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Received for review October 26, 1978. Accepted January 8, 1979. Oregon Agricultural Experiment Station Technical Paper No. 4993. This study was conducted in cooperation with the USDC, NOAA, Institutional Sea Grant 04-7-158-44085 and a grant from the National Fisheries Institute.

Determination of Chromium in Selected United States Diets

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As a basis for estimating mean daily chromium intake in the United States, chromium contents of 28 selected daily diets of different compositions were determined by graphite furnace atomic absorption utilizing a continuum source, echelle, wavelength modulated atomic absorption spectrometer system for improved background correction. These diets were prepared for a human metabolic study of high fat (43%) and low fat (25%) content diets at the Nutrition Institute, Beltsville, MD. The 43% fat diets which were "typical American diets" with regard to fat and calories contained less chromium (62 ± 28 μg/day) than the 25% fat diets (89 ± 56 μg/day). Fifty-seven percent (8/14) of the high fat and 21% (3/14) of the low fat diets were at or below the minimum of the recently proposed range of chromium intake of 50–200 μg/day.

Recent studies in research on chromium nutrition have confirmed earlier findings with experimental animals which suggested that chromium was essential for the normal glucose tolerance of man (Mertz, 1969; Hopkins et al., 1971; Jeejeebhoy et al., 1977; Mertz et al., 1978; Glinsmann et al., 1966). As a result, a provisional recommendation for chromium intake has been proposed for the next revision of the Recommended Dietary Allowances (RDA) (Mertz, 1979).

In order to determine whether or not the chromium intake of people will meet the recommended level, we need reliable data on the level of chromium supplied by diets of different compositions. Only scattered information is available on the daily chromium intake in the United States. Levine et al. (1968) analyzed seven diets, of elderly and of young subjects eating institutional diets, and found that the daily chromium intake varied from 5 to 115 μg. Mean daily chromium intake was 52 μg for elderly subjects and 65 μg for young subjects. Schroeder et al. (1962) found 70 μg/day chromium intake in a typical institutional diet. These values are lower than the reported average daily chromium intake in Japan, which was determined as 130–140 μg (Murakami et al., 1965). In West Germany, Schelenz (1977) recently estimated the dietary intake of 25 elements, including chromium, based on analyses of the total daily diet of four adult males during 1 week. Intake of chromium averaged 62 μg/day, ranging from 11 to 195 μg/day. The analytical validity of these data is, however, difficult to evaluate because suitable Standard Reference Materials (SRM) for chromium were not available at the time of these analyses. Only recently has the National Bureau of Standards (NBS) issued the chromium certified brewer's yeast (SRM-1569) and also certified the previously issued bovine liver (SRM-1577) for chromium. Orchard leaves (SRM-1571) had been certified for Cr, but as a plant material it contains high levels of Cr in a different form. Several additional chromium certified plant material SRM are now available from NBS including spinach (SRM-1570), pine needles (SRM-1575), and tomato leaves (SRM-1573).

Several laboratory comparison studies indicate that the present state of chromium analysis in biological and environmental materials is not yet satisfactory (Mertz et al., 1977; McClendon, 1974; Parr, 1978; Kumpulainen and Koivistoinen, 1977; Schelenz, 1977; Scott, 1978). For analysis of chromium in a water matrix, graphite furnace atomic absorption spectrophotometry is relatively reliable, sensitive, and rapid. However, for the analysis of complex matrices such as food, errors are apparently introduced during the digestion of organic matter and other steps in the preparation of samples for the instrumental analysis. In wet digestion, large amounts of acids may be a significant source of chromium contamination even when reagents of the highest attainable purity are used.

There is also some evidence that chromium may form volatile compounds during some wet digestions, especially if the mixture contains perchloric acid (Gorsuch, 1959; McClendon, 1978). Chromium may be lost by volatilization during dry ashing at temperatures of 700 °C or higher (Shapcott et al., 1977; Koirtyohann and Hopkins, 1976). Results may also be erratic due to adsorption of Cr on the walls of crucibles during dry ashing (Koirtyohann and Hopkins, 1976; Shapcot et al., 1977; Jones et al., 1975). Low-temperature ashing would eliminate most of these problems, but we found that it was ineffective for the digestion of bovine liver (SRM-1577). Furthermore, in low-temperature ashing, the sample size is more limited than in dry ashing and use of relatively high amounts of hydrogen peroxide are often needed as an ashing aid.

