Mauger, A. B., Irreverre, F., Witkop, B., *J. Amer. Chem. Soc.* **88,** 2019 (1966).
Mauger, A. B., Katz, E., Mason, K. T., *J. Chromatogr.* **85,** 167 (1973).
McLafferty, F. W., "Interpretation of Mass Spectra," W. A. Benjamin, New York, N.Y., 1967.
Moore, S., Spackman, D. H., Stein, W. H., *Anal. Chem.* **30,** 1185 (1958).
Nakanishi, K., "Practical Infrared Absorption Spectroscopy," Holden-Day, San Francisco, Calif., 1962.
Nozoe, S., Machida, Y., *Tetrahedron Lett.* **14,** 1177 (1970).
Piez, K. A., *J. Biol. Chem.* **207,** 77 (1954).
Piez, K. A., Irreverre, F., Wolff, H. L., *J. Biol. Chem.* **223,** 687 (1956).
Plattner, P. A., Nager, U., *Helv. Chim. Acta* **31,** 2203 (1948).
Richard, J. L., Engstrom, G. W., Pier, A. C., Tiffany, L. H., *Mycopathol. Mycol. Appl.* **39,** 231 (1969a).
Richard, J. L., Pier, A. C., Tiffany, L. H., *Mycopathol. Mycol. Appl.* **40,** 161 (1970).
Richard, J. L., Tiffany, L. H., Pier, A. C., *Mycopathol. Mycol. Appl.* **38,** 313 (1969b).
Robertson, A., Smithies, W. R., Tittensor, E., *J. Chem. Soc.,* 879 (1949).
Russell, D. W., *Quart. Rev., Chem. Soc.* 559 (1966).
Scott, A. I., Sutherland, S. A., Young, D. W., Guglielmetti, L., Arigoni, D., Sim, G. A., *Proc. Chem. Soc.,* 19 (1964a).
Scott, A. I., Young, D. W., Hutchinson, S. A., Bhacca, N. S., *Tetrahedron Lett.* **15,** 849 (1964b).
Sheehan, J. C., Mania, D., Nakamura, S., Stock, J. S., Maeda, K., *J. Amer. Chem. Soc.* **90,** 462 (1968).
Sheehan, J. C., Zachau, H. G., Lawson, W. D., *J. Amer. Chem. Soc.* **80,** 3349 (1958).
Spackman, D. H., Stein, W. H., Moore, S., *Anal. Chem.* **30,** 1190 (1958).
Suzuki, A., Kuyama, S., Kodaira, Y., Tamura, S., *Agr. Biol. Chem.* **30,** 517 (1966).
Svec, H. J., Junk, G. A., *J. Amer. Chem. Soc.* **86,** 2278 (1964).
Taylor, A., *Advan. Appl. Microbiol.* **12,** 189 (1970).
Voelter, W., Jung, G., Breitmaier, E., Bayer, E., *Z. Naturforsch. B* **26,** 213 (1971).
Waddell, W. J., *J. Lab. Clin. Med.* **48,** 31 (1956).
Waldman, A. A., Nishimura, J. S., *J. Biol. Chem.* **243,** 1833 (1968).
Wellner, D., Meister, A., *J. Biol. Chem.* **235,** 2013 (1960).
Whalley, W. B., Green, B., Arigoni, D., Britt, J. J., Djerassi, C., *J. Amer. Chem. Soc.* **81,** 5520 (1959).
Whetzel, H. H., *Ont. Nat. Sci. Bull.* **5,** 3 (1909).
Whittaker, V. P., Wijesundera, S., *Biochem. J.* **51,** 348 (1952).
Wulfson, N. S., Puchkov, V. A., Bochkarev, V. N., Rozinov, B. V., Zyakoon, A. M., Shemyakin, M. M., Ovchinnikov, Yu. A., Ivanov, V. T., Kiryushkin, A. A., Vinogradova, E. I., Feigina, M. Yu., Aldanova, N. A., *Tetrahedron Lett.* **17,** 951 (1964).
Wulfson, N. S., Puchkov, V. A., Rozinov, B. V., Zyakoon, A. M., Shemyakin, M. M., Ovchinnikov, Yu. A., Kiryushkin, A. A., Ivanov, V. T., *Tetrahedron Lett.* **32,** 2793 (1965).

