cipitated by phosphotungstic acid. In continuing this research, Peters' eluate [*Biochem. J.*, **27**, 225 (1933)] was used as a potent concentrate of B_6, in batches usually containing 10 to 50 thousand B_6 units. As the first step toward purification, vitamin B_6 was adsorbed on fuller's earth at *p*H 1.4, using 1 g. of earth to 10 units of B_6. Adsorption was repeated three times. The combined adsorbates were washed by grinding in a mortar with 2 ml. of 0.1 *N* hydrochloric acid per gram of fuller's earth used. The adsorbate was then eluted twice with 0.1 *N* barium hydroxide (12 ml. per gram of earth) by grinding in a mortar and letting stand overnight in the refrigerator. The filtrates were precipitated immediately with sulfuric acid and filtered. This filtrate was adjusted to *p*H 6.8 to 7 with 10% sodium hydroxide and evaporated to dryness. The residue was extracted five times with 95% ethyl alcohol, using 100 ml. each time, and the filtrate was evaporated to 50 ml. and treated with 450 ml. of ethyl acetate. After standing overnight in the refrigerator, the precipitate was filtered off and the filtrate evaporated to dryness. The residue was then taken up in 200 ml. of water, filtered, and the filtrate subsequently precipitated (1) with platinum chloride (activity remaining in the filtrate), then (2) with phosphotungstic acid (20% in 1 *N* sulfuric acid). The phosphotungstate was decomposed with barium hydroxide. The combined filtrates were precipitated with sulfuric acid, filtered, and the filtrate (neutralized with sodium hydroxide) evaporated to dryness. The residue was extracted several times with 95% ethyl alcohol to make 100 ml., filtered, and the extract precipitated with 400 ml. of ether and let stand overnight in the refrigerator. The ether was then filtered, evaporated, and the residue taken up in water. Activity of this concentrate corresponded to 20 to 100 γ of solids for one "rat day dose," with a total yield of 10 to 30% of the original strength. Repeated precipitation with phosphotungstic acid followed by regeneration gave aqueous solutions from which crystalline preparations were obtained having an activity of 5 γ per "rat day dose." Daily administration of 15 γ cured rat acrodynia in two weeks, of 5 to 10 γ in three to four weeks. Crystals were colorless rods of varying size, with rounded ends. They seemed to have a tendency to coalesce in rosets or fan-shaped formations.

The curative influence of these crystalline preparations was confined to disappearance of the specific skin symptoms. Growth was not promoted. Even the skin effect was not regularly attained unless a further supplement, corresponding to the so-called "filtrate factor" [*J. Biol. Chem.*, **114**, 109 (1936)], was added.

BABIES AND CHILDRENS HOSPITAL AND DEPARTMENT OF PEDIATRICS OF WESTERN RESERVE UNIVERSITY SCHOOL OF MEDICINE CLEVELAND, OHIO

PAUL GYÖRGY

RECEIVED FEBRUARY 21, 1938

THE PRODUCTION OF AN ANTIRACHITIC PROVITAMIN FROM CHOLESTEROL

Sir:

It has already been reported [F. C. Koch, M. E. Koch and Ragins, *J. Biol. Chem.*, **85**, 141 (1929); Waddell, *ibid.*, **105**, 711 (1934); Hathaway and Lobb, *ibid.*, **113**, 105 (1936); Haman and Steenbock, *ibid.*, **114**, 505 (1936)] that the natural antirachitic provitamin D may be related to cholesterol rather than to ergosterol, and Windaus, Lettre and Schenck [*Ann.*, **520**, 98 (1935)] actually prepared from cholesterol by a number of difficult steps 7-dehydrocholesterol which upon irradiation with ultraviolet light acquired strong antirachitic properties.

If the precursor of the animal antirachitic provitamin is cholesterol, then its formation in the body may result from the partial dehydrogenation of the latter under the influence of dehydrogenating enzymes or under that of light in the presence of hydrogen acceptors. Some time ago we decided to test this view chemically by allowing cholesterol acetate to react with equimolecular proportions of hydrogen acceptors in the presence or absence of dehydrogenating catalysts and of light.

When cholesterol acetate, spectroscopically free from the antirachitic provitamin, was allowed to react with benzoquinone in a sealed tube at 120–130° for about two hours and the product subsequently freed from quinhydrone, unconverted quinone, etc., it was found to contain substantial quantities of 7-dehydrocholesterol. The crude mixture was then irradiated in pure ethyl ether with a quartz mercury lamp for four hours and the resulting product assayed biologically for us by Professors Robert S. Harris and J. W. M. Bunker of this Institute. They reported an antirachitic potency of considerably more than 6500 U. S. P. vitamin D units per gram, whereas a blank with our purified cholesterol acetate had a

potency of only about 2 U. S. P. vitamin D units per gram.

