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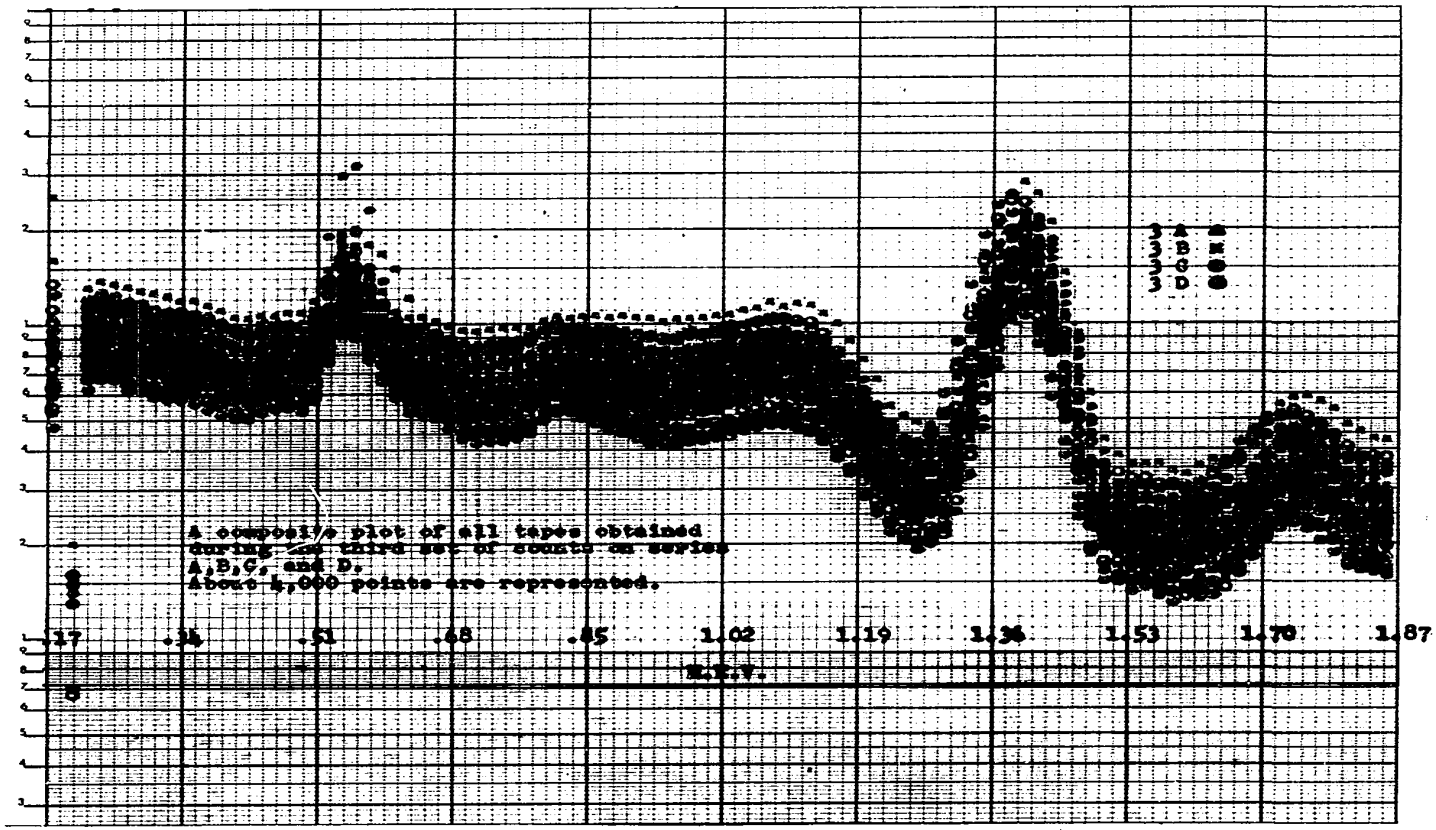
Many other friends have contributed in no small way to this paper but it is always impossible to mention them all. To Dr. R.E. Jervis must go the credit of instigating and encouraging our interest in this field.


A composite plot of all tapes obtained
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About 4,000 points are represented.
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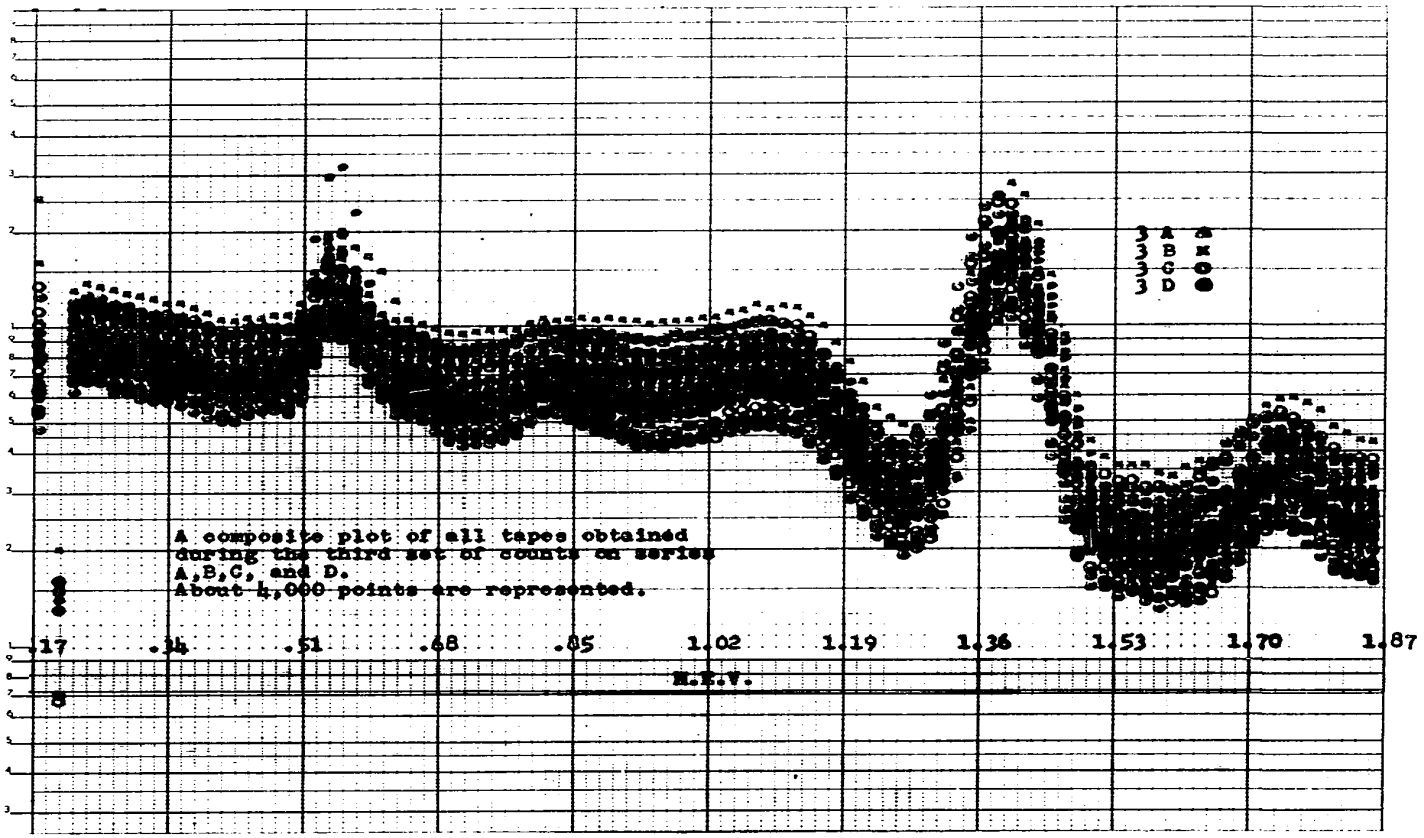
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I N D E X

LIST OF TABLES, CHARTS AND GAMMA SPECTROGRAMS

INTRODUCTION

PART I

Biological Considerations

Hair is the keratinous proliferation arising from follicles, distributed in a characteristic manner over most of the dermal surfaces of the mammalian body. The morphology of the hair is indicative of the region of origin, i.e. pubic, scalp, axillary, etc. Genetic factors modify or directly control; colour, density (of distribution), mean diameter, patterns of distribution and balding, etc. (1, 2, 3).

Hair and its growth characteristics can be used to demonstrate certain nutritional anomalies, particularly in animals where diet may be more easily manipulated and regulated. The specific effects of faulty diets on hair are not well understood, but include certain peculiarities arising from deficiencies or excesses of metals, notably copper, zinc and manganese (1, 2).

The effects of disease, (particularly fevers), and senility, on hair growth and hair morphology are even less well understood but could be considered to be the after-effects of disruption of, or interference

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with, certain general (or particular) metabolic processes and hence with the nutritional factors responsible for hair growth (2).

It appears from the evidence available, that the trace element composition of hair will include those elements entering the metabolic and nutritional pathways of the body as well as those arising from internal and external contamination. Some elements detected in hair have no known metabolic function and are as yet considered merely adventitious; others have been assigned definite functions in the body, particularly the enzyme co-factors including zinc, copper, etc. (6). Only a few elements are thought to have any specific function in the formation of hair (1, 2).

Hair is a preferred route of excretion for certain of the more toxic, adventitious elements, in particular, arsenic and thallium. The disulphide linkages of keratin protein are, presumably, a readily accessible means of withdrawing these poisonous materials from the circulation and immobilizing them in a fashion probably less harmful to the organism than any alternative method of excretion available. Radio-active tracer studies have shown that the rate of incorporation of some ingested elements into hair is relatively rapid,

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such, that in a matter of a few minutes, certain elements can be detected in the growing regions of the hair (12). However, some days must elapse before the emergent hair shaft will contain these radio-active tracers, due to the time required for the shaft to lengthen enough so that the region in question will appear above the scalp or epidermal layers. Scalp hair grows at the rate of 1/3 to 1/2 mm. a day (1, 12).

There is a good base in fact for the assertion that, incorporation of some trace elements into hair is a continuing process, and furthermore, that there will be a direct relationship between these trace elements and the nutritional and metabolic history of the person or animal concerned.

Trace elements can enter the hair by several main pathways. One route is by direct metabolic involment resulting ultimately in incorporation as cellular debris. A less significant (perhaps) manner is by chance diffusion into or entrainment by the growing region of the hair bulb. A scarcely less important way is by external contamination, particularly in view of the fact that the hair is exposed to it for such long periods. This is due in no small measure to the various cosmetic and aesthetic practices indulged in by humans.

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Keratin is known to absorb metals from solutions of their salts, in some cases irreversibly. For instance, arsenic is known to be absorbed into hair from arsenic dust and other environmental arsenic sources or solutions (20). Copper is absorbed from dust and solution; and can combine with cystine by breaking the disulphide bonds. Iron and zinc have likewise been shown to be absorbed from solutions. Lead is readily absorbed, either from dust or solution, and cannot be removed by thorough washing since it too is chemically bound to the keratin. There seems little doubt that many of the trace elements found in hair, enter the hair, to some extent at least, via this route of external contamination.

The hair follicle is nourished directly by a rather extensive capillary network so that the same elements will be available to the hair roots as to any other parts or portion of the body. Nevertheless, there are frequently, striking differences in the relative proportions of some elements found in hair, compared to other body tissues (including skin, another keratinized tissue) either in the natural course of events or following chronic or intermittent exposure to these elements (1, 2, 8, 13, 18). This would seem to indicate some process of

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selection is occurring over and above that due to the availability of disulphide linkages. Perhaps some of the enzyme systems responsible for keratin or pigment production are the reason for these unusually high concentrations of some trace elements found in hair. On the other hand it may be that these high concentrations are caused by a form of ion-exchange within the molecular structure of the hair keratin not directly associated with the disulphide linkages (31). The already established prescence of salt cross-linkages, and hydrogen bonding would facilitate such ion exchanging. The physical structure of the keratin molecule may even permit (to some extent) a phenomena similar to molecular sieving. The degree of hydration would no doubt be an important factor in such a case, affecting as it does the inter-molecular attractions and binding forces, and the intra-molecular spacings, as shown by the work of Astbury (32, 33) and others, (34) concerning the a-keratin and b-keratin configurations.

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INTRODUCTION

PART II

Hair Cycle

In any research involving hair, due consideration must be given to the influence, if any, of the Hair Cycle on the results of the research. All too frequently this influence is obviously unappreciated or even apparently entirely unknown to the researcher with consequent invalidation of the analytical results (25). Even as recent a review as that of Niyogi, S.K. (29) fails to mention the Hair Cycle.

The hair cycle may be defined as a period beginning with the commencement of the growth of a new hair (anagen), in a follicle, followed by a resting period (telogen) and subsequent loss of the hair, the cycle ending with the beginning of a new hair in the same follicle. Criticism of this perhaps oversimplified scheme known as "Dry's System", (26), has been voiced by B.K. Davis who introduces another step known as metagen (27), which would refer to that phase beginning with keratinization of the hair shaft and ending with the beginning of the catagen phase when synthesis of hair ceases.

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In humans, each follicle is autonomous and shows no relationship to neighbouring follicles other than in a very general sense. Each follicle determines its own growing periods and resting periods within a general average for the particular type of hair involved, scalp, pubic, etc. The total length of the cycle varies with the type of hair involved. Scalp hair has the longest cycle, averaging twenty months or more. Vellus hair has the shortest cycle, of only a few months from start to finish. Hair cycle lengths are known only approximately and in some cases are doubtful to say the least.

In the scalp (the hair of most interest to forensic examiners), each follicle shows a remarkable constancy in the length of its particular cycle. This rhythm may be broken by plucking during anagen forcing the start of the telogen phase with the result that a new time baseline is established, the cycle frequency being the same as formerly. There is some suggestion of a seasonal fluctuation in the growth rate of the follicles, although definite proof seems lacking. It is postulated that the increased incidence of ultraviolet light in summer is the causative factor, based on indications that the follicles are slightly influenced

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by U.V. light. Otherwise they are influenced by very few external agents, an exception being hormones. Hormonal control is demonstrable and indeed is predominant in certain types of hair, e.g., axial hair. As a consequence, hairs may be differentiated into three broad classes on the basis of this hormonal control:-

Class (1) Hairs essentially the same in male and female, evidently uninfluenced by hormones, e.g., hair of forehead, infra-orbital region, body vellus. To demonstrate association with this class, hairs from male, female, hypogonadic and castrate persons should be substantially the same.

Class (2) Hairs similar in both sexes but hormone dependent; evidently the hormones are equivalent - in effect, whether male or female. Hairs of axillary and pubic regions fall in this class. These are hairs of an ambosexual nature.

Class (3) Hairs in which full development is normally limited to one sex, e.g., beard hair in men and perhaps the terminal hair of chest, shoulders, etc. in males.

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A random sample of hair, gathered from a normal individual, from one area of the scalp, will include hairs representing many phases of the growth and resting stages.

It should be specified, in any research involving hair, whether cut or plucked hair samples are being used. It is manifestly difficult to obtain numerous, sizeable, plucked samples from live humans. For this reason cut samples are customarily employed. These are satisfactory for some purposes, however, it must be pointed out that the only way to differentiate hairs as anagen or telogen phase is by reference to the roots. In cut samples the roots are necessarily lacking and no such separation will be possible. It should also be mentioned at this point, that, particularly in studies of hair with possible forensic applications in mind, the hairs most likely to fall from the normal head under the usual circumstances and hence, those most likely to be found at the scene of a crime, would be those of the dormant, telogen phase and that these are not the most suitable for techniques involving sectioning, as is explained later. It has been reported that most of the scalp hairs are in anagen phase (85-90%) but that the range of variability is great and the percentage may be as low as 65% in some and as high as 95% in others. Limited studies by this author

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lead to figures of 30% anagen at least, when employing only those hairs at least 6 inches long, as used in the later experiments. Catagen hairs, the transition state between anagen and telogen, are very infrequent comprising as they do only some 1% of the total. The catagen phase is quite short compared to the other phases (27).

If a cycle of 20 months is assumed for the sake of argument and a sample of some 20 hairs is considered and if the anagen phase constitutes 80% of the total hairs, then the sample will have about 16 hairs from the growing phase and 4 from the resting stages, respectively. If the anagen phase is some 16 months and the telogen phase approximately 4 months, the 4 resting hairs will represent hairs ranging from 16 months to 20 months of age approximately and the 16 growing hairs would be 16 months or less in age.

The diet changes daily, even from meal to meal. It follows, that the amounts of adventitious trace elements ingested must fluctuate correspondingly and hence, the trace element concentration in the hair must vary along the length of the hair in like manner, at least for some elements.

It seems obvious, that for the adventitious trace elements at least, there will be only a limited correlation between the amounts in neighbouring hairs.

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The exact effect will be governed by the relative proportion of growing period to resting period, a somewhat unsettled matter at present.

If two adjacent hairs are both in the growing phase (anagen) the adventitious trace elements (internal) will be incorporated in equivalent amounts, at any particular time.

If the adjacent hairs are in opposite phases, then obviously the adventitious trace element (internal) will only be incorporated into a growing hair and none should appear in the resting hair (4, 5).

