Phosphorus was not determined on the EDTA extracts because most of them were colored. The amounts extracted with TCA and HOAc were nearly the same for all samples tested, as was shown earlier in Figure 1.

The charcoal (Fisher, C-179) used in this work is no longer available. It was used without purification and gave blanks that were near zero for all elements determined. Rather than use impure charcoal, it is recommended that it be omitted, despite the resulting colored extracts. Analysis of ten samples with and without charcoal (Table III) indicates that charcoal did not affect the recovery of Ca, Mg, K, Zn, and Mn. It is likely that results for Na and Cu would likewise be unaffected. However, for colorimetric determination of P, aliquots must be decolorized before developing the color (Johnson and Ulrich, 1959).

Other work (data not given) indicates significant variation in extractability of some elements from some plant materials. For example, at 25° Mn extraction was incomplete from hop leaves and bean plants after 4 hr, but was complete from the other samples listed in Table II after only 1 hr, and was complete from all samples listed after 1 hr at 60°. Moreover, for sorghum tops and corn leaves, all elements studied, except Fe, were quantitatively extracted in 1 hr at 25°.

An interesting feature of TCA extraction is that extractability is not dependent on element concentration. For example, all of the Mg was extracted in 1 hr at 25° from hop leaves containing 1.75% Mg, as well as from sorghum tops containing only 0.21% Mg. Similarly, the extraction of Zn was quantitative from wheat straw (7 ppm), potato tops (56 ppm), and potato tops (419 ppm). Again, Mn was completely extracted from corn leaves (235 ppm) in 1 hr at 25°, but was only 83% complete from beans (81 ppm) after 1 hr and 95% after 4 hr. Thus, the extractability of the elements seems to be more related to the plant material than to the element concentration.

The precision of the TCA extraction method is well within acceptable limits for the various elements (Table IV). The high coefficient of variation for Cu results from the low concentrations; the limits of determination for this element were approached with the techniques used. Certainly the precision of measurement on the TCA extracts is as good as that for wet ashing. No difficulty in determining Ca in the presence of TCA was encountered, as was reported by Baker *et al.* (1969).

The results of the studies indicate that TCA extraction provides adequate analytical answers for most purposes. The method should prove most useful in laboratories routinely analyzing large number of plant samples for several elements. The procedure is simple and rapid, and it avoids the hazards associated with $HClO_4$ and the special manifolds and fume hoods required in wet ashing. Likewise, it eliminates the transfer of samples and thus decreases the risk of contamination, and also eliminates the need for platinum labware used for removing silica after dry ashing.

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LITERATURE CITED

Baker, J. H., Greweling, T., *J. Agr. Food Chem.* **15**, 340 (1967).
Baker, R. A., Hartshorne, D. J., Wilshire, A., *At. Absorption Newslett.* **8**, 44 (1969).
Barr, C. R., Ulrich, A., *J. Agr. Food Chem.* **11**, 313 (1963).
Greweling, T., *J. Agr. Food Chem.* **10**, 138 (1962).
Johnson, C. M., Ulrich, A., *Calif. Agr. Exp. Sta. Bull.* 766 (1959).
Kitson, R. E., Mellon, M. G., *Ind. Eng. Chem. Anal Ed.* **16**, 379 (1944).
Knezek, B. D., Maier, R. H., *J. Agr. Food Chem.* **14**, 266 (1966).
Nicholas, D. J. D., *J. Hort. Sci.* **24**, 72 (1948a).
Nicholas, D. J. D., *J. Hort. Sci.* **24**, 260 (1948b).
Nicholas, D. J. D., *Soils Fert.* **14**, 191 (1951).

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Chromium in Foods in Relation to Biological Activity

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Chromium was determined in foods and in extracts of selected samples of foods using modification of the colorimetric procedure to stabilize color development. Extracts were prepared both by hydrolysis of samples to obtain total chromium and by alcohol extraction. Biological activities of these extracts were determined using the glucose oxidation procedure in the presence of insulin. No significant relationship was found between total chromium and biological activity. However, there was a significant relationship for chromium in alcohol extracts of meats, fungi, seeds, and seafoods, excluding fruits and vegetables. A proposed evaluation of the foods was based on these data.

