Nutrition investigations on the carbohydrates of lichens, algae, and related substances / by Mary Davies Swartz.

Contributors

Rose, Mary Swartz, 1874-1941.
Augustus Long Health Sciences Library

Publication/Creation

New Haven, Conn. : Yale university press, 1911.

Persistent URL

https://wellcomecollection.org/works/z28hk5k4

License and attribution

Nutrition Investigations

on the

Carbohydrates

of

Lichens, Algae, and Related Substances

BY
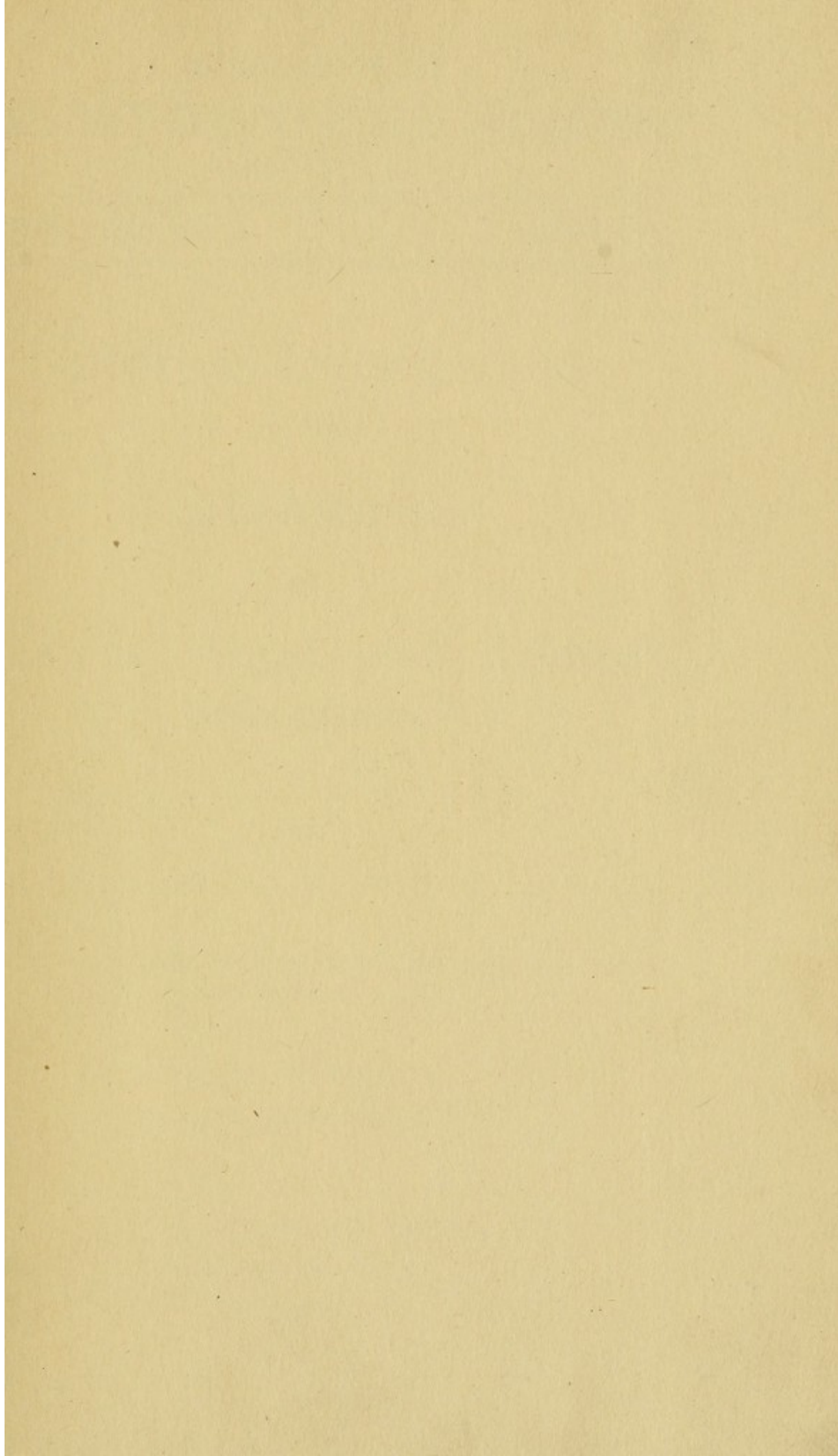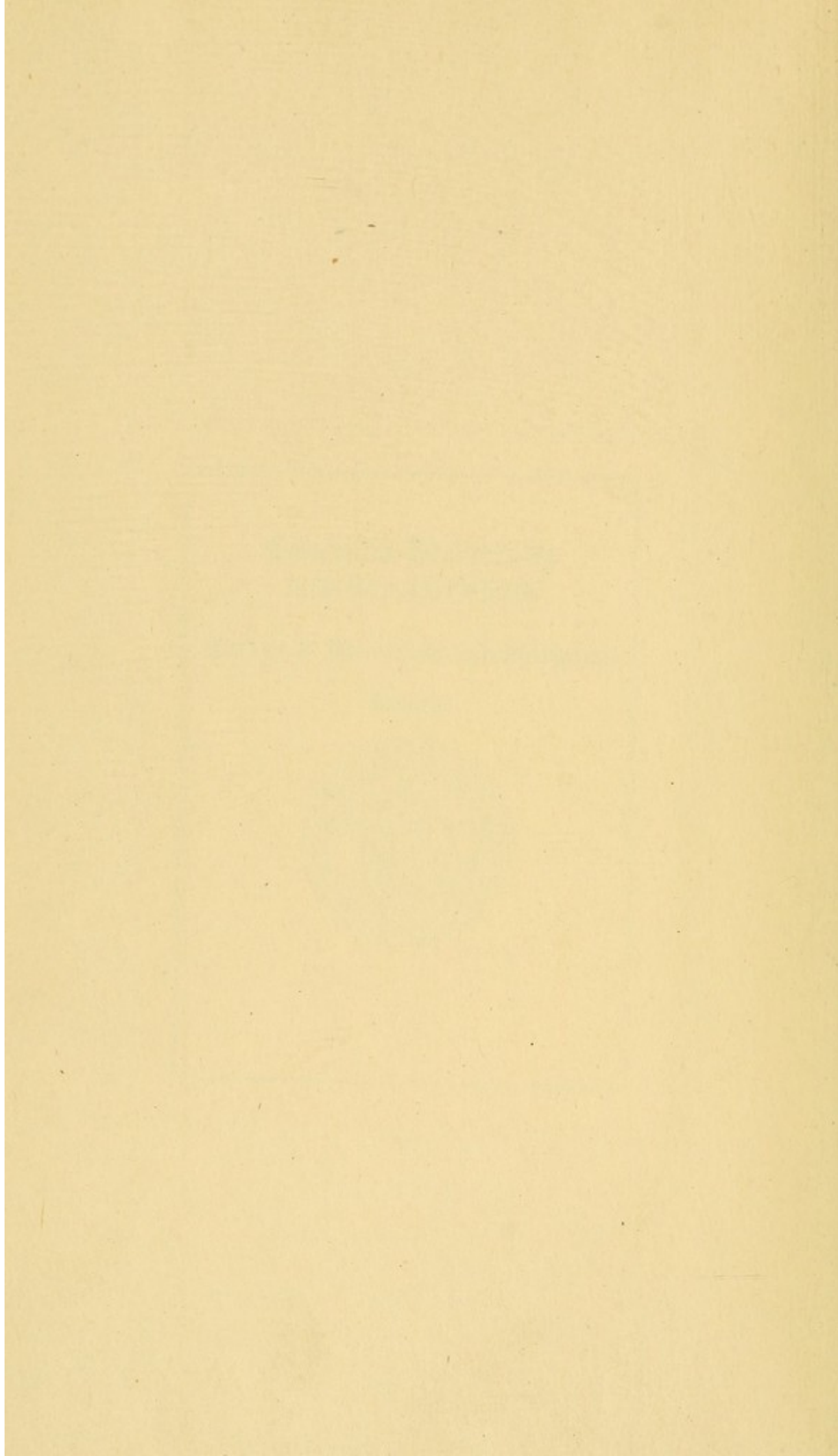
MARY DAVIES SWARTZ

FROM THE LABORATORY OF PHYSIOLOGICAL CHEMISTRY
SHEFFIELD SCIENTIFIC SCHOOL

CONTENTS.

I. INTRODUCTION.

II. HISTORICAL PART.

III. EXPERIMENTAL PART.

CHEMICAL INVESTIGATIONS.

BACTERIOLOGICAL INVESTIGATIONS.

PHYSIOLOGICAL INVESTIGATIONS.

IV. CONCLUSIONS.

V. BIBLIOGRAPHY.

I. INTRODUCTION.

LICHENS, ALGAE, TREE BARK AND CERTAIN TUBERS AS FOODSTUFFS.

From the earliest times, the food of man has included lichens and algae, and even the tender branches and inner bark of certain trees and shrubs, such as elm, birch, pine, and the staff-tree or bitter-sweet (*Celastrus scandens*). When the bark of trees is so used, it is freed from cork and the hard outer rind; is cleaned, dried, mixed with more or less meal, and made into "bark bread." Such substitutes for bread are commonly resorted to only in northern lands where there is scarcity of cereal crops, or in other regions during periods of famine. Johnson (7) records that elm bark is so employed in some continental countries, and Dillingham (4) relates that certain tribes of North American Indians, 'in times of extreme dearth, were accustomed to keep body and soul together by boiling and eating the bark of the staff-tree.' Poulsson (17) states that in Finland and northern Russia, sphagnum mosses are similarly employed; and Schneider (21) agrees with these other writers, saying that in general lichens are used as articles of diet only in cases of special need, principally because all lichens contain a bitter principle, which not only gives an unpleasant flavor and is difficult to remove, but also exerts an irritating effect upon the digestive tract, causing inflammation. Nevertheless, in the northern parts of the Scandinavian Peninsula, where cereal crops are always scanty or uncertain, great interest attaches to two species of lichen widely distributed through Europe, and through Arctic and Antarctic regions: namely, *Cetraria islandica* and *Cetraria nivalis*, which, as Poulsson (17) observes, 'have been considered nutritive and easily digestible since olden times.' *Cetraria islandica*, whitened and freed from its bitter principle by washing with dilute alkali, is a rather appetizing substance; it has sometimes been used as a foodstuff by Polar navigators, and Dr. Hansteen, chief lecturer in the Agricultural school at Aas, Norway, has gone so far as to prophesy that moss is destined to become the great popular food for the masses, because of its cheapness and nutritive properties.

Of marine algae, many tons are gathered and eaten annually in various parts of the world, the largest quantities being consumed

by the Japanese, Chinese, and Hawaiians. These algae are found in great variety and widely distributed. In Japan, the general name applied to them is "Nori," which is also given to several prepared products. According to H. M. Smith (23), the most important Japanese seaweed preparations are: "Kanten," or seaweed isinglass, made from various species of *Gelidium*, the principal one being *Gelidium corneum*, often adulterated with similar seaweeds; "Kombu" made from Kelps, especially numerous species of *Laminaria, Arthothamnus,* and *Alaria;* "Amanori," from species of *Porphyra;* and "Wakame," from *Undaria pinnatifida.*

Kanten is used largely for food, in the form of jellies, and as an adjuvant of soups and sauces. According to H. M. Smith (23), it is also employed in foreign countries 'in jellies, candies, pastries, and many desserts, in all of which it is superior to animal isinglass.' It has recently also attained popularity as a therapeutic agent in chronic constipation, being sold under various trade names, either plain or impregnated with laxative drugs, as cascara or phenolphthalein.[1] Kombu enters into the dietary of every Japanese family, being cooked with meat, soups, etc., and also served as a vegetable, or made into a relish with Soy-bean sauce. Amanori is eaten fresh or else is chopped and sun-dried in thin sheets, which are toastsd over a fire before eating. The crisp amanori is crushed between the hands and dropped into sauces or soups to impart flavor; or broken into pieces, dipped in sauce and eaten alone. Sheets of amanori, spread with boiled rice and covered with strips of meat or fish, are rolled and cut into transverse slices, and take the place of the American sandwich. Wakame is eaten as a salad, or cooked like amanori.

In Hawaii, edible algae are called "limu." Of these there are over seventy distinct species used for food, more than forty being in general use (18). Tons of limu are gathered for eating in Hawaii annually, and large quantities are also imported from the Orient and San Francisco. Some idea of the extent of their use may be gained from the following statement by Miss Reed (18): "Ancient Hawaiians probably seldom ate a meal without some kind of limu, and even today no Hawaiian feast is considered quite complete without several varieties served as a relish with meats or poi."[2] Since, with the exception of a few experiments reported by Oshima (15) and Saiki (20), there are no

[1] Cf. Galactans, p. 283.

[2] Poi is a thick paste made from the root of the taro plant, and takes the place of rice or bread in the native diet.

data upon the digestibility of marine algae, an investigation of some of these Hawaiian limu seemed highly desirable; and through the kindness of Miss Reed, a number have been obtained for this purpose. Their occurrence and uses will therefore be described in some detail.[1]

These limu are washed carefully after gathering, salted, and usually broken, pounded, or chopped into small pieces. They may then be eaten uncooked, as a relish with poi, meats or fish; boiled with meats; put into soups for thickening or flavoring; or roasted with pig in a pit. Served raw and crisp, they take much the same place in the diet as our salads. Among the most popular varieties are *Limu Eleele* (*Enteromorpha* of various species), *Limu Kohu* (*Asparagopsis sanfordiana*) and *Limu Lipoa* (*Haliseris pardalis*). Next in favor come *Limu Manauea* (*Gracilaria coronopifolia*), *Limu Huna* (*Hypnea nidifica*) and *Limu Akiaki* (*Ahnfeldtia concinna*). *Limu Pahapaha* (*Ulva fasciata* and *Ulva lactuca*) is widely distributed but not very popular. *Limu Uaualoli* (*Gymnogongrus vermicularis americana* and *Gymnogongrus disciplinalis*) is limited to certain islands, and hence not in such general use and favor as some of the others.

Limu eleele is a great favorite, forming a part of every native feast. It is generally eaten uncooked, sometimes being dropped into hot gravy, broth or meat stews just before serving. Limu kohu is always pounded in cleaning to free it from bits of coral and soaked 24 hours in fresh water to remove the bitter iodine flavor. It becomes slightly fermented and acquires a somewhat sour taste. Limu lipoa is popular on account of its penetrating spicy flavor, and is frequently used as a condiment, taking the place of sage and pepper in Hawaiian foods. Limu huna is especially prized for boiling with squid or octopus, though limu manauea and limu akiaki are often used as substitutes. These limus, as well as limu kohu, yield large amounts of mucilaginous extract on boiling, limu manauea being considered especially fine for thickening chicken broth.

Many of the seaweeds used in Hawaii and Japan occur also along the coasts of the United States and Europe, and are to some extent used as food in both regions. The very species of *Gelidium* from which the Japanese prepare their Kanten grow in abundance on our Pacific coast. Irish moss (*Chondrus crispus*), the "Tsunomata" of Japan, has long had considerable commercial value as a foodstuff in Ireland. In this country it is found from North Carolina to Maine, being especially abundant north of Cape Cod. After cleansing, cur-

---

[1]For fuller description see Reed (18).

ing, and bleaching it is to some extent used for making blanc mange
or a demulcent for coughs. Through the kindness of Dr. C. F. Lang-
worthy, Nutrition Expert, United States Department of Agriculture,
I have obtained the following interesting data concerning the use of
Irish moss, from the Journal of the South-Eastern Agricultural Col-
lege, Wye, Kent (1): "Professor D. Houston, of the Royal College of
Science, Dublin, has favored us with the following notes on this sub-
ject:

Chondrus crispus (carrageen, or Irish moss) is a seaweed plentifully distributed
along our northern, western and southern coasts. It is gathered and sold to local
chemists, who retail it, in some parts at all events, at 6d. per pound. It is used by
many people as an article of food in the west, and generally for colds, for which pur-
pose it is boiled in milk.

Several of my students tell me that it is used for feeding weak calves and with
striking results, bringing about an alteration of condition within four days. One
student tells me that in one case at his own farm a batch of twelve calves took a
kind of wasting disease, and nine died; the other three on the verge of death were
given this plant, and all three recovered. It is prepared by putting one pound of
the "weed" in a net bag and boiling in a gallon of water. The water on cooling
sets to a jelly. The calves are given one glass of jelly in their milk each meal
and wonderful results are said to be obtained."

The high proportion of mineral matter is noteworthy;[1] but without
making a fuller investigation, it is impossible to say precisely wherein
lies the value of this seaweed.

Purple laver (*Porphyra laciniata*), a source of Japanese amanori,
is found in abundance on the rocky shores of America and Europe
generally; but it is not used in this country save sparingly by the Chi-
nese, who usually import it directly from China, and by some of the
Indians of our northwest coast. In Ireland it is known as 'sloak,'
and is boiled and served with butter, pepper, and vinegar as an ac-
companiment of cold meats, or is served with leeks and onions.

Dulse (*Rhodymenia palmata*) is found abundantly on rocky shores
both in this country and in Ireland. It is very abundant in New
England, where it is rough-dried in the sun and eaten as a relish. In
Philadelphia it is called sea-kale and eaten as a vegetable. In Scot-
land it has long been used both in the fresh state and dried. In the
Scotch Highlands, "a dish of dulse boiled in milk is," it is said, "the
best of all vegetables." In Ireland, it is eaten with fish or boiled in
milk with rye flour. Purple dulse (*Iridea edulis*), which occurs on
the Pacific coast, is often eaten like Rhodymenia palmata.

---

[1]Cf. Analysis of *Chondrus crispus*, p. 254.

Besides such lichens and algae, and the bark of trees, various tubers are used as food for man. In Japan, the tubers of *Hydrosme rivieri* (Conophallus Konjaku) are extracted with lime water, and the resulting gelatinous mass is cut into small cakes. These, cooked with "shoyu" or Soy-bean sauce form a common article of diet. The tubers of many species of *Orchis* and *Eulophia*, native to Turkey, the Caucasus, Asia Minor and the greater part of Central and Southern Europe, furnish a food material known as *Salep*. The small ovoid, oblong or palmate tubers are decorticated, washed, heated till horny and semi-transparent, and finally dried. An abundant mucilaginous extract is obtained by macerating the bulbs in water. Frequently the tubers are ground to powder, and the powder used like sago or tapioca. Royal salep, said to be used as food in Afghanistan, is prepared from *Allium Macleanii*. A former instructor in the American College for Girls, in Constantinople, reports that salep is a very common article of diet in Turkey. It is sold in the markets in powdered form, and is made into a sort of sweetened gruel with milk. Not only is it used as a warm drink in the household, much as we use cocoa or chocolate, but it is also sold in the streets by venders, who either stand in booths along the way, or go about carrying huge brass urns strapped to their shoulders, clinking their cups and calling "Tazé-Sahlep!"[1] It is especially popular in districts of the city where people work late at night. In the month of Ramazon, the time of all-day fasting, hot salep finds a ready sale at night. It is no uncommon thing to see the workman standing with his salep cup in hand, waiting for the firing of the sunset cannon.

In spite of the fact that there have been almost no scientific investigations as to the digestibility of such mucilaginous plant substances there seems to be a special virtue attached to mucilages in the popular mind. The prevailing impression is shown in some of the following remarkable statements. The United States Dispensatory, 1908, not only says that the mucilaginous extract of slippery elm bark (*Ulmus fulva, Michaux*) is nutritious, but adds, "We are told that it has proved sufficient for the support of life in the absence of other food." Of salep Smith (25) says in his dictionary of economic plants: "It contains a chemical substance called bassorin, which is said to contain more nutritious matter than any other vegetable product, *one ounce per diem* being sufficient to sustain a man"! The United States Dispensatory also assures us that salep is "highly nutritious." Johnson

---

[1] Fresh salep.

(7) particularly recommends Iceland moss (*Cetraria islandica*) as a diet for consumptives, as "it seems to be both extremely nutritious and very easy of digestion, though of course, only capable of use as a substitute for starchy matters." In regard to Irish moss (*Chondrus crispus*), he is a little more uncertain. "It is much used for invalids, especially in cases of consumption, but with doubtful advantage when substituted for more nutritious food." Schneider (21) says of Iceland moss: "Inhabitants of Iceland, Norway, and Sweden mixed this lichen with various cereals and mashed potatoes, from which an *uncommonly healthful* bread was prepared." Until the matter has been thoroughly investigated, we must suspend our judgment as to the accuracy of such statements. After a few metabolism experiments, Oshima (15) far more conservatively remarks concerning the algae of Japan: "Their actual value doubtless depends in considerable measure upon the mineral salts they contain."

In view of the scarcity of any scientific investigations as to the behavior of all these substances in the body, further experiments upon their nature and digestibility seem highly desirable, since they are not only widely distributed, and already form a considerable portion of the diet of many persons; but because, if they possess any real nutritive value, a wider use of such comparatively cheap materials would be an economic advantage; and because, under the prevailing notions as to their food value, they are sometimes relied upon as a source of nutriment in diseases (as diabetes) where the character of the diet is particularly important. The present work has been undertaken to throw some light on this interesting subject. A survey of the literature shows that even the chemical nature of many of these algae has scarcely been investigated; and if this were known, we should still be under the necessity of studying their behavior in the animal body, for it is impossible to tell from chemical analysis alone whether a given substance will or will not prove digestible, as Rubner has long since warned us.

II. HISTORICAL PART.

INTRODUCTION.

According to the current practice of agricultural analysts, the carbohydrates of plants are reported as crude fiber and nitrogen-free extract. Crude fiber is the term applied to the resistant mixture forming the mature cell wall, shown as long ago as 1864 by Henneberg and Stohman (41) to have no definite chemical composition. It is therefore not identical with cellulose, but consists of a mixture of cellulose with incrusting substances, lignin and cutin, the relative proportions of which have recently been exhaustively studied by König (51), Fürstenberg (39), and Murdfield (63). Cellulose is the chief constituent; the other two are usually present in varying proportions.

Schulze (74) to whom much of our knowledge of the composition of the plant cell wall is due, has classified the carbohydrates of the nitrogen-free extract as follows:

I. Water-soluble carbohydrates. To this class belong the mono-, di-, and tri-saccharides, and some soluble polysaccharides.

II. Carbohydrates insoluble in water, but yielding sugar under the action of diastase. The chief member of this group is starch.

III. Carbohydrates insoluble in water and resistant to the action of diastase, never being changed by it into sugar. This group is called the Hemicelluloses.

The term hemicellulose, as used by recent writers[1] seems to be interpreted to include some polysaccharides of the first group. It is therefore used here as a group name for those carbohydrates which are distinguished from cellulose by being capable of hydrolysis on boiling with dilute mineral acids, and from the other polysaccharide carbohydrates by not being readily digested by diastase. According to the kind of sugar yielded on hydrolysis, the hemicelluloses are designated as Pentosans or Hexosans, the latter including Galactans, Mannans, Dextrans, Levulans, etc. After a general review of the chemical

---

[1] e.g., Lohrisch.

nature of lichens and algae, each of these classes will be discussed separately in detail.

The percentage composition of some common species of algae is shown in the following table:

FOOD MATERIAL.	WATER.	PROTEIN.	FAT.	CARBOHYDRATES.		ASH.
				Nitrogen-free Extract.	Crude	
I.* Cystophyllum fusiform, dried...............	15.74	11.37	.49	54.84		17.56
Ecklonia bicyclis, dried..	18.75	9.58	.46	51.63	9.79	9.79
Enteromorpha linza, dried (Limu eleele)...	13.53	19.35	1.73	46.18		19.21
Laminaria sp., dried....	23.08	7.11	.87	47.70		21.24
Porphyra laciniata, dried...............	13.98	33.75	1.30	41.22		9.75
Ulopteryx pinnatifida, dried...............	18.92	11.61	.31	37.81		31.35
II.† Ahnfeldtia concinna, fresh (Limu akiaki)...	80.00	1.4	0.0	14.4		4.2
Ulva fasciata and U. lactuca, fresh (Limu pahapaha)	80.00	3.7	0.0	12.5		3.8
Gracilaria coronopifolia, fresh (Limu manauea)	80.00	1.8	0.0	14.1		4.1
III.‡ Chondrus Crispus, dried.	13.40	13.06	2.59	54.16	2.57	14.22‖
IV.§ Cetraria islandica......		0.32	1.2	43.3	5.3	2.2

* Oshima (15).

† Reed (18), (calculated on uniform water basis).

‡ Annet (1).

§ Schmidt (24), first studied the ash and reported a notable amount of calcium and potassium phosphates. He found no nitrogen. Blondeau (3) reported 21.36 per cent nitrogen.

‖ Brown (334).

Until 1905 the chemical nature of the constituents of algae had received little attention. Analyses of many species of algae from Japan and China were reported recently by König and Bettels (8), the results of which are given in the following table on page 255.

According to Oshima and Tollens (16) the carbohydrates of *Porphyra laciniata* consist largely of anhydrides of *d*-mannose and *i*-galactose. Müther and Tollens (13) studying various species of Fucus (*F. vesiculosus, F. nododus, F. serratus*), Laminaria, and Chondrus crispus, found a methyl-pentosan (*fucosan*), in Fucus and Laminaria; and glucose, fructose, galactose and pentose groups in Chondrus Krefting reports a reserve carbohydrate in *Laminaria digitata* in win-

NO.	SPECIES OF ALGAE.	ANHYDRIDES OF HEXOSES.			ANHYDRIDES OF PENTOSES.	
1	Porphyra	Galactose	Glucose	Fructose		Pentoses
2	Porphyra tenera	Galactose	Glucose	Fructose		Pentoses
3	Gelidium Raw	Galactose		Fructose		Pentoses
4	Gelidium Bleached	d-Galactose		Fructose		Pentoses
5	Celidium cartilagineum	d-Galactose		Fructose	Methyl pentoses	Pentoses
6	Laminaria japonica		Glucose	Fructose		Pentoses
7	Other Laminaria		Glucose	Fructose		Pentoses
8	Cystophyllum			Fructose	Methyl pentoses	Pentoses
9	Cystophyllum fusiforme			Fructose	Methyl pentoses	Pentoses
10	Enteromorpha compressa		Glucose	Fructose	Rhamnose	Pentoses
11	Ecklonia bicyclis		Glucose			Pentoses
12	Undaria finnatifida	d-Galactose	Glucose	Fructose	Methyl pentoses	Pentoses

ter only, which yields *d*-glucose. The algae investigated are thus all seen to yield pentoses, very frequently fructose and methyl-pentose, sometimes glucose and galactose.

Lichens are symbiotic forms embracing algae and fungi. Because of this symbiotic nature, they exhibit great variety in composition. From the investigations of Escombe (6), Ulander and Tollens (27), Karl Müller (11), Nilson (14), Wisselingh (29) and others,[1] it appears that the cell walls are usually of cellulose, but occasionally of chitin.[2] Many species yield on extraction with hot water a gelatinizing substance, which Berzelius (2) in 1808 named "Flechtenstärke" (*lichenin*), but which later investigators[3] have shown to be, not a single substance, but a number of related carbohydrates yielding dextrose, such as *lichenin* from *Cetraria* and *Ramalina fraxinea*, and *evernin* from *Evernia prunastre, usnin* from *Usnea barbata*. Other species, on the contrary yield little dextran, but mannan, galactan, pentosan and methyl-pentosan in varying proportions. The table on page 257 showing the hemi-celluloses occurring in a number of lichens, has been compiled from data given by Karl Müller (11) and Ulander and Tollens (27).

OCCURRENCE AND NATURE OF CELLULOSE.

Cellulose is said to occur in pure form in the wall of the young plant cell. With increasing age, modifications take place by which the true cellulose becomes more and more encrusted with lignin and cutin, two substances shown by König (52), Fürstenberg (39), and Murdfield (63) to be almost entirely indigestible. According to Wielen (87) and Hofmeister (43), even pure cellulose is not a simple substance, but can be separated into soluble and insoluble portions.[4] Much of our information regarding the nature of cellulose is due to the work of Schulze and his pupils. Schulze (75) has defined cellulose as that part of the cell wall giving the typical cellulose reactions,[5] and yielding dextrose on hydrolysis with concentrated sulphuric acid.

---

[1]For early literature see Czapek, Biochemie der Pflanzen, Vol. I, pp. 514–516.

[2]Chitin occurs in *Peltigera canina* and *Evernia prunastre*.

[3]Cf. Müller (11) and Ulander (26).

[4]According to its behavior in sodium hydroxide solutions, the quantitative relations depending upon the source of the cellulose and the concentration of the solution.

[5]Insolubility in dilute acids and alkalies; solubility in ammoniacal copper oxide solutions; and production of a blue color with iodine and sulphuric acid.

NO.	SPECIES OF CRYPTOGAM.	ANHYDRIDES OF HEXOSES.			ANHYDRIDES OF PENTOSES.	
1	Cladophera glomerula	Glucose			Xylose	
2	Cladonia rangiferina	Glucose	Galactose	Mannose	Pentoses	Methyl pentoses
3	Cetraria islandica	Glucose	Galactose		Pentoses	Methyl pentoses
4	Evernia prunastre	Glucose	Galactose		Pentoses	Methyl pentoses
5	Leiscyphus taylori (Hook)				Xylose, Arabinose	Methyl pentoses
6	Mastigobryum trilobatum				Xylose, Arabinose	Methyl pentoses
7	Sphagnum trilobatum				Xylose, Arabinose	Methyl pentoses
8	Polytrichum commune				Xylose	Methyl pentoses
9	Stereocaulon pascale L.		*d*-Galactose	*d*-Mannose	Pentoses	Methyl pentoses
10	Peltigera aphtosa		*d*-Galactose	*d*-Mannose	Pentoses	Methyl pentoses
11	Usnea barbata	Glucose	*d*-Galactose	*d*-Mannose	Pentoses	Methyl pentoses
12	Cornicularia aculeata		*d*-Galactose	*d*-Mannose	Pentoses	Methyl pentoses

CYTASES IN THE VEGETABLE KINGDOM.

By the early investigators, Haubner (40), Henneberg and Stohman (41), Kühn, Aronstein, and Schulze (54), it was accepted without much question that, since cellulose disappeared from the alimentary tract of herbivora, it is digested like starch, and equally valuable as a nutrient. But after Tappeiner (78), in 1884, showed that cellulose could be decomposed by micro-organisms, and promulgated his theory that this was the only way to account for the disappearance of cellulose from the alimentary canal of ruminants, the matter fell into great dispute,[1] and the question is not yet definitely settled as to how cellulose is digested and what are the products of its digestion. A diligent search has been made for enzymes capable of attacking it (*cytases*), but so far, such cytases have been proved to exist only in plants and lower animals. Many of these so-called cytases act upon hemicellulose rather than true cellulose, and will be discussed in connection with the hemicelluloses, though it is not always possible to make a sharp distinction between the two. A careful review of the subject of cytases in plant physiology up to 1898, has been made by Biedermann and Moritz (34), from which it appears that the penetration of wood by the mycelia of moulds is due to such cytases, and that a powerful cellulose-dissolving enzyme has been derived from *Peziza sclerotium* by de Bary (37) and from another botrytis (presumably a *Peziza*) by Ward (84), while Brown and Morris (36) have described cytases existent in germinating grasses which dissolve their cell walls. That this is anything more than a diastatic enzyme is denied by Reinitzer (67); but Newcombe (64) considers the assumption of the identity of all cell-wall dissolving enzymes with diastase as far from justifiable. Bergmann (32) reports such cytases in hay and straw. Scheunert and Grimmer (71), on the contrary, find none in oats, corn, horse-beans, lupine seeds, buckwheat or vetch. Thus we see that even in the case of plants, these enzymes need to be isolated and identified before we can arrive at any satisfactory conclusions.

That cellulose can be dissolved by bacteria has been demonstrated for such forms as *Amylobacter butyricus*, *Vibrio regula* and *Clostridium polymyxa* (34). Omelianski (65) has described two organisms which ferment cellulose, and Ankersmit (31) finding Omelianski's bacteria on hay, has studied their behavior when introduced into the alimentary canal of the cow on its food. He finds that they do not increase

---

[1] For a review of this discussion cf. Lohrisch (56).

in number during their passage through the digestive tract, and therefore concludes that they play a very inconsiderable rôle in the decomposition of cellulose. According to Van Iterson (81), certain aerobic bacteria, attacking cellulose, form from it products which nourish other forms (*spirilla*); certain anaerobes are also shown to attack it. Eberlein (38), finding in the first stomach of herbivora Infusoria which utilize cellulose for food, suggests that these protozoa, digested farther along in the alimentary tract, serve as means of transformation of cellulose into products which the animal can digest; but there is nothing to indicate that such forms occur in sufficient numbers to be worthy of much consideration.

Since 1906 three investigators have given the problem careful attention. Scheunert (68) has concluded from experiments *in vitro* that bacteria play an exclusive rôle in the solution of crude fiber in the coecal contents of horses, swine, and rabbits. He found that filtered coecal fluid acted on cellulose much less than unfiltered or simply strained coecal contents. This is contrary to the opinion of Hofmeister (45) and Holdefleiss (48), who attribute the phenomenon to the action of enzymes, and explain the loss of power occasioned by filtering as due to the effect of exposure to the air upon the enzymes. Lohrisch (57) has reported that fresh coecal fluid is effective in destroying cellulose while heated fluid is not. On the other hand, implanting the sterilized fluid with coecal bacteria and protozoa would not restore its activity. Coecal fluid kept at 38° C. any length of time gradually lost its cellulose-dissolving power, while that kept on ice remained active. v. Hoesslin and Lesser (47) have attempted to explain these apparent contradictions, and conclude from their own experiments that anaerobic bacteria are the most effective agents in cellulose decomposition in the intestine. Equal volumes of non-sterilized and sterilized coecal fluid of the horse, to which weighed amounts of cellulose had been added, were suspended in sterile physiological salt solution under practically anaerobic conditions and digested for periods of from 9 to 35 days. The disappearance of cellulose with the non-sterilized coecal fluid amounted to from 55.7 per cent to 71.2 per cent; with sterilized fluid, to from 6.2 per cent to 42.4 per cent. It was also found that the addition of 1–5 grams of dextrose would effectively protect the cellulose from digestion by the non-sterilized fluid, the bacteria preferring the more easily attacked carbohydrate. The gases evolved in these fermentations were characteristic of bacterial action, being chiefly methane, carbon dioxide, and hydrogen. The retarding effect of exposure to the air is explained by the theory that anaerobes are

the effective agents.   So, also, the fact that Lohrisch was unable to get cellulose digestion in sterilized fluid again inoculated with unsterilized fluid is attributed to the medium's being an unfavorable one for the development of these organisms, inasmuch as the addition of peptones to similar preparations caused in several cases an increased decomposition.   It seems fairly well established, therefore, that the action of the coecal fluid of the horse is due to enzymes of bacterial origin.

CYTASES IN LOWER ANIMALS.

There is no doubt that cytases occur in some of the lower forms of animal life.   Biedermann and Moritz (34) found a powerful cellulase in the secretion of the liver of the common snail (*Helix pomatia*), and their observation was verified by E. Müller (61), also by Lohrisch (57) who reports two series of experiments in which snails fed tender lettuce leaves digested from 40.1 per cent to 81.6 per cent of the cellulose present.   On the other hand, Müller (61) could not verify Knauthe's report of a cellulase in the hepato-pancreas of the carp (50); Pacault found none in the saliva of Helix pomatia (66); and Biedermann none in the digestive juice of the meal worm (*Tenebrio molitor*) or of the cabbage worm (*Pieris brassica*) (34).   Biedermann also examined the faeces of the cabbage worm microscopically and found unaltered particles of leaves, from which he concluded that much of the plant food eaten is excreted unchanged.   Lohrisch (56) has obtained similar results with caterpillars of sphinx moths (*Sphinx euphorbiae*), not only in experiments with intestinal juice *in vitro*, but also in feeding experiments in which the cellulose was quantitively excreted.

Sellière (75–76) has recently added some interesting contributions to this subject, showing that cotton treated in various ways; namely, that recovered after solution in Schweitzer's reagent, that treated with concentrated zinc chloride, or with 25 per cent caustic alkali hot or cold until the fibers are swollen, and subsequently washed with 1 per cent acetic acid and water, is attacked by *Helix pomatia* much more readily than the untreated substance.   Subsequent drying of the treated cotton diminished its digestibility somewhat, suggesting that the physical condition of the cellulose is a definite factor in its utilization.   Sellière believes that only the more tender portions of plant cellulose are attacked by the digestive juice of this snail.   It would seem that the previous treatment of the cellulose is a factor to be kept in mind in the interpretation of the results of feeding experiments.[1]

---

[1] Cf. the experiments on cellulose utilization in the dog, p. 263.

CYTASES IN HIGHER ANIMALS.

There is at present no proof of the existence of cytases in any of the higher animals. The literature on the subject has been exhaustively reviewed by Bergmann (32), and Lohrisch (55, 56, 57) and it appears that there is no cellulase in the saliva or pancreatic juice of swine, horses, cattle, or sheep. The old observation by MacGillawry[1] (cited by Biedermann and Moritz(34) that a cytase can be extracted from the vermiform appendix of the rabbit has been denied by Zuntz and Degtiareff (88). Schmulewitsch's[2] statements (also cited by Biedermann and Moritz) are worthless because he employed no antiseptics. E. Müller (61) found no sugar formed from the decomposition of cellulose in the stomach of the goat, and Lusk (59) observed no increase in sugar elimination after feeding a phlorhizinized dog 20 grams of cauliflower, or a phlorhizinized goat 10 grams of paper. Lohrisch (57) fed pure cellulose (5–20 grams) to a phlorhizinized rabbit and found that it had no marked influence on the sugar output, and no nitrogen-sparing effect. Scheunert (70) has made further investigation on the action of the saliva and salivary glands in sheep, and confirms the earlier experiments with the saliva of this animal. On the other hand, Sellière (77) reports that the specially treated cellulose mentioned above is converted into dextrose by the intestinal secretions of the guinea pig in some instances.

