Reports

Iodine-131 in the Thyroids of North American Deer and Caribou: Comparison after Nuclear Tests

Abstract. *Concentrations of I^{131} in the thyroids of deer from Washington, Colorado, and Maryland, and of caribou from Alaska were measured after the resumption of nuclear weapon tests by the U.S.S.R. on 1 September 1961. Maximum concentrations occurred nearly 2 months after the first nuclear test and then decreased at an effective half-time of about 15 days. Thyroids from Washington and Colorado deer showed the highest concentrations, thyroids from Alaskan caribou the lowest.*

During the initial fallout period after the U.S.S.R. nuclear-weapon tests which began on 1 September 1961, radioiodine concentrations were measured in thyroids of wild deer and caribou from several widely separated areas in the United States. Since these herbivores are native residents upon the landscape, their organs should reflect the pattern of vegetation contamination resulting from radioactive fallout.

Thyroids of mule deer (*Odocoileus hemionus*) from southeastern Washington and north-central Colorado, white-tailed deer (*O. virginianus*) from Maryland, and barren-ground caribou (*Rangifer arcticus*) from northwestern Alaska were analyzed for iodine-131 (*1*). Entire thyroids were macerated, a weighed sample was placed in 1-dram vials, and individual vials were inserted into the well of a NaI(Tl) crystal coupled to a photomultiplier and pulse-height analyzer. Iodine-131 was identified by its gamma-ray photopeak at 0.36 Mev. Data were obtained by counting the pulses with a decade scaler.

Sampling was started in the middle of October 1961, a time before maximum values occurred after the Soviet nuclear test series was concluded. The study was continued until February 1962, when all values were below the detection limit (about 0.1 nanocuries of I^{131} per gram of wet thyroid tissue).

Results are presented in Fig. 1. Thyroids from Washington deer contained the highest I^{131} concentrations on any given sampling day, but the concentrations were generally comparable to the values for thyroids from Colorado animals. Thyroids from Maryland deer contained concentrations slightly greater than those of Alaskan caribou. The uptake of I^{131} by the thyroids of deer and caribou was grossly similar to that of the thyroids of Nevada cattle (*2*). Maximum concentrations occurred on 25 to 26 October in caribou and during the period 31 October to 8 November in Colorado mule deer. Similarly, Blincoe and Bohman (*2*) reported that maximum concentrations in thyroids of domestic cattle from the vicinity of Reno, Nevada, occurred during this period; however, the concentrations were one-third of those in the Colorado deer we examined. Eisenbud *et al.* (*3*) reported that maximum I^{131} concentrations in the thyroids of people in New York City occurred in late October. For these people the main source of radioiodine was concluded to be milk. This would indicate that the thyroidal I^{131} peak in dairy cattle of the New York City milkshed occurred several days earlier than it occurred in the Colorado and Nevada animals.

The concentration of fission products in air samples taken in September along the 80th meridian (west) in the eastern United States increased markedly on 12 September and reached a peak during the period 19–25 September (*4*). The radioactivity in the rainfall in England showed a similar pattern (*5*).

The rate of decrease from the period of maximum I^{131} concentrations in thyroids is indicated in Fig. 1 by lines fitted by the least squares method. The apparent 14- to 16-day effective half-life of radioiodine in samples from Alaska, Colorado, and Washington indicates that the animals received decreasing increments of radioiodine during this period. It is noteworthy that thyroidal I^{131} concentrations in the Nevada cattle (*2*) decreased at a rate twice that of deer and caribou thyroids. This suggests that the cattle received a short exposure to the radioiodine fallout, after which they were placed on a maintenance ration of uncontaminated feed. This may also explain the much lower concentrations in cattle thyroids compared with those in Colorado deer during the same period.