Referring to Chart I, it is apparent that external contamination will affect all exposed hairs in a reasonably uniform manner regardless of the particular period of the cycle the hair may be in, since, to all intents and purposes, the hairs exposed above the scalp are similar. A growing hair may be perhaps a little less horny in the immediate root region, but this will be probably a negligible factor for most external contaminations. Immediately following a single exposure to external contamination, analysis should show a similar concentration of the element in each hair, as shown in Chart I, Initial Phase. Yet, in a very few months there will be a significant number

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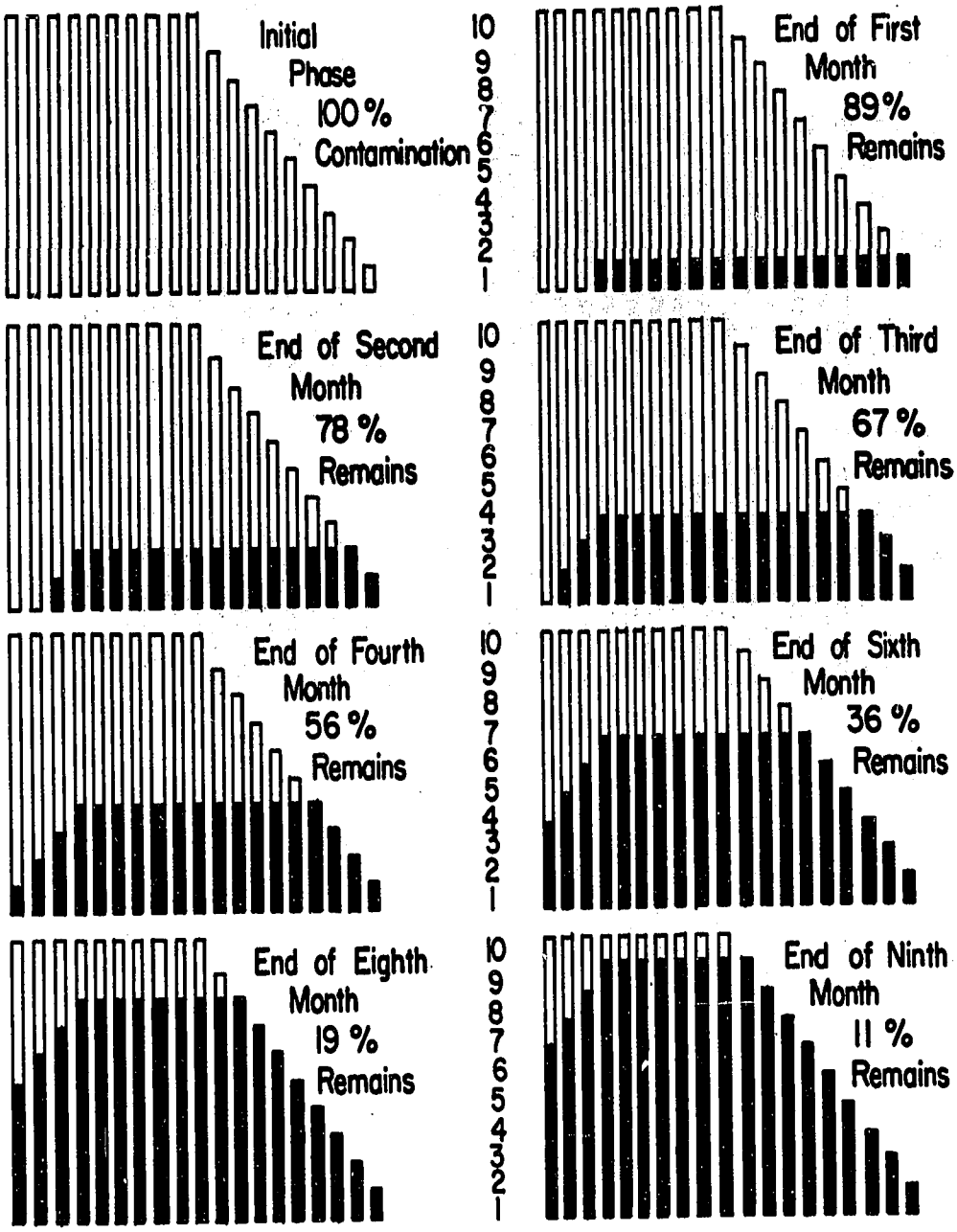
Chart # 1



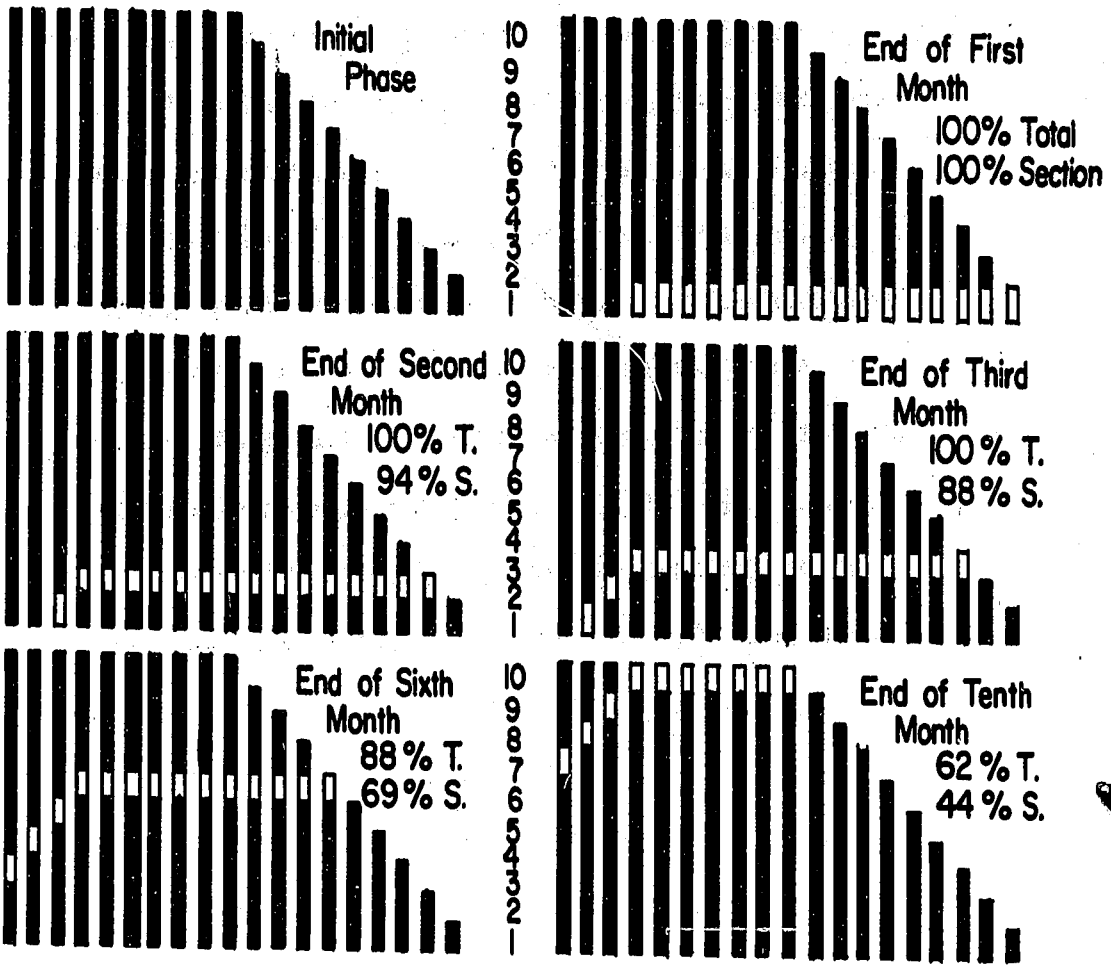
Dilution effect following single instance of external contamination.

Graphical representation of the effects of adding monthly increments to hair, removing equivalent amounts from tip to compensate. The first hair on the left is lost each month, a new one commencing on the right.

A horizontal section taken at level 10 of the ideal hair bundle at the end of the ninth month would show only some 7% of the initial contamination.

A similar sample at level 8 would show 1½% of the initial contamination and a lower section at say, level 6 would show no contamination.

Chart # 2



Attenuation following single dose of internal contamination

A graphical representation of the effect of adding monthly increments to hair, removing equivalent amounts from tips to compensate. The first hair on the left is lost each month, a new one commencing on the right.

A horizontal section at the 10 level of the ideal bundle at the end of the tenth month will include only 44% of the initial contamination. Similarily, a section taken at level 8 will show only approx. 6% of the initial contamination.

of hairs with concentrations of varying total amounts less than the maximum (Charts: I, Second Month; I, Third Month etc.) due to the fact that continued growth has exposed uncontaminated lengths of hair. The overall effect is, then, that some hairs which were in telogen (resting) phase will have a much higher concentration of that trace element than other hairs that were, for instance, only partially grown at the time of exposure, if the whole hair is analyzed (see Chart I). Clearly then, discretion must be employed in drawing conclusions from data gathered from analysis of unselected bundles of whole hairs, as a study of the change in percentages illustrated, will show.

Chart 2 illustrates the percentage concentration changes which can be expected using a similar 20 hair sample and observing the changes evidenced at one month intervals following a single instance of internal contamination. Some interesting work has been done recently by Smith (30), illustrating some of the difficulties experienced when attempting analysis of unselected hair bundles.

Earlier data will have to be re-assessed with these effects in mind (20). For the same reason, caution must be exercised in using any information resulting from sectioning of bundles of hair. This

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technique has been used in the past on the assumption that equal lengths of hair represent similar definite periods in the history of the grower. As has now been established, there may be no particular relationship between the chronological ages of adjacent hairs. Only hairs in anagen phase, of equal length, can be safely assumed to be approximately the same age. The sectioning technique could be used in such a case. In any other instance the hair bundle sections would inevitably contain sections from anagen and telogen hairs, which would be chronologically unrelated. Naturally, such cautions would not apply to cases of uniform chronic exposure extending over many months. Nevertheless, any previous data which presumed chronological relationship (20), will have to be invalidated until such time as the mode and duration of entrance of the trace elements into the hair is established. Such techniques as sectioning can be used, but each hair in a bundle should be examined to determine whether it is in the anagen or the telogen phase. A separate bundle containing only anagen hairs could then be formed for analysis. This preliminary sorting can be done without difficulty by a trained hair examiner provided the roots are intact. It should be evident that only hairs from the anagen

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phase may be employed since no definite age can be assigned to a telogen hair, in normal circumstances.

Knowing the average rate of scalp hair growth, the length of the hair and the fact that it was actively growing (in anagen phase) one may reasonably assign certain regions of that hair to certain chronological periods of the individual's recent past.

It follows, that, some conclusions, drawn from previous work in this field without consideration of and due allowance for Hair Cycle effects, will necessarily be open to question in these respects (20).

The author is of the opinion that many of the anomalies observed in previous analyses by himself and others, can be explained satisfactorily as being due to effects of the Hair Cycle. For instance, suspected cases of arsenic poisoning frequently show only some 4 to 10 ppm. in the hair, (e.g. as reported by Jervis and Mackintosh and Lenihan, etc.) (13, 16, 18, 19, 23) instead of the higher levels one might reasonably expect in some cases of arsenic poisoning. In view of the fact that bundles of hair were used in most, (if not all) instances for these analyses and since out of every 100 scalp hairs in a bundle only a few are less than one month old and many are more than 10 months old and no longer growing (telogen phase), it

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becomes obvious that in some of these (acute poisoning) cases, the arsenic content of some of the anagen hairs is probably significantly high but is being averaged into the low, normal, insignificant arsenic content of the rest of the hair sample. This "Dilution Effect", leads to a lower average value. If anagen hairs were preselected, from the scalp, the arsenic values would doubtless be much higher. (See Chart 2.)

It should also be obvious from a study of Charts I and 2, why it should not be surprising to get quite different analytical results from successive single unselected hairs. For example, if we happened to be examining single hairs from the individual represented by Chart I (end of fourth month), it is apparent that concentrations ranging from 10% to 100% can be expected. Similarly from Chart 2, first month, concentrations ranging from 10% to 100% can also be anticipated. Great caution must, therefore, be observed when interpreting data from single hair studies. In fact, it is difficult to see how very much success (regarding hair identification) can be anticipated from the analysis of single hairs except in certain circumstances, where the source and age of the hair are known and controlled and where con-

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tamination has also been controlled. Data from single hair analysis would appear to be unreliable if these factors are uncontrolled as they would be in most forensic cases.

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INTRODUCTION

PART III

A Definition of Trace Element

Since, for some elements, reliable data is extremely scarce, especially as concerned with human functions and requirements, information from animal studies must be used to supplement data from human studies. There seems to be no particular objection to this as in most cases nutritional requirements are similar, but obviously some care must be observed lest too sweeping assumptions and generalizations are implied or expressed, because there do exist some highly specific peculiarities in the field of nutritional requirements of trace and other elements. One notable instance is the probable use of vanadium instead of iron by a group of marine organisms (Ascidian),as an oxygen storage complex or respiratory pigment (24).

One of the primary problems in discussing trace elements is to satisfactorily define the term. The principal common ground of all definitions is that trace means small amount. From this point divergencies of opinion arise. Some authors favour a rigorous quantitative statement, such as "less than .005% of

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an element calculated on a dry weight basis". Probably the principal drawback to such a restrictive specification lies in the inherent lack of flexibility. Difficulties invariably arise because some species have very different quantitative requirements when essential trace elements are concerned. It follows then that a definition including a, "less than specific quantity", clause is too restrictive for general usage. Furthermore, one must specify what particular valence form of an element is being discussed as well as which organism is being referred to. The additional restrictions stipulated by some authors, defining mode of action or deficiency-syndrome manifestations, are frequently vague and often ambiguous. The phenomena of; interaction, antagonism and sparing-effects, (until recently unappreciated), demonstrated by various elements have done nothing to clarify the problem of defining an essential trace element.

In general terms it might be said that an essential trace element is any element occurring constantly in a particular organism in minute amounts, the lack of which, (or decrease below a certain amount) results in some generally deleterious effect on the health, appearance or well-being of the organism, which effect is removed by the administration of said

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element in certain small amounts. Other parameters may have to be defined in some cases where antagonisms or sparing-effects are suspected or known.

Even such a general statement is to some extent worthless, since there are good grounds for expecting that, in the future, additional essential trace elements will be elucidated. These, until then, unknown co-enzymes, etc., will be required in such extremely low concentrations that it will be impossible to deplete normal body reserves or to remove them completely from food-stuffs and hence the arguments concerning amount and specificity will probably never be completely resolved.

Only when an enzyme is isolated in pure form and the prosthetic group or co-enzyme identified, can a function for it be proven and even then the possibility exists that another element may replace or "spare", the effects of deprivation of one element.

The interaction existing between or among various trace elements further complicates the issue. Molybdenum, for instance, has an inhibiting effect on copper retention that is markedly influenced by inorganic sulfate. A well substantiated association exists between iron and copper. Recent research using rats has shown a relationship between copper and zinc.

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It follows, therefore, that a study of, for instance, iron requirements of an animal, is to some extent meaningless unless copper, molybdenum, sulfate and zinc are also studied and discussed at the same time. Such relatively recent innovations and discoveries have rendered invalid much of the early work done on essential trace element requirements.

Since essential trace elements function in such minute amounts, with such dramatic effects, there appears little doubt that they function primarily as catalysts. In most cases such roles are readily demonstrated, as for example, the role of cobalt as a complex in Vitamin B12, or that of zinc in the enzyme carbonic anhydrase. In these instances the association is a highly specific one and other elements are largely unsuccessful as replacements if indeed they will function at all. At the other end of the scale, however, there are what might be called, "non-specific activators". These include the peptidases and phosphatases wherein the metallic portion or prosthetic group is readily removable by dialysis, the enzyme becoming somewhat less active but still functioning. Replacement by a totally different cation is possible in some extreme instances.

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INTRODUCTION

PART IV

Sources of Trace Elements

Insofar as humans are concerned, trace elements are commonly ingested in food and beverages. The mass production farming and the mechanical-scientific transport, storage and processing methods of modern society are rendering it more and more difficult to find deficiencies in man in the more mechanized countries.

Vegetable sources account in the first instance for most of the trace elements found in humans as in other animals. In vegetables as in all plants there occur carbon, hydrogen, oxygen, nitrogen, phosphorous, and sulphur associated with potassium, magnesium, calcium and silicon. In lesser amounts (micro nutrients) are found iron, manganese, copper, zinc, molybdenum, sodium, vanadium, boron, chlorine and cobalt.

Those trace elements known and demonstrated to be essential to man are: iron, iodine, copper, zinc and manganese. Other elements suspected to be essential include: fluorine, bromine, selenium, molybdenum, barium, strontium and perhaps cobalt.

Since modern man obtains his nutrients from a wide variety of sources; it is obvious that regional

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or geographical deficiencies tend to become obscured readily. Goitre, the deficiency syndrome of iodine, is perhaps the only good example of a regional deficiency still demonstrable in man. Even here, the widespread use of iodized salt is rapidly diminishing the incidence of goitre.

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INTRODUCTION

PART V

Nutritional Functions of Some of the Trace Elements Discussed

Magnesium functions catalytically with the following enzymes: phosphatases, phosphorylases, leucylpeptidases, enolase, glucophosphomutase and carboxylase.

Zinc is a constituent or cofactor for several enzymes: carbonic anhydrase, uricase, phosphatases, carboxypeptidases and some pyridine nucleotide dehydrogenases.

Copper is essential in hematopoiesis and is a cofactor in several enzymes: ascorbic acid oxidase, laccase, uricase, tyrosinase, butyryl CoA dehydrogenase and phenolase complex. Copper deficiency graying has been noted in rabbits, cats and cattle. Deficiency causes loss of crimp in sheep.

Iron deficiency caused hairlessness in rats. Iron is a co-enzyme in cytochromes, catalase, peroxidase, succinic dehydrogenase, xanthine oxidase, aconitase transferrin, cytochrome C reductase and fumaric hydrogenase.

Cobalt deficiency in sheep produced less wool with weak fibres. Also cobalt is associated with Vit. B12.

Manganese is a cofactor with arginase and prolidase.

Molybdenum acts as a cofactor with xanthine oxidase, aldehyde oxidase and nitrate reductase.

Iodine is essential for the production of thyroxine or triiodothyronine.

Vanadium has a possible role in mineralization of tooth and bone.

Selenium acts like Vit. E in many functions.

Specific Functions Associated with Hair

Copper plays a role in pigmentation and is essential for keratinization. If deficient, graying occurs in rabbits, cats and cattle and causes loss of crimp in sheep's wool.

Zinc is suspected to be essential for hair formation, since hair is notably high in zinc, averaging 200 ppm. If deficient, pellegra-like lesions occur and a form of Keratosis.

Iron appears to play a role in the formation of pigment in red hair.

Manganese - large amounts are found in hair but no specific function is as yet known. In cattle a deficiency leads to dead, dry and brownish hair.

