47.7% in the high protein dietary lot to 55.0% in the low protein group receiving Aureomycin. From these data the high-protein dietary levels appear to decrease the total solids content of hams; however, the variance mean squares were not significant, but appreciable. The observation that the hams having the greatest protein content also have the greatest water (volatile matter) content is in agreement with the knowledge that muscle contains more water than nonmuscular tissues, such as fat.

Conclusions

The data present concentrations of several phosphorus and nitrogen compounds in hams that apparently have not previously been reported. The values obtained show that an appreciable range of protein in the ration, with or without Aureomycin, does not affect either the concentrations of acid soluble and nucleic acid phosphorus, or nucleic acid and ammoniacal nitrogen and total solids, at statistical levels of significance. The interaction effect of protein and Aureomycin on the lipid phosphorus may be associated with the fact that the swine receiving the antibiotic had greater back fat thickness (*5*). The dietary protein and Aureomycin effects on the phosphoprotein phosphorus and nitrogen suggest that phosphoprotein metabolism is a determining factor in the amount of total protein found in the hams. In general, the level of dietary protein as indicated by the data has a greater influence on nitrogen compounds than the antibiotic.

Acknowledgment

The writers wish to thank the Lederle Laboratories for supplying the Aureomycin and B complex vitamins used in the rations, and the U.S. Public Health Commission for the grant-in-aid financial assistance during the study.

The authors also wish to acknowledge the technical assistance in this study of Mike Milicevic, Dave O'Connor, Robert Johnson, and Jon Herring.

Literature Cited

(1) Assoc. Offic. Agr. Chemists, "Official and Tentative Methods of Analysis," 7th ed., 1950.
(2) Mirone, Lenora., J. AGR. FOOD CHEM, **1**, 519 (1953).
(3) Schneider, Walter C., *J. Biol. Chem.*, **161,** 293 (1945).
(4) Snedecor, George W., "Statistical Methods Applied to Experiments in Agriculture and Biology," Ames, Iowa, Collegiate Press, Inc., 1946.
(5) Wallace, H. D., Milicevic, M., Pearson, A. M., Cunha, T. J., and Koger, M., *J. Animal Sci.*, **13,** 177 (1954).

Received for review April 29, 1954. Accepted July 22, 1954. Authorized for publication as Paper 270, journal series, Florida Agricultural Experiment Station.

ATMOSPHERIC FLUORINE

Fluorine Acquired by Forage Cultures in Outdoor And Washed Atmospheres at Columbia, Tenn.

W. H. MacINTIRE, L. J. HARDIN, and MARY HARDISON
The University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.

Multiple pot cultures of red clover and of rye grass were grown 6 weeks at Knoxville, without any additive, in normal atmosphere on soil low in fluorine content, and then were transported to Columbia, where the atmosphere was purported to have higher occurrences of fluorides. After 21 days, the fluorine contents of the plants grown outdoors and in the washed atmosphere of an adjacent closed chamber were compared to the fluorine content of check plants that were retained at Knoxville. Sudan grass cultures were used in similar comparisons in two subsequent experiments. In all three experiments the transported plants acquired substantial increases in fluorine contents during their growth outdoors at Columbia, in comparisons with the check plants that were kept outdoors at Knoxville. The removal of the fluorine content of the Columbia atmosphere was reflected in the relatively low content of fluorine in the transported plants that were grown in the washed atmosphere of the nearby chamber. When forage cultures were grown in an atmosphere contaminated with ionized fluorides, the plants acquired more fluorine than they acquired in the washed atmosphere and the amounts derived from the atmosphere exceeded the uptakes of fluorine from the soil. However, the results do not demonstrate that the increases in fluorine content acquired by the plants grown outdoors were due solely to ionized fluorides.

