TABLE 11 (*Continued*).—No Crystals of Calcium Oxalate or of Any Substance Resembling It Present.

Sinapis Alba	Taraxacum	Verbasci Flores
Sinapis Nigra	Thuja	Verbasci Folia
Solanum	Thymus	Verbena
Spigelia	Trillium	Zea
Staphisagria	Triticum	Zedoaria
Strophanthus	Valeriana	Zingiber
Sumbul		

SACCHARIN FEEDING OF RATS.*,[1]

B. FANTUS AND L. HEKTOEN.

The importance of saccharin as a sweetening agent for medicine and in diabetes mellitus justified, it seemed to us, the undertaking of a prolonged feeding experiment.

EXPERIMENT I.

To determine the effect of prolonged ingestion of relatively enormous quantities of saccharin, parallel observations were made on 25 young rats (5 males, 20 females), in each of three good-sized cages. Group I A received 10% saccharin in its food, Group I B received 1% saccharin in food, Group I C served as control.

After preliminary trials, the following food formula was adopted: Cornmeal, 200 Gm.; White Flour, 100 Gm.; Meat, 50 Gm.; Water, 200 cc. This was given the control animals, while for the other groups the required amount of soluble saccharin (sodium benzoylsulphonicimide—Heyden Chemical Works) was added. The mixture was then molded into cakes. The only other food the animals received was a small amount of lettuce or carrots, once a week. More food was given than could be eaten, though the exact amount of food consumed was determined only at the beginning of the experiment, until the animals had attained full growth and the food consumption became fairly constant.

The collective weight of the animals in each cage was determined daily at first, and at weekly intervals later on in the experiment. From Chart 1, which gives the average weight of the animals of each group, it will be seen that the animals fed on food containing 10% saccharin did not do as well as the controls. The weight curve of the animals receiving 1% saccharin was practically equal to that of the controls up to the 24th week, when the saccharin-fed animals commenced to lag slightly behind. This difference in weight might be due to difference in food consumption, which probably explains the lower weight curve of the animals that received 10% saccharin, who did not consume as much food as the animals of the other two groups. The animals that received 1% saccharin consumed practically the same amount of food as the controls.

During the course of the experiment, animals died from time to time, practically always from acute or chronic bronchopneumonia. A few animals died from cerebral abscesses. By the end of the 36th week, 16 animals had survived in Group I A, 13 animals had survived in Group I B, and 19 animals in Group I C.

All the rats were now killed by chloroform; and it was found that all of the rats of the saccharin groups and most of the rats of the control group had more or

* From the John McCormick Institute for Infectious Diseases.

[1] Scientific Section, A. Ph. A., Cleveland meeting, 1922.

less extensive pulmonary involvement. The difference in the findings of the three groups is shown in Table 1.

TABLE 1.

Lung Involvement of Animals Killed at the End of Experiment I.

Rat No.	Group I A. 10% saccharin rats.	Group I B. 1% saccharin rats.	Group I C. controls.
1	Extensive	Extensive	None
2	Extensive	Moderate	Quite extensive
3	Small acute focus	Slight	Quite extensive
4	Extensive	No evident change	Quite extensive
5	Extensive	Very extensive chronic	None
6	Extensive	Quite extensive	Extensive
7	Small acute focus	Quite extensive	Very extensive
8	Moderate	Rather slight	Extensive
9	Extensive	Not very extensive	None
10	Moderate	Entire right lung	None
11	Extensive	Slight	None
12	Quite extensive	Moderate	Slight
13	Quite extensive	Slight tumor of left chest wall	None
14	Extensive		None
15	Moderate		Moderate
16	Moderate		Rt. lung involved
17			Slight
18			Quite extensive
19			Moderate

From this tabulation it will be seen that the controls suffered much less from the pulmonary disease than did the others, and that the 1% saccharin-fed rats showed considerably less pulmonary disease than did the rats given food containing 10% saccharin. Indeed, the difference in the amount of lung involvement was so marked that it would easily account for the difference in the weight and size of the three groups of animals at the end of the experiment. The various other organs of the animals showed no gross evidence of disease; and the kidney, liver, spleen, pancreas, and stomach of each of the rats submitted to microscopic examination did not reveal any special changes.

