tions may be estimated and the solution made in a 10 ml. vial.)

Because of the small quantity used and the highly volatile nature of solution A and the relatively wide range in strength which may be employed in either solution, an exact formula is not necessary. The tendency is to make the solutions too thick. Best results are obtained with freshly prepared solutions. It should be remembered that solutions A and B are soluble in 95 per cent. alcohol. Whether Mayer's albumin or water alone is used in stretching the sections is immaterial.

After the stretched sections have been thoroughly dried each slip is flooded with solution B and placed face up on a level surface for half an hour, or until the sections are sufficiently free of paraffine to appear nearly clear. Then, two or more layers of fine filter paper (Munktell's No. 3 is excellent) are placed over the tissue and rolled vigorously with a smooth bottle to absorb the surplus solution and flatten any sections which may have buckled. The sections are immediately flooded with solution A and the slip stood on end against a staining jar until the celloidin attains a glistening surface and toughened texture. The slip is then placed in 70 per cent. alcohol for several minutes, then passed rapidly (to prevent loss of the celloidin) through 95 per cent. alcohol and into carboxylol, where it should remain several hours, or be placed into xylol, to remove the paraffine. The slip is passed from carboxylol through 95, 70 or 50 per cent. alcohol to water and stained. After being stained and cleared in carboxylol the sections should remain in xylol sufficiently long (24 hours is not injurious) to insure removal of all remaining paraffine.

By replenishing, and thus prolonging, the application of solution B the sections may be passed from 70 per cent. alcohol to water and stained, instead of going to carboxylol before being stained. In general this shorter method is not recommended, for staining is often faulty due to incomplete removal of paraffine from the sections.

This method lends itself admirably to routine work in preparing numbers of slides. To facilitate handling a considerable number of slips the writer uses $1 \times 4 \times 12$-inch soft pine boards, each of which easily accommodates ten slips. Such a board of slips is placed upon an empty board, which raises the slips nearly two inches above the table top, and each slip is then flooded with solution B. Five of the ten are blotted at one time, hastily flooded with solution A and slanted against the two boards to allow the solution to drain and evaporate to the proper consistency before the slip is placed in 70 per cent. alcohol.

The celloidin film employed in this method does not retain stains as does that to which clove oil has been added. Preliminary experiments showed that with iron hematoxylin three-hour mordanting and three-hour staining, or three-hour mordanting and twenty-hour staining, the stain in the film was removed by iron alum solution before sections of striated muscle were properly destained. Mann's eosin-methyl blue was retained by the celloidin nearly as readily as by the sections of nerve trunk tissue, but the depth of the stain was not great enough to be objectionable. Mallory's phosphotungstic acid hematein ammonium staining method did not discolor the celloidin in over a hundred slides of rat tissues.

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SPECIAL ARTICLES

LAKE VEGETATION AS A POSSIBLE SOURCE OF FORAGE*

THE past summer, with its high temperatures and low rainfall, has accentuated the drought conditions which for the past several years have been more or less prevalent over considerable portions of the Great Plains Region. The ground water table has been generally greatly lowered throughout the region. Many lakes and streams have either disappeared or have dwindled to a remnant of their former size, and in many areas there is insufficient forage to maintain through the winter even the reduced number of live stock which remain in the area. In certain areas all local forage of every kind has already been consumed.

In the glaciated area of this region there exist tens of thousands of lakes. Many of these under the influence of deficient rainfall have become shallow and large areas of them are literally choked with masses of lake vegetation. Other deeper lakes have large shallow encircling areas or bays in which a rank growth of lake "weeds" is present.

It occurred to the writer that perhaps such lake vegetation might be utilized as a source of forage in the present emergency and, in fact, might represent a new natural resource which could contribute to the future agricultural wealth of the region. Accordingly, samples of the dominant vegetation types were collected during September and early October from representative Minnesota lakes, including both those with sand and mud bottoms. Those samples were analyzed with the results shown in Table I. In Table

* Paper No. 1313, Journal Series, Minnesota Agricultural Experiment Station.