FLUORINE OCCURRENCES IN THE atmosphere are minute at points distant from industrial operations. Hence, under normal atmospheric conditions, field crops derive their fluorine content from the mineral fluorides that occur discretely in most soils, and according to soil pH and abilities of particular plant roots to effect uptake of fluorine. However, abnormal occurrences of fluorine may occur as particulates in localities where rock phosphate is mined and processed, and as gaseous phases in those locales where fluoric emissions come from phosphate, phosphorus, and other manufacturing operations (*1*, *3*). Such occurrences have been determined through 3-year analyses of a succession of replicated exposures of Spanish moss at different points in Tennessee (*5*).

Of particular concern are contentions

that forage crops acquire abnormal content of fluorine from the atmosphere in two widely separated Tennessee counties in which livestock show symptoms of fluorosis. As one phase of a project assigned jointly to the Departments of Chemistry and Animal Husbandry of The University of Tennessee Agricultural Experiment Station, fluorine occurrences in forage vegetation on fluorine-enriched soils have been studied through pot culture and lysimeter experiments (*6*, *9*). Experimentally grown crops did not acquire abnormal contents of fluorine from rational incorporations of fluorides in soils adequately supplied with calcium through inputs of either wollastonite or limestone (*8*). The same proved true of the quenched slag, which contained nearly 4% fluorine and imparted relatively high concentrations of fluorides to the free water of the soil (*6*, *7*). The Maury silt loams of Tennessee and Kentucky are relatively rich in their contents of native fluorides. The Kentucky formation is richer, but neither soil imparted abnormal content of fluorine to vegetation grown on it after the soil had been transferred to large lysimeters at Knoxville. Although the Kentucky soil had a fluorine content almost five times that of the Tennessee soil, the two Maury soils imparted fluorine contents of only 6 to 8 p.p.m. to eight successive crops grown on them at Knoxville. Moreover, the fluorine content of the alfalfa grown on the two Maury soils in the lysimeters at Knoxville was virtually identical to the content of fluorine in the alfalfa grown in the field on the Maury soil at Lexington, Ky. But, in each case where alfalfa was grown at Knoxville on the Maury soils from Columbia and Kentucky, and also grown at Lexington, Ky., the fluorine content was only a sixth as much as the fluorine content of the alfalfa that was grown outdoors on the Maury soil at Columbia.

Therefore, it was planned to reverse the order of transfer so that plants would be grown experimentally at Knoxville and then transferred to Columbia for further growth of 3 weeks, so as to ascertain whether those plants and those retained at Knoxville would acquire different contents of fluorine. An additional objective was to compare further growth of the transported plants in outdoor and washed atmospheres at Columbia.

Figure 1. Over-all view of opened chamber, showing exhaust tube, intake, baffle, and watering tubes, with an example of a clover culture

The present paper reports findings from three experiments as to fluorine acquired by three types of vegetation in pot cultures during early growth at Knoxville on soils of meager content of fluorine, and during 21 days further growth there and at Columbia. The pot cultures of red clover, rye grass, and Sudan grass were grown in respective parallels, outdoors, and in the washed atmosphere of an adjacent chamber at the Middle Tennessee Experiment Station at Columbia.

Experiment I, Red Clover and Rye Grass

Soil A Fullerton silt loam was used in the comparisons of Table I. That soil had a fluorine content of 0.015%, or 150 p.p.m., pH of 5.3, and cation content of 6.02 meq. per 100 grams of dry soil, accounted for by the milliequivalent values of 2.02 for calcium, 0.33 for magnesium, 0.17 for potassium, and 3.50 for hydrogen. Twenty-four 2-gallon pots of soil were seeded to red clover, and 24 to rye grass, at Knoxville in April 1952, and the resultant seedlings were allowed to grow 6 weeks. Twelve pot cultures of both plants then were transferred to the Middle Tennessee Station, where the atmosphere was deemed representative for that locality. There, four cultures of each crop were placed near the edge of a field, in the sparce marginal shade of a nearby tree, and in the adjacent gas-tight chamber (Figures 1, 2, and 3).

