The melting points, percentage yields, and analytical results for the eleven new hydantoins prepared have been collected in Table III.

Summary

1. *sym*-Di-allyloxypropanol and three representatives of the *sym*-alkoxypropanol series have been prepared, one of which, the *sym*-di-*n*-heptyloxypropanol, has not been previously reported in the literature.

2. Three new members of the *sym*-dialkoxypropanone type of compounds have been prepared, and, in addition, the *sym*-diallyloxypropanone has been synthesized—not, however, in an analytically pure state.

3. Two new members of the *unsym*-dialkoxypropanol series have been synthesized and adequately characterized.

4. Attempts to obtain pure *unsym*-dialkoxypropanones resulted in failure.

5. Utilizing the series of ten *sym*-dialkoxypropanones and *sym*-diallyloxypropanone, Bucherer's method has been extended by the synthesis of eleven hydantoins of a new type.

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Studies in the Activation in Sterols

BY JOHN W. M. BUNKER, ROBERT S. HARRIS AND L. MALCOLM MOSHER

The two important provitamins D in nature are ergosterol and 7-dehydrocholesterol. Is there a difference in their activation by ultraviolet light?

To find an answer to this question we have irradiated ether solutions of crystalline preparations of these provitamins with measured doses of substantially monochromatic ultraviolet through a large quartz monochromator. The activated products have been fed concurrently to large groups of rachitic animals and data sufficient to warrant statistical evaluation have been obtained.

Activation of Ergosterol.—The procedure for the irradiation of ergosterol has been published.[1] Since each irradiation yielded activated sterol sufficient for the feeding of only ten experimental animals, the irradiation procedure was repeated five times for each wave length. The repetitive irradiations were scattered at random through the whole series to distribute any undetected variations in attendant conditions. Each rat was fed an equivalent of 8 mg. of ergosterol that had been exposed to 300×10^{12} quanta, which recent experience showed to be appropriate for the severity of rickets at that time. By the use of a large number of animals in each group in a concurrent test, 48 or 49 observations were obtained for each of the five wave lengths studied.

In an earlier publication[1] we concluded that "the photochemical activation of ergosterol in ether by monochromatic light of 2537, 2652, 2804, 2967 or 3025 Å. is substantially uniform per quantum of energy applied," and in our discussion we confessed to an impression that 2804 Å. appeared to be very slightly more efficient. By statistical analysis this tendency could not be proved to be significant.

In a confirmatory test herein reported, line 2537 Å. was replaced by 2894 Å. because the latter wave length is of much more physiological importance, being at the edge of the solar spectrum while 2537 Å. is in the abiotic region of the ultraviolet, and also because the new line fills in the gap between 2804 and 2967 Å., a region particularly interesting.

A summary of our findings is presented in Table I. The spread in the response of the animals in each group to identical feedings of antirachitic substance is reasonable for the bioassay. However, the concentration of the consistent responses at a mode for each group presents a picture unusually favorable for statistical analysis. The apparent similarity in response which is indicated by the arithmetic means was tested for reality by the "*t*" test[2] which is statistically appropriate for the study of this type and volume of data.

The data essential to the interpretation of the "*t*" test are presented in Table II. A "*t*" value of 2.5 has a probability of 19 out of 20 chances of being a real difference. This analysis indicates that the activation of ergosterol by 3025 Å. is decidedly less efficient than by the other lines tested

(1) Harris, Bunker and Mosher, THIS JOURNAL, **60**, 2579 (1938).

(2) Bunker, Harris and Mosher, *ibid.*, **62**, 508 (1940).

TABLE I

SUMMARY OF EXPERIMENTAL DATA FROM IRRADIATED ERGOSTEROL (ALL ANIMALS FED CONCURRENTLY)

Wave length	Number of rats	Av. wt. (in g.) First	Av. wt. (in g.) Last	Healing response	Arithmetic mean response
2652 Å.	1	56	77	0.0	
	1	51	66	.5	
	5	54	75	1.0	
	8	54	73	1.5	
	18	53	74	2.0	
	13	53	72	2.5	
	3	55	76	3.0	
	0	..	..	3.5	1.9388
Total	49				
2804 Å.	0	..	..	0.0	
	3	53	69	.5	
	5	53	73	1.0	
	8	54	78	1.5	
	16	54	74	2.0	
	11	54	72	2.5	
	4	54	66	3.0	
	1	50	58	3.5	1.9479
Total	48				
2894 Å.	0	..	..	0.0	
	3	51	79	.5	
	3	51	72	1.0	
	7	54	77	1.5	
	17	53	73	2.0	
	11	54	74	2.5	
	7	53	69	3.0	
	1	56	74	3.5	2.0612
Total	49				
2967 Å.	0	..	..	0.0	
	2	51	64	.5	
	1	56	73	1.0	
	5	51	72	1.5	
	18	54	75	2.0	
	14	53	72	2.5	
	7	54	74	3.0	
	2	54	67	3.5	2.2143
Total	49				
3025 Å.	1	51	61	0.0	
	8	54	79	.5	
	10	53	76	1.0	
	8	53	72	1.5	
	13	52	70	2.0	
	7	54	68	2.5	
	2	54	76	3.0	
	0	..	..	3.5	1.5408
Total	49				

(2967, 2894, 2804, 2652 Å.), and that there is no significant difference between the efficiency of these four other lines, under the conditions of these experiments.

