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ANALYSIS OF HUMAN FECAL COMPONENTS AND STUDY OF METHODS FOR THEIR RECOVERY IN SPACE SYSTEMS

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FOREWORD

This investigation was carried out by Drs. S. A. Goldblith and E. L. Wick of the Department of Nutrition, Food Science and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts, under contract AF 33(616)-6136, by authority of Project 6373, "Equipment for Life Support in Aerospace," Task 63122, "Waste Recovery and Utilization." Mr. Richard E. Bennett Applied Ecology Section, Sustenance Branch, Life Support Systems Laboratory of the Aerospace Medical Laboratory, served as contract monitor.

Contractual work began 15 December 1958, with a literature survey of the chemical constituents of human feces. Isolation and chemical and microbiological analyses of a fecal protein fraction were carried out from March 1959 to February 1960. Fecal minerals and trace elements were investigated from February 1960 to February 1961. A preliminary study of fecal fatty acids was made from January 1961 to 15 March 1961.

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ABSTRACT

Human fecal constituents were investigated for possible use in waste recovery systems aboard a space vehicle during a journey of long duration (1-3 months). In an attempt to investigate possible methods for recovering these constituents, it was necessary to first isolate them and then to evaluate their potential usefulness for waste recovery systems.

A review of the literature indicated that there is very incomplete knowledge of human fecal components, that detailed knowledge of the chemical nature of only a small proportion of fecal components exists, and that these have rarely been isolated in any quantity. However, protein, minerals and trace elements present in feces are possibly useful materials.

Investigation indicated that use of fecal protein or its component amino acids as nutrients for man would require supplementation of the diet. A number of minerals were isolated from feces. If found to have nutritional value, feces could possibly serve as an available source of their supply.

Insufficient information (e.g., authentic space diets and identification of useful fecal components) exists to compare or recommend feasible methods for recovery of usable materials from feces in space vehicles.

PUBLICATION REVIEW

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A STUDY OF POSSIBLE METHODS FOR RECOVERY OF USABLE MATERIALS FROM WASTE

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SECTION I. - INTRODUCTION

An investigation of possible methods of recovering usable materials from fecal material aboard a space vehicle has been carried out. It was initiated with a critical review of the literature for possible methods of recovering usable products from fecal matter with careful consideration being given to methods for recovery of materials potentially usable as nutrients for algae or for human consumption.

The literature review was carried out with particular attention being given to obtaining as complete knowledge as possible of the known components of human feces and of methods by which these components might be isolated. It was found that complete knowledge of the fecal components was not available. Only approximately 32% of fecal dry matter is identified in any way and 16% of the total feces is completely unaccounted for. It was also found that detailed knowledge of the chemical nature of only a small proportion of fecal components exists, and that these have rarely been isolated in any quantity from feces. Since exact knowledge of the chemical structure, purity and nutritional value of any substance which is to be used in a closed ecological system as a nutrient for men or algae is required, it was necessary to critically review available knowledge and to make a practical and somewhat empirical choice of components which might be most useful during space travel.

Such a choice would appear to depend upon the quantity of the particular component available, its ease of separation from feces and its usefulness once isolated. In view of the nature of feces, the dependence of their composition on diet and the fact that the diet of space travelers is unknown, it was immediately recognized that knowledge of the identity of fecal components was probably of greater importance than was the absolute quantity of any one component found to be present in feces resulting from a normal diet. The yield in which fecal components were obtained during the investigation could thus not necessarily be assumed to be attainable from feces resulting from a space traveler's diet. This limitation to the overall value of the investigation appeared to be unavoidable.

Judgement of the usefulness of fecal components to men or algae in a closed ecological system during space flight was also very difficult to achieve. It is conceivable that a substance which has great use in daily living on earth might have little or no use in a closed ecological system. In the absence of the knowledge required to evaluate the potential usefulness of fecal components under conditions of space flight, it was concluded that such judgements must be based on standards of life as it is known on earth.

On the basis of these considerations and a critical review of the literature protein, minerals and trace elements appeared to offer the most practical and profitable areas for investigation. Therefore the identity and quantity of these substances in the human adult male feces have been investigated and a general evaluation of their nutritive value has been made. A preliminary study of the fatty acids of fecal lipid was also carried out.

In view of the exploratory nature of this work it was necessary to determine what analytical technique best applied to the problem as the work progressed. Insufficient knowledge exists to allow recommendation of methods which would allow isolation of materials usable in space vehicles.

SECTION II. - LITERATURE REVIEW

1. Chemical Constituents of Human Feces

Since the ultimate purpose of this investigation was to obtain usable substances in reasonable quantities from feces, it was of first importance to determine what the chemical constituents of feces are and in what quantities they are present.

Feces are generally known (19, 5, 2) to contain indigestible food residues (e.g., cellulose), small quantities of undigested and unabsorbed foodstuffs, remains of mucosal cells, digestive fluids, bacteria and unabsorbed products of bacterial activity. Additional substances known to be present are indole, skatole, hydrogen sulfide, methylmercaptan (all odorous materials), methane, hydrogen, carbon dioxide, ammonia, proteoses, peptones, peptides, fats, minerals and trace elements, and vitamins.

The relative importance of the above substances as potentially usable materials which might be obtained from feces during space travel depends fundamentally upon the quantity of each found to be present (30). The composition and amount of feces vary (30) depending upon the composition of the diet and on the physical condition of the individual from whom they are obtained. The quantitative composition of human feces is thus reported in the literature as average normal values. These values may be considered to be a measure of the theoretical availability of substances which might subsequently be converted to usable products.

It has been assumed throughout this investigation that the gross bulk as well as the content of human feces will be the same under conditions of space travel as under conditions of the normal well-balanced diet of the present day. This assumption may not be valid in view of the probability that food used during space travel may be in concentrated form and differ in bulk, if not in other respects, when compared with conventional diets. However, for the purposes of this investigation there is no alternative to this assumption. The work must be carried out on the basis of current knowledge and with readily available starting material.

A resume of the major chemical components of human feces is given in Table I (5). The quantities given represent the average production of one man in 24 hrs. and are reported in terms of per cent of total fecal material. It is noteworthy that only approximately 84% of the total feces is accounted for and only 32% of the dry matter is identified. Substances listed in Table I which appear to provide a source of materials potentially suitable for use by men or algae during space travel are fat, carbohydrate, protein, water, vitamins and minerals.

In order to obtain a conception of the theoretical availability of such substances, the information in Table I has been used to determine the approximate quantity of each which should be present in feces produced by 3 to 20 men during each 24 hr. of a space flight. These figures are given in Table II. It is seen that any methods devised for the separation of useful materials from feces must be adequate for processing from 450 gm. (3 men) to 3000 gm. (20 men) of material per 24-hr. day. From these quantities approximately 81 gm. and 540 gm. respectively of dehydrated feces could be obtained for further processing into possibly useful substances.

The use of fecal components as possible nutrients for algae would appear to depend primarily upon their possible use as sources of nitrogen, minerals and trace elements. Since the presence of algae in a space ship is proposed primarily for the conversion of expired carbon dioxide to oxygen, it seems likely that the use of fecal components as carbon sources would be of little importance. In fact, in order to be considered as a possible algal nutrient, any substance must be demonstrated to contribute to the efficiency of this conversion. On this basis a substance which was "used" by the algae but which caused a decrease in the carbon dioxide to oxygen conversion must be considered harmful to the ecological system within the space ship, and could not be considered as a useful nutrient.

Problems involved in the use of algae for the conversion of carbon dioxide to oxygen in a closed ecological system have been studied extensively by other workers (3, 18, 21). On the basis of their work it appears that the use of fecal components as algal nutrients would require rather exact knowledge of the identity of the components and extensive treatment of feces to produce the components in adequately pure form.

In the event that algae were used as part of the waste disposal system in a space ship it is probable that whole, unprocessed feces would be treated with algae. This possibility has been studied by others (21, 29) and has not been considered as part of this investigation.

In view of the possible use of fecal components as nutrients for men, it is of interest to consider their potential caloric value. The caloric value of feces has been reported (41) to be 70 to 140 calories per man per day. This means that the maximum caloric value of 3000 gm. of feces (20 men), assuming complete conversion to useful substances, would be only 1400 to 2800 calories! This is an extremely small yield.

In view of the small caloric yield which theoretically can be obtained by complete isolation of fecal components and by their complete conversion to useful substances, and in view of the complex problems involved in accomplishing such isolations and conversions, it is extremely important that a practical choice of the components to be isolated and of the methods to be used be made. Substances which appear to provide a possible source of nutrient material are fat, carbohydrate, protein, water, vitamins and minerals. A discussion of the composition of these fecal components and of some of the possible problems associated with their isolation, separation, and conversion to usable materials follows.

A. Water

On the basis of quantity (approximately 66%) and relative ease of isolation, water may be the most important component of feces. Because of the probable need for conservation of all usable materials during space flight, the water present in feces must be isolated and purified. Problems associated with the recovery of water from human waste have been considered by other workers (21, 42) and therefore have not been a major concern of this project. Removal of water would, however, probably be an integral operation in any method proposed for the isolation and conversion of fecal components to useful substances.

B. Lipid

Although lipid comprises approximately 3% of the feces and is the most abundant of the known organic components, it does not appear to be a promising source of usable substances. Approximately 30% of the lipid is unsaponifiable material which consists mainly of sterols such as cholesterol and its reduction products, β-coprosterol and β-cholestanol (12, 8). Neither of the latter two compounds is absorbed by the intestine and therefore each would require conversion to a usable form. The saponifiable fraction of fecal fat is made up (31) of approximately 7.3% neutral fat, 5.6% free fatty acids, and 4.6% soaps. Other fatty substances present in small quantity in human feces are certain bile acids, cholic acid, deoxycholic acid and lithocholic acid (28).

The nature of the free and bound fatty acids in the fecal lipid of human beings with normal fat absorption has been determined (23). The fatty acid composition and the identity of the individual fecal acids is given in Table III. It is seen that palmitic acid (16:0, 45.2%) is the major acid component followed by stearic (18:0, 22.3%), oleic (18:1, 8.7%), myristic (14:0, 6.6%), isomers of oleic (18:1, 5.0%), lauric (12:0, 3.3%) and linoleic (18:2, 2.0%) acids. Worthy of note is the fact that the isomers of oleic acid contain the double bond at the 4, 5, 6, 7, 8, 9, 10, 11 and 12 positions, and that about half of these isomers possess the trans configuration about the double bond. Of equal interest is the presence of 10-hydroxystearic acid.

Although knowledge of the composition of fecal fatty acids is of great physiological and biochemical interest, it is difficult to conceive of them or of the other lipid components as sources of useful nutrients. Isolation of these substances and their conversion to useful forms by methods simple enough to be carried out under conditions of space travel seems unlikely.

C. Carbohydrate

Feces contain (38) minimal quantities of carbohydrate since only a very small portion of that ingested is not absorbed. Any carbohydrate which is not absorbed is generally changed by intestinal bacteria into absorbable substances. Therefore the carbohydrate present in feces consists of undigestible cellulose and vegetable fibers (pentosans) and serves as a source of bulk or roughage. In man the nutritional significance of dietary cellulose is considered to be negligible.

No definite quantity of cellulose or pentosan has been reported to be present in feces. This is presumably due to the variability of the amount of cellulosic material in the diet. It is thus difficult, without knowledge of diets to be used, to predict the importance of fecal cellulose as a source of usable compounds during space travel. However, fecal cellulose, if present, is a potential source of glucose. The glucose could be obtained by either chemical or enzymatic hydrolysis of cellulose.

D. Protein

Protein is usually not present (15, 5) in the feces of healthy adult humans. However, approximately 1.5 gm. of nitrogen is excreted (5) (see Table I) in the feces each day (24 hrs.). About half of this nitrogen is believed to be of bacterial origin with the remainder representing small quantities of unabsorbed intestinal secretions

and digestive fluids, mucus, food residues, intestinal enzymes and the like (8). Bacteria, mostly nonpathogenic, compose about one-third of the dry weight of the feces under average dietary conditions (8). Escherichia coli, the most common bacteria found in man, is located mainly in the large intestine (39). Porter (32) reports that the chemical composition of microorganisms includes a variety of proteins such as glycoproteins, lecithoproteins, phosphoproteins, and nucleoproteins. It is thus quite certain that protein is present, although no definite total quantity has been reported in the literature.

Nitrogenous compounds other than protein which are present in feces are indole, skatole, histamine and tyramine. These make up only a very small amount of the total fecal nitrogen. Other nitrogenous substances which must be present are bacterial nucleic acids.

The quantity of amino acids excreted in the protein of feces has been observed to remain comparatively constant in spite of relatively large changes in the individual amino acid content and pattern of the diet (35). The results of the microbiological determination of eight amino acids in feces which had been homogenized and hydrolyzed by autoclaving with 4 N hydrochloric acid for 10 hrs. at 120°C. are shown in Table IV. The quantity of feces examined was a sample collected during 6 days. Their total nitrogen content was not determined.

Since the quantities of amino acids shown in Table IV (0.5 to 2.9 gm.) were determined microbiologically on an approximately 900 gm. sample of feces homogenate, they must be considered to be the maximum quantities present. They cannot be considered to be quantities which could be obtained in useful form after chemical isolation and purification.

The practical accomplishment of the separation of protein from the complex mixture present in feces, hydrolysis of the protein, isolation and purification of the component amino acids probably cannot be expected to be carried out in large yield. However, the protein or its hydrolyzate might possibly be used as nutrients for intermediary conversion agents such as algae. In any case, the identity and amount of each acid present must be determined in order to ascertain the amino acid pattern. It is not known whether the actual isolation is dispensable.

Albanese, Davis, Lun and Smetak (1) have reported the isolation of protein from infant's feces by isoelectric precipitation at pH 6.0 from an alkaline ethanol extract of an aqueous homogenate. They found that 22.4% of the total fecal nitrogen was present in the protein and that fluctuations from this value were spontaneous rather than related to the diet.

Purification of the protein by electrodialysis, and granulation in acetone solutions was followed by hydrolysis in 6 N hydrochloric acid for 6 hrs. Analysis of the resulting hydrolyzate showed the presence of the quantities of amino acids presented in Table V. These results were obtained from infants which had been fed an evaporated milk diet. Since only very slight differences were noted when other diets were used, these results are considered representative of infant fecal protein.

It is recognized that the work of Albanese (1) and coworkers might have little relationship to the nature of adult fecal protein. Their results are, however, of interest.