Practically nothing is known concerning the way in which cellulose disappears from the alimentary tract of man. Schmidt and Lohrisch (73) fed pure cellulose to diabetics and observed a disappearance averaging 77.7 per cent, and no increase in the elimination of sugar. They believe that most of it is absorbed in soluble form and not destroyed by fermentation in the intestines. Lohrisch, having fed cellulose in various diseases of the alimentary tract,[3] calls attention to the fact that in constipation, where there is the least bacterial action, the utilization of cellulose is highest, while in fermentation dyspepsia, in which one might expect a marked disappearance, the utilization is lowest. He therefore considers the digestion of cellulose as due at least in part to enzymes.

---

[1]Archiv Neerland, Vol. XI.

[2]Über das Verhalten der Verdauungssäfte zur Rohfaser der Nahrungsmittel. Bulletin de l'Academie Imperial de St. Petersburg, 1879.

[3]See results, p. 264.

DIGESTION AND UTILIZATION OF CELLULOSE BY ANIMALS.

The literature on the digestion of cellulose up to 1909 has been so exhaustively reviewed by Lohrisch that it is unnecessary to enter into a detailed discussion of it. From tables (55) showing the results of all previous experiments on the utilization of crude fiber in herbivora, carnivora, and birds, it appears that in the case of herbivora, especially ruminants, 20–28 per cent of the crude fiber ingested with food disappears from the alimentary canal; that in case of carnivora[1] and birds[2] there is no utilization whatever. Lohrisch (56) himself reported three experiments in which dogs were fed pure cellulose and digested 31.1 per cent, 37.45 per cent and 5.4 per cent respectively, but Scheunert and Lötsch (72) repeating Lohrisch's work with a somewhat different method of determining cellulose found that the administration of 40 grams of prepared white cabbage, containing 7.37 grams of pure cellulose, resulted in the recovery of the total amount ingested. Cooking the cabbage in bouillon did not increase its digestibility. They attribute the apparent utilization in the preceding experiment to destruction of cellulose by the reagents used for its purification. Since the publication of their paper, Lohrisch has repeated his work with the dog (57), and reports complete recovery of the cellulose fed. He explains the error in the earlier investigation as due to the fact that the ingested cellulose was twice subjected to purification (before feeding and in faeces) with consequent increase in percentage of loss, which was not taken into account. He points out the inevitable loss of some cellulose by any method at present in use for its determination, and defends his own as sufficiently accurate for all practical purposes if conditions are carefully observed.[3]

---

[1]The only experiments on record are by Voit and Hoffmann on the dog and by von Knieriem on the hen.

[2]Experiments by Weiske on the goose, and by von Knieriem on the hen.

[3]Lohrisch used the method of Simon and Lohrisch, in which the cellulose is dissolved by heating for an hour on a water bath with 50 per cent potassium hydroxide, then adding $\frac{3}{4}$ cc. of 30 per cent hydrogen peroxide, and digesting from $\frac{1}{2}$ to $\frac{3}{4}$ hour longer if necessary. The cellulose is then precipitated by adding to the solution one half its volume of 96 per cent alcohol and 6–7 cc. of concentrated acetic acid; filtered off, washed with water, dilute acetic acid, alcohol and ether, dried and weighed.

Scheunert and Lötsch mix the substance to be analyzed with 100 cc. of cold water, add 100 grams of potassium hydroxide and heat for an hour on a water bath, then filter through a hard filter paper, wash the residue on the paper with boiling water till only a trace of alkali remains, transfer it to a beaker and thence to a weighed

Cellulose digestion in the dog has been almost simultaneously studied by v. Hoesslin (46). Two dogs on a meat-fat diet to which was added daily 2 grams of specially prepared white cabbage (containing 63.25 per cent of pure cellulose), for five periods of five days each, excreted on the average 99.7 per cent and 94.5 per cent respectively. This long experiment is significant as showing no adaptation of the digestive glands to the type of food. By these independent workers it seems now well established that the dog is unable to utilize cellulose.

Hoffmann (42) has just published the results of some investigations on the influence of cellulose on the nitrogen balance and on phlorhizin-diabetes in the rabbit, from which it appears that after ingestion there is no increase of sugar excretion, and no glycogen formation, yet he thinks that cellulose and hemicelluloses have a favorable influence in phlorhizin-diabetes.[1] It seems to follow from this, that even in case of herbivora cellulose is not utilized in the manner customary for starch and sugar.

DIGESTION AND UTILIZATION OF CELLULOSE BY MAN.

A similar tabulation of results of feeding experiments on man, shows that cellulose is not so well utilized as by herbivora, but does disappear in appreciable amounts. With one exception, the cellulose in all these experiments was administered as crude fiber. Hofmeister (43) fed pure cellulose and reported 75.7 per cent soluble cellulose and 5.6 per cent insoluble cellulose digested. König and Reinhardt (53) added to a diet rich in protein and fat, but free from cellulose, in several experiments, green peas and ripe shelled peas, red cabbage, white

---

filter, on which it is washed successively with hot water, dilute acetic acid, hot water, alcohol and ether, and finally weighed.

Scheunert and Lötsch claim that by Lohrisch's method the cellulose is altered in character, and as much as 40 per cent lost in the process; and that subsequent treatment of the recovered material causes an even greater per cent of loss, while by their method the loss in the first case is not over 6.8 per cent, and that in the second case even less.

For the details of this controversy over method see the following: Simon and Lohrisch; Zeitschrift für physiologische Chemie, Vol. 42, p. 55, (1904). Scheunert; Berliner tierärztliche Wochenschrift, No. 47, p. 826, (1909). Scheunert and Lötsch; *Ibid.*, p. 867, (1909); also Biochemische Zeitschrift, Vol. 20, p. 10, (1909); and Zeitschrift für physiologische Chemie, Vol. 65, p. 219, (1910). Scheunert and Grimmer; Berliner tierärztliche Wochenschrift, No. 48, p. 152, (1910). Lohrisch; Zeitschrift für physiologische Chemie, Vol. 69, p. 143, (1910).

[1] Unfortunately the original paper was not accessible.

beans, graham and soldiers' bread and found 30.27 per cent to 76.79 per cent of the added cellulose digested. Lohrisch (55) finds that the cellulose of a common vegetable diet disappears from the alimentary tract in large amounts, the actual quantity varying with the age, source and tenderness of the cellulose. Thus he finds that for normal individuals, of cellulose from lentils, 45 per cent is digestible; from kohlrabi, 79.1 per cent; from white cabbage, 100 per cent. Under abnormal conditions in the digestive tract, he has obtained the following results:

CONDITION.	CELLULOSE UTILIZATION IN PER CENT.
Normal...	57.9
Chronic Constipation............................	81.4
Fermentation Dyspepsia..........................	37.8
Gastrogenic Diarrhea............................	29.5
Fatty Faeces in Icterus.........................	27.8
Fatty Faeces in Disease of Pancreas.............	20.9

According to Lohrisch, two diabetics on a cellulose-free diet, to which white cabbage was added in quantities to yield about 6 per cent of cellulose per day, digested 68.6 per cent and 84.5 per cent respectively, without increased output of sugar in the urine.

Since the only way to determine definitely the energy value to the organism of such amounts of cellulose as are absorbed, is by means of respiration experiments, Lohrisch (57) has performed such an experiment on man, using the Zuntz-Geppert apparatus. In fasting, the respiratory quotient averages about 0.76. After ingestion of carbohydrates such as starch, it rises gradually in two to three hours, to 0.9–1.0, and when the carbohydrate has been consumed, sinks again to a lower level. Since the respiratory quotient for fat is 0.7 and for protein about 0.8, it is possible to determine in this way to what extent the carbohydrate replaces protein and fat in metabolism. Hence if cellulose is absorbed and oxidized as a carbohydrate, the respiratory quotient should rise. If it is decomposed by bacteria, the respiratory quotient should not rise, since the theoretical respiratory quotient for fatty acids, such as butyric and acetic, is, according to Munk (62) and Mallèvre (60), 0.6 and 0.5 respectively. Now Lohrisch, feeding a man moist cellulose equivalent to 73.6 grams of dry substance, of which 25 per cent was digested (18.5 grams) obtained the following results:

NUMBER OF EXPERIMENT.	BEGINNING OF EXPERIMENT. Hour.	DURATION OF EXPERIMENT IN MINUTES.	VOLUME INHALED PER MINUTE. ccm.	CO₂ CONSUMPTION. Per cent.	CO₂ PRODUCTION. Per cent.	R. Q.	O₂ CONSUMPTION PER MINUTE. ccm.	CO₂ PRODUCTION PER MINUTE. ccm.	O₂ CONSUMPTION. Per cent.	CO₂ PRODUCTION. Per cent.	AFTER BEGINNING OF CELLULOSE INGESTION. Hours.
1	6.43	24	4695	4.14	3.33	0.804	194.37	156.34			
2	7.25	27	3981	4.77	3.68	0.772	189.89	146.50			
Av.						0.788	192.13	151.42			
3	10.11	26	4237	4.59	3.37	0.734	194.47	142.79	+1	−5½	2
4	11.27	27	4230	4.43	3.40	0.792	187.38	143.82	−2½	−5	4
5	12.36	24	4649	4.36	3.23	0.740	202.69	150.16	+5½	−1	5
6	1.30	24	4768	4.27	3.10	0.726	203.49	147.81	+6	−2½	6
7	2.58	25	4390	4.63	3.22	0.700	203.26	141.36	+6	−6½	7
8	4.40	22	5041	4.44	3.10	0.700	223.82	157.78	+16½	+4	9
9	6.22	23	4859	4.35	3.01	0.692	211.37	146.26	+10	−3½	11
10	7.53	21	5290	4.15	3.01	0.725	219.53	159.23	+4	+5	12

The respiratory quotient attains its highest value in the fourth hour, instead of the second or third, showing that cellulose is absorbed more slowly than starch. The rise is too slight to indicate that cellulose exercises any considerable protein- or fat-sparing effect. It is unfortunate that the amount of cellulose absorbed was so small. It is striking that the O_2-consumption decreases at the very time that the respiratory quotient rises, and the CO_2-production scarcely increases. Lohrisch interprets this as indicating that the increased O_2-consumption required for oxidation of the cellulose is compensated by a sparing of protein and fat. The differences seem too small to draw any satisfactory conclusions as to the energy value of cellulose. The low respiratory quotient in the later hours of the experiment, together with the increased O_2-consumption, indicates the utilization of some of the cellulose in the form of fatty acids. We must bear in mind that no formation of sugar or glycogen from cellulose, in men or animals, has been demonstrated. Further investigations would seem to be necessary before we can agree with Lohrisch in saying, "*Wir wissen, dass Cellulose und Hemi-cellulosen vom Menschen reichlich verdaut werden, wir haben allen Grund anzunehmen, dass ihre Verdauung nach Analogie der Stärke abläuft . . . Die resorbirten Mengen werden im menschlichen Organismus vollständig verbrannt. Dabei wird Eiweiss und Fett von der Verbrennung geschützt.*" In any event, the quantities of cellulose which the alimentary tract of man is capable of absorbing are, apparently, too small for it to play a rôle of any importance in the diet of a normal individual.

OCCURRENCE AND NATURE OF PENTOSANS.

The anhydrides of the 5-carbon sugars are collectively designated as pentosans. These are not reported to occur in the animal kingdom, but the pentose sugars are found forming a part of the nucleic acid radical of the nucleo-protein molecule. In the vegetable kingdom, pentosans are very widely distributed, as has been shown by many investigators, especially Tollens and his pupils.[1] They occur in all kinds of plants, from the lowest to the highest, and are limited to no

[1] Tollens, Landw. Vers., V. 39, p. 401, (1891); Tollens, Jour. f. Landw., Vol. 44, p. 171 (1896).

For an exhaustive review of the literature on the occurrence of the pentosans see v. Lippmann, Chemie der Zuckerarten, 3rd Edition, Vol. I, pp. 44–60; 116–123; and Czapek, Biochemie der Pflanzen, Vol. I, pp. 537–545 (1905).

particular organ or tissue, being found abundantly in roots, stems, leaves or seeds.

In regard to solubility in water, pentosans show all possible variations. De Chalmot (108) found them present in the watery extract of the leaves of many plants; Winterstein (167) in the somewhat mucilaginous hot water extract of the seeds of *Tropaeolum majus;* Schulze (146), in both soluble and insoluble form in the cotyledons and endosperms of the seeds of *Lupinus luteus* and other legumes, where they are doubtless stored as reserve material for the growing plant; and in the cell walls of the mature plants, where in most cases they approach true cellulose in character. It is difficult to differentiate these highly resistant pentosans of the cell wall, which are commonly included in the term crude fiber, from the ligno-celluloses and oxycelluloses also found there, which as Cross, Bevan and Beadle (104) have shown,[1] are like true pentosans in yielding furfurol on distillation with dilute hydrochloric acid. Besides hemicelluloses yielding pentoses (*xylose and arabinose*) exclusively, occur many yielding also methyl-pentoses (*fucose, rhamnose*). These yield on distillation with dilute hydrochloric acid, methyl-furfurol, which is precipitated by phloroglucin, and hence included in quantitative estimations of pentosans by the method of Tollens and Kröber (121). The distribution of methyl-pentosans has been studied especially by Tollens and his pupils. Japanese "Nori" (*Porphyra laciniata, Laminaria,* and other seaweeds) (129), tragacanth and many other gums (163) contain fucosan. Rhamnose occurs also widely distributed in the plant kingdom, but more frequently in the form of a glucoside. Röhmann (134) reports a rhamnosan in *Ulva lactuca.*

It is a very common thing to find pentosans and hexosans occurring together. In fact, it is absolutely impossible, in treating of hemicelluloses, to draw any sharp dividing lines, for they are not only intimately associated, but frequently chemically combined. Schulze (146) has given the name paragalactan to the carbohydrate yielding arabinose and galactose, which occurs in the seeds of many legumes. Winterstein (167) finds galacto-xylan in the water extract of *Tropaeolum majus,* and numerous other examples of such combinations might be cited.

A class of substances to which has been given a distinctive name because of their peculiar gelatinizing property, is the Pectins. As Czapek[2] remarks, "It is uncertain whether they form a definite

---

[1] For further details see v. Lippmann; Chemie der Zuckerarten, Vol. I, pp. 160–169.
[2] Die Pektin-Substanzen; Czapek, Biochemie der Pflanzen, Vol. I, p. 545.

class of cell wall substances, or whether they should be classified as 'hemicelluloses' or 'pentosans.'" In 1868, Scheibler (141) found a sugar which he called pectinose, but which was later shown to be arabinose (142). In 1875, Reichardt (132) obtained a pectin body from carrots and beets, which he called 'pararabin,' expressing the view that pectins should hardly be considered as a special class of carbohydrates. Tromp de Haas and Tollens (160) have found from numerous analyses, that the pectins do not differ from other carbohydrates in their relative proportions of hydrogen and oxygen so much as earlier workers supposed, and hence they may be classified with other hemicelluloses according to the products of their hydrolysis (pentoses; galactose and other hexoses). Cross (106) believes them to be allied to the ligno-celluloses. The whole matter is still in a state of uncertainty. Herzfeld (116) has shown that arabinose can be obtained from most pectins, and consequently they have been included among the pentosans, though from the frequency with which they yield galactose, they might equally well be discussed with the galactans. According to Czapek while pectins occur frequently in phanerogams, ferns and mosses, their presence in algae is doubtful, although it is possible that soluble carbohydrates of algae yielding arabinose or galactose are closely related to the pectins of other plants.[1]

RÔLE OF THE PENTOSANS IN PLANT PHYSIOLOGY.

Comparatively little is known of the rôle of pentosans in plant physiology. De Chalmot's (108) observation that they decrease in quantity in seeds — peas and corn — during germination, and reappear in the stems and roots of the growing plant, would seem to indicate that they form a part of the reserve material in the seed; but Schöne and Tollens (145), finding no diminution in the amount of pentosans in grains during germination, but rather a slight increase, declare that they do not belong to the reserve-stuff of the seed; so the question may be regarded as still unsettled. Changes in the relative amounts of pentosan in plants at different stages of growth, studied by Cross, Bevan and Smith (105), Götze and Pfeiffer (113), Calabresi (98), and others, show that the increase of pentosans runs parallel to the formation of the skeletal substance; and have led to the idea that they arise through the transformation of a part of the cellulose, and along with lignin and cutin, take part in wood formation. Ravenna and Cereser

---

[1]Cf. also Bigelow, Gore, and Howard (92).

(131) find in the case of dwarf beans that when the food is wholly dextrose administered to the leaves, pentosans increase greatly, especially in the light, and that when the functioning of chlorophyll is prevented for long periods the amount of pentosans decreases. They conclude that the simple sugars exert a preponderating influence in pentosan formation, and that these serve as a reserve material when the plant has exhausted its more readily available food materials.

PENTOSANASES IN THE VEGETABLE KINGDOM.

Our knowledge of enzymes inverting pentosans is meager, and rather indefinite. The action of such forms as *Hymenomycetes* upon wood seems to be of chemical nature. At any rate it is evident (107–146) that they are able to utilize xylan. Bourquelot and Hérissey (95) have isolated an enzyme from malt diastase which produces reducing sugar from pectins, and call it pectinase. This is not to be confused with the so-called pectase which causes the coagulation of pectin bodies. Bigelow, Gore and Howard (92) also find that the enzymes of *Aspergillus* partially hydrolyze the pectin of gentian root. According to Harrison (114), *Bacillus oleraceæ* produces a cytase capable of dissolving the cell walls of potatoes, turnips, cauliflower and allied plants, which acts particularly on the middle lamella, the supposed seat of pectin.[1] The latter is not an inverting enzyme. In Persian Berries (*Rhamnus*) (162), in *Penicillium glaucum*, and *Botrytis cinerea* (90), an enzyme (*rhamnase*) has been found which splits off rhamnose from some of its glucosides (*rhamnetin and rhamnazin*). An early observation of the presence of rhamnase in the rutin of garden rue was made by Bornträger (94). That some of the so-called cytases described under cellulose[2] may act on pentosans seems possible, but there is no direct evidence that such is the case. On the contrary, Cross and Bevan (105) believe that pentosans once formed in the plant, remain thenceforth unaltered.

Tollens and Glaubitz (159) assert that the pentosans do not undergo lactic or butyric acid fermentation, and are otherwise unaffected by yeast, as has also been shown by Lintner and Düll (125). The pentosans are very resistant toward the action of bacteria. Slowtzoff (154) found that a small amount of pure xylan in a putrefying mixture,

---

[1] Cf. Czapek, Biochemie der Pflanzen.

[2] Cf. Biedermann and Moritz (34), Brown (35), Brown and Morris (36), Bergmann (32), Grüss (184), Newcombe (64).

kept at a temperature of 40° C., did not entirely disappear from the solution before the ninth or tenth day. Two widely distributed fermenting agents acting on hemicellulose (*Bacillus asterosporus Arth. Meyer,* and *Bacillus clostridieforme, Burri and Ankersmit*), studied by Ankersmit (89), are said by him to occur in insufficient numbers to make their activity of any significance in the alimentary canal of the cow.

PENTOSANASES IN LOWER ANIMALS.

Extensive investigations regarding the occurrence of pentosan-splitting enzymes in lower animals, have been made by Sellière since 1905. The secretion of the hepato-pancreas of the common snail (*Helix pomatia*) not only digests cellulose *in vitro*,[1] but also xylan, according to this writer (148). In feeding experiments, analyses of the food (oak wood) and excreta of these xylophages showed a higher percentage of xylan in the former than in the latter (149). Hence xylan must have been digested. In 1907, he showed that pentoses were actually liberated and absorbed, by testing the blood of these snails, which gave the phloroglucin reaction (151). That sugar can be found in their blood is denied by Couvreur and Bellion (99), but this Sellière attributes to the fact that the sugar content is much less than in higher animals, and hence has been entirely overlooked.

Xylanase also occurs in other species of snail (150) such as *Helix aspera Müll., Helix nemoralis L., Limax arborum Bouch., Limax variegatus Drap., Arion rufus L., Patella vulgata L., Littorina littorea L., Littorina littoralis L.,* and in a representative of the *Coleoptera, Phymatodes variabilis L.* The presence of a xylanase in *Patella vulgata* and the *Littorinae* is especially significant, as their food consists in pentosan-rich algae. Sellière (150) and Pacault (130) have independently discovered a xylanase in the salivary glands of *Helix pomatia.* According to Röhmann(134), *Aplysia,* which subsist largely upon *Ulva lactuca,* do not, digest the soluble methyl-pentosan (*rhamnosan*) present in this alga. He finds this carbohydrate present in the glands of the midgut, but regards it as a food residue.

PENTOSANASES IN HIGHER ANIMALS.

There have been only a few investigations as to the presence in higher animals of enzymes hydrolyzing pentosans. Slowtzoff (154)

---

[1]Cf. Biedermann and Moritz (34).

found that pure xylan was not digested by saliva, gastric or pancreatic juice, but could be gradually hydrolyzed (in two or three days) by 0.2 per cent hydrochloric acid. Bergmann (91) digested pure xylan with extracts of the intestines of many animals (hen, goose, guinea-pig, sheep, ox, horse), and of the vermiform appendix of rabbits, but in no case found a xylanase. These experiments were performed with suitable antiseptics and controls in all cases. An old experiment by Fudakowski (112), attributing an inverting action upon gum arabic to pepsin, and another by Schmulewitsch (144), attributing such an action upon crude fiber to pancreatin, must be disregarded, as no antiseptics whatever seem to have been used. According to Sellière (152), neither the pancreatic juice of rabbits, nor a mixture pancreatic and intestinal juices, will hydrolyze xylan. Negative results were also obtained by him with macerated intestines of these animals. On the other hand, chloroform extracts of the intestinal contents of rabbits and guinea-pigs fed fresh hay and bread, produced pentoses in a 5 per cent xylan solution after 48 hours digestion at 37 degrees C., while negative results were obtained with boiled controls. This indicates that the enzymes causing hydrolysis were of bacterial origin, a conclusion substantiated by later work of the same author (153). No xylanase was detected in the excreta of carnivora such as the lion, panther, and wolf. From a centrifugalized extract of human faeces and soluble xylan, digested under aseptic conditions, xylose was obtained after 15–20 hours; but in meconium of calves and human beings in which bacteria were absent no xylanase could be found, although the intestinal glands were functioning. McCollum and Brannon (126) have shown that in the case of the cow intestinal bacteria destroy pentosans under anaerobic conditions, the degree of destruction varying with the kind of plant. Corn, wheat and oat feeds were incubated with fecal bacteria of this animal, and digestions continued 14 days in atmospheres both of carbon dioxide and hydrogen, with the following average results:

MATERIAL.	ATMOSPHERE.	PER CENT OF PENTOSANS DISAPPEARING.
Corn Fodder.............	CO_2	51.78
Corn Fodder.............	H	76.13
Wheat Straw............	CO_2	28.09
Wheat Straw............	H	37.99
Oat Straw..............	CO_2	30.66
Oat Straw..............	H	54.00

From this review it is evident that the presence of pentosanases in the higher animals has not yet been demonstrated.

DIGESTION AND UTILIZATION OF PENTOSANS BY ANIMALS.

In the case of men and animals subsisting on a mixed diet, the hexoses and their derivatives so overbalance the pentosans, under normal conditions, that the utilization of the latter is a question of theoretical rather than of practical importance. But in the case of herbivora, limited to a diet in which pentosans occur in considerable amounts, the extent of pentosan utilization becomes a question of economic importance. It is not surprising to find, therefore, that since the development of satisfactory methods of quantitative determination, a considerable number of investigations have been made upon such utilization by animals. The results of these experiments are shown in tables on pages 274 and 275.

The results in these experiments were obtained by analysis of food and faeces. Lindsey (123) Götze and Pfeiffer (113) and Tollens (157) found no measurable amount of pentoses or pentosans excreted in the urine of sheep, but Neuberg and Wohlgemuth (128) state that pentosans always occur in the urine of rabbits, only disappearing when the vegetable diet is compensated by pentose-free material. They report that 9 per cent of soluble araban (cherry gum) fed to rabbits was excreted in the urine. Slowtzoff (154) found 1.4–4.5 per cent of xylan in the urine of rabbits, but no reducing sugar. He also found that if the animal were killed shortly after xylan feeding, xylan could be detected in blood, liver and muscles. Hence xylan must have been absorbed from the digestive tract.

The feeding experiments show that herbivora digest, on the average, 55–60 per cent of the pentosans in their diet, but since no animal enzymes hydrolyzing pentosans have been demonstrated, and there is always the possibility of bacterial decomposition in the intestines, the most conclusive experiments as to the actual nutritive value are those of Kellner (118) with the respiration calorimeter. From the slight difference in loss of potential energy, when the furfurol-yielding rye straw preparation was substituted for starch, he concludes that furfurol-yielding substances participate in the formation of fat in the animal body.

DIGESTION AND UTILIZATION OF PENTOSANS BY MAN.

We have seen that pentosans can be digested by herbivora to a considerable extent. Can they be digested by man? The only feeding experiments on record are by König and Reinhardt (120).

In 1902, they conducted researches on two men whose main diet consisted of meat and butter or other fat, and beer; to this, in the various experiments, were added respectively (along with sugar, butter, beef extract, etc., used in preparing them) the following substances:

Experiment I. Green Peas. Experiment II. Ripe Shelled Peas. Experiment III. Red Cabbage. Experiment IV. Canned White Beans. Experiment V. Soldiers' Bread. Experiment VI. Graham Bread.

From analyses of food and faeces the following results were obtained:

TOTAL PENTOSANS IN GRAMS.	EXP. I.	II.	III.	IV.	V.	VI.
In Food	15.55	23.15	14.01	12.80	52.64	41.26
In Faeces	0.79	0.59	0.70	1.12	8.66	4.06
Per cent not utilized, estimating Pentosans in Beer as unutilized.......	5.08	2.55	5.0	8.75	16.45	9.84
Total per cent not digested...........	7.47	3.24	7.75	14.32	20.24	12.97

Hence we see that of the total pentosans in the diet 3.24–20.24 per cent were excreted. Only a little furfurol-yielding substance was found in the urine. From the small percentage recovered in these experiments, König and Reinhardt (120) conclude that the pentosans are to a high degree utilized by man, but they take no account of possible destruction by bacteria.[1]

Since pentosans do disappear from the alimentary tract of men and animals, it behooves us to consider whether, on the assumption that they are hydrolyzed like starch, the pentose sugars so produced are as well utilized as dextrose. König and Reinhardt (120) found some furfurol-yielding substance in the urine, and Blumenthal (93) observes that after eating huckleberries, cherries and prunes, pentosans are excreted, but no reducing sugar. Cominotti (100) finds pentoses absent from the urine of man on a meat diet, but always present on a mixed diet. He agrees with König and Reinhardt that the output in the urine is small compared with the amount of pentosans in the food, and proposes to investigate the possibility of glycogen formation from pentosans.

The behavior of pentoses in the body has been exhaustively reviewed by Neuberg(127).[2] It appears from the work of Cremer (102, 103),

---

[1] Cramer (101) has shown (according to a recent review, the original paper was not accessible) that bacteria are essential to hemicellulose transformation.

[2] For a recent discussion of the absorption and utilization of pentoses see A. Magnus-Levy, Oppenheimer's Handbuch der Biochemie der Menschen und der Tiere, 1909, Vol. IV, pp. 395–407.

NAME OF INVESTIGATOR.	DATE.	SPECIES OF ANIMAL.	MATERIAL FED	AVERAGE PER CENT PENTOSANS DIGESTED.
Stone (155)	1892	Rabbits	Corn meal, Wheat bran	40–60
Stone and Jones (156)	1893	Sheep	Various kinds hay and grasses	60.3
Weiske (166)	1895	Sheep	Oats	65.1
		Rabbits	Oats	53.8
		Rabbits	Wheat bran	53.9
Lindsey and Holland (124)	1895	Sheep	Hay and different grasses	55–90
Götze and Pfeiffer (113)	1896	Sheep	Luzerne hay, Cherry gum	54.3
		Sheep	Luzerne hay	44.6
Sherman (143)	1897	Steer	Wheat bran	66.2
Fraps (110)	1900	Sheep	Timothy hay	53.9
			Feeding stuffs	68.5
			Average all materials	64.2
Kellner and Köhler (118)	1900	Oxen	A 3 Kg. Rye straw extracted with dilute sodium hydroxide, containing 31.1% furfurol-yielding substance added to a basal ration	Loss of potential energy 14.0%
			B 2.5 Kg. starch added to basal ration	Loss of potential energy 10.1%
Slowtzoff (154)	1901	Rabbits	Pure xylan	55.78
Weiser (164, 165)	1903	Oxen	Hay, rape	63.4
		Horses	Rape, corn, oats	45.5
		Swine	Rape	47.9
		Sheep	Rape	55.1
		Fowls	Corn, rape	23.9
Lindsey (123)	1903	Sheep	Hay, grains, by-products	40–90

	Year	Animal	Diet	Value
Rudzinski (136)	1904	Sheep	A Rye straw + starch and sugar	46.82
			B Straw + grain and staff + starch and sugar	39.89
			C Threshed straw	70.20
			D Straw + 1.57 lb. sugar and 6.29 lb. starch per Kg. of body weight	12.56
Zuntz-Utzjanzew (168)	1905	Rabbits	Oats and hay	(With coecum) 17.3–50.0 (Without coecum) 13.2–40.0
Bergmann (91)	1906	Rabbits		Heated / Ordinary
			A Wheat straw	22.26 / 19.91
			B Wheat straw	29.30 / 19.72
			C Meadow hay	38.95 / 36.07
			D Meadow hay	45.69 / 33.54
Utzjanzew (161)	1907	Rabbits	Wheat and hay	(With coecum) 35.8
			Oats and hay or oats alone	(Without coecum) 27.6
König (119)	1907	Sheep	Hay cut before blooming	82.06
			Hay cut in bloom	68.52
			Hay cut after blooming	53.6
		Swine	Pea bran	94.01
			Buckwheat bran	54.75
			Barley bran	51.45
			Wheat bran	57.77
		Rabbit	Wheat bran	49.7
McCollum and Brannon (126) .	1909	Cow	A Wheat straw / Whole wheat / Wheat gluten	50.86
			B Oat straw / Rolled oats	54.69
			C Corn meal / Corn stover / Corn gluten feed	67.21

Ebstein (109), Frantze (111), Neuberg and Wohlgemuth (128), Salkowski (137), v. Jacksch (117), Lindemann and May (122), Brasch (96) and others, that the pentoses and methyl-pentoses (*rhamnose*) are excreted more readily than the hexoses; that they exert an unfavorable effect in diabetes; and that there is no evidence of their acting as glycogen-formers in man. Consequently, even if further experiments justify König and Reinhardt's conclusions, the pentosans must apparently still play a very small part in the nutrition of man.

OCCURRENCE AND NATURE OF GALACTANS.

Next to the pentosans, no hemicelluloses seem to be so widely distributed as the galactans; both occur together in the plant cell, and often in a more or less intimate chemical combination. The pure galactans, i.e., those yielding exclusively galactose upon hydrolysis, have been differentiated into several classes, chiefly by differences in solubility or specific rotation, namely:

1. α-galactan, so named by Müntz (199), the first to identify galactan as an anhydride of galactose; it composes 42 per cent of luzerne seeds and occurs also in beans, barley, and malt.

2. β-galactan, isolated from the lime residues in the sugar beet industry by Lippmann (192).

3. γ-galactan, first isolated from Chinese moss (*Sphaerococcus lichenoides*) by Payen (262), in 1859, and by him called "gelose." He also identified it in agar-agar[1] (*Gelidium corneum*) and other algae. The carbohydrates of agar-agar were again studied by Reichardt in 1876, who obtained a substance of the formula $C_{12}H_{22}O_{11}$ and considered it identical with the "pararabin" which he found in carrots and beets.[2] In 1881 and 1882, Greenish (180, 181) investigated the carbohydrates of *Fucus amylaceus* (Ceylon agar-agar) and obtained on hydrolysis a sugar-yielding mucic acid (galactose). From *Sphaerococcus lichenoides* he also obtained a substance resembling Payen's "gelose." In 1884, Bauer (169) showed that agar-agar yields galactose; and in 1905, König and Bettels (190) gave the following percentage composition of Japanese agar-agar from Gelidium:

	Per cent.		Per cent.
Galactans	33	Ash	3.5
Water	20	Pentosans	3.1
Protein	2.6	Crude fiber	0.4

---

[1]The term agar-agar is applied to the hot water extract of various red algae, mainly species of Gelidium.

[2]See Pentosans, p. 268.

Another species of marine algae in which galactan has been fully identified, is *Chondrus crispus* (Irish moss). This is also a red alga. C. Schmidt (210) first examined it, in 1844; he demonstrated that the gelatinizing substance was a carbohydrate and yielded sugar on hydrolysis. Flückinger and Mayer (178), in 1868, discovered that the water extract of this alga yielded considerable mucic acid. In 1875, Bente (171) obtained levulinic acid from the products of its hydrolysis, and in 1876, reported that it yielded a non-crystallizing syrup (172). The first quantitative analysis was made by Hädike, Bauer and Tollens (185), who showed that the water extract yielded mucic acid corresponding to about 25 per cent of galactan. Sebor (220), in 1900, found in the products of hydrolysis, glucose, fructose and a small quantity of pentose. These observations were verified by Müther (200) in 1903, who further identified the galactose as a *d*-galactose. From the large yield of mucic acid, the water extract of *Chondrus* may therefore be regarded as chiefly galactan, together with some dextran and levulan, and a very little pentosan; groups which, according to Hädike, Bauer and Tollens (185), may be partly or entirely bound into ester-like compounds.

Examples of galactans occurring in combination, or close association with other hemicelluloses are numerous. Lupeose, from luzerne seeds, originally called β-galactan, yields 50 per cent galactose and 50 per cent fructose (214). The tuberous roots of *Stachys tuberifera* contain a soluble crystallizable carbohydrate yielding 37 per cent mucic acid, along with an unidentified sugar (225). Para-galactan (*galacto-araban*) forms a large proportion of the reserve material of many seeds.[1] Rothenfusser (204) finds that the mucilaginous extract of flaxseed yields equal parts of pentosans and hexosans, the latter being mainly galactose. Galactans and pentosans, as already indicated,[2] occur together in many lichens and algae, and also in the pectins.[3] Hérissey (187) has shown that the "galactine" of Müntz (199) yields equal parts of galactose and mannose. Galacto-mannans also frequently occur in the reserve material of seeds, as in those of the date and other species of palm, and in coffee beans; in the American honey

---

[1]Cf. Schulze (215), Schulze, Steiger and Maxwell (217), Schulze and Castoro (218), Castoro (176), and Goret (179). Also Schulze and Godet, Zeitschrift für physiologische Chemie, V. 61, p. 279, for a very complete review of the work of Schulze and his pupils.

[2]See Chemical Nature of Lichens and Algae:- König and Bettels (8), Escombe (6), K. Müller (11), Ulander (26).

[3]Cf. Pentosans, p. 268.

locust (*Gleditschia triacanthus*), Goret (179) found the albumen to yield 66–70 per cent galactose and 22–23 per cent mannose; he has shown, in fact, that the carbohydrate reserve of almost all seeds with horny albumen consists largely of a mixture of mannans and galactans.[1]

GALACTANASES IN THE VEGETABLE KINGDOM.

The hydrolysis of the paragalactan of lupine seeds during germination was first observed by Schulze and his co-workers. That ordinary diastatic enzymes do not form sugar from the para-galactan of *Lupinus hirsutus* was demonstrated by Schulze and Castoro (218). Ptyalin, pancreatin, malt diastase and "taka" diastase, will, however, in the course of 5 or 6 days' digestion at 35–40° C. render this carbohydrate soluble in water to the following extent:

	Per cent.		Per cent.
Malt diastase	38	Ptyalin	40
Taka diastase	35	Pancreatin	15

Grüss (184) has made exhaustive microchemical investigations upon the germinating date endosperm, in which he has been able to observe the solution of the galactans by enzymes developed during germination. Bourquelot and Hérissey (174) find a soluble enzyme hydrolyzing galactan,[2] produced by the germinating embryos of the seeds of the carob, Nux vomica, fenugrec and luzerne. Shellenberg (208), studying the action of moulds on hemicelluloses, found at least four different ferments showing considerable specificity in their action; seeds of *Lupinus hirsutus* (containing paragalactan) were attacked by most of these moulds (*Mucor neglectus, Mucor piriforme, Rhizopus nigricans, Thamnidium elegans, Penicillium glaucum*). Similarly, Hérissey (187) found galactose produced from manno-galactans by *Aspergillus niger* and *Aspergillus fuscus;* Saiki (205) obtained sugar from Irish moss by digesting it with inulase prepared from *Aspergillus niger* and *Penicillium glaucum;* and with "taka" diastase prepared from another mould, *Eurotium oryzae.*

Little is known of the action of bacteria upon galactans. Gran (182) found sugar produced from agar-agar by *Bacillus gelaticus*, through the action of an enzyme which he calls "gelase." Saiki (105),

---

[1] Cf. Mannans, p. 283; for a further discussion of the occurrence of Galactans see v. Lippmann, Chemie der Zuckerarten, Vol. I, pp. 686–697.

[2] Cf. Mannans, p. 284.

in experiments with *B. coli communis*, on culture media containing different kinds of comminuted seaweed, found a slight gas production in one culture, in media with agar-agar and Irish moss.

GALACTANASES IN THE ANIMAL KINGDOM.

