The Nontoxicity of Levulinic Acid*†

By R. G. Tischer, C. R. Fellers and B. J. Doyle

The discovery of a practical method for the preparation of levulinic acid (β-acetylpropionic acid) from corn at a very low cost has resulted in consideration of this acid as an element in the production and preservation of foods. Experiments conducted in this laboratory show that levulinic acid may be used successfully as an acidulant for carbonated and fruit beverages, jams, jellies and mayonnaise. Before industrial use could be made of this acid, it was necessary to obtain information as to its action in the human body. On the basis of chemical structure it should act similarly to the well-known acetic and propionic acids. A very complete bibliography of 423 references covering levulinic acid and its salts has just been compiled by the Division of Research Development of the A. E. Staley Manufacturing Company (1).

The literature reveals only a few references of a physiological nature. Gisselsson and Sylvan (2), in self-experiments using the calcium salts of gluconic and levulinic acids, caused a slight acidosis by the administration of massive doses of these salts.

Gordon, Kough and Proskouriakoff (3) have concluded that calcium levulinate administered orally, intravenously or subcutaneously is nontoxic for rats and human tuberculosis patients.

Greville and Dodds (4) injected intravenously 20 cc. of a 25% solution of calcium levulinate. Their subjects were one normal person, four tetany patients, and five jaundiced patients (preoperative treatment). No reactions whatsoever were observed.

Proskouriakoff (5) states: "Calcium levulinate was thoroughly tested by us on animals, and its extensive trial on tuberculosis patients showed no untoward symptoms . . . . As the low toxicity of levulinic acid in its salts was well established by previous investigators (Weintraud, W., *Arch. exptl. Path. Pharmakol.*, 34, p. 367), as well as by our own experiments on animals and men, it seemed to be desirable to prepare and try the levulinates of some other metals, the inorganic salts of which were tested and were shown to have some therapeutic value."

Although these reports indicate the nontoxicity of levulinates, and in particular that of calcium, there seems to exist no definite proof of the nontoxicity of levulinic acid itself. It was to answer this question that the present investigation was undertaken.

EXPERIMENTAL

Animal Experiments.—Preliminary toxicity tests on rats were used to determine the advisability of further experiments with human subjects. Three groups of rats (three in each group) were fed 0, 1 and 2% of the diet as levulinic acid for a period of 16 days. The acid was thoroughly mixed with a full nutrient ration and fed without restriction of amount. The results are presented in Table I and show no indication of toxicity in the concentration used.

Guinea pigs and chicks were also used to investigate the toxicity of levulinic acid. The acid was fed to guinea pigs in graded amounts varying from 0.5 to 5.0 cc. of 10% levulinic acid per day by means of a 1-cc. pipette or a stomach tube. Autopsy findings on these animals (after etherization) indicated no change in the appearance of the internal organs. The chicks survived similar treatment without apparent change.

Experiments with Human Subjects.—All the tests used in this investigation are standard procedures, details of which are found in "Approved Laboratory Technique" by Kolmer and Boerner (6).

Blood sugar, non-protein nitrogen, and creatinine were determined by the Folin-Wu technique. The carbon dioxide combining power of the plasma was determined by the Van Slyke and Cullen method, using a Van Slyke gas analysis apparatus.

In the urinalysis, acidity was determined using alk-acid paper (Fisher Scientific Company); sugar was determined by Benedict's qualitative test and albumin by Roberts' qualitative test.

The six normal male adults selected for the test ingested 3 cc. of pure levulinic acid daily for a period of 30 days, Sundays excluded. The acid was diluted with 150 to 400 cc. of fruit juice to disguise the acid taste.

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† The levulinic acid used in these experiments was kindly supplied by the A. E. Staley Manufacturing Company, Decatur, Ill.

TABLE I.—EFFECT OF LEVULINIC ACID ON GROWTH RATE OF RATS (16 DAYS)

Number of Rat	Levulinic Acid in Feed, %	Original Weight, Gm.	Final Weight, Gm.	Total Gain in Weight, Gm.	Total Food, Gm.	Gain in Weight per Gram of Food, Gm.
1	0	90	125	35	230	0.152
2	0	120	175	55	250	0.220
3	0	106	144	38	264	0.144
4	1	96	140	44	205	0.215
5	1	88	143	55	211	0.260
6	1	88	148	60	232	0.259
7	2	100	190	90	292	0.316
8	2	112	202	90	306	0.294
9	2	104	193	89	275	0.324

