Safety of Adipic Acid as Compared with Citric and Tartaric Acid

HENRY J. HORN, EMILY G. HOLLAND,[1] and LLOYD W. HAZLETON
Hazleton Laboratories, Falls Church, Va.

Increased usage of adipic acid as a food additive has prompted the comparison of it with citric and tartaric acids. Acute and chronic administration to laboratory animals has shown that adipic acid is comparable to these acids and is a safe food additive.

Adipic acid (1,4-butanedicarboxylic acid), citric acid (2-hydroxy-1,2,3-propanetricarboxylic acid), and tartaric acid (1,2-hydroxy-1,2-ethanedicarboxylic acid) are straight chain organic acids of 6, 5, and 4 carbon atoms, respectively. Adipic acid has no substituted groups, citric acid has both a hydroxy and carboxylic group substituted on the second carbon, while tartaric acid has a substituted hydroxy group on both the first and second carbons. Of these acids, only adipic is nonhygroscopic. Because of the increased interest in their use as food additives, the following work was undertaken.

Review of Available Literature

Rose (*10*, *11*) is responsible for several investigations in the course of which he found that, following subcutaneous administration, adipic acid was mildly irritating to the kidneys, while glutaric acid (1,3-propanedicarboxylic acid) was nephrotoxic. In 1925, Rose and coworkers (*12*) concluded from their investigations that none of the higher homologs were irritating to the kidneys. Corley and Rose (*1*) examined 19 different acids for nephrotoxicity but found that only three—tartaric, mucic, and glutaric—exerted pronounced toxicity. Mucic acid is tetrahydroxyadipic acid. These authors concluded that: The number of carbon atoms, per se, present in a dicarboxylic acid has no relation to its toxicity; the introduction of a hydroxy or ketonic group on the first carbon of glutaric acid destroys its nephrotoxic effects; and the introduction of a hydroxy or ketonic group on the first carbon of adipic acid does not influence the toxicity.

Somewhat later, Harding and Nicholson (*8*) directed studies toward evaluating this apparent discrepancy in toxicity in acids which possessed rather similar properties. Their studies indicated that following either subcutaneous or intramuscular administration, glutaric acid was readily absorbed with a minimum of local reaction, whereas adipic acid caused marked local reaction at the site of injection and higher homologs of the series could be definitely identified as retained crystals at the site. Based on these observations, at least a portion of the difference in renal toxicity might be due to poor absorption of adipic acid and the higher homologs; their work confirmed the nephrotoxicity of glutaric acid. However, following subcutaneous administration, Flaschenträger (*4*) recovered approximately 50% of administered adipic acid in the urine.

In 1942, Enders (*2*) reported that adipic acid, azelaic acid (1,7-heptane dicarboxylic acid), and sebacic acid (1,8-octanedicarboxylic acid) are only slightly toxic when given in large single oral doses to rabbits, or when fed daily to rats over a long period of time. Excretion of these acids in the urine of rats showed adipic acid to be more slowly excreted than the others.

Simola and Kosunen (*13*) fed the sodium salts of a series of organic acids in single doses to adult rats and analyzed the urine for increased citric and ketonic acid excretion. All of the acids studied increased both the citric and ketonic acids, but the increase was slight with adipic acid.

Both Hanson (*7*) and Weitzel (*14*), in their studies on the urinary recovery of orally administered adipic acid to humans, concluded that decomposition took place in the body with small amounts being more completely catabolized than large amounts.

An unpublished report by Foulger (*5*) is of particular interest from a practical standpoint as it presents the results following the repeated administration of relatively large doses of adipic acid. Immature rats failed to gain weight properly when given 638 to 1332 mg. per kg. Fitzhugh and Nelson (*3*) reported 2-year rat feeding experiments on several acids, including tartaric acid, which they found was not toxic in concentrations up to 1.2% of the diet.

More recently, Gruber and Halbeisen (*6*) reported that, following intraperitoneal administration to rats, adipic acid appears to be more toxic than citric. They reported deaths from citric acid up to 1 week but could not associate this with postmortem findings. The intraperitoneal administration of adipic acid resulted in extensive irritation and adhesion of visceral organs. A rapid intravenous injection of citric acid in mice resulted in an LD_{50} of 0.22 millimoles per kg. When the injection was made at the rate of 1.5 millimoles of acid per minute until the animals died, the average LD_{100} was 2.08 millimoles per kg.

