REFERENCES

(1) Robbins, E. B., THIS JOURNAL, **44**, 497(1955).
(2) Pohland, A., and Sullivan, H. J., *J. Am. Chem. Soc.*, **77**, 3400(1955).
(3) Marrs, J., Glass, W., and Silvani, J., *Am. J. Pharm.*, **131**, 271(1959).
(4) Gruber, C. M., *et al.*, *J. Pharmacol. Exptl. Therap.*, **118**, 280(1956).
(5) Van Bergen, W., and North, W., *J. Am. Med. Assoc.*, **172**, 1372(1960).
(6) Cann, H. M., and Verhulst, H. L., *U. S. Bull. Natl. Clearinghouse Poison Control*, Washington, D. C., March 1960.
(7) Hyatt, H. W., *New Engl. J. Med.*, **267**, 710(1962).
(8) Frasier, S. D., Crudo, F. S., and Johnson, D. H., *J. Pediat.*, **63**, 158(1963).
(9) Cann, H. M., and Verhulst, H. L., *op. cit.*, July 1960.
(10) Cann, H. M., and Verhulst, H. L., *A. M. A. J. Diseases Children*, **99**, 380(1960).
(11) Chapman, J. E., and Walaszek, E. J., *Toxicol. Appl. Pharmacol.*, **4**, 752(1962).
(12) Litchfield, J. T., and Wilcoxon, F. J., *J. Pharmacol. Exptl. Therap.*, **96**, 99(1949).
(13) Simpson, G. G., Roe, A., and Lewontin, R. C., "Quantitative Zoology," Harcourt, Brace, and Co., New York, N. Y., 1960, pp. 176, 186.
(14) Costa, P. J., and Bonnycastle, D. D., *J. Pharmacol. Exptl. Therap.*, **113**, 310(1955).
(15) Jalavisto, E., Kantero, I., and Leikola, Y., *Acta Physiol. Scand.*, **23**, 212(1951).
(16) Philpot, F., and Cantoni, G., *J. Pharmacol. Exptl. Therap.*, **71**, 95(1941).
(17) West, G., *J. Pharm. Pharmacol.*, **3**, 656(1951).
(18) Strawitz, J., Temple, R., and Heft, H., *Surg. Forum*, **9**, 54(1958).
(19) Gertler, M., and Karp, M., *Proc. Soc. Exptl. Biol. Med.*, **64**, 213(1947).

Distribution of Glucose-1-C-14 in Gold Thioglucose Obese and Normal Nonobese Mice

By D. L. ARNEY* and H. A. SWARTZ

Normal nonobese and gold thioglucose obese mice show similar patterns of residual tissue activity following glucose-1-C-14 administration in controls, glucose pretreated, and 48-hour starvation. Glucose pretreatment lowered liver and muscle levels and elevated blood and kidney levels; starvation elevated liver and muscle but lowered kidney and blood levels. The obese mice showed greater liver activities associated with increased glycogen formation. Hypothalamic levels were higher than the cerebrum and hind brain in all instances with the obese mice but only after starvation in the nonobese mice. Selected anorectic agents had little effect on general tissue activities but caused significant increases in hypothalamic levels in normal nonobese mice.

THE REGULATION of food intake by centers in the hypothalamus has been established in several species (1), including dogs (2, 3), rats (4–6), monkeys (7, 8), cats (9), and mice (10). These studies involved the production of surgical lesions into specific hypothalamic nuclei. In all instances where the ventro medial nuclei were destroyed completely or partially, hyperphagia was observed, while destruction of the lateral hypothalamic nuclei resulted in a temporary aphagia. Administration of LD_{50} doses of gold thioglucose to mice was reported to result in hyperphagia and subsequent obesity (11, 12). Histological examination within 3 days of administration revealed hypothalamic lesions associated predominately with the ventro medial nuceli (13) but also in other neighboring nuclei. Examination after a period of 3 months revealed that permanent damage was restricted to the ventro medial nuclei. With rats, the destruction of the ventro medial cells was fatal in all instances (14). Administration of similar gold compounds, such as gold thiomalate, gold thiosorbitol, gold thioglycerol, gold thiocaproate, and gold thioglycoanline, did not produce hypothalamic lesions in either rats or mice or result in hyperphagia.