The only discovered instance of a galactanase in lower animals is cited by Bierry and Giaja (173), who found that the hepato-pancreatic juice of *Helix pomatia* produced galactose from extracts of carob seeds (*Ceratonia siliqua*); later experiments upon agar-agar, with extracts from a number of crustaceans (*Astacus fluviatilis* Rondel., *Homarus vulgaris* Bel., *Maja squinado* Rondel., *Carcinus moenas* L., and *Platycarcinus pagarus* L.) were entirely negative; the galactans of luzerne and fenugrec were attacked with difficulty by the extract from *Astacus*. Strauss (221) could find no enzyme attacking agar-agar, in the larvae and puppae of various species of *Lepidoptera* and *Diptera*.

No galactanases have been found in higher animals. Bierry and Giaja (173), using extracts of luzerne seeds, got negative results with digestive juices of dogs and rabbits, and Sawamura (207) obtained similar results with extracts of different sections of the alimentary canal of swine and horses. Saiki (205) found saliva, pancreatic, and intestinal juices unable to hydrolyze Irish moss.

DIGESTION AND UTILIZATION OF GALACTAN BY ANIMALS AND MAN.

The first study of the digestibility of galactans in higher animals was made in 1903, by Lindsey (191). Alsike clover-seed, containing 8 per cent galactan, was fed in connection with hay, the digestibility of which had been previously determined; from analyses of food and faeces, the galactan in the hay (1.72 per cent) was found to be 75 per cent digestible, and that in the clover 95.78 per cent digestible. Saiki (205) fed agar-agar and Irish moss to dogs and recovered a large part in the faeces, as shown by the increased amount of carbohydrate excreted. Lohrisch (194) fed dogs and rabbits agar-agar in its usual form, and also "soluble-agar" prepared from ordinary agar by Dr. Karl Dieterich of Dresden, Director of the Helfenberg Chemical Factory. This product seems to be partially hydrolyzed in its preparation, since it is not only readily soluble in water, but has slight reducing action; it yields on boiling with Fehling's solution, 3.5–4.1 per cent sugar, and if a watery solution is allowed to stand 18 hours at

37° C., it is further hydrolyzed and yields then 16.9–20.4 per cent sugar. The results of Lohrisch's experiments appear in the folowing table:

ANIMAL.	FOOD.	HEMICELLULOSE EQUIVALENT OF AGAR FED.	HEMICELLULOSE EXCRETED.	HEMICELLULOSE DIGESTED.
				Per cent.
Rabbit I..........	Ordinary agar	18.77 = 14.48	7.1	50.9
Rabbit II.........	Ordinary agar	11.8 = 9.11	4.71	48.3
Rabbit III........	Soluble agar (given in 9 days)	95.9 = 65.02	14.2	78.1
Dog..............	Same as III	53.0 = 35.9	11.7	67.3

Lohrisch (194) has also studied the utilization of agar-agar in starving herbivora. In two experiments, rabbits starved for two days were fed ordinary agar as long as they would eat it, other animals of the same weight being kept in starvation as controls; in a third experiment, "soluble agar" was fed. Urine and faeces were collected and analyzed. Of the ordinary agar, about 50 per cent was excreted in the faeces; of "soluble agar," about 25 per cent. No positive evidence of any change in nitrogen excretion attributable to the agar fed, can be drawn from the protocols. One animal died through accident, another survived its control but one day, and the third, in spite of its apparently good digestion of the "soluble agar," died a week before its control.

In the case of rabbits made diabetic with phlorhizin and then fed 20–40 grams of both ordinary and soluble agar, Lohrisch (194) found that the D : N ratio remained fairly constant throughout each experiment, showing no marked increase in sugar excretion. We see, therefore, no grounds for assuming that agar-agar (*galactan*) forms glycogen in rabbits.

The first studies on the utilization of galactan by man were made by Saiki (205) (1906). In feeding experiments in which various carbohydrates were at different times added to a uniform diet, consisting of 513 grams beefsteak, 500–600 grams bread, 40 grams sugar, 31 grams butter, 2 eggs and 2 apples — a diet on which over 98 per cent of the carbohydrates were digested, he obtained the following results:

NO.	SUBSTANCE ADDED TO DIET.	EQUIVALENT OF SUBSTANCE IN DEXTROSE.	CARBOHYDRATES IN FAECES CALCULATED AS DEXTROSE.	HEMICELLULOSE DIGESTED.
		Grams.	*Grams.*	*Per cent.*
1	20 grams agar................	10	9.2	8
2	24 grams agar................	12	8.8	27
3	40 grams wakame.............	4.7	3.4	28
4	45 grams kombu.............	11.4	2.5	78

Lohrisch has also studied the digestibility of "soluble agar" in man. Sometimes it is not well borne, especially if given in quantities over 50–60 grams per day and causes gas formation, diarrhoea, and other intestinal disturbances; in other cases, large amounts (100 grams per day) cause no unpleasant symptoms whatever. The agar was dissolved in some beverage, and the diet was otherwise carbohydrate-free. Some of the results are shown in the following table (194):

NO.	DURATION OF EXPERIMENT.	AMOUNT DIGESTED.		HEMICELLULOSE EXCRETED.	HEMICELLULOSE DIGESTED.	HEMICELLULOSE DIGESTED.
		As Air Dry Soluble Agar.	As Hemicellulose.			
		Grams.	*Grams.*	*Grams.*	*Grams.*	*Per cent.*
1	1 day	100	61.9	46.06	15.84	25.6
2	1 day	100	61.9	39.1	22.8	36.8
3	3 days	235	145.4	90.5	54.9	37.7
4	3 days	240	148.5	40.8	107.7	72.5
5	1 day	100	61.9	25.4	36.5	58.9
6	1 day	110	67.8	23.4	44.4	65.5

No. 4 was a case of chronic constipation; the high percentage of hemicellulose digested is in accordance with the observations of Lohrisch (193) and Pletnew (203), on the extraordinarily good utilization of all foodstuffs in chronic constipation. Two of these experiments were on diabetics, and showed that the 18.36 grams of "soluble agar" absorbed per day caused no increase of sugar in the urine, and had no noticeable effect on nitrogen metabolism.

From these experiments, we see that ordinary agar is digestible to a very small extent, and that even when changed to an easily hydrolyzed form, it is only digested to about 50 per cent. Is the part digested absorbed and utilized as galactose? The recent exhaustive

discussion of the behavior of galactose in the animal body by Brasch (175) renders any details on the utilization of this sugar unnecessary. Hofmeister (188) showed that of all sugars it is most readily excreted. That galactose can form glycogen in dogs and rabbits, has been shown by Weinland (226), Kausch and Socin (189), Cremer (177), Voit (223), Brasch (175), and others.[1] Brasch (175) has shown that the assimilation limits for galactose lie, for normal man, between 30 and 40 grams, while for dextrose they lie between 100 and 150 grams. Voit (224), Sandmeyer (206), Bauer (170), and others have shown that galactose, even in small amounts increases the sugar excretion in diabetes. It would seem, therefore, that if soluble agar were absorbed as sugar, it would increase the sugar output in the urine. To throw some light on this problem Lohrisch (194) has conducted three respiration experiments on men after ingestion of 100–110 grams of soluble agar, of which, on the average, about 63 per cent was absorbed. The changes in the respiratory quotient are shown in the following table:

Respiratory Quotient.

NO.	IN FASTING.	NUMBER OF HOURS AFTER INGESTION OF SOLUBLE AGAR.						
		1	2	3	4	5	6	7
I	0.768		0.766	0.835	0.860	0.770	0.735	0.724
II	0.786	0.768		0.794	0.825	0.767		0.714
III	0.739			0.815	0.800	0.774		

NO.	IN FASTING.	NUMBER OF HOURS AFTER INGESTION OF SOLUBLE AGAR.					
		8	9	10	11	12	13
I	0.768						
II	0.786		0.693	0.730		0.618	
III	0.739			0.703			0.669

The distinct rise in the respiratory quotient in the fourth hour (beginning in the third hour in Experiment I) would indicate that carbohydrate was being oxidized, which in this case must come from the agar. The low value in the later hours seems due to the oxidation of fatty acids;[2] that such acids may be formed from soluble agar by bacteria, appears probable also from the intestinal fermentation pro-

---

[1] Cf. Magnus-Levy, Verwerthbarkeit der Galactose in normalen Organismus: Oppenheimer's Handbuch der Biochemie der Menschen und der Tiere, Vol. IV, p. 379.
[2] Cf. respiration experiments described under Cellulose.

duced when large amounts of this preparation are taken. A slight increase in acetone output, shown in the metabolism experiments with diabetics, points to the same conclusion. Perhaps, as Lohrisch suggests, the very slow digestion of the carbohydrate, may enable the organism to utilize the galactose formed, and account for its non-excretion, but this requires further demonstration.

According to these experiments by Lohrisch, cellulose and the soluble galactan show little difference in their physiological behavior. Both can be digested to about 50 per cent. Ordinary agar, as Saiki's experiments show, is largely recovered in the faeces; in fact, a therapeutic practice which has been recently established is based upon the recognized indigestibility of agar, namely, its employment as a remedy in cases of chronic constipation. It is especially valuable, as Mendel (196) points out, in those cases where the difficulty is due to an extremely complete digestion and absorption of all foodstuffs from the alimentary tract, which causes the formation of dry, hard faecal masses (*scyballa*) difficult to evacuate. The agar, remaining undigested and retaining a high percentage of water, gives bulk and softness to the faeces, and facilitates their daily elimination. Being resistant towards bacterial action, it causes neither gas formation nor production of harmful decomposition products. According to A. Schmidt (209), it can be advantageously taken in quantities up to 25 grams per day, part with the breakfast cereal, and part with sauce or cream, at another meal. In view of such facts as these, we are hardly prepared to agree with Lohrisch, that 'Cellulose and Hemicelluloses are readily digested.'

OCCURRENCE AND NATURE OF MANNANS.

As widely diversified in origin and character as the galactans, and very intimately associated with them are the Mannans. They show all possible degrees of solubility, from the readily soluble mucilage found in certain legumes, to the completely insoluble "reserve-cellulose," which forms the horny albumen in such seeds as the date, and which was long confused with true cellulose.

A few examples will serve to show the diverse places in which mannans may be found. They occur in yeast:[1] (258) in algae, as *Porphyra laciniata*; (278) in moulds, as *Penicillium glaucum*; (285) in the leaves and roots of the Japanese plant, Conophallus konjaku (280); in the bark and wood of many American trees (272).

---

[1] For further discussion see v. Lippmann, Chemie der Zuckerarten, Vol. I, pp. 641–649, and Czapek, Biochemie der Pflanzen, pp. 325–329.

The most extensive study has been given to the mannans of various seeds, in which, as already shown,[1] mannans and galactans seem almost invariably to occur together. The seeds of the carob tree (*Ceratonia siliqua*) contain a hemicellulose originally called "caruban" by Effront (241) (1897), but shown by van Ekenstein (282) to yield mannose, and by Bourquelot and Hérissey (232) (1899), *d*-galactose. The first elaborate studies of "reserve-cellulose" were made by Reiss (264), who showed that the horny albumen of the seeds of *Phytelepas macrocarpa*, *Phoenix dactylifera* and other species of palm, *Allium cepa*, *Asparagus officinalis*, *Iris pseudacorus*, *Strychnos nux vomica* and *Caffea arabica*, differed chemically from true cellulose in their color reactions, in the ease with which they can be hydrolyzed, and in yielding, instead of dextrose, a sugar which he called "seminose," but which proved to be identical with Fischer and Hirschberger's (242) previously described mannose.

Mannan also occurs richly in the tubers of the many species of *Orchis* and *Eulophia* which are the source of commercial salep. On extraction with water, they yield a mucilaginous extract which was first studied by C. Schmidt (270) in 1844, and called by him "salepbassorin"; on hydrolysis with dilute sulphuric acid he obtained, besides some gummy substance and cellulose, a fermentable sugar which he thought to be dextrose. Mulder (259) considered the salep mucilage a mixture of starch and gum or pectin acids, while Franck (243) thought it a modification of cellulose, and Girand (248) a transformation of a starchy substance into a variety of dextrin swelling in water. Pohl (263) by precipitation with neutral salts, distinguished an "α-Schleim" and a "β-Schleim." According to Thamm (276), who has made the most recent investigations, "α-Schleim" does not occur in German salep. Tollens and Gans (277) showed that on hydrolysis, besides dextrose, mannose or, as they called it, "isomanitose" was formed, but this was shown by Fischer and Hirschberger (242) to be identical with *d*-mannose. Thamm (276) and Hilger (254) have shown conclusively, that the starch-free water extract contains an anhydride of mannose only.

A very resistant type of mannan occurring in some plants, has been designated as manno-cellulose by Schulze (273). Bertrand (227) finds it taking the place of xylan in the woody tissues of gymnosperms.

---

[1] Cf. Schulze and his coworkers, and Goret, under Galactans. Also Schulze and Godet, Zeitschrift für physiologische Chemie, V. 61, p. 279, for a very complete review.

MANNANASES IN THE VEGETABLE KINGDOM.

There is very little literature concerning the action of bacteria upon mannans. Sawamura (267) observed that extracts of *Hydrangea paniculata*, used in the manufacture of Japanese paper, which contain mannan (along with galactan and araban), became liquefied on standing. In bacteriological studies with extracts of this plant, and of roots of *Conophallus konjaku*, he found that only *B. mesentericus vulgatus* dissolved these mannans. The action was greatly facilitated, and sugar formation increased if a certain wild yeast, in itself inactive, were present. Traces of a similar enzyme seem to occur in *B. prodigiosus*.

In his studies of the action of moulds on hemicelluloses, Schellenberg (269) found that the seeds of *Ruscus aculeata*, which yield almost exclusively mannose (237–240), were attacked only by *Penicillium glaucum*. Hérissey (253), using pure cultures and water extracts of cultures of *Aspergillus niger* (grown on media rich in mannose and galactose to incite the development of mannanase and galactanase), with suitable antiseptics and controls, obtained mannose — and galactose — from seeds of *Ceratonia siliqua* and *Gleditschia triacanthus*, and an abundant yield of mannose from salep; similar results were obtained with *Aspergillus fuscus*.

As early as 1862, Sachs (266) observed the change of the thickened cell-walls of the date endosperm into sugar during germination. The cytases producing this change in 'reserve-cellulose' were later carefully investigated by Reiss (264), Brown and Morris (230), Newcombe (261), Grüss (251), and others. Still more recently, Bourquelot and Hérissey have made many studies on the specific characteristics of these plant enzymes. An exhaustive review of the literature on mannans and the action of enzymes upon them has been published by Hérissey (253), consequently this subject will only be reviewed very briefly here.

Grüss (251) has demonstrated that the solution of the date embryo (*Phoenix dactylifera*) is due to a ferment, the product of whose activity is galactan and mannose. Effront (241) (in 1897) attributed the solution of the albumen of carob seeds (called by him caruban) to a "caroubinase," but thought that the product of its activity was not identical with the products of hydrolysis; in 1899, however, Bourquelot and Hérissey (233) showed the possibility of obtaining mannose by the action of a soluble ferment derived from these seeds, which they called "seminase." Shortly afterwards, a similar enzyme was

isolated by them from the seeds of *Phoenix canariensis*. Hérissey (253) has been able to show that seeds of such legumes as luzerne, fenugrec, and common genet have, at least at the time of germination, ferments capable of transforming mannans—and galactans—into their corresponding sugars. Experiments *in vitro* show that they are not limited to action upon the seeds by whose embryos they are produced; but act on the reserve-cellulose of seeds from very distinct groups of plants. However, the luzerne ferment does not digest all mannans and galactans; it will hydrolyze the mannans of the tubers of the Orchis family (and commercial salep prepared from them), but not those of the albumen of palm seeds.

Grüss (251) has also shown that the enzyme of the date endosperm hydrolyzes starch, although this does not occur in the date seed, and that malt diastase works on α-mannan (the soluble mannan of date seeds, according to Grüss) which does not occur in the barley endosperm. Grüss considers diastatic enzymes a group working not only on starch, but also on hemicelluloses. Hérissey thinks that diastase and seminase are found together in varying proportions in barley, legumes, carob seeds, etc., and that neither is a simple ferment, but a "superposition de ferments," and defines "seminase" as a "ferment or group of soluble ferments, causing the transformation of the carbohydrates of horny albumens of the seeds of *Leguminosae* into assimilable sugars." Gatin (247) has made further researches upon the nature of seminase, and states that during the germination of certain seeds whose reserve is in the form of mannan, the presence of mannose is exceptional, but dextrose occurs in abundance. This phenomenon he attributes to a "manno-isomerase," which transforms the mannose, as fast as formed by the seminase, into dextrose. Experiments *in vitro* seem to indicate that this is a soluble ferment.

MANNANASES IN THE ANIMAL KINGDOM.

There are only a few instances on record of mansases occurring in lower animals. Bierry and Giaja (228, 229) found that the hepatopancreatic juice of *Helix pomatia* was capable of producing mannose from extracts of carob seeds and salep; that of *Astacus fluviatilis*, *Homarus vulgaris*, and *Maja squinado*, from the ivory nut (*Phytelepas macrocarpa*), the two latter hydrolyzing it at ordinary room temperature. On the other hand, the mannans of fenugrec and luzerne were hydrolyzed with difficulty, or not at all, by very pure gastro-intestinal juice. No mannanase was found by Strauss (275) in the larvae and

puppae of *Lepidoptera* and *Diptera*. Similar negative results have been obtained with the digestive enzymes of higher animals. Kinoshita (257) found that emulsin and invertin did not hydrolyze the mannans of *Conophallus konjaku* and Gatin (245, 246) tried the blood of rabbits, chicken serum, the pancreatic juice of dogs, the macerated intestines and pancreas of chickens and cattle, upon salep and carob seeds with negative results; on the other hand, Sawamura (268) reports a mannanase in the extracts from different sections of the alimentary tract of swine and horses.

DIGESTION AND UTILIZATION BY ANIMALS AND MAN.

There are also very few records in the literature of feeding experiments with mannans. In a paper in the Zeitschrift für Biologie, Voit (283) in 1874[1] described one by Hauber, who fed a medium sized dog 390 grams of dry salep powder in the course of eight days. The faeces of the feeding period were roughly marked off, and Hauber reported no unchanged salep present in them, because there was no swelling in water as with the original powder. Calculations based on the yield of sugar from the faeces on hydrolysis showed that at least 50 per cent of the salep was absorbed. This seems to have been a very crude experiment, and cannot be considered of convincing value.

In 1879, Weiske (284) fed carob-beans (*Ceratonia siliqua*) to sheep, along with meadow hay, and compared the nutritive value of this ration with one in which the carob-beans (210 grams) were replaced by an equivalent weight of starch, sugar and protein (from crushed peas). The coefficients of digestibility and nitrogen balance were so nearly the same on the two rations, that Weiske pronounced "Johannisbrod" (carob beans) an acceptable and digestible feed for sheep.

In 1890, Schuster and Liebscher (274) tried feeding the sawdust of ivory nut (*Phytelepas macrocarpa*) to sheep, having previously found that it had a favorable effect on cattle. Merino sheep gained considerable fat when fed oat straw and vetch fodder, plus ivory nut sawdust furnishing 50 per cent of the digestible carbohydrates. The ration, exclusive of the ivory nut, did not yield enough energy for such a result to be possible, hence the latter must have been utilized. The coefficient of digestibility, both for the nitrogen-free extract and crude fiber of this material, was at the same time shown by Niebling (262) to be 82 per cent for sheep.

---

[1]This paper reviews the early literature on gums.

From these experiments, mannan would seem to be well utilized by herbivora. The only experimental data regarding the nutritive value of mannans to man, are cited by Oshima (15) from work by Kano and Iishima (255), who found the coefficient of digestibility of konjaku 82 per cent (prepared from *Conophallus konjaku*). Further investigations seem highly desirable, in view of the fact that in certain regions food stuffs like salep and konjaku, consisting of almost pure mannan, are among the chief articles of the poor man's diet. It is also a question whether the nutritive value of bark, especially of coniferous trees, is due to mannan present. According to Dillingham (239) the quantity of mannan present does not justify such an assumption, aside from the question of its digestibility.

We have finally to inquire whether mannan can be hydrolyzed within the organism, and if so, whether the mannose produced can be retained and form glycogen. From the literature on the subject, it appears that mannose is well utilized by rabbits, dogs and men. According to Neuberg and Mayer (260), the *d*-form is better utilized than the *l*- or *i*-form. Mannose is readily converted to dextrose in the organism; thus Neuberg and Mayer found that a rabbit, receiving 10 grams of *l*-mannose *per os*, excreted 1 gram *l*-mannose and 4–5 grams *l*-glucose; 10 grams of *d*-mannose given rabbits *per os*, or subcutaneously, were almost completely oxidized. Rabbits fed 30 grams *d*-mannose by Cremer (238) excreted 3-4 grams in the urine, and dogs given 20 grams by Rosenfeld (265), excreted over 4 grams. This is somewhat more than would be excreted on giving equally large quantities of dextrose or levulose. Cremer (238) found no sugar in the urine of a man after feeding 3-12 grams of mannose.

That mannose can act as a glycogen former in rabbits, has been demonstrated by Cremer (238) and also by Rosenfeld (265). Neuberg and Mayer (260) found only a small amount of glycogen in the livers of starving rabbits after feeding *l*-mannose, but even this form is utilized to some extent. There is good reason for assuming, therefore, that if mannans can be converted into mannose in the process of digestion, they may be considered as true nutrients for the organism, the mannose being to a high degree capable of absorption and conversion into glycogen.

OCCURRENCE AND NATURE OF LEVULANS.

A number of polysaccharide carbohydrates yielding levulose on inversion have been described. They are all levo-rotatory, more or less soluble in cold water and insoluble in alcohol, and easily hydrolyzed by dilute acid, but have not been investigated sufficiently to permit any conclusion to be drawn respecting their relation to one another. The most important of these substances and their sources are shown in the following table:*

NAME.	SOURCE.	INVESTIGATOR.
Inulin	Tubers of dahlia, artichoke, Jerusalem artichoke, elecampane; bulbs of onion, garlic, narcissus, hyacinth, and tuberose; flowers, seed, etc., of various compositae	Tanret (321) Chevastelon (291)
Pseudo-inulin Inulenin Helianthin Synanthrin	Tubers of dahlia, artichoke, Jerusalem artichoke, elecampane; bulbs of onion, garlic, narcissus, hyacinth, and tuberose; flowers, seed, etc., of various compositae	Tanret (321, 322)
Levulin	Tubers of Helianthus tuberosus (Jerusalem artichoke)	Reidemeister (314) and others
Phlein	Rootstalks of Phleum praetense (Timothy)	Ekstrand and Johanson (296)
Cerosin	Unripe grains	Tanret (320)
Graminin	Rootstalks of various grasses, e.g., Trisetum alpestre	Ekstrand and Johanson (296) Harlay (301)
Triticin	Dracaena australis and rubra, Triticum repens (couch grass)	Reidemeister (314)
Sinistrin	Bulbs of Scilla Maritima (Sea onions or squills)	Schmiedeberg (318) Reidemeister (314)
Levulan	Molasses in beet-sugar industry	v. Lippmann (309)

* Cf. v. Lippmann, Chemie der Zuckerarten, Vol. I, pp. 795–807.

The best known member of this group is inulin,[1] closely associated with which are the four levulans described by Tanret; these seem to be intermediate products between inulin and levulose, all having greater solubility than inulin, but less levo-rotatory power. The other carbohydrates mentioned are also more soluble than inulin, but have higher specific rotation.

LEVULANASES IN THE VEGETABLE KINGDOM.

Comparatively few studies have been made upon the action of enzymes on the levulans, and these have been for the most part limited to inulin. Certain micro-organisms as B. Coli communis (295), Clostridium pastorianum (328), and several Schizomycetes, decompose inulin, but without any production of sugar. Yeast, according to Tanret (321) does not ordinarily ferment it, but Lindner (308) asserts that certain forms of top yeast change it readily. Levulin is fermented by yeast, according to Lévy (307), and triticin, in the course of four or five days, according to Reidmeister (314); but it seems probable that the first changes are due to gradual hydrolysis on standing in water, or to other organisms.

The effect of vegetable enzymes on these carbohydrates, as far as they have been studied, is shown in the following table:

NAME OF LEVULAN.	INVERTIN OF YEAST.	MALT DIASTASE.	"TAKA" DIASTASE.	INULASE OF ASPERGILLUS.
Inulin	− (8)	− (3)	− (3)	+ (7)
Levulin	+ (1)			
Graminin		− (4)		+ very slowly (4)
Triticin	− (2)	+ (5)		
Sinistrin		− (6)		

(1) Levy (307)
(2) Reidemeister (314)
(3) Chittenden (292)
(4) Harlay (301)
(5) Reidemeister (314)
(6) Schmiedeberg (318)
(7) Dean (293) and others
(8) Komanos (303)

Discovery of the best known ferment for any levulan is due to Green (300) who, in 1888, extracted such an enzyme from the tubers of the Jerusalem artichoke (*Helianthus tuberosus*), and named it "in-

---

[1]For description and early literature see Kiliani (302) and Dean (294).

ulase." Subsequently, Bourquelot (289) found inulase in *Aspergillus niger* and *Penicillium glaucum;* and Chevastelon (291) showed that this enzyme would hydrolyze the inulin of the monoctyledons. Dean (293) has studied the properties of inulase exhaustively, and shown that in *Aspergillus* and *Penicillium* it exists only as an endo-enzyme. Went (327) has found inulase also in *Monilia sitophila* and other *Amylomyces.*

LEVULANASES IN ANIMALS.

The first instance of an inulase in an animal organism has been cited by Strauss (319). In 1908, he reported studies on the enzymes of seven species of *Lepidoptera* and *Diptera*, during their various stages of development (*Euproctis chrysorrhea, Ocneria disparata, Bombyneustria, Bombyx mori, Galleria melonella, Hyponomenta, Calliophera vomitoria*), but found inulase present only in the eating larvae of *Bombyx mori* and *Hyponomenta*. No inulase was present in the larvae of these species after they had ceased eating, nor in the pupae and imagines.

The results of Kobert (304) in 1903, with extracts of May beetles, cross spiders, scorpions, cockroaches, ascarides, pupae of pine spiders, and house flies, were entirely negative; so also have been the experiments *in vitro* with digestive juices of higher animals, as shown by table on following page.

DIGESTION AND UTILIZATION BY ANIMALS.

Inulin is hydrolyzed by very dilute acid (0.05–0.2 per cent at 40° C. according to Chittenden), so that its more or less complete inversion by the gastric juice is possible, and has led many to believe that in spite of the negative results obtained with amylolytic enzymes shown above, it might be converted into levulose, and as such be readily utilized by the animal organism. It has therefore frequently been recommended for the diet of diabetics, who show a special tolerance for levulose; in fact, simply because inulin did not reappear in the urine as sugar, when fed to diabetics, its utilization has been assumed by many, no account being taken of its possible reappearance in the faeces. This reappearance is well demonstrated in an experiment of Sandmeyer (317) in which, after feeding 80 grams of inulin to a diabetic dog, over 46 grams were recovered in the faeces.

AUTHORITY.	DATE.	SOURCE OF ENZYME.	KIND OF LEVULAN.	RESULT.
Komanos (303).......	1875	Saliva	Inulin	—
		Pancreatic juice	Inulin	—
Schmiedeberg (318)....	1879	Saliva	Sinistrin	—
Chittenden (292)......	1898	Saliva	Inulin	—
		Pancreatic juice	Inulin	—
Bierry and Portier (288)	1900	Macerated pancreas and intestines of dog, rabbit and seal	Inulin	—
Bierry and Portier (288).	1900	Macerated pancreas and intestines of dogs, rabbits; fed three months on artichokes to induce formation of an inulase*	Inulin	—
Harlay (301).........	1901	Saliva	Graminin	—
Bierry (286)..........	1905	Pancreatic juice of dog	Inulin	—
		Pancreatic juice of dog + macerated intestines of dogs and rabbits	Inulin	—
Bierry (287)..........	1910	Pancreatic juice of dog from pancreatic fistula after injection of secretin	Inulin	—
		Same pancreatic juice added to macerated intestines of dog and rabbit, in slightly acid, slightly alkaline and neutral solutions	Inulin	—
		Hepato-pancreatic juice of Helix pomatia	Inulin	Levulose
		Enzyme prepared from hepato-pancreatic juice of Helix pomatia	Inulin	Levulose
Weinland (326).......	1905	Extract of small intestine of dog	Inulin	—

* Cf. Richaud, (326).

Attempts to induce glycogen formation in rabbits have not justified the hopes of the dieto-therapists in regard to inulin as a food for diabetics. The earlier experiments were either negative or open to criticism on account of faulty technique. The more discriminating work of recent investigators (Miura [313]; and Mendel and Nakaseko [312]), has shown that little glycogen is formed from inulin, even under the most favorable circumstances. A brief survey of the experiments in this field is given in the following table:

Observer	Date	No.	Dose	Grams.	Remarks
Luchsinger (310)	1874	1	40 gms. in 6 injections	0.53	Estimated on basis of glycogen [a] D=130 degrees, hence figure is too high
Komanos (303)	1875	1	20 gms.	0.835	
Külz (305)	1875	5	40 gms. in 5 injections	1. 0.475 2. 0.280 3. 0.724 4. 0.204 5. 0.362	Strikingly less than in Fructose feeding
Frerichs (299)	1876	3	1. 50 gms. in 5 injections 2. 50 gms. in 3 injections 3. 50 gms. in 3 injections	0.1241 0.828 0.1395	Unaltered inulin found in faeces
von Mering (324)	1877				Says results verify those of earlier experimenters, hence no figures given
Finn (297)	1877	7	25–35 gms.	Trace in 5 exp. 0.124 in 1 exp. 0.196 in 1 exp.	
Miura (313)	1895	19	10–25 gms., 10–12 injections in ½- to 1-hour periods	Total glycogen content of livers: 0.123— 3.1468	In all cases examined large amounts inulin and levulose in the alimentary tract
Mendel and Nakaseko (312)	1900	7	18–33 gms. in doses of 2.8 gms.	0.0223 0.0518 0.2049 0.4066 0.5087 0.7555 0.2411	Estimating the starvation maximum for rabbits, starved 5½–7 days, as 0.252 g. per kilo, in only 3 cases did the glycogen content exceed the limit

Excluding the experiment of Luchsinger (310) which was estimated on a very low specific rotation for glycogen, only four out of the 17 experiments before Miura's (313) are positive, and in these the glycogen was estimated without purification, so that the figures are probably high.   In more reliable experiments of Miura (313), and Mendel and Nakaseko (312), the glycogen content of the rabbits' livers was as low or lower than the starvation maximum for the rabbit, as estimated by Külz (309), so that glycogen formation from inulin must be regarded as doubtful, or very slight.

When inulin is introduced parenterally into the organism, there is no inversion or utilization, as shown by the experiments of Mendel and Mitchell (311).   They injected warm solutions into the peritoneal cavity, and determining the output of inulin in the urine (which was sugar-free) by calculations from the specific rotation, recovered 2.2 grams of 2.8 grams injected.   In an experiment in which the sugar-free urine was hydrolyzed, and the output of inulin calculated from the amount of reducing sugar obtained, 1.43 grams were recovered out of 2.2 grams injected.   Weinland (326) after subcutaneous injections of inulin into dogs, continued for a month, found no inulase produced thereby.   On the other hand, Saiki (316) succeeded in producing a definite anti-inulase in rabbit's serum.

We see, therefore, that inulin is not attacked by animal enzymes, as far as investigated, with the possible exception of two species of invertebrates; and by a very few vegetable enzymes.   It appears to a considerable extent in the faeces after being fed *per os* in spite of the ability of the gastric juice to hydrolyze it.   In spite of the accepted fact that levulose is capable of being directly utilized by the animal body there is no conclusive evidence of glycogen formation from inulin. Whether other levulans resemble this hemicellulose in these respects has not been investigated.

OCCURRENCE AND NATURE OF DEXTRANS.

In the higher plants, starch, dextrin, and cellulose occur almost to the exclusion of other anhydrides of dextrose. A few hemicelluloses yielding dextrose have been described, however, such as "α-amylam" (soluble in hot water) and "β-amylam" (soluble in cold water), discovered by O'Sullivan (343) in wheat, rye and barley; those in the mucilaginous extracts of flax-seed and fleabane, described by Bauer (329) and Rothenfusser (345); and that in *Colocasia antiquorum*, described by Yoshimure (352).

Even in the lower plants, dextrans do not occur to any great extent. They have been observed in bacteria (338), yeast (339), fungi (350), and liverworts (337), but occur most abundantly in lichens and algae[1] the lichens, as already stated, yielding dextrans to which the names lichenin, isolichenin, usnin, everniin, etc., have been given. Especial interest is attached to the dextrans of Cetraria islandica (lichenin and isolichenin) which together form 80–90 per cent of the total carbohydrates of this lichen, because of its abundance in northern lands and its use there as a foodstuff; hence these carbohydrates have received more attention from chemical investigators than any other dextrans. Ever since Berzelius (333), in 1808, studied the hot water extract of *Cetraria islandica,* and called the carbohydrate mixture so extracted "moss-starch," on account of its giving a blue color with iodine, the idea that it is, like starch, a valuable nutrient, has prevailed. That this hot water extract contained two carbohydrates, one soluble in cold water (isolichenin) and the other in hot, was demonstrated by Berg (332) in 1873, who also showed that the blue coloration with iodine was a property of isolichenin, but not of lichenin. Lichenin was first found to yield dextrose by Klason, in 1886 (337). The next year the two carbohydrates were more fully investigated by Hönig and St. Schubert (336), who have carefully reviewed the earlier literature on this subject. That lichenin and isolichenin yield dextrose on hydrolysis, has been verified by Karl Müller (341), Brown (334), and Ulander (348), who have also shown the hemicelluloses of the water-insoluble part to consist of dextran, mannan, and galactan, with a small amount of pentosan. Escombe's (335) observation that lichenin yields galactose has proved to be incorrect.

DEXTRANASES IN THE VEGETABLE KINGDOM.

Hönig and St. Schubert (336) subjected isolichenin to the action of malt diastase, and observed a rapid disappearance of the iodine color reaction, and the formation of a dextrin-like substance precipitable by alcohol — a result verified by Brown (334) in 1898. Berg (332) treated lichenin with malt diastase but was unable to observe any change produced in it; his results also have been verified by Brown (334). The only experiments in which sugar has been obtained from lichenin by the action of vegetable enzymes have been carried out by Saiki (346) with "Taka" diastase from *Eurotium oryzae* and inulase from *Aspergillus niger.*

[1]Cf. p. 255, also v. Lippmann, Chemie der Zuckerarten, Vol. I, pp. 215–220.

DEXTRANASES IN THE ANIMAL KINGDOM.

Attempts to hydrolzye lichenin by animal enzymes have been uni-
formly unsuccessful. The most exhaustive researches were made by
Nilson (342), in 1893, partly with pure lichenin and partly with the
powdered lichen itself. Digestions were made with human gastric juice
for 24 hours, in neutral, acid, and alkaline solutions; with pancreatic
extracts; with gastric juice followed by pancreatic extract; and with
these same extracts, using preparations treated with $\frac{1}{4}$ per cent sodium
hydroxide solution for 24 hours before the digestion. Nilson signifi-
cantly remarks that this resistance to sugar-forming enzymes is worthy
of note, inasmuch as certain lichens have been considered valuable food
for man, and that it is hard to understand how reindeer utilize the car-
bohydrates of lichens. His negative results with animal enzymes have
been substantiated by Brown (334)—who found digestion with 0.2 per
cent to 0.4 per cent hydrochloric acid equally ineffective — and by
Saiki (346). Torup (347) reports that the dextran isolated from *La-
minaria digitata* by Krefting is not hydrolyzed by ptyalin, amylopsin
or diastase.

DIGESTION AND UTILIZATION IN ANIMALS AND MAN.

Interest in the digestibility of lichenin arises, not only from its use
in the diet of normal individuals, but in the possibility of its furnish-
ing a substitute for other carbohydrates in the diet of diabetics.
After this idea was set forth by Külz (305), in 1874, it is not surprising
to find, in 1879, the Italian physician Cantani,[1] and the Norwegian
physician Bugge[2] reporting experiments in the use of Cetraria bread
for diabetics. Without any further observations than that the sugar
in the urine was not increased, the idea prevailed which Voit expres-
sed in his monograph on Nutrition in 1881 (348) and Poulsson repeated
in 1906 (344), that in some way or other, the "moss-starch," or
lichenin, was changed into sugar in the alimentary tract, and served as
a true nutrient. Poulsson undertook to verify this by feeding experi-
ments with two diabetics, but as Mendel (340) has taken pains to
point out, the results obtained, namely that 45–49 per cent of the car-
bohydrates of the Cetraria bread eaten were utilized, are unreliable,
since the carbohydrates of the faeces were calculated by difference,
instead of being determined directly by analysis.

---

[1]Cited by Poulsson (344).

[2]Bugge, Förhandlingar i det medicinske selskap, Kristiania, 1879, p. 179 (cited by
Poulsson).

The few feeding experiments made with animals do not sustain the claims made for the value of Cetraria as a foodstuff. Brown (334) found only 1.25–0.7 per cent glycogen in the livers of rabbits after Cetraria feeding, but these results are not very satisfactory, since the rabbits would not eat it very well. An old experiment by von Mering (351), in which 16 grams lichenin were fed to each of two rabbits, shows 0.56–0.63 grams of glycogen in the liver, but Miura (313) has pointed out that his glycogen estimates were probably too high. Saiki (346) fed Cetraria extract, containing 2 per cent dry matter, in portions of 292 cc. and 300 cc. on two successive days, to a meat-fed dog. The faeces of the feeding period were marked off at the beginning of the Cetraria diet by fine quartz, and at the end by cork. Their composition is shown in the following table:

DIET		WEIGHT AIR DRY, GRAMS.	CARBOHYDRATE.	AS DEXTROSE.
Meat....................	2 days	10	5.8	0.68
Meat + Cetraria extract..	2 days	15*	25.8	3.90
Meat....................	2 days	5*	24.5	1.20
Meat....................	2 days	6	3.2	0.19

* Faeces of Cetraria Period.