TABLE I

ANALYSES OF SAMPLES OF LAKE-WATER VEGETATION FROM REPRESENTATIVE MINNESOTA LAKES[1]

No.	Dry matter content Per cent.	Dry basis analyses: Ash Per cent.	Crude protein Per cent.	Ether extract Per cent.	Crude fiber Per cent.	N-free extract Per cent.
1	13.6	20.26	17.65	1.28	11.15	49.66
2	....	17.08	17.53	1.21	14.93	49.25
3	....	17.08	17.34	1.65	13.19	50.74
4	22.7	18.29	13.68	1.45	23.01	46.03
5	....	38.75	10.41	0.91	16.52	33.41
6	....	30.17	12.26	0.87	17.69	39.01
7	....	13.03	19.03	1.36	19.36	47.22
8	....	18.38	11.49	2.07	22.63	45.43
9	14.2	22.84	14.44	1.82	15.20	45.70
10	14.6	17.26	12.72	3.08	17.59	49.35
11	....	23.90	13.62	2.18	18.70	41.60
12	....	21.76	14.11	1.13	21.04	41.96
13	....	18.39	13.38	2.75	22.65	42.83
14	18.4	27.86	12.13	1.71	16.20	42.10
15	14.4	22.89	19.25	2.36	12.30	43.20
16	....	23.69	15.74	2.50	20.73	37.34
17	14.2	20.21	14.81	1.44	17.51	46.03
18	....	28.63	15.03	1.29	18.26	36.79
19	....	28.44	13.29	1.27	13.67	43.33
20	....	7.95	17.03	2.79	13.75	58.48
21	....	30.37	5.21	0.78	15.23	48.41
22	....	14.85	18.92	1.92	18.69	45.62
23	....	14.98	13.01[2]	1.17	17.42	53.42
24	....	16.93	13.94	2.22	18.28	48.63
25	10.8	12.60	14.75	....	21.56	51.09[3]
26	16.9	13.66	13.90	....	22.38	50.06[3]
27	15.4	14.91	13.72	....	22.49	48.88[3]
28	15.0	17.63	14.60	....	20.34	47.43[3]

No.
1–3. *Myriophyllum spicatum* (water millfoil).
4–5. *Potamogeton amplifolius* (large leaved pond weed).
6. *Potamogeton Richardsonii* (Richardson's pond weed).
7. *Potamogeton pectinatus* (Sago).
8. *Potamogeton zosteraefolius* (eelgrass).
9–13. *Najas flexilis* (Naiad).
14. *Elodea canadensis* (water thyme).
15–17. *Ceratophyllum demersum* (hornwort).
18. *Vallisneria spiralis* (water celery).
19. *Heteranthera dubia* (water star grass).
20. *Nymphaea advena* (yellow pond lily).
21. *Chara* sp.
22. *Ruppia occidentalis* and *Potamogeton pectinatus.*
23. Largely *Myriophyllum spicatum.*
24. Largely *Najas flexilis.*
25. Largely *Elysma plantago-aquatica* (water plantain).
26–28. Random mixed sample.

TABLE II

AVERAGE PROXIMATE ANALYSIS OF THE DRY MATTER IN REPRESENTATIVE FORAGES[4]

Type of forage	Ash Per cent.	Crude protein Per cent.	Crude fiber Per cent.	Carbo-hydrates and fat Per cent.	Number of analyses
Corn fodder	7.14	8.57	29.8	54.4	56
Sorghum fodder	8.64	8.2	28.9	54.2	22
Kentucky bluegrass	7.6	9.56	32.6	50.2	26
Timothy	5.5	7.0	33.7	53.7	221
Sudan grass	7.9	9.26	30.4	52.4	44
Prairie hay	8.2	8.55	32.6	50.3	42
Alfalfa hay	9.4	16.3	31.0	43.3	250
Cow-pea hay	12.96	21.4	24.9	40.5	35
Soy-bean hay	9.4	17.5	27.2	45.8	23

II are shown corresponding analyses of the commonly used forage plants.

It will be observed that with but a single exception (*Chara* sp.) the lake vegetation is characterized by (1) a high ash content, (2) a high protein content and (3) a very low "crude-fiber" content, and that the legume hays are the only commonly used forages which even approximate the lake weeds in these constituents. The *Chara* sp. was analyzed simply from curiosity. It does not ordinarily occur in any great abundance and was found growing in very shallow water on a sandy beach.

Preparation for Analysis. The plants were brought to the laboratory soon after collecting, washed with tap water from a hose while whirling them in a perforated basket centrifuge. The whirling in the centrifuge was continued until the surface moisture had disappeared. The green weight was then taken and the samples dried in a current of air at about 65° C.

The Dry Matter Content. The table shows that the dry matter content can be expected to range from 13.6 to 22.7 per cent. of the green weight of the plants (excepting the leaves of the water plantain and perhaps other lily forms). These values approximate what might be expected of succulent upland grasses.

The Ash Content. The ash content of the lake vegetation is unusually high for forage plants. However, many of the lake plants are marl-formers and

[1] Botanical identification was kindly made by Dr. C. O. Rosendahl of the Department of Botany, University of Minnesota.

[2] Dried as "hay" in the open. Subjected to severe rains during curing.

[3] Includes "ether extract."

