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REPORT

STUDIES OF RADIOACTIVITY AND HYDROTHERMAL PROCESSES IN PROTOBIOCHEMISTRY ON EARTH AND THE MOON

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ABSTRACT

Under Contract No. NASW-1508, with the National Aeronautics and Space Administration, the Nuclear Science & Engineering Corporation has performed studies related to abiogenic synthesis of protobiochemicals in a hydrothermal model system under the influence of ionizing radiation. The performance on the contract was from October 3, 1966 to March 31, 1967. During the contract, a successful demonstration of the abiogenic formation of sulfur-containing organic compounds including sulfur amino-acids as well as a number of non-sulfur organic compounds was made. The model system concept envisions chemical abiogenesis proceeding on the primitive Earth in hot aqueous environments subjected to ionizing radiation. It is proposed that similar propitious geochemical events and conditions on the Moon and Mars, similar to those operative early in the history of the Earth, may be conducive to the formation of extraterrestrial organic compounds at the present time.

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INTRODUCTION

The work reported in this document was performed under contract with the National Aeronautics and Space Administration to conduct studies of radioactivity and hydrothermal processes in protobiochemistry on Earth and the Moon.

Prior to the initiation of this contractual effort, considerable work on the role of radioactivity and hydrothermal processes in protobiochemistry had been carried out by Nuclear Science & Engineering Corporation under Contract NASW-989 (1). The earlier work had demonstrated that an abundance of biochemically significant organic compounds can be formed from the simplest chemical resources in hot, aqueous environments under the influence of ionizing radiation. Furthermore, it was shown that high-molecular weight peptides and proteinoids can be produced in the same unique model system thus providing mechanisms for the generation of macromolecules, an essential development for the evolutionary progression from chemical simplicity to prebiotic molecular complexity. Such experimental evidence for the possible geological origin of biochemical substances (chemical abiogenesis) suggested that spontaneous primordial chemical reactions might well have led to the production and proliferation of organic molecules in favorable local environments, e.g. hot springs, early in the history of the Earth. Results of ground-based protobiochemical studies assume an immediate significance for lunar and planetary exploration in view of the fact that propitious geochemical events and conditions on the Moon and Mars, similar to those present earlier in the history of the Earth, may provide primitive hydrothermal radioactive systems in which abiogenic formation of extraterrestrial organic compounds may have proceeded.

The specific purpose of this contract was to further pursue the formation of organic sulfur compounds in the model system since the earlier work had indicated the presence of sulfur amino acids in the hot irradiated aqueous solutions.

An extensive background and discussion of the NSEC model systems which embody ionizing radiation treatment of warm, aqueous solutions containing fairly complex mixtures of simple primitive chemical resources is contained in the previous report (1) and in the proposals to NASA dated April 24, 1963 (2), May 28, 1965 (3), and February 28, 1966 (4).

EXPERIMENTAL

I. Methods

A. Reaction Mixtures. Model systems containing sulfur in the primitive chemical forms of sulfide and thiocyanate were employed. Methanol was chosen as the principal source of carbon, ammonium chloride as the principal source of nitrogen, and pyrophosphates as the source of phosphorus. The compositions and treatments of the six model samples selected for investigation under the contract are given in Table I. A comparison is also provided between irradiated solutions and identically handled unirradiated solutions in both the sulfide series and the thiocyanate series. These unirradiated samples, termed "control", are in fact hydrothermal model systems in which prebiological syntheses may also proceed. In the previous study at NSEC (1) it was demonstrated that syntheses proceeded in both irradiation and hydrothermal systems, but that ionizing irradiation was much more productive.

The sample solutions were labeled with radioactive isotopes to facilitate the location and identification of separated compound spots on paper chromatograms. For each primitive form of sulfur studied, namely sulfide and thiocyanate, two identical solutions were used; one labeled with sulfur-35 to show the sulfur compounds formed and the other labeled with carbon-14 in the methanol carbon source to show the carbon compounds. Thus the finding of both sulfur-35 and carbon-14 at one spot indicates that a sulfur-containing organic compound was formed and is present. Location of sulfur-35 spot with no carbon-14 indicates that the compound is an inorganic form of sulfur. In the case of thiocyanate, however, the carbon of the thiocyanate was not labeled and a sulfur-labeled spot may be inorganic or organic sulfur since the carbon could originate from thiocyanate rather than from the labeled methanol. Finding a carbon-labeled spot with no sulfur indicates a non-sulfur organic compound originating from radioactive methanol.

For each primitive sulfur source studied, a third solution, termed the hydrothermal or control sample, was subjected to the same temperature treatment (55°C) but was not irradiated. These were labeled with both sulfur-35 and methanol-carbon-14. Previous experience indicated that the production of compounds under these conditions would be very much less than in the irradiated samples.

The reaction solutions, shown in Table I, were prepared in carefully cleaned and sterilized Pyrex glassware. Glassware was cleaned with detergents and with aqua regia, thoroughly washed, and rinsed with glass redistilled water. Before and after irradiation the solutions and fractions were stored in sterilized screw-cap vials at -15°C. They were thawed only for the minimum working period, and all glassware was heat-sterilized. These precautions were designed to minimize the possibility of bacterial and dust contaminations. In addition, the irradiation treatment itself was a sterilization process.

B. Irradiation Method. High voltage electron irradiation was achieved with a Van de Graaf accelerator at the Western New York Nuclear Research Center, Buffalo, New York. The solutions were irradiated at 50-55°C with 2×10^8 rads of 1.5 Mev electrons. The sample consisted of a 20 ml aqueous solution in a 75 mm diameter aluminum dish covered with a thin aluminum foil to reduce evaporation losses. All samples were stored and transported frozen at all times.

C. Analytical Procedures. The samples were fractionally distilled in sterilized glass apparatus under vacuum, and two successive volatile fractions were trapped at dry ice and liquid nitrogen temperatures; the residual fraction was expected to contain salts and inorganic compounds plus higher molecular weight organics. This fraction is particularly suitable for paper chromatography. The volatile fractions were analyzed by gas chromatography, along with some volatile derivatives which could be made from the undistilled material.

The fractionated samples were numbered by the following system. Series XII contained sulfide and Series XIII contained thiocyanate as the sulfur sources. Sample -A was sulfur-35 labeled and irradiated, -B was carbon-14 labeled and irradiated, and -C was the unirradiated control. Fraction -0 was the original sample, -1 and -2 were halves of the unvolatilized residue, -3 and -4 were halves of the volatile fraction trapped at dry ice temperature, -5 was the volatile fraction trapped at liquid nitrogen temperature. All fractions were stored in screw-capped, sterilized vials in a freezer at -15°C.