The Cetraria extract contained 6.3 grams carbohydrate estimated as dextrose, the faeces 5.1 grams.

Feeding experiments on man, in which the intake and output of carbohydrate have been carefully determined by direct analysis of the carbohydrate as dextrose, have recently been conducted in Professor Mendel's laboratory. The data have not yet been published in detail, but from a preliminary description given by Mendel (340) is taken the following report of one experiment*:

	FAECES. Weight Air Dry.	CARBOHYDRATE. As Dextrose.		CETRARIA FED.
	Grams.	*Grams.*	*Per cent.*	
I. Fore period = 3 days.............	35	2.1	1	
Cetraria period = 3 days..........	146	38.0	56	80 g. = 56g. as dextrose
II. Fore period = 2 days.............	68	6	4	
Fore period = daily..............	34	6	2	
Cetraria period = 1 day	53	24	13	20 g. = 14½ g. as dextrose
After period = 2 days............	29	6	2	

* From unpublished experiments by Dr. V. C. Meyers, Sheffield Laboratory of Physiological Chemistry.

In this experiment, the Cetraria islandica was carefully washed, extracted with a dilute solution of potassium carbonate, to remove the bitter principle; again thoroughly washed, dried and ground to a powder. This preparation contained 72.5 per cent carbohydrate as dextrose. The carbohydrates of the diet, throughout the experiment, were limited to fine white bread and zwieback, forms in which they are utilized in man to 98 per cent. The faeces were hydrolized with dilute acid, and the carbohydrates determined as dextrose by Allihn's gravimetric method. It is evident that nearly all of the Cetraria carbohydrate escaped digestion and was recovered in the faeces.

Through the kindness of Professor Mendel, the protocol of a similar experiment, by Mr. S. W. MacArthur, is also reproduced, in which the technique was practically the same as described for Dr. Myers's experiment.

PERIODS.	DIET.	COMPOSITION OF THE FAECES.			
	Cellulose-Free.	Weight Moist.	Weight Air Dried.	Dextrose.	Dextrose.
		Grams.	Grams.	Per cent.	Grams.
Fore = 3 days....	Meat, etc.	281	90.0	4.4	3.96
Mid = 3 days....	Meat + Cetraria*	542	149.0	27.6	34.5*
After = 3 days....	Meat, etc.	284	87.5	4.9	4.2

* Amount Cetraria eaten = 47 grams, which would be equivalent to 34.1 grams of dextrose in faeces.

It is evident that the results of this experiment simply confirm those of Dr. Myers, and demonstrate that uncooked Cetraria, although taken in a form as favorable as possible for its digestion, is scarcely affected by its passage through the alimentary canal, and must be classed among the indigestible carbohydrates. Very desirable experiments on the digestibility of the peculiar carbohydrate of Cetraria — lichenin — are also being conducted, which may throw new light on the digestibility of the dextrans, but at present we certainly have no grounds for assuming that this group of hemicelluloses deserves to be classed with the true nutrients; all experiments show that they are not attacked by animal enzymes, and are recovered unchanged in the faeces after feeding.

In conclusion, attention may be called to certain data from Japanese dietary studies, given by Oshima (15), as to the digestibility of

some dried marine algae, which have not been mentioned in connection with the different classes of hemicelluloses. The coefficient of digestibility for each species studied is given in the following table:

ALGAE DRIED.	OTHER SUBSTANCES IN DIET.	COEFFICIENT OF DIGESTIBILITY (Carbohydrates including crude fiber).
Ecklonia bicyclis...............	Shoyu* and sugar	36.2
Laminaria sp...................	Shoyu	75.2
Laminaria sp...................	Shoyu and cleaned rice	55.0
Ulopteryx pinnatifida..........	Cleaned rice, shoyu, sugar	72.3
Average..		67.7

* Soy-bean sauce.

III. EXPERIMENTAL PART.

INTRODUCTION.

The foregoing review has emphasized the limits of our knowledge, both in regard to the chemical composition of marine algae, and their fate in the alimentary tract of men and animals, as determined by actual measurement of intake and output, and as explained by the action of bacteria and enzymes *in vitro*. Ten species of marine algae have, therefore, been made the basis of the present investigations. Eight of them were Hawaiian Limu, obtained, as already stated, through the kindness of Miss Minnie Reed, Science teacher in the Kamehameha Boys' School, Honolulu. They were dried in the sun, with the salt water adhering to them, before shipping to America. The other two (dulse and Irish moss) were easily obtained in our Eastern markets.

That the carbohydrates of algae are chiefly hemicelluloses, is indicated by the analyses which have already been made; that in many species, these are to a great extent water-soluble, is also well known. In as much as such soluble forms are thus particularly well adapted for nutrition investigation on account of their freedom from all incrusting substances, which end to interfere with digestion, the present studies have been confined as far as possible to them. Since it was desirable to study the different groups of hemicelluloses, and mannans and levulans were not found in the seaweeds in sufficient quantities for metabolism experiments, these were obtained from other sources; a mannan from salep, and a levulan (sinistrin) from squills (*Scilla maritima*).

Other investigators in this laboratory are working on a dextran which would naturally be included here, namely lichenin from *Cetraria islandica*; consequently no experimental studies on this group of hemicelluloses have been made. In considering any classifications of these materials, it must be borne in mind that most of these carbohydrates are more or less complex in nature, and can be grouped only with reference to what appears to be the chief constituent in any given case. The following list comprises al the species examined, arranged upon this plan:

I. THE PENTOSANS:
    Dulse (*Rhydomenia palmata*),
    Limu Lipoa (*Haliseris pardalis*),
    Limu Eleele (*Enteromorpha intestinalis*),
    Limu Pahapaha (*Ulva lactuca laciniata* and *Ulva fasciata*).

II. THE GALACTANS:
    Irish Moss (*Chondrus crispus*),
    Limu Manauea (*Gracilaria coronopifolia*),
    Limu Huna (*Hypnea nidifica*),
    Limu Akiaki (*Ahnfeldtia concinna*),
    Limu Uaualoli (*Gymnogongrus vermicularis americana* and
        Gymnogongrus disciplinalis),
    Limu Kohu (*Asparagospis sanfordiana*),
    Slippery Elm (*Ulmus fulva*).

III. THE MANNANS:
    Salep (Species of *Orchis* and *Eulophia*).

IV. THE LEVULANS:
    Sinistrin (*Urginea* or *Scilla maritima*).

The primary object of these investigations has been to determine the fate of these substances in the alimentary canal of man, since they are all used as foodstuffs except sinistrin, and are all representative of a large class of materials so employed. The experiments conducted have been CHEMICAL, BACTERIOLOGICAL and PHYSIOLOGICAL in character, and each of these phases will be taken up separately in turn in the following pages.

CHEMICAL INVESTIGATIONS.

The aim of the experiments was to isolate, identify, and prepare for bacteriological and physiological experiments, any water-soluble carbohydrates present in sufficient amount in the materials under consideration; and to determine such of their properties as would facilitate their detection, isolation, and quantitative estimation in these experiments.

GENERAL METHODS.

All the seaweeds, with the exception of Irish moss, were washed repeatedly in cold tap water, to remove salt, sand, and other foreign substances, and for convenience, dried by spreading in thin layers

over steam radiators. The Irish moss, being comparatively free from salt, etc., and largely soluble in pure water, was quickly washed once, and extracted immediately.

All hydrolyses of carbohydrates were made with 2 per cent hydrochloric acid, by boiling with a reflux condenser over a free flame. After cooling, the acid was neutralized with potassium hydroxide, using phenolphthalein as an indicator, when the solutions were sufficiently light in color; in other cases, litmus paper was employed. When the products of hydrolysis served to determine the nature of the carbohydrates, they were evaporated on a water bath nearly to dryness, the residues extracted with hot 95 per cent alcohol the alcohol removed from the filtered solution by evaporation, the residues frequently taken up in a little water and decolorized with charcoal, concentrated, and again extracted with absolute alcohol.

All qualitative tests for reducing sugar were made with Fehling's solution; all quantitative tests by Allihn's gravimetric method for dextrose, the results being calculated as dextrose in view of the complex nature of most of the products, and the advantage of uniformity. On all preparations used for feeding experiments, the length of time in which the maximum yield of sugar could be obtained has been determined, as a criterion in analyses of faeces. Five grams of dry air material were hydrolyzed in 500 cc. of 2 per cent hydrochloric acid, 50 cc. being removed at intervals of one or more hours, cooled, neutralized, made up to 100 cc. and reducing power determined as dextrose by Allihn's gravimetric method.

Tests for the presence of fermenting sugars have been made in fermentation tubes with fresh compressed yeast, using as controls solutions of the substance to be tested, without yeast, and dextrose solutions with yeast.

All carbohydrate solutions for polariscopic examination have been clarified by addition of an equal volume of alumina cream.

Qualitative tests for pentosans have been made by boiling the substance to be tested in a small Erlenmyer flask with 12 per cent hydrochloric acid and testing for furfurol with anilin-acetate paper.

Quantitative tests for pentosans have been made by the furfurol-phloroglucin method.[1]

Tests for galactans or galactose have been made by oxidation with nitric acid to mucic acid, and the mucic acid identified by its melting point (212° C.–215° C.).

---

[1] Described in "Official and Provisional Methods of Analysis," United States Department of Agriculture, Bureau of Chemistry, Bull. No. 107, 1907.

Qualitative tests for mannose have been made by Storer's (271) method. The products of hydrolysis, freed from the greater part of the salts, gums, etc., in the manner already described, were taken up in a little water, and portions of 1 cc. or 2 cc. placed in test tubes. The reagent for testing was freshly prepared by shaking together 1 cc. of phenylhydrazin, 2 cc. of glacial acetic acid, and 10 cc. of distilled water. 3–16 drops of this reagent were added to each of the test tubes, and after standing several hours at room temperature, they were examined for precipitates of mannose-hydrazone. These precipitates were examined under the microscope, because they usually contained considerable amorphous matter. The mannose-hydrazone itself does not come down as colorless rhombic plates at first, but as globules of greenish-yellow or brownish-yellow color, sometimes smooth and resembling large yeast cells in the way they c uster together, and at other times covered with blunt points or spines. When these globules were observed, the precipitate was carefully washed with water, sometimes without removing from the test-tube, the last drops being taken up with filter paper, and then dissolved in warm diluted alcohol (3 parts of 95 per cent to 1 part water), which was not filtered, but decanted from the amorphous insoluble portion, and allowed to evaporate slowly to facilitate the formation of crystals. Unless these crystals could be obtained, the tests were considered negative, although Storer has pointed out that they are sometimes difficult to obtain, even when true mannose-hydrazone balls are present.

All quantitative determinations have been made in duplicate unless otherwise stated.

PENTOSAN PREPARATIONS.

Dulse.

A pure, water-soluble pentosan-preparation has been obtained from dulse (*Rhodymenia palmata*). After boiling in water, in an open vessel, with occasional stirring, for several hours, this dark, reddish-brown seaweed yielded a carbohydrate, non-mucilaginous in character, which could be precipitated from its solutions by alcohol. About 12 hours' boiling proved to be necessary for complete extractions. The hot, brown, watery extract was first filtered through gauze, and then through cotton, as it clogged up filter paper very quickly. This filtrate, concentrated to a syrup on a water bath, was poured while

still warm into about three times its volume of acetone, which expe⁻
rience showed to be a more satisfactory precipitant than alcohol·
Most of the carbohydrate came down very soon, in large, flocculent,
yellowish-white masses, but a portion remained in suspension as a
fine white powder, which made filtration difficult. The bulk of the
precipitate was therefore removed by filtering through three or four
thicknesses of fine gauze, and the rest obtained by distilling off the ace-
tone, concentrating the residue, and reprecipitating the carbohydrate
in solution with acetone. This precipitate was very hydroscopic, and
was therefore transferred immediately to 95 per cent alcohol. This
was replaced by fresh alcohol after a few hours, and the whole boiled
on a reflex condenser for half an hour. A yellowish, granular powder
was thus obtained, which was filtered, washed with ether, and the ad-
herent ether allowed to evaporate. It was then redissolved in a small
volume of water, filtered hot through paper, on a jacketed funnel,
reprecipitated with acetone, again put into 95 per cent alcohol, and
finally into absolute alcohol, in which it was allowed to stand for
several weeks. It was then filtered off, washed with ether, and dried *in
vacuo* over sulphuric acid. The product was a cream-white powder,
and apparently not at all hydroscopic. From about two kilograms of
crude commercial dulse, approximately 75 grams of this material were
obtained, and used subsequently for feeding experiments.

An attempt made to remove the dark red coloring matter by extrac-
tion with 1 per cent sodium carbonate, led to the discovery that this
carbohydrate is readily extracted by dilute alkaline solutions. For
preparations on a large scale, it was therefore found more satisfactory
to use the following method, based on Salkowski's method (139, 140)
of obtaining xylan and araban by precipitation with Fehling's solu-
tion. This method could be applied exactly as described, but there
was an evident tendency for the carbohydrate to dissolve in the Feh-
ling's solution.

The dulse was accordingly extracted with 1 per cent potassium
hydroxide solution for 48 hours, with occasional stirring, the extract
removed by a hand press, and the extraction with fresh alkali repeated
for 24 hours.[1] These extracts were filtered through several thicknesses
of gauze, and to this filtrate a solution of copper sulphate was added
till the reaction was just neutral. A flocculent, bluish-green precipi-
tate formed. Into this solution was stirred carefully the alkaline
Rochelle salt-potassium hydroxide solution used for Fehling's solu-
tion, until the precipitate clumped together in heavy granular masses.

[1] A third extraction contained so little of the material that it was discarded.

This was easily filtered off through gauze, as much liquid as possible removed by pressure, and the precipitate washed quickly with a little water to remove the excess of alkali. The carbohydrate was freed from its copper compound just as described by Salkowski (140). The precipitate was placed in a mortar and rubbed to a cream with diluted hydrochloric acid (1 volume of water to 1 volume of concentrated acid) the acid being added until all blue particles had disappeared. It was then poured into 90 per cent alcohol, the precipitate filtered off upon plaited paper and washed with 50 per cent alcohol, replaced in 90 per cent alcohol acidified with hydrochloric acid, and allowed to stand several hours to dissolve out the copper. It was then filtered, dissolved in dilute potassium hydroxide, and the dark brown, muddy solution filtered through paper on a hot funnel, the carbohydrate reprecipitated with acid alcohol, and redissolved and reprecipitated until free from copper. When it no longer came down readily in alcohol, acetone was substituted, in which it formed white fibrous masses resembling paper pulp. Washed with absolute alcohol and ether, and dried *in vacuo* over sulphuric acid, it became a cream-white powder. Both of these methods yielded a product readily soluble in cold water, forming a clear, limpid, amber-colored solution. It gave no color reaction with iodine, and contained no reducing substance. In Fehling's solution it formed a very flocculent white precipitate, was not precipitable by lead acetate, neutral or basic, in neutral solution, but formed a precipitate in alkaline solutions. A test for mucic acid gave negative results, but a strong furfurol reaction was obtained on boiling with hydrochloric acid, indicating the presence of pentosans. A 1-gram sample of material, prepared by the method first described, was tested quantitatively for pentosans. It contained 26.8 per cent moisture, and 2.48 per cent ash, and yielded 0.076 grams of phloroglucid, from which the yield of pentosans, according to Kröber's tables,[1] is calculated as 72 per cent. The phoroglucid precipitates were afterwards extracted with 95 per cent alcohol, according to Ellett and Tollen's[2] method for quantitative determination of methyl-furfurol. The Gooch crucibles containing the precipitates were warmed 10 minutes to 60° C. with 15–20 cc. of alcohol, the extract filtered off, and the extraction repeated till the alcohol was colorless. The precipitates were then dried at 100° C. and weighed. The loss of weight was 0.0047 grams or 6 per cent of the original precipitate. The dulse preparation therefore contained a small amount of methyl-pentosan

[1]Zeitschrift für physiologische Chemie, XXXVI, appendix.
[2]Berichte der deutschen chemischen Gesellschaft, Vol. 38, p. 492 (1905).

The products of hydrolysis were tested for fermenting sugar, with negative results, but after heating with phenyl-hydrazin-hydrochloride and sodium acetate, an abundant yield of osazones was obtained. These crystallized out only on cooling, were pale yellow, soluble in hot water only with great difficulty, but very soluble in alcohol, acetone, or pyridin. After four or five recrystallizations from alcohol, they melted at 152° C. and this melting point remained constant after ten or twelve recrystallizations. However, there were very minute points at which melting seemed to occur about 140° C. Under the microscope, clusters of long needles were seen, each with a tuft of small fine needles springing from its very tip. Dissolved in glacial acetic acid, and examined in a 100 mm. tube, these osazones showed no rotation of polarized light.

A very white sample of the dulse carbohydrate was used to determine its specific rotation. It contained 7.1 per cent moisture and 1.68 per cent ash. Two determinations were made, one on a 0.6 per cent solution and the other on a 1.0 per cent solution for which the polariscope readings in a 200 mm. tube were respectively $-0.90°$ and $-1.52°$. The specific rotation, calculated from these readings was therefore $[\alpha]_D = -75.2°$ and $-76.2°$, or corrected for moisture and ash, $[\alpha]_D = -82.4°$ and $-83.6°$, average, $-83°$.

The rate of hydrolysis and maximum reducing power were determined as follows: 5 grams of the material dissolved in 500 cc. of 2 per cent hydrochloric acid were boiled in the usual way. At the end of two hours, and at intervals of one hour thereafter, 50 cc. portions were removed, neutralized and made up to 100 cc., and the amount of reducing sugar present determined as dextrose. The following results were obtained:

TIME OF BOILING.	SUGAR AS DEXTROSE.
Hours.	*Per cent.*
2	87.2
3	87.2
4	89.4
5	89.5

That the results vary greatly with the concentration, is shown by the fact that a 0.3 per cent solution boiled 5 hours yielded 67.1 per cent of sugar as dextrose.

Having established the fact that this dulse preparation consists of pentosans, with the properties described, further investigations into the exact chemical nature of the carbohydrates composing it were not considered within the province of this work.

Hawaiian Seaweeds.

Beside the dulse preparation, three seaweeds have been included in this group which yielded little or no soluble carbohydrates, namely, Limu Lipoa (*Haliseris pardalis*), Limu Eleele (*Enteromorpha intestinalis*) and Limu Pahapaha (*Ulva lactuca*, etc.).

Limu Lipoa. Limu Lipoa contained a small amount of non-mucilaginous carbohydrate, soluble in cold water as well as hot. It was precipitated by alcohol, in which it came down as a white fibrous mass. On hydrolysis, it yielded a dextro-rotatory fermenting sugar; a test with phenylhydrazin acetate for mannose was negative, as were tests for pentosans. The total amount of this carbohydrate was so small as to be almost negligible, as far as feeding experiments were concerned, hence the original washed material was used, after grinding to a powder in a coffee mill. It contained a very high percentage of inorganic matter because the thalli were so encrusted with calcareous substances, that it was impossible to remove them entirely by washing. This preparation gave a strong furfurol test, and a single quantitative test for pentosans gave the following results:

The sample, weighing 1 gram, contained 10.5 per cent moisture and 18.5 per cent ash. It yielded 0.161 grams of phloroglucid, which according to Kröber's tables [1] is equivalent to 0.147 grams pentosans, or 14.7 per cent of the crude substance.

Tests for starch and reducing sugar were negative. Only a minute quantity of mucic acid was obtained; a quantity too small to purify and determine the melting point. The products of hydrolysis showed slight fermentation, which was doubtless due to the mannan of the water-extract.

A determination of the reducing power made in the same manner as already described, gave the results:

TIME OF BOILING.	SUGAR AS DEXTROSE.
Hours.	*Per cent.*
$1\frac{1}{2}$	Very little
3	14.3
4	14.7
6	12.9
8	12.8

Limu Eleele. Limu Eleele yielded no appreciable amount of water-soluble carbohydrate, even after boiling 3 or 4 hours. The dried

---

[1] Zeitschrift für physiologische Chemie, XXXVI, appendix.

seaweed was therefore simply finely ground for use in feeding experiments.

It gave a strong furfurol test, but yielded a mere trace of mucic acid. Tests for starch and reducing sugar were negative. The products of hydrolysis contained no fermenting sugar. From this it was evident that the hemicelluloses were chiefly pentosans.

Determination of the reducing power gave the following results:

TIME OF BOILING.	SUGAR AS DEXTROSE.
Hours.	Per cent.
2	16.8
3	16.9
4	18.1
5	16.8

Limu Pahapaha. Ulva lactuca is said by Röhmann (134) to contain a water-soluble methyl-pentosan, rhamnosan; but if this occurs in Limu Pahapaha, it must be in very small amount, as an extract of 50 grams of the dried seaweed, made by boiling 3 or 4 hours, gave very little residue on evaporation to dryness. For feeding experiments, the dry crude substance was simply ground to a powder. Like Limu Eleele, it gave a strong furfurol test, but yielded no mucic acid. Starch was present, but no reducing sugar. Fermentation with yeast was marked in 12 hours, probably due chiefly to the hydrolysis of the starch.

Determination of reducing power gave the following results:

TIME OF BOILING.	SUGAR AS DEXTROSE.
Hours.	Per cent.
2	28.8
4	31.8

GALACTAN PREPARATIONS.

Irish Moss.

The carbohydrates of Irish moss are, as already noted, readily soluble in cold water, after the salt has been removed from the seaweed. By allowing the moss to stand for 24 hours in cold water (about 10 liters to 250 grams of dry substance), an almost colorless, semitransparent, mucilaginous extract was obtained. By straining this off through gauze, and allowing it to stand over night, for minute particles of cellulose held in suspension to settle, a solution almost entirely free from insoluble material was obtained by decantation.

This was considered sufficiently pure for feeding experiments, and was quickly dried by pouring into broad shallow dishes and placing over a steam radiator. It formed yellowish, translucent scales, which were easily removed, and finely ground.

Subsequent extractions were made in a steam sterilizer, heating several hours at a time. Tests showed that the carbohydrate was not hydrolyzed by this repeated subjection to high temperature. The several extracts were first strained off through gauze and then filtered hot through cotton, to remove the cellulose particles. As these clogged even cotton filters very rapidly, it was found most satisfactory to let the extracts stand over night, decant off the supernatant fluid as far as possible, and filter in a water-jacketed funnel. Solutions containing over 1 per cent dry substance could not be filtered through paper. For experiments where a perfectly clear fluid was desired, a ½ per cent solution was filtered hot through plaited paper, and then concentrated on a water bath to the desired strength. One per cent solutions formed a soft jelly on cooling; 2 per cent solutions, a firm jelly.

Even when evaporated to a thick syrup, the carbohydrates of the Irish moss extract are not readily precipitated by comparatively large volumes of 95 per cent alcohol, but form a voluminous, transparent, gelatinous mass. This was found to be more or less characteristic of all the galactans examined. They could be brought down most satisfactorily by addition of sodium chloride to the extract before pouring it into the alcohol. In this way a white precipitate of fine fibers was obtained from the moss. The carbohydrate could also be precipitated by saturation with potassium acetate, and freed from inorganic salts by dialysis, according to the method described by Pohl (263). It could not be precipitated by Fehling's solution, nor by lead acetate in neutral solution.

Owing to the opacity of its solutions, and to the fact that its gelatinizing property made the use of very dilute solutions necessary, no satisfactory determination of its specific rotation could be obtained. A 0.5 per cent solution, clarified with alumina cream, and examined in a 200 mm. tube, showed a rotation of $+0.34°$, and other trials gave positive evidence that it was dextro-rotatory. The products of hydrolysis were also dextro-rotatory, and yielded osazones, which after one recrystallization from alcohol, had a melting point of $184°–185°$ C.

The carbohydrate gave a red-violet color with iodine, and contained no reducing sugar. A faint furfurol test was obtained. Oxidation with nitric acid gave a rich yield of mucic acid. Since Hädike,

Bauer and Tollens (185), and Müther (200) have already shown that Irish moss contains galactan, levulan, dextran and pentosan groups, these tests were simply verifications of some of their observations. Determination of the reducing power gave the following results:

TIME OF BOILING.	SUGAR AS DEXTROSE.
Hours.	*Per cent.*
2	45.6
3	48.6
4	45.8

Hawaiian Seaweeds.

Limu Manauea (*Gracilaria coronopifalia*),
Limu Huna (*Hypnea nidifica*),
Limu Akiaki (*Ahnfeldtia concinna*),
Limu Kohu (*Asparagopsis sanfordiana*),
Limu Uaualoli (*Gymnogongrus*).

These five seaweeds all contained soluble carbohydrates, which were extracted by boiling in water in an open vessel over a free flame for two hours or longer. Limu Manauea, Limu Huna, and Limu Akiaki, which consist largely of soluble gelatinizing hemicelluloses, yielded most of these on boiling two or three hours. The extracts were strained off through gauze, filtered hot through cotton, and dried in thin sheets as described for Irish moss. While the preparations were dark colored, and had a decided "sea" flavor, they were not unpleasant, and were used in feeding experiments without further purification. As already stated, the carbohydrates were not easily precipitated with alcohol unless a neutral salt (as sodium chloride) was present.

Limu Kohu and Limu Uaualoli contained only a small proportion of soluble hemicelluloses, and this was obtained only after boiling 8 to 24 hours. The extracts were also much less gelatinous in character. The thalli of Limu Kohu are almost like wire when dry, and remain tough and hard even after many hours' boiling. The extracts of these two species were more readily precipitated by alcohol than the others, but the precipitation was greatly facilitated by adding sodium chloride. The carbohydrate of Limu Kohu was precipitated as a white cheese-like cake, floating on the surface, while that of Uaualoli came down as a mass of coarse white fibers. These precipitates were transferred to absolute alcohol, and after standing several days, were filtered off, washed with ether and dried at 40°–50° C. The

Kohu preparation should have been dried *in vacuo*, for it proved to be slightly hydroscopic, and instead of remaining a fine white powder, became somewhat brownish. The Uaualoli preparation dried easily to a grayish white, light, fibrous mass.

Tests for starch and reducing sugar were negative on all these substances. Tests for galactans and pentosans were positive in every case. Three-gram samples of the air-dry preparations of Limu Akiaki, Limu Uaualoli and Limu Kohu respectively yielded 0.53 grams, 0.92 grams and 0.64 grams of mucic acid, recrystallized once from ammonium carbonate.[1] The products of hydrolysis in no case contained fermenting sugars. It is evident therefore, that these five preparations from the foregoing Hawaiian seaweeds consisted chiefly of galactans, accompanied by some pentosan-groups. From the frequency with which methyl-pentosans have been shown to occur in all seaweeds previously investigated, it is very likely that they occur in all these varieties and it would be desirable to make tests for methyl-pentosans.

Determinations of the reducing power were made, as shown in the following table:

SPECIES OF SEAWEED.	SUGAR AS DEXTROSE.			
	1 Hour.	2 Hours.	3 Hours.	4 Hours.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Limu Manauea............		41.9	44.6	39.8
Limu Huna..............	43.6	58.4	55.6	30.8
Limu Akiaki.............	36.0	36.0	34.0	

Slippery Elm. For the preparation of the carbohydrate which forms the mucilaginous extract of slippery elm bark, pieces of the latter were torn into narrow strips and allowed to stand over night in cold water,[2] and then the mucilage expressed by squeezing through gauze. This process was repeated until the bark became a mass of separate fibers. The mucilaginous principle swells in cold water to a transparent jelly, but is soluble only to a very limited extent. It was found impossible to filter it, even through gauze, and therefore, although it contained small particles from the disintegrated bark

---

[1] For method cf. Bull. No. 107, p. 55, Bureau of Chemistry, United States Dept. of Agriculture.

[2] It was found impossible to extract the mucilaginous principle in hot water.

fibers, the carbohydrate was precipitated by pouring the thick slimy mass into about six times its volume of 95 per cent alcohol. After standing some hours, a transparent, gelatinous precipitate settled to the bottom, and was filtered off through several thicknesses of gauze. Dehydrated by means of absolute alcohol and ether, it formed a grayish-brown powder. This was found to be soluble in dilute alkali, and was subsequently purified by dissolving in 1 per cent potassium hydroxide, filtering through cotton and reprecipitating with 95 per cent alcohol. The product was somewhat lighter in color than at first, but still far from white. It was soluble in hot Fehling's solution, but precipitable with lead acetate. It gave no color with iodine, although a small amount of starch was present in the original bark.

Furfurol tests were faint showing only traces of pentosans, but the yield of mucic acid was large, 0.15 grams of mucic acid being obtained from 1 gram of the air dry powder.

The products of hydrolysis were dextro-rotatory and contained no fermenting sugars. Hence this preparation consisted chiefly of galactan.

A MANNAN PREPARATION.

Since none of the algae which form the basis of these studies yielded mannan, save Limu Lipoa, and that in amounts inadequate for the experiments proposed, this hemicellulose was obtained in soluble form from salep. Both the small, horny dried tubers and the grayish-white powder made from them, were purchased from Schieffelein & Co., New York.

A preparation of pure mannan was made in the following way: The tubers were soaked in cold water 24 hours, washed thoroughly and ground in a meat chopper. To this mass, cold water was added in large volume, and the whole allowed to stand over night, then the dissolved mannan filtered off through gauze. According to Hilger (254), the extract made in this way should contain no starch. But when the tubers are heated before drying, the starch is made soluble, and in this instance the cold water extract gave a blue color with iodine.[1] Hence subsequent extractions were made with hot water on a water bath, for several hours. The salep swells very much in water so that a very large portion was required to get the mannan all into

---

[1]Salep tubers purchased since this work was done yielded only a trace of starch in the cold water extract.

solution.[1] The extracts, strained through cheese cloth, were digested 24 hours with malt diastase to free from starch, then concentrated to a thick syrup on a water bath, and poured into three times their volume of 95 per cent alcohol. A voluminous, flocculent, and somewhat fibrous, snow-white precipitate formed, which was filtered off, pressed free from alcohol, redissolved in hot water, and reprecipitated. (This was done largely to free it from sugar produced by the digestion of the starch.) It was then transferred to absolute alcohol and allowed to stand three or four days, after which it was washed with ether, and dried in a vacuum desiccator. A somewhat coarse white powder resulted, containing 6.94 per cent moisture and 0.74 per cent ash.[2] It swelled up very readily in water, but dissolved exceedingly slowly to a colorless, semi-transparent mucilaginous solution, which did not reduce Fehling's solution, and examined in the polariscope, after clarification with alumina cream, appeared optically inactive. However, on reprecipitating the carbohydrate with alcohol, and examining the alcoholic filtrate, sugar was found to be present in small amount. A solution absolutely sugar-free became optically active. A sample in which the sugar had been removed by fermentation with yeast, was used to determine the specific rotation. The following results were obtained: (1) A 2 per cent solution in a 200 mm. tube read $-1.59°$; applying corrections for moisture and ash, $[a]_D = -43.1°$. (2) A sample containing in 100 cc. 0.5868 grams mannan dried to constant weight at 105° C. read $-0.48°$; corrected for 0.4 per cent ash, $[a]_D = -43.8°$. According to Thamm (276), salep extract is inactive. In the above experiments, the levo-rotatory nature of the mannan was at first obscured by the presence of traces of reducing sugar formed by the hydrolysis of the starch, which could not be detected by testing directly by Fehling's solution. Thamm, however, in several ways carefully tested salep hydrolysis products for dextrose with negative results, so that the only way to account for these conflicting results seems to be to attribute it to difference in the specimens of Orchis which furnished the mannan.

Salep-extract is readily precipitated by Fehling's solution in flocculent white masses. It is not precipitated by lead acetate in neutral solution (nor, according to Thamm [276], in solutions of other neutral salts), but is precipitated by basic lead acetate.

A furfurol test was faintly positive, verifying the report of traces of pentosans by Tollens and Widtsoe (163), and also by Thamm (276).

---

[1] 15 liters of water to 100 grams salep powder, according to Thamm (276).
[2] Thamm found 0.483 per cent.

The products of hydrolysis were dextro-rotatory and contained sugar fermentable with yeast. A rich yield of mannose-hydrazone was obtained with phenyl-hydrazine acetate, melting on recrystallization at 188° C. According to Thamm (276), salep extract yields exclusively mannose on complete hydrolysis.

Hydrolyzed for three hours, the reducing power of this mannan was 91.6 per cent.

Determinations of ash, moisture, starch, and mannan were made on the salep obtained in the form of a powder. Starch and mannan were determined as follows: 1 gram of air dry powder was boiled in 250 cc. water, and after cooling to 37.5° C., the starch hydrolyzed with malt diastase, dialyzed sugar-free. The solution was then filtered, concentrated to small volume, and the mannan precipitated with absolute alcohol. The precipitate was filtered off, dissolved in a little water and reprecipitated, to obtain any sugar retained in the first precipitation. The mannan was then dried at 100° C. and weighed. The filtrates were combined, freed from alcohol, hydrolyzed with 2 per cent hydrochloric acid 45 minutes to convert all the maltose to dextrose, and sugar determined by Allihn's method. The results of these analyses are shown in the following table:

	Per cent		Per cent
Moisture	0.77	Starch	26.4
Ash	8.9	Mannan	19.5

According to Dragendorf[1] the composition of Orchis tubers is as follows:

	Per cent		Per cent
Starch	27.3	Protein	4.9
Mucilage	48.1	Cellulose	2.4
Sugar	1.2		

Thamm also reports a yield of 40–45 per cent mucilage from the salep powder used in his investigations. Hence the powder used in this the present experiment was for some reason very deficient in mannan.

Its reducing power was as follows:

TIME OF BOILING. Hours.	SUGAR AS DEXTROSE. Per cent
2	74.2
3	75.8
5	75.8

[1]Cited in the National Dispensatory (1884), also by Thamm (276).

A LEVULAN PREPARATION.

Commercial Squills, consisting of the dried and broken leaves of the bulbs of Scilla maritima (or *Urginea Scilla Stenh.*) yield, as discovered by Schmiedeberg (318), the levulan sinistrin. They were finely ground in a coffee mill, and the sinistrin prepared according to Schmiedeberg's directions. To the dry powder sufficient water was added to make a thin cream, and then a saturated lead acetate solution until further addition produced no precipitate. To the clear, straw-colored filtrate, freed from lead with hydrogen sulphide, was added freshly prepared milk of lime, with constant stirring, until a somewhat creamy consistency was produced. To facilitate the formation of sinistrin-calcium carbonate, this mixture was concentrated on the water bath for some time (as suggested by Reidemeister) [314]. The precipitate was then sucked dry on a Büchner funnel, washed thoroughly with cold water (being rubbed up in a mortar for the purpose), again sucked dry, rubbed to a cream with water, and treated with carbon dioxide until the fluid was no longer alkaline to litmus. After heating to facilitate the complete separation of the calcium carbonate, the sinistrin in solution was filtered off, a little oxalic acid carefully added to remove the last traces of lime, and the solution then decolorized with charcoal, and evaporated to a syrup at a temperature of about 40° C. From this solution the sinistrin was precipitated with 95 per cent alcohol, as a white gummy mass. Transferred to absolute alcohol, and allowed to stand 24–36 hours it became very tenacious, but on longer standing, with occasional stirring, it grew brittle, and finally crumbled to a coarse white powder, which was dried in a vacuum desiccator. This material was readily soluble in cold water. (According to Schmiedeberg [318], even solutions of 20–30 per cent are not syrup-like.) It gave no color with iodine, did not reduce Fehling's solution, and was not precipitated by it. This preparation, at first, contained 13 per cent moisture and 0.76 per cent ash. Determination of the specific rotation then gave the following results: A 2 per cent solution in a 200 mm. tube, read −1.32°; corrected for moisture and ash, $[a]_D = -38.2°$. After longer standing (three months) over sulphuric acid, the moisture content was 4.8 per cent, and determination of specific rotation gave the following results: A 1 per cent solution in a 200 mm. tube, read −0.55°; corrected for moisture and ash, $[a]_D = -29.1°$. Schmiedeberg (318) found the average for $[a]_D = -41.4°$, and Reidemeister (314), $[a]_D = -34.6°$. It is impossible to account for these differences. Reidemeister claims

that the rotation increases on standing, but in these solutions there was no change in 48 hours, at room temperature.

On hydrolysis, sinistrin yields a levo-rotatory, reducing sugar, fermenting with yeast. Schmiedeberg (318) reports this as a mixture of levulose and an inactive sugar, but Reidemeister (314) declares that it is neither a mixture of levulose and an inactive sugar, nor of levulose and dextrose, in spite of the fact that he found for it $[a]_D = -88°$, while for levulose, $[a]_D = -106°$, a difference for which he is unable to account.

SUMMARY.

The composition of the preparations which have been described is best shown in the following table:

SOURCE OF MATERIAL.	NATURE OF CARBOHYDRATES PRESENT.				
	Pentosans.	Galactan.	Mannan.	Levulan.	Dextran.
Dulse (*Rhodymenia Palmata*)	+				
Limu Lipoa (*Haliseris Pardalis*)	+				
Limu Eleele (*Enteromorpha intestinalis*)	+				
Limu Pahapaha (*Ulva lactuca*, etc.)	+				(Starch)
Irish Moss (*Chondrus crispus*)	Trace	+		+	+
Limu Manauea (*Gracilaria coronopifolia*)	+	+			
Limu Huna (*Hypnea nidifica*)	+	+			
Limu Akiaki (*Ahnfeldtia concinna*)	+	+			
Limu Uaualoli (*Gymnogongrus*)	+	+			
Limu Kohu (*Asparagopsis sanfordiana*)	+	+			
Slippery Elm (*Ulmus*)		+			
Salep (*Orchis.*)	Trace		+		
Squills (*Urginea scilla*) [Sinistrin]				+	

The foregoing observations correspond with those of König and Bettels (8), in that the marine algae all yield pentosans, and frequently galactans. The gelatinizing principle in every case appears to be due to the galactan groups. No specific tests have been applied

for fructose, the polysaccharide of which also appears to be common in algae, but the absence of fermenting sugar in all the algae except Limu Lipoa, indicates that if present, it is in too small amount to be detected in the hydrolysis products of 5–10 grams of crude material.