The occurrence and function of chromium in biological systems have been summarized by Mertz (1969). Among the properties reported was the *in vitro* potentiation of insulin-stimulated oxidation of glucose in the presence of rat fat pad tissue (Roginski *et al.*, 1970, 1971). In phosphate buffer, chromium as an inorganic salt gave some increase in glucose oxidation in this system. However, the rate of oxidation was greatly increased when extracts of Brewers yeast containing chromium were added. On the basis of extracts containing chromium complexed in organic structures capable of restoring serum glucose levels in deficient animals, such compounds became known as the chromium glucose tolerance factor (GTF). As part of the research on chromium distribution, the total chromium in a food sample could be determined by chemical analysis, but only part of that chromium was available for

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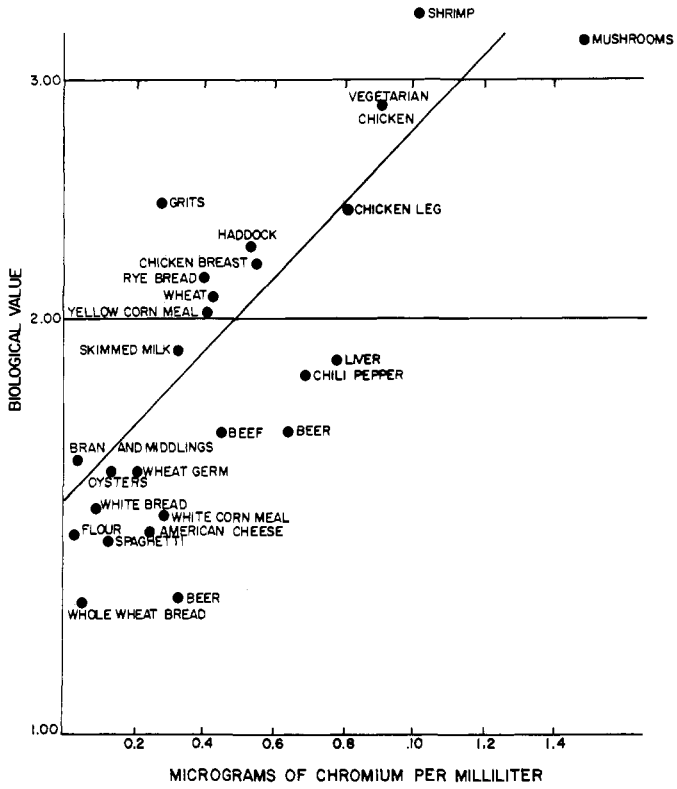

Figure 1. Relationship between chromium contents per milliliter of aqueous alcohol extracts of foods and the corresponding biological activities.

glucose oxidation in the *in vitro* system, as described by Toepfer *et al.* (1971) and Roginski *et al.* (1971). This paper reports the results of chromium analyses of selected food samples and of the extracts prepared from these samples. Relationships between chromium in food extracts and their effects on glucose oxidation activity are shown. The procedure for conducting analyses for chromium was applicable to foods, since size of sample was not a factor and permitted determinations with a lower limit of 10 ng of chromium per gram of samples. The amount of chromium per milliliter of extract as used for relative biological activity determinations required estimations in this order of magnitude.

EXPERIMENTAL SECTION

Source of Samples. Food items and related materials were obtained from local sources. The edible portion was prepared and the material was reduced by grinding or blending in order to withdraw representative material for analysis. Wet samples were frozen. All were refrigerated in sealed containers. Liquid samples were evaporated to small volumes for convenience, keeping track of evaporation losses.

