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Increase in B-Vitamin Concentrations in Digestive Tract Contents of Young Dairy Calves.* (19280)

E. M. KESLER AND C. B. KNODT.

From the Department of Dairy Husbandry, Pennsylvania State College, State College, Pa.

In a previous report(1) the authors presented data which indicated that levels of certain of the B vitamins in the contents of the digestive tract of calves were not related to the age of the animal. Concentrations were as high or higher in calves 2 and 4 weeks of age as in older calves up to 14 weeks, and these levels were greater generally than those in the feed consumed. It seemed desirable to make additional studies using the younger calf in an attempt to determine whether the indicated high values were a consistent phenomenon.

Experimental. Male Holstein calves were quartered and fed in individual pens in an artificially heated and ventilated barn. A medium grade of alfalfa hay and a calf starter were fed *ad libitum* to the animals from the time they entered the barn. Each calf had received colostrum for approximately 3 days, then was placed on a milk feeding schedule which would allow 270 lb of Holstein herd milk during the ensuing 36 days. Twenty calves were divided at random into age groups for slaughter, 5 being sacrificed at each of the following ages: 8, 16, 24 and 32 days. The final feeding of milk occurred 6 hours prior to slaughter; however, hay and grain were accessible to the calf until the time of slaughter. The contents of the rumen, abomasum, small intestine and large intestine were collected in sample jars, iced, taken to the laboratory and blended into a slurry. Chemical assays for thiamine, riboflavin, niacin and pteroylglutamic acid were performed on the slurried contents of the digestive tracts of these calves. Thiamine was determined by the thiochrome method(2), riboflavin by a modification of the fluorometric procedure as set forth by Loy(3), niacin by the cyanogen bromide-metol method(4) and pteroylglutamic acid (PGA) analyses were performed according to the fluorometric method as outlined by Alfrey, *et al.*(5). The milk, grain, and hay which were being fed were similarly assayed several times during the trial. Concentrations of all factors were calculated on a dry matter basis. Since records had been made of the amounts of milk, hay and grain which each calf had received, it was possible to calculate an average level of vitamin intake for each calf on a dry matter basis. This level of intake varied slightly among calves, depending upon the relative proportions of the three feedstuffs consumed.

Results and discussion. The results of the assays for the four vitamins studied are pre-

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TABLE I. Levels of Thiamine, Riboflavin, Niacin and PGA in Digestive Tract Contents of Dairy Calves Compared to Levels in the Feed Consumed.* (μg/g of dry matter).

	Age group (days)	Intake	Rumen	Abomasum	Small intestine	Large intestine
Thiamine	8	5	15.4	2.3	8.3	15
	16	5.1	16.4	4.5	7.9	14.4
	24	5.2	9.7	2.8	6.3	15.3
	32	5.5	10.1	3.9	7.9	12.4
Riboflavin	8	15.8	45.6	13.6	74	256.8
	16	15.3	34.9	13	46.5	153.9
	24	15.3	26.4	14.7	45.3	93.4
	32	14.7	21.6	12.9	42.9	61.7
Niacin	8	7.6	40.9	26	86.6	88.7
	16	7.9	40	8.6	55.5	42.4
	24	9.8	45.2	19.5	103.5	77.4
	32	15.7	36.3	21.8	74.4	39
PGA†	8	7.7	15.6		9.4	55.1
	16‡	6.8	48.4		21.2	66.1
	24	10.6	23.8		26.9	52.4
	32	18	14		31.9	65.3

* Data presented are means for 5 calves slaughtered at each age.
† PGA analyses were run on contents of rumen, small intestine and large intestine only.
‡ PGA data at 16 days on 3 calves only.