The integrity of the ventro medial nuclei appeared essential for food intake regulation; as only the gold thioglucose molecule was capable of producing lesions, glucose was considered to be involved in the physiological action of these cells. It was postulated that the ventro medial nuclei had an affinity for available glucose and acted as a food intake satiety center in response to blood glucose levels (15–17). This postulation resulted in the glucostatic theory of food intake regulation. Sulfur-35 and gold-198 labeled gold thioglucose were reported to concentrate in the hypothalamus, as observed from autoradiogram and tissue activity studies (18).

The lateral hypothalamic nuclei have been characterized as appetite or feeding centers (19–21), and stimulation of these cells results in hyperphagia and their destruction in aphagia. The ventro medial nuclei, in response to blood glucose, were shown to inhibit the lateral feeding center (22, 23). Hence, the destruction of the ventro medial nuclei prevents the indirect inhibiting action of glucose on the feeding center, and hyperphagia occurs.

Gold thioglucose obese mice offered an excel-

Received August 26, 1964, from the College of Pharmacy, Butler University, Indianapolis, Ind.
Accepted for publication September 29, 1964.
Presented to the Scientific Section, A.PH.A., New York City meeting, August 1964.
* Present address: Long Hospital, Indiana University Medical Center, Indianapolis.

lent avenue in the study of the etiology of obesity and food intake regulation; several reports have been observed. The degree of obesity was found to be directly proportional to the extent of ventro medial cell destruction (24). Gold thioglucose obese mice exhibit hyperphagia during the development and maintenance of the obese state (25–27), with rapid weight gain on a high lipid diet and a loss occurring on the high protein diets. Weight loss readily occurs upon starvation. Carcass examinations have shown that the increase of body weight is due to increased lipid deposition (28). The metabolism of C-14 carboxy labeled acetate is similar to normal nonobese mice, but the turnover is less in the obese mice. Lipogenesis is greater in both peripheral tissue and the liver of the obese mice, with a disproportion of the fat synthesis occurring in the adipose tissue (29–32). Blood glucose levels and glucose tolerance do not differ from controls (12), but increased liver glycogen levels and intestinal absorption are observed. Obese mice normally are resistant to the effect of the growth hormone and sensitive to insulin (33). Cholesterol levels were normal (28). Selected anoretic agents were observed to reduce the daily food intake, with a subsequent loss in body weight in both the gold thioglucose obese and normal nonobese mice (34). Phenmetrazine and dextroamphetamine were most effective and, as with the other agents employed, had a greater effect on the normal mice.

Anorectic agents have been widely used as food intake depressants. One of the most common is dextroamphetamine, a sympathomimetic amine, or one of its analogs. The mechanism of appetite depression or food intake inhibition of the anorectic agents is not fully established, but the general belief is that they possess a central action. Dextroamphetamine has been reported by several workers to act *via* stimulation of the satiety center, the ventro medial nuclei (35–37). This location of action was indicated in the study on daily food intake reduction (34), where dextroamphetamine gave a 27.4% reduction in the normal nonobese mice, compared to a 19.3% reduction in the gold thioglucose obese mice. One report stated that dextroamphetamine acted as an inhibitor of the lateral feeding center since food intake reduction was observed in rats upon dextroamphetamine administration after destruction of the ventro medial nuclei (38). No comparison to controls was made in this study. It was not considered that total destruction of the ventro medial nuclei was not achieved, as stated in a previous report (24). Other reports on dextroamphetamine have shown that there is no elevation of blood sugar (39, 40) and that gastroenteric effects are slight and variable (41–43).

A study of the distribution of glucose or tissue activity after the administration of labeled glucose in gold thioglucose obese mice, compared to normal nonobese mice, appeared to be of considerable value. The present study involves the determination of the residual activity of various tissues in both groups of mice following pretreatment with glucose, starvation, and selected anorectic agents. Particular attention is given to brain tissue.

EXPERIMENTAL

Administration of the Gold Thioglucose.—A saline solution of gold thioglucose[1] (100 mg./ml.) was administered i.p. to female Rockland Swiss mice of 8 to 10 weeks in doses of 0.8 mg./Gm. The mice were starved for 48 hours prior to administration. A total of 10 to 15% of the fatalities occurred within 3 days of treatment. The surviving mice and nontreated litter mates were maintained in separate groups for a period of 3 to 4 months until a weight plateau was observed. Final weights ranged from 42 to 66 Gm. for the obese mice and 24 to 32 Gm. for normal nonobese mice. Of the initial number treated with the gold thioglucose, from 64 to 80% attained weights of 42 Gm. or greater.