Iodine has its effect on the integument and its outgrowths, hair, fur and feathers, in that, normal

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functioning of the thyroid is essential for the adequate growth of hair. Malfunction leads to Cretinism or Myxedema, associated with dry, scanty hair or shedding of the hair.

(1, 2, 6, 7, 8, 9, 10, 11).

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METHODS AND APPARATUS (I)

Trace Element Measurement

Trace element measurement and detection are processes almost invariably attended by several serious obstacles. Accidental contamination before and during an analysis is probably responsible, in part, for the customarily widely varied results quoted in the literature. A lack of sensitivity for some elements limits, frequently, the amount of element detectable unless very large quantities of raw material are used. In human hair studies, the size of suitable sample available (as already has been discussed) is rather restricted and in some cases quite small.

Spectrographic results are frequently open to question when careful consideration is given to the methods of sample preparation used. Hair is one of the more difficult of biological materials to ash and is very subject to contamination from various sources. Results will vary depending on the method adopted. Some representative data (Table #I) from the literature and other sources demonstrates the very wide variations found in spectrographic hair analysis.

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The sensitivity for most elements in a spectrographic analysis approaches about 10^{-8} to 10^{-9} grams per gram of dry material. (Refs. 2, 6, 7, 8, 9, 10, 11, Ashley, A.E.C.L., Chalk River). Colorimetric processes can in some instances exceed this figure but the purification procedures and hence the contamination problems become unwieldy, particularly when several or many elements are to be determined from one necessarily restricted sample.

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TABLE #I

TRACE ELEMENT COMPOSITION OF HAIR
AS DETERMINED BY
SPECTROGRAPHIC AND OTHER MEANS

	Ashley*	Rothman (2)	Cremer HD (10)	Spector**
Ag	.5 (ppm)	.0048 - .045 (ppm)		
Al	6	.00002 - 36		26 - 32 (ppm)
As	X		.5	2.2
Au	X			
Ba	1.5			
Be	.16			
Bi	2			
Ca	250	188 - 4,900		188 - 212
Co	X	14.2 - 18.1		18
Cr	.4	2		2
Cu	13	4.3 - 128		108
Fe	24	.84 - 170		141
Mg	11	10 - 101		
Mn	2	.00001 - 46		25 - 38
Ni	5	5.4 - 8.2		5 - 8
Pb	15	17 - 284	15	21 - 284
Sb	X			
Sn	.7			
Ti	1.4			
Zn	250	9 - 562		116 - 212
Si	1	.000022 - .091		
Hg	X			
Tl	X		.05	
U	X	.000127	40	.000127

X - not detected (average of 3 samples).

* - Ashley. Unpublished data AECL October 1960.

** - Spector Handbook of Biological Data, Saunders & Co. 1956.

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As a method of trace element analysis, Neutron Activation possesses several significant advantages. The sensitivity for most elements is 10^3 or 10^4 times greater than the comparable spectrographic sensitivity. Contamination problems can be very much lessened by performing all chemical operations subsequent to the irradiation so that only very gross chemical contamination will have any significant result. Only contamination by radioactive species is of any particular consequence once irradiation is a fact. A limitation to Neutron activation analysis is the restricted precision obtainable. Since the process depends on the assessment of truly random events (radioactive disintegrations) statistical considerations must be borne in mind and in any event will limit the practicable precision to a few percent (19).

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METHODS AND APPARATUS (2)

Neutron Activation Theory

Many elements when subjected to a flux of suitable neutrons are able to form artificially radioactive isotopes of the parent element, irrespective of the nature or composition of the sample or the chemical combination state of the element. Each isotope produced displays a unique mode of disintegration, rate of decay and energy or energies associated with the disintegration. These unique characteristics can be used to identify, unambiguously, the isotopes present. The amount present can be readily determined by suitable counting procedures.

The most probable reaction during bombardment by thermal neutrons is the capture of a neutron by the nucleus of the stable element. By consideration of the properties of these elements and the conditions of irradiation the expression

$$A = \frac{f \cdot w \cdot N^{0} \cdot \theta \cdot \sigma_{ac} \cdot}{M} \left(1 - e^{\frac{-.693\,t}{T_{\frac{1}{2}}}}\right)$$

may be employed to estimate the activity expected, where;

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A is the disintegration rate of the activated atoms, (d/sec.)

f is the neutron flux, ($n/cm^2/sec.$)

σac is the activation cross section, ($x10^{-24} cm.^{2*}$)

W is sample wt, (gms.)

N^o is Avogadro's number, (atom/gm atom)

M is atomic wt.

Θ is isotopic abundance factor.

$T\frac{1}{2}$ is half life, (hours)

t is time of irradiation, (hours)

Under similar circumstances of irradiation the induced radioactivity will be a function of; the mass of the element present, its isotopic abundance and its activation cross-section. Since wide differences have been found in effective cross-sections and isotope abundances, naturally the sensitivity of the method will vary from one element to another.

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* It must be emphasized that this is not the actual physical dimensions but rather represents, as it were, the apparent willingness of a nucleus to accept neutrons.

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The calculated order of sensitivity for several elements using an NRX flux of 7×10^{13} N/cm^2/sec. is:

Cu^{64}	about	1.2×10^{-11}	gms.
Fe^{59}	"	1×10^{-8}	"
Mn^{56}	"	2×10^{-12}	"
Zn^{65}	"	1.7×10^{-10}	"
As^{76}	"	8×10^{-12}	"

using the following expression to calculate the sensitivity of detection of an element under specified conditions

$$m\ (grams) = \frac{at.\ wt \cdot rate\ of\ disintegration}{f \cdot N^{\circ} \cdot \theta \cdot \sigma ac \cdot \left(1 - e^{-\frac{.693\,t}{T_{\frac{1}{2}}}}\right)}$$

Assuming: (1) no decay period,

(2) irradiation period equal to one half-life at 7×10^{13} N/cm.^{2}/sec.,

(3) a disintegration rate of 200 dpm.

Under these conditions a five hour delay in counting will reduce sensitivity for Mn^{56} by a factor of about 4X or to about 8×10^{-12} gms.

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No consideration has been given to detector efficiencies nor to proportion of usable radiation available. The half-life of the isotope produced limits the sensitivity only insofar as practical considerations such as chemical manipulation are concerned. Any isotope with a half-life greater than 30 minutes can usually be satisfactorily utilized using routine methods. Special procedures are available for the other very short half-life elements, e.g., strictly automated/instrumental procedures.

Herein, the basis of selection of the elements listed (Table 2, page 35) is governed by the half-life, the cross-section and isotopic abundance, the presence of suitable gamma-ray energies and the reported occurrence in human hair. On this basis the elements listed in Table 2 were selected for investigation.

Sodium and potassium were not considered for investigation due to the enormous problems associated with contamination by absorption of sweat, etc. The sheer ubiquity of these two elements prevents their routine use in the same manner as the other elements.

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Preliminary studies were undertaken to determine which of these listed elements could be detected in large samples of hair (4 - 10 gms.) in amounts which would indicate the feasibility of extending the research to progressively smaller hair samples. The ultimate goal would be the satisfactory analysis of a single human head hair.

Work had previously been done on certain isolated aspects of neutron activation analysis of hair, notably in the case of arsenic. Jervis and Mackintosh at A.E.C.L. (Canada), have developed a satisfactory procedure for arsenic determination on small amounts of human hair (13). Lenihan, et al, also developed a rapid method for arsenic determination in hair (17). These methods, while satisfactory for this particular element, are too restrictive for general consideration and a different method was considered advisable in the present case. Arsenic determination by Activation Analysis is the method of choice of the RCMP Crime Detection Laboratories and several cases have been done; these determinations have been done on unselected hair bundles and were not corrected for hair cycle effects.

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TABLE #2

SOME CHARACTERISTICS OF RADIOACTIVE ISOTOPES FREQUENTLY ENCOUNTERED IN ACTIVATED HUMAN HAIR

Isotope	$T_{\frac{1}{2}}$	Parent isotope abundance %	Principal Gamma Energy M.E.V.	x sect. Barns Cm^{-24}	Reported* in hair p.p.m.
Ag^{110}	270days	48.6	M(many)	3.2	.5
Ba^{139}	84mins.	72	.163	.5	1.5
Cr^{51}	27.8d	4.3	.325	13.5	2
Cu^{64}	12.8hrs.	69.1	1.34	4.3	100
Fe^{59}	45.1d	.38	1.1 - (57%) 1.3 - (43%)	.98	140
Mn^{56}	2.6h	100	M(.822)	13.3	40
Ni^{65}	2.6h	1.16	M	1.6	8
Zn^{65}	245d	48.9	1.12	.44	200
As^{76}	26.4h	100	.549	5.4	.5 average
Au^{198}	2.7d	100	.412	96	?
Co^{60}	5.24yrs.	100	1.33 1.17	20	18

* By various methods, by various authors (See Table I particularly)

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In addition to the simple case of the n reaction mentioned above, there are frequently other processes occurring during the irradiation, which must be taken into consideration. The following reactions might complicate the determination of arsenic, (13, 14).

(1) Se^{76} (np) As^{76}

(2) Se^{77} (γp) As^{76}

(3) Br^{79} (na) As^{76}

(4) Ge^{74} (nγ) Ge^{75} β^- As^{75}, As^{75} (nγ) As^{76}

It has been shown that, under the conditions specified, these four additional reactions, while possible, occur only to an insignificant extent and need not be considered in the final calculations.

Similarly in the case of Cu^{63} (nγ) Cu^{64} some possible side reactions are:

(1) Zn^{64} (np) Cu^{64}

(2) Ni^{62} (nγ) Ni^{63} β^- Cu^{63} (nγ) Cu^{64}

These reactions, again, will only be significant when zinc or nickel are present in very large amounts compared to the copper. Interfering or secondary reactions are possible in almost any case but

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are usually of very minor significance; the cross sections for these reactions being very small. Nevertheless they must be considered, especially when working with very low concentrations of an element when contributions from side reactions will be most evident.

It is possible to use Neutron Activation as an absolute method of measurement. Precision is difficult to obtain because several of the governing factors in the equation $A = 6 \times 10^{23} f \sigma ac \frac{W}{M} \times \theta (1-e^{-\frac{.693T}{T\frac{1}{2}}})$, are known only approximately, e.g. cross sections. In addition, the neutron flux of reactors cannot by any means be considered to be constant. Consideration must also be given to attenuation in the sample, non-homogenity of flux, self-shielding, matrix effects and even to the supposed thermal distribution or spectrum of the neutron energies available. For these reasons, among others, absolute determinations are rarely completely satisfactory. In any event, the irradiation of proper standards along with the sample, as in the almost universally employed comparative method, minimizes any deficiencies in flux estimation, matrix or self-absorption or shielding effects and for most purposes is a much more satisfactory procedure for quantitative studies. (13, 14, 15, 16, 17, 18).

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METHODS AND APPARATUS (3)

(1) Irradiation, facilities and procedures used

In some irradiations the NRX reactor facilities were employed using a self-serve irradiation position yielding a nominal flux of 2×10^{13} thermal neutrons per square centimeter per second.

The customary procedure for self-serve irradiations is to weigh the cleaned raw hair into an aluminum capsule with or without polythene or nylon inserts. These capsules are then cold-weld sealed and irradiated as a routine irradiation at A.E.C.L.

Following the specified irradiation, the radio-active capsules and contents are opened in the "active" laboratory for the necessary chemical manipulations prior to the counting procedures, the capsule being discarded at this stage.

In other phases of the work, particularly when short irradiations were required, the "Rabbit" was used. For "Rabbit" irradiations the cleaned hairs were wrapped in foil and enclosed in an iron capsule which was then directed by compressed air into an unused fuel rod position in NRX having a nominal flux of $7 \times 10^{13} N/Cm^2/sec.$ in one position or $1 \times 10^{13} N/Cm^2/sec.$ in another position. The irradiated capsules are then directed back to the "hot" lab for unwrapping and repackaging for counting.

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METHODS AND APPARATUS (4)

(2) Counting Apparatus and Procedures

For virtually all of the later work and in particular for the Hair Cycle Study, an automated assembly was used consisting of a 100 channel C.D.C.* Kicksorter from which the data was routed through a Chalk River transistorized typewriter and tape punch control into an IBM electric typewriter and a Friden tape punch. In this manner, simultaneous tapes and conventional, digital, tabular presentations of the data were prepared. The tapes were thus available for use on an XY plotter so that a final semi-log presentation was readily achieved. The XY plotter used was part of a Chalk River assembly.

The scintillation detectors used, varied, depending on the information being sought. Typically (as in the Hair Cycle study) the detector was a 2" thallium activated NaI crystal used with a 2" photo-multiplier. Following initial amplification in a pre-amplifier the pulses were further amplified in a Victoreen Model 851A linear amplifier and thence were transmitted to the Kicksorter. An Atomic Instrument Co. high voltage power supply was used to power the

*C.D.C. - Computing Devices of Canada.

photomultiplier, which was contained in a very large castle, a 3' hollow cube of 3" thick lead shielding.

In some cases the radio-active source was counted while in direct contact with the housing of the crystal. This, simplified handling and reproducibility and was permissible for less active sources where counting losses were not excessive. In this hair cycle study losses averaged 20% for the first count dropping to a few percent within 24 hours. Losses averaged over 80% when the cobalt monitors* were counted this way. In this case, the simple expedient was used of raising the source some 6" above the crystal by placing it on a cardboard carton. Losses then dropped to the tolerable level of approximately 15%.

The Kicksorter was ordinarily used at a setting of 17 K.E.V. per channel, with channel 1 at a level of 170 K.E.V. Sensitivity was adjusted by controlling the gain of the amplifying systems so that the Cs^{137} photopeak came at channel 30-31.

In some instances difference spectra** were plotted by hand, primarily, wherever it was desired to remove background interferences as much as possible.

The factors to be considered in the counting procedures were; statistical reliability, detector efficiency, background and resolution.

*Page 57.

**Obtained by subtracting background counts from each channel.

Statistical reliability is achieved in counting random events by increasing the total events recorded. Practical considerations limit the time available for counting. Increasing the activity of the sample or decreasing the distance between source and detector is not always the best way to increase counts per unit time. A point is reached where the detector is, in effect, saturated and can no longer discriminate between successive gammas, due to the small, but finite, dead time characteristic of each type of crystal. Such coincident pulses may not be registered, introducing what can be a large error into the counts registered. The error will, of course, be proportional to events per unit time and be a function of the type of crystal used. Corrections may be applied provided various factors are known.

Detector efficiency depends on the shape and size of the crystal phosphor. Shielding and other surrounding materials, background conditions and electrical systems - resolving time, dead time etc., all affect performance. These effects can be greatly minimized by using samples of about the same size, the same source geometry and the same operating conditions, as was done during the research whenever possible.

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The background, (counts registered in the system when no deliberate source is present) for critical counting periods must be monitored or at least randomly sampled. Usually areas, in which it is intended that accurate counting be performed, are selected on a basis of negligible background.

Resolution (as distinguished from resolving time) is a function of the crystal and phosphor used. The photomultiplier and associated circuitry can contribute thermal noise in poorly designed or operated equipment, principally when measuring low energies.

Counting times varied and were chosen with a view to obtaining statistically reliable counts without unduly prolonging counting periods. Sample sizes and irradiation times were selected whenever possible so that counting periods of less than 20 minutes were satisfactory. When dealing with single hairs the primary consideration was to obtain sufficient counts, convenience was a secondary consideration and for this reason, counts of one hour or more were commonly employed.

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METHODS AND APPARATUS (5)

(3) Cleaning Hair

The importance of a cleaning process can hardly be emphasized enough. The problem is not so much in deciding whether or not cleaning should be done, rather, the difficulties consist in determining how much cleaning should be done and the most suitable way to do it.

Hair is very susceptible to contamination from external sources, due to its physical structure and chemical nature. Fine, particulate matter is readily entrained in the natural, waxy, sebaceous covering of hair and in the interstices of the cuticular scales. Under certain conditions a pronounced electrostatic field can exist on a head of hair, a matter of common experience. Cosmetic and aesthetic practices encourage the liberal use of nostrums and potions at frequent intervals, all containing oils, greases and scents. These factors all contribute to the usual presence of an extremely tenacious film or layer of a dirty, greasy nature on each hair. The film can be removed completely, by drastic cleaning methods, resulting, usually, in

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actual damage to or removal of the cuticular layer. Any other, less extreme, cleaning procedure must necessarily be considered incomplete, as can readily be proven by microscopic examination. On the other hand, removal of the cuticular layer can hardly be justified on any other grounds. It could be, that certain elements are concentrated in the cuticular layer for some reason and removal of this cuticle could thus affect the completeness or validity of a trace element analysis.

The cleaning problem is perhaps unresolvable, in a practical sense. It can be minimized, at least, by careful selection of cleaning agents and procedures and by adhering to a rigid time schedule so as to ensure a measure of reproducibility. Since some hairs are more coarse than others, the question arises, "Is the same washing time suitable for all hair samples?". The answer to that question will not be found in this paper or anywhere else. All reagents must be screened carefully since it is reported by Lenihan and Smith (20) that some detergents contain arsenic which is found to be readily absorbed into hair by soaking. It was concluded by those authors that the arsenic was present as an

impurity in the sulfuric acid used in the manufacture of the detergents. Only sulfuric acid of the lead-chamber process variety appears to suffer from this type of contamination, due to the presence of arsenic in the pyrites used as a sulfur source in this case. No doubt arsenic could appear in many products made with this particular acid.

There can be little reason to suppose that arsenic is unique as a contaminant. As is mentioned elsewhere in this paper, many elements are known to be absorbed by hair from solutions, aerosols, dusts, etc. It just happens that arsenic has been intensively studied in this regard recently and the data are available.

Some limited experiments were conducted to determine a permissible washing procedure. These studies resulted in the six stage procedure outlined below:

Stage 1 - soaking in ether, 1 minute with agitation.

Stage 2 - soaking in acetone, 1 minute with agitation.

Stage 3 - soaking in distilled water, 1 minute with agitation.

Stage 4 - soaking in acetone, 1 minute with agitation.