sented in Table I, where comparisons are made between levels of each vitamin found in the different regions of the digestive tract and the intake levels in the feed consumed. With the exception of the abomasum, concentrations of thiamine were higher in the material after ingestion than in the feed. Concentrations of this vitamin in the rumen and large intestine were greater than those in the abomasum and small intestine. Levels of thiamine in the samples from the 8-day-old calves were as high or higher than those from the older calves. This lack of difference due to increasing age was noted for all vitamins studied. The results of riboflavin analyses were similar to those for thiamine. Greatly increased levels over those in the feed were found in the rumen, a decrease in the abomasum, then an increase in the small intestine followed by very high concentrations in the large intestine. Much higher levels of niacin were noted throughout the digestive tract than were present in the feed, with the highest concentrations usually in the intestines. PGA determinations were made on the contents of the rumen, the small intestine and the large intestine, the abomasum being omitted. Data presented for the 16-day group are the average of only three calves, the data for the other two being unavailable. With the exception of the 32-day group, concentrations of PGA in the rumen contents were higher than in the feed. The contents of the large intestine exhibited the highest concentrations of this vitamin.

These data show that levels of the vitamins studied were higher in the contents of the digestive tracts of young calves than in the feed. However, no positive proof of microbiological synthesis is offered, since selective absorption of nutrients other than the vitamins studied could result in a concentrating effect of the vitamins in the unabsorbed material in the tract. The differences noted between levels in the digestive contents and the feed are of similar magnitude to those noted by other workers using older ruminants. In addition to whole milk, animals in this experiment had access to hay, grain and bedding. This, plus the fact that they had been with their dams for 3 days after birth, offered opportunity for vitamin synthesizing organisms to develop.

Summary. Concentrations of thiamine, riboflavin, niacin and pteroylglutamic acid were found to be higher in the contents of various regions of the digestive tract of young dairy calves than in the feed consumed. The regions studied were the rumen, abomasum, small intestine and large intestine. Concentra-

tions did not change with increasing age of the calf from 8 to 32 days.

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Development of Tolerance and Cross Tolerance by Mephobarbital.* (19281)

M. I. GLUCKMAN AND CHARLES M. GRUBER.

From the Department of Pharmacology, The Jefferson Medical College, Philadelphia, Pa.

A review of the literature on the development of tolerance to the hypnotic action of many barbituric acid derivatives in experimental animals has been presented by Gruber and Keyser(1). That cross-tolerance between different barbiturates can also occur has been demonstrated by the same investigators. Since that publication further evidence on the development of tolerance and cross-tolerance between the barbituric acid derivatives has been demonstrated by Carmichael(2), Hubbard and Goldbaum(3) and Holck and his associates(4). There have been reports in the literature, however, which indicate that no tolerance can be developed with mephobarbital (Mebaral) 5-ethyl-1-methyl-5-phenylbarbituric acid(5-8) but we have been unable to find any report in which a study has been made to determine actually whether there can or can not be developed tolerance to this chemical in experimental animals. This investigation was therefore undertaken to answer this question and also if tolerance can be developed with mephobarbital in experimental animals does it also produce cross-tolerance.

Methods. Seventeen rabbits, 8 males and 9 females, weighing between 2.1 and 2.6 kg were used. The sodium salt of mephobarbital was employed for injection. This salt was formed by neutralizing the acid with sodium hydroxide and back-titrating with hydrochloric acid to the lowest pH possible without precipitation. Solutions were made up in distilled water and the concentrations adjusted so that 2 cc/kg were given as the final dose. Mephobarbital (40 mg/kg once daily for three days) was injected rapidly, intravenously and the length of induced sleeping time noted following each injection. On the fourth day butabarbital sodium (36 mg/kg) was similarly administered. The same dose of butabarbital was injected on the fourteenth day as a control for this drug. The criterion for development and abolition of sleep was the loss and gain of the righting reflex respectively(9).

Results. Evidence recorded in the literature indicates that there is no essential sex difference in rabbits in response to the action of certain barbiturates(1,9,10). Our results are in accord with these findings. Following the injections of mephobarbital on the first, second and third days, the males slept an average of 19.5, 7.7 and 7.3 minutes respectively while the females slept an average of 16.2, 10.1 and 4.8 minutes respectively. The results with the use of butabarbital also showed no significant difference in the sleeping times between the males and females. Therefore the results obtained from the male and female animals were combined. The mean sleeping times for the first, second and third daily injections of mephobarbital were 17.8, 8.9 and 5.9 minutes respectively and two times the standard errors were $\pm$ 5.4, $\pm$ 1.9

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