Glucose-1-C-14 Tissue Distribution.—Separate groups of five gold thioglucose obese and five normal nonobese mice were administered an aqueous solution of labeled glucose (20 mg./ml.) i.p. in doses of 100 mg./Kg. and a radioactive dose[2] of 0.05 μc./Gm. after the following treatments: (*a*) free access to food, (*b*) 48-hour starvation, and (*c*) 30-minute pretreatment with an aqueous solution of glucose (20 mg./ml.) 100 mg./Kg. i.p. Selected anorectic agents were administered i.p. in saline to normal nonobese mice 30 minutes prior to the administration of glucose-1-C-14. The agents administered were dextroamphetamine sulfate[3] (1 mg./Kg.), phenmetrazine hydrochloride[4] (4 mg./Kg.), diethylpropion hydrochloride[5] (5 mg./Kg.), and benzphetamine hydrochloride[6] (6 mg./Kg.). The individual mice were sacrificed by decapitation, 30 minutes after the administration of the glucose-1-C-14. Blood was collected in a porcelain dish previously moistened with a saturated solution of sodium citrate to prevent coagulation. Blood samples of 0.25 ml. were obtained with micropipets, previously rinsed with the saturated sodium citrate. Accurately weighed[7] wet tissue samples of the adrenals, kidney, liver, and muscle were obtained. The intact brain was isolated and chilled in a deep freeze. Three areas were then dissected—the cerebrum,[8]

[1] Supplied by the Schering Corp., Bloomfield, N. J.
[2] Marketed as Glucose-1-C-14 by the Nuclear Chicago Corp.
[3] Dextroamphetamine (*d*-methyl phenethylamine). Supplied by Smith Kline and French Laboratories, Philadelphia, Pa.
[4] Phenmetrazine (3-methyl-2-phenyltetrahydro-2H-1,4-oxazine). Supplied by the Geigy Co., Ardsley, N. Y.
[5] Diethylpropion (diethylaminopropionphenone). Supplied by the Wm. S. Merrell Co., Cincinnati, Ohio.
[6] Benzphetamine (*n*-benzyl-*n*-dimethyl-phenethylamine). Supplied by the Upjohn Co., Kalamazoo, Mich.
[7] Precision Balance, model LG, Federal Pacific Electric Co.
[8] Cerebrum, prosencephalon minus the diencephalon.

TABLE I.—TISSUE RESIDUE IN PER CENT OF ADMINISTERED RADIOACTIVE DOSE OF GLUCOSE-1-C-14 PER GRAM OF DRY TISSUE AND MILLILITER OF BLOOD

Tissue	Treatment Prior to Administration of Glucose-1-C-14: Controls $\bar{X}$	Controls S_x[b]	Glucose, 100 mg./Kg.[a] $\bar{X}$	Glucose, 100 mg./Kg.[a] S_x[b]	Starvation, 48 Hr. $\bar{X}$	Starvation, 48 Hr. S_x[b]
Gold Thioglucose Obese Mice						
Blood	1.87	0.03	2.76	0.25	0.94	0.17
Adrenal	8.89	1.15	8.12	0.7	13.66	1.75
Kidney	11.53	0.48	17.49	1.37	6.08	1.06
Liver	19.41	0.64	16.05	0.48	23.08	0.74
Muscle	3.86	0.75	3.22	0.47	5.83	0.42
Cerebrum	18.25	5.07	9.84	0.93	14.62	0.56
Hind brain	12.97	1.32	9.72	1.15	16.47	1.77
Hypothalamus	19.92	1.33	10.37	0.4	19.48	1.42
Nonobese Mice						
Blood	0.94	0.1	5.51	0.51	0.23	0.14
Adrenal	7.8	0.74	6.38	0.99	8.52	0.2
Kidney	18.83	1.46	21.19	1.04	11.49	1.35
Liver	18.27	1.08	10.06	1.67	20.41	0.76
Muscle	11.04	1.75	6.01	0.55	8.67	1.31
Cerebrum	23.60	5.98	18.19	1.11	18.96	0.77
Hind brain	22.73	0.98	15.45	1.31	19.49	2.14
Hypothalamus	11.63	0.88	7.95	0.87	22.84	1.27

[a] Glucose administered 30 minutes prior to the administration of glucose-1-C-14. [b] Standard deviation: $S_x = \sqrt{\frac{n\Sigma x^2 - (\Sigma x)^2}{n(n-1)}}$.