This conclusion departs in only one respect from that previously derived which was based on data from successive tests on small groups of animals which were difficult to analyze because of large group-to-group variations.

TABLE II

ESSENTIAL "*t*" TEST DATA (IRRADIATED ERGOSTEROL)

Wave lengths compared (Å.)	Difference of arithmetic means	$S\sqrt{\frac{1}{N_1}+\frac{1}{N_2}}$	"*t*"	Interpretation
2652–2804	0.0091	0.1349	0.07	
2652–2894	.1224	.1349	.91	
2652–2967	.2755	.1285	2.14	
2652–*3025*	.3980	.1411	3.54	Significant
2804–2894	.1531	.1349	1.14	
2804–2967	.2664	.1353	1.97	
2804–*3025*	.4071	.1473	2.75	Significant
2894–2967	.1531	.1349	1.14	
2894–*3025*	.5204	.1470	3.54	Significant
2967–*3025*	.6735	.1414	4.76	Significant

Photoactivation of 7-Dehydrocholesterol.— By the same technique and procedure, the relative effectiveness of the dominant mercury lines in the region 2483–3025 Å. in the activation of 7-dehydrocholesterol in 0.1% ether solution was measured on 328 rats.[2]

By applying the "*t*" test it was demonstrated that 2967 Å. is significantly more efficient than any of the other lines tested (2483, 2537, 2652, 2804, 2894 and 3025 Å.) with the possible exception of 2894 Å. which had a value between the plateau and the peak of responses.

These two studies have provided the answer to the initial question: there is a difference in the ultraviolet activation of ergosterol and 7-dehydrocholesterol, in respect to wave length 2967 Å.

Discussion

Our reliance upon statistical evaluations of experimental findings in preference to analyses by inspection merits some explanation. In the ordinary bioassay for vitamin D, it is the custom in our laboratory and in others to use groups of not over ten animals at a time for concurrent feedings of a given test dosage. It is well known that the distribution of responses of animals within any group is subject to variations due to uncontrollable animal inconsistencies, and it is obvious that the chance variation of an extreme degree will influence the picture in a small group more than in a large one.

Moreover, the mathematical symbols customarily used to represent degrees of healing response do not bear to each other the simple linear arithmetic relation which they appear to carry. The

objection to treating so small a number of observations as 10 statistically, because of undue apparent significance of chance variation, is removed if a test group be sufficiently numerous. In view of our records of behavior of a large number of groups, and with the advice of a statistician in the Department of Mathematics at the Massachusetts Institute of Technology, it was decided that 50 constitutes a group size sufficient to overcome the above handicap of the bioassay. According to Bills[3] the expected probable error in a group of 50 is about 7%, in our groups it is about 3.5%.

In tests designed to compare responses of different antirachitics occurring in a restricted region of the line test values of the Bills scale, the relative significance of numerical values in any other part of the scale becomes less.

In every laboratory, the severity of experimental rickets varies from season to season, in an unpredictable fashion, which militates against the reliability of successive bioassays to detect with precision small differences of response. This handicap is overcome by concurrent feeding of dosages to be compared in large groups.

In a previously reported test on ergosterol[1] we used successive groups of 7 or 8 each, and interpreted results by inspection. A repetition with concurrent feedings of 50 animals per group has been interpreted statistically. The two sets of results agree quite well, except that the more efficient test relegates wave length 3025 Å. to an inferior level of activation efficiency.

Any statistical method can be at best only a computation of the probability that some hypothesis is correct. In the absence of any reason to the contrary, one must assume that the effects of different wave lengths are equal. Upon this premise, our findings indicate that it is highly probable that activation of ergosterol under the particular conditions of our experiments is effected with equal quantum efficiency for the wave lengths specified, except for 3025 Å. which is low, and that 7-dehydrocholesterol behaves differently in response to wave length 2967 Å.

In 1937 a study[4] from these Laboratories showed that the number of quanta required to effect a standard degree of rachitic healing in epilated rats irradiated with monochromatic ultraviolet, varies with the wave length. Line 2967 Å. was significantly more efficient than 3025, 2804, 2652 or 2537 Å. Wave length 3130 Å. is ineffective presumably because of the transparency of the skin provitamin to this wave length, and 2483 Å. is relatively inactive, perhaps because of the opacity of the rat skin to this wave length.

The absence of correlation between the results with ergosterol and skin irradiation agrees with the presumption that ergosterol is not a normal constituent of animal skin. On the other hand, the superiority of 2967 Å. in the activation of 7-dehydrocholesterol and in the healing of experimental rickets by direct irradiation of the skin conforms with a prevalent belief that 7-dehydrocholesterol may be the significant provitamin D of the skin.

Conclusion

In that portion of the active region of the mercury arc spectrum between 2537 and 2967 Å., inclusive, the equal quantum efficiency of conversion of ergosterol into vitamin D_2, the superiority of 2967 Å. in activation of 7-dehydrocholesterol to vitamin D_3, and the greater effectiveness of 2967 Å. in the treatment of rachitic rats by direct skin irradiation, strengthen the assumption that 7-dehydrocholesterol is the significant provitamin D of the skin.

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(3) Bills, *J. Biol. Chem.*, **90**, 619 (1931).

(4) Bunker and Harris, *New Engl. J. Med.*, **216**, 165 (1937).