On the basis of the above information, isolation of fecal protein appeared to be feasible, and methods for its isolation, purification, and conversion to useful substances were worthy of practical investigation.

E. Vitamins

The following quantities of B Vitamins have been detected (40) in feces collected over a period of 24 hrs.

p-Aminobenzoic Acid	0.246 mg.
Biotin	0.133
Folic Acid	0.304
Pantothenic Acid	2.20
Pyridoxin	0.38
Nicotinic Acid	3.63
Thiamine	0.548
Riboflavin	1.029

Small quantities of Vitamins C, K, E and A (2) are also found. As indicated in Table I, the vitamins account on the average for about 0.01% of the feces. The greater part of this fecal vitamin content is believed to be of bacterial origin. Thiamine is in the form of cocarboxylase (40) while little is known of the chemical form in which the others exist.

In view of the lack of knowledge of the exact nature of the vitamins as they exist in feces, readily available methods for their isolation from feces do not exist. For this reason and because the total quantity present is so small, the vitamins do not appear to be a practical source of materials which might be used during space flight.

F. Minerals and Trace Elements

Minerals and trace elements are excreted in feces in small but widely varying quantities. Certain of those which are found in relatively large amounts are given below (5).

	Weight excreted in 24 hrs.
Sodium	0.12 gm.
Potassium	0.47
Calcium	0.64
Magnesium	0.20
Chloride	0.09
Phosphorus	0.51
Sulfur	0.13

Many other substances such as arsenic, copper, iron, lead, manganese, nickel and zinc are present (2) in trace quantities (see Table VI).

As a group, the minerals and trace elements are potentially useful substances. Certain of them such as calcium and phosphorus are present in relatively large quantities. However, as seen in Table VI the quantities reported to be present vary greatly.

This variation is not surprising since it is well known (19, 5, 2, 14) that the mineral content of feces is directly influenced by variable dietary, metabolic, and gastrointestinal factors. A study (2, 11) of the pathways of mineral metabolism showed that relatively few minerals are more than 70% absorbed by man and are thus excreted in the urine and feces.

It is difficult to predict the potential usefulness of minerals and trace elements as nutrients for man during extended space travel. Man's minimum daily requirements for these substances is largely unknown. This is particularly true in the case of the trace elements since little is known of the quantitative levels necessary to assure their physiological functions, or of their desirable dietary levels in man. It is, however, possible that minerals and trace elements could be useful to algae during space flight.

Since the mineral content of feces is known to be so variable, knowledge of the identity of the major mineral components of human feces is of much greater importance to the current investigation than is a quantitative determination of the absolute amount of each metal present. Each individual fecal sample investigated could be expected to differ in the exact quantity of each mineral or trace element present, but the general composition of different fecal samples should be roughly similar.

G. Bile Pigments

Although the bile pigments have no obvious nutritional potential as useful substances, they are present in feces in approximately 0.1% (5) (see Table I). They have a tetrapyrrole structure and are responsible for the color of feces. From the point of view of the project under investigation, they appear to be of little use.

2. Conclusions

On the basis of the above general review, it was concluded that the following components of human feces were possible sources of substances which might be converted to forms having value during space travel: water, fecal protein, and minerals and trace elements. Consideration of methods required for the isolation of these components led to the conclusion that two general schemes could result in rough separation and isolation, in the one case, of water, lipid, and protein; and in the other case, in separation and isolation of water and fecal ash which contained minerals and trace elements. Both separation schemes involved initial removal of water from feces by lyophilization. The resulting dehydrated feces could then be stored or treated in any manner desired.

The remainder of this report describes investigations carried out on the protein minerals and trace elements present in feces. Since lipid is present in feces in relatively large amounts (3%) and since knowledge of fatty acids derived from lipid may be relatively easily obtained by gas chromatography, a preliminary investigation of fecal fatty acids is also described.

SECTION III. - FECAL PROTEIN

EXPERIMENTAL PROCEDURES

1. Lyophilization of Feces Homogenate

A. Preparation of the Sample

Normal human male adult feces was processed either immediately after collection or within an hour (stored at about 4°C.). The feces (50 gm.) was diluted with water (1:1) and thoroughly homogenized in a Waring blendor for 2 min. Acidification of the feces homogenates with approximately 5-7 ml. of concentrated hydrochloric acid to pH 1.0 as determined by Hydrion pH paper was carried out to lessen the odor, kill bacteria and facilitate lyophilization.

B. Procedure

Acidified feces homogenate (80 gm.) was placed in a 2-l. lyophilization flask having a 24/40 ground-glass joint, completely frozen by swirling in an ethanol-dry ice bath, then immediately attached to the lyophilization trap and evacuated. Lyophilization required approximately 8 hrs. The dried product, a brown granular solid, was obtained in 16.1% yield (Table VII) and its nitrogen content determined. A 40 x 600 mm. chromatographic tube filled with coarse activated charcoal and connected between the lyophilization trap outlet and the vacuum pump adsorbed all odor.

2. Removal of Lipids

Feces lyophilizate (approximately 2.4 gm.) was continuously extracted with 80 ml. of solvent in a Soxhlet apparatus for 16 hrs. After extraction, the thimbles were dried to constant weight. The solvent was evaporated on a steam bath and the residue dried in a vacuum desiccator until constant weight was obtained.

Extraction was carried out by three different solvent systems and the results compared. These systems were: (1) anhydrous, peroxide-free, ethyl ether for 16 hrs. (2) absolute ethanol for 16 hrs. and, (3) anhydrous ethyl ether for 8 hrs. followed by absolute ethanol for 8 hrs.

Nitrogen determinations were carried out on both the lipid-free residues and the lipid extracts. Yields of lipid-free material obtained and the per cent nitrogen present are given in Table VIII.

3. Isolation of Protein Fraction from Solvent Extracted Feces Homogenates

The procedure was based on the method of Koch and Hanke (24) and is outlined in Figure 1. The lipid-free sample (1gm.) was thoroughly mixed with 100 ml. of 2 N sodium hydroxide in a 250 ml. centrifuge bottle, let stand 10 min. and centrifuged

for 20 min. at 2200 r.p.m. The brown supernatant liquid was decanted into a 250 ml. volumetric flask. Sodium hydroxide (2 N, 50 ml.) was added to the residue in the centrifuge bottle, the resulting suspension was mixed with a glass rod and centrifuged for 30 min. The resulting supernatant liquid and the supernatant liquids from two more washings with 40-ml. portions of 2 N sodium hydroxide were combined with the original liquid and brought to a final volume of 250 ml. with 2 N sodium hydroxide solution (Solution 1, Figure 1). The alkali insoluble residue (Residue 1, Figure 1) was refrigerated.

A. Precipitation of Protein Fraction

Solution 1 (200 ml.) was placed in a 300 ml. Erlenmeyer flask and adjusted to pH 3.8 (Hydrion pH paper) with concentrated hydrochloric acid. The resulting suspension was divided between two 250 ml. centrifuge bottles and centrifuged for 15 min. at 2200 r.p.m. The insoluble residues were transferred to a single centrifuge bottle, and were washed twice by suspension in 50-ml. portions of Walpole's acetate buffer pH 3.80 and by centrifugation. The protein fraction thus obtained (Residue 2, Figure 1) was stored under refrigeration. It gave a negative molybdate test for phosphate. The combined supernatant liquids were brought to a final volume of 500 ml. with the buffer (Solution 2, Figure 1).

Nitrogen determinations were made on Residues 1 and 2, Solutions 1 and 2 to evaluate the efficiency of the separation. The yields of these isolates as well as their nitrogen content are presented in Table IX. A summary of the fractionation of dehydrated feces is given in Table X.

4. Investigation of Fecal Protein

A. Hydrolysis of Crude Protein Fraction

Prior to hydrolysis the Molisch test was performed on the crude protein fraction to determine if any carbohydrate was present. The presence of carbohydrate in protein may cause from negligible to very large destruction of amino acids during hydrolysis (4). In all cases the presence of carbohydrate was indicated.

Hydrolysis of the crude protein fraction was carried out by treatment of 1.5 gm. of the protein with 25 ml. of 2 N hydrochloric acid and autoclaving the resulting mixture for 16 hrs. at 121°C. and 15 p.s.i.

For purposes of microbiological and paper chromatographic analysis, the hydrolyzate was treated with 4 ml. of 2.5 N sodium acetate solution, filtered and diluted to 100 ml. in a volumetric flask. It was preserved with toluene and stored at approximately 3°C.

For purposes of quantitative column chromatographic analysis, the fecal protein hydrolyzate was thoroughly mixed with 100 mg. of Darco G 60 and then filtered by suction through a layer (1/8 in.) of filtercel on highly retentive, acid-washed, blue ribbon filter paper to remove the large quantity of humin present.

Samples of the hydrolyzate before and after Darco treatment and of the filter-cake were analyzed for nitrogen by the micro-kjeldahl method. The filtered hydrolyzate was examined for the presence of halogen (chloride) by sodium fusion (36) and by

the Beilstein test (36). Chloride was removed from the hydrolyzate by ion-exchange chromatography on a 0.85 x 7.75 in. column of Dowex, 50W-X8, cation exchange resin, mesh 200-400, in the acid form.

Elution with distilled water was carried out until a negative silver nitrate test for chloride was obtained. The amino acids were eluted from the column by 300 ml. of 2 N ammonium hydroxide. Lyophilization of the desalted protein hydrolyzate (125 ml.) was carried out in a 2-l. flask in the manner described previously. The solid residue (0.5 gm.) thus obtained was light tan and almost odorless. It contained no chloride ion.

B. Paper Chromatographic Analysis of Fecal Protein Hydrolyzates

A schematic representation of the chromatogram obtained by separation of the hydrolyzate in a n-butanol : acetic acid : water (250:60:250 V/V) system is given in Figure 2. It appears that the fecal protein hydrolyzate (UNK) is separated into at least 10 amino acids. Comparison of the distance moved by each spot in the fecal hydrolyzate (UNK) with the acids present in the known mixture (MIX), and with the individual acids, shows that spot 1 may contain lysine (LYS) and histidine (HIS), spot 2 may contain arginine, spots 3, 4, 5, 6 and 7 are unidentified, spot 8 may be a mixture of methionine (MET) and valine (VAL), spot 9 may be phenylalanine and spot 10 probably contains leucine and iso-leucine.

The probable presence of a number of other amino acids is shown by the chromatogram (see Figure 3) obtained by separation of the hydrolyzate (UNK) with a phenol (88%) : 2-propanol : water (5:1:1, V/V) system. In this case it appears that spot 1 may contain aspartic acid (ASP), spot 2 may contain glutamic acid (GLU), spot 3 may contain serine (SER), spot 4 may contain glycine (GLY), spot 5 may contain threonine (THR), spot 6 may contain alanine (ALA), while spots 7 and 8 are unknown.

C. Microbiological Analysis

The presence of lysine, leucine, iso-leucine, phenylalanine, methionine, arginine, valine, histidine and threonine was confirmed by microbiological assay of the fecal protein hydrolyzate. The results of these determinations are shown in Table XI. The presence of tyrosine, in addition to the above amino acids, was indicated. It was probable that tryptophan and cystine were not present.

D. Ion Exchange Chromatographic Analysis

Detailed quantitative knowledge of the composition of purified human fecal protein was obtained by means of a Beckman/Spinco Amino Acid Analyzer. The results of this analysis are given in Table XII. By this means the presence of all the amino acids indicated to be components of the protein by microbiological and paper chromatographic analyses was confirmed. In addition, proline, allo iso-leucine, and methionine sulfoxide were found to be present. No cystine or tryptophan was found. The absence of tryptophan was believed to be due to the acid hydrolysis conditions used rather than to its natural absence in the fecal protein. A schematic representation of the chromatogram of the amino acid components obtained by the Amino Acid Analyzer is given in Figure 4.

RESULTS AND DISCUSSION

1. Isolation of Fecal Protein

Fecal protein from normal human male adult feces has been isolated in about 75% purity and in reproducible yields of about 12% on a dry basis.

A. Lyophilization of Feces

Dehydration of feces was successfully carried out by lyophilization. The water thus removed was colorless and had the typical fecal odor. The remaining residue was a brown, easily handled, hygroscopic powder which also had the typical fecal odor. It contained approximately 4.5% moisture and 12.0% ash. A total of 347 gm. of feces was lyophilized producing an average yield of 16.1% (range 12.1 to 22.2%) of dried feces, (Table VII). This yield is in close agreement with that reported in the literature (5) (Table I). Dried feces were found to have an average nitrogen content of 6.0% (range 5.6 to 6.1%).

On the basis of these results, it appeared that a successful lyophilization of fecal matter can be easily carried out. There should be no major problem encountered in the lyophilization of larger amounts of feces homogenate. The use of this method has been suggested previously for the recovery of water from waste in space ships (42).

B. Lipid Removal from Dehydrated Feces

Lyophilization of the acidified feces probably effected a denaturing of the fecal protein (17). As a result, solvent extraction was expected to remove both free and bound proteins. The efficiency of several solvents or solvent mixtures was investigated. The results of solvent extraction (Table VIII) were found to be reproducible and revealed that ethanol extracted the largest amount of material. Ethanol-extracted residues were obtained in yields of 54.35%, 52.8% and 64.1% and they contained 9.42%, 9.49% and 9.49% nitrogen respectively. On the other hand, diethyl ether extraction gave the largest yields (88.14% and 83.3%) of extracted residues but these residues contained the least amount of nitrogen (7.08% and 7.6%). The "lipid free" products in all cases were light tan, odorless, less hygroscopic than the lyophilized samples and easily handled. They contained approximately 4.4% moisture and 12.2% ash.

The results from the lipid separations were calculated on a yield basis rather than on a per cent extracted basis, because of interest in the protein remaining after lipids had been extracted. However, calculations on a per cent extracted basis were found to be in close agreement with those on a yield basis.

C. Isolation of the Protein Fraction

The procedure for the isolation of the protein fraction from the lipid-free material was based on the method of Koch and Hanke (24) (Figure 1). The alkali insoluble fraction (R-1) was considered to be made up largely of cellulose and fibers whereas the supernatant liquid (solution I) was probably composed of nucleic acids,

salts, protein and other soluble substances. The protein fraction (R-2) was precipitated at its isoelectric point (pH 3.80) from Solution I. A negative test for phosphate indicated it to be free of nucleic acids. A nitrogen balance of the whole operation was carried out to determine possible experimental losses.

