g. of dry or 8 cc. of fluid skimmed milk per rat per day when fed separately from the basal ration, or 25% of the total food mixture when the skimmed milk powder was mixed with the basal diet.

By feeding the diets described to experimental animals of suitable age and size and sufficiently matched as to nutritional history and litter controls, it is believed to be possible, when dealing with averages of ten or more rats on each diet, to detect a diminution certainly of 25% and probably of 15% in the vitamin B content of the food tested.

There was no evidence of any measurable diminution of vitamin B in milk powder heated dry with free access of air at 100° even when this heating was continued for 48 hours. When the milk was heated in the fluid state for 6 hours at 100° there was an apparent diminution in its vitamin B content; probably about 1/4 of the vitamin was thus destroyed. Vitamin B in the form in which it exists in milk is, therefore, comparatively stable to heating at 100° in the dry state, but less stable when heated at the same temperature in water solution.

New York, N. Y.

[Contribution from the Department of Chemistry, Columbia University, No. 425]

A QUANTITATIVE STUDY OF THE DESTRUCTION OF VITAMIN B BY HEAT

By H. C. Sherman and M. R. Grose

Received August 6, 1923

In the study of vitamins the property of thermostability bears important relations both to the behavior of the vitamin in attempts to isolate it and to the conservation of the vitamin values of foods. Hence, statements regarding this property are frequently met, but most of the work which has been done to determine the effect of heating upon vitamins A and B has been qualitative, or little more than qualitative, in nature.

Building upon the work described in the two preceding papers[1,2] we have attempted in the experiments here recorded to establish a quantitative relation between temperature and the rate of destruction by heat of vitamin B, somewhat as had been done in the previous work of this Laboratory with vitamin C.[3,4]

Vitamin B is more stable than vitamin C both toward heat and toward oxidation but its destruction by heat can be studied on the same general principle by the use of higher temperatures. Using tests of the amounts of food required to cure polyneuritis in fowls or pigeons as the measure of

[1] Sherman and Edgeworth, This Journal, **45**, 2712 (1923).

[2] Sherman and Spohn, *ibid.*, **45**, 2719 (1923).

[3] Sherman, LaMer and Campbell, *Proc. Nat. Acad. of Sci.*, **7**, 279 (1921).

[4] LaMer, Campbell and Sherman, This Journal, **44**, 172 (1922).

vitamin B content, Chick and Hume reported[5,6,7] results which have been widely quoted as indicating that vitamin B is stable at 100° but rapidly destroyed at temperatures around 120°. This would seem to imply, chemically, a marked increase in the temperature coefficient of the process of heat destruction in the neighborhood of 120° and, nutritionally, a marked diminution of vitamin B value in foods which have been subjected to the temperatures commonly used in processing canned goods as compared with those which have been heated only to the ordinary cooking temperature of about 100°. For both of these reasons and also because the behavior on heating constitutes an interesting point of comparison of vitamins with enzymes, the present investigation was undertaken with the general plan of determining the percentage destruction of vitamin B when heated in solution for four hours at 100°, 110°, 120° and 130° at the hydrogen-ion concentration equivalent to P_H 4.3.

Methods and Materials Employed

For reasons explained in previous papers from this Laboratory[1,2] we have employed the rat-growth method for the detection and measurement of any diminution of vitamin B which might result from destruction by heat. Young rats of an initial age of 4 weeks were used and the experimental period was continued for 8 weeks. The initial weights of individual rats were usually between 40 and 55 g. The precautions developed in the work of Sherman and Spohn[2] were observed in the selection of animals of sufficient uniformity and the arrangement of litter-controls. The same basal diet was also employed, namely, purified casein, 18%; butter fat, 8%; cod liver oil, 2%; Osborne and Mendel salt mixture,[8] 4%; starch, 68%. This basal diet was given freely, the food which furnished the vitamin B being always fed separately. All experimental animals were kept in individual cages having raised floors of wire screen so that the animals did not have access to their excreta. The construction and the frequent cleaning of the cages both served as precautions against the possibility of the animals obtaining any significant amount of vitamin from their own urine or feces. No bedding was used and the technique of the handling of the animals was as previously described.[2]

The juice of canned tomatoes was selected as the source of vitamin B in these experiments. Osborne and Mendel have shown[9] that tomatoes are a rich source of vitamin B. There is every reason to suppose that canned tomatoes of a given brand will be fairly constant in this respect, and the use of this material makes the present work on vitamin B directly