TABLE II.—TISSUE RESIDUES IN ANORECTIC TREATED NORMAL NONOBESE MICE

Tissue	%/Gm. Dry Tissue and ml. Blood[a]: Dextroamphetamine $\bar{X}$	Dextroamphetamine S_x	Diethylpropion $\bar{X}$	Diethylpropion S_x	Phenmetrazine $\bar{X}$	Phenmetrazine S_x	Benzphetamine $\bar{X}$	Benzphetamine S_x
Blood	1.54	0.35	1.24	0.42	1.03	0.2	1.31	0.31
Adrenal	8.31	0.95	7.79	0.93	7.73	0.72	7.54	0.31
Kidney	15.41	0.89	18.64	0.91	17.6	1.58	17.53	1.14
Liver	12.23	0.63	19.13	0.68	18.37	1.22	15.92	1.28
Muscle	12.29	1.19	11.18	0.99	12.17	1.46	11.67	0.73
Cerebrum	22.99	1.02	24.01	1.73	22.68	1.60	24.05	1.74
Hind brain	18.25	1.26	21.30	1.35	19.59	1.46	19.40	0.57
Hypothalamus	13.74	0.84	12.80	0.95	13.30	1.37	11.62	0.89

[a] Anorectics administered 30 minutes prior to glucose-1-C-14.

hypothalamus,[9] and the hind brain.[10] Weighed samples of each area were obtained. Exterior blood was removed by absorbent paper from all tissue samples prior to weighing. The blood and all tissue samples were added to glass counting vials[11] containing 1 ml. of 1 *M* Hyamine[12] base in methanol (44). The vials were sealed with screw caps and incubated for 24 hours in an oven at 55° to facilitate solution of the samples. The vials and contents were cooled to room temperature, and 10 ml. of a liquid scintillation solvent system was added (45). The solvent system contained 2,5-diphenyloxazole (PPO) (0.4%), naphthalene (8.0%), 1,4-dioxane (300 ml.), ethylene glycol mono ethyl ether (cellusolve) (300 ml.), and toluene *q.s.* to 1000 ml. The vials were agitated, sealed, and washed thoroughly to remove any possible external contamination. The samples were placed into the freezer cabinet of the detector unit at −20° and stored for 48 hours for dark adaptation and temperature adjustment. Standards of the glucose-1-C-14 solutions used were prepared by diluting 0.1-ml. aliquots to 100 ml. and adding 0.1 ml. of these dilutions to 10 ml. of the liquid scintillation solvent system in counting vials. The activity in counts per minute of the blood, tissue samples, and the glucose-1-C-14 standards was determined by a liquid scintillation detector and associated beta spectrometer.[13] The activities were measured at the established C-14 balance point (eff. in per cent)²/Bg. with a discriminator range of 10 to 40. The quenching effect on the true count rate was corrected by means of a C-14 internal standard[14] (46). A blank sample, containing only the solvent system, was used to determine the background count. The net activity of each blood and tissue sample in counts per minute was compared to the calculated administered radioactive dose in counts per minute, and the per cent uptake or residue per gram of dry tissue and milliliter of blood was determined. The dry tissue weights were calculated from the wet weights on the basis of determined moisture contents for each group of mice and for each treatment. Weighed wet samples of each tissue after administration of each agent and treatment, as previously described, were

[9] Hypothalamus, diencephalon area.
[10] Hind brain, complete rhombencephalon area.
[11] American Tradair Corp., Long Island City, N. Y.
[12] *p*-(Diisobutylcresoxethyl)-dimethyl benzyl-ammonium chloride. Marketed as Hyamine by Rohm and Haas, Philadelphia, Pa.
[13] Ekco Liquid Scintillation Detector, model N664A, and Scaler, model N610A, American Tradair Corp., Long Island City, N. Y.
[14] Internal standard, hexadecane-1-C-14; Nuclear Chicago Corp.

dried to a constant weight in an oven at 55°. Five individual mice were employed for each treatment; the mean moisture values were calculated. The mean tissue activities for both groups of mice are listed in Table I; those for anorectic-treated normal mice are listed in Table II.