Extraction. Samples were hydrolyzed with hydrochloric acid or extracted with 50% ethyl alcohol. Acid hydrolysates were obtained by refluxing usually 10 g of dry weight of sample with 200 ml of 5 *N* HCl for 18 hr. Most of the HCl was removed by rotary vacuum distillation, including redistillation after adding water. Solutions in water were made to pH 7 with KOH and made to 25-ml volume. Aliquots were taken for analyses. For the alcohol extracts, usually 25 g of dry weight of sample were suspended in 500 ml of 50% of volume ethyl alcohol. The mixture in a covered container was placed in an autoclave set to steam heat at 80° for 5 min. Loss of volume was small during the heating and cooling times and losses due to spontaneous boiling were avoided. After cooling, the mixture was filtered on a Büchner funnel and the residue was washed with 50% alcohol. Alcohol was removed by rotary vacuum distillation. Sample solutions with appreciable fat were ether extracted and residual ether was removed by evaporation. Samples were neutralized and made to 25-ml volume. Aliquots were taken for analyses.

Biological Activity. The relative biological activity of sample extracts was obtained by measuring the CO_2 production from glucose oxidation using glucose-1-^{14}C in the presence of rat epididymal tissue and 100 microunits of insulin, as described by Mertz (1969) and Roginski *et al.* (1970). Male Wistar rats of 21 days were placed on a low chromium Torula yeast diet. They were sacrificed at 9–10 weeks of age to obtain epididymal tissue for immediate use. Pieces of fat tissue weighing approximately 100 mg were incubated in Krebs–Ringer phosphate medium (pH 7.4) with 100 microunits of insulin plus 10 mg of bovine serum albumin and 1 mg of glucose (in part glucose-1-^{14}C) per ml. After 2 hr of incubation at 37° with continuous shaking, H_2SO_4 was added to stop the reaction and the CO_2 evolved was collected in a suspended cup containing hydroxide of hyamine [*p*-(diisobutylcresoxyethoxyethyl)-dimethylbenzylammoniumhydroxide] and measured by scintillation counting. Results were expressed in terms of relative counts per 100 mg of tissue in triplicate for the sample as compared to controls. The usual test procedure required the determinations on extracts undiluted and diluted five and ten times. Typically, the addition of active GTF preparations to chromium-deficient adipose tissue resulted in a steeper slope of the response to graded doses of insulin, as compared to the response in the absence of GTF. The factor by which the slope of insulin response was increased was used as an expression of GTF activity; *i.e.*, a factor of one would denote a slope identical with that obtained without insulin (no bioactivity), whereas a factor of 2 or 3 expressed a two- or threefold increase of the slope. Complete dose–response curves were determined only for a few of the samples tested. The biological values reported for most extracts were derived from triplicate determinations on one level of insulin.

Chromium Analyses. A modified colorimetric procedure for chromate, described by Dean and Beverly (1958), was used for chromium analyses. As originally described, the color developed by *s*-diphenylcarbazide was unstable in the methyl isobutyl ketone (MIBK) solution used to extract chromate. The procedure was modified to reextract the chromate with 0.1 *N* KOH and develop the color in aqueous solution stabilized with phosphate buffer. In preparing solutions for chromium analysis, samples containing chlorides would lose chromium during dry ashing. In order to remove chlorides, samples of usually estimated 1 μg of chromium were weighed in Vicor dishes or crucibles. Wet samples were treated with 2 ml of concentrated HNO_3 and evaporated. Dry samples were charred by placing the crucible in a cold muffle set for 500° and removing it when that temperature was reached. When cool, the char was wet with water, treated with 2 ml of concentrated HNO_3, and evaporated. Both types of samples were dissolved or suspended in a small amount of water (5 ml) and 1 g of potassium persulfate ($K_2S_2O_8$) was added. The solution was evaporated slowly to dryness. The crucibles were now placed in a cold muffle set for 932° and removed when that temperature was reached. Further treatment was not usually necessary. A resuspension in water, evaporation, additional $K_2S_2O_8$, and heating in the muffle would be sufficient. The dry material was easily dissolved in water plus 1 ml of concentrated HNO_3 and made to a known volume containing at least an estimated 0.1 μg of chromium per ml.