Received for review August 5, 1974. Accepted November 12, 1974.

Metabolism of Carbaryl (1-Naphthyl *N*-Methylcarbamate) in Human Embryonic Lung Cell Cultures

Tsu H. Lin, Hanson H. North, and Robert E. Menzer*

Carbaryl (1-naphthyl *N*-methylcarbamate) was metabolized oxidatively by primary human embryonic lung cells in culture. After incubation of [^{14}C]carbaryl (300,000–400,000 dpm, about 3 μg) for 72 hr, 70% of the recovered radioactivity resulted from oxidative metabolites, with 30% being water soluble. The total recovery of added radioactivity was over 99%. Protein and nucleic acids determinations on cellular materials revealed that carbaryl at the applied dosage level did not inhibit cell growth and enzyme syntheses. Unconjugated metabolites from both cells and cell sonicates were identified as 1-naphthol, 5-hydroxycarbaryl, 4-hydroxycarbaryl, and 5,6-dihydro-5,6-dihydroxycarbaryl. The water-soluble materials released after HCl hydrolysis were identified as 4-hydroxycarbaryl, 1,4-naphthalenediol, and 5,6-dihydro-5,6-dihydroxycarbaryl. Several unknowns remained to be identified. The conjugated metabolites were possibly not *O*-glucuronides because β-glucuronidase treatment did not free aglycones from conjugation. Furthermore, the addition of UDPGA and other cofactors to the cell sonicates did not effect any increase in conjugations.

The metabolism of carbaryl (1-naphthyl *N*-methylcarbamate) has been studied extensively in plants (reviewed by Kuhr, 1970) and animals (reviewed by Dorough, 1970) by many investigators. Presently, however, only one paper on the metabolism of carbaryl in cell cultures has been published, indicating that investigators in insecticide toxicology and chemistry have just begun to use tissue or cell culture systems as a research tool. Cell cultures provide a means for investigating the direct action of pesticides on cells or tissues in the absence of the complex system of a whole organism (Rosenoer, 1966). Baron and Locke (1970) first published work on the metabolism of carbaryl in a cell culture system using an established cell line, L-132 human embryonic lung cells. They identified three aglycones of carbaryl as 1,4-naphthalenediol, 4-hydroxycarbaryl, and 5,6-dihydro-5,6-dihydroxycarbaryl; 1-naphthol was not found among the aglycones. These authors also suggested that the water-soluble metabolites were *N*-glucuronides instead of *O*-glucuronides because the water-soluble metabolites could not be hydrolyzed by β-glucuronidase. Many water-soluble metabolites remained unidentified.

The metabolic fate of carbaryl may vary with the age, stage of development, and type of cells. The present study reports the investigaton of carbaryl metabolism by primary human embryonic lung (HEL) cell cultures.

MATERIALS AND METHODS

Materials. [^{14}C]Carbaryl (1-[^{14}C]naphthyl *N*-methylcarbamate) was synthesized by reacting equimolar quantities of methyl isocyanate in toluene (1:2, v:v) and 1-[^{14}C]naphthol (20.8 mCi/mmol, Amersham/Searle Corp.) at 80° for 2 hr (Leeling and Casida, 1966). The product was identified by cochromatography with authentic standard carbaryl (Union Carbide Corporation) and had a specific activity of 12.16 mCi/mmol.

Several derivatives of carbaryl were also supplied by Union Carbide Corporation. Beef liver β-glucuronidase

Department of Entomology, University of Maryland, College Park, Maryland 20742.

