THE SIMULTANEOUS MULTI-ELEMENT ANALYSIS OF FORENSIC SAMPLES BY ATOMIC ABSORPTION SPECTROMETRY

by

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ABSTRACT

A multi-element atomic absorption spectrometer is described together with modifications that enable one centimetre sections of hair to be analysed for nine trace elements simultaneously. A survey of hairs from four individuals has been made and the results presented here. The information found is essentially individual in nature and although some longitudinal concentration trends in hair are found there does not appear to be any specific trend that is found for all elements and all individuals. The discrimination test has shown that existing statistical methods are not suitable for treating the data obtained. A preliminary statistical method developed to treat this data has shown great promise. A study has also been made of the analytical usefulness of carbon furnace atomic emission. It has been found that nearly all the elements investigated emit atomic lines. A calibration curve for aluminium has shown that a better detection limit and longer working range than atomic absorption using a similar atomiser can be obtained. A multichannel optical analyser was used as the photo-electric detector in an investigation of simultaneous multi-element analysis using carbon furnace atomic emission.

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A.J.S.

Imperial College
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"But meanwhile we must be vigilant for
truth, and keep proportion, that we
may distinguish certain from uncertain,
day from night."

Thomas Burnet (1635?-1715)

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Chapter 1

INTRODUCTION

1.1 The Forensic Problem

The accurate comparison of samples from crime and suspect has been a goal of forensic science since its beginning. This area of comparative analysis extends over a range of forensic samples some of which are hair, glass, paint fragments and fibres. Unless any given parameter can be measured precisely, and its range in any sample is very small compared to its range in the population of samples, then good discrimination cannot be obtained. To improve such a situation it is necessary to measure more than one parameter provided that they themselves are not interdependent, the more correlated the variables the less useful the extra information.

The trace element composition of material can be used to discriminate between samples (1). The measurement of more than one constituent on a single sample can be achieved by simultaneous multi-element analysis and there are several techniques capable of this. In most cases only small samples such as single hairs or mg size glass fragments are available. The technique must therefore be sensitive and to this end neutron activation analysis (NAA) and mass spectrometry (MS) have been exploited. One of the most difficult comparisons of this type is that of hair and this thesis will deal primarily with methods for the trace element analysis of hair.

1.2 Hair

Hair has been mentioned as an example of a material of forensic importance and its identification has been used as important circumstantial evidence in criminal cases over the past century. An example of its earlier use as evidence can be found in the case: Reg. v. Teague (Cornwall Assizes 1851). In this case medical evidence was given that two short hairs found on a hammer were eyebrow hairs similar to those of the deceased and could not have been goat hairs as was suggested by the defence (the hammer was normally used to beat goat skins). Thus the hair of interest may not be human head hair, but may be from other parts of the body or may even be animal. The range of criminal cases involving hair as evidence is considerable, particularly those involving violence e.g. murder, rape and assault where hairs are easily removed and transferred between assailant and victim.

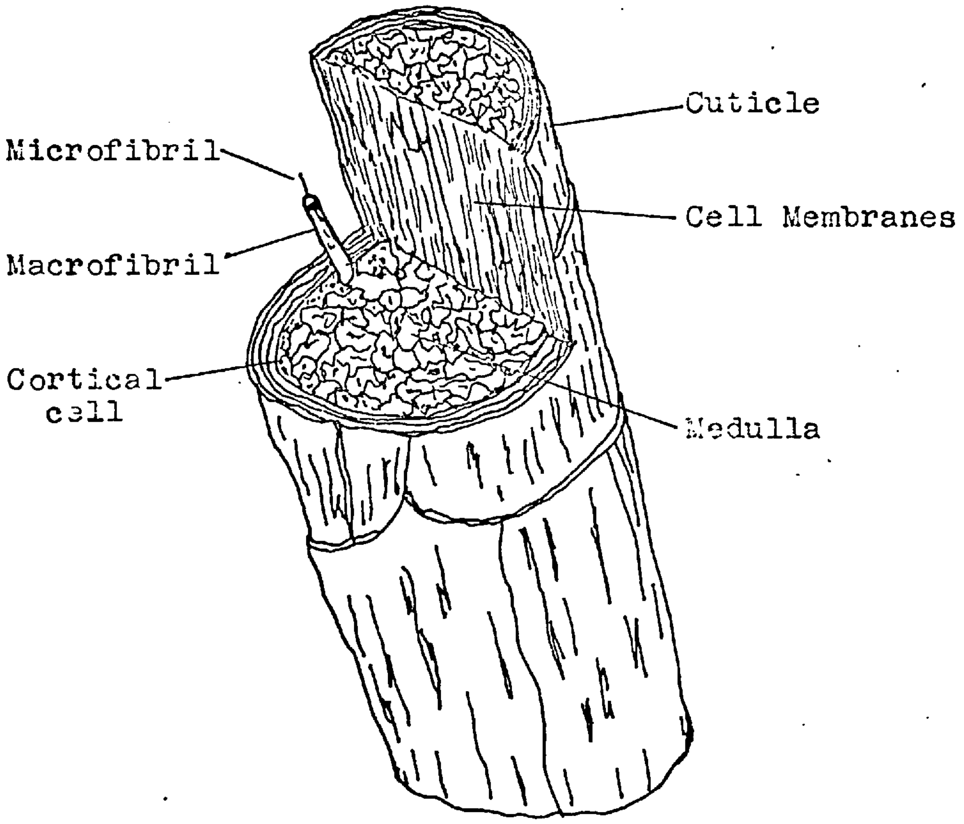
1.3 Structure and Composition of Hair

Hair belongs to a group of proteins called keratins which also form the basis of horn, quill, wool, beak, hoof, feather, scale, nail, claw and the outer layer of skin. As the vertebrates changed to life on the land, keratin was evolved to protect the animal against loss of body fluid (2). The vertebrates diversified through evolution and their keratinous protection took on many forms.

The structure of keratins has been an area of considerable research (3) and was reviewed recently by Leon (4). The keratin fibre consists of three major parts: (a) the cuticle cells which are like scales and wrap around the fibre; (b) the cortical cells which are thin fibrous cells aligned along the fibre; (c) the cell membrane, which separates the cells. In human head hairs a medulla is usually present which consists of air-filled cells (Fig. 1).

Keratin, like other proteins, is composed of polypeptides which are made up of 18 major α-L-amino acid residues. A notable feature of these is the relatively high concentration of the sulphur containing groups cysteine and cystine (2). The sulphur atoms form di-sulphide linkages across the

Fig. 1 Schematic diagram of human head hair structure



chains of polypeptides and give the fibres their strength and resistance to chemical attack. The di-sulphide bonds can be broken by oxidation and then re-made when the hair is in a different shape. This forms the basis of the cosmetic treatment known as permanent waving.

1.4 Methods of Comparing Hairs

The most extensively exploited method of comparing hairs has been microscopy (5,6). Two of the parameters measured are diameter and medullary index (ratio of width of medulla to width of hair); they are, however, of limited use in distinguishing between human hairs. Scale counts and scale patterns have been shown to be of little value in distinguishing between human hairs (7,8) but are of use in distinguishing between species (9). The colour of hair has recently been investigaged (10) by matching hair fibres with dyed nylon tufts. It was found that only 9 different colour groups could be used to obtain the minimum ambiguity of colour assignment. The variation in colour over an individual could be as much as 3 successive groups so many contròl hairs were needed in order to establish the range of colours from one individual. It was found that useful discrimination could be obtained for lighter coloured hairs only. The refractive index of hair has also been measured (11) but found to give limited information.

The cosmetic treatment undergone by a hair can be of some use in discrimination and methods are available for determining whether a hair has been dyed, bleached, rinsed or lacquered and whether a medicated shampoo has been used (10).

Several chemical methods have been investigated to decide whether they are capable of discriminating between the hair of different heads. These include: amino-acid analysis, pyrolysis gas chromatography and determination of fatty acids and glycerides by esterification and gas liquid chromatography. Insufficient variation has been found between individuals for these methods to be useful for comparative purposes. The most promising chemical method of hair comparison is by trace element analysis.

1.5 Trace Elements in Hair

1.5.1 General.

At least 47 trace elements have been found in hair and their determination has been the subject of a considerable volume of research. The major recent surveys of trace elements are given in Table 1. It should be noted that some of the values quoted, e.g. Goldblum (20) et al. for Sn, Ti, Al, Mn and Ag are well below the values found by other authors

Table 1. Range of elements found in hair

Ref.	12	13	14	15	16	17	18	19	20	21
Ag	D.L.-18		D.L.-0.65				0.12	0.4-2.6	4.8×10^{-3} 4.5×10^{-2}	
Al							1-9.5	1-9	5.5×10^{-8} 2.1×10^{-5}	
As	0.5-150	D.L.-4					0.4-8	0.06-1.2		
Au	D.L.-6	0.5-5.6	0.005-0.36		D.L.-0.6		$(0.08-27) \times 10^{-2}$			
B									2-8 ppm	
Ba								0.46-5.6		0.14-16.1
Bi+										
Br	D.L.-1600	0.2-18	4.6-12.4		D.L.-90			0.17-2.5		0.82-12.2
Ca			250-400		500-10,000			135-1150	700-4900	1238-2366
Cd				D.L.-11		0.1-10.0		0.34-1.8		D.L.-0.4
Cl					D.L.-7,000					

Table 1 cont.

Ref.	12	13	14	15	16	17	18	19	20	21
Co	D.L.-8		D.L.-0.22	D.L.-0.6				0.24-0.34		
Cr	0.1-22	D.L.-35	0.5-8	D.L.-25	D.L.-20			0.6-33		
Cs	D.L.-5									0.14-0.68
Cu	D.L.-500	21-400		D.L.-250	D.L.-100	11-60	7-234	8-130	31.2-128	2.5-19.3
F+										
Fe	10-250		D.L.-65					2.7-110	0.84-11	
Ga	D.L.-250							D.L.-0.14		
Ge		3-126						0.9-3.7		
Hg	D.L.-19	1.1-55	1-6		D.L.-35	0.1-140	0.1-34			
I					D.L.-90			0.03-.14		
K								72-900		1496-2965
La	D.L.-4							D.L.-0.83		

Table 1 cont.

Ref.	12	13	14	15	16	17	18	19	20	21
Mg				10-240				7-22	10-101	
Mn	D.L.-30	D.L.-22			D.L.9.0	1-2.2	0.08-22	1.7-8	$5.5x10^{-8}$ $1.0x10^{-5}$	0.27-3.1
Mo							$(0.4-13.0)$ $x10^{-2}$	0.1-3		
Na	D.L.-5,500	12-600			D.L.-4,000			500-1200		
Ni	D.L.-250			D.L.-11		0.7-7.5		0.45-28		D.L.-0.68
P								120-210	25.8-140	
Pb				D.L.-95		6-60		14.5-52	41-100	
Pd+										
Pt+										
Rb	D.L.-17							0.2		
S								440-840		
Sb	D.L.-65		D.L.-0.1		D.L.-3		0.1-4.5			
Sc			0.0022 ±0.00012							

Table 1 cont.

Ref.	12	13	14	15	16	17	18	19	20	21
Se	D.L.-7.5	0.23-7	D.L.-90				0.4-125	D.L.-0.8		
Si								10-28	300-3600	
Sn								0.4-1.6		D.L.-0.95
Sr	D.L.-0.37							0.75-12	2.2×10^{-4} 9.1×10^{-2}	0.54-3.5
Ti								4-24	0.032- 0.064	
Tl*										
V+										
W							$(0.3-6.4)$ $\times10^{-2}$			
Y										D.L.-174.9
Zn	50-3800	217-1270	150-220	D.L.-320	50-700	65-300	50-600	122-246	64-562	10.9-490
Zr								1.0-2.3		

\+ Detected by W.J. Richardson (22)

* Reported by Renshaw (23) below detection limit by non-flame A.A.S. in normal hair found *ca*. 30 ppm in a case of poisoning.

D.L. = detection limit

and the ranges quoted by Jervis (12) are rather larger than other workers particularly for Ni, Zn, As, Ga and Br. These workers have been mainly working within forensic science and have been attempting to use these analyses to identify individuals. Trace metal analysis of hair has also been used to investigate correlation with disease, metabolism, toxicology, pollution, sex, age and hair colour. There seems to be considerable conflict in the literature on the value or existence of some of these correlations. These will be considered in more detail.

1.5.2 Disease and Metabolism

Several workers have investigated the relationship between concentration of zinc in hair and zinc deficiency. Strain et al. (24,25) reported that the zinc content of hair was significantly reduced in ten zinc-deficient Egyptian male dwarfs but increased with zinc sulphate therapy. The comparisons were made against a control group of males from Rochester, New York. They also found that the zinc concentration in hair was highest during the summer months for both groups. Pories and Strain (25) also showed by means of hair analyses that burns produce zinc deficiency. Reinhold et al. (26) suggested that hair zinc level was a reliable indicator of zinc status in human nutrition and that a value of 127ppm or less in hair indicated a deficiency. Eminions et al. (27) and McBean et al. (28), studying Iranian children, have however shown that the zinc levels in hair and blood plasma are not significantly correlated. This would not support the suggestion that the zinc content of hair is a reliable indicator of body zinc stores. Klevay (29) also found no correlation between hair and blood plasma zinc levels.

Copper concentration in hair and its relationship to Wilson's disease has been investigated. Rice and Goldstein (30) and Martin (31) found no abnormal copper concentration in diseased patients' hair, unlike most other tissues. MacDonald and Warren (32) found the copper level in hair of patients with "Kwashiorkor" was lower than normal whereas Lea and Luttrell (33) found that the levels in hair were elevated during this disease. Klevay (34) showed that the copper levels in hair and blood plasma are correlated.

Chromium is related to the utilization of carbohydrates in body metabolism. Hambidge and Baum (35) have investigated the changes of chromium in hair during the first few months and found it to be higher then than at any other time of life. They also found that the hair chromium level reflected the blood plasma level. Hambidge and Rodgerson (36) discovered that the chromium concentration in hair of pregnant women was lower than that of non-pregnant women and suggested that the foetus only obtains its required chromium by severe depletion of the maternal chromium stores. Hambidge et al. (37) also investigated the chromium level in hair of normal children and those with juvenile diabetes mellitus. They found chromium levels significantly lower in children with diabetes.

Schroeder et al. (38) have determined cobalt in a vast range of natural materials. They found no common chronic disease was related to cobalt but they suggested there was some correlation of increased cobalt with increased immunity to infectious disease. Kopito et al. (39) have found that the lead concentration in hair could be used as a diagnostic aid for chronic, mild or subacute lead poisoning in children. Briggs et al. (40) analysed Cu, Fe and Zn in hair for a range of population but found the results to be of little medical diagnostic aid. Schneider and Anke (41) have also investigated the mineral content in human hair in different pathological cases.

The assignment of essential or non-essential characteristics to elements has been attempted through hair analysis by several workers. Liebscher and Smith (42) based their decisions of essentiality on the distribution curves of elements in the population and suggested that essential elements have normal and non-essential elements log-normal distributions. They showed that As, Sb and Hg had log-normal distributions whereas copper and zinc had normal distributions and were thus essential. Renshaw et al.(43), however, found copper and lead to be log-normally distributed. Hammer et al. (44) found zinc to have a normal distribution and As, Cd, Pb and Cu had a positively skewed distribution approximating to log-normal. Perkons and Jervis (45) found that Na, Zn, Cu, Au, Hg, Br, Cr and As were log-normally distributed in hair but gallium and manganese appeared to be normally distributed. They also obtained some results for antimony and lanthanum, but their distribution was less well defined. Goldblum et al. (20) analysed metals related to enzyme processes: Zn, Mg, Cu, Fe and Mn and those thought to be inert: Si, B, Pb, Ti, Sr, Ag and Al; they did not, however, consider the distribution of these elements. Smith (46) studied the distribution of As, Sb, Cu and Zn in a large range of human tissue; he noted the importance of environmental contamination of hair, nails, skin and lung tissue in the interpretation of trace element analysis of these tissues.

1.5.3 Poisoning and Pollution

Arsenic has been determined in hair by many workers (47-54), both for investigation into pollution and in cases of poisoning. Bencko et al. (51) determined the arsenic content of the hair of boys living near a power station known to emit 1 ton of arsenic per day; they found a high arsenic content of the hairs in comparison to a non-exposed group. The use of arsenic in criminal poisoning cases has led to several papers on its determination in hair (52-54). Of special interest is the determination of arsenic in the remaining hair of Napoleon Bonaparte (55-57) which has shown that he may have met his death by arsenic poisoning.

Trace elements other than arsenic have been determined in hair, particularly to determine if people have been exposed to environmental pollution. Weiss et al. (58) found that the lead content of antique (1871-1923) hair was more than an order of magnitude greater than that of modern (1971) hair, contrary to what might be expected from the increased lead in the environment from the use of petrol (containing lead). Coleman et al. (16) found that 5% of the population had high trace metal content in hair which would be attributable to their occupation. Hammer et al. (44,59) analysed hair for Cu, Zn, As, Cd and Pb and found that As, Cd and Pb levels accurately reflected community exposure whereas copper and zinc did not. Nord et al. (60) found the mercury level in hair for women was higher in the industrialised area of Pasadena than in the urban Los Alamos. Mercury in hair has been extensively studied (17,61-68) particularly because of its toxic nature and the recent interest in pollution control.

1.5.4 Age and Sex

The most comprehensive study of the variation of trace metals with age is that carried out by Cornelis (69) who analysed the samples collected by a doctor from his two sons over a period of twenty five years. He took fourteen samples per year except for the period of the World War 1914-1918. He found that zinc was virtually constant to within experimental error over the whole period and copper was only marginally less so. Hg, As, Sb, Mn and Au were also determined, gold showing the greatest variation being up to four times greater in one brother than the other. Petering et al. (70) found that for males the lead level in hair decreased with age whereas female hair showed a maximum at ca. 35 years followed by a dramatic decrease. They also showed that for cadmium both men and women had an increase from birth reaching a maximum at ca. 10 years for men and at ca. 47 years for women. The same authors also studied copper and zinc (71). Schroeder and Nason (15) found that magnesium and zinc showed no significant changes with age, but cadmium declined after the seventh decade and lead after the fifth decade. In females the copper level fell after the fifth decade of life; in males it gradually declined after the second decade. Eads and Lambdin (17) found that lead increases with age in the hair of men but Cd, Ni, Zn, Cu, Hg and Mn show no significant trends with age. Women show a decline in Cd, Pb, Zn, Cu and Hg after ca. 37 years. Hambidge and Baum (35) found that the chromium level of hair was highest during the first few months of life and thereafter declined. Coleman et al. (16) found that there was sufficient difference between male and female head hair to permit sexing in 90% of the cases.

Coleman et al. also studied the variation of trace metals with time and found that the variation over a week is usually much less than that over several months after which time the hair can have a very different trace element composition. In common with Cornelis, Coleman et al. found that gold had a very high variability. Jervis et al. (13) have studied the short term variation of elements in hair and found that the gamma-ray spectra of hairs collected over 14 months were similar but noted trends in Se, Cu and Au. The variation over 14 years was studied for one female head, Hg, Se, Cu, Br and Cr showed considerable variation; germanium increased with age. It should be noted that Jervis's values for copper are much higher ca. x 5 than other authors (see Table 1) and also that they report germanium in hair which few other authors have found and none at the levels reported.

It should be pointed out that several workers have sampled male and female heads in different ways. These mainly involve taking whole short hairs from men and ends of hairs from women. It will be shown in section 1.5.8 that this inaccurate sampling will lead to false conclusions about the concentration variation with sex. Schroeder and Nason, and Petering et al. both make this sampling error; Eads and Lambdin took samples from local hairdressers which presumably were cut from the ends of hairs and would thus be a biased sample if on average the women had longer hair.

1.5.5 Colour

Probably the earliest chemical investigation of hair colour was made by Sorby (72) in 1878 who extracted a bright pink pigment from red human hair; this was later shown to contain iron. The relationship between red hair and iron content was later studied by Flesch (73,74). The same author also studied the role of copper (75) and advanced the theory that copper plays a role as a local catalyst in mammalian pigmentation. Eads and Lambdin (17) related the concentration ratio of zinc and copper to hair pigmentation, the larger the ratio zinc:copper the darker the hair. Kakkawa et al. (76) found significantly higher copper content in pigmented hair and less in the non-pigmented hair of animals and men. Green and Cross (77) however found no relationship between hair pigmentation and copper. Schroeder and Nason (15) found that women with brown hair often had high copper content. They also studied Mg, Zn, Co, Cd, Pb, Ni and Cr and found a complex relationship between trace elements and hair colour.

1.5.6 Washing Procedures

A major problem that exists before a hair can be analysed is that of washing and it is one that has been investigated by many research teams. Harrison et al. (78) compared an organic wash (2 hr. soxhlet extraction using

50:50 v.v. ethanol:acetone) with that of a non-ionic detergent (1% 7 X 0-matic). They found that the copper and zinc levels in hair were nearly the same by either wash whereas the magnesium and iron were appreciably removed by the non-ionic detergent. Bate and Dyer (18) compared two washing procedures, an organic ethanol-acetone wash and a non-ionic detergent Kyro EO, in both cases followed by a distilled water rinse. They found that the water alone did not remove a significant part of the inorganic material. They concluded that the non-ionic detergent was probably the best cleaning procedure since this matched normal hair shampoo. Bate (79) found detergents more effective at cleaning hair than organic washes, and suggested that an ionic detergent should be used to wash hair prior to analysis by NAA; this would remove sodium and bromine which otherwise interferes with the determination of Al, V and Mn.

Bate (80) has studied the adsorption and elution of trace elements on human hair to investigate the effects of possible environmental contamination. He found that Cl, Br, Cu, Zn, Au, Se, Cr, I, As, Ag, Sb, Co, Fe, Mn, Co, Ba and Sr all adsorbed onto hair. Cations adsorbed to a greater extent when the pH of the solution was closer to neutral and anions adsorbed most at low pH values. The elements were not easily removed from the hair; on washing with various complexing agents Au, Ag and Ba were retained more than 80%. In the case of zinc, the natural hair lost zinc faster to the eluting solution (0.1M EDTA) than did the hair with adsorbed zinc.

Perkons and Jervis (81) used distilled water, acetone-ethanol washes and showed that a prolonged wash in either of these or ionic detergents greatly disturbed the trace metal level in hair. Hammer _et al_. (44) used a severe washing procedure consisting of detergent, distilled water, ethanol and boiling in EDTA solution; they found that this resulted in considerable loss of trace metals from the hair. Obrusnik et al. (14) investigated two washing procedures: boiling in distilled water followed by rinsing in distilled water, and successively with diethyl ether, acetone, water, acetone, ether, acetone, water, acetone and finally ether. They showed that there was little difference between the washing techniques and between no washing at all, thus concluding that their hair samples must have been clean when taken! Hilderbrand and White (82) investigated several washing methods and found them all to affect the trace metal content of hair, but recommended the use of an ion-free detergent, "Snoop". During their study of trace metals in hair by NAA, Coleman et al. (83) used a 2 hr. diethyl ether Soxhlet extraction of hair. They reached this decision after investigating a non-ionic detergent, several organic washes, several combinations of organic washes and the effect of time on the ether extraction. The 2 hr. ether extraction was chosen for five reasons: (a) the variation in trace element concentration on a single head is minimised; (b) it was shown that this method removed the excretion products of the scalp and also, presumably, dust entrained in these products; (c) the method can be easily and reproducibly performed routinely, up to 20 samples simultaneously; (d) the solvent is continuously purified by distillation reducing any risk of it contaminating the hair; (e) the trace element content of hair is not appreciably altered by this washing method. Van den Berg et al. (84) have examined the effect of washing procedures on hair and have attempted to quantify the ways in which trace elements, particularly arsenic, can be incorporated into the hair. Van den Berg et al. (85) also studied the sorption behaviour of Na, Cu, Zn, Cr^{III}, Mn^{II}, Au^{I} and As^{V}. They found sodium and manganese could be easily introduced into or removed from hair by solutions; Cu, Zn and Cr ions can also move within hair but more slowly. Arsenic ion mobility is very dependent on pH. They suggested that gold is reduced and thus fixed when it enters the hair and that there is a part of the hair (medulla) where ions can only penetrate with great difficulty and would thus contain only trace elements that had been grown into the hair. Hambidge et al. (86) have investigated the effects of washing on the determination of chromium in hair. Tolgyesi and Cottington (87) studied the adsorption of aluminium by hair and found that at normal temperatures the adsorption process is mainly diffusion controlled.

1.5.7 Large Scale Studies of Variation of Trace Metals in Hair over the Population

Jervis (12) has analysed 1500 samples from 600 individuals. Coleman et al. (16) have surveyed 750 individuals from England and Wales. Schroeder and Nason (15) analysed hair from 126 males and 55 females and related trace metals to hair colour, age and sex. Bate and Dyer (18) used a population of 99 individuals from USA and New Zealand. Perkons (88) studied the trace element concentrations in the hair of a population of 1200 people.

1.5.8 The Variation of Trace Elements in Hair

Trace metals in hair are known to vary considerably from person to person, over the head and down the length of the hair. Head hair can also be found in two states, anagen or growing and telogen or resting phase. All of these sources of variability conspire to ensure that hair comparison by trace metal analysis is very difficult. Some of the more important findings in the literature will now be discussed.

Obrusnik et al. (14) found no definite differences in trace element concentration between hairs in the anagen and telogen phases and concluded that more work would be needed to distinguish them apart. They also showed that there is usually an increase in trace elements towards the distal end of the hair. Selenium was 20 times higher at the distal end than near the root; Cr, Fe, Co, Ag, Sb, Au and Hg also demonstrated this behaviour but with a less dramatic increase towards the distal end. The exception was zinc which remained relatively constant. This work, however, was carried out on very long hairs up to 51 cm. and 10 cm. sections of these were analysed, thus little detail of variation down the length was obtained. They found that Cr, Fe, Ag, Sb, Au and Se varied from hair to hair on one head as much as previously reported values for the population as a whole, but Co, Hg and Zn showed much less variation than the population. Kerr (89) has measured the trace elements in 80 hairs from the same head to investigate any differences between anagen and telogen phases. His results were of analog form as gamma-ray spectra (after NAA) and the variability was not calculated but the similarity of the spectra indicated that the variation between hairs was small. Lima et al. (90) considered the effect of the growing cycle and presented information on variation of trace elements down the length of hairs from several individuals. They also compared hairs from different parts of the body and observed the variation of trace elements down the length of a pubic hair, finding them to increase for some elements. The results were displayed as channel number rather than element and only some of these were identified in the text. They could find no close group relationship between trace element level and workers at the same industrial plant, people in the same family, etc. Coleman et al. (16) carried out a study on the variation of trace elements across the head and found that the amount of variation was itself subject to considerable variation among individuals. They also analysed some hairs for variation down the length and found that the variation was of the same order as that found for different single hairs on the same head. They concluded that in matching crime samples similar portions of hair should be analysed but that if this was not possible then the use of whole hairs for the control sample is not likely to introduce large errors. Bate and Dyer (18) measured the variation along the length of segmented strands of women's hair; they found that Zn, Hg, Au and Cu increased from scalp to end by a factor of about 2. It should be noted that no other workers have found zinc to vary along the length of hair. Jervis <u>et</u> <u>al</u>. (13) carried out replicate analyses of different samples from one individual and found them to be reproducible to within experimental error. They also took hair from different areas of the scalp from one individual and found them to be fairly constant, mercury and arsenic showing most variation. Valkovic <u>et</u> <u>al</u>. (91) have measured the ratio of trace elements Ca, Sr, Cu, Br and Fe with respect to zinc concentration down the length of a female head hair. Measurements were made on 3 cm. sections and the hair was washed in triply distilled water. They used the technique of proton-induced X-ray emission. For Ca/Zn, Sr/Zn, Cu/zn and Br/Zn they found an increase in value towards the distal end. The normal value of zinc in hair is <u>ca</u>. 200ppm; if this value is used to interpret their results then the respective level of Ca, Sr, Cu, Br and Fe at the distal end is 20,000, 300, 150, 20 and 24 ppm. The value for calcium is higher by a factor of two than the previously reported maximum and is especially difficult to accept since the hair was washed with water which would be likely to reduce the level of calcium. The level of strontium is also remarkably high; the previously reported maximum is 12 ppm (19). Bromine and iron are within other reported values; see Table 1. If a lower value of zinc is taken, say 100 ppm, the calcium and strontium values would still be very high and Reinhold <u>et</u> <u>al</u> (26) considered that a value of 127 ppm Zn or below in hair would indicate zinc deficiency. Schlesinger <u>et</u> <u>al</u>. (92) determined Cu, Mn, Zn, Na, Cl, Br and I in hair but found no significant trends of the elements down the length of the hair.

Renshaw et al. (43,93) have published the most detailed study so far of the variation of lead and copper down the length of head hair, sampling every 1 cm. in the case of lead analyses and every 0.3 or 0.5 cm. in the case of copper. They found that the concentration of both metals increased towards the distal end of the hair; for one individual the lead concentration was nearly constant between 3 and 27 cm. from the root and then increased rapidly towards the tip. Renshaw et al. (43) also showed that six hairs from the same head had a similar trace metal profile down the length. They found that the variation of both lead and copper over the population was about twice the variation within an individual.

Arsenic has been determined in hair to prove cases of chronic arsenic poisoning. There is, however, some evidence to show that arsenic appears at the tip and in the body of the hair shaft only hours, in some cases, after ingestion. Smith (50) showed that arsenic ingested orally (3mg As_2O_3) increased in the hair roots only ½ hr. after ingestion and after 4 hrs. had increased up to 20-fold. He suggested that the hair root was in relatively close equilibrium with trace elements in the blood stream. Lander et al. (53) found anomalous results, some cases of acute poisoning showing arsenic in the tips of hair within a few hours, others showing arsenic only in the proximal sections of the hair. They concluded that little significance could be attached to the distribution of arsenic along the shaft of the hair. Lima (94) examined the level of arsenic along the length of hair after ingestion of 2mg arsenic trioxide per day. He found that increased arsenic was seen in one case at 4 cm. from the scalp after only 10 days; this is more than x 10 the distance expected by normal growth. He postulated that the mechanism for this was capillary action of sweat containing arsenic and its deposition on evaporation. In cases of acute arsenical poisoning there is usually profuse sweating and this would probably account for the reports of arsenic in the tips of hair shortly after ingestion. Lima showed that this mechanism is feasible by demonstrating capillary action of an arsenic solution along the shaft of a hair. Wyttenbach _et al._ (54), however, found no abnormal concentrations of arsenic in sections of hair beyond 5 mm. from the scalp in a case of acute arsenical poisoning.

Hambidge _et al._ (95) determined the changes in hair chromium with increasing distance from the root. They found no increase of chromium towards the tips of hairs and concluded that environmental contamination was not usually a significant factor. They considered that the chromium level in hair reflected the nutritional status of the element in the individual. Giovanoli-Jakubczak _et al._ (68) examined mercury in the hair of individuals who had been exposed to methyl mercury; they showed that the changes in mercury level along the hair agreed with a theoretical model of exposure to mercury.

1.6 Methods for the Trace Element Analysis of Hair

The determination of trace elements in head hair, particularly on small samples, demands a sensitive analytical technique and most of those capable have been used. For the purposes of this research a technique capable of determining 12 trace elements in 1 cm. sections (_ca._ 100 μg) of hair was required; thus the techniques available are strictly limited. The analytical methods that have been used to determine trace elements in hair will thus be discussed with this criterion in view.

1.6.1 Neutron Activation Analysis

This is the most widely used technique, particularly by forensic investigators. The method was reviewed by Coleman and Pierce (96) and also by Girardi (97) and its application to forensic investigation by Lyon and Miller (1), Coleman (98), Forslev (99) and Jervis (12,100). A bibliography of work done in the field of NAA up to 1971 can be found in the National Bureau of Standards (NBS) publication (101). Reference to Table 2, which is Coleman's list of sensitivities for 100 hr. irradiation (or to saturation, whichever is smaller) at a flux density of 10^{12} n s^{-1} cm^{-2} shows that the highest sensitivity occurs mainly for elements that have either not been found in hair or are at a low concentration (Table 1).

Table 2

Element	Sensitivity μg
Au,Dy,Eu,In,Mn	10^{-5} - 10^{-6}
Hf,Ho,Ir,La,Re,Rh,Sm,V	10^{-4} - 10^{-5}
A,Al,As,Ba,Br,Co,Cu,Er,Ga, Hg,I,In,Na,Pd,Pr,Sb,Sc,U, W,Yb	10^{-3} - 10^{-4}
Cd,Ce,Cl,Cs,Gd,Ge,Mo,Nd,Os Pt,Ru,Sr,Ta,Tb,Th,Tm	10^{-2} - 10^{-3}
Ag,Ca,Cr,Mg,Ni,Rb,Se,Te,Ti,Zn	10^{-1} - 10^{-2}
Zr	1 - 10^{-1}
Fe,S	10 - 1

Although the irradiation time, and thus sensitivity, can be increased, the length of time required for an analysis is already a major disadvantage of NAA. It is for these reasons, together with cost and reactor availability, that a detailed multi-element study of 1 cm. sections of hair by this technique is not feasible.

Workers using NAA have employed different ways of interpreting their results. Coleman et al. (16), Perkons and Jervis (13) and Bate and Dyer (18), the main workers in the forensic application, have obtained concentration values for the trace elements and in the case of Coleman's team developed mathematical methods for the comparison of such data (102-104). Guin et al. have used a comparison of gamma ray spectra as a criterion of similarity between samples. Lima et al. (90) presented their data as ratios either as elemental ratios, e.g Cu/Na, against sample length, or as the ratio of succeeding segments to the first segment against concentration.

1.6.2 Spark Source Mass Spectrometry

This technique has been applied to the analysis of hair (19,22). By using only tipped electrodes these workers were able to use as little as 0.5 mg. of ash, samples being ashed in a muffle furnace (19) or with a low temperature oxygen asher (22). They required, however, several milligrams of hair to perform a single analysis and so this technique is not suitable for the analysis of 1 cm. (ca. 100μg) lengths of hair. Williamson and Harrison (106) compared NAA and MS as applied to hair analysis. They needed a few strands to analyse for 8 elements by NAA; the analysis took about a week. They required $\frac{1}{2}$ g of hair for MS analysis but had quantitative answers for 22 elements within two days. They needed however to calibrate the MS results against one element which they subsequently determined by AAS.

Table 3 Detection limit (by flameless AAS) and range in hair

ELEMENT	DETECTION LIMIT for 100 μg of hair /ppm	CONCENTRATION RANGE /ppm
Ag	0.06	D.L. - 18
Al	0.3	1 - 50
As	0.2	D.L. - 150
Au	0.3	D.L. - 6
Ba	3.0	0.14 - 16
Bi	0.4	0.1 - 10
Ca	0.4	135 - 10,000
Cd	0.01	D.L. - 11
Co	0.25	D.L. - 8
Cr	0.10	D.L. - 33
Cs	1.0	D.L. - 5
Cu	0.5	D.L. - 500
Fe	0.3	D.L. - 250
Ga	23	D.L. - 250
Hg	110	D.L. - 140
K	0.25	50 - 3000
Mg	0.03	7 - 240
Mn	0.03	D.L. - 22
Mo	0.9	0.004 - 3.0
Na	0.1	D.L. - 5,500
Ni	1.4	D.L. - 250
Pb	0.1	D.L. - 100
Rb	0.5	D.L. - 17
Sb	2.6	D.L. - 65
Se	30	D.L. - 90
Si	1.2	10 - 28
Sn	0.1	D.L. - 1.6
Sr	2.0	D.L. - 12
Ti	20	0.032 - 24
Tl	1.3	D.L. - 30
V	3.5	Detected
Zn	0.01	D.L. - 3,800

1.6.3 Emission Spectroscopy

This has been used by a few workers. Goldblum et al. (20) in 1953 used emission spectroscopy; 100 mg of hair were dissolved in nitric acid and the digest placed on the carbon electrode by a Pyrex rod. Their results for Al, Ag, Mn, Si and Sr disagree with most other published data (Table 1). More recently, emission spectroscopy has been used (24,35,86,95,108) to determine zinc and chromium. Hambidge (35,86,95,108) used a D.C. arc with a static argon atmosphere and achieved 6% r.s.d. for triplicate analyses of chromium at 5 - 35 ng/ml of serum. Although this method is sensitive it does not have the absolute sensitivity required to analyse 100 μg of hair for analytes at the ppm level.

1.6.4 X-ray Methods

Valkovic et al. (91) have examined trace elements in hair using proton induced x-ray emission photometry; they have examined a single hair and studied the variation of ratios of elements with respect to zinc. Although some of their results are a little high judged by previously reported values, the technique appears to offer potential in the study of the variation of trace elements in single hairs. Zeitz et al. (109) have used x-ray fluorescence to determine zinc and calcium in hair using 1 cm. strands. Their detection limits were 24 and 20 ppm respectively for Zn and Ca. This method also shows promise in the detailed study of trace elements in hair.

1.6.5 Atomic Absorption Spectrometry

Conventional AA using burner-nebuliser systems has been a popular tool for the investigation of trace metals in hair. It has been used by workers investigating effects of pollution, body mineral metabolism, the relationship between trace elements and disease, age, sex, colour etc., but it has not been used by forensic investigators to compare hairs. Conventional flame AAS does not have the sensitivity for a detailed study of multi-element analysis of hair. Mercury does not lend itself to determination by electrothermal atomisation AA because of its high volatility. It is usually necessary to remove solvent or matrix by a preheating step and this would remove mercury. Thus cold vapour techniques have been developed and several workers have used these to determine mercury in hair. The cold vapour method is not generally extendable to multi-element mode working.

Renshaw et al. (43,93) have shown the feasibility of lead and copper analyses in small sections, 1 cm. or less, of hair by AA with electrothermal atomisation of sample using a commercially available furnace and power supply. Based upon available sensitivities it can be seen that many elements are capable of detection by electrothermal atomisation AA in 100 μg hair samples (Table 3). Sensitivities in Table 3 are from Perkin-Elmer (PE) figures for their HGA series of electrothermal atom cells. In the experience of the author using PE atomisers and other makes of spectrometer (see later) it is usually possible to improve upon these figures by a factor of two. Thus this technique offers in single element mode the detailed examination of possibly 23 elements in hair.

Chapter 2

GLASS ANALYSIS

2.1. Introduction

The trace element composition of glass is of importance to the forensic scientist as a method of comparison of glass fragments found at the scene of crime and on the suspect; it may also provide information as to the site of manufacture and type of glass: window, container, head-lamp etc. Generally, forensic samples of glass are examined by the physical measurement of density and refractive index, which are usually correlated. Where this is found not to be the case good discrimination against other glasses is obtained, but for a large number of glasses the refractive index falls within a very limited range: 1.516 - 1.519. This group forms ca. 40% of the glass samples handled by the Home Counties Regional Forensic Laboratory (110). Refractive index can be measured with a precision of ±0.001 resulting in poor discrimination within this range. It is particularly for this group of glasses that multi-element trace analysis would be of most use. The physical properties of density and refractive index are related to the major constituent composition of glass; trace element analysis will complement these methods and provide further non-correlated discriminatory information.

Coleman and Wood (111) have discussed methods of glass fragment comparison and the application of emission spectrography, NAA and MS in particular to this problem. Campbell and Adams (112) have reviewed the methods available for trace metal analysis of glass. The two techniques that have been most thoroughly exploited in this field are NAA and MS using a spark source. Both can be used in the multi-element mode determining 15-30 elements and allowing the accumulation of much data from one analysis. This point is important since only small (ca. 1 mg.) fragments are usually available from clothing (113). NAA and MS are both costly in capital investment, running and maintenance and usually require several skilled operators. In both techniques a precision better than 10% can be obtained for 60% of the elements determined in glass (114,115). This thesis describes analytical methods for the determination of two elements in glass: lead and iron with a working precision of ca. ± 10%. They are based on electrothermal vaporisation of samples in an atomic absorption spectrophotometer. This is simpler and cheaper than NAA or MS and for comparative work no sample pretreatment, other than cleaning, is required.

2.2 Trace Elements in Glass

The trace metal content of glass has been shown to be dependent on the type of glass and on its site of manufacture (114), and it usually depends on the trace element composition of the raw materials. The majority of flat glasses in this country are manufactured by Pilkington Brothers. They have three manufacturing sites: St. Helens, Queenborough and Pontypool. Some examples of trace elements that can be used as discriminatory indicators between samples of glass are given below in Table 4.

Table 4. Trace elements of discriminatory importance

Element	Comment	Concentration
As	Batch refining agent, found in container and lamp glasses	10^2-10^3 ppm
Rb	High in St.Helens glass, low in other glasses	D.L.-30 ppm
Sr	Low in St.Helens glass, higher in other glasses	D.L.-80 ppm
V	Variation is thought to be correlated to concentration in fuel oil used	D.L.-10 ppm

/cont.

Table 4 (cont.)

Element	Comment	Concentration
Fe	Depends on furnace lining condition. Varies daily	ca 10^3 ppm
Sb	Low in Queenborough glass	D.L.-30 ppm.
Ba	Low in Queenborough glass	D.L.-300 ppm
Sn	Present in float glass	D.L.-30 ppm
Pb	Show a useful variation with type of glass and with site of manufacture.	D.L.-30 ppm
Zn		D.L.-20 ppm
Mn		30-400 ppm

D.L. = Detection limit

The trace metal composition of glass is of particular importance where the glass is to be used in fibre optic waveguides for laser telecommunications (116). Most transition metals in concentrations above 5 ppm will affect the transmittance of light through glass in the spectral range 2000 - 20,000 Å (112). Some work on the determination of the concentration of iron in such glasses is also presented here.

2.3 Direct Analysis of Lead in Glass

The direct analysis of glass, weighing fragments of glass into the furnace with subsequent atomisation of sample, is the most convenient method of analysis by AA. The results of these analyses are compared with those using solution techniques. The samples used had been previously analysed by spark source MS at HOCRE and will be referred to by their codes.

(a) Sample Preparation

The glass samples were washed with trichloroethylene and then soaked in 10% v/v nitric acid (Aristar grade, 0.005 ppm Pb) for 15 minutes. The glass was repeatedly washed in water followed by acetone. The sample was then broken into fragments in an agate pestle and mortar. For the experiments where powder was required the glass was further ground in the mortar under acetone (low in lead), the acetone was finally removed by evaporation under an infra-red lamp.

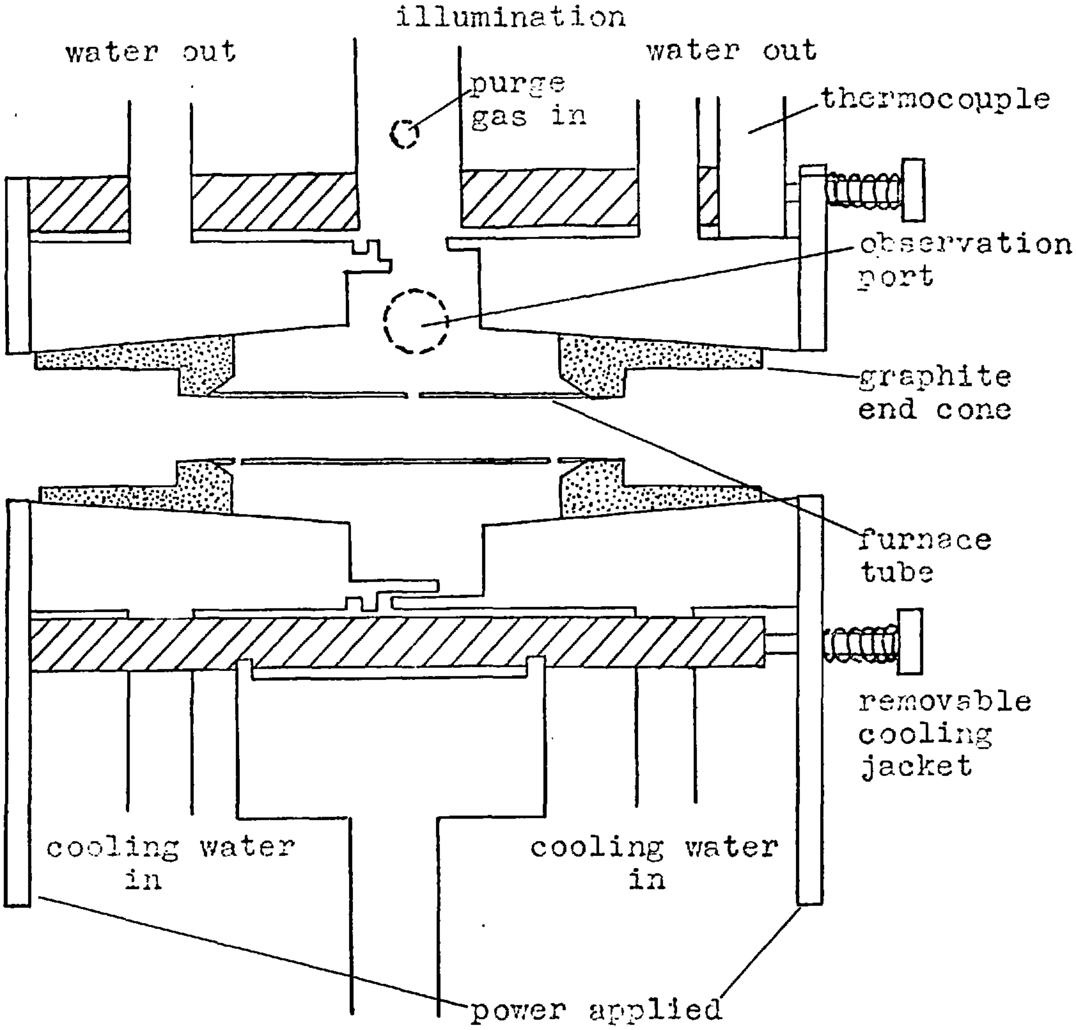
(b) Equipment

A Perkin Elmer (PE) AA spectrophotometer model 303 fitted with an HGA 70 furnace atomiser and a Telsec chart recorder readout was used for this work. A diagram of the furnace is shown in Fig. 2. Argon was used as the purge gas.

(c) Method

The glass fragments were weighed on an electronic microbalance weighing down to 1μg and introduced into the furnace using a vacuum tweezer. The lead concentration was determined by observing atomic absorption at the ground state 2833.0 Å line. The PE 303 has no background correction facility and therefore a nearby non-ground state line 2873 Å was used to check for non-atomic absorption. None was observed under the conditions of this experiment. The samples were atomised at full power (10v on HGA 70 power supply *ca.* 2500°C) with no ashing step; this gave maximum sensitivity. The absorption pulse, as recorded on the chart recorder was fast (Fig. 3) and was followed by a series of smaller absorption peaks probably due to particle scatter. If after cooling the sample was re-atomised no atomic absorption pulse was seen at the same time in the cycle as the initial absorption pulse although some later peaks

Fig. 2 HGA 70 Graphite Furnace



probably due to scatter are seen. The glass bead was removed from the furnace after analysis by use of a metal rod, the furnace then being flashed at full power to remove any contamination. The samples were reproducibly positioned in the centre of the furnace using the vacuum tweezer; several blank runs ensured that this did not introduce any lead contamination.

Several glasses were analysed by this method and it was found that in all cases a straight line calibration curve could be constructed for mass of glass vs. absorbance (Figs. 4,5).

Fig. 3 Repeated atomisation of one glass sample

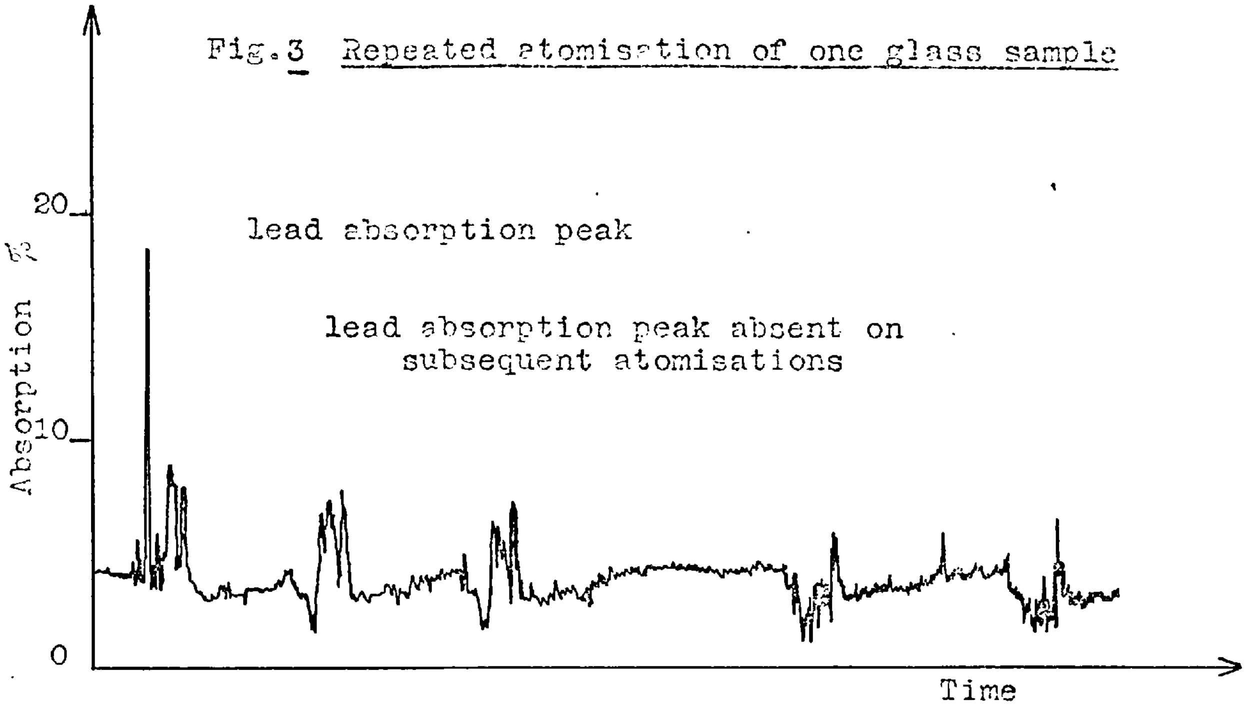
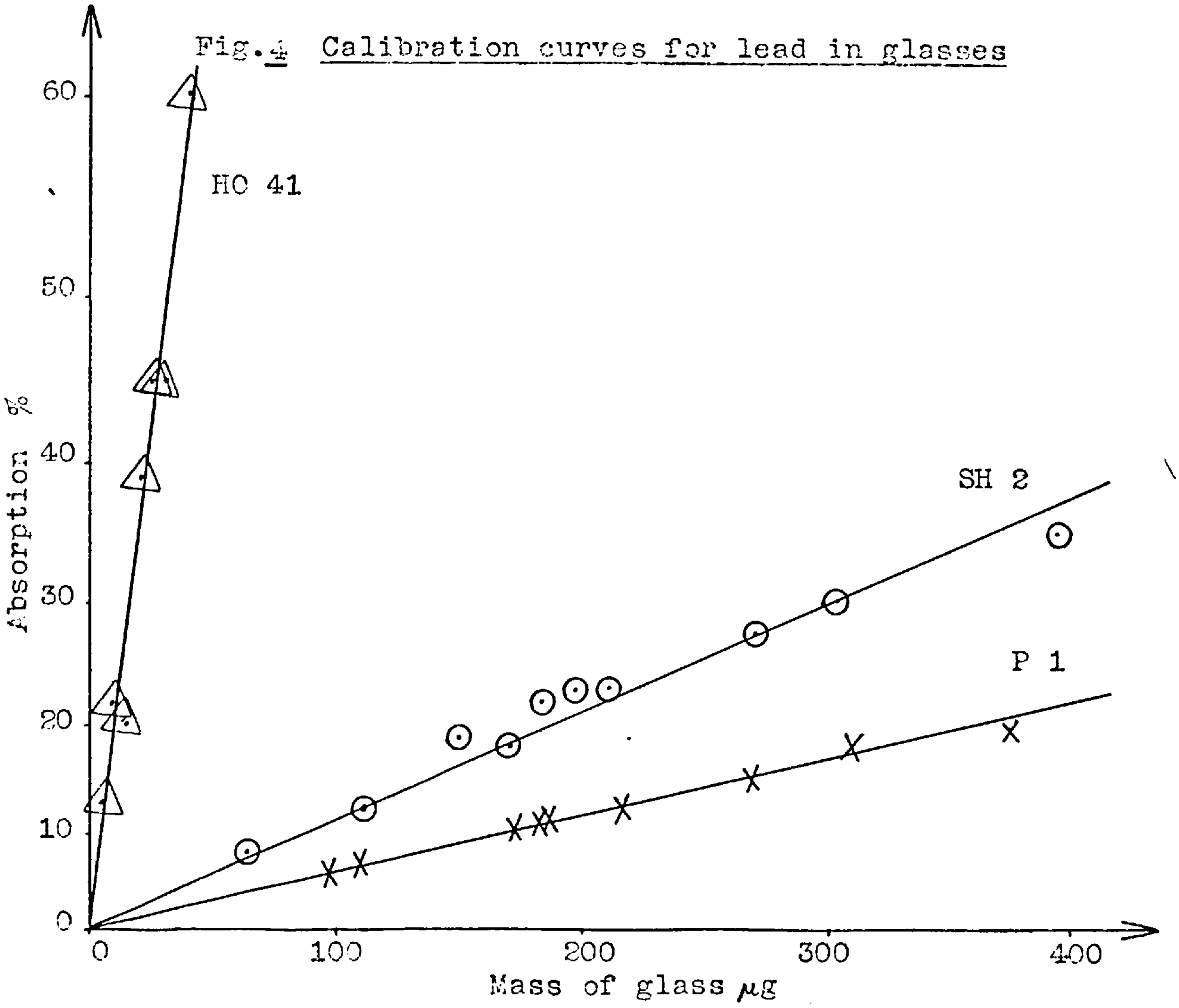


Fig. 4 Calibration curves for lead in glasses



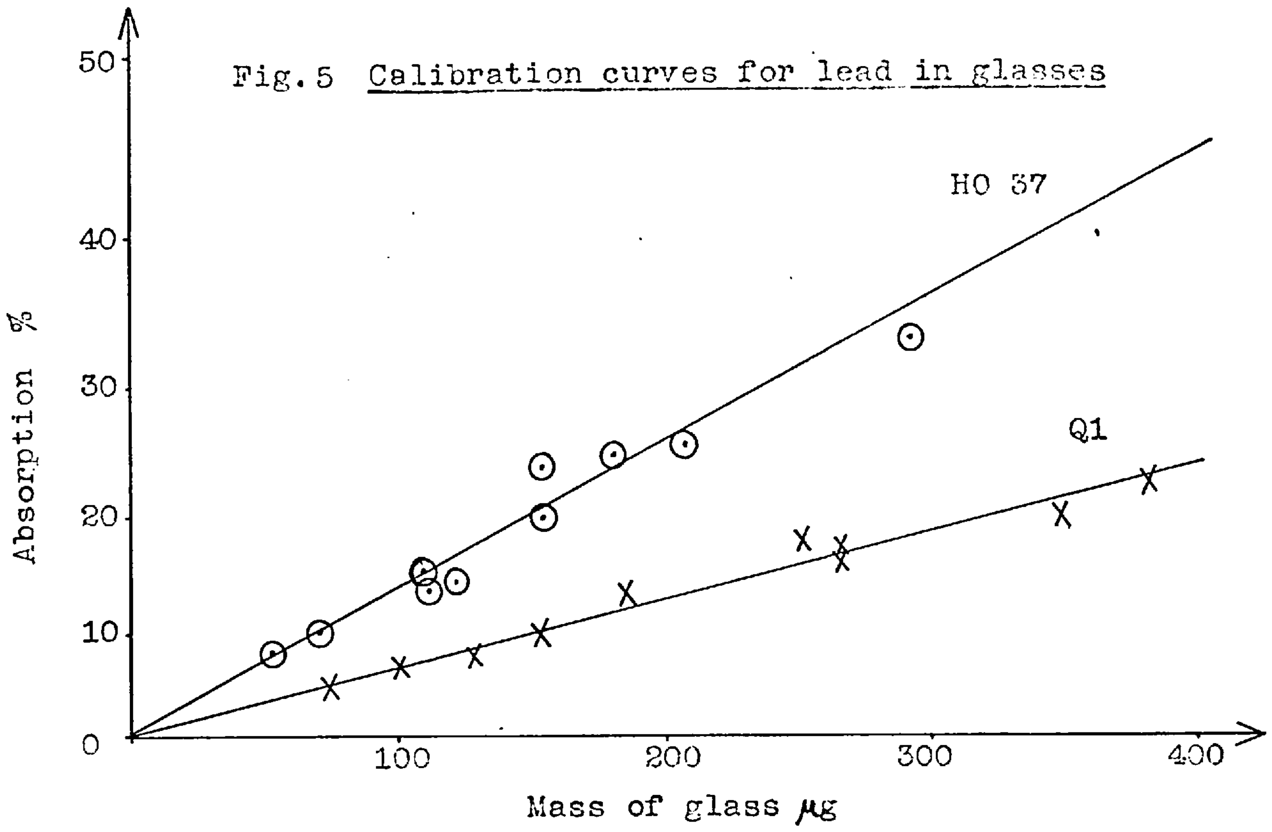


Fig. 5 Calibration curves for lead in glasses

By comparing the slope of such a graph against that for lead solution it was possible to determine the concentration of lead in these glasses. Samples of more than ca. 400 µg caused erratic signals probably due to analyte at surface and centre atomising at different times.

Table 5 Results of direct analysis

Glass	Code	Mass for 20% Abs. µg	Concentration ppm	Relative Standard deviation%	Concentration from MSppm
St. Helens	SH2 17.8.67 7m/C	150	2.6	7	18
Queenborough	Q2 8.10.67	310	1.3	8	N.D.
Pontypool	2.8.67 13m/C	355	1.1	4	N.D.
	HO 41	9.5	41	12	189
	HO 37	145	2.7	10	20

N.D. = not determined

(d) Lead Calibration Curve

A lead calibration curve was constructed using 10μl aliquots of sample. They were dried for 20 secs and atomised at full power (10v on meter, ca. 2500°C). The calibration curve was linear to 2ng of lead and the detection limit was 0.05ng of lead. The precision was better than ±5% for analyses of more than 0.15ng.

(e) Conclusion

It is possible using this method of direct atomisation to distinguish between the glasses analysed except for samples HO 37 and SH2 which had the same concentration by this method and MS within experimental error. There is poor agreement however between the actual values of concentration between this method and MS and it was decided to analyse the glass for lead by a wet chemical method.

2.4 Analysis for Lead in Glass by Wet Methods

To determine whether the direct method of analysis was accurate the glass was dissolved in acid and the lead extracted and determined by the standard addition method.

A 20mg sample of the ground glass was placed in a platinum crucible and 1 ml. of 72% $HClO_4$ and 3 ml. of 48% HF were added, the solution was heated on a steam bath adding HF as necessary until the glass was dissolved. On complete dissolution the crucible was transferred to a hot-plate and carefully evaporated to dryness. After cooling the residue was dissolved in a few drops of $HClO_4$ and made up to 5 ml. in a graduated flask with distilled water. A 10μl aliquot of this solution when atomised in the furnace gave non-atomic absorption signals on the nearby non-ground state line at ca. 2873Å; this could not be eliminated by ashing without loss of lead. Sodium fluoride has a similar boiling point to lead and was possibly the cause of this non-atomic absorption. The problem was overcome by extracting the lead as the PbI_4^{2-} complex into methyl iso-butyl ketone (MIBK). The lead was complexed by the addition of 5% HCl-KI solution which had previously been cleaned of its Pb contamination by extraction with MIBK (AnalaR KI contains ca. 5 ppm Pb). The extraction procedure is very efficient and only one extraction was required to remove the Pb from the KI to a level below the detection limit for Pb. To 500 μl of glass solution was added 500μl of acidified KI and 25ml of 30% HCl to ensure sufficient acid strength. This mixture was then extracted into one 500μl aliquot of MIBK and the analytical samples taken from the organic layer. The process was repeated twice using added standards. The MIBK extracts were ashed in the furnace at 750°C for 30 seconds. There was no scatter signal observed on subsequent atomisation of the sample.

A sample of glass analysed by this method gave the following results.