Some biological materials such as brewer's yeast (SRM-1569), contain acid-insoluble material, apparently silicates, that can strongly adsorb chromium. Therefore,

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the sample digest has to be shaken vigorously to insure a homogeneous suspension of the insoluble material before injection into the graphite furnace (Kumpulainen, 1978). The precipitate can also be solubilized in a Teflon container by adding hydrofluoric acid.

In the present study, we have been able to minimize the above-mentioned sources of error and to optimize a digestion procedure using dry ashing at 500 °C with sulfuric acid and hydrogen peroxide as ashing aids. We have used this procedure to determine the chromium in selected U.S. daily diets.

MATERIALS AND METHODS

Twenty-eight diets prepared for a human metabolic diet study by the Lipid Nutrition Laboratory, Nutrition Institute, Beltsville, MD, were analyzed for chromium. In half of these diets, 43% of the total caloric content was derived from fats and the ratio of polyunsaturated to saturated fatty acids (P/S) was 0.3. The other half of the diets contained 25% fat calories and a P/S ratio of 1.0. The high fat diet is a "typical American diet" with regard to its fat-caloric content. The diets were purposely made to meet established RDA's for the nutrients, which is not necessarily true of the typical American diet. The subjects were fed a diet of caloric intake to maintain weight, but only one calorie level, 2800 cal, was selected for analysis. The diets were composed of commonly used U.S. foods purchased on the open market. They were prepared from standard recipes or from recipes on the packages. No salt was added during cooking except when called for in a recipe. Vegetables and meats common to the low and high fat diets were prepared together. Each serving was individually weighed and reheated in a microwave oven just prior to serving. Juices or cooking water was minimal because calculated servings were without juices. All food in the composites was either weighed or measured. This included all beverages such as milk, juices, etc., but did not include coffee or tea which were permitted ad libitium. Alcoholic beverages were not permitted. The diets were composited and frozen in plastic containers until homogenization.

In the laboratory, the samples were thawed, cut in pieces and transferred with a stainless steel fork and knife into plastic jars. The possible chromium contamination from the cutlery thus represented that of a normal eating situation. For homogenization of samples, a blender blade assembly (Osterizer, Model 857-01J, Oster Corporation, Milwaukee, WI) was fitted to the plastic jar. Chromium contamination during homogenization, was avoided by use of blender blades that were specially fabricated of low-chromium steel (Brown and Sharp, Ground Flat Stock, 0.5% Cr). The apparent chromium content of a diet did not differ when analyzed after either 3 or 11 min of homogenization. Small aliquots of homogenized diets in plastic containers were dried in a vacuum oven at 70 °C for 18 h, then stored refrigerated in tightly covered plastic bottles.

Diet samples of 150–200 mg dry weight were weighed in 1.5-mL Coors porcelain crucibles and ashed overnight at 500 °C in a muffle furnace (Model 51894, Lindberg, Watertown, WI). After samples cooled, 10 μL of concentrated H_2SO_4 (sub-boiling distilled, National Bureau of Standards, Gaithersburg, MD) and 20 μL of 50% H_2O_2 (Fisher Scientific) were added and the samples very carefully evaporated to dryness on a hot plate in a Class 100 clean air hood (EACI, Hagerstown, MD). The crucibles were heated for two additional hours at 500 °C. The acid and muffle furnace treatments were repeated until the ash was white. Usually only one acid treatment was necessary for the completion of the digestion.

The ash was dissolved in 1.0 mL of 1 N HCl (prepared by isothermal distillation; Alvarez et al., 1969) and analyzed for chromium by use of a continuum source, echelle, wavelength modulated, atomic absorption spectrophotometer (CEWM-AA) equipped with a Perkin Elmer HGA 2100 graphite furnace and Model 56 recorder. Improved background correction capability of the CEWM-AA is critical for determination of chromium by graphite furnace atomic absorption spectrometry (Guthrie et al., 1978B). Detailed information on background correction by CEWM-AA was published by Harnly and O'Haver (1977).