As the average food consumption of these animals was 11.2 Gm. for the first 6 weeks, and then rose to an average of 13.2 Gm. during the remainder of the experiment, and the total food consumption during the 252 days of the experiment approximated 3100 Gm., *each of the 10% saccharin rats consumed about 310 Gm. of saccharin during the course of the experiment; or about twice its maximal weight.* Yet, be it noted, no evidence of definite damage could be found in the kidney, liver, spleen, pancreas, and stomach on either macroscopic or microscopic study.

EXPERIMENT II.

Inasmuch as organs might undergo changes of such nature as not to be discoverable even by microscopic examination, the next experiment was planned to continue for the natural life-time of the animals. In an attempt, unfortunately unsuccessful, to protect at least some of the rats against infection, three of the seven animals of each group were kept in separate cages, while one male and three females were kept in one cage.

The animals were fed a ration, the formula for which was kindly furnished us by E. V. McCollum consisting of the following:

Sugar	140 Gm.
Whole wheat	2240 Gm.
Powdered milk	350 Gm.
Calcium carbonate	42 Gm.
Sodium chloride	28 Gm.

Enough water to make into cakes.

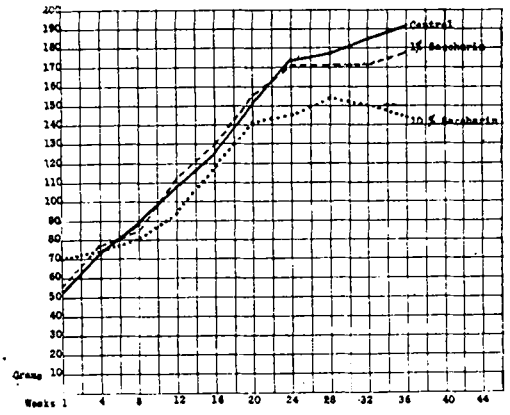

Chart 1. Experiment I.—Average weight curve of rats fed 10% saccharin (dotted line); 1% saccharin (broken line); controls (continuous line). Animals killed at end of 36th week.

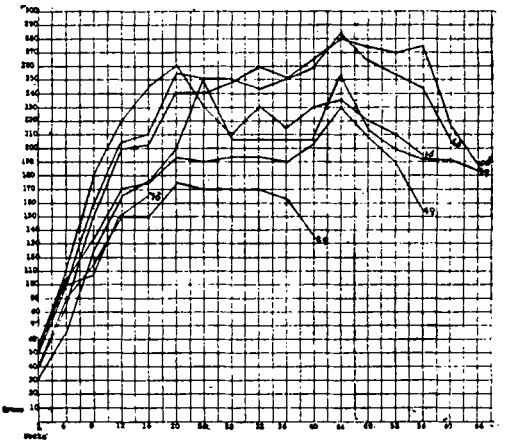

Chart 2. Experiment II.—Weight curve of individual rats given food containing 1% saccharin. Animals permitted to live until natural death occurred.

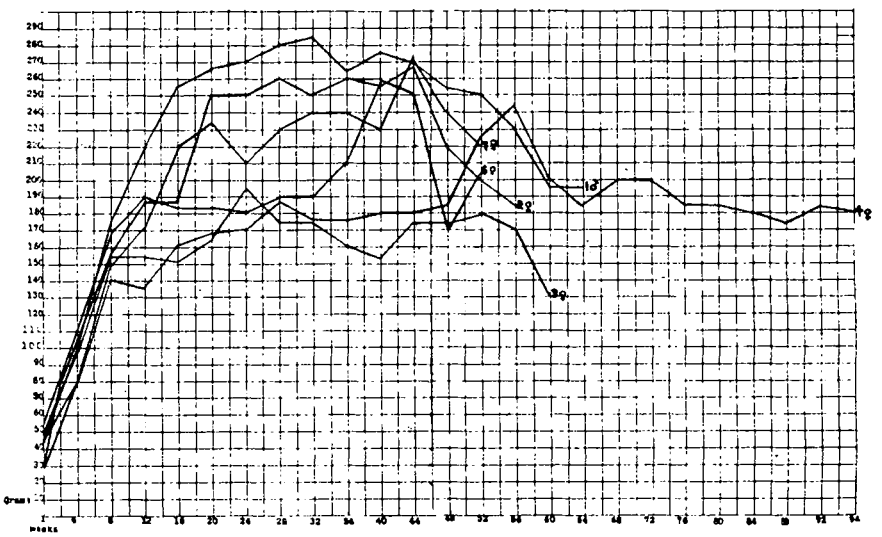

Chart 3. Experiment II.—Weight curve of individual rats given food containing 0.1% saccharin. Animals permitted to live until natural death occurred.