[4] Calculated from data in "Feeds and Feeding," by W. A. Henry and F. B. Morrison. The Henry-Morrison Company, Madison, Wis. (1923).

the ash is composed to a very considerable extent of lime, in part deposited as incrustations on the surface of the leaves and stems. In the high-ash samples of *Potamogeton* and *Chara* the lime incrustation was especially marked. It is generally recognized that the cereal grains are deficient in calcium, so a high-lime forage should admirably supplement such feeds.

The "Crude Protein." With the exception of the *Chara* sp. the "crude protein" of all samples compares favorably with the "crude protein" content of legume hays. No great species differentiation is seen in the protein content, neither does the "crude protein" content differ markedly between samples of the same species collected from mud-bottom and sand-bottom lakes. It seems probable that the high "crude protein" content is the resultant of an unusually favorable environment. The best top soil of the vicinity washes into the lake, therefore the lake bottom should possess high fertility. In addition the nitrates from the surrounding lands wash into the lake with the drainage water and provide continuous fertilization. Lastly microscopic forms of life and the lake fauna die and decompose and their nitrogen contributes to the fertilization of the plants.

The "Crude Fiber." The crude fiber represents the cellulose and other supporting elements of the plant. In every instance it is lower than the average analyses of the conventional forage crops. The low fiber content is presumably due to the fact that the lake plants do not need supporting structural elements—the buoyancy of the water serving in place of cellulose strands.

The Feeding Value. Literature on the feeding value of water vegetation is apparently extremely meager. *Elodea canadensis* has been reported by both German[5] and Holland[6] workers to be an excellent food for cattle and swine, being fed either green or as ensilage. Apparently no other forms have been studied. There is, however, no *a priori* reason to suspect that many of the types of lake vegetation will not serve as suitable feeding stuffs. The analyses appear to indicate that they may be superior to much of the forage which is now used on farms in the Great Plains area.

Representative bulk samples have been collected, and their feeding value, including palatability, vitamin content, digestibility, protein quality, nitrogenous constituents, types of carbohydrate present, etc., will be studied in these laboratories during the coming winter. If the drought conditions should continue through another year the lakes of the glaciated region may provide the necessary forage.

In any event, the uniformly high "crude protein" content of lake vegetation suggests the possibility of growing suitable non-leguminous plants in our shallow lakes and preparing therefrom what is essentially a "concentrate" for animal feeding. The high-protein, high-lime, low-fiber meal may well become an article of commerce.

It is generally recognized in the thickly populated areas of Asia that "an acre of water will produce more human food than an acre of land." Aquiculture, in suitable areas, may well become a part of our changing agriculture.

ADDENDUM

Since writing the above the analyses of certain aquatic plants by H. J. Harper and H. A. Daniel (*Bot. Gaz.*, 96: 186 (1934)) has appeared. These authors note that aquatic plants are likely to have a higher nitrogen content than upland vegetation but make no suggestions of the possibility of using aquatic plants as a source of forage.

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THE STRUCTURE OF THE CARDIAC AGLUCONES

IN our recent preliminary note in SCIENCE[1] we have described the degradation of digitoxigenin to an acid, $C_{20}H_{32}O_2$, which corresponded in properties with aetiocholanic acid obtained by Wieland, Schlichting and Jacobi[2] by the degradation of cholic acid. Through the generous cooperation of Professor Wieland we have been able to make direct comparisons of the melting points of our ethyl and methyl ester with the esters of aetiocholanic acid prepared by the German workers. This comparison as well as mixed melting points has shown their identities. At the end of our previous note we briefly mentioned that "should the identity of these substances be verified, the conclusions are obvious which can be drawn in regard to the structure of the cardiac aglucones." Shortly after our note appeared, the current number of *Angewandte Chemie* reached us, which contains a preliminary article by R. Tschesche[3] on the similar degradation of another cardiac aglucone, uzarigenin. The appearance of the latter requires that we be more explicit in regard to the conclusions which can be drawn from our work.

Tschesche has succeeded in degrading uzarigenin likewise to an acid, $C_{20}H_{32}O_2$, which, however, did not prove to be aetiocholanic acid but appeared from the melting points of the acid and its ester to be identical with aetioallocholanic acid obtained by degradation of hyodeoxycholic acid. These results simul-

[5] F. R. Ferle, *Fühlings. Landw. Ztg.*, 53: 549–58, 1904.

[6] Anon., *Bull. Agr. Intelligence*, 9: 1079–80, 1918; *Jour. Board Agr.* (London), 26: 321–2, 1919.

[1] W. A. Jacobs and R. C. Elderfield, SCIENCE, 80: 434, 1934.

[2] *Zeits. physiol. Chem.*, 161: 102, 1926.

[3] *Angewandte Chem.*, 47: 729, 1934.