Chromium was read at 357.9 nm and sample size for analysis was 30 μL using a program cycle of: drying, 30 s at 110 °C (ramped 20 s); ashing, 20 s at 1100 °C (ramped 15 s); and atomization, 9 s at 2700 °C. Argon was used as the purge gas and gas flow was interrupted during the atomization cycle. Scale expansion was 5X. Working standards in the range of 4–40 ng/mL were prepared twice a week in 1 N HCl from 1 μg/mL of chromium stock standard (Alpha, Ventron).

In order to find a suitable dry ashing procedure, recovery studies were carried out by adding aliquots of ^{51}Cr to samples of NBS brewer's yeast and bovine liver SRM which had been weighed into 1.5-mL Coors porcelain crucibles. The samples were dry ashed at 500 °C, acid treated, and dissolved as described above for the diets. Various acid treatments of the ash were tested using H_2SO_4, H_2O_2, and/or HNO_3 (subboiling distilled, NBS). ^{51}Cr activity of the dissolved samples was measured using a small whole-body γ counter (Nuclear Chicago).

The yeast extracts were prepared as follows: 20 mL of 0.1 N NH_4OH was added to 2 g of dried yeast, followed by shaking for 60 min at 30 °C. The mixture was then centrifuged at 1500*g* for 15 min. The clear extract was separated and stored in plastic bottles at 4 °C. An aliquot of the extract was assayed for its insulin-potentiating activity, as described by Anderson et al. (1978). For chromium analyses of the extract, 1.0 mL was measured into 1.5-mL Coors porcelain crucibles and slowly evaporated to dryness on a hot plate. Low-temperature oxygen plasma ashing was done by keeping crucibles for 6 h in the asher (Trapelo 505, LFE Corporation, Waltham, MA) set at 1-mm O_2 pressure and 400-W power. After samples cooled, 100 μL of 50% H_2O_2 was added, and the samples were slowly evaporated to dryness on a hot plate. Extracts were dry ashed as described for the diet samples. We analyzed all of the chromium extracts for total chromium as described for the diet samples but used pyrolytic graphite tubes to improve the sensitivity of the instrumental analysis.

RESULTS AND DISCUSSION

The recoveries of added ^{51}Cr after dry ashing with no acid treatment were 99.7 and 100.6%, respectively, for NBS bovine liver and NBS brewer's yeast (Table I). Upon acid treatment of the ash to ensure complete digestion, lower recoveries were obtained (Table I). For bovine liver the best digestion mixture was H_2O_2 and H_2SO_4 with a mean recovery of 95.2%. Use of HNO_3 resulted in a 10–15% loss of added Cr. Complete recovery of endogeneous Cr in biological samples was ascertained by analyses of NBS bovine liver and NBS brewer's yeast (Table II). These data suggest no significant loss of endogeneous Cr with different acid treatments in contrast to the data for added Cr.

One source of error in Cr analysis has been attributed to the adsorption of chromium on the digestion crucible walls (Jones et al., 1975; Shapcott et al., 1977). To study

Table I. Recovery of Added ^{51}Cr after Different Combinations of Dry and Wet Ashing

sample	no. of samples	mean recov. after dry ashing at 500 °C (% ± SD)	acid treatment	recov. after complete digestion (% ±SD)
NBS brewer's yeast	7	100.6 ± 0.62	1 × 20 μL of $HNO_3 + H_2O_2$	98.6 ± 0.76
NBS bovine liver	8	99.7 ± 1.7	4 × 20 μL of $HNO_3 + H_2O_2$	89.8 ± 2.15
NBS bovine liver	4		3 × 0.25 mL of 50% H_2O_2	85.0 ± 1.9
NBS bovine liver	4		3 × 20 μL of $H_2SO_4 + H_2O_2$	95.2 ± 1.27

Table II. Chromium Concentration in NBS Bovine Liver and NBS Brewer's Yeast

sample	mean Cr, μg/g ± SD	no. of samples	acid treatment
NBS brewer's yeast	2.10 ± 0.003 (2.12 ± 0.05)[a]	6	$HNO_3 + H_2O_2$
NBS bovine liver	0.095 ± 0.014[b]	10	$HNO_3 + H_2O_2$
NBS bovine liver	0.092 ± 0.005	8	$H_2SO_4 + H_2O_2$
NBS bovine liver	0.085 ± 0.003 (0.088 ± 0.012)[a]	6	$HNO_3 + H_2O_2$

[a] NBS certified value. [b] Method of standard additions.