Table 6 Results of wet analysis

S.H.2	Sample 1	16.4	16.4	ppm Pb
	2	11.0	19.7	ppm Pb
	3	18.6	16.4	ppm Pb

Mean 16.4 ppm

Conclusion

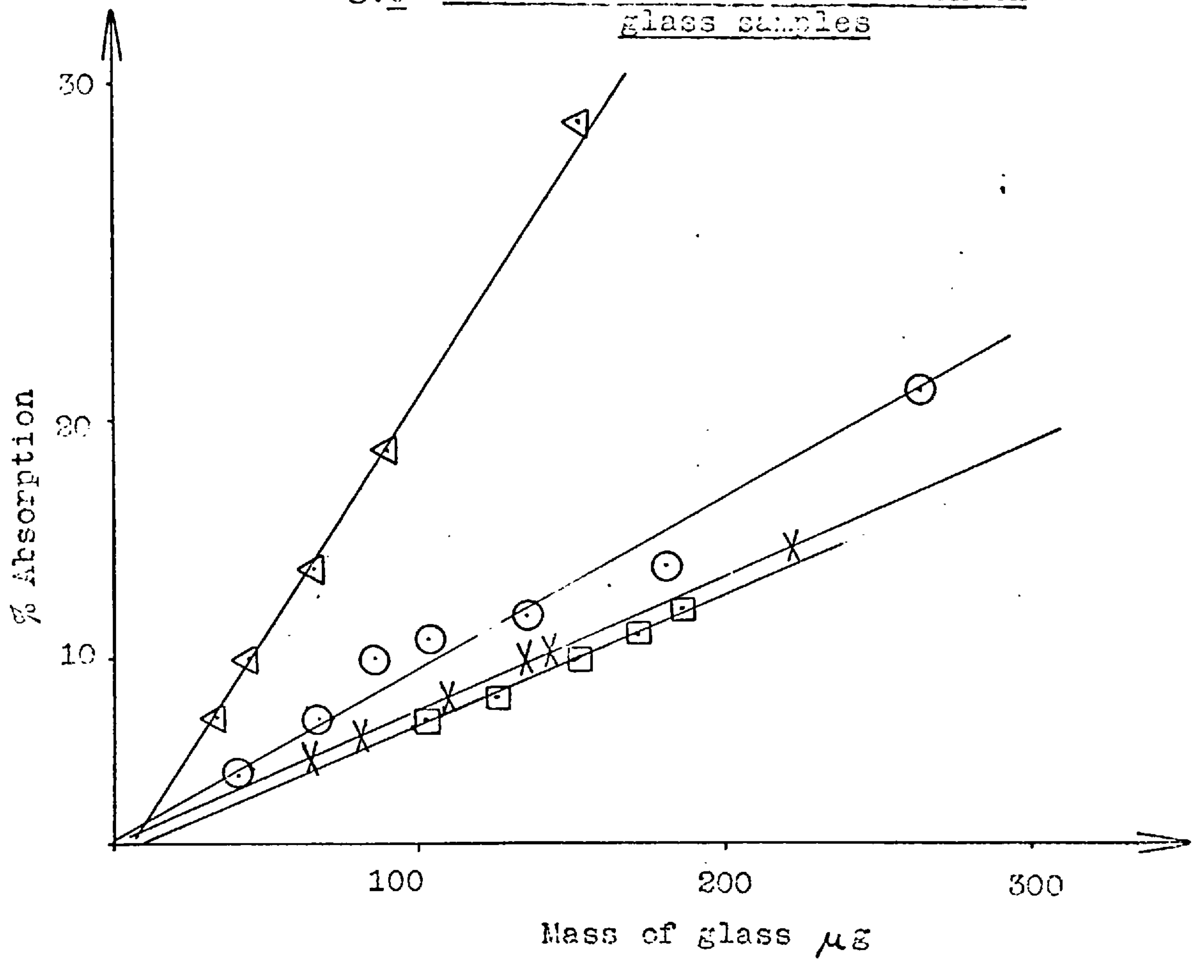
This wet analysis agrees within experimental error with the result obtained by MS but suggests that there is a severe depression of signal during the direct analysis of lead in glass. This is perhaps not surprising in view of the involatile matrix of the sample.

2.5 Analysis for Low Level Iron in Glass

Some samples of silicate and glass material, used in the manufacture of glass fibres for laser telecommunications, and consequently low in iron, were analysed to determine this relative iron concentration. The vapour phase interference during atomisation of the sample was studied by a method of standard additions.

A single channel AA spectrometer system was used in this work the details of which are given later (see page 43). Samples of glass were weighed on a microbalance (Oertling S1) and introduced into the furnace atomiser using a vacuum tweezer. Observation was made at the iron ground state 2483.3 Å line and the samples were atomised at full power (ca. 2500°C). The instrumentation used did not have background correction and non-atomic absorption was measured for several samples on a nearby non-ground state line at ca. 2460 Å. A modified vacuum tweezer with a glass capillary tube as the needle reduced iron contamination to a negligible level. A calibration curve for iron solution was constructed using 10μl aliquots; the samples were dried at 100°C for 20s and atomised at ca. 2500°C. Several glasses were analysed by directly weighing fragments into the furnace and atomising the samples at ca. 2500°C; there was no measurable non-specific absorption during atomisation. Straight line calibration curves for the glass samples were obtained (Fig. 6) and a comparison of slopes with the solution calibration curve gave the concentration of iron. A precision of ±10% or better was obtained for the glass analysis.

Fig.6 Calibration curves for iron in glass samples



(a) Purge Gas

Argon and nitrogen were separately used as purge gases and it was found that argon gave a higher (x 2.5) sensitivity than nitrogen (Fig. 7). This has been observed for many elements in AA and also in studies of atomic emission. This will be discussed later in more detail (see page 169).

(b) Gas Phase Interference

It was decided to introduce a 10μl aliquot of 0.1 ppm standard iron solution with the glass samples into the furnace. These were dried for 20s at 100^{o}C and atomised at full power ca. 2500^{o}C. A single absorption

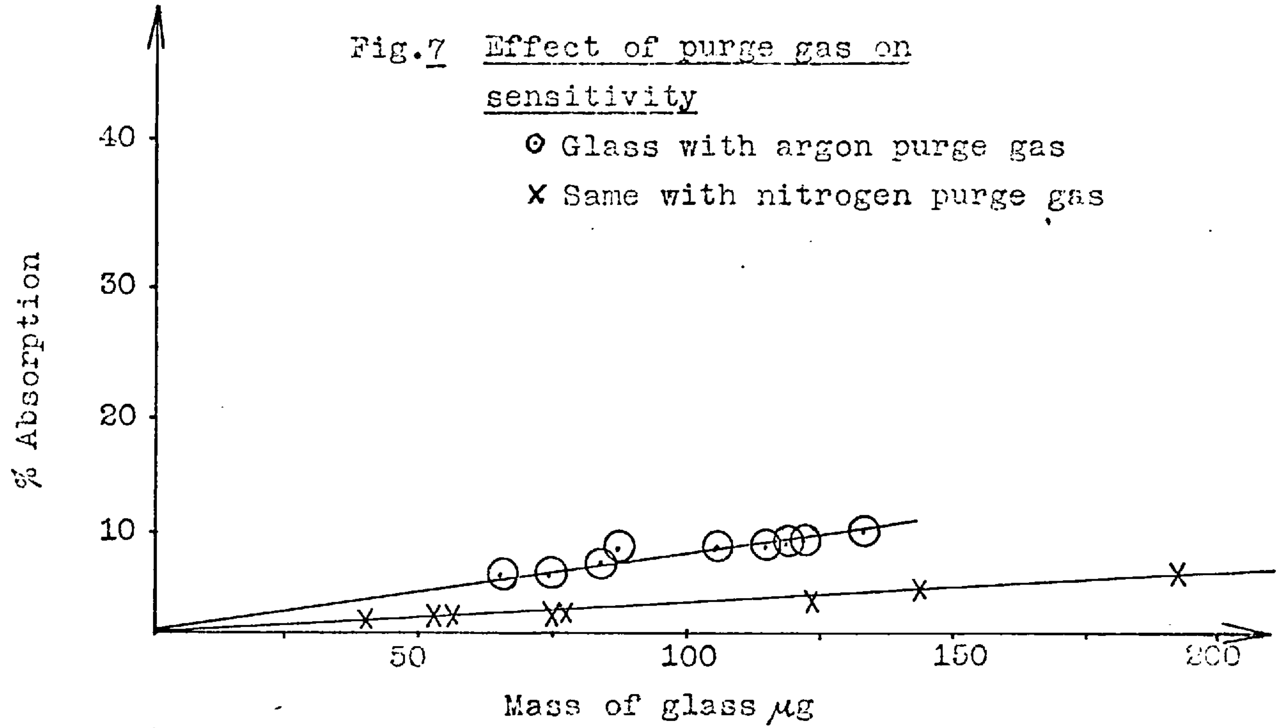


Fig.7 Effect of purge gas on sensitivity

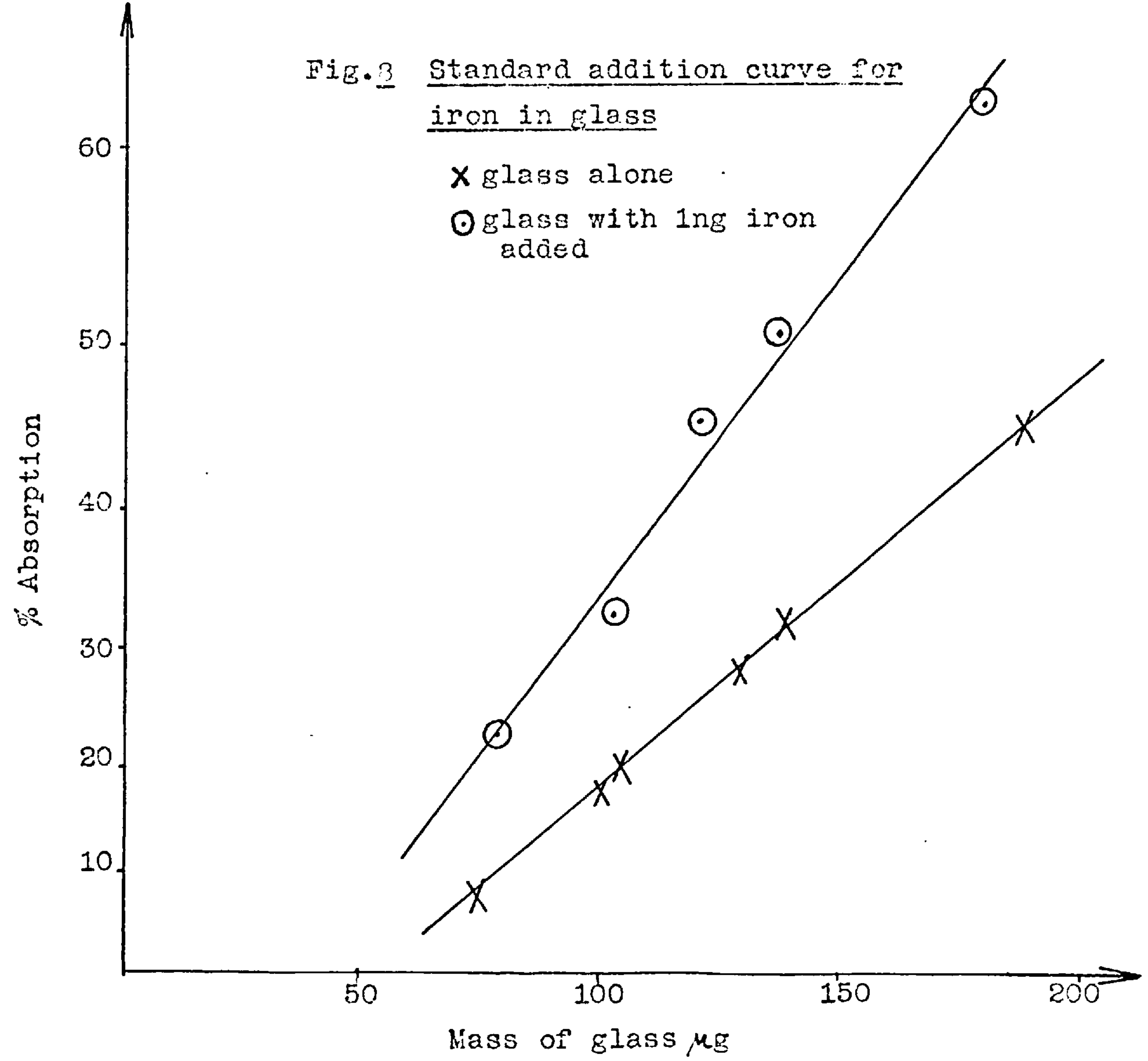


Fig.8 Standard addition curve for iron in glass

peak was seen showing that the atoms from analyte and standard were formed coincidentally in time and thus experienced the same vapour phase conditions and interferences. A straight line standard additions curve was obtained with a steeper gradient than the calibration curve for the glass, indicating that the interference was an enhancement of signal (Fig. 8). Both curves intersect the abscissa, this was not observed for the same glass at a later analysis and was possibly due to incorrect alignment of the furnace allowing some light from the furnace walls to reach the PMT and cause this effect.

2.6 Conclusion

For the purposes of some analyses it is necessary only to determine relative concentration. This is particularly true in forensic science where a comparison between crime and suspect samples is made. The technique of AA using electrothermal vaporisation of sample allows a comparative analysis of this kind to be made. It has the advantages of being rapid, simple and cheap compared with NAA and MS.

The analyses appear to suffer from matrix effects of the glass which have been shown to be a depression overall for lead and a vapour phase enhancement in the case of iron. As such these matrix effects prohibit accurate analyses. It is possible to use a dissolution technique together with solvent extraction to obtain better results and it should be possible to adapt the wet method described to analyse mg samples.

Chapter 3

Single Element Analysis of Trace Metals in Hair

3.1 Introduction

3.1.1 General

The operating parameters necessary for the analysis of individual elements in hair were investigated and a single set of conditions devised for simultaneous multi-element analysis. At the beginning of this work it was realised that a compromise between length of hair and detection limit of analyte would have to be made. One centimetre of hair weighs 50 - 150μg; it is convenient to handle and permits the analysis of up to 23 elements (Table 3), 13 of which have been determined in this study. This length of hair also allows a reasonably detailed trace metal profile to be obtained on whole head hairs of normal length.

It was considered that the best method of analysis would be direct introduction of the 1 cm. sample of hair into the furnace,followed by ashing and atomisation. The ashing step removing organic material which would otherwise cause non-atomic absorption.

3.1.2 Matrix Effect

Atomic absorption signals obtained from a sample can differ from the signals obtained from the same quantity of pure analyte element. The signals can undergo either attenuation or enhancement. During atomisation, a cloud of material other than the analyte is often produced and this may induce condensation of the metal atoms and reduce the atomic absorption. The cloud of material produced may also cause non-atomic absorption to occur, giving false high readings.

In any analysis it is necessary to know whether the signals obtained have come from analyte which is experiencing a matrix effect. Enhancement can be checked for by observing on a near-by line which is not an absorbing line for the element of interest. Some types of enhancement might escape notice by this method. A depression of signal has in this case to be checked for by using an alternative analytical method; several wet methods have been used and are discussed in detail under the appropriate element.

3.1.3 Standard Hair

The ideal way of determining the accuracy of the method is to analyse a standard hair with known trace metal contact. Chapter 1 should convince the reader that no natural standard hair is to be found but attempts have been made to produce one using a silk matrix and the results of these experiments are discussed in Appendix 1.

3.1.4 Washing Procedure

The washing pretreatment given to hair prior to analysis has been the subject of much research and is discussed in Chapter 1. Most workers have used a washing procedure and a compromise between removing contaminating dust and grease and removing trace elements more closely bound to the hair has to be made. The washing procedure adopted by Coleman et al. (83) and later by Renshaw et al. (43,93) was a 2h. Soxhlet extraction using diethyl ether and this was also the method used in this study. It removes the surface grease and dirt but, being non-ionic, it does not remove the more firmly bound ions. Coleman et al. (83) found that this method retained all the elements studied better than 70% and Cu, Cl, Na, Zn, Br, Au, I, Ca and Sb were retained more than 81% (117).

3.2 Equipment

A single channel system was used (Fig. 9) comprising a D 330 Hilger and Watts monochromator fitted with a Jobin-Yvon 1200 lines/mm plane grating blazed at 3300 Å. An EMI 6256B photomultiplier tube powered by a Brandenberg power supply (max 2 kV) and Servoscribe strip-chart recorder (response time 250 ms for F.S.D.) provided photo-electric detection and readout. The HGA 70 graphite furnace and power supply was used as the atom cell (see Fig. 2). Various hollow cathode lamps were used powered by a power supply built in the Departmental Electrical Workshop. An Oertling QS1 quartz fibre microbalance weighing down to ca. 0.1μg was used to weigh the samples of hair. The reproducibility of the balance was found to be better than ½%. A solids injector consisting of a borosilicate capillary and platinum wire plunger was used to introduce the hair samples into the furnace. A 10μl Eppendorf micropipette was used to deliver the solution samples.

3.3 Copper

3.3.1 Dry ashing

The samples of hair were washed, weighed and introduced into the furnace using the solids injector. The operating conditions are given in Table 16. Atomisation of an ashed sample gave an absorption signal of less than 0.3% on the non-absorbing line at 3283 Å, thus, non-atomic absorption was considered insignificant under these conditions. Analyses were carried out with nitrogen purge gas. A solution calibration curve was constructed using identical conditions, it was found to be linear up to 4 ng of copper. There was no loss of copper under these ashing conditions.

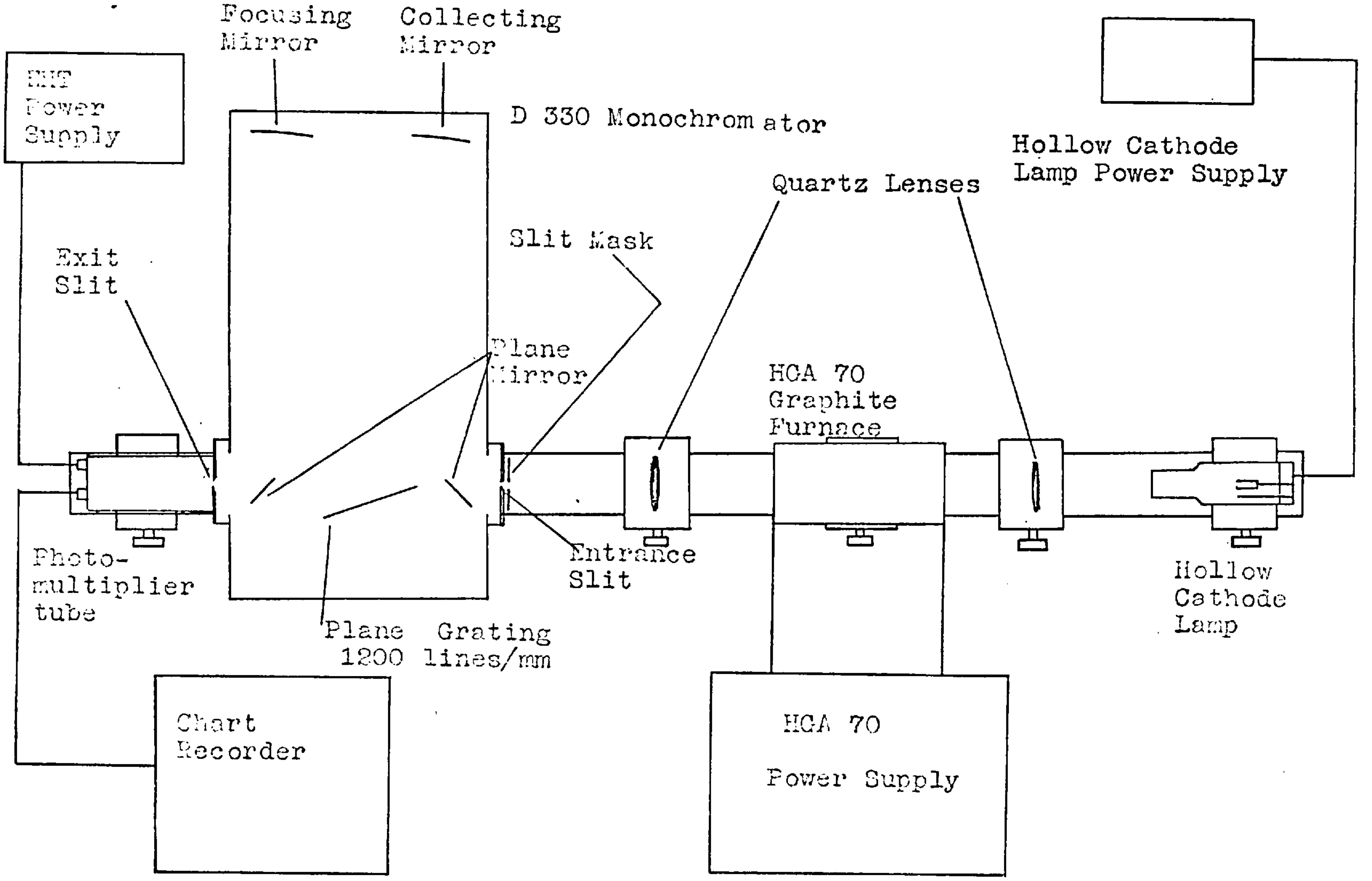


Fig.9. Layout of Single Channel AA Spectrometer

3.3.2 Wet ashing

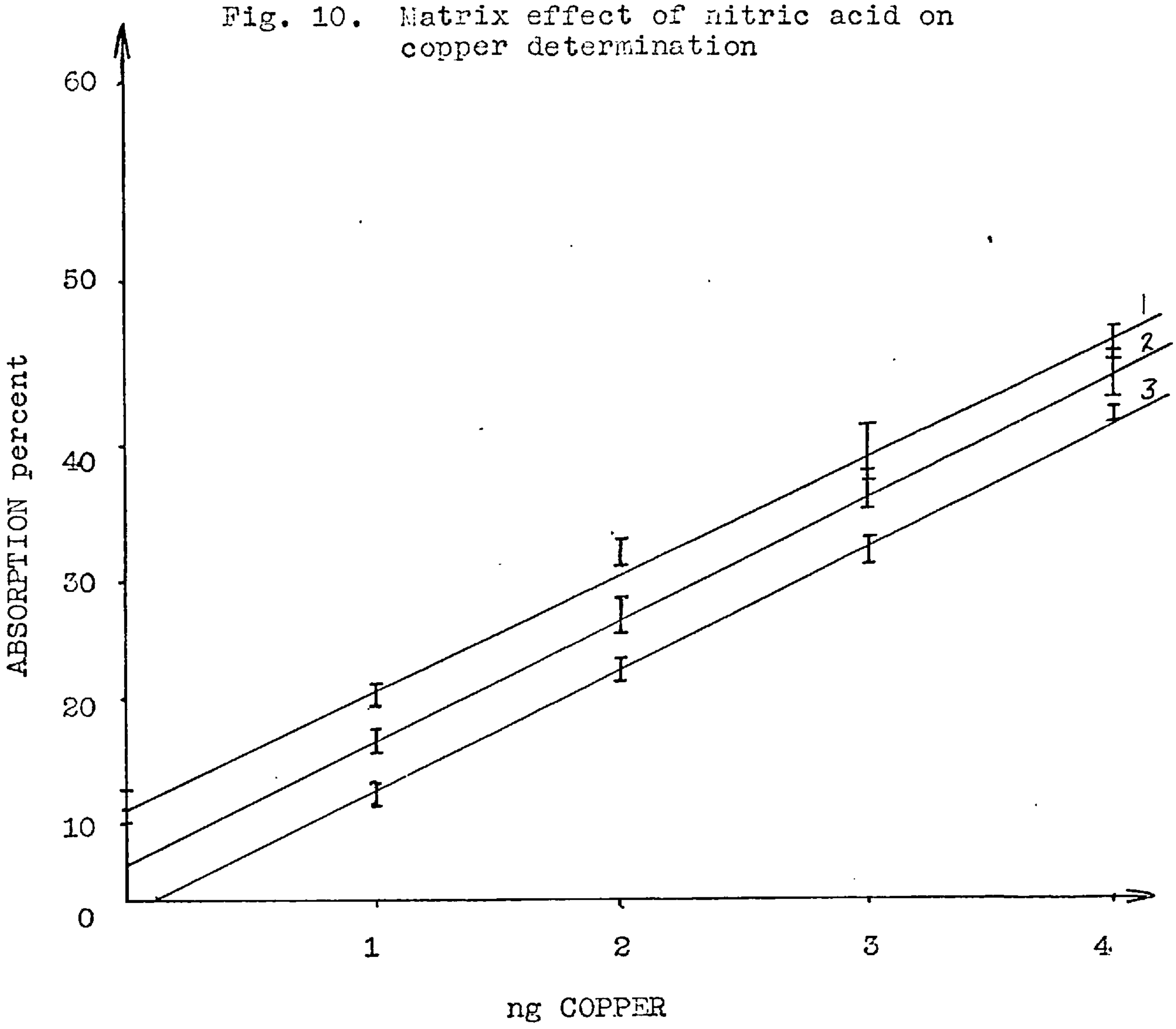
A method of breaking down the organic matrix whilst keeping contamination to a minimum was sought. Hair dissolves rapidly in 70% nitric acid if heated. A micro-dissolution technique was therefore developed using nitric acid. A weighed section of hair (ca. 1cm.) was inserted into the end of a glass capillary micro-pipette ($5\mu l$ volume) which had been previously scribed in the middle. Nitric acid (70%) was drawn into the pipette to the mark and the pipette was warmed to ca. 60°C. The nitric acid was moved to and fro to facilitate dissolution. The dissolved sample was injected into the furnace and the pipette rinsed in. The sample was then dried, ashed and atomised (Table 16) and the absorption signal measured. The nitric acid blank was determined and found to be 0.01 ppm Cu; the use of plastic containers introduces less contamination than glassware.

3.3.3 Alternate wet and dry ashing

The hair when analysed by the dry ashing method is heated to a maximum temperature of 1100°C prior to atomisation to destroy most of the organic matrix. A black residue is left after ashing, indicating that a complete removal of organic material does not occur. By dissolving the hair samples in nitric acid it was hoped that the organic material could be oxidised and evaporated leaving only inorganic salts. This would decrease any matrix effect.

Several hairs were then analysed, alternate sections by wet and dry ashing respectively. The arithmetic means and standard deviations for the copper concentration by the two methods were determined. The results are shown in Table 7 for the determination of 40 sections of hair from the same head.

Fig. 10. Matrix effect of nitric acid on copper determination



1. Standard addition curve for hair dissolved in nitric acid
2. Standard addition curve for pure nitric acid
3. Calibration curve for copper.

Table 7. Copper in hair

Dry ashing	13.1 ± 2.9 ppm Cu
Wet ashing	12.2 ± 4.2 ppm Cu

These results are in agreement with each other and with other published data (Table 1). The standard deviations include the error due to the method together with any variation along the length.

The matrix effect of nitric acid on the determination of copper was investigated by dissolving some hair in nitric acid and determining the copper by standard addition (Fig. 10). The slopes of solution calibration curve, nitric acid standard addition and hair digest standard addition agree within 5% and thus nitric acid has no appreciable matrix effect on the determination of copper.

It was concluded that the dry ashing method is accurate for copper. Several hairs were then analysed along their length using ca. 1cm. samples. The results are shown in Figs. 11,12. There is no trend common to all the samples although head hair Fig. 12 shows an erratic decrease in copper towards the root. The pubic hair Fig. 12 has a similar copper concentration to the head hair.

3.4 Lead

3.4.1 Dry ashing

Lead was determined in hair by the dry ashing method already discussed, under conditions that are given in Table 16. The atomic line chosen was 2833.0 Å; this is slightly less sensitive than the 2170.0 Å line but is emitted more strongly (x 10) by the hollow cathode lamp and therefore a better signal:noise is obtained. It was found from

Fig. 11 Copper concentration variation along length for two head hairs, Male 1.

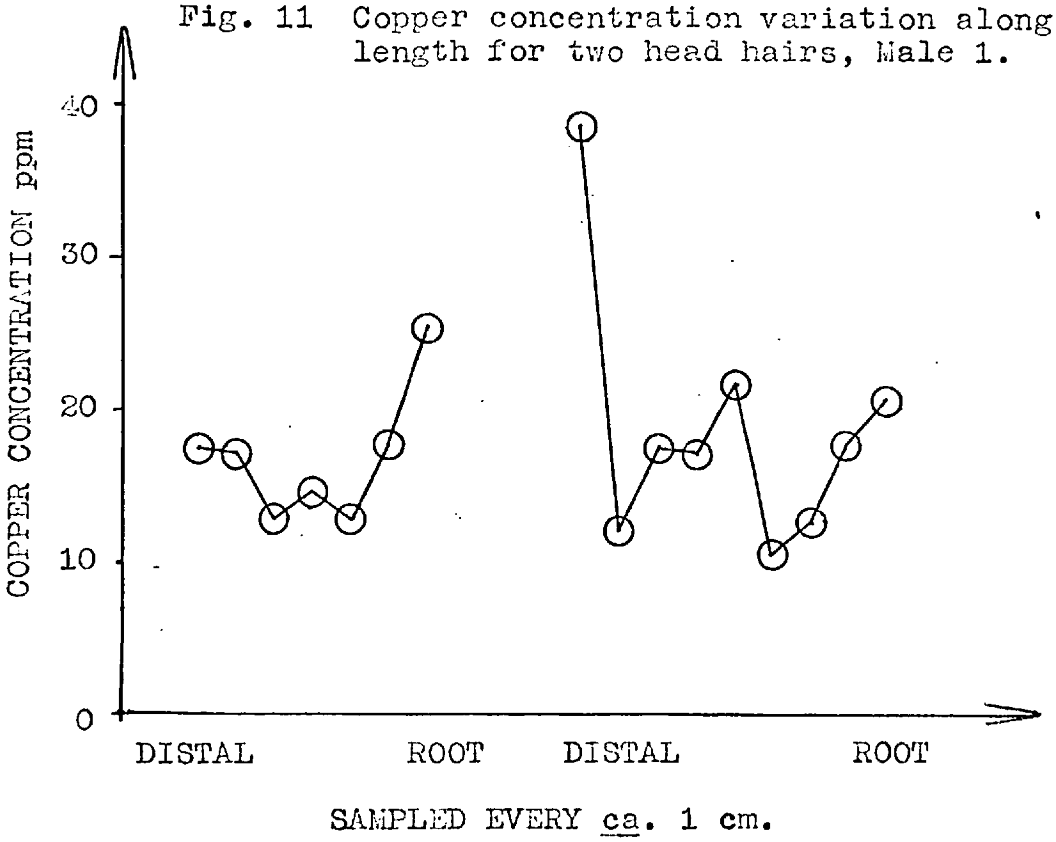


Fig. 12. Copper concentration variation along length for a head hair and a pubic hair, Male 1.

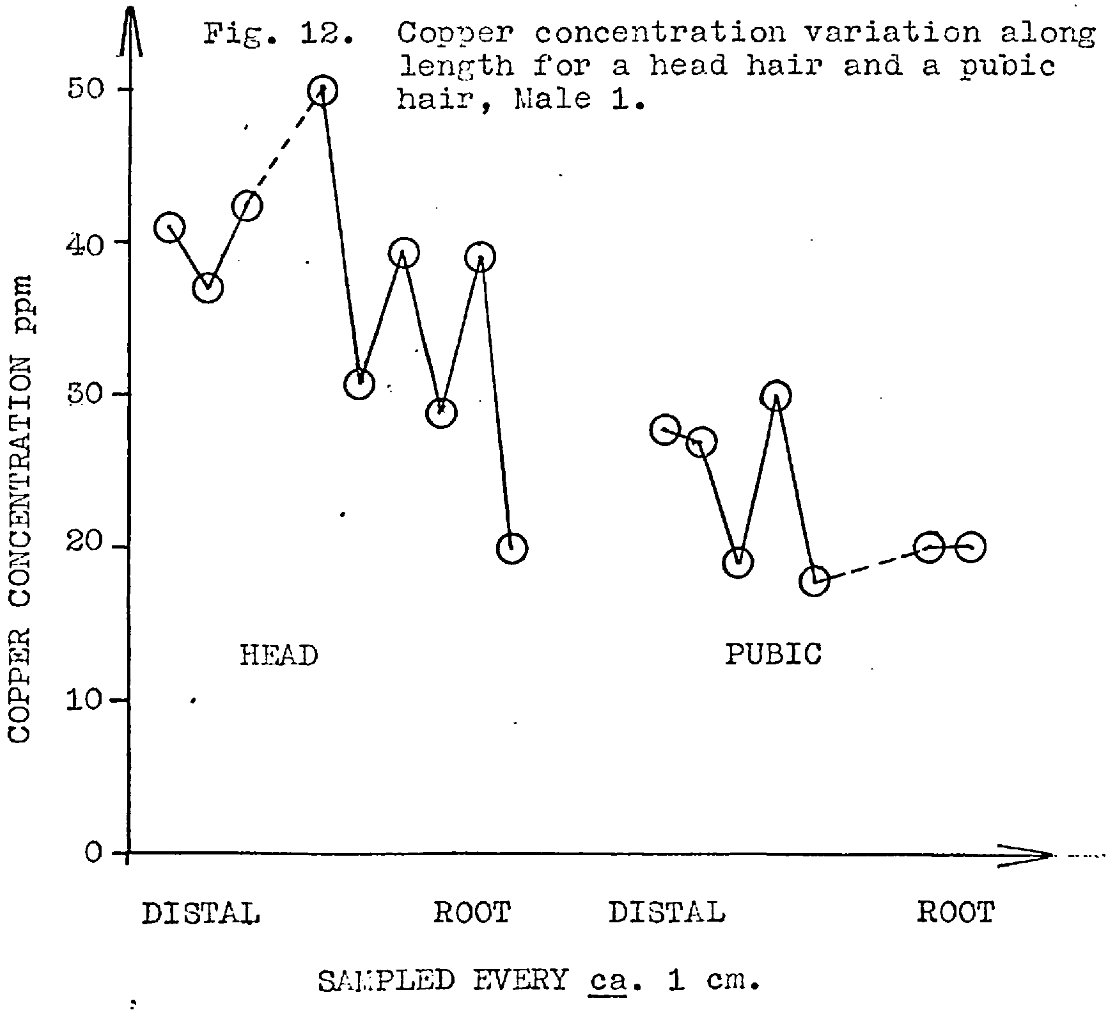
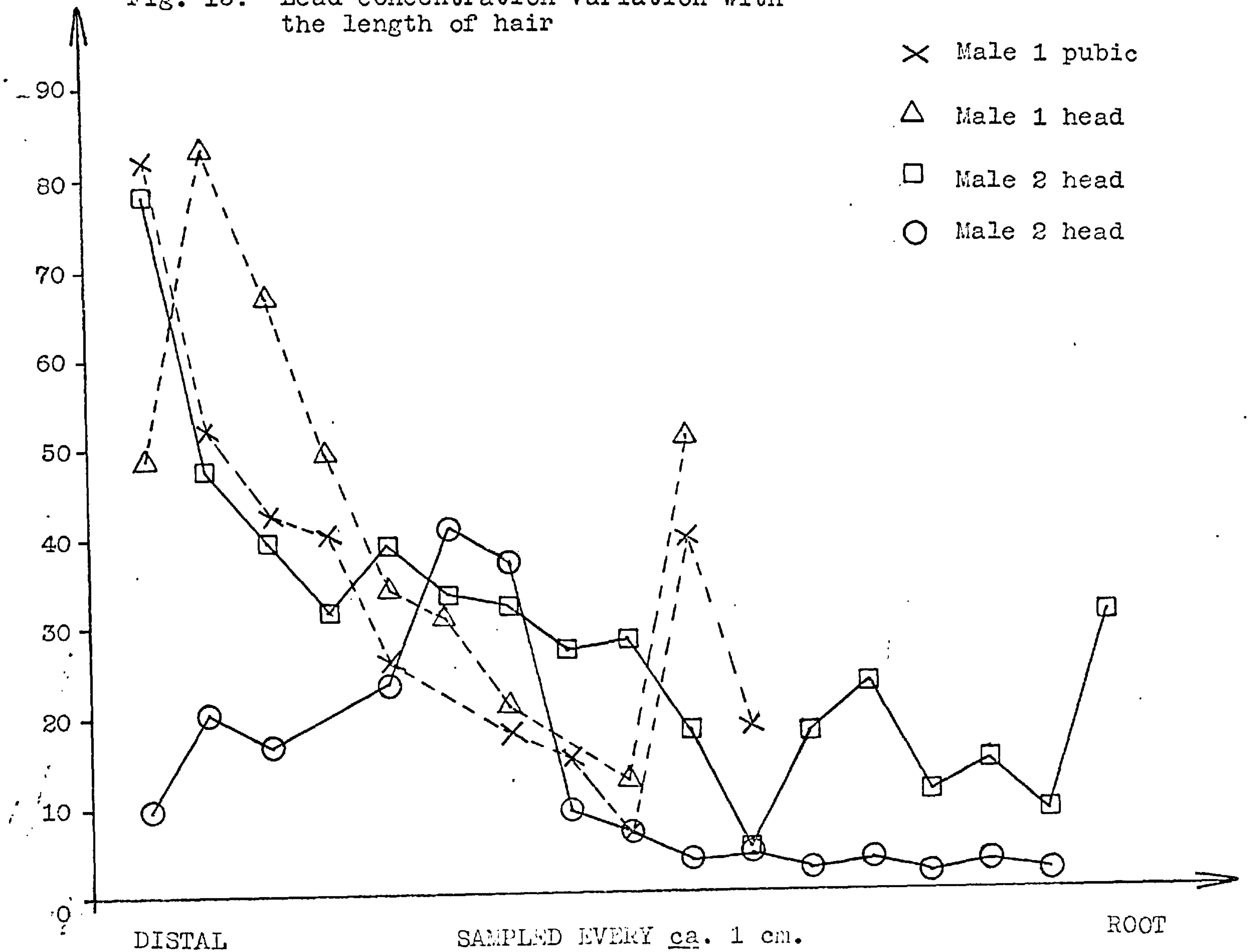


Fig. 13. Lead concentration variation with the length of hair

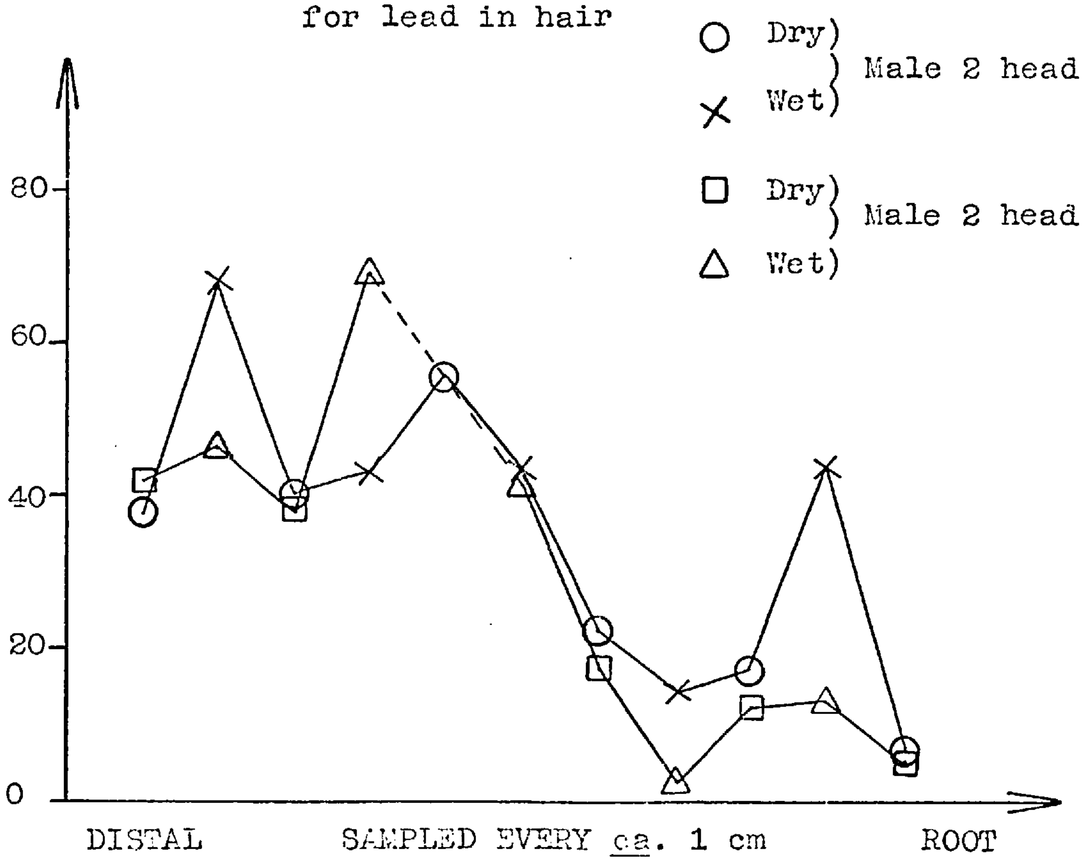


the analysis of several hairs that the lead concentration increases from root to tip (Fig. 13), this was also found by Renshaw and co-workers (43,93). Non-atomic absorption was checked for by observing on the nearby non-absorbing line 2873 Å; none was found under these conditions. A solution calibration curve was obtained and found to be linear up to 5 ng of lead.

3.4.2 Alternate wet and dry ashing

A similar procedure for lead was used, alternate 1cm. sections being analysed by wet and dry ashing. The results for some typical analyses are shown in Fig. 14.

Fig. 14. Alternate wet and dry ashing for lead in hair



It can be seen that there is a trend of increasing concentration towards the hair tip. This effectively prohibits any simple statistical analysis. A similar profile of lead concentration along hairs from the same head is obtained by dry ashing alone and by alternate wet and dry ashing (Figs. 13,14). This is a rather unsatisfactory method of determining the accuracy of the dry ashing method but is the result of the lack of a standard hair and the inherent increase in lead towards the distal end in natural hair. The concentration values found however are in agreement with those published in the literature (Table 1).

3.4.3 Matrix effect of nitric acid

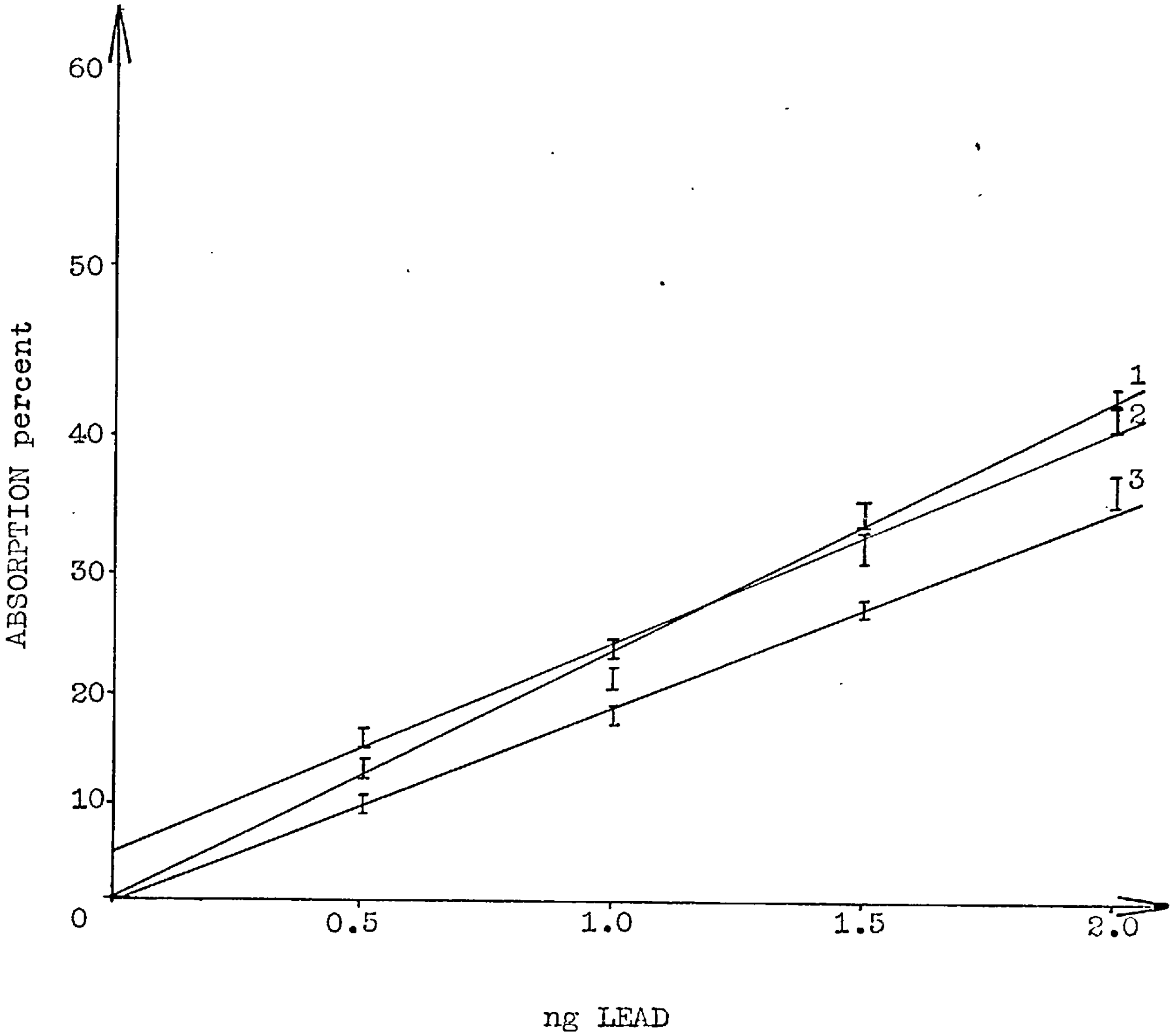
The matrix effect of nitric acid was found by dissolving several milligrams of hair in concentrated "Aristar" nitric acid and determining the lead by standard addition. A standard addition curve for the pure acid was also obtained. These two curves were then compared with the lead solution calibration curve (Fig. 15). It can be seen that the standard addition curves suffer a depression compared with the calibration curve.

These two experiments, alternate wet and dry analysis and standard addition analysis of the acid, were intended to demonstrate that the dry ashing technique gave accurate results. They have shown that there is no major difference between wet and dry ashing methods. The results also agree with literature values (Table 1). This would indicate that the dry ashing technique for lead analysis gives reasonably accurate results. A better comparison cannot be made by this approach due to the variation down the length of the hair.

3.5 Zinc

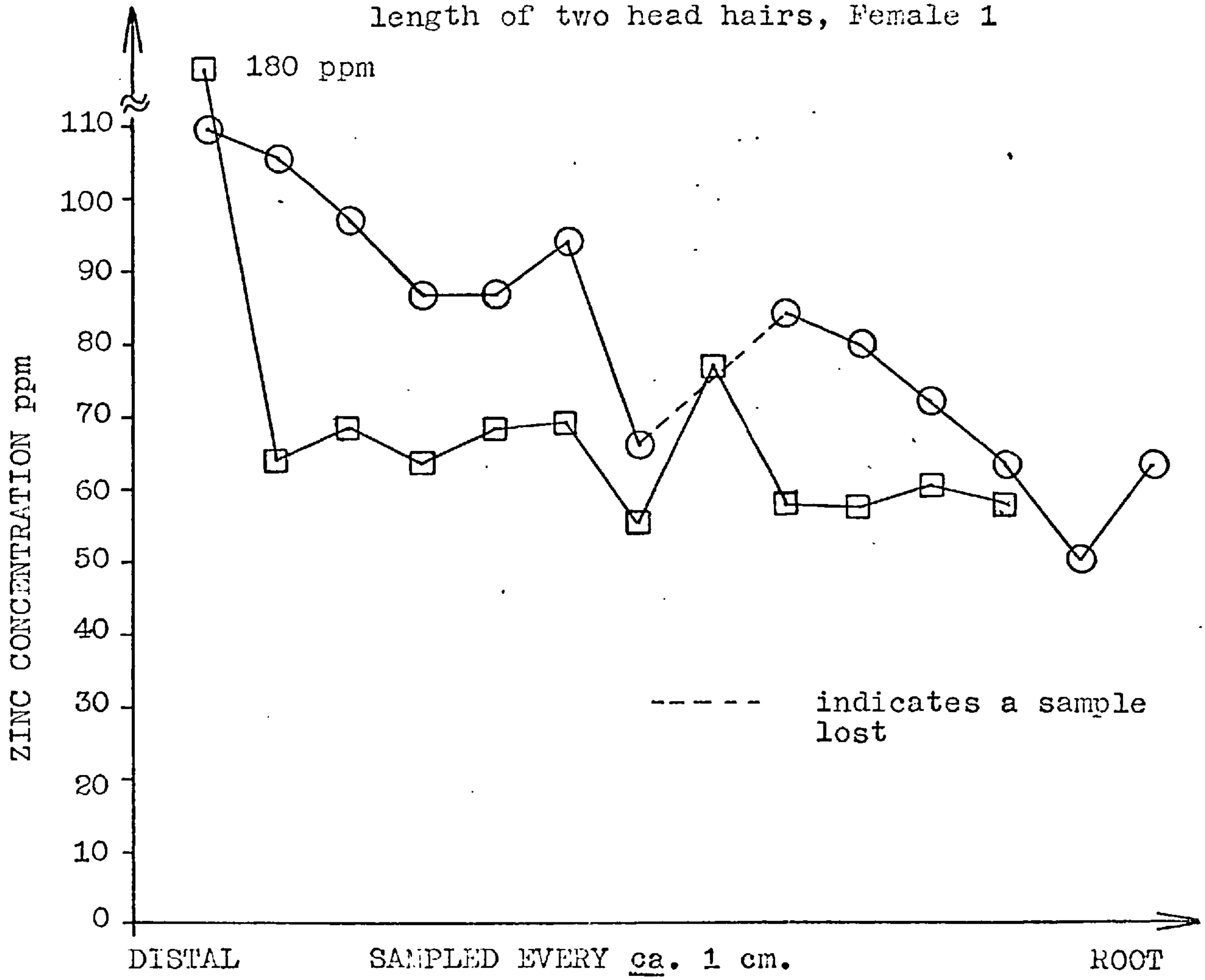
The optimum conditions for the analysis of zinc in hair can be found in Table 16. The 3075.9 Å Zn line has been used to ensure that the measurements are made on the linear

Fig. 15. Matrix effect of nitric acid on lead determination



1. Lead calibration curve
2. Standard addition curve for hair dissolved in nitric acid
3. Standard addition curve for pure nitric acid

Fig. 16. Variation of zinc concentration along length of two head hairs, Female 1



part of the calibration curve. The 2138.6 Å Zn line is far too sensitive in absorption to be used for this analysis, owing to the high zinc concentration in hair. The nitrogen purge gas supply was stopped during atomisation; this was necessary to give adequate sensitivity (2-fold increase). The minimum ashing conditions necessary to remove all organic matter and prevent non-atomic absorption were: ashing for 30 seconds at 750°C followed by 10 seconds at 1100°C. Two hairs from a female head were analysed for zinc along their length (Fig. 16). It can be seen that for both hairs there is an increase in concentration towards the distal end. This does not agree with data published by Obrusnik *et al.* (14) but their study involved 10 cm. sections of hair and thus little detail for hair was obtained whereas this analysis sampled the hair every centimetre. It should be noted that the actual values obtained are very low compared with literature values (Table 1).

To compare the accuracy of this method a bulk sample of washed hair ends, ca. 3-4 cm. long, was analysed by flame AA and by the above dry ashing procedure. 60 mg, approximately, of this hair sample, was dissolved in 1 ml of 70% nitric acid, with heating; after dissolution $\frac{1}{2}$ ml of 70% perchloric acid was added and the solution evaporated to $\frac{1}{2}$ ml in volume then made up to 5 mls. with distilled water in a graduated flask. This was analysed by AA in an air-acetylene flame using a Varian Techtron VA 1000 spectrometer. The method of standard additions was used to overcome any matrix effect due to the acids present. A large number of ca. 1 cm. sections of hair were then analysed by dry ashing under conditions described above.

Table 8. Zinc in hair

Method	Concentration: S.D.	R.S.D.
Dry Ash	60.5 ± 5.5 ppm	± 9%
Wet Ash	240.5 ± 10.0 ppm	± 4%
Repeat Wet Ash	216.2 ± 8.7 ppm	± 4%

S.D. = Standard Deviation
R.S.D. = Relative Standard Deviation

The wet ash results (Table 8) are in agreement with published data (Table 1) on zinc levels in hair. The dry ash results (Table 8) are 3-4 fold lower and this is possibly due to loss of zinc on ashing. The boiling point of zinc is 913°C and the furnace reaches a maximum temperature of 1100°C. The dry ash results are given for 33 analyses; the R.S.D. is the combined error introduced by the error in the method and any variation in the sample thus the method must have a precision of better than 9%. This is of little use however, since the accuracy of the method is so low.

3.6 Aluminium

The optimum conditions for the analysis of aluminium in hair by the dry ashing method are given in Table 16. It was found that there was a 3.5 fold increase in sensitivity by using argon instead of nitrogen as the purge gas. The difference in thermal properties of the gases may account for part of this increase, argon has a lower thermal conductivity than nitrogen and the furnace will not be cooled as much by a given flow of argon. Aluminium can also form a nitride and may do so when nitrogen is used; the nitrogen might also contain sufficient oxygen to reduce the atomic population by oxide formation.

A sample of female head hair was obtained by brushing and analysed using 1 cm samples by the dry ashing method (see Table 16 for conditions). The aluminium concentration was found to be 15.5 ± 3.9 ppm. The total error is 25%. This contains the component errors of precision, over the head and down the length variations.

A larger sample of male head hair, clipped from the ends, was analysed by both wet and dry techniques. The dry technique used was as above. In the wet digestion 4 g. of hair was dissolved in 7 ml of a mixture of nitric, sulphuric and perchloric acids, 3:1:1 v/v. This mixture was diluted with an equal volume of water and 1% molybdenum (as MoO_3) added to speed the dissolution. The solution was evaporated to $\frac{1}{2}$ ml and 30 ml of 72% nitric acid added slowly to digest the remaining organic matter. The solution was fumed to dryness and the residue dissolved in

98% sulphuric acid and made up to 25 ml with water, in a graduated flask. The aluminium content was then determined in the HGA 70 furnace by direct standard addition; a 10 μl aliquot of sample followed by a 10 μl aliquot of standard. This method has been found to give precise results; no double absorption peaks being seen. An acid blank was also determined.

Table 9. Aluminium in hair

Dry Ashing	6.3 ± 3.6 ppm
Wet Ashing	6.5 ± 0.4 ppm

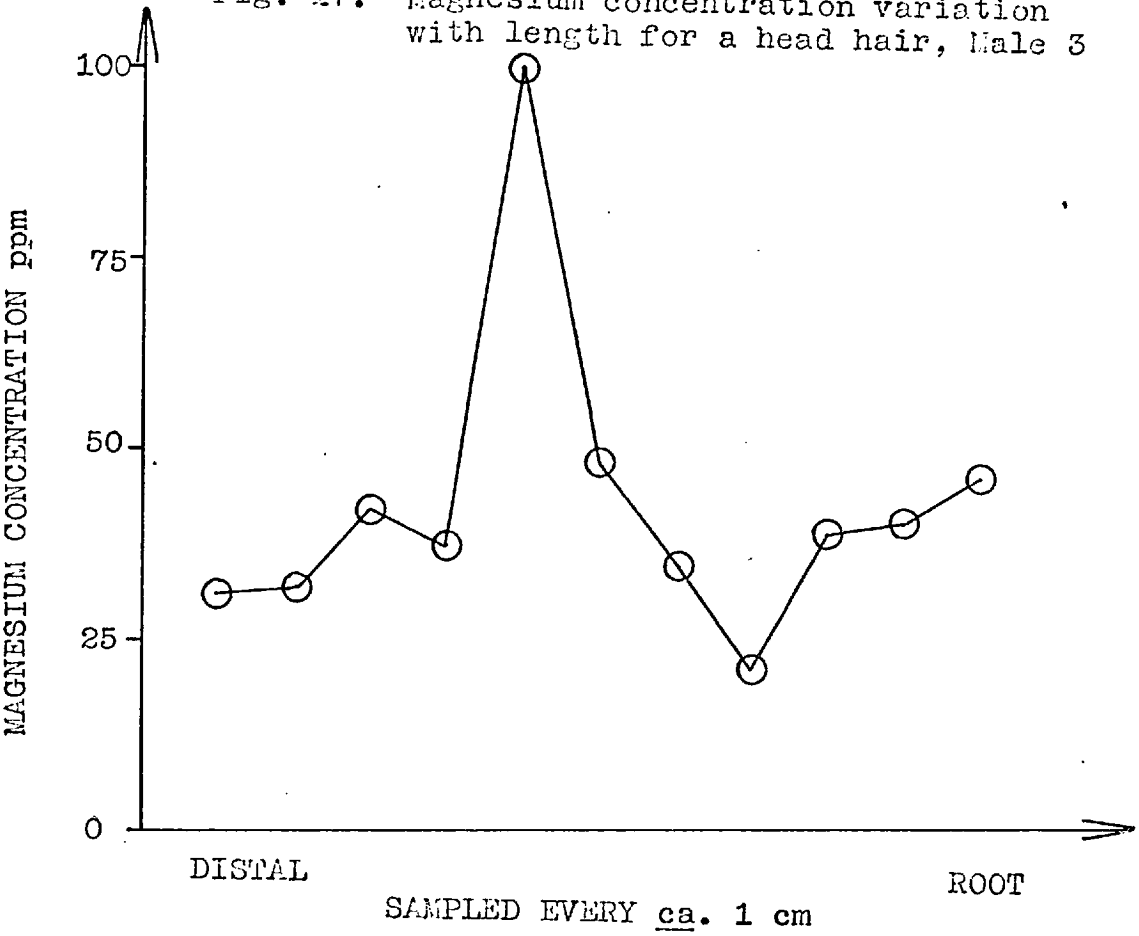
The total variation of the dry ashing method is 57%. It was concluded from the above results (Table 9) that there is no significant difference between them and that the dry ashing method is accurate for the determination of aluminium in hair.

3.7 Magnesium

It was necessary to use the 2025.8 Å line for the determination of magnesium because of its relatively high concentration in hair: 10-100 ppm. No non-atomic absorption was seen at a nearby line when the conditions in Table 16 were used.

A single male head hair was then analysed, approximately 1 cm at a time, by the dry ashing method (Table 16). A plot of concentration against length was obtained; the concentration increased slightly towards the root (Fig. 17).

Fig. 17. Magnesium concentration variation with length for a head hair, Male 3



A sample of female head hair obtained by brushing was analysed by wet and dry ashing. The dry ashing method is described above. A sample of hair (ca. 100 mg) was dissolved in nitric acid and determined by direct standard addition in the HGA 70 furnace.

Table 10. Magnesium in hair

Dry ashing	37.8 ± 10.8 ppm	Mg
Wet ashing	35.0 ± 5 ppm	Mg

From the figures in Table 10 it can be seen that there is no significant difference between the results of the wet and dry ashing techniques. The dry ashing method is therefore considered accurate. The relatively poor precision of the wet ashing method was due to the high concentration of magnesium in the "Aristar" nitric acid used.

3.8 Silicon

The sensitivity of silicon determinations in a new graphite furnace fell to a plateau after several atomisations. This may be due to carbide formation, a change in surface on ageing facilitating this process. A liner was made and fitted in a furnace (118). It was found that although the lined furnace gave a 60% increase in sensitivity, the liner became brittle, distorted and increased the continuum background observed by the photomultiplier tube. The analyses of silicon in hair were therefore performed with unlined tubes.

A sample of female head hair, cut from the ends (1-4 cm. long), was collected, part analysed by the dry ashing method and part (700 mg) dissolved in 3 ml tetramethyl ammonium hydroxide (TMA). The solution was made up to 10 ml and analysed. A corresponding blank was determined.

Table 11. Silicon in hair

Dry Ashing	14.7 ± 2.7 ppm Si
Wet Ashing	12.6 ± 0.6 ppm Si

It was concluded that there is no significant difference between these two results and that the dry ash results are accurate.

3.9 Cobalt

A comparison was made between the wet and dry ashing methods for cobalt in hair. A sample of female head hair (850 mg) was dissolved in 3 mls of TMA (25% w/w) and made up to 10 mls in a graduated flask. The solution was then analysed by direct standard addition in the furnace. A TMA blank was also determined in this way. The results of this analysis were compared with dry ashing results (Table 12) on 1 cm segments of the same hair sample.