The fractionation procedure was performed twice on lipid-free materials obtained from the three solvent systems previously described. These separations are summarized in Table IX. More than 90% of the total nitrogen was recovered from fractionation I of the ethanol and ethanol-ether extracted materials. Only 83.8% of the total nitrogen was recovered from the ether extracted material of fractionation I. In fractionation 2, an average of about 80% recovery of the total nitrogen was realized. In all cases, the loss of nitrogen occurred during the precipitation of the protein fraction from solution I (Table IX). The losses of nitrogen incurred are not considered to be of significance for the following reasons. The yield of the protein fraction obtained from the lyophilized feces is approximately 12.3% for all three solvent systems used for fat removal. The nitrogen content of all the protein fractions from the three solvent systems is approximately 12.0%. Thus the solvent systems tested were equally effective in accomplishing the isolation of the protein fraction from dehydrated feces. Ethanol was selected as the most practical solvent to be used for the removal of lipids because it was easier to handle than ether, was not used in a solvent mixture and is nontoxic under conditions of use.

2. Investigation of Fecal Protein

A positive Molisch test was obtained on all crude protein fractions indicating the probable presence of carbohydrates. A negative result by this general test is good evidence of the absence of carbohydrates, but a positive test is merely an indication as to their probable presence. The presence of carbohydrates in protein can cause from negligible to very large destruction of the amino acids during subsequent hydrolysis. However, hydrolysis without further purification would, if successful, save much time. It was thus decided to proceed with the hydrolysis. If no major problems developed due to the presence of impurities, separation and analysis of the component amino acids would be carried out by ion exchange chromatography.

Acid hydrolysis of the crude protein fraction was carried out. Nitrogen determinations on the protein itself and on three hydrolyzates revealed a 97.4% average recovery of the total nitrogen in the hydrolyzate. Removal of humin and of inorganic salts from the protein hydrolyzate was accomplished. The resulting solution of amino acids was lyophilized. Nitrogen determinations revealed that the removal of humin, desalting and lyophilization resulted in an 89% recovery. This represents a 72.8% recovery of nitrogen based on the original crude protein fraction.

The purified amino acid lyophilizate was not at all hygroscopic but remained an easily handled off-white powder which possessed the typical protein hydrolyzate odor and contained no halide. On the basis of the above work, quantitative chromatographic analysis of the amino acid content of the purified hydrolyzate was warranted.

A. Paper Chromatographic Analysis of Amino Acids in Fecal Protein Hydrolyzates

A schematic representation of the actual chromatograms developed in n-butanol: acetic acid:water mixture is presented in Figure 2. It appears that the fecal

protein hydrolyzate (UNK) has been separated into at least 10 amino acids. Comparison of the R_f values of the known amino acid mixture (MIX), known amino acids and of the components of the hydrolyzate, indicated that spot 1 probably contained lysine (LYS) and histidine (HIS) and that spot 2 probably contained arginine (ARG). Spots 3, 4, 5, 6 and 7 were unknown while spot 8 probably contained methionine (MET) and valine (VAL), spot 9 probably contained phenylalanine (PHE) and spot 10 probably contained a mixture of iso-leucine (ISO) and leucine (LEU).

A diagram representing the actual chromatograms developed in 88% liquid phenol: 2-propanol:water mixture is presented in Figure 3. It appears that the fecal protein hydrolyzate (UNK) has been separated into at least eight amino acids. Comparison of R_f values revealed that spot 1 may contain aspartic acid (ASP), spot 2 may contain glutamic acid (GLU); spot 3 may contain serine (SER), spot 4 may contain glycine (GLY), spot 5 may contain threonine (THR), spot 6 may contain alanine (ALA) while spots 7 and 8 were unknown.

Data (Table XI) from microbiological assays of component amino acids in the fecal protein verified the presence of lysine, leucine, iso-leucine, phenylalanine, methionine, arginine, valine, histidine and threonine.

B. Ion Exchange Chromatography of the Purified Protein Hydrolyzate

A sample of purified amino acid hydrolyzate was examined by means of a Beckman/ Spinco Amino Acid Analyzer. The results obtained from this analysis (Figure 4, Table XII) confirmed the presence of arginine, aspartic acid, threonine, serine, proline, glutamic acid, glycine, alanine, valine, methionine, iso-leucine, leucine, tyrosine, and phenylalanine. In addition, it was found that the hydrolyzate contained lysine, histidine, arginine, methionine sulfoxide and allo iso-leucine.

3. An Evaluation of the Nutritional Value of Human Fecal Protein

A review of the composition of fecal protein (Table XII) shows that 22.6% of the crude fecal protein is made up of essential amino acids. Total amino acids make up 47.0% of the crude fecal protein. On the other hand, based on the purified fecal protein hydrolyzate, 48.0% of the total fecal amino acids are essential acids, while the total fecal acids represent 82% of the purified hydrolyzate. As the result of the ion exchange chromatographic analysis, 89.9% of the fecal protein hydrolyzate was accounted for.

An evaluation of the nutritional value of the purified fecal protein was made by comparison of its composition with that of egg albumin, a protein of high nutritional value (Table XIII). Egg albumin is the most complete protein available in our food supply because it contains a high proportion (48.1%) of essential acids and an excellent balance of essential amino acids. Fecal protein is deficient to egg albumin in both respects. It contains only 22.6% of essential amino acids and its overall essential amino acid balance is inferior (Table XIII).

It is evident that use of fecal protein or its component amino acids as nutrients for man would require supplementation of the diet.

SECTION IV. - MINERALS AND TRACE ELEMENTS

EXPERIMENTAL PROCEDURES

1. Lyophilization of Feces Homogenate

A. Preparation of the Sample

Normal human adult male feces were lyophilized in the manner described above. The product, a brown granular solid, obtained in 25.6% average yield (see Table XIV) was ground in a mortar and stored in a desiccator. This yield differed from that obtained (16.1%) during work on fecal protein. Since the feces in each case were obtained from different persons, the difference is not unusual.

2. Preparation of a Composite Fecal Sample

Approximately 10 gm. from four different (II, III_1, IV_a, and V_a, Table XIV) preparations of lyophilized feces were combined, ground in a mortar, mixed thoroughly and stored. This composite sample (35.1 gm.) was the source of all subsequent investigations.

3. Moisture Determination of Lyophilized Feces

Seven portions (about 0.50 gm. each) of the ground, composite, lyophilized feces sample were accurately weighed in dry porcelain coors crucibles (30 ml.) and dried in an electric oven, at 100-110°C. for 22 to 24 hrs., cooled in a desiccator for 30 to 60 min. and weighed. The yields of dried feces thus obtained (average 86.7%) are given in Table XV.

4. Isolation of Fecal Ash

Each moisture-free sample was ignited at red heat over a bunsen burner, then over a Meker burner until a near-white ash was obtained, and finally held overnight (22 to 24 hrs.) in a muffle oven at 600°C. Each crucible was cooled in a desiccator and weighed. The yields of ash obtained (average 14.1%) are given in Table XV. The average yield of ash from raw feces is 3.1%.

5. Separation of Manganese, Cobalt, Copper and Iron by Anion Exchange Chromatography

A. Preparation of the Column

A 10-ml. Kimax burette (diameter 0.8 cm.) was packed with 24.5 cm. of Dowex 1-X8 (Cl^-), 100 to 200 mesh, anion exchange resin. The column was washed with 100 to 150 ml. of distilled water and 10 ml. of 12 M hydrochloric acid before addition of a sample.

B. Preparation of Sample

a. Known Mixture

Standard solutions were used to prepare the known mixture. An appropriate volume for the p.p.m. desired (see Table XVI) was pipetted from the standard solution of each metal into a 125 ml. Erlenmeyer flask. This solution was carefully evaporated to dryness over an open flame and the residue dissolved in 1 ml. of 12 M hydrochloric acid.

b. Fecal Ash

Samples number 1, 6, and 7 (see Table XV) were treated with 1.5 ml. of 12 M hydrochloric acid and the combined solution evaporated carefully to dryness over an open flame. The residue in each case was redissolved in 1 ml. of 12 M hydrochloric acid and the resulting clear yellow-green solution added to the column.

C. Separation by Anion Exchange Chromatography

The method of Kraus and Moore (25) was used for the separation of the anionic chloride complexes of manganese (II), cobalt (II), copper (II) and iron (III). The solution (1 ml.) of fecal ash or of the known mixture of metals was added to the column. The flask was rinsed with two 1 ml. portions of 12 M hydrochloric acid, which were added successively to the column. Cations were removed from the column by elution with 7 ml. 12 M HCl at a flow rate of 0.3 to 0.35 ml./min. Successive elution at room temperature with 15 ml. of 6 M hydrochloric acid, 15 ml. of 4 M hydrochloric acid, 15 ml. of 2.5 M hydrochloric acid, and 20 ml. of 0.5 M hydrochloric acid, at a slightly increasing flow rate of 0.35 to 0.7 ml./min., brought about separation of manganese (II), cobalt (II), copper (II) and iron (III). Elution diagrams of the separation of these ions from the known mixture and from fecal ash are given in Figures 5 and 8 respectively.

The effluent was collected by means of a "Rinco" automatic fraction collector in 1-ml. fractions which were then analyzed for their respective metal content by means of flame spectrophotometry. The initial 10 ml. of effluent containing the cationic portion of the samples was collected and set aside for further examination.

6. Separation of Lithium, Sodium, Potassium, Magnesium, Nickel, Calcium, and Strontium by Cation Exchange Chromatography

A. Preparation of the Column

A burette (diameter 1.2 cm.) was packed to a height of 11.6 cm. with Dowex 50W-X8 (H^+), 200 to 400 mesh, cation exchange resin. The resulting column was washed with 100 to 150 ml. of distilled water and 30 ml. of 0.15 M hydrochloric acid before addition of a sample.

B. Preparation of Cation Fraction for Separation

The first 10 ml. of effluent (eluted by 12 M hydrochloric acid) from the anion exchange chromatography were combined and rinsed into a 125 ml. Erlenmeyer flask by

means of 1 to 2 ml. of 12 M hydrochloric acid and 2 to 3 ml. of distilled water. This solution was gently evaporated to dryness over an open flame. The resulting white residue was dissolved in 3 ml. of 0.15 M hydrochloric acid.

C. Separation of Cations by Cation Exchange Chromatography

The method of Cohn and Kohn (9) was modified and used for the separation of lithium (I), sodium (I), potassium (I), magnesium (II), nickel (II), calcium (II), and strontium (II). The sample (3 ml.) obtained either from fecal ash or from the known mixture was added to the column. The flask was rinsed with two 3-ml. portions of 0.15 M hydrochloric acid which were added to the column. The metals were removed from the column by elution with 400 ml. 0.15 M hydrochloric acid, 400 ml. 0.5 M hydrochloric acid and 400 ml. 1.0 M hydrochloric acid, or by linear gradient elution. In the latter case elution was carried out from a beaker which contained 550 ml. of 1.5 M hydrochloric acid, connected by means of a small glass tube to another similar beaker containing 550 ml. of 0.15 M hydrochloric acid. The latter was connected to the column by a glass tube so that the initial concentration of eluant was 0.15 M. Continuous mixing of the eluant was accomplished with a magnetic stirrer. The flow rate was 0.3 to 0.6 ml./min. The metals were eluted in the following order: lithium (I), sodium (I), potassium (I), magnesium (II), nickel (II), calcium (II), and strontium (II). Separation accomplished with the known sample by stepwise and gradient elution methods are shown in Figures 7 and 8. Separation of the cations of fecal ash by gradient elution is shown in Figure 9. Effluents from the known sample were collected in 5 ml. portions by means of the automatic fraction collector. Effluents from fecal ash were collected in 10 ml. portions in the same manner. The metal content of all fractions was determined by flame spectrophotometry.

7. Flame Spectrophotometric Determination of Certain Minerals Derived from Feces

A. Apparatus

A Beckman Model DU Spectrophotometer fitted with a Flame Photometer Attachment No. 9200, a photomultiplier attachment (No. 4300) and an oxygen-hydrogen atomizer-burner (No. 4020) was used for the quantitative analysis of certain minerals in fecal ash. The instrument was set up, adjusted, and its reproducibility checked in accordance with the Beckman Instruction Manual.

B. Preparation of Standard Solutions

Standard stock solutions (1-l.) containing 1000 p.p.m. of each of the eleven minerals selected for determination were prepared from reagent grade chloride salts and distilled water. Serial dilutions of 400-1000 ml. down to 1 p.p.m. covering the range in which the metal was found were made as required. All standard solutions were kept in polyethylene bottles, and prepared fresh about every month.

C. Analytical procedure

a. The following instrumental conditions were used during this investigation:

Sensitivity control	Maximum
Selector switch, position	0.1

Phototube resistor	22 megohms
Zero suppression	3
Sensitivity (photomultiplier)	Full
Oxygen pressure	10 p.s.i.

b. The wave length, slit width and hydrogen pressure used for determination of each metal were the following:

Metal	Wave-length (mμ)	Slit Width (mm.)	Reference Concentration of Standards (p.p.m.)	Hydrogen Pressure (p.s.i.)
Lithium	670.8	0.028	25	4
Sodium	589.3	0.007	250	4
Potassium	768.0	0.260	500	5
Magnesium	371.0	0.026	250	5
Nickel	341.5	0.050	25	5
Calcium	554.0	0.009	250	4
Strontium	460.5	0.015	25	4
Manganese	403.6	0.018	25	5
Cobalt	341.1	0.070	25	5
Copper	327.6	0.032	25	5
Iron	372.0	0.03	250	5

The flame size was adjusted to give the best readings for each metal.

c. A series of standard calibration curves covering the concentration range found in fecal ash were prepared for each metal. In each case emission intensity was plotted on the ordinate and concentration (p.p.m.) on the abscissa of regular graph paper. Background readings for each metal were determined with distilled water.

Since experimental conditions must be constant in order for results to be valid, calibration curves were prepared immediately prior to flame spectrophotometric analysis of chromatographic fractions. Comparison of emission intensity for each metal in fractions obtained from the separation of the known mixture and samples of fecal ash with the calibration curve for that metal indicated the concentration present. Background readings for each metal (anion exchange) were determined on the hydrochloric acid solution used to elute the metal. In the case of linear gradient elution background readings were determined on fractions collected immediately before and after elution of the particular metal.

Each fraction and each standard solution was analyzed at least twice or as many times as required to obtain consistent readings. A number of brief readings were found to yield the best results. The burner capillary was rinsed with distilled water between readings of the fractions. The null meter was checked after each reading and brought to zero if necessary.

d. Results of the flame spectrophotometric determination of ten metals in a known mixture are presented in Table XVI. Results of analysis of three samples of fecal ash for the same ten metals and lithium are presented in Table XVII.