Table III. ^{51}Cr Retained in Crucibles after Dissolution of Ash in Hydrochloric Acid[a]

sample	no. of samples	mean ^{51}Cr retained in crucibles (% ± SD)
NBS brewer's yeast	7	1.75 ± 1.28
NBS bovine liver	8	2.00 ± 1.46

[a] Ash dissolved in 1.0 mL of 10 N HCl and crucible rinsed 3 × 1.0 mL of 1 N HCl.

this effect we added ^{51}Cr to NBS brewer's yeast and bovine liver samples in the crucibles and ashed them, and the ash was dissolved by addition of 1.0 mL of 10 N HCl, followed by rinsing three times with 1 N HCl after 0.5 h. The crucibles were then counted and found to retain 2% or less of the added chromium (Table III). Subsequent studies showed identical results with 1 N HCl.

There is some evidence that chromium may volatilize during dry ashing (Wolf et al., 1974; Behne et al., 1976). The volatile chromium has been hypothesized to be the biologically active form of chromium called "glucose-tolerance factor" (GTF) (Tuman et al., 1978). Brewer's yeast is the richest known source of GTF (Toepfer et al., 1973). To test the volatility of GTF in the dry ashing procedure compared to low-temperature ashing, ammonium extracts of NBS brewer's yeast and of another commercial yeast were analyzed for chromium by using both low-temperature and dry ashing. These extracts, which contained less than 0.1% of the total chromium of the yeasts, were found to be highly active biologically in chromium bioassay by the method of Anderson et al. (1978). Chromium values were slightly higher from dry ashing than from low-temperature ashing (Table IV). That finding suggests that chromium was not volatile in dry ashing if we assume no volatility in low-temperature ashing. Blanks were also consistently lower in dry ashing than in low-temperature ashing because much smaller amounts of H_2O_2 were needed to complete the ashing. In the studies of Wolf and Greene (1976) in this laboratory, dry ashing blanks were higher and more variable than reported in this work. This might be attributed to use of an older muffle furnace in the earlier work whose heating coils are not as well isolated, leading to increased airborne levels of chromium from Nichrome heating wire. Also changes in general laboratory procedures regarding "clean air" techniques have been implemented since the prior studies were carried out.

Table IV. Chromium Concentrations of Ammonium Extracts of NBS Brewer's Yeast and of a Commercial Yeast

sample	biological activ.[a]	dry ashing, of ng Cr/mL ± SD	low-temp ashing of ng Cr/mL ± SD
NBS brewer's yeast	4.4	6.2 ± 0.6 (3)[b]	4.5 ± 0.8 (5)
commercial yeast	5.0	8.0 ± 0.2 (5)	7.2 ± 0.7 (5)

[a] Biological activity of $CrCl_3$ was 1.0. [b] Number of samples is in parentheses.

Table V. Chromium Content of Selected Diets

25% fat diet, PS/1.0, μg/day		43% fat diet, PS/0.3, μg/day	
1	96 ± 7	1	79 ± 4
2	51 ± 5	2	66 ± 1
3	103 ± 9	3	130 ± 3
4	74 ± 1	4	54 ± 4
5	224 ± 6	5	41 ± 5
6	30 ± 3	6	107 ± 1
7	181 ± 4	7	40 ± 2
8	58 ± 7	8	43 ± 5
9	73 ± 6	9	49 ± 8
10	126 ± 1	10	45 ± 7
11	25 ± 2	11	50 ± 7
12	41 ± 7	12	46 ± 8
13	82 ± 8	13	87 ± 8
14	76 ± 3	14	37 ± 1
$\bar{X}$ ± SD	89 ± 56	$\bar{X}$ ± SD	62 ± 28

The results of our methodological experiments are in agreement with the findings of Rook and Wolf (1977) who reported that only a very small fraction of the Cr is thermally distilled from SRM-1569 at 300 °C. Studies by Versieck et al. (1978), Koirtyohann and Hopkins (1976), Christensen et al. (1976), Shapcott et al. (1977), Jones et al. (1975), Kumpulainen (1977), and Cary and Olson (1975) also detected no significant volatility of chromium in dry ashing. The present study also confirms the results of the study of Kumpulainen (1977) in terms of adsorption of chromium on the walls of crucibles in dry ashing. The high adsorption rates reported by Jones et al. (1975), Koirtyohann and Hopkins (1976), and Shapcott et al. (1977) apparently were due to use of excessively high dry ashing temperatures (700–800 °C).