Experimental

Acute Oral Administration. Male albino mice were used in this study. A 3% aqueous solution of adipic acid, kept at body temperature, was tried but proved impractical as sufficiently large doses to determine an LD_{50} could not be administered. Therefore, a 6% suspension of adipic acid in 0.5% methyl cellulose was administered orally, resulting in an LD_{50} of 1900 mg. per kg. or 13.0 millimoles per kg. (Table I). Autopsy of the animals that died showed distention of the stomach and small intestine, with a spastic concentration of the caecum. Irritation and hemorrhage of the intestines were noted. Initial mortality developed overnight and deaths continued throughout the first week, after which survivors appeared normal. All animals were sacrificed after 10 days.

Acute Intraperitoneal Administration. A few mice were given lethal doses (600 and 900 mg. per kg.) of a 3% aqueous solution of adipic acid intraperitoneally. These mice showed depression immediately and, at autopsy, the intestines appeared irritated and the lungs appeared hemorrhagic.

Male albino rats were given a 3% aqueous solution of adipic acid intraperitoneally (Table I). Mortality occurred during the first 5 days. The LD_{50} was 275 mg. per kg. (1.88 millimoles per kg.). Animals that succumbed showed hemorrhagic lungs and irritation of the intestines. The survivors, sacrificed 1 week after administration, showed extensive irritation and adhesions of the visceral organs.

Acute Intravenous Administration. Intravenous injection to mice at various dosage levels, at a rate of 0.01 ml. per second, with 2% solutions of adipic,

[1] Deceased.

Table I. Acute Toxicity of Adipic, Citric, or Tartaric Acid to Male Albino Mice or Rats

(Dosages are as the acid. Values are the number of animals dead per number of animals tested)

Dose, Mg./Kg.	Adipic			Citric,	Tartaric,
	Oral[a], mice	Intraperitoneal[b], rats	Intravenous[c], mice	Intravenous[c], Mice	Intravenous[c], Mice
175				2/13	
200		1/7		6/13	0/3
225				10/13	
250				3/3	
300		4/7			
350		6/7			
400					0/2
450					1/13
475					2/13
500					9/13
650			4/13		
675			7/13		
700			8/13		
1500	3/13				
2000	8/13				
2500	9/13				
LD_{50}, mg./kg.	1900	275	680	203	485
Confidence limits, mg./kg.	1640–2200	193–392	653–708	190–217	462–509
LD_{50}, millimoles/kg.	13.0	1.88	4.65	1.07	3.23

[a] 6% suspension in 0.5% methyl cellulose.
[b] 3% aqueous solution.
[c] 2% aqueous solution.

Table II. Summary of Average Body Weights of Albino Rats

(Controls received the basal diet. Other animals received the basal diet containing the indicated percentage of the adipic acid or citric acid)

	Average Body Weight in Grams								
	Males							Females	
		Adipic Acid				Citric Acid			Adipic acid,
Week	Control	0.1%	1%	3%	5%	3%	5%	Control	1%
0	59	61	63	61	57	62	61	49	48
8	269	280	265	224	182	239	225	178	175
16	325	333	320	276	233	298	278	222	213
24	361	374	354	309	264	329	320	242	233
32	377	391	376	329	291	328	339	257	249
40	397	407	401	357	314	370	361	279	263
48	423	433	421	372	322	393	377	275	270
56	428	447	436	380	336	400	388	286	277
64	426	455	436	385	339	407	401	295	284
72	407	447	431	385	336	400	389	301	288
80	408	441	430	383	349	411	391	313	301
88	413	448	432	398	344	411	389	309	303
96	432	424	436	396	354	409	393	318	308
104	440	417	437	400	360	417	397	321	304

Table III. Summary of Data for Albino Rats Receiving Basal Laboratory Diet or Basal Diet of Adipic or Citric Acid for 2 Years

(Per cent of survival based on length of survival as well as number of animals)

