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Received September 27, 1955. P.S.E.B.M., 1955, v90.

Metabolism of Trichloroethylene in the Bovine.* (22019)

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It has been suggested(1) that the aplastic anemia which is observed in the bovine(2) fed certain specimens of trichloroethylene-extracted soybean oil meal is induced by residual trichloroethylene in the toxic meal. Prolonged feeding of relatively large amounts of trichloroethylene however failed to produce harmful effects in calves(3). This suggests that the bovine, like some other species which are relatively resistant to the toxic effects of trichloroethylene-extracted soybean oil meal (4) can metabolize trichloroethylene. In fact, urochloralic acid has recently been isolated from the urine of calves fed trichloroethylene and identified through degradation and synthesis of a derivative as 2′, 2′, 2′-trichloroethyl-β-D-glucopyranosiduronic acid(5). It was thus established that the bovine, like the dog(6), can metabolize trichloroethylene to trichloroethanol and excrete the latter as a conjugate. It remained to be determined however, to what extent this occurs and if trichloroacetic acid, a major metabolite of trichloroethylene in mice(7), rats(8), dogs (9) and man(10) is also produced by the bovine.

Methods. Four female Holstein calves weighing from 100 to 112 lb were placed into individual metabolism cages, fed 5 lb of whole cows' milk twice daily and given water *ad libitum.* Trichloroethylene was administered orally in gelatin capsules twice daily with the milk in the following total daily amounts: Calf A, 3 g for 5 days; calf B, 12 g for 5 days; calves C and D, 12 g for 4 days. The urine was collected in brown bottles under toluene. Separate 24-hour specimens were obtained before, during and after the period of administration of trichloroethylene. Aliquots of the urine were used for determination of trichloroacetic acid, "total trichloro compounds" and trichloroethylene; the latter was also determined in the toluene layer. The analytical methods used have been described recently(11). Briefly they consist in the quantitative application of the Fujiwara alkali-pyridine reaction. In our modification trichloroacetic acid, trichloroacetaldehyde, chloroform and trichloroethanol do not interfere with the determination of trichloroethylene. The latter as well as chloroform can be removed from the urine prior to the determination of trichloroacetic acid or of "total trichloro compounds." Trichloroethanol and urochloralic acid do not interfere with the determination of trichloroacetic acid but all 3 compounds are included in the determination of "total trichloro compounds." The difference between "total trichloro compounds" and trichloroacetic acid (calculated on a molar basis as trichloroethanol) corresponds to free and combined "trichloroethanol" including urochloralic acid. Other products of metabolism of trichloroethylene which no doubt exist have not been definitely recognized. If, after oxidation they would give a positive Fujiwara reaction they would be included as "total trichloro compounds" and would thus contribute

* Paper No. 3414 Scientific Journal Series, Minnesota Agri. Exp. Station. This is paper No. 11 of a series "Studies on Trichloroethylene-extracted Feeds." Financial assistance for part of this work from E. I. DuPont de Nemours and Co., Inc., is gratefully acknowledged. Dr. V. Perman, School of Veterinary Medicine, kindly assisted with handling of animals and collection of urine.

TABLE I. Trichloroacetic Acid and "Trichloroethanol"* in Urine.

24 hr interval	Calf A (3 g/day) TCA†	Calf A (3 g/day) TCE‡	Calf B (12 g/day) TCA†	Calf B (12 g/day) TCE‡	Calf C (12 g/day) TCA†	Calf C (12 g/day) TCE‡	Calf D (12 g/day) TCA†	Calf D (12 g/day) TCE‡
	mg							
1	11.8	500	16.2	1169	0	793	0	911
2	18.6	596	43.1	1339	7.3	613	14.6	692
3	33.6	848	109	2487	15.8	1453	37.4	2116
4	48.3	1475	78.0	1346	37.2	2350	59.3	2595
5	46.6	576	145	1973	79.3	1651	97.9	1697
§								
6	36.5	75	121	116	20.7	39.5	28.2	53
7	25.0	8.6	73.5	16.4				
8	16.9	0	37.2	5.5				
9	16.6	0	38.1	0				
10	11.7	0	16.8	0				

* Includes urochloralic acid and other compounds which give a positive Fujiwara reaction after oxidation.

† Trichloroacetic acid.

‡ "Trichloroethanol" corrected for trichloroacetic acid excreted.

§ Daily dose of trichloroethylene given in parentheses below letter of each animal; fed on days above horizontal line.

to the fraction which, with this reservation in mind, is referred to as "trichloroethanol."