After we showed in these methodological studies that chromium could reliably be analyzed in SRM, the chromium levels of the selected diets were determined. Table V shows the chromium levels of the diets. The mean chromium content in the low-fat diets was higher (89 ± 56 μg) than in the high-fat diets (62 ± 28 μg), but the difference was not quite statistically significant ($P = 0.1$). The distribution of the diets according to their chromium content is presented in Figure 1. A provisional recommendation for chromium RDA has been suggested at 50–200 μg/day (Mertz, 1979). The chromium content of the diets we analyzed in the present study was relatively low: 39% of the diets contain chromium at or below that

Table VI. Composition of a 2800-cal, 43% Fat Diet Containing 45 μg of Cr

food	amount, g
breakfast	
orange juice	249
soft boiled egg	50
corn muffin	84
milk, whole	244
lunch	
meat loaf	90
spaghetti	150
tomato puree	135
Parmesan cheese	20
French bread	25
salad	
lettuce	50
celery	20
cucumber	25
salad dressing	
corn oil	20
vinegar	30
cup cake with icing	36
dinner	
roast turkey breast	90
gravy	60
boiled potato	122
lima beans	85
roll, dinner	28
desert	
jello	65
milk, whole	244
whipped cream	30
salad	
lettuce leaf	25
cream cheese	20
sliced pineapple	244
coffee cream (20%)	45
butter	30
sugar	10

of the lowest proposed recommended intake, 50 μg/day. For the low fat diets, only 3 of 14 were below the 50 μg/day. In the more "typical" U.S. diet of 43% fat, 8 of 14 or 57% were at or below the lowest recommended level. This suggests that some U.S. population groups may consistently have low or marginal chromium intakes. This also suggests that a lower fat diet may be more beneficial in respect to supplying adequate amounts of the trace nutrient chromium. The main sources of chromium in these diets are not known. Possibly some of the chromium in the diets was introduced from stainless steel cooking vessels, but this would be typical of most home prepared foods in the United States. Table VI shows the composition of the 2800-cal, 43% fat diet that contained 45 μg of Cr. This diet represents a low quartile diet in Cr content. These diets were formulated in the summer months and there may be some seasonal variations.

The mean chromium intake that we found agrees with published mean chromium intakes in the United States (Levine et al., 1968; Schroeder et al., 1962). Furthermore, both the range and mean of our chromium levels agree with the chromium intake from self-selected diets in West Germany (Schelenz, 1977).

By use of $^{51}CrCl_3$, absorption of chromium in human subjects has been demonstrated to be about 1% (Doisy et al., 1968). Urine is thought to be the main excretion route of absorbed chromium (Mertz, 1969). Urinary excretion of chromium for 12 healthy male adults in the United States was 0.8 ± 0.4 μg/day (Guthrie et al., 1978a). Thus, the mean intake of 78 ± 43 μg/day, found in our study, agrees with the aforesaid excretion value, assuming the mean absorption to be about 1%.