For a standard curve, six 125-ml Erlenmeyer flasks containing 0, 1, 2, 3, 4, and 5 μ of chromium were used. The solution was standard $K_2Cr_2O_7$ (28.28 mg per 100 ml and

Table I. Chromium Content of Selected Food Samples and Biological Value of Extracts Containing Chromium

Sample	Total solids, %	Chromium: Total: Wet wt, μg/g	Chromium: Total: Dry wt, μg/g	Chromium: Extracted: %	Chromium: Extracted: Dry wt, μg/g	Biological activity: Acid hydrolysis: Biological value	Biological activity: Acid hydrolysis: Cr, μg/ml	Biological activity: Extract: Biological value	Biological activity: Extract: Cr, μg/ml
Meats and fish									
Liver, calf's	30.2	0.55	1.77	33.6	0.59	2.95	0.220	1.88	0.077
Beef, round	34.1	0.57	1.67	18.7	0.31	2.60	0.220	1.65	0.044
Chicken, breast	29.2	0.11	0.37	24.6	0.09	2.42	0.086	2.19	0.054
Chicken, leg	26.2	0.18	0.70	21.9	0.15	1.99	0.146	2.41	0.080
Shrimp	15.2	0.07	0.48	69.4	0.33	0.91	0.059	3.46	0.101
Haddock	19.8	0.07	0.34	51.5	0.18	1.24	0.053	2.26	0.052
Lobster tail	19.9	0.05	0.23	93.6	0.22	2.06	0.007	5.09	0.065
Oysters	12.2	0.26	2.16	30.2	0.65	1.95	0.089	1.56	0.013
Grains, grain products									
Wheat, grain	88.7	0.28	0.32	37.8	0.12	2.29	0.022	2.08	0.042
Wheat, germ	94.6	0.23	0.24	85.0	0.20	1.82	0.024	1.55	0.020
Wheat, bran and middlings	91.1	0.38	0.42	15.8	0.07	2.08	0.030	1.58	0.003
Wheat, flour	90.2	0.23	0.25	14.3	0.04	1.86	0.018	1.40	0.002
Bread, white	62.2	0.26	0.42	42.9	0.18	2.98	0.077	1.47	0.008
Bread, whole wheat	64.5	0.42	0.66	32.8	0.22	2.26	0.023	1.25	0.005
Bread, rye	62.7	0.30	0.49	29.7	0.15	1.70	0.027	2.15	0.039
Spaghetti	91.4	0.15	0.16	75.0	0.12	2.34	0.006	1.38	0.012
Cornflakes	94.3	0.14	0.15	80.0	0.12	1.60	0.014	1.09	0.011
Cornmeal, yellow	91.2	0.10	0.11	74.8	0.08	3.18	0.021	2.02	0.040
Cornmeal, white	91.1	0.12	0.13	47.0	0.06	1.80	0.024	1.44	0.028
Grits	90.0	0.05	0.06	90.0	0.05	2.19	0.011	2.43	0.027
Fruits									
Bananas	27.3	0.10	0.38	99.5	0.38	2.02	0.056	0.99	0.057
Apple, peel	18.6	0.27	1.48	52.5	0.78	2.65	0.258	1.04	0.064
Apple, pared	14.4	0.01	0.09	40.0	0.04				
Oranges	14.7	0.05	0.31			2.30	0.064		
Strawberries	8.5	0.03	0.34	80.7	0.27	1.95	0.029		
Blueberries	22.0	0.05	0.22			2.96	0.060		
Vegetables									
Carrots	11.6	0.09	0.78	26.5	0.21	1.92	0.101		
Green beans, snap	8.2	0.04	0.48	100	0.48	2.33	0.192		
Potatoes, old	47.4	0.27	0.55	48.0	0.26	1.75	0.076		
Potatoes, new	37.0	0.21	0.57	53.6	0.31	1.72	0.077		
Spinach	9.4	0.10	1.03			1.91	0.200		
Miscellaneous									
Mushrooms	3.7	0.04	1.27	86.6	1.10	1.88	0.169	3.19	0.147
Yeast, Brewers	95.8	1.12	1.17	48.2	0.56	3.34	0.070	4.06	0.007
Cheese, American	61.9	0.56	0.92	30.4	0.28	1.39	0.034	1.40	0.024
Beer	4.6	0.03	0.61	100	0.61	3.60	0.083	1.66	0.063
Vegetarian									
Choplets	20.1	0.10	0.48	24.6	0.12	1.18	0.077	3.72	0.047
Chicken slices	21.5	0.07	0.32	87.8	0.28	1.15	0.055	2.86	0.090
Pepper									
Black, table	90.5	0.35	0.38	100	0.38	2.35	0.076	1.99	0.076
Chili, fresh	23.4	0.30	1.28	22.5	0.29	2.81	0.120	1.82	0.068
Butter	85.4	0.13	0.15	80.0	0.12	1.32	0.030	1.20	0.010
Margarine	90.9	0.18	0.20	50.0	0.09	1.35	0.070	1.28	0.008
Milk, skimmed	9.5	0.01	0.13	84.4	0.11	1.00	0.030	1.90	0.032
Ginger ale	10.5	<.01	0.05			2.74	0.023		
Sugar, cane	100		0.02						
Egg white	12.2	0.08	0.65	36.0		1.20	0.088	1.08	0.048
Egg yolk	47.8	1.83	3.84	50.4		1.59	0.065	0.96	0.025

