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Presence of Endogenous Digitalis-Like Factors in Milk

To the Editor:

Endogenous factors with biological and immunological properties similar to digitalis drugs (endogenous digitalis-like factors, EDLF) have been found in several tissues and body fluids of animals and humans, and higher concentrations are generally observed in newborns (*1–3*). We recently reported that EDLF are present in human milk (*4*). To verify whether EDLF are normal constituents of milk in other mammals, we measured digoxin-like immunoreactivity in milk pools from some domestic animals (horse, goat, sheep, rabbit, dog, and cow) by a previously described solid-phase RIA method, which uses an anti-digoxin antibody (*4, 5*). Significant EDLF concentrations were found in the milk of all species studied (Table 1), thus suggesting that EDLF may play an important physiological role in the first days of life in mammals and confirming previous data obtained in humans (*3, 4*).

Table 1. EDLF Concentrations Measured by RIA in Pooled Milk Samples

Source of milk	EDLF conc, ng/L de
Human	61
Cow	47
Dog	179
Goat	67
Horse	64
Sheep	157
Rabbit	150

Moreover, we measured the EDLF concentrations in 30 milk specimens from 12 cows (from 6 cows, milk specimens were collected for 4 successive days); the mean (± SD) EDLF concentration found in milk specimens of these cows was 46.2 ± 10.8 ng/L digoxin equivalents (de) (range 20.5–60.0 ng/L de). We did not find any increase in EDLF concentrations after boiling the milk specimens, thus suggesting that EDLF are not (or weakly) bound to milk proteins, as also previously reported for human milk (*4*).

Finally, to determine whether industrial techniques for preparing artificial milk formulas for babies could affect the EDLF concentrations, we assayed three different types of milk formulas for neonates, choosing the most popular commercial products in Italy: five formulas for preterm babies (ESPGAN 1982), eight adapted formulas (ESPGAN 1977), and five cow-milk-based formulas (CODEX 1976) (all are powdered milk; reconstitution with water to the desired dilution was done as suggested by the manufacturers). No significant difference was observed among EDLF concentrations in the different formulas assayed; a mean EDLF value of 42.7 ± 16.9 ng/L de was found, which is very similar to that of native cow milk (46.2 ± 10.8 ng/L de), but lower than that previously observed in human milk (60.6 ± 4.9 ng/L de) (*1*).

In conclusion, our data indicate that (*a*) EDLF are normal constituents of milk from humans and several types of domestic animals; (*b*) industrial techniques for the preparation of artificial milk formulas for babies do not affect EDLF concentrations present in the native cow milk used.

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Cost-Effective Method for Detection of "Hook Effect" in Tumor Marker Immunometric Assays

To the Editor:

Under certain conditions, immunometric assays can give inaccurate measurements when the concentration of the analyte is greatly in excess of that of the antibody. This phenomenon, termed the "hook effect," occurs when the apparent concentration of an undiluted specimen with a high concentration of analyte falls within the calibration curve of the assay. Sandwich-type immunoassays, in which all components are added simultaneously, are particularly susceptible to this effect. Although most analytes do not reach sufficient serum concentrations to be affected by the hook effect, some tumors secrete extremely large amounts of specific proteins, used as tumor markers, and give rise to this phenomenon, in which the serum concentration of the analyte may appear to be normal. In this laboratory we have previously experienced the hook effect with specimens analyzed for prolactin and prostate-specific antigen (PSA) (*1–4*). For example, the concentration of PSA in a specimen that was 50 660 μg/L was initially found to have a concentration of 5.4 μg/L when undiluted (*4*).

Although underestimations of tumor marker concentration due to the hook effect occur infrequently, the consequence of such an error has serious medical implications. We have tried several approaches to detect samples that display the hook effect while maintaining cost-effectiveness. Our initial strategy was to analyze all patients' specimens at a minimum of two dilutions; however, this increased the cost of analysis by ≥100%. As an alternative, we use the following strategy: all specimens in a run are batched in groups of 10. As the specimens are prepared for analysis, an aliquot from