The reducing power has been determined on each substance used in feeding experiments; the results of all determinations are summarized in the following table:

SUBSTANCE.	SUGAR AS DEXTROSE AFTER BOILING.						
	1 Hour.	2 Hours.	3 Hours.	4 Hours.	5 Hours.	6 Hours.	8 Hours.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Dulse..........................		87.2	87.3	89.4	89.5.		
Limu Lipoa....................			14.3	14.7		12.9	12.8
Limu Eleele...................		16.8	16.9	18.1	16.8		
Limu Pahapaha................		28.8		31.8			
Irish Moss....................		45.6	48.6	45.8			
Limu Manauea.................		41.9	44.6	39.8			
Limu Huna....................	43.6	58.4	55.6	30.6			
Limu Akiaki...................	36.0	36.0	34.0				
Salep (Powder)................		74.2	75.8		75.8		
Salep (Pure mannan)...........			91.6				

BACTERIOLOGICAL INVESTIGATIONS.

INTRODUCTION.

It is an accepted fact that even cellulose, with its high powers of resistance, is to some extent decomposed in the alimentary tract by bacteria. It is therefore reasonable to expect that the less resistant hemicelluloses will also be attacked and decomposed by bacteria. The object of these experiments has been to throw some light on the problem as to what organisms are most likely to effect such a decomposition, and whether there is an appreciable production of sugar as a result of bacterial activity. The four classes of hemicelluloses under special investigation have been represented by the following substances:

Pentosans......Dulse.   Mannans........Salep.

Galactans......{ Irish Moss.   Levulans........Sinistrin.
                { Limu Manauea.

Both aerobic and anaerobic cultures have been made, in neutral, faintly alkaline, and faintly acid reaction, with solutions made from the carbohydrates alone, and with the addition of small amounts of such nutrients as beef extract or peptone to facilitate the growth of the organisms.

Anaerobic cultures in test tubes have been made by the Wright method; anaerobic cultures in Erlenmeyer flasks, by passing a stream of hydrogen through for half an hour, and then sealing hermetically.

The aerobes which have been employed all occur in the human digestive tract. Both aerobic and anaerobic cultures from the faeces of human subjects have also been used, in conjunction with soil bacteria from street sweepings.

Tests for the presence of reducing sugar have been made by precipitating the carbohydrates in solution with absolute alcohol, evaporating the alcoholic extract to dryness, taking up the residue in 2 or 3 cc. of water, and boiling two minutes with Fehling's solution.

Suitable controls have been used in all cases.

TRIALS WITH PURE CULTURES OF AEROBES.

One per cent solutions of the preparations from dulse, Irish moss and salep, neutral, acid, and alkaline in reaction, and consisting of, (1) pure carbohydrate; (2) carbohydrate plus $\frac{1}{4}$ per cent beef extract and $\frac{1}{4}$ per cent sodium chloride; (3) carbohydrate plus 1 per cent peptone and $\frac{1}{4}$ per cent sodium chloride, have been used as culture media. Five cc. portions of each of these solutions were placed in test-tubes with a pipette, and inoculated with the following organisms: B. Coli communis, B. Pyocyaneus, B. Prodigiosus, B. Proteus vulgaris, B. Pyogenes foetidus.

To approximate the conditions in ordinary digestion of these carbohydrates, they were incubated for three days at a temperature of 37.5° C. At the end of this time, nearly all gave evidence of some bacterial growth. Salep-peptone cultures of B. Pyocyaneus showed a brilliant green; salep solutions containing B. Pyogenes foetidus, and B. Coli in alkaline-beef extract media, had changed from transparent colorless solutions to an opaque white jelly insoluble in water.

The carbohydrates were then precipitated with alcohol, and after standing several days were compared with controls similarly prepared, to see whether any change could be observed in the nature or amount of carbohydrate. The results were in all cases negative. These precipitates were then transferred to small folded filter papers of uniform

weight, previously prepared. The alcoholic filtrates were tested for sugar; the precipitates were dried, and their weight compared with that of the control. It was thought that this rather crude method would show whether any considerable amount of the carbohydrate had disappeared. The results were so largely negative that weighings of every precipitate were not made. There seemed to be a slight loss of dulse, in some of the cultures of B. Proteus vulgaris, B. Pyogenes foetidus, and B. Coli communis, but repetition of these experiments allowing the organisms in question to grow two weeks, not only in dulse but also in salep media, did not justify any conclusion that an appreciable amount of carbohydrate had disappeared.

All tests for reducing sugar were negative.

Four per cent solutions of Irish moss, and two per cent solutions of limu manauea were then prepared, with reactions and additions of nutrient material as described in the first series of experiments. These formed firm jellies, which were used to study the possibility of lique-faction or gas formation. Stab cultures were made, and grown at a temperature of 25°–30° C. for one to three weeks. No liquefaction or gas formation was observed in any case.

TRIALS WITH MIXTURES OF AEROBES.

Mixtures of B. Pyocyaneus, B. Prodigiosus, B. Proteus vulgaris, and B. Pyogenes foetidus, were used, also mixtures of faecal and soil bacteria. These were first inoculated into nutrient bouillon, the former from pure cultures, the latter from human faeces and street sweepings, and incubated 24 hours. Five cc. portions of these cultures were then introduced into 50 cc. of neutral solutions of each of the different carbohydrates, in small Erlenmeyer flasks, and these cultures allowed to grow for four weeks at 37.5° C. At the end of this time, no marked change had taken place save in the salep culture of B. Pyocyaneus, B. Proteus vulgaris, B. Pyogenes foetidus and B. Prodigiosus. This had changed from a colorless, semi-transparent, slightly mucilaginous fluid, to a firm, white opaque jelly, insoluble in water, but readily soluble in dilute alkali; a phenomenon already observed with this carbohydrate in cultures of B. Coli communis and B. Pyogenes foetidus. No liquefaction had taken place with Irish moss nor limu manauea.

The carbohydrates were then precipitated with alcohol, the alcoholic extracts tested for sugar, and the precipitates hydrolyzed by boiling with 2 per cent hydrochloric acid, neutralized, made up to a

definite volume, and examined in a polariscope. The results of these experiments are shown in the following table. Mixtures of B. Pyocyaneus, B. Prodigiosus, B. Proteus vulgaris and B. Pyogenes foetidus are designated A, and mixtures of faecal and soil bacteria, B.

SUBSTANCE	BACTERIAL CULTURE	REDUCTION OF FEHLING'S SOLUTION.	ROTATION AFTER HYDROLYSIS.	
			Experiment.	Control.
Dulse.............	B	....	+0.13°	+0.20°
Irish Moss.........	A	....	+0.20°	
Irish Moss.........	B	....	+0.27°	+0.20°
Limu Manauea	A	....	Not determined.	
Salep	A	....	Not determined.	
Salep	B	+	+0.17°	+0.20°
Sinistrin...........	A	....	−0.97°	−0.97°

The action of putrefactive organisms upon the dulse preparation was also studied, according to the method used by Slowtzoff (154) in the case of xylan. One hundred grams of chopped lean beef and 10 grams of sodium carbonate were added to 1 liter of water, and the mixture allowed to stand in a warm place for three days. Two hundred and fifty cc. were then removed for a control, and to the remainder 0.5 gram of dulse was added. This solution gave a strong pentosan reaction; the control was pentosan-free. The two solutions were put in a warm place, and tested daily for pentosans. After five days' digestion, the reaction of the dulse solution was very much fainter than at first, but it did not entirely disappear till the twelfth or thirteenth day. Slowtzoff found that xylan disappeared in nine or ten days, but his solution was kept at a temperature of 40° C., while these mixtures remained at a temperature of from 30° to 35° C., a condition less favorable for rapid decomposition.

Solutions of Irish moss were digested with faecal mixtures in the following manner: Human faeces were rubbed to a mud with water. Ten cc. portions of this material were added to flasks containing 50 cc. of a 1 per cent "moss" solution, and allowed to digest in a warm place for 24 hours. A portion of water inoculated in the same way was used as a control. Small portions of these solutions were then evaporated nearly to dryness, extracted with alcohol, and tested for reducing sugar. The results were wholly negative.

That limu manauea is not entirely resistant to the action of putrefying organisms is shown by the following: A solution was made

up to contain 2 per cent of the air dry extract, 1 per cent peptone, ¼ per cent beef extract and ¼ per cent sodium chloride. This could be filtered through paper only on a hot, water-jacketed funnel, from which it dropped as a clear, amber-colored jelly. After standing unsterilized over night in a warm room, this was found to be entirely broken up by the formation of gas throughout the whole mass. The reaction, which had been neutral, was now acid to litmus. This material was placed in a flask and allowed to stand for two months, at the end of which time, the greater portion was liquefied, the former lumps of jelly being reduced to small particles distributed throughout the liquefied portion. Alcoholic extracts did not reduce Fehling's solution. A sterile preparation of the plain manauea extract in test tubes was inoculated with some of this material, but without producing the same striking results. There were evidences of growth, but none of liquefaction or gas formation, in the course of two weeks.

TRIALS WITH ANAEROBES.

The action upon Irish moss of pure cultures of the powerful putrefactive organisms B. Putrificus, Bienstock, B. Maligni œdematis, and B. Anthracis symptomatici, was tried in the following way. A 4 per cent solution of the moss was prepared, which would not become liquefied at a temperature of 30°–35° C. From this material culture media were prepared, neutral, alkaline, and acid in reaction, using the solution plain, and with the addition of ¼ per cent beef extract and ¼ per cent salt, or 1 per cent peptone and ¼ per cent salt. Test tubes were inoculated from fresh, active cultures, and the organisms allowed to grow for one to three weeks, being examined at first daily, and later every three or four days, for liquefaction and gas formation. The results were negative in all cases, save that in the peptone media an occasional small bubble was seen, with cultures of the bacilli of malignant œdema and symptomatic anthrax. However, the same phenomena were observed in peptone-agar tubes used as controls.

Mixtures of B. Anthracis symptomatici and B. Maligni œdematis were tried upon solutions of dulse, Irish moss, salep and sinistrin, in the following way: Small Erlenmeyer flasks containing 50 cc. of 1 per cent solutions of each of these carbohydrates, and 5 cc. of ordinary nutrient bouillon, were inoculated with fresh cultures of these organisms, rendered anaerobic, and incubated for four weeks at 37.5° C. On inspection, no change was apparent. The carbohydrates were removed, the alcoholic extracts examined for reducing sugar, and

the carbohydrate residues hydrolyzed and examined in the polari-scope, as in similar trials with aerobes. The results are shown in the following table:

NAME OF SUBSTANCE.	REDUCTION OF FEHLING'S SOLUTION.	ROTATION AFTER HYDROLYSIS.	
		Experiment.	Control.
Dulse................................		Lost by	accident
Irish Moss...........................		+ 0.24°	+ 0.20°
Salep................................	+	+ 0.13°	+ 0.20°
Sinistrin............................	+	− 0.27°	− 0.97°

Mixtures of soil and faecal bacteria were also tried, the experiments being carried out just as described for mixtures of the bacilli of symptomatic anthrax and malignant œdema. The results are shown in the following table:

NAME OF SUBSTANCE.	REDUCTION OF FEHLING'S SOLUTION.	ROTATION AFTER HYDROLYSIS.	
		Experiment.	Control.
Dulse................................		+ 0.13°	+ 0.20°
Irish Moss...........................		+ 0.20°	+ 0.20°
Salep................................	+	+ 0.03°	+ 0.20°

DISCUSSION AND SUMMARY.

It seems reasonable to expect, that if the hemicelluloses used in these trials were readily attacked by micro-organisms, there would have been some evidence of change in three days, if conditions for growth were favorable as regards reaction and temperature; but although the concentration of the solutions was moderate, the reaction varied, and temperature 37.5° C., results were negative, even in the cases where nutrients were added to facilitate bacterial growth. Apparently all of the material was recovered in unaltered condition, save in certain instances where salep underwent an insoluble modification.

In trials where the cultures were allowed to grow from one to three weeks, no difference in the results could be detected, by the methods employed. In solid media there was no liquefaction and practically no gas formation, except in the case of the peptone-beef extract preparation of limu manauea, on exposure to the air.

Marked evidences of change were observed in one trial with a putrefactive mixture (on dulse), and in some of the four-week cultures.

Irish moss was the most thoroughly investigated and proved the most resistant. In the long experiments (4 weeks) where the other carbohydrates suffered more or less change this one remained apparently unaltered. The results of this series are summarized in the following table:

Irish Moss.

CULTURES USED.	REDUCTION OF FEHLING'S SOLUTION.	ROTATION OF UNALTERED CAR-BOHYDRATE AFTER HYDROLYSIS.	
		Irish Moss.	Control.
Mixture of Pure Aerobes.............		$+0.20°$	$+0.20°$
Mixture of Faecal and Soil Bacteria (aerobic)...........................		$+0.27°$	$+0.20°$
Mixture of Bacilli of Malignant Oedema and Symptomatic Anthrax..		$+0.24°$	$+0.20°$
Mixture of Faecal and Soil Bacteria (anaerobic)........................		$+0.20°$	$+0.20°$

The single experiment with the galactan, limu manauea, under the same conditions, with the mixture of pure aerobes, gave similar results, but the fact that liquefaction occurred in the peptone-beef extract culture medium after exposure to the air, shows that general conclusions as to the behavior of galactans cannot be drawn from study of a single representation of the class. We have, however, further proof that the galactans are not easily decomposed by bacteria, in the fact that aqueous solutions of all the galactans included in the present series, could be left several days in the warm atmosphere of the laboratory without any apparent change taking place; and in the fact that agar-agar, so widely used in bacteriological laboratories on account of its indifference to bacterial action, is a member of the galactan group. It has been suggested[1] that extracts of other seaweeds might prove good substitutes for agar-agar as culture media, if fully investigated. So far, the greatest objection to use of Irish moss in this way is that it tends to liquefy at body temperature; strong solutions (4 per cent) can, however, be kept fairly firm at a

[1]Cf. Reed (18).

temperature of 30° C. The extract of limu manauea is free from these objections, but extensive experiment is still necessary to demonstrate its powers of resistance.

The soluble dulse pentosan is certainly decomposed not only by putrefactive organisms under the most favorable conditions (*e.g.*, in meat mixtures), but by aerobes and anaerobes in solutions where the carbohydrate is the chief source of nutriment. The results of the four weeks' digestions are summarized in the following table:

Dulse.

CULTURES USED	REDUCTION OF FEHLING'S SOLUTION.	ROTATION OF UNALTERED CARBONYDRATE AFTER HYDROLYSIS.	
		Dulse.	Control.
Mixture of Faecal and Soil Bacteria (aerobic)	....	+0.13°	+0.20°
Mixture of the Bacilli of Malignant Oedema and Symptomatic Anthrax..	....	(Lost by Accident)	+0.20°
Mixture of faecal and Soil Bacteria (anaerobic)	....	+0.13°	+0.20°

In the present studies, this pentosan stands second to the galactans in degree of resistance.

Sawamura (267) thought that he observed a slight hydrolysis of mannan by B. Prodigiosus, an observation which has not been verified in these experiments. No reducing substance was detected in the three-day cultures nor the four-weeks cultures, in which this organism was present. The opaque jelly, insoluble in water, formed from salep by the action of B. Coli communis, B. Prodigiosus, and mixed cultures containing these organisms, resembles an intermediary product of the acid hydrolysis of salep-mannan described by Thamm (276). He isolated and examined two such products, one forming an opalescent solution in water, the other insoluble, but passing over into the soluble form by treatment with dilute alkali; both were anhydrides of mannose. It seems reasonable to inquire whether this insoluble material produced by bacterial action may not be regarded as an early stage in the hydrolysis of the carbohydrate under consideration, especially in view of the fact that in all the other four-week trials a very definite reduction of Fehling's solution was noted, corresponding

in strength with the loss of unaltered carbohydrate, as shown in the following summary:

Salep.

CULTURES USED.	REDUCTION OF FEHLING'S SOLUTION.	ROTATION OF UNALTERED CARBO-HYDRATE AFTER HYDROLYSIS.	
		Salep.	Control.
Mixture of Pure Aerobes.............	(Insoluble jelly)	Not determined	
Mixture of Faecal and Soil Bacteria (aerobic)...........................	+	+ 0.17°	+ 0.20°
Mixture of the Bacilli of Malignant Oedema and Symptomatic Anthrax..	+	+ 0.13°	+ 0.20°
Mixture of Faecal and Soil Bacteria (anaerobic)........................	+	+ 0.03°	+ 0.20°

These experiments give some grounds for expecting the hydrolysis of salep in the alimentary tract, through the action of bacteria.

Two experiments with sinistrin gave the following results:

Sinistrin.

CULTURES USED.	REDUCTION OF FEHLING'S SOLUTION.	ROTATION OF UNALTERED CAR-BOHYDRATE AFTER HYDROLYSIS.	
		Sinistrin.	Control.
Mixture of Pure Aerobes............		− 0.97°	− 0.97°
Mixture of Bacilli of Malignant Oedema and Symptomatic Anthrax.........	+	− 0.27°	− 0.97°

Sinistrin is therefore hydrolyzed by the anaerobic putrefactive organisms, but further experiments are necessary to determine how readily this change takes place.

PHYSIOLOGICAL INVESTIGATIONS.

In the physiological experiments, attempts have been made to answer the following questions: (1) To what extent are hemicelluloses digested by animal and vegetable enzymes? (2) Can they be absorbed and utilized without intervention of the alimentary tract?

(3) Do they reappear in the faeces after administration *per os?* The various experiments will accordingly be discussed in these three groups: (1) Trials with Enzymes; (2) Parenteral Trials; (3) Feeding Experiments.

TRIALS WITH ENZYMES.

Approximately 1 per cent solutions of the various hemicelluloses (with the exception of Limu Lipoa, which was finely ground and suspended in water), have been digested for 24 hours at 37.5° C. in the presence of toluene, with the following enzymes: (1) Filtered human saliva. (2) Malt diastase, dialyzed sugar-free. (3) "Taka" diastase (*Eurotium oryzae*). (4) Chloroform extract of pig's pancreas. (5) Fresh pancreatic juice of dogs. (6) Chloroform water extract of dog's intestines. (7) Glycerol extract of pig's stomach.

Digestions have also been made with 0.2 per cent hydrochloric acid, to determine whether any of the action of the artificial gastric juice might be due to the acid present. The activity of the amylolytic enzymes has always been tested first with starch paste, and that of the gastric extract with fibrin. Boiled controls have been employed in every instance, and all trials have been made in duplicate.

Tests for reducing sugar have been conducted in the following manner: At the end of 24 hours the solutions were evaporated to thick syrups on the water bath, to free from toluene and to concentrate so that the undigested hemicelluloses could be readily precipitated by absolute alcohol. The alcoholic extracts were filtered off and evaporated to dryness; the residues were taken up in a few drops of water and tested for sugar with Fehling's solution. The results of all digestion trials are shown in the table on opposite page.

PARENTERAL INJECTIONS.

Methods and Technique.

Small dogs were used for all injections, after a confinement in cages long enough to obtain samples of normal urine. The carbohydrates employed in these experiments were preparations of dulse,[1] Irish moss,[2] salep,[3] and sinistrin.[4] They were introduced *subcutaneously*, by means

---

[1]Cf. p. 303.
[2]Cf. p. 308.
[3]Cf. p. 312.
[4]Cf. p. 315.

HEMICELLULOSE.		ENZYME.							
CLASS.	SOURCE.	SALIVA.	MALT DIASTASE.	"TAKA" DIASTASE.	PANCREATIC EXTRACT.	PANCREATIC JUICE.	INTESTINAL EXTRACT.	GASTRIC EXTRACT.	0.2 PER CENT. HCl.
Pentosan......	Dulse	—	—	+	—				—
Pentosan......	Limu Lipoa	—	—	—	—	—		—	—
Galactan......	Irish Moss	—	—	—		—	—	—	—
Galactan......	Limu Manauea	—	—	—		—	—		+
Galactan......	Limu Huna	—	—	—		—	—	+	—
Galactan......	Limu Akiaki	—	—	—		—	—		+
Galactan......	Limu Uaualoli	—	—	—		—	—	+	—
Galactan......	Limu Kohu	—	—	+	—	—	—		+
Galactan......	Slippery Elm	—	—	—		—	—	—	+
Mannan.......	Salep	—	—	(+ in 3 days)		—		+	—
Levulan......	Sinistrin	—	—	+		—	—		+ + (½ hr. at 37° C.)

of a syringe, or *intraperitoneally*, by means of a needle and burette with pressure-bulb attached, always under aseptic conditions. After receiving injections, the animals were replaced in cages, and the urine collected under toluene. The excess of toluene was removed, at the time of examination, by means of a separatory funnel, and the urine measured, filtered, and tested for reducing substances with Fehling's solution.

Qualitative tests for the carbohydrates were made in the following manner: (1) for dulse and salep, by boiling a few drops of urine with Fehling's solution, from which these hemicelluloses were precipitated in fine white flocks, even if only traces were present; (2) for Irish moss, by the reduction of Fehling's solution after hydrolysis of the urine with dilute hydrochloric acid;[1] (3) for sinistrin, by the marked increase in the levo-rotation of the urine.

Isolation of the carbohydrates was accomplished by freeing the urine from inorganic salts with lead acetate, removing the excess of lead with hydrogen sulphide, and concentrating the salt-free solutions to a small volume. Dulse and Irish moss were then precipitated with absolute alcohol; salep with alcohol or Fehling's solution; sinistrin with milk of lime, being freed from its calcium compound by the method used in its preparation.[2]

These substances were identified as carbohydrates, by their yielding reducing sugar on hydrolysis; salep and sinistrin were further identified by their levo-rotation, Irish moss by testing for mucic acid, and dulse by testing for furfurol.

Quantitative determinations of dulse, salep and sinistrin were made by polariscopic examination in a 200 mm. tube, all samples of urine being clarified with equal volumes of alumina cream. A satisfactory quantitative method for the determination of Irish moss was not developed. It proved impossible to estimate any of these carbohydrates quantitatively by the method of acid hydrolysis. In some instances, especially with Irish moss, a trace of reduction was obtained, but in most cases, the results were negative, although the hemicellulose was known to be present.[3]

---

[1] Trial was made of Bauer's method (Zeitschrift für physiologische Chemie, 51, p. 158, 1907) of determining galactose in urine as mucic acid, by concentrating 100 cc. of urine with 25–35 cc. of concentrated nitric acid (sp. gr. 1.4) to a volume of 20 cc., but owing probably to the low percentage of galactose from the small amount of Irish moss present, this test was unsatisfactory.

[2] Cf. p. 315.

[3] Samples were removed and tested every half hour for $2\frac{1}{2}$ hours. At the end of 1 hour they were usually neutral, or slightly alkaline in reaction. Addition of suf-

INJECTIONS OF DULSE.

1. *Subcutaneous.*

A dog weighing 11 kg. received 60 cc. of a dulse solution containing 0.9 grams of pure substance. No reduction of Fehling's solution was observed at any time. The time and rate of dulse excretion are shown in the following table:

Examination of Urine.

TIME.	VOLUME.	ROTATION.	ESTIMATED EXCRETION OF DULSE.*
	cc.		Grams.
February 1, 12:30 P.M..........		−0.14°†	
February 1, 1 P.M.............	Injection		
February 2, 10 A.M.............	226	− 0.62°	0.61
February 3, 10 A.M.............	250	− 0.55°	0.57
February 4, 10 A.M.............	150	− 0.41°	0.21
February 5, 10 A.M.............	210	− 0.34°	0.21
February 6, 10 A.M.............	310	− 0.28°	0.04
February 7, 10 A.M.............		− 0.20°	
		Total........	1.64

2. *Intraperitoneal.*

The same dog received in this experiment 75.6 cc. of a dulse solution containing 1.4 grams of pure substance. No reduction of Fehling's solution was observed before or after the injection. The time and rate of dulse excretion are shown in the following table:

Examination of Urine.

TIME.	VOLUME.	ROTATION.	ESTIMATED EXCRETION OF DULSE.*
	cc.		Grams.
December 3, 2 P.M.............		−0.14°†	
December 3, 3 P.M.............	Injection		
December 4, 10 A.M.............	133	− 0.62°	0.36
December 5, 10 A.M.............	200	− 0.52°	0.42
December 5, 12 M..............	115	− 0.28°	0.05
December 6 and 7.............	383	− 0.48°	0.69
December 8, 10 A.M............	520	− 0.28	0.24
December 9, 10 A.M............	350	− 0.20	
		Total......	1.76

ficient hydrochloric acid to make the strength 2 per cent caused no subsequent production of sugar:

* All readings have been taken on the Ventzke scale, and calculated as angular degrees.
† Estimating normal rotation of urine as −0.17° (average).

In both these experiments, the presence of dulse was readily detected by Fehling's solution in every urine which showed a high rotation. From the samples of the first 48 hours after injection, a considerable amount was isolated and identified as carbohydrate. It is evident that the excretion of this pentose-carbohydrate is gradual, commencing soon after the injection, and continuing from four to five days. While any quantitative estimate of the amount excreted, based on the changes in rotation, is subject to a high percentage of error, owing to normal fluctations in the rotation of the urine, as well as to analytical discrepancies unavoidable in dealing with solutions containing only minute quantities of the substance under investigation, it is evident that most of the dulse must have been excreted, and that, too, without any essential change in character.

INJECTIONS OF IRISH MOSS.

1. *Subcutaneous.*

A dog weighing 9.4 kg. received 100 cc. of Irish moss solution, containing 1.5 grams of dry substance. No reducing substance occurred in the urine. Changes in rotation, due to the injection, are shown in the following table:

Examination of Urine.

TIME.	VOLUME.	ROTATION.	IRISH MOSS.
	cc.		
May 18, 9 A.M......................		$-0.04°$	—
May 18, 4 P.M......................	Injection		
May 19, 9 A.M	128	$+0.34°$	—
May 20, 9 A.M......................	226	$+0.06°$	—
May 21, 11 A.M.....................	330	$-0.20°$	—
May 22, 9 A.M......................	370	$-0.14°$	—

Tests for Irish moss on May 19th were negative, but on May 20th–22nd they were faintly positive. The experiment was discontinued at this point. The injection was not very well borne, the dog remaining lethargic throughout the period.

2. *Intraperitoneal.*

Experiment A. A dog weighing 10 kg. received 160 cc. of an Irish moss solution containing 1.3 grams air dry material. Examination

for the presence of carbohydrate was made by testing the urine for reducing substances, before and after hydrolysis. The results are shown in the following table:

Examination of Urine.

TIME.	VOLUME.	REDUCTION OF FEHLING'S SOLUTION.	
		Before Hydrolysis.	After Hydrolysis.
	cc.		
October 13, 11 A.M....................		—	—
October 13, 12:30 P. M...............	Injection		
October 13, 2 P.M....................	27	—	—
October 13, 5 P.M....................	60	—	+
October 14, 9 A.M....................	450	—	+
October 15, 5 P.M....................	45	—	—
October 16, 9:30 A.M.................	—	—	—

The urine before the injection showed a rotation of −0.14°, a sample of the mixed urines of October 13, 5 P.M., and October 14, 9 A.M., showed a rotation of −0.034°, the diminished levo-rotation undoubtedly due to the presence of this dextro-rotatory carbohydrate. On hydrolysis, 50 cc. of this mixed sample yielded sugar equivalent to 0.035 grams of dextrose (by Allihn's method). From the remainder of this sample, Irish moss carbohydrate was isolated; it formed a grayish-white powder, swelling in water, and yielding mucic acid on oxidation with nitric acid.

Experiment B. A dog weighing 9 kg. received intraperitoneally 100 cc. of a 2 per cent solution of Irish moss preparation. Examination for carbohydrate was made as in the preceding experiments. The results appear in the following table:

Examination of Urine.

TIME.	VOLUME.	REDUCTION OF FEHLING'S SOLUTION.	
		Before Hydrolysis.	After Hydrolysis.
	cc.		
October 30, 1 P.M....................		—	—
October 30, 2:30 P.M.................	Injection	—	—
October 31, 9 A.M..................	250		
November 1, 10 A.M.................	200	—	+
November 2, 10 A.M.................	115	—	—

Irish moss was isolated and identified in the urine of November 1st.

338 *Mary Davies Swartz,*

INJECTIONS OF SALEP.

1. Subcutaneous.

A dog weighing 7.2 kg. received 56 cc. of salep solution, containing 0.75 grams of pure mannan. No reducing substance was found in the urine. The changes in rotation, due to salep, are shown in the following table:

Examination of Urine.

TIME.	VOLUME.	ROTATION.	ESTIMATION OF AMOUNT OF SALEP EXCRETED.
	cc.		*Grams.*
May 17,............................		$- 0.17°$	
May 18, 3:30 P.M.................	Injection		
May 19, 9 A.M....................			
May 20, 9 A.M....................	138	$- 0.27°$	0.3
May 21, 9 A.M....................	132	$- 0.27°$	0.3
May 22, 9 A.M....................	114	$- 0.20°$	0.04
May 22, 5 P.M....................	127	$- 0.14°$	

Salep was isolated and identified in the urines of May 20, 21, and 22.

2. Intraperitoneal.

Experiment A. A dog weighing 7 kg. received 68 cc. of salep solution, containing 1.2 grams of air dry mannan. No reducing substance was present in the urine at any time. Tests for the presence of salep by means of Fehling's solution, gave the following results:

Examination of Urine.

TIME.	VOLUME.	SALEP PRESENT.
	cc.	
October 21, 12 M........................		
October 21, 2:30 P.M....................	Injection	
October 22, 9 A.M.......................	125	+
October 23, 9 A.M.......................	190	+
October 24, 9 A.M.......................	140	—

The salep was easily isolated and identified in the urine of October 22 and 23, the sugar obtained on hydrolysis being equivalent to 0.33 grams salep.

Experiment B. A dog weighing 9.2 kg. received 80 cc. of salep so-

lution, containing 1.4 grams of air dry substance. No reducing substance was detected in any of the urines. Tests for salep with Fehling's solution gave the following results:

Examination of Urine.

TIME.	VOLUME.	SALEP PRESENT.
	cc.	
October 24, 11 A.M.		
October 24, 12 M.	Injection	
October 25, 12 M.	155	+
October 26, 10 A.M.	180	+
October 27, 10 A.M.	180	+
October 28, 10 A.M.		—

From the urine of October 25, salep was isolated, which yielded on hydrolysis 0.39 grams reducing sugar as dextrose; it was also isolated from the urines of the next two days, but was not estimated quantitatively.

Experiment C. A dog weighing 9.2 kg. received 90 cc. of salep solution, containing 1.8 grams of pure mannan. No reduction of Fehling's solution occurred with any of the samples. Tests for salep with Fehling's solution gave the following results:

Examination of Urine.

TIME.	VOLUME.	ROTATION.	SALEP PRESENT.
	cc.		
December 2, 10 A.M.		−0.17°	
December 2, 2:30 P.M.	Injection		
December 3, 10 A.M.	960		+
December 4, 10 A.M.	234	− 0.41°	+ (0.6 gm.)
December 5, 10 A.M.	520	− 0.27°	+ (0.5 gm.)

Unfortunately this experiment was unavoidably interrupted at this point. The salep was precipitated from 50 cc. of the urine for December 3, hydrolyzed, and sugar determined gravimetrically as dextrose, from which the total amount of salep in this day's urine was calculated as 0.67 gram. Salep determined in the same way on December 4, showed an elimination of 0.18 gram; hence 0.85 gram was actually recovered in these two days. The influence of the levorotatory carbohydrate on the rotation of the urine was marked.

Experiment D. A dog weighing 6.4 kg. received 98 cc. of salep solution containing 1 gram of pure mannan. No reduction of Fehling's solution was observed throughout the experiment. The changes in rotation due to the salep are shown in the following table:

Examination of Urine.

TIME.	VOLUME.	ROTATION.	SALEP PRECIPITATED BY FEHLING'S SOLUTION.
	cc.		
January 31......................	116	− 0.41°	
February 1.....................	Injection		
February 2.....................	152	− 0.41°	—
February 3.....................	238	− 0.13°	+
February 4.....................	154	− 0.13°	—
February 5.....................	137	− 0.20°	—

The results in this experiment are very puzzling. The normal rotation was high (−0.41°) for several weeks before this experiment but fairly constant, averaging −0.44°. If salep were excreted as mannan, the levo-rotation should have increased, yet it was decidedly low on a day when salep was shown to be present, and also on a day when none could be detected. The absence of any positive tests for sugar, excluded the idea that the salep was being excreted in this form, but finally a sample of February 4, was tested with yeast, and marked fermentation observed. Unfortunately, this was after all the other samples had been discarded, hence no further tests could be made.

Experiment E. A dog weighting 9.8 kg. received intraperitoneally 97.5 cc. of salep solution containing 1.3 grams pure mannan. No reduction of Fehling's solution was observed. The changes in rotation are shown in the first table on the next page.

Salep was isolated and identified as carbohydrate, in the urines of May 19, 20, and 21, although the amount in the last two days was apparently too small to be detected by any change in the rotation.

INJECTIONS OF SINISTRIN.

1. Subcutaneous.

A dog weighing 6.5 kg. received 49 cc. of sinistrin solution, containing 3.3 grams pure substance. This solution showed a rotation of

Examination of Urine.

TIME.	VOLUME.	ROTATION.	ESTIMATION OF AMOUNT OF SALEP EXCRETED.
	cc.		*Grams.*
May 17, 10 A.M..............		− 0.14°	
May 18, 10 A.M..............		− 0.14°	
May 18, 3 P.M..............	Injection		
May 19, 9 A.M..............	165	− 0.34°	0.4
May 20, 9 A.M..............	250	− 0.14°	Salep present—precipitated by Fehling's Solution.
May 21, 11 A.M..............	405	− 0.14°	Salep present.
May 22, 9 A.M..............	200	− 0.14°	No Salep present.

−3.88° in a 200 mm.   tube. The urine contained no reducing substance at any time.   The changes in rotation, due to sinistrin injection, are shown in the following table:

Examination of Urine.

TIME.	VOLUME.	ROTATION.	ESTIMATION OF AMOUNT OF SINISTRIN EXCRETED.[*]
	cc.		*Grams.*
January 15, 12 M..............		− 0.41°	
January 15, 2:30 P.M...........	Injection		
January 16.....................	260	− 0.97°	2.5
January 17 and 18..............	165	− 0.41°	
January 19.....................	60	− 0.41°	
January 20.....................	108	− 0.47°	

[*] Calculating for sinistrin $[\alpha]_D = -29.1°$.

2. Intraperitoneal.

Experiment A.   A dog weighing 6.5 kg. received 110 cc. of sinistrin solution, containing 2 grams pure substance. This solution showed a rotation of −1.18° in a 200 mm. tube.   No reducing substance was found in the urines examined.   The changes in rotation, due to sinistrin injection, are shown in the following table:

Examination of Urine.

TIME.	VOLUME.	ROTATION.	ESTIMATION OF AMOUNT OF SINISTRIN EXCRETED.*
	cc.		*Grams.*
January 11, 10:30 A.M..........		− 0.48°	
January 11, 3 P.M.............	Injection		
January 12, 9:30 A.M..........	88	− 2.04°	2.7
January 13, 9:30 A.M..........	127	− 0.48°	
January 14, 9:30 A.M..........	116	− 0.48°	

* Calculating for sinistrin $[\alpha]_D = -29.1°$.

Experiment B. A dog weighing 4.6 kg. received 108 cc. of sinistrin solution, containing 2.3 grams pure substance. The rotation of this solution was −1.38° in a 200 mm. tube. No reducing substance was detected in the urine at any time. The changes in rotation are shown in the following table:

Examination of Urine.

TIME.	VOLUME.	ROTATION.	ESTIMATION OF AMOUNT OF SINISTRIN EXCRETED.*
	cc.		*Grams.*
January 26.....................		− 0.14°	
January 27, 9:30 A.M..........	Injection		
January 27, 5:P.M.............	148	− 1.38°	2.1
January 28, 9:AM.............	95	− 0.41°	0.4
January 29, 9:A.M.............	155	− 0.14°	

* Calculating for sinistrin $[\alpha]_D = -29.1°$.

In all these experiments, the sinistrin was isolated and identified as a levo-rotatory carbohydrate, yielding reducing sugar on hydrolysis. It was apparently excreted quantitatively in every case.

FEEDING EXPERIMENTS.

Methods and Technique.

Feeding experiments were conducted with dogs and human subjects, under conditions as nearly normal as possible. The *dogs* were kept in metal cages, arranged for the separate collection of urine and faeces. They were fed once a day, on a uniform weight diet, consisting of chopped lean meat, lard, and cracker meal, in suitable portions

and amounts to maintain a constant body weight. The carbohydrate under investigation was dissolved or suspended in water, and mixed with this basal ration. In the earlier experiments the periods were divided as follows: Fore = 3 days on the basal ration; mid = 3 days in which some preparation was added, the amount being the same each day; after = 3 days like the fore period. Separation of the periods in the faeces was accomplished by marking with soot or carmine capsules. In all later experiments, two days constituted the fore period, and a day on the normal diet was included at the beginning and end of the mid period, making thus four days, to insure against any of the material under investigation being carried into the faeces of the after period.

In several cases, the presence or absence of galactans or mannans in the faeces has been verified by testing the hydrolyzed material for mucic acid or mannose-hydrazone.