TABLE II.—RESULTS OF THE BLOOD CHEMISTRY TESTS ON HUMAN SUBJECTS

Subject	Test No.[a]	Sugar, Mg./100 Cc.	Non-Protein Nitrogen, Mg./100 Cc.	Creatinine, Mg./100 Cc.	CO_2 Combining Power, Vol. %
R.G.T.	1	79.7	28.5	1.64	62.0
	2	80.5	30.1	1.59	66.0
	3	71.4	26.3	1.46	65.7
	4	81.2	29.4	1.57	70.0
C.R.F.	1	94.9[b]	31.3	1.69	65.0
	2	83.8	30.8	1.74	63.1
	3	68.2	26.3	1.70	61.8
	4	85.1	29.5	1.66	64.5
R. S. L.	1	81.9	32.7	1.14	59.4
	2	84.0	30.9	1.23	60.2
	3	64.1	27.2	1.19	60.1
	4	85.0	31.0	1.25	58.5
J. J. P.	1	79.4	31.7	2.00	67.1
	2	70.4	33.1	2.70	65.0
	4	75.2	30.0	1.86	69.4
A. S. L.	1	76.3	27.9	1.58	71.2
	2	73.5	30.0	1.24	69.0
	4	78.5	26.3	1.49	67.1
W.B.E.	1	83.0	26.9	1.23	73.0
	2	81.2	28.0	1.27	70.2
	4	84.5	29.3	1.29	70.5

[a] The dates on which the tests were made were: No. 1, October 30, 1941; No. 2, November 13; No. 3, November 13 (about 40 min. after test No. 2 and 30 min. after taking 3 cc. of levulinic acid in 200 cc. of water); No. 4, November 27. All tests were made at about 8:00 a. m.

[b] A small bowl of oatmeal with sugar was eaten 1 hr. before the test.

The control medical and laboratory examinations were made on all the subjects before the administration of the acid was begun. The same tests were repeated after two weeks and four weeks.

At the end of the two-week period, all the tests were repeated on three of the subjects 30 to 50 min. after the ingestion of 3 cc. of levulinic acid in 200 cc. of water. This extra test was included to discover any possible immediate effects of the acid.

Blood samples were taken at about 8:00 a. m., the subjects having fasted since the previous night. The urine sample was collected on the morning of the test. Two 10-cc. samples of blood were taken, one from each arm. One sample was mixed with lithium oxalate to prevent coagulation and was then used in the preparation of the Folin–Wu protein-free filtrate for determination of blood sugar, non-protein nitrogen and creatinine. The remaining 10-cc. sample was used in determining the carbon dioxide combining power of the plasma.

RESULTS

The blood sugar results shown in Table II may appear to be rather low unless it is remembered that all determinations were made on fasting samples. As the first tests were made before the administration of acid was begun, these values are considered to be normal for each individual.

All of the values included in Table II appear to be in close agreement with the normal values expressed in test No. 1 except in the three cases where an additional test (No. 3) was made about 40 min. after ingestion of the daily dose of 3 cc. of acid. In these tests, the blood sugar, non-protein nitrogen and creatinine decreased a little in all three subjects, but not to an extent that would indicate a pathological condition. As tests No. 2 and No. 4 were performed less than 24 hrs. after the preceding dose of acid, the normal results indicate a complete recovery from the depressing effects noted in test No. 3.

There was no depressing effect whatever on the carbon dioxide combining power of the blood even

when the blood sample was taken within one hour of the time of ingestion of 3 cc. of pure levulinic acid in water. Likewise the non-protein nitrogen and creatinine values are all well within the normal range and show nothing of physiological significance.

The results of the urinalyses are recorded in Table III. The tests for sugar and for albumin were negative. Color, *p*H and specific gravity did not show significant variation in any of the tests.

TABLE III.—RESULTS OF URINALYSES

Subject	Test Number	*p*H	Specific Gravity
R.G.T.	1	5.0	1.012
	2	5.0	1.016
	3	5.0	1.023
	4	7.5	1.020
C.R.F.	1	5.0	1.024
	2	5.0	1.031
	3	5.0	1.032
	4	5.0	1.018
R. S. L.	1	4.0	1.024
	2	5.0	1.031
	3	6.0	1.030
	4	5.0	1.025
J. J. P.	1	4.0	1.030
	2	5.0	1.030
	4	5.0	1.030
A. S. L.	1	4.0	1.020
	2	6.0	1.030
	4	5.0	1.030
W.B.E.	1	4.0	1.012
	2	6.0	1.016
	4	5.5	1.014

The physical examinations of the men were performed in accordance with accepted medical practice. The results shown in Table IV indicate no significant variations in any of the measurements. The condition of the heart was observed by means of stethoscopic examinations, while "symptoms" were observed as general variation from normal health. Both were negative in every case. Careful and complete personal health diaries for each subject were kept before, during and following the period of this experiment. There was nothing of physiological significance in these data.