The first step of analysis was to achieve separation of each sample mixture, which contained both unconsumed reactants and products, into its chemical constituents. These separations were achieved principally by gas and paper chromato-

graphy. The second step was to identify as many as possible of the separated compounds. This was achieved by combinations of chemical, radioisotope and physicsl methods depending on the characteristics of each individual compound.

1. Paper Chromatography and Paper Electrophoresis. One of the analytical techniques selected was paper chromatography. It has the particular advantage of completeness when a radioactive tracer is used and is located by autoradiography. If a labeled compound is present in the sample its presence will necessarily be evident on the paper, with the sole exception of losses by volatilization. The sensitivity of detection is several orders of magnitude higher than chemical detections. The subsequent identification of located spots required comparisons with known standards, which are identified on paper by chemical tests or by radioactive labels.

A number of solvent systems for paper chromatography were considered. These included alcohol-ammonia and alcohol-acid types for amino acid and sugar separations. Three systems, used by others for separations of biological sulfur compounds, were tested. These were Solvent F (34) ethanol: tert-butanol:58% NH_4OH:water (60:20:5:15, v/v/v/v); Solvent G (34) tert-butanol:88% formic acid:water (14:3:3, v/v/v); and Solvent H [(35) - p. 165, (36)] 2, 6-lutidine: collidine: NH_4OH:water (20:20:0.3:20, v/v/v/v). To move lower Rf compounds and inorganics Solvent I (36) was also tested: phenol:water (80%:20%, w/v). Of these systems Solvents F and G were selected for two-dimensional separations, Solvent H for supplementary one-dimensional separations, and Solvent I was found of little value. There may be some acid degradation of certain sulfur forms in Solvent G. Whatman No. 1 paper was used throughout.

Amino acid standards were run as well as various available organic sulfur compounds. Several difficulties were encountered in the identification of standard compound spots on the paper chromatograms. Some of the color reactions in the literature did not work well, and considerable methods development time was required.

Tables II and III list standard amino acids and sulfur compounds, respectively, with sulfur amino acids duplicated in both tables. The amino acids were identified with the standard ninhydrin test; dipping the paper in 0.5% ninhydrin in

acetone, letting stand at room temperature overnight and finally heating 20 min. at 60°C. Table III lists other tests which were used. The neutral bromcresol green dip, 0.2% in acetone, was used for acids and some amines. However, its sensitivity was relatively low. Palladous iodide [(35) p. 131] showed white or gray spots on a gray background, with some compounds requiring several days for the spot to develop. The periodate-benzidine spray test (37) did not work well, apparently because of insolubility of the reagents. By increasing the concentration of periodate and benzidine and reducing the acetic acid, a transient blue was produced plus some development of gray spots on a gray-brown background after several days. The N-ethyl maleimide color [(35) p. 132)] seems the most promising test for sulfhydryl but its use was not initiated soon enough to check all of the standards.

When radioactive standards were available, they were run and were detected by autoradiography. The radioactive sulfide-S^{35} and thiocyanate-S^{35} standards were from the labeled substrates used in these experiments. Thiocyanate showed no impurities. The sulfide, however, showed a number of spots, indicating either labeled impurities or variations in chemical form such as oxidation products or possible polysulfide formation. The other radioactive standards used were labeled with carbon-14.

Autoradiographic exposures of the papers to Eastman Non-Screen or Industrial Type KK films required 11 to 27 days. Development was standard, using Liquid X-ray Developer and Liquid Fixer.

A number of reference standard non-radioactive sulfur compounds were not commercially available. Maps of the amino acids and the sulfur compounds are given in Figures 1 and 2. The corresponding identification symbols are listed in Tables II and III. Comparing the distributions on the two maps, the general patterns are similar, and the only serious overlaps are in the region near the origin. If more standards were added the number of coincidences would increase. In particular it is known from other work that maps of the important sugars and bio-organic acids would show patterns quite similar to these. Time precluded our preparing such additional maps.

The amino acid standards, Figure 1, represent biologically important members of the class. The sulfur standards, Figure 2, are much less comprehensive. They were limited by availability of chemicals and of identification reactions. The number of chemical possibilities for sulfur structures is very great.

On the sulfur map, Figure 2, two compounds varied in location depending on concentration and on the presence of other compounds. These were thiocyanate and taurine (symbols 7 and 4). Dotted lines for some spots indicate either uncertainty as to location or a lower detection sensitivity. Thiosemicarbazide and mercaptoacetic acid (symbols 10 and 12) produced two spots each, and sulfide (symbol 6) gave several as already mentioned.

On the non-sulfur map, Figure 1, tryptophan (symbol N) shifted location somewhat, and ethylene glycol (symbol Z) was doubled.

2. Electrophoresis. Paper electrophoresis offers an alternative separation to paper chromatography, based on migration in an electric field in ionized buffer solution. Molecular size, hydration and ionic charge contribute to differential movement rates between various ionizable solutes. A Spinco-Durrum apparatus (35) was used with 30 cm strips at 290 volts, and about 10.5 milliamps when 8 strips were in position. The pH 2.0 buffer (38) contained 31.2 ml formic acid plus 59.2 ml glacial acetic acid per liter. The pH 8.6 buffer was the standard Spinco barbital buffer used for serum proteins. Spot detections were by ninhydrin or Pd iodide color, and autoradiography of sulfur-35. Data are presented in Table IX.

3. Gas Chromatography. Considerable time was spent in attempting to achieve gas chromatographic separation and identification of the more volatile sulfur compounds. An F and M Gas Chromatograph with programmed temperature and flame ionization detector was used. The sample fractions which were analyzed were those volatile fractions from the vacuum distillation which condensed at dry ice temperatures. Amino acid analyses were achieved using the method of Lamkin and Gehrke (39) for the preparation of volatile n-butyl N-trifluoracetyl esters. One-half of the nonvolatile fraction of each experimental sample was used to prepare the derivatives.

II. Results

A. Paper Chromatography. Autoradiograms of two-dimensional chromatograms of the six experimental reaction mixtures in Table I were prepared. Typical photographic prints of these are shown in Figures 3 through 8, and a tabulation of the approximate numbers of spots detected in Table IV. The prints suffer loss of detail in reproduction. Several findings are immediately noticeable. The presence of sulfide and thiocyanate (Figures 3 and 6) resulted in many coincident sulfur spots plus a number that were different. The carbon-14 patterns for sulfide and thiocyanate (Figures 4 and 7) similarly showed many coincidences but also a surprising number of differences, including the formation of different non-sulfur organics. The hydrothermal nonirradiated samples (Figures 5 and 8) also show a number of compounds. Where these coincide with irradiated compounds the hydrothermal spots revealed that distinctly smaller quantities were formed. The double labeling with both carbon-14 and sulfur-35 must be taken into account in interpreting these two figures.

