THE EFFECT OF SUNSHINE UPON THE ANTIRACHITIC POTENCY OF BUTTER

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Long before the discovery of vitamin D it was believed that sunshine and fresh air had some definite relationship to the prevalence of rickets. Extensive work upon this subject has established a direct connection between sunlight and vitamin D. Huldschinsky (1919) working with German children after World War I, was the first to demonstrate that ultraviolet irradiation constitutes a specific cure for rickets. McCollum and his associates (1939) showed that there are two kinds of rickets: one due to a diet deficient in calcium but containing a normal amount of phosphorus, and the other due to a diet deficient in phosphorus but containing a normal amount of calcium. These are characterized as low calcium and low phosphorus rickets. Hess (1924) and Steenbock (1924), working independently, induced antirachitic properties in rickets-producing diets by means of ultraviolet irradiation. Further work by these investigators led to the discovery that only cholesterol and other sterols develop antirachitic potency upon irradiation.

Luce (1924) found that the antirachitic properties of milk are dependent upon the diet of the cow and the amount of sunshine to which the cow is exposed. Betchel and Hoppert (1936) found that winter milk contained only five International Units of vitamin D per quart, while summer milk contained forty International Units. Champion, Henry, Kon and McIntosh (1937) concluded that direct exposure of the cow to sun and skyshine contributes all, and pasture none, of the vitamin D potency of summer milk.

The greater amount of sunshine in Oklahoma, linked with the knowledge that exposure of the cow to sunlight increases the antirachitic properties of milk, suggested that butter produced in this state might prove to be a better source of vitamin D than that produced in a state having less sunshine.

A northern state, noted for its dairy products and known to have less sunshine than Oklahoma, was selected as the source of butter for comparison. Butter for the present study was obtained from the Department of Agriculture of the State University in this northern state, and from a local dairy. Both butters used in the experiment were made during the months of March and April 1940 from milk produced in the regions under investigation.

United States Weather Bureau Reports (1940) showed Oklahoma City to have more sunshine than the northern area. The actual percent of sunshine for the northern area was thirty-eight for March and forty for April, while the actual percent for Oklahoma City and vicinity was seventy-six for March and seventy-four for April 1940.

Relative humidity is also a factor in the amount of sunshine available,

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as an increase in humidity would shut off some of the sunshine. The northern area was found to have a larger percent of relative humidity than Oklahoma City.

EXPERIMENTAL

Six litters of twenty-two to twenty-five-day-old albino rats of good nutritional background, containing six animals to the litter, were used. They were placed in individual mesh wire cages and fed a rachitogenic diet. The animals were continued upon this diet until they developed rickets.

The test period lasted eight days. One animal of each litter was used as a negative control and continued on the rachitogenic or basal diet. Another one, a positive control, was given a total of thirty milligrams of reference cod liver oil during the eight-day period, in addition to the weighed portions of the basal diet. According to Simms, this amount of reference oil should give + + healing.

Of the four remaining rats of each litter, two were given different amounts of butter produced in the area having more sunshine, and two were given the same amounts of butter produced in the area having less sunshine, in addition to the weighed portions of the basal diet.

Three levels of butter fat were fed, namely, 0.5 gram, 1.5 grams, and 2.0 grams. The amounts of butter fat fed were decided upon after careful study of the fat content of a large number of experimental diets for albino rats. The weighed amounts of butter fat for each rat per day were mixed with a small amount of the basal diet and fed in castor cups. When the butter fat was eaten the remainder of the basal diet was given.

As a means of distinction, the experimental animals fed Oklahoma butter fat are referred to as Oklahoma rats, and the others, northern rats.

RESULTS AND DISCUSSION

The "line test", developed by McCollum and associates and still the most satisfactory method for testing the potency of antirachitic substances, revealed severe rickets in all of the negative control animals except that of litter II. This animal reached a stage somewhere between severe rickets and a + healing. The positive control of each litter, except that of litter V, showed a + + healing or better. The excepted animal showed approximately a + healing.

While the difference in the degree of healing produced by the lowest compared to the intermediate, and the intermediate compared to the highest level of butter fat fed is noticeable, there is a very marked difference between the healing at the lowest and the highest levels.

Since the degree of healing reached by the experimental animals did not resemble, in all details, the degree of healing exhibited by the pictures supplied by other workers in this field, an individual method of grading the specimens were developed. In an attempt to show comparisons between the stages of healing, a series of plusses is used. When the degree of healing seems to be somewhat better than the lower stage, but not as good as the next higher stage of healing, some part of the plus sign is added. As the degree of healing becomes more like the next stage, more parts of the plus sign are shown. (Table I).