Table 12. Cobalt in hair

Dry Ashing	0.38 ± 0.10 ppm Co
Wet Ashing	0.31 ± 0.03 ppm Co

It was concluded that there is no significant difference between the results of wet and dry analysis. The results are also in agreement with published data (Table 1) on cobalt levels in hair. The dry ashing technique was therefore considered to yield accurate results for the analysis of cobalt in hair.

A long, single female head hair, plucked from the head, was analysed to determine the variation of cobalt along its length. It can be seen from the plot of cobalt concentration against length (Fig. 18) that there is no noticeable trend. The concentration was not constant, however; the coefficient of variation was found to be 68% over 38 samples.

3.10 Nickel

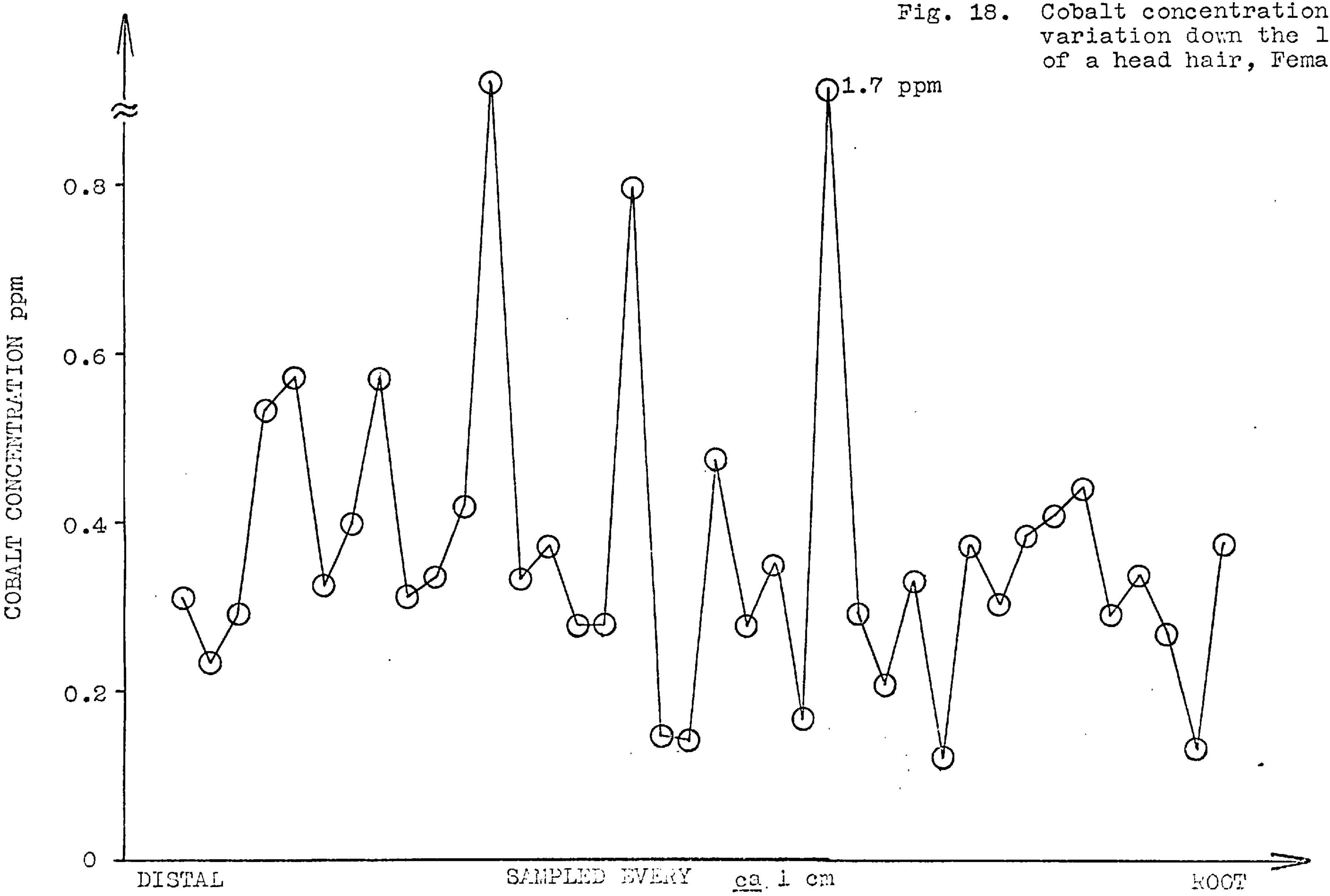
A sample of female head hair was analysed for nickel by the dry ashing method as shown in Table 16. A second sample of the same hair was dissolved in TMA and analysed by direct standard addition as in the case of cobalt previously described.

Table 13. Nickel in hair

Dry Ashing	2.3 ± 0.4 ppm Ni
Wet Ashing	2.0 ± 0.3 ppm Ni

It was concluded from the results in Table 13 that there is no significant difference between the wet and dry ashing methods and they agree with published data (Table 1)

Fig. 18. Cobalt concentration variation down the length of a head hair, Female 2



for nickel. The dry ashing method is an accurate method for the determination of nickel in hair.

The variation of nickel along the length of a hair was then investigated by the dry ashing method (Table 16). A female head hair was sampled every centimetre and the results are shown in Fig. 19. There is an increase towards the root and the coefficient of variation was found to be 25%. The coefficient of variation of a random sample from another person's head was found to be 15%,thus the precision of the method is better than 15%.

3.11 Chromium

A sample of female head hair was analysed for chromium by the dry ashing technique as in Table 16. A sample of the same head hair was dissolved in TMA and determined by direct standard addition in the furnace.

Table 14. Chromium in hair

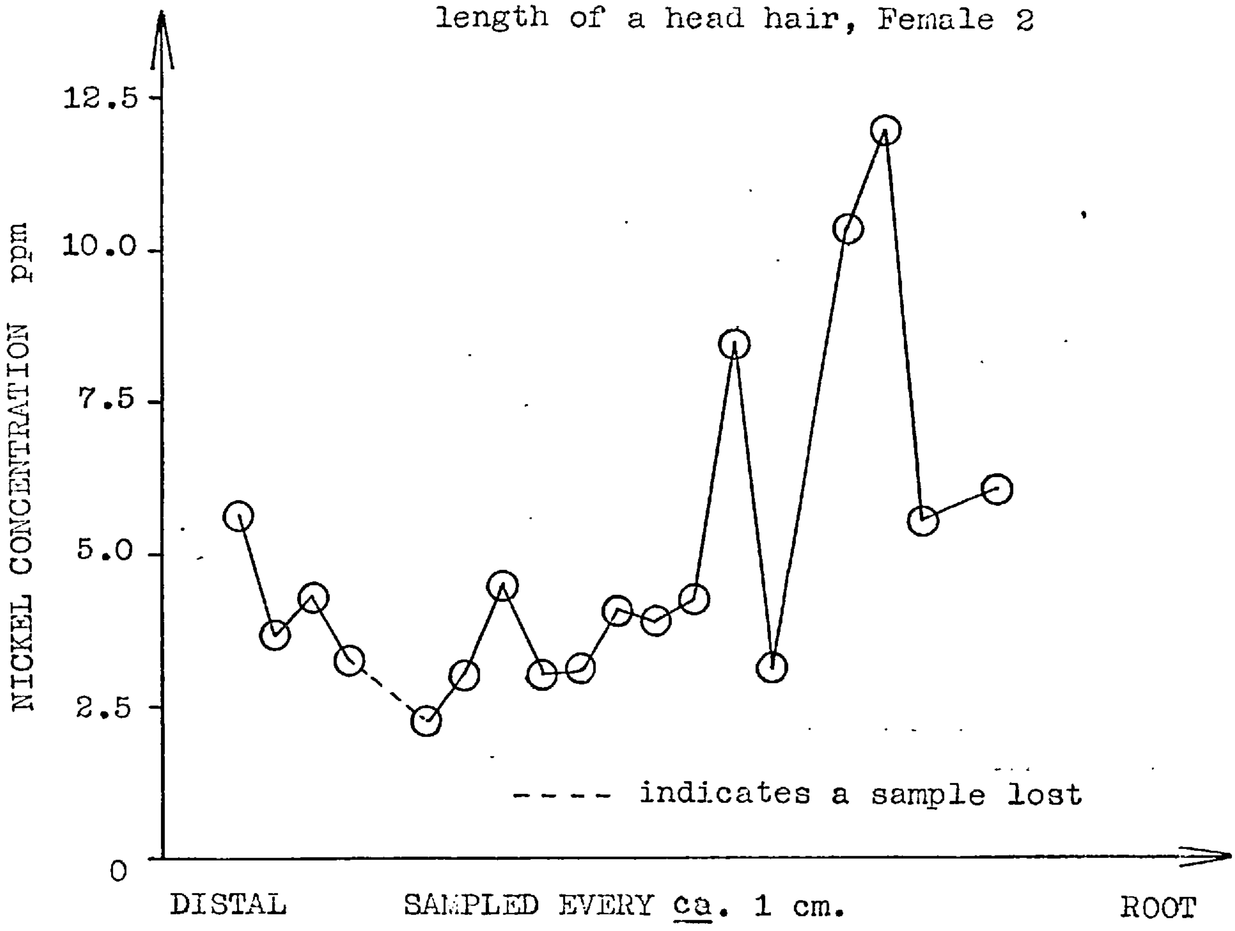
Dry Ashing	0.33 ± 0.07 ppm Cr
Wet Ashing	0.33 ± 0.01 ppm Cr

The results in Table 14 are in agreement with each other and with those published in the literature (Table 1). The dry ash technique was therefore considered to be an accurate technique for the determination of chromium in hair.

3.12 Bismuth

A sample of female head hair was analysed by the dry ashing method using the conditions described in Table 16. Most bismuth salts are insoluble so a sample of hair (ca. 3 g) was dissolved in 6 ml of 72% nitric acid and the solution made up to 25 ml. The excess nitric acid

Fig. 19. Nickel concentration variation down the length of a head hair, Female 2



maintained the bismuth in solution. The sample was then analysed by direct standard addition as described before, allowance being made for the nitric acid blank. The results for wet and dry analysis are not in agreement (Table 15) and a second wet analysis by standard addition was performed using a Varian Techtron VA 1000 flame AA spectrometer using an air-acetylene flame. The determination was performed close to the detection limit of the instrument.

Table 15. Bismuth in hair

Dry Ashing	1.5 ± 0.5	ppm Bi
Wet Ashing	11.2 ± 0.8	ppm Bi
Flame AAS	14.4 ± 3.0	ppm Bi

The results in Table 15 show a considerable discrepancy between the dry ashing and the wet methods. Bismuth is a fairly volatile metal, B.P. 1560°C, and its loss on ashing (1100°C) probably accounts for the large discrepancy in the results.

3.13 Iron

The optimum conditions for the determination of iron in hair are given in Table 16. A sample of female head hair was analysed by the dry ashing technique and it was found to contain 21.5 ± 2.7 ppm iron. The relative standard deviation of this result is 12.3%, the variation of the method must be better than this figure. The value obtained agrees with values published in the literature (Table 1).

3.14 Manganese

A sample of female head hair was analysed by the dry ashing technique (Table 16). A value of 0.67 ± 0.18 ppm manganese was obtained. This value agrees with literature values (Table 1).

3.15 Silver

Four short male head hairs were analysed using the conditions in Table 16. The results are shown in Fig. 20; it can be seen that there is considerable variation within the four samples but the range of values is in agreement with those published in the literature (Table 1)

Fig. 20. Silver concentration variation with length for four head hairs, Male 3

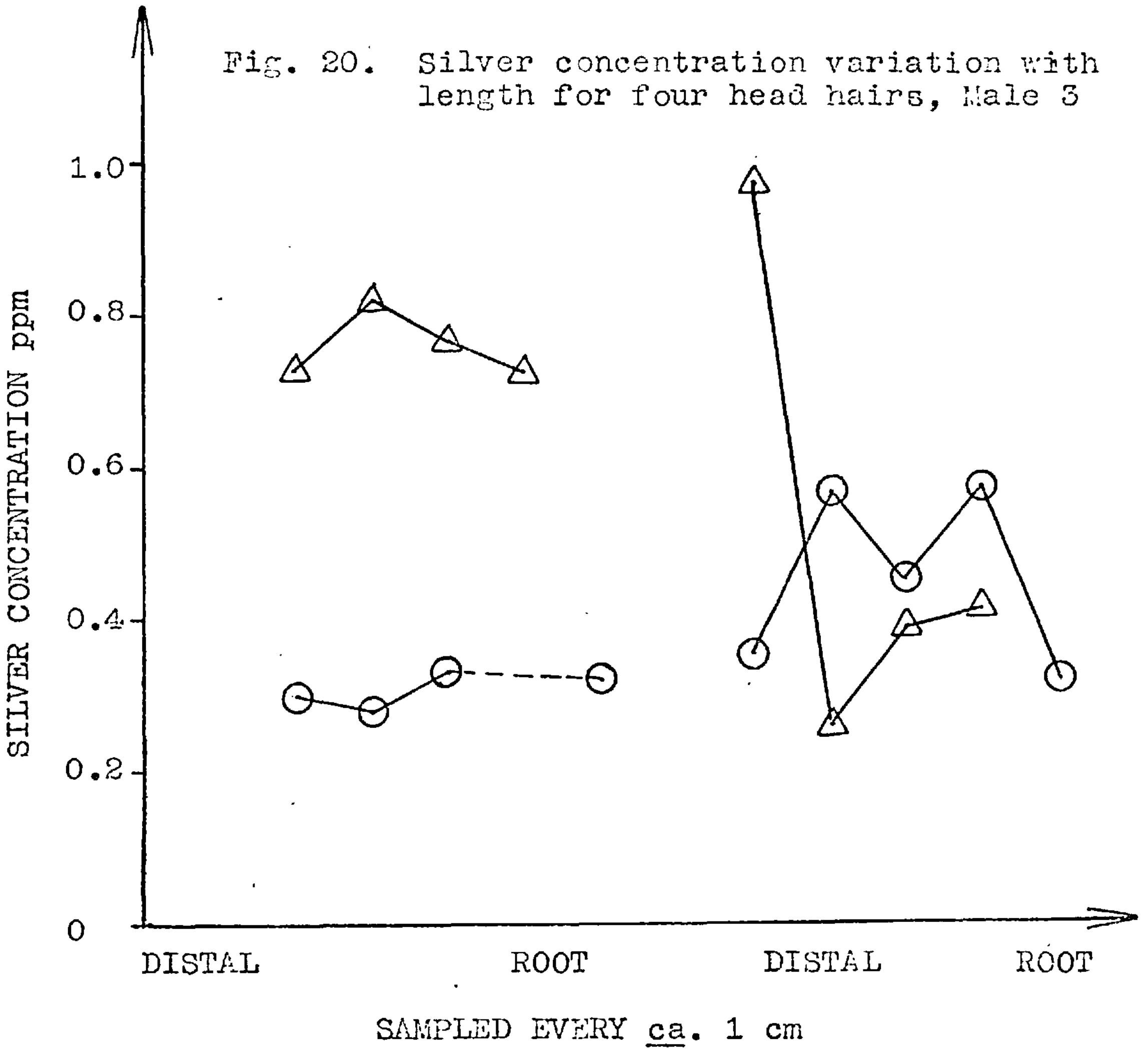


Table 16

Table of Working Conditions

Element	Line Å	Detection Limit in 100µg of hair ppm	Ashing Method Time secs	Programme	Atomisation Temperature °C
Cu	3247.5	0.3	30	7	2500
Pb	2833.0	0.1	30	6*	2500
Zn	3075.9	10	30 10	6 7	2300
Al	3092.7	0.3	30	7	2600
Mg	2025.8	0.1	30	7	2400
Si	2516.1	1	30	7	2600
Co	2407.3	0.1	30	7	2600
Ni	2320.0	0.5	30	7	2600
Cr	3578.7	0.1	30	7	2600
Bi	2230.6	0.3	30 10	6 7	2600
Fe	2483.3	0.2	30	7	2400
Mn	2794.8	0.03	30	7	2600
Ag	3280.7	0.05	30 10	6 7	2600

Programme 6 = 750°C
7 = 1100°C

The ashing conditions were chosen to prevent non-atomic absorption on nearby non-absorbing lines.

* The lead concentration in hair was such that it could be determined with minimum instrumental gain and thus non-atomic absorption was less noticeable and the ashing conditions used kept it at an insignificant level.

3.16 Conclusion

The philosophy behind the work presented in this chapter was that the dry ashing method of analysis would be the best method of analysis for single centimetre segments of hair provided that it could be shown to yield accurate results. Any wet ashing technique used to analyse 1 cm of hair would probably introduce an unacceptable level of contamination with a subsequent reduction in precision. It has been shown that for the elements:Co, Ni, Cr, Si, Al, Mg, Pb and Cu the dry ashing method gives accurate results. The results for Fe, Mn and Ag have been compared with the literature values for these elements in hair (Table 1) and found to be in broad agreement, as have the results for Co, Ni, Cr, Si, Al, Mg, Pb and Cu. Bismuth and zinc determinations have been found to differ markedly both giving lower results by dry ashing than by a comparative wet ashing analysis. The zinc values obtained are also low compared with most workers' values (Table 1). Reinhold _et_ _al_. (26) state that 127 ppm zinc in hair represents the threshold of zinc deficiency, the values obtained by dry ashing in this study were nearly all well below this value, more than one person's hair being examined. Both bismuth and zinc are fairly volatile elements and the probable explanation of the low dry ashing results is that the analyte is partially lost during the ashing process. The calibration curves were obtained under similar ashing conditions so possibly the elements are lost as more volatile organic compounds.

It has been shown that for the more involatile elements the method of dry ashing yields reasonably accurate results, thus the method should be extendable to the analysis of other non-volatile elements in hair such as Ba, Be, Ca, Cs, Mo, Ti and V. More volatile elements are liable to be lost during the ashing process necessary to remove the organic matrix.

The data obtained for the variation of trace metals down the length of hair for magnesium and nickel were not previously available, data on Ag, Zn and Co were available (14) but not in such detail. Detailed variation of lead and copper in hair was reported by Renshaw et al. (43,93). The variation of magnesium down the length of a hair (Fig. 17) shows a slight increase towards the root although few meaningful observations can be made on the analysis of a single hair. It was found from other analyses, however, that magnesium shows a considerable variation within a single individual. The single hair analysed for nickel also shows an increase towards the root (Fig. 19) but again this is a single analysis so few conclusions can be drawn. Ag, Zn and Co have been studied by Obrusnik et al. (14) who showed zinc to be relatively constant with a slight decline towards the tip and cobalt to have a steady, nearly five-fold, increase towards the tip. Silver was shown to increase towards the tip but with less uniformity than cobalt. In this study silver was found to show considerable variation from hair to hair (Fig. 20) but less so within an individual hair. One sample examined (from a different individual) contained no detectable silver; most of the determinations of silver in hair were close to the detection limit; this lowers its usefulness as a diagnostic element since as the detection limit is approached the precision worsens. Zinc was shown to increase towards the distal end (Fig. 16), although one hair is nearly constant. The results for cobalt (Fig. 18) show a nearly constant overall concentration along the length. The results for copper do not indicate that copper always increases towards the tip, unlike the work of Renshaw et al. (43) using similar equipment. They did not however present results for many copper analyses. In the case of lead both this work and that of Renshaw and co-workers shows that lead increases towards the distal end. The profile of lead concentration is not identical from hair to hair from the same head and the increase towards the distal parts may not be uniform; in some hairs a dip in concentration near the tip is seen and a sharp change at the root end is also often found. It is not possible that an increase at the tip could be grown into a hair, thus a mechanism of contamination must be considered. It seems feasible that the basis of this might be that sweat from the apocrine glands' at the base of the hair follicle may travel down the hair by capillary action and then evaporate leaving a deposit of trace metals from the sweat. Should it,however,just be a deposit resting on the hair surface then shampooing would remove the trace metals. It is known that hair can show ion-exchange type properties (80,85) and it is more likely that the trace metals become firmly bound into the keratin matrix,thus not being easily removed by shampooing.

Chapter 4

The Multi-element Atomic Absorption Spectrometer

4.1 Introduction

Single element AA using electrothermal pulse vaporisation of sample is well known and the theory and practice have been well documented (119,120,121). The proposition of multi-element AA has been less researched and at the present time there are no commercial instruments available that are capable of such analyses.

In 1965 Butler and Strasheim (122) described a multi-element spectrometer; using only half the exit slit of a medium quartz spectrometer they were able to use lines much closer together than with the conventional instrument. With this system and a multi-element hollow cathode lamp as the source they were able to analyse up to four elements simultaneously. Strasheim and Human (123) in 1967 used a time-resolved spark as the primary light source for multi-element AA. The system had the advantage of easy change of element but suffered from poorer detection limit and lower precision than a conventional AA spectrometer. Brech (124) described a system using 45° semi-silvered mirrors in order to synthesize a single beam. In 1968 Mavrodineanu and Hughes (125) constructed a multi-channel AA spectrometer using two grating spectrometers; one was used to synthesize a composite beam from three separate hollow cathode lamps, and the other was employed in the normal manner to resolve the spectral lines. Mg, Ni and Cr were determined simultaneously in a flame by AA, together with K, Ca and Na by flame emission spectrometry. This system is best suited to widely spaced spectral lines, since closely spaced absorption lines would necessitate more complex synthesiser optics, and the light intensity at the observing spectrometer would be reduced. Pickford and Rossi (126) have described a system for the simultaneous determination of Co, Cr, Cu, Fe, Mn, Ni and Pb by AA using an electrothermal atom cell. Background absorption was simultaneously corrected for on each channel by means of seven reference channels. A single multi-element hollow cathode lamp was used as the spectral source.'

Walsh (127,128,129) has described several multi-element AA spectrometers. He has constructed a six channel AA spectrometer using resonance detectors and a multihole burner as the atom cell. This mode of operation has the advantage that the resonance detectors, effectively acting as monochromators, have very narrow band-passes and their operational wavelength is independent of changes in room temperature and pressure, unlike normal monochromators. The multihole burner allows different sensitivities to be obtained by adjustment of the path length through the flame. The resonance detector tube, however, suffers from a relatively short life-time and the whole system is less sensitive than a conventional flame AA spectrometer. Walsh has also discussed the use of selective modulation in multi-element AA spectrometers.

More recently multi-element spectrometry has been achieved using vidicon tubes; these are discussed in more detail in Chapter 6. Aldous et al. (130-134) have carried out multi-element analyses using a flame and detecting the radiation using a 500-channel vidicon tube. This system observes a continuous region of the spectrum, the bandwidth of which is dictated by the dispersing power of the grating or prism used. The absorption lines of the elements of interest must lie within this bandwidth. Aldous and co-workers have used this system to determine Zn, Cd, Ni, Co, Fe, Mn and Cu in potable waters. Horlick and Codding (135) have described a similar system with a bandwidth of 130 Å, giving simultaneous determination of nickel and chromium and of chromium and iron.

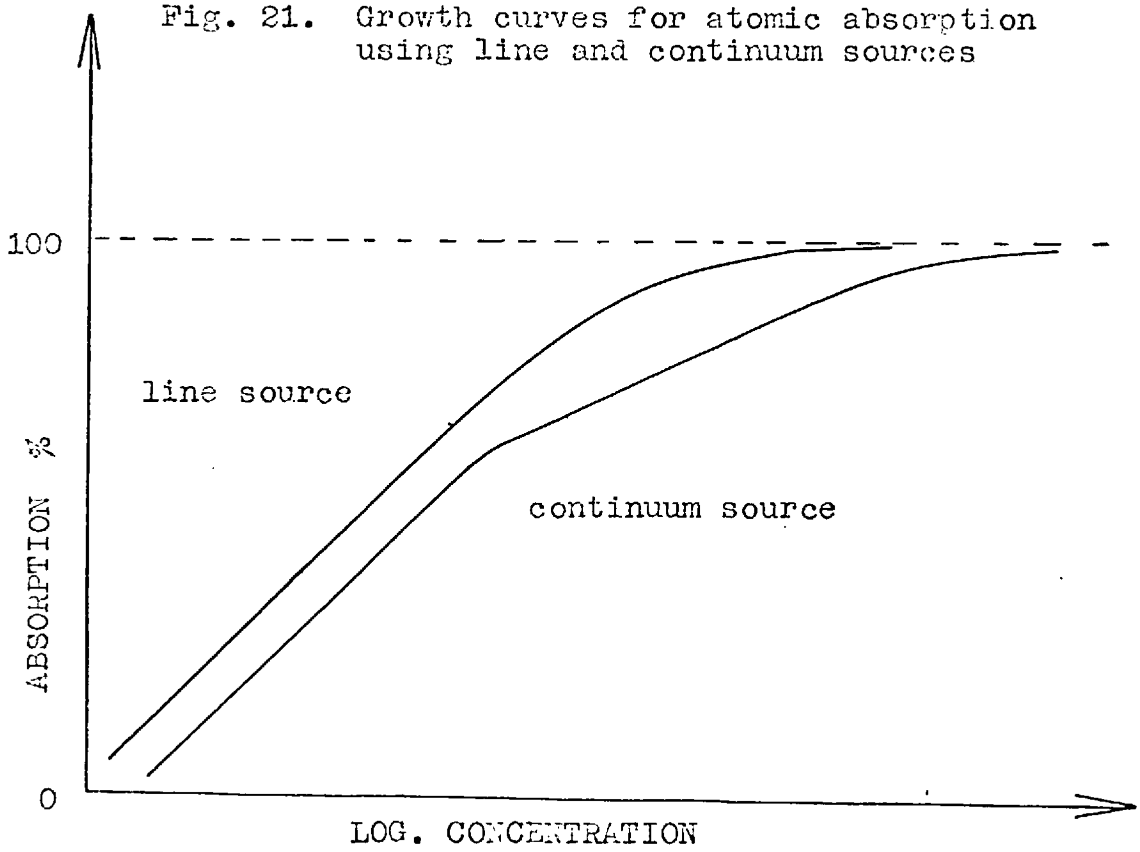
Theory imposes certain restrictions on multi-element

AAS, the most important of which is the limited working range and the attendant problem of elemental concentration ratios. Optical design is another major problem, as is electronic signal handling and presentation. These problems and their solutions will now be dealt with in greater detail and a practical multi-element AA spectrometer will be described.

4.2 Theoretical Limitations of Atomic Absorption in the Multi-element Mode

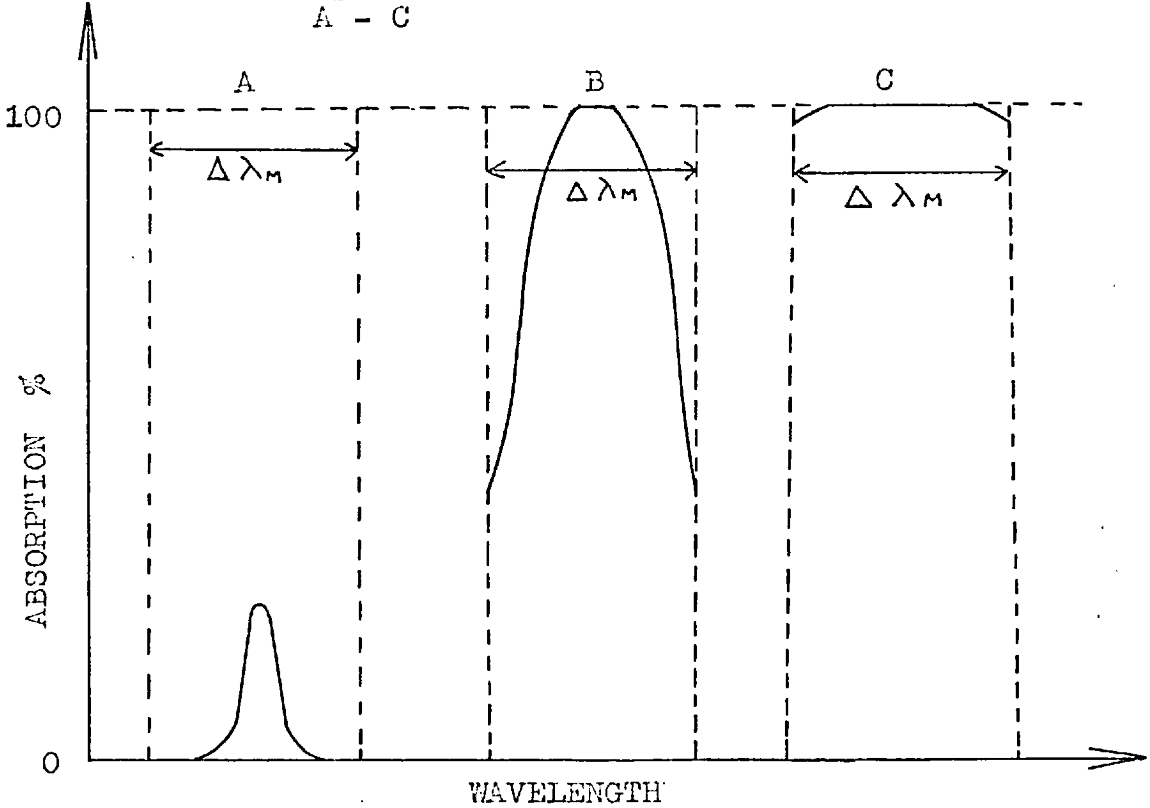
Measurement of AA is limited at high absorption by increasing absorption line width. At low absorption it is limited by the ability of the photo-electric detection system to measure small changes in the light intensity. When the species in the atom cell is optically thin then the curve of growth for a line source is linear (Fig. 21).

Fig. 21. Growth curves for atomic absorption using line and continuum sources



As the species concentration increases, the optical density increases and the line profile for the absorbing species widens (Fig. 22); absorption begins to occur in the wings of the spectral line, and deviation from linearity occurs (Fig. 21). For a more complete and mathematical treatment the reader is referred to references 119-121 and 136.

Fig. 22. Spectral profile for the absorbing species with increasing concentration A - C

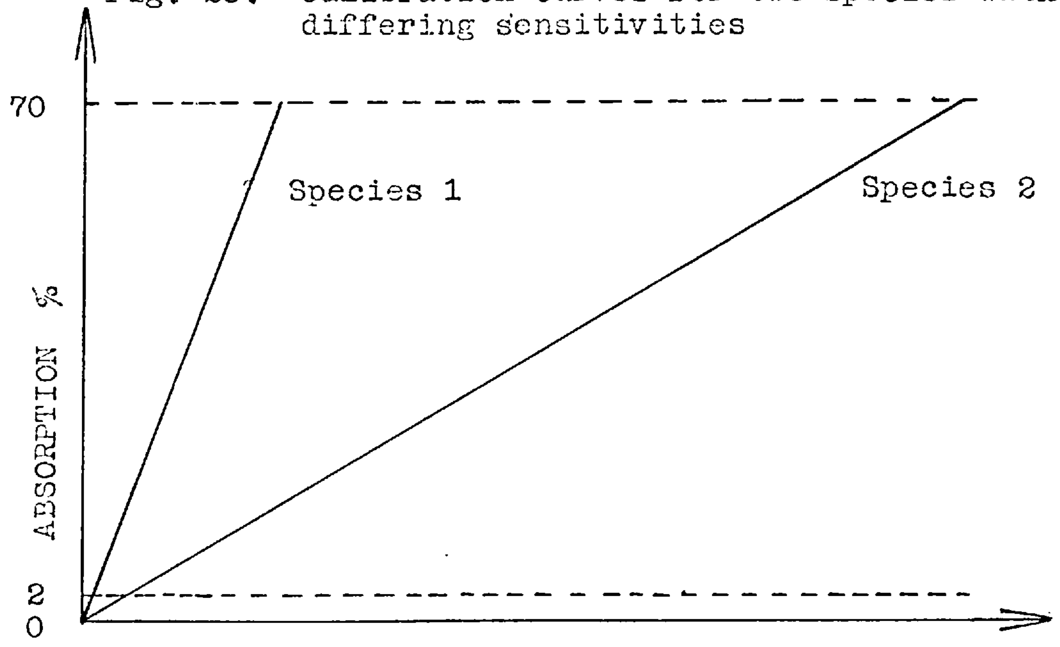


$\Delta\lambda_M$ is the bandpass of the monochromator

The accurate measurement of light absorption at low optical density, when less than 2% of the incident radiation is being absorbed, is difficult. The problem is that a small difference signal is being sought on a large background and this is further compounded when the absorption signal is transient, such as the absorption signal from an electrothermal atom cell.

These limitations apply to all AA measurement but in single element analyses other parameters can be altered such as concentration or absorbing line. In simultaneous multi-element analyses these limitations constrain the whole system. If, in a general case, two elements are to be determined simultaneously and their sensitivities differ markedly (Fig. 23) then it is clear that unless element 1 always lies within a small low concentration range compared to element 2, the system will not be measuring under optimum conditions. Should the elemental concentrations differ considerably from this then simultaneous analysis would not be feasible. Thus the concentration ratios of elements in the sample have to be limited for simultaneous analyses to be viable. The use of more than one atomic line for each element in order that a wide range of concentration can be determined would prohibitively increase the complexity of the instrumentation, particularly if ten elements or more are to be determined.

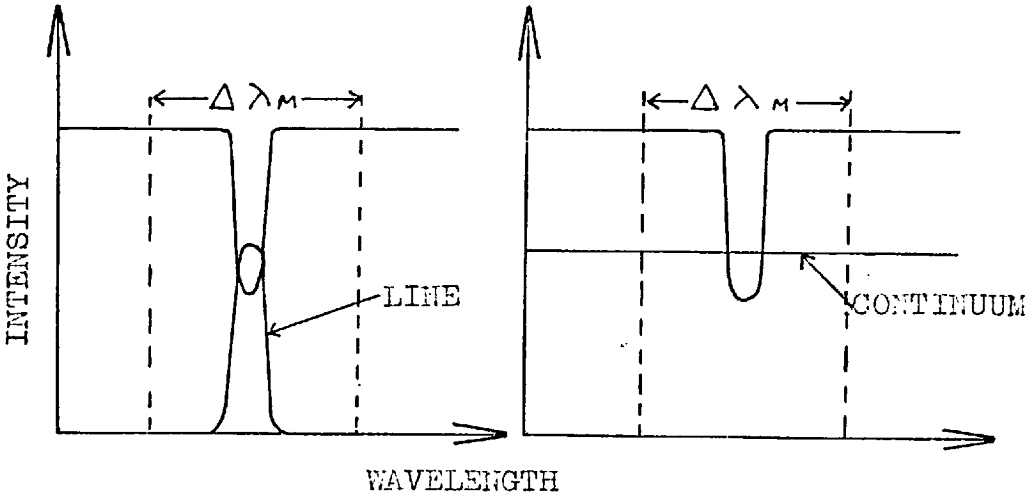
Fig. 23. Calibration curves for two species with differing sensitivities



4.3 Sources

In a multi-element AA spectrometer it would be desirable to keep the number of sources to a minimum in order to keep the optics simple. Thus a continuum source or a multi-element line source might be envisaged. The continuum source offers the advantage of being commercially available and providing radiation for all channels. It suffers, however, from even more limited range than a line source because the radiation is continuous with wavelength and thus the light seen at the exit slit cannot all be absorbed by the species; this results in a reduced linear range (Fig. 24)(136).

Fig. 24. Absorption of line and of continuum radiation



$\Delta\lambda_M$ is the bandpass of the monochromator

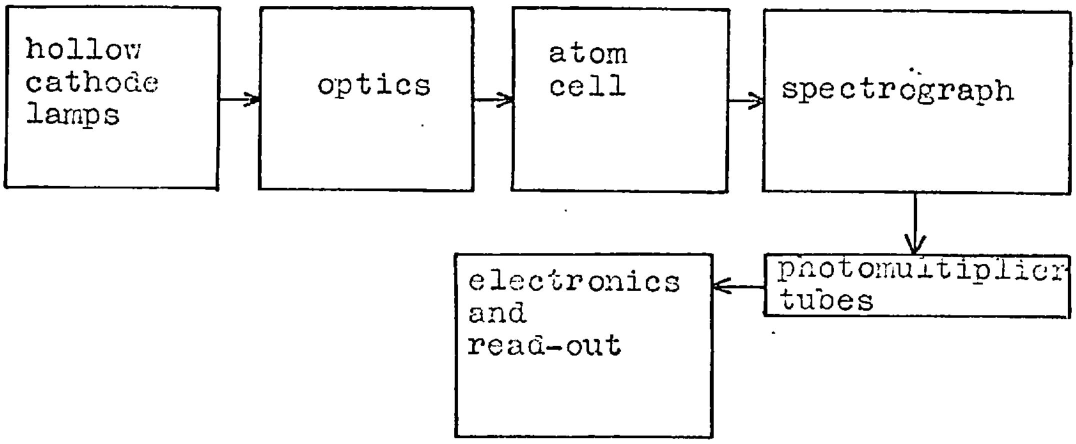
The continuum source is thus rejected on the grounds that it would restrict the linear range. A multi-element spectral line source would be the ideal source but at the present time this is not commercially available for the number of elements of interest i.e. up to twelve. Multi-element electrodeless discharge lamps (mainly dual-element) have been constructed by Norris and West (138,139) and others (140,141) but prove to be difficult to construct for many elements (142). Multi-element hollow cathode lamps are readily available for up to seven elements, although the combination of elements available in a single lamp is rather restricted. Their output intensity, stability and life-time are acceptable and thus are useful as sources in multi-element AA.

At the beginning of this project it was intended that the elements of interest would be changed from time to time; thus in the original spectrometer the optics reflect the desire to use single element hollow cathode lamps.

4.4 Optics

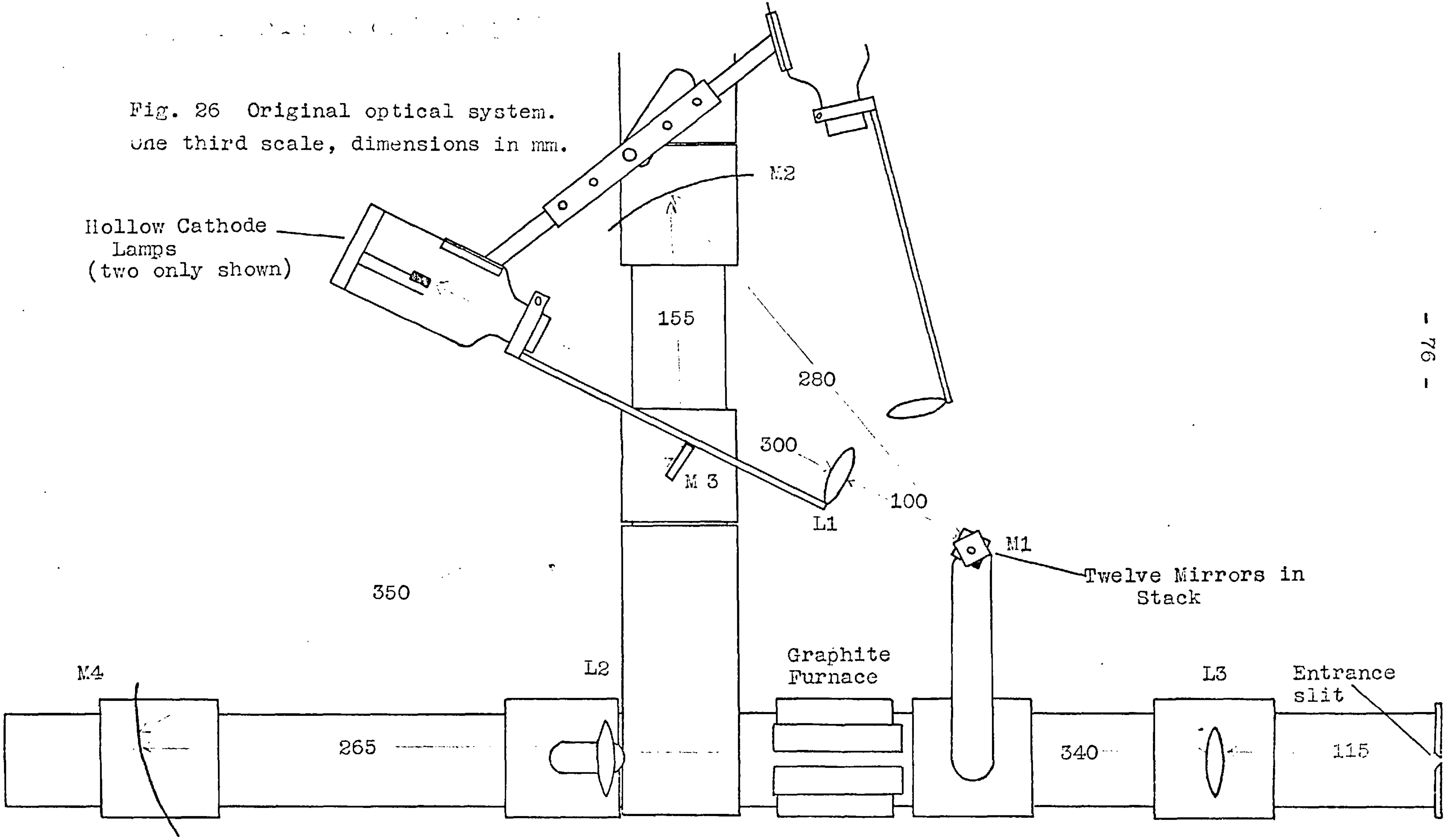
A Hilger and Watts medium quartz direct reading spectrometer with transistorised electronics E 549 was used as the basis of the instrument. A standard Perkin-Elmer HGA 70 furnace (see Fig. 2) was used as the atom cell, with the end cones drilled out to 9 mm to increase the aperture. A simplified block diagram (Fig. 25) shows the main components of the spectrometer.

Fig. 25. Simplified block diagram



The light from the hollow cathode lamps passes through the furnace and into the vertical slit of the spectrometer. The light from nine hollow cathode lamps, whatever the physical arrangement in which they are placed, presents an awkward object. If the total light from the lamps is treated as coming from one object then manipulation by lenses and mirrors will produce an image similar to the object. If the hollow cathode lamps are in a circular array then the image will be a circular array of images of their cathodes and only the top and bottom images will pass through the vertical slit. The light from each lamp has therefore to be manipulated separately, and this was achieved by using a small stack of mirrors, each individually adjustable about horizontal and vertical axes. The original optical system is shown in Fig. 26. In order to keep light losses to a minimum wide aperture optics are

Fig. 26 Original optical system.
One third scale, dimensions in mm.



required but the spectrometer and graphite furnace are both low aperture ca.f/12 and ca.f/8 respectively and thus impose considerable problems. Wide aperture short focal length silica lenses are very expensive whereas similar mirrors are relatively cheap. The focal length of mirrors is also independent of wavelength and the use of mirrors allows a more compact system to be devised. Thus mirrors were used for the optics, a long light path system with wide aperture mirrors keeping the light losses to a minimum.

A lens was fixed in front of each hollow cathode lamp using a rigid arm and an image of the lamp was focused on a mirror in the stack; 3-fold diminution of object size occurred. The mirror stack provides an object only 35 mm high with the hollow cathode images in a vertical alignment. The light from the stack of mirrors M1 in Fig. 26 is then collected by M2, the large collecting mirror; this reflects and focuses the light onto the plane mirror M3 which reflects it to the large collecting mirror M4. The light is then focused and passed through the furnace and into the spectrometer slit through the quartz lenses L2 and L3. The Specifications of the components used in the original system is given in Table 17.

4.5 Electronics

The absorption pulse recorded by the photomultiplier tube consists of a short (typically less than 1s) +ve going pulse on a steady -ve DC background. It is often necessary to measure very small absorption pulses; this is difficult, largely due to the standing DC potential.

The existing electronics with the Hilger and Watts E 549 12-channel direct reading spectrograph was designed for the measurement of continuous emission signals over periods of 5, 10, 15 .... etc. up to 60 second periods and this is not suited for handling transient absorption signals on high DC backgrounds. An electronic device capable of interfacing between photomultiplier tubes and the integrators of the E 549 was designed and constructed by

Table 17 Specifications of the components used in the original system

Hollow Cathode Lamp Supply

12 channel 2-50 mA continuously variable at 150-250V D.C. constant current supply. Open circuit voltage greater than 350V D.C.

Hollow Cathode Lamps

Hilger and Watts 'FL 2000' series
Westinghouse high intensity lamps

Optical Components

M1	Twelve 1.5 x 4 mm plane front surface mirrors mounted to permit movement about the vertical and horizontal axes perpendicular to the light path
M2	100 mm dia. x 100 mm focal length front surfaced spherical mirror.
M3	Plane 40 mm dia. front surfaced mirror.
M4	100 mm dia. x 150 mm focal length front surfaced spherical mirror.
L1	40 mm dia. x 75 mm focal length. Spectrosil B lens.
L2	50 mm dia. x 75 mm focal length. Spectrosil B lens.
L3	50 mm dia. x 75 mm focal length. Spectrosil B lens.
L4	25 mm dia. x 50 mm focal length. Spectrosil B lens.

Table 17 cont.

Monochromator

Hilger and Watts medium quartz 12 channel direct reading spectrograph

Entrance slit 50 m x 10 mm
Exit slits 35 m x 10 mm
Reciprocal linear dispersion of spectrograph at 20°C

2000 Å: 4 Å/mm
2200 Å: 6 Å/mm
2800 Å: 12.2 Å/mm
3200 Å: 17 Å/mm
4000 Å: 32 Å/mm

(see Fig.31)

Graphite Furnace

Perkin-Elmer HGA 70 graphite furnace and power control unit.

End cones drilled out to 9 mm internal diameter

Purge gas: Argon 1.5 l/min.

Signal Processing

Photomultiplier Tubes EMI 9781 A and B (low noise types)

EHT. Stepwise 400-900 v

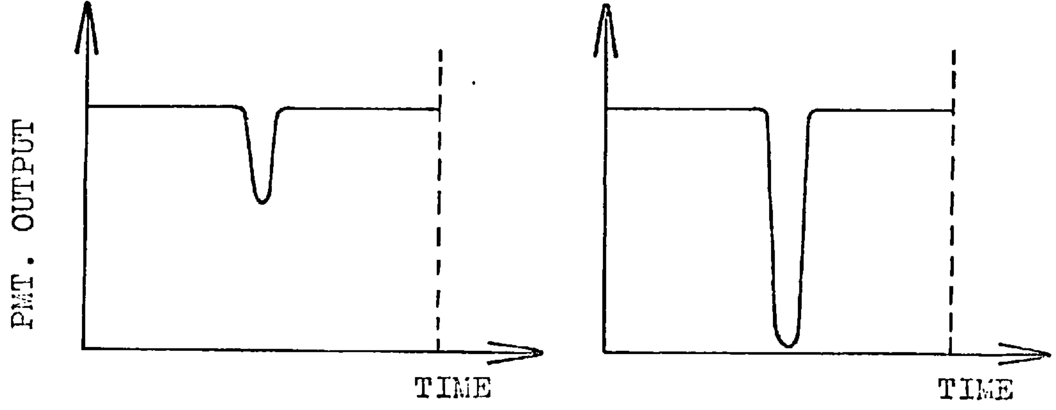
ATSP Band Pass. 0.4 - 38 Hz 3dB limits
AC Gain ca. 66dB

(see Figs. 28,29)

Hilger and Watts electronics, standard electronics for E 549 Polyspek (12 channel).

Mr. D. Alger of the Departmental Electrical Workshops. The effect of observing a transient pulse on a high DC background whilst integrating over a much longer period than the pulse is shown in Fig. 27; at 100% peak absorption there is, still a considerable area that is not absorbed.

Fig. 27. Effect of integration with original system



Thus the absorption signal would be measured as the difference between the steady DC signal, i.e. 100%, and the integrated signal less the absorption pulse. This limits the dynamic range and reduces the sensitivity. The Alger Transient Signal Processor (ATSP) is shown in Fig. 28. It consists of an impedance convertor and high impedance load (10 MΩ) for the photomultiplier tube, followed by a differentiator and a buffer stage and then an integrator. The final output is through a diode. The high photomultiplier load resistance provides large voltage changes for changes in incident light. The high impedance buffer ensures the load is not shunted by any following circuitry. The next stage is a differentiator, the frequency response vs. gain of which is shown in Fig. 29. The system acts as a differentiator up to ca. 2 Hz, then for a short region as a band-pass filter and for frequencies above this it acts as an integrator; the ingetration of noise is effectively zero for components above the band-pass frequencies ca. 40 Hz. For absorption pulses of the type obtained with the electrothermal cell used the differentiator's frequency range ca. 0.04-2 Hz should be sufficient to handle more than 97.5%

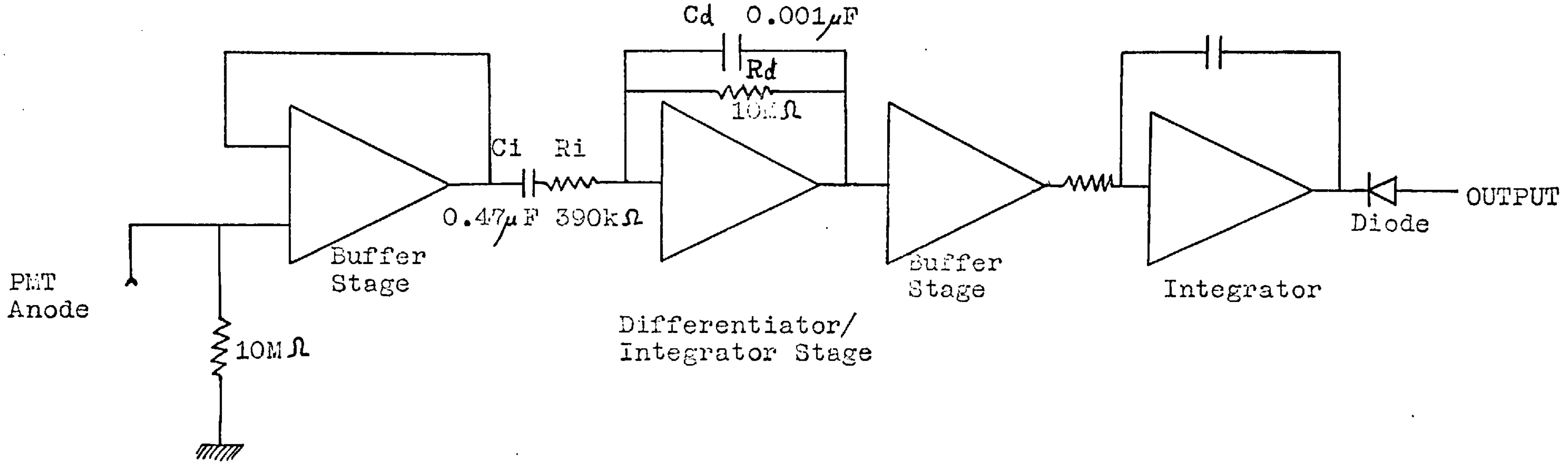


Fig. 28. ATSP circuit (simplified)

Fig. 28a. Diode Characteristic

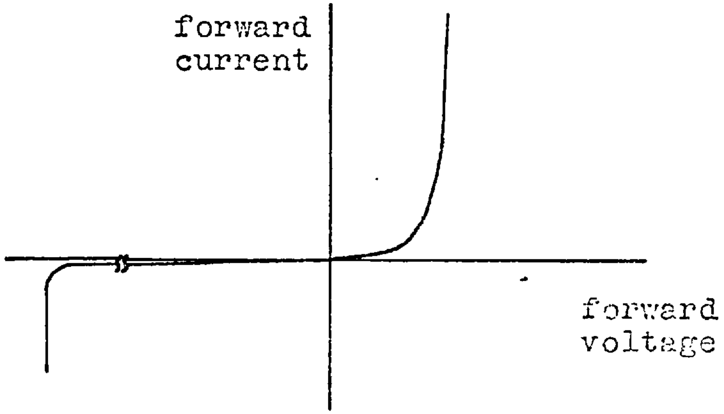
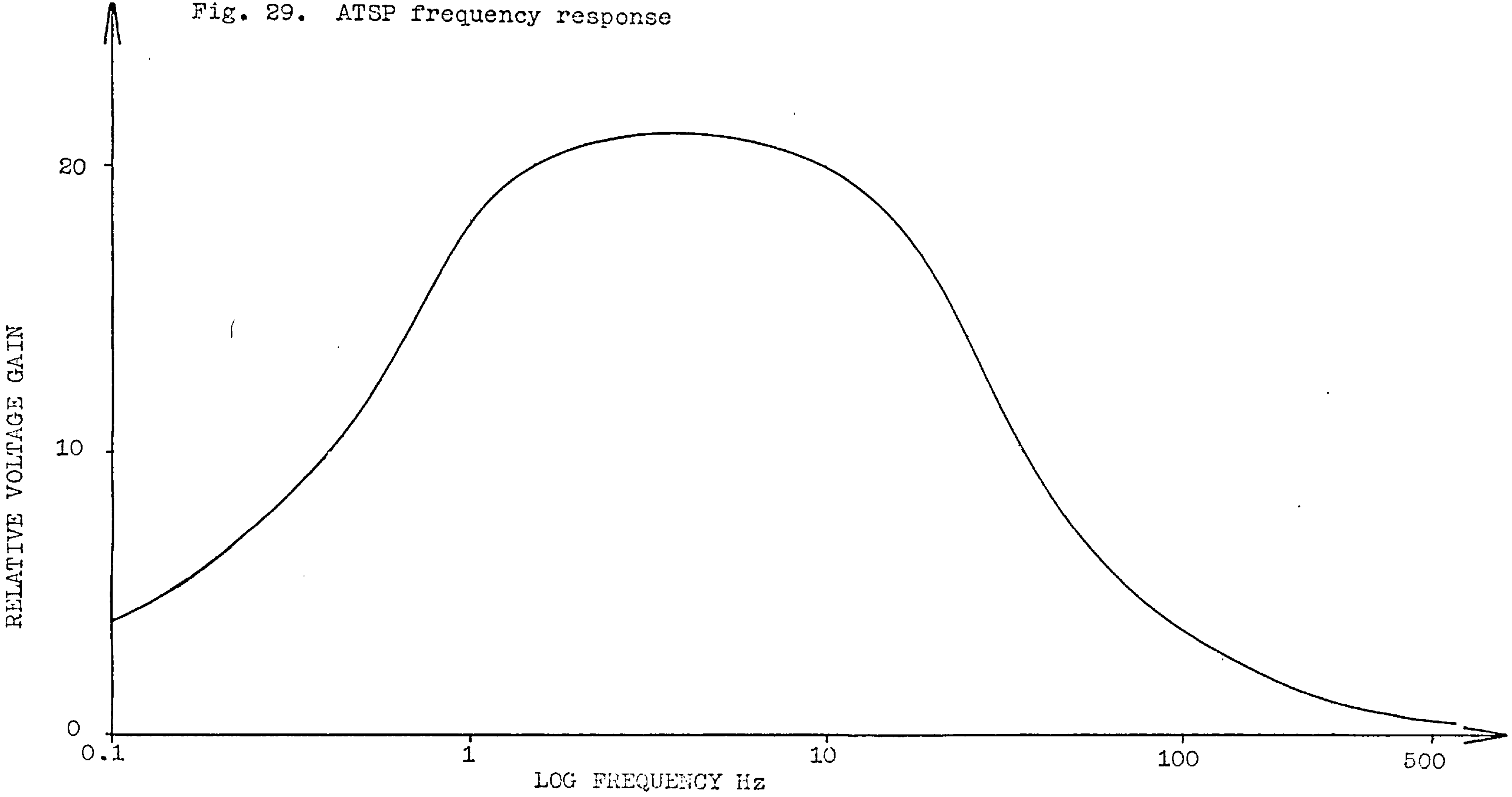


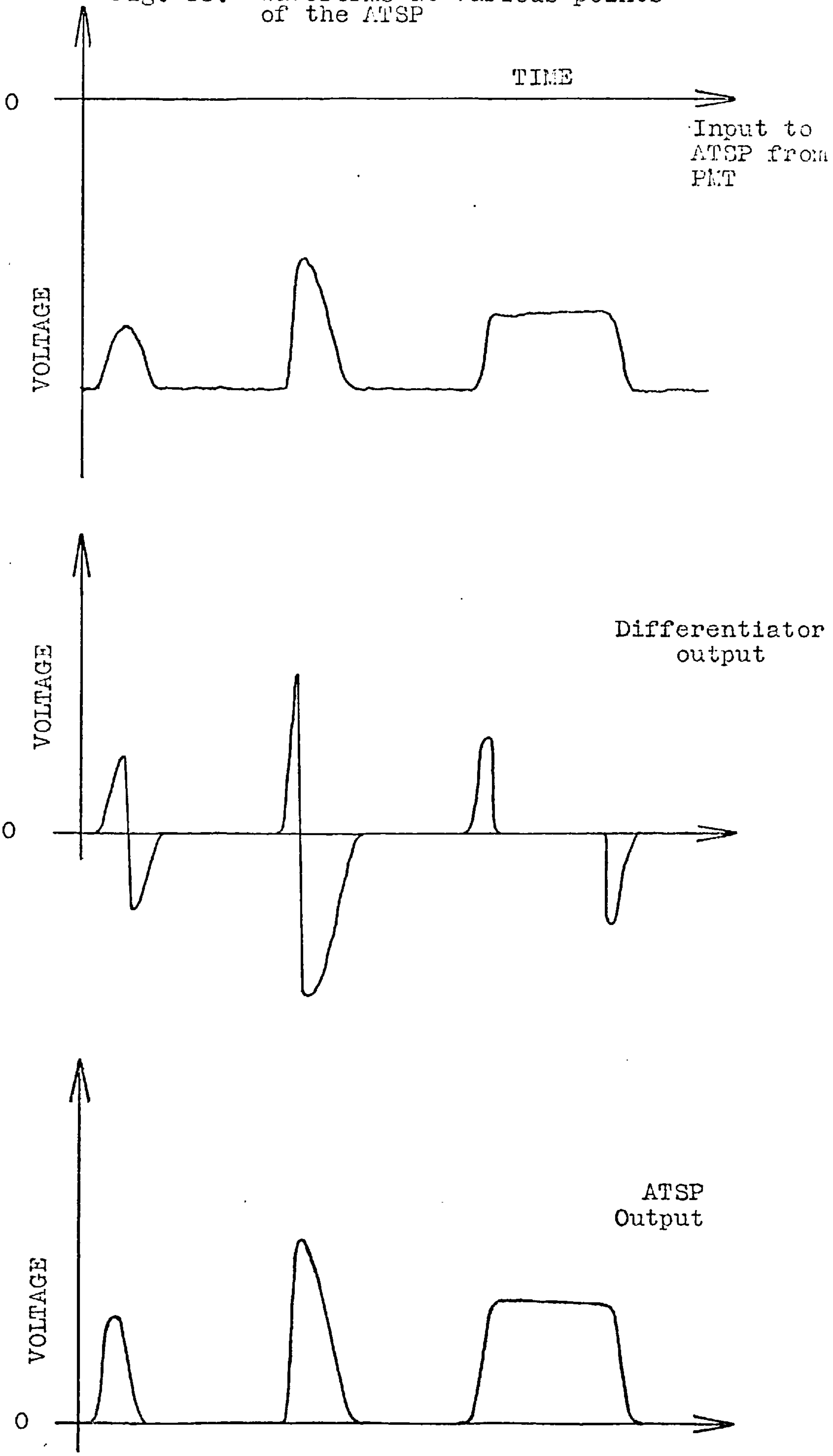
Fig. 29. ATSP frequency response



of the information from the fastest pulse likely to occur with this atom cell. For slower pulses the retention of information will be better than 97.5%. For frequencies below the centre frequency Cd and Rd (Fig. 28) will be the dominant impedances and the circuit will behave as a differentiator; above the centre frequency Ri Ci will be the dominant impedances and the circuit will behave as an integrator. The differentiator is followed by a buffer which isolates the stages, and the pulses are then fed to an integrator which restores the original pulse shape; the pulse is then fed through a diode to the input of the E 549 where it is stored and then sequentially read out. The waveforms at various points of the ATSP circuit are shown in Fig. 30. It should be noted that the final stage inverts the signal so as to present pulses of the correct polarity for the E 549 unit, i.e. as if the PMT was recording emission signals; these are -ve going pulses. It was found that the calibration curves obtained using this signal processor were linear when signal output was plotted against concentration. The reason for this lies in the output diode; pulses below 0.7v are not seen because of the silicon diode threshold potential and in the region just above this the impedance characteristic of the diode is logarithmic (Fig. 28a). Thus pulses which occur in this region will be subject to an impedance which varies logarithmically with their peak value. The absorption signals are therefore effectively converted into absorbance readings. The diode also prevents pulses with the opposite polarity from getting into the E 549; emission signals, and in particular continuum background emission, are not observed.