RESULTS AND DISCUSSION

1. Isolation and Determination of Fecal Minerals

Methods for the isolation, identification and quantitative determination of fecal minerals were suggested by a review of investigations of minerals in other biological materials (20, 6, 10, 11). The wide use of flame spectrophotometry for analysis of such mixtures either before or after separation of the minerals by ion exchange chromatography indicated that this was the method of choice. A review of the literature concerned with ion exchange separation of metals (cations) showed that the fecal minerals should be successfully separated by this technique. The availability of several general texts (27, 7, 13) which reviewed the method aided in this review. Of particular interest was the work of Kraus (25, 26) and co-workers who studied the separation of alkali metals and alkaline earths by cation exchange chromatography and separation of divalent transition metals by anion exchange chromatography.

On the basis of their work it was concluded that separation and determination of ten of the minerals reported (Tables I, VI) to be present in feces could be carried out by ion exchange chromatography and flame photometry. These minerals were sodium, potassium, magnesium, calcium, strontium, nickel, manganese, cobalt, copper and iron.

Separation of these substances should be possible by a combination of anion and cation exchange chromatography in which manganese, cobalt, copper and iron would be separated and removed as anionic chloride complexes (MCl_3^- and $MCl_4^=$) from cations, and the cations would in turn be separated on a cation exchange resin.

It was anticipated that samples of fecal minerals, suitable for analysis by the above methods, could be obtained by lyophilization of feces and ignition (ashing) of the resulting dry product.

A. Lyophilization, Dehydration and Ashing of Feces

Lyophilization proved to be a practical and successful method for preparation of dehydrated feces. The yield of lyophilized feces obtained was 25.6% (Table XIV). The yield differed from that obtained (16.1%) during work on fecal protein. Since the feces in each case were obtained from different persons, the difference is not unusual. The product was a brown, easily handled solid which contained approximately 13% moisture (Table XV). Removal of moisture yielded dried feces in 22.1% yield. They possessed a typical fecal odor, as did the water which was removed. A total of 293 gm. of raw feces were processed yielding 74.8 gm. of lyophilized material. A portion (35.1 gm.) of the total product was combined and mixed thoroughly to provide a homogeneous sample from which aliquots could be taken for further investigation. This was of fundamental importance if significant analyses for metals were to be carried out since considerable daily variation in composition of feces may be expected.

The fact that a truly homogeneous sample had been obtained was illustrated by the similar moisture content and quantity of ash isolated from seven aliquots (approximately 0.5 gm.) of the sample (Table XV). On the basis of these results the raw feces were found to contain approximately 3.1% ash. This value is higher than that reported by Boerner and Sunderman (Table I). However, due to the well known wide variability in composition of fecal samples this difference is not considered to be of importance.

Consideration of the procedure for isolation of fecal minerals (ash) from the point of view of its possible use aboard a space ship indicates that it might be suitable for this purpose. It requires two relatively simple operations: the removal of water, and the combustion of the lyophilized product. In both cases the product could be stored in a small area and used when desired.

2. Separation of the Mineral Components of Fecal Ash

The ready availability of knowledge concerning the separation of inorganic substances by ion exchange chromatography allowed straightforward application of the method to the selected components of fecal ash. The method of Kraus and Moore (25) for separation of Mn, Co, Cu, Fe and Zn, and of Cohn and Kohn (9) for separation of the alkali metals were used with slight modification.

Separation of a known mixture of the metals to be determined in fecal ash was carried out successfully as is shown by the elution diagrams given in Figures 5, 7 and 8. Complete separation of all components except magnesium and nickel was accomplished. Incomplete separation of these substances may have contributed to the somewhat low recovery of nickel found by flame spectrophotometry (Table XVI). Separation of the samples of fecal ash was also carried out successfully (Figures 6 and 9) although complete overlapping of magnesium and nickel was observed.

Identification of each of the metals of interest in the known mixture as well as in the fecal ash samples was accomplished by comparison of the wavelength of maximum emission intensity of each peak with the known characteristic wavelengths of metals present.

It was recognized that minerals and trace elements other than those selected for analysis might be present in the fecal ash. In fact lithium was found to be present in fecal ash though it had not been included in the known mixture. The fact that the elution diagrams of the fecal ash contain peaks which have shoulders indicated that additional substances might indeed be present. However, review of the elution diagrams of the known mixture also shows the presence of unsymmetrical peaks. It is therefore probable that the lack of symmetry in both cases is due to interference effects in the flame spectrophotometric analyses. Further investigation would be required to determine the validity of this probability.

3. Flame Spectrophotometric Determination of Certain Fecal Minerals

The results of flame spectrophotometric analysis of chromatographic fractions obtained from the known mixture and from three samples of fecal ash are given in Tables XVI and XVII respectively.

Recovery of the components of the known mixture was good except for the case of nickel (89.0%), strontium (73.3%) and cobalt. The relatively low recovery of strontium and the failure to obtain reliable results for cobalt are believed to be due to the fact that the quantities of these substances present in the chromatographic fractions studied were too small for accurate detection. These levels, however, corresponded to the levels of strontium and cobalt actually observed to

be present in fractions obtained from samples of fecal ash (see Table XVII). It thus appeared that accurate quantitative determination of these trace elements would require separation of much larger samples of fecal ash and a consequent scale-up of the whole chromatographic procedure.

The relatively low recovery of nickel (89.0%) may be due to the fact that it was eluted in essentially the same fractions as magnesium, and that the presence of magnesium may have interfered in its accurate determination.

Although the determinations of strontium, nickel and cobalt were not completely satisfactory, it was not considered necessary to investigate them further for the following reason. The major goal of this investigation of fecal minerals was to determine what substances might be present in quantities large enough to warrant their eventual isolation, and not primarily to determine the exact amount of each metal present. It has been pointed out previously that wide variation in the composition of fecal samples is to be expected. Thus intensive determination of the exact quantity of each component present was not warranted.

The important observation which resulted from analysis of the known mixture was the fact that the determinations were reproducible. It was thus concluded that analysis of samples of fecal ash could be carried out with reliable though, in some cases, somewhat inaccurate results, and that the presence of reasonable quantities of any of the metals investigated could be reliably determined.

Review of the results of determinations of metals (Table XVII) in fecal ash showed good agreement among the three samples. In each case approximately 40% of the ash appeared to be accounted for. Comparison of the results of this investigation with those of other workers (see Tables I and V) is difficult since all have been calculated on different bases: i.e., weight excreted in 24 hrs., µg./kg. of body weight per day, and µg./gm. of dried feces. Recalculation of all results on the basis of gm. of mineral excreted per day in 150 gm. of feces by a 158 lb. average man, yielded the figures shown in Table XVIII. The great similarity between columns (1) and (2) indicates that certain of these results may be from the same source. They differ from those of the present investigation in that much less calcium, iron nickel and cobalt were found. No information was given for lithium and strontium. The large quantity of calcium found in the current work can probably be explained in that the subject from which the feces was collected drinks at least a quart of milk a day.

The quantity of each mineral found in fecal ash is given below.

Sodium		3435	µg./gm. dried feces
Potassium		8824	µg./gm. dried feces
Magnesium		7657	µg./gm. dried feces
Calcium		35740	µg./gm. dried feces
Nickel		88.5	µg./gm. dried feces
Lithium		79.0	µg./gm. dried feces
Strontium	(one determination)	17.9	µg./gm. dried feces
Manganese		104.1	µg./gm. dried feces
Cobalt		42.5	µg./gm. dried feces
Copper		31.5	µg./gm. dried feces
Iron		873.3	µg./gm. dried feces

It is recognized that analysis of other fecal samples can be expected to yield quite different quantities of these substances. It is, however, believed that a successful method for their isolation and determination has been established.

4. The Possible Nutritional Value of Human Fecal Minerals

It is generally stated (33) that the animal body requires calcium, magnesium, sodium, potassium, phosphorus, sulfur, chlorine, iron, copper, iodine, manganese, cobalt and zinc. This investigation has been concerned with only 9 of these substances.

Serious consideration of the possible value of these minerals and trace elements as nutrients for man during extended space travel is very difficult since man's minimum daily requirements for these substances are largely unknown.

The Recommended Daily Dietary Allowances of the Food and Nutrition Board-National Research Council (1958) for calcium and iron are 0.8 gm. and 10 mg. respectively for adult human males. A daily allowance for copper of 1 to 2 mg. for adults is suggested. It is well known that sodium, potassium and chlorine are essential dietary constituents. However, particularly in the case of the trace elements, little is known about the quantitative levels that are necessary for their essential physiological functions, or about their desirable dietary levels in man. This is, of course, the information which is necessary in order to determine the actual usefulness of these substances under the very limited and controlled conditions of a closed ecological system that must exist during space flights of very long duration. The fact that many of the trace elements are widely distributed in nature, and are thus constituents of man's ordinary diet, has made determination of minimum daily requirements an extremely difficult problem.

It is therefore clear that an assessment of the degree of usefulness of fecal minerals and trace elements during space flights of long duration cannot be made at this time.

SECTION V. - LIPIDS

EXPERIMENTAL PROCEDURES

1. Extraction of Lipid

Lyophilized feces (2 gm.) were treated according to the method of Folch, Ascoli, Lees, and Meath (16) with a total of 80 ml. of chloroform-methanol (2:1 V/V). The resulting mixture was filtered to remove insoluble nonlipid material, the filtrate was poured into 500 ml. of distilled water, and the mixture allowed to stand 4 hrs. at room temperature. The aqueous layer was removed and 30 ml. of absolute methanol was added to the chloroform solution. The resulting homogeneous solution was evaporated to dryness on a steam bath.

2. Saponification of Lipid

The dark colored lipid extract was treated with 20 ml. of 0.5 N methanolic sodium hydroxide and heated under reflux for 1 hr. The resulting mixture was extracted with petroleum ether to remove nonsaponifiable substances. The aqueous layer was stored overnight in refrigerator and then acidified while ice-cold, with 2 to 5 ml. of 5 N hydrochloric acid. The free acids were extracted with ether and the resulting ether solution evaporated just to dryness.

3. Preparation of Methyl Esters

The fatty acid isolate was immediately treated (37) with 20 ml. of 5% hydrochloric acid in dry methanol (dried by refluxing with and distillation from calcium oxide) and heated under reflux for 1 hr. Ethyl ether (40 ml.) was added to the reaction solution at room temperature and the total solution was then extracted with distilled water until neutral. The ether solution was evaporated on a steam bath and the light brown residue stored under refrigeration for analysis.

4. Gas Chromatographic Analysis of the Methyl Esters of Fecal Fatty Acids

Gas chromatographic separation was carried out with a conventional apparatus fitted with 100,000 ohm thermistors (Victory Engineering Corp., Union, New Jersey, Type A177). A 6-ft. 6 mm. i.d. glass column containing Craig polyester succinate on chromosorb W (60-80 mesh) (Wilkens Instrument Company, Walnut Creek, California) was used at 186°C., Helium pressure 19 p.s.i., flow 94 ml./min., detector temperature 197°C.

Separation of standard mixtures (Applied Science Laboratories, Inc., State College, Pa.) of authentic methyl esters (1 to 3 µl. samples) was carried out initially in order to determine that satisfactory analyses would be obtained. The retention volumes found for the known esters are given in Table XIX. Figure 10

shows the expected linear relationship between the chain length of the esters and the $\log_{10}$ of their retention volumes.

Separation of 3 µl. samples of methyl esters of fecal fatty acids showed the presence of the components shown in Table XIX. Comparison of retention volumes with those of known methyl esters indicated the presence of 12:0, 14:0, 15:0, 16:0, 18:0 and $18:1^9$ acids.

RESULTS AND DISCUSSION

1. Investigation of Methyl Esters of Fecal Fatty Acids

A brief preliminary investigation of fecal fatty acids was carried out. Extraction and saponification of lipids, and preparation and separation of the fatty acid esters were carried out according to well known procedures (16, 37, 22).

By comparison of the retention volumes of the fecal acids with those of known samples (Table XIX) it was concluded that palmitic (16:0) acid, oleic ($18:1^9$) acid, stearic (18:0) acid, myristic (14:0) acid, lauric (12:0) acid, and n-pentadecanoic (15:0) acid were present. Palmitic, oleic and stearic acids were the major components present, while the other acids were present in very minor quantities.

2. Evaluation of Fecal Fatty Acids

The results of this preliminary investigation are in agreement with those of James (23) who has reported the nature of the free and bound fatty acids in human fecal lipid. They are by no means as extensive. Since it is difficult to imagine that fecal fatty acids could be useful sources of nutrients and since James (23) has provided knowledge of their composition, it was not considered worthwhile to pursue their investigation further. Isolation of these substances and their conversion to useful forms by methods simple enough to be carried out under conditions of space travel seems unlikely.

SECTION VI. - GENERAL SUMMARY AND REVIEW OF REMAINING PROBLEM AREAS

A literature survey and laboratory investigations of human fecal minerals and trace elements and of a crude fecal protein fraction have been carried out. These substances were chosen for study because they appeared to be possible sources of materials which could be considered to have potential "use" for men or plants during space flights of long duration.

A major limitation of this investigation has been the inability to judge the "usefulness" of fecal components. Substances must be of great use to the closed ecological system which must exist in a spaceship if their isolation and purification for use by men or plants can be justified. One cannot assume that fecal components resulting from today's normal diet will be obtained in the dietary regime of the space

traveler. It has, therefore, been necessary to devise methods for the separation and purification of, at present, qualitatively and quantitatively unknown substances.

In view of the complex biochemical system existing in feces and the inherent difficulties encountered in isolation and identification of their components, it is suggested that any future investigations should be carried out on feces resulting from authentic space diets of the type that persons on flights of long duration would eat. If this were done a relatively realistic idea of the nature of available fecal components could be obtained and better criteria of their "usefulness" could be devised.

TABLE I

Proximate Composition of Feces (5)

Component	Weight (gm.)	Per cent of Total
Bulk	150	
Water	99	66.0
Dry Matter	27	17.8
Fat	4.7	3.0
Protein	?	?
Nitrogen	1.5	1.0
Carbohydrate	?	?
Minerals	2.1	1.4
Sodium	0.12	
Potassium	0.47	
Calcium	0.64	
Magnesium	0.20	
Chloride	0.09	
Phosphorus	0.51	
Sulfur	0.13	
Trace Elements: (2)		
Copper		
Iron		
Lead		
Manganese		
Nickel		
Zinc		
Arsenic		
Vitamins (15)	0.015	0.01
Bile Pigments	0.15	0.1

TABLE II

Approximate Quantity of Fecal Components to be Processed

Period of Time: 1 Day (24 hrs.)