1 ml diluted to 100 ml). Solutions were made to about 10 ml with water, 1 ml of concentrated HNO_3 was added, and the solutions were heated gently for a few minutes on the hot plate. To these flasks were added 0.2 ml of concentrated H_3PO_4, 2.0 ml 0.1 *N* $AgNO_3$, and 1 g of $K_2S_2O_8$ in three additions with shaking. In 7.5 min, the flasks

Table II. Calculated Chromium Biological Values of Selected Foods, Edible Portion as Purchased

Sample	Relative biological value	Sample	Relative biological value
Yeast, Brewers (dried)	44.88	Peppers, chili (fresh)	2.27
Pepper, black	10.21	Wheat bran and middlings	2.21
Liver, calf's	4.52	Vegetarian chicken	2.16
Cheese, American	4.39	Cornmeal, white	2.09
Wheat germ	4.05	Shrimp	2.03
Bread, whole wheat	3.59	Grits	1.97
Cornflakes cereal	3.01	Lobster	1.95
Bread, white	2.99	Mushrooms	1.92
Spaghetti	2.89	Chicken leg	1.89
Beef round	2.89	Haddock	1.86
Wheat grain	2.96	Patent flour	1.86
Butter	2.81	Beer	1.77
Bread, rye	2.67	Egg white	1.77
Margarine	2.48	Chicken breast	1.75
Oysters	2.43	Vegetarian choplets	1.72
Cornmeal, yellow	2.35	Skimmed milk	1.59

were boiled gently for 15 min, washing down the sides of the flasks. One milliliter of 10 M H_3PO_4 was added and the flasks were placed in crushed ice for 5 min. Cold centrifuge tubes, 50 ml, were kept in crushed ice. To each were added 1.5 ml of 10 N HCl. The cold solutions from the flasks were poured into the centrifuge tubes in ice. Tubes were equalized in volume and centrifuged for 5 min at 1500 rpm to remove AgCl. Cold 125-ml separating funnels were placed in the rack and 15 ml of cold methylisobutylketone (MIBK) were added. The solutions were decanted from the centrifuge tubes into the funnels and placed on the shaker for 5 min. The water layer was drawn off and discarded. The MIBK layer was shaken 1 min with 15 ml of cold 1 N HCl, and the HCl was discarded. The chromates were extracted from the MIBK layer with 2-min shakings with 5 ml of 1 N KOH, twice, followed by 5 ml of H_2O. These were drawn off into cold test tubes in ice. At this time the tubes were removed from ice, acidified with 1.5 ml of 8 N H_2SO_4, and 1 ml of the color reagent (0.125 g of *s*-diphenylcarbazide in 50 ml of acetone) was added and mixed. After 3 min, 2 ml of 4 M NaH_2PO_4 were added. The solutions were filtered through dry Whatman No. 40 filter paper (9 cm) into clean tubes and read in 5-cm cells at 540 mm in a Beckman DU. The color was stable as long as overnight. Samples were treated the same way, using sufficient solution for 1 μg in 10 ml and preferably 2 to 3 μg.