The electronics succeed in overcoming some major problems associated with AA, and with simultaneous multi-element AA in particular. The differentiator-integrator system transforms the small pulse on a large DC background into a pulse on a zero DC background. This is simpler to handle and easier to amplify. Two particular problems are overcome: in any system of this type where several sources are combined

Fig. 30. Waveforms at various points of the ATSP



then increases in background light and the possibility of line overlap (with wide band-pass instruments) becomes more significant; both phenomena reduce the line:background and hence reduce sensitivity and working range. By only observing the absorption pulse this reduction can be overcome but the noise contribution from non-absorbed light is still present (although noise above 40 Hz is reduced). In addition, the integration cycle of the E 549 only records a signal when an absorption pulse is present, thus keeping the working range to a maximum. The continuum background is of less importance since the output circuit only "sees" absorption-going pulses. The signals have effectively gone through a logarithmic element and thus are linear with respect to concentration.

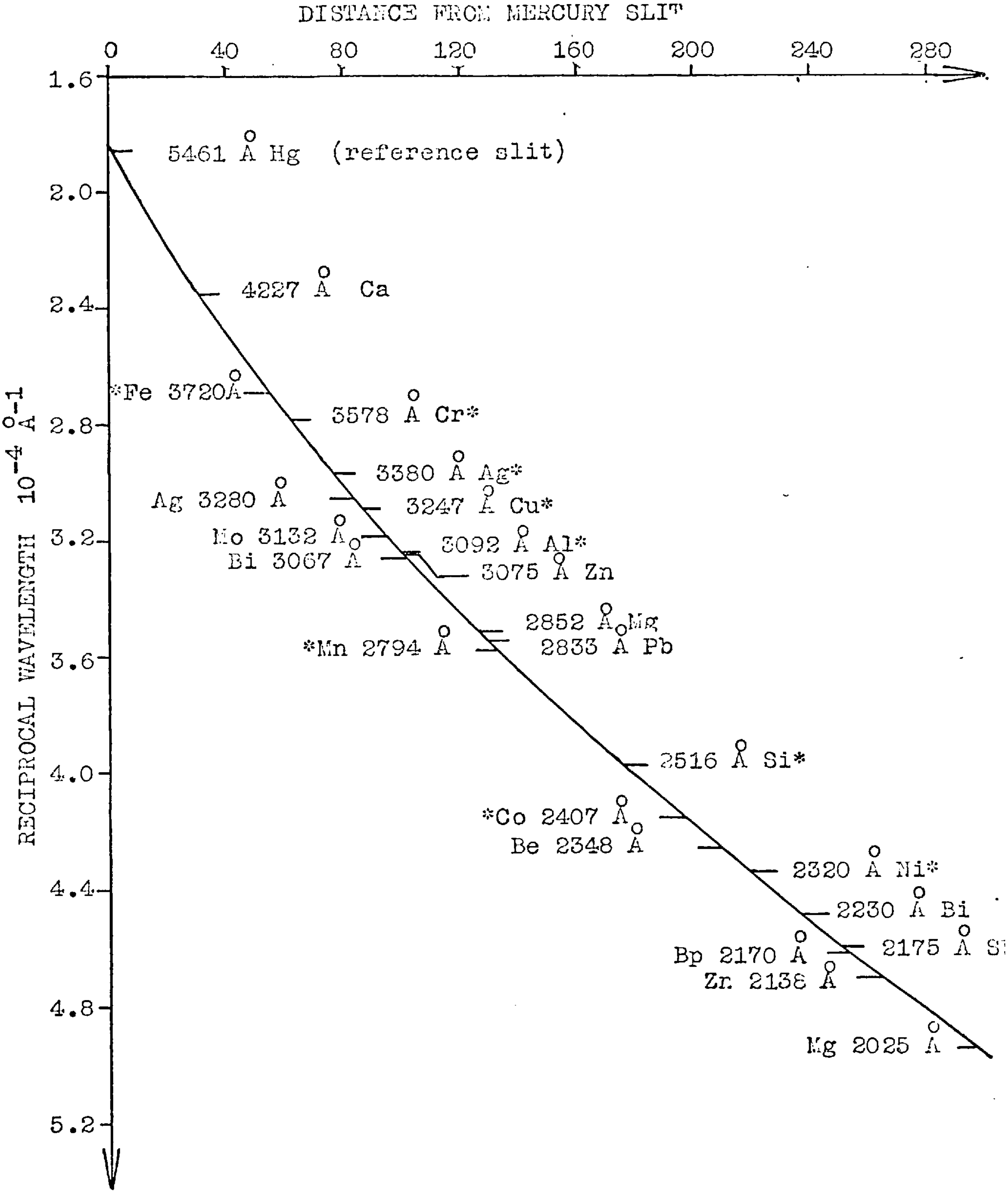
4.6 Atomisation Difficulties

A problem that could occur when multi-element AA with electrothermal pulse vaporisation is considered is that each element will have a different rate of evaporation from the carbon furnace and thus their absorption peaks will be observed at different times of the atomisation cycle. This is overcome by integration over 10s; the electronics removing the DC background component.

4.7 Line Selection

Thirteen elements were studied, in Chapter 3, in single element mode; of these zinc and bismuth were found to give low results by the direct dry ashing method. Furthermore the zinc line at 3075.9 Å is too close to the aluminium line at 3092.8 Å for them both to be included in the instrument. For these reasons zinc was not included in the multi-element system. Of crucial importance in deciding which lines can be incorporated in the system is the relationship between dispersion and wavelength and this is shown in Fig. 31. The centre of the slits at the focal plane of the spectrometer cannot be closer than 4 mm apart. Thus certain

Fig. 31. Dispersion Curve for Spectrometer



* indicates line selected for the system

lines will be mutually exclusive, e.g. Pb 2833.0 Å and Mn 2794.8 Å. The final choice of lines is shown in Table 21. It was hoped to include the magnesium line at 2025.8 Å but this line fell outside the wavelength range of the slit mask.

4.8 Evaluation of initial system

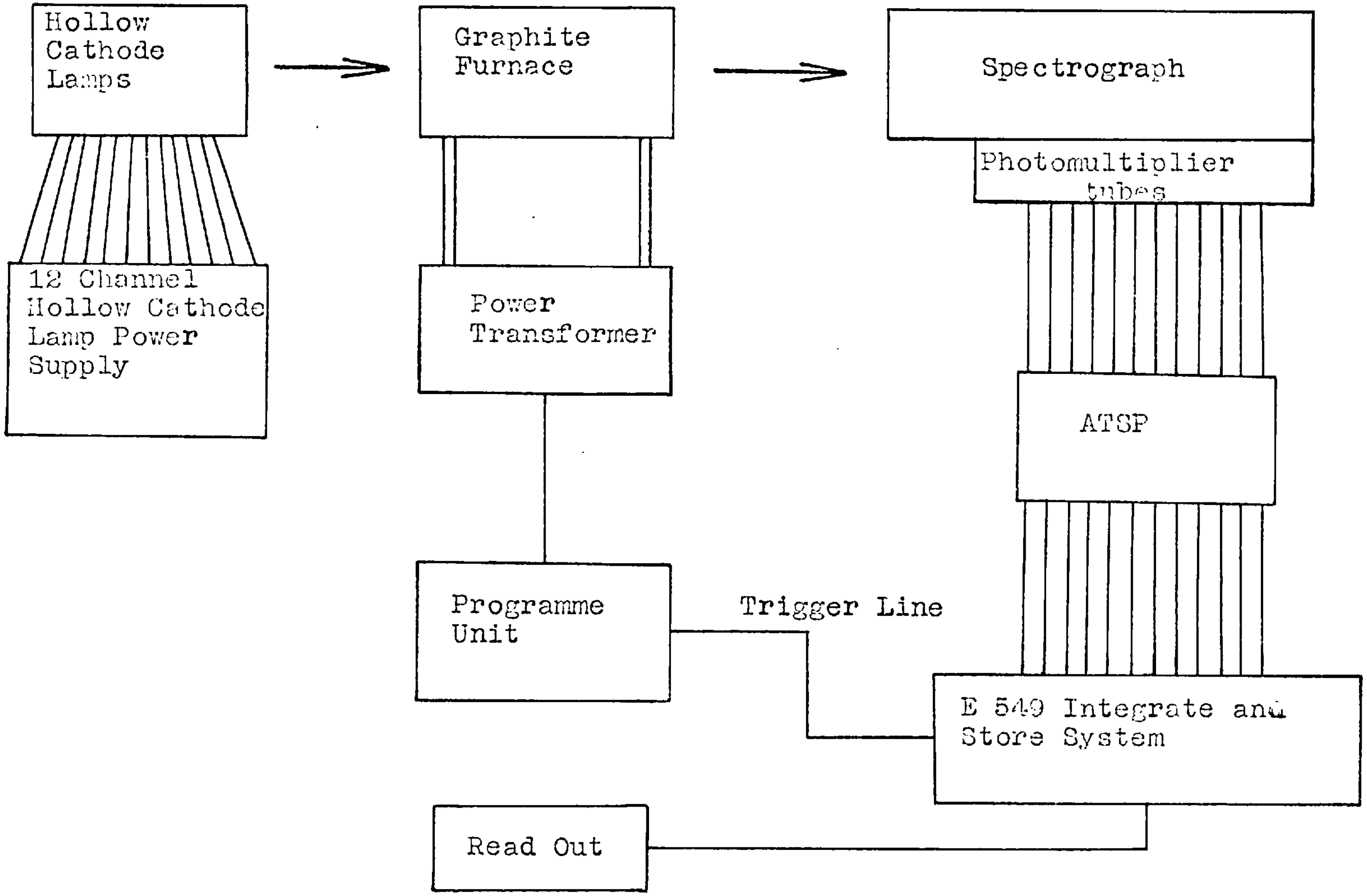
The complete system is shown in block diagram form in Fig. 32. A trigger line connects the starting circuits of the E 549 unit and the HGA 70 power supply. The time cycles of both electronic system are adjusted so that the integration cycle (10s) of the E 549 unit starts at the beginning of the atomisation cycle of the HGA 70. While the sample is being ashed in the furnace the E 549 unit is running through its pre-spark cycle. The initial series of calibration curves are shown in Fig. 33. Table 18 gives the concentrations of the solutions used to construct the calibration curves in Fig. 33.

Table 18. Weight of element in ng per 10μl aliquot

Solution	Ni	Co	Si	Mn	Al	Cu	Ag	Cr	Fe
S 1	0.3	0.2	2.5	0.05	0.5	1.0	0.1	0.2	1.0 ng
S 2	0.6	0.4	5.0	0.1	1.0	2.0	0.2	0.4	2.0 ng
S 3	0.9	0.6	7.5	0.15	1.5	3.0	0.3	0.6	3.0 ng
S 4	1.2	0.8	10.0	0.2	2.0	4.0	0.4	0.8	4.0 ng

It can be seen that not all of the calibration curves pass through the origin; this is probably due to the integrators (Fig. 28) drifting. Drift is a common property of operational amplifiers; any small difference "seen" (by the amplifier) across the two outputs will be amplified, will appear at the output and will be integrated. There are pre-set controls on the ATSP to adjust the zero on each channel; zeroing is performed as part of the initial setting up but

Fig. 32. Complete block diagram of Spectrometer



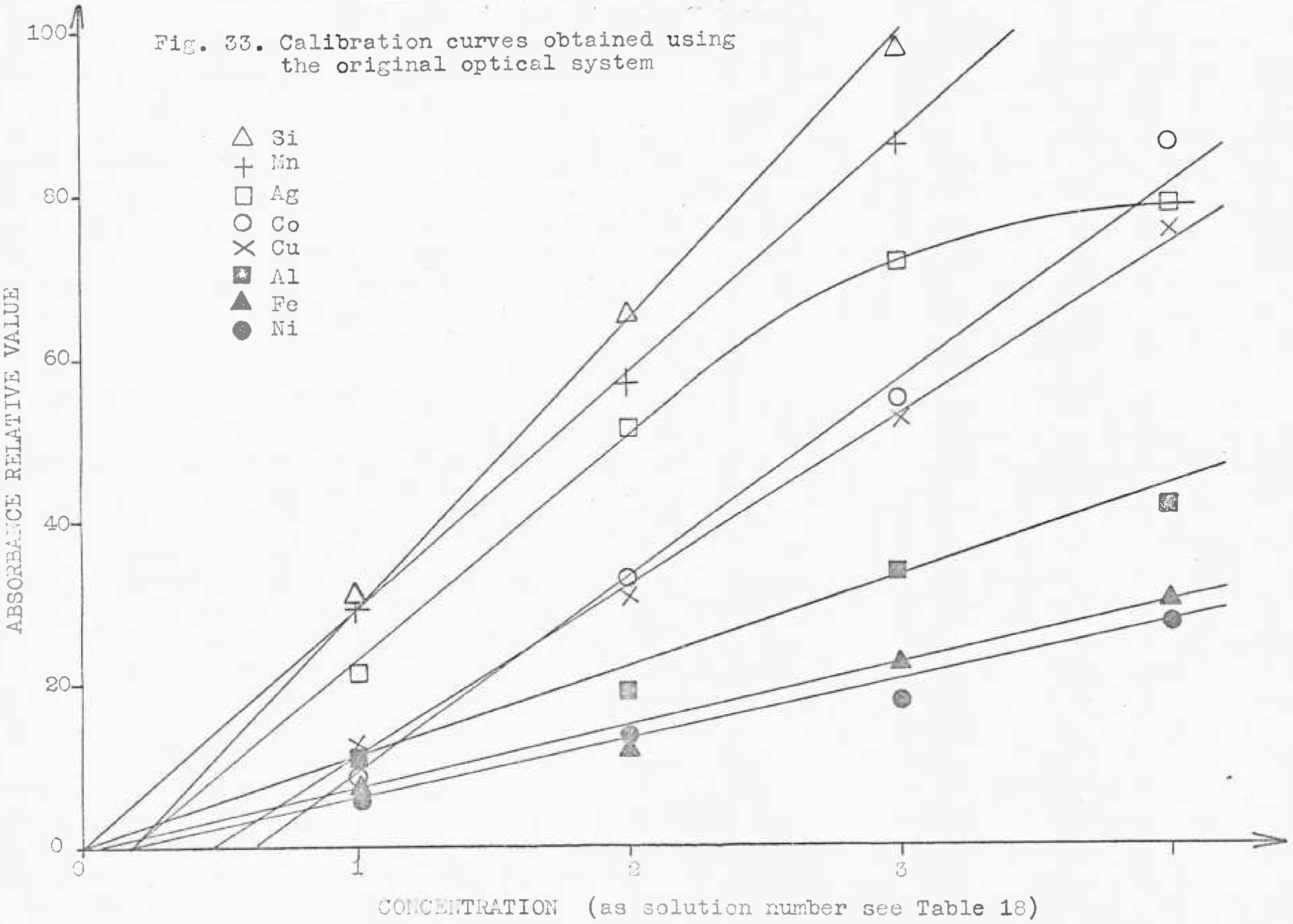


Fig. 33. Calibration curves obtained using the original optical system

drift can occur during operation and can be of either polarity. The drift will prevent the calibration curves passing through the origin. A calibration curve for chromium could not be obtained at this stage; it was found later that the position of the furnace along the light path was critical for the operation of the chromium channel. It was also necessary to introduce a diaphragm between furnace and entrance slit, to reduce the amount of continuum radiation reaching the spectrometer. The system was operated with stopped purge gas (argon) during atomisation; this causes a certain amount of air to be entrained into the furnace. The combination of nitrogen with the hot carbon gives rise to cyanogen and the emission of very strong CN bands; these can be seen on the plates (p.162-4) in Chapter 6. The CN bands are particularly close to the chromium ground state line at 3578.7 Å and spectral overlap is thought to be the cause of the initial non-function of the chromium channel, emission from the CN bands swamping any small absorption changes due to chromium atoms.

The precision of the initial system is given in Table 19. It was felt that the precision could be improved by increasing the light intensity reaching the photomultiplier tubes. Thus two further optical systems were tried using multi-element lamps.

Table 19. Relative standard deviations obtained with first optical system

	Ni	Co	Si	Mn	Al	Cu	Ag	Cr	Fe
S 1	36	31	26	21	37	46	19	-	22 %
S 2	37	38	14	23	32	43	23	-	35 %
S 3	26	13	4	11	34	20	11	-	19 %
S 4	32	7	3	11	3	5	8	-	13 %
*EHT setting	9	9	9	9	9	8	7	8	8

* The EHT was adjustable from 400-900 V in 10 steps.

4.9 Modifications to the Original Optical System

The original system using complex optics and single element hollow cathode lamps succeeded but due to the low light levels involved a number of the channels needed very high EHT on the photomultiplier tubes. This high EHT introduced noise and in turn this led to poor reproducibility at low absorbance. It was decided to explore methods of increasing the light throughput of the system and a simple system using one seven-element hollow cathode lamp was used. Of the seven elements, Si, Al, Cu, Fe, Mn, Ca and Zn, only Si, Al, Cu and Fe channels were aligned on the spectrometer (Table 21). It was found that some light was being recorded by the chromium channel; it was later shown that the lamp also emitted the chromium spectrum although rather weakly. Therefore five elements were determined using this lamp: Si, Al, Cu, Cr and Fe. The precision of this system was better than the original, and it is shown in Table 20. Linear calibration curves were obtained, passing through the origin, for the solution concentrations determined (Table 18). It can be seen from Table 20 that the EHT levels used were much lower than for the first system (Table 19) and thus the better precision figures were obtained.

Table 20. Relative standard deviations obtained using one multi-element lamp

	Si	Al	Cu	Cr	Fe	
S 1	15	5	5	-	4	%
S 2	6	5	7	25	6	%
S 3	4	5	6	12	6	%
S 4	8	4	8	13	3	%
EHT setting	4	0	4	5	1	

Table 21. Working Conditions

Element	Wavelength Ångstroms	Limit of detection ng.	Linearity max. range ng.	Range in hair (found in this study) ppm.
Ni	2320.0	0.25	1.5	D.L. 0 - 15
Co	2407.3	0.05	1	D.L. 0 - 3
Si	2516.1	0.1	10	2 - 60
Mn	2794.8	0.002	0.2	D.L. - 4
Al	3092.8	0.05	2	1 - 50
Cu	3247.5	0.01	4	1 - 60
Ag	3382.8	0.005	0.3	D.L. - 3
Cr	3578.7	0.005	1	D.L. - 10
Fe	3719.9	0.02	4	5 - 40

Gas flow 1.5 l/m of argon vented during atomisation

Programme sequence	HGA 70			E 549	
	dry	100°C	20s	pre-spark	50s
	ash	(750°C	15s	integrate	10s
		(1100°C	15s		
	atomise	2600°C	15s		
	cool down		30s		
total cycle time			95s		

After the success of this system it was decided to expand it to include the original nine elements. Since no nine-element hollow cathode lamps are commercially available, a Ni, Co, Mn, Cu, Cr and Fe lamp was used together with three single-element lamps. A simplified optical system using the small stack of mirrors was developed (Fig. 34). This retains a certain degree of flexibility since a maximum of five single-element lamps can be used in the vertical array shown and alternative lamps can be quickly fitted. The hole in M4 only slightly reduces the light reflected since the image of the hollow cathode lamps fills the whole of M4. A higher light throughput was obtained for all channels with this modification than in the original design; this is reflected in the EHT settings given in Tables 19 and 22.

The final working conditions and concentration ranges are given in Table 21. The precision of the system for 45 replicate analyses is given in Table 22; it can be seen that in general poorer precision is obtained when the EHT is high (this is a measure of the light throughput). These precision figures are considerably better than those obtained using the original optical system (Table 19). The majority of the analyses presented in Chapter 5 were carried out using this modified optical system.

4.10 Conclusion

The proposition of multi-element AAS is constrained by limitations of concentration range that can be analysed, usually less than two orders of magnitude. This imposes a limitation on the range of concentration ratios of elements in the sample if simultaneous multi-element AAS is contemplated. Thus as a general technique an emission mode of spectroscopy would be inherently better. It is possible, however, for specific samples and specific elements, to design a multi-element AA spectrometer that can be used for the simultaneous determination of, in this case, nine elements in hair. Some of the difficulties associated with

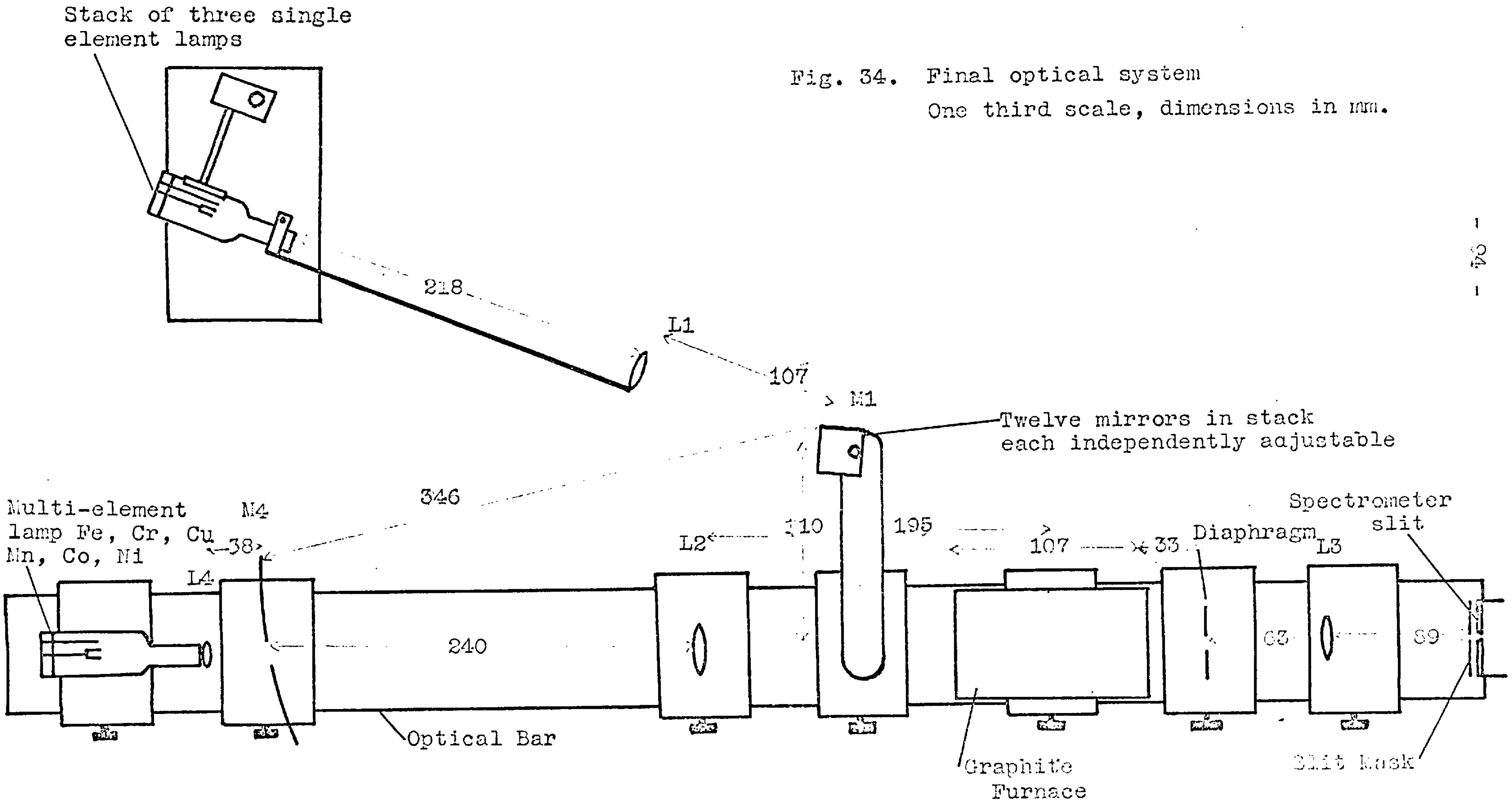


Fig. 34. Final optical system
One third scale, dimensions in mm.

Table 22 45 replicate analyses of a solution (S 2) containing Ni Co Si Mn Al Cu Ag Cr Fe

	Ni	Co	Si	Mn	Al	Cu	Ag	Cr	Fe	samples
$\bar{x}$	39	64	55	57	84	69	61	81	34	1 - 10
s	7	8	4	1	4	2	8	5	1	
rsd %	18	12	7	3	4	3	13	6	3	
$\bar{x}$	30	59	50	55	82	70	63	84	32	10 - 20
s	10	7	3	1	4	5	7	6	1	
rsd %	34	13	6	2	5	8	11	8	3	
$\bar{x}$	31	54	49	54	82	66	63	76	29	20 - 30
s	7	9	4	1	4	2	5	5	1	
rsd %	22	17	8	2	5	3	8	6	5	
$\bar{x}$	30	59	52	58	85	67	68	80	31	30 - 40
s	8	10	4	4	10	7	10	4	2	
rsd %	26	17	9	7	12	10	14	5	8	
$\bar{x}$	32	64	48	56	86	68	66	76	29	40 - 45
s	9	10	3	1	8	1	4	4	1	
rsd %	29	16	6	2	9	2	6	6	4	
mass of analyte ng.	0.6	0.4	5.0	0.1	1.0	2.0	0.2	0.4	2.0	
EHT setting	7	8	7	2	6	4	6	3	3	

$\bar{x}$ = mean value

s = standard deviation

rsd = relative standard deviation

the measurement of the electrical signals from photomultiplier tubes in electrothermal AA have been overcome by differentiation of the pulses to remove the standing background potential followed by integration to re-constitute the original pulse shape. This manipulation greatly simplifies signal manipulation and it overcomes premature curvature, which would have been caused by high background or line overlap, since only the absorption pulse is measured. The contributions from high background and line overlap serve to degrade the signal:noise and thus the precision and detection limit. The original optical system using single element lamps was found to yield rather imprecise results. This was attributed to the low light levels and the necessarily high EHT settings. Modifications were made by the author to the system so that more light would reach the photomultiplier tubes. This involved the use of multi-element hollow cathode lamps. The increased light throughput improved the precision. In Chapter 5 the application of the spectrometer to the simultaneous multi-element analysis of hair is discussed.

The original spectrometer optics (Section 4.4) were designed and assembled by Dr. J.F. Alder, Chemistry Department, Imperial College.

Chapter 5

Simultaneous Multi-element Analysis of Hair

5.1 Introduction

The trace metal analysis of hair has been of use in several fields of investigation, such as toxicology, physiology and pollution control, but has been mainly used for forensic purposes, viz. the comparison of hairs left at the scene of crime and those found on suspects. The methods used for the multi-element analysis of hair have, however, not been sensitive enough to obtain any detailed profile of trace-elements along the length of the hair. There are also very few data in the literature on trace elements in hair from different parts of the human body. The work presented in this chapter goes some way to filling this gap, at least for the elements investigated.

It is in the field of forensic analysis that the basis of this study lies and it is pertinent to consider, at this stage, what might be expected of the multi-element analysis of hair. The most detailed studies of trace elements along the length of hair by Renshaw et al. (43,93) and by Obrusnik et al. (14) have both shown certain features of the trace metal profiles, along the length of individual hairs, that are worthy of further discussion. A number of the elements investigated by Obrusnik et al. (14) and both of those investigated by Renshaw et al. (43,93) showed that an increase in element concentration occurred in the distal parts of the hair and that the distribution of trace element concentration in hair was not usually a normal distribution but positively skewed and in the case of Renshaw et al. shown to be a log-normal distribution. Both research groups have related the variation of trace metal concentration (in hair) within an individual to the variation of the trace metal concentration (in hair) over the population. Renshaw et al. (43) showed that, in the case of lead and copper, the variation within an individual was approximately half the variation with the population as a whole. They concluded that the copper and lead concentration in hair would be of use in discriminating between hair samples from different sources. It might at first be anticipated that a variation within an individual of half the variation over the population would lead to a discrimination factor of 1 in 2 (per element). Further analysis of the results reveals that this is, however, not the case; the discrimination factor depends upon where the mean value for the individual lies on the frequency distribution curve. If the mean for the individual lies at the centre of the curve then with a variation of half that of the whole curve the discrimination will be worse than 1 in 2 (say 2 in 3); whereas if the individual mean occurs at a concentration much displaced from the centre of the distribution then a discrimination of better than 1 in 2 will be obtained (say 1 in 5). This is a rather unsatisfactory situation and can only be improved if more elements are determined, provided that their concentrations are not correlated. If an average discrimination of 1 in 2 is obtained per element and ten non-correlated elements are determined then an overall discrimination of just better than 1 in 1000 would be obtained; this would be useful but would still leave much to be desired for forensic applications. Coleman and co-workers have used statistical methods developed by Parker (102-104) to compare hairs. These methods employ a discrepancy index which is a measure of the difference between the two sets of attributes, one from the crime sample and one from the control sample. It was initially envisaged that Parker's methods might be used with data from this study to compare hairs. This method demands an accurate knowledge of the errors involved, these are error due to measurement and error due to variation of trace element in hair. The discrepancy index C is defined by,

$$C = \sum_{i=1}^{N} (Di/\lambda i)^2 \qquad (102)$$

N = number of attributes (i = 1, 2 ... N)

D = difference between measure means of attributes

$$\lambda i = \sqrt{\sigma i^2 + \theta i^2 + \phi i^2}$$

where

θi = error due to variation of attribute

σi = error due to measurement

$\phi i = \frac{\sigma i}{\sqrt{n}}$

n = number of measurements made.

It can be seen that λi is the combined error and if the variation of trace metal in the hair is large compared with the measurement error, then the contribution to the total error by the measurement error will be small and the ϕi contribution insignificant. If the total error is 20% and the error due to measurement 8% then the variation of the trace metal will be 18.3%. The variation of concentration of trace elements in hair is shown in Figs. 35-77; it can be seen to be large. It can also be seen that the variation along the length is not always random and trends and peaks can be distinguished. The determination of an "error" as the variation down the length of a hair is therefore meaningless since it is a non-random distributed population. Furthermore the averaging of the concentrations down the length of the hair degrades the extra information obtained by this method of analysis. Thus the use of Parker's method in this case is wasteful and, if the variation down the length is employed in the calculation as θi, then it is inaccurate.

A different method of comparing the data is required if full advantage of the extra information is to be made.

5.2 Results of the multi-element analysis of hair

5.2.1 Introduction

A comparative analysis using wet and dry ashing was carried out in order to determine whether the dry ashing method was giving accurate results under the conditions of multi-element analysis. A survey was then made of trace elements in hair of a small population. Two long hairs from a female head and two long hairs from a male head were analysed one centimetre at a time to discover whether long hairs showed any notable trends. Two females and two males were then sampled in different areas of the body; head, pubic, axillary, arm, leg, chest, beard, eye-lash and eyebrow (where available). These were then analysed. The results are presented in Figs. 35-77.

A discrimination test was also carried out to discover whether the method was suitable for the comparison of single crime and control hairs.

5.2.2 Comparative wet and dry ashing

To check the accuracy of the analysis a comparative wet and dry ashing analysis was carried out. A sample of female head hair, cut from the ends, was divided into three portions; a random sample of the first was analysed by dry ashing using the conditions in Table 21 (Chapter 4), the second 3g approximately was dissolved in 8 ml of TMA and analysed by direct standard addition and the third, 4g approximately, was dissolved in 5 ml of nitric acid and analysed by direct standard addition. The analysis conditions in Table 32 were used. In addition to analysis by standard addition the solutions were analysed by the intercept method and the values obtained included for comparison. The results are shown in Table 23. Dilutions of twenty times were made in order to determine the more concentrated species; this diluted some of the analytes below their detection limits. The solution of hair in TMA was made up in a conventional graduated glass flask and was allowed to stand for some time prior to analysis and the high silicon and aluminium values obtained are probably a result of the residual TMA dissolving some of the glass vessel. TMA solution is very alkaline. The Cr, Ni and Co concentrations were below the detection limit in the solution of hair in nitric acid used in the standard addition but with less dilution were determined by interpolation.

There is broad agreement with the dry ashing results except in the cases already discussed above and in the case of silver which is probably lost during the ashing process. A similar discrepancy for the volatile elements zinc and bismuth was noted in Chapter 3. The relatively wide spread of results is due in part to the low precision (these results were obtained using the original optical design) and partly due to the dilution necessary to enable the more concentrated elements to be determined. Also at the levels at which these analytes are determined the blanks represented a considerable fraction of the total analyte concentration and this introduces further error.

Table 23. Results of comparative wet and dry ashing analysis

		Ni	Co	Si	Mn	Al	Cu	Ag	Cr	Fe	
DRY ASHING	$\bar{x}$	4.5	3.9	9.6	0.15	7.9	12.7	0.10	2.5	17.7	ppm
	s	2.4	0.8	3.6	0.16	1.5	5.0	0.02	1.4	2.6	ppm
	rsd	55	21	38	110	19	39	20	56	15	%
HNO_3 WET ASHING	S.A.	-	-	23.9	0.18	4.9	8.6	0.3	-	11.1	ppm
	INT.	-	-	12.8	0.17	2.4	5.6	0.3	2.7	9.1	ppm
TMA	S.A	4.0	3.1	83.1	0.33	28.0	9.5	0.91	-	18.3	ppm
	INT.	7.6	4.9	34.2	0.24	8.6	9.3	0.36	3.8	18.1	ppm

$\bar{x}$ = mean

s = standard deviation

rsd = relative standard deviation

S.A.= Standard Addition

INT.= Interpolation

5.2.3 Trichogram survey

Figs. 35-43

Trichogram of two female (2) and two male (4) head hair. The cobalt and nickel channels were not operational when the first female head hair was analysed.

Figs. 44-50

Trichogram of two head hairs, three pubic hairs and three eyebrow hairs from one female (3). Chromium and silicon were below the detection limit for nearly all samples.

Figs. 51-59

Trichogram of two head, two pubic, two axillary, four arm, four leg, two beard, two chest and three eyebrow hairs from one male (5).

Figs. 60-68

Trichogram of three head, two pubic, one axillary, three arm, three leg, three chest, one eye-lash and two eyebrow hairs from one male (6).

Figs. 69-77

Trichogram of two head, three pubic, four arm and four leg hairs for one female (4).

----- indicates sample lost

All trichograms are plotted with the distal end of the hair on the left.

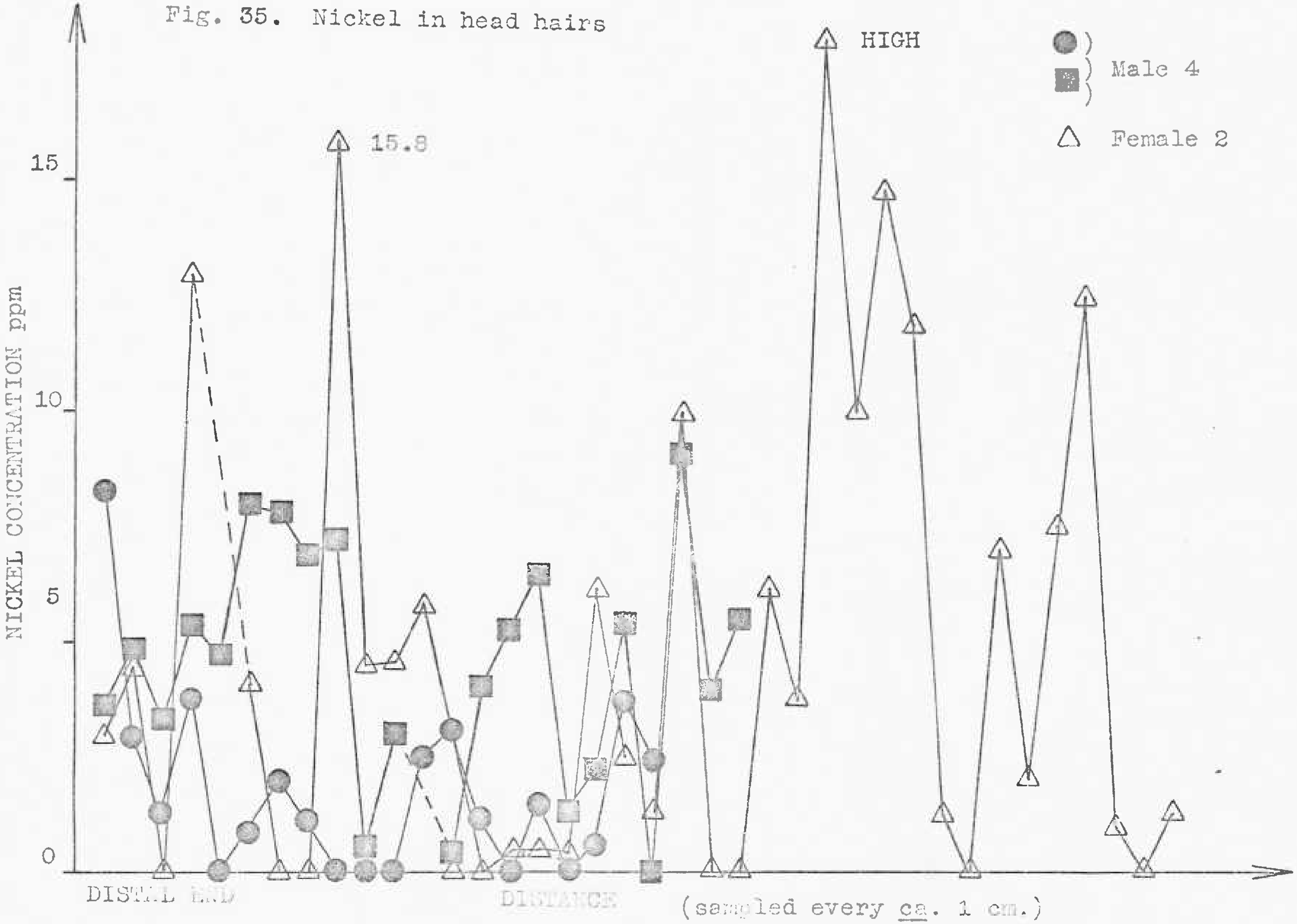
All head hairs were sampled every <u>ca</u>. 1 cm.

/cont.

Pubic, axillary and chest hairs were sampled every *ca*. ½cm.

Arm, leg, eye-lashes and eyebrow hairs were usually sampled whole.

Fig. 35. Nickel in head hairs



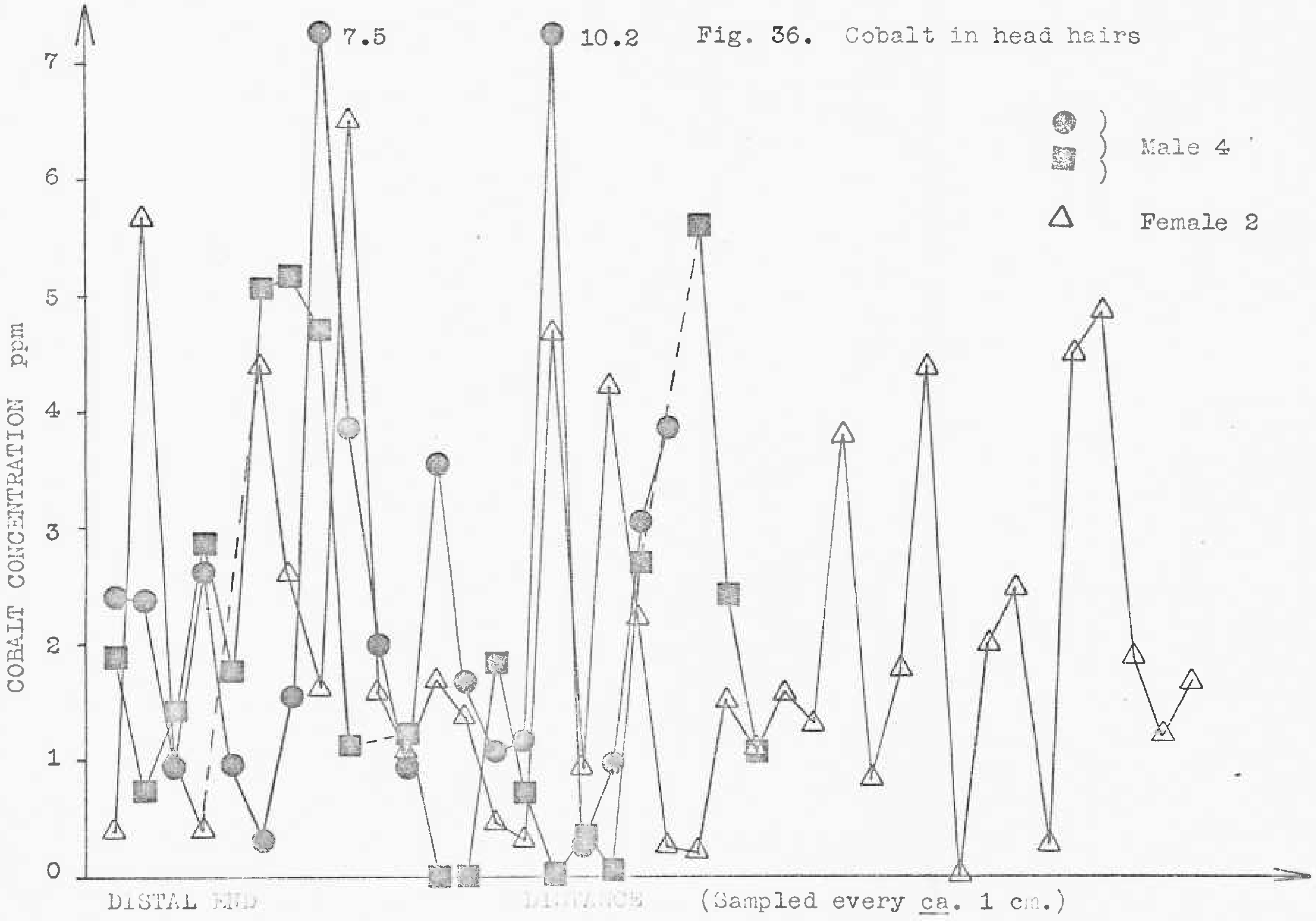


Fig. 36. Cobalt in head hairs

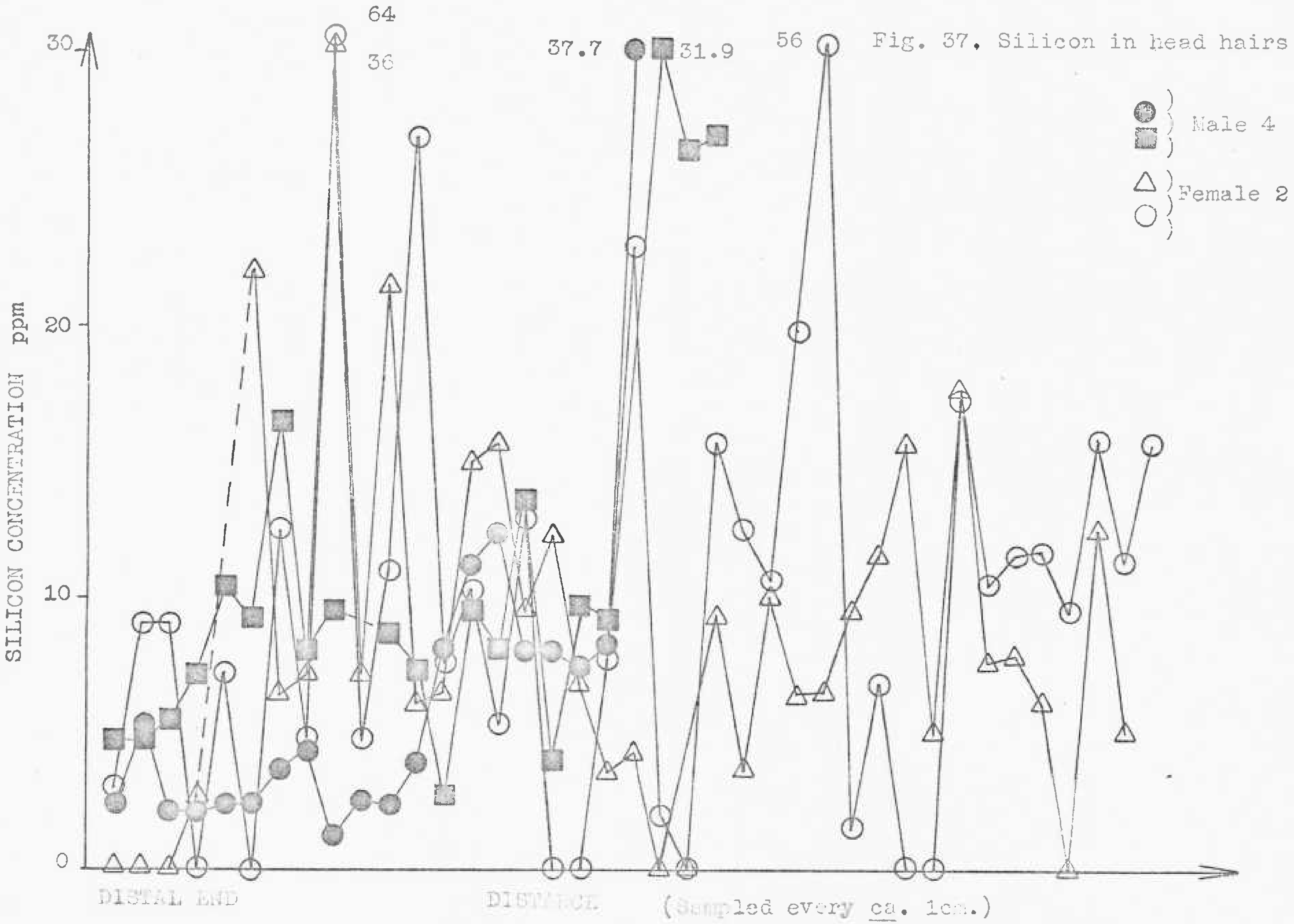


Fig. 37. Silicon in head hairs

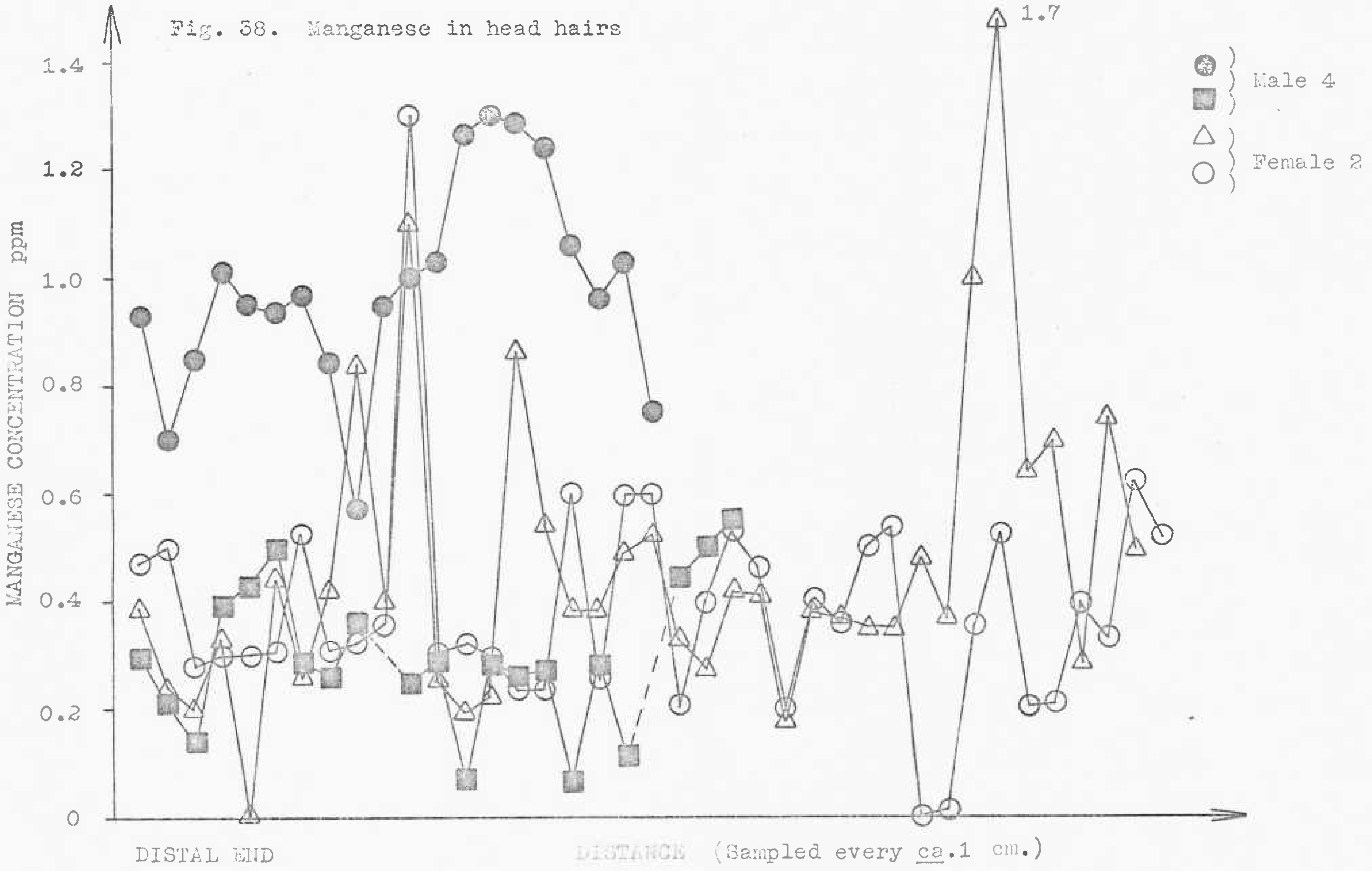
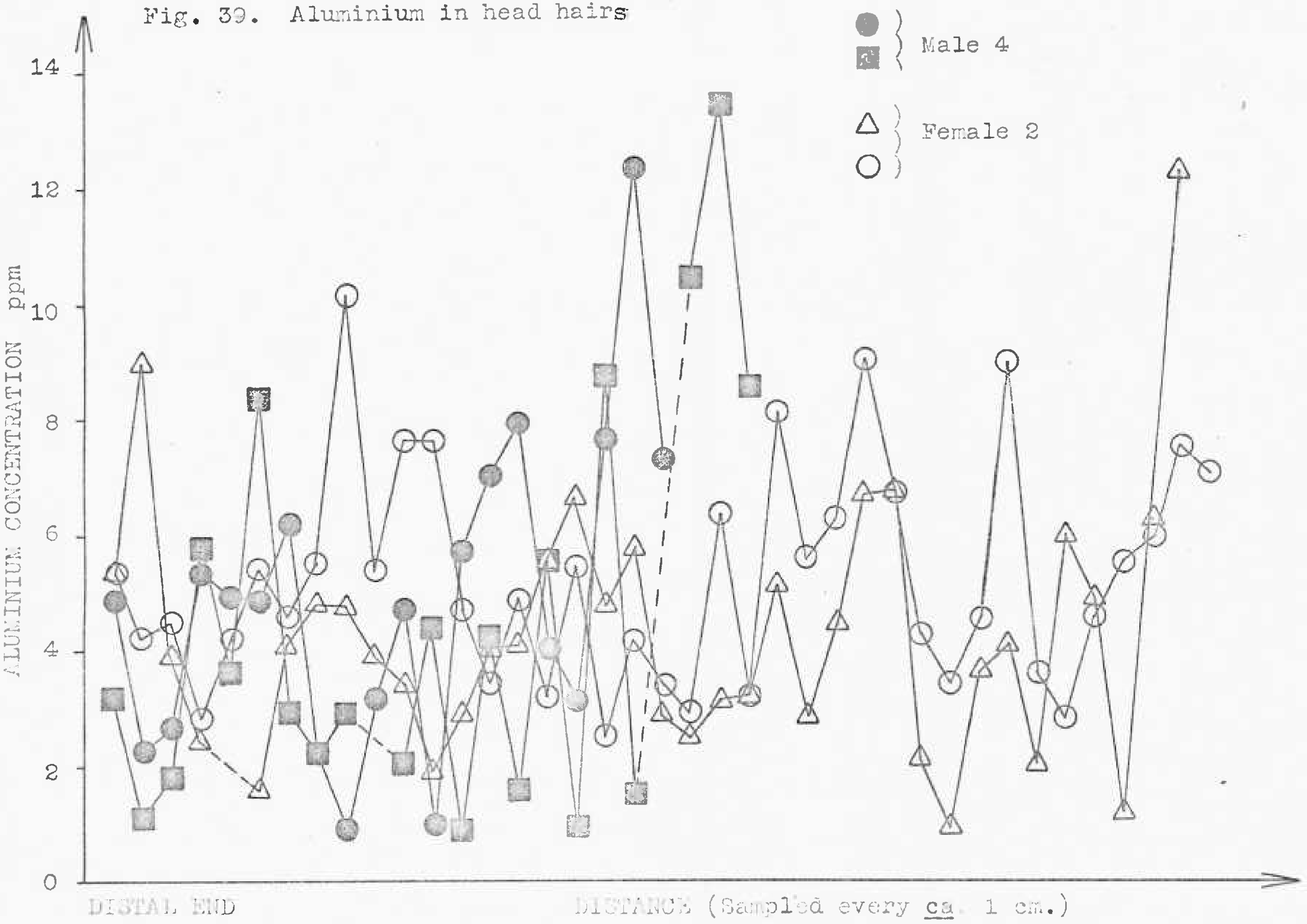