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TABLE #3

SHOWING EFFECT OF WASHING PROCESS
ON SODIUM CONTENT OF HAIR

Sample #		.51 MEV Peak Ht.	Sample Wt. in Grams	Corrected* PK Ht.	Average	%
Raw	1	2100	.26	8064)	7700	100
	2	1600	.22	7280)		
Washed Once	3	1150	.184	6210)	6150	80
	4	1050	.177	5985)		
Washed Twice	5	830	.186	4457)	4650	62
	6	980	.207	4802)		
Washed Thrice	8	980	.193	5096	5100	66

* Corrected means that all peak heights are extrapolated back to immediate end of irradiation = 0 decay time and that all sample weights are equated.

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Stage 5 - soaking in ether, 1 minute with agitation.

Stage 6 - repeat from stage 2.

Stage 7 - air drying, indefinite (at least several hours).

The reagents were available for this procedure in a state of satisfactory purity as far as could be determined by routine chemical analysis. Samples of hair were prepared using the above procedure in various ways, the results of which are shown in Table 3.

These studies were conducted using the level of sodium activity as an index of washing efficiency since sodium had proven to be troublesome during earlier irradiations and all efforts were subsequently bent to minimizing the interference from sodium ^{24}. It appears that two washing cycles abstracted all the sodium that could be readily removed, being due, presumably, to surface contamination. Since it was desirable to perform the least washing permissible, to minimize leaching and contamination, the cleaning process was standardized at two wash cycles, each cycle comprising all six steps, with the exception, that step 7 was eliminated between cycles 1 and 2 and step 6 after cycle 2.

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Earlier work on this project had demonstrated the desirability of a water washing to remove as much sweat as possible, otherwise very high, unrealistic values for sodium and chloride were obtained. It was found, however, that preliminary treatment with ether was necessary to remove natural and introduced hair oils prior to water washing, otherwise the water was unable to wet the hair satisfactorily. The acetone is employed primarily as an agent miscible with both ether and water. Detergents and other wetting agents were avoided to minimize contamination (20).

Probably any similar washing procedure would prove as suitable, providing care were taken to ensure reproducibility. Jervis et al, (19) used water and a mixture of acetone and methanol. No tests were conducted to determine the thoroughness of this system. In another paper, Mackintosh and Jervis used acetone followed by three water washings. This system was not tested either. Cleaning data is lacking in papers from most other authors. *

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* Although Baychi and Ganguly (8) appear to have used a very elaborate cleaning procedure which while probably efficacious in one way could hardly fail to grossly contaminate the hair in other ways.

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It is difficult to establish whether cleaning was even attempted in some earlier work. Since hair is so easily contaminated, making washing obligatory, apparently some previous work must be viewed with reserve and even suspicion. Most of the trace elements reported earlier may have been actually external to the hair in the form of adhering or introduced contamination.

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METHODS AND APPARATUS (6)

(4) Chemical Procedures

It was originally intended to perform the counting procedures on the irradiated hair without chemical manipulation but it was found that the Na^{24} activity seriously hampered the acquisition of useful data by purely electronic processes for a lengthy period following irradiation.

Necessarily then, a method of removing the Na^{24} activity was desirable particularly if the shorter lived isotopes were to be studied, e.g. Cu^{64}, Mn^{56}, etc. (12.8h and 2.6h respectively).

Many procedures were studied and rejected either because of the tediousness associated with the process and hence its unsuitability for a number of routine analysis or because reproducibility appeared to be a problem. In the end two methods were adopted for further consideration: "Modified Sulphide Precipitation" and "Anion Exchange Using Chloride Complexes".

A) The Sulphide Precipitation as ultimately adopted consists of the following steps:

a) Dissolve the washed, weighed, irradiated hair using 10 x the wt. of the hair of conc. sulfuric acid.

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Add an accurately known amount of arsenic carrier (pentavalent) (about 10 mg. As.) Heat to fuming and add 30% H_2O_2 until a clear, pale yellow solution is attained. (Total time about 30-35 minutes.)

b) Dilute to an acid concentration of about 2 M. Add H_2S gas. (saturate) Filter through a millipore filter. The dried precipitate is weighed for chemical yield and examined by gamma spectroscopy. (fraction I).

c) The filtrate is boiled to remove free H_2S. The filtrate is treated with NH_4OH to pH 9 after adding Fe. carrier (about 10 mg.).

d) The hydroxide-oxide ppte. is filtered off, weighed and dried and examined by gamma spectroscopy. (fraction II).

e) The filtrate is then treated with H_2S at about pH 9 after adding Zn carrier (10 mg.) The ppte. is filtered, dried, weighed and examined by gamma spectroscopy. (fraction III).

f) The filtrate is evaporated and examined for Na^{24} and other activity (fraction IV), as a check on the completeness of the various precipitations and filtrations.

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This procedure was expected to yield these fractions: (See also accompanying gamma spectrographs pp. 52A and 52B.)

(I) the acid sulphide fraction to contain; As, Mo, Cu, Au, Sb, Pb, Cd, Re, and Sc.

(II) the hydroxide-oxide fraction to contain; Ca, Al, Fe, Cr, Mg, Sr, and Barium in the form of the daughter La^{140}.

(III) the basic sulphide fraction to contain; Zn, Co, Ni, and Mn.

(IV) the filtrate fraction to contain; Na and K.

Chemical yields calculated as As, Fe, and Zn averaged 60-80%.

As was expected, very little Na^{24} was detected in the pptes. It appeared principally in the filtrate (IV).

Separations were on the whole satisfactory although some elements apparently were incompletely precipitated on occasion, appearing in subsequent precipitations, as carry over.

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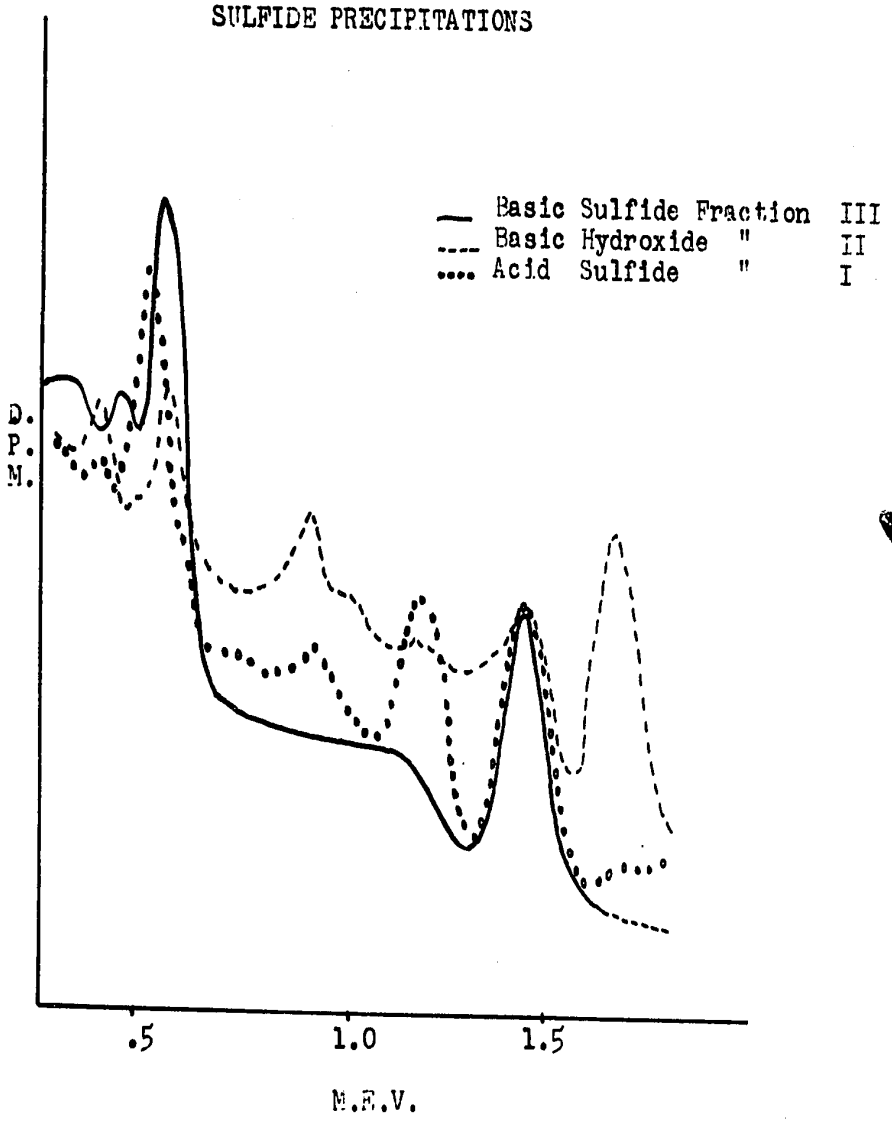
SULFIDE PRECIPITATIONS



Approximately 6 hours after end of irradiation.

SULFIDE PRECIPITATION

Acid Sulfide Fraction

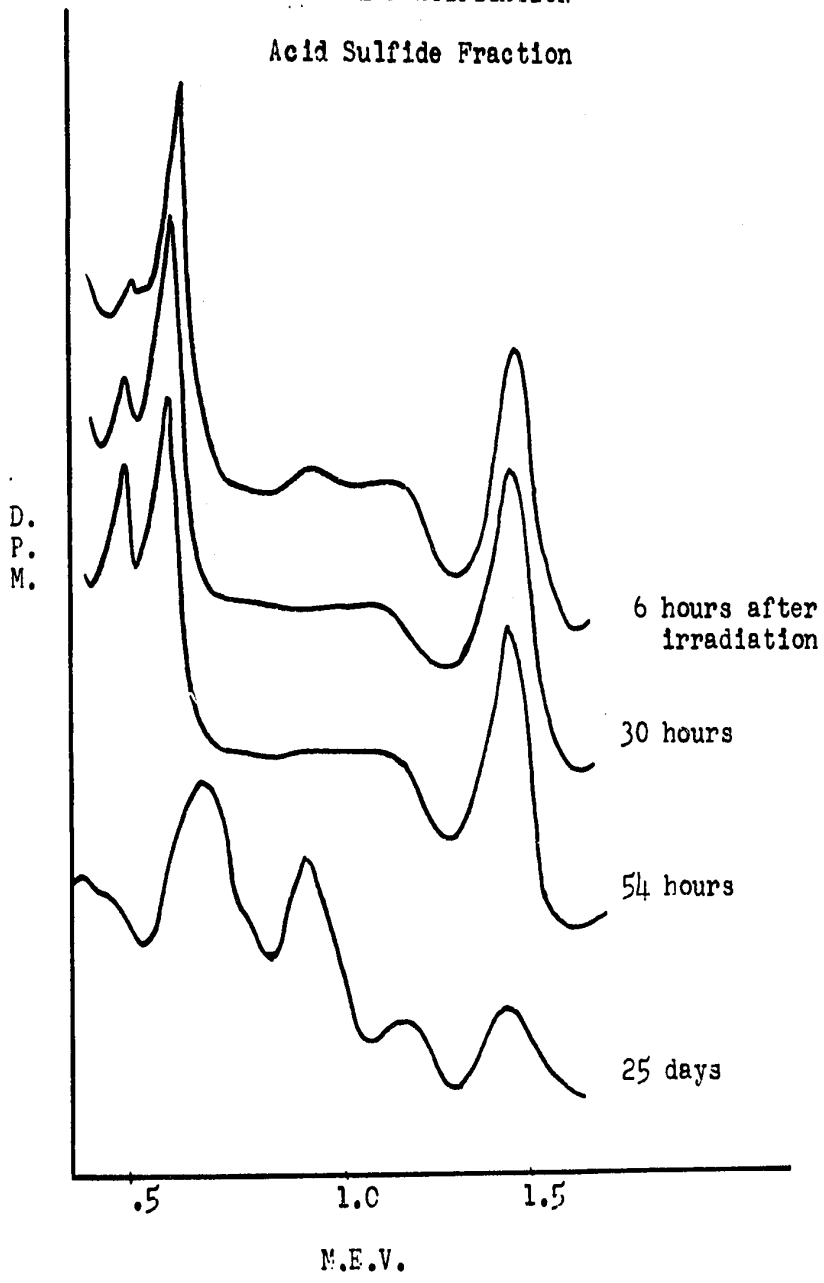


TABLE #4

SOLUBILITIES OF SOME SULFIDE SALTS**

		Cold Water	Hot Water
Magnesium Sulfide		dec	
Aluminium	"	sol	
Antimony	"	.000175	
Arsenic	" *	insol	
Cadmium	"	.00013	
Chromium (ic)	"	insol dec	
Cobalt	"	insol	
Copper (ic)	" *	.000033	
Gold (ic)	"	insol	
Iron (ic)	" *	v sls (dec)	.001
Lead (ic)	"	insol	
Manganese (ic)	" *	insol	
Mercury (ic)	"	.000001 or insol	
Nickel	"	insol	
Selenium	"	insol	
Silver	"	.00002	
Strontium	"	sol	
Tin (ic)	"	.00002	
Zinc (ic)	" *	.00069	.000065

** (Handbook of Chemistry and Physics, 40th Edition).

* Elements listed in Tables 2 and 3.

dec = decomposes
sol = soluble
insol = insoluble
v = very
sl = slightly

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The alternate procedure considered and tested was

B) Anion Exchange Using Chloride Complexes (21, 22).

The principal obstacle in this method was the conversion of the sulfuric acid oxidation solution to a hydrochloric solution for the formation of the chloride complexes. This was accomplished principally by vigorous evaporation and addition of HCl conc. The HCl solution, adjusted to about 8 M. with 10 mg. each of several carriers added, was run through a column of Dowex 1, prepared according to Nelson and Kraus (21), and the successive fractions were eluted with progressively weaker acid solutions ranging from 11 N, 9.6, 8.2, 6.9, 5.5, 4.1, 2.7 to 1.4 N to distilled water, followed by conc. NH_4OH. There was, of course, some degree of overlap in such a series of serial elutions and it was found that several fractions could be combined without materially altering the separations obtained which, on the whole, were quite good and roughly as expected. The appended charts demonstrate the effects of elution with progressively more dilute acid. Chart 5 shows the gamma spectrograms obtained from each eluted fraction immediately following elution of all fractions. Chart 6 shows the gamma spectrometer scans

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obtained from the same fractions four days later. Chart 7 shows the scans obtained after a four months decay period. These demonstrate first of all, the sometimes remarkable chemical separations obtainable with ion exchange procedures. In the second place, the four day and four month scans demonstrate that certain elements, at first prominent, soon decay to insignificance and in their place certain long lived isotopes achieve prominence after a period of time.

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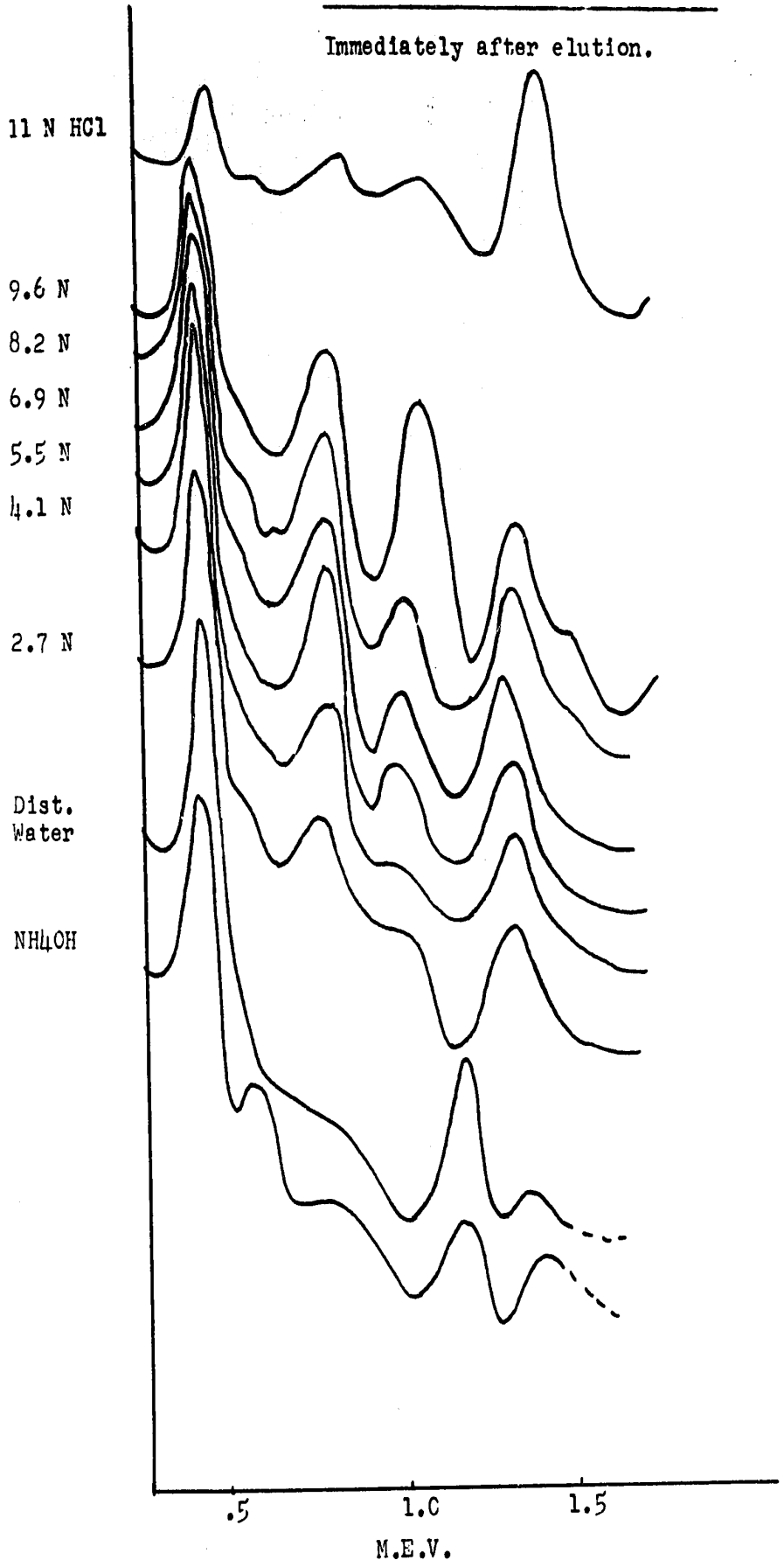
ION EXCHANGE CHLORIDE COMPLEXES