RESULTS AND DISCUSSIONS

The methods described for the chromium analyses required the elimination of chloride. Nitric acid and potassium persulfate proved useful in this respect and oxidized organic material left a white residue, without prolonged use of high muffle temperatures. In this particular analysis the use of perchloric acid was avoided. The stable color developed in aqueous solutions contributed to the usefulness of this assay. Percentage recoveries of the standard averaged 100.2, with a standard deviation of 4.18. The standard curve obtained from optical density (y) and micrograms of chromium (x) in the volume of the solution used for readings was represented by $y = 0.020 + 0.212x$. The method was intended for use with samples where size was not a limiting factor and where representative sample material would be drawn from a correspondingly sized lot. The colorimetric procedure could be used to estimate chromium in less than one part-per-million with the usual equipment in the laboratory. Much more sophisticated procedures have been reported recently by Wolf *et al.* (1972) capable of determining chromium in picogram quantities with a relative standard deviation of 6%.

The data on chromium content of foods are shown in Table I on a wet sample as received, and prepared for analysis basis and on a dry weight basis. Groupings were made for similar samples and to permit comparisons. There were no significant relationships between chromium per ml in the acid-hydrolyzed material and biological values according to the *in vitro* test. Letting y represent the biological value of the solutions tested and x be the chromium content per ml of the solution tested, a nonsignificant relationship was found. The regression equation was $\log y = 0.260 + 0.607x$, with $\bar{x} = 0.0708$, $\log \bar{y} = 0.302$. Solutions resulting from acid hydrolysis of the samples contained all the chromium of the sample so treated.

When the data from alcohol extracts were similarly treated, a highly significant relationship was found between the biological values of the solutions (y) and the chromium content per ml, as shown in Figure 1. The regression equation was $\log y = 0.171 + 2.726x$, $\bar{x} = 0.042$, $\log \bar{y} = 0.285$, F = regression ms/residual ms = 0.215445/0.013719 = 15.70. F at 1/25 = 7.7 at $p = 0.01$. Apparently, the alcohol extracts contained GTF and chromium in amounts resulting in a highly specific response in the glucose oxidation procedure used for the test. It is doubtful that the alcohol extraction selectively extracted only that chromium associated with GTF. However, the percentages of chromium extracted from foods, as shown in Table I, indicated those portions related to biological values. These values for chromium could be the basis for evaluating different foods. Only the meats, fungi, grains, and seeds gave biological values greater than the controls. Notably, egg yolk alcohol extract did not yield a GTF value, although egg yolk was found to contain 3.84 μg of chromium on a dry weight basis, and the alcohol extract contained over half of this chromium. Vegetarian choplets and chicken slices contained soybean products, as well as yeast concentrates for flavoring purposes, and could be expected to show a biological response. Using the relationship between alcohol-extractable chromium and biological value of corresponding extracts, a means of expressing biological values of foods could be proposed. A major criticism would be the extrapolation beyond the limits of the experimental data and therefore the calculation can only be considered temporary and subject to reservations for

the values above 3. The data in Table II can serve as a useful means for comparative ranking until a better system can be contrived. Thus the extractable chromium content per gram of food items on a fresh weight basis of the edible portion as prepared for analysis was used. The data were arranged in descending order and showed most of the grain and cereal products in the middle group.

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LITERATURE CITED

Dean, J. A., Beverly, M. L., *Anal. Chem.* **30**, 977 (1958).
Mertz, W., *Physiol. Rev.* **49**, 163 (1969).
Roginski, E. E., Toepfer, E. W., Polansky, M. M., Mertz, W., *Fed. Proc. Abstr.* 695 (1970).
Roginski, E. E., Toepfer, E. W., Polansky, M. M., Mertz, W., *Fed. Proc. Abstr.* 238 (1971).
Toepfer, E. W., Mertz, W., Roginski, E. E., Polansky, M. M., Abstracts, 162nd National Meeting of the American Chemical Society, Washington, D. C., Sept. 12–17, 1971, AGFD No. 42.
Wolf, W. R., Taylor, M. L., Hughes, B. M., Tiernan, T. O., Sievers, R. E., *Anal. Chem.* **44**, 616 (1972).