[5] Chick and Hume, *Proc. Royal Soc.*, **90B,** 60 (1917).
[6] Chick and Hume, *Trans. Soc. Trop. Med. Hyg.*, **10,** 141 (1917).
[7] Chick and Hume, *J. Roy. Army Med. Corps*, **29,** 121 (1917).
[8] Osborne and Mendel, *J. Biol. Chem.*, **37,** 572 (1919).
[9] Osborne and Mendel, *ibid.*, **39,** 29 (1919).

comparable in all respects with our previous work on vitamin C.[3,4] The canned tomatoes used in this study were all of the same brand. They were purchased as needed and in the course of the work the products of two seasons were used. We encountered no indication of material variation in the vitamin content of the canned tomatoes as purchased. In preparing the juice for feeding, either with or without heat treatment, the contents of the can was first thrown on cheese cloth and the juice separated from the bulk of the pulp by suction and pressure. The resulting juice was then passed through filter paper, yielding a light yellow liquid, clear or slightly opalescent, which yielded a slight coagulum when boiled, and a little carbonized material at 130°.

The heating at 100° was carried out in Erlenmeyer flasks immersed to the necks in a bath of boiling water heated by live steam. Loosely fitting glass stoppers with flanges that rested on the tops of the flasks rendered the evaporation negligible. Tests showed that such an arrangement gave a temperature within the flask about 0.2° lower than that of the boiling water without.

For the three higher temperatures a steam-heated autoclave was employed, the juice being contained in large Erlenmeyer flasks, closed with inverted beakers. For each heating the pressure was rapidly increased until the gage indicated that the desired temperature had been reached. The exhaust was then adjusted and the whole held at constant pressure for 4 hours, measured from the time of turning on the steam to the time of turning it off. The air was always blown out of the autoclave carefully in order that the pressure-temperature relation, as expressed on the gage, should not be in error. The accuracy of the temperature control was checked by readings of a thermometer inserted in the well of the autoclave. It was not difficult to regulate the autoclave so that the variations were not greater than 1° on either side of the desired temperature. Both the heated and the unheated portions of juice were consumed readily by the experimental animals. The "daily" doses were fed for six days each week.

A series of over 100 experiments, each involving the feeding of an experimental animal for eight weeks, was devoted to the quantitative determination of the relation between the amount of unheated tomato juice fed daily as sole source of vitamin B and the resulting gain or loss of weight during the experimental period of eight weeks. It was found that the most regular results were obtained at a level of vitamin intake which resulted in little or no net gain in body weight during the eight weeks of the experiment. This is usually 4 or 5 cc. of tomato juice per day depending upon the initial weight of the rat. The animals used in the series of experiments represented in Fig. 1 averaged somewhat larger than those used in the subsequent series. Because of the greater regularity of results in the region of maintenance or of only slight gain, this was adopted as the basis of comparison to be used in the study of the effects of heating in this investigation, the experimental method thus differing slightly at this point from that employed in the preceding investigation.[2] In the main series of experiments to determine the effects of heating, 4 cc. per day of unheated juice was fed to the "positive control" animals, and different doses of the heated juices were fed to other animals from the same litter as the control to determine what amount of heated juice was equivalent in vitamin B content to 4 cc. of unheated juice.

Experimental Results

Unheated Tomato Juice.—Various quantities of unheated tomato juice up to 14 cc. were fed, but few experiments were made with more than 10 cc. as this was about as much as the animals would readily consume. The larger amounts also tended to diminish the consumption of solid food, so that with quantities greater than 8 cc. the growth, although still below normal, did not increase in proportion to the increased vitamin intake. The average-weight curves of the rats receiving daily doses of 0, 2, 4, 5, 6, 7, 8, 9 and 10 cc. of unheated juice are shown in Fig. 1 and the average initial weights and total gain or loss in weight during the eight weeks of experimental feeding are given in Table I.