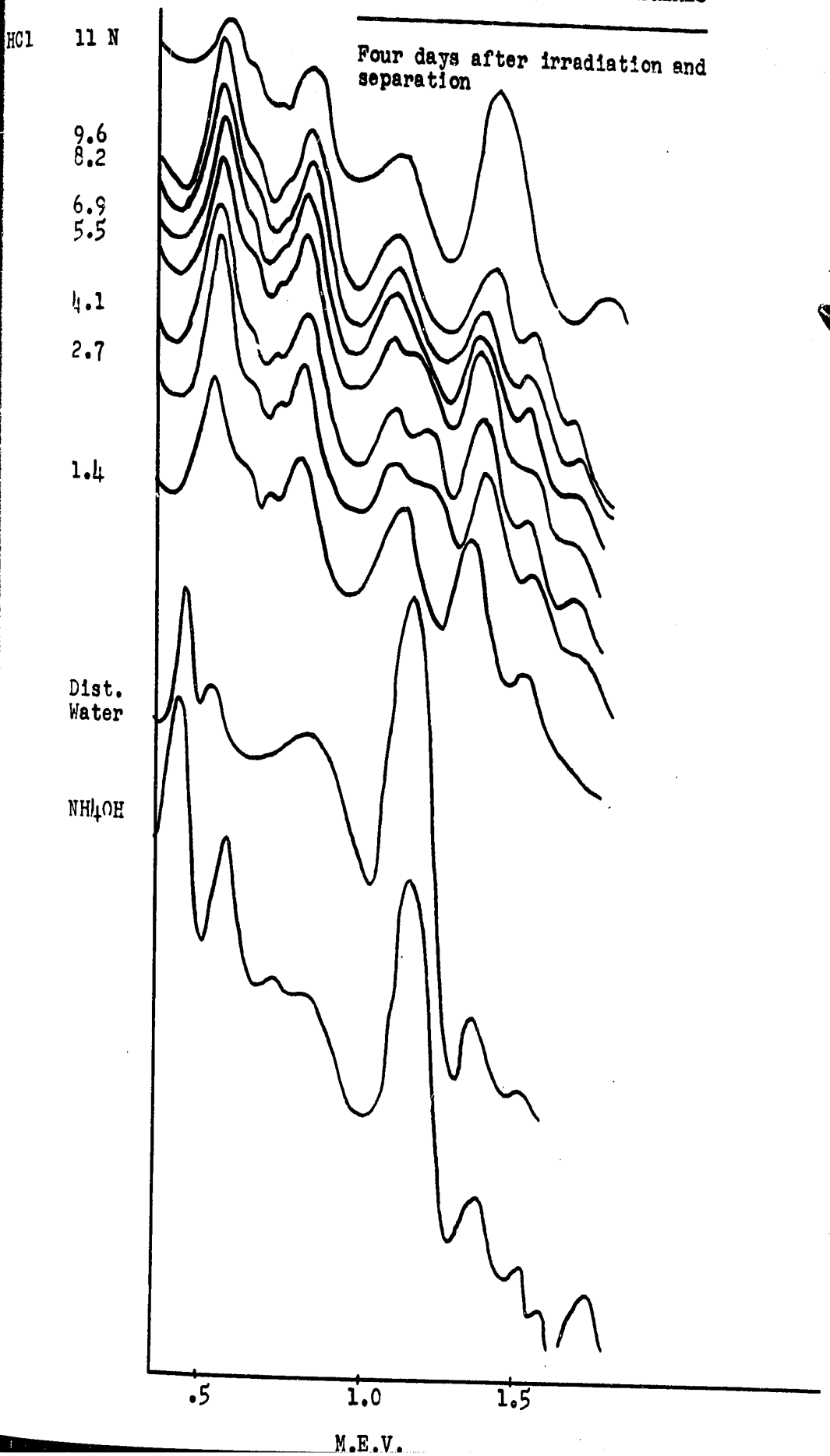
Immediately after elution.



55 B

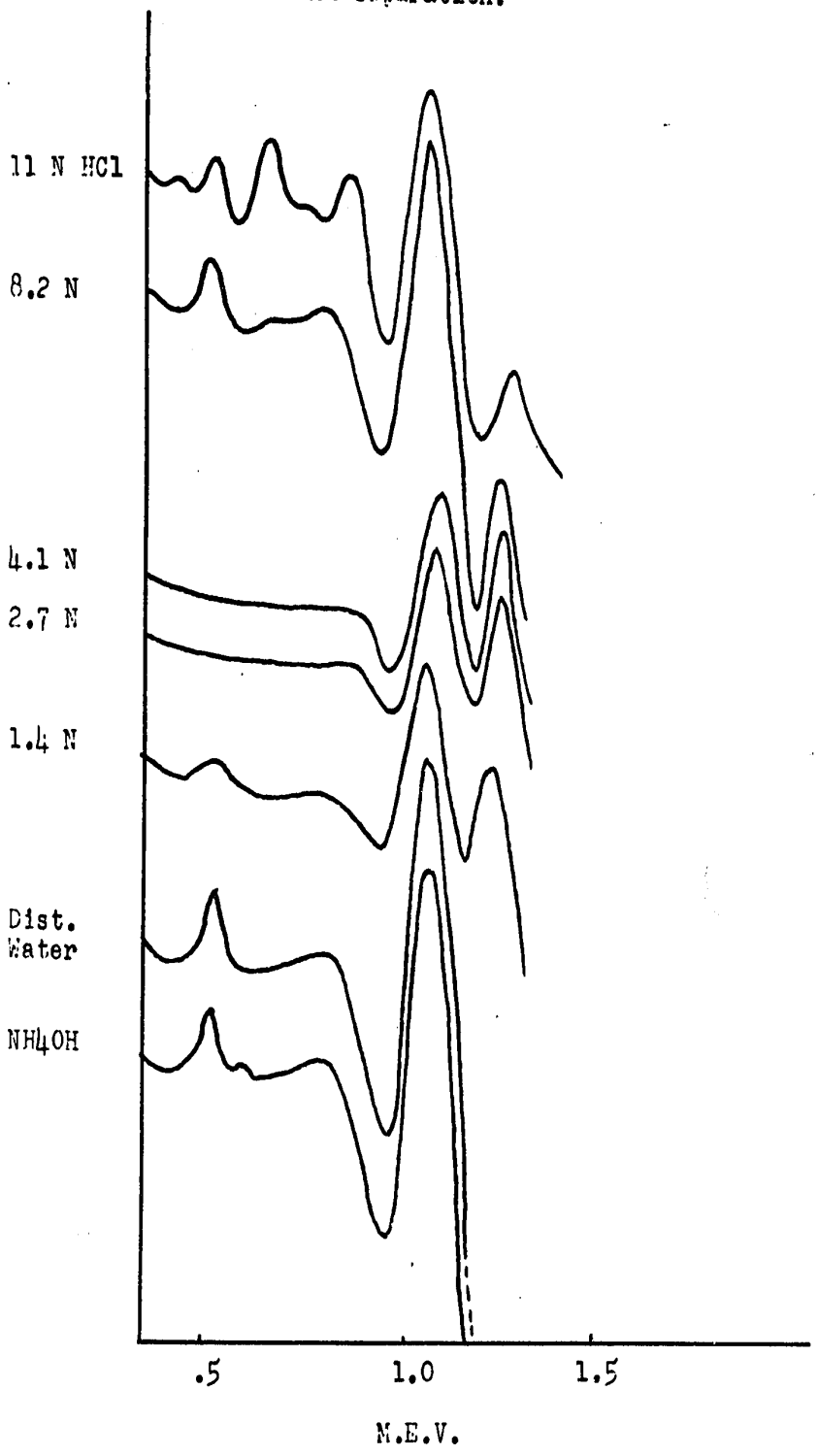
Chart 6

ION EXCHANGE CHLORIDE COMPLEXES



ION EXCHANGE CHLORIDE COMPLEXES

Four months after irradiation and separation.



An Experiment Designed to Illustrate the Effects of the Hair Cycle on Data Obtainable from Human Hair by Neutron Activation Analysis

For this study, 80 hairs were selected from a batch of some 123 which had been individually and randomly plucked at one time from the same area of the scalp of a 24 year old woman. The diet, environment and cosmetic practices of this subject are considered typical, with the reservation, perhaps, that she had experienced very few professional hair treatments of any sort during her lifetime.

The 80 hairs mentioned consisted of two lots. One lot of 40 hairs represented almost the entire content of anagen hairs found in the 123 hair sample (46 were definitely classed as anagen on the basis of a microscopic study of the roots). All the other hairs were identified as "not definitely anagen hairs", and included those hairs definitely "telogen", as well as those whose roots had broken off on plucking in addition to those hairs which were tentatively classed as "doubtful anagen". From these 77 hairs, 40 were selected and called "the telogen series".

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The entire sample of 123 hairs was washed using the procedure outlined on page 45. The 80 further selected (40 plus 40) were then each trimmed to a length of 6" by removing hair from the tip end only. The tip end can be readily determined (if there is any doubt) by pulling the hair between the fingers, the direction of easiest pull is from root to tip.

Sixty of the 80 hairs were individually weighed (Table 6, p. 63) and then each of them was inserted into individual one cm. lengths of polyethylene tubing (approx. 1 mm. ID). Ten of these packed hairs were inserted into a standard iron capsule used for pneumatic facility (rabbit) irradiations in the N.R.X. reactor. Into each capsule also was inserted a 1 cm. length of an Al/Co alloy of 1% Co content, to serve as a neutron flux monitor. The weights and activities of some flux monitors are seen in Table 5, appended. The gamma spectrograms are also appended (see Appendix, last page).

Irradiations were of $16\frac{1}{2}$ hours duration in the "J" rod position of N.R.X. reactor (nominal flux of 1×10^{13} $N/cm^2/sec.$). Following irradiation, using the rabbit facility which happened to be convenient for the purpose and was available at this time, the capsule was opened and discarded and the hairs were transferred to fresh, labelled sections of tubing of somewhat longer length to facilitate handling.

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TABLE 5

Wts. and Peak Hts. of Cobalt Monitors

	wt. gms.	Counts/Unit Time at 1.19 MEV and 1.33 MEV	
A series	.03446 ± .00005	13960	10600
B "	.03450 "	14240	10900
C "	.03446 "	14120	10800
D "	.03360 "	13600	10400

Since the weights and activities correspond within a few percent no corrections were deemed necessary to the values obtained by counting the respective series A, B, C and D. Had extreme accuracy been necessary the flux could have been considered to have been some 2 - 3% lower during irradiation of sample D and corresponding increases could be made in the data for that series. This was not done however for reasons stated elsewhere.

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Each capsulefull of 10 samples was designated with an alphabetical letter A, B, C, etc., and each hair was further labelled with a number 1 to 10, so that each hair was identified thusly; A1, A2, A3 etc. = A series. Since there were 40 hairs in the anagen group there were four series, A, B, C and D. The telogen group consisted of series E, F and G. The E and F series were prepared, irradiated and counted in as nearly identical fashion to A, B, C and D as was possible.

G series, on the other hand, was treated somewhat differently. In this case it was desired to examine the longer half-life elements in the (unfortunately unrealized) hope that the similarities and differences were to be found therein rather than in the short half-life material examined thus far. The G series was irradiated for some 10 days using the self-serve irradiation facilities available at N.R.X. Since more space is available in a self-serve capsule than in a "rabbit" capsule, twenty hairs were irradiated together along with the cobalt monitor. Several of the resulting "gammagrams" are appended for the sake of completeness, (see Appendix).

The A, B, C, D series were irradiated on successive days and in each case counting was commenced (gamma spectroscopy) at 11:00 a.m. of the same morning

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irradiation was terminated. Each hair was counted three times the first day after irradiation, once a day for the next three days and once again following a lapse of one day, once again after a further lapse of two days etc. The E series was irradiated and counted some four weeks after A, B, C and D series. The F series were examined some nine days after that.

The counting schedule followed may be seen in Table 7. Initially, counts were of 10 minute duration, however, after about 6 days it was necessary to lengthen counting periods to one hour to accumulate sufficient counts. In all cases, counting was performed with the sample in contact with the crystal shield. Losses were high (20%) for early counts but, since all samples were counted the same way, such losses were ignored.

The CDC Kicksorter was used, as before described, with the addition of a Friden tape punch (Datatron format) so that a tape was punched while the printout phase was being performed. These tapes were then put through an XY recorder so that the linear readout from the Kicksorter was plotted in a semi-log presentation. Since some 400 graphs were prepared for this study it can be appreciated what a time-saver this recorder was. The frontispiece graph contains something like 4,000 points and would never have been considered had manual plotting been required.

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Table 7

Counting Schedule

SERIES.	DAYS 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
A#	1A 4A 5A 6A 7A 8A 9A 10A X 2A 3A
B	1B 4B 5B 6B 7B 8B 9B 10B X 2B 3B
C	1C 4C 5C 6C 7C 8C 9C 10C X 2C 3C
D	1D 4D 5D 6D 7D 8D 9D 10D X 2D 3D
E##	1E 4E 5E 6E 7E 8E X 2E 3E
F###	

\# Irradiated June 3/63, beginning at 1600 hours.
\## Irradiated July 8/63, beginning at 1600 hours.
\### Irradiated July 17/63, beginning at 1600 hours, counted once Sept 17/63.

1A, 2A etc. indicates first count of A series ($1A_1$ to $1A_{10}$), second count ($2A_1$ to $2A_{10}$), etc.

No corrections for weight or flux difference were applied in any instances, although the data that would be required for these corrections, if necessary, was obtained when possible. The principal reason mitigating against such corrections was, of course, that the XY plotter could not be used without the time-consuming and expensive step of repunching all tapes on a properly programmed and informed computer. The advantages accruing from these corrected plots would be tenuous in any event, due to the possibility of a flux variation of some 10% along the length of the irradiation capsule which, if present, could completely conceal any quantitative variations of lesser magnitude found from otherwise completely corrected data.

It was originally anticipated that the primary objective of this particular study could be adequately served by a strictly qualitative comparison. This anticipation has not been completely borne out as a study of the graphs and charts will reveal.

A few hairs were selected at random and the corrections were applied to some of the data to attempt to visualize the magnitude of adjustment indicated by these cases. The flux differences were ignored since no appreciable flux difference was found to exist. Weight corrections were tested, however, and were found in most cases to accentuate, even more so, the similarities between the anagen samples.

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The weights of the hairs are shown below since it is interesting in itself to see the range encountered (or available perhaps) in semi-random scalp hair sampling. These hairs are washed and trimmed to 6".

TABLE 6

A	B	C	D	E	F
.00059	.00080	.00066	.00082	.00107	.00098
.00088	.00110	.00068	.00091	.00096	.00085
.00066	.00100	.00100	.00071	.00085	.00107
.00082	.00081	.00096	.00112	.00072	.00104
.00085	.00087	.00075	.00077	.00100	.00103
.00070	.00050	.00077	.00073	.00099	.00119
.00100	.00079	.00078	.00070	.00087	.00093
.00086	.00092	.00103	.00110	.00102	.00094
.00090	.00059	.00112	.00083	.00083	.00095
.00084	.00081	.00100	.00057	.00095	.00076

Anagen, average wt. .00082 gms.

Mostly telogen, average wt. .00095 gms.

Insofar as was possible - every effort was made to schedule counting periods so that comparisons between series would be facilitated as much as possible, i.e. sample A1 was counted for the first time

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at 11:01 a.m., sample B1 was counted 24 hours later at 11:01 a.m., C1 was 24 hours later etc. After several days had elapsed following irradiation it was no longer necessary to be quite so rigid in observing counting periods since the rapidly decaying short half-lived materials had diminished by then to a great extent. The cesium 137 standard was counted at appropriate intervals (2 or 3 times a day) so that drift in the Kicksorter would be kept to a minimum by frequent minor corrections to the fine gain control (drift was generally less than 1 channel in a four or six hour period).

It had been predicted, from considerations of the hair cycle, etc., that the degree of correlation among the 40 anagen hairs (A, B, C and D) would be very close, in fact, no significant differences were to be expected. Allowing for the quantitative differences already mentioned, and expected, due to the lack of correction for weight, etc., an examination of the appended graphs and spectrograms shows that the expected similitude is indeed realized. The only stipulation observed in selecting these spectrograms is that they, of necessity, must represent nearly similar stages of the radio-active decay scheme of the hairs. It is not permissible, obviously, to compare a hair freshly irradiated to a hair irradiated some days or weeks previously.

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The simple expedient of plotting together the graphs of all the hairs of a similar relationship, for instance; 2A2, 2B2, 2C2, and 2D2 (see Appendix) shows that these curves could be readily superimposed with only minor quantitative adjustments. Similarly; 4A2, 4B2, 4C2 and 4D2 show the same degree of similarity, at a later count.

Many plots were done, (see Appendix) choosing hairs more or less at random and arranging the data in many ways. If, for example, all of the hairs in any one series were plotted together; 3A1, 3A2, 3A3, 3A4 — etc., once again a high degree of similarity is encountered (bearing in mind that 3A10 would have been counted some 2 hours after 3A1 and thus had decayed two hours more than 3A1).

In short, no matter which hairs or series of hairs were compared, a very remarkable relationship could be readily seen, bearing in mind the restrictions regarding matching decay periods.

Unfortunately, the foregoing observations regarding similarity among the anagen hairs were also found to hold true of series E, F and G of the "telogen series". A study of the spectrograms appended will show no obvious differences between these telogen series and the earlier series, in fact the similarity, on the contrary is remarkable.

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DISCUSSION

Investigations to date, using whatever neutron activation procedures were most suitable, have shown the following elements to be present in human hair: arsenic, copper, zinc, iron, silicon, sodium, vanadium, gold, cobalt, manganese, molybdenum, selenium, chlorine, bromine, iodine, mercury, germanium, chromium and manganese (19). Barium in the form of the daughter La^{140} was found by Kerr in material fractionated by sulfide precipitations. Some later studies showed antimony to be present in significant amounts. These 20 odd elements, while representing those most readily observable using routine methods, are probably only a small part of those actually present. Bear in mind, also, that frequently one or more of the above listed elements may be absent from a hair sample. Mercury, for instance, is by no means always detected, at least using present procedures. Particularly when dealing with small samples or single hairs, it is not uncommon to find that many of the elements are difficult, or impossible, to detect.