Received for review May 31, 1972. Accepted September 6, 1972. Presented at Division of Agricultural and Food Chemistry, 162nd Meeting, ACS, Washington, D. C., Sept. 12–17, 1971.

Partition Column Chromatography for Quantitating Effects of Fertilization on Plant Acids

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A method for determining organic acid levels in biological materials by silica gel partition column chromatography (pcc) is described. It uses tetrabromophenolphthalein ethyl ester, which has a high molar absorbency, for quantitating the acids in the column effluent. The concentrations of several organic acids were determined in two species of crested wheatgrass, Nordan *(Agropyron desertorum)* and Fairway *(A. cristatum)*, grown under different conditions of K fertilization. For comparison, aconitic, citric, and malic acids were also determined by polarography, spectrophotometry, and fluorometry, respectively. Results obtained by these methods agreed with the data obtained by pcc. The concentration of *trans*-aconitic acid averaged about 96 and 21 mequiv/kg of dry matter in Nordan and Fairway, respectively, when grown in the greenhouse without K. K fertilization (312 kg/ha) approximately doubled the *trans*-aconitic acid concentration in both species. Fairway contained 363 mequiv/kg of malic acid, while Nordan contained 280 mequiv/kg. K fertilization increased these concentrations to 611 and 446 mequiv/kg, respectively. Citric acid was increased by K fertilization, but there was no significant difference between species and in no case was the citric acid concentration greater than 80 mequiv/kg of dry matter.

The influence of fertilization on organic acid concentrations in plants has been a subject of considerable interest in recent years (Grunes *et al.*, 1970). In a field study of southern Indiana, Teel (1966) found that fertilization with NH_4NO_3 at the rate of 168 kg N/ha increased the concentration of total organic acids in tall fescue *(Festuca arundinacea)*. Such elevated concentrations of organic acids are believed to cause toxic responses in animals (Burau and Stout, 1965; Grunes *et al.*, 1970; Stout *et al.*, 1967). For instance, grass tetany has occurred in animals grazing California spring grasses high in *trans*-aconitic acid (Burau and Stout, 1965; Stout *et al.*, 1967). Stout *et al.* (1967) suggested that this acid was forming complexes with Mg which decreased the availability of Mg^{2+} to the animal and that 1% *trans*-aconitic acid (172 mequiv/kg dry matter) was potentially toxic. Additional evidence for the role of organic acids in grass tetany was provided by Bohman *et al.* (1969), who produced toxic symptoms in cattle by oral administration of KCl with *trans*-aconitic acid or citric acid. In later work, Scotto *et al.* (1971) found that citric acid in the blood of cattle rose as the quantity of KCl, combined with an oral dose of citric acid, was increased.

The above evidence has emphasized the need for analytical methods capable of rapidly quantitating organic acids in biological material. Enzymatic assays (Bergmeyer, 1963), gas chromatography (Alcock, 1969; Clark, 1969; Hautala and Weaver, 1969), thin-layer chromatography (Myers and Huang, 1966), paper chromatography (Barness *et al.*, 1970; Kennedy and Barker, 1951), polarography (Burau, 1969), and silica gel partition column chromatography (Barness *et al.*, 1970; Bulen *et al.*, 1952; Dijkshoorn and Lampe, 1962; Kesner and Muntwyler, 1966, 1969a,b; Marvel and Rands, 1950; Neish, 1949; Rosevear *et al.*, 1971) have been used. For our studies we have modified the silica gel partition column chromatography method (pcc) developed by Kesner and Muntwyler (Kesner, 1965; Kesner and Muntwyler, 1966; Kesner and Muntwyler, 1969b). It has also been successfully employed for analyses of organic acids in excreta and tissues of animals. Presented herein are data for organic acid concentrations in crested wheatgrass grown in the greenhouse and in pastures of the western United States. Results obtained with pcc are compared with those obtained by other established methods.

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