4.14 ± 0.71 g). The differences between experimental and control animals are not significant.

Of the 508 21-day fetuses, 504 (409 dissected and 95 cleared for skeletal examination) were normal and only four were considered abnormal. One had hydrocephalus, but 12 of its littermates were normal; its mother had been injected once on the 7th day with 50 μg of LSD. Another fetus had short extremities and syndactylism; there were nine normal littermates; the mother had been injected daily from the 7th to the 11th day with 50 μg of LSD. Two fetuses were small, weighing 1.7 and 2.1 g; they were from a litter of six, the other four being normal; the mother had been injected on the 7th and 9th days with 50 μg of LSD. There was no common pattern in the abnormalities of the four young and there was no dose-dependence.

The four treated rats that delivered had 44 apparently normal young, some of which could be raised and bred. Since an incidence of about 1 percent of congenital malformations in the offspring of untreated Wistar rats is not unusual, our pilot experiments did not prove that LSD is teratogenic in rats. Even dosage as great as 300μg to rats did not harm their embryos. Such dosage is comparable to human dosage; on a weight basis it is more than 200-times larger.

Later we learned of the results of Alexander *et al.* (*2*) who gave single injections of LSD to rats early in pregnancy (4th day of gestation) and observed resorption of one litter and some stillborn, stunted young in others. On the 4th or 5th day of pregnancy we gave 34 rats doses ranging from 1 to 100 μg of LSD (Delysid), and obtained from 32 females a total of 335 young. Of these, 296 were removed on the 21st day by cesarean section; with the exception of one fetus that was small, all were normal on external inspection, dissection, or clearing. Thirty-nine young were delivered and raised; they remain alive and healthy. Two of the 34 rats had no litters. A resorption rate of 5.9 percent is not different from that for pregnant Wistar rats injected with saline solution.

Although most of the doses administered in these experiments were very large (the highest single doses administered by us were about 80-times larger than those given by Alexander *et al.*), we found no abnormalities other than reduction in size of one of the young. Alexander *et al.* (*2*) used a dry LSD dissolved in saline, while we administered Delysid. The stocks of rats used in their and our experiments also must differ since they mention that their control offspring weighed an average of 64 g at 10 days; rats in our laboratory weigh about 19 g at 10 days. Donaldson (*3*) cites weights of 15 g for 10-day-old rats. If the LSD-treated rats weighed 44 to 46 g at 10 days, as Alexander *et al.* (*2*) state, it would appear that the drug did not reduce the weight of these animals but more than doubled it.

Thus we did not find LSD teratogenic during the organogenetic period and found no abnormalities in the offspring of rats injected on the 4th or 5th day of pregnancy, although the doses administered to some of the pregnant rats were as high as those used by human beings.

We draw no conclusions from the negative results with rats, concerning teratogenicity of LSD in man, since it is known that a drug teratogenic in one species may not be so in another; this general rule applies to results with mice (*4*) and hamsters (*5*) also.

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Carbon-14 Milk Constituents from Cows Fed Carbamate Labeled with Carbon-14 on the Carbonyl

Abstract. *Oral administration to a dairy cow of Furadan insecticide (2,2-dimethyl-2,3-dihydro-7-benzofuranyl* N*-methylcarbamate) labeled with carbon-14 on the carbonyl produced in the milk certain radioactive materials which were not Furadan metabolites. The data suggest that these products were natural milk constituents containing only the carbon-14 atom from the Furadan molecule. Carbon-14–labeled carbon dioxide formed by the hydrolysis of the carbamate insecticide is the apparent precursor of these radiolabeled constituents of the milk.*

Carbamic acid esters act as parasympathomimetic agents as a result of their ability to inhibit acetylcholinesterase. In summarizing their many clinical uses, Casida (*1*) also pointed out that their pesticidal applications were equally varied.

Metabolism of carbamate insecticides in mammals, plants, and insects has been explored by radiotracer techniques. The use of carbamates labeled on the carbonyl carbon with carbon-14 has become a standard procedure in investigations of the general metabolic fate of these chemicals (*2*). We can easily estimate the degree of hydrolysis by collecting and quantitating the labeled carbon dioxide released from the treated organism. Radioactive residues remaining in the organism, or in its excretory products in the case of animals, are considered as carbamate metabolites.

The possibility that rapid hydrolysis of a carbonyl-C^{14} carbamate in an animal might lead to the incorporation of labeled carbon dioxide into naturally occurring products has been considered. An investigation (*3*) in which eight carbonyl-C^{14} carbamates were administered to rats revealed that 25 to 77 percent of the dose was expired as labeled carbon dioxide.

In order to support the assumption that the expired radioactivity originated from hydrolysis of the carbamate or its metabolites, Krishna and Casida administered sodium carbonate-C^{14} to the rats; they reported that the radioactivity was rapidly and almost quantitatively eliminated as C^{14}-carbon dioxide. Four hours after the animals were treated, 96 percent of the dose was expired as C^{14}-carbon dioxide, 2 percent was excreted in the urine, and 3 percent remained in the body.

The nature of the radioactive material in the urine and body was not determined. The findings suggested that the use of carbamates labeled with carbonyl-C^{14} would not complicate a study of metabolism by allowing in-

corporation of C^{14}-carbon dioxide into natural body constituents.

