clavin, which when purified free from ash had the properties described by Vahlen, consisted entirely of these three amino acids, the latter being isolated analytically pure: 2.02 grams of clavin gave 0.79 gram of leucin, 0.45 gram isoleucin, 0.75 gram valin. We have not yet determined whether any of these amino acids has the pharmacological effect assigned to clavin.

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Some effects of sodium benzoate.

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This research was suggested to me by different experiences with sodium benzoate when taken by mouth in the following ways: A. Pure (1) as crystalline salt, or (2) in aqueous solution. B. In neutral or alkaline solutions, or in mixtures rich in fat, carbohydrate or protein, *e. g.*, milk. C. With vegetable or fruit acids (1) hot, as in tomato soup, or (2) cold, as in canned plums, oranges, lemons, etc. D. In beverages containing high percentages of organic acids, *e. g.*, cider, lemonade, grape juice, wine, etc. E. In mixtures containing inorganic acids, *e. g.*, artificial gastric juice.

Brunton has studied the effects of benzoic acid on enzymes and bacteria. The strong inhibiting effect of this substance on their activity is in striking contrast to the slight effect of sodium benzoate. Doepner has shown that fairly large quantities of sodium benzoate (2 per cent.) did not prevent the development of *Proteus vulgaris* and, in strengths equal to 0.5 per cent., only slightly retarded the development of *B. enteritidis*, *B. fluorescens* and *B. coli.* Fleck found that benzoic acid in concentrations equal to 0.6–0.7 per cent. caused marked inhibition of yeast fermentation and that the inhibiting action was markedly decreased by the amount of protein present. Lehman observed that meat extract putrefies in the presence of 1 to 2 per cent. of sodium benzoate, but less benzoic acid acts more strongly antiputrefactive when the reaction of the medium is markedly acid. The action of sodium benzoate under markedly acid conditions is the same as the action of benzoic acid. Under such conditions the action of the benzoate diminishes with decrease of

acidity. Chassevant and Garnier found that 1.4 gram of benzoic acid per kilo was fatal to guinea pigs in five to seven hours; larger doses (2 grams per kilo) did not necessarily kill sooner.

The results of my own work may be briefly summarized as follows:

Effects on microörganisms.—Sodium benzoate, in concentrations of about 1 per cent., preserves fruits and vegetables which are *strongly* acid. Crystals of free benzoic acid often appear in such mixtures. Sodium benzoate (1 per cent.) added to *weakly* acid fruits and vegetables does not preserve them well. Sodium benzoate (1 per cent.) added to fruits and vegetables, the acidity of which has been *neutralized,* does not preserve them. Pure apple juice, containing 0.1 per cent. of sodium benzoate, developed mould after ten days; commercial benzoated cider under the same conditions, without the further addition of benzoate, did not develop mould or otherwise undergo degeneration.

Effects on taste. — Acid fruit juices containing 1 per cent. of sodium benzoate, have a biting taste, an effect due to the liberated benzoic acid. Milk or alkaline vegetables treated with sodium benzoate (1 per cent.) do not taste of benzoic acid at any time during the first twenty-four hours after the treatment. After twenty-four hours, however, acid decomposition begins in milk in spite of the presence of 1 per cent. of sodium benzoate, when the mixture tastes distinctly of benzoic acid. Sips of 1 c.c. of orange juice, to which 1 per cent. of sodium benzoate has been added, cause burning in the posterior part of the mouth, the throat, the esophagus and stomach, with gastric discomfort, belching, uneasiness of the bowels and excessive passage of gas by rectum.

Experiments on men with cider. Pure cider. — Pure apple juice tastes sweet, bland; produces no stinging sensation in the throat; and is ordinarily enjoyed and well borne in volumes equal to 1,000 to 2,500 c.c. (ingested during a period of two or three hours). It is diuretic in action and, in amounts varying from 1,000 to 2,000 c.c., causes laxation of the bowels. This effect frequently depends on the rapidity with which it is ingested; it does not ordinarily cause laxation even when taken in large amounts, if ingested little by little. The average amount of pure apple juice consumed during an evening by adult males who

had free access to it was about 1,200 c.c. (twenty subjects). When from 1,000 to 2,500 c.c. of pure apple juice are consumed neither headache, nausea, albuminuria nor sub-normal temperature is produced. The specific gravity of the urine is greatly decreased when a liter of pure unfermented cider is consumed but the volume is markedly increased within forty-five minutes after its ingestion. The forty gallons of pure apple juice consumed by the human subjects of my investigation contained considerable apple pulp and 2.716 grams of free acid (calculated as acetic acid) per 1,000 c.c.