The chamber was an air-tight wooden box 2 feet × 5 feet × 18 inches, with capacity for 10 2-gallon pots. Its removable Plexiglas top was sealed after introduction of the pots. Each enclosed culture was watered by means of a sealed-in glass tube that passed through a long side of the box and reached to the center of the culture. Delivery of water was into a small glass funnel, the stem of which was joined to the outer end of the glass tube by means of rubber tubing, with a clamp for closure. However, because of the humidity imparted to the water scrubbed air, the enclosed cultures required only occasional watering.

Air was blown into the chamber by means of a tank-type electric vacuum cleaner. Inblow was governed by means

Table I. Fluorine Acquired by Transported Cultures of Red Clover and Rye Grass

(After growth in outdoor and washed atmospheres at Middle Tennessee Experiment Station)

	Fluorine (Air-Dry Vegetation), P.P.M.											
	Red Clover After						Rye Grass After					
	21 Days at Columbia			63 Days at Knoxville			21 Days at Columbia			63 Days at Knoxville		
				Outdoors						Outdoors		
Culture No.	In open field	Under tree	In chamber	Initial[a]	Final	In greenhouse[b]	In open field	Under tree	In chamber	Initial[a]	Final	In greenhouse[b]
1	18	20	15	7	4	8	21	32	17	9	11	12
2	18	24	15	7	5	10	19	31	17	13	12	13
3	17	24	13	7	5	7	22	31	17	10	8	12
4	17	25	19	7	7	6	17	30	17	7	11	12
Mean	18[c]	23	16	7	5	8	20[d]	31	17	10	11[b]	12

[a] At time of transfer of 6-week old cultures to Columbia and to Crossville.

[b] Retained control cultures were kept outdoors and in greenhouse throughout 9 weeks between seeding and harvest, May 28, 1952.

[c] Corresponding four-unit cultures of two crops were grown simultaneously at Crossville, Tenn., on Cumberland Plateau and analysis of composited crops showed fluorine content of 4 p.p.m.

[d] The cultures were grown 6 weeks at Knoxville and then transferred to Middle Tennessee Station.

L.S.D. at 5% level for red clover and rye grass, 2 p.p.m.; at 1% level, 3 p.p.m.

Table II. Increases in Fluorine Concentration Acquired by Transported Sudan Grass

(After growth in outdoor and washed atmospheres at Middle Tennessee Experiment Station[a])

	Fluorine in Harvests of Air Dry Sudan Grass, P.P.M.					
	After 21 Days Growth at Columbia				After 42 Days Growth at Knoxville	
	In June 1953		In Sept. 1953			
Culture No.	Outside	In chamber	Outside	In chamber	In June 1953 outside	In Sept. 1953 outside
1	34	11	40	10	4	10
2	37	12	51	10	7	13
3	37	9	45	8	2	15
4	39	9	47	10	..	14
5	36	7	51	12	..	14
Mean	37	10	47	10	4[b]	13
Increases[c]	27	..	37	..	..	..

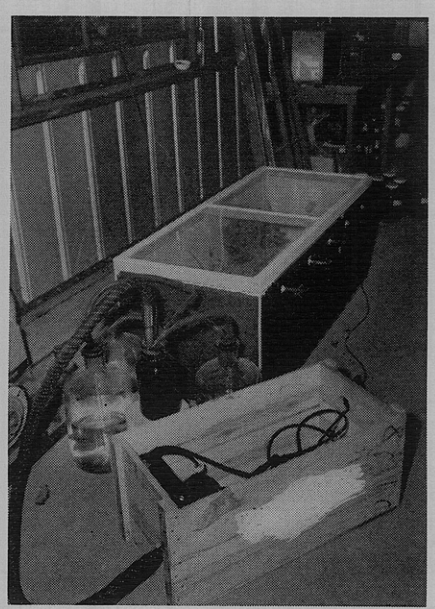
[a] The cultures were grown 21 days at Knoxville before transfer to Middle Tennessee Experiment Station.
[b] From triplicate determinations on composite from growth on three pots.
[c] Over the concentration of fluorine in chamber plants

of a conventional domestic stoker-control device for delivery of 21 cubic feet of air per minute and a Variac for 5-minute operation per 0.5 hour. Blower, stoker device, and Variac were housed in a wooden box for their protection and to facilitate portability (Figure 3).