Fig. 38. Manganese in head hairs

Fig. 39. Aluminium in head hairs



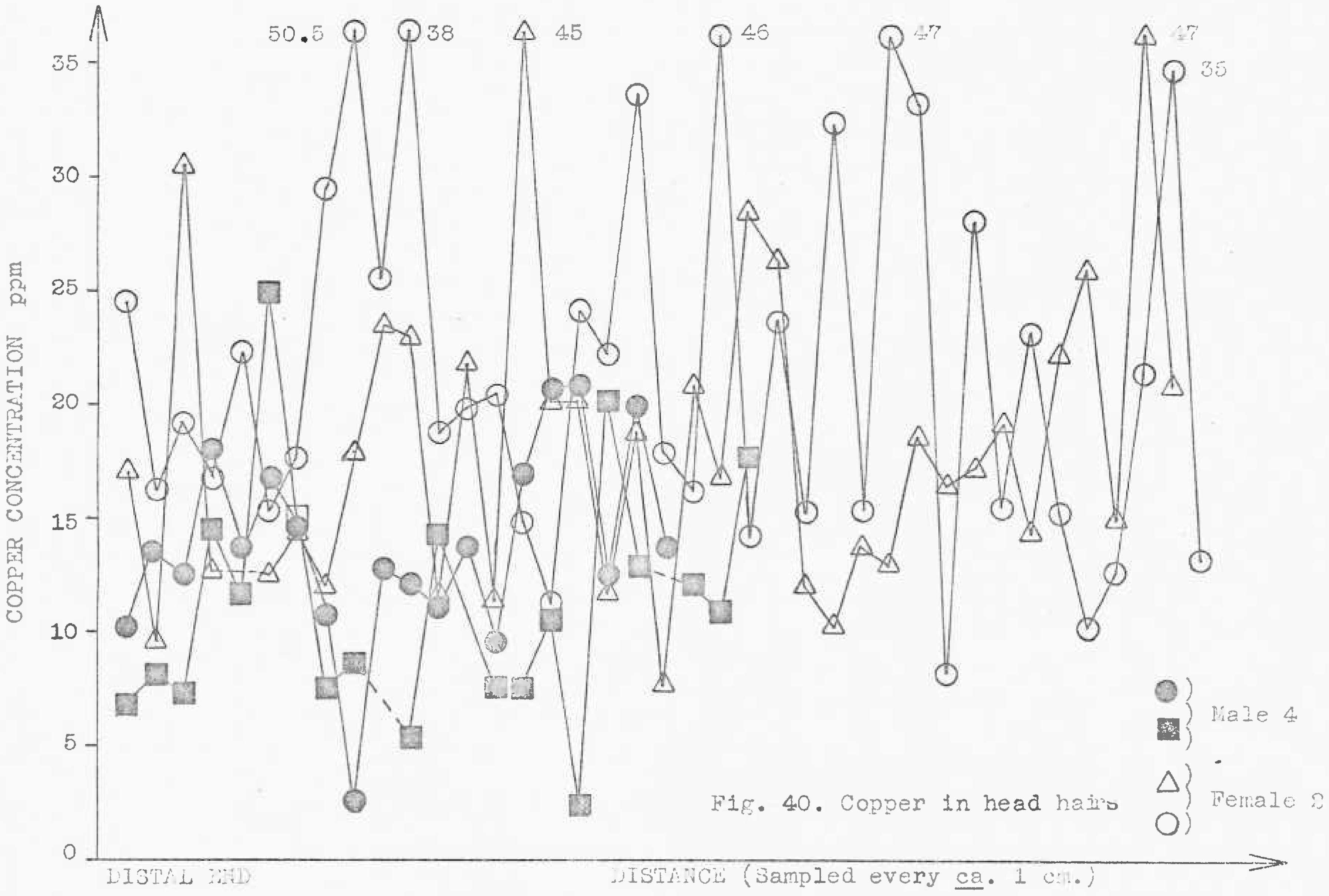


Fig. 40. Copper in head hairs

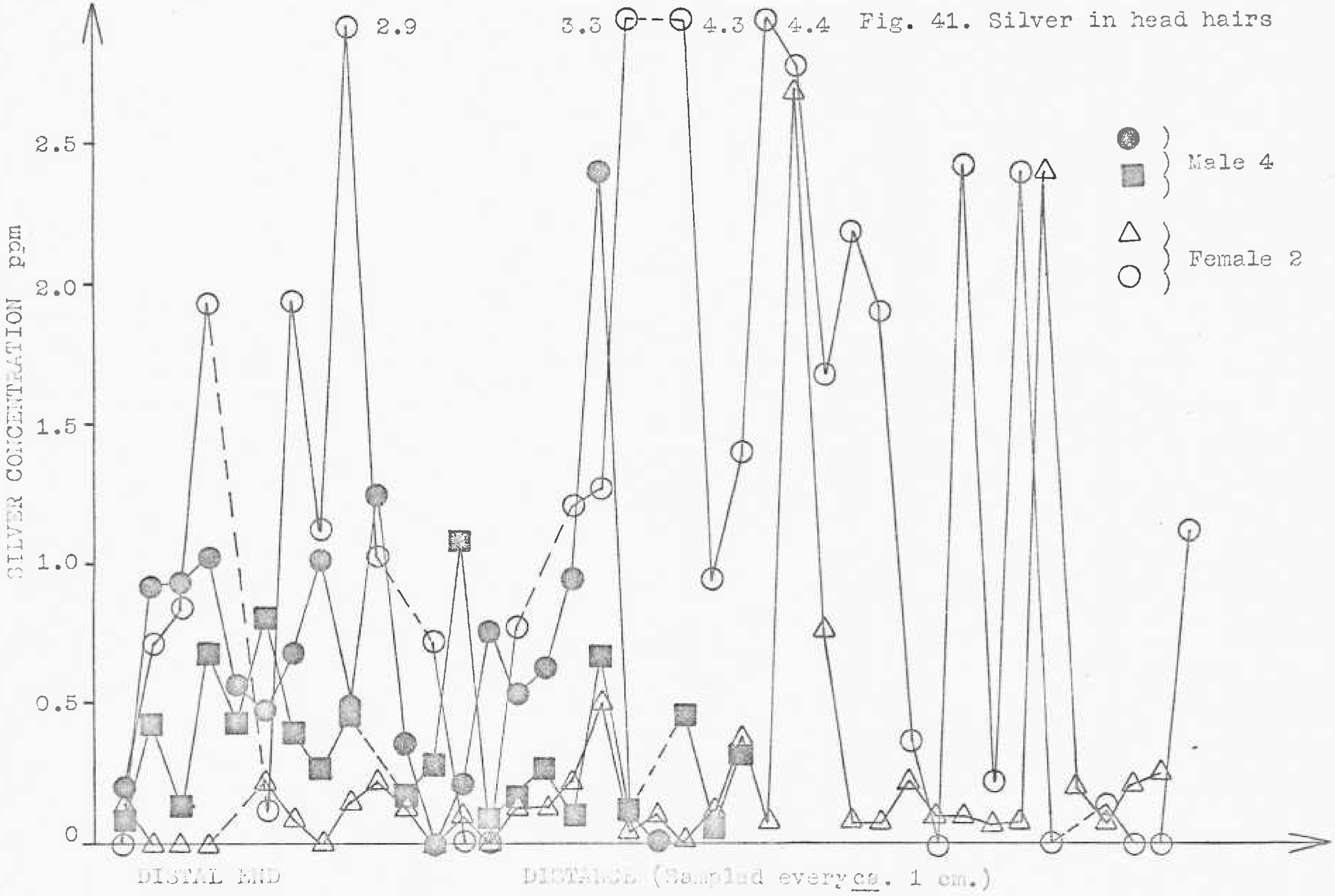


Fig. 41. Silver in head hairs

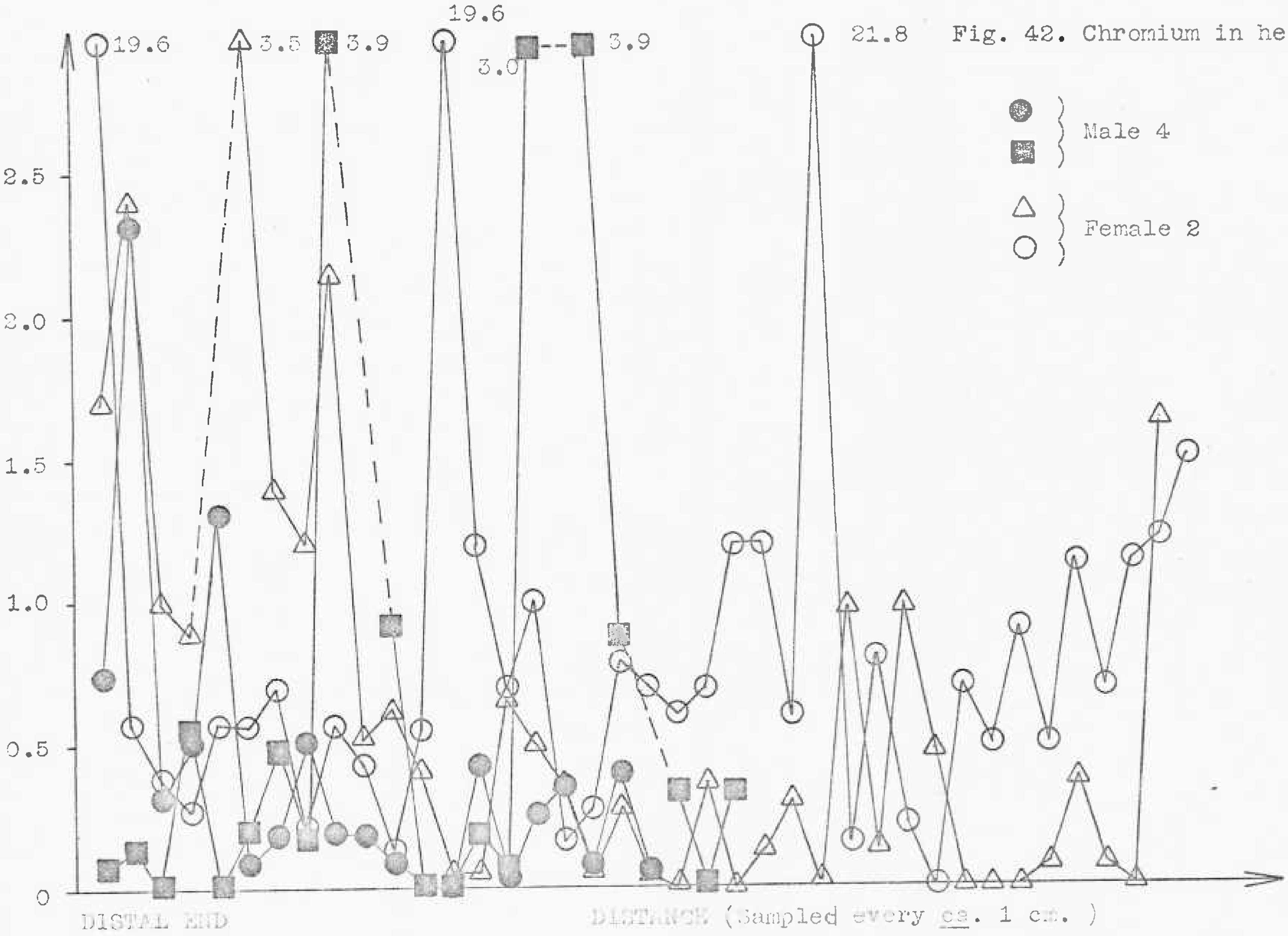


Fig. 42. Chromium in head hairs

Fig. 43. Iron in head hairs

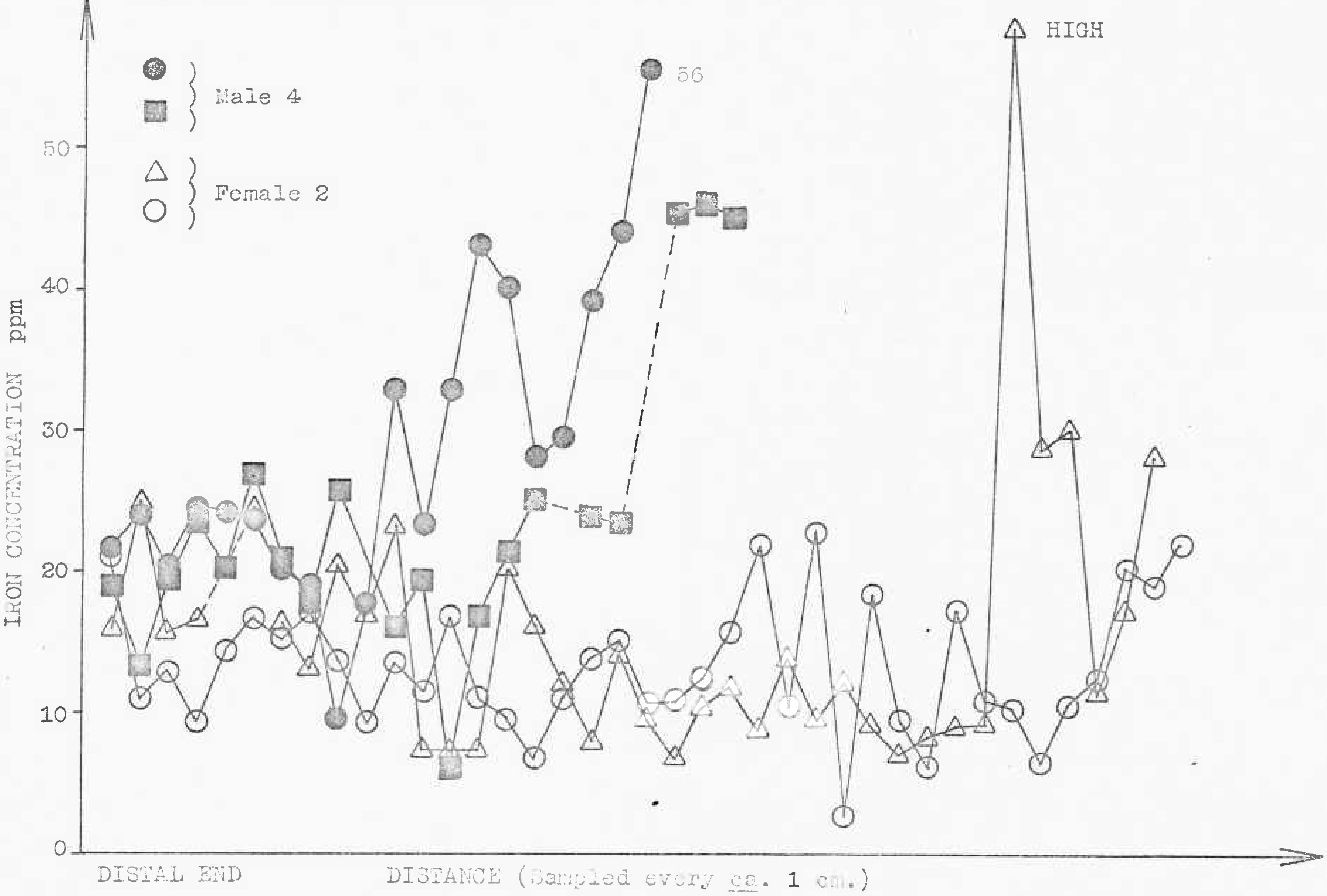


Fig. 44. Nickel in hair. Female 3

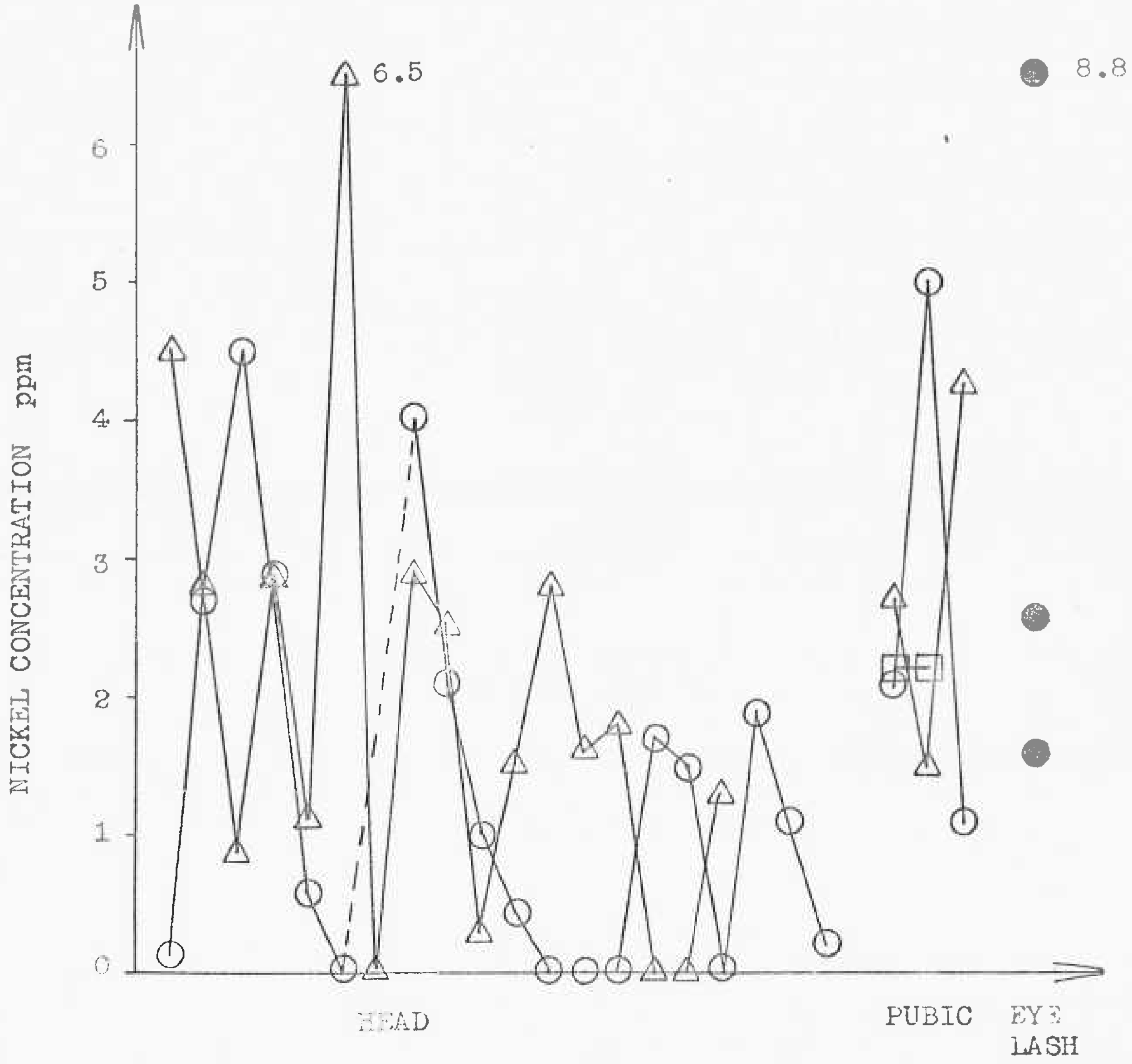


Fig. 45. Cobalt in hair. Female 3.

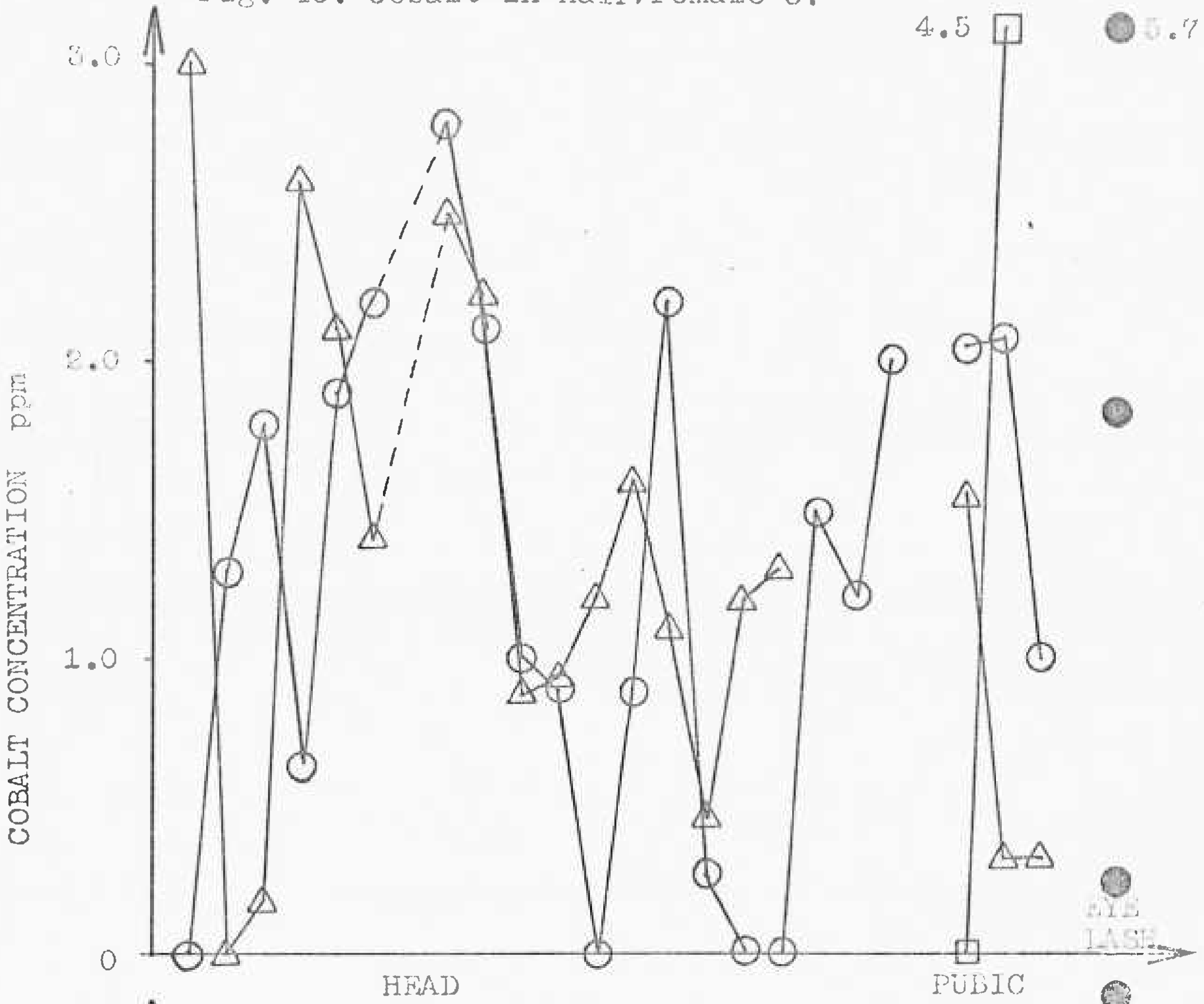
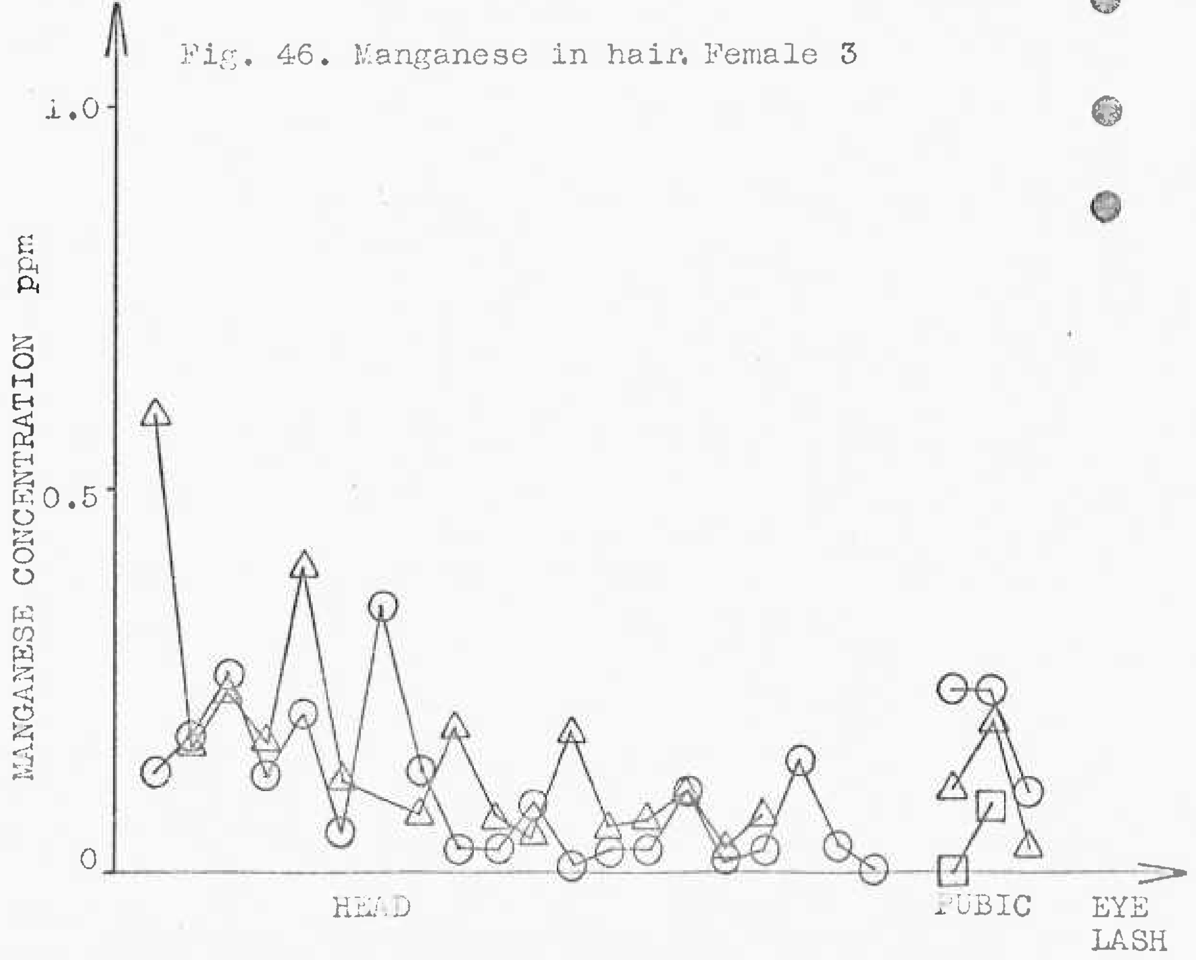


Fig. 46. Manganese in hair. Female 3



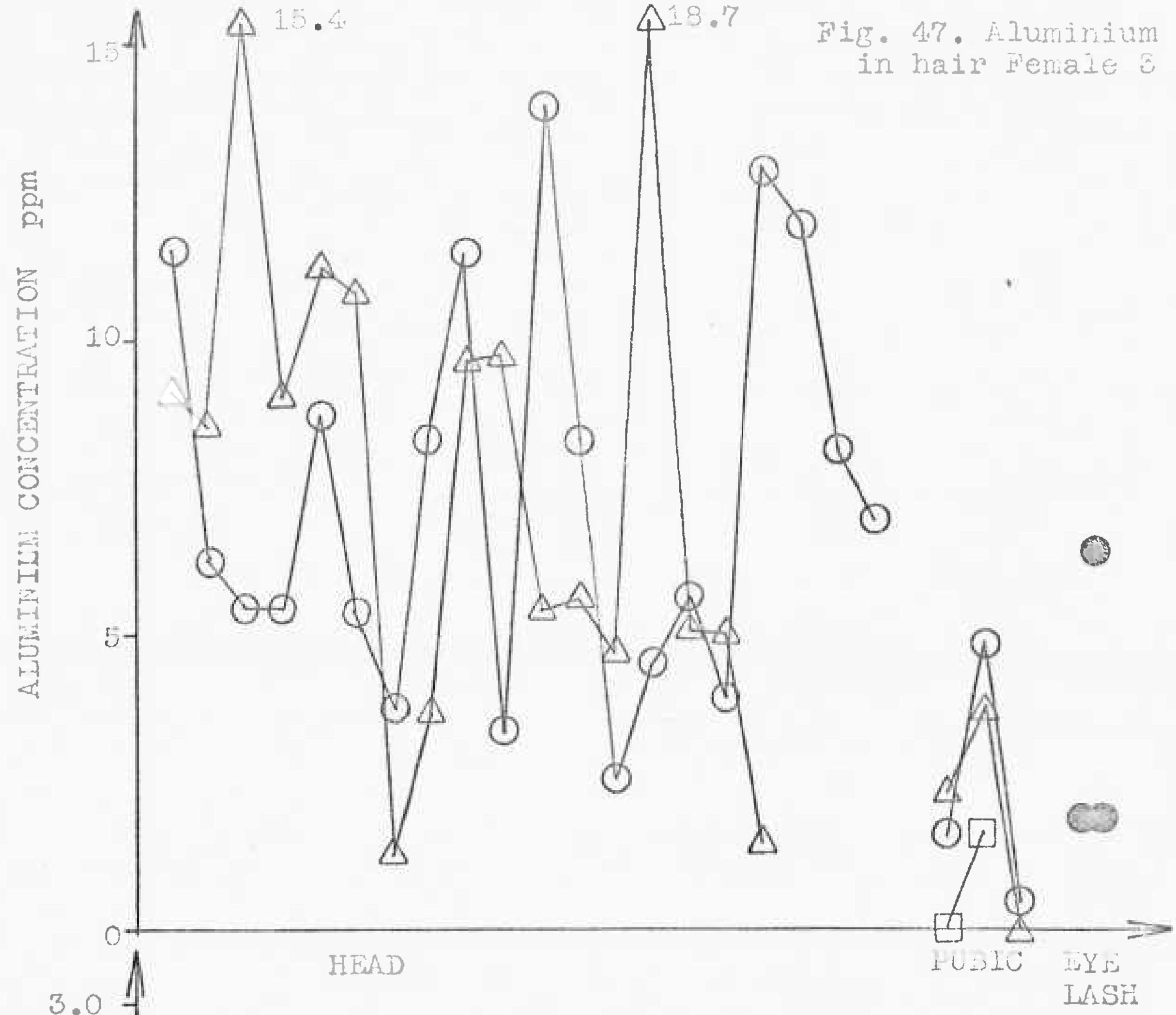


Fig. 47. Aluminium in hair Female 3

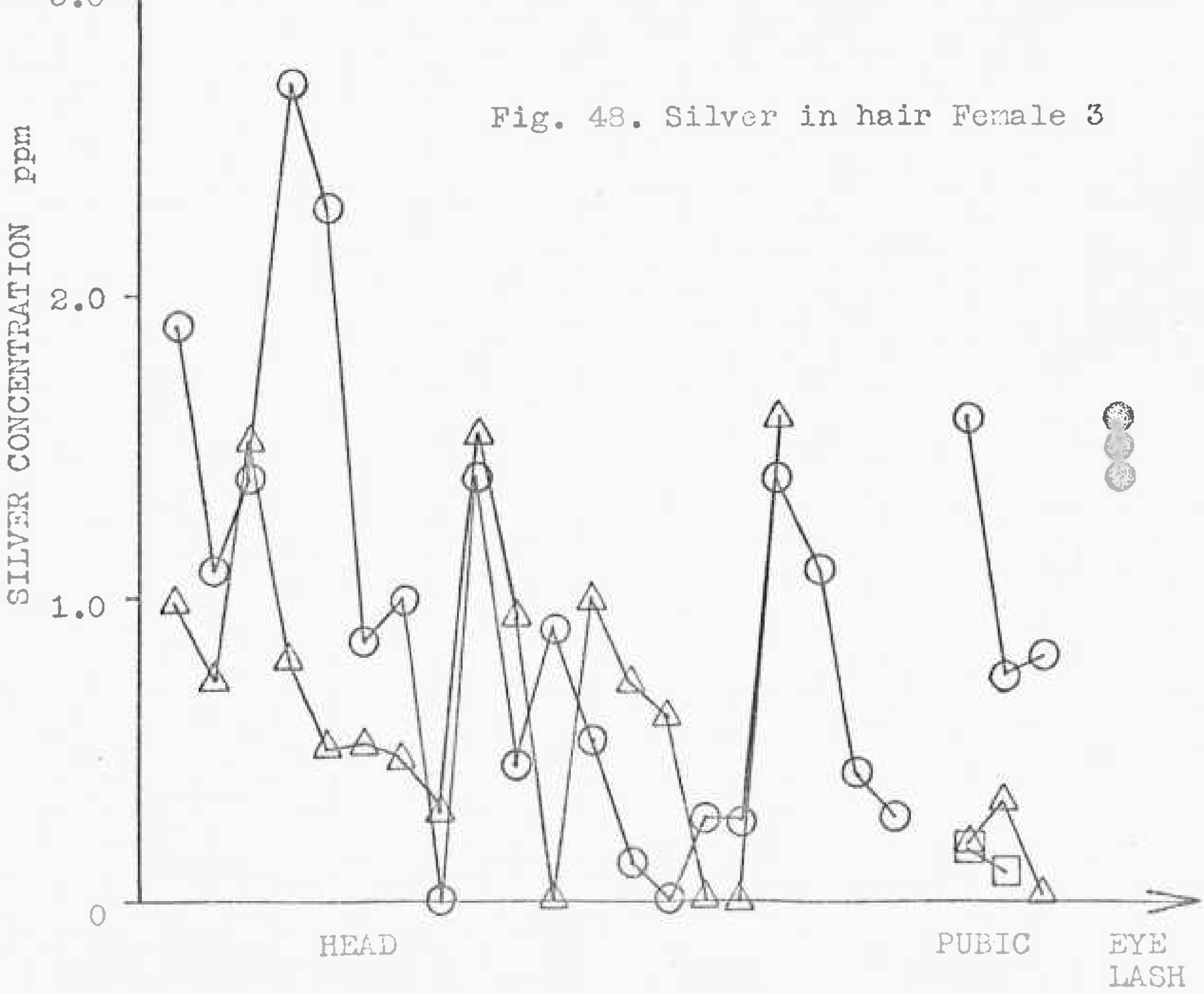
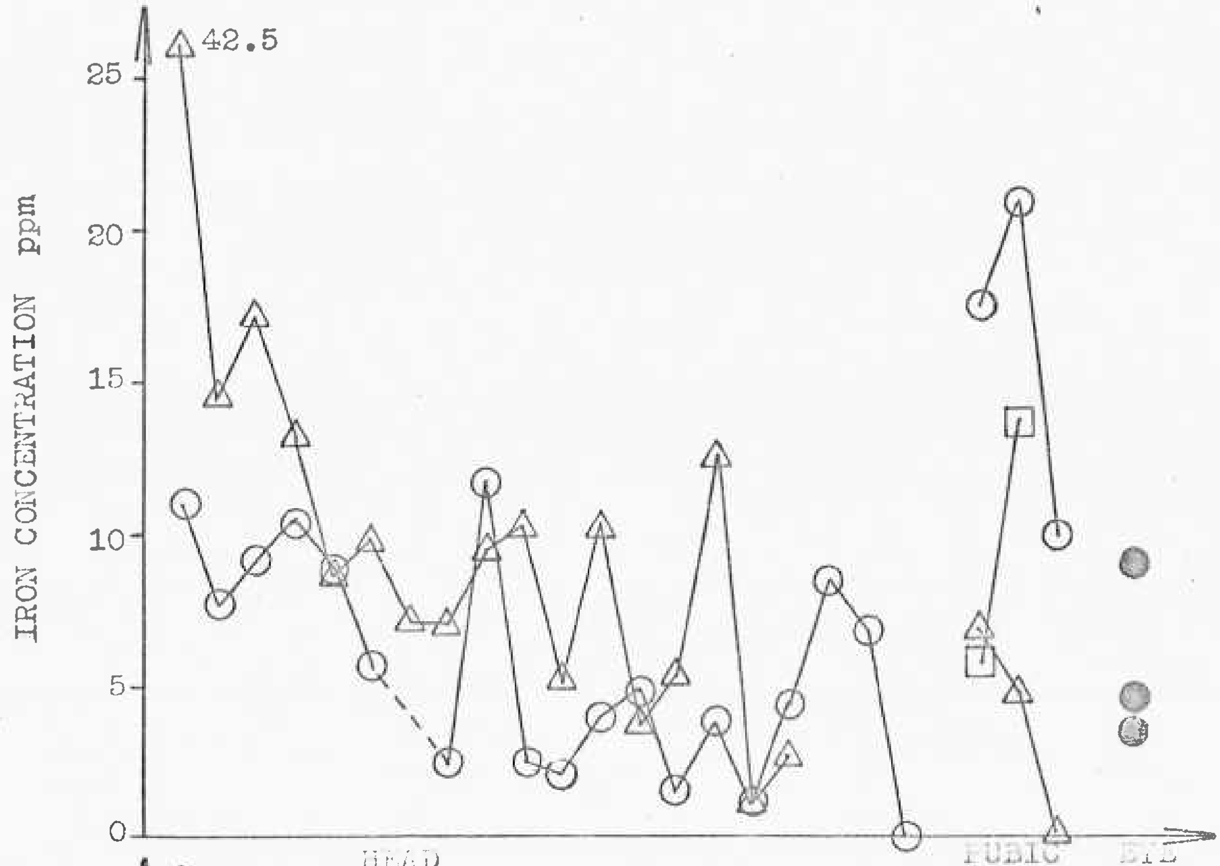


Fig. 48. Silver in hair Female 3

Fig. 49. Iron in hair Female 3



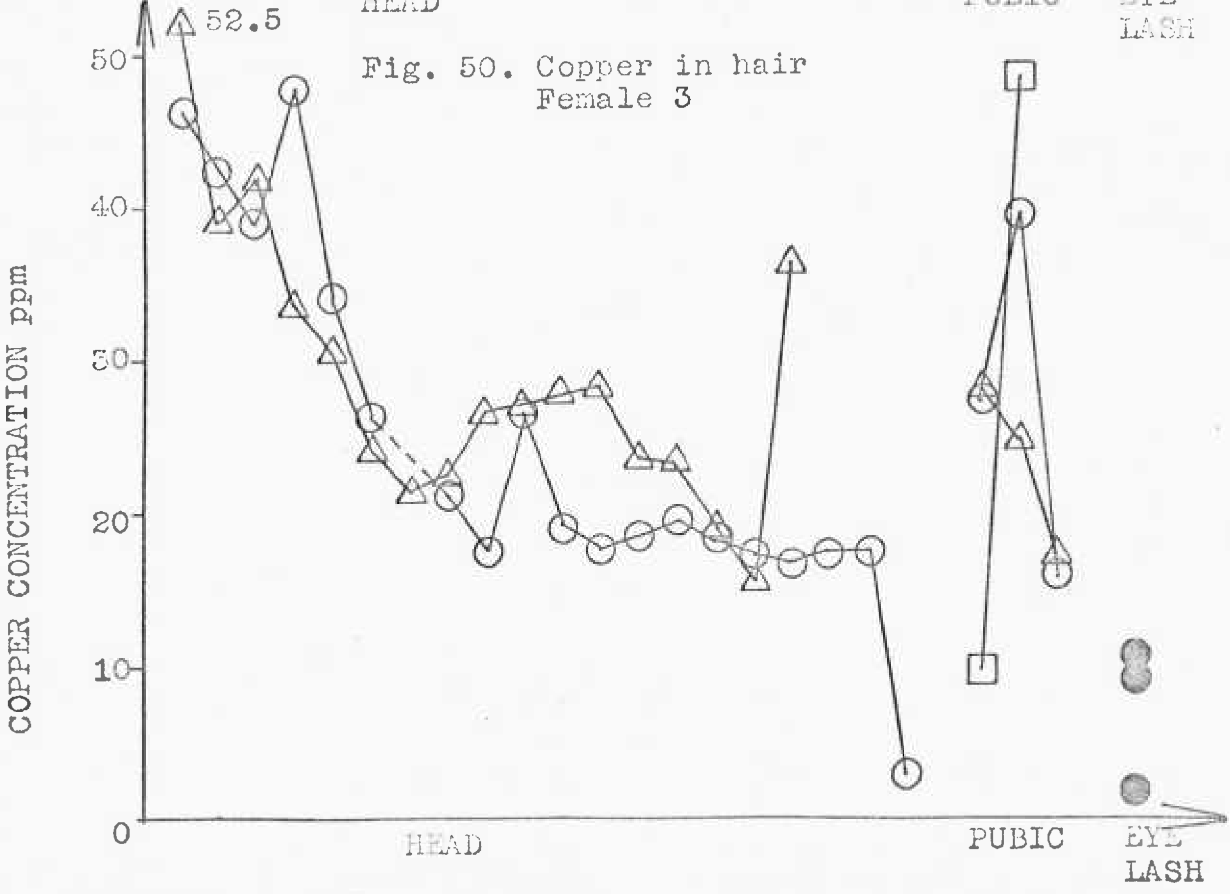


Fig. 50. Copper in hair Female 3

Fig. 51. Nickel in hair Male 5

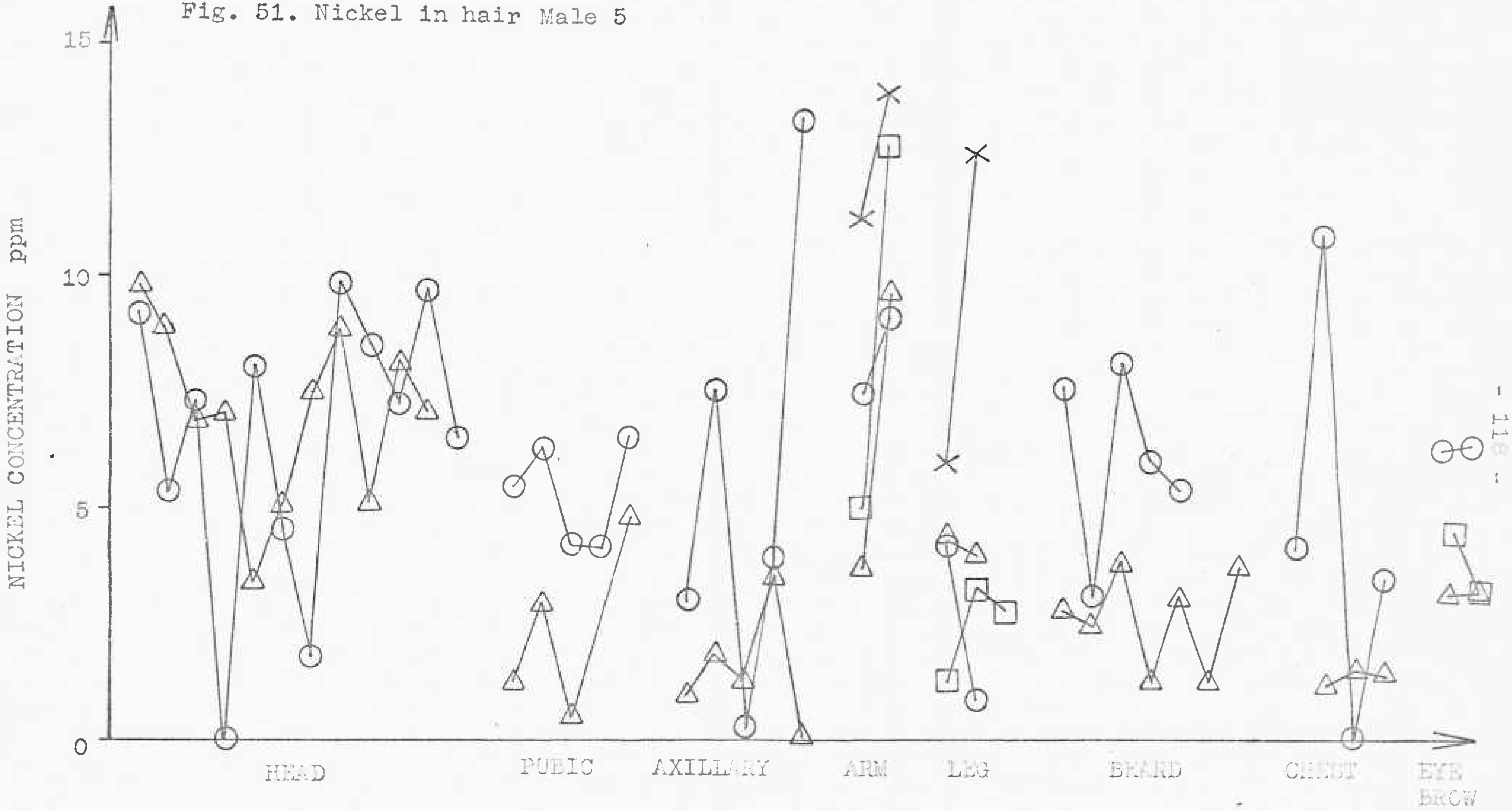


Fig. 52. Cobalt in hair Male 5

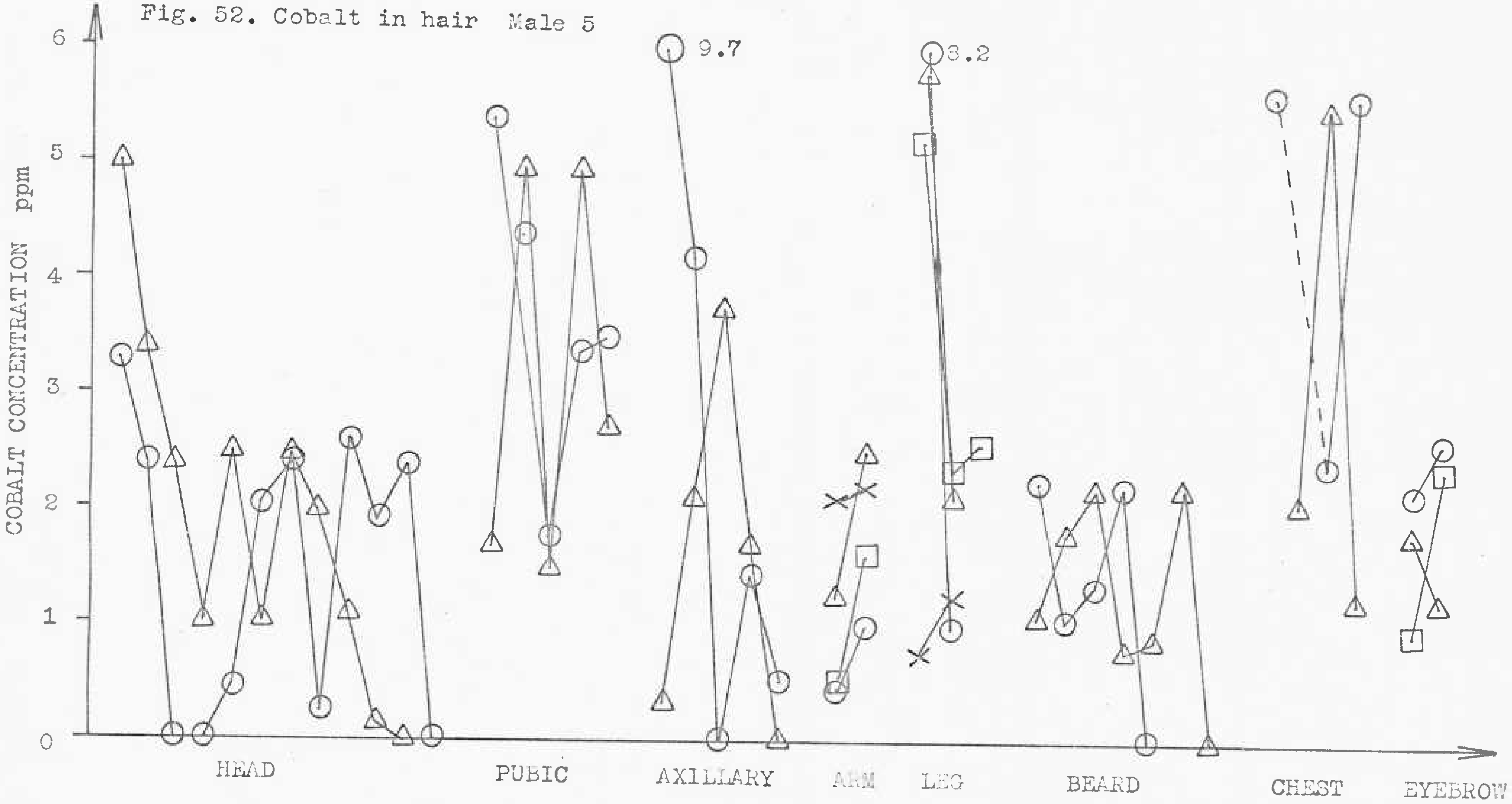


Fig. 53. Silicon in hair. Male 5

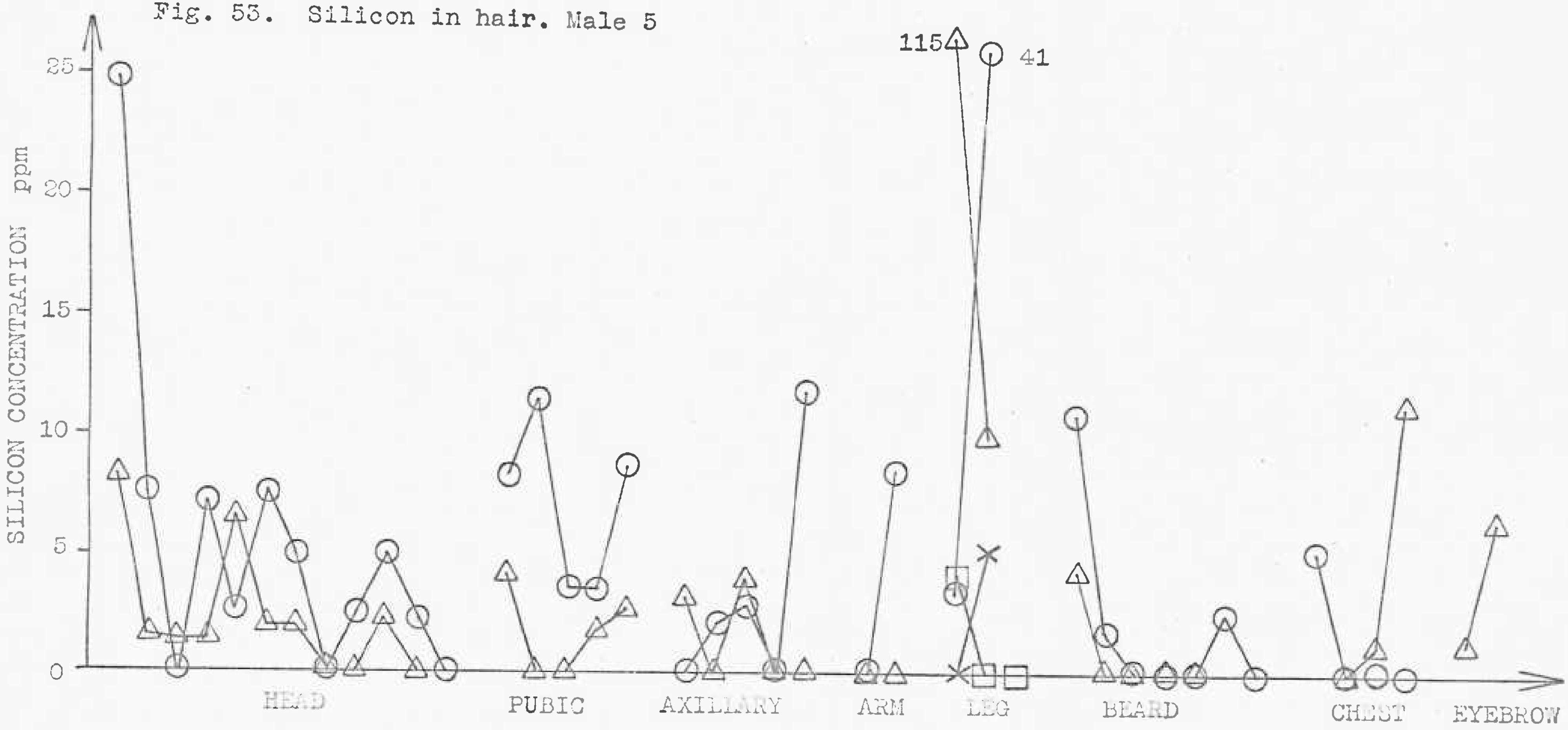


Fig. 54. Manganese in hair. Male 5

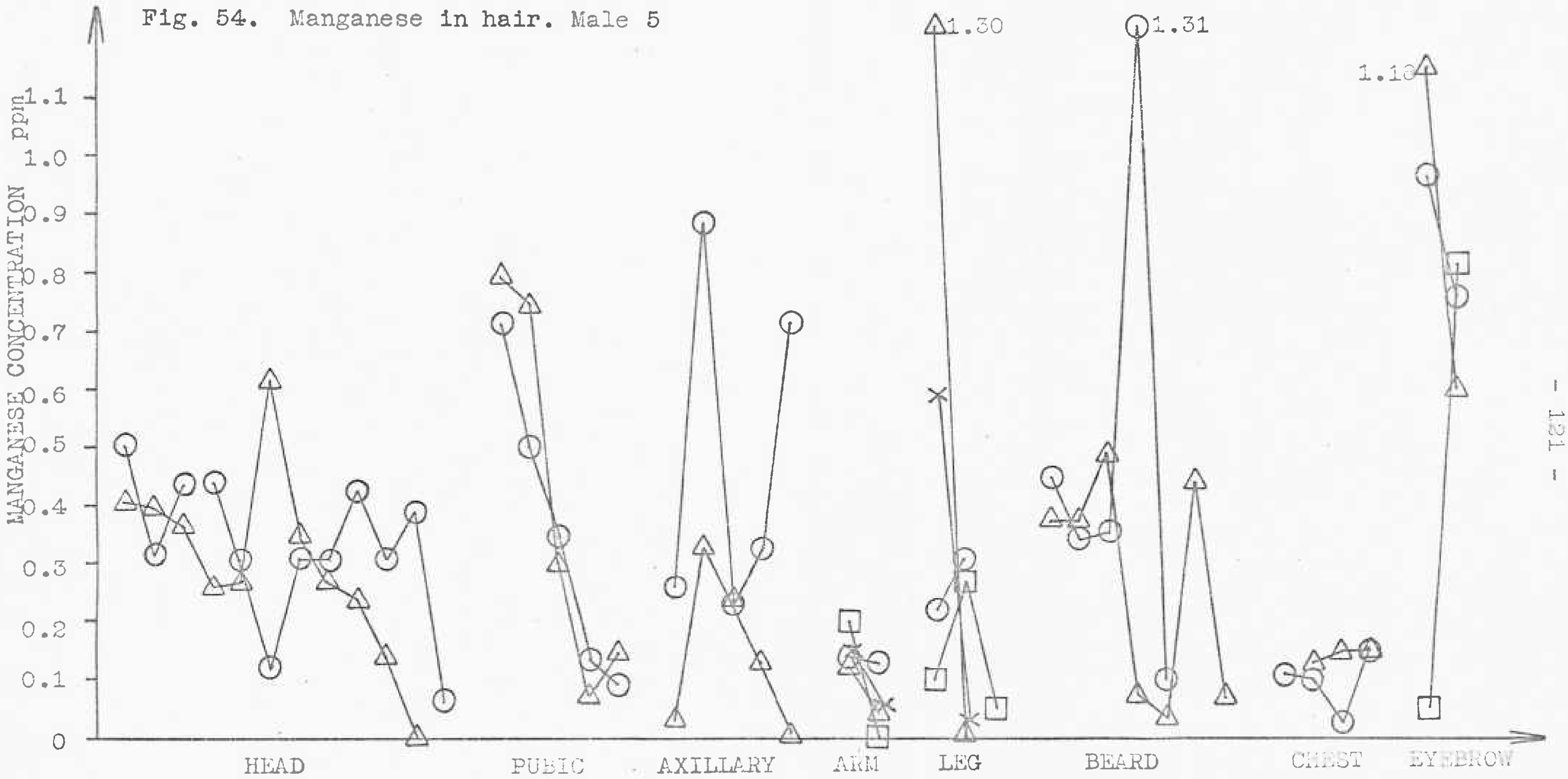


Fig. 55. Aluminium in hair. Male 5

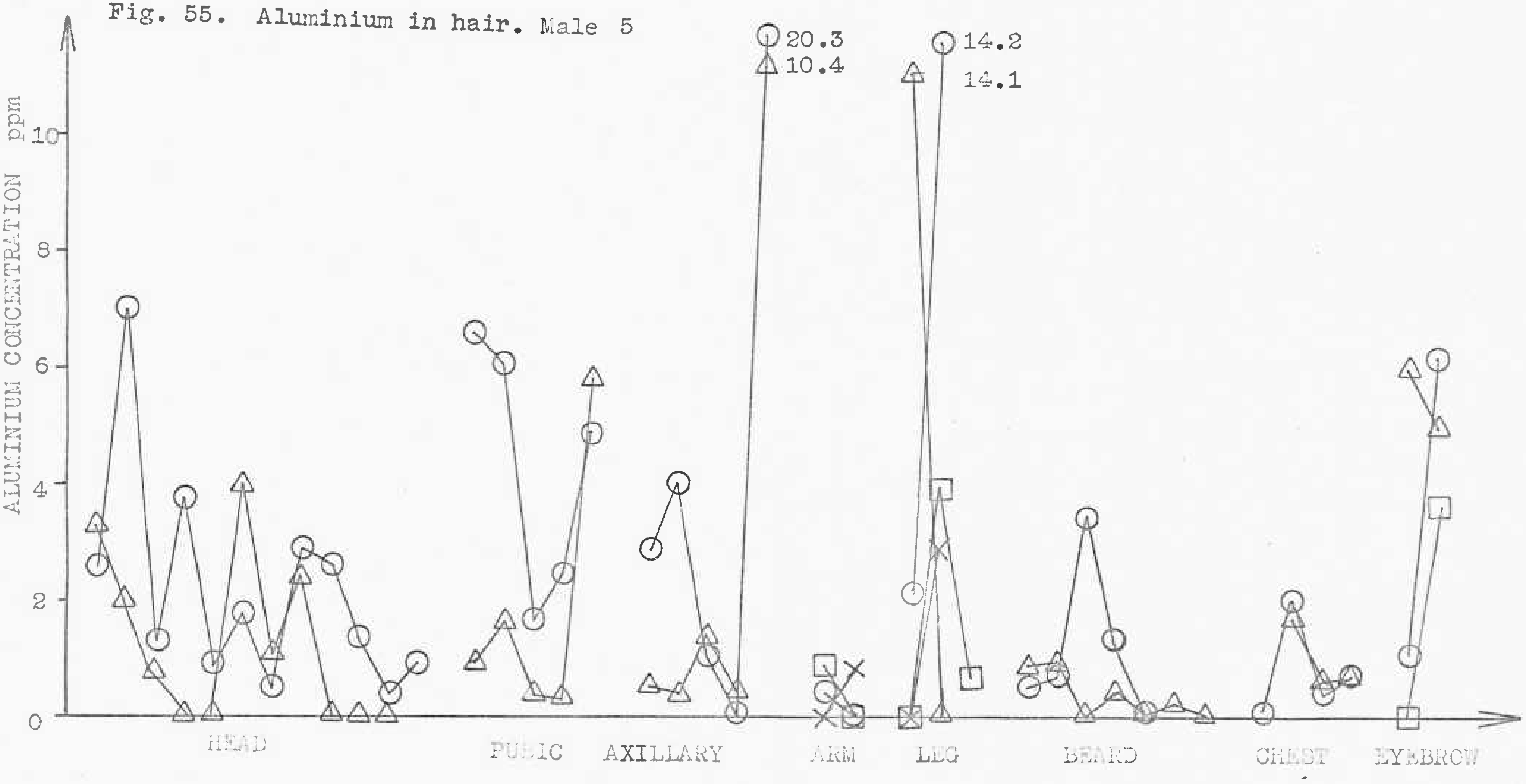


Fig. 56. Copper in hair. Male 5

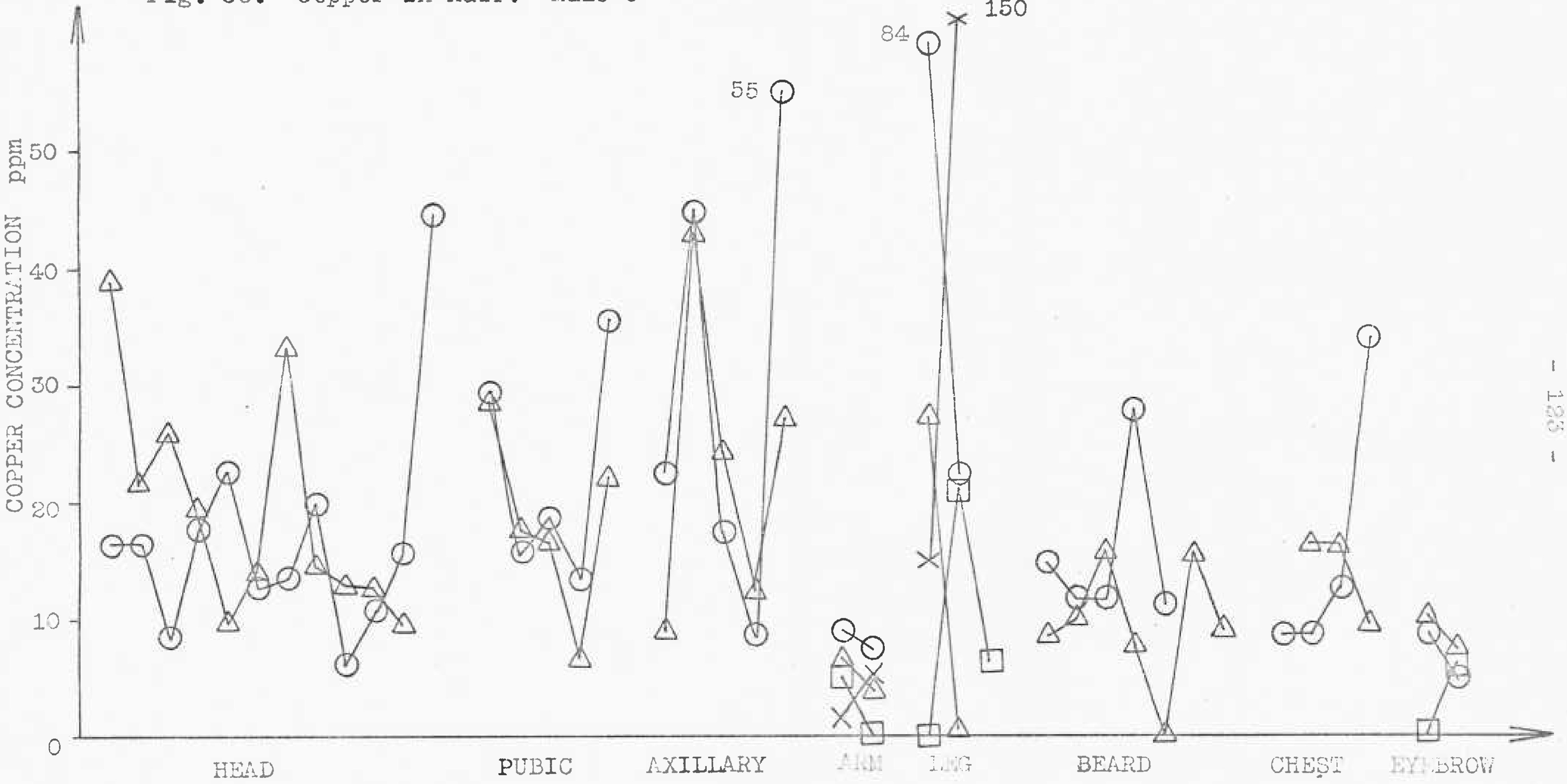
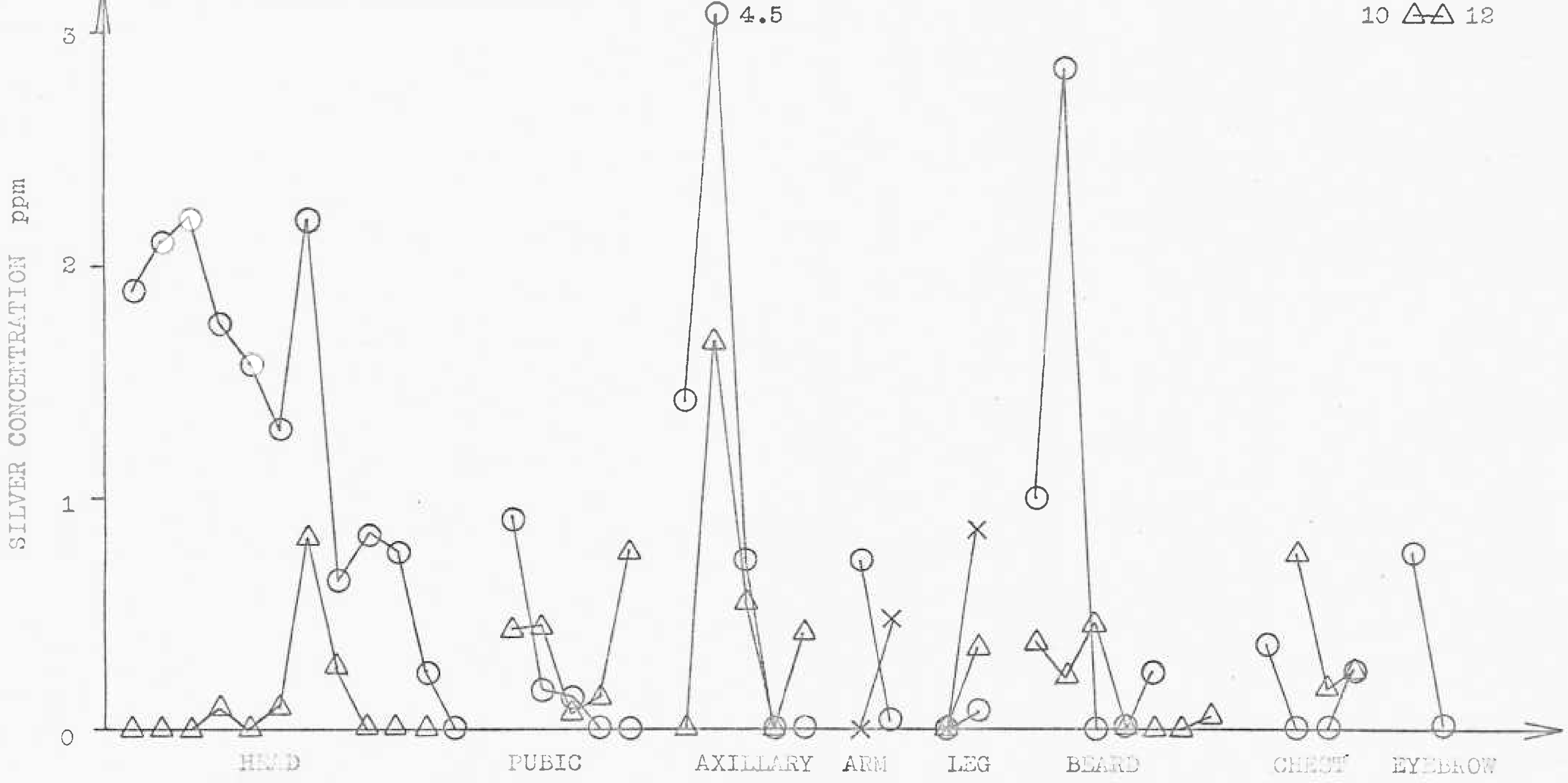