Table IV lists the approximate numbers of spots visible on the original autoradiograms discussed above. There is some uncertainty in cases of overlaps, and there may be some very faint traces which were overlooked. In the sulfide group of experiments, irradiation resulted in 52 sulfur-35 spots and 40 carbon-14 spots with about 15 of these being coincident, giving a total of 77 spots. The hydrothermal sample showed a combined total of 22 spots. Subsequent studies, discussed below, made it evident that there is substantial overlapping in some of these spots. There is some chemical difficulty in the handling and chromatography of sulfide solutions. The standard sulfide tends to form both polymers in alkaline solutions with ammonia and to alter valence states, principally via oxidation. Thus numerous inorganic sulfur forms are present and complicate the interpretations. Some of these forms are also either produced or altered by the irradiation treatment of the original sample solutions.

In the thiocyanate group of Table IV there were 45 sulfur and 43 carbon spots after irradiation, with 13 or 14 coincident for a total of about 74 compounds. The hydrothermal sample showed 16 spots. The standard thiocyanate

appears as a large central spot plus a number of others, but is not nearly as complex as the sulfide.

The large number of sulfur-35 spots in the upper right areas of the chromatograms (Figures 3-8) posed special problems of identification. These compounds were relatively soluble in the solvents used, and none of the standard reference compounds examined nor those reported in the literature are located in this area.

In summary, the two irradiated-hydrothermal protobiochemical model systems containing sulfur as either sulfide or thiocyanate and having methanol as the primary source of carbon were shown to have formed at least 75 spots representing abiogenic compounds in each, including many organic and inorganic forms of sulfur and also non-sulfur organic compounds. The heated, non-irradiated hydrothermal samples produced a much smaller number of compounds. However, as is shown later, interpretation is complicated by the presence of chromatography artifacts.

A comparison between an aliquot of the original solution and the nonvolatile sample fraction was made for each of the six reaction mixtures. The original solution contained the volatile compounds, which presumably would be lost during chromatography. The nonvolatile fraction was more concentrated, and the possibility exists that some further hydrothermal reactions could have occurred during the vacuum fractionation process. The original sample autoradiograms were essentially identical to the nonvolatile fractions but did show a few additional or more intense spots, particularly in the areas of high Rf. These additional compounds were assumed to be in the volatile fractions which were analyzed by gas chromatography. No further attention was given to the separation of the original solutions by paper chromatography.

Because of the lability of inorganic and organic sulfur compounds and because of the well known air oxidation tendency during paper chromatography, a test was made of the oxidation of the two hydrothermal control solutions and of the two sulfur-35 standards. Aliquots of the standards and of the control nonvolatile fractions were spotted on paper and allowed to stand 24 hours in air. Other aliquots were freshly spotted just before the solvent runs. The pairs of papers were run one dimensionally in tanks flushed with nitrogen gas. The autoradiographs were compared.

The air-oxidized sulfide standard and sample showed some increase in tailing and minor spot production in the region of Rf 0.30 to 0.70. Thiocyanate showed little difference between treatments except for the presence of a light spot at Rf 0.4 in the fresh sample. This was presumably a volatile material. These differences were not considered sufficiently great to warrant routine use of a nitrogen atmosphere. This conclusion should be carefully reviewed in light of the extensive artifact production described in the section on S-C bonding below.

The next step was to attempt identification of individual compounds.

When the sample autoradiograms were superimposed on the map of amino acids, Figure 1, there was a surprising lack of coinciding spots. There was a spot labeled with carbon-14 only near urea in the thiocyanate series. In the sulfide series of unknowns the area near the origin was too complex to interpret. The thiocyanate set and the other sulfide sample spots fell near to but not on some amino acids. This was interpreted initially as absence of amino acids. Subsequent results from gas chromatographic studies suggests that small distortions in the Rf of the unknowns may have obscured the identifications. Such distortions are common in the chromatography of complex samples. They could be checked by rechromatography of unknowns with added internal standards, but this was not attempted within the time available.

Similarly, the map of sulfur compounds, Figure 2, did not clearly indicate many coincidences for the same reasons. There were several autoradiographic spots in the region of taurine, more prominent in the case of thiocyanate samples than with sulfide samples.

The crowded areas near the origin (area A, Figure 9) which included the locations for cysteine, cysteic and homocysteic acids were cut out and eluted. The eluted samples along with standards were used for electrophoresis (see below) and for one dimensional chromatography in solvent G for an extended time of 96 hours. The area A fractions of both the sulfide-S^{35} and thiocyanate-S^{35} samples separated well. Both produced the same 5 chromatographic spots, which are listed along with the standards in Table V. Of the 5 spots, No. 4 coincided with homocysteic acid and the others remain unknown.

In a similar manner the thiocyanate paper areas near taurine and urea were cut out, eluted, and rechromatographed in Solvent H; Table VI gives these results. The urea area produced two sulfur-35 labeled spots, one of which was very volatile and actually moved ahead of the solvent front plus two carbon-14 labeled spots. None coincided with standard urea. The taurine area produced two sulfur-35 labeled spots, one of which coincided with standard taurine. The lack of a carbon-14 labeled taurine spot indicates that the carbon precursor had to be solely the thiocyanate carbon, with no contributions from labeled methanol. Further taurine separations were made by electrophoresis and are described later.

B. S-C Bonded Compounds. A direct comparison was made between pairs of sulfur-labeled and carbon-labeled spots which seemed to correspond to each other on the pairs of the dimensional chromatograms. The autoradiogram pairs were superimposed and the irregular areas which were blackened on both the S^{35} and the C^{14} autoradiograms were marked. This was done with both the sulfide series and the thiocyanate series. Figure 9 gives a map locating 12 to 15 approximate areas which did so coincide.

The marked areas were then cut from the corresponding papers, eluted with 10% isopropanol, and concentrated by evaporation with a stream of nitrogen to about 0.1 ml. Each sample was divided into about half. One half was spotted on paper and the second half mixed with its corresponding other eluate and then spotted. These papers were run one dimensionally in Solvent H. Thus the Areas C from the sulfide pair of chromatograms gave three new samples: S^{35} labeled, C^{14} labeled, and a mixed spot with S^{35} plus C^{14}. Similarly each other area pair was run. Then the completed papers were autoradiographed for 24 days. (It should be noted that the entire procedure consumed about 2 weeks for sample preparation and chromatography plus two consecutive 3-week autoradiogram exposures, for a total of 8 weeks elapsed time.) Time prevented comparing every possible spot and precluded any reruns.