Air inblow was through a vacuum-cleaner hose of 1.25-inch diameter and into a stoppered bottle that contained 4 gallons of water and thence through three outlets that served as a manifold through which the air passed into three 3.5-gallon bottles, each of which contained 2 gallons of water. The three air inlets were fronted by a perpendicular baffle plate to assure that the washed air would be distributed uniformly within the chamber. Exhaust was through a 1.25-inch sealed-in metal tube that was joined to a 6-inch section of Gooch rubber tubing. Under pressure the tubing was tubular; upon release of pressure it collapsed and folded on its horizontal axis, and thus prevented backflow of unwashed air.

Analyses After 21 days of growth, the clover and rye grass of the 1952 experiment were harvested simultaneously, and conveyed to Knoxville for fluorine determinations. The samples were dried, ground in a Wiley mill, and 5-gram charges were mixed with lime water and calcined at 500° C. The calcines were fused with sodium hydroxide, and the cooled fusions were subjected to perchloric acid distillation at 135° C., and the resultant, 500-ml. distillates, were titrated with thorium nitrate, as in the method of Willard and Winter (*12*). In their 1933 proposal these workers demonstrated that full recoveries of fluorine were obtained from certain pure fluorides, and they stated that full recoveries were not obtained by the perchloric distillation of the ash of vegetation. Several laboratories adopted the fusion of the calcines by means of sodium hydroxide pellets to effect the formation of fluorides that yield fluorine to perchloric acid distillations. The necessity for such fusion and their efficacy were demonstrated in the papers by Remmert *et al.* (*10*) and Rowley *et al.* (*11*) in 1953. The Willard and Winter technique was modified also through the use of a current of steam, instead of the initial direction for dropwise additions for temperature control of the digestion. In 1939, MacIntire and Hammond (*4*) showed that the passage of a balanced current of steam accelerated fluorine removal to a 50% saving in duration of distillation and also eliminated "bumping." That technique is now in general use, and it may have been adopted in other laboratories prior to 1939.

Figure 2. Over-all view of closed chamber, showing filter bottles, manifold, watering tubes on the long sides, and a clover culture



Fluorine Acquired by Red Clover and Rye Grass Cultures

After additional growth outdoors for 21 days and in the chamber at Columbia (in May 1952), the two sets of transported plants were comparable in growth and appearance. The data of Table I show that while growing outdoors, the red clover and rye grass cultures attained fluorine contents that were substantially greater than the corresponding contents of the cultures that were retained at Knoxville. After initial growth of six weeks at Knoxville, the red clover contained 7 p.p.m. of fluorine, against respective final occurrences of only 5 and 8 p.p.m. in the outdoor and indoor cultures at the greenhouse. In contrast, the outdoor cultures of red clover at Columbia had acquired fluorine occurrences of 23 and 18 p.p.m., which were far greater than the occurrences acquired by parallel controls during their entire 63-day growth. In corresponding transported cultures that were grown for 21 additional days at Crossville on the Cumberland Plateau, the clover showed a final fluorine content of only 4 p.p.m.

The fluorine content of the red clover cultures that stood in the field was somewhat less than the content of the cultures that were under the tree. The variation may not be significant; but, in case it is real, the difference may be attributable to a combination of effects, greater washing action by direct rainfall on the plants in the field, and the impeded rainfall through the tree leaves, which, however, may have yielded some of their fluorine to the clover below. The chambered clover also showed a gain in fluorine during the final 21 days of its growth and obviously, such gain represented an enhancement in uptake from the soil, the moisture content of which was maintained more effectively than was possible for the moisture in the exposed cultures.