Fig. 57. Silver in hair. Male 5



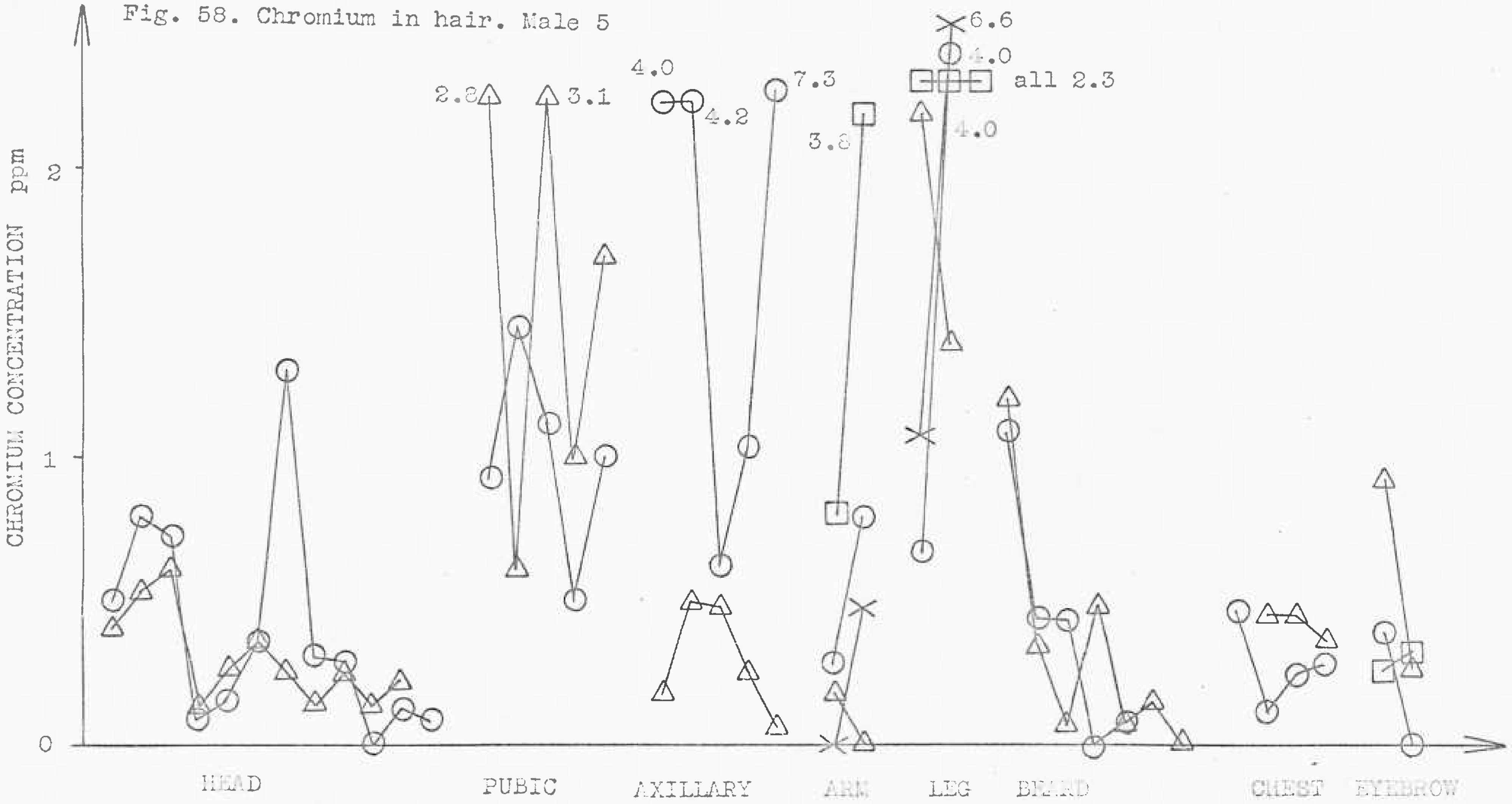
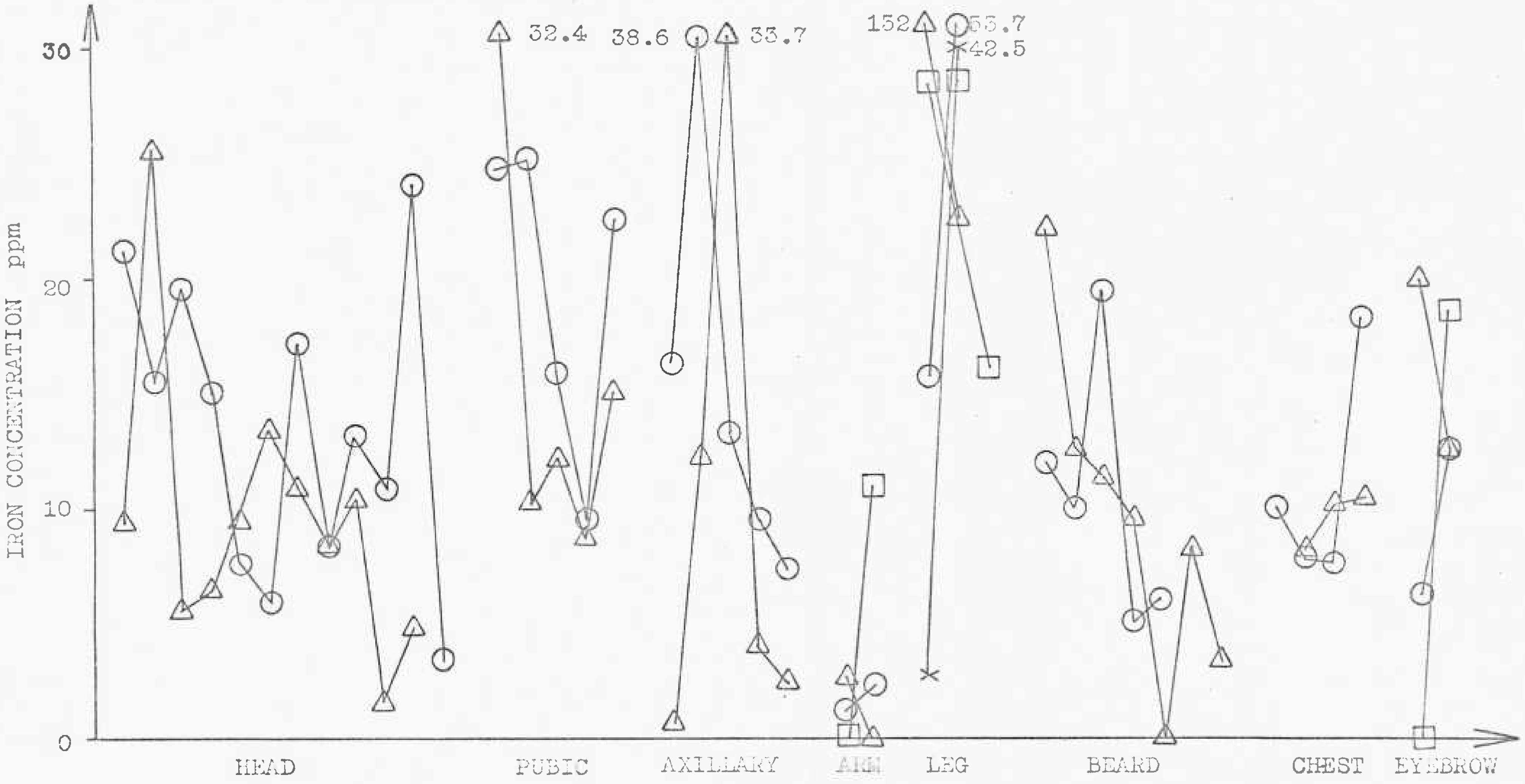


Fig. 58. Chromium in hair. Male 5

Fig. 59 Iron in hair. Male 5



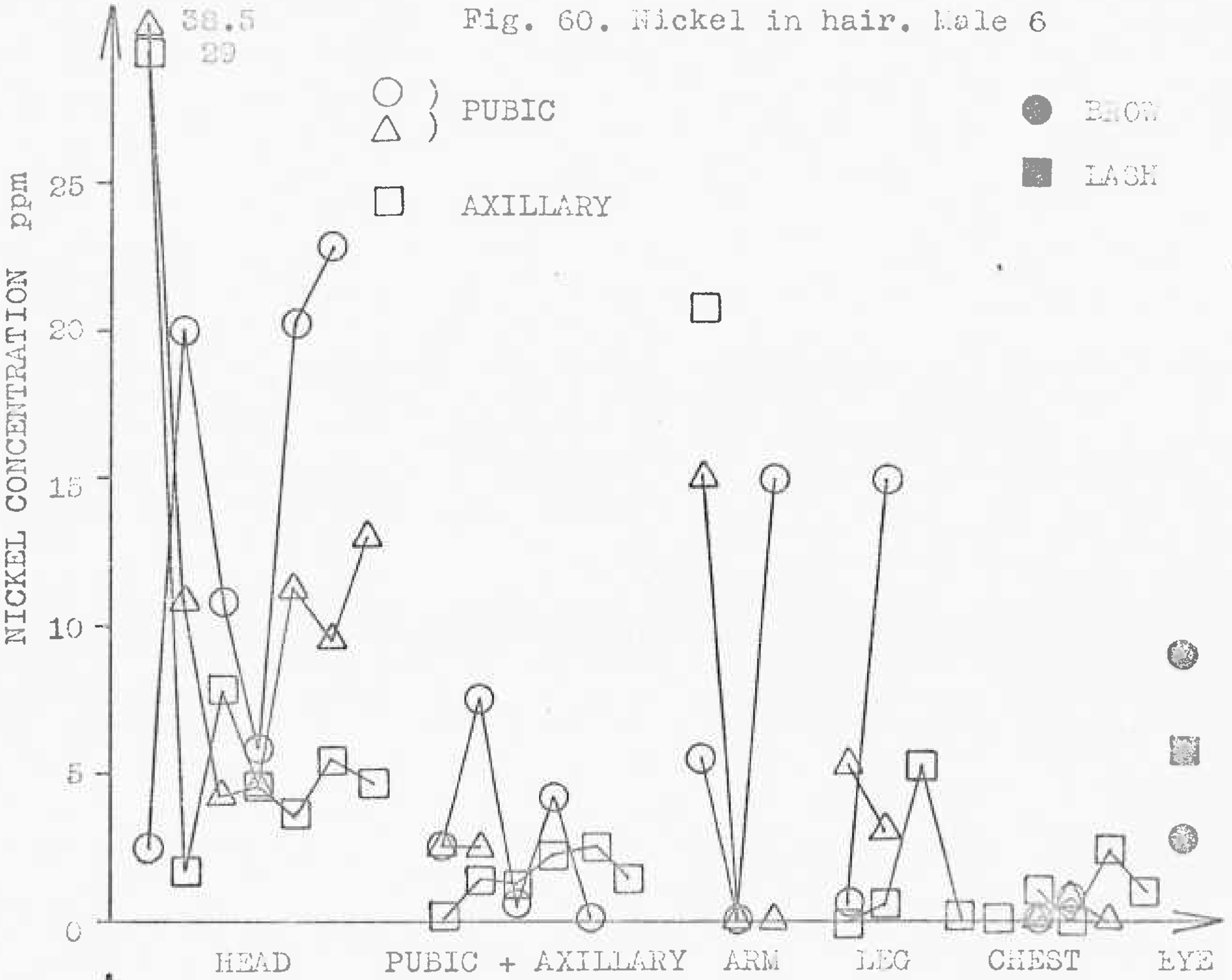


Fig. 60. Nickel in hair. Male 6

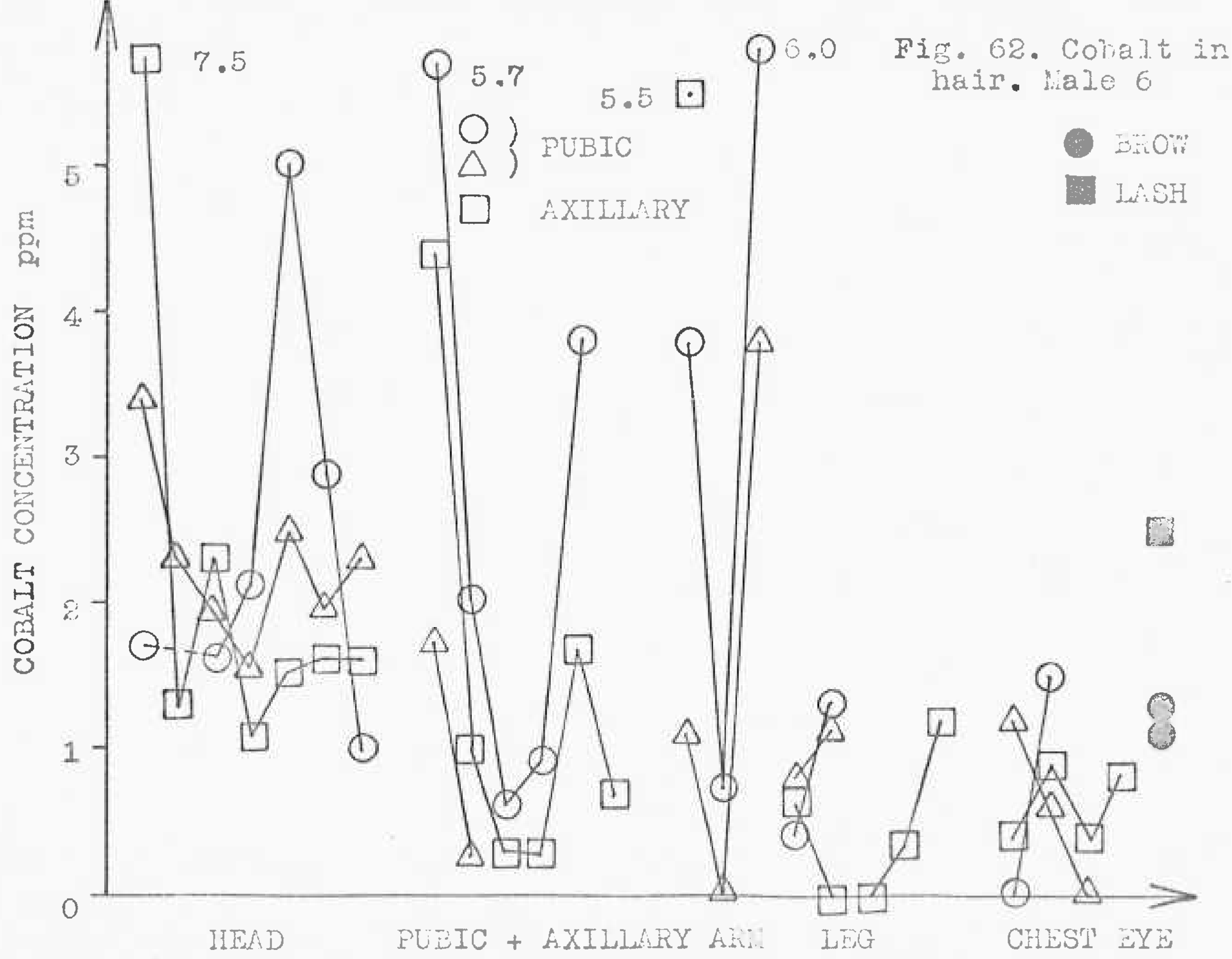


Fig. 62. Cobalt in hair. Male 6

Fig. 62. Manganese in hair. Male 6

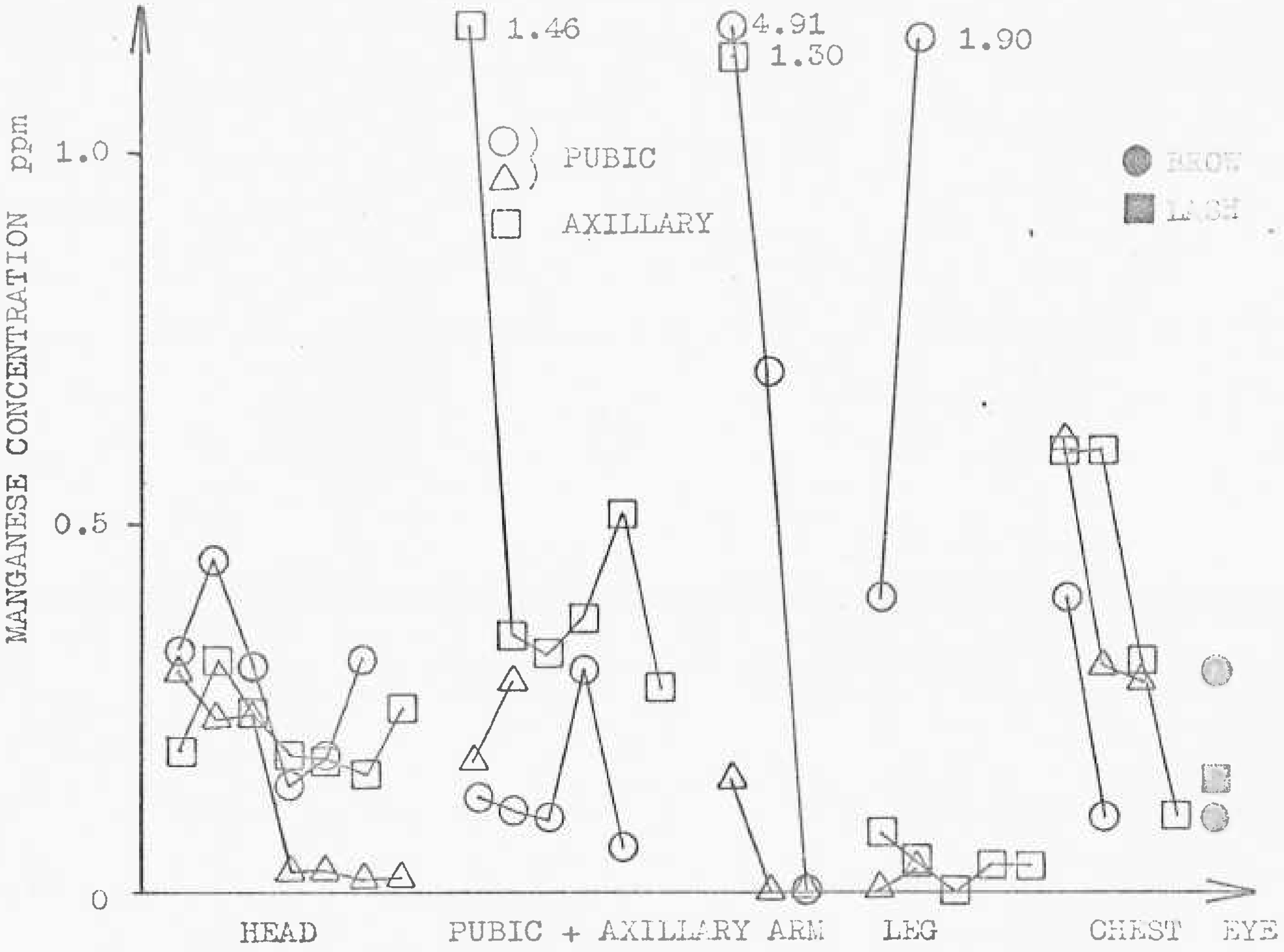


Fig. 63. Silicon in hair. Male 6

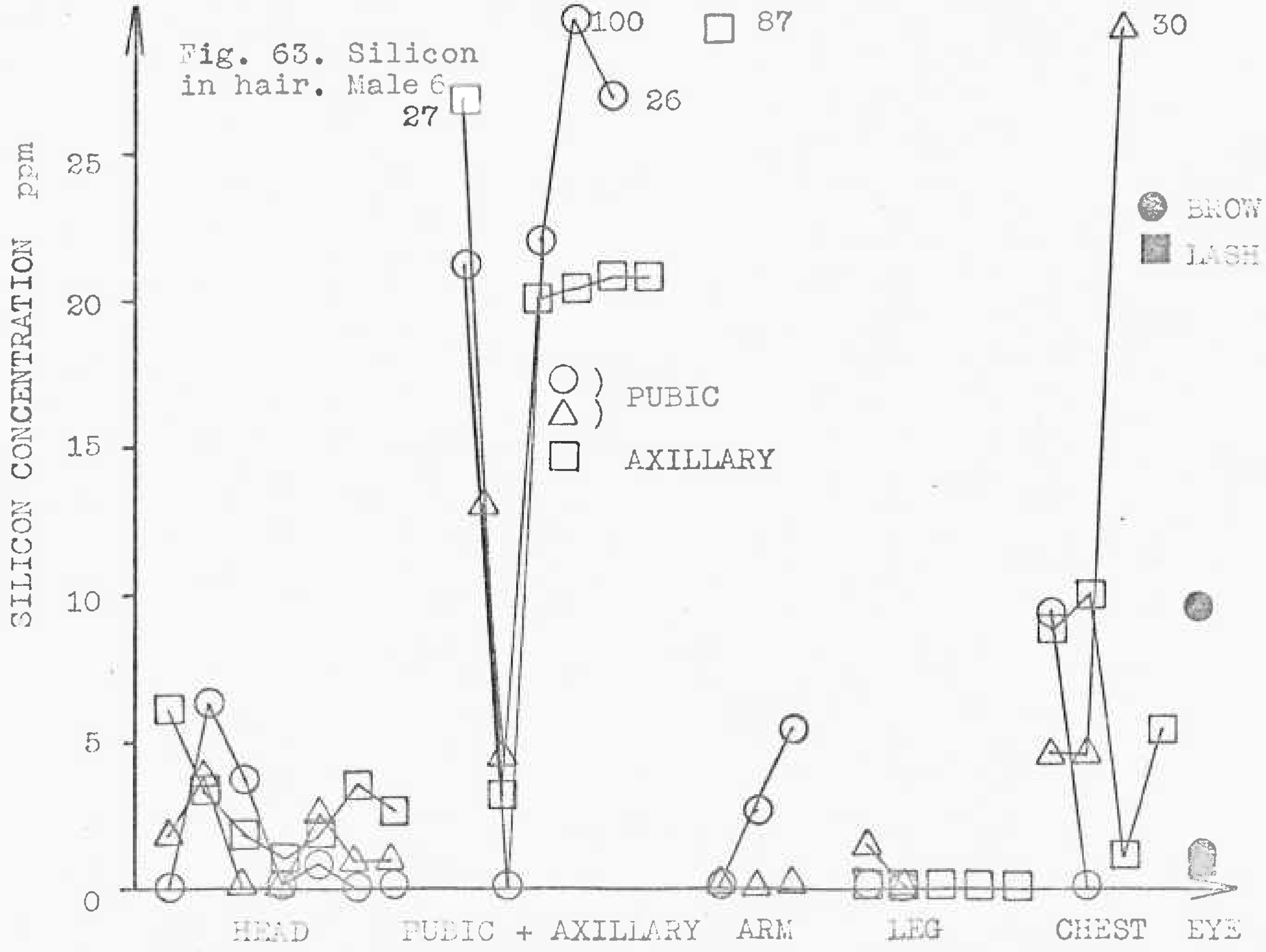


Fig. 64. Aluminium in hair. Male 6

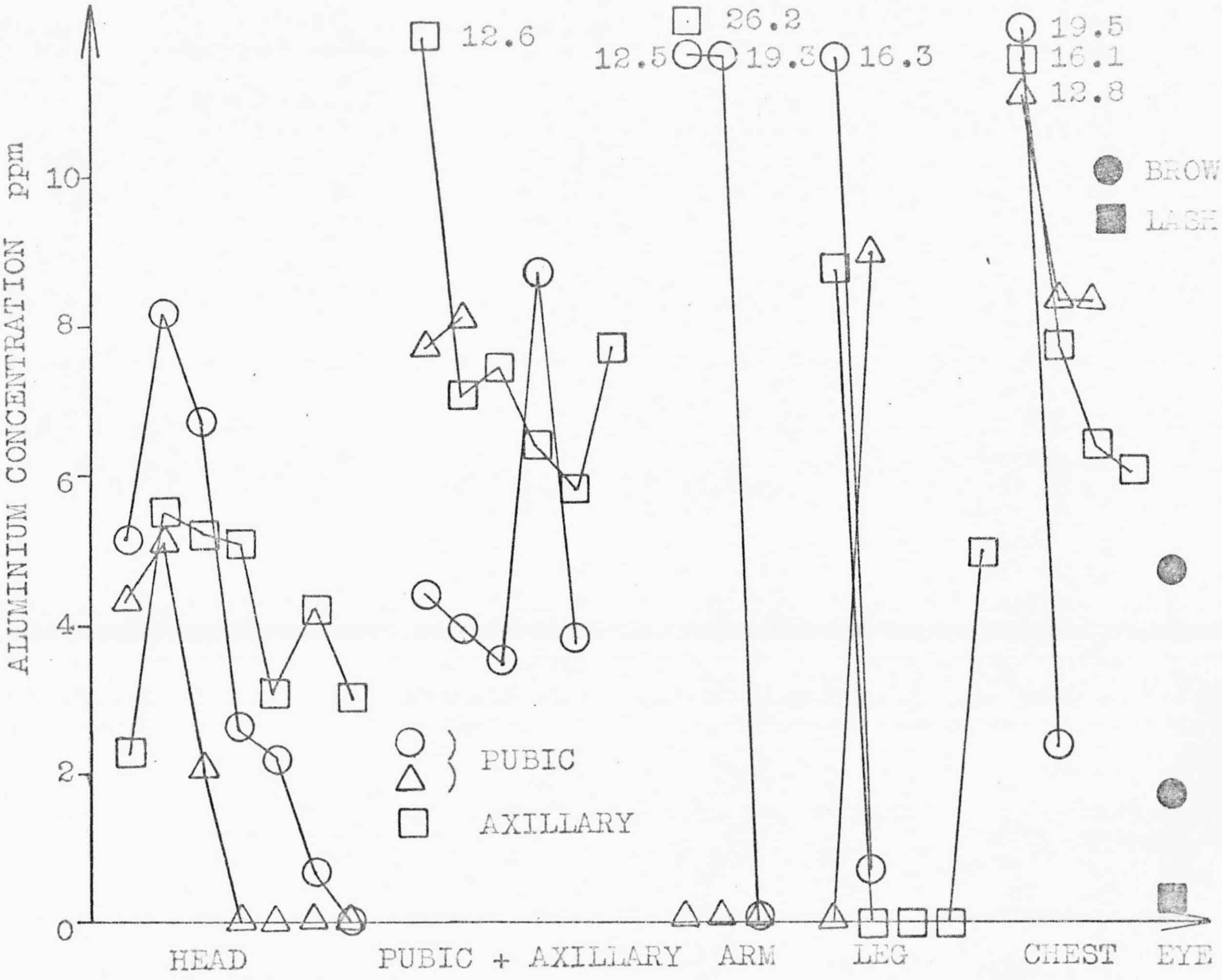
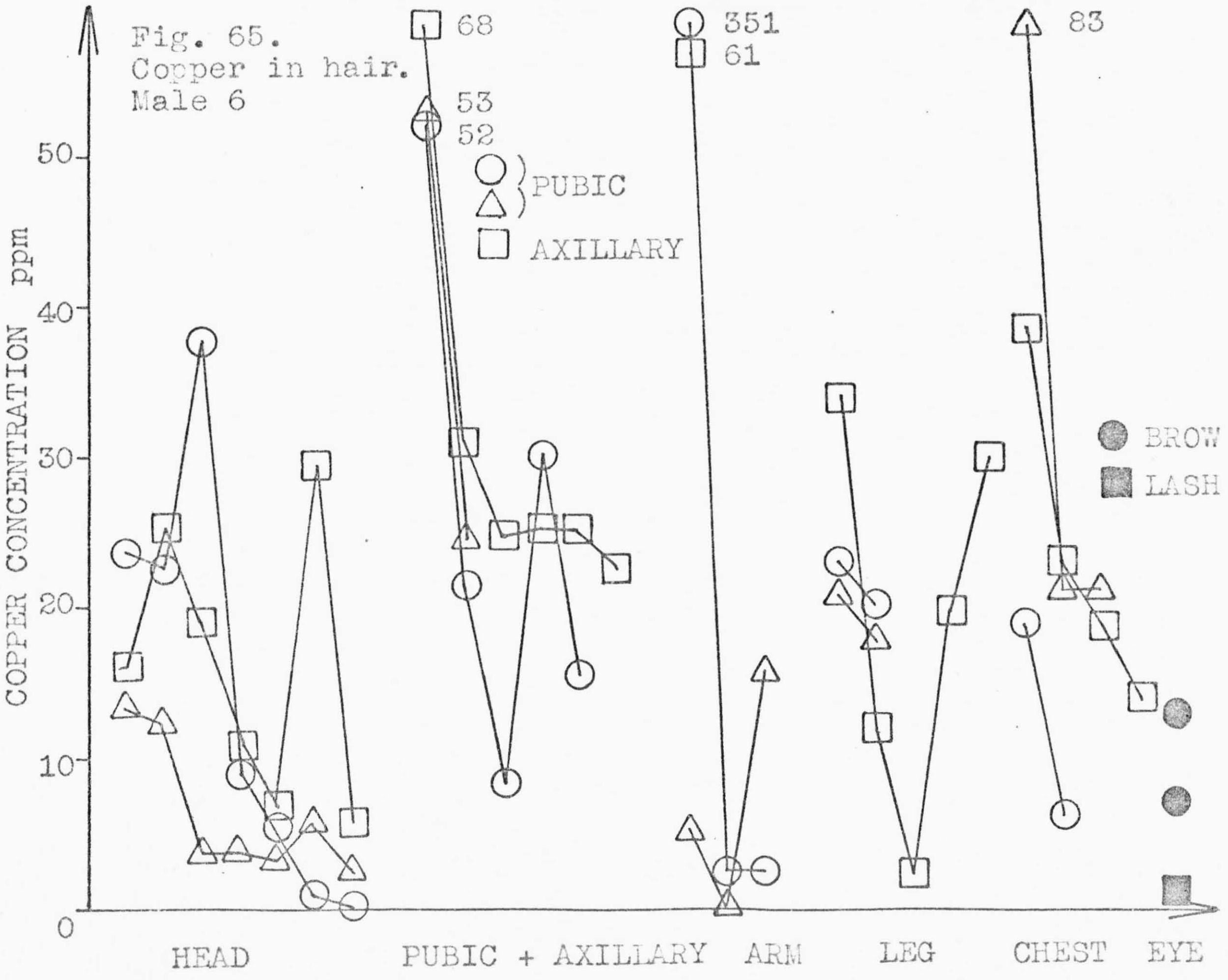