The amount of C^{14}-carbon dioxide incorporation may be insignificant in a general metabolism study. However, incorporation does occur and may be of great importance in instances where minute quantities of residues must be considered. This was revealed by our study of the metabolism of Furadan insecticide (carbonyl-C^{14} 2,2-dimethyl-2,3-dihydro-7-benzofuranyl *N*-methylcarbamate) in a lactating cow. The radioactive dose (2.7 mc) was administered to the animal orally by gelatin capsule, and samples of milk, urine, and feces were taken at frequent intervals after treatment.

Because the milk was of primary importance, we closely observed any quantity of detectable radioactivity. Radioassay by liquid-scintillation counting demonstrated that 2 percent of the administered dose was eliminated in the milk. Extraction of the milk by a procedure (*4, 5*) that separated the radioactive residues into organoextractable, water-soluble, and unextractable fractions resulted in a distribution of radioactivity markedly different from that found with other carbamates. Great quantities of labeled residues could not be extracted from the milk solids, and most of the radioactivity extractable by organic solvents could not be separated from lipid materials. These characteristics of the radioactive residues in milk were not noted with the carbamates previously used. However, these other carbamates [carbaryl (*5*) and 2-methyl-2-(methylthio)-propionaldehyde-*O*-(methylcarbamoyl) oxime (Temik)] were not labeled on the carbamate moiety. With both of these compounds, the radioactive residues in the milk of treated cows were present primarily as organoextractable and water-soluble fractions. The organoextractable fractions were easily separated from the lipids, and the major metabolites were identified. Very little radioactivity was detected as unextractable fractions from milk solids. These differences suggested that some of the radioactive material in the milk of the cow treated with carbonyl-C^{14} Furadan was not metabolites of the administered carbamate but actually resulted from naturally occurring chemicals that had incorporated only the carbon-14 atom from the insecticide molecule.

Treatment of the same cow 3 weeks later with Furadan labeled in the ring with C^{14} confirmed our suspicion that incorporation of labeled carbon dioxide had occurred in the earlier treatment. Radioassay showed that only 0.2 percent of this dose was eliminated in the milk. Changing the radiocarbon from the carbonyl position to one in the benzofuranyl ring resulted, therefore, in the residue secreted in milk being ten times less radioactive. Residues in milk from treatment with Furadan labeled on the ring carbon were fractionated in the same manner as those for the first treatment were. The radioactivity was found mostly in water-soluble conjugates that could be converted to organoextractable metabolites identified as oxidative or hydrolytic products of Furadan. Approximately 85 percent of the water-soluble metabolites were extractable from the aqueous phase after acid treatment, whereas only 5 percent of the water-soluble radioactivity in milk from the cow treated with carbonyl-C^{14} Furadan partitioned into organic solvents after an identical procedure.

A small amount of the ring-C^{14} residues in the milk was extractable directly with organic solvent. These products were separable from the milk lipids and were identified as Furadan-type metabolites. Carbonyl-C^{14} residues which were extractable directly with organic solvents contained less than 50 percent of actual Furadan-related products. There were only trace quantities of radioactivity in the milk solids after treatment with Furadan labeled in the ring, but these unextractables constituted a major portion of the carbonyl-C^{14} residues in the milk.

Analysis of the cow urine after each treatment with labeled Furadan suggested the same conclusion as that from the analysis of milk. Less than 25 percent of the water-soluble residues in the urine after treatment with Furadan labeled on the ring carbon remained in the aqueous layer after acid treatment and extraction with organic solvent. These products were characterized as typical Furadan metabolites. More than 50 percent of the radioactivity remained in the water phase as a result of the same treatment of the carbonyl-C^{14} water-soluble residues in the urine.

After the milk, urine, and feces were free of all radioactivity from the treatment with the Furadan labeled in the ring, the cow received sodium bicarbonate-C^{14} orally. The amount of radioactivity (3.0 mc) was approximately the same as that from the carbonyl-C^{14} Furadan treatment. Analysis showed that 3.7 percent of the dose was eliminated in the milk, almost twice that which resulted from treatment with carbonyl-C^{14} Furadan. The radioactivity was distributed among the organoextractable, water-soluble, and unextractable fractions of the milk. All the radioactivity in the organic extract stayed with the milk lipids regardless of the techniques used to separate them. The water-soluble milk fraction contained labeled material that could not be converted to organoextractable products by acid treatment. Analysis of the urine gave similar results.

We concluded that in the dairy animal the rapid production of C^{14}-labeled carbon dioxide from hydrolysis of a carbamate labeled with carbonyl-C^{14} can result in the incorporation of carbon-14 into body chemicals normally found in the milk and urine. Although these chemicals have not yet been identified, they may be in the form of carbohydrates and lipids. Incorporation of the carbon from carbon-14 dioxide into glucose has been demonstrated (*6*). Glycerol labeled with C^{14} produced from the glucose by glycolytic reactions could permit biosynthesis of other C^{14}-carbohydrates and could also result in the presence of radioactive glycerides in the body.

Because the development of carbamate pesticides and pharmaceuticals continues at a rapid pace, we must have proper regulations for their use. Our study points out a possible source of error or complication, or both, that could result from the use in metabolic investigations of carbamates labeled with carbonyl-C^{14}.

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