14002

Activation of Pro-Vitamin D_3 in the Rat.

E. GEIGER AND S. LASSEN.

From the Van Camp Laboratories, Terminal Island, California.

The experiments of Boer *et al.*,[1] who isolated 7-dehydro-cholesterol from cholesterol of animal origin were followed by the discoveries of Brockman, Drummond, Simons *et al.*,[2-4] who identified activated 7-dehydro-cholesterol as the main constituent of the natural Vitamin D in fish liver oils, indicating that the Vitamin D found in the animal organism is Vitamin D_3 in contra-distinction to Vitamin D_2 which is formed by activation of ergosterol.

In view of the ability of the animal organism to form biologically highly active sterol derivatives of a hormone nature, it was considered of importance to determine whether the animal organism, *per se*, possesses the means of transforming cholesterol or cholesterol-derivatives into Vitamin D_3. Several of these compounds are now being investigated, namely, 7-keto-cholesterol, 7-hydroxy-cholesterol and 7-dehydro-cholesterol. This report concerns the fate of 7-dehydro-cholesterol in the rat.

A sample of 7-dehydro-cholesterol was prepared from cholesterol by the method of Windaus.[5] The product was repeatedly recrystallized from methyl alcohol in darkness; 0.0053 g of the purified substance was finally dissolved in 50 ml of propylene-glycol, C.P.

The activated 7-dehydro-cholesterol used in these experiments was made by irradiating an aliquot of the solution of the provitamin by pouring the solution into a Petri dish. The source of irradiation was a 2-arc therapeutic lamp held over the dish at a distance of 12 in for 30 min. The arc was held in such a position that the incident rays formed an angle of 45° with the surface of the solution.

Forty-two rats which had been on a rachitogenic diet for 21 days and which showed to be fully depleted by appearance as well as by examination of control rats were divided in groups of 7, continued on the rachitogenic diet and treated as follows:

Group I—Injected subcutaneously with 0.1 ml propylene-glycol daily and kept in total darkness throughout the experiment.

Group II—Injected subcutaneously with 0.1 ml propylene-glycol daily and irradiated daily for 4 min with a 2-arc therapeutic carbon lamp at a distance of 12 in. The animals were also daily exposed to sunshine for 3 hr.

Group III—Given daily 0.1 ml orally of propylene-glycol and irradiated as under Group II.

Group IV—Injected subcutaneously every other day with 0.2 ml of the 7-dehydro-cholesterol solution in propylene-glycol and exposed to irradiation and sunlight as under Group II.

Group V—Injected subcutaneously every other day with 0.2 ml of an *activated* 7-dehydro-cholesterol dissolved in propylene-glycol and kept in total darkness during the 8 days' experiment.

Group VI—Given orally every day 0.1 ml of activated 7-dehydro-cholesterol and kept in total darkness.

Group VII—Given subcutaneously every other day 0.2 ml of 7-dehydro-cholesterol in propylene-glycol and kept in darkness.

After 8 days all the rats were killed, the tibiæ were removed and sectioned, and the degree of calcification was evaluated by the line-test. The results are seen in Table I.

[1] Boer, A. G., Reerink, E. H., van Wijk, A., and van Niekerk, J., *Proc. Acad. Sci. Amsterdam*, 1936, **39**, 622.

[2] Haslewood, G. A. D., and Drummond, J. C., *J. Soc. Chem. Ind.*, 1936, **55**, 598.

[3] Brockmann, H., *Ztft. Physiol. Chem.*, 1936, **241**, 104; 1937, **245**, 96.

[4] Simons, E. J. H., and Zucker, T. F., *J. Am. Chem. Soc.*, 1936, **58**, 2655.

[5] Windaus, A., Lettre, H., and Schenck, F., *Ann.*, 1935, **98**, 520.

TABLE I.

Group of 6 rats	Dosage per rat	Treatment of rats during 8-day assay period	Degree of healing observed
I	0.1 ml daily, subcutaneously, of propylene-glycol	Kept in darkness	0
II	Same	Irradiated daily	0.1
III	Same, orally	" "	0.2
IV	0.2 ml every other day subcutan. of 7-dehydro-cholesterol in propylene-glycol	" "	1.86
V	Same of *activated* 7-dehydro-cholesterol	Kept in darkness	3.21
VI	Same but 0.1 ml daily orally	" " "	3.43
VII	Same but 0.2 ml every other day subcutan.	" " "	0

The experiments demonstrate the following:

1—Activated 7-dehydro-cholesterol, as expected, shows a strong curative effect on rachitic rats when it is injected subcutaneously.

2—The subcutaneous injection of provitamin D to rachitic rats kept in darkness is totally ineffective.

3—The subcutaneous injection of provitamin D to depleted rats is effective only if the rats receive ample irradiation.

4—Irradiation of rachitic rats alone under the conditions of our experiments does not prove curative.

5—There is no essential difference in the curative effect of activated 7-dehydro-cholesterol whether it is given the rat subcutaneously or *per os*.

The failure of irradiation to produce any healing effect in the rachitic rat makes it doubtful whether the mammalian organism can produce any appreciable amount of provitamin D. It seems possible, therefore, that the 7-dehydro-cholesterol isolated by several authors from mammalian tissue was not synthesized in the body but was present as a result of previously ingested food containing it or was perhaps produced by oxidation of the cholesterol in the course of its preparation.[6]

[6] Wintersteiner, O., and Bergstrom, S., *J. Biol. Chem.*, 1941, **137**, 785.

14003

Prothrombin Studies: III. Effect of Vitamin K upon Hypoprothrombinemia Induced by Dicumarol* in Man.

SHEPARD SHAPIRO, MILTON H. REDISH AND HAROLD A. CAMPBELL.† (Introduced by J. Murray Steele.)

From the Department of Medicine, New York University, and the Third (New York University) Division, Welfare Hospital, Welfare Island, New York.

The recent work of Overman, Field, Baumann and Link[1] and of Overman, Stahmann and Link[2] has established that in animals vitamin K administered in adequate dosage will counteract the hypoprothrombinemic effect of Dicumarol (3, 3′ methylene-bis[4-hydroxycoumarin]).

In the work reported in man this has not

* Dicumarol is the collective trade-mark of the Wisconsin Alumni Research Foundation which controls the use thereof.

† Present address: Central Laboratories, General Foods Corporation, Hoboken, N.J.

[1] Overman, R. S., Field, J. B., Baumann, C. A., and Link, K. P., *J. Nutrition*, 1942, **23**, 589.

[2] Overman, R. S., Stahmann, M. A., and Link, K. P., *J. Biol. Chem.*, 1942, **145**, 155.