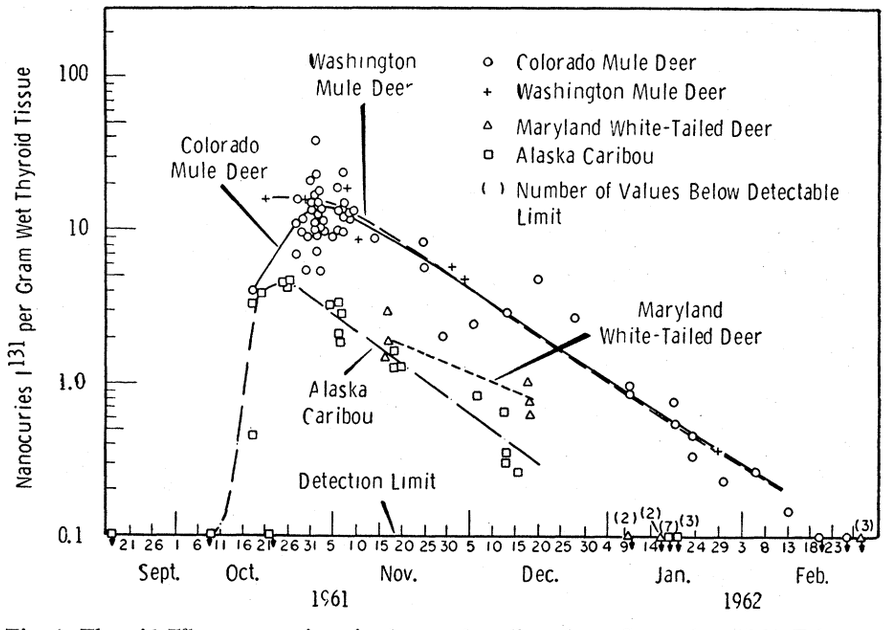

Fig. 1. Thyroid I^{131} concentrations in deer and caribou from September 1961–February 1962.

Differences in grazing or browsing habits of the species in this study are regarded as having little effect upon the sample values because the short physical half-life of I^{131} allows only recently contaminated plant surfaces to contribute to the concentration of the nuclide in thyroids (*6*).

W. C. HANSON
Hanford Laboratories, General Electric Company, Richland, Washington
F. W. WHICKER
A. H. DAHL
Radiology Section, Colorado State University, Fort Collins

References and Notes

1. Thyroids of Maryland deer contributed by Dr. V. Flyger of the University of Maryland, those of Alaska caribou by P. Lent and O. Lønø of the University of Alaska working under the direction of Dr. W. O. Pruitt, Jr., and those of Colorado deer by D. E. Medin and A. E. Anderson, Colorado Department of Game and Fish. The assistance is gratefully acknowledged.
2. C. Blincoe and V. R. Bohman, *Science* **137**, 690 (1962).
3. M. Eisenbud *et al.*, *Science* **136**, 370 (1962).
4. L. B. Lockhart, Jr., in *U.S. At. Energy Comm. Publ. HASL-117*, (1961), p. 163.
5. K. Boddy, *Nature* **192**, 443 (1961).
6. Work performed under contract AT(45-1)-1350 between the Atomic Energy Commission and the General Electric Company.

20 February 1963

Albumin Replacement by Fatty Acids in Clonal Growth of Mammalian Cells

Abstract. *Clonal growth of Chinese hamster strain CHD-3 cells in a synthetic nutrient mixture supplemented with 10 micrograms of purified fetuin per milliliter exhibits an apparent requirement for serum albumin. The albumin can be replaced by linoleic acid, which occurs as a tightly bound component of most albumin preparations. Linolenic acid and corn oil can also replace albumin, while oleic acid and esters of linoleic and linolenic acids cannot.*

Synthetic nutrient mixture F10, supplemented with purified serum albumin and fetuin, will support the clonal growth of Chinese hamster strain CHD-3 cells (*1*). Minor modifications of the plating technique and the composition of the nutrient mixture permit reduction of the protein concentrations required for clonal growth to 10 μg of fetuin and 20 μg of albumin per milliliter. Under these conditions, serum albumin appeared to be essential for clonal growth.

However, the serum albumin can be replaced by an unsaturated fatty acid which normally occurs tightly bound to it. To show this, the nutrient mixture F10 (*1*) was modified by increasing the calcium chloride concentration to $1.5 \times 10^{-3}M$ and the magnesium sulfate concentration to $1.0 \times 10^{-3}M$. These concentrations were the best in titrations at the reduced protein concentrations, and represent substantial improvements over the calcium and magnesium concentrations in F10.