Table I. [^{14}C]Carbaryl Metabolism by HEL Cells: Percent of Each Metabolite Present in Recovered Radioactivity at Corresponding Periods of Incubation

Metabolites	6 hr		12 hr		24 hr		48 hr		72 hr	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
1,4-Naphthalenediol	0	5.6	0	7.2	0	6.6	0	3.4	0	0.9
1-Naphthol	0.4	50.5	0.5	59.0	0.7	66.4	0.9	71.2	1.2	73.2
1,5-Naphthalenediol	0	1.8	0	1.5	0	1.8	0	0.6	0	0.5
Carbaryl	98.6	20.8	98.3	8.4	97.5	3.3	97.3	0.6	96.3	0.2
5-Hydroxycarbaryl	0	1.2	0	1.9	0	1.1	0	0.6	0	0.2
4-Hydroxycarbaryl	0	0.7	0	2.4	0	0.8	0	0.5	0	0.1
5,6-Dihydro-5,6-dihydroxycarbaryl	0	0.6	0	1.4	0	0.7	0	0.3	0	0.3
Unknown (origin)	0.3	17.4	0.3	14.0	0.3	6.8	0.2	3.6	0.3	1.5
Total organosoluble	99.3	98.6	99.0	96.0	98.5	87.7	98.4	80.8	97.8	77.4
Total water soluble	0.6	1.2	1.0	3.6	1.5	12.1	1.6	19.0	2.2	22.4
Washed cell pellet	0	0.1	0	0.12	0	0.12	0	0.20	0	0.20

(70,000–100,000 units/g), serum bovine albumin, serum DNA, yeast-soluble RNA, NADPH, and UDPGA (ammonium salt) were all obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.

Cell Cultures. Human embryonic lung (HEL) cells were obtained from Flow Laboratories, Rockville, Md. These cells were freshly explanted from in vivo sources and seeded in tissue culture flasks with nutrient medium. The general procedures of North and Menzer (1972) for cell culture studies were used in the present investigation.

Incubation Conditions. Radioactive carbaryl (300,000–400,000 dpm, 2.2–3.0 μg) in diethyl ether was applied to a culture flask by dispersing evenly over the bottom of the flask upon which the cells would be seeded. A gentle stream of sterile air was used to evaporate the solvent.

Two-week-old cells were dislodged by trypsinization from a donor flask and together with 3 ml of nutrient medium (pH 7.4) were added to the flasks which were previously inoculated with carbaryl. The flasks were stoppered and the cultures were maintained in an incubator at 37° for periods of 6 hr to 3 days. Control flasks containing all the above components except cells were prepared and incubated in the same manner.

The concentration of nucleic acids and protein concentrations in relation to the progression of cellular growth were also studied. Culture flasks were inoculated as described, but they contained nonradioactive carbaryl with a concentration identical with those flasks containing the labeled carbaryl. For determinations of nucleic acids and protein, cells were first dislodged from the flask walls as described, and then were carefully washed 3 times with 2 ml of 0.85% saline by centrifugation at 275*g* at 5° for 10 min. Nucleic acids (RNA and DNA) were extracted and determined according to the procedure of Schneider (1957). Protein concentration was determined by the method of Lowry et al. (1951) using bovine serum albumin as the standard.

Carbaryl metabolism was also investigated by using HEL cells which had been sonically disrupted. Primary HEL cells were allowed to grow to maximum density and then were scraped into suspension in the nutrient medium. Culture medium containing cells was centrifuged at 275*g* at 5° for 10 min. The supernatant was decanted and the cells resuspended in a pH 7 Tris-HCl buffer (0.05 *M*) with subsequent recentrifugation. This washing process was repeated 4 times to remove serum protein in the medium from the cells. The cells were then resuspended in 15 ml of Tris-HCl buffer and transferred to a Branson rosette container to disrupt the cells by sonic vibration for 1 min from the microtip of a Branson sonifier (Branson Sonic Power, Danbury, Conn.). The cells in a Branson rosette were immersed in ice during sonication. The sonicate (containing 25 μg of albumin equivalent protein) was decanted into a 25-ml erlenmeyer flask which contained 200,000–300,000 dpm of [^{14}C]carbaryl as previously described, 36 μmol of NADPH, and 3 μmol of magnesium chloride. Control flasks contained the same components except that cell sonicates were boiled prior to use. Test and control flasks were incubated at 37° in a water bath with constant shaking for 3 hr.