Comparisons of the new autoradiographic spots in the above sets of 3 were then made as to Rf and to relative blackening (relative label concentrations). Tables VII and VIII summarize these data. Comparisons were also made to standard sulfide-S^{35} and standard thiocyanate-S^{35}. If within a set there is produced

a newly separated spot corresponding to S^{35} and an identically behaving spot corresponding to C^{14} then the assumption is made that the spot represents a single organic compound containing sulfur. If there is no such correspondence then it is assumed that the original eluted area contained two or more compounds, one sulfur-labeled and one carbon-labeled. Comparisons to the standards helps identify unreacted standard and other inorganic forms.

In Solvent H the standard sulfide-S^{35} gave 3 spots near the origin and 7 other spots from Rf 0.25 to 0.90. For both substrates the eluted samples showed numerous correspondences to standard sulfur spots, invariably including the 3 origin spots. In the "only S" column in Tables VII and VIII about half the spots coincide with standards. This indicates that the paper chromatographic method is inducing artifacts of interconversions of chemical forms of sulfur. The cause may possibly be air oxidations plus changes in S-S type polymerizations. Air oxidation might be preventable by working in inert gas atmospheres, as was tried initially. Unfortunately these results were not available until near the end of the experimental period, precluding modification or studies of the methods. Lack of time also eliminated studies of the chromatography of inorganic sulfur forms, which might have indicated the chemistry of the conversions.

The thiocyanate-S^{35} standard showed two trace spots near the origin and one major one at Rf 0.89. In the experimental samples, Table VIII, there was some apparent exchange labeling of thiocyanate with substrate carbon-14 from the labeled methanol (samples marked with *). There was also chromatographically induced degradation of sulfur forms to give some spots corresponding to the sulfide standard. This latter effect is not due to radiation decomposition products from the original substrates, which would not be present in these eluted samples in the patterns observed.

The above complex chemistry is quite in accord with the chemistry of sulfur. It makes interpretation of the identify of original irradiation products much more difficult. On the other hand it indicates that an irradiated original solution contains a very complex set of quite reactive molecules and consequently represents many possible intermediate and final products.

Several effects in addition to the above artifacts were considered in interpreting the data in Tables VII and VIII. Usually only one sulfur atom per molecule is expected, except in polymers. More than one carbon atom per molecule is quite likely, but in the thiocyanate set, Table VIII, the thiocyanate carbon was not labeled originally while the methanol carbon was. Therefore, the C-S ratio within the structures of two different compounds may vary. The initial specific activity of sulfur was higher than carbon, but sulfur has an 87 day half-life. The quantity of various products may range from faint traces to a substantial amount. All these factors influence the autoradiographic image intensity and the subsequent interpretation.

From the sulfide data, Table VII, it can be seen that of 12 areas studied, 8 contained compounds relatively heavily labeled with both S and C. Several of the areas contained more than one such spot, but a conservative assumption would be that there was only one such compound originally and this broke down during chromatography in Solvent H. It would appear to be quite difficult to test this assumption. The column "light C - light S" may be similar but containing those products present in much lower yield. The column "heavy C - light S" could include compounds with high C-S ratio, that is longer carbon chains. The "light C - heavy S" column is more difficult to explain unless it contains compounds such as polysulfides. In all four columns, of course, there remains a possibility that a spot contains two or more different compounds which happen to chromatograph similarly. The "only S" column includes chromatographic degradation products as already discussed. The "only C" column represents non-sulfur organics derived from the labeled methanol substrate. These methanol products were discussed in more detail in the report of Contract NASW-989 (1). Comparing across the columns for each area, it is confirmed that coincident C^{14} and S^{35} spots do represent sulfur-organic compound syntheses in almost every case.

A similar situation and similar comparisons were observed in the thiocyanate studies, Table VIII. Approximately 15 coincident S and C spots were found which represent organic sulfur compounds. The picture is somewhat complicated by the unlabeled thiocyanate carbon atom, and its exchange with methanol-C^{14} (indicated by the *). The production of "only C" compounds was more pronounced than in the sulfide series.

C. Paper Electrophoresis. Paper electrophoresis was used in an attempt to improve the separations in the sample areas near the origin on the two-dimensional chromatograms. Since this area includes the sulfides and other inorganics, it was thought that the rapid movement in electrophoresis might move them away from the organic sulfur. Two pH's were used; that of the original irradiation, pH 8.6, and one strongly different and recommended for amino acid separation, pH 2.0.

Data from the autoradiograms of paper electrophoretic strips are shown in Table IX. The heaviest bands are underlined. In order to improve the separations of the crowded spots near the origin of the two-dimensional paper chromatograms the area (Area A of Figure 9) was cut out of the paper, eluted with 10% isopropanol, reconcentrated and applied to the electrophoresis strip. Thus, the final separation is the total of two paper solvents plus the electrophoresis run.

By comparing the Sample XII-A, Area A (irradiated sulfide-S^{35}) in Table IX with the standards, it is seen that there is some residual sulfide, for example, the band at +4.0 for pH 8.6. However, most of the sample bands do not coincide with either sulfide or any of the organic standards. It is noteworthy that at pH 2.0 the amino acid standards ran in the negative direction while the sample radioactivity ran in the positive direction. The very fast bands at pH 2.0 may be other inorganic forms, such as sulfate.

Sample XIII-A, Area A (irradiated thiocyanate-S^{35}) showed the +4.0 band at pH 8.6 which was also characteristic of sulfide residue, indicating possible degradation of thiocyanate. The sample showed no correspondences to any of the standards listed. The listed standards are those compounds falling in or near Area 1 on the sulfur chromatogram map, Figure 2.

Thus, the compounds in Area A for both samples remain unidentified, but with a number of possible sulfur compounds eliminated.

In a similar manner the chromatogram areas near taurine (symbol 4, Figure 2) were tested. The sulfur spots in this area were not taurine in either the sulfide or thiocyanate experiments. This contrasts slightly with the paper chromatographic results in Table VI which showed possible taurine-S^{35}.

D. Gas Chromatography. The results of gas chromatographic separations and identifications are presented in Table X which shows the retention times and elution temperature of peaks observed on a carbowax column which affords good separations of sulfur compounds. Fourteen to twenty-eight peaks were seen in each irradiated sample and eight to eleven in the hydrothermal samples. More compounds were formed from sulfide than from thiocyanate.