Level	Sex	No. of Rats		Av. Body Weight, G.		Food Consumed, G.,	Compound Consumed, Mg.,	Survival,
		Start	Finish	Initial	Final	Av./Rat/Day	Av./Rat/Day	%
Control	M	20	8	59	440	16.8		82.5
	F	10	8	49	321	14.2		98.9
Adipic acid								
0.1%	M	20	13	61	417	17.0	17.0	87.7
1%	M	20	15	63	437	17.5	175	94.7
	F	19	17	48	304	15.8	158	96.3
3%	M	20	16	61	400	16.8	505	94.5
5%	M	20	15	57	360	15.8	814	97.2
Citric acid								
3%	M	20	14	62	417	17.1	512	92.6
5%	M	20	16	61	397	15.7	784	95.0

citric, or tartaric acid yielded LD_5 values of 680, 203, and 485 mg. per kg. respectively (4.65, 1.04, and 3.23 millimoles per kg.). The results of these experiments are presented in Table I. These acids caused immediate, convulsive deaths, probably due to acute acidosis as the pH of the solutions was 3.08, 2.50, and 2.53, respectively. Autopsy showed hemorrhagic lungs but no other gross pathology. In survivors, recovery was apparently complete and there were no latent deaths. Statistical analysis was done by the method of Litchfield and Wilcoxon (*9*).

Chronic Feeding. Young male and female albino rats of the Carworth Farms strain, having approximate mean initial weights of 60 and 50 grams, respectively, were selected at random for use in these studies. All of the rats were housed individually in cages with wire mesh floors elevated above the droppings. The animals had free access to food and water at all times.

Groups of rats were placed on either the basal laboratory diet or the basal diet containing either adipic acid or citric acid, as follows:

Group	Males	Females
Basal laboratory diet used as control	20	10
Basal diet containing 0.1% adipic acid	20	0
Basal diet containing 1% adipic acid	20	19
Basal diet containing 3% adipic acid	20	0
Basal diet containing 5% adipic acid	20	0
Basal diet containing 3% citric acid	20	0
Basal diet containing 5% citric acid	20	0

The body weights and food consumption of all rats were recorded at weekly intervals during the course of the study. In addition, weekly observations were made of the general appearance and condition of each animal. Whenever possible, gross autopsy was performed on those animals that died during the course of the experiment.

After 2 years on the respective diets, the surviving rats were weighed, sacrificed by a blow on the head, and examined for gross and microscopic pathology. The brain, thyroid, lungs, heart, liver, spleen, kidneys, adrenals, stomach, and testes of approximately half of each group of males were weighed. The kidneys, spleen, liver, and heart of each female were weighed. Microscopic examination of the following tissues were done on a representative number of animals of each group: thyroid, lungs, heart, liver, spleen, kidneys, adrenals, stomach, small intestine, large intestine, pancreas, bone marrow, testes or ovaries, and uterus.

Results

Males. The average body weights for the male rats are tabulated in Table II for each 8-week interval. Throughout the entire 2-year study, the 0.1 and 1% adipic acid groups were comparable with the control groups. During the rapid growth period, the weight gains of the 3 and 5% adipic acid and the 3 and 5% citric acid groups were significantly less than the control groups; however, there was no significant difference among these four test groups. Throughout the latter half of the study, the average body weights of the various test groups were not remarkable—although the 5% adipic acid group was consistently the lowest.

Table III presents a summary of food and compound consumed and survival data for the entire 2-year feeding period. There was only a slight, but consistent, reduction in food consumption by the 5% adipic acid and 5% citric acid groups. Other test groups were comparable to the control group. The per cent survival for each test group was better than the control group.

Autopsy data for the male animals that died during the course of the 2-year feeding program and for the sacrificed rats were analyzed for incidence of tumors and/or lung pathology. To be included in the following table, a tumor must have presented gross evidence of being a new growth.

	Deaths				Sacrificed	
Male Group	Lung pathology	Tumors	Other causes	Total deaths	Lung pathology	Tumors
Control	7	3	3	12	4	1
Adipic						
0.1%	3	2	3	7	7	2
1%	1	2	2	5	7	2
3%	3	..	1	4	3	..
5%	..	4	1	5	4	..
Citric						
3%	1	2	3	6	1	..
5%	1	2	1	4	4	1

These findings appear not to be related to the compounds under study as an equivalent incidence was observed in the controls.