The results quoted by Jervis, Perkons, Mackintosh and Kerr (19) are a good example of what may be accomplished by instrumental means. Very little chemistry was employed with the resultant advantages accruing thereto. There are limitations to such a procedure, in that, some elements

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may not be satisfactorily worked with, due to masking, spectra overlap, etc. On the other hand, chemical separations are at best tedious and at worst, can be a source of error.

A judicious blend of both procedures would appear to have merit in some cases. The increased time expended is perhaps justified by the additional elements utilizable and the extra sensitivity and accuracy possible on occasion.

Some of the more disturbing variations found in the literature are; values for calcium ranging from 188 ppm to 4900 ppm; manganese values from 1×10^{-5} ppm to 46 ppm; strontium varying from 23×10^{-6} ppm to 91×10^{-3} ppm. One author, Cremer (10) reports 40 ppm of uranium; another, Rothman, quotes 12.7×10^{-5} ppm for the same element. Whether these remarkable variations represent true extremes of concentration or merely the effects of gross contamination, before or during analysis, is a moot point.

Ashley (unpublished data A.E.C.L. see Table I) reports the presence of beryllium in one sample of hair. It was detected in a hair sample taken four years previous to analysis. More recent samples from the same source do not show this element. In general, Ashley's results appear typical of those reported elsewhere. As in most spectrographic analyses, Ashley's samples are

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large, unselected, random samples of hair from an entire head. They were washed as outlined on page 45. Ashley reports the presence of tin and bismuth as well as barium, elements which do not appear to have been mentioned elsewhere as yet, with the exception of barium which was reported by Kerr (19).

The theoretical aspects of the Hair Cycle and its effects on trace element content of hair are based on reasonable and sound, premises and postulations. There can be little doubt concerning the mechanics of trace element absorption into, and excretion by, the body. These are reasonably straightforward processes. Much doubt still remains, however, regarding the actual incorporation into, or diffusion into, or contamination of, the hair by these trace elements. It appears most likely that no one single process is at work here, but rather that several possibilities must be considered, and it appears probable that, under different circumstances, different processes will predominate.

Although there is, as yet, no direct evidence to indicate positively that it must be so, it appears likely that a significant part (if not most) of these trace elements enter the hair by a process of external contamination. The exact mechanism will vary depending on the material and the circumstances. By the same token, the problem of adequately cleaning the hair prior to analysis raises several insurmountable difficulties.

That cleaning is mandatory can be established by even a cursory microscopic examination. The most desirable cleaning method is, of course, the one which causes the least contamination in itself. For this reason it appears necessary to forego the undoubted advantages of detergents and rely principally on the more volatile, organic solvents from which metallic contaminants are more readily removed. At least one wash with distilled water is necessary to remove adhering salt and similar detritus. Reproducibility of the washing process is important in view of the fact that any of these washing procedures results in either leaching materials from the hair or adding contaminants to the hair. All that can be done is to minimize this as much as possible and attempt to reproduce that which is unavoidable. It is unfortunate, that (as far as can be determined) most previous chemical analyses of hair have overlooked, or unduly minimized, this very important aspect of hair cleaning. Cosmetic cleaning will not suffice.

Much time and effort was expended in an endeavor to amass sufficient data to demonstrate that the Hair Cycle did, in fact, govern the incorporation of elements into the hair and also, that a predictable pattern of incorporation would result from a given type of contamination.

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Unfortunately, this attempt has been, to date, largely unsuccessful. Several reasons suggest themselves. The sample material may have been gathered from the "exception" rather than from the "rule". The donor of the hair used leads a rather sedentary life and, in fact, has not, in the last ten years, been more than, at most, a few hundred miles from her birth place and then only for very brief periods. Her diet also is unspectacular. These factors may have a bearing on the lack of difference between her anagen and telogen hairs.

It is a possibility also, that the method of analysis used was inadequate in some respect. The sensitivity of activation analysis is good but nevertheless - one 6" hair is a rather restrictive sample to work with. Of necessity, the work was done using the most prevalent, (highest concentration) elements and the differences expected may not, in fact, lie therein.

For these, and other, reasons, it appears that there is no other satisfactory alternative than to use what might be termed a "doped" sample. Time has not permitted such a study to be commenced but all logical considerations point to such a study as being a very desirable one and even a necessary one. The project, as envisaged by this author, would consist, simply, of deliberately contaminating a scalp by, perhaps, shampooing with a soap to which a small

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amount of gold chloride had been added. This would constitute a, "single instance of external contamination". This scalp would then be sampled at appropriate intervals and analysed for the remaining gold. The pattern of gold distribution should eventually resemble that of Chart I. In like manner, a scalp could be prepared showing the effects of a single dose of internal contamination using some suitable easily analysed material - zinc for instance.

For the sake of completeness, samples could be collected for a period of 24 months following treatment. If the trace element pattern found, corresponds to Charts I and II then it would indicate almost complete vindication of the predictions set forth in the section concerning the Hair Cycle. The reservations regarding the use of single hairs would also be substantiated.

If all these surmises are found to be true, it will indicate that altogether too much stress has been placed on the chemical aspects of the trace element problem in the past and insufficient study has been concentrated on the biological facets involved. These two fields must be integrated for truly meaningful interpretations to emerge from this and similar research projects involving biological materials.

Neutron Activation Analysis remains a very promising tool both for routine analysis and for such highly

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specific employment as that involved in forensic hair identification. It is the only feasible analytical method suitable for analysis of a single hair. To use the process for this particular application one must be, however, fully cognizant of the problems involved, in order that the reputation of the method will not be jeopardized by a too-hasty or ill-considered, erroneous or misleading conclusion. It is unfortunate, but true, that such a mishap would tarnish the method to some extent rather, perhaps, than the user.

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SUMMARY

Nutrition, metabolism and contamination play the significant parts in determining the trace element composition of hair. Definite correlations between certain elements and particular morphological manifestations in hair have long been established. Other factors influencing metabolism and morphology are, as yet, less well correlated.

Most of these trace elements are normally present in hair in very small amounts, of the order of a few parts per million or less. Assessment of such trace elements is hampered by the lack of suitably sensitive methods of analysis. Sensitivity is necessitated by the limited amounts of suitable hair available from a specific area of an individual at any one time.

Activation analysis, employing thermal neutrons to determine these trace elements, is discussed, along with some results obtained using this procedure. Sulphide precipitation and ion exchange of chloride complexes are the two chemical separation methods employed. The major portion of the work was conducted using strictly instrumental means.

Eventually, it is anticipated that such analyses may help to identify the individual from whom the hair

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was obtained. The importance of the role that the Hair Cycle plays in such an analysis is discussed and suggestions are presented in an attempt to circumvent the many problems presented by the effects on the cycle.

Experiments designed to test the effects of the Hair Cycle are outlined. The results of one such test are appended and a discussion of the significance of the test is included. It would appear that much of the previous data regarding hair analysis should be viewed with a great deal of caution.

The problem of adequate hair cleaning is also mentioned as are some of the difficulties involved. A compromise cleaning method is detailed.

It is increasingly obvious that much more attention must be paid to the biological aspects of hair growth if truly meaningful interpretations are to be gathered from any analysis involving hair. In the case of single hairs it is at least theoretically essential to be cognizant of the immediate past history of the hair involved. An assessment of the elements present can be quite meaningless unless certain pertinent facts are known concerning the involvement of the Hair Cycle.

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APPENDIX

1st page — Composite of Gamma spectrograms of C_3 and C_4 showing almost perfect superimposition, both initially, and following many hours of decay.

2nd page — Gammagrams of sample #2 from series A, B, C and D, second count.

3rd page — Gammagrams of sample #2 from series A, B, C and D, fourth count.

4th page — Gammagrams of samples 1-5, A series, third count.

5th page — Gammagrams of samples 1-5, B series, first count.

6th page — Gammagrams of samples 1-5, C series, first count.

7th page — Gammagrams of samples 1-5, D series, first count.

8th page — Gammagrams of samples 1-5, E series, first count.

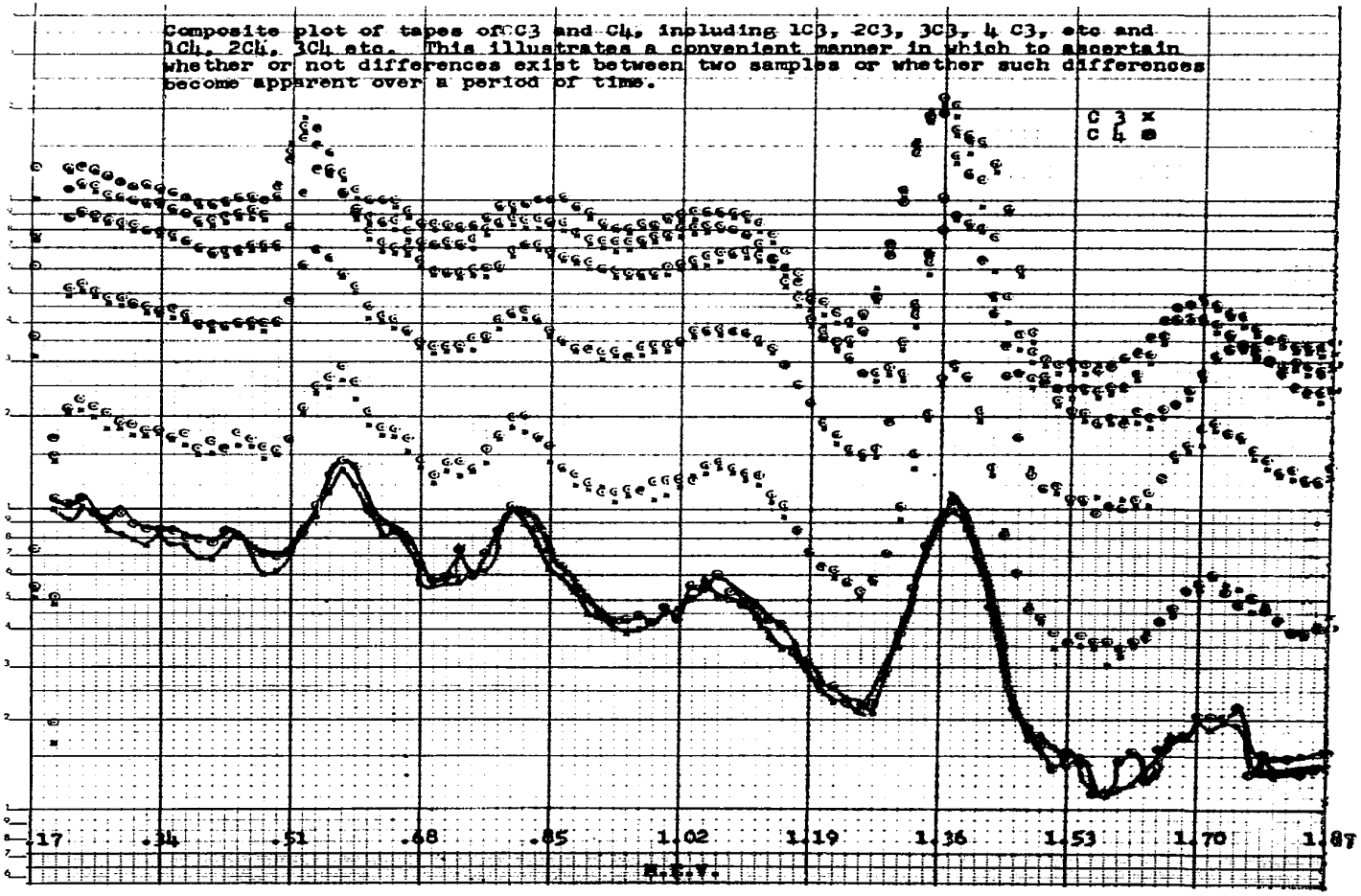
9th page — Gammagrams of samples 1-4, G series, first count.

10th page — Gammagrams of samples 6-10, G series, first count.

11th page — Gammagrams of samples 11-15, G series, first count.

12th page — Gammagrams of samples 16-20, G series, first count.

13th page — Gammagrams of cobalt monitors A, B, C and D showing similarity.


Composite plot of tapes of C3 and C4, including 1C3, 2C3, 3C3, 4 C3, etc and 1C4, 2C4, 3C4 etc. This illustrates a convenient manner in which to ascertain whether or not differences exist between two samples or whether such differences become apparent over a period of time.
C 3 x
C 4 o
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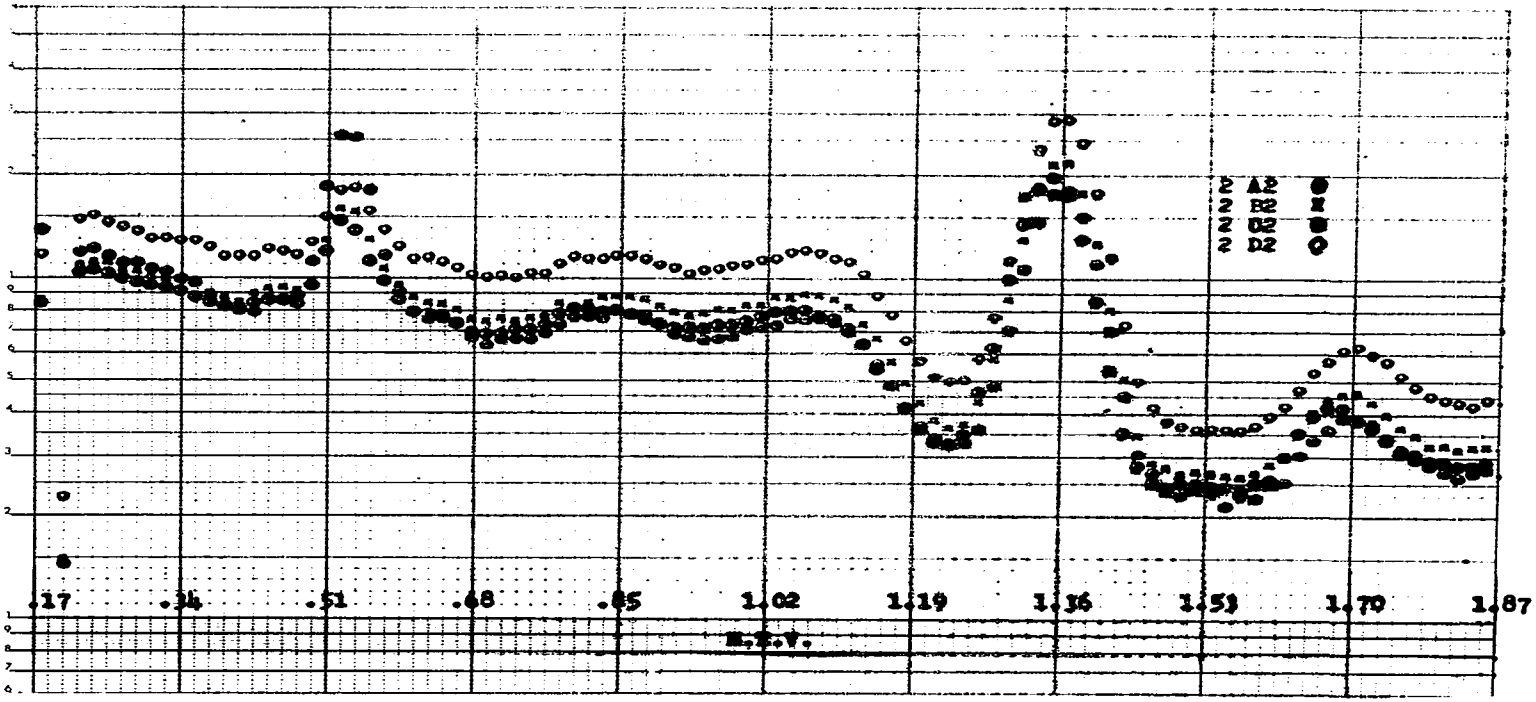