Benzoated cider. — Twenty four subjects were observed in the first experiment. Twelve received *pure* apple juice; twelve received samples of the same apple juice containing 0.1 per cent. of sodium benzoate. As none of the subjects knew that they were to receive at that time anything but pure apple juice, unfavorable psychological influences were eliminated from the experiments. Each subject received three question blanks to be filled out by himself daily as long as any symptoms lasted, which, I am assured, was done faithfully in every instance.

In comparison with those who received pure cider, the men who drank the benzoated apple juice exhibited the following special symptoms: Burning taste, fulness in the head, headache, nervousness, nausea, vomiting, itching of the skin, unusual perspiration, irregularity of bowels (*constipation* usually) *decreased* flow of urine, *increased* specific gravity of the urine, and albuminuria. Excessive amounts of hippuric acid were eliminated, especially during the first few hours, after ingestion of the benzoated apple juice.

Apple juice to which a small amount of sodium benzoate is added becomes sweeter to the taste, but astringent, stinging, and irritating to mucous membranes. The presence of 0.5 per cent. of sodium benzoate renders cider quite unpalatable, but the presence of 0.1 per cent. may be overlooked by subjects not acquainted with the taste of pure apple juice.

If the apple pulp is previously filtered from the juice the effects of added benzoate become much more evident. A liter of such filtered cider, containing 0.2 to 0.3 per cent. of sodium benzoate, caused albuminuria within three hours almost without exception in the largest and soundest picked subjects. However, I myself

was able to ingest 1,000 c.c. of apple juice containing 0.5 per cent. of sodium benzoate, without any albuminuria arising. The amount of hippuric acid in the urine was very large for the first few hours. The secretion of urine was very much reduced for twelve hours, while I suffered from some of the other symptoms above mentioned, *although as a subject in a former investigation I ingested as much as 6 grams per day, for three successive days, in milk on a full stomach, without the slightest discomfort.*

Small doses of sodium benzoate given with *acid* substances to patients with albuminuria aggravated this condition and caused alarming symptoms, classical of nephritis—for six days thereafter in one subject.

Experiments on dogs. First experiment. — One dog weighing 3.5 kilos fasted for twenty four hours and was then given 1 gm. of sodium benzoate, decomposed with the theoretical amount of hydrochloric acid to form sodium chloride and free benzoic acid, with no excess of hydrochloric acid. In thirty minutes the animal showed evidences of muscular weakness and nausea, lay quietly and breathed in a laborious manner. This continued for six hours. On the next day, twenty four hours after the previous dose, the animal was given 4 gm. of sodium benzoate with a sufficient amount of hydrochloric acid to decompose it into benzoic acid, plus 120 c.c. of 0.2 per cent. citric acid. The animal became very weak in one hour, respirations were reduced to nine per minute, and were very labored. Tonic and clonic convulsions began one hour and fifteen minutes after the dose was given. The animal, after several hard convulsions, died two hours and twenty minutes after administration.

The autopsy showed congestion of various organs. There was very pronounced congestion of the kidneys, stomach and intestines, with ulceration in places. The liver and lungs also showed evidences of infarcts.

Second experiment. — Two dogs had been fed on dog biscuits and water for several weeks, and then fasted for thirty six hours.

Animal No. 1. — The first animal was a male, weighing 3.5 kilos. He was given a mixture of 3.5 gm. of sodium benzoate, 50 c.c. of water, 0.65 c.c. of concentrated hydrochloric acid (sp. gr. 1.19) and 100 c.c. of citric acid (0.2 per cent.). The animal

became quite uneasy after receiving the dose. At the end of an hour he showed great muscular weakness and tremor.

Animal No. 2. — The weight of the second animal was 4.25 kilos. It was given a mixture of 100 c.c. of 0.2 per cent. citric acid, 50 c.c. of water and 0.85 c.c. of concentrated hydrochloric acid (sp. gr. 1.19). This animal was entirely unaffected.

The same experiments were repeated on the same animals the next day; the results were practically identical. The animal (1) that received the free acid-benzoic acid mixture, however, was more prostrated than on the previous day and showed general stiffness of the muscles. At the end of six hours it was chloroformed and autopsied, when it was found that the stomach contained "coffee ground" material. There were ecchymotic areas and some places appeared to be ulcerated slightly. The intestines showed marked congestion here and there and appeared to be slightly ulcerated in places throughout. The grumous material in the stomach and intestines gave a strong guaiac test and was undoubtedly modified blood. The liver and lungs showed considerable congestion with some evidences of infarcts. The kidneys were cyanotic, the cortex very much congested, while the medulla was pale and anemic.

Further investigation is contemplated, especially on the influence of nephrectomy on the toxicity of benzoic acid.

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