The compounds identified are listed in Table XI. Allyl sulfide, $(CH_2=CH-CH_2-)_2S$, and diethyl disulfide, $(CH_3-CH_2-)_2S_2$, are the two sulfur derivatives found. The alcohols include n-propanol, n-butanol, tert-butanol, and isopentanol. Other probable non-sulfur compounds include semicarbazone and a group of acids (acetic, propionic, and butyric). In addition, there is a group of interesting compounds which make a single broad peak on the columns tried so far; this group includes 2-aminoethane thiol, mercaptoacetic acid, thioacetic acid, semithiocarbazole and three alcohols (methanol, ethanol, and isopropanol). Since methanol was the original carbon source all the experimental samples have a large peak at this point, and the four irradiated samples have a double peak.

The identifications were hampered by the unavailability of many desired reference standards. Table XII lists sulfur and alcohol standards for this study. In summary, numerous peaks were separated from the volatile fraction of each irradiated sample and a few peaks from the hydrothermal samples. Several peaks were identified.

No further attempt was made to separate the group of compounds mentioned above which contained alcohols, thio-acids, etc. Instead, the available time was directed to amino acid analyses using the method of Lamkin and Gehrke for preparation of volatile n-butyl N-trifluoracetyl esters (39). The n-butyl n-trifluoroacetyl esters of several standard amino acids were freshly prepared (Table XIII) for gas chromatography (39). In addition, other amino acids may be identified by interpolation using the relative retentions given by Lamkin and Gehrke. In the present study, the glycine-alanine peaks were interchanged from their values given by Lamkin and Gehrke. Moreover, variable results at the position of tyrosine were obtained and the possibility that either a false peak or no tyrosine derivative formation occurred was acknowledged. Standard cysteine was very difficult to prepare.

The three sulfur-containing amino acids, cysteine, methionine, and ethionine, were of particular concern. The latter two, with more complex molecular structures, are expected to occur in much lower yield. The alternate oxidation states, the sulfoxides and sulfones, of methionine and ethionine appeared as the parent acids in this method. Peaks appearing before glycine were thought to be amines and esters. Results are given in Table XIII. The irradiated samples contained more compounds than the hydrothermal ones, and appeared to contain higher yields. Although cost and time limitations precluded running blanks on freshly prepared original solutions, previous experiences suggest that peaks labeled "trace" are products rather than contaminants.

In the two sulfide group samples (Table XIII, the irradiated sample contained 7 major and 26 minor peaks while the hydrothermal sample contained 7 major and 19 minor ones. Glycine and possible tyrosine were present in both; alanine was present only in the irradiated sample. The irradiated sample contained apparent traces of all 3 sulfur-amino acids while the hydrothermal sample only had a small trace of cysteine. There are also many differences in the unidentified peaks.

The two thiocyanate group samples, Table XIII, show more total peaks and more major peaks than the sulfides. Irradiated thiocyanate samples yielded 13 major and 27 minor peaks while the hydrothermal thiocyanate has 4 major and 22 minor peaks. Both samples contained glycine, alanine, tyrosine (or artifact), cysteine, and methionine. The irradiated sample also has major leucine and serine peaks and a trace of ethionine. Furthermore, large quantities of cysteine and methionine were found. Hydrothermal cysteine is the largest peak (Table XIII) and glycine is the second largest. By contrast, the irradiated methionine is high and cysteine low. There is a possibility that irradiated cysteine is slightly accelerated in its progress through the column and is really the peak at 834 sec-152°C identifed as serine. Sample XIII-B yielded a spectrum essentially identical to that of XIII-A in Table XIII. These two samples are identical in composition and treatment except for radioisotope labeling.

In summary, gas chromatographic separations of amino acid derivatives showed 33 and 40 products formed from irradiated sulfide and thiocyanate solutions and lesser numbers (26) from hydrothermal solutions. The sulfur amino acids cysteine, methionine and probably ethionine were formed. Non-sulfur glycine, alanine, and probably leucine, serine and tyrosine were also identified.

More reliance is placed on the amino acid identifications via gas chromatography than on the paper chromatographic studies. The latter were obscured by extensive artifact formations on the paper plus interferences by inorganic forms. The gas chromatographic method will detect smaller quantities than ninhydrin on paper.

DISCUSSION AND SUMMARY

The general literature on prebiological chemistry and exobiology has been reviewed in the previous NSEC proposals and reports (1-4). The results of the initial study on the role of radioactivity and hydrothermal processes in protobiochemistry demonstrated that an abundance of organic micromolecules as well as complex macromolecules can be produced in hot aqueous solution subjected to the influence of ionizing radiation. The present study confirmed that sulfur-containing organic compounds including sulfur amino acids can be formed in similar model systems from sulfide or thiocyanate sources of sulfur.

In present day biology sulfur enters the biocycle in inorganic forms and is converted principally to active sulfhydryl compounds such as the sulfur amino acids and mercaptans. Plants principally absorb sulfate, the most oxidized natural form, and convert it by a series of reduction reactions to the most reduced sulfhydryl form (5, 6, 7, 8, 9). Various microorganisms, however, exhibit a much wider range of reactions; and specific species can either oxidize or reduce all the natural inorganic forms of sulfur, including sulfide, elemental sulfur, thiosulfate, sulfite and sulfate (10).

The most important biochemical function of sulfur is its role in the structure and reactivity of protein, especially enzymes and structural proteins such as keratin and collagen (5, 11, 12). Here the reactivity of the sulfhydryl groups of cysteine and methionine and their propensity for forming S-S bonded bridges between segments of the peptide chain are dominant. Some chemotrophic microorganisms utilize the oxidation of inorganic sulfur as their primary source of chemical energy, and other microorganisms require inorganic sulfur as part of their electron transport system in photosynthesis (9, 13). Numerous other functions of sulfur are indicated by its presence in the molecules of coenzyme A, insulin, mustard oil gluosides, djenkolic acid, thetin, sulfa drugs, glutathione, thiamine, biotin, and chondroitin sulfate (5, 6, 14, 15, 16).

The numbers of compounds of sulfur, their varieties of organic structural classes, and the reactivity of the compounds, including their oxidation state shifts, all indicate the complexity of the problem in the detection and identification of unknown sulfur compounds. The general chemistry of organic sulfur compounds has been

described extensively (14, 17, 18, 19), and the inorganic forms are described in numerous inorganic texts. The reactions resulting from ionizing radiation would be expected to result in the production of very large numbers of compounds falling into most of the chemical classes. These would total many hundreds, even thousands of possibilities. In a study such as the present contract it is only feasible to examine a selected few examples in the classes closely related to known biochemically important substances.

The forms of sulfur available on the primitive earth have received less attention than other biologically essential elements. Vinogradov (20) considers most sulfur to be in the form of iron and heavy metal sulfides in the mantle with volcanic hydrogen sulfide and elemental sulfur emitted into the atmosphere. These atmospheric forms became subject to oxidation as soon as oxygen became available. Rubey (21) also postulates atmospheric H_2S present at 2% in the atmosphere and in equilibrium a solution of 12×10^{20} grams in the ocean. Various sulfide minerals have been found in meteorites, including the iron sulfides (22), and the recently reported niningerite (23) which is a mixed Fe-Mg-Mn-Ca-Cr sulfide.