For analysis, the faeces, collected and weighed, were rubbed to a thin mud with alcohol, dried to constant weight on a water bath, weighed air dry, and ground finely in a coffee mill. The portions constituting each period were thoroughly mixed, and from 2 to 5 grams taken for hydrolysis, according to the yield of carbohydrate anticipated. The samples were boiled on a reflex condenser with 100 cc. of 2 per cent hydrochloric acid, for two hours; or longer if thought to contain a carbohydrate which previous analysis[1] had shown to require more time for complete hydrolysis.

The products of hydrolysis, cooled and neutralized, were made up to 250 cc. and sugar determined as dextrose by Allihn's gravimetric method. It was found that the copper reduction was often very incomplete, and that much more satisfactory results came from clarifying the solutions with charcoal after making up to volume. Not only were duplicate analyses in closer agreement, but in some cases the yield of cupric oxide was two or three times greater than before this treatment. Owing to the complexity and diversity of the products of hydrolysis, results are at best only approximate.

In experiments with dulse, the pentosans were determined by the phloroglucin method.[2]

The *human subjects* were healthy, active young women. Their diet was not weighed, but was kept as uniform as possible. All cel-

---

[1] Cf. table, p. 317.

[2] Cf. Official and Provisional Methods of Analysis, Bulletin No. 107 (1907), Bureau of Chemistry, United States Department of Agriculture.

lulose-containing foods, such as nuts, fruits, green vegetables, peas and beans, coarse bread and cereals, were carefully avoided; so that the carbohydrates were limited almost entirely to bread and crackers made from fine white flour, a small quantity of potato, and sugar. To this diet the gelatinizing carbohydrates were added in the form of blanc mange or jelly; dulse was dissolved in some beverage, and the insoluble preparations boiled half an hour in a little water and eaten as a vegetable, seasoned with salt, butter, and vinegar. The blanc manges or jellies made from the Hawaiian seaweed preparations were equally attractive in texture and flavor with those made from Irish moss.

Periods were marked, and the analyses of faeces conducted in the manner already described for the experiments with dogs.

The Digestibility of Pentosans.

Four preparations were fed, Dulse,[1] Limu Eleele,[2] Limu Lipoa,[2] and Limu Pahapaha,[3] without production of unpleasant symptoms in any case. The results of all trials are shown in the tables on the following pages.

---

[1] Cf. p. 303.
[2] Cf. p. 307.
[3] Cf. p- 308.

Dulse.

| SERIES. | NO. | SUBJECT. | WEIGHT. Kg. | PERIOD. | DIET. | COMPOSITION OF FAECES. | | | | DULSE FED (As Pentosan). | DULSE RECOVERED. |
						WEIGHT MOIST. *Grams.*	WEIGHT AIR DRY. *Grams.*	PENTOSAN (As Phloroglucid). *Per cent.*	PENTOSAN (As Phloroglucid). *Grams.*	*Grams.*	*Per cent.*
A	1	Dog III	7	Fore = 3 days	Meat, 150 gms. Lard, 30 gms. Cracker meal, 75 gms.		13.4	2.3	0.31		
				Mid = 3 days	Same + 45 gms. dulse preparation		28.3	29.6	8.4	32.5	20
				After = 3 days	Same as Fore Period		20.0	8.0	1.6		
A	2	Dog I	9.2	Fore = 2 days	Meat, 150 gms. Lard, 30 gms. Cracker meal, 30 gms.		13.8	1.1	0.15		
				Mid = 4 days	Same + 20 gms. dulse preparation		26.6	19.9	5.3	14.4	34
				After = 2 days	Same as Fore Period		11.8	1.8	0.2		
A	3	Z (Woman)	50	Fore = 2 days	Cellulose-free	62.6	27.8	1.8	0.5		
				Mid = 4 days	Same + 10 gms. dulse preparation	87.7	32.8	2.4	0.8	7.2	
				After = 2 days	Same as Fore Period	243.6	70.0	2.2	1.5		
A	4	P (Woman)	56	Fore = 2 days	Cellulose-free	209.1	46.1	2.7	1.2		
				Mid = 4 days	Same + 10 gms. dulse preparation	191.1	48.6	2.4	1.2	7.2	
				After = 2 days	Same as Fore Period	150.4	37.2	2.4	0.9		

Limu Eleele (Enteromorpha intesinalis).

SERIES.	NO.	SUBJECT.	WEIGHT. Kg.	PERIOD.	DIET.	COMPOSITION OF FAECES.				LIMU FED (As Dextrose). Grams.	LIMU RE-COVERED. Per cent.
						WEIGHT MOIST. Grams.	WEIGHT AIR DRY. Grams.	PENTOSAN (As Dextrose). Per cent.	PENTOSAN (As Dextrose). Grams.		
A	5	Dog V	11.2	Fore = 2 days	Meat, 200 gms. Lard, 25 gms. Cracker meal, 30 gms.	34.0	15.5	4.9	0.7		
				Mid = 4 days	Same + 20 gms. powdered Limu Eleele boiled ½ hr.	65.3	36.6	9.3	3.4	3.6	50
				After = 2 days	Same as Fore Period	24.6	14.5	6.7	0.9		
A	6	Dog IV	13.6	Fore = 2 days	Meat, 250 gms. Lard, 40 gms. Cracker meal, 30 gms.	30.3	14.3	8.3	1.2		
				Mid = 4 days	Same + 30 gms. powdered Limu Eleele boiled ½ hr.	221.7	88.2	8.1	7.1	5.4	80
				After = 2 days	Same as Fore Period	27.3	17.3	9.0	1.6		
A	7	Z (Woman)	52	Fore = 2 days	Cellulose-free	150.9	41.7	4.3	1.8		
				Mid = 4 days	Same + 30 gms. powdered Limu Eleele boiled ½ hr.	300.3	89.2	5.4	4.6	5.4	31
				After = 2 days	Same as Fore Period	65.7	22.5	4.8	1.0		

Limu Pahapaha (Ulva Lactuca, etc.).

SE-RIES.	NO.	SUBJECT.	WEIGHT.	PERIOD.	DIET.	COMPOSITION OF FAECES.					LIMU FED (As Dextrose).	LIMU RECOVERED.
---	---	---	---	---	---	WEIGHT MOIST.	WEIGHT AIR DRY.	PENTOSAN (As Dextrose).				
								Per cent.	Grams.		Grams.	Per cent.
			Kg.			Grams.	Grams.					
A	8	Z (Woman)	52	Fore = 2 days	Cellulose-free	65.7	22.5	4.8	1.0			
				Mid = 4 days*	Same + 30 gms. Limu Paha-paha, boiled ½ hr.	95.4	46.4	8.1	3.8		1.0	66
				After = 2 days	Same as Fore Period	193.4	26.8	8.2	2.1			

* Some intestinal fermentation was noticed on Limu days.

Limu Lipoa (Haliseris Pardalis)

SE-RIES.	NO.	SUBJECT.	WEIGHT.	PERIOD.	DIET.	COMPOSITION OF FAECES.					LIMU FED (As Dextrose).	LIMU RECOVERED.
---	---	---	---	---	---	WEIGHT MOIST.	WEIGHT AIR DRY.	PENTOSAN (As Dextrose).				
								Per cent.	Grams.		Grams.	Per cent.
A	9	Dog V	11.2	Fore = 2 days	Meat, 200 gms. Lard, 25 gms. Cracker meal, 30 gms.	35.8	19.4	5.5	1.0			
				Mid = 4 days	Same + 30 gms. Limu Lipoa boiled ½ hr.	184.0	64.4	8.6	5.5		4.4	84
				After = 2 days	Same as Fore Period	24.2	14.5	6.7	0.9			

The coefficients of digestibility of the pentosan preparations, as determined in the usual way from the preceding experiments, are set forth in the following table:

SERIES A. EXPERIMENT NO.	PENTOSAN.	COEFFICIENT OF DIGESTIBILITY.	
		For the Dog.	For Man.
1	Dulse	80	
2	Dulse	66	
3	Dulse		100
4	Dulse		100
5	Limu Eleele	50	
6	Limu Eleele	20	
7	Limu Eleele		69
8	Limu Pahapaha		34
9	Limu Lipoa	16	

It is evident from these figures, that pentosans in soluble form disappear from the alimentary tract of dogs to a very considerable extent (average 73 per cent), and that small quantities, ingested by man, do not reappear in the faeces. The insoluble limu preparations appear much more indigestible, an average of 28 per cent being digested by dogs, and 51 per cent by man.

It must be borne in mind, in interpreting the results of these metabolism experiments, that they are at best only approximate. The difficulty of strict separation of the faeces, the fact that the human subjects were not kept on a uniform weighed diet, and the errors unavoidably introduced by determining many different kinds of sugar as dextrose, make all of the figures given as "coefficients of digestibility," in this and succeeding sections, comparative rather than absolute.

The Digestibility of Galactans.

In these experiments, preparations of the water extracts of Irish moss, Limu Manauea, Limu Huna and Limu Akiaki have been fed, without any disagreeable symptoms. The results are given in the tables which follow:

Irish Moss (Chondrus Crispus).

SE-RIES	NO.	SUBJECT.	WEIGHT.	PERIOD.	DIET.	COMPOSITION OF FAECES.					
						WEIGHT MOIST.	WEIGHT AIR DRY.	CARBOHYDRATES (As Dextrose).		LIMU FED (As Dextrose).	LIMU RECOVERED.
			Kg.			Grams.	Grams.	Per cent.	Grams.	Grams.	Per cent.
B	1	Dog I	8.4	Fore = 3 days	Meat, 200 gms. Lard, 40 gms. Cracker meal, 100 gms.		29.1	9.4	2.7		
				Mid = 2 days	Same + 20.3 gms. Irish moss preparation		42.5*	25.5	10.8	9.8	54
				After = 3 days	Same as Fore Period		15.6	19.0	2.9		
B	2	Dog II	10.0	Fore = 3 days	Meat, 200 gms. Lard, 40 gms. Cracker meal, 100 gms.		16.9	8.6	1.4		
				Mid = 3 days	Same + 45 gms. Irish moss preparation		67.7*	26.4	17.8	21.8	80
				After = 3 days	Same as Fore Period		21.0	6.0	1.3		
B	3	X (Woman)	42.0	Fore = 2 days	Cellulose-free	57.9	12.5	6.4	0.8		
				Mid = 4 days	Same + 30 gms. Irish moss preparation	458.9	98.8†	14.8	14.6	14.5	89
				After = 2 days	Same as Fore Period	150.7	35.8	9.1	3.2		
B	4	Z (Woman)	52	Fore = 2 days	Cellulose-free	115.7	35.3	5.7	2.0		
				Mid = 4 days	Same + 30 gms. Irish moss preparation	367.6	95.0‡	21.0	20.0	14.5	100
				After = 2 days	Same as Fore Period	133.6	34.6	9.6	1.3		

* Rich yield of mucic acid from faeces of this period.
† Mucic acid obtained from these faeces.
‡ Ten grams faeces yielded 0.26 grams mucic acid.

Limu Manauea (Gracilaria Coronopifolia).

SE-RIES.	NO.	SUBJECT.	WEIGHT.	PERIOD.	DIET.	COMPOSITION OF FAECES.				LIMU FED (As Dextrose).	LIMU RECOVERED.
						WEIGHT MOIST.	WEIGHT AIR DRY.	CARBOHYDRATES (As Dextrose).			
			Kg.			Grams.	Grams.	Per cent.	Grams.	Grams.	Per cent.
B	5	Dog IV	13.6	Fore = 2 days	Meat, 250 gms. Lard, 40 gms. Cracker meal, 40 gms.	30.3	14.3	8.3	1.2		
				Mid = 4 days	Same + 14 gms. Limu Manauea preparation	94.6	31.0	18.0	5.6	6.2	45
				After = 2 days	Same as Fore Period	27.3	17.3	9.0	1.6		
B	6	Dog V	11.2	Fore = 2 days	Meat, 200 gms. Lard, 25 gms. Cracker meal, 30 gms.	34.0	13.5	4.8	0.7		
				Mid = 4 days	Same + 10 gms. Limu Manauea preparation	95.7	34.7	11.5	4.0	4.4	68
				After = 2 days	Same as Fore Period.	35.8	19.4	5.4	1.0		
B	7	P (Woman)	56	Fore = 2 days	Cellulose-free	158.9	40.0	6.2	2.4		
				Mid = 5 days	Same + 15 gms. Limu Manauea preparation	297.7	83.9	11.1	9.3	6.7	70
				After = 2 days	Same as Fore Period	150.4	37.2	3.8	1.4		
B	8	Z (Woman)	52	Fore = 2 days	Cellulose-free	150.9	41.7	4.3	1.8		
				Mid = 5 days	Same + 10 gms. Limu Manauea preparation	536.6	99.7	7.8	7.8	4.4	100
				Mid = 4 days	Same + 14 gms. Limu Manauea preparation	242.5	67.9	7.1	4.8	6.2	40
				After = 2 days	Same as Fore Period	90.8	24.9	6.4	1.6		

RSE- RIES	NO.	SUBJECT.	WEIGHT.	PERIOD.	DIET.	COMPOSITION OF FAECES.					
						WEIGHT MOIST.	WEIGHT AIR DRY.	CARBOHYDRATES (As Dextrose).		LIMU FED (As Dextrose).	LIMU RECOVERED.
			Kg.			Grams.	Grams.	Per cent.	Grams.	Grams.	Per cent.
B	9	Dog IV	13.6	Fore = 2 days	Meat, 250 gms. Lard, 40 gms. Cracker meal, 30 gms.	30.3	14.3	8.3	1.2		
				Mid = 4 days	Same + 20 gms. Limu Huna preparation	126.1	53.9	16.1	8.6	11.6	60
				After = 2 days	Same as Fore Period	27.3	17.3	9.0	1.6		
B	10	Dog V	11.2	Fore = 2 days	Meat, 200 gms. Lard, 25 gms. Cracker meal, 30 gms.	35.8	19.4	5.4	1.0		
				Mid = 4 days	Same + 7 gms. Limu Huna preparation	57.8	40.5	7.7	3.1	4.0	17
				After = 2 days	Same as Fore Period	34.0	15.5	4.9	0.7		
B	11	X (Woman)	42	Fore = 2 days	Cellulose-free	143.4	38.2	7.3	2.8		
				Mid = 5 days	Same + 10 gms. Huna preparation	245.1	94.8	7.6	7.0	5.8	90
				After = 2 days	Same as Fore Period	135.7	23.7	9.9	2.3		

Limu Akiaki (Ahnfeldtia concinna).

B	12	B (Woman)	62	Fore = 2 days	Cellulose-free	147.8	24.5	6.0	1.6		
				Mid = 4 days	Same + 20 gms. Limu Akiaki preparation	451.1	72.2	8.3	6.0	7.2	40
				After = 2 days	Same as Fore Period	Not weighed	24.5	10.4	2.5		

The coefficients of digestibility of the galactan preparations are given in the following table:

SERIES B. EXPERIMENT NO.	GALACTAN.	COEFFICIENT OF DIGESTIBILITY.	
		For the Dog.	For Man.
		Per cent.	*Per cent.*
1	Irish Moss	46	
2	Irish Moss	20	
3	Irish Moss		11
4	Irish Moss		0
5	Limu Manauea	55	
6	Limu Manauea	12	
7	Limu Manauea		30
8	Limu Manauea		30 (av.)
9	Limu Huna	30 (20 gms. fed)	
10	Limu Huna	83 (7 gms. fed)	
11	Limu Huna		10
12	Limu Akiaki		60

Although these preparations were administered in small quantities, under the most favorable conditions for digestion in, the only instance where the utilization in any degree approaches that of starch (Limu Huna), the quantity fed (7 grams) was so small that this experiment can hardly be taken as a criterion of digestibility. Exclusive of this experiment, the average of five trials with dogs is 32 per cent, while that of six trials with human subjects is 23 per cent. In both cases, the averages are lower than that of Lohrisch (194) for "soluble agar," 50 per cent.

Where the quantity of galactan fed was 10 or more grams, the influence on the character of the faeces was usually noticeable. The increase in bulk, after ingestion of 45 grams of Irish moss, is well illustrated in a photograph of the dried and ground faeces of the dogs used in experiments 1 and 2:[1]

A represents the fore-period (3 days), B the mid-period, during which 15 grams of moss were ingested daily (3 days), and C the after-period (3 days). The separation of the faeces at the beginning of experiment 1 (on the right) was not very satisfactory. The dog had previously been fed bone-ash, and the marked faeces were undoubtedly contaminated with this, so that they appear unusually bulky. Experiment 2 is typical of the results obtained in most of the experiments

[1] Cf. p. 343.

with human subjects. In these, the undigested hemicelluloses gave frequently a peculiar, wax-like consistency, especially noticeable with Limu Huna in the experiment recorded,[1] and in another not reported, because the faeces for part of the time were lost. In the experiment with Limu Akiaki (No. 12),[1] the galactan was excreted after the first day's feeding, in a tough mass almost impossible to break up with a
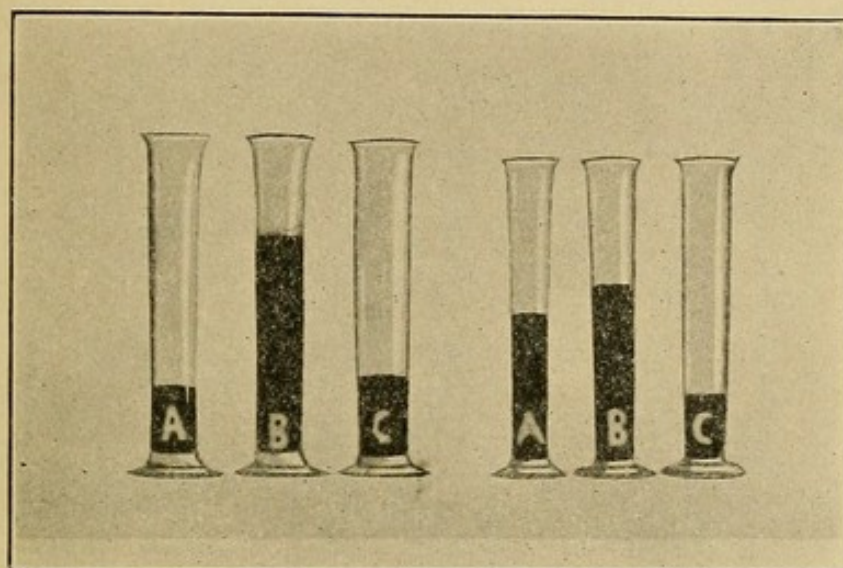
EXP. 2   EXP. 1

I.

The Influence of Irish Moss upon the Mass of the Faeces.
   A. Fore Period: 3 Days on a Cellulose-free Diet.
   B. Mid Period: 3 Days on a Cellulose-free Diet to Which 15 grams of
                   Irish Moss were Added Daily.
   C. After Period: 3 Days of a Cellulose-free Diet.

spatula. That of the second day was not excreted till the third day after feeding, the subject being inclined to constipation. It seems likely that the high coefficient of digestibility is due to this fact, or else to the method of determination, which is not altogether satisfactory, in view of the complexity of the products of hydrolysis, the danger of decomposing a part of the sugar from the easily inverted polysaccharides by the long boiling necessary for the more resistant, and the great difference in reducing power of the sugars so produced.

The Digestibility of Mannan.

In four experiments, the commercial salep powder (containing 19 per cent mannan and 26 per cent starch) was administered; in the others, pure mannan prepared from the Orchis tubers. The results of seven trials are tabulated on the following pages.

[1] Cf. p. 345.

Salep.

SE-RIES.	NO.	SUBJECT.	WEIGHT Kg.	PERIOD.	DIET.	COMPOSITION OF FAECES		CARBOHYDRATES (As Dextrose).		SALEP FED (As Dextrose).	SALEP RECOVERED.
						WEIGHT MOIST. Grams.	WEIGHT AIR DRY. Grams.	Per cent.	Grams.	Grams.	Per cent.
C	1	Dog III	7	Fore = 3 days	Meat, 150 gms. Lard, 30 gms. Cracker meal, 75 gms.		13.4	5.7	0.7		
				Mid = 3 days	Same + 45 gms. Salep Powder		31.5	37.9	11.9	34.0	30
				After = 3 days	Same as Fore Period		6.9	17.1	1.2		
C	2	Dog I	9.2	Fore = 3 days	Meat, 200 gms. Lard, 40 gms. Cracker meal, 75 gms.		29.1	9.4	2.7		
				Mid = 3 days	Same + 45 gms. Salep Powder		30.2	18.3	5.5	34.0	
				After = 3 days	Same as Fore Period		21.8	15.0	3.2		
C	3	Z (Woman)	52	Fore = 2 days	Cellulose-free	115.7	35.3	5.7	2.0		
				Mid = 4 days	Same + 30 gms. Salep Powder	178.2*	53.1	8.6	4.5	22.7	
				After = 2 days	Same as Fore Period	150.5	49.4	4.3	2.1		
C	4	X (Woman)	42	Fore = 2 days	Cellulose-free	57.9	12.5	6.4	0.8		
				Mid = 4 days	Same + 30 gms. Salep Powder	265.5†	61.7	9.3	5.7	22.7	6
				After = 2 days	Same as Fore Period	262.3	57.1	6.4	3.6		

* Tests for mannose-hydrazone in hydrolyzed faeces were negative.

† Tests for mannose-hydrazone were negative.

Salep.

SE-RIES.	NO.	SUBJECT.	WEIGHT.	PERIOD.	DIET.	COMPOSITION OF FAECES				SALEP FED (As Dextrose).	SALEP RECOVERED.
						WEIGHT MOIST.	WEIGHT AIR DRY.	CARBOHYDRATES (As Dextrose).			
			Kg.			*Grams.*	*Grams.*	*Per cent.*	*Grams.*	*Grams.*	*Per cent.*
C	5	Dog I	9.2	Fore = 2 days	Meat, 150 gms. Lard, 30 gms. Cracker meal, 30 gms.		11.8	8.4	1.0		
				Mid = 3 days	Same + 10 gms. Salep-Man-nan		32.3*	27.5	9.8	9.0	90
				After = 2 days	Same as Fore Period		12.3	6.2	0.7		
C	6	Z (Woman)	52	Fore = 2 days	Cellulose-free	62.6	27.8	5.4	1.5		
				Mid = 4 days	Same + 20 gms. Salep-Man-nan	243.5†	70.0	6.0	4.2	18.0	
				After = 2 days	Same as Fore Period	218.7	59.7	3.9	2.3		
C	7	P (Woman)	56	Fore = 2 days	Cellulose-free	209.1	46.1	6.3	2.5		
				Mid = 4 days	Same + 20 gms. Salep-Man-nan	173.2†	42.6	7.0	3.0	18.0	
				After = 2 days	Same as Fore Period	150.4	37.2	3.8	1.4		

* A large yield of mannose-hydrazone from hydrolyzed faeces.
† No mannose-hydrazone obtainable from hydrolyzed faeces.

The coefficients of digestibility of the salep preparations are shown in the following table:

SERIES C. EXPERIMENT NO.	MANNAN.	COEFFICIENT OF DIGESTIBILITY.	
		For the Dog.	For Man.
		Per cent	*Per cent*
1	Salep Powder	70	
2	Salep Powder	100	
3	Salep Powder		100
4	Salep Powder		94
5	Salep Mannan	10	
6			100
7			100

Thus we see that in every case, except that in which a dog received, in one day, 10 grams of pure mannan, the greater portion of the salep fed was digested, the coefficient of salep powder for dogs averaging 85 per cent, and for man, 97 per cent; while that of pure mannan for man is 100 per cent, in spite of the fact that it is not attacked by digestive enzymes!

The contrast between the volume of faeces produced when a galactan such as Irish moss was fed, and that when a more digestible hemicellulose was given, is shown in the photograph of the faeces from experiments Nos. 1 and 2 of Series C,[1] on the next page, in which A represents the fore-period, B the mid-period, and C the after-period, each period being three days in duration. The group on the right represents experiment No. 1, in which 70 per cent of the hemicellulose and starch of the salep powder was digested, and that on the left, experiment No. 2 in which apparently all of these were digested.

DISCUSSION AND SUMMARY.

A glance at the table on page 327 clearly shows that none of the hemicelluloses under consideration are readily attacked by the ordinary animal or vegetable enzymes. The results are for the most part entirely negative. Even where there has been hydrolysis with 0.2 per cent hydrochloric acid, the amount of sugar produced in 24 hours was relatively small. The hydrolyzing action of the gastric juice is probably largely due to the presence of acid, although no comparison of the relative amounts of sugar produced by gastric juice or by

[1] Cf. p. 348.

0.2 per cent acid alone has been made.   It is noticeable that even the very soluble hemicellulose, sinistrin, which is so speedily hydrolyzed by acid (in $\frac{1}{2}$ hour at 37° C. with 0.2 % hydrochloric acid) is not attacked by ordinary diastatic enzymes within 24 hours.

The parenteral introduction of these carbohydrates has resulted in their speedy and apparently complete elimination through the kidneys without any change in character.   The carbohydrates prepared from Dulse, Irish Moss, Salep and Sinistrin have all been isolated and identified in the urine, after subcutaneous and intraperitoneal injections.   These results are not surprising, in view of the commonly ac-
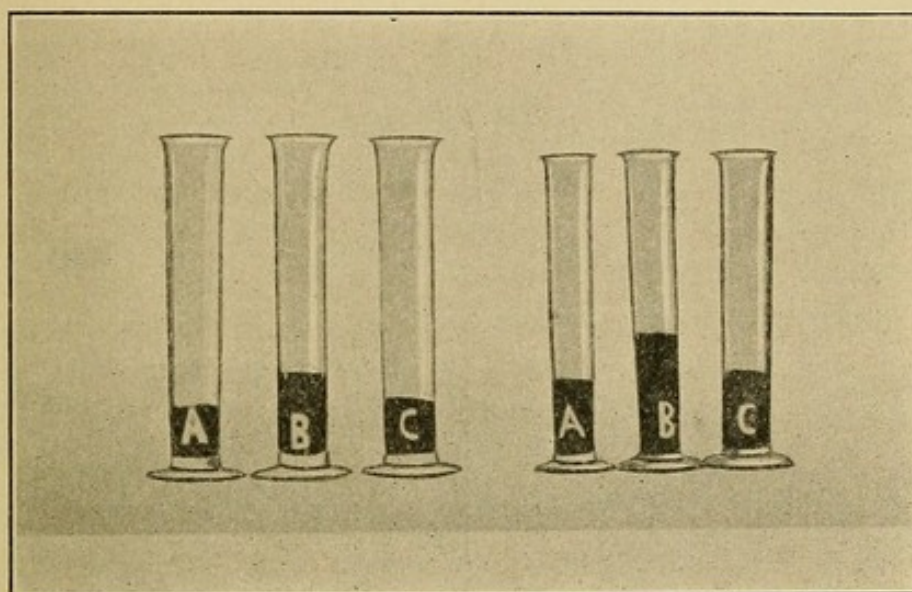
EXP.   EXP. 1

II.

The Influence of Salep upon the Mass of the Faeces.
    A. Fore Period: 3 Days on a Cellulose-free Diet.
    B. Mid Period: 3 Days on a Cellulose-free Diet to Which 15 grams of
                            Salep Powder were Added Daily.
    C. After Period: 3 Days on a Cellulose-free Diet.

cepted fact that carbohydrates must be converted into monosaccharides before they can enter into the processes of intermediary metabolism.

Experimental evidence in support of this fact is given by such investigators as F. Voit[1] and Blumenthal,[2] who found that even disaccharides, as lactose and saccharose, were eliminated almost quanti-

---

[1] Münchener medicinische Wochenschrift, 1896, p. 717; Deutsches Archiv für klinische Medicin, v. 58, p.521 (1897).

[2] Beitäge zur chemischen Physiologie, v. 6, p. 329 (1905).

tatively after subcutaneous injection in man and the rabbit; or as Mendel and Mitchell,[1] who have shown that polysaccharides like dextrin, soluble starch, glycogen, inulin, and isolichenin are recovered to a considerable extent in the urine, whether introduced subcutaneously, intraperitoneally, or intravenously.

In the present experiments, the dulse pentosan was the most slowly eliminated, being found in the urine four or five days after injection; Irish moss and salep were not detected after the third day; while sinistrin seemed to be all excreted within the first 24 hours.

The average coefficients of digestibility for the ten preparations which have formed the basis of the feeding experiments, are summarized in the following table:

Coefficients of Digestibility of Hemicelluloses.

HEMICELLULOSE.		SUBJECT OF EXPERIMENT.	
Class.	Source.	Dog.	Man.
		Per cent.	*Per cent.*
Pentosan..........	Dulse	73 (2 exp.)	100 (2 exp.)
Pentosan..........	Limu Eleele	35 (2 exp.)	9 (2 exp.)
Pentosan..........	Limu Pahapaha		34 (1 exp.)
Pentosan..........	Limu Lipoa	16 (1 exp.)	
Galactan..........	Irish Moss	33 (2 exp.)	6 (2 exp.)
Galactan..........	Limu Manauea	33 (2 exp.)	30 (3 exp.)
Galactan..........	Limu Huna	56 (2 exp.)	10 (1 exp.)
Galactan..........	Limu Akiaki		60 (1 exp.)*
Mannan..........	Salep Powder	85 (2 exp.)	97 (2 exp.)
	Salep Mannan	10 (1 exp.)	100 (2 exp.)

* Subject with chronic constipation.

That the low coefficients enumerated above are not due to inability of the various subjects to utilize carbohydrates, is shown by the following figures.

The coefficient of digestibility for cracker meal in the experiments on dogs, determined by taking the average of all the fore-periods of the feeding trials, in which five different dogs were used, was 99.0 per cent. This is much higher than London and Polowzowa's[2] coefficient for carbohydrate digestibility in dogs on a bread diet, 96 per cent.

[1] American Journal of Physiology, v. 14, p. 239 (1905).
[2] Zeitschrift für physiologische Chemie, 56, 513 (1908).

For the four women who were subjects of feeding experiments, the average daily amount of carbohydrate excreted in the faeces, on a cellulose-free diet, estimated as dextrose, by averaging the fore-periods of all trials, was 0.8 gram. The utilization of carbohydrates was therefore unusually good, since Atwater and Bryant's[1] coefficient of digestibility for such a diet is 98 per cent, and undoubtedly every one of these individuals consumed over 50 grams of carbohydrate per day. With the exception of the subject of a single experiment who had chronic constipation, these were all normal, healthy individuals, free from disturbances of the alimentary tract.

The three seaweeds fed *in toto*, Limu Eleele, Limu Pahapaha, and Limu Lipoa, show an average digestibility of 51 per cent. This is higher than that obtained in Professor Mendel's laboratory for uncooked *Cetraria islandica*[2] (average of three experiments, 15 per cent) and much lower than that reported by Oshima for dried marine algae[3] (average 77 per cent).

In man, with the exception of dulse and salep, which almost entirely disappeared in the alimentary tract, the average digestibility of all preparations is only 34 per cent, a figure in contrast to those of Lohrisch (194), who finds cellulose and hemicellulose 50 per cent digestible. In dogs, the average of all preparations is 42 per cent.

Considering that the pentosan of dulse was in a form most favorable for digestion, the results with this hemicellulose are in harmony with those of König and Reinhardt (120) who reported 75 per cent of the pentosans as disappearing from the alimentary tract in man; and with the averages obtained by the various investigators on animals, which show these carbohydrates 40–70 per cent digestible in herbivora.[4] It would be desirable to repeat the experiments with larger quantities, although the process of preparing the material is rather laborious. It must be borne in mind, that the dulse pentosan is not attacked by ordinary diastatic enzymes, but can be decomposed by soil and faecal bacteria; and although this decomposition did not occur readily in pure solutions of the carbohydrate, or even in a putrefying mixture, it still remains to be demonstrated whether the complete disappearance from the alimentary tract is not largely due to the more favorable conditions for bacterial activity within the

[1] Report Storr's Agricultural Experiment Station, 1899, p. 86.
[2] Cf. pp. 297–298.
[3] Cf. p. 299.
[4] Cf. pp. 274–275.

organism. While we have, in the case of herbivora, some convincing evidence that the pentosans are a true source of energy,[1] we have as yet no real grounds for this assumption in the case of man.

The insoluble pentosans of the Hawaiian algae are manifestly less digestible than the soluble forms. The coefficient of digestibility is approximately the same as Slowtzoff's (154) average for pure xylan in rabbits, 55 per cent. While it would be perhaps desirable to determine the pentosans directly by the furfurol-phloroglucin method, rather than by estimation of sugar after acid hydrolysis, a trial with dulse by both methods gave practically identical results: hence, considering that the hemicelluloses of these algae are chiefly pentosans, it seems safe to assume that the results reported represent the amount of pentosan excreted, within the limits of error for all of the feeding experiments.

The galactans were all soluble, and were ingested in quantities not exceeding 15 grams per day, yet the coefficient of digestibility is lower than for any other hemicellulose group (26 per cent). The resistance of Irish moss is particularly striking, but is not surprising in view of its utter indifference to attacks of digestive enzymes or bacteria. Its influence on the character of the faeces was not so marked as that of Limu Huna, owing probably to a greater tendency to liquefy at body temperature. The latter would seem to be a very effective agent in constipation; a comparison of its efficiency with that of agar-agar would be extremely interesting. Saiki (205) found the coefficient of digestibility for agar (average of two experiments) 17 per cent.

In view of the negative results of digestions *in vitro* and of trials with bacteria, we can scarcely be surprised at the results of these metabolism experiments, especially as we recall that Lohrisch (57) found that his "soluble agar," already partially hydrolyzed, was only digestible to 50 per cent (average).

The mannans stand in striking contrast to the galactans. In the present studies, 99 per cent of the salep administered has been utilized, a result in accordance with Kano and Iishima's (255) coefficient of digestibility for the Japanese mannan, Konjaku, 82 per cent. Pure mannan fed to a dog, was excreted the succeeding day, seemingly unaltered, since it formed a semi-transparent gelatinous mass in the faeces, from which, later, a rich yield of mannose-hydrazone was obtained. The very different result with salep powder, of which 85 per cent was digested by dogs, may perhaps be accounted for by the

---

[3] Cf. Kellner, p. 274.

fact that it contained a high percentage of starch (26 per cent). The amount of undigested carbohydrate excreted in the faeces is in close agreement with the quantity of pure mannan ingested. However, as tests for mannose-hydrazone were negative in these cases, further experiments are necessary before an authoritative statement can be made in regard to this question.

It is manifestly possible for faecal and soil bacteria to produce sugar from mannan;[1] hence it is not unlikely that hemicelluloses of this group are inverted in the intestines through the activity of micro-organisms, and that the sugar so produced is absorbed and becomes a true source of energy for man, in spite of the resistance of mannans to the action of digestion enzymes. Further investigations to determine its exact nutritive value seem highly desirable.

In considering the proper place in the dietary for marine algae, lichens and similar substances, we must not disregard the possibility of their having a valuable function entirely aside from the question of energy production. As Oshima (15) points out, they may be valuable for their inorganic salts. The non-irritating, laxative properties of many species make them desirable adjuncts to the diet of persons with a tendency to constipation;[2] and even if they disappear, in marked degree, from the alimentary tract during the process of digestion, they may perhaps still play an important rôle as stimulants to intestinal activity, being in fact what Prausnitz[3] calls "faeces-forming foods." An illustration of this effect is afforded by the experiments in which salep powder was fed to dogs.[4] The periods were equal in length, and in one case (No. 2 in photograph) the utilization of carbohydrates was equally good for all three; yet in the mid-period there is a decided increase in the bulk and weight[5] of the faeces, not more than 1 gram of which is by any possibility attributable to the cellulose of the salep powder, and in the other experiment, the increased amount of faeces cannot be wholly accounted for by the amount of undigested carbohydrate present.

Mendel (196) has already sounded a warning against the hasty assumption that every carbohydrate, by virtue of its ultimate chemical composition, stands in the category of true nutrients for the human organism. The results of the present investigations emphasize the

[1] Cf. Sawamura (267).
[2] Cf. p. 283.
[3] Zeitschrift für Biologie, v. 35, p. 335 (1897).
[4] Cf. pp. 348-349.
[5] Cf. Table, p. 348, Series C, Experiments Nos. 1 and 2.

necessity of drawing our final conclusions only from exact metabolism experiments. The soluble hemicelluloses show great diversity of behavior in the alimentary tract, although equally resistant to digestive enzymes *in vitro;* some disappear entirely, others reappear in the faeces in varying degree, up to 100 per cent. It is evident that the latter do not constitute a source of energy for the organism: how far the former actually do so, remains to be demonstrated.

IV. CONCLUSIONS.

1.   The hemicelluloses of the ten species of marine algae included in these investigations are chiefly pentosans and galactans.   The pentosans are largely insoluble in water, but a soluble form in considerable quantity has been isolated from *Rhodymenia palmata*.   The galactans are soluble in hot water, and are characterized by their gelatinous nature.   Small quantities of soluble pentosans have been found associated with them in every case.

2.   In order of resistance to the action of bacteria, the hemicellulose groups studied stand as follows, — galactans, pentosans, levulans, mannans, the galactan of *Chondrus crispus* being entirely unaffected by common micro-organisms.

3.   Aerobic and anaerobic cultures of soil and faecal bacteria, and cultures of *B. anthracis symptomatici* and *B. maligni oedematis*, caused inversion of salep mannan, with actual production of reducing sugar. The latter cultures also hydrolyzed the pentosan of *Rhodymenia palmata*, and the levulan, sinistrin.   In a mixture of aerobes, salep appeared to be partially hydrolyzed, forming an insoluble transition product.

4.   Digestion experiments *in vitro*, continued for 24 hours at body temperature under antiseptic conditions, have been almost entirely negative in result.   The only exceptions are the hydrolysis of the pentosan of dulse, the galactan of limu kohu, and the levulan, sinistrin, by "Taka" diastase; and of sinistrin, and the galactans of limu kohu, limu akiaki, and slippery elm bark, by artificial gastric juice or 0.2 per cent hydrochloric acid, the action of the gastric juice being in all probability due to its acidity.