Component	1 Man	3 Men	20 Men
Bulk	150 gm.	450 gm.	3000 gm.
Water	99	297	1980
Dry Matter	27	81	540
Fat	4.5	13.5	90
Protein		Not Reported	
Nitrogen	1.5	4.5	30
Carbohydrate		Not Reported	
Minerals and Trace Elements	2.1	6.3	42
Vitamins			
B Vitamins	0.015	0.045	0.3
Bile Pigments	0.15	0.45	3.0

TABLE III

Comparison of Composition of Bound, Free, and Total Fatty Acids in Fecal Lipid for a Normal Human (23)

Acid**	Percentage of Acids in C_6 - C_{20} Range Free	Bound	Total
10:0	0.6	0	0.3
12:0	4.3	2.2	3.3
14:0	8.9	4.4	6.6
Branched 15:0	0.7	1.1	0.9
15:0	1.4	1.1	1.2
16:0	55.2	35.3	45.2
Branched 17:0	0.9	1.4	1.2
17:0	0.4	0.8	0.6
18:0	12.9	31.8	22.3
10-Hydroxy 18:0	0.7	0.9	0.8
14:1	0	0	0
16:1	1.4	2.1	1.8
18:1	6.8	10.7	8.7
Isomer 18:1	3.5	6.5	5.0
18:2	1.3	2.8	2.0
18:3	0	0	0
20:3	Trace*	Trace*	Trace*
20:4	Trace*	Trace*	Trace*
Other unsaturated C_{20} acids	Trace*	Trace*	Trace*

*Trace indicates less than 0.5%.
**Number of carbons and double bonds.

TABLE IV

Some Microbiologically Available Amino Acids in Feces (33)

Amino Acid	Wt. Present in Feces (approx. 900 gm.)
Methionine	0.5 - 0.8 gm.
Lysine	1.9 - 2.9
Arginine	1.2 - 2.1
Histidine	0.6 - 0.8
Leucine	1.8 - 2.9
Iso-Leucine	1.4 - 2.3
Valine	1.5 - 2.6
Threonine	1.4 - 2.2

TABLE V

Analysis of Protein Isolated from Infant Feces (1)

Amino Acid	Amino Acid Nitrogen (Per cent of Total Protein Nitrogen)
Threonine	6.0 %
Arginine	12.7
Histidine	5.4
Lysine	16.4
Cystine	1.3
Methionine	1.9
Iso-Leucine	4.6
Phenylalanine	2.9
Tyrosine	2.1
Tryptophan	3.1

TABLE VI

Results of Solvent Extraction of Dehydrated Feces

Solvents	Time (hrs.)	Yield of Defatted Product (%)	Nitrogen in Defatted Product (%)	Per cent of Total Fecal Nitrogen
I. Diethylether	16	1) 88.14 2) 83.3	1) 7.08 2) 7.67	1) 96.74 2) 104.0
II. Diethylether followed by Absolute ethanol	8 8	1) 71.94 2) 69.4	1) 8.26 2) 8.28	1) 92.13 2) 93.58
III. Absolute ethanol	16	1) 54.35 2) 52.8	1) 9.42 2) 9.49	1) 79.37 2) 81.60

TABLE VII

Minerals and Trace Elements Excreted in Human Feces (2)

Substance	Amount: Mean Quantity and Range (µg./kg. of body wt. per day)
Aluminum	0.6
Arsenic	33(1-116)
Calcium	7490 (5000-10,000)
Chloride	(210-500)
Cobalt	0.007 (0.002-0.02)
Copper	27 (23-37)
Iron	120 (65-208)
Lead	4.2 (2.2-19.8)
Magnesium	2500 (1510-3185)
Manganese	(18-120)
Mercury	0.14
Nickel	(1.2-2.5)
Phosphorus	9860 (7100-20,000)
Potassium	6,700
Silver	0.8
Sodium	1,700
Sulfur, total	2000
Tin	(170-450)
Zinc	100 (58-144)

TABLE VIII

Isolation of Dehydrated Feces Homogenate

Lyophilization	Raw Feces Weight (gm.)	Feces Homogenate (gm.)	Dried Feces (gm.)	Per cent Yield	Dried Feces Per cent N	
A	30.3	60.5	11.8	19.5	5.9	6.1
B	40.0	80.0	14.4	18.0	6.2	
C	20.6	41.2	9.2	22.2	6.5	
	18.5	36.9	7.5	20.3	6.5	
D	41.3	82.5	10.0	12.1	6.1	
	42.8	85.6	11.4	13.2	6.1	
E	37.5	75.0	11.1	14.8	5.9	
	35.5	71.0	10.5	14.8	5.9	
F	40.0	80.0	10.4	13.0	5.6	
	40.0	80.0	10.8	13.5	5.6	
Total	346.5	692.7	107.1	161.4	60.3	
Average	34.7	69.3	10.7	16.1	6.0	

Average % N in feces on wet basis is 1.85% N.

TABLE IX

Fractionation of Solvent-extracted (Defatted) Dehydrated Feces

Solvent	Fraction	Yield (%)	Nitrogen (%)	Nitrogen (mg.)	Per cent of Total Nitrogen in Defatted Fecal Material
I. Diethyl-ether (16 hr. extraction)	A. Alkali Insoluble	1) 27.65 2) 27.72	1) 5.50 2) 4.42	1) 15.2 2) 12.2	1) 21.5 2) 15.9
	B. Solution I	---	---	1) 42.5 2) 63.5	1) 60.1 2) 82.8
	Nitrogen recovery from A(1) and B(1) = 81.6% Nitrogen recovery from A(2) and B(2) = 98.7%				
	C. Protein Fraction	1) 14.43 2) 13.98	1) 13.80 2) 11.27	1) 20.7 2) 19.8	1) 29.3 2) 25.8
	D. Solution II	---	---	1) 23.4 2) 29.5	1) 33.1 2) 38.4
	Nitrogen recovery from C(1) and D(1) = 103.8% Nitrogen recovery from C(2) and D(2) = 77.3% Total nitrogen recovery in Fractionation 1 = 83.9% Total nitrogen recovery in Fractionation 2 = 80.1%				
II. Ether (8 hrs.) followed by Ethanol (8 Hrs.)	A. Alkali Insoluble	1) 34.24 2) 26.86	1) 5.89 2) 4.80	1) 20.2 2) 12.9	1) 24.4 2) 15.6
	B. Solution I	---	---	1) 56.0 2) 71.3	1) 67.8 2) 86.1
	Nitrogen recovery from A(1) and B(1) = 92.2% Nitrogen recovery from A(2) and B(2) = 101.7%				
	C. Protein Fraction	1) 16.89 2) 18.52	1) 13.40 2) 11.10	1) 23.5 2) 25.5	1) 28.4 2) 30.8
	D. Solution II	---	---	1) 39.6 2) 29.5	1) 47.9 2) 35.6
	Nitrogen recovery from C(1) and D(1) = 112.6% Nitrogen recovery from C(2) and D(2) = 77.1% Total Nitrogen Recovery in Fractionation 1 = 100.7% Total Nitrogen Recovery in Fractionation 2 = 82.0%				
III. Absolute Ethanol (16 hrs.)	A. Alkali Insoluble	1) 35.81 2) 26.36	1) 6.51 2) 4.98	1) 23.3 2) 10.2	1) 24.8 2) 13.9
	B. Solution I	---	---	1) 67.0 2) 68.4	1) 71.1 2) 93.2
	Nitrogen recovery from A(1) and B(1) = 95.9% Nitrogen recovery from A(2) and B(2) = 107.1%				
	C. Protein Fraction	1) 25.7 2) 21.07	1) 12.52 2) 11.26	1) 33.5 2) 23.0	1) 35.6 2) 31.4
	D. Solution II	---	---	1) 29.5 2) 23.2	1) 31.3 2) 31.6
	Nitrogen recovery from C(1) and D(1) = 94.1% Nitrogen recovery from C(2) and D(2) = 67.6% Total nitrogen recovery in Fractionation 1 = 91.7% Total nitrogen recovery in Fractionation 2 = 76.9%				

TABLE X

Summary of Fractionation of Dehydrated Feces

Solvent	Defatted Material (A)			Alkali Insoluble Fraction				Protein Fraction			
	Yield (%)	Nitrogen (%)	Per cent Total N in dry Feces	Yield from A (%)	Nitrogen (%)	Yield from Dry Feces (%)	Per cent Total N in Dry Feces	Yield from A (%)	Nitrogen (%)	Yield from Dry Feces (%)	Per cent Total N in Dry Feces
Ethyl-ether	1) 88.14	1) 7.08	1) 96.74	1) 27.65	1) 5.50	1) 24.37	1) 20.0	1) 14.43	1) 13.80	1) 12.72	1) 26.3
	2) 83.3	2) 7.67	2) 104.0	2) 27.72	2) 4.42	2) 23.09	2) 16.6	2) 13.98	2) 11.27	2) 11.64	2) 21.3
Ether-ethanol	1) 71.94	1) 8.26	1) 92.13	1) 34.24	1) 5.89	1) 24.63	1) 22.4	1) 16.89	1) 13.40	1) 12.15	1) 25.1
	2) 69.4	2) 8.28	2) 93.58	2) 26.85	2) 4.80	2) 18.64	2) 14.5	2) 18.52	2) 11.10	2) 12.85	2) 23.1
Ethanol	1) 54.35	1) 9.42	1) 79.37	1) 35.81	1) 6.51	1) 19.46	1) 19.6	1) 25.7	1) 12.52	1) 13.96	1) 27.1
	2) 52.8	2) 9.49	2) 81.60	2) 26.36	2) 4.93	2) 13.92	2) 11.2	2) 21.1	2) 11.26	2) 11.12	2) 20.3
	3) 64.1	3) 9.49	3) 105.4	3) 21.9	3) 6.28	3) 14.1	3) 15.3	3) 18.6	3) 12.23	3) 11.9	3) 25.2
								Average 12.0%		Average 12.32%	

TABLE XI

Microbiological Determination of Component Amino Acids of Crude Fecal Protein

Acid	Per cent Amino Acid in protein	Per cent Amino acid N of total N	Amino acid (gm.) per 16 gm. N
Lysine	6.0	9.6	8.0
	6.6	10.5	8.7
Leucine	5.9	5.3	7.9
	5.8	5.2	7.8
	5.5	4.9	7.3
Iso-Leucine	4.7	4.2	6.3
	4.7	4.2	6.3
Phenylalanine	3.5	2.5	4.7
	3.5	2.5	4.7
Methionine	1.9	1.5	2.6
	2.0	1.6	2.7
Arginine	4.3	11.5	5.7
	4.3	11.5	5.7
Valine	6.4	6.4	8.3
	6.6	6.5	8.8
Histidine	1.9	4.2	2.5
	1.9	4.2	2.5
Threonine	2.8	2.8	3.7
	2.9	2.8	3.9
Tryptophan	0.53	0.61	0.71
	0.50	0.57	0.67
Tyrosine	1.4	0.90	1.9
	1.4	0.90	1.9
Cystine	0.45	0.44	0.60
	0.45	0.44	0.60
	0.48	0.47	0.64

TABLE XII

Component Amino Acids of Fecal Protein
Determined by Amino Acid Analyzer

Amino Acid	µ Mole	Mg.	Per cent of Protein	gm. Amino Acid per 16 gm. N
Lysine	0.816	0.150	3.8	5.1
Histidine	0.240	0.050	1.3	1.7
Ammonia	3.350	0.178	4.6	-
Arginine	0.439	0.093	2.4	3.2
Aspartic acid	1.800	0.250	6.4	8.5
Methionine sulfoxide	0.009	0.004	-	-
Threonine	0.803	0.096	2.5	3.3
Serine	0.784	0.082	2.1	2.8
Glutamic acid	1.740	0.129	3.3	4.4
Proline	0.938	0.108	2.8	3.7
Glycine	1.290	0.097	2.5	3.3
Alanine	1.560	0.139	3.6	4.8
Valine	1.060	0.124	3.2	4.3
Methionine	0.359	0.053	1.4	1.9
Iso-Leucine	0.800	0.105	2.7	3.6
Leucine	1.275	0.167	4.3	5.7
Tyrosine	0.508	0.092	2.4	3.2
Phenylalanine	0.594	0.098	2.5	3.3
Allo Iso-Leucine	0.015	0.002	-	-
TOTALS		2.017 mg.	47.2%	

Quantity of Crude Fecal Protein Hydrolyzed = 3.91 mg.
Quantity of Fecal Protein Hydrolyzate Analyzed = 2.25 mg.
Quantity of Fecal Protein Hydrolyzate Recovered = 2.01 mg.
Quantity of Component Amino Acids Recovered = 2.01 - 0.178 = 1.84 mg.
Quantity of Component Essential Amino Acids Determined = 0.855 mg.

Per cent Essential Amino Acids in Crude Fecal Protein

$$\frac{0.855}{3.91} \times 100 = 22.6\%$$

Per cent Total Amino Acids in Crude Fecal Protein

$$\frac{1.84}{3.91} \times 100 = 47.0\%$$

Per cent Essential Amino Acids in Total Acids

$$\frac{0.855}{1.84} \times 100 = 48.0\%$$

Per cent Total Amino Acids in Fecal Protein Hydrolyzate

$$\frac{1.84}{2.25} \times 100 = 82.0\%$$

Per cent of Fecal Protein Hydrolyzate Accounted For

$$\frac{2.01}{2.25} \times 100 = 89.9\%$$

TABLE XIII

Comparison of the Essential Amino Acid Composition of Fecal Protein and Egg Albumin

Essential Amino Acids for Nitrogen Balance in Man	Essential Acids per 16 gm. of N		gm. per 100 gm. of Essential Acids		Ratio for (34) N Balance in Man
	Fecal Protein*	Egg Albumin (4)	Fecal Protein	Egg Albumin	
iso-Leucine	3.6	6.8	12.1	14.1	11.0
Leucine	5.7	9.0	19.1	18.7	17.3
Lysine	5.1	6.5	17.0	13.6	12.6
Methionine	1.9	4.9	6.5	10.2	17.3
Phenylalanine	3.3	7.1	11.1	14.8	17.3
Threonine	3.3	4.2	11.1	8.7	7.9
Tryptophan	1.2**	1.5	4.0	3.1	4.0
Valine	5.7	8.1	19.1	16.8	12.6
TOTALS	29.8	48.1	100.0	100.0	100.0

*Composition from Beckmen/Spinco Amino Acid Analyzer.