Throughout the study, especially the final 6 months, the following signs were observed among all the groups, including the controls: wheezing, blood-tinged crust about the noses and eyes, and body sores. The incidence of these findings did not appear to be significantly different among the groups although a lower incidence of signs indicative of respiratory infection and body sores occurred in the 5% adipic acid group.

When the surviving males were sacrificed at the end of the 2-year period, there was no significant gross pathology that could be related to either compound. Soft edematous testes were noted at least as frequently in the controls as in the experimental animals. There was no significant difference in organ weights of the experimental groups *vs.* the controls.

Microscopic examination of thyroid, lungs, heart, liver, spleen, kidneys, adrenals, stomach, pancreas, bone marrow, and large and small intestines revealed these tissues to be within normal limits in all groups of male rats.

Females. The average body weights for the female rats are tabulated in Table II for each 8-week interval while food and compound consumption, together with survival data, are presented in Table III. There was no significant difference between the body weight gains or food consumption for the two groups.

In the last 6 months, the animals exhibited signs normally associated with advancing senility in rats. There was an equal incidence of blood-tinged crust about the eyes and noses, unthriftiness, and body sores in both groups. A few control and experimental animals had alopecia, and one experimental rat appeared to develop a middle ear infection during the 102nd week. The average weight of the kidneys, spleen, liver, and heart, together with organ-to-body weight ratios, appeared to be within normal limits.

One experimental and two control animals died during the final 6 months. All three exhibited diarrhea, respiratory infection, and loss of weight prior to death. Upon autopsy, one control rat and one experimental rat were found to have tumors, while the other control animal had a granular liver and dark red apexes on both lungs.

When the surviving animals were sacrificed at the end of the 2-year period, there was no significant gross pathology that could be related to ingestion of the compound. There was an equal incidence of mottled, granular livers with peripheral thickening in both the control and experimental animals. Two of the surviving control rats and one experimental animal had ovarian tumors; ovarian cysts were noted in both control and experimental rats.

Discussion

The results of the above experiments indicate that adipic acid is significantly less toxic than tartaric or citric acid following intravenous administration to mice. The doses were calculated as milligrams per kg. and as millimoles per kg. The action of these three acids appears comparable.

No direct comparison of the intravenous toxicity of citric acid in these tests and those reported by Gruber and Halbeisen (*6*) is possible because they used a faster rate of injection. The LD_{50} values of 1.06 millimoles per kg. obtained in this experiment is midway between the LD_{50} and the LD_{100} of Gruber and Halbeisen.

Single oral administrations of an almost saturated solution (*3%*) of adipic acid did not cause appreciable mortality in tolerable volumes. With a 6% suspension, the LD_{50} approximated 2 grams per kg. Comparable values for citric and tartaric acids are not available.

Following intraperitoneal administration to rats, adipic acid appears to be more toxic than citric (*6*). The intraperitoneal administration of adipic acid resulted in extensive irritation and adhesion of visceral organs.

During the rapid growth period of the 2-year feeding studies, weight gains for the male rats receiving 3 or 5% adipic or citric acid was significantly less than the male controls; however, there was no significant difference among these four experimental groups. Growth for other groups—0.1 and 1.0% male and 1.0% female—was comparable to that of the respective controls. There was no evidence of gross pathology associated with the feeding of either acid. There was no significant difference in survival among the various groups from the controls. The incidence of lung pathology, tumors, or soft testes was observed at least as frequently in the controls. The organ-to-body weight ratios appeared to be within normal range. The results of microscopic examination appeared to be within normal limits for the representative tissues studied.

Comparison of the chronic feeding of adipic acid with citric acid (herein reported) and also with tartaric acid in an equivalent study (*3*) indicates that adipic acid is comparable with citric and tartaric acids.

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DEHYDRATION TEMPERATURE EFFECTS

Effect of Temperature of Dehydration on Proteins of Alfalfa

ROY E. BEAUCHENE[1] and H. L. MITCHELL

Kansas Agricultural Experiment Station, Manhattan, Kan.