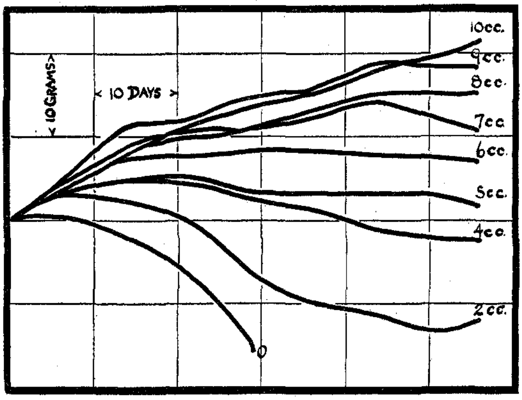


Fig. 1.—Weight curves of young rats showing the effect of graduated doses of vitamin B in the form of unheated tomato juice. Rats shown in curve marked O received the basal diet only, while those whose averages are shown by the other curves received this ration supplemented by daily doses of 2 cc. to 10 cc. of the unheated tomato juice as indicated on the curve. Table I shows the number and the average initial weight and gain in weight of each group. The curves in this and following figures cover an experimental period of eight weeks

Heated Tomato Juice.—The effect of heating for 4 hours at 100°, 110°, 120° and 130° was studied by means of feeding experiments employing a total of about 50 animals for testing each temperature and varying the doses of the heated juices over a considerable range until the amount equivalent to 4 cc. (or to 5 or 6 cc.) of unheated juice had been approximated and then the experiment was repeated with doses at this level until averages establishing the equivalent quantities with sufficient precision were obtained.

TABLE I

EFFECTS OF DIFFERENT DOSES OF UNHEATED TOMATO JUICE AS SOLE SOURCE OF VITAMIN B

Amount of juice fed daily Cc.	Number of rats	Average initial weight G.	Gain in weight in 8 weeks G.	Probable error[10]
10	10	55	21.4	±1.1
9	5	58	18.2	±3.4
8	10	53	15.3	±2.1
7	7	45	10.7	±2.6
6	21	48	7.0	±0.2
5	11	42	1.6	±1.4
4	25	48	− 2.2	±0.9
2	6	54	−12.0	±1.1
0	22	47	(−16.0)[a]	(±0.8)[a]

[a] The rats receiving none of the juice and whose food intake therefore was presumably entirely devoid of vitamin B showed an average survival period of 29 days and an average weight at death of 31 g.

Tables II, III, IV and V summarize the initial weights, food intakes and net changes in weight of those individual rats whose records are used in drawing the final averages. The left half of each table gives the record of the "positive controls" or rats receiving the unheated juice, while opposite these in the right half of the tables are given the records of the comparison animals receiving the heated juices, the two animals thus directly compared being always from the same litter. In this way physio-

TABLE II

EXPERIMENTS WITH TOMATO JUICE UNHEATED AND HEATED FOR FOUR HOURS AT 100°

	Rats fed unheated doses				Rats fed heated doses			
	Dose Cc.	Initial weight G.	Food intake G.	Gain G.	Dose Cc.	Initial weight G.	Food intake G.	Gain G.
	4	52	268	−10	5	51	257	− 3
	4	44	226	− 1	5	53	232	− 7
	4	23	205	11	5	23	179	+ 9
	4	44	219	2	5	45	227	− 7
	4	37	215	4	5	32	171	6
	4	40	211	4	5	40	217	4
	4	31	213	14	5	31	231	9
	4	43	201	3	5	38	223	10
Av.				+ 3.4				+ 2.6
	6	39	234	9	7	40	230	5
	6	58	356	13	7	43	299	6
	6	56	252	12	7	48	246	7
	6	47	223	5	8	49	207	6
	6	45	215	13	8	45	220	13
	4	42	252	− 4	6	38	229	1
Total Av.	4.7	43	235	+ 5.4	5.9	41	227	+ 4.2

[10] Computed according to the classical method as described, for example, by Jevons, "Principles of Science," The MacMillan Co., New York and London, **1905**, p. 387.

logical variation from litter to litter is excluded as a source of error in the comparison of the average results.

In case a test animal died during the experimental period neither its record nor that of its direct control is included in the averages. Such premature deaths were somewhat more frequent among the rats getting the more highly heated juices but did not constitute an important factor in any case.

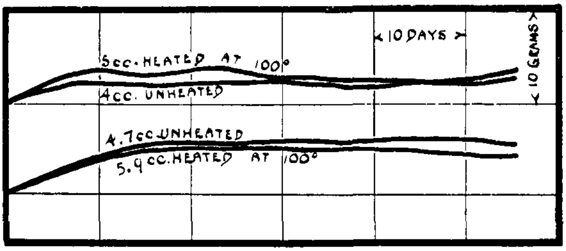


Fig. 2.—The upper curves show weights of rats receiving 4 cc. of unheated tomato juice in comparison with 5 cc. heated for 4 hours at 100°. The lower pair of curves represents the rats of Table II taken as a whole, and receiving an average dose of 4.7 cc. of unheated juice in comparison with 5.9 cc. heated for 4 hours at 100°