**Tryptophan was not found due, it is believed, to destruction during acid hydrolysis. Thus, the quantity of tryptophan necessary for nitrogen balance in man was assumed.

TABLE XIV

Lyophilization of Raw Feces

Preparation	Raw Feces Weight (gm.)	Feces Homogenate (gm.)	Lyophilized Feces Weight (gm.)	Per cent Yield
II	36.0	71.9	8.9	24.7
III_1	45.4	90.7	11.6	25.6
III_2	39.2	78.3	9.7	24.8
IV_a	39.4	78.7	10.3	26.2
IV_b	43.3	86.6	12.3	28.4
V_a	45.2	90.3	11.6	25.7
V_b	44.5	89.0	10.4	23.4
Total	293.0	585.5	74.8	178.8
Average	41.8	83.6	10.7	25.6

TABLE XV

Determination of Dried Feces and Fecal Ash

Sample Number	Weight of Lyophilized Feces (gm.)	Weight of Dried Feces (gm.)	Yield (%) of Dried Feces	Moisture (%) in Lyophilized Feces	Weight of Ash (gm.)	Per cent Ash in Dried Feces
1	0.5133	0.4418	86.3	13.9	0.0616	13.9
2	0.5278	0.4507	85.5	14.6	0.0605	13.4
3	0.5050	0.4346	86.0	13.9	0.0621	14.3
4	0.5106	0.4450	87.2	12.8	0.0659	14.8
5	0.5242	0.4529	86.6	13.6	0.0624	13.8
6	0.5038	0.4438	88.1	11.9	0.0607	13.7
7	1.0237	0.8901	86.9	13.0	0.1300	14.6
Total	4.1084	3.5589	606.6	93.7	0.5032	98.5
Average	0.5869	0.5084	86.7	13.4	0.0719	14.1

TABLE XVI

Flame Spectrophotometric Determination of Certain Metals in Chromatographic Fractions Obtained from a Known Mixture

Metal	Amount Present (µg.)	Amount Found (µg.)	Per cent Recovery
Sodium	2,000	2,013	101
Potassium	5,000	5,007	100
Magnesium	8,000	7,696	96.1
Nickel	200	178	89.0
Calcium	12,000	11,833	98.7
Strontium	50	36.6	73.3
Manganese	50	46.9	93.8
Cobalt	50	Not	Reproducible
Copper	50	53.6	107
Iron	400*	377	94.5
Lithium	—	—	—

* Not in known mixture as was not expected to be found.

TABLE XVII

Flame Spectrophotometric Determination of Certain Metals in Chromatographic Fractions Obtained from Three Samples of Fecal Ash

Metal	Sample 1 Found*	Sample 1 Elution Range**	Sample 6 Found*	Sample 6 Elution Range	Sample 7 Found*	Sample 7 Elution Range	Average*	Per cent Deviation
Lithium	84.5	4-5	77.0	8-9	75.4	4-6	79.0	±7.0
Sodium	3,380	4-7	3,400	10-14	3,526	6-9	3,435	±2.6
Potassium	8,620	8-12	8,470	17-23	9,382	9-13	8,824	±6.3
Magnesium	7,910	12-18	7,651	30-37	7,410	14-19	7,657	±3.3
Nickel	83.6	13-17	86.9	32-36	95.0	13-19	88.5	±7.3
Calcium	36,800	21-40	34,820	39-53	35,600	20-41	35,740	±3.0
Strontium***	____	____	17.9	55-58	____	____	17.9	
Manganese	102	11-19	107	11-18	103	10-19	104	±2.9
Cobalt	42.6	24-28	45.5	23-27	39.4	23-28	42.5	±7.3
Copper	30.6	36-44	31.4	36-43	32.4	36-45	31.5	±2.9
Iron	850	54-64	885	50-63	885	48-62	873	±2.6
Per cent of Ash Recovered	41.1		40.6		39.2		40.3	

* µg./gm. dried feces

** Elution ranges include tubes which contain significant amount of metal.

*** Only one determination made.

TABLE XVIII

Comparison of Experimental Results with Results Found in the Literature*

Metal	Boerner and Sunderman (5)	Handbook of Biological Data (19)**	Experimental Results***
Sodium	0.12 gm.	0.122 gm.	0.116 gm.
Potassium	0.47 gm.	0.482 gm.	0.291 gm.
Calcium	0.64 gm.	0.549 gm.	1.180 gm.
Magnesium	0.20 gm.	0.180 gm.	0.252 gm.
Nickel	---	0.130 mg.	2.900 mg.
Iron	---	8.640 mg.	28.800 mg.
Manganese	---	4.760 mg.	3.430 mg.
Copper	---	1.940 mg.	1.020 mg.
Cobalt	---	0.00049 mg.	1.400 mg.
Lithium	---	---	2.600 mg.
Strontium	---	---	0.590 mg.

* Calculated as the weight of minerals in 150 gm. of feces excreted per day by an average man of 158 lb.

** Original results multiplied by 72.

*** Original results multiplied by 33.

TABLE XIX

Gas Chromatographic Separation of Methyl Esters of Known and of Fecal Fatty Acids

Acid	Shorthand Designation	t_R (min.)	Retention Volume (V_R) (ml.)	$\log_{10} V_R$
n - Hexanoic	6:0	1.85	179.0	2.253
n - Octanoic	8:0	3.20	398.0	2.600
n - Nonanoic	9:0	4.30	416.0	2.619
n - Decanoic	10:0	5.85	660.0	2.820
n - Undecanoic	11:0	8.05	778.0	2.891
n - Dodecanoic	12:0	11.25	1089.0	3.036
n - Tridecanoic	13:0	15.60	1510.0	3.179
n - Tetradecanoic (Myristic)	14:0	21.80	2109.0	3.340
n - Pentadecanoic	15:0	30.40	2940.0	3.468
n - Hexadecanoic (Palmitic)	16:0	41.90	4060.0	3.680
n - Heptadecanoic	17:0	58.00	5610.0	3.743
n - Octadecanoic (Stearic)	18:0	81.60	7890.0	3.897
n - Octadecenoic (Oleic)	$18:1^{9}$	90.40	8740.0	3.941
n - Octadecadienoic (Linoleic)	$18:2^{9,12}$	108.40	10490.0	4.023
n - Nonadecanoic	19:0	102.70	9920.0	3.996
Fecal Acids				
1		11.05	1069.0	3.290
2		21.15	2045.0	3.311
3		29.35	2840.0	3.453
4		41.15	3980.0	3.600
5		79.75	7720.0	3.906
6		88.75	8580.0	3.934

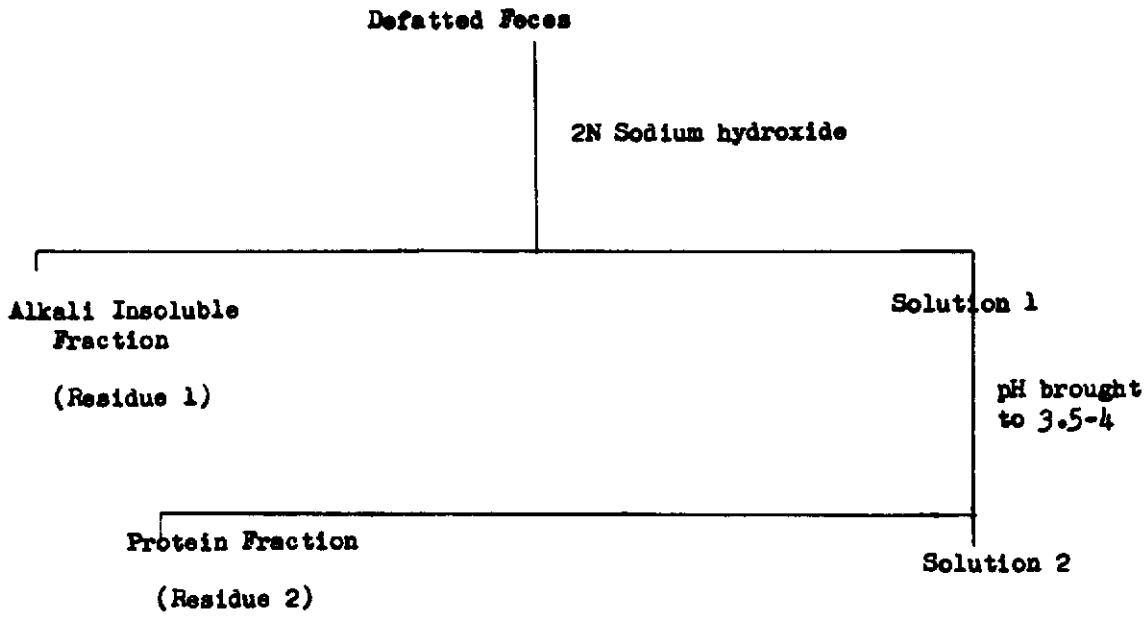

Figure 1. Procedure for Isolation of the Protein Fraction from Solvent-extracted Dehydrated Feces

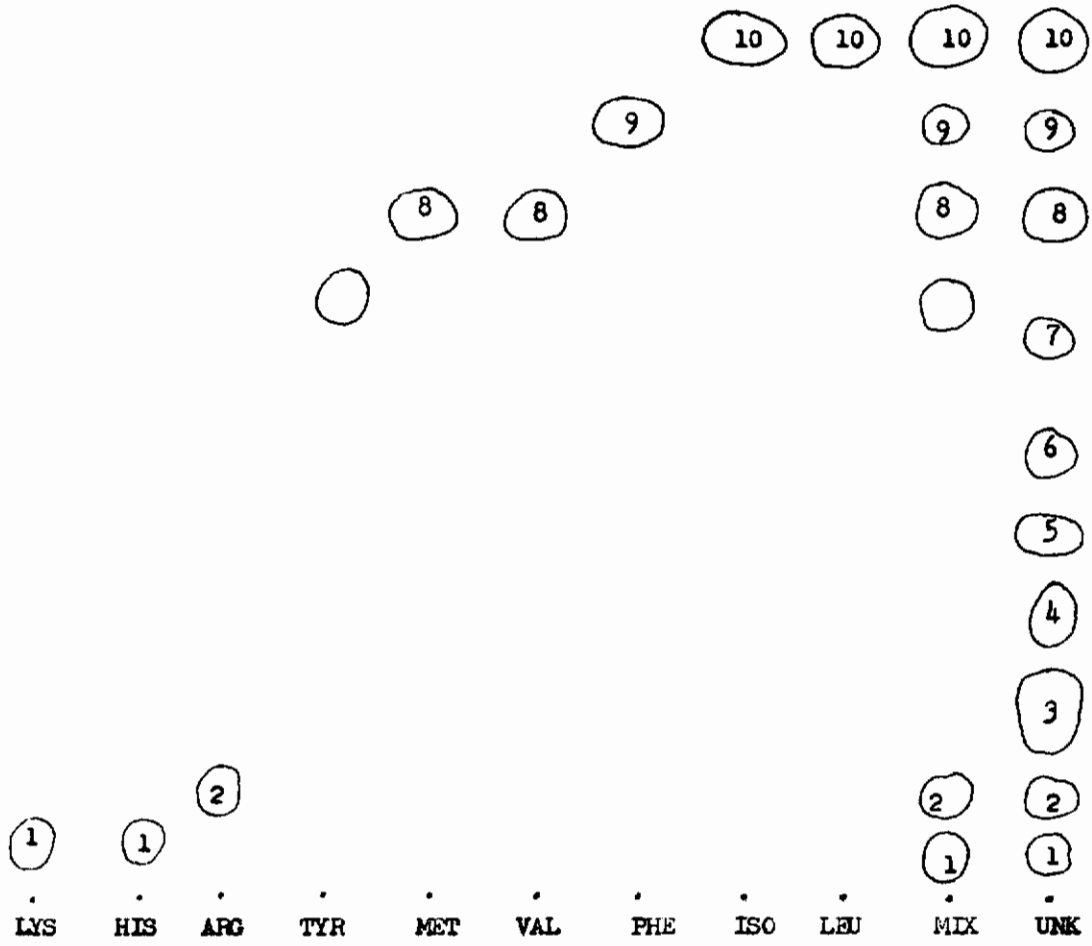

Figure 2. Schematic Representation of a Paper Chromatographic Separation of the Component Amino Acids of Fecal Protein

Solvent: n-Butanol:acetic acid:water (25:6:25, v/v)

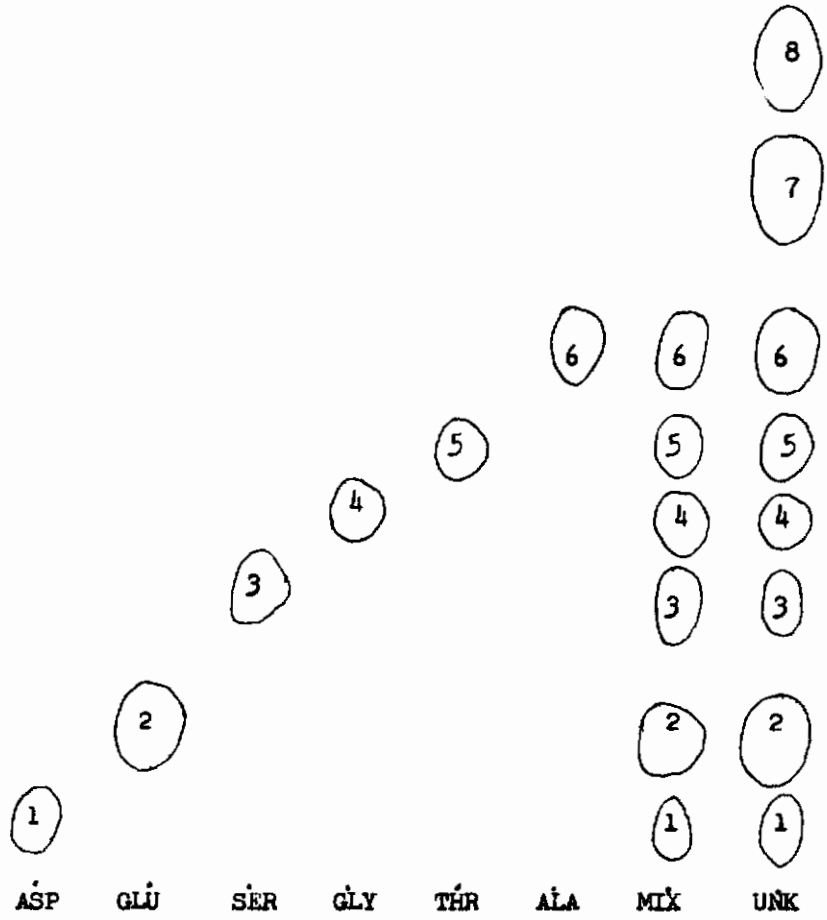

Figure 3. Schematic Representation of a Paper Chromatographic Separation of the Component Amino Acids of Fecal Protein