RESULTS AND DISCUSSION

The tissue residues are not necessarily due to glucose-1-C-14 uptake alone since labeled metabolic products could be released into the circulating blood from various tissues, such as skeletal muscle. Upon penetration into the cell cytoplasm, these products could contribute to the observed activity. It is believed, though, that glucose-1-C-14 uptake was the major source of the tissue residual activity and an exclusive or near exclusive source in brain tissue. Independent of the labeled product involved in the tissue uptake, the observed tissue residues are indicative of the distribution of the administered glucose-1-C-14 as the label at carbon 1 is maintained in glucose metabolites. A notable exception is in the Embden-Meyerhof pathway which occurs essentially in the liver. Any variation from controls is indicative of altered glucose or glucose metabolite uptake and in either instance reflects on glucose metabolism.

Compared to the controls of each group, the blood and kidney levels were markedly higher following pretreatment with glucose and lower after 48 hours of starvation. Liver levels were higher upon starvation and reduced upon prior administration of glucose. Muscle levels followed a pattern similar to liver in the obese mice; but in the normal nonobese mice, both starvation and pretreatment with glucose gave lower tissue residual activity. Adrenal levels were not altered greatly by glucose pretreatment but were higher following starvation. This effect was probably related to activation of the adrenal cortex antistress action. In general, the values are indicative of the normal response of gold thioglucose obese mice to glucose, with the blood and tissue levels varying as in normal nonobese mice.

A comparison of individual tissue levels of the two groups supports the previous reports (12, 33) of increased liver glycogen formation. In all treatments and controls the liver levels of obese mice were higher than those of nonobese mice, while muscle and kidney levels were lower. Blood levels of the obese mice were higher in the controls and after starvation, but lower after glucose administration. Adrenal levels indicate increased adrenal metabolism in the obese mice which might possibly involve the hypothalamic-hypophyseal-adrenal system (47, 48).

Hypothalamic levels were lower than the cerebrum and hind brain in the controls and glucose pretreated with the normal nonobese mice but were higher following starvation. In the gold thioglucose obese mice, the hypothamalic levels were higher than both the cerebrum and hind brain in all instances and were higher than the hypothalamic levels of the normal nonobese mice, except for the starved animals. A comparison of the hypothalamic levels of the treated mice to the controls in each group by the Student-Fisher t test[15] indicated a significant decrease ($p > .99$) for both the obese and nonobese mice after glucose pretreatment and a significant increase ($p > .99$) in the nonobese after starvation. The observed values lend support to the glucostatic theory (15–17) and the postulated ventro medial nuclei affinity for available glucose. The normal nonobese had significantly higher hypothalamic residues after 48 hours of starvation, when the administered glucose-1-C-14 would be the major source of available glucose. After glucose pretreatment, where tissue uptake and blood levels would be saturated or at a maximum, indicated by increased excretion *via* the kidney, both groups had a significant decrease in hypothalamic residues. The higher hypothalamic levels for all treatments observed in the obese mice could be due to an inability to utilize glucose or an altered glucose uptake or penetration into the hypothalamus due to the previous destruction of the ventro medial nuclei. The differences in hypothalamic residues between the obese and nonobese mice is undoubtedly related to the anatomical variation in the number of functioning hypothalamic ventro medial nuclei. In the normal mice, the hypothalamic residues exceeded the cerebrum, hind brain, and control hypothalamic levels only after starvation, when the administered glucose-1-C-14 was the major source of glucose for the tissues. The glucose uptake by the hypothalamus would result in inhibition of food intake through the activation of the ventro medial nuclei, as previously reported (22, 23). The brain values clearly illustrate the greater fluctuation or sensitivity of the hypothalamic area to glucose levels of the body, particularly in the normal nonobese mice with intact ventro medial nuclei.

Selected anorectic agents were observed to have little effect on the general tissue residues of administered glucose-1-C-14. Blood levels were higher with all compounds employed and, as previous studies reported no effect on blood sugar levels (39, 40), the increased activities over the controls could have been due to glucose metabolite uptake. Adrenal levels were similar to controls, with dextroamphetamine causing a slight increase. Kidney levels were slightly lower with dextroamphetamine and produced the greatest change from the controls. Liver levels were lower in the dextroamphetamine and benzphetamine treated mice, similar with phenmetrazine, and slightly higher with diethylpropion. Muscle levels varied little from the controls. Cerebrum levels were quite similar to the controls, with diethylpropion and benzphetamine showing the greatest increase. Hind brain levels were all lower, with dextroamphetamine the lowest. Hypothalamic levels were significantly increased ($p > .95$), except for the benzphetamine treated mice, which gave values identical to the controls. The increased hypothalamic glucose uptake following the administration of the anorectics could be due to increased cell activity associated with the stimulation of the ventro medial nuclei (34–37). But it is interesting that the anorectics reported to cause the maximum food intake reduction (34) also caused the highest increases in hypothalamic glucose uptake—namely dextroamphetamine and phenmetrazine. It would appear then that the increase in glucose uptake is involved in the mechanism of food intake inhibition.