Investigations of conjugation of carbaryl by HEL cell sonicates were also conducted. Two-milliliter cell sonicates (containing 25 μg of protein) in Tris-HCl buffer were added to 50-ml erlenmeyer flasks which contained 200,000–300,000 dpm of [^{14}C]carbaryl added as previously described, 36 μmol of NADPH, and 3 μmol of magnesium chloride. The final volume in each flask was adjusted to 5 ml with 0.05 *M* Tris-HCl buffer (pH 7.4). The flasks and contents were incubated at 37° in a water bath with constant shaking for 3 hr. At this point, 12 nmol of UDPGA in Tris-HCl buffer was added to each flask except the control flasks. The final volume in each flask was 6 ml with the pH value maintained at 7.4. The flasks and contents were incubated further at 37° with shaking for 20 min (Dutton, 1966). The metabolites were extracted and separated for identification.

Extraction Procedures. At the end of incubation, the cell culture medium (pH 7.6–8.0) was neutralized and an equal volume of diethyl ether was added to each flask to stop cell metabolic activities. The ether layer was removed and the remaining aqueous solution was extracted 4 times with additional equal volumes of diethyl ether. Finally, the ether extracts from each single flask were combined into one fraction for each flask. The remaining aqueous solution was adjusted to pH 2.0 by adding 0.1 *N* HCl and then was further extracted 4 times with diethyl ether to yield the ether extract and the aqueous fraction.

The last aqueous solution was adjusted to pH 4.4 with 0.1 *N* HCl followed by incubation with β-glucuronidase (1750 units/ml) at 37° for 24 hr in order to free possible conjugates of carbaryl (Kuhr and Casida, 1967; Lucier and Menzer, 1970). The solution was then extracted with diethyl ether as before. Radioactivity in the ether extract and aqueous solution was assayed. Aliquots of the water phase were also subjected to acid hydrolysis by adding 0.1 *N* HCl to pH 1.0 and heating at 100° for 6 hr. The freed metabolites were extracted into diethyl ether and radioassayed.

Chromatography. Commercially prepared TLC plates (silica gel F-254, 0.25 mm thickness, E. Merck) were used to separate ether-extractable metabolites of carbaryl. Because of insufficient radioactivity, the water-soluble metabolites were not subjected to separation. Two-dimensional chromatography was used for cochromatography in the identification of ether-extractable metabolites. Thin-layer plates were developed in solvent systems as listed in

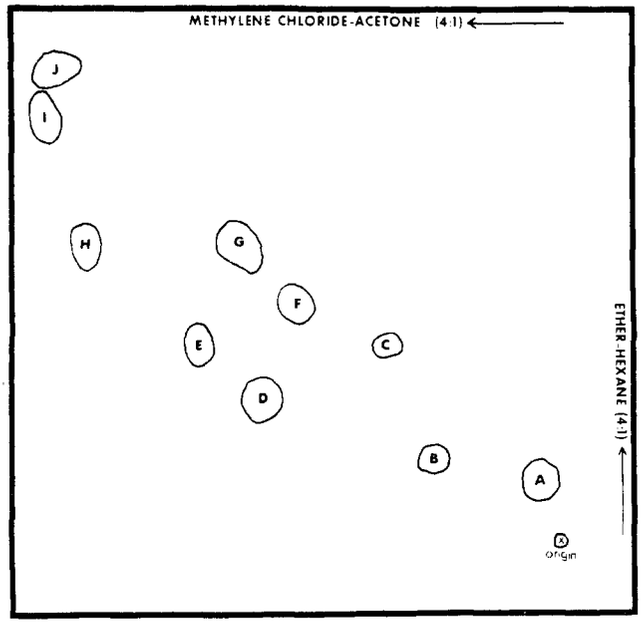

Figure 1. Two-dimensional thin-layer chromatogram of carbaryl and some possible metabolites on a silica gel F-254 plate developed in solvents as shown. Letters represent the following compounds: (A) 5,6-dihydro-5,6-dihydroxycarbaryl; (B) 5,6-dihydro-5,6-dihydroxynaphthol; (C) 1-naphthyl *N*-hydroxymethylcarbamate; (D) 4-hydroxycarbaryl; (E) 5-hydroxycarbaryl; (F) 1,3-naphthalenediol; (G) 1,5-naphthalenediol; (H) carbaryl; (I) 1-naphthol; (J) 1,4-naphthalenediol.