Group II A was fed with 1% saccharin added to the above mixture; Group II B was given 0.1% saccharin, Group II C served as control. The animals ate the saccharin food greedily and thrived as well as the control animals, as may be seen from the growth curves of the individual rats shown on Charts 2, 3, and 4.

TABLE 2.

The Necropsy Findings and Duration of Life of Rats in Experiment II.

Animal number.	Group II A. 1% saccharin. Necropsy findings.	Lived.	Group II B. 0.1% saccharin. Necropsy findings.	Lived.	Group II C. Controls. Necropsy findings.	Lived.
1	Hepatization of middle lobe of left lung	14 mo.	Chronic pneumonia	16 mo.	Infection of left middle ear	3 mo.
2	Chronic pneumonia	10 mo.	Chronic and acute pneumonia	14 mo.	Large abdominal growth, and pneumonia	16 mo.
3	Chronic pneumonia abscess of lung	16 mo.	Acute pneumonia	15 mo.	Pneumonia	14 mo.
4	Chronic and acute pneumonia	14 mo.	Negative was killed	26 mo.	Abscess in right post. fossa of skull	8 mo.
5	Pneumonia	15 mo.	Lost	...	Very extensive chronic pneumonia	11 mo.
6	Chronic pneumonia	16 mo.	Very extensive bilaterial broncho-pneumonia chronic	13 mo.	Pneumonia	17 mo.
7	Overloaded stomach, no other indications of disease	4 mo.	Old abscess of lung	13 mo.	Chronic pneumonia, abscess of lung	14 mo.
	Av.	$12^5/_7$ mo.	Av.	$16^5/_7$ mo.	Av.	$11^6/_7$ mo.

The necropsy findings and duration of life of these animals are shown in Table 2. It will be seen that the 0.1% saccharin group (II B) showed the longest average life period. This was due to one animal that lived for 26 months; and which, when finally killed, was found to be free from disease. Eliminating this animal from calculation, gives an average life period for Group II B, of $14^1/_5$ months. This is practically the same as the average life period of the animals of the control group, if we eliminate the two animals that died from infection within the skull, which greatly shortened life. It will then be seen that the average duration of the remaining animals that died of pulmonary infection was $14^2/_5$ months. In Group II A, fed on 1% saccharin, it is but fair to eliminate No. 7 which had an unusually short duration of life and died from some obscure cause. The average duration of life of the remaining animals is $14^1/_6$ months.

The conclusion is, therefore, forced upon us that saccharin, in the quantities used in this experiment, did not shorten the lives of those animals that ultimately succumbed to the pulmonary disease. The quantities of saccharin given were much in excess of those that would ever be likely to be consumed by man. Figuring on the relative weights, the quantity would approximate 3 Gm. per day for

a 60 Kg. person in the 0.1% saccharin experiment, and 30 Gm. per day in the 1% saccharin experiment.

EXPERIMENT III.

To study the question whether saccharin had any detrimental effect upon the progeny, groups of 4 of the young rats of saccharin-fed animals were continued on the same food as that given the parents, and compared with the young of control rats kept under similar conditions.

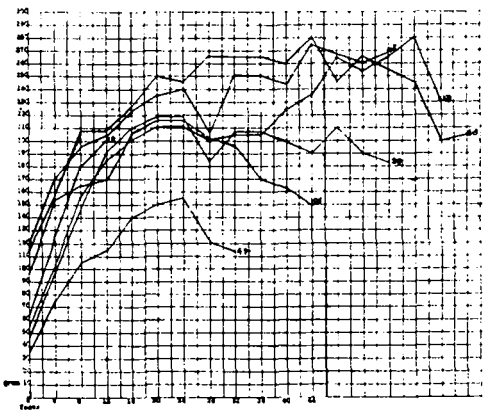

Chart 4. Experiment II.—Weight curve of individual rats serving as control. Animals permitted to live until natural death occurred.

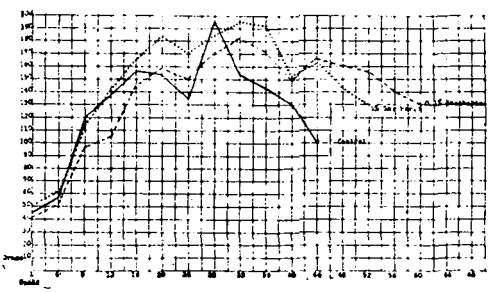

Chart 5. Experiment III.—Average weight curve of progeny of saccharin-fed rats continued on same food their parents received. Rats given 1% saccharin in food (dotted line); 0.1% saccharin (broken line); compared with that of control rats (continuous line). Animals permitted to live until natural death occurred.