Oklahoma rat four of litter IV showed better than a + + + healing (table I). Next in order and ranked according to their degree of healing are: Oklahoma rat five, litter IV; Oklahoma rat three, litter V; Oklahoma rats two and four, litter VI: northern rats five and six, litter II; and northern rat six, litter III.

The conclusions regarding the degree of healing of each of the animals were not reached by the writer alone, but were verified by three people expert in the use of the microscope on biological material.

Since one gram of reference cod liver oil contains 95 United States Pharmacopoeia Units of vitamin D per gram, the thirty milligrams administered to the positive control contained 2.85 United States Pharmacopoeia Units. As judged by the stages of healing reached by the animals, 5.0 grams of Oklahoma butter are indicated to be equivalent to thirty milligrams of reference oil, or to contain 0.57 United States Pharmacopoeia Units per gram, and therefore 57 units are present in 100 grams of the butter fat.

The results of this experiment show an appreciable difference in the antirachitic potency of Oklahoma butter fat and that of butter fat from a region with less sunshine. The values found for the two butters studied show that of Oklahoma to be approximately four times as potent a source of vitamin D as the northern butter. These results, it is believed, can be attributed to the fact that Oklahoma had more sunshine during the months of March and April 1940 than did the northern states.

The daily vitamin D requirement of adults has not been determined. Some think that adults should have as much as the present suggested standard for young children (400 International Units). One hundred grams of butter fat (approximately one-fourth pound of butter) supplies about one-seventh of this.

If further experimental work gives comparable results, it can be concluded that butter produced in Oklahoma has a superior vitamin D content to that made in regions where there is less sunshine. This fact should stimulate its production and consumption.

LITERATURE CITED

Betchell, H. E., and C. W. Hoppert. 1936. A study of seasonal variation of vitamin D in normal cows' milk. J. Nutrition 11: 537-547.

Champion, J. E., Kathlene Mary Henry, S. K. Kon, and James MacIntosh. 1937. The source of vitamin D in summer milk. Biochem. J. 31: 81.

Hess, A. F. 1924. Trans. Am. Ped. Soc. 36: 57; Am. J. Diseases Children 28: 256.

Huldschinsky. 1919. Deutsche Med. Wochenschr. 45: 712.

Luce, E. W. 1924. Further observations on the influence of sunlight upon the growth-promoting and antirichetic properties of milk. Biochem. J. 18: 1279-1288.

McCollum, E. V., Elsa Orent-Keiles, and Harry G. Day. 1939. The newer knowledge of nutrition, fifth ed. New York: The Macmillan Company.

Simms, Helen Davison, University of California. Letter to Laura Adele Miller, University of Oklahoma.

Steenbock, H. 1924. Induction of growth-promoting and calcifying properties in a ration by exposure to light. Science 60: 224-5.

United States Weather Bureau Reports for March and April 1940. Communicated in letters to author from officials in charge.

TABLE I

Degrees of healing in relation to dietary supplements

Litter number	Rat number	Positive control		Oklahoma rat			Northern rat		
		Supplement ref. oil	Degree of healing	Rat number	Supplement butter fat	Degree of healing	Rat number	Supplement butter fat	Degree of healing
		mg			*g*			*g*	
I	3	30	| ++	1	0.5	-| +	4	0.5	-|
II	2	30	+++	3	0.5	-| ++	5	0.5	-|
III	1	30	| ++	3	1.5	-| ++	5	1.5	-|
VI	6	30	| ++	2	1.5	-| ++	3	1.5	| ++
IV	1	30	++	4	2.0	-| +++	2	2.0	| ++
V	6	30	-| +	1	2.0	++	4	2.0	++

Poor = Less than one plus. (| or -|)
Fair = one plus.
Good = above one plus, including two plus.
Very good = between two plus and three plus, including three plus.
Excellent = above three plus.
Part of a plus indicates an intermediate stage.

TABLE I—Continued

Litter number	Rat number	Oklahoma rat		Rat number	Northern rat		Rat number	Negative control	
		Supplement butter fat	Degree of healing		Supplement butter fat	Degree of healing		Supplement	Degree of healing
		g			*g*			*g*	
I	2	0.5	++	5	0.5	-|	6	0	None
II	4	0.5	-| ++	6	0.5	|	1	0	Slight
III	4	1.5	+++	6	1.5	|	2	0	None
VI	4	1.5	| ++	5	1.5	++	1	0	None
IV	5	2.0	+++	3	2.0	++	6	0	None
V	3	2.0	+++	5	2.0	++	2	0	None