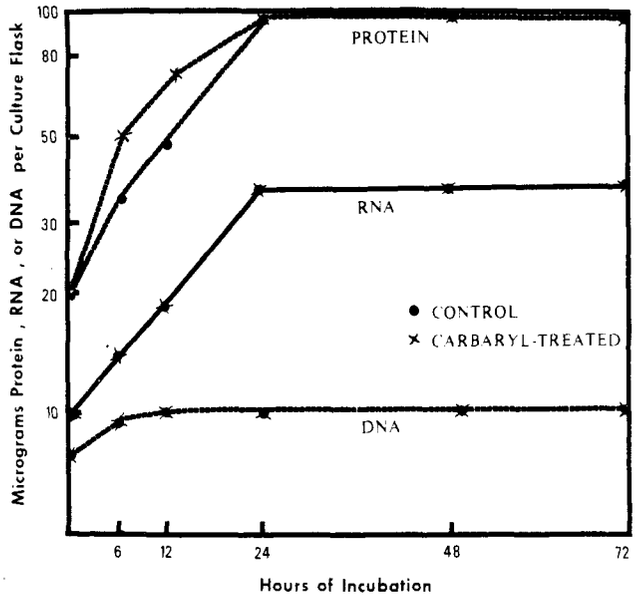

Figure 2. *Changes in protein, DNA, and RNA concentrations in* HEL cell cultures after treatment with a single dose of 3 μg per culture flask of carbaryl. Incubation at 37° for indicated periods.

Table I. For the two-dimensional system, the plate was first developed in ether–hexane (4:1), and then in methylene chloride–acetonitrile (4:1).

Detection and Identification of Metabolites. After resolution of the metabolites on TLC plates, the locations of radioactive carbaryl metabolites were determined by radioautography using Kodak No-Screen X-ray film. The locations of the unlabeled reference carbaryl derivatives were observed on the TLC plates by visualizing fluorescence under uv light.

Radioassay Procedures. A Packard Tri-Carb liquid scintillation spectrometer (Model 3375) was used for radioassays, using the techniques described by North and Menzer (1972). The external standard method was used to correct for quenching.

RESULTS

Carbaryl Metabolism by HEL Cell Cultures. The total recovery of radioactivity from cell cultures after incubation at all time intervals was over 99% for both the control and test flasks. The radioactivity remaining in the washed cell pellets constituted only 0.1 to 0.2% of the administered dose. The ether-extractable metabolites decreased in the longer incubation periods, with 98.6% of applied dose being recovered after 6 hr of incubation and 77.4% after 72 hr of incubation. The relative concentration of water-soluble metabolites, conversely, increased with prolongation of incubation time; only 1% of the dose was present in the post-extraction aqueous phase after 6 hr of incubation, and by 72 hr of incubation, the formation of water-soluble metabolites had increased nearly 22-fold (Table I). No significant radioactivity could be extracted into ether after the aqueous fraction was adjusted to pH 4.4 with 0.1 *N* HCl followed by digestion with β-glucuronidase at 37°. In contrast treatment with 0.1 *N* HCl for 6 hr in a boiling water bath without added enzyme converted 30 to 50% of the radioactivity in the aqueous phase into ether-extractable materials. All radioactivity from the control flasks was extracted into ether.