SUMMARY

Tissue and blood residues of administered glucose-1-C-14 in gold thioglucose obese mice were ob-

[15] Student-Fisher t test: $t = \bar{d}/S\bar{d}$.

served to vary slightly but to follow a pattern similar to that of normal nonobese mice.

Brain tissue residues of glucose-1-C-14 vary considerably in the gold thioglucose obese and normal nonobese mice, and the observed values support the glucostatic theory of the food intake regulation.

The administration of selected anorectics to normal nonobese mice caused a significant increase in hypothalamic glucose uptake. This effect is possibly involved in the mechanism of food intake inhibition.

REFERENCES

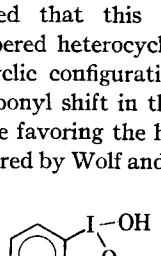
(1) Houssay, B. A., "Human Physiology," McGraw-Hill Book Co., Inc., New York, N.Y., 1955, p. 1081.
(2) Bailey, P., and Bremer, F., *Arch. Intern. Med.*, **28**, 773 (1921).
(3) Solari, L. A., *Acta V. Cong. Nac. Med.*, **3**, 303(1934).
(4) Smith, R. E., and Greenwood, C. P., *Anat. Record*, **29**, 373(1925).
(5) Hetherington, A. W., and Ranson, S. W., *Am. J. Physiol.*, **136**, 609(1942).
(6) Hetherington, A. W., and Ranson, S. W., *Anat. Record*, **78**, 149(1940).
(7) Ranson, S. W., Fisher, C., and Ingram, W. R., *Endocrinology*, **23**, 175(1938).
(8) Brooks, C. M., Lamber, E. F., and Bard, F., *Federation Proc.*, **1**, 11(1942).
(9) Wheatley, M. D., *Arch. Neurol. Psychiat.*, **52**, 296 (1944).
(10) Mayer, J., Zomzely, C., and Shull, E., *Compt. Rend.*, **242**, 928(1956).
(11) Hetherington, A. W., and Ranson, S. W., *Endocrinology*, **31**, 30(1942).
(12) Brecher, G., and Waxler, S. H., *Proc. Soc. Exptl. Biol. Med.*, **70**, 498(1949).
(13) Drachman, R. H., and Tepperman, J., *Yale J. Biol. Med.*, **26**, 394(1954).
(14) Marshall, N. B., Barnett, R. J., and Mayer, J., *Proc. Soc. Exptl. Biol. Med.*, **90**, 240(1955).
(15) Mayer, J., *J. Physiol. Rev.*, **33**, 472(1953).
(16) Mayer, J., *J. Clin. Res. Proc.*, **5**, 123(1957).
(17) Bates, H., *Am. J. Physiol.*, **168**, 472(1953).
(18) Swartz, H. A., Christian, J. E., and Andrews, F. N., *ibid.*, **199**, 67(1960).
(19) Wyrwicka, W., and Dobrzecka, C., *Science*, **132**, 805 (1960).
(20) Anand, B. K., China, G. S., and Sengh, B., *ibid.*, **138**, 597(1962).
(21) Anand, B. K., *J. Physiol. Rev.*, **41**, 677(1961).
(22) Anand, B. K., Dua, S., and Sengh, B., *Electroencephalog. Clin. Neurophysiol.*, **13**, 54(1961).
(23) Omura, Y., *et al.*, *Science*, **143**, 484(1964).
(24) Liebelt, R. A., and Perry, J. H., *Proc. Soc. Exptl. Biol. Med.*, **95**, 774(1957).
(25) Waxler, S. H., and Brecher, G., *Am. J. Physiol.*, **162**, 428(1950).
(26) Marshall, N., and Mayer, J., *ibid.*, **178**, 271(1954).
(27) Mayer, J., and Anliker, J., *J. Appl. Physiol.*, **8**, 1106 (1956).
(28) Bates, M. W., *et al.*, *Am. J. Physiol.*, **180**, 301(1955).
(29) Bates, M. W., Mayer, J., and Nauss, S., *ibid.*, **180**, 304 (1955).
(30) *Ibid.*, **180**, 309(1955).
(31) Bates, M. W., Zomzely, C., and Mayer, J., *ibid.*, **181**, 187(1955).
(32) Mayer, J., *et al.*, *ibid.*, **181**, 501(1955).
(33) Mayer, J., and Zighera, C. Y., *Science*, **119**, 96(1954).
(34) Cullen, P. D., and Swartz, H. A., *Can. Pharm. J.*, **97**, (7), 33(1964).
(35) Leake, C., "The Amphetamines," Charles C Thomas, Springfield, Ill., 1958, p. 52.
(36) Anand, B. K., Dua, S., and Schoenberg, K., *London J. Physiol.*, **127**, 143(1955).
(37) Brobeck, J., *Ann. N. Y. Acad. Sci.*, **63**, 44(1955).
(38) Stowe, F. R., and Miller, A. T., *Experientia*, **13**, 114 (1957).
(39) Dill, D., Johnson, R., and Daly, C., *Am. J. Med. Sci.*, **198**, 102(1939).
(40) Donley, D., *Ohio Med. J.*, **33**, 1229(1957).
(41) Sangster, W., and Grossman, M., *Am. J. Physiol.*, **153**, 259(1948).
(42) Beyer, K., and Meek, W., *Arch. Intern. Med.*, **63**, 752 (1939).
(43) Meyersom, A., and Rituo, M., *J. Am. Med. Assoc.*, **107**, 24(1936).
(44) Passman, J. M., Radin, N. S., and Cooper, J. A. D., *Anal. Chem.*, **28**, 484(1956).
(45) Swartz, H. A., *Can. Pharm. J.*, **96**, 433(1963).
(46) Radin, N. S., "Liquid Scintillation Counting," Pergamon Press, New York, N. Y., 1958, p. 123.
(47) Syer, G., Redgate, E. S., and Royce, P. C., *Ann. Rev. Physiol.*, **20**, 243(1958).
(48) George, R., and Leong, E., *J. Pharmacol. Exptl. Therap.*, **125**, 111(1959).