The effect of temperature of dehydration on the proteins of alfalfa was investigated. There was no difference in the total nitrogen contents of meals dehydrated at 50° C. and dehydrated commercially. The low temperature meal, as opposed to the high temperature meal, contained more α-amino nitrogen and less protein nitrogen, contained some water-soluble protein, and possessed proteolytic activity. Digestion by pepsin, trypsin, and erepsin, or by high levels of pancreatin, showed no differences in the digestibilities of the meals. Suboptimum quantities of pancreatin liberated α-amino nitrogen at a greater rate from low temperature meal than from commercially dehydrated meal. Extracts of the meals inhibited the action of trypsin on casein and the inhibition was not decreased by autoclaving the extracts.

About a million tons of dehydrated alfalfa meal are produced each year. Dijkstra (*6*) reported that the commercial dehydration of grass reduced the in vivo digestibility of its protein. Brew (*5*) showed that the temperatures used in dehydrating alfalfa commercially were sufficient to alter the solubility of the proteins. This is a report of a further investigation of the protein changes caused by commercial dehydration of alfalfa meal and their possible effect on the nutritive value of the meal.

Experimental

Samples. Three alfalfa meals were prepared by subjecting freshly chopped, first-cutting alfalfa to different heat treatments. One portion was dried at 50° C. in a circulating air oven. A second portion was dehydrated in a commercial dehydrator operating with an air inlet temperature of approximately 900° C. and an air outlet temperature of about 175° C. High temperature–pelleted meal was prepared by passing a portion of this meal through a California pelleting machine and regrinding it to pass through a 20-mesh screen. The latter sample was prepared because the meal is subjected to additional heat in the pelleting operation.

[1] Present address, Department of Household Arts and Sciences, Texas State College for Women, Denton, Tex.

Nitrogen Fractions. The effect of the heat treatments on certain nitrogen fractions was studied. The total nitrogen content of each of the three meals was determined by the Kjeldahl procedure (*1*). To measure the water-soluble nitrogen, 5 grams of meal were extracted with 200 ml. of cold water for 10 minutes in a Waring Blendor, the extract was filtered, and a 50-ml. aliquot was analyzed for nitrogen as before. A second portion of the filtered extract was adjusted to pH 4.5, heated to boiling, and filtered again. Fifty milliliters of this solution were analyzed for nitrogen also. The difference between the nitrogen values of the two aliquots represents soluble protein nitrogen. The remainder of the heated solution was neutralized and was analyzed for α-amino nitrogen by a flame photometric procedure (*2*).

Table I shows that there was little difference in the total nitrogen content of the three meals. However, differences between the low and high temperature meals were noted in the nitrogen breakdown. The conversion of soluble to insoluble protein by the heat of the commercial dehydration process confirms the qualitative data of Brew (*5*). The higher α-amino nitrogen and lower protein nitrogen of the low temperature meal indicate that proteases were active during low temperature drying.

Proteolytic Activity of Meals. The meals were tested for the presence of proteolytic activity by observing their effect on casein solutions. Ten milliliters of a 1% casein solution, 20 ml. of water, and 1 or 2 crystals of thymol were placed in each of two beakers. One gram of low temperature meal was added to one of the beakers and 1 gram of high temperature meal to the other.

Table I. Quantity of Nitrogen in Some Fractions of Alfalfa Meals Subjected to Different Heat Treatments

Meal[a]	Total Nitrogen (A)	Water-Soluble Nitrogen (B)	Nonprotein Nitrogen (C)	Soluble Protein Nitrogen (B − C)	Total Protein Nitrogen (A − C)	α-Amino Nitrogen (D)	Unidentified Nitrogen (C − D)
	Mg./G.						
1	33.6	11.4	9.7	1.7	23.9	3.7	6.0
2	32.8	5.2	5.2	0.0	27.6	2.7	2.5
3	32.9	5.6	5.4	0.2	27.5	2.6	2.8

[a] 1, low temperature meal; 2, high temperature meal; 3, high temperature–pelleted meal.