5.   After parenteral injection, whether subcutaneous or intraperitoneal, the hemicelluloses are excreted through the kidneys, and can be recovered unaltered in the urine.   The pentosan of dulse is completely eliminated in four to five days, and the carbohydrates of Irish moss, salep and sinistrin, in one to three days.

6.   Feeding experiments show that those hemicelluloses most readily attacked by bacteria disappear most completely from the alimentary tract.   The average coefficient of digestibility for man is, in the case of the pentosan of dulse and the mannan of salep, 99 per

cent notwithstanding their apparent resistance to amylolytic enzymes and the hydrolyzing influence of the gastric juice; their disappearance seems therefore directly attributable to bacterial activity, and the possibility of sugar formation by this agency having been demonstrated, it remains to be shown by means of respiration experiments to what extent materials so hydrolyzed can serve as true nutrients for the organism.   Dogs can also utilize the dulse pentosan to a considerable degree, but their power to digest mannan is still an open question.

In striking contrast to the above hemicelluloses stand the galactans, with their high degree of resistance to bacterial decomposition; they show in man, an average digestibility of approximately 25 per cent, in dogs of 45 per cent.   It is manifestly impossible to treat of the digestibility of hemicelluloses as a class, in view of such diversity in the groups.   Not only must each type receive special consideration, but distinction must be drawn between soluble and insoluble forms, as is illustrated by the pentosans, the ratio of the digestibility coefficient of the former to the latter being approximately 100 to 50 in man, and 75 to 25 in dogs.   We may, however, say in general, that they disappear from the alimentary tract of men and animals to an extent seemingly proportional to their susceptibility to attacks of micro-organisms, and give little justification for any high claims made for them as sources of energy in nutrition.   They may, however, have a valuable function as adjuvants in the dietary, as therapeutic agents in constipation, or as sources of inorganic salts.

V. BIBLIOGRAPHY.

LICHENS AND ALGAE.—COMPOSITION AND USES.

(1) ANNETT, DARBISHIRE AND RUSSELL: The Journal of the South-Eastern Agricultural College, Wye, Kent, No. 16, pp. 204, 205, (1907). (Extract obtained from Dr. C. F. Langworthy, U. S. Dept. of Agriculture.)

(2) BERZELIUS: Recherches sur la nature du lichen d'Islande et sur son emploi comme aliment. Annales de Chimie, V. 90, p. 277, (1814).

(3) BLONDEAU: De la Goëmine, substance neutre extraite du Goëmon (Fucus crispus). Comptes Rendus, V. 60, p. 860, (1865).

(4) DILLINGHAM: The Staff Tree as a Former Food Supply of Starving Indians. The American Naturalist, June, 1907.

(5) DILLINGHAM: A Contribution to the History of Bark Bread. Bulletin of the Bussey Institution, Vol. III, Part V, p. 120, (1906).

(6) ESCOMBE: Chemie der Membranen der Flechten und Pilze. Zeitschrift für physiologische Chemie, V. 22, p. 288, (1896).

(7) JOHNSON, C. PIERPONT: The Useful Plants of Great Britain, (1862).

(8) KÖNIG AND BETTELS: Die Kohlenhydrate der Meeresalgen und daraus hergestellter Erzeugnisse. Zeitschrift für Untersuchung der Nahrungs- und Genussmittel, V. 10, p. 487, (1905).

(9) KREFTING: A New Carbohydrate from Laminaria Digitata. Biochemisches Centralblatt, V. 8, p. 770 (1909). (From Pharmacia, V. 6, p. 151.)

(10) MENDEL: Das Verhalten einiger unverdaulicher Kohlenhydrate im Verdauungstrakt. Zentralblatt für die gesammte Physiologie und Pathologie des Stoffwechsels, No. 17, p. 1, (1908).

(11) MÜLLER, KARL: Die chemische Zusammensetzung der Zellmembranen bei verschiedenen Kryptogamen. Zeitschrift für physiologische Chemie, V. 45, p. 264, (1905).

(12) MÜTHER: Untersuchungen über Fucusarten, Laminaria und Carragheenmoos, sowie die hydrolytisch daraus entstehenden Substanzen und über Derivate derselben, besonders Fucose und Fuconsäure. Inaugural Dissertation, Göttingen, (1903). Berichte der deutschen chemischen Gesellschaft, V. 37, p. 298, (1904).

(13) MÜTHER AND TOLLENS: Über die Producte der Hydrolyze von Seetang (Fucus), Laminaria, und Carragheenmoos. Zeitschrift des Vereins der Deutschen Zucker Industrie. Band 54, Heft 576, s. 59, (1904).

(14) NILSON: Zur Kenntniss der Kohlenhydrate in den Flechten. Upsala Läkareförenings Förhandlingar, Vol. 38, also Jahresbericht für Thierchemie, V. 23, p. 53, (1893).

(15) OSHIMA: A Digest of Japanese Investigations on the Nutrition of Man. Office of Experiment Stations, United States Department of Agriculture, Bulletin 159, p. 34, (1905).

(16) OSHIMA AND TOLLENS: Über das Nori aus Japan. Berichte der deutschen chemischen Gesellschaft, V. 34, p. 1422, (1901).

(17) Poulsson: Untersuchungen über das Verhalten einiger Flechten-Kohlenhydrate im menschlichen Organismus und über die Anwendung derselben bei Diabetes mellitus. Festschrift für Olof Hammersten, XIV, pp. 1–25. Upsala Läkareförenings Förhandlingar, (1906), supplement.

(18) Reed, Minnie: The Economic Seaweeds of Hawaii and Their Food Value. Report of Hawaiian Agricultural Experiment Station, (1906).

(19) Richards: Some Edible Seaweeds. Science, N. S., V. 21, p. 895, (1905).

(20) Saiki: The Digestibility and Utilization of some Polysaccharide Carbohydrates Derived from Lichens and Marine Algae. Journal of Biological Chemistry, V. 2, p. 251, (1906–7).

(21) Schneider: A Text-book of Lichenology, p. 23, (1897).

(22) Setchell: Limu. University of California Publications, Botany, Vol. 2, No. 3, p. 91, (1905).

(23) Smith, H. M.: The Seaweed Industries of Japan; and the Utilization of Seaweed in the United States. Bulletin of Bureau of Fisheries, V. 24, p. 133, (1904).

(24) Schmidt, C: Über Pflanzenschleim und Bassorin. Liebig's Annalen, V. 51, p. 56, (1844).

(25) Smith: Dictionary of Popular Names of Economic Plants, p. 418, (1882).

(26) Ulander: Untersuchungen über die Kohlenhydrate der Flechten. Dissertation, Göttingen, (1905).

(27) Ulander and Tollens: Untersuchungen über Kohlenhydrate der Flechten. Berichte der deutschen chemischen Gesellschaft, V. 39, p. 401, (1906).

(28) Winterstein: Zur Kenntniss der in den Membranen der Pilze enthaltenen Bestandtheile. Zeitschrift für physiologische Chemie, V. 19, p. 521, (1895).

(29) Wisselingh: Microchemische Untersuchungen über die Zellwände der Fungi. Jahrbücher für wissenschaftliche Botanik, V. 31, p. 619, (1897).

(30) Yendo: Uses of Marine Algae in Japan. Postelsia, 1901.

II. CELLULOSE.

(31) Ankersmit: Untersuchungen über die Bakterien im Verdaungskanal des Rindes. Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten. Abt. I, V. 40, p. 100, (1905).

(32) Bergmann: Studien über die Digestion der Pflanzenfresser. Skandinavisches Archiv für Physiologie, V. 18, p. 119, (1906).

(33) Biedermann: Beiträge zur vergleichenden Physiologie der Verdauung. Pflüger's Archiv für Physiologie, V. 72, p. 105, (1898); V. 75, p. 1, (1899).

(34) Biedermann and Moritz: Über ein Celluloselösendes Enzym im Lebersecret der Schnecke, (Helix Pomatia). Pflüger's Archiv für Physiologie, V. 73, p. 236, (1898).

(35) Brown: On the Search for a Cellulose-dissolving (Cyto-hydrolytic) Enzyme in the Digestive Tract of Certain Grain-feeding Animals. Journal of the Chemical Society, London, V. 61, p. 352, (1892).

(36) Brown and Morris: On the Existence of a Cellulose-dissolving Enzyme (Cyto-hydrolyst) in the Germinating Seeds of the Grasses. Journal of the Chemical Society, London, V. 57, p. 497, (1890).

(37) De Bary: Über einige Sclerotinien und Sclerotienkrankheiten. Botanische Zeitschrift, V. 44, p. 377, (1886).

(38) EBERLEIN: Ueber die im Wiederkauermagen vorkommenden ciliaten Infusorien. Zeitschrift für wissenschaftlichen Zoologie, V. 49, p. 233, (1895). (Cited by Scheunert, Berliner tierärztliche Wochenschrift, No. 47, p. 826, (1909).

(39) FÜRSTENBERG: Das Verhalten der pflanzlichen Zellmembran während der Entwicklung in chemischer und physiologischer Hinsicht. Zentralblatt für Physiologie, V. 22, p. 567, (1908).

(40) HAUBNER: Journal für Landwirtschaft, 1855, p. 177. (Cited by Lohrisch.)

(41) HENNEBERG AND STOHMAN: Beiträge zur Begründung einer rationellen Fütterung der Wiederkauer. Heft. 2, 1864. Cited in Zeitschrift für Biologie, V. 21, p. 613, (1885).

(42) HOFFMANN: Über den Einfluss von Hemizellulosen und von Zellulose auf die Stickstoffbilanz und den Phlorhizindiabetes von Kaninchen. Dissertation, Halle, 1910. (Cited in Zentralblatt für Biochemie und Biophysik, V. 10, p. 794, (1910).

(43) HOFMEISTER: Die Rohfaser und einige Formen der Cellulose. Landwirtschaftliche Jahrbücher, V. 17, p. 239, (1888).

(44) HOFMEISTER: Die Cellulose und ihre Formen. Ibid., V. 18, p. 767, (1889).

(45) HOFMEISTER: Über Celluloseverdauung beim Pferde. Archiv für wissenschaftliche und practische Thierheilkunde, V. 11, p. 46, (1885).

(46) v. HOESSLIN: Zur Kenntnis der Zelluloseverdauung. 2. Mitteilung. Die Ausnützung der Zellulose beim Hunde. Zeitschrift für Biologie, V. 54, p. 395, (1910).

(47) v. HOESSLIN AND LESSER: Ueber die Zersetzung der Zellulose durch den Inhalt des Coecums des Pferdes. Zeitschrift für Biologie, V. 54, p. 47, (1910).

(48) HOLDEFLEISS: Die Bedeutung des verdauten Antheils der Rohfaser für die tierische Ernährung. Berichte aus dem physiologischen Laboratorium und der Versuchsanstalt des Landwirtschaftlichen Instituts der Universität Halle. Heft. 2, (1895). (Cited by Lohrisch.)

(49) KELLNER: Untersuchungen über den Stoff- und Energieumsatz der erwachsenen Rinder bei Erhaltungs und Productionsfutter. Landwirtschaftliche Versuchs-Stationen, V. 53, p. 457, (1900).

(50) KNAUTHE: Über die Verdauung und den Stoffwechsel die Fische. Archiv für Anatomie und Physiologie, Physiologische Abtheilung, V. 22, p. 149, (1898).

(51) KÖNIG: Zur Bestimmung der Rohfaser und zur Trennung von Cellulose, Lignin und Cutin in derselben. Berichte der deutschen chemischen Gesellschaft, V. 41, p. 46, (1908).

(52) KÖNIG: Die Zellmembran und ihre Bestandtheile in chemischer und physiologischer Hinsicht. Landwirtschaftliche Versuchs-Stationen, V. 65, p. 55, (1907).

(53) KÖNIG AND REINHARDT: Über die Ausnutzung der Pentosane beim Menchen. Zeitschrift für Nahrungs- und Genussmittel, V. 5, p. 110, (1902).

(54) KÜHN, BRONSTEIN AND SCHULZE: Bericht über die auf der landwirtschaftlichen Versuchs-Station Weende ausgeführten Versuche. Journal für Landwirtschaft, V. 14, p. 269, (1866).

(55) LOHRISCH: Über die Bedeutung der Cellulose im Haushalte des Menschen. Zeitschrift für physiologische Chemie, V. 47, p. 200, (1907).

(56) LOHRISCH: Über die Verdauung und Verwertung der Rohfaser und Zellulose im tierischen und menschlichen Organismus. Zentralblatt für die gesammte Physiologie und Pathologie des Stoffwechsels, V. 21, (1907).

(57) LOHRISCH: Der Vorgang der Cellulose und Hemicellulosen Verdauung beim Menschen, und der Nährwerth dieser Substanzen für den menschlichen Organismus. Zeitschrift für experimentelle Pathologie und Therapie, V. 5, p. 478, (1908).

(58) LOHRISCH: Bemerkungen zur Frage der Celluloseverdauung beim Hund und über die Methoden der quantitativen Cellulosebestimmung. Zeitschrift für physiologische Chemie, V. 69, p. 143, (1910).

(59) LUSK: On the Question Whether Dextrose Arises from Cellulose in Digestion. American Journal of Physiology, V. 6, p. xiii, (1901–2).

(60) MALLEVRE: Der Einfluss der als Gährungsprodukt der Cellulose gebildeten Essigsäure auf den Gaswechsel. Pflügers Archiv für Physiologie, V. 49, p. 460, (1891).

(61) MÜLLER, E.: Ein Beitrag zur Frage der Cellulose verdauung im Darmcanale. Pflügers Archiv für Physiologie, V. 83, p. 619, (1901).

(62) MUNK: Der Einfluss des Glycerins, der flüchtigen und festen Fettsäuren auf den Gaswechsel. Pflüger's Archiv für Physiologie, V. 46, p. 303, (1890).

(63) MURDFIELD: Das Lignin und Kutin in chemischer und physiologischer Hinsicht. Inaugural Dissertation, Münster, 1906. (Cited by König, Fürstenberg and Murdfield.)

(64) NEWCOMBE: Cellulose Enzymes. Annals of Botany, V. 13, p. 49, (1899).

(65) OMELIANSKI: Sur la Fermenentation Formique de la Cellulose. Centralblatt für Physiologie, V. 16, p. 522, (1902). (From Archives des Sciences Biologiques, St. Petersbourg, V. IX, No. 3, p. 251.)

(66) PACAULT: Sur deux propriétés diastatiques de la salive de l'escargot. Comptes Rendus de la Société de Biologie, V. 59, p. 29, (1909).

(67) REINITZER: Über das Zellwandlösende Enzym der Gerste. Zeitschrift für physiologische Chemie, V. 23, p. 175, (1897).

(68) SCHEUNERT: Beiträge zur Kenntniss der Zelluloseverdauung im Blinddarm und des Enzymgehaltes des Caecalsekretes. Zeitschrift für physiologische Chemie, V. 48, p. 9, (1906).

(69) SCHEUNERT: Über die Celluloseverdauung bei den Haustieren. 2. Mitteilung. Vermag der Hund Cellulose zu verdauen? Berliner tierärztliche Wochenschrift, No. 47, p. 867, (1909).

(70) SCHEUNERT: Vermag Schafspeichel Zellulose zu lösen? Berliner tierärztliche Wochenschrift, No. 48, p. 113, (1910).

(71) SCHEUNERT AND GRIMMER: Zur Kenntniss der in den Nahrungsmitteln enthaltenen Enzyme und ihrer Mitwerkung bei der Verdauung. Zeitschrift für physiologische Chemie, V. 48, p. 27, (1906).

(72) SCHEUNERT AND LÖTSCH: Vermag der Hund Cellulose oder Rohfaser zu verdauen? Biochemische Zeitschrift, V. 20, p. 10, (1909).

(73) SCHMIDT AND LOHRISCH: Über die Bedeutung der Cellulose für den Kraftwechsel der Diabetiker. Deutsche medicinische Wochenschrift, No. 47, p. 1938, (1907).

(74) SCHULZE: Über die zur Gruppe der stickstofffreien Extractstoffe gehörenden Pflanzenbestandtheile. Die Landwirtschaftliche Versuchs-Stationen, V. 52, (1904).

(75) SCHULZE, STEIGER AND MAXWELL: Zur Chemie der Pflanzenzellmembranen. Zeitschrift für physiologische Chemie, V. 14, p. 227, (1890).

(76) SELLIÈRE: Remarques sur l'hydrolyse diastatique de la Cellulose du Coton et de quelques autres Polysaccharides. Comptes Rendus de la Société de Biologie, V. 63, No. 34, (1907); Centralblatt für Biochemie, V. 7, p. 88, (1908).

(77) SELLIÈRE: Sur la digestion de la cellulose. Comptes Rendus de la Société de Biologie, V. 68, p. 107, (1910).

(78) TAPPEINER: Untersuchung über die Gärung der Cellulose, insbesondere über deren Lösung im Darmkanale. Zeitschrift für Biologie, V. 20, p. 52, (1884).

(79) TOLLENS: Untersuchungen über Kohlenhydrate. Die Landwirtschaftliche Versuchs-Stationen, V. 39, p. 401, (1891).

(80) USTJANZEW: Zur Frage über die Rolle der Rohfaser in dem Stickstoffumsatz des tierischen Organismus. Die Landwirtschaftliche Versuchs-Stationen, V. 56, p. 463, (1902).

(81) VAN ITERSON. Die Lösung der Cellulose durch aërobe Microorganismen. Biochemisches Zentralblatt, V. I, p. 678, (1903).

(82) VOIT AND HOFFMAN: Sitzungsberichte der Bayerischen Akademie, 1869. (Cited by Tappeiner.)

(83) VON KNIERIEM: Über die Verwertung der Cellulose im tierischen Organismus. Zeitschrift für Biologie, V. 21, p. 67, (1885).

(84) WARD: On a Lily Disease. Annals of Botany, V. 2, p. 319, (1889).

(85) WEISKE AND MEHLIS: Über das Verhalten der Rohfaser im Verdauungsapparate der Gänse. Landwirtschaftliche Versuchs-Stationen, V. 21, p. 411, (1878).

(86) WEISKE: Beeinflussen die in Vegetabilien vorkommenden Fermente der Ausnutzung der Nahrung im Organismus? Zeitschrift für physiologische Chemie, V. 19, p. 282, (1894).

(87) WIELER: Analysen der Jungholzregion von Pinus Silvestris und Salix Petendra nebst einem Beitrag zur Methodik der Pflanzenanalyse. Landwirtschaftliche Versuchs-Stationen, V. 32, p. 307, (1886).

(88) ZUNTZ: Gesichtspunkte zum Kritischen Studium der neueren Arbeiten auf dem Gebiete der Ernährung. Landwirtschaftliche Jahrbücher, V. 8, p. 65, (1879).

III. PENTOSANS.

(89) ANKERSMIT: Untersuchungen über die Bakterien im Verdauungskanal des Rindes. Centralblatt für Bakteriologie, Parasitenkunde und Infectionskrankheiten, V. 40, Abt. I, p. 100, (1905).

(90) BEHRENS: Beiträge zur Kenntnis der Obstfäulnisse. Chemisches Centralblatt, V. 98b, p. 1027, (1898).

(91) BERGMANN: Studien über die Digestion der Pflanzenfresser. Skandinavisches Archiv für Physiologie, V. 18, p. 119, (1906).

(92) BIGELOW, GORE AND HOWARD: Studies on Apples. United States Department of Agriculture, Bulletin of the Bureau of Chemistry, No. 94, (1905).

(93) BLUMENTHAL: Die Pentosurie, Deutsche Klinik, Vol. 3, (1903). (Cited by Lohrisch.)

(94) BORNTRÄGER: Über die Rutinsäure. Liebig's Annalen, V. 53, p. 385, (1845).

(95) BOURQUELOT AND HERISSEY: Sur l'existence dans l'orge germée d'un ferment soluble agissant sur la pectine. Comptes Rendus, V. 127, p. 191, (1898).

(96) BRASCH: Über das Verhalten nicht-gährungsfähiger Kohlenhydrate im tierischen Organismus mit Berücksichtigung des Diabetes. Zeitschrift für Biologie, V. 50, p. 113, (1907).

(97) BROWN: On the Search for a Cellulose-dissolving (Cytohydrolytic) Enzyme in the Digestive Tract of Certain Grain-feeding Animals. Journal of the Chemical Society, London, V. 61, p. 352, (1892).

(98) CALABRESI: Über die Bildung und die physiologische Aufgabe der Pentosane in den Pflanzen. Centralblatt für Agriculturchemie, V. 37, p. 93, (1908).

(99) COUVREUR AND BELLION: Sur le sucre dans le sang d'escargot. Comptes Rendus de la Société de Biologie, V. 63, p. 339, (1907).

(100) COMINOTTI. Ueber das Vorhandensein der Pentosen im Harne des Menschen und der Tiere. Biochemische Zeitschrift, V. 22, p. 106, (1909).

(101) CRAMER: Untersuchungen über Hemicellulosevergärung durch menschlichen Kot. Dissertation, Halle, 1910. (Cited in Zentralblatt für Biochemie und Biophysik, V. 10, p. 800, (1910).

(102) CREMER: Über das Verhalten einiger Zuckerarten im thierischen Organismus. Zeitschrift für Biologie, V. 29, p. 484, (1892).

(103) CREMER: Über die Verwertung der Rhamnose im tierischen Organismus, und einige damit zusammenhängende Fragen der Physiologie der Kohlehydrate. Zeitschrift für Biologie, V. 42, p. 428, (1901).

(104) CROSS, BEVAN AND BEADLE: Die natürlichen Oxycellulosen. Berichte der deutschen chemischen Gesellschaft, V. 27, p. 1061, (1894).

(105) CROSS, BEVAN, AND SMITH: Über einige Vorgänge in der Gerstenpflanze. Berichte der deutschen chemischen Gesellschaft, V. 28, p. 2604, (1895).

(106) CROSS: Über die Constitution der Pektinstoffe. Ibid., V. 28, p. 2609, (1895).

(107) CZAPEK: Zur Biologie der holzbewohnenden Pilze. Berichte der deutschen botanischen Gesellschaft, V. 17, p. 166, (1899).

(108) DE CHALMOT: Pentosans in Plants. American Chemical Journal, V. 15, p. 276, (1893).

(109) EBSTEIN: Einige Bemerkungen zum Verhalten d l Pentosen im menschlichen Organismus. Virchow's Archiv, V, 129, p. 401, (1892).

(110) FRAPS: The Digestibility of some Non-nitrogenous Constituents of Certain Feeding Stuffs. North Carolina Experiment Station, Bulletin No. 172, (1900).

(111) FRENTZEL: Über Glycogenbildung im Thierkörper nach Fütterung mit Holzzucker. Pflüger's Archiv für Physiologie, V. 56, p. 273, (1894).

(112) FUDAKOWSKI: Spaltung der Arabinsäure durch Pepsin. Berichte der deutschen chemischen Gesellschaft, V. 11, p. 1069, (1878).

(113) GÖTZE AND PFEIFFER: Beiträge zur Frage über die Bildung resp. das Verhalten der Pentaglykosen in Pflanzen- und Thierkörper. Die Landwirtschaftliche Versuchs-Stationen, V. 47, p. 59, (1896).

(114) HARRISON: A Bacterial Disease of Cauliflower (Brassica and Oleraceae), and Allied Plants. Centalblatt für Bakteriologie, Abtheilung II, V. 13, p. 185, (1904).

(115) HOFMEISTER: Die quantitative Trennung von Hemicellulose, Cellulose, und Lignin, und das Vorkommen der Pentosane in diesen. Die Landwirtschaftliche Versuchs-Stationen, V. 50, p. 347, (1898).

(116) HERZFELD: Die Pektinsubstanzen. Chemisches Centralblatt, V. II, p. 618, (1891).

(117) V. Jacksch: Über Alimentäre Pentosurie. Zeitschrift für Heilkunde-V. 20, p. 195, (1899).

(118) Kellner and Köhler: Untersuchungen über den Stoff- und Energie-umsatz des erwachsenen Rindes bei Erhaltungs- und Productionsfutter. Die Landwirtschaftliche Versuchs-Stationen, V. 53, p. 457, (1900).

(119) König: Die Zellmembran und ihre Bestandteile in chemischer und physiologischer Hinsicht. Die Landwirtschaftlichen Versuchs-Stationen, V. 65, p. 55, (1907).

(120) König and Reinhardt: Ausnutzung der Pentosane beim Menschen. Zeitschrift für Untersuchung der Nahrungs- und Genussmittel, V. 5, p. 110, (1902); V. 7, p. 729, (1904).

(121) Kröber: Untersuchungen über die Pentosanbestimmungen mittelst der Salzsäure-Phloroglucin Methode, nebst einiger Anwendungen. Journal für Landwirthschaft, V. 48, p. 357, (1900).

(122) Lindemann and May: Die Verwerthung der Rhamnose vom normalen und vom diabetischen menschlichen Organismus. Deutsches Archiv für klinische Medicin, V. 56, p. 283, (1895–6).

(123) Lindsey: The Pentosans. Fifteenth Massachusetts State Report, p. 69, (1903).

(124) Lindsey and Holland: Twelfth Massachusetts State Report, p. 175, (1900). (Cited by Lindsey.)

(125) Lintner and Düll: Über die chemische Natur des Gerstengummi. Chemiker Zeitung, V. 15, Repertorium p. 266, (1891).

(126) McCollum and Brannon: The Disappearance of Pentosans from the Digestive Tract of the Cow. Journal of the American Chemical Society, V. 31, p. 1252, (1909).

(127) Neuberg: Die Physiologie der Pentosen und der Glukuronsäuren. Ergebnisse der Physiologie, V. 3, p. 421, (1904).

(128) Neuberg and Wohlgemuth: Über das Verhalten stereo-isomer Substanzen im Tierkörper. Zeitschrift für physiologische Chemie, V. 35, p. 41, (1902).

(129) Oshima and Tollens: Über das Nori aus Japan. Berichte der deutschen chemischen Gesellschaft, V. 34, p. 1422, (1901).

(130) Pacault: Sur deux propriétés diastatiques de la salive de l'escargot. Comptes Rendus de la Société de Biologie, V. 59, p. 29, (1905).

(131) Ravenna and Cereser: Origin and Physiological Function of Pentosans in Plants, Journal of the London Chemical Society, V. 96, p. 1946, (1909). (From Atti R. Accad. Lincei, 1909 [V] *18*, ii, p. 177.)

(132) Reichardt: Pararabin, ein neues Kohlehydrat. Berichte der deutschen chemischen Gesellschaft, V. 8, p. 807, (1875).

(133) Reinhardt: Die Bestimmung der Cellulose und ihr Verhalten, sowie das der Pentosane im Darmcanal des Menschen. Dissertation, München, (1903).

(134) Rohmann: Einige Beobachtungen über die Verdauung der Kohlehydrate bei Aplysien. Centralblatt für Physiologie, V. 13, p. 455, (1899).

(135) Rubner: Über den Werth der Weizenkleie für die Ernährung des Menschen. Zeitschrift für Biologie, V. 19, p. 45, (1883).

(136) Rudzinski: Über die Bedeutung der Pentosane als Bestandtheile der Futtermittel, insbesondere des Roggenstrohes. Zeitschrift für physiologische Chemie, V. 40, p. 317, (1904).

(137) Salkowski: Über die Gährung der Pentosen. Zeitschrift für physiologische Chemie, V. 30, p. 478, (1900).

(138) SALKOWSKI: Über das Verhalten der Pentosen im Thierkörper. Centralblatt für die medicinische Wissenschaften, No. 11, p. 193. (1893).

(139) SALKOWSKI: Über das Verhalten des Arabans zu Fehling'scher Lösung. Zeitschrift für physiologische Chemie, V. 35, p. 240, (1902).

(140) SALKOWSKI: Ueber die Darstellung des Xylans. Ibid., V. 34, p. 162, (1901–2).

(141) SCHEIBLER: Über den Pectinzucker (Pectinose), eine neue durch Spaltung der Metapectinsäure entstehende Zuckerart. Berichte der deutschen chemischen Gesellschaft, V. 1, p. 108, (1868).

(142) SCHEIBLER: Über das Vorkommen der Arabinsäure (Gummi) in den Zuckerrüben und über den Arabinzucker (Gummizucker). Berichte der deutschen chemischen Gesellschaft, V. 6, p. 612, (1873).

(413) SHERMAN: The Insoluble Carbohydrates of Wheat. Journal of the American Chemical Society, V. 19, p. 308, (1897).

(144) SCHMULEWITSCH: Bulletin de l'Académie de St. Petersbourg, V. 25, p. 549, (1879). (Cited by Bergmann.)

(145) SCHÖNE AND TOLLENS: Untersuchungen über Kohlehydrate. Die Landwirtschaftlichen Versuchs-Stationen, V. 40, p. 377, (1892).

(146) SCHORSTEIN: Zur Biochemie der Holzpilze. Centralblatt für Bakteriologie, Abtheilung II, V, 9. p. 446, (1902).

(147) SCHULZE, STEIGER AND MAXWELL: Zur Chemie der Pflanzenzellmembranen. Zeitschrift für physiologische Chemie, V. 14, p. 227, (1892).

(148) SELLIÈRE: Sur la présence d'une diastase hydrolysant dans le suc gastrointestinal d'escargot. Comptes Rendus de la Société de Biologie, V. 58, p. 409, (1905).

(149) SELLIÈRE: Sur une diastase hydolysant la xylane dans le tube digestif de certaines larves de Coléoptères. Ibid., V. 58, p. 940, (1905).

(150) SELLIÈRE: Sur la présence de la xylanase chez différents mollusques gastéropodes. Ibid., V. 59, p. 120, (1906).

(151) SELLIÈRE: Sur l'absorption et la présence dans le sang chez l'escargot des produits de l'hydrolyse digestion de la xylane. Comptes Rendus de la Société de Biologie, V. 63, No. 36, (1907).

(152) SELLIÈRE: Sur la digestion de la xylane chez quelques mammifères herbivores. Ibid., V. 64, p. 941, (1908).

(153) SELLIÈRE: Sur la digestion de la xylane chez les mammifères. Comptes Rendus de la Société de Biologie, V. 66, p. 691, (1909).

(154) SLOWTZOFF: Über das Verhalten des Xylans im Thierkörper. Zeitschrift für physiologische Chemie, V. 34, p. 181, (1901).

(155) STONE: Die Verdaulichkeit der Pentosane. Berichte der deutschen chemischen Gesellschaft, V. 25, p. 563, (1892).

(156) STONE AND JONES: Verdaulichkeit der Pentosane. Centralblatt für Agriculturchemie, V. 22, p. 677, (1893).

(157) TOLLENS: Über die in den Pflanzenstoffen und besonders den Futtermitteln enthaltenen Pentosane, ihre Bestimmungsmethoden und Eigenschaften. Journal für Landwirthschaft, V. 44, p. 171, (1896).

(158) TOLLENS: Untersuchungen über Kohlenhydrate. Die Landwirtschaftlichen Versuchs-Stationen, V. 39, p. 401, (1891).

(159) TOLLENS AND GLAUBITZ: Über den Pentosan-gehalt verschiedener Materialien, welche zur Ernährung dienen, und in den Gärungs-Industrieen ange-

wendet werden, und über den Verbleib des Pentosans bei den Operationen, welcher die obigen Materialien unterworfen werden. Journal für Landwirthschaft, V. 45, p. 97, (1897).

(160) TROMP DE HAAS AND TOLLENS: Untersuchungen über die Pectionstoffe. Zeitschrift des Vereins für die Rübenzucker-Industrie der Deutschen Reiches, V. 45, p. 473, (1895). Liebig's Annalen, V. 286, p. 278, (1895).

(161) UTZJANZEW: Zur Physiologie des Blinddarmes bei den Pflanzenfressern. Biochemische Zeitschrift, V. 4, p. 154, (1907).

(162) WARD AND DUNLOP: On Some Points in the Histology and Physiology of the Fruits and Seeds of Rhamnus. Annals of Botany, V. I, p. 1, (1887).

(163) WIDTSOE AND TOLLENS: Über Arabinose, Xylose und Fucose aus Traganth. Berichte der deutschen chemischen Gesellschaft, V. 33, p. 132, (1900).

(164) WEISER: Über die Verdaulichkeit der Pentosane. Die Landwirtschaftlichen Versuchs-Stationen, V. 58, p. 238, (1903).

(165) WEISER AND ZAITSCHEK: Beiträge zur Methodik der Stärkebestimmung und zur Kenntnis der Verdaulichkeit der Kohlenhydrate. Pflüger's Archiv für Physiologie, V. 93, p. 98, (1903).

(166) WEISKE Über die Verdaulichkeit der in den vegetabilischen Futtermitteln enthaltenen Pentosane. Zeitschrift für physiologische Chemie, V. 20, p. 489, (1895).

(167) WINTERSTEIN: Über das pflanzliche Amyloid. Zeitschrift für physiologische Chemie, V. 17, p. 353, (1893).

(168) ZUNTZ AND UTZJANZEW: Zur Bedeutung des Blinddarmes für die Verdauung beim Kaninchen. Archiv für Anatomie und Physiologie, physiologische Abtheilung, 1905, p. 403.

IV. GALACTANS.

(169) BAUER: Ueber den aus Agar-agar entstehenden Zucker, über eine neue Säure aus der Arabinose nebst dem Versuch einer Classification der Gallertbildenden Kohlehydrate nach den aus ihnen enstehenden Zuckerarten. Journal für praktische Chemie, V. 30, p. 36, 7, (1884).

(170) BAUER: Weitere Untersuchungen über Alimentäre Galaktosurie. Zentralblatt für innere Medizin, 1906, p. 1176.

(171) BENTE: Ueber anderweitige Darstellung von Levulinsäure. Berichte der deutschen chemischen Gesellschaft, V. 8, p. 417, (1875).

(172) BENTE: Zur Darstellung der Levulinsäure und über Carragheenzucker. Berichte der deutschen chemischen Gesellschaft, V. 9, p. 1157, (1876).

(173) BIERRY AND GIAJA: Digestion des mannanes et des galactanes. Comptes Rendus, V. 148, No. 8, p. 507, (1909).

(174) BOUREUELOT AND HÉRISSEY: Germination de la graine de Caroubier. Comptes Rendus, V. 129, p. 614, (1899).

(175) BRASCH: Ueber das Verhalten nicht-gärungsfähiger Kohlehydrate im tierischen Organismus mit Berücksichtigung des Diabetes. Zeitschrift für Biologie, V. 50, p. 113, (1907).

(176) CASTORO: Beiträge zur Kenntnis der Hemicellulosen. Zeitschrift für physiologische Chemie, V. 48, p. 96, (1905); V. 49, p. 96, (1906).

(177) CREMER: Ueber das Verhalten einiger Zuckerarten im tierischen Organismus. Zeitschrift für Biologie, V. 29, p. 484, (1892).

(178) FLÜCKINGER AND MAYER: Neues Repertorium für Pharmacie, 1868, p. 350. (Cited by Haedike, Bauer and Tollens.)

(179) GORET: Étude chimique et physiologique de quelques albumens cornés de graines de Légumineuses. Thèse, Paris, 1901. (Cited by Hérissey.)

(180) GREENISH: Untersuchung von Fucus Amylaceous. Berichte der deutschen chemischen Gesellschaft, V. 14, p. 2253, (1881).

(181) GREENISH: Die Kohlehydrate des Fucus Amylaceous. Ibid., V. 5, p. 2243, (1882).

(182) GRAN: Die Hydrolyse des Agars durch ein Enzym. Centralblatt für Bacteriologie, Abtheilung II, V. 9, p. 562, (1902).

(183) GRÜSS: Studien über Reservecellulose. Botanisches Centralblatt, V. 70, p. 242, (1897).

(184) GRÜSS: Ueber den Umsatz bei der Keimung der Dattel. Berichte der botanischen Gesellschaft, V. 20, p. 36, (1902).

(185) HÄDIKE, BAUER AND TOLLENS: Ueber Galactose aus Carragheenmoos. Liebig's Annalen, V. 238, p. 302, (1887).

(186) HÉRISSEY: Isolément du galactose crystallisé dans les produits de digestion par la seminase, des galactans, des albumens cornés. Comptes Rendus de la Société de Biologie, V. 54, p. 1174, (1902).

(187) HÉRISSEY: Recherches chimiques et physiologiques sur la digestion des mannanes et des galactanes, par la seminase, chez les végétaux. Revue Générale de Botanique, 1903, p. 345.

(188) HOFMEISTER: Ueber Resorption und Assimilation der Nährstoffe. Archiv für experimentelle Pathologie und Pharmakologie, V. 25, p. 240, (1888).

(189) KAUSCH AND SOCIN: Sind Milchzucker und Galactose directe Glycogenbildner? Archiv für experimentelle Pathologie und Pharmakologie, V. 31, (1893). (Cited by Lohrisch.)

(190) KÖNIG AND BETTELS: Die Kohlenhydrate der Meeresalgen und daraus hergestellter Erzeugnisse. Zeitschrift für Untersuchung der Nahrungs- und Genussmittel, V. 10, p. 487, (1905).

(191) LINDSEY: The Digestibility of Galactan. 17th Annual Report, Massachusetts State Experiment Station, p. 78, (1905).

(192) LIPPMANN: Ueber ein neues Galactan und einige Eigenschaften der Galactose. Berichte der deutschen chemischen Gesellschaft, V. 20, p. 1001, (1890).

(193) LOHRISCH: Die Ursachen der chronischen habituellen Obstipation im Lichte systematischer Ausnutzungsversuche. Deutsche Archiv für klinische Medizin, V. 79, p. 383, (1904).

(194) LOHRISCH: Der Vorgang der Cellulose- und Hemicellulosenverdauung beim Menschen und der Nährwerth dieser Substanzen für den menschlichen Organismus. Zeitschrift für experimentelle Pathologie und Pharmakologie, V. 5, p. 478, (1908).