Solvent: Phenol (88%): 2-propanol:water (5:1:1, v/v)

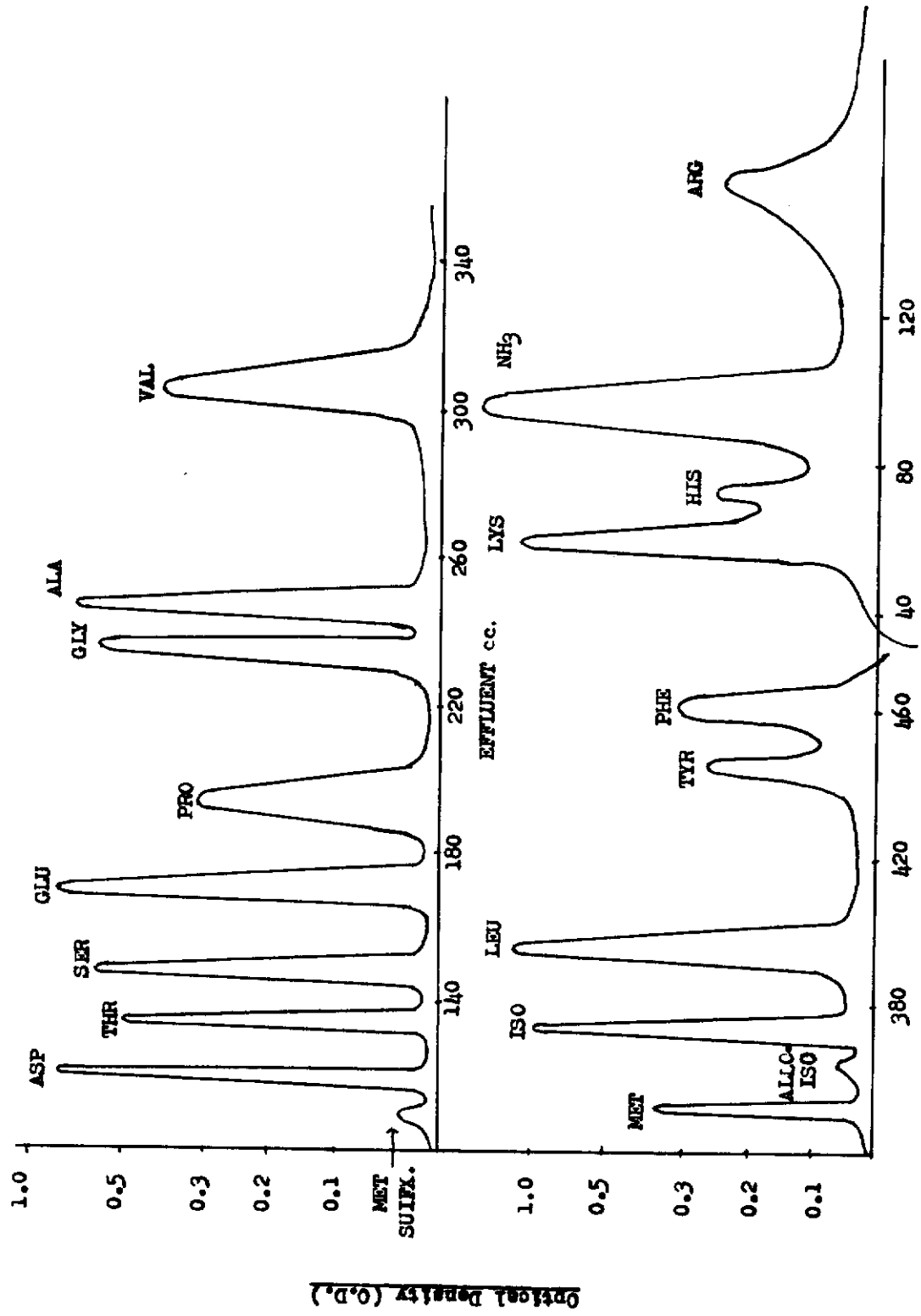

Figure 4. Schematic Representation of the Separation of the Component Amino Acids of Fecal Protein by Means of an Amino Acid Analyzer

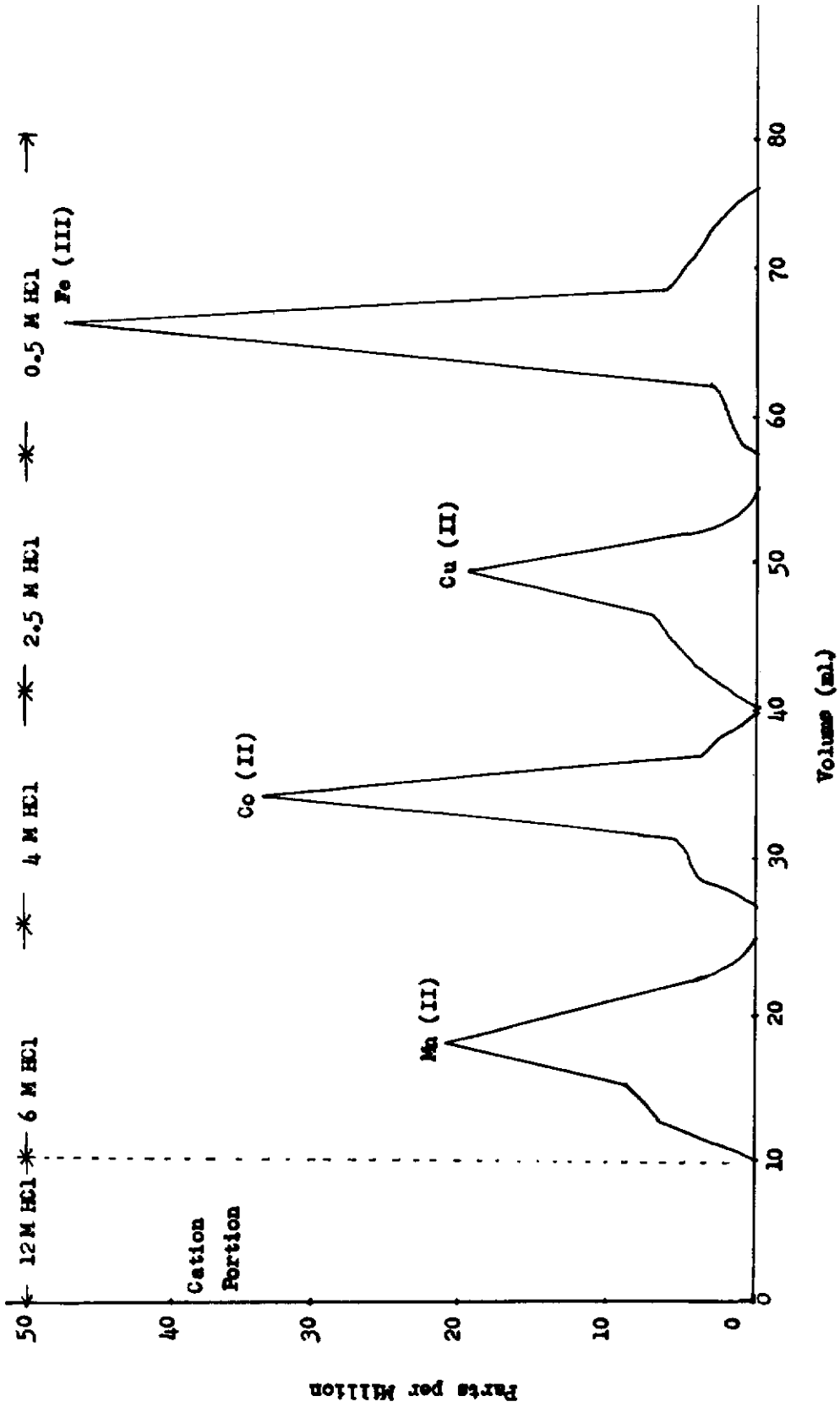

Figure 5. Anion Exchange Chromatography – Separation of a known Mixture
(Dowex 1-X8, 100-200 mesh, Column 0.8 cm x 24.5 cm, Flow Rate 0.3 to 0.7 ml/min.)

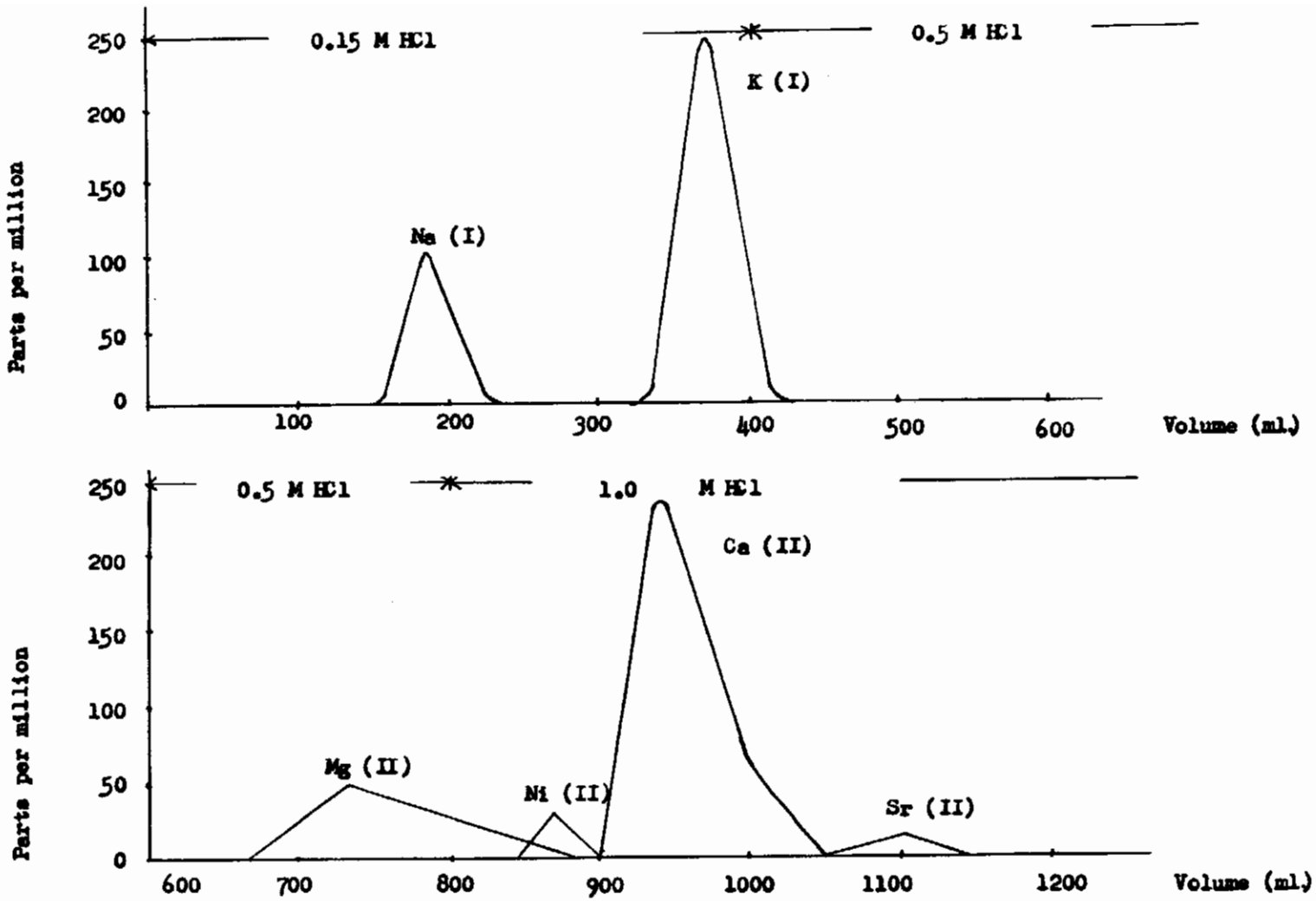

Figure 6. Cation Exchange Chrometography — Separation of a Known Mixture by a Stepwise Elution Procedure

(Dowex 50W-X8, 200-400 mesh, Column 1.2 cm.x 11.6 cm, Flow Rate 0.3 to 0.6 ml/min.)