Of Tables II, III and V, each includes (1) a number of tests at a fixed ratio of dosage, (2) additional tests at varying ratios which latter are grouped together in the lower part of the table. Averages with or without these latter tests lead to the same conclusions. Feeling it somewhat safer in

Table III

Experiments with Tomato Juice Unheated, and Heated for Four Hours at 110°

	Rats fed unheated doses				Rats fed heated doses			
	Dose Cc.	Initial weight G.	Food intake G.	Gain G.	Dose Cc.	Initial weight G.	Food intake G.	Gain G.
	4	38	189	− 3	6	39	221	− 2
	4	44	193	−13	6	46	215	−10
	4	51	274	2	6	50	259	− 5
	4	62	230	− 4	6	55	263	− 3
	4	44	224	4	6	38	212	6
	4	40	214	2	6	37	243	− 1
	4	46	245	0	6	47	274	1
	4	23	205	11	6	26	181	13
	4	31	213	14	6	32	211	11
	4	43	201	3	6	45	234	6
Av.				+ 1.6				+ 1.6
	5	49	230	− 1	6	49	253	− 4
	4	51	263	− 2	7	54	252	−11
	4	38	204	− 1	7	36	189	5
	4	35	192	3	5	42	171	− 5
	6	36	258	15	8	48	289	9
	4	30	182	3	7	26	219	5
Total Av.	4.2	42	220	+ 1.8	6.3	42	230	+ 0.9

general to use the data of as many individuals as possible, we have made use chiefly of the "total average" from each table. In Fig. 2 are shown the

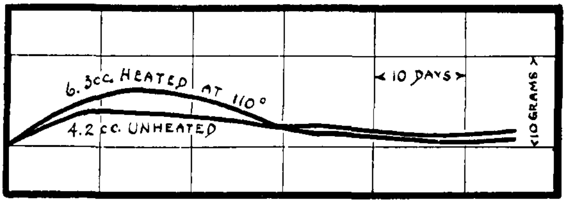


Fig. 3.—Weight curves of rats receiving an average dose of 4.2 cc. of unheated tomato juice in comparison with those receiving an average dose of 6.3 cc. heated for 4 hours at 110°

weight curves both of the averages of the rats receiving 4 cc. of unheated and 5 cc. of heated juices, respectively, and also the averages for all cases

Table IV

Experiments with Tomato Juice Unheated, and Heated for Four Hours at 120°

	Rats fed unheated doses				Rats fed heated doses		
Dose Cc.	Initial weight G.	Food intake G.	Gain G.	Dose Cc.	Initial weight G.	Food intake G.	Gain G.
4	38	204	− 1	8	35	200	12
4	44	219	2	8	48	213	1
4	40	211	4	8	28	214	13
4	57	308	−12	8	57	254	−12
4	37	215	4	8	30	161	3
4	25	200	8	8	25	183	7
4	51	263	− 2	8	56	227	−11
4	50	250	− 4	8	65	287	− 3
4	45	254	0	7	37	223	− 1
4	46	245	0	7	45	235	− 5
4	23	205	11	7	26	202	13
4	40	211	4	7	38	208	10
4	43	201	3	7	47	229	0
Total Av. 4	41	230	+ 1.3	7.6	41	219	+ 2.1

in the table. Figs. 3, 4 and 5 show composite weight-curves for all the animals included in Tables III, IV and V, respectively.

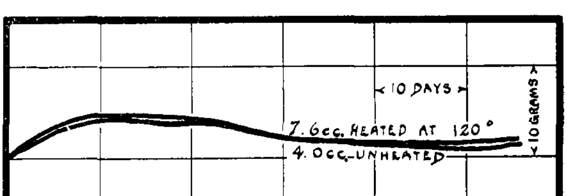


Fig. 4.—Weight curves of rats receiving 4 cc. of unheated tomato juice in comparison with those receiving an average dose of 7.6 cc. heated for 4 hours at 120°

TABLE V

EXPERIMENTS WITH TOMATO JUICE UNHEATED AND HEATED FOR FOUR HOURS AT 130°

	Rats fed unheated doses				Rats fed heated doses			
	Dose Cc.	Initial weight G.	Food intake G.	Gain G.	Dose Cc.	Initial weight G.	Food intake G.	Gain G.
	4	38	204	− 1	9	36	202	2
	4	44	219	2	9	42	267	7
	4	23	203	11	9	27	171	8
	4	57	308	−12	9	47	229	− 7
	4	33	222	3	9	35	177	− 5
Av.				+ 0.6				+ 1.0
	4	37	204	− 1	10	40	223	7
	4	32	205	− 2	12	29	187	7
	4	43	201	3	8	48	175	−11
	4	44	226	− 1	7	59	229	− 7
	4	43	225	− 1	7	52	229	−12
Total Av.	4	39	222	+ 0.1	8.9	41	209	− 1.1

