three assistant state geologists and at least five other members of state geological surveys: three members of the U. S. Geological Survey, eighteen college and university professors, three members of museum staffs, two engineers, one mining engineer, one member of a state highway commission and an uncounted number of laboratory research investigators and graduate students. The attendance, according to unofficial accounts, was approximately one hundred for a part of the trip and approximately one half that number registered for one of the optional day trips, following the formal adjournment of the conference. The post-conference trips offered either the country of the iron ranges of northeastern Minnesota or the Keweenawan sediments and lava beds of northern Wisconsin, according to the option of the participant.

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SPECIAL ARTICLES

FRACTIONATION STUDIES ON PRO-VITAMIN D

PREVIOUS work from these laboratories[1,2,3] demonstrated: (1) that cholesterol which had been purified by methods which destroyed ergosterol still retained some residual provitamin-D activity; (2) that cholesterol purified by different methods varied somewhat in the amount of residual provitamin D; (3) that the provitamin D of cholesterol purified through the dibromide could be enhanced 100 times by heating this cholesterol at 185°–200° for one hour in the presence of traces of oxygen; and (4) that boiling in an alcoholic solution of sodium hydroxide also enhances the provitamin-D factor, but to a lesser degree. Table I gives a summary of these findings.

TABLE I
EFFECT OF VARIOUS PURIFICATION METHODS ON PROVITAMIN D OF CHOLESTEROL

Sample	Treatment	Daily dose to produce 2+ cure in 10 days	Absorption spectra
Cholesterol W	Commercial product	0.1 mg	Ergosterol bands and general absorption
Cholesterol A	Cholesterol W treated with boiling 10 per cent. NaOH in alcohol, dissolved in ether, washed with water, ether evaporated, residue recrystallized 5X from acetone	2.5 mg	No absorption
Fraction II	Cholesterol separating in emulsion layer between ether solution and water washing	0.1 mg	General absorption
Cholesterol A sublimate	Cholesterol A heated 200°–210° three hours at 7–10 mm pressure	0.1 mg	General absorption
Cholesterol A residue	Residue left in tube after heating	0.1 mg	General absorption
Purified cholesterol	(1) Through the dibromide (2) Boiling 3X with $KMnO_4$ in alcohol (3) Acetylation and acid hydrolysis (4) Acetylation and alkaline hydrolysis	7–10 mg 3 mg 4 mg 1.5 mg	No absorption No absorption No absorption No absorption
Alkaline-treated cholesterol	Cholesterol treated 3X with $KMnO_4$ and boiled 5 hours in 5 per cent. NaOH in alcohol	0.75 mg	Slight general absorption

These findings led to the conclusion that provitamin-D activity is not limited to ergosterol, but that cholesterol itself or a modification of it produced by heat or by alkali or both of these forms also have provitamin-D potency.

Waddell's[4] findings that crude cholesterol and cholesterol purified by boiling in an alcoholic solution of potassium hydroxide, when irradiated, was more effective in preventing leg weakness in chicks than an equivalent number of rat units of irradiated ergosterol led us to test our heated cholesterol in the same manner. Ten groups of white Leghorn chicks were fed the basal ration described by Lachat, Halvorson and Palmer.[5] The following additions were made to the diets of nine of the groups: 0.25 per cent. cod liver oil, 0.25 per cent. 10,000X viosterol diluted 1,000 times, 0.01 per cent., 0.03 per cent., and 0.06 irradiated, heated, purified cholesterol, 0.01 per cent. irradiated filtrate residue from heated cholesterol, 0.1 per cent. irradiated purified cholesterol, and 0.01 per cent. and 0.03 per cent. commercial cholesterol. Table II gives the results obtained.

[1] Fred C. Koch, Elizabeth M. Koch and Ida Kraus Ragins, *Jour. Biol. Chem.*, 85: 141–158, 1929.

[2] Elizabeth M. Koch, Fred C. Koch and Harvey B. Lemon, *Jour. Biol. Chem.*, 85: 159–167, 1929.