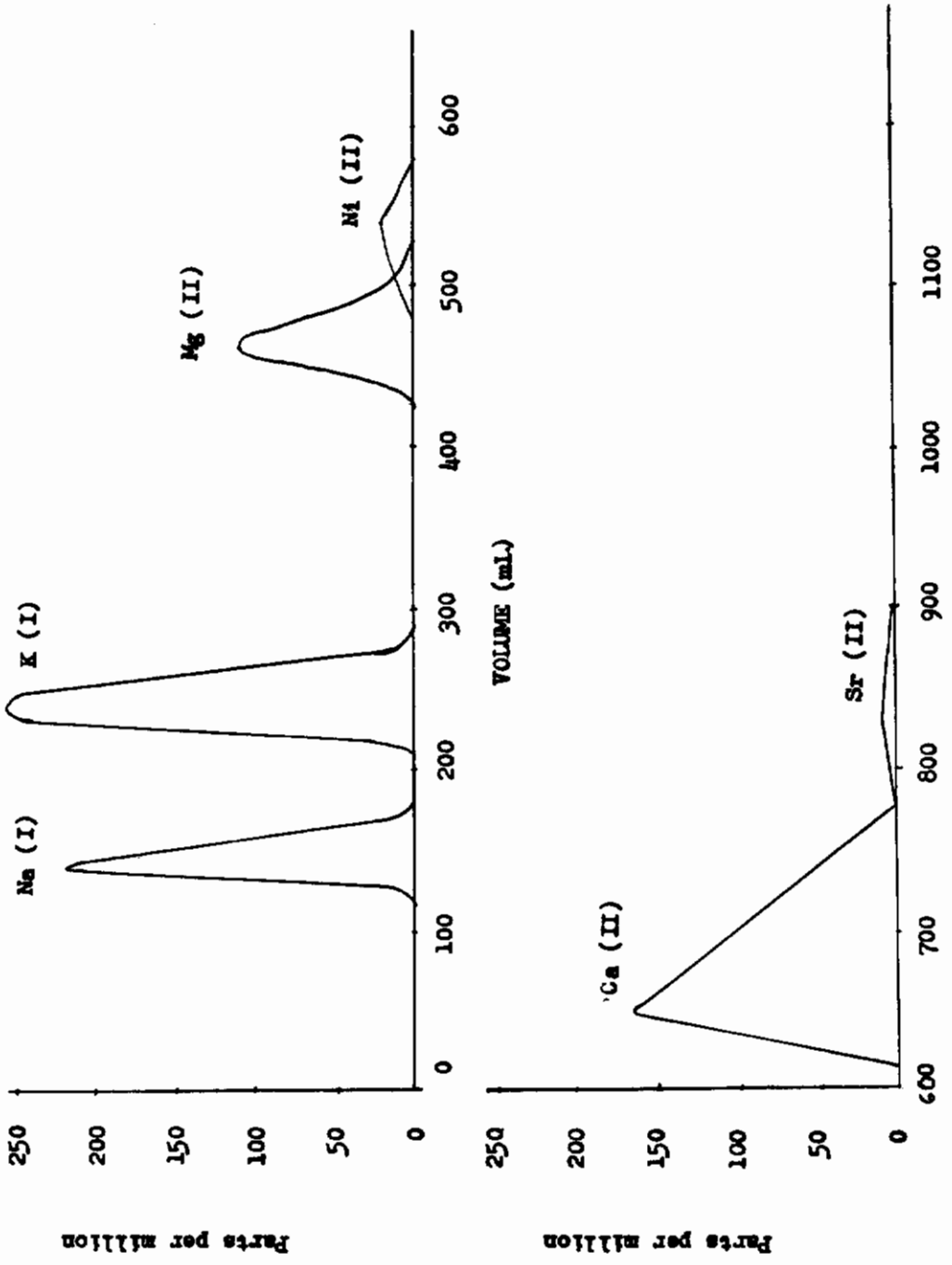

Figure 7. Cation Exchange Chromatography —Separation of a known Mixture by Linear Gradient Elution Procedure

(Dowex 50W-X8, 200-400 Mesh, Column 1.2 cm.x 11.6 cm, Flow Rate 0.3 to 0.6 ml/min.)

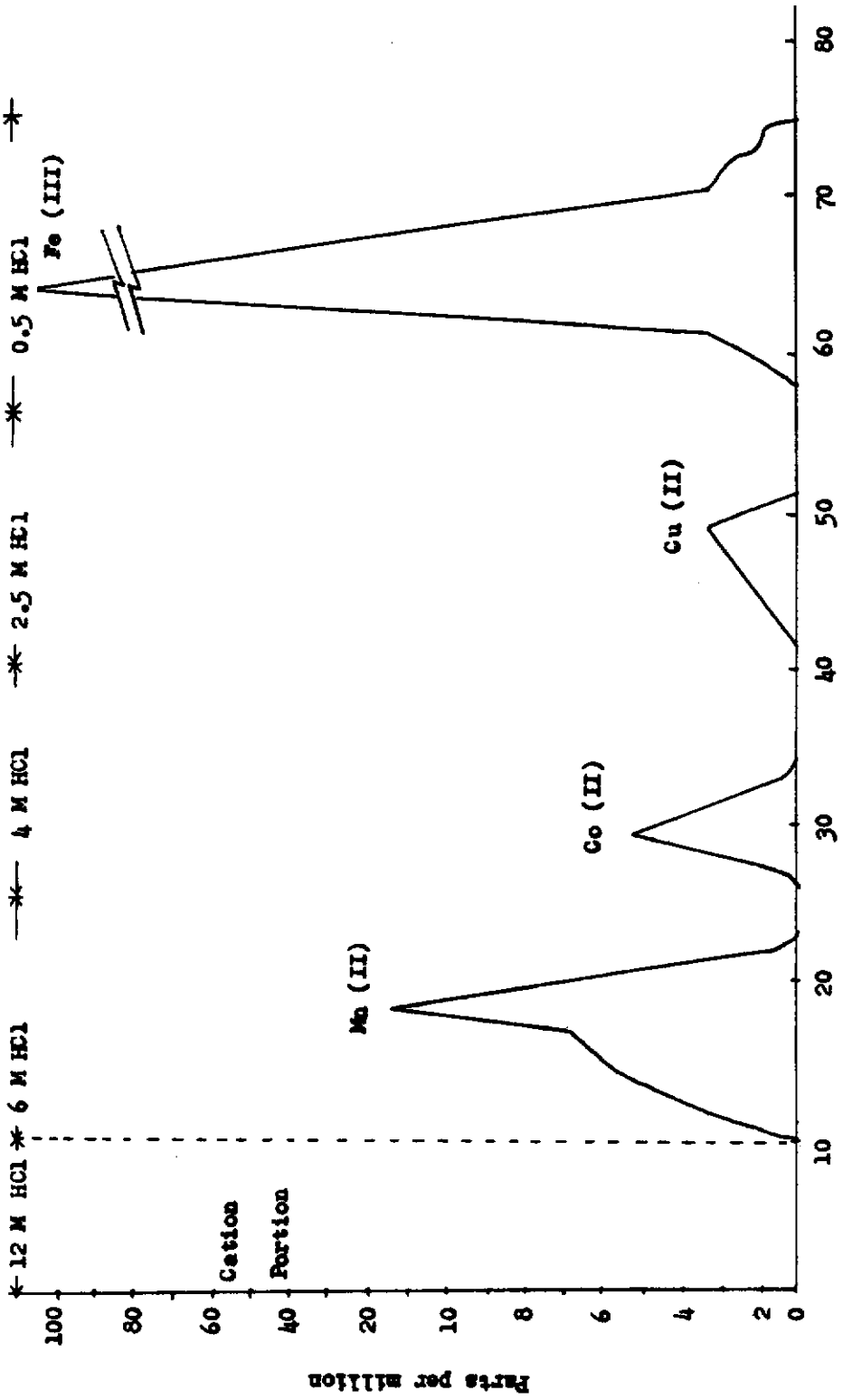

Figure 8. Anion Exchange Chromatography — Separation of Fecal Ash

(Dowex 1-X8, 100-200 Mesh, Column 0.8 cm.x 24.5 cm, Flow Rate 0.3 to 0.7 ml/min.)

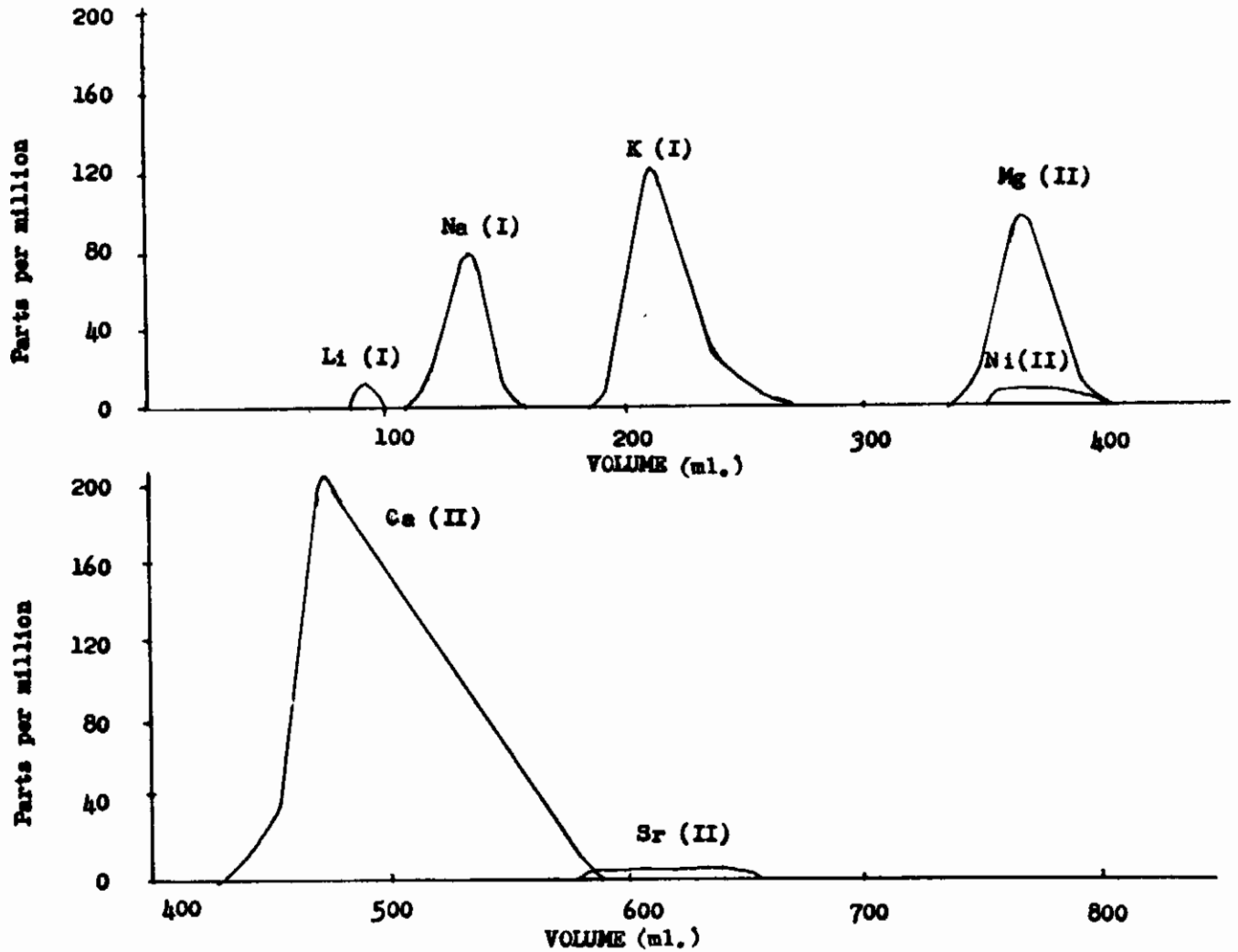

Figure 9. Cation Exchange Chromatography — Separation of Fecal Ash by Linear Gradient Elution Procedure

(Dowex 50W-X8, 200-400 Mesh, Column 1.2 cm.x 11.6 cm. Flow Rate 0.3 to 0.6 ml/min.)

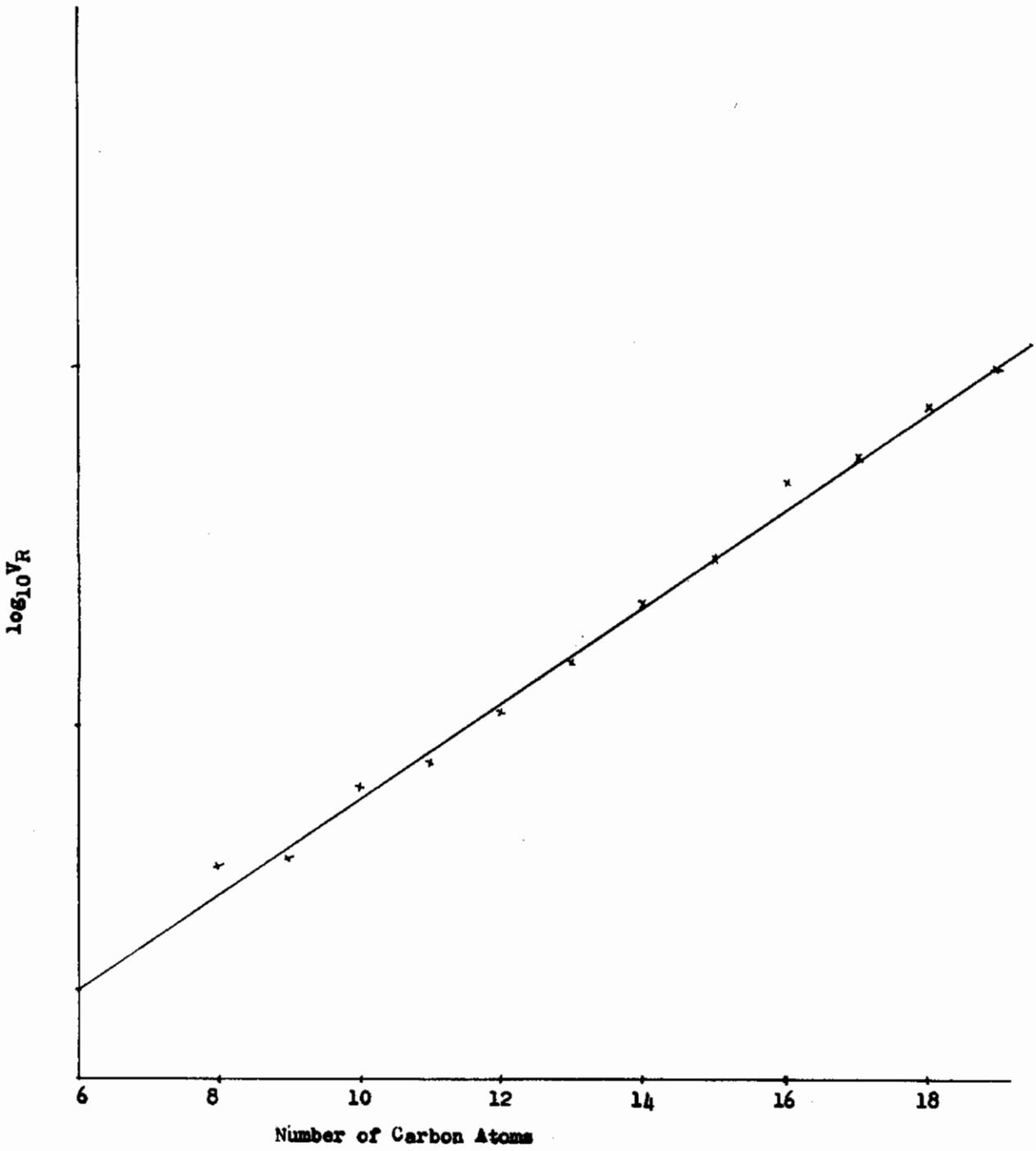

Figure 10. Relation Between $\log_{10}$ Retention Volume and Chain Length for Methyl Esters of Known Fatty Acids

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