It will be seen that throughout the four series of experiments summarized in Tables II to V, each experimental animal is paralleled by a litter control, and in each series the average initial weights of experimental and control animals are practically equal. This being the case, it has not seemed

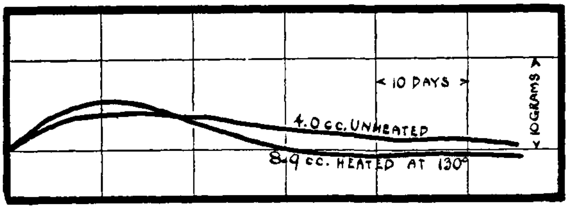


Fig. 5.—Weight curves of rats receiving 4 cc. of unheated tomato juice in comparison with those receiving an average dose of 8.9 cc. heated for 4 hours at 130°

essential to the purpose of this paper to discuss the relation of dosage to initial weights of individual experimental animals. From the data given this can easily be computed by any reader who desires to do so.

Discussion and Interpretation

The Percentage of Vitamin B Destroyed.—In each of the four tables given above the gain in weight of the animals receiving the heated juice is approximately the same as that of the positive controls. The curves of these sets of animals given in Figs. 2 to 5 further show that the two sets of animals closely parallel each other throughout the experimental period. It may, therefore, be concluded that the average doses of heated and unheated juice as given in each table are essentially equal in vitamin content. This being the case, the percentage of destruction by 4 hours' heating can be derived directly from the data of each table as follows.

Cc.	Temperature of heating °C.	Equivalent to unheated juice Cc.	Destruction %
5.0	100	4.0	20
5.9	100	4.7	20
6.0	110	4.0	33
6.3	110	4.2	33
7.6	120	4.0	47
8.9	130	4.0	55

The data as obtained and employed in these tables are thought to involve a minimum of error, and the method of arriving at the percentage of destruction is both direct and simple.

In deriving the figures for destruction just given, the curves for the two sets of animals have been taken as substantially identical. If desired, one may compute them to coincidence in a comparatively simple manner, but it is open to question whether figures so obtained are of any higher order of precision.

To do this it is necessary only to compute the change required in the unheated doses to bring the two comparison curves into coincidence at the termination of the eight-weeks period. This is done by using the relation established between growth and unheated juice in the experiments summarized in Table I. The method of computing may be illustrated using the figures from the table for 100°. Here the positive controls give a curve terminating 1.2 g. (5.4–4.2) above that for the animals receiving heated juice; or, better, taking only those receiving doses of 4 as against 5 cc., the difference is 0.8 g. Since a difference of 1 g. in weight at the end of the eight weeks corresponds to about 0.25 cc. of unheated juice per day, the curves may be brought into coincidence by reducing the unheated dose of the positive controls from 4.7 to 4.4 cc. as against 5.9 cc., or better to 3.8 cc. against 5 cc., and the destruction at 100° becomes 24%. Carrying out similar computations for the averages of Tables IV and V gives indication of a destruction of 45% at 120° and 58% at 130°.

For destruction at 110°, the best data are probably those of the upper groups only in Table III which show exact agreement at 4 as compared with 6 cc. or a destruction of 33%.

Hence, according as we take the average data as they stand or the revised data resulting from the assumptions and calculations just explained, we obtain the two following series of estimates for heat destruction of vitamin B in 4 hours at the temperatures stated: at 100°, 20% or 24%; at 110°, 33%; at 120°, 47% or 45%; at 130°, 55% or 58%. The temperature coefficients computed for the three 10° intervals are:

Q_{10} (100–110°) 33/20 = 1.6 or 33/24 = 1.4
Q_{10} (110–120°) 47/33 = 1.4 or 45/33 = 1.3
Q_{10} (120–130°) 55/47 = 1.2 or 58/45 = 1.3

Q_{10} (Av. 100–130°) 1.4 or 1.33

Viewed from any standpoint the results plainly show that about $^1/_5$ to $^1/_4$ of the vitamin is destroyed by being heated for 4 hours at 100° in slightly acid medium and that (the other conditions remaining the same) about $^1/_3$ is destroyed at 110°, slightly less than $^1/_2$ at 120°, slightly more than $^1/_2$ at 130°. The temperature coefficient of the destructive process is distinctly lower than that of most chemical reactions, resembling in this respect that of vitamin C, and there is no rapid rise in the rate of destruction at temperatures around 120° as had been inferred from earlier work.