In the revised plating technique, the modified nutrient mixture is placed in the petri dishes, and the protein supplements are added to it with the cells. Modified nutrient, with 500 μg of fetuin (*2*) and 1000 μg of albumin per milliliter (*3*), is used to dilute the cell suspension obtained by trypsinization of a stock culture. The first stage of the serial dilution is made as quickly as possible after the cells are released from the surface of the culture bottle. A hemocytometer count is then made, and this is followed by additonal dilutions to yield a final concentration of 2000 cells per milliliter. One-tenth of a milliliter of the final dilution is then added to 5 ml of the modified nutrient in each petri dish, resulting in a suspension of 200 cells per dish, and a final protein concentration of 10 μg of fetuin and 20 μg of albumin per milliliter.

Plastic tissue-culture petri dishes (Falcon No. 3002) were used. Carefully cleaned glass dishes may also be used, but at low protein concentrations they tend to yield less consistent results. Except for these modifications, all procedures were as previously described (*1*).

In albumin replacement tests, the albumin is omitted from the diluent, and albumin replacements are added to the nutrients in the petri dishes. Fatty acids are dissolved in absolute ethanol at $1.0 \times 10^{-3}M$ and added directly to the modified nutrient mixture in the petri dishes or else are first serially diluted with the modified mixture. Care is taken never to add a total of more than 1 percent ethanol to the final medium.

Serum albumin binds fatty acids so tightly that even crystalline preparations retain them unless specially treated (*4*). In a recent gas-liquid chromatographic analysis of the fatty acids bound to a preparation of commercial albumin similar to that used in this laboratory for cell growth, Saifer and Goldman reported the presence of 43 different fatty acid peaks (*5*). One of the major peaks,

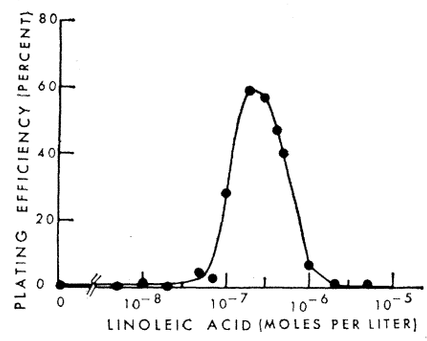

Fig. 1. Response of strain CHD-3 to linoleic acid in the absence of serum albumin. Basal medium. Modified F10 plus 10 μg of fetuin per milliliter.

comprising 20 percent of the total bound fatty acids, was linoleic acid, an essential nutrient for at least some mammals (*6*).

Linoleic acid (Calbiochem, "A grade") was tested as a replacement for serum albumin and was effective in promoting clonal growth of strain CHD-3 within a narrow range of concentration (Fig. 1). The optimum concentration is approximately $2.5 \times 10^{-7}M$, with essentially no growth occurring below $1 \times 10^{-7}M$ or above $1 \times 10^{-6}M$. Near the optimum concentration of linoleic acid, well-formed colonies are obtained which are almost as large as those obtained with 20 μg of serum albumin per milliliter. At 10 days, the colonies grown with $2.0 \times 10^{-7}M$ linoleic acid averaged 440 cells per colony, compared to 640 cells per colony with 20 μg/ml of albumin.

Linolenic acid, which will replace

Table 1. Replacement of serum albumin for clonal growth of Chinese hamster strain CHD-3 cells. Results are expressed as percentages of the plating efficiency obtained with 20 μg of albumin per milliliter. (The average plating efficiency obtained with albumin is 70 percent.) The esters and the corresponding free acids are at the same molar concentrations for direct comparisons. All other additives are at optimum concentrations.

Additive	Amount (μg/ml)	Relative plating efficiency
None	——	0.2
Albumin*	20	100
Linoleic acid†	0.07	83
Ethyl linoleate‡	0.08	1
Linolenic acid‡	0.03	58
Methyl linolenate‡	0.03	0
Oleic acid†	0.08	4
Corn oil§	1.0	85

* Normal human serum albumin, "Albumisol" Merck, Sharpe and Dohme. † Calbiochem "A grade". ‡ Nutritional Biochemicals "Highly purified" grade. § "Mazola" Best Foods Division, Corn Products Co.