Very little has been reported on the protobiochemical synthesis of sulfur compounds, for the reason that most workers have not included sulfur in their reaction systems. Oro' (24) has speculated briefly on possible mechanisms leading to cysteine from thioformaldehyde, and to methionine from methyl mercaptan. Dauvillier (25) mentions thiophene synthesis by two possible routes. Wald (26) discusses the role of sulfur high energy bonding in metabolism. Snell (27) describes thermal synthesis of cysteine from serine plus H_2S catalyzed by pyridoxal.

The radiation chemistry of sulfide minerals and other sulfur forms was treated by Nanobashvili, et al (28), who showed the production of a variety of inorganic oxidation products. Na_2S and mineral sulfides were oxidized to sulfate. Thiocyanate behaved similarly to sulfide but included a greater variety of products. Carbon disulfide, thiophene, various mercaptans and thiourea were irradiated both pure and in aqueous suspensions to produce oxidations of the sulfhydryl grouping to give disulfides and sulfo compounds with an accompanying large decrease in pH. The amino acids cysteine and cystine were interconverted by x-irradiation (29). Krampitz and Knappen achieved thermal copolymerization of protein hydrolysates labeled with sulfur-35 (30) and fed the products to rats (30, 31).

The most direct study of sulfur protobiochemistry has been by Choughuley and Lemmon (32) who irradiated a primitive atmospheric mixture containing hydrogen sulfide, methane, ammonia and water vapor with 10^9 rads from an electron beam. The H_2S was sulfur-35 labeled. Cysteic acid, taurine and cystamine were produced. There was also some evidence for cysteine and cystine, which probably were oxidized to cysteic acid during chromatography; paper chromatography and autoradiography were the analytical methods employed. A previous study by Heyns et al (33) under somewhat similar conditions failed to produce amino acids, but ammonium thiocyanate was tentatively identified. More recently, an attempt to produce cysteine under simple primitive conditions by sparking a mixture of alanine and sodium sulfide was not successful (40).

The previous study at NSEC (1) on irradiated aqueous systems incorporated sulfur, as sulfide, in the reaction mixtures. Indications of five product sulfur compounds were found, viz. cysteine, methionine, propyl mercaptan, allyl sulfide and thiophene. The carbon source for all of these was methanol.

In the current study, sulfur sources selected for study were sulfide and thiocyanate. These forms of sulfur are considered to represent primitive earth resources under reducing conditions. Methanol was chosen as the principal carbon source because of its demonstrated reactivity in the previous study (1); carbon was also provided by thiocyanate in one-half of the reaction mixtures.

The irradiation of warm aqueous solution of the simple chemical resources shown in Table I yielded an abundance of organic compounds detected by paper chromatography, paper electrophoresis and gas chromatography. From sulfide sources, approximately 77 irradiation products and 22 hydrothermal products were detected by paper methods whereas gas chromatography showed the volatile fractions to contain fourteen and eight compounds, respectively. Thiocyanate sources of sulfur yielded 74 irradiation and 16 hydrothermal products by paper techniques and 28 irradiation and 11 hydrothermal peaks by gas chromatography. However, the paper separations were found to be subject to chemical changes during chromatography which seemed to interconvert some related sulfur compounds. On the other hand, some of the paper spots also may have represented two or more compounds. The gas chromatographic separations may contain some overlaps with the paper spots; therefore, the total number of compounds formed cannot be ascertained.

The most difficult aspect of the project was compound identification after separation. Three major problem areas were contributory. The complexities of sulfur chemistry permitted reaction or change during the analytical procedures. There were large numbers of possible sulfur products, so that only relatively few could be sought. Methods were frequently inadequate, requiring much development time. There were also practical hinderances such as instrumentation problems, procurement delays, and difficulties in scheduling the sequential steps in the program.

The sulfur-containing compounds which were definitely identified or presumably detected included cysteine, methionine, ethionine, homocysteic acid, allyl sulfide, diethyl disulfide, and possibly taurine. Non-sulfur products included n-propanol, n-butanol, tert-butanol, isopentanol, and in addition probably semicarbazone, several lower fatty acids and additional simple alcohols. The formation of S-C bonds was shown in a dozen or more paper spots in both the sulfide and the thiocyanate series.

It is clear that the magnitude of detailed investigations of sulfur protobiochemistry is much more comprehensive than was possible under the present scope of work. Additional analytical effort would be required to identify additional unknown compounds found and clarify some of the uncertainties.

The productivity in protobiochemical syntheses of irradiated aqueous model systems has been demonstrated to be very great. The analogous hydrothermal models are only slightly productive. There are good arguments for considering radioactive hydrothermal models at least as prominently as atmospheric models in theories of chemical abiogenesis and molecular evolution.

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TABLE I

COMPOSITION OF REACTION MIXTURES

Millimoles per 20 ml aqueous solution (pH adjusted to 8.5 with NH_4OH)

Chemical Composition	Solution XII (sulfide) Irradiated A (S-35)	Irradiated B (C-14)	Hydro-thermal C (control)	Solution XIII (thiocyanate) Irradiated A (S-35)	Irradiated B (C-14)	Hydro-thermal C (control)
Methanol	23.6	23.6	23.6	23.6	23.6	23.6
Methanol-C-14	-	3.0 mc	2.0 mc	-	3.0 mc	2.0 mc
Na_2S	1.0	1.0	1.0			
Na_2S-S^{35}	5.0 mc	-	5.0 mc			
K thiocyanate				1.0	1.0	1.0
K thiocyanate-S^{35}				5.0 mc	-	5.0 mc
K_2HPO_4 Na_2HPO_4	1.0	1.0	1.0	1.0	1.0	1.0
NH_4Cl	5.0	5.0	5.0	5.0	5.0	5.0
Elemental Composition						
C	23.6	23.6	23.6	23.6+1.0	23.6+1.0	23.6+1.0
N	5.0	5.0	5.0	5.0+1.0	5.0+1.0	5.0+1.0
S	1.0	1.0	1.0	1.0	1.0	1.0
P	1.0	1.0	1.0	1.0	1.0	1.0
K	2.0	2.0	2.0	1.0	1.0	1.0
Na	2.0	2.0	2.0	2.0	2.0	2.0
Cl	5.0	5.0	5.0	5.0	5.0	5.0