Results. No ill effects on the calves were observed as a result of the administration of trichloroethylene. No compounds giving a positive Fujiwara test were detected in the urine collected before the administration of trichloroethylene.

Trichloroethylene could not be detected in the aqueous phase of the collected specimens even after extensive aeration and collection of the volatile components in toluene. However, while trichloroethylene was fed, the toluene layer used to preserve the urine contained from 3 to 6 micromoles of trichloroethylene per day which in the case of calves B to D is less than 0.01% of the dose fed.

The presence of trichloroacetaldehyde, an assumed product of metabolism of trichloroethylene(6) and the probable direct precursor of trichloroacetic acid and trichloroethanol could not be detected by a test(11) which is sensitive to at least 15 μg; lack of evidence for the presence of trichloroacetaldehyde is in accord with the observations of Butler with dogs(6).

Table I summarizes the data obtained with determinations of trichloroacetic acid and "trichloroethanol" in the urine. Although trichloroacetic acid was not identified as such in the urine it was detected through its behavior in the Fujiwara reaction(11). During the administration of trichloroethylene, the concentration of trichloroacetic acid in the urine increased gradually; it decreased again gradually after feeding of trichloroethylene had ceased. The molar recovery of trichloroacetic acid in the urine of calves A and B was only 1.4 and 1% respectively of the total amount of trichloroethylene administered and only 5.6 and 7.2% respectively of the "total trichloro compounds" found in the urine. Continued excretion of trichloroacetic acid after exposure to trichloroethylene has also been observed in humans and in dogs(6). However, in those species the proportion of trichloroethylene metabolized to trichloroacetic acid appears to be much higher than in the bovine. Butler estimated that in dogs (6) trichloroacetic acid was excreted to the extent of about $\frac{1}{3}$ to $\frac{1}{2}$ of the amount of trichloroethanol. In the urine of rats(8,12) however, exposed to 80 mg of trichloroethylene per liter of air, the concentration of trichloroacetic acid was only 1.2 mg per liter. Ahlmark and Forssman(8) found that man excreted from 6 to 18% of inhaled trichloroethylene as trichloroacetic acid in the urine. The highest concentration of trichloroacetic acid (50 mg per liter) was found in calf B on the fifth day. In man, Grandjean *et al.* (13) have found that a mean of 13% of the

trichloroethylene inhaled during a working day was excreted as trichloroacetic acid; the concentration of trichloroacetic acid in the urine of their subjects ranged from 8 to 440 mg per liter with a mean of 84.7 mg.

"Trichloroethanol" was excreted by the calves rapidly and extensively after administration of trichloroethylene. The largest amount, 2.6 g was found in the urine of calf D on the fourth day after feeding trichloroethylene; the concentration was 940 mg per liter in this specimen of urine. When feeding of trichloroethylene was discontinued "trichloroethanol" disappeared from the urine within 2 to 3 days. Feeding of trichloroethylene at the higher level increased the excretion of "trichloroethanol" but not proportionately. The recovery of "total trichloro compounds" in the urine during the whole period was only 25.4, 13.3, 13.5 and 15.2% respectively for calves A through D, calculated on a molar basis. Our analytical procedure did not permit differentiation between free and combined trichloroethanol. Butler (6) has reported that dogs excreted from 42 to 66% of trichloroethanol which was injected intramuscularly, mostly in conjugated form.

Discussion. We are not aware of experiments in which the metabolism of trichloroethylene was studied after oral administration; for this reason and because the bovine has not previously been used for studies of the metabolism of trichloroethylene comparisons with other species are somewhat tenuous. However, these experiments show that some of the products of metabolism of trichloroethylene in the bovine are similar to those observed in other species. While it is evident that the calf can "detoxify" trichloroethylene to a considerable extent and excrete urochloralic acid as well as trichloroacetic acid a major fraction of the trichloroethylene administered remained unaccounted for. It is possible that this portion may have been in part exhaled or excreted with the feces, or metabolized to monochloroacetic acid(14) or to inorganic chloride(15) and thus would have escaped detection by the Fujiwara test.

Summary. Following the oral administration of 3 to 12 g of trichloroethylene to calves weighing about 100 lb, about 1% of this compound was excreted in the urine as trichloroacetic acid and from 13 to 25% as trichloroethanol (free and combined). Only traces of trichloroethylene were excreted in the urine. No trichloroacetaldehyde could be demonstrated in the urine with the method used. Some of the metabolic products of trichloroethylene in the bovine are the same as those produced by man and dogs.

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