[3] Milicent L. Hathaway and F. C. Koch, *Jour. Biol. Chem.*, 108: 773–782, 1935.

[4] J. Waddell, *Jour. Biol. Chem.*, 105: 711–729, 1934.

[5] L. L. Lachat, H. A. Halvorson and L. S. Palmer, *Jour. Assoc. Official Agr. Chem.*, 15: 660–675, 1932.

TABLE II

COMPARATIVE ANTIRACHITIC POTENCY OF COD LIVER OIL, VIOSTEROL AND IRRADIATED, HEATED CHOLESTEROL IN RATS AND CHICKS

Supplement to basal ration	Rat units per 100 gm diet	Chick assay				Remarks
		Av. wt. at 30 days	Av. gain in wt.	Line test	Bone ash per cent.	
		gm	gm			
None	0	116	66	Rickets	29.9	All symptoms of leg weakness; marked tendency to squat at 2½ weeks.
0.25 per cent. cod liver oil	10	196	146	Good calcification	44.7	Chicks strong; active.
0.25 per cent. viosterol 1–10,000X diluted 1,000 times	100	108	63	Rickets	33.3	All symptoms of leg weakness; marked tendency to squat at 2½ weeks.
0.01 per cent. irradiated, heated, purified cholesterol	10	204	159	Good calcification	44	Chicks strong; very active.
0.03 per cent. irradiated, heated, purified cholesterol	30	195	144	Good calcification	47	Chicks strong; very active.
0.06 per cent. irradiated, heated, purified cholesterol	60	169	121	Good calcification	45.5	Chicks strong; very active.
0.1 per cent. irradiated, purified cholesterol	1	133	86	Rickets	28.6	All symptoms of leg weakness; tendency to squat at 3 weeks.
0.01 per cent. irradiated filtrate residue from heated cholesterol	>10	110	63	Rickets	29.6	All symptoms of leg weakness; tendency to squat at 3 weeks.
0.01 per cent. irradiated cholesterol W	10	174	129	Good calcification	44.8	Chicks strong; very active.
0.03 per cent. irradiated cholesterol W	30	164	117	Good calcification	43	Chicks strong; very active.

The purified cholesterol was prepared through the dibromide. Irradiated, this unheated product produced a 2+ cure when fed to rats in 10-mg daily doses for 10 days. The Steenbock rat unit is therefore 100 mg. The heated cholesterol was prepared by placing 3 gm of the purified cholesterol in a 250-cc flask with two capillary openings and heating this in an oil bath to 185°–195° C. for one hour. The solidified mass was dissolved in boiling alcohol, and the crystals which separated at room temperature were filtered off, dried and irradiated. A daily dose of 0.1 mg produced a 2+ cure in rats. The semi-crystalline residue left after evaporation of the filtrate was also irradiated. Cholesterol W was a fairly pure product obtained from The Wilson Laboratories.

The results show unmistakably the superior effectiveness of cholesterol W and the heated purified cholesterol over viosterol or the purified, unheated cholesterol in preventing leg weakness. Ten rat units of either preparation per 100 gm of diet were equivalent to 0.25 per cent. or 10 rat units of cod liver oil. The provitamin D of heated cholesterol evidently separates readily in the crystallization from alcohol, since the filtrate residue has no potency when given in comparable doses to rats or chicks. The chicks receiving 100 rat units of viosterol per 100 gm of diet were in no better condition than those receiving the basal ration only.

This is a preliminary report. Efforts to concentrate the provitamin-D factor of heated cholesterol are under way.

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THE BLUE MUD-DAUBER AS A PREDATOR OF THE BLACK WIDOW SPIDER

DURING the past few months, a study of the black widow spider, *Latrodectus mactans* Fabr., was made to find a method of securing a large quantity of venom for experimental work. It was finally decided to breed black widows in large numbers. The question of food for the breeding stock and for growing young spiders became a problem. In the building where the cages were kept, many mud-dauber nests were observed, which suggested feeding spiders on the young mud-daubers found in them. This type of food was satisfactory—both young and adult spiders ate the larvae of the mud-daubers.