,5-triiodobenzoic acid and/or metabolites was observed in the cold-stressed pregnant rats in relation to pregnant controls, the increased loss of compound had no obvious effect on 2,3,5-triiodobenzoic acid and/or metabolite levels in cold-stressed rats.

The data obtained from the determination of adrenal ascorbic acid levels indicate that rats on Day 19 of gestation do not exhibit an apparent condition of stress from pregnancy. However, the additional insult to the pregnant animal from cold apparently resulted in a stressed condition. These data imply that cold stress and pregnancy act synergistically, suggesting that stress must be considered in the determination of the potential toxicity of an environmental contaminant as well as a medicinal agent.

REFERENCES

(1) A. A. Goldberg, *Quart. J. Pharm. Pharmacol.*, **19,** 483 (1946).

(2) A. K. Saz and F. Bernheim, *Science*, **93,** 622(1941).

(3) A. K. Saz and F. Bernheim, *J. Pharmacol.*, **73,** 78(1941).

(4) B. Nelson, *Science*, **166,** 977(1969).

(5) G. C. Fuller, Ph.D. thesis, Purdue University, Lafayette, Ind., 1966.

(6) D. E. Bass and A. Henschel, *Physiol. Rev.*, **36,** 128(1956).

(7) H. Barry, III, and J. P. Buckley, *J. Pharm. Sci.*, **55,** 1159 (1966).

(8) L. A. Spitznagle, J. E. Christian, A. J. Ohlrogge, and C. E. Breckenridge, Jr., *ibid.*, **57,** 764(1968).

(9) R. H. Jarboe, J. B. Data, and J. E. Christian, *ibid.*, **57,** 323 (1968).

(10) G. A. Bruno and J. E. Christian, *Anal. Chem.*, **33,** 1216 (1961).

(11) C. H. Wang and D. L. Willis, "Radiotracer Methodology in Biological Science," vol. 1, Prentice-Hall, Englewood Cliffs, N. J., 1965, p. 294.

(12) O. Weininger, *Science*, **119,** 285(1954).

(13) D. P. Mork, W. V. Kessler, and S. M. Shaw, *J. Pharm. Sci.*, **59,** 803(1970).

(14) R. P. Maickel, *Anal. Biochem.*, **1,** 498(1960).

(15) "Analysis of Variance for Factorial Design," (BMD2V), Purdue University Statistical Laboratory, Lafayette, Ind.

(16) N. M. Downie and R. W. Heath, "Basic Statistical Methods," 2nd ed., vol. 1, Harper and Row, New York, N. Y., 1965, p. 138.

(17) S. Katsh, G. F. Katch, and P. Osher, *Amer. J. Physiol.*, **178,** 457(1954).

(18) L. P. Dugal and M. Therien, *J. Endocrinol.*, **44,** 420(1949).

ACKNOWLEDGMENTS AND ADDRESSES

Received May 11, 1970, from the *Bionucleonics Department, School of Pharmacy and Pharmacal Sciences, Purdue University, Lafayette, IN 47907*

Accepted for publication December 10, 1970.

This investigation was supported in part by the National Institute of Environmental Health Sciences, U. S. Public Health Service, and was conducted under the auspices of the Institute for Environmental Health.

* Present address: Department of Biology, South Campus of Allegheny County Community College, West Mifflin, PA 15122

Crystal Structure of a 1:1 Aminopyrine–Barbital Complex

SETSUO KIRYU

Abstract □ The crystal structure of a 1:1 complex of aminopyrine and barbital was determined from three-dimensional X-ray diffraction data. The unit cell dimensions are $a = 11.915$, $b = 26.922$, $c = 7.199$ Å, and $\beta = 97° 17'$. The space group is $P2_1/c$; $Z = 4$. The intensity data were collected photographically, and the structure was solved directly by the symbolic addition method. Molecules in the crystal are arranged in the series of aminopyrine–barbital–barbital–aminopyrine linked by hydrogen bondings. The ethyl groups and phenyl groups are in contact with the same groups of other molecules, with normal van der Waals' interactions.