The crops from the two sets of outdoor cultures of rye grass at Columbia showed means of 20 and 31 p.p.m. of fluorine, whereas the corresponding values for cultures retained at Knoxville were only 11 and 12 p.p.m.

Statistical treatment for the fluorine contents of the harvest of clover and rye grass at Columbia registered significance for the rye grass, but not for the clover in comparison between the minimal occurrence of fluorine in the plants grown in the chamber and the occurrence in the plants grown in the field.

Experiments II and III

The Clarksville silt loam used in these experiments had fluorine content of 169 p.p.m., or 0.0169%, and pH of 5.6. An exchange capacity of 6.9 meq. per 100 grams of dry soil was accounted for by milliequivalents of 1.74 for calcium, 0.48 for magnesium, 0.08 for potassium, and 4.6 for hydrogen. Fifteen cultures of Sudan grass were grown to height of 6 inches in 2-gallon pots in successive series at Knoxville in June and in September 1953. Ten cultures of each set in both series then were transported to Columbia for 3 weeks' additional growth of five cultures outdoors and five within the described and illustrated chamber, which was located under the lightly shaded border of a tree to protect the plants against full sunlight. The third five-unit set of each experiment was retained at Knoxville and exposed outdoors. As in the 1952 experiment, the air was drawn into the scrubbers from a point near the outdoor cultures by means of a plastic hose or $^5/_8$ inch in inside diameter.

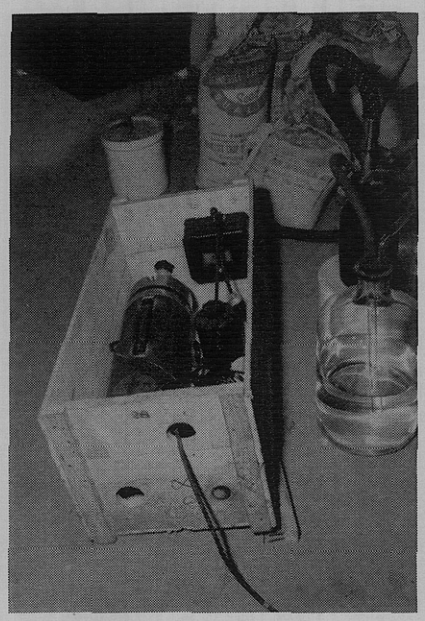


Figure 3. Close-up view of blower, control assembly, manifold, and wash bottles

The fluorine determinations on the crops of the two 1953 experiments (Table II) were analyzed as described for the comparison of the 1952 experiment, except for the use of potassium hydroxide instead of sodium hydroxide in the ash fusions, as described by Hardin, MacIntire, and Hardison (*2*). In 48 parallel analyses for fluorine contents of eleven samples of alfalfa, Bermuda, orchard, and crab grasses, fescue, and Spanish moss, in fluorine content range between 7 and 137 p.p.m., those workers found virtually identical mean values of 55.5 and 55.4 p.p.m., respectively, of fluorine for sodium hydroxide and potassium hydroxide fusions of the limed ash calcines. The perchloric acid distillations were made with the passage of "a balanced current of steam" as cited (*4*) instead of dropwise water replacement.

Fluorine in Sudan Grass

Within the 21-day growth at Columbia in June 1953, the transported Sudan grass cultures acquired a mean increase of 27 p.p.m. of fluorine over the content of the plants that grew twice as long at Knoxville (Table II). Within the corresponding duration of growth outside at Columbia in September, the Sudan grass attained a fluorine content of 47 p.p.m., which represented a gain of 37 p.p.m. over the content of the plants that had been retained at Knoxville.