Fig. 65.
Copper in hair.
Male 6



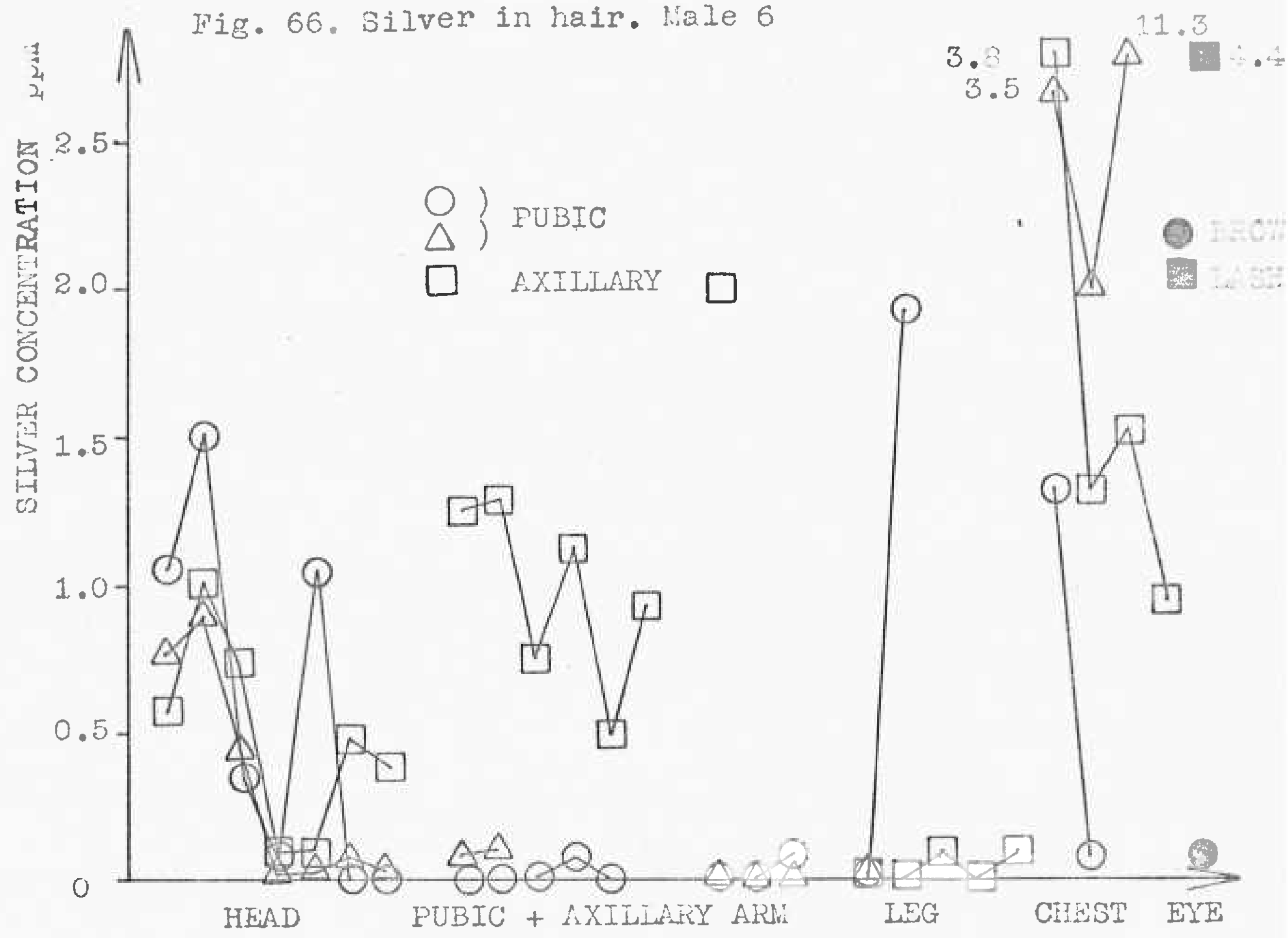


Fig. 66. Silver in hair. Male 6

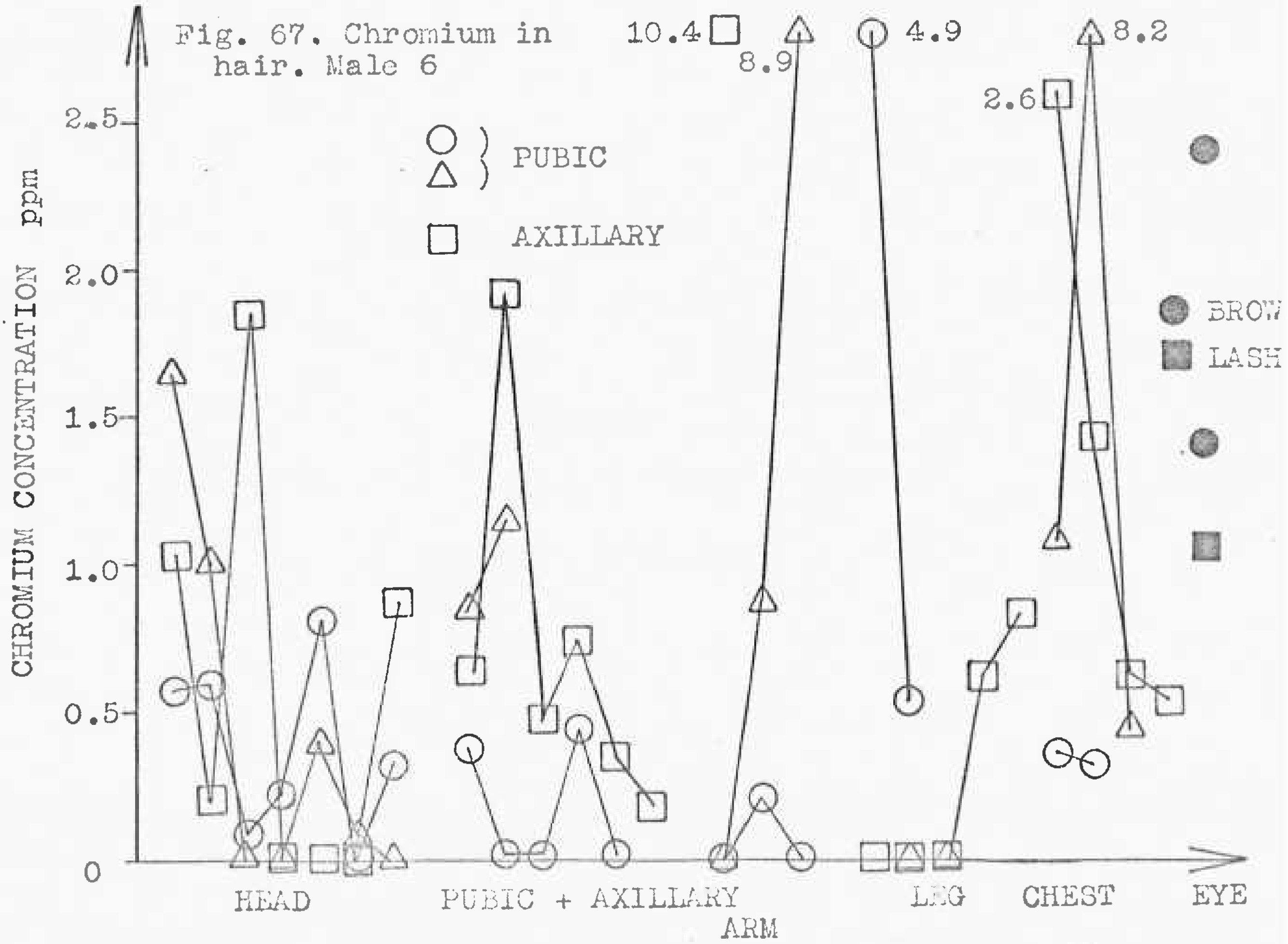


Fig. 67. Chromium in hair. Male 6

Fig. 68. Iron in hair. Male 6

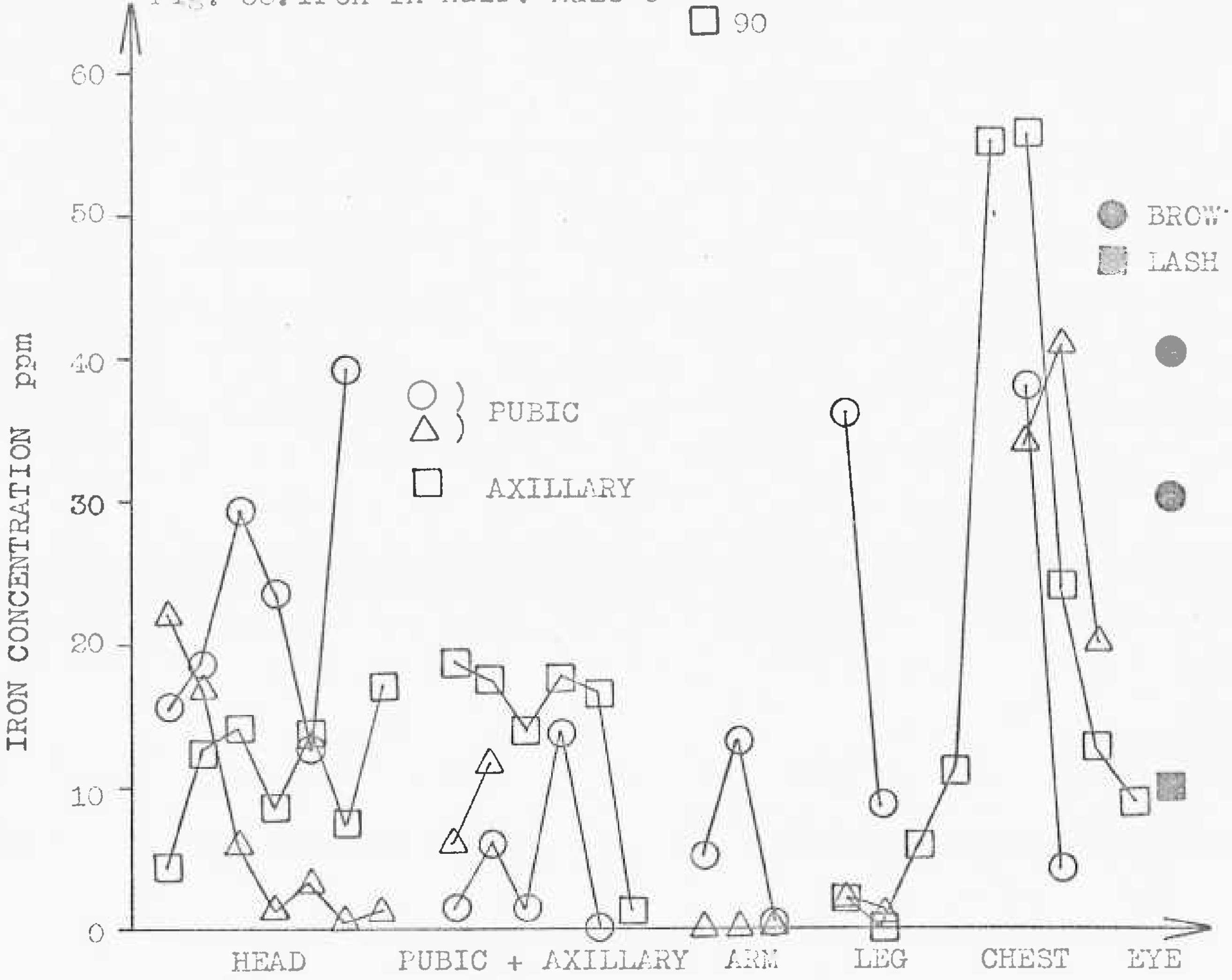


Fig. 69. Nickel in hair. Female 4

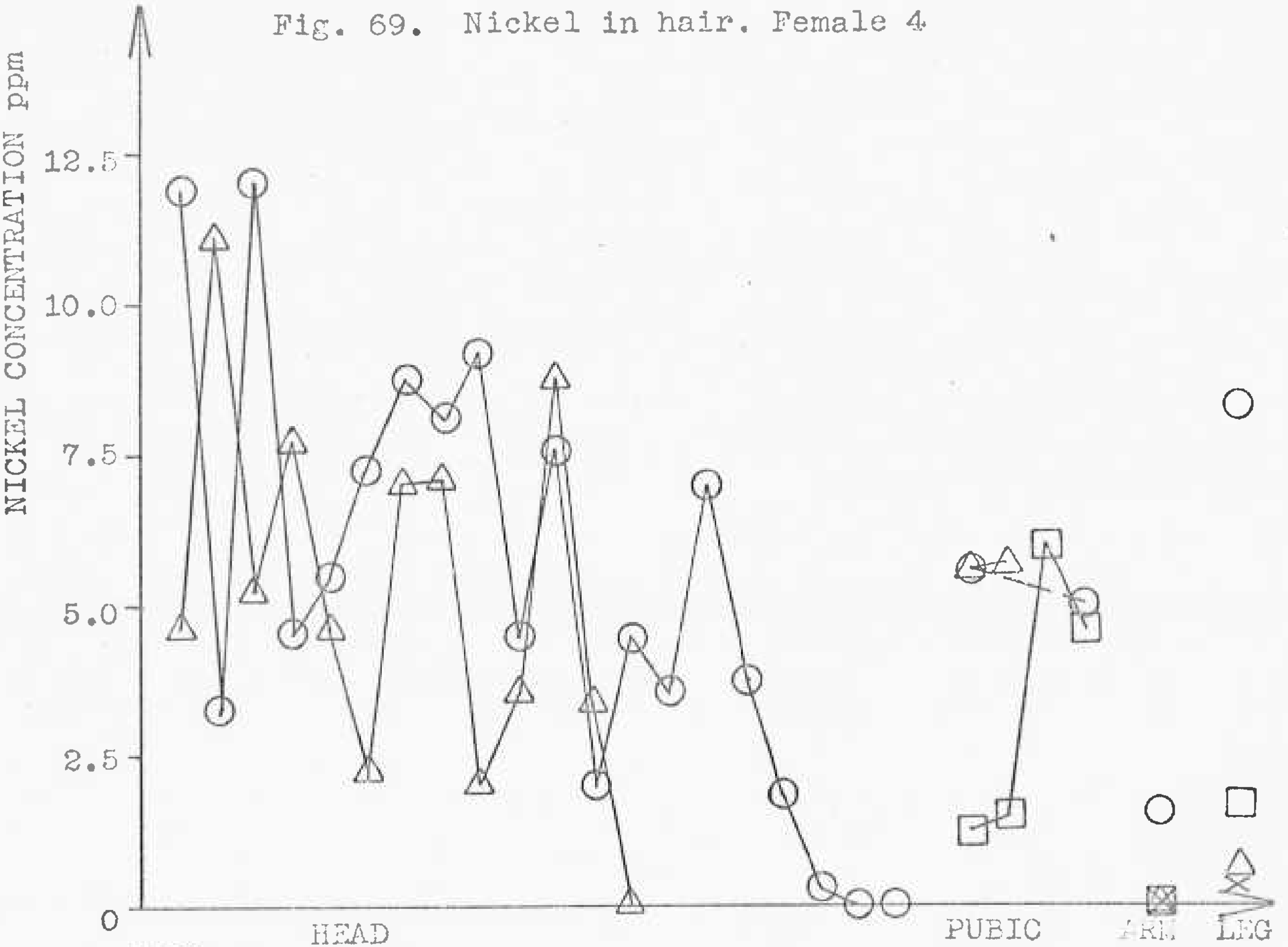


Fig. 70. Cobalt in hair. Female 4

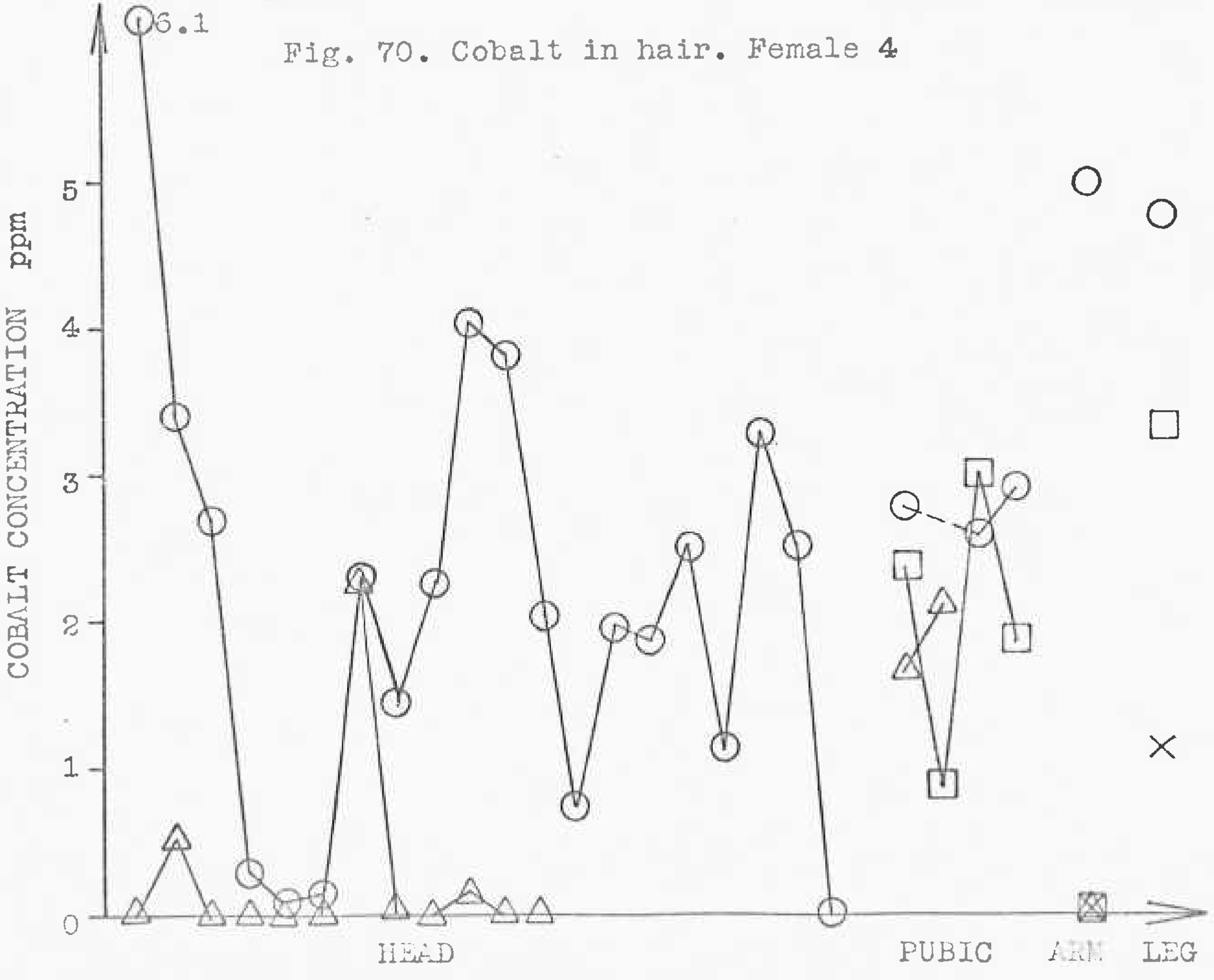


Fig. 71. Silicon in hair. Female 4

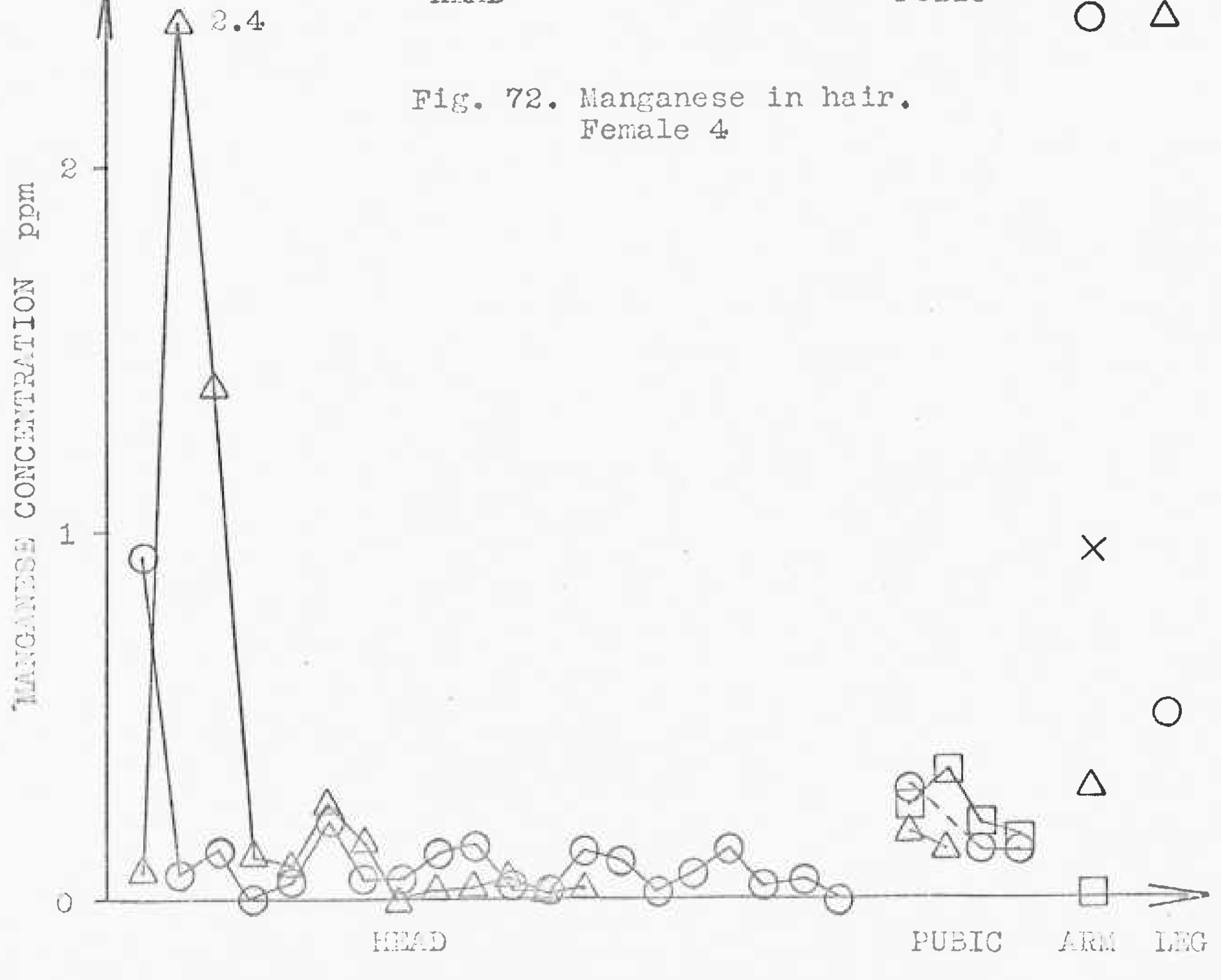


Fig. 72. Manganese in hair. Female 4

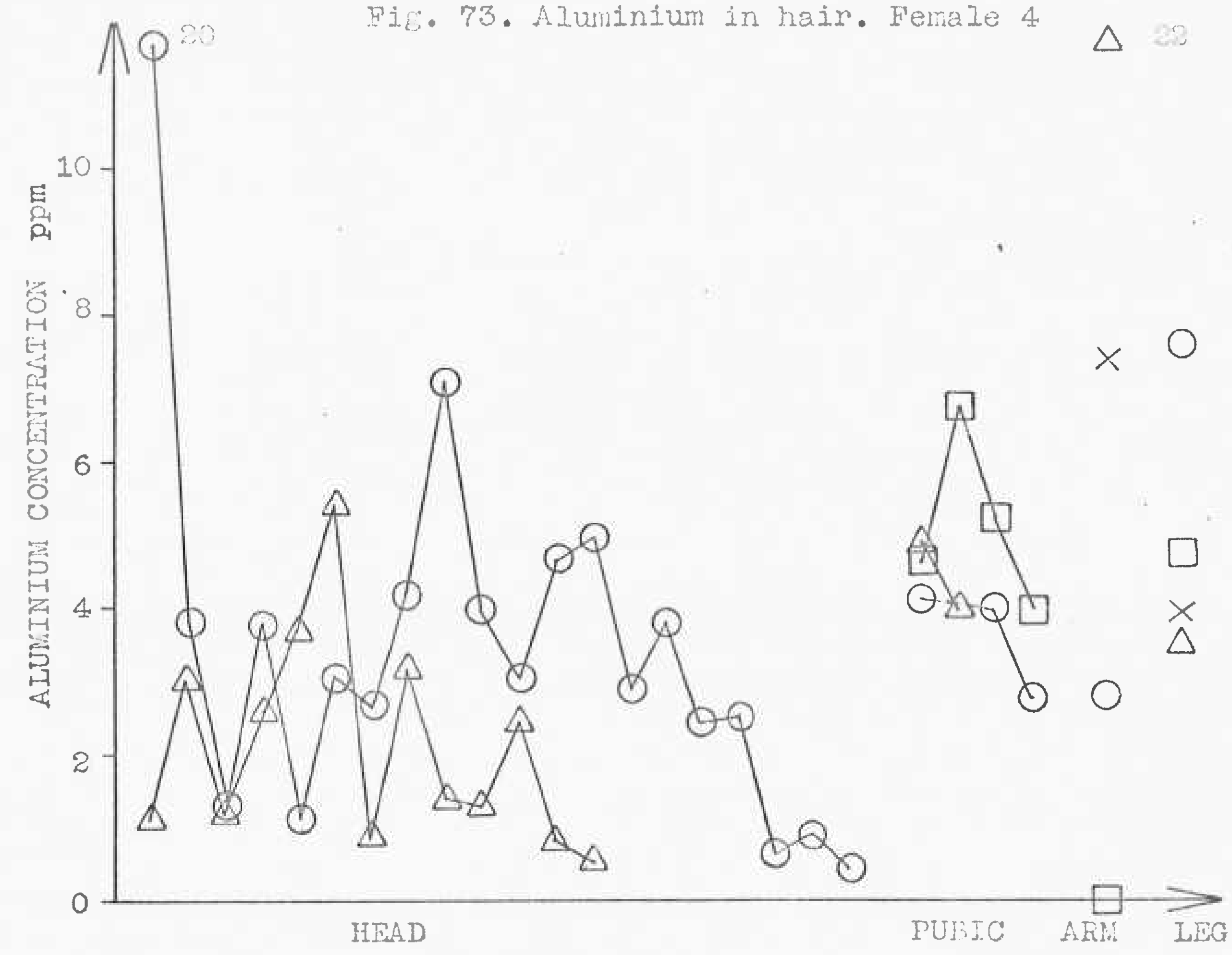


Fig. 73. Aluminium in hair. Female 4

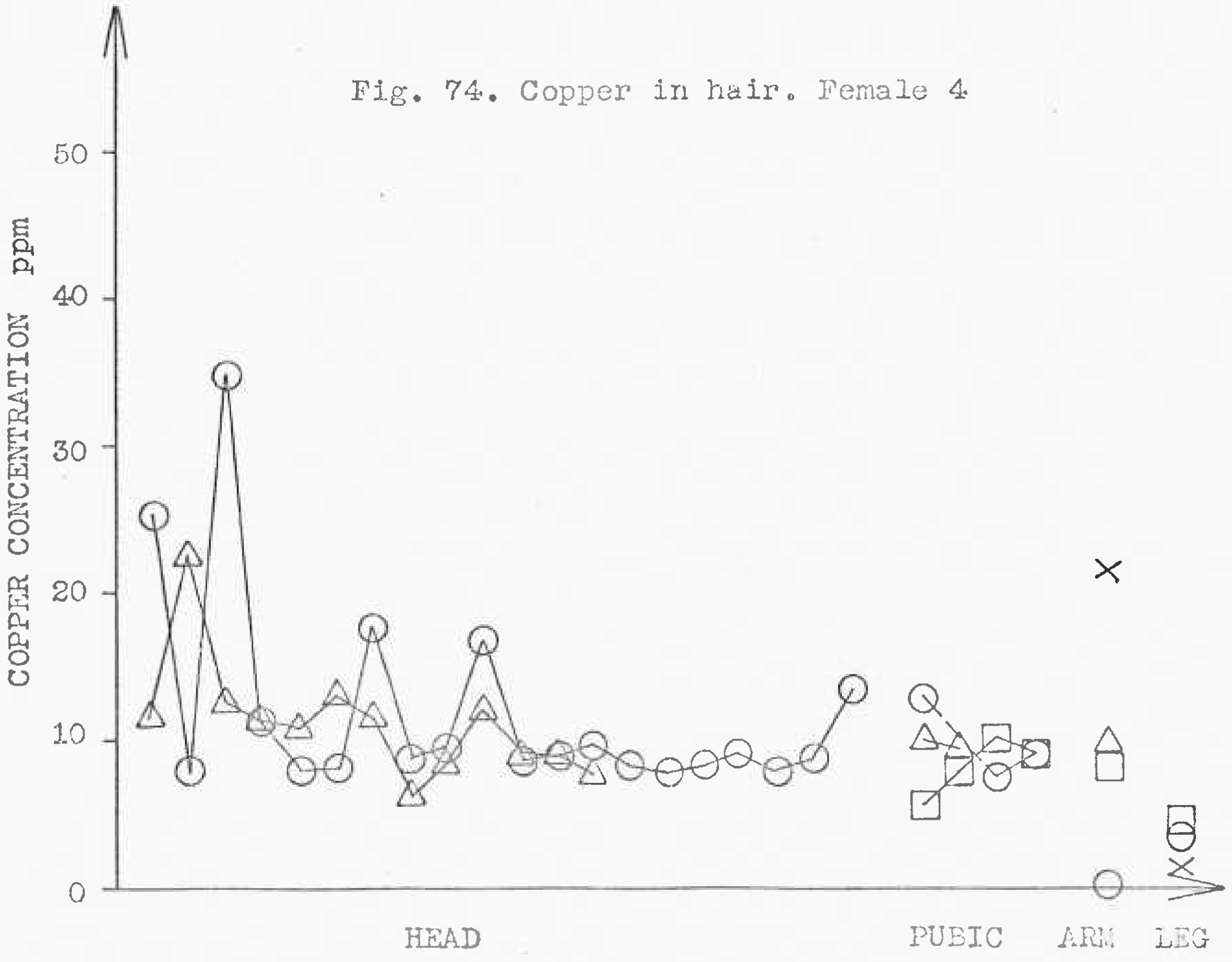


Fig. 74. Copper in hair. Female 4

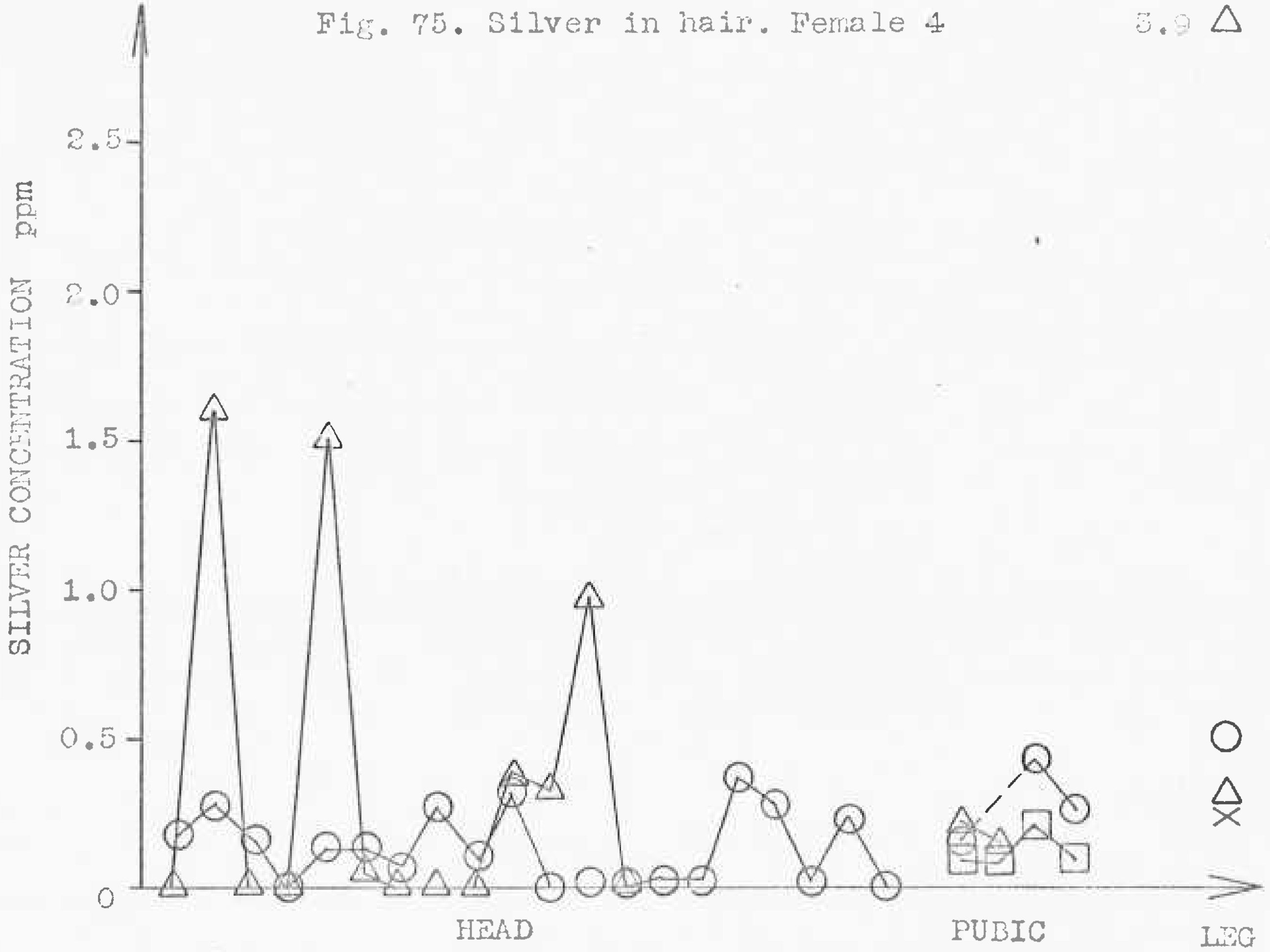


Fig. 75. Silver in hair. Female 4

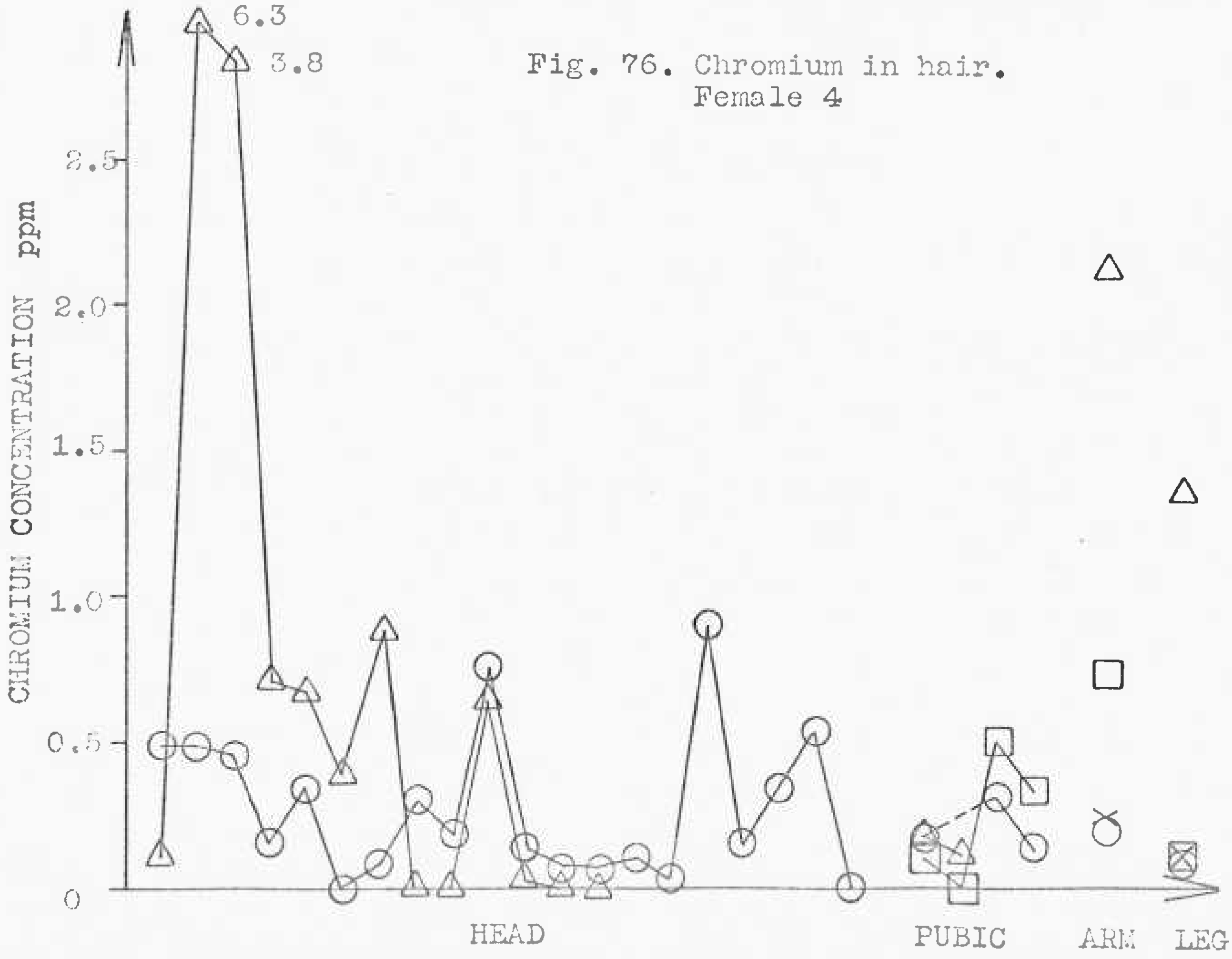
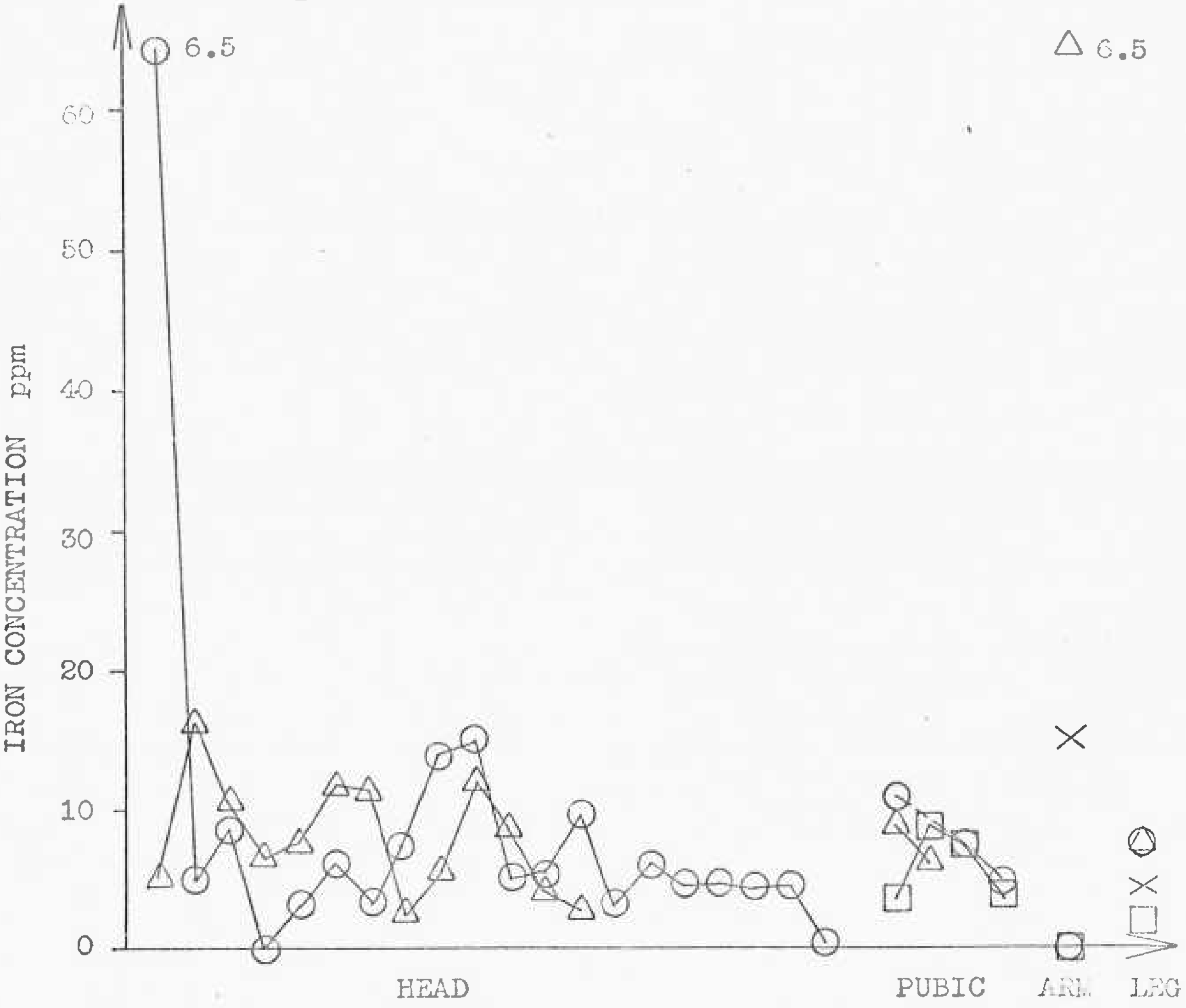


Fig. 76. Chromium in hair. Female 4

Fig. 77. Iron in hair. Female 4



5.2.4 Discussion

Nickel

Some head hairs show an erratic decrease to the root and there are some hairs from the same head that have similar nickel concentration profiles down the hair, but not all the hair samples show these features. In general there are no trends in concentration in the body hairs sampled and there are few correlations between profiles of hairs from the same area on an individual.

Cobalt

There is considerable variation in the patterns from different individuals. Male 4 shows a rapid rise near the root, Female 3 shows a rise in the middle section, Male 5 shows a rapid decrease from the tip. The two hairs for Male 4 show correlations in their profiles, and similar patterns between the head hairs of Female 3 are found. The body hairs show considerable variation from individual to individual with few noteworthy trends.

Silicon

Three of the individuals' head hairs examined (Male 5, Male 6 and Female 4) showed an increase towards the distal end. The hair from Female 2 and Male 4 showed an increase towards the root. Many of the body hairs had concentration of silicon at or below the detection limit; no notable trends were seen.

Manganese

This element showed that for most individuals there was no trend but sharp changes at root and distal ends occurred. For Male 4 one head hair had nearly 3x more manganese than the other. Pubic hairs for Male 5 and Female 4 showed a decrease towards the root; other body hairs showed no specific trends.

Aluminium

Four of the head hair samples show a decrease towards the root. Male 4 hairs are alone in increasing near the root, both hairs having similar profiles. Female 2 shows little overall trend. The body hairs in general show correlation i.e. profiles for hairs from the same area of the individual are similar. Of note is the dramatic increase in concentration in the aluminium concentration of the axillary hairs of Male 5 at the root.

Copper

The copper concentration in head hairs often shows a decrease towards the root. Female 3 shows reasonably well correlated patterns for the two hairs, the value falling to a plateau region followed by a sharp change at the root. Female 4 shows similar behaviour but the decrease from the distal end is more erratic. The head hairs of Male 4 and Female 2, however, show no overall trend. The pubic hairs of Male 5 appear to have correlated profiles as do the profiles of the axillary hairs. The other body hairs in the survey have no notable patterns.

Silver

The head hairs of Males 5 and 6 and Females 3 and 4 mainly show a decrease from tip to root. The head hairs of Female 2 and Male 4 show no trend along their lengths. Most of the body hairs studied in this survey had very low concentrations, many below the detection limit.

Chromium

No clear trend was observed in samples from Males 4 and 6 or Female 2. The chromium concentration in the hair of Female 3 was mainly below the detection limit. The head hair of Female 4 shows some evidence of a decrease towards the root. Male 5 shows an erratic decrease towards the root, both hairs (apart from one sample) showing a similar pattern. The body hairs show no notable trends.

Iron

Head hairs from Male 4 show an increase towards the root. Whereas head hairs from Females 2 and 3 and Male 5 show a decrease towards the root, Male 6 and Female 4 show no overall trend. The chest hairs of Males 5 and 6 show a decrease towards the root. Other body hairs have in general no notable features.

General.

Thus for the elements considered all but manganese show some evidence of a decrease towards the root. This, however, is not found in all samples for any element and considerable variation between hairs from the same head for the same element can be found. The body hairs in general have similar trace metal concentrations to head hairs but show more variation between samples from the same individuals than head hairs.

There is a number of cases where similar profiles are found for the same element for different hairs from a common area on an individual; these occur for all the elements investigated. This suggests that the dips, peaks and trends in concentration are not random behaviour but are a reflection of true changes in concentration that occur in the hairs. So far only correlations of position and concentration for single elements have been considered. When interelement correlations are investigated even in this qualitative way the situation becomes extremely complicated. The head hair of Male 4 shows no real evidence of decreasing towards the root for any element, but Co, Si, Al and Fe all show a rapid rise near the root; no other individual investigated shows this pattern. Other inter-element correlations can be seen but appear to be restricted to the individual rather than as general phenomena.

The simultaneous multi-element analysis of small sections of hair provides a considerable amount of information and the results of this survey would indicate that the character of the information is essentially individual. This would be of great help in identifying individuals for cases of comparison in forensic work. The nature of the information is however very complex and as has been shown in the introduction to this chapter beyond the existing statistical methods of comparing hairs (102-104). It is not within the area of this research to make a detailed investigation of suitable statistical methods for comparing the data obtained from multi-element hair analysis.

5.2.5 Discrimination Test

A test was carried out for HOCRE to discover whether the simultaneous multi-element analysis of hair was capable of discriminating between head hairs. Ten control hairs were supplied together with ten sample hairs each in a separate marked envelope. The hair samples were all from male heads and were pulled out to ensure that they were all in the same growth phase. The males were aged 22-58 and had approximately the same coloured hair (mousey). The hairs were washed separately for two hours using diethyl ether in a Soxhlet extraction apparatus as throughout this study. The majority of the hairs were of similar length and seven consecutive portions were cut from the ends of each and separately weighed and then analysed as previously described.

The results were sent to HOCRE where they were analysed using Parker's method (102-104) and they were also analysed by the author (who was ignorant, at the time, of the identity of the suspect hair in the batch of unknowns) by comparing the mean concentration of each element for each sample hair with that of the mean for all the controls. The answers obtained were not encouraging (see Table 24). The true identities of the control hairs in the batch of unknowns were numbers three and seven.

It has been shown in 5.1 that Parker's method and that of averaging are neither adequate nor accurate for the comparison of the data obtained here. Thus before any decisions, concerning the ability of trace metal analysis to discriminate between hairs of different heads, are taken, methods of statistical analysis that are not demonstrably inaccurate must be sought. To this end there seem to be two areas worthy of exploration. These are comparison of the profiles of the trace metals down the length and investigation of inter-element correlation coefficients. The discrimination test carried out involved the analysis of ten controls and ten crime samples for nine elements. The construction and inspection of 180 trace-metal profiles seemed a considerable task and so a preliminary study of the trace-element correlations was undertaken. It was decided to concentrate on those elements that showed the least variation; these were Mn, Cu, Co and Fe. Correlation plots of Cu vs. Fe, Mn vs. Fe, Cu vs. Mn, Mn vs. Co, Fe vs. Co and Cu vs. Co were constructed for unknowns and controls and are shown in Figs. 78-83. It can be seen from these plots that certain samples have definite correlations, e.g. Fig. 78, S 10 shows a tight group of points, whereas S 8 points nearly all have a constant copper concentration ca. 15 ppm. The control

Table 24. Discrimination Test

	Averaging Test	HOCRE Test	
	Rank	Rank (a)	Rank (b)
S1	4	3	6
S2	6	8	4
S3	3	7	7
S4	9	6	8
S5	10	10	10
S6	8	4	3
S7	6	2	2
S8	2	9	9
S9	4	4	5
S10	1	1	1

(a) Parker test as applied to average concentrations for whole hair i.e. all the data from the segments of one hair averaged.

(b) Parker test applied to a single segment nearest to the scalp

Fig. 78. Correlation between Copper and Iron

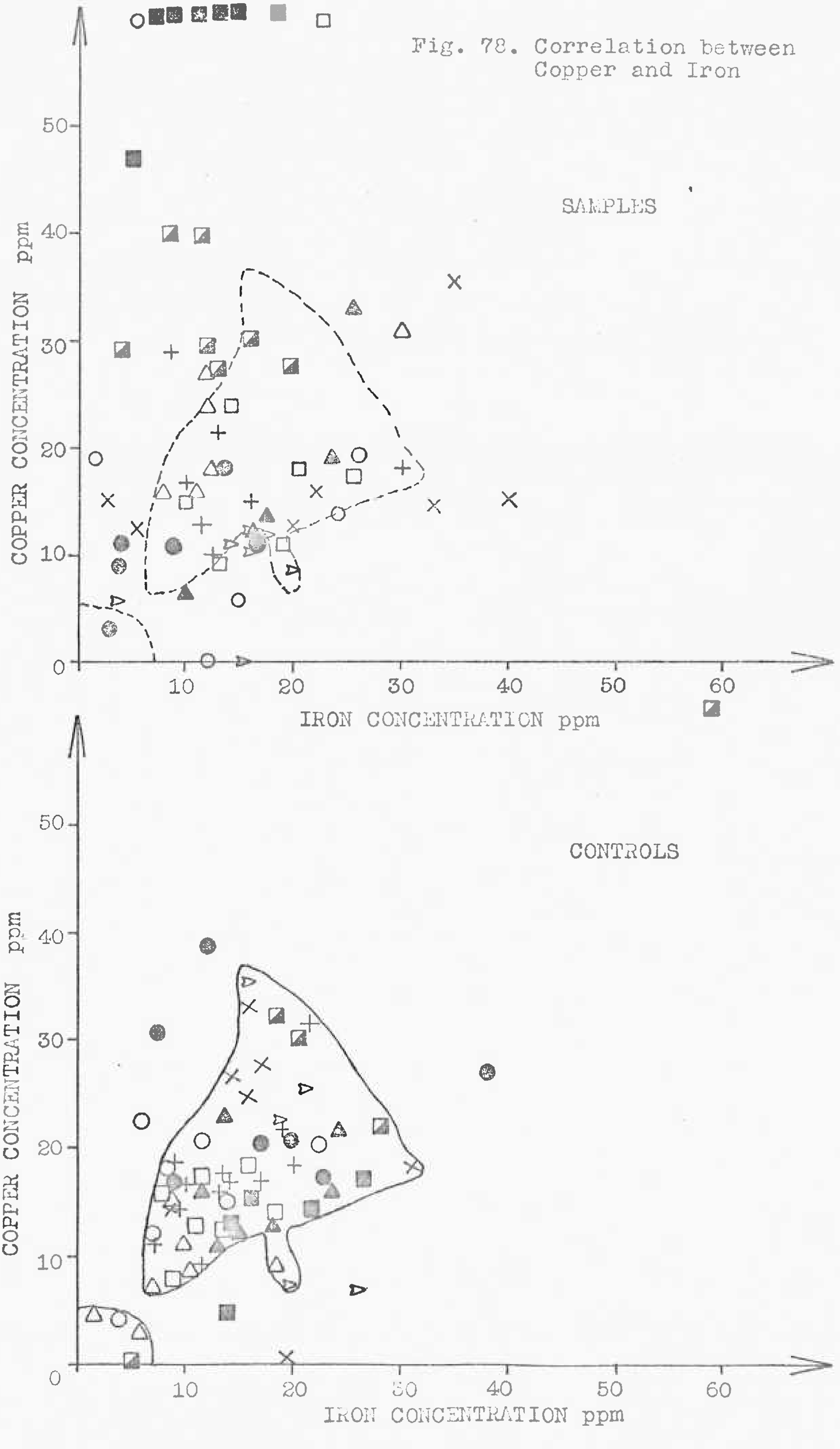


Fig. 79. Correlation between Copper and Manganese

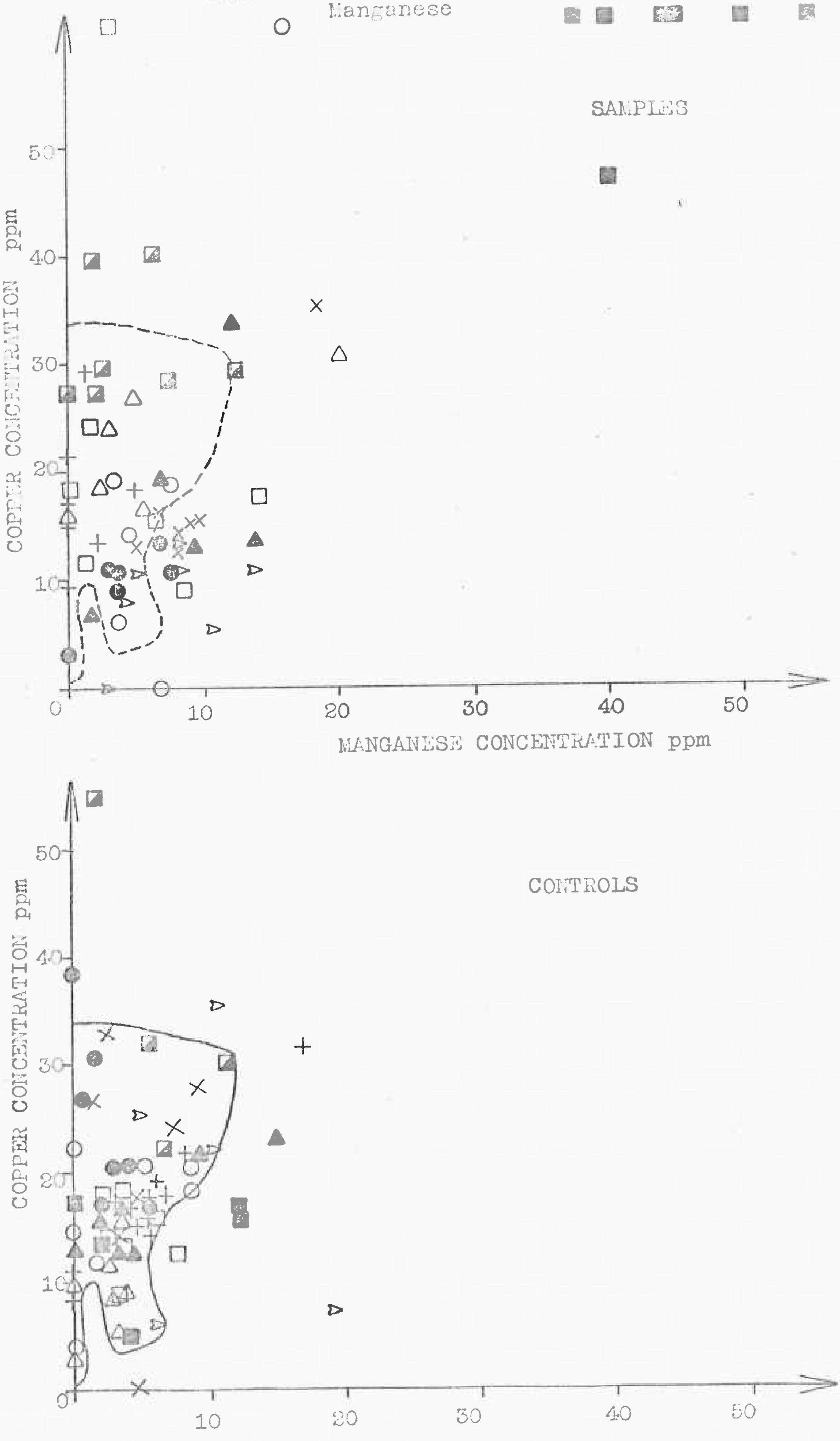


Fig. 80. Correlation between Manganese and Iron

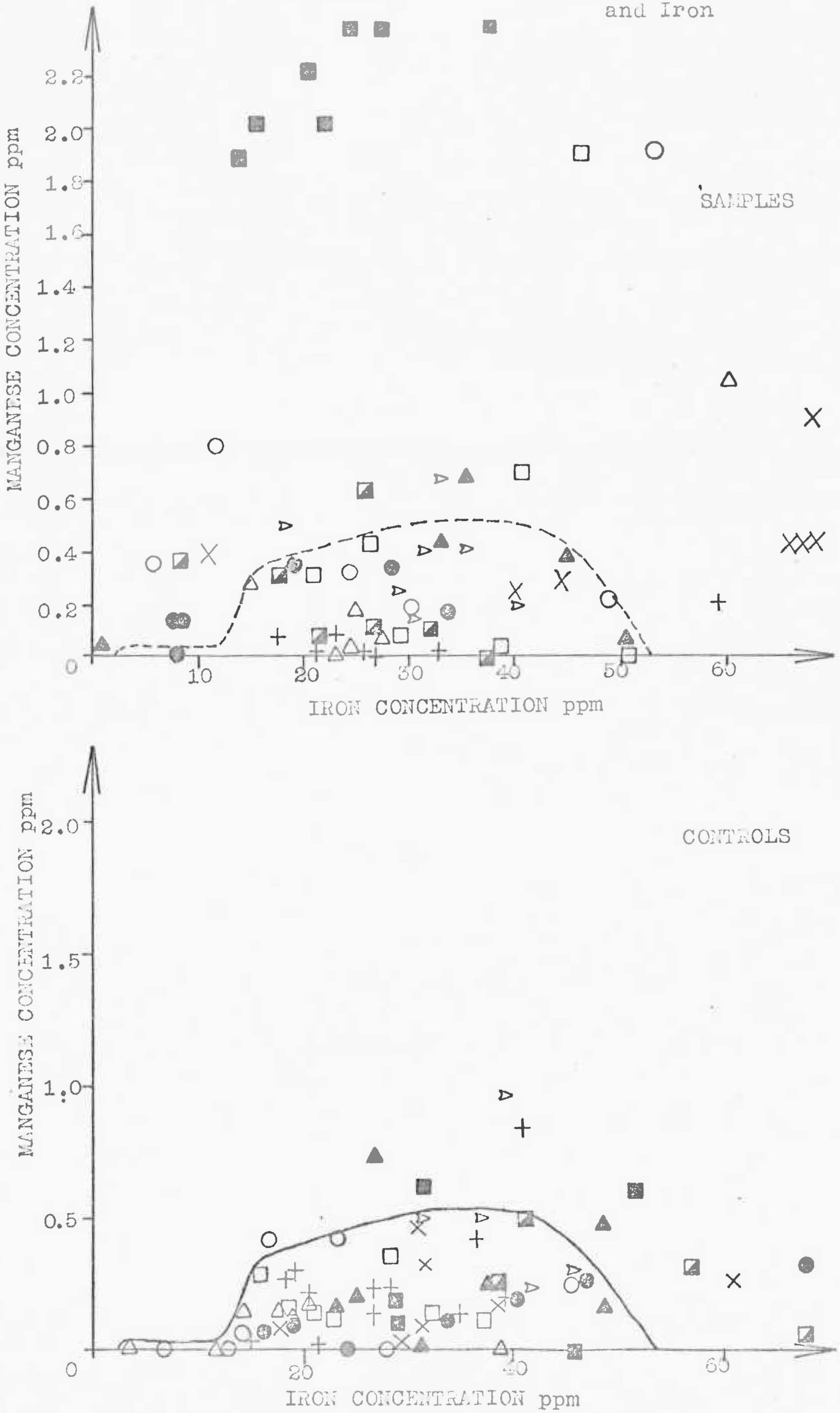


Fig. 81. Correlation between Manganese and Cobalt

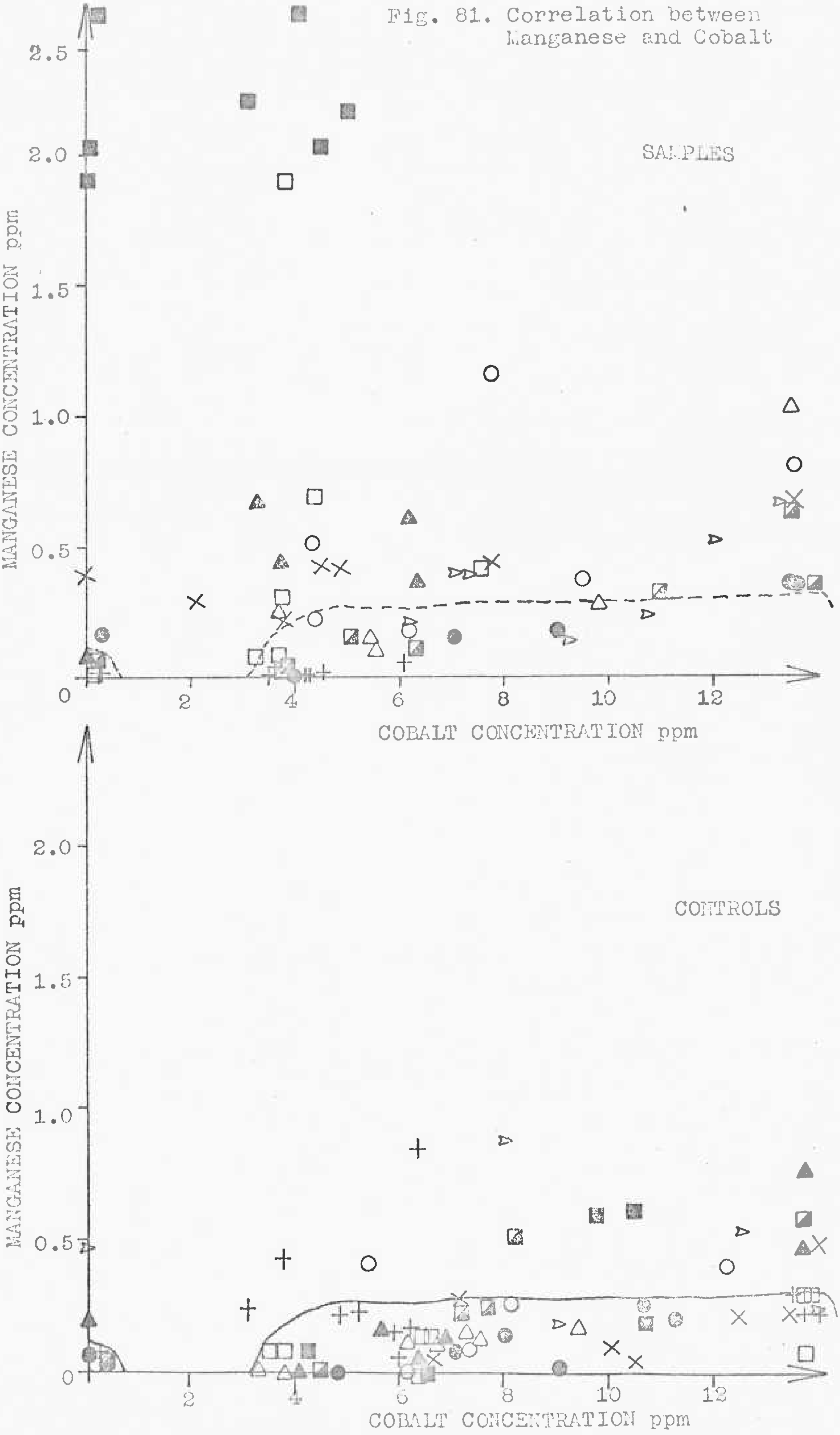


Fig. 82. Correlation between Iron and Cobalt

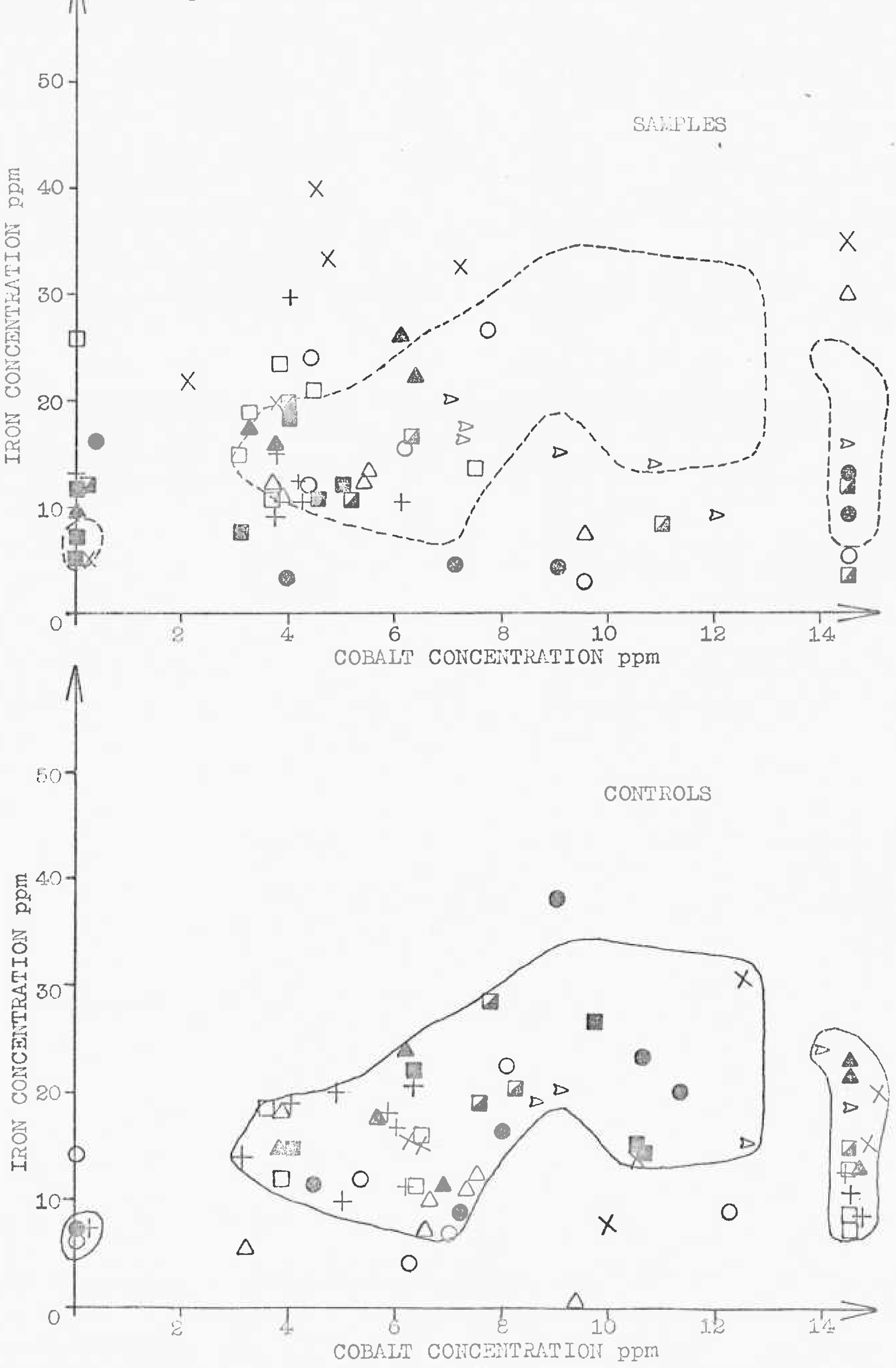
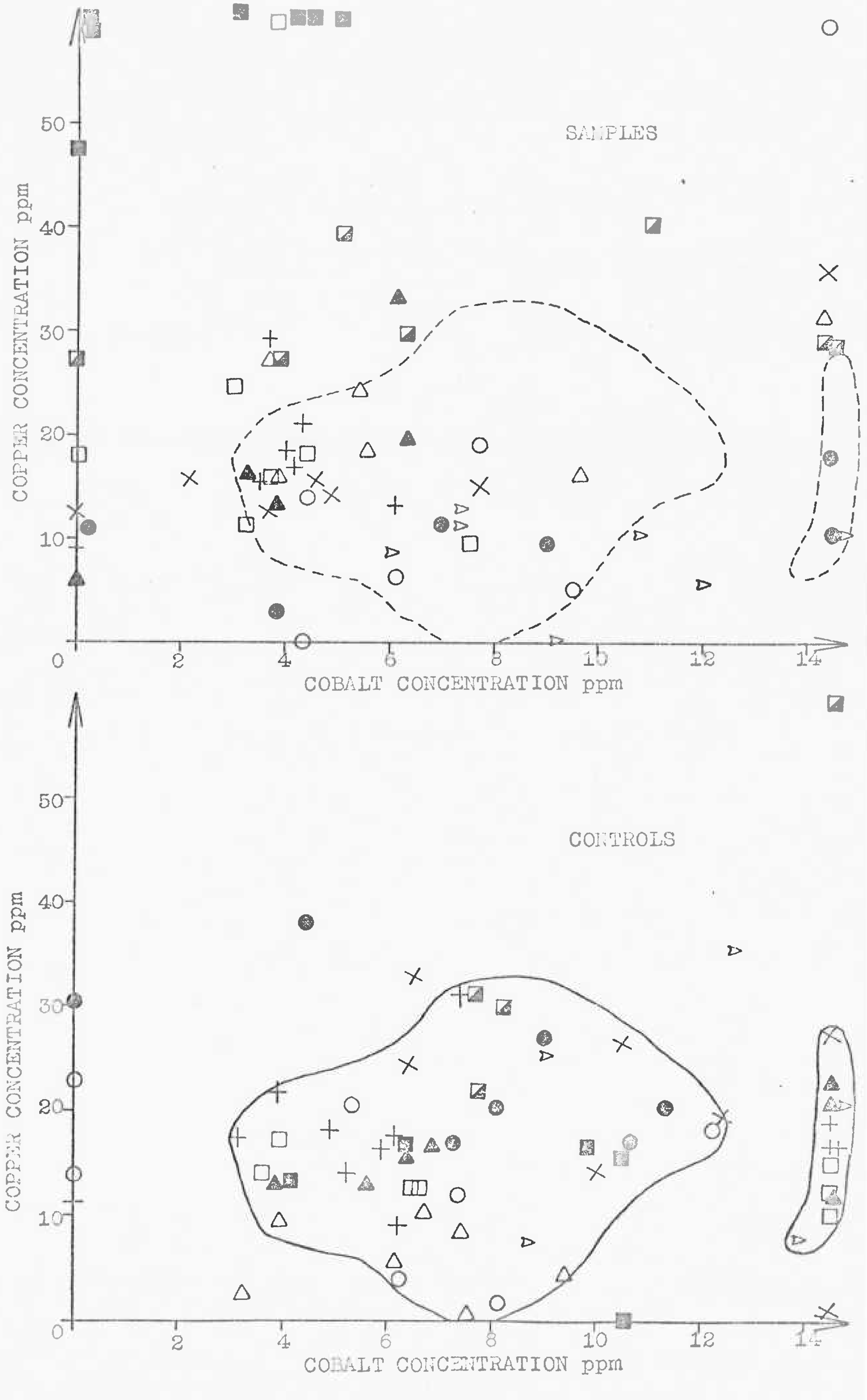


Fig. 83. Correlation between Copper and Cobalt



samples however fall in a reasonably well-defined area. This area, chosen so that it covered most of the points of the controls whilst maintaining maximum discrimination against all the samples, was superimposed on the samples (dotted line). The number of points for each sample falling within this area and those outside it were counted separately. This was repeated for the controls in order to find the spread in the percentage of controls that fall in the enclosed areas. The total results are summarised in Tables 25-26. It can be seen from these that on average 84% of the controls fall within the areas superimposed. Samples 7 and 3 fall within the range of the controls whilst all the other samples lie well away. Now if the distribution of the controls between the area and the outside is random i.e. Gaussian statistics can be applied, then the standard deviation calculated from the controls is 8.8. Thus Sample 7 lies within 0.14 standard deviations of the mean, Sample 3 within 1.4 standard deviations, but Sample 4, the next nearest, within 3.2 standard deviations and thus has only 72 chances in 100,000 of being similar to the controls. The other samples have even less chance of being similar to the controls. Thus some measure of discrimination has been achieved albeit of an empirical nature. This method can be further extended since only 6 correlations have been examined and for nine elements there are 36 possible correlations. Furthermore this method should be readily adaptable to analysis by computer and the area superimposed on the suspect samples determined by the minimum area necessary to cover say 80% of the control points. Further work will be necessary to establish the optimum percentage of control (area) that would give maximum discrimination. The use of a computer would prevent any subjective bias which will otherwise be introduced by an operator. A similar procedure has been successfully used, for the discrimination of white bond papers, by Drewer and Kowalski (143). They also used a "pragmatic segmentation of the individual concentration ranges".

Table 25. Number of Sample points falling inside and outside area.

	Cu/Fe		Cu/Mn		Mn/Fe		Mn/Co		Fe/Co		Cu/Co		Total		%
Symbol	IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN
○ S1	2	4	4	2	3	3	2	4	3	3	4	2	18	18	50
□ S2	5	2	4	3	4	3	2	5	2	5	3	4	20	22	48
△ S3	4	2	5	1	5	1	4	2	4	2	4	2	26	10	72
◎ S4	3	3	4	2	4	2	3	3	2	4	4	2	20	16	56
▨ S5	0	7	0	7	0	7	0	7	5	2	0	7	5	37	12
▲ S6	2	3	1	4	1	4	1	4	2	3	3	2	10	20	37
+ S7	6	1	7	0	6	1	7	0	4	3	5	2	35	7	83
X S8	2	5	2	5	2	5	0	7	1	6	4	3	11	31	29
◩ S9	2	5	4	3	5	2	4	3	4	3	0	7	19	23	45
▷ S10	3	4	2	5	3	4	3	4	5	2	4	3	20	22	48

Table 26. Number of Control points falling inside and outside area.

	Cu/Fe		Cu/Mn		Mn/Fe		Mn/Co		Fe/Co		Cu/Co		Total		%
Symbol	IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN
○ C1	6	1	7	0	6	1	5	2	4	3	5	2	33	9	79
□ C2	7	0	6	1	7	0	7	0	7	0	7	0	41	1	98
△ C3	7	0	7	0	7	0	7	0	5	2	6	1	39	3	93
● C4	4	3	6	1	6	1	7	0	6	1	5	2	34	8	81
▩ C5	4	1	3	2	3	2	3	2	5	0	4	1	22	8	73
▲ C6	7	0	6	1	5	2	4	3	7	0	7	0	36	6	86
+ C7	12	0	11	1	11	1	9	3	12	0	11	1	66	6	92
× C8	6	1	6	1	6	1	6	1	6	1	5	2	35	9	83
☒ C9	3	1	3	1	2	2	2	2	4	0	3	1	17	7	71
⊳ C10	4	1	3	2	4	1	2	3	5	0	4	1	22	8	73

Mean of % IN (Total) = 84.2 ± 8.8 (omitting C9
4 points are not
representative)

5.3 Conclusion

It has been shown that the simultaneous multi-element analysis of hair yields reasonably accurate results both from the results presented in Chapter 3 and the results presented in Section 5.2.2 of this chapter. Silver, in common with the other volatile elements zinc and bismuth, yields rather low answers using the dry ashing technique. The concentration of silver in human hair is usually close to the detection limit for one centimetre samples, therefore the accuracy and sensitivity, for silver, need to be improved to increase its usefulness for forensic discrimination purposes.

The survey of trace elements in human hair has shown some evidence of longitudinal trends in concentration for the hair samples examined, but more importantly it has shown the essentially individual nature of the information; trends being shown within an individual that are not repeated among samples from other individuals. Also, of importance in the statistical handling of the data, is the fact that the longitudinal concentration profiles are not merely random but often contain features that are found in other samples from the same individual (and same area of body). This then requires that new methods of handling the data be developed.

The discrimination test carried out showed again that the existing statistical methods are not accurate when used with the data from this study. A preliminary investigation of the correlation between trace-elements has shown promise as a method for distinguishing between hairs from different heads. Using the pragmatic assignment of areas to cover the maximum number of controls whilst maintaining maximum discrimination and assuming that the percentages of the number of points of each control lying within the areas are normally distributed then a good discrimination has been obtained and the two suspect hairs accurately identified. The method should easily be adaptable to analysis by computer to eliminate bias. The discrimination test dealt with scalp hairs but from the information given in Section 5.2.3 it should be possible to discriminate between the pubic hairs from different individuals (pubic hairs usually having detectable amounts of analyte for the elements investigated).

Chapter 6

Investigation of Atomic Emission from the Carbon Furnace

6.1 Introduction

Emission signals were observed on the aluminium channel of the multi-element spectrometer (Chapter 4) during the setting-up procedure. Further investigation indicated that these might be atomic and so a study was undertaken to prove this. Spectra were obtained from the carbon furnace HGA 70 on a conventional spectrograph using repeated shots of aluminium. The spectra showed lines of the aluminium spectrum. A calibration curve was then constructed using a conventional monochromator system. Further elements were then investigated and it has been shown that atomic emission from the carbon furnace is a general phenomenon. For nearly all the elements so far investigated characteristic lines have been found on the spectrum plates. In order to find the mechanism of this emission the temperature of the atoms was determined by spectroscopic means and this was compared to the temperature of the furnace walls.

An optical multi-channel analyser was also used to investigate the usefulness of the technique in multi-element analysis and briefly its application to hair analysis.

At the start of this investigation there was no published work on the use of the carbon furnace as a source for quantitative atomic emission in analytical chemistry. The original use of the carbon furnace by King (144-7) was for the production of spectra similar to those of stellar atmospheres rather than for chemical analysis. Two papers have appeared this year dealing with carbon furnace atomic emission. The first by Massman and Gücer (148) included spectra of rare earths but did not show lines below 3800Å nor was the feasibility of quantitative analysis demonstrated. Ottaway and Shaw (149) also published a paper demonstrating atomic emission from the furnace, although spectra were not presented to prove the atomic nature of the emission signals. They studied K, Li, Na, Cr, Al and Mg and found that the calibration curves were curved from the origin; this was ascribed to absorption of the radiation by atoms at the cooler ends of the tube. The sodium curve was non-linear at the sub-picogram level. The effect of different purge gases (Ar, He and N_2) was investigated: they found that argon gave the best sensitivity in all cases.

6.2 Preliminary Study

6.2.1 Equipment

The Perkin-Elmer graphite furnace HGA 70 (Fig. 2) was used in all experiments. The monochromator system described in chapter 3 (Fig. 9) was used for some of the experiments. A conventional Hilger and Watts medium quartz spectrograph was used to obtain spectra of the emission from the furnace. Kodak spectrum plates were used; B 10 spectrum plates for Plates 1 and 2 and No. 1 spectrum plates for Plates 3-6. They were developed for 3 minutes and fixed for 6 minutes (ca. 25°C) using recommended solutions. Temperatures were measured using a Northrup-Leeds disappearing filament optical pyrometer.

6.2.2 Procedure

It was necessary in the first instance to show that the emission being observed was atomic line emission and not molecular band emission. This was achieved by atomisation of aliquots of sample repeated several times to obtain a good spectrum. The aluminium lines at 3082.16, 3092.71, 3944.03 and 3961.53 Å were clearly visible together with a band system, probably the CN band, band-head at 3883.3 Å. The purge gas which was vented during atomisation was argon. Nitrogen was also used but found to give much weaker emission. A blank spectrum of emission from the carbon tube using argon and nitrogen purge gases (separately) was obtained and is shown in Plate 1.

A calibration curve for aluminium emission was then constructed (Fig. 84) using the single channel spectrometer previously described. A detection limit of approximately 5 pg of aluminium was obtained; this was approximately the level of aluminium in 10μl of the distilled water (5×10^{-4}ppm Al) used to make up the solutions. It can be seen from the calibration curve that a linear response is maintained over nearly four orders of magnitude of mass. This compares with 2-2.5 orders of magnitude normally available with atomic absorption. The limit of detection is also better than Perkin-Elmer's published figures for absorption under similar conditions (30 pg Al).

A survey was then carried out to determine whether this was a general phenomenon or was restricted to aluminium. Spectra were obtained for several elements using the spectrograph method just outlined and several elements were found to emit using the single channel spectrometer as detector system. A complete list of the elements found to emit with the main emission lines found is shown in Table 27 (the spectrum from iron contained too many lines to list all of them here). Plates 3-6 are also shown to indicate the type of spectra obtained, usually each element emits only a few lines, i.e. those associated with the lowest energy levels, thus the spectra are usually simple compared to those obtained using arc or spark excitation. It is of note that some of the transitions involve considerable energy e.g. Be 2348.61 Å (5.27 eV).

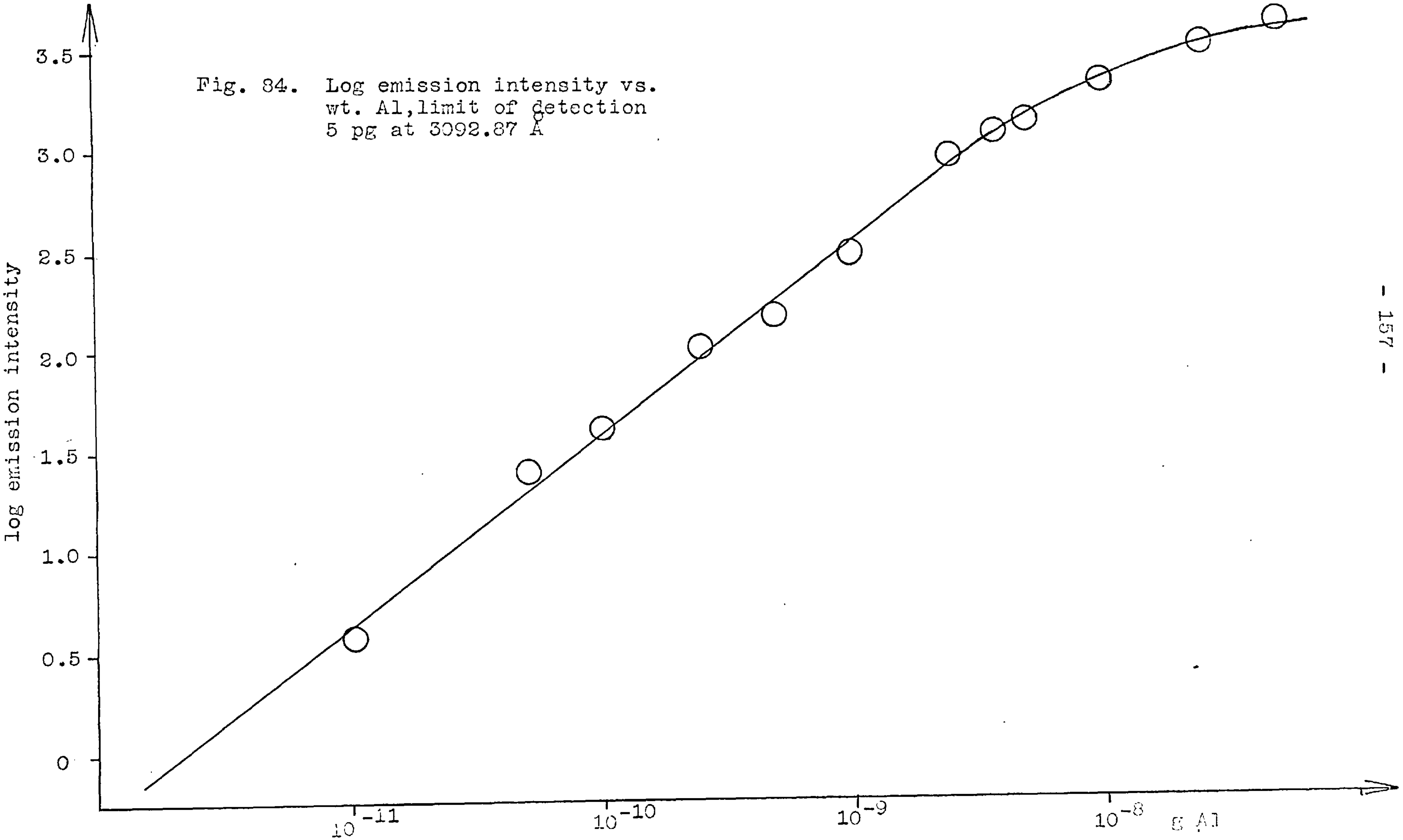


Fig. 84. Log emission intensity vs. wt. Al, limit of detection 5 pg at 3092.87 Å

Table 27. Lines observed in emission from the graphite furnace

Element	Wavelength Å
Ag	3382.89, 3280.68
Al	3961.52, 3944.01, 3092.72, 3082.16, 2575.11, 2568.00
Be	2348.61
Bi	3067.72
Co	3137.33, 3098.20, 3072.34, 3061.82, 3044.01
Cr	3578.69
Cu	3273.96, 3247.54
Fe	3749.48, 3745.56, 3737.13, 3734.86, 3733.32, 3722.56, 3719.94, 3631.46, 3618.77, 3586.11, 3570.10, 3490.57, 3020.49, 3008.14, 3000.95, 2994.43, 2983.57, 2973.13, 2966.90, 2720.90, 2719.02, 2522.84
Ga	4172,06, 4032.98, 2943.64, 2874.24
Mo	3864.11, 3624.46, 3456.39, 3237.08, 3208.83, 3193.97, 3170.35, 3158.16, 3147.35, 3132.59, 3112.12
Na	3302.94/.34, 2853.03, 2852.83
Ni	3414.76, 3050.82 (+ many others)
Pb	2833.06
Pt	3064.71
Si	2528.51, 2524.11, 2519.21, 2516.11, 2514.32, 2506.90
Sn	3801.02, 3330.62, 3262.34, 3175.05, 3034.12, 3009.14, 2863.33, 2839.99
Tl	3775.72
Zn	3075.90
Mg	2852.12

List of Plates

Spectra listed from top, wavelength scales in 100 Å.

Plate 1.

(a) blank with argon flowing
(b) blank with argon vented: notice appearance of CN band
(c) blank with argon flowing
(d) 10 shots of 10μl, 10 ppm Al solution with nitrogen gas vented (note the CN bands and Al emission at 3961 and 3944 Å)
(e) repeated with nitrogen gas flowing: note no Al emission.
(f) repeat of (e)

Plate 2.

(a) background emission from walls of furnace with nitrogen vented
(b) repeat of (a) with nitrogen flowing
(c) 10 shots of 10μl 10 ppm Al with argon vented: note 3082, 3092, 3944, 3961 Å lines.
(d) Aluminium hollow cathode lamp
(e) 10 shots of 10μl 10 ppm Al with argon flowing

Plate 3.

(a) 10 shots of 10μl 100 ppm Cu argon vented
(b) 10 shots of 10μl 100 ppm Mg argon vented
(c) 10 shots of 10μl 100 ppm Sn argon vented

/cont.

(List of Plates cont.)

Plate 4.

(a) 10 shots of 10µl 100 ppm Ga argon vented (lines 4033, 4172 Å swamped by background)

(b) 10 shots of 10µl 100 ppm Tl argon vented (line at 3776 Å swamped by background)

(c) 10 shots of 10µl 100 ppm Bi argon vented (line at 3067 Å very weak)

Plate 5.

(a) 10 shots of 10µl 100 ppm Al argon vented

(b) 10 shots of 10µl 100 ppm Ag argon vented (Ag lines 3383, 3281 Å very weak also weak Al lines at 3082, 3092 Å)

(c) 10 shots of 10µl 100 ppm Fe argon vented

Plate 6.

(a) 10 shots of 10µl 100 ppm Ge argon vented no Ge lines seen but strong Na doublet (unresolved) 3303 Å (the scale is slightly displaced away from the visible on this plate) Germanium dissolved in alkaline medium.

(b) 10 shots of 10µl 100 ppm Ta (Ta dissolved in NaOH) argon vented. No Ta lines seen but there is the strong Na doublet (unresolved) at 3303 Å.

(c) 10 shots of 10µl 100 ppm Ta (Ta dissolved in HF) argon vented. No Ta lines see but lines of Si, Al, Mg and Fe all dissolved out of the glass flask by the excess HF. The silicon lines are very weak but visible on the original plate.

/cont.

(List of Plates cont.)

