XII. NOTE ON THE QUANTITY OF THEOBROMINE IN THE MILK OF COWS FED ON A DIET INCLUDING THIS ALKALOID

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UNTIL the discovery by Knapp & Coward [1934] that cacao shell is rich in vitamin D, this by-product of the cocoa and chocolate industry never achieved more than a casual interest as an accessory cattle food. Kon & Henry [1935] reported that the feeding of 2 lb. of shell daily for a month during the winter raised the vitamin D content of the milk of stall-fed cattle to the normal summer level, whilst Golding & Burr [1937] found that the fat content of these milks was increased during the period of shell feeding. Because of these facts it appears probable that cacao shell will occupy a much more prominent place as a cattle fodder than it has done in the past.

Cacao shell contains approximately 1% of theobromine, an alkaloid valued by pharmacologists for its properties as a stimulant and diuretic. It is natural to enquire how much, if any, of this alkaloid reaches the milk of cattle fed with shell. An opportunity to throw some light on this matter was recently presented when an experiment was conducted at this Institute to determine whether the increase in the milk-fat yield, which had been observed when cacao shell was fed, was due to the theobromine alone. In this experiment, theobromine was fed to a small group of dairy cows for a period of 3 weeks; the daily weight administered was 9 g., which is the quantity contained in 2 lb. of cacao shell. Samples of milk were taken from three cows from this group on the last day of the feeding and an estimate made of their theobromine contents. The results obtained (Table I) and the chemical procedure adopted are given below.

Table I

Animal	Average daily milk yield lb.	Yield on last day of experiment lb.	"Theobromine" in milk mg. per l.
Profit	35.06	8½	4.71
Lead 46	19.44	6½	2.05
Sainfoin	17.75	6	4.72

Method of analysis. The method adopted was that of Kunz [1935]. The proteins in 1 l. of milk were precipitated with trichloroacetic acid. After filtration, the filtrate was neutralized with ammonia, conc. *in vacuo* to about 100 ml., seeded with lactose crystals, and left for 24 hr. The crystals of lactose which formed were removed by centrifuging, the solution made slightly alkaline with ammonia and the precipitate again removed by centrifuging. The solution was conc. to 40 ml. and continuously extracted with chloroform for at least 8 hr. About 10 ml. of water were floated on the surface of the CHCl_3 which was then distilled off. To the resulting aqueous solution was added excess 30% phosphotungstic acid, the mixture was left overnight in an ice chest, and the bases regenerated from the precipitate by means of baryta. After removal of excess

Ba with CO_2 , the solution was boiled, filtered and conc. to 20 ml. 1 ml. each of 10% acetic acid and satd. sodium acetate were added, the solution was heated to boiling and 1 ml. each of 40% NaHSO_3 and 13% CuSO_4 were added. After boiling 5 min., it was filtered directly into the extraction apparatus and extracted for 5 hr. with CHCl_3 . The extract was transferred to aqueous solution as before and brought to 25 ml. From the N content of this solution, determined by micro-methods, the concentration of theobromine was calculated (1 ml. $N/100$ acid $\equiv$ 0.4499 mg. theobromine). This figure would include any caffeine that might be present, as it was considered unnecessary further to complicate the analysis with another precipitation.

Controls. A blank determination on normal milk gave a "theobromine" content as 0.99 mg. per l. Kunz [1935] obtained a blank of 0.2–0.4 mg. per l. as caffeine in woman's milk, but gives no similar figure for theobromine. In normal cow's milk to which had been added 10.37 mg. theobromine per l. I could detect only 6.89 mg. per l. using the technique described above.

Isolation and identification of theobromine in milk. Seven hundred ml. of milk from Sainfoin were treated according to the above method as far as the second chloroform extraction, when the residue after evaporation of CHCl_3 amounted to 3.9 mg., equivalent to a concentration of 5.57 mg. per l. of milk, which exceeds the value calculated from the N-determination by only 0.85 mg. per l.

An attempt was made to recrystallize this residue from water, but the crystals so obtained were of irregular shape although of the same general (minute) size as theobromine crystals. They gave a definitely positive reaction to the following test for theobromine, caffeine and theophylline: To 0.5 mg. theobromine on a watch-glass add 2 drops of a mixture of conc. HCl diluted with 2 vols. water. Boil, add 5 drops Br-water, and evaporate carefully to dryness. Dissolve the residue in 5 drops water, add 1 drop 1% FeSO_4 , place 1 drop N ammonia by the side of the solution, and allow slowly to mix. An intense dark blue coloration forms, which quickly fades on standing or with excess ammonia.

DISCUSSION

It is evident that a very small quantity of theobromine has passed into the milk of these cows, the maximum concentration being approximately 7 mg. per l.

The British Pharmaceutical Codex and Squire's Companion to the British Pharmacopoeia give the medicinal dose of theobromine as 0.3–0.6 g.; taking the case of a child of under 12 months, for which the dose would be about 1/12th of that prescribed for an adult, the minimum dose is contained in $6\frac{1}{4}$ pints of this milk. Perhaps a better idea of the harmlessness of this concentration of theobromine is obtained when it is considered that the average plain eating chocolate contains 0.3% and that it is not uncommon for $\frac{1}{4}$ lb. of this chocolate (containing 0.3 g. of theobromine) to be eaten by an adult at one time. It is unlikely, therefore, that the proportion of theobromine appearing in these milks could have any noticeable effect on the most delicate of constitutions.

One would expect both the health of the animal and the period of feeding to influence the proportion of alkaloid appearing in the milk, and a more comprehensive experiment on these lines seems desirable.

SUMMARY

9 g. of theobromine were fed daily to each of three cows for a period of 3 weeks. The maximum concentration of theobromine appearing in the milks yielded on the last day was approximately 7 mg. per l., an amount which may be presumed to be entirely innocuous.

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REFERENCES

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