When grown inside the chamber at Columbia in June, and in September, the transported cultures of Sudan grass attained fluorine contents that were, respectively, 27 and 37 p.p.m. less than the contents of the companion crops that were grown outdoors nearby (Table II). The apparent gain in the fluorine content of the grass cultures in the chamber in June crop over the content of the cultures retained at Knoxville (10 p.p.m. *vs.* 4 p.p.m.) is offset by corresponding comparison of 10 p.p.m. *vs.* 13 p.p.m. for the September crop.

Those larger and statistically significant differences in the two experiments of 1953 lend support to the trend that was indicated by the smaller differences in the findings for fluorine occurrence in the transported cultures of clover and rye grass that were grown in parallel, outdoors and in the chamber at Columbia, in the comparisons for the clover and rye grass crops of 1952.

Implications

The emissions of fluorine from the several thermal operations for production of fused phosphates and phosphorus in Maury County are chiefly hydrofluoric acid (*3*), to which is attributed the 0.18 p.p.m. of fluorine and the 6.82 mgm. content in the composite of the clear distilled water washings of the inblown air of the June experiment. At that concentration, the vapor pressure of hydrofluoric acid is virtually nil, and that highly desiccant acid could not have been swept through the scrubbers. Since the amount of fluorine trapped during the 21 days was determined, and since the pump registered the quantity of air that was pumped into the chamber during the second experiment, the occurrence of fluorine in the atmosphere then could be computed in terms of parts per billion.

Even though the increases in fluorine content acquired by the plants grown outdoors at Columbia came from the atmosphere, those plants could have become contaminated by dusts of high fluorine content, as well as by the gaseous phases of fluorine known to be emitted into the atmosphere at nearby points (*3*), and established through analyses of filtered rainwaters (*5*). Although the higher content of fluorine acquired by the plants grown outdoors at Columbia cannot be proportioned absolutely to solid and gaseous contaminants, the lesser content of fluorine in the plants grown in the chamber can be accounted for by the fact that the plants were breathing in an atmosphere from which gaseous and particulate fluorides had been removed.

Literature Cited

(1) Churchill, H. V., Rowley, R. J., and Martin, L. N., *Anal. Chem.*, **20,** 69–71 (1948).
(2) Hardin, L. J., MacIntire, W. H., and Tubb, Mary Ellen, *J. Assoc. Offic. Agr. Chemists*, **37,** 552–3 (1954).
(3) Hignett, T. P., and Siegal, M. R., *Ind. Eng. Chem.*, **41,** 2493 (1949).
(4) MacIntire, W. H., and Hammond, J. W., *J. Assoc. Offic. Agr. Chemists*, **22,** 234 (1939).
(5) MacIntire, W. H., Hardin, L. J., and Hester, Winnifred, *Ind. Eng. Chem.*, **44,** 1365–70 (1952).
(6) MacIntire, W. H., Shaw, W. M., Robinson, Brooks, and Sterges, A. J., *Soil Sci.*, **65,** 321–41 (1948).
(7) MacIntire, W. H., and Sterges, A. J., J. Agr. Food Chem., **1,** 370–8 (1953).
(8) MacIntire, W. H., Winterberg, S. H., Clements, L. B., Jones, L. S., and Robinson, Brooks, *Ind. Eng. Chem.*, **43,** 1797–9 (1951).
(9) MacIntire, W. H., Winterberg, S. H., Thompson, J. G., and Hatcher, B. W., *Ibid.*, **34,** 1469–79 (1942).
(10) Remmert, L. F., Parks, T. D., Lawrence, Alice M., and McBurney, E. H., *Anal. Chem.*, **25,** 450 (1953).
(11) Rowley, R. J., Grier, J. G., and Parsons, R. L., *Ibid.*, **25,** 1061 (1953).
(12) Willard, H. H., and Winter, O. B., *Ind. Eng. Chem., Anal. Ed.*, **5,** 7–10 (1933).

Received for review December 7, 1953. Accepted July 8, 1954.