Group III A, consisting of 4 young rats of one litter of a rat that had been fed 1% saccharin in its food for $7^1/_2$ months, was continued on this food for the whole life-time. The average weight of these rats (dotted line, Chart 5) became higher than that of the other animals and continued so until near the end when it dropped, obviously due to disease. All of these animals died of pneumonia. The average duration of life of these animals was 11 months (Table 3).

TABLE 3.

The Necropsy Findings and Duration of Life of Animals in Experiment III.

Animal number.	Group III A. 1% saccharin. Necropsy findings.	Lived.	Group III B. 0.1% saccharin. Necropsy findings.	Lived.	Group III C. Controls. Necropsy findings.	Lived.
1	Pneumonia	9 mo.	Pleurisy. Lung collapsed	5 mo.	Pneumonia	4 mo.
2	Pneumonia chronic-acute	11 mo.	Pneumonia	10 mo.	Necropsy lost	5 mo.
3	Pneumonia	11 mo.	Chronic pneumonia	14 mo.	Pneumonia	7 mo.
4	Extensive nodular pneumonia	13 mo.	Chronic pneumonia	15 mo.	Pneumonia	11 mo.
		Av. 11 mo.		Av. 11 mo.		Av. $6^3/_4$ mo.

Group III B was made up of four young of a rat fed 0.1% saccharin and of practically the same age as those of Group III A, having been born only a few days later. Their weight curve (broken line, Chart 5) continued a little lower than that of the animals that were fed ten times as much saccharin, except toward the end. Some of the animals lived longer; but the average duration of life was the same as that of Group III A.

In this experiment, the animals of the control group (III C) suffered more severely from pneumonia than did the saccharin-fed animals, as is shown by the progressive drop (continuous line, Chart 5) in weight commencing with the 28th week and by the fact that these animals lived an average of only $6^3/_4$ months as compared with an average of life of 11 months of the saccharin-fed animals.

DISCUSSION.

If Experiment I stood alone, the impression would be gained that the animals fed saccharin were less resistant to pneumonia than the controls; and that the diminution in resistance was in proportion to the amount of saccharin: though it must be admitted that the quantities used were so large as to render this experiment of no practical value as far as consumption of saccharin by human beings is concerned. Curiously enough, in Experiment III, the relations as to weight and duration of life are exactly the reverse. This probably shows that the incident and severity of the pneumonic involvement had nothing to do with the saccharin given; but was due to some other factor, such as possibly the location of the cages in relation to the window. It so happened that in Experiment I Cage A, the animals in which suffered most from pneumonia, was nearest to the window; while in Experiment III Cage C containing the controls, which suffered most from pneumonia, was nearest the window. This window was kept slightly open at all times, summer and winter.

While the gross figures of the average life span in Experiment II show quite a difference, decidedly unfavorable to the control animals, it would be unfair to conclude that saccharin lengthened the life-time of the animals that received it. Correcting the figures in the light of the necropsy findings gives a remarkably close correspondence of the average life period.

CONCLUSIONS.

Feeding saccharin to rats in even relatively enormous doses, and for a life-time of these animals, does not produce lesions appreciable either macroscopically or microscopically, nor does it interfere with the development of these animals or of their progeny, or shorten their span of life.

THE RESORCIN TEST FOR METHYL ALCOHOL.*

BY A. B. LYONS.

The tests most commonly used for the detection of methyl alcohol depend on the conversion into formaldehyde by oxidation of a portion at least of that compound, formaldehyde itself being easily recognized by characteristic reactions.

In case ethyl alcohol also is present the acetaldehyde which results from its oxidation may interfere with some of the distinctive tests for formaldehyde, as in the case of the very striking morphine test, where the purple color produced by formaldehyde is masked by the brown due to acetaldehyde.

The test which is the subject of this paper is not wholly free from this objection, yet fallacious conclusions from its indications are not liable to be reached. Although it has been somewhat neglected of late years, it is one of unique intrinsic interest.

* Scientific Section, A. Ph. A., Cleveland meeting, 1922.