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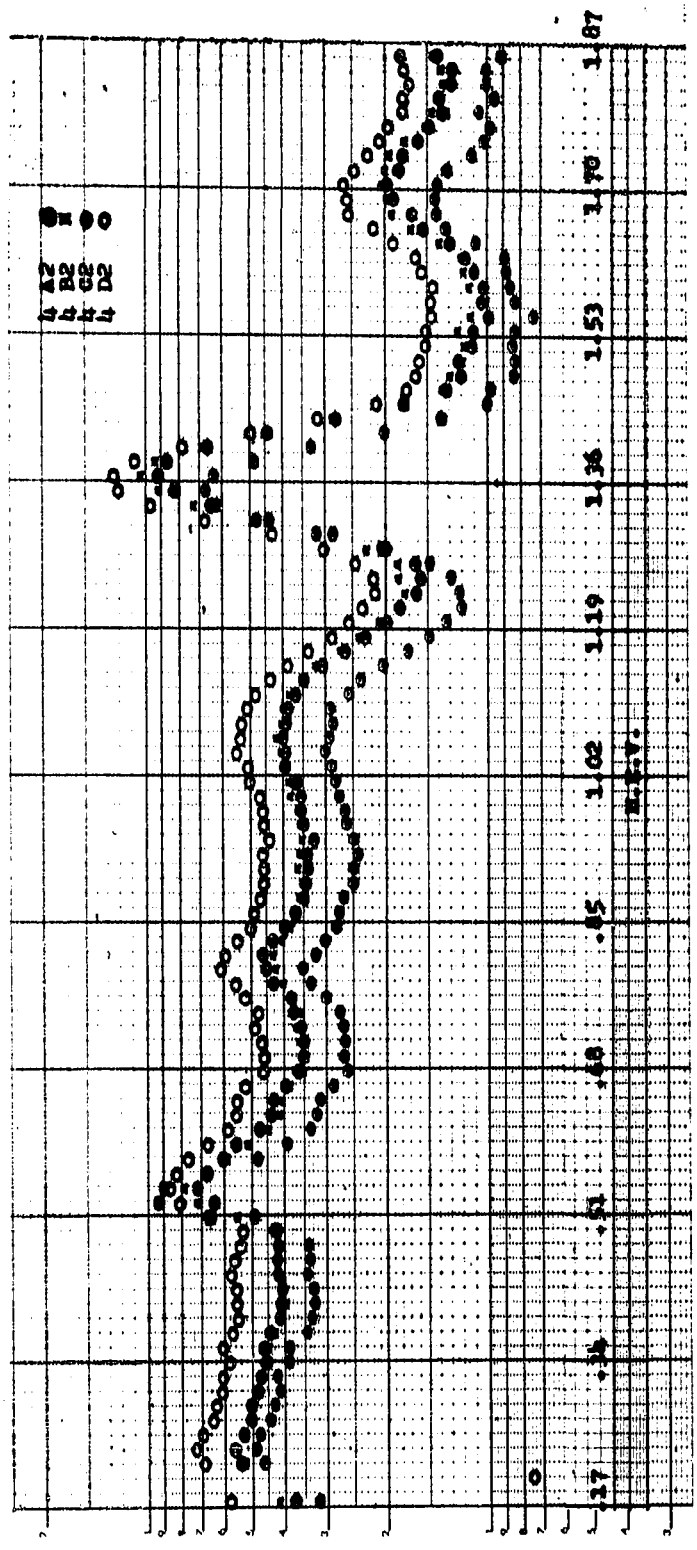
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B2
C2
D2
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1.02
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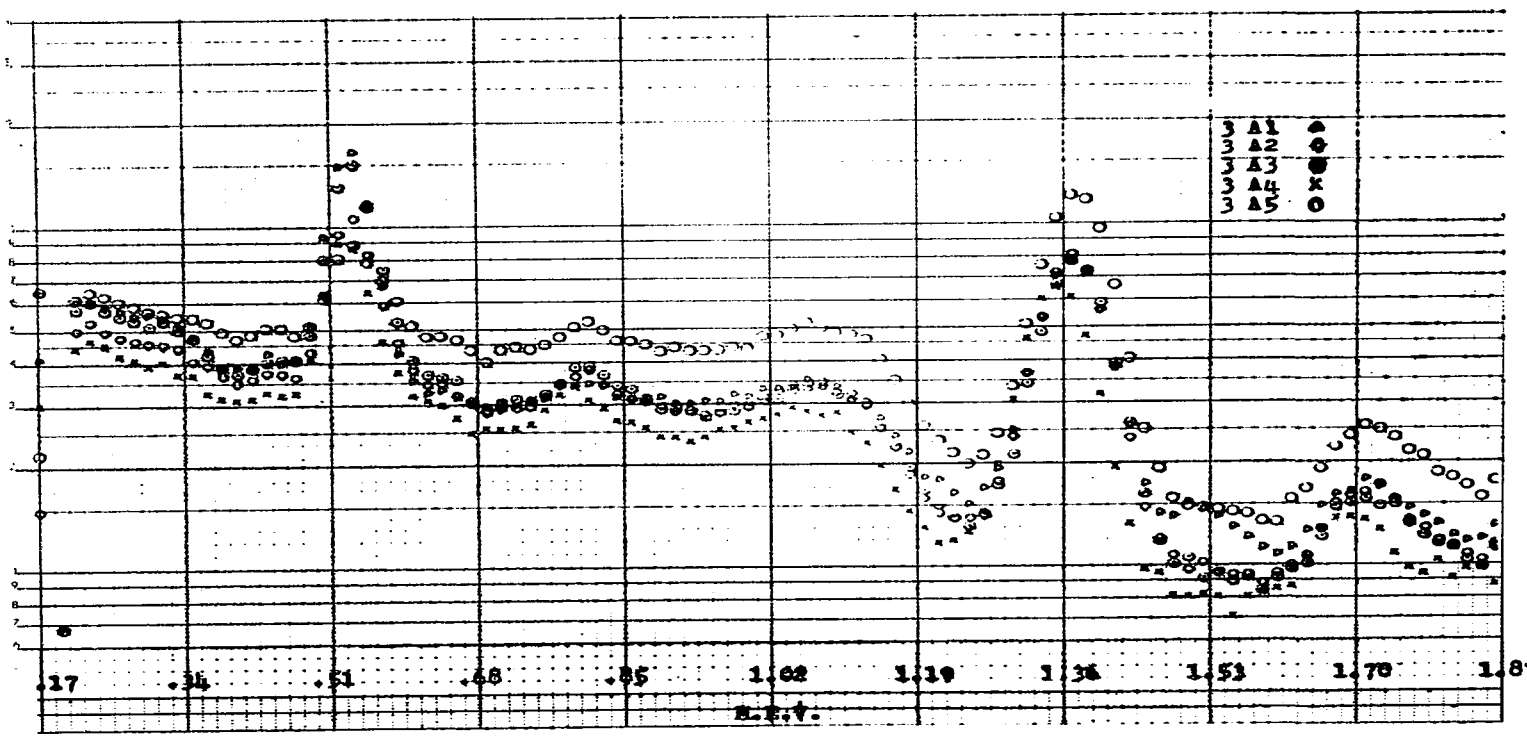
1.34
1.53
1.48
1.45
1.02
1.17


3 A1
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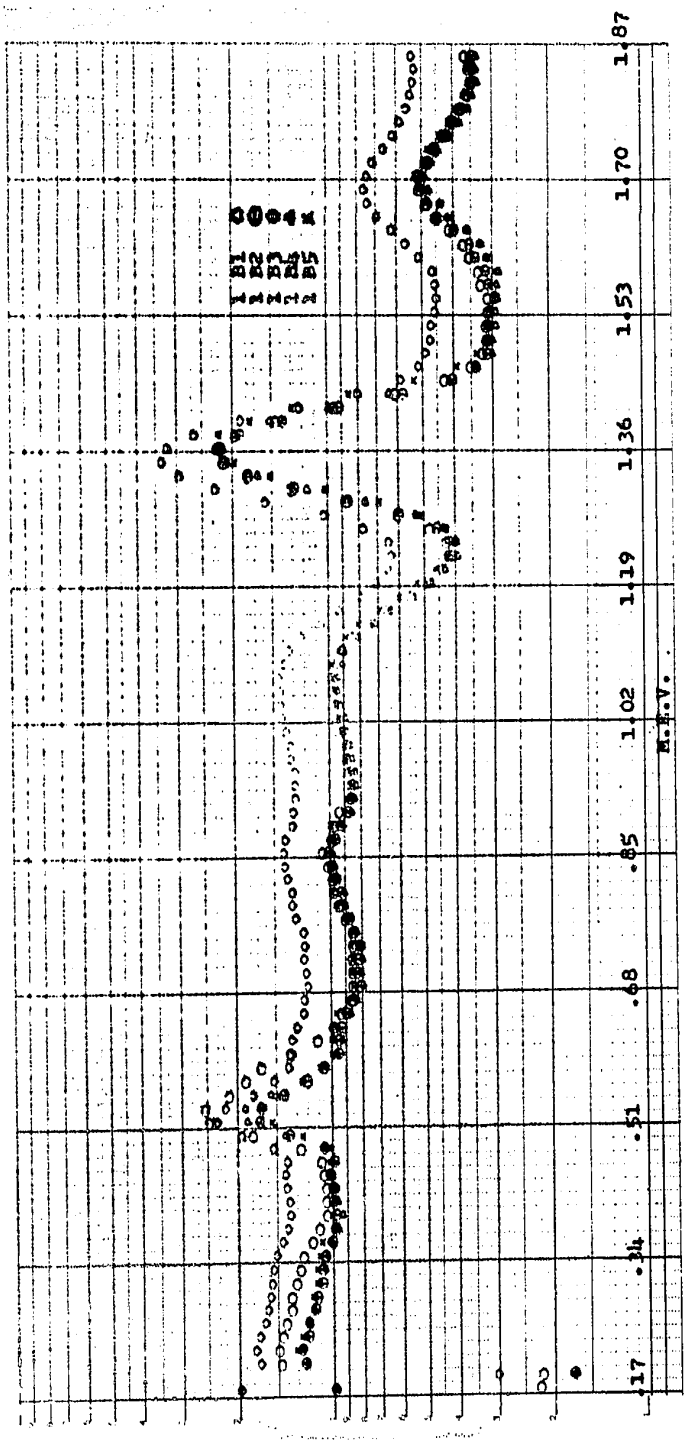
R.E.V.


B1
B2
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B4
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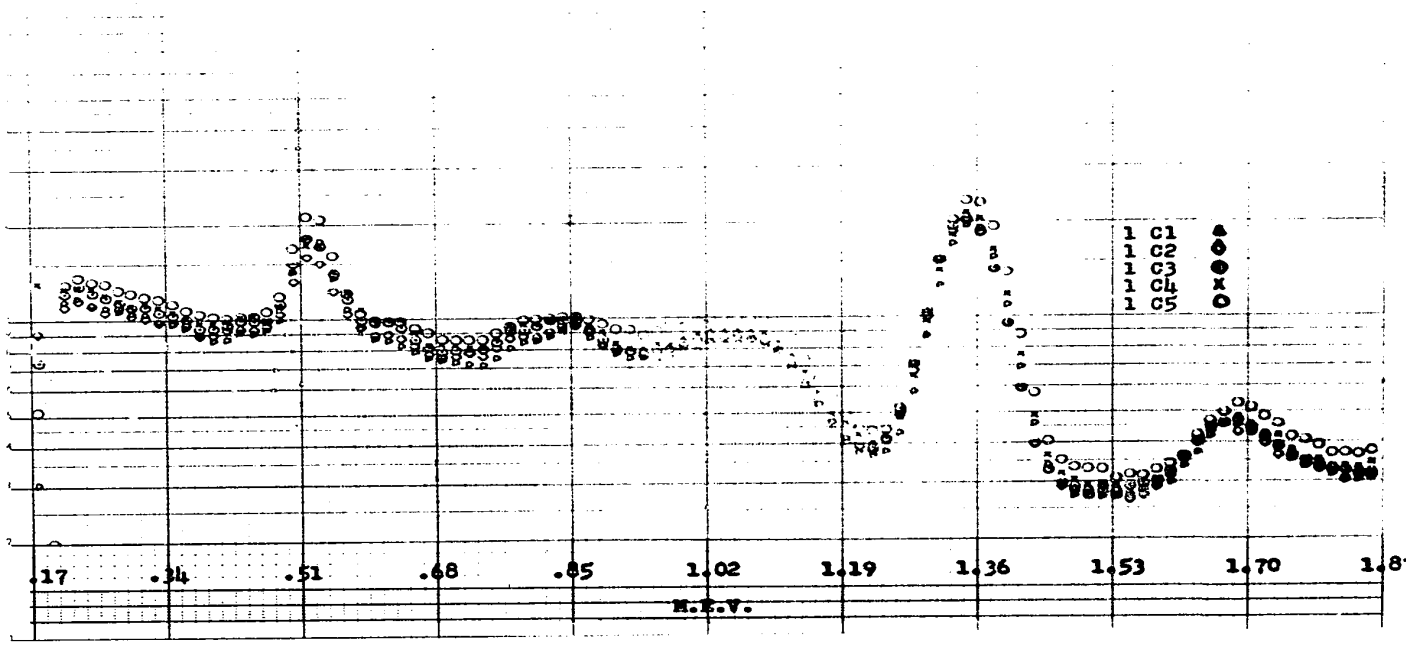
1.87
M.E.V.