Crystal and Molecular Structure of 1,3-Dihydro-1-hydroxy-3-oxo-1,2-benziodoxole

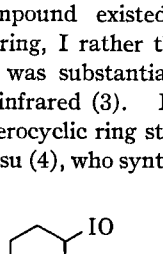
By ELI SHEFTER* and WALTER WOLF

The crystal and molecular structure of 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole (I) has been determined by X-ray crystallographic methods. The compound crystallizes as monoclinic needles, in the space group $P2_1/c$ with four molecules in a unit cell of dimensions $a_o = 12.89$, $b_o = 4.10$, $c_o = 14.05$ Å., and $\beta = 96.73°$. The geometry observed around the iodine atom appears to be trigonal bipyramid. The 11 atoms composing the molecule were found to be coplanar. The C(1)-I, O(3)-I, and O(2)-I bond distances are 2.16 ± 0.05 Å., 2.00 ± 0.05 Å., and 2.30 ± 0.05 Å., respectively, confirming the heterocyclic nature of I. An abnormally short intermolecular I-O distance at 2.90 Å. suggests that the iodine may be acting as a Lewis acid.

o-IODOSOBENZOIC ACID (I) has been recognized as an atypical compound ever since it was first synthesized (1). Askenasy and Meyer (2) suggested that this compound existed as a 5-membered heterocyclic ring, I rather than II. This cyclic configuration was substantiated by the carbonyl shift in the infrared (3). Further evidence favoring the heterocyclic ring structure was offered by Wolf and Hsu (4), who synthesized



I



II

Received August 26, 1964, from the Department of Chemistry, University of California, Los Angeles, and the School of Pharmacy, University of Southern California, Los Angeles.

Accepted for publication October 6, 1964.

Presented in part to the Scientific Section, A.PH.A., New York City meeting, August 1964.

This investigation was supported in part by grant A-3909 from the Institute of Arthritis and Metabolic Diseases and grant GM-10721 from the Institute of General Medical Studies, U. S. Public Health Services, Bethesda, Md.

The authors acknowledge discussions with Professors K. N. Trueblood, J. D. McCullough, J. A. Biles, L. F. Dahl, and Dr. P. Goldstein and Dr. M. Laing.

* Present address: Chemical Crystallography Laboratory, Oxford University, Oxford, England.