The low temperature coefficient of destruction by heat makes it improbable that the vitamin is an unstable protein, and inappropriate to group it with enzymes since the typical enzymes which have been studied in this respect have shown rates of destruction by heat with high temperature coefficients up to the point at which the enzymic activity was entirely destroyed.

Summary and Conclusions

The effect of 4 hours' heating upon vitamin B of tomato juice has been investigated at 10° intervals over the range of 100° to 130° using the growth of young rats during an experimental period of eight weeks as a measure of vitamin B content.

For this purpose a dosage giving approximate maintenance has been found to be the best level of feeding. The disturbing factors are thus minimized and the sensitivity of the method is apparently at a maximum.

The results show that when heated at a temperature of 100° for 4 hours in slightly acid medium there is an appreciable destruction of vitamin B, a fact in agreement with previous results obtained in this Laboratory, using milk in the liquid state as the source of vitamin B.

That this destruction is due to the effect of the hot water upon the vitamin, and not to the possible presence of dissolved air is indicated by the fact that much longer heating at 100° in the dry state with free access of air resulted in no appreciable destruction of vitamin B. (Experiments of Sherman and Spohn.)

The average figures for destruction of vitamin B in tomato juice, derived directly by determining the size of heated doses at each temperature necessary to give weight curves approximately coinciding with those of positive controls fed 4 cc. of unheated juice are for a period of 4 hours' heating, as follows: at 100°, 20%; at 110°, 33%; at 120°, 47%; at 130°; 55%.

By a further simple mathematical computation bringing the comparison curves into exact coincidence, the following slightly different figures have been obtained: at 100°, 24%; at 110°, 33%; at 120°, 45%; at 130°, 58%.

These figures establish a low temperature coefficient of heat destruction as one of the characteristics of vitamin B, a property possessed also by vitamin C as shown by the previous experiments of Delf and of LaMer, Campbell and Sherman. For an increase of 10° in the temperature range of 100° to 130°, the rate of heat destruction of vitamin B in solutions such as here studied is increased only 1.3–1.4-fold, as compared with a 2-fold increase in most chemical reactions.

In the work here presented there was no indication of an increased temperature coefficient of heat destruction at temperatures around 120°. We find no evidence of any departure from the orderly course of a chemical reaction under the accelerating influence of heat but with a less than average temperature coefficient.

In this respect the heat destruction of the vitamin is in marked contrast with the heat coagulation of typical proteins and with the heat destruction of such typical enzymes as have been investigated.

As previously suggested in the case of vitamin C, the low temperature coefficient of the heat destruction of vitamin B may perhaps be due to the reaction being one which involves two phases of a heterogeneous system, the vitamin being in combination with or adsorbed upon colloidal material rather than in true solution in the hot water by which it is destroyed.

New York, N. Y.

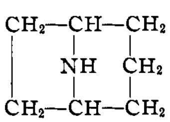
[Contribution from the Chemical Laboratory of the University of Illinois]

SYNTHESIS OF A NEW BICYCLIC NITROGEN RING. ISOGRANATANINE DERIVATIVES. PREPARATION OF AN ISOMER OF HOMOCOCAINE

By S. M. McElvain[1] with Roger Adams

Received August 9, 1923

Molecules containing bicyclic rings of an aliphatic character with a nitrogen atom in common occur in many of the natural alkaloids. Of these the most important are dihydro-nortropidine (I), quinuclidine (II), and granatanine (III), the basic nuclei of cocaine, quinine and pseudopelletierine respectively. The method described in this communication has been developed for the preparation of derivatives of a nucleus isomeric with granatanine, to which has been given the name isogranatanine (IV); a new ring resembling quinuclidine fairly closely.



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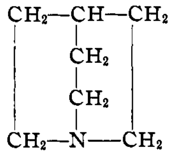

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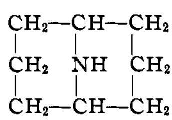

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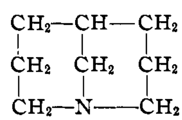

IV

[1] This communication is an abstract of a thesis submitted by S. M. McElvain in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Chemistry at the University of Illinois.