Keyphrases □ Aminopyrine–barbital complex—crystal structure □ Crystal structure—1:1 aminopyrine–barbital complex □ Molecular structure—aminopyrine, barbital □ X-ray diffractometry—analysis

Since aminopyrine diethylbarbiturate appeared as a potent analgetic about half a century ago, numerous physicochemical as well as pharmacological investigations of this well-known system have been reported.

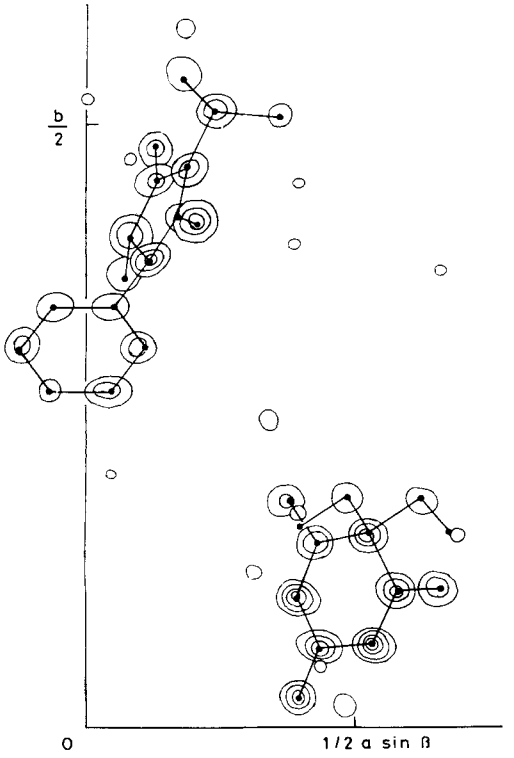

Figure 1—*Sections from a three-dimensional E-map projected along the c-axis. The contours are at equal intervals on an arbitrary scale.*

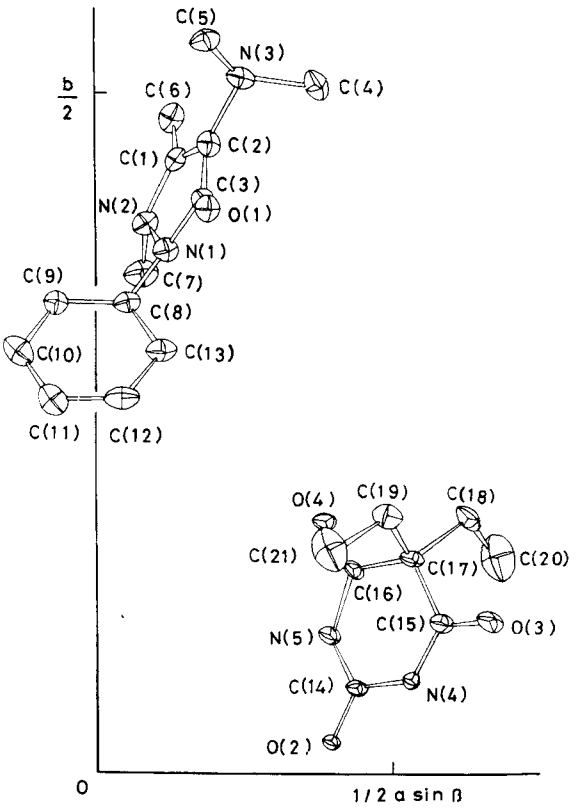

Figure 2—*Ellipsoidal representations of atomic thermal parameters and the atomic numbering scheme.*

Today a variety of information on this compound, such as its thermal or spectroscopic properties, is available; but the only study on its crystal structure was reported by Hertel in 1931 (1), and it does not offer sufficient information. Therefore, structure analysis by the X-ray diffraction method was undertaken to determine the exact crystal structure of this compound.

Table I—Initial Phase Assignments

h	*k*	*l*	|*Fo*|	|*E*|	Sign
5	10	5	35.2	4.34	+
−2	1	1	148.5	2.59	−
8	2	5	23.0	3.75	+
1	22	0	40.2	3.56	a
8	11	2	35.2	3.53	b
6	1	5	28.5	3.27	c