(195) MALLÈVRE: Der Einfluss der als Gährungsprodukt der Cellulose gebildeten Essigsäure auf dem Gaswechsel. Pflüger's Archiv für Physiologie, V. 49, p. 460, (1891).

(196) MENDEL: Das Verhalten einiger unverdaulicher Kohlehydrate im Verdauungstrakt. Zentralblatt für die gesammte Physiologie und Pathologie des Stoffwechsels, No. 17, p. 1, (1908).

(197) MÜLLER, KARL: Die chemische Zusammensetzung der Zellmembranen bei verschiedenen Kryptogamen. Zeitschrift für physiologische Chemie, V. 45, p. 264, (1905).

(198) MUNK: Der Einfluss des Glycerins, der flüchtigen und festen Fettsäuren auf den Gaswechsel. Pflüger's Archiv, V. 46, p. 303, (1890).

(199) MÜNTZ: Sur la Galactine. Comptes Rendus, V. 94, p. 453, (1882).

(200) MÜTHER: Untersuchungen über Fucusarten, Laminaria und Carragheenmoos, sowie die hydrolytisch daraus entsehenden Substanzen und über Derivate derselben, besonders Fucose und Fuconsäure. Inaugural Dissertation, Göttingen, 1903.

(201) MÜTHER AND TOLLENS: Ueber die Producte der Hydrolyse von Seetang (Fucus) Laminaria, und Carragheen-Moos. Berichte der deutschen chemischen Gesellschaft, V. 37, p. 298, (1904).

(202) PAYEN: Sur la gélose et les nids de salagane. Comptes Rendus, V. 49, p. 521, (1859).

(203) PLETNEW: Vergleichende Ausnutzungsversuche an normalen und habituell obstipirten Menschen. Zeitschrift für experimentelle Pathologie und Therapie, V. 5, p. 186, (1908).

(204) ROTHENFUSSER: Der Schleimkörper des Leinsamens. Jahresbericht für Thierchemie, V. 34, p. 78, (1904).

(205) SAIKI: The Digestibility and Utilization of some Polysaccharide Carbohydrates derived from Lichens and Marine Algae. The Journal of Biological Chemistry, V. 2, p. 251, (1906).

(206) SANDMEYER: Ueber die Folgen der partiellen Pankreasextirpation beim Hund. Zeitschrift für Biologie, V. 31, p. 32, (1895).

(207) SAWAMURA: Über Enzyme im Verdauungskanal. Bulletin of the College of Agriculture, Tokyo Imperial University, V. No. 2, (1902); Jahresbericht für Thierchemie, V. 32, p. 419, (1902).

(208) SCHELLENBERG: Untersuchungen über das Verhalten einiger Pilze gegen Hemicellulose. Flora, V. 98, p. 257, (1908). Biochemisches Centralblatt, V. 7, p. 767, (1907).

(209) SCHMIDT, A.: Neue Beobachtungen zur Erklärung und rationellen Behandlung der chronischen habituellen Obstipation. Münchener medizinische Wochenschrift, V. 52, p. 1970, (1905).

(210) SCHMIDT, C.: Ueber Pflanzenschleim und Bassorin. Liebig's Annalen, V. 51, p. 56, (1844).

(211) SCHMIDT AND LOHRISCH: Weitere Beobachtungen über die Bedeutung der Zellulose (Hemicellulose) für die Ernährung der Diabetiker. Deutsche mediziische Wochenschrift, No. 47, p. 1, (1908).

(212) SCHULZE: Ueber die Zellwandbestandtheile der Cotyledonen von Lupinus luteus und Lupinus angustifolius, und über ihr Verhalten während des Keimungsvorganges. Zeitschrift für physiologische Chemie, V. 21, p. 392, (1896).

(213) SCHULZE: Über die zur Gruppe der stickstofffreien Extractstoffe gehörenden Pflanzenbestandtheile. Journal für Landwirtschaft, V. 52, (1904).

(214) SCHULZE: Zur Kenntnis des β-Galactans. Berichte der deutschen chemischen Gesellschaft, V. 25, p. 2213, (1893).

(215) SCHULZE: Ueber das Vorkommen eines unlöslichen Schleimsäure gebenden Kohlehydrats in den Rothklee- und Luzerne-pflanzen. Die Landwirtschaftliche Versuchs-Stationen, V. 42, p. 9, (1893).

(216) SCHULZE: Zur Kenntnis der in den Leguminosensamen enthaltenen Kohlehydrate. Ibid., V. 41, p. 207, (1892).

(217) SCHULZE, STEIGER AND MAXWELL: Zur Chemie der Pflanzenzellmembranen. Zeitschrift für physiologische Chemie, V. 14, p. 227, (1890).

(218) SCHULZE AND CASTORO: Beiträge zur Kenntnis der Hemicellulosen. Ibid., V. 37, p. 41, (1902); V. 39, p. 318, (1904).

(219) SCHULZE AND STEIGER: Untersuchungen über die stickstofffreien Reservestoffe der Samen von Lupinus luteus und über die Umwandlungen derselben während des Keimungsprozesses. Die Landwirtschaftliche Versuchs-Stationen, V. 36, p. 391, (1889).

(220) SEBOR: Ueber die Kohlenhydrate des Carragheen-Moos. Botanisches Centralblatt, V. 86, p. 70, (1901).

(221) STRAUSS: Ueber das Vorkommen eini ger Kohlehydratefermente bei Lepidopteren und Dipteren in verschiedenen Entwicklungsstadien. Zeitschrift für Biologie, V. 52, p. 95, (1908).

(222) ULANDER: Untersuchungen über die Kohlenhydrate der Flechten. Dissertation, Göttingen, 1905.

(223) VOIT: Ueber die Glykogenbildung nach Aufnahme verschiedener Zuckerarten. Zeitschrift für Biologie, V. 28, p. 245, (1891).

(224) VOIT: Ueber das Verhalten der Galactose beim Diabetiker. Zeitschrift für Biologie, V. 29, p. 147, (1892).

(225) VON PLANTA AND SCHULZE: Ueber ein neues krystallisirbares Kohlehydrat. Berichte der deutschen chemischen Gesellschaft, V. 23, p. 1692, (1890).

(226) WEINLAND: Ueber die Bildung von Glykogen nach Galactose-fütterung. Zeitschrift für Biologie, V. 40, p. 374, (1900).

V. MANNANS.

(227) BERTRAND: Sur la presénce de la mannocellulose dans le tissu ligneux des plantes gymnospermes. Comptes Rendus, V. 129, p. 1025, (1899).

(228) BIERRY AND GIAJA: Sur la digestion des mannanes et des galactanes. Comptes Rendus de la Société de Biologie, V. 60, p. 945, (1906).

(229) BIERRY AND GIAJA: Digestion des mannanes et des galactanes. Comptes Rendus, V. 148, pp. 735 and 507, (1909).

(230) BROWN AND MORRIS: On the Existence of a Cellulose-dissolving Enzyme (Cytohydrolyst) in the Germinating Seeds of the Grasses. Journal of the Chemical Society, London, V. 57, p. 497, (1890).

(231) BOURQUELOT AND HÉRISSEY: Sur la composition de l'albumen de la graine du Phoenix canariensis et sur les phénomènes chimiques qui accompagnent la germination de cette graine. Comptes Rendus, V. 133, p. 302, (1901).

(232) BOURQUELOT AND HÉRISSEY: Sur la composition de l'albumen de la graine de caroubier: production de galactose et de mannose par hydrolyse. Ibid., V. 129, p. 228, (1899). Sur la composition de la graine de caroubier. Ibid., V. 129, p. 391, (1899).

(233) BOURQUELOT AND HÉRISSEY: Germination de la graine de caroubier: production de mannose par un ferment soluble. Ibid., V. 129, p. 614, (1899).

(234) BOURQUELOT AND HÉRISSEY: Sur les ferments solubles produits pendant la germination par les graines a albumen corné. Comptes Rendus, V. 130, p. 42, (1900).

(235) BOURQUELOT AND HÉRISSEY: Sur l'individualité de la seminase, ferment soluble secrété par les graines de legumineuses à albumen corné pendant la germination. Ibid., V. 130, p. 340, (1900).

(236) BOURQUELOT AND HÉRISSEY: Sur la mecanisme de la saccharification des mannanes du corrozo par la séminase de la luzerne. Ibid., V. 136, p. 404, (1903).

(237) CASTORO: Beiträge zur Kenntnis der Hemicellulosen. Zeitschrift für physiologische Chemie, V. 49, p. 96, (1906).

(238) CREMER: Verhalten einiger Zuckerarten im tierischen Organismus. Zeitschrift für Biologie, V. 29, p. 484, (1892).

(239) DILLINGHAM: A Contribution to the History of the Use of Bark Bread. Bulletin of the Bussey Institution, Vol. III, Part V. 120, (1906).

(240) DUBAT: Composition des hydrates de carbone de réserve de l'albumen des graines de quelques Liliacées et en particulier du Petit Haux. Comptes Rendus, V. 133, p. 942 (1901).

(241) EFFRONT: Sur une nouvelle enzyme hydrolytique, "la caroubinase." Ibid., V. 125, pp. 38 and 116, (1897). Sur la caroubinase. Ibid., V. 125, p. 309, (1897).

(242) FISCHER AND HIRSCHBERGER: Ueber Mannose. Berichte der deutschen chemischen Gesellschaft, V. 21, p. 1805, (1888); V. 22, pp. 365 and 1155, (1889).

(243) FRANCK: Ueber die anatomische Bedeutung und die Enstehung der vegetabilischen Schleime. Jahrbücher für wissenschaftliche Botanik, V. 5, p. 161, (1866).

(244) GANS AND TOLLENS: Ueber die Bildung von Zuckersäure aus Dextrose haltenden Stoffen, besonders aus Raffinose, und über die Untersuchung einiger Pflanzenschleimarten. Liebig's Annalen, V. 249, p. 215, (1888).

(245) GATIN: Action de quelques diastases animales sur certaines mannanes. Comptes Rendus de la Société de Biologie, V. 58, p. 847, (1905).

(246) GATIN AND GATIN: Über die Verdaulichkeit der Mannanen durch Diastasen der höheren Tiere. Chemisches Centralblatt, 1907 (2), p. 1181.

(247) GATIN: Isomérisation de mannose en glycose sous l'action d'un ferment soluble. Comptes Rendus de la Société de Biologie, V. 64, p. 903, (1908).

(248) GIRAND: Étude comparative des gommes et des mucilages. Comptes Rendus, V. 80, p. 477, (1875).

(249) GORET: Sur la composition de l'albumen de la graine de févier d'Amérique (Gleditschia triacanthos L., Legumineuses). Comptes Rendus, V. 131, p. 60, (1900).

(250) GRÜSS: Studien über Reserve-Cellulose. Botanisches Centralblatt, V. 70, p. 242, (1897).

(251) GRÜSS: Ueber den Umsatz bei der Keimung der Dattel. Berichte der deutschen botanischen Gesellschaft, V. 20, p. 36, (1902).

(252) HÉRISSEY: Sur la digestion de la mannane des tubercules d'Orchidées. Comptes Rendus, V. 134, p. 721, (1902).

(253) HÉRISSEY: Recherches chimiques et physiologiques sur la digestion des mannanes et galactanes par la seminase chez les végétaux. Revue Génerale de Botanique, 1903, p. 345.

(254) HILGER: Zur Kenntnis der Pflanzenschleime. Berichte der deutschen chemischen Gesellschaft, V. 36, p. 3198, (1903).

(255) KANO AND IISHIMA: Bulletin of the College of Agriculture. Tokyo Imperial University, II, No. 2, 1894. (Cited by Day, Bulletin 202. Office of Experiment Stations, United States Department of Agriculture.)

(256) KINOSHITA: On the Occurrence of two kinds of Mannan in the Root of Conophallus Konjaku. Bulletin of the College of Agriculture, Tokyo Imperial University, No. V, p. 205, (1902).

(257) KINOSHITA: On the Occurrence of Mannan. Ibid., No. V, p. 253, (1902).

(258) MEIGEN AND SPRENG: Ueber die Kohlehydrate der Hefe. Zeitschrift für physiologische Chemie, V. 55, p. 48, (1908).

(259) MULDER: Ueber Pflanzenschleim. Journal für praktische Chemie, V. 37, p. 334, (1846).

(260) NEUBERG AND MAYER: Schicksal der drei Mannosen im Kaninchenleib. Zeitschrift für physiologische Chemie, V. 37, p. 530, (1902).

(261) NEWCOMBE: Cellulose Enzymes. Annals of Botany, V. 13, p. 49, (1899).

(262) NIEBLING: Untersuchungen über die künstliche Verdauung landwirthschaftlicher Futtermittel nach Stutzer, und Pepsinwirkungen im allgemeinen. Landwirtschaftliche Jahrbücher, V. 19, p. 149, (1890).

(263) POHL: Ueber die Fallbarkeit colloider Kohlenhydrate durch Salze. Zeitschrift für physiologische Chemie, V. 14, p. 159, (1890).

(264) REISS: Ueber die Natur der Reserve-Cellulose. Berichte der deutschen botanischen Gesellschaft, V. 7, p. 322, (1889).

(265) ROSENFELD: Untersuchungen über Kohlehydrate. Centralblatt für innere Medizin, V. 21, p. 177, (1900).

(266) SACHS: Zur Keimungsgeschichte der Dattel. Botanische Zeitung, 1862, p. 241.

(267) SAWAMURA: On the Liquefaction of Mannan by Microbes. Centralblatt für Bakteriologie, Abtheilung II., V. 11, p. 21, (1903–4).

(268) SAWAMURA: On the Digestive Power of the Intestinal Canal. Bulletin of the College of Agriculture, Tokyo Imperial University, No. V, p. 155, (1902).

(269) SCHELLENBERG: Untersuchungen über das Verhalten einiger Pilze gegen Hemicellulosen. Flora, V. 98, p. 257, (1908).

(270) SCHMIDT, C.: Ueber Pflanzenschleim und Bassorin. Liebig's Annalen V. 51, p. 29, (1844).

(271) STORER: Testing for Mannose. Bulletin of the Bussey Institution, Vol. III, Part II, p. 13, (1902).

(272) STORER: Notes on the Occurrence of Mannan in the Wood of Some Kinds of Trees, and in Various Roots and Fruits. Ibid., V. III, Part III, p. 47, (1903).

(273) SCHULZE: Zur Chemie der pflanzlichen Zellmembranen. Zeitschrift für physiologische Chemie, V. 16, p. 387, (1892).

(274) SCHUSTER AND LIEBSCHER: Der Nährwerth der Steinnussspähne. Landwirthschaftliche Jahrbücher, V. 19, p. 143, (1890).

(275) STRAUSS: Ueber das Vorkommen einiger Kohlehydratefermente bei Lepidopteren und Dipteren in verschiedenen Entwicklungsstadien. Zeitschrift für Biologie, V. 52, p. 95, (1908).

(276) THAMM: Ein Beitrag zur Kenntnis der Pflanzenschleime. Dissertation, München, 1903.

(277) TOLLENS AND GANS: Mannose oder Isomanitose aus Salepschleim. Berichte der deutschen chemischen Gesellschaft, V. 21, p.2150, (1888).

(278) TOLLENS AND OSHIMA: Über das Nori aus Japan. Berichte der deutschen chemischen Gesellschaft, V. 34, p. 1422, (1901).

(279) TOLLENS AND WIDSTOE: Über die Reactionen des Methyl-Furfurols und der Methyl-Pentosane. Ibid., V. 33, p. 132, (1900).

(280) TSUJI: Mannan as an Article of Human Food. Bulletin of the College of Agriculture, Tokyo Imperial University, No. II, p. 103, (1894).

(281) TSUKAMATO: Ueber die Bildung von Mannan in Amorphophallus Konjak. Chemisches Centralblatt, V. 97a, p. 930, (1897).

(282) VAN EKENSTEIN: Sur la caroubinose et sur la d-mannose. Comptes Rendus, V. 125, p. 719, (1897).

(283) VOIT: Ueber die Aufnahme des Pflanzenschleims und des Gummis aus dem Darme in die Säfte. Zeitschrift für Biologie, V. 10, p. 59, (1874).

(284) WEISKE: Versuche über die Verdaulichkeit und den Nähreffect des Johannisbrodes. Journal für Landwirthschaft, V. 27, p. 321, (1879).

(285) ZANOTTI: Untersuchungen über einige zusammengesetzte Kolhehydrate. Chemisches Centralblatt, V. 99a, p. 1209, (1899).

VI. LEVULANS.

(286) BIERRY: Recherches sur la digestion de l'inuline. Comptes Rendus de la Société de Biologie, V. 59, p. 256, (1905).

(287) BIERRY: Recherches sur la digestion de l'inuline. Comptes Rendus, V. 150, p. 116, (1910).

(288) BIERRI AND PORTER: Recherches sur la digestion de l'inuline. Ibid., V. 52, p. 423, (1900).

(289) BOURQUELOT: Inulase et fermentation alcoholique indirecte de l'inuline. Comptes Rendus, V. 116, p. 1143, (1893).

(290) BOURQUELOT: Remarques sur les ferments solubles sécrétés par l'Aspergillus et le Pénicillium. Comptes Rendus de las Société de Biologie, V. 9, p. 653, (1893).

(291) CHEVASTELON: Sur l'inuline d'ail, de la jacinthe, de l'asphodèle et de la tubéreuse. Journal de Pharmacie, V. 4, p. 2, (1895). (Cited by Dean.)

(292) CHITTENDEN: The Behavior of Inulin in the Gastro-intestinal Tract. American Journal of Physiology, V. 2, p. XVII, (1898).

(293) DEAN: Experimental Studies on Inulase. Botanical Gazette, V. 35, p. 24, (1903).

(294) DEAN: On Inulin. American Chemical Journal, V. 32, p. 69, (1904).

(295) DUCAMP: Beitrag zum Studium der Unterscheidung des Colibazillus, Wirkung der Bazillen der Colityphusruhrgruppe auf die Kohlehydrate. Jahresbericht für Thierchemie, V. 37, p. 952, (1907).

(296) EKSTRAND AND JOHANSON: Zur Kenntnis der Kohlehydrate, I. Berichte der deutschen chemischen Gessellschaft, V. 20, p. 3310, (1887). Zur Kenntnis der Kohlehydrate, II. Ibid., V. 21, p. 594, (1888).

(297) FINN: Experimentelle Beiträge zur Glycogen- und Zuckerbildung in der Leber. Arbeiten aus dem physiologischen Laboratorium, Würzburg, 1877. (Cited by Miura.)

(298) FITZ: Ueber Schizomycetengährungen. Berichte der deutschen chemischen Gesellschaft, V. 11, p. 42, (1878).

(299) FRERICHS: Zur Glykogenbildung in der Leber. Dissertation, Würzburg, 1876. (Cited by Miura.)

(300) GREEN: On the Germination of the Jerusalem Artichoke (Helianthus tuberosus). Annals of Botany, V. I, p. 223, (1888).

(301) HARLAY: De l'hydrate de carbone de réserve dans les tubercules de 'avoine à chapelets. Comptes Rendus. V. 132, p. 423, (1901).

(302) KILIANI: Ueber Inulin. Liebig's Annalen, V. 205, p. 145, (1880).

(303) KOMANOS: Ueber die Verdauung des Inulins und seine Verwendung bei Diabetes Mellitus. Dissertation, Strassburg, 1875. Jahresbericht für Thierchemie, V.6, p. 180, (1876).

(304) KOBERT: Ueber einige Enzyme wirbelloser Tiere. Pflüger's Archiv für Physiologie, V. 99, p. 116, (1903).

(305) KÜLZ: Beiträge zur Pathologie und Therapie des Diabetes Mellitus. Jahresbericht für Thierchemie, V. 4, p. 448, (1874).

(306) KÜLZ: Beiträge zur Kenntnis des Glycogens. Centralblatt für Physiologie, 1890, p. 789.

(307) LÉVY: De la fermentation alcoholique des topinambours, sous l'influence des levures. Comptes Rendus, V. 116, p. 1381, (1893).

(308) LINDNER: Gärversuche mit verschiedenen Hefen und Zuckerarten. Chemisches Centralblatt, 1901, p. 56.

(309) v. LIPPMANN: Ueber das Lävulan, eine neue, in der Melasse der Rübenzucker-fabriken vorkommende Gummiart. Berichte der deutschen chemischen Gesellschaft, V. 11, p. 57. (1881).

(310) LUCHSINGER: Zur Glykogenbildung in der Leber. Pflüger's Archiv für Physiologie, V. 8, p. 289, (1874).

(311) MENDEL AND MITCHELL: On the Utilization of Various Carbohydrates without Intervention of the Alimentary Digestive Processes. American Journal of Physiology, V. 14, p. 239, (1905).

(312) MENDEL AND NAKASEKO: Glycogen Formation after Inulin Feeding. Ibid., V. 4, p. 246, (1900).

(313) MIURA: Wird durch Zufuhr von Inulin die Glykogenbildung gesteigert? Zeitschrift für Biologie, V. 32, p. 255, (1895).

(314) REIDEMEISTER: Sinistrin, Levulin, und Triticin. Chemisches Centralblatt, 1880, p. 808; Jahresbericht für Thierchemie, V. 11, p. 68, (1881).

(315) RICHAUD: Sur quelques points relatifs à l'histoire physiologique de l'inuline chez les animaux. Comptes Rendus de la Société de Biologie, V. 52, p. 416, (1900).

(316) SAIKI: Anti-inulase. Journal of Biological Chemistry, V. 3, p. 395, (1907).

(317) SANDMEYER: Ueber die Folgen der partiellen Pancreasextirpation beim Hund. Zeitschrift für Biologie, V. 31, p. 32, (1895).

(318) SCHMIEDEBERG: Ueber ein neues Kohlehydrat. Zeitschrift für physiologische Chemie, V. 3, p. 112, (1879).

(319) STRAUSS: Ueber das Vorkommen einiger Kohlehydrate fermente bei Lepidopteren und Dipteren. Zeitschrift für Biologie, V. 52, p. 95 (1908).

(320) TANRET: Sur la levulosane, nouveau principe immédiat des céréales Comptes Rendus, V.: 112, p. 293, (1891).

(321) TANRET: Sur l'inuline et deux principes immédiats nouveaux. Ibid., V. 116, p. 514, (1893).

(322) TANRET: Sur les hydrates de carbone du topinambour. Ibid., V. 117, p. 50, (1893).

(323) TANRET: Sur une nouvelle glucosane, la lévoglucosane. Ibid., V. 119, p. 158, (1894).

(324) VON MERING: Zur Glykogenbildung in der Leber. Pflüger's Archiv für Physiologie V. 14, p. 274, (1877).

(325) WALLACH: Zur Kenntnis der Kohlehydrate. Liebig's Annalen, V. 234, p. 364, (1886).

(326) WEINLAND: Ueber das Auftreten von Invertin im Blut. Zeitschrift für Biologie, V. 47, p. 280, (1906).

(327) WENT: Monilia sitophila (Mont.) Sacc., ein technischer Pilz. Chemisches Centralblatt, V. (1901) b, p. 650.

(328) WINOGRADSKY: Clostridium pastorianum, seine Morphologie und seine Eigenschaften als Buttersäureferment. Chemisches Centralblatt, V. 1902, b, p. 709.

VII. DEXTRANS.

(329) BAUER: Ueber eine aus Leinsamenschleim entstehende Zuckerart. Die Landwirtschaftlichen Versuchs-Stationen, V. 40, p. 480.

(330) BAUER: Ueber eine aus Laminariaschleim entstehende Zuckerart. Berichte der deutschen chemischen Gesellschaft, V. 22, p. 618, (1899).

(331) BAUER: Ueber die Arabonsäure und die aus Lichenin entstehende Zuckerart. Journal für praktische Chemie, V. 34, p. 46, (1886).

(332) BERG: Jahresbericht für Chemie, 1873, p. 848. (From Russ. Zeitschr. Pharm., 1873, pp. 129 and 161.)

(333) BERZELIUS: Recherches sur la nature du lichen d'Islande, et sur son emploi comme aliment. Annales de Chimie, V. 90, p. 277, (1814).

(334) BROWN: Notes on Cetraria Islandica. American Journal of Physiology, V. I, p. 455, (1898).

(335) ESCOMBE: Chemie der Membranen der Flechten und Pilze. Zeitschrift für physiologische Chemie, V. 22, p. 288, (1896).

(336) HÖNIG AND ST. SCHUBERT: Ueber Lichenin. Monatshefte für Chemie, V. 8, p. 452, (1887).

(337) KLASON: Über die durch Inversion von Lichenin entstehende Zucker. Berichte der deutschen chemischen Gesellschaft, V. 19, p. 2541, (1886).

(338) MEYER: Ueber Reservestoffe, Kerne und Sporenbildung der Bakterien. Chemisches Centralblatt, V. 1900, b. p. 56, (1900).

(339) MEIGEN AND SPRENG: Ueber die Kohlehydrate der Hefe. Zeitschrift für physiologische Chemie, V. 55, p. 49, (1908).

(340) MENDEL: Das Verhalten einiger unverdaulicher Kohlehydrate im Verdauungstrakt. Zentralblatt für die gesammte Physiologie und Pathologie des Stoffwechsels, No. 17, p. 1, (1908).

(341) MÜLLER, KARL: Die Chemische Zusammensetzung der Zellmembranen bei verschiedenen Kryptogamen. Zeitschrift für physiologische Chemise, V. 45, p. 264, (1905).
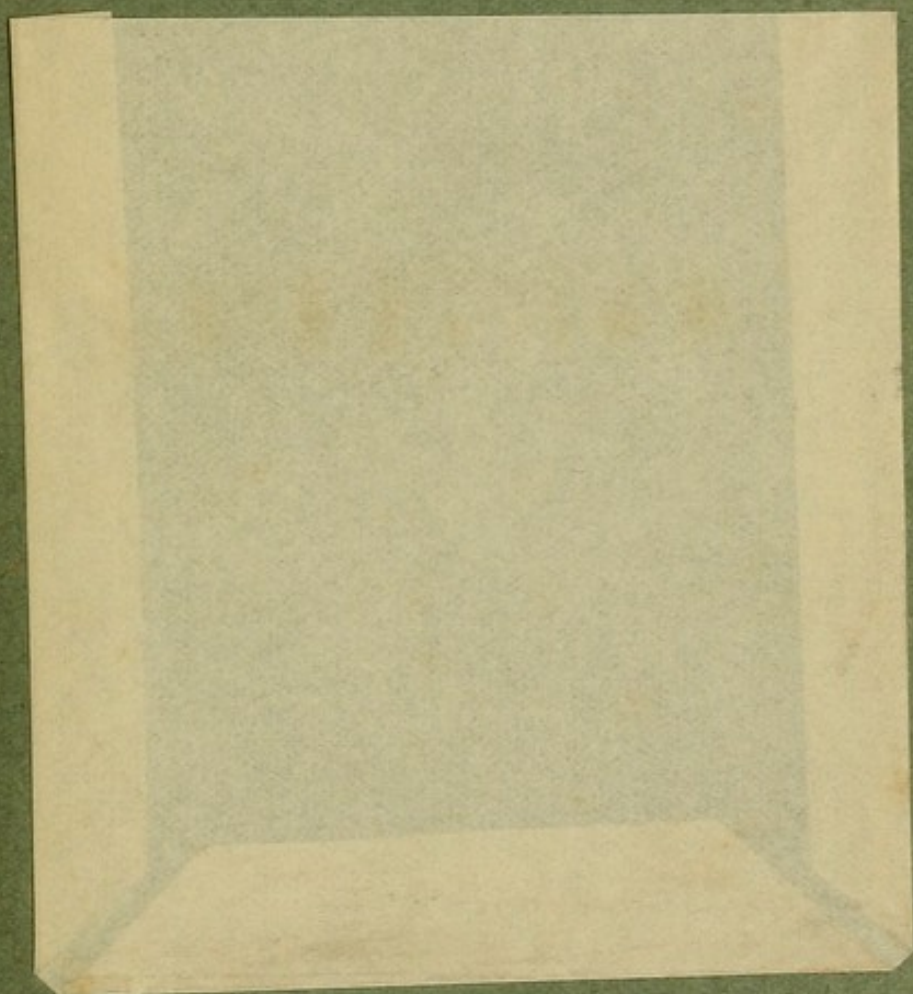
(342) NILSON: Kenntnis der Kohlenhydrate in den Flechten. Upsala Läkareförenings Förhandlingar, V. 28. (Jahresbericht für Thierchemie, V. 23, p. 53, (1893).
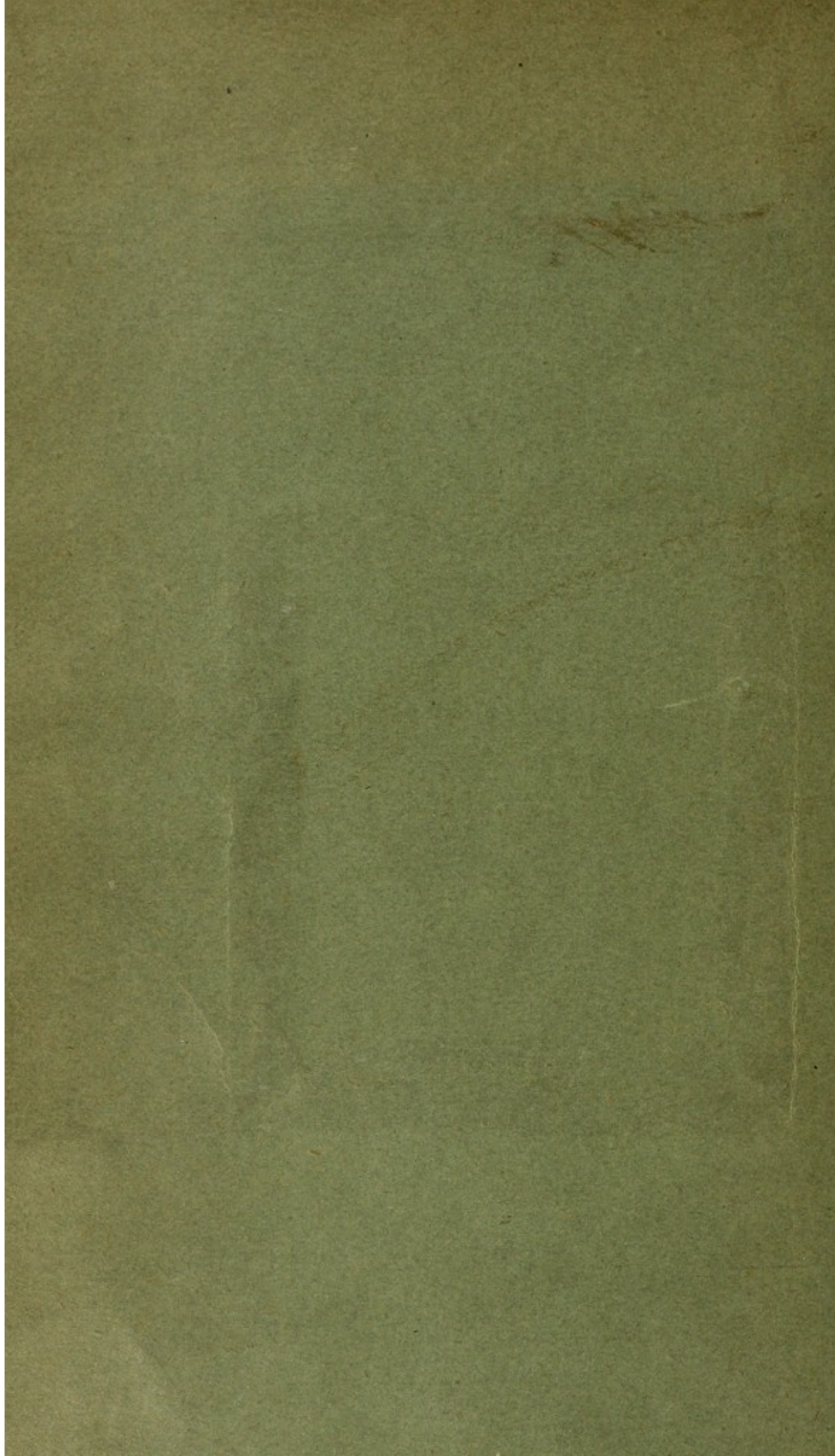
(343) O'SULLIVAN: Amylam in Wheat, Rye and Barley. Chemical News, V. 44, p. 258, (1881).
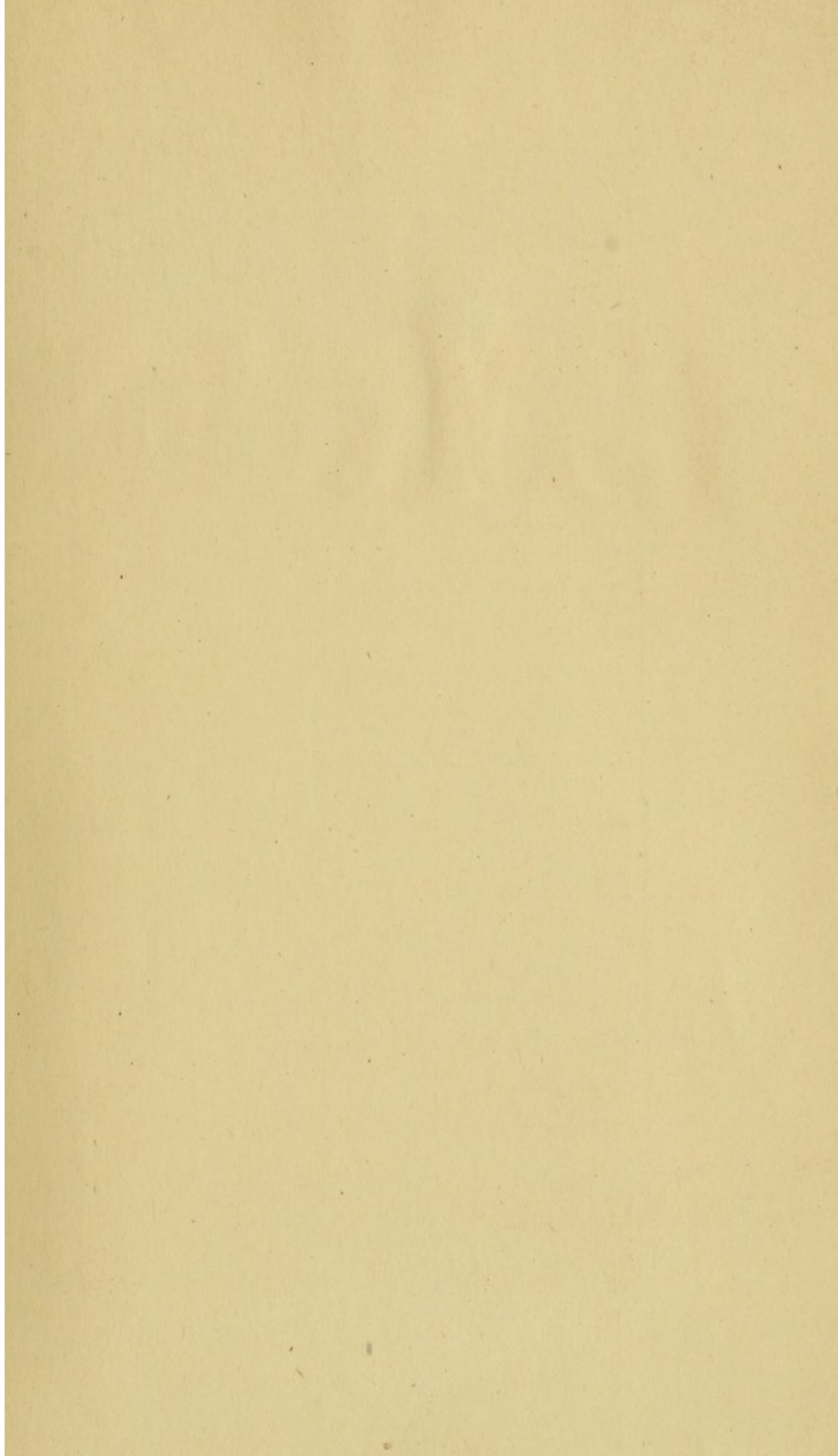
(344) POULSSON: Untersuchungen über das Verhalten einiger Flechtenkohlehydrate im menschlichen Organismus und über die Anwendung derselben bei Diabetes-Mellitus. Festschrift für Olof Hammarsten XIV, Upsala Läkareförenings Förhandlingar, (1906).

(345) ROTHENFUSSER: Der Schleimkörper des Leinsamens. Dissertation, München, 1903. (Jahresbericht für Thierchemie, V. 34, p. 78, 1904.

(346) SAIKI: The Digestibility and Utilization of Some Polysaccharide Carbohydrates derived from Lichens and Marine Algae. Journal of Biological Chemistry, V. 2, p. 251, (1906).

(347) TORUP: A New Carbohydrate from Laminaria Digitata. Biochemisches Centralblatt, V. 8, p. 770, (1909). (From Pharmacia, V. 6, p. 153.)

(348) ULANDER: Untersuchungen über die Kohlenhydrate der Flechten. Dissertation, Göttingen, (1905).

(349) VOIT: Hermann's Handbuch der Physiologie, V. 6, p. 413, (1881).

(350) WINTERSTEIN: Zur Kenntnis der in den Membranen der Pilze enthaltenen Bestandtheile. Zeitschrift für physiologische Chemie, V. 19, p. 521, (1895). V. 21, p. 152, (1897).

(351) VON MERING: Zur Glykogenbildung in der Leber. Pflüger's Archiv für. Physiologie, V. 14, p. 274, (1877).

(352) YOSHIMURA: Ueber einige Pflanzenschleime. Jahresbericht für Thierchemie, V. 25, p. 51, (1895). (Bulletin II, No. 4, College of Agriculture, Tokyo Imperial University.)