Some additional preliminary experiments are described in Table I. The 7-dehydrocholesterol was determined spectroscopically and the percentage conversion calculated from the results obtained. The absorption spectrum of our purified product showed two prominent absorption bands the peaks of which were at 269 and 281 mμ, identical with those of 7-dehydrocholesterol and of ergosterol.

TABLE I

THE PARTIAL DEHYDROGENATION OF CHOLESTEROL ACETATE

Dehydrogenating agent	$E^{1\%}_{1\text{ cm.}}$ at 269 mμ	Conversion, %
Methylene blue + light (25°, 30 days)	6.5	2.3[a]
Benzoquinone + Pd + light (25°, 30 days)	2.5	0.9
Benzoquinone + Pd (120–130°, 2 hrs.)	3.0	1.1
Benzoquinone (120–130°, 2 hrs.)	5.0	1.8
Benzoquinone (120–130°, 6 hrs.)	56.0	20.0
Chloranil (120–130°, 2 hrs.)	22.0	7.8(?)
Succinodehydrogenase (beef heart)	0.42	0.15

[a] This gives the percentage of a purified material which was approximately one-tenth of the original crude product.

In the case of methylene blue the dehydrogenation was carried out in benzene solution which was rapidly stirred while it was exposed to light from a 250-watt lamp for thirty days. A similar experiment in benzene was performed without stirring using benzoquinone and palladium black. The succinodehydrogenase was prepared from beef heart in accordance with the method of Thunberg [*Biochem. Z.*, **285**, 48 (1933)].

This work is being continued actively in this Laboratory with cholesterol, stigmasterol, and sitosterol, using various hydrogen acceptors and dehydrogenating catalysts under diversified conditions, and a more complete report will be published in the future.

CONTRIBUTION NO. 166 FROM THE RES. LAB. OF ORG. CHEM. MASSACHUSETTS INST. OF TECH. CAMBRIDGE, MASS.

NICHOLAS A. MILAS
ROBERT HEGGIE

RECEIVED MARCH 21, 1938

RESTRICTED ROTATION IN ETHYL ALCOHOL, ACETONE AND ISOPROPYL ALCOHOL

Sir:

The entropies given in Table I, column 2, were calculated from molecular data, assuming free rotation for the perfect gases at one atmosphere.[1]

(1) Details will appear in a forthcoming publication by the authors.

TABLE I

	Temp., °K.	S Free rot.	S 3rd law	S Rest. rot.	Potentials
C_2H_5OH	351.6	73.1	69.7	69.7	3000, 9000
i-C_3H_7OH	355.4	82.6	78.3	78.3	3400, 3400 6000
$(CH_3)_2CO$	329.2	73.3	72.6	72.6	1250, 1250

It is doubtful whether an error of more than 0.3 e. u. could result from the choice of the vibration frequencies, including those similar to the ${}^2\nu_\alpha M$ vibrations in ethane. Column 3 of Table I lists the experimental third law entropies of the gases at their boiling points from thermal data down to 16°K. (Kelley). Column 4 gives the values of the entropy, calculated using the empirical restricting potentials in column 5 and the method of Pitzer.

Table II, column 2, gives ΔS values for the reaction

$$CH_2{=}CH_2(g) + H_2O(g) \longrightarrow C_2H_5OH(g)$$

calculated from remarkably good equilibrium data[2-4] using ΔH values from Rossini's accurate data on the heat of formation of ethylene, ethyl alcohol, and water.

TABLE II

Temp., °K.	ΔS Equilib. data	ΔS 3rd law	ΔS Free rot.	ΔS Rest. rot.
498	−31.13 −31.29	−31.27	−28.58	−31.27
548	−31.08	−31.16	−28.62	−31.16
593	−30.86	−31.13	−28.75	−31.13

The equilibrium data are probably reliable to about 5% in K, corresponding to 0.1 e. u. Column 3 gives values of ΔS calculated from the accurate experimental third law entropy data (see also Egan and Kemp), and heat capacities from the Raman frequencies (using the above restricting potentials for ethyl alcohol) to extrapolate above the boiling point. Columns 4 and 5 give, respectively, ΔS values from molecular data assuming free and restricted rotations with the above potentials.

Table III gives a similar comparison for the equilibrium

$$(CH_3)_2CHOH(g) \longrightarrow (CH_3)_2CO(g) + H_2(g)$$

with the equilibrium data probably accurate to 7% or 0.15 e. u.

The results leave little doubt that neglect of potentials restricting internal rotations is the cause of the discrepancies in Table I, between values cal-

(2) Stanley, Youell and Dymock, *J. Soc. Chem. Ind.*, **53**, 105T (1934).

(3) Applebey, Glass and Horsley, *ibid.*, **56**, 279T (1937).

(4) Dodge and Bliss, *Ind. Eng. Chem.*, **29**, 19 (1937).