(Plate 6)

(d) 10 shots of 10μl 100 ppm Mo argon vented. Na doublet unresolved at 3303 Å also present. (MoO_3 dissolved in NaOH)

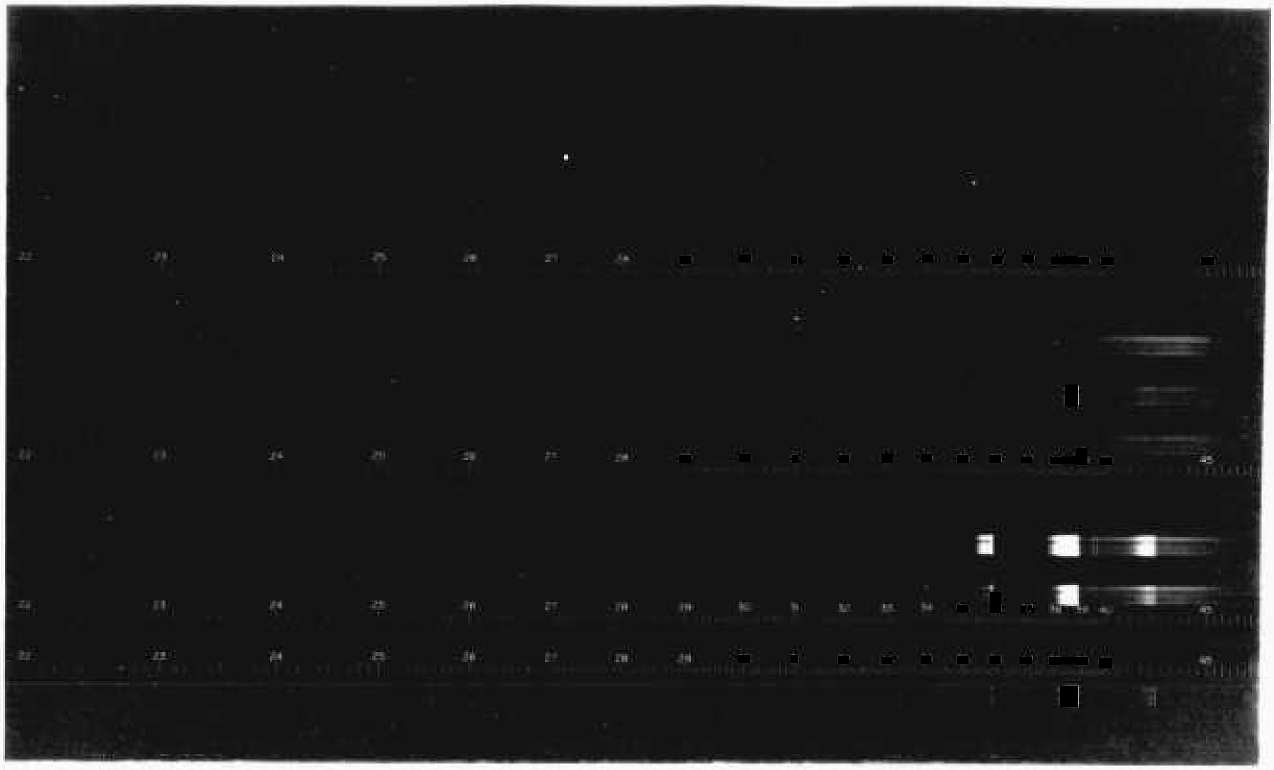


PLATE 1

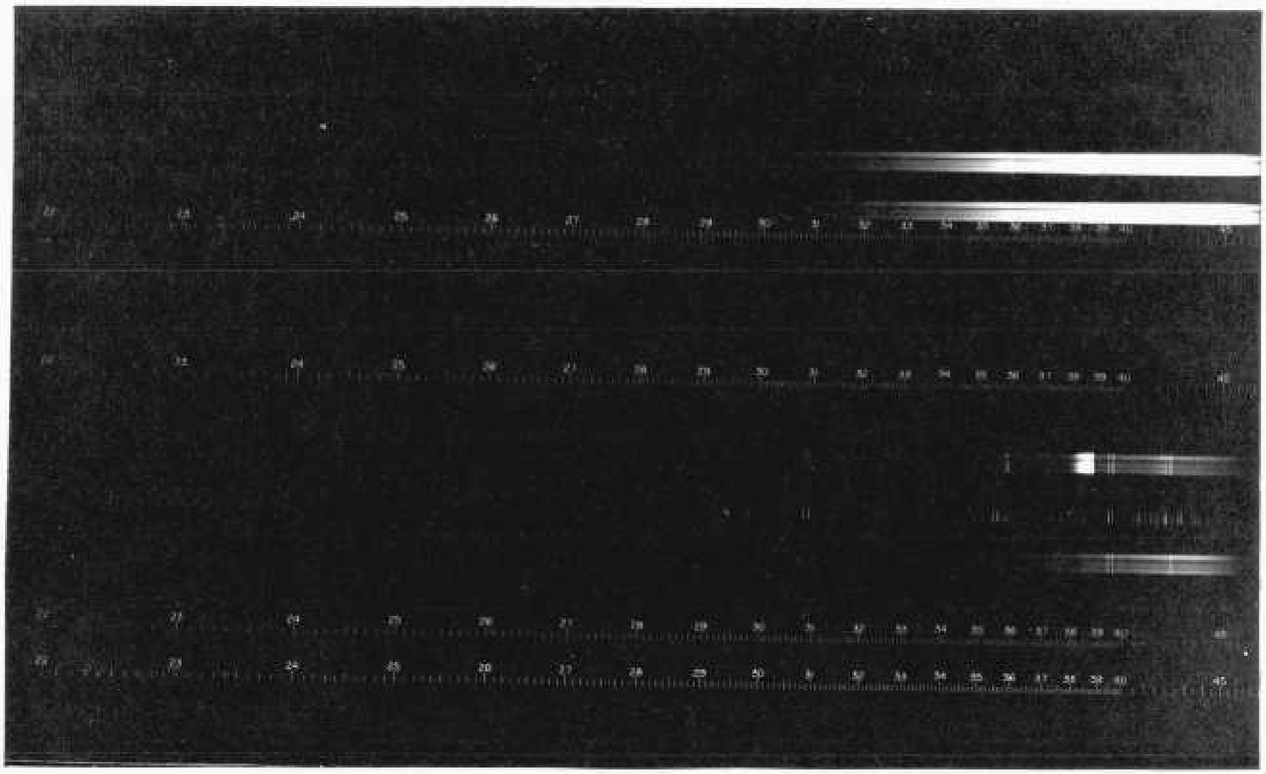


PLATE 2

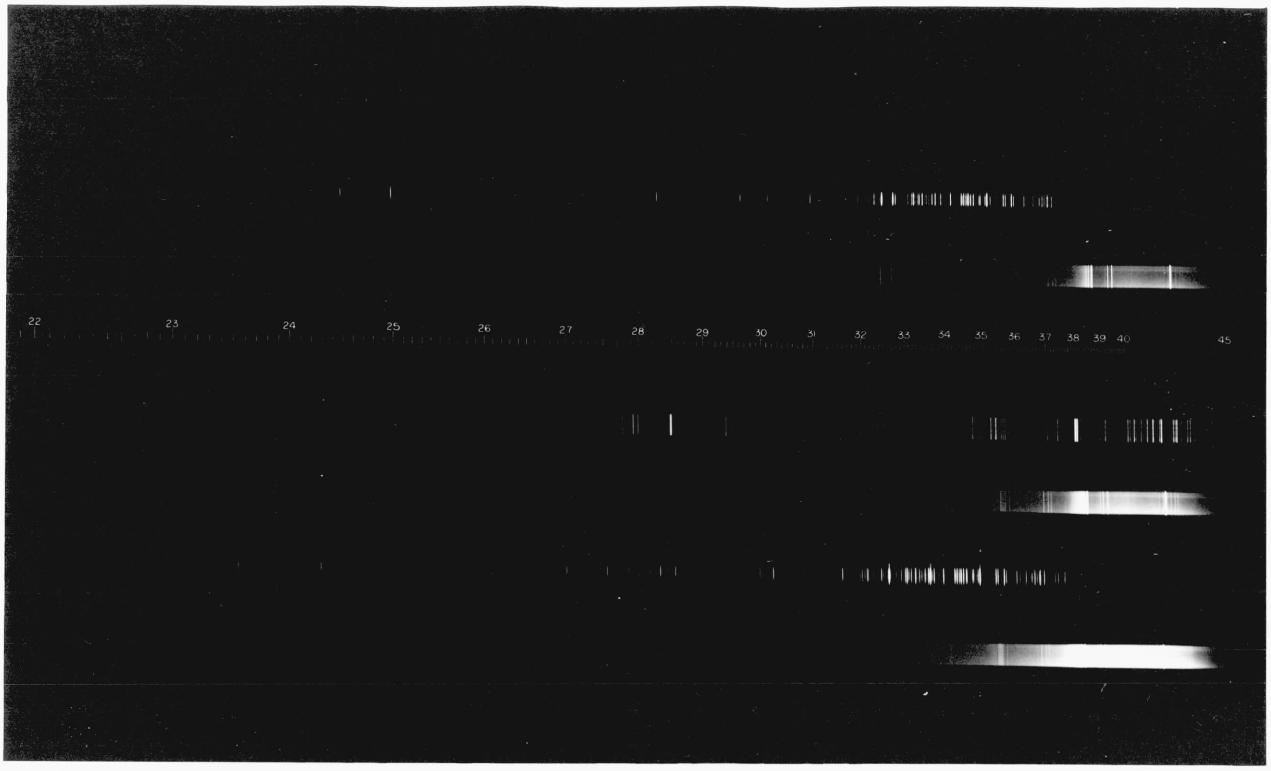


PLATE 3

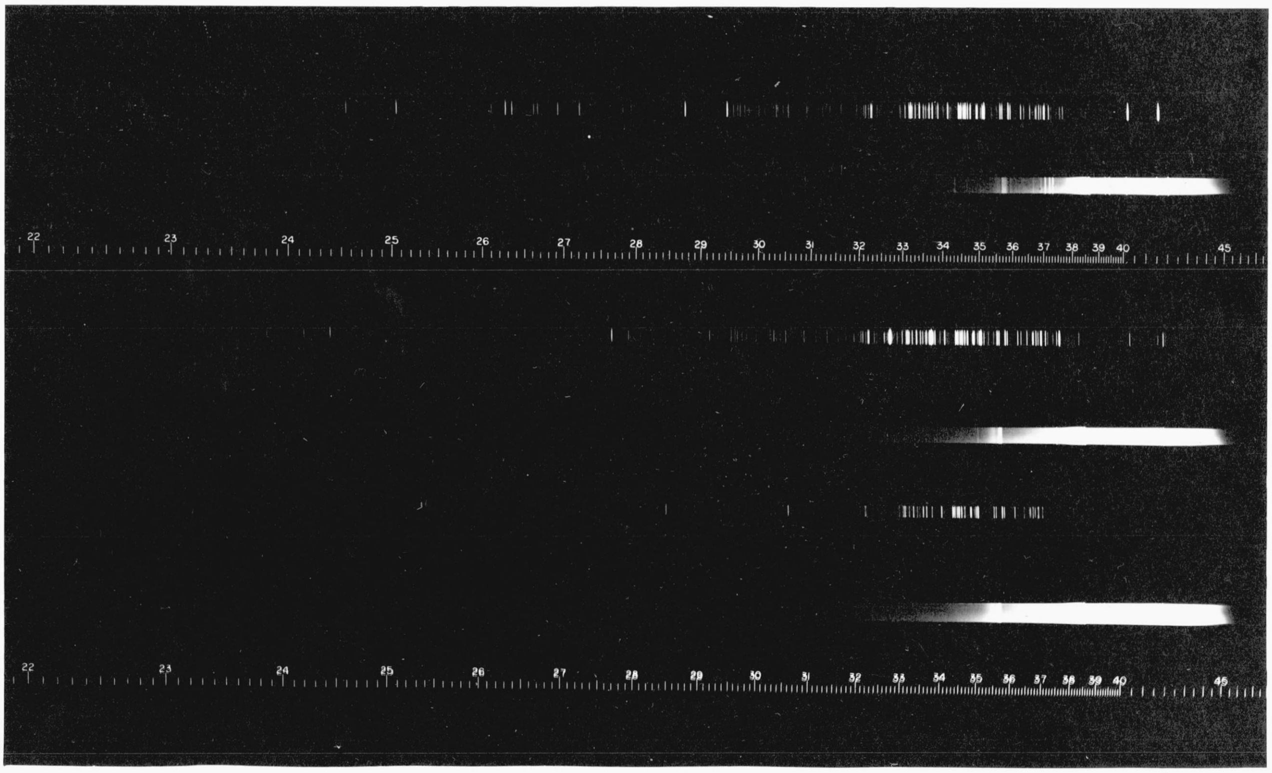


PLATE 4

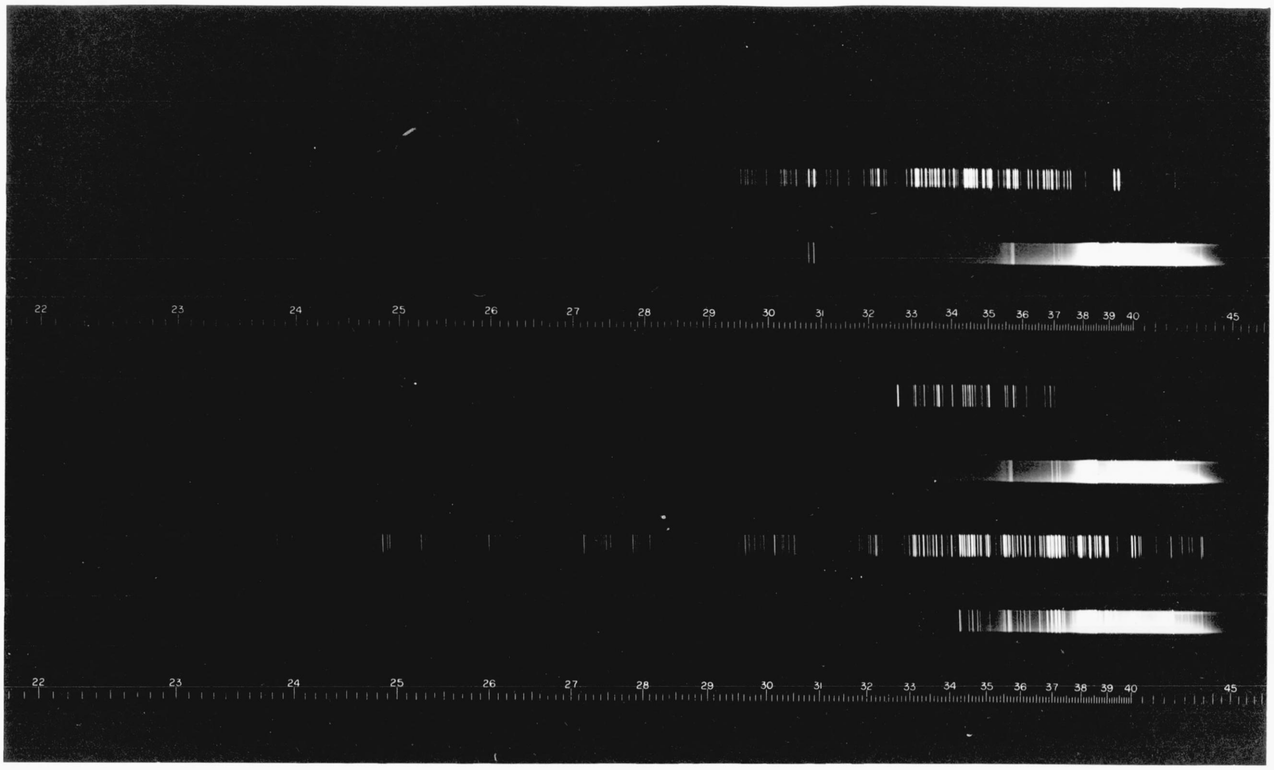


PLATE 5

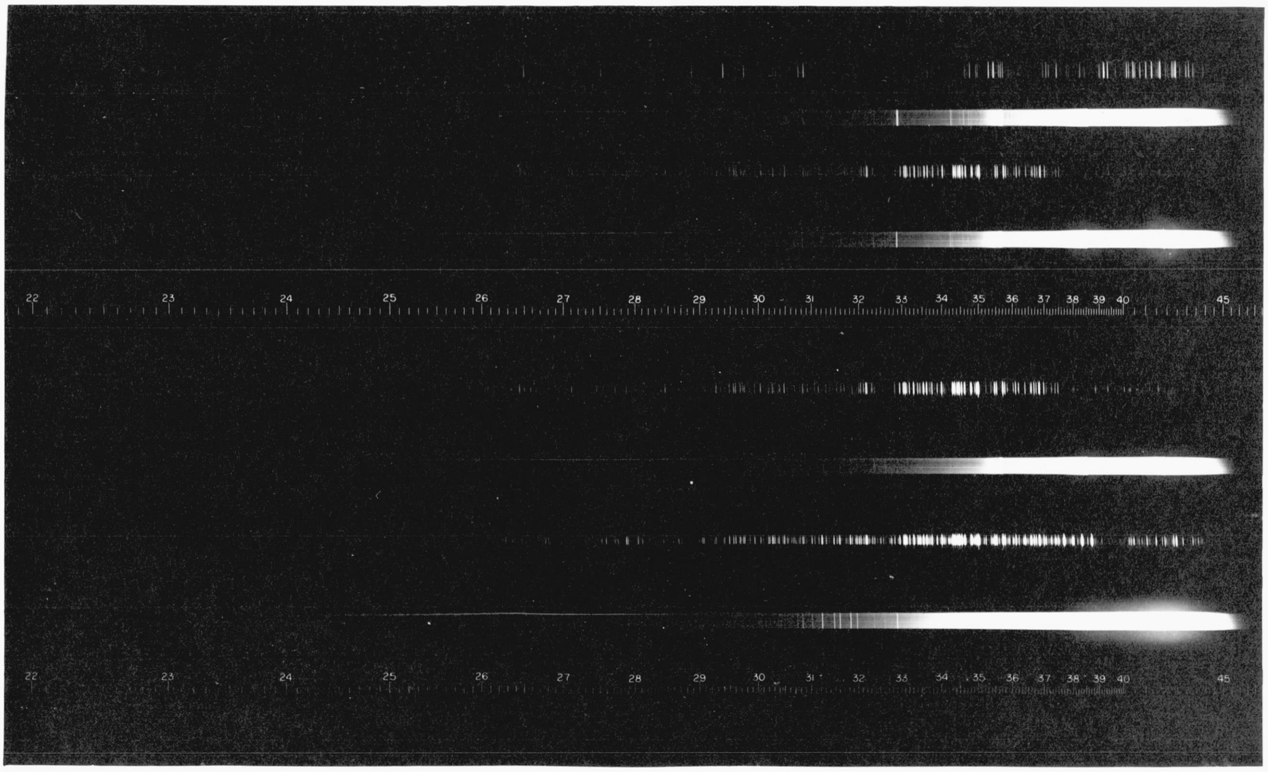


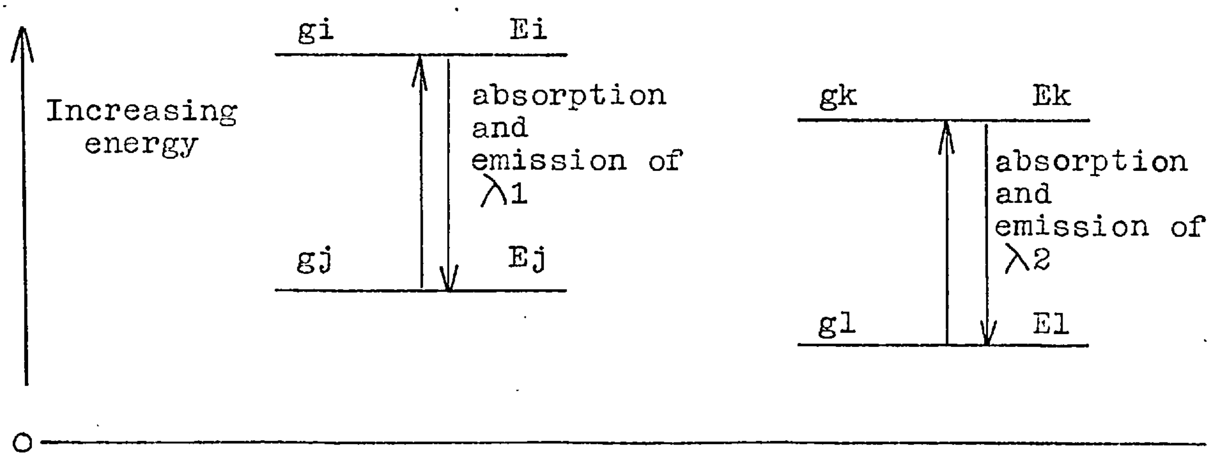
PLATE 6

Of all the elements so far investigated only tantalum and germanium do not emit lines of their spectrum when atomised (2600°C) in the furnace.

6.2.3 Temperature Measurements

To discover whether the atoms were obtaining the necessary energy for emission by some chemiluminescent process the temperatures of the emitting atoms and their surroundings were measured. The temperature of the walls of the furnace was measured using an optical pyrometer and the temperature of the atoms (iron) by the "two-line" method. The theoretical derivation of the formula used for calculating the temperatures can be found in Sargent's Ph.D. Thesis (150) and suitable line pairs in Vetter's Ph.D. Thesis (151). The nomenclature and formula used for calculating the temperatures are given below in Fig. 85.

Fig. 85. Nomenclature for the two spectral lines used in the temperature measurement.



$$\frac{I_1}{I_2} = \frac{g_1}{g_k} \frac{f_{ij}}{f_{kl}} \cdot \frac{\lambda_2^{3}}{\lambda_1^{3}} \exp\left[\frac{-(E_i - E_k)}{kT}\right] \qquad \text{from (150)}$$

I = intensity of line

gn = statistical weight of the level n

f = oscillator strength

λ = wavelength of line

En = energy level of level n (eV)

k = Boltzmann's constant

T = absolute temperature

The line pairs used were those calculated by Vetter (151) to have the least self-absorption over given temperature ranges,

$$\text{i.e.} \quad \frac{k_1}{k_2} = 1 = \frac{g_i\ f_{ij}}{g_k\ f_{kl}} \exp\left[\frac{-(E_j - E_l)}{kT}\right] \qquad \text{from (150)}$$

k = integrated absorption coefficient

Line intensity ratios were measured from spectrum plates, obtained as previously, and from data obtained using the spectrometer.

The temperature of the walls of the furnace as measured using the optical pyrometer was 2700 ± 100 K. This was measured by focusing the pyrometer through the observation port (Fig. 2) of the furnace and through the sample hole of the carbon tube so that the temperature obtained was that of the inside of the carbon walls at the centre of the furnace. A list of the temperature values obtained using measurements from the spectrographic plates and from the spectrometer is given in Table 28.

Table 28

Line Pair	Optimum Range	Temperature	
		Monochromator	Plate
Å	K	K	K
3722.56 - 3734.87	2340 - 2540	2060	2590 2070 2230
3733.32 - 3734.87	2070 - 2230	2280	2100 2269
3733.32 - 3758.24	2740 - 2990	2470	
3733.32 - 3749.49	2400 - 2600		2100
3705.57 - 3349.49	2700 - 2900		2200

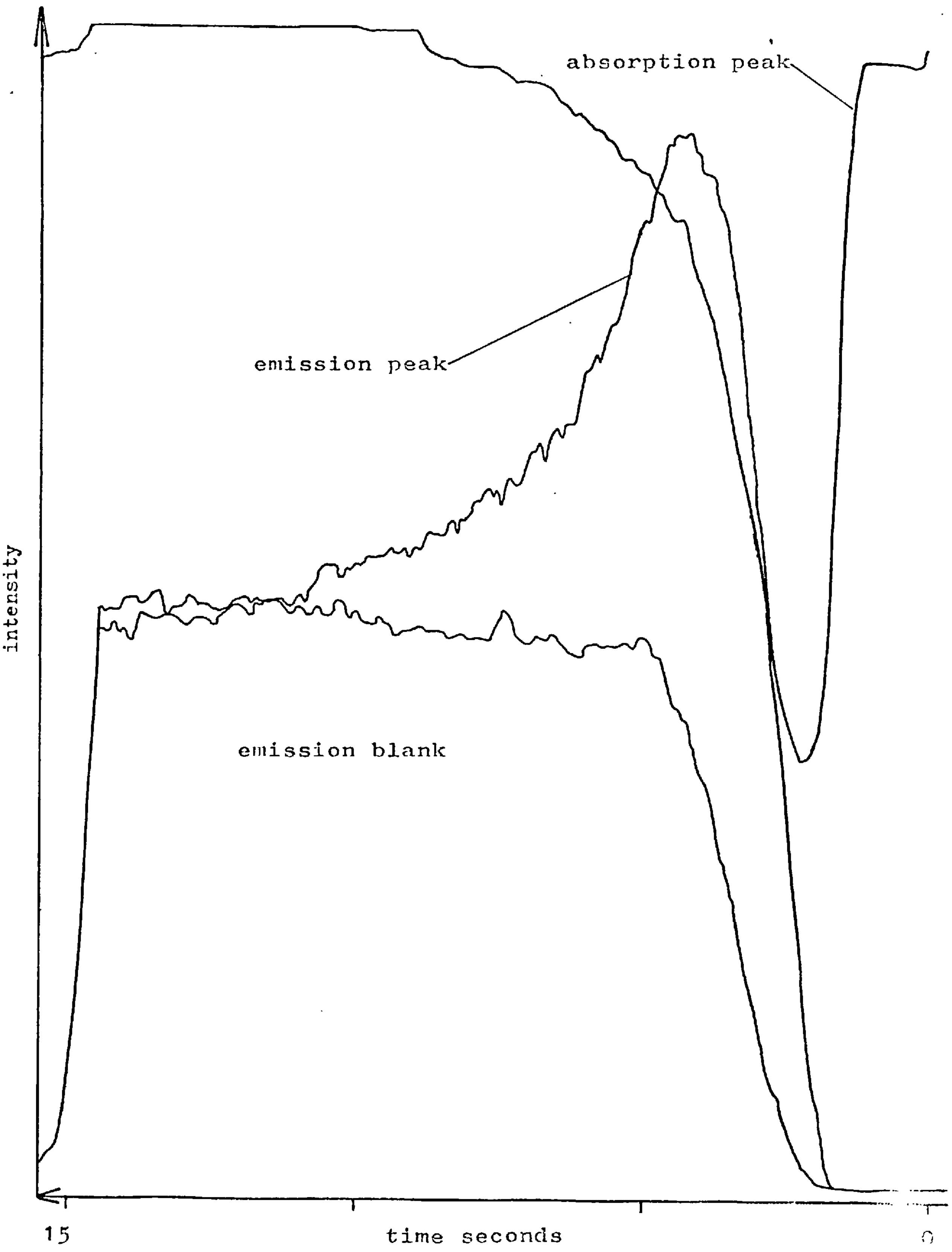
The line densities on the spectrographic plates were measured using a Hilger Open densitometer. The relatively large spread of results is a reflection of the difficulty of accurately measuring the densities of the lines and of the inaccuracies in the values of transition probabilities used. The transition probabilities are those used by Vetter (151) who in turn used the values previously compiled by Corliss and Tech (152).

Measurement of the absorption and emission of 1 ng of iron together with background emission over the same time period (Fig. 86) shows that the emission peak reaches a maximum after the maximum of the absorption peak. The emission peak also corresponds to the maximum temperature of the furnace tube as shown by the background emission curve.

6.3 Discussion

Atomic emission from the carbon furnace has been shown to be a general phenomenon for most elements, the exceptions being tantalum and germanium. Tantalum has a

Fig. 86. Absorption and emission of Fe at 3720 Å in a carbon furnace



very high boiling point (5425°C) and is sometimes used as a liner for this type of electrothermal furnace tube, thus it would not be expected to atomise readily. Germanium forms a stable oxide and it is difficult to reduce the oxide in the electrothermal furnace. Thus it is perhaps not surprising that emission from these two elements is absent. Aluminium is very sensitive in emission and shows a reasonably long linear working range. The temperature measurements have shown that the walls of the furnace and the emitting species (iron) have similar values. The measurement of emission and absorption over the same time scale (Fig. 86) shows that the emission peak corresponds to the maximum temperature of the furnace as shown by the background curve. This would lend support to the excitation mechanism being simply due to energy received from the tube walls rather than from a chemical reaction. The large energies required for some of the observed lines e.g. Be 2348.61 Å (5.27 eV) and Si 2516.11 Å (4.95 eV) might suggest, however, that a chemiluminescent process be responsible for excitation. Measurement of the temperature of other emitting species in the furnace by spectroscopic means may help to elucidate the excitation mechanism.

The effect of purge gases on atomic absorption has been briefly mentioned earlier (Chapter 3, Section 3.6) and for aluminium argon was found to give a x3.5 better sensitivity than nitrogen. Although no comparative quantitative work in atomic emission has been carried out here, it was found that the atomic lines from aluminium were very considerably reduced when similar spectra were obtained using argon and nitrogen purge gases. Plates 1 and 2 show this, Plate 1 showing considerably stronger CN bands with nitrogen purge than with argon. Ottaway and Shaw (149) also observed this phenomenon, finding argon to be x4 more sensitive for lithium and x2 more sensitive for aluminium than nitrogen, but offered no explanation. Nitrogen can react with the species in the tube to form nitrides unlike argon which does not readily form compounds. Nitrogen has a higher thermal conductivity than argon, thus cooling the analyte atoms and reducing the population in the excited state more than with argon as the purge gas. These two effects may be responsible for the discrepancy between the two gases with nitride formation probably being responsible for any large differences observed.

6.4 Simultaneous multi-element atomic emission using a vidicon detector

An optical multi-channel analyser has been used to investigate the possibility of simultaneous multi-element analysis in the atomic emission mode using a carbon furnace source. A Hilger and Watts medium-quartz spectrograph was used as the dispersion device with the vidicon tube attached to the spectrograph in its focal plane centred on *ca*. 3050 Å. A survey of several elements (that had lines close to ground state) was made and a multi-element solution was then used to obtain simultaneous multi-element analysis. The effect of excess phosphate on aluminium emission was briefly investigated as was the application of this technique to the analysis of hair.

The vidicon tube has been used in atomic spectroscopy (130-135) but this is the first time it has been used as the detector for atomic emission using the carbon furnace source. The vidicon can be used to observe a spectral band-pass (rather than a single line) thus facilitating simultaneous multi-element analysis.

6.4.1 Equipment

The detector was an SSR model 1205D Optical Multi-channel Analyzer (OMA). The normal electronics supplied with this device was used. The detector was placed in the focal plane of the Hilger and Watts medium quartz spectrograph and centred on 3050 Å. The HGA 70 carbon furnace (Fig. 2) was used as the atom cell. The output from the OMA was fed to a Servoscribe chart recorder.

6.4.2 Tube Detector System

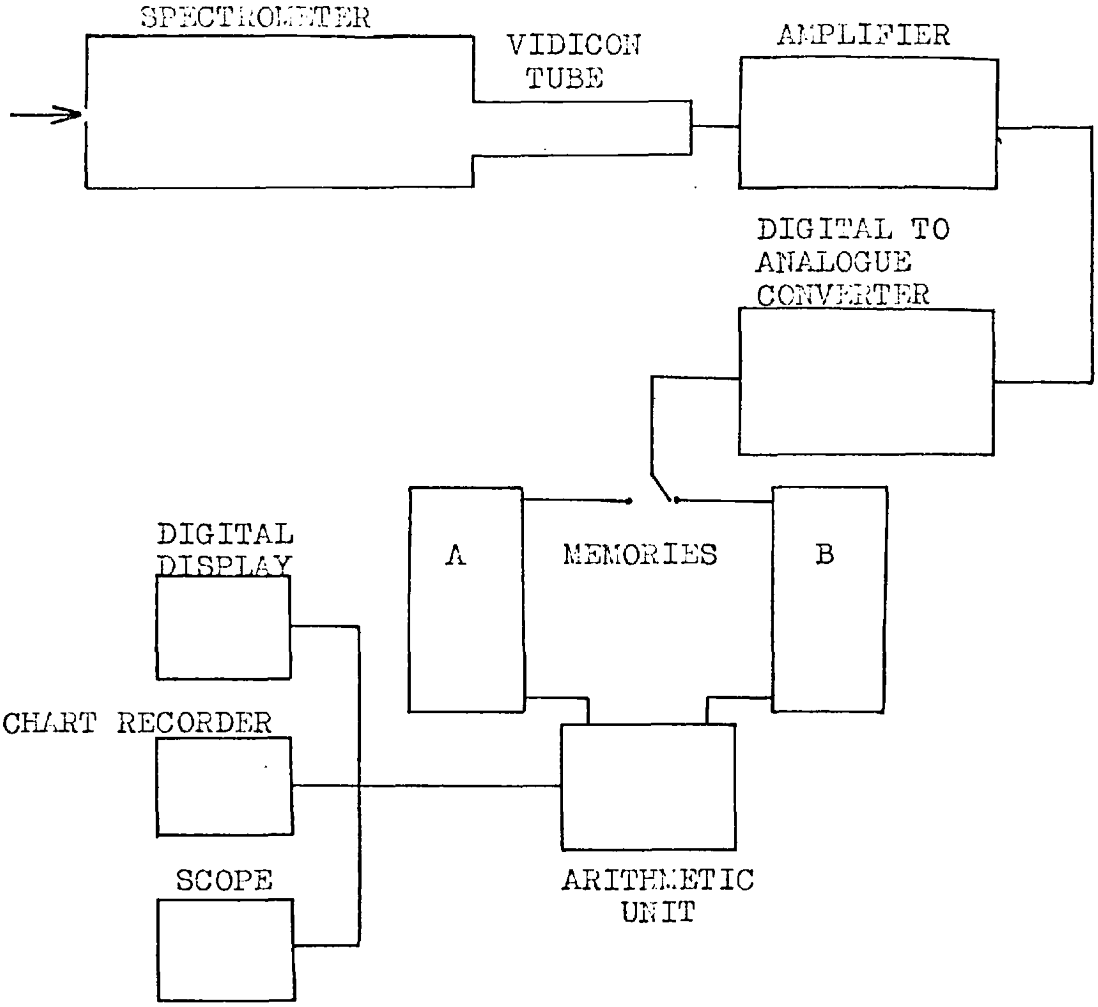
A block diagram of the complete system is shown in Fig. 87. The light from the spectrograph was focused on to the target area of the vidicon tube. The target consists of an array of reverse-biased silicon diodes which are maintained at a constant potential by a scanning electron beam. Any photon incident on the diode will generate positive carriers which will migrate and discharge the diode. The current required to recharge the diode from the scanning electron beam is proportional to the incident radiation that has previously fallen on the diode and constitutes the signal.

The target (0.5 x 0.4") consists of 500 vertical channels (25μm apart) and this whole target area is scanned every 32 ms. The area of the spectrum focused onto the vidicon is thus divided into 500 channels. The signal from each channel is amplified and converted to a digital signal. In these experiments the signals obtained from atomisation of the analyte were stored in memory A and the background signals from furnace alone stored in memory B. Memory B was then subtracted from A and the resulting spectrum displayed on the oscilloscope and fed to the chart recorder.

6.4.3 Procedure

The system was firstly calibrated for wavelength using various hollow cathode lamps. The signal:background for emission was then optimised by adjustment of the furnace position. A brief survey of elements with strong

Fig. 87. Block Diagram of the detector system



lines was made. A multi-element solution was then used to construct calibration curves for Al, Ni, Mo and Pt simultaneously. The effect of excess phosphate on the emission from this solution was also briefly investigated. Several samples of hair were atomised in the furnace and their composite signal stored and subtracted from an equivalent background.

Samples were injected into the furnace using plastic tipped micro-pipettes dried at 100°C (until all the solvent had been removed) and atomised <u>ca</u>. 2600°C. Argon was used as the purge gas but was vented during atomisation to increase the residence time of the atoms in the tube and hence the observed emission intensity. Continual cleaning of the furnace, by heating to maximum temperature, was necessary to prevent the build up of any residual analyte. Molybdenum was a particular problem in this respect probably due to the formation of its carbide (153).

6.4.4 Results and Discussion

The calibration curve of wavelength <u>vs</u>. channel number is shown in Fig. 88. The region 2940 - 3140 Å was originally chosen to cover lines from a wide range of elements with different chemistries to discover the full range of the technique. It is also important that the range be away from the strong CN band emission at <u>ca</u>. 3883 Å and at as low a wavelength as practical to ensure that the background is low. The particular vidicon tube was a 1205D type which was not coated with a scintillator and thus did not respond below <u>ca</u>. 3040 Å and only half of the chosen band-pass could be examined. Several elements were found to emit in the band-pass under investigation and the elements and their wavelengths are listed in Table 29. A multi-element solution was then analysed and the results are shown in Figs. 89-92. It can be seen that for all the

Fig. 88. Wavelength vs. OMA channel number

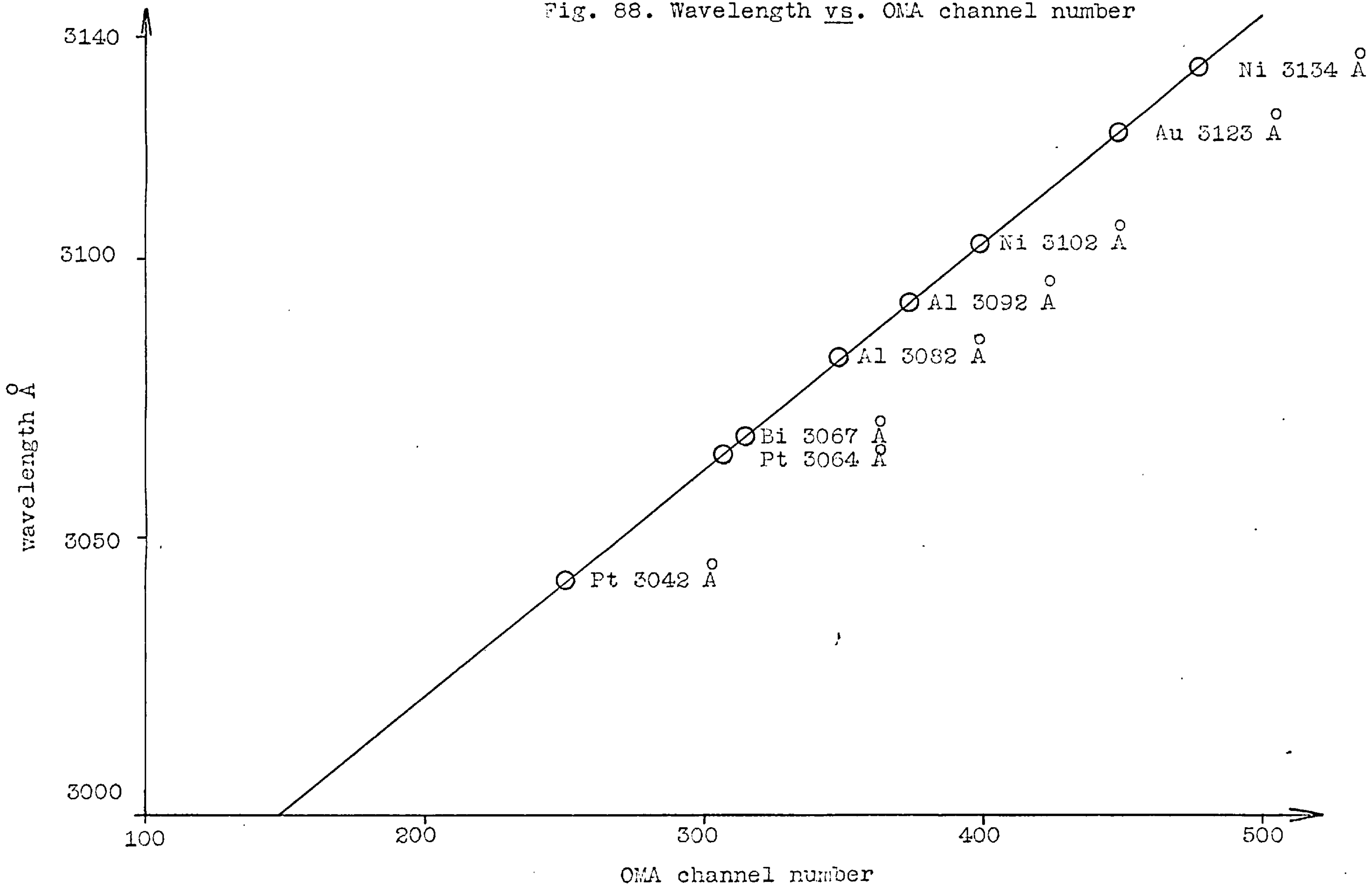


Table 29. Elements found to emit from the carbon furnace using the OMA as detector

Element	Wavelength Å
Al	3082.16, 3092.87
Bi	3067.72
Co	3044.01, 3061.82, 3072.34, 3098.20, 3137.33
Fe	3047.61, 3059.09, 3067.24, 3100.67
Mo	3132.59
Ni	3037.94, 3057.64, 3101.88, 3134.11
Pt	3064.71
Zn	3075.90

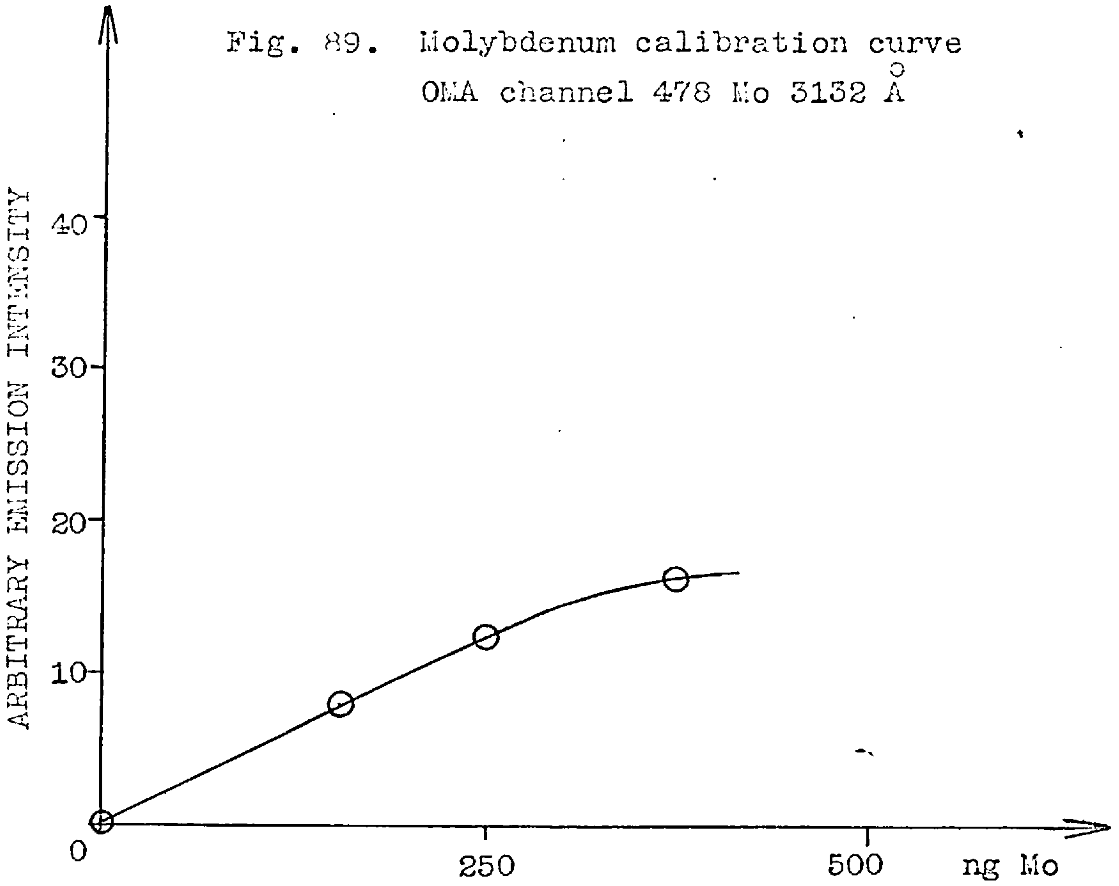


Fig. 89. Molybdenum calibration curve
OMA channel 478 Mo 3132 Å

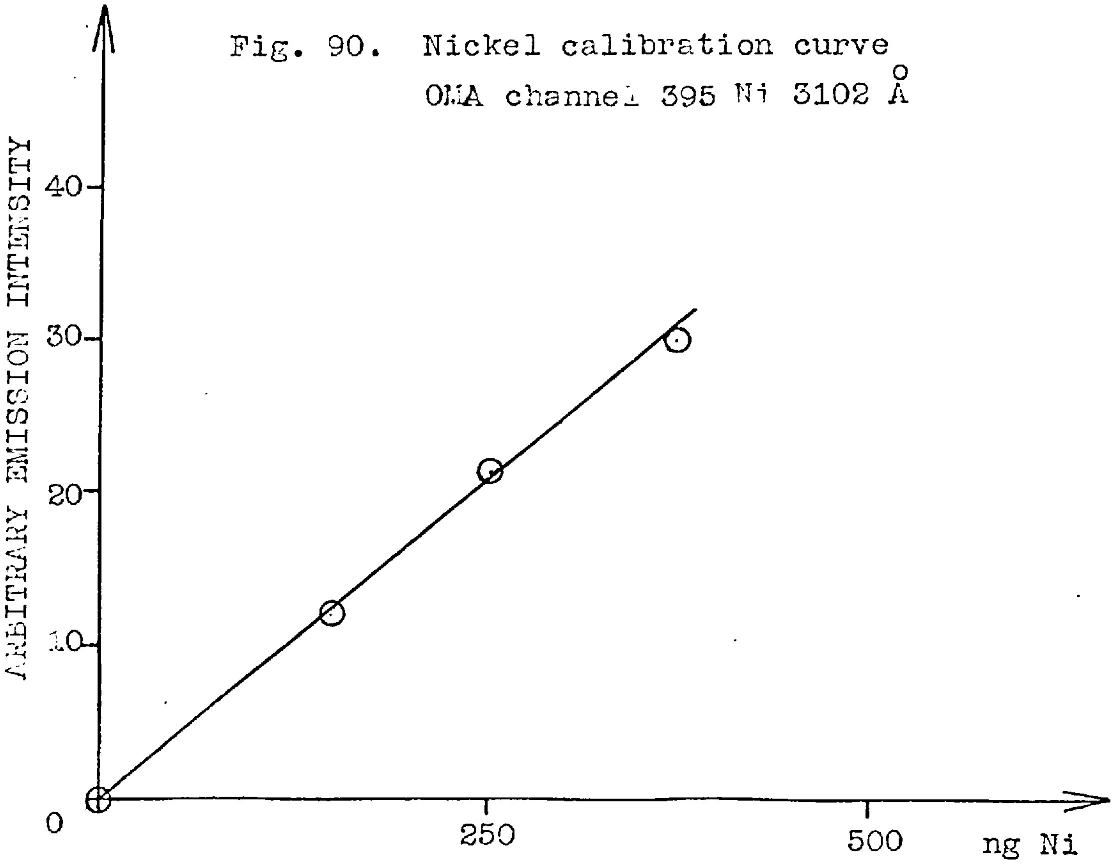


Fig. 90. Nickel calibration curve
OMA channel 395 Ni 3102 Å

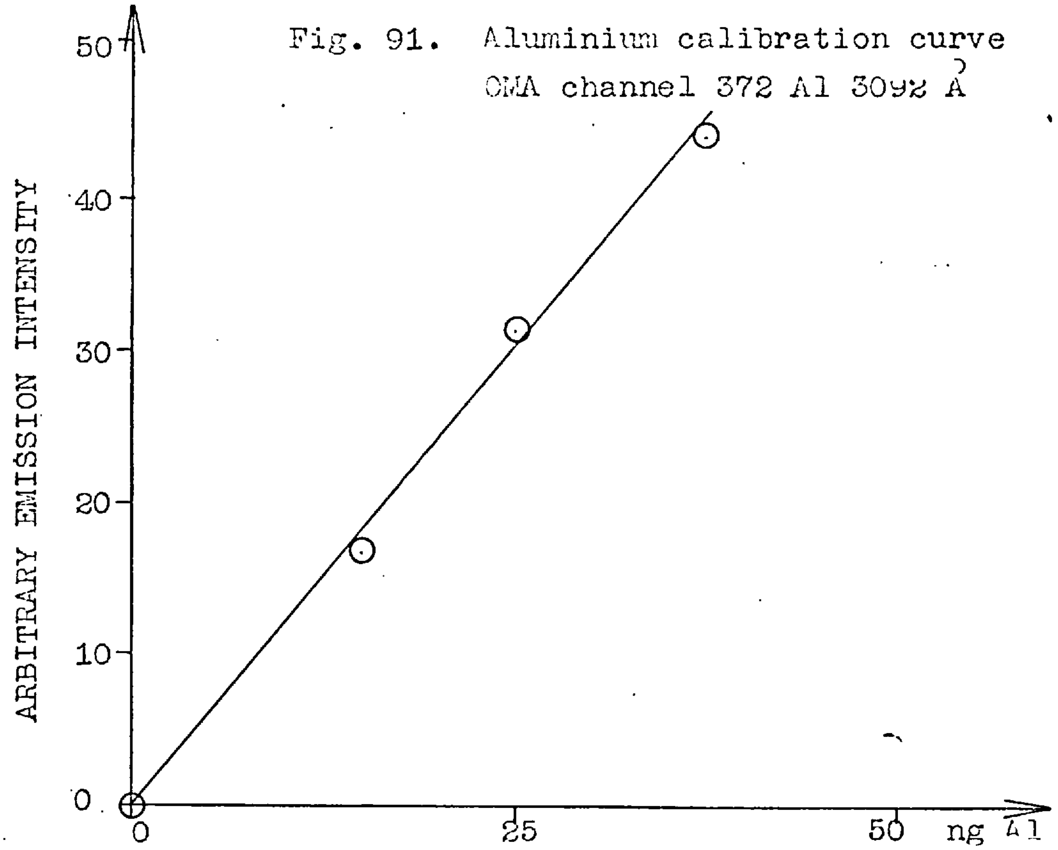


Fig. 91. Aluminium calibration curve
OMA channel 372 Al 3092 Å

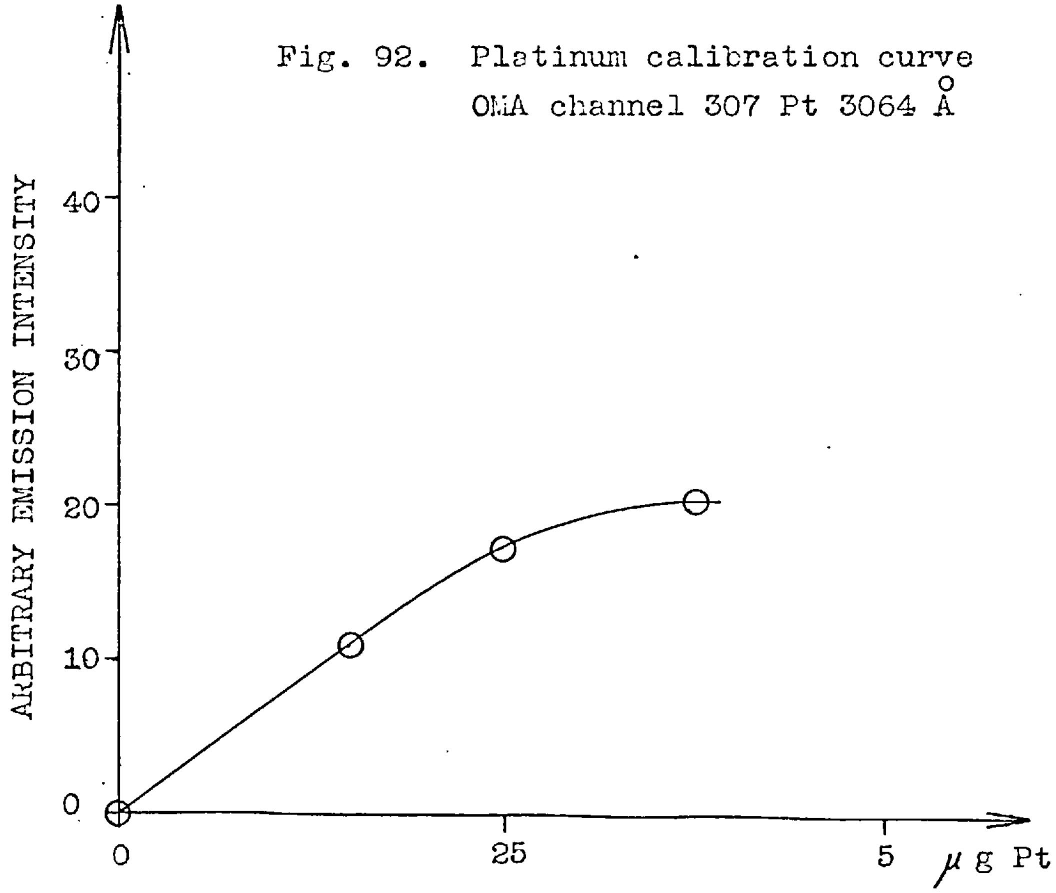


Fig. 92. Platinum calibration curve
OMA channel 307 Pt 3064 Å

elements a linear calibration curve is obtained over the range examined.

The effect of excess sodium phosphate solution on the emission from the multi-element solution is given in Table 30.

Table 30.

	No added PO_4^{3-}	With added PO_4^{3-}	xs mass of PO_4^{3-}	PO_4^{3-} blank
Al	14	22	x 2000	8
Ni	12	11	x 20	0
Pt	9	9	x 200	0
Mo	4	3.5	x 200	0

It can be seen that the excess phosphate has made negligible difference to the emission intensity for all the elements investigated.

Three hair sections ca. 1.5 cm. long were separately introduced into the furnace, ashed and atomised as in the multi-element atomic absorption analysis. The signals were all stored in memory A and three blanks stored in memory B, the memories were subtracted A-B and the resulting spectrum recorded. This repeated atomisation caused the residual signal to be rather noisy and no clear line intensities could be determined but there was evidence of the two aluminium peaks at 3092.72 and 3082.16 Å.

The vidicon tube contains no amplifying chain of dynodes like a photomultiplier and therefore all the extra gain required has to be by means of electronic amplification external to the tube and thus a poorer signal: noise is obtained. This fact coupled with the restricted and non-optimum spectral range chosen means that the application of the carbon furnace atomic emission using a vidicon photodetector has not been properly evaluated with respect to hair analysis. However, it does seem unlikely that the vidicon will be capable of detecting the very low light levels emitted from analyte in ca. 100μg of hair unless the gain of the vidicon tube can be substantially improved. This work has shown that for relatively high concentrations the vidicon can be used for simultaneous multi-element analysis. The absence of significant interference from excess phosphate is also a useful feature; although the effect of interferences on the phenomenon of atomic emission from the carbon furnace needs to be much more extensively studied.

6.5 Conclusion

Atomic emission from the carbon furnace appears to be a general phenomenon for the elements so far investigated, germanium and tantalum being the exceptions. The calibration curve for aluminium shows that good sensitivity and long linear working range can be obtained. Preliminary study of the excitation mechanism suggests that the mechanism is thermal rather than chemiluminescent, although more work is needed to check this.

The OMA has shown that it is capable of detecting the radiation from several elements when atomised simultaneously in the carbon furnace. The low internal gain of the vidicon tube, however, means that the detection limit for the system is not as good as can be obtained in single channel using a photo-multiplier tube. Preliminary study of interference by phosphate has shown little effect for Al, Ni, Mo and Pt but a more extensive study is required.

6.6 Acknowledgements

The temperature measurements and spectra (plates 3-6) presented in this chapter were obtained jointly by the author and Mr. R.D. Snook as part of his M.Sc. Research project (154). The author would also like to thank E.D.T. Supplies Ltd. (Ivy Crescent, Chiswick, W.4.) for the loan of the OMA type SSR 1205D.

Chapter 7

Suggestions for Further Work

The work presented in this thesis shows that simultaneous trace metal analysis of centimetre sections of hair is possible and that the information thus obtained can be useful in distinguishing between the hairs from the heads of different individuals. Using the same instrumentation it would be useful to extend this work in several directions. A repeated discrimination test using a larger number of samples and controls would be very useful, so that the statistical method can be evaluated without operator bias. It is also important to know the full range of concentrations of trace elements that can be expected over the head of any individual and to this end a detailed study of many hairs from the heads of several individuals would be very instructive. Probably the most profound part of the problem of hair analysis is that of interpretation of the data and development of statistical methods capable of treating the data. The proposed statistical treatment described in Chapter 5 is useful but any information contained in the data about specific longitudinal trends in the hair is lost with this treatment.

There are other areas to which the simultaneous trace metal analysis of small samples is of interest and the application of this technique to identification of glasses, paints and fibres is of considerable interest.

The instrument might also undergo profitable modifications that would improve its usefulness. The investigation of other suitable elements for determination in hair has been outlined in Chapter 3. The instrument in its present atomic absorption mode is restricted in its dynamic range and modifications to overcome this would be desirable.

The use of an emission mode would facilitate this and at the present time it would appear that atomic emission, using an inductively coupled radio frequency plasma with development of a method of introducing the sample into the plasma, such as electrothermal pulse vaporisation, would offer the most advantages (155,156).

Although this work has been chiefly concerned with forensic problems the simultaneous analysis of small samples for several elements may have benefits for many fields of interest, particularly in biological research. A more detailed picture of trace element metabolism should be obtainable with this type of analysis.

The work on carbon furnace atomic emission has shown it to be a promising technique for many elements. The work on the mechanism of excitation is not yet conclusive and it may be instructive to repeat the "two-line" temperature measurements using other elements to discover if they give values similar to those obtained for iron. Calibration curves should be obtained for several elements in order to assess the general sensitivity of the technique and a detailed study of interference effects should be undertaken.

Appendix 1

A.1 Preparation of a Standard Hair

A recurrent problem in analytical chemistry is that of standard materials; these are required to check the accuracy of an analytical technique for a given sample. No standard material (hair) has been available during this study and the accuracy and precision of the methods of analysis employed have had to be checked in alternative ways. It should be clear to the reader from Figs. 35-77 that natural hair varies considerably from centimetre to centimetre and therefore in its normal state cannot be used as a standard material. It is necessary, therefore, to manufacture a standard hair and there seem to be several possible ways of doing this.

A.1.1 Human Hair

(a) Doping

Hair is known to show ion-exchange properties (79, 85) and these could be exploited to provide a uniform standard hair. A large sample of head hair could be washed in boiling ammonical EDTA to remove the existing trace elements; fresh elements could then be doped into the hair by soaking in an appropriate multi-element solution. This method has some advantages, particularly that the hair remains essentially unaltered and thus the matrix effect, if any, will be similar to that found in real samples and also the convenience in handling is not lost by the process of manufacture. A recent communication between Maes and Pate and HOCRE (157) has suggested that at least some of the longitudinal concentration features found in hair (for Cu) may be related to structural damage to the hair allowing elements to be absorbed, from the environment, sweat, etc., selectively at these sites. The evidence however is not conclusive. Should this prove to be the case then the foregoing method of preparation of a standard hair would be of limited use.

(b) Homogenising

Another method of obtaining hair with uniform trace element concentration might be to dissolve the hair and then to reprecipitate it. There are few substances capable of readily dissolving hair that do not at the same time destroy its components, but performic and peracetic acids will partly dissolve hair leaving an insoluble residue (β Keratose). The dissolved material could then be precipitated and the amorphous mass used as a standard material, if the trace metals are found to be uniformly distributed throughout. However this amorphous mass would have to be divided into sections of the correct weight ca. 100μg and this would mean considerable manipulation with the attendant increase in risk of contamination.

(c) Tissue Culture

The use of a culture medium to grow a standard hair has also been suggested to the author. Tissue culture, however, is not easy and a research programme would be required to achieve this.

A.1.2 Animal Hairs

The possibility that animal hairs might be useful as standards has been briefly examined using the hair of a domestic dog and cat. However it was found that for the elements silicon and copper there was as much variation from segment to segment as there was for human head hairs. However, the hair of animals living under controlled conditions, such as those kept for scientific research, may have much more uniform trace element concentrations than those of domestic pets whose diet can be subject to considerable change. This possibility was not investigated in this study.

A.1.3 Silk

The possibility of using silk as a material into which trace metals could be doped to produce an artificial hair has been the subject of two research projects at Imperial College (158,159). The part of natural silk that is used for spinning, tyrosine, is a protein and is similar to wool keratin (though it contains no sulphur). It is known to possess ion-exchange properties (160) and these have been used to dope silk to the desired concentration of several trace elements. It was found that Cu, Ag, Ni and Co could be simultaneously introduced into the silk to the level normally found in human scalp hair. Iron was found to be present in silk at the level normally found in scalp hair ca. 20 ppm. The introduction of lead into the silk by the same method of soaking was tried but without success; the failure of lead to ion-exchange into the silk was attributed to its large ionic radius. A sample of the multi-element doped silk was analysed using the multi-element AA spectrometer described in Chapter 4, under similar conditions to those used for hair analysis. The results are shown below in Table 31.

Table 31. Results of silk analyses

	Original Study (159) ppm	Multi-element analysis ppm	R.S.D%
Ni	0.4	3.4 ± 2.5	73
Co	1	3.2 ± 2.7	84
Cu	20	21.5 ± 19.7	94
Ag	7	5.2 ± 1.8	35
Fe	20	14.9 ± 6.3	42

Nickel and cobalt give rather higher values but the others are in broad agreement between the single element analysis of the first study (159) and the multi-element analysis of this study. However, the precision of the analysis is very poor and this is a reflection of the biggest physical difference between silk and hair. The silk used was from untreated woven cloth (Pongees 504 1oz/yd^2) and each strand consisted of many fibres. The handling of the silk in one centimetre strands is very difficult because of the tendency of the strand to fray; this can lead to loss between balance and furnace unless the operator is extremely careful. Once the strand has been introduced into the furnace and is undergoing ashing it does not always melt into one spot but often remains stretched across the tube leading to only partial removal of organic material on ashing and poor atomisation. This is probably the cause of the poor precision and counts heavily against the use of silk as a standard hair.

A.2 Summary

The most extensively researched method of producing a standard hair has shown that it is possible to dope silk to the required level in up to five elements but that the silk itself is not suitable physically because of difficulties associated with its handling and with its ashing and atomisation in the furnace. Other ways of producing a standard hair must therefore be investigated and the most promising of these appear to be doping of human scalp hair and the use of animal fur.

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-24-	line 11 for Guin read Bryan *et al.*(105).
-49-	Ordinate should read LEAD CONCENTRATION ppm.
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