TLC resolved ether-extractable radioactivity from HEL cell incubations into eight spots (Table I; see Figure 1). These spots were identified by cochromatography with standard compounds as carbaryl, 1,4-naphthalenediol, 1-naphthol, 1,5-naphthalenediol, 5-hydroxycarbaryl, 4-hydroxycarbaryl, and 5,6-dihydro-5,6-dihydroxycarbaryl. One spot (the origin) was not identified. The major metabolite was 1-naphthol, whose formation seemed to be rapid and continuous as long as carbaryl still existed in the medium. TLC resolution of radioactivity extracted from control incubations revealed minute quantities of 1-naphthol (less than 1.5%), indicating very little degradation of carbaryl in the controls (Table I). The rate of disappearance of carbaryl may be taken as the rate of metabolism of carbaryl by HEL cells. The fastest metabolic rate for carbaryl in HEL cells was found to take place in the first 6 hr of incubation.

TLC resolved ether-extractable radioactivity from the acid hydrolysates into six radioactive spots. Three of these spots were identical with 5,6-dihydro-5,6-dihydroxycarbaryl (1.3% of administered dose), 4-hydroxycarbaryl (2.2%), and 1,4-naphthalenediol (6.6%). Three other spots remained unidentified (1.2%).

Carbaryl Metabolism by HEL Cell Sonicates. After incubation of carbaryl with sonicates of HEL cells and cofactors for 3 hr as described, metabolites were extracted into ether. About 10% of the applied carbaryl was converted to water-soluble materials, with the remainder being extracted into ether. TLC separated the ether-soluble radioactivity into six spots. Cochromatography revealed that five of the spots were 1-naphthol, carbaryl, 4-hydroxycarbaryl, 5-hydroxycarbaryl, and 5,6-dihydro-5,6-dihydroxycarbaryl. 1-Naphthol in small percentage (less than 3%) was the only decomposition product found in the controls (boiled sonicates). Assays of the aqueous phase following incubation of HEL cell sonicates with [^{14}C]carbaryl, UDPGA, and other cofactors as described revealed no more radioactivity than that found in the incubation medium containing no UDPGA. This suggested that no glucuronic acid conjugation took place in the HEL cell cultures.

Protein and Nucleic Acid Contents in HEL Cell Cultures. Carbaryl metabolism in test and control cultures was determined concomitantly with assays for cellular protein and nucleic acids. There were faster increases in protein concentrations in the cells with carbaryl than in controls without carbaryl up to 24 hr after a single dose treatment with carbaryl (1 μg/ml) and incubation at 37°.

Nucleic acid concentrations were identical in tests and controls under the same conditions. This indicated that cells were growing normally despite the treatment with carbaryl (Figure 2).

DISCUSSION

Carbaryl was almost completely degraded by primary HEL cell cultures after 72 hr of incubation. The recovery of applied radioactivity was high indicating that the HEL cells did not convert carbaryl to carbon dioxide. Baron and Locke (1970) reported 33% loss of $^{14}CO_2$ formation when ring-labeled [^{14}C]carbaryl was incubated for 72 hr with L-132 human embryonic lung cell cultures, an established cell line. The primary HEL cells which were used in the present investigation may not possess the enzyme system necessary for breaking down the naphthalene ring of carbaryl to form carbon dioxide.

The major metabolite of carbaryl from HEL cell cultures was found to be 1-naphthol at all incubation intervals. Although 1-naphthol presumably results from hydrolysis of carbaryl, its production in this system is not surprising because 1-naphthol has been found to be a major metabolite of carbaryl degradation in many organisms studied. Other metabolites of carbaryl found in the first ether extracts included 1,4-naphthalenediol, 1,5-naphthalenediol, 5-hydroxycarbaryl, 4-hydroxycarbaryl, and 5,6-dihydro-5,6-dihydroxycarbaryl. However, the two diols, 1,4-naphthalenediol and 1,5-naphthalenediol, could be formed through hydrolysis of 4- and 5-hydroxycarbaryl, respectively, during pH adjustments of culture media with 0.1 *N* HCl.

Relatively high amounts of radioactivity remained at the origin after TLC separation, but the amount was not random at each study interval. It decreased with prolongation of incubation periods. The unidentified material at the origin is, therefore, carbaryl metabolite(s) of a more polar nature, which are not extractable by diethyl ether or are metabolized further with prolonged incubation to materials which do migrate in the systems used.