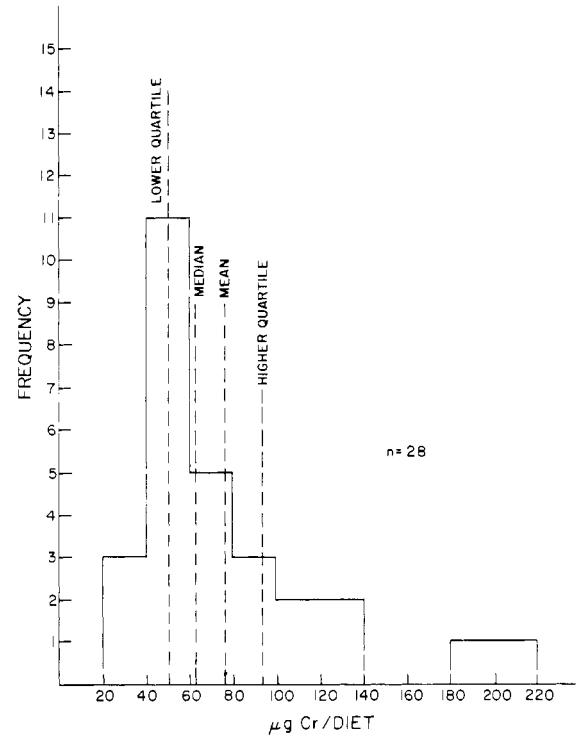

Figure 1. Distribution of diets according to chromium content.

ACKNOWLEDGMENT

The authors thank Mary Marshall for supplying the diet samples, Richard Anderson and Marilyn Polansky for the chromium bioassays, and James Harnly for technical advice concerning the use of CEWM-AA.

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Received for review October 11, 1978. Accepted January 26, 1979. Mention of a trade mark of proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be available. Supported in part by a grant from W. K. Kellogg Foundation. This paper was presented in part at the 62nd Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, NJ, April 9–14, 1978, Program No. 1014.

Chemistry of Toxic Range Plants. Determination of Pyrrolizidine Alkaloid Content and Composition in *Senecio* Species by Nuclear Magnetic Resonance Spectroscopy

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The pyrrolizidine alkaloid and corresponding *N*-oxide contents of small samples of *Senecio longilobus*, *S. riddellii*, *S. jacobaea*, and *S. vulgaris* have been measured using nuclear magnetic resonance spectroscopy. The percentages of the individual alkaloids seneciphylline, senecionine, riddelliine, and retrorsine in the total alkaloid mixture have also been determined from the same NMR spectra. Exceptionally high total alkaloid contents were found for *S. longilobus* and *S. riddellii* relative to most *Senecio* sp. The technique provides a rapid, facile method for examination of plant samples in order to evaluate the hepatotoxicity hazard to animals and humans and for regulation of dose rate of pyrrolizidine alkaloids in animal feeding experiments.

The hepatotoxic pyrrolizidine alkaloids (PAs) present in *Senecio* and several other plant species have been implicated in the poisoning of animals and, on occasion, humans in many parts of the world (Bull et al., 1968; Mattocks, 1972). *Senecio* species present a toxicity hazard to cattle, horses, and, to a lesser extent, sheep on ranges and pastures in time of drought or overgrazing when other food is scarce or after spring rains when these plants exhibit lush growth that precedes growth of more palatable and nutritious species. In addition, concern has arisen that the pyrrolizidine alkaloids may enter the human food supply in milk (Johnson, 1976; Dickinson et al., 1976), in honey (Deinzer et al., 1977), and through contamination of grains by seeds.

The effect upon animals of consumption of *Senecio* is generally chronic, characterized by a cirrhosis-like condition of the liver. Signs of intoxication are frequently slow to appear, occurring weeks or even months after ingestion of the plant, resulting in poisoning being falsely attributed to infectious diseases or to toxic plant species most visible on the range at the time the symptoms are observed. Moreover, the insidious and irreversible nature of the hepatotoxicity results in unexpected fatality when the animals are subjected to any of a variety of severe, subsequent stresses.

On ranges in the western United States, three *Senecio* species are of particular concern: *S. jacobaea* (tansy ragwort), an introduced plant which presents a problem in coastal areas of the Pacific Northwest, *S. longilobus* (threadleaf groundsel), and *S. riddellii* (Riddell's groundsel), native species in semidesert areas of the Southwest. In connection with animal experiments designed to evaluate the effect of sublethal doses of the latter two species on cattle and synergism with other toxic plants such as locoweed which may be consumed concurrently, it became essential to determine the level of PAs present in a large number of plant samples. In addition to measuring the total level of PAs, it was considered necessary to determine the amount existing in the *N*-oxide form, since the relative toxicities of the alkaloid vs. *N*-oxide are not known, and also to obtain the relative proportions of at least the major individual alkaloids present.

To date, the only satisfactory method for estimation of PAs in biological materials has been a spectrophotometric method (Mattocks, 1967, 1968; Bingley, 1968) based upon the Polonovsky reaction. In this method the alkaloidal

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