DISCUSSION

Of the three sets of data tabulated in Table IV, variation from normal conditions is observed only in the biochemical tests made on the blood drawn within one-half hour after the sample of acid was taken. Although a depression of the blood sugar level, non-protein nitrogen and creatinine is evident in these cases, these are not considered significant deviations from the normal because all other tests indicate the ability of the body mechanism to overcome the effect of the acid quickly and completely in every case. The absence of change in the alkali reserve balance as shown by the carbon dioxide combining power of the plasma only adds further weight to the evidence that the levulinic acid, at the level used in these tests, has only an insignificant effect, if any, on the human system.

CONCLUSIONS

1. No significant changes in the blood and urine, nor in general physical well-being resulted from the administration to men of 3 cc. of levulinic acid per day for 30 days.

2. The immediate effects on the sugar, non-protein nitrogen and creatinine content of the blood are well within the normal variations which might accompany the ingestion of ordinary foods.

3. No cumulative toxic effects are indicated.

TABLE IV.—MEDICAL EXAMINATION DATA

Subject	Age	Test Number	Weight, Lb.	Blood Pressure, Mm.	Pulse per Min.
R.G.T.	29	1	161	128/80	72
		2	165	138/78	72
		4	168	130/80	78
C.R.F.	47	1	169	136/84	66
		2	170	132/88	66
		4	172	132/88	72
R. S. L.	26	1	210	122/84	90
		2	214	128/86	84
		4	214	126/84	90
J. J. P.	23	1	117	110/70	78
		2	115	118/68	84
		4	116	116/70	74
A. S. L.	28	1	160	104/60	60
		2	159	108/66	48
		4	161	114/68	60
W.B.E.	29	1	164	138/88	72
		2	165	136/84	72
		4	165	130/84	66

4. Laboratory tests on albino rats, guinea pigs and chicks show that levulinic acid is nontoxic to these animals when fed to the extent of 5% of the food intake.

5. These preliminary studies suggest that levulinic acid in small amounts may be used safely to acidulate foods or beverages.

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Nomenclature Confusion in the Case of the Balsam Poplar or Tacamahac*†

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Populus balsamifera Linné was the binomial invariably applied to the Balsam Poplar or Tacamahac until Farwell (1) took exception to the name in 1919, and said that "the binomial. . . belongs to the Carolina Poplar, as usually understood. . . ." The confusion in the literature regarding the taxonomical status of these poplars following Farwell's article seems to be due in part, at least, to Linné's error in botanical synonyms for *P. balsamifera* in his "Species Plantarum" (2). In this connection, Farwell (3) has pointed out that the technical description of Linné is from the "Hortus Cliffortianus" (4), and that ". . . reference to the latter publication shows that species No. 4, *Populus foliis cordatis crenatis*, is the one referred to. This is founded solely on *Populus nigra, folio maximo, gemmis balsamum odoratissimum fundentibus* Catesby (5), . . . a Carolina species. There is therefore no question . . . that the binomial *Populus balsamifera* belongs to the Carolina Poplar, as usually understood, since in the last analysis the Linnæan species is founded upon that of Catesby."

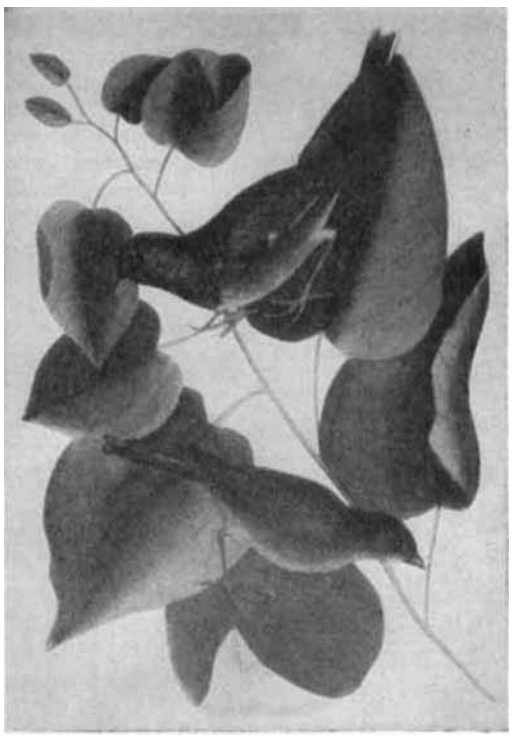

Fig. 1.—Catesby, M., "The Natural History of Carolina, Florida and the Bahama Islands," 1731, t. 34.

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