The aqueous phase was subjected to acid hydrolysis, ether extraction, and TLC analysis. Three metabolites of carbaryl freed from water-soluble conjugates were characterized as 4-hydroxycarbaryl, 1,4-naphthalenediol, and 5,6-dihydro-5,6-dihydroxycarbaryl. Since 1,4-naphthalenediol was a hydrolytic product of 4-hydroxycarbaryl, 4-hydroxycarbaryl could be the major carbaryl metabolite which was conjugated by the cells. These results agreed with those of Baron and Locke (1970). Sullivan et al. (1970) found 5,6-dihydro-5,6-dihydroxycarbaryl to be the principal aglycone of carbaryl isolated from acid hydrolysate of rat urine water-soluble materials. Such was not found to be the case in the HEL cell culture system. Species specificity may account for the discrepancy.

β-Glucuronidase treatment did not free aglycones from conjugation. This result also agreed with the report by Baron and Locke (1970). Furthermore, the addition of UDPGA and other cofactors to the cell sonicates did not increase the radioactivity in the aqueous phase. These facts plus the absence of 1-naphthol (a compound known to form an *O*-glucuronide conjugate readily) in the ether extracts of acid hydrolysate strongly suggested that the water-soluble conjugates of carbaryl metabolites were not *O*-glucuronides. Although Dorough and El-Shourbagy (1974) reported a high degree of 1-naphthol *O*-glucuronide formation in adult rat lung tissues, the human embryonic lung cells in culture lacked the ability to form *O*-glucuronides of carbaryl metabolites. Perhaps the embryonic cells have not developed the enzyme system for *O*-glucuronide formation. The salient suggestion of Baron and Locke (1970) on the nature of conjugates of carbaryl metabolites is supported here. However, whether the conjugates were *N*-glucuronides cannot be affirmed. Acid hydrolysis did not produce 100% conversion of water-soluble materials into aglycones of carbaryl. There was additional radioactive material remaining unextracted and unidentified. It appears that a wider variety of enzymes should be employed to free the metabolites from conjugation in order to further characterize these unknown metabolites. It is also possible that cultured cells conjugate hydroxylated metabolites of carbaryl with moieties not found in previous carbaryl studies.

LITERATURE CITED

Baron, R. L., Locke, R. K., *Bull. Environ. Contam. Toxicol.* **5**, 287 (1970).
Dorough, H. W., *J. Agric. Food Chem.* **18**, 1015 (1970).
Dorough, H. W., El-Shourbagy, N. A., *J. Econ. Entomol.* **67**, 344 (1974).
Dutton, G. J., "Glucuronic Acid," Academic Press, New York, N.Y., 1966, p 281.
Kuhr, R. J., *J. Agric. Food Chem.* **18**, 1023 (1970).
Kuhr, R. J., Casida, J. E., *J. Agric. Food Chem.* **15**, 814 (1967).
Leeling, N. C., Casida, J. E., *J. Agric. Food Chem.* **14**, 281 (1966).
Lowry, A., Rosebrough, N. J., Farr, A. L., Randall, R. J., *J. Biol. Chem.* **193**, 265 (1951).
Lucier, G. W., Menzer, R. E., *J. Agric. Food Chem.* **18**, 698 (1970).
North, H. H., Menzer, R. E., *Pestic. Biochem. Physiol.* **2**, 278 (1972).
Rosenoer, Y. M., in "Cells and Tissues in Culture," Vol. 3, Willmer, E. N., Ed., Academic Press, New York, N.Y., 1966, p 351.
Schneider, W. C., *Methods Enzymol.* **3**, 680–681 (1957).
Sullivan, L. J., Eldridge, J. M., Knaak, J. B., Tallant, M. J., FMC Corporation, Niagara Chemical Division, Middleport, N.Y., 1970, 32 pp.

Received for review June 24, 1974. Accepted October 21, 1974. Scientific article no. A2011, Contribution no. 4959, of the Maryland Agricultural Experiment Station, Department of Entomology. This investigation was supported in part by research Grant No. ES-00121 from the National Institute of Environmental Health Sciences.