* Not including NH_4OH for pH adjustment

TABLE II

STANDARD AMINO ACIDS AND NON-SULFUR COMPOUNDS

* sulfur containing
** mapped with radioactive compound

Compound		Map Symbol (Figure 1)
cysteine	*	C
homocysteine	*	X
methionine	*	M
methionine sulfoxide	*	MX
methionine sulfone	*	MO
ethionine	*	E
arginine		A
ornithine		O
lysine		L
histidine		H
aspartic acid		K
asparagine		J
glutamic acid		G
glycine		Y
serine		S
threonine		T
β-alanine		B
proline		P
tyrosine		R
tryptophan		N
valine		V
phenylalanine		Q
leucine		I
isoleucine		IL
urea	**	U
lactic acid	**	W
ethylene glycol	**	Z

TABLE III

MAP SYMBOLS AND COLOR TESTS FOR STANDARD SULFUR COMPOUNDS ON PAPER CHROMATOGRAMS

positive = +, doubtful or partial = (+), negative = 0

Compound	Structure	Map Symbol (Fig. 2)	Color Tests (a)				
			Ninhydrin	Bromcresol Green	Pd Iodide	Benzi-dine	Malei-mide (C)
cysteine	$HS-CH_2-CHNH_2-COOH$	C	+				
homocysteine	$HS-CH_2-CH_2-CHNH_2-COOH$	X	+				
methionine	$CH_3S-CH_2-CH_2-CHNH_2-COOH$	M	+				
methionine sulfoxide	$CH_3SO-CH_2-CH_2-CHNH_2-COOH$	MX	+				
methionine sulfone	$CH_2SO_2-CH_2-CH_2-CHNH_2-COOH$	MO	+				
ethionine	$CH_3CH_2S-CH_2-CH_2-CHNH_2-COOH$	E	+				
L-cysteic acid	$HOSO_2-CH_2-CHNH_2-COOH$	1	+	(+)	0	0	
homocysteic acid	$HOSO_2-CH_2-CH_2-CHNH_2-COOH$	2	+	(+)	0	0	+
cysteine sulfinic acid	$HOSO-CH_2-CHNH_2-COOH$	3	+	+	+	+	
taurine	$HOSO_2-CH_2-CH_2NH_2$	4	+	(+)			
methyl sulfuric acid	$HOSO_3-CH_3$	5		+	0	(+)	+
sulfide (b)	S^-	6	0	0	0	0	
thiocyanate (b)	SCN^-	7	0	0	0	0	
thiourea (b)	$NH_2-CS-NH_2$	8	0				
methyl thiourea (b)	$NH_2-CS-NHCH_3$	9	0				
thiosemicarbazide	$NH-CS-NHNH_2$	10	0	+	+	(+)	
thiol acetic acid	CH_3-COSH	11		(+)	0	0	+
mercaptoacetic acid	$HS-CH_2-COOH$	12		(+)	0	0	+
aminoethane thiol (mercaptoethylamine, thioethanolamine)	$HS-CH_2-CH_2NH_2$	13	0	+	+	+	
thioacetamide	$CH_3-CS-NH_2$	14	0	(+)	+	0	
thiazole	$CH=CH-N=CH-S$	15	0	(+)	0	(+)	+
thiolactic acid (d)							+

(a) see text for methods
(b) radioactive standard was used
(c) Maleimide data incomplete
(d) tested only for maleimide

TABLE IV

NUMBERS OF SPOTS SHOWN BY 2-DIMENSIONAL CHROMATOGRAPHY-AUTORADIOGRAPHY OF THE NON-VOLATILE FRACTION OF SIX MODEL SOLUTIONS

Sample No.	Form of sulfur	Isotope present	Treatment	Number of spots	Spots in common
XII-A-1	sulfide	S-35	irradiated	52	15
XII-B-1	sulfide	C-14	irradiated	40	
XII-C-1	sulfide	S-35+C-14	hydrothermal	22	
XIII-A-1	thiocyanate	S-35	irradiated	45	13-14
XIII-B-1	thiocyanate	C-14	irradiated	43	
XIII-C-1	thiocyanate	S-35+C-14	hydrothermal	16	

TABLE V

RECHROMATOGRAPHED AREA A FRACTIONS AND STANDARDS

Distance of spots from origin after 96 hours in Solvent G

Spots	Standards	Irradiated sulfide-S^{35}	Irradiated thiocyanate-S^{35}
Cysteine	9 cm		
Unknown No. 1		11 cm	11 cm
Unknown No. 2		14	14
Cysteic acid	16.5		
Unknown No. 3		17.5	17.5
Homocysteic acid	21.5		
Unknown No. 4		21.5	21.5
Unknown No. 5		26	26
Homocysteine	27.5		
Cysteine sulfinic acid	28		

TABLE VI

RECHROMATOGRAPHY OF TAURINE AND UREA AREAS IN THE THIOCYANATE SAMPLES, USING SOLVENT H

	Rf		
	Standard	S^{35} sample	C^{14} sample
Urea area (see map Fig. 1 for location)			
Urea-C^{14}	.48		
Unknown No. 1		.56	
Unknown No. 2		(1.0+)	
Unknown No. 3			.27 trace
Unknown No. 4			.40
Taurine area (see map Fig. 2 for location)			
Taurine	.35		
Unknown No. 1		.10	
Unknown No. 2		.36	

TABLE VII

SOLVENT H SEPARATIONS OF ELUTED SULFIDE CHROMATOGRAM AREAS

Numbers of labeled spots

Area Fig. 9	Heavy C Heavy S	Heavy C Light S	Light C Heavy S	Light C Light S	Only S	Only C
B	1		3		7	
C (below)			1		7	
C (above)	2			4	2	2
E			1	1	8	
F	1		2		11	
G		2			10	
H	4	1	2	1	1	
I			1	2	5	3
J	3				8	
K	2			1	5	
M	1		1		6	
N	2				7	

TABLE VIII

SOLVENT H SEPARATIONS OF ELUTED THIOCYANATE CHROMATOGRAM AREAS

Numbers of labeled spots. p = major spot, * = thiocyanate-C^{14} in one spot.

Area Fig. 9	Heavy C Heavy S	Heavy C Light S	Light C Heavy S	Light C Light S	Only S	Only C
A	2			2	7	
C-D	2		1	3	4	6
F	2*		7		3	
H-J	1*	3		2	3	1
I	1		1*	1	5	4 (1 p)
L			1	2	8	4 (1 p)
M	2*		1	2	3	1 (1 p)
N	1*				4	

TABLE IX

PAPER ELECTROPHORESIS OF STANDARDS AND EXPERIMENTAL SAMPLES

cm per hour at 10 V/cm. See text for methods. Heavy bands are underlined.