1 C1
1 C2
1 C3
1 C4
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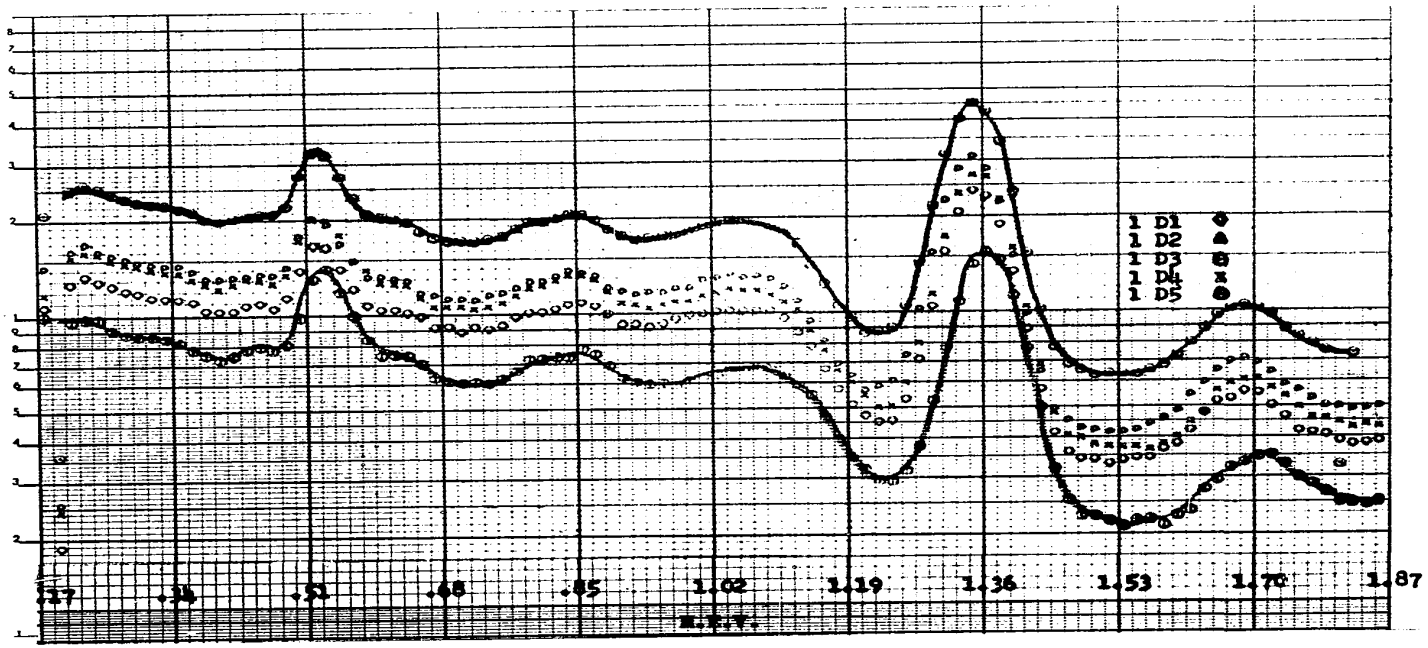
M.E.V.



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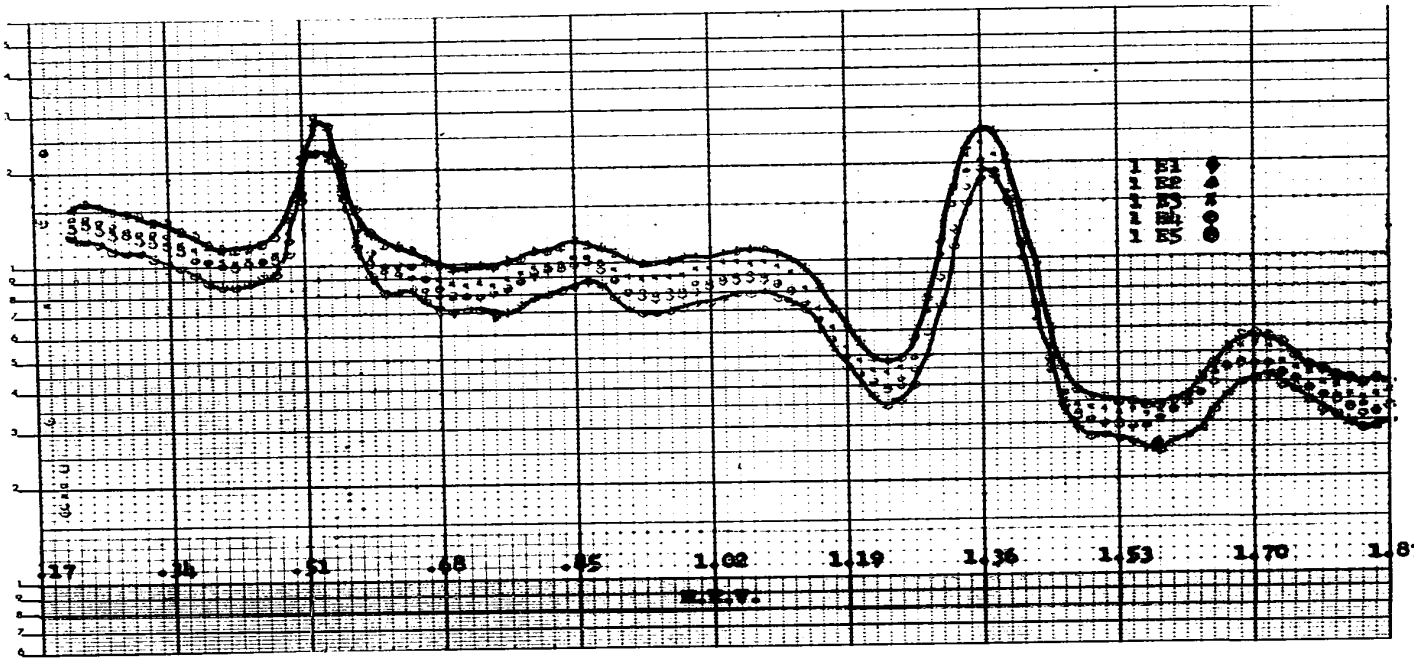
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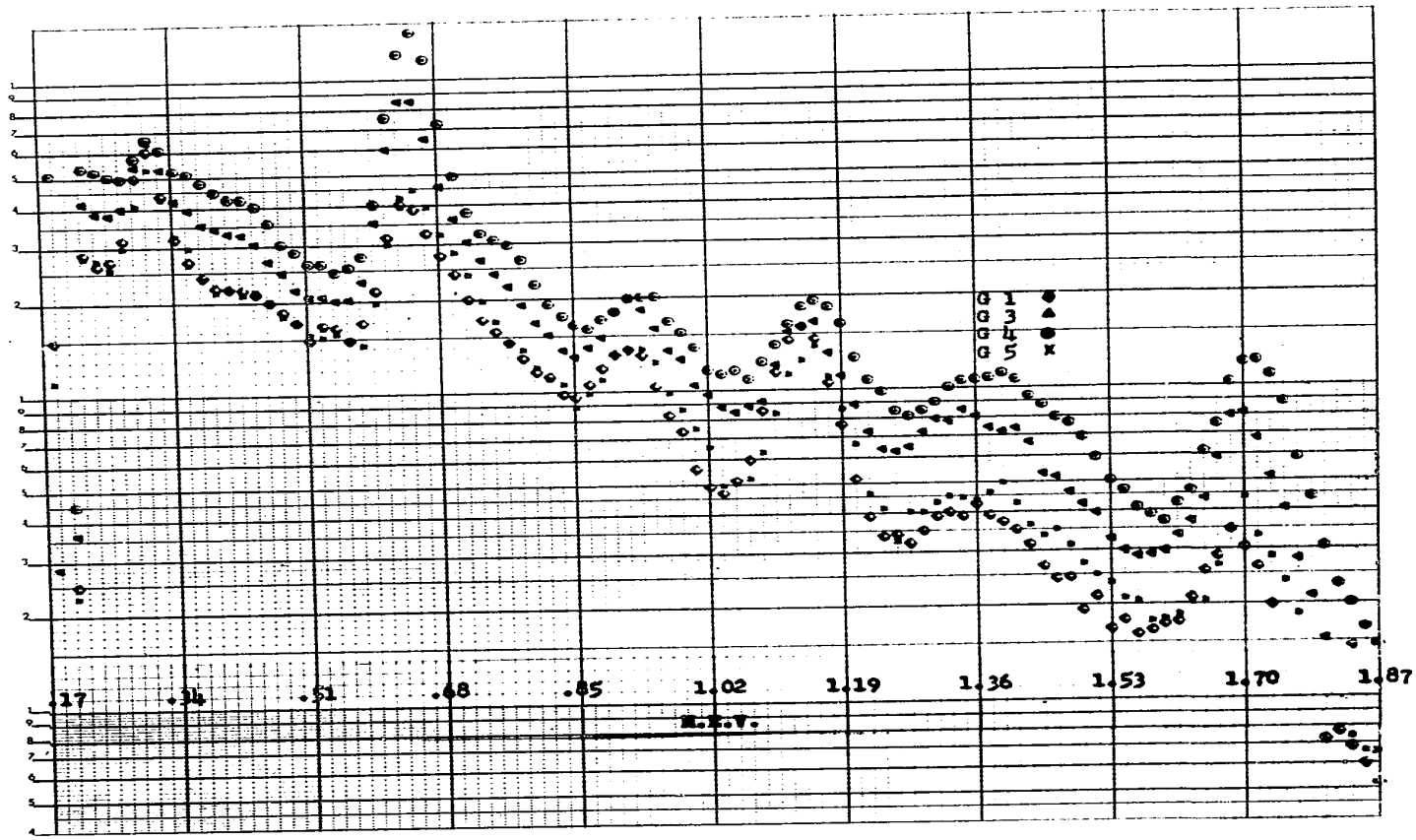
G 1
G 3
G 4
G 5
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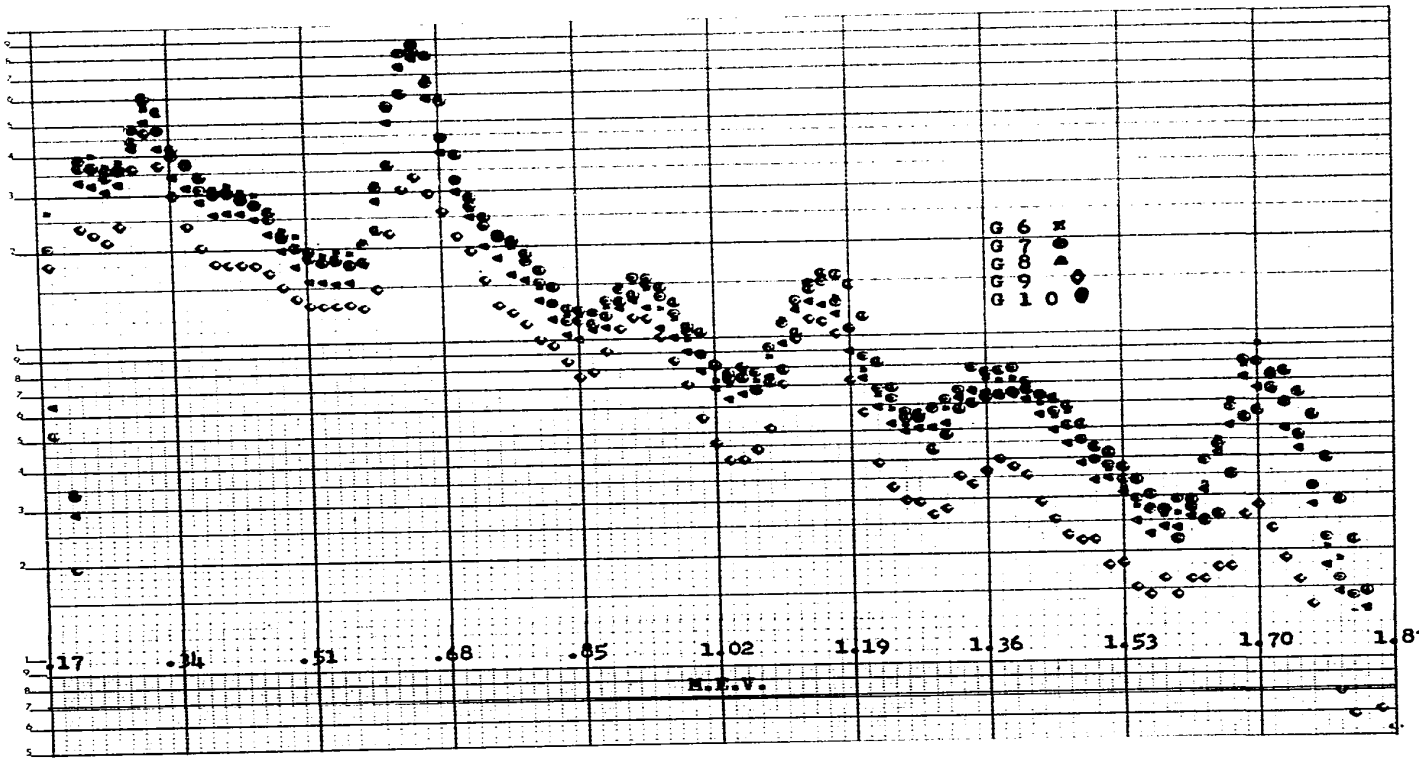
R.I.V.



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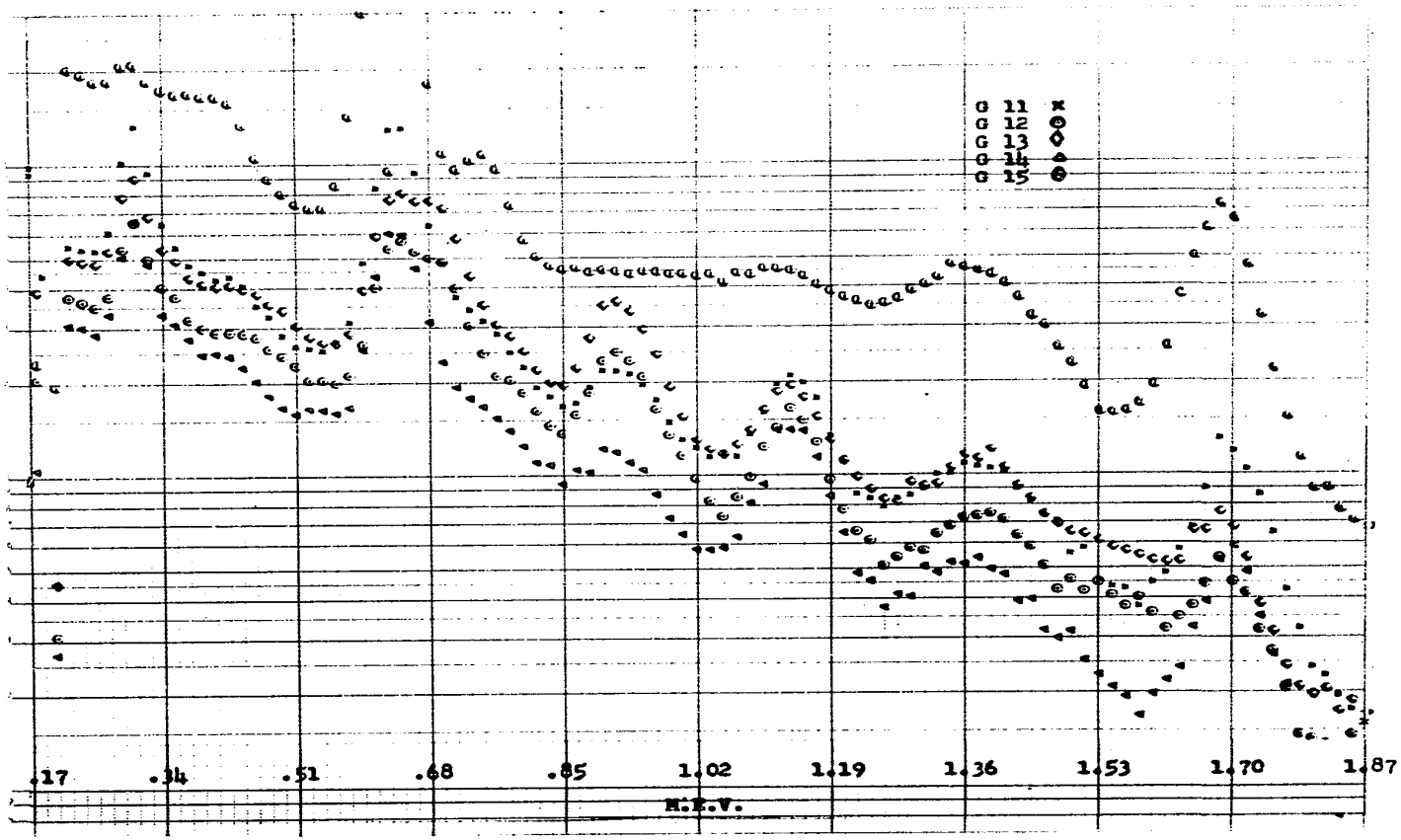
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G 12 ⊕
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G 15 ⊖
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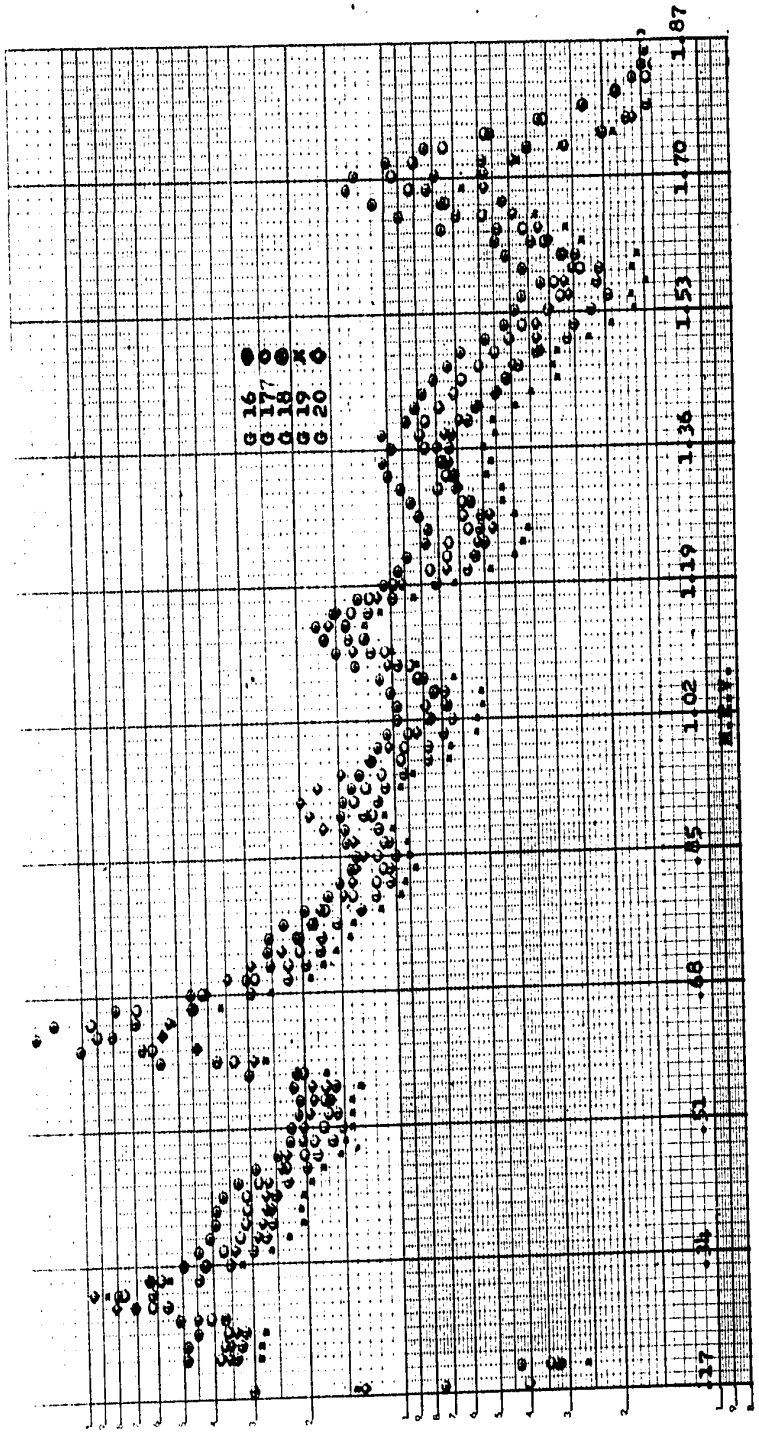
M.E.V.


G 16
G 17
G 18
G 19
G 20
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1.36
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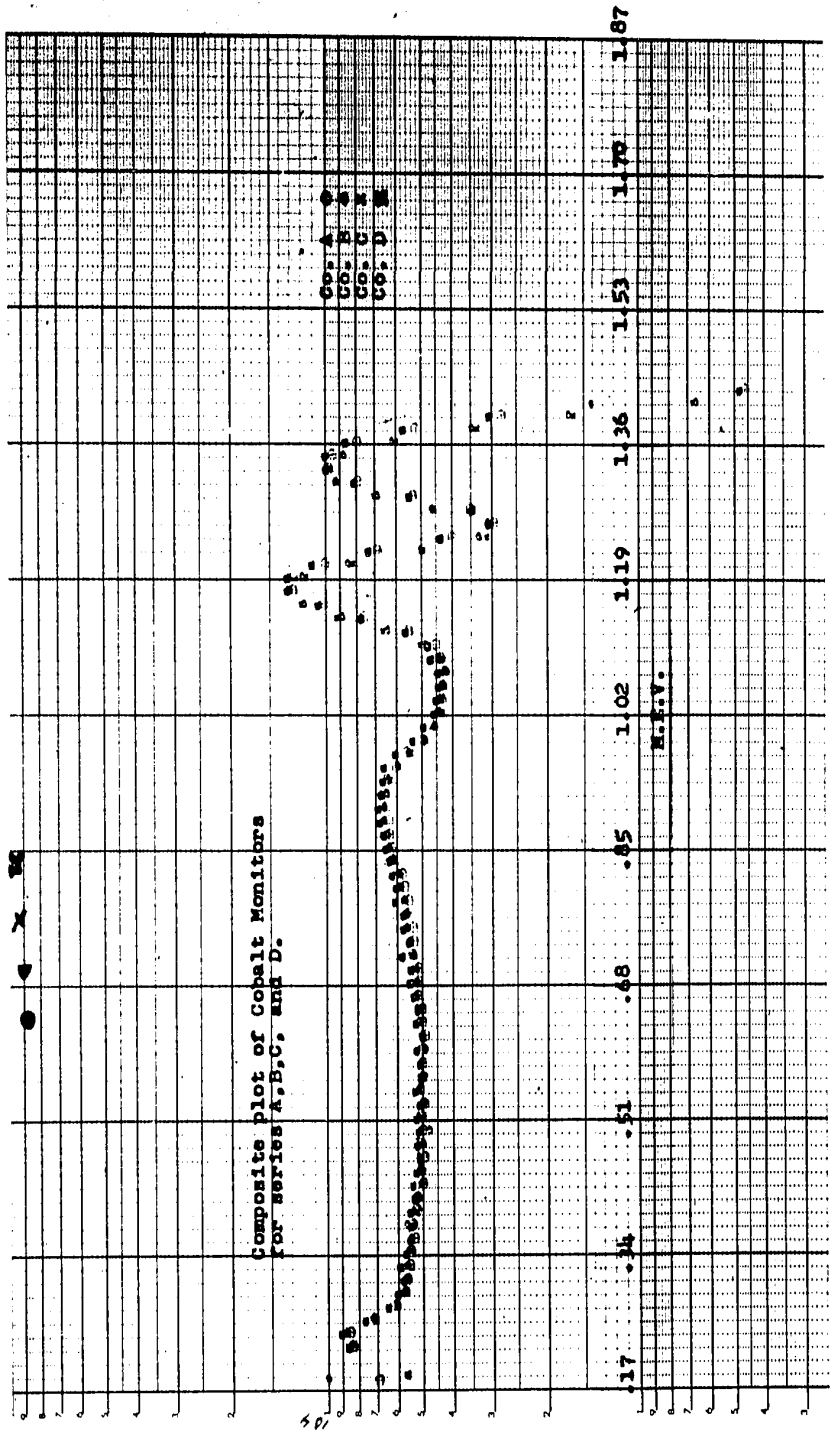
Composite plot of Cobalt Monitors
for series A,B,C, and D.
Co. A
Co. B
Co. C
Co. D
.17
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1.53
1.70
1.87
M.E.V.