Sample	pH 8.6	pH 2.0
cysteine	0.0	-3.7
homocysteine	0.0	+1.0
cysteic acid	+3.3	+0.9
homocysteic acid	+3.7	-0.9, -2.4
cysteine sulfinic acid	+3.0	+1.7
thiosemicarbazone		-2.0
taurine		0.0
17 amino acids		-2.2 to -6.4
sulfide-S^{35}	+4.0 (tailing)	-7.5 (trace)
	+6.8	0.0 - +2.7 (trace)
	+8.3	+2.8 (trace)
		+3.8 - +10.0
		+10.5
thiocyanate-S^{35}	0.0 (trace)	
	8.3	
Sample XII-A, area 1 (Irradiated sulfide-S^{35} near origin)	-0.5 (trace)	-18.0
	0.0	-12.0
	+1.5 - +3.0 (trace)	- 0.5
	+3.2	+ 2.0
	+4.0	+ 4.2
	+4.8	+ 9.5 (trace)
	+5.5	+13.0 (trace)
	+5.6 - 8.0	+33.6
		+34.0 - + ?
Sample XIII-A, area 1 (Irradiated thiocyanate-S^{35} near origin)	0.0 (trace)	0.0
	2.2	+ 3.8
	4.0 (tailing)	+ 4.5 edge effects
	4.8 (tailing)	+ 5.5 " "
	5.3	+10.0 " "
	5.4 - 7.5	+12.5 " "
		+12.5 - + ? (trace)
Sample XII-A, area 2 (Irradiated sulfide-S^{35} (near taurine)		- 0.4
		+ 1.0
		+ 5.6
		+13.5 (trace)
Sample XIII-A, area 2 (Irradiated thiocyanate-S^{35} near taurine)		- 0.4
		+ 1.8
		+ 2.8

TABLE X

GAS CHROMATOGRAPHIC PEAKS FROM VOLATILE FRACTIONS OF SIX MODEL SOLUTIONS

Column: 8' x 1/8", 10% carbowax-20M. Temperature program, 5°C per min. Sample fraction trapped at dry ice temperature. Numbers are: retention time in seconds, and elution temperature in °C. Brackets indicate broad double peaks.

	Sulfide			Thiocyanate	
XII A-3 irradiated	XII-B-3 irradiated	XII-C-3 hydrothermal	XIII-A-3 irradiated	XIII-B-3 irradiated	XIII-C-3 hydrothermal
44 (63°)	40 (63°)	47 (63.5°)	50 (64°)	51 (64°)	70 (65°)
47 (64°)	48 (64°)	151 (71.5°)	79 (66°)	74 (66°)	78 (66°)
64 (65.5°)	91 (67.5°)	160 (72.5°)	98 (68°)	95 (67.5°)	99 (68°)
84 (67°)	132 (71°)	{200 (75°)	144 (72.5°)	140 (71.5°)	159 (72.5°)
99 (68°)	151 (72.5°)	231 (78.5°)}	163 (74°)	159 (73°)	{200 (76°)}
158 (73.5°)	181 (75°)	409 (93.5°)	190 (76°)	243 (80°)	{233 (79°)}
171 (74.5°)	{200 (77°)	527 (103.5°)	{215 (78°)	261 (81.5°)	455 (97°)
197 (77°)	224 (78.5°)}	604 (110°)	241 (80.5°)}	379 (91°)	608 (109°)
231 (79°)	248 (80.5°)	521 (103°)	265 (82.5°)	406 (93°)	631 (112°)
244 (80°)	367 (90°)	{641 (112°)	384 (92.5°)	422 (95°)	
271 (83°)	381 (91.5°)	658 (113.5°)}	409 (94.5°)	482 (100°)	
388 (92°)	486 (100°)	845 (129°)	654 (114°)	535 (104°)	
399 (92.5°)	518 (102°)	1049 (146°)	874 (133°)	610 (110°)	
420 (95.5°)	541 (104.5°)		940 (138°)	652 (114°)	
509 (103°)	600 (110°)		1062 (147°)	862 (130°)	
542 (106°)	637 (112.5°)			906 (135°)	
{596 (109°)	709 (119°)			1013 (144°)	
605 (111°)}	740 (120.5°)				
639 (112.5°)	919 (136°)				
850 (131°)	975 (140.5°)				
750 (139°)	1095 (150.5°				
1048 (147°)	1125 (153.5°)				
1111 (152.5°)	1185 (158°)				
1229 (163°)	1216 (160.5°)				
1331 (171°)	1283 (166°)				
	1304 (167.5°)				
	1338 (171.5°)				
	1422 (178.5°)				
	1456 (180°)				
Total peaks					
24	28	11	14	17	8

TABLE XI

VOLATILE COMPOUNDS IDENTIFIED BY GAS CHROMATOGRAPHY

Column: 8' x 1/8", 10% carbowax-20M. Temperature program 5°C per min. Complete list of peaks observed is in Table IX. Numbers are retention time in seconds and elution temperature in °C.

SULFIDE			THIOCYANATE		
XII-A Irradiated	XII-B Irradiated	XII-C Hydrothermal	XIII-A Irradiated	XIII-B Irradiated	XIII-C Hydrothermal
semicarbazone 231 sec, 79°	n-butanol 600 sec, 110°		allyl sulfide 654 sec, 114°	2-amino ethane thiol 261 sec, 81.5°	
tert-butanol 420 sec, 95.5°	allyl sulfide 637 sec, 112.5°				
				n-butanol 610 sec, 110°	
n-propanol 509 sec, 103°	ethyl disulfide 709 sec, 119°				
allyl sulfide 639 sec, 112.5°	isopentanol 740 sec, 120.5°				
methanol + others 230-258 sec, 78-81°	methanol + others	methanol	methanol + others		methanol
	acetic, propionic, butyric acids 4 peaks: 1304-1456 sec, 167-180°				

TABLE XII

GAS CHROMATOGRAPHIC STANDARD VOLATILE SULFUR COMPOUNDS AND ALCOHOLS

Column: 10% carbowax-20M. Initial temperature 60°C, increasing 5°/min; He rotameter 0.6. Flame ionization detector. Sulfur compounds underlined.

Standard	Peak Seconds	Peak Temp. (C)
ethyl sulfide	224	78°
thioacetic acid	239	79
methanol	230	78
	239-245	79.5
ethanol	239	80
mercaptoacetic acid	244	79.5
isopropanol	266	82
semithiocarbazone	266	82
thioethanolamine	277	82.5
thiophene	384-419	91-94.5
tert-butanol	424	95
n-propanol	430	95.5
water	-	-
n-butanol	630	111
allyl sulfide	645	113
ethyl disulfide	713	118
isopentanol	736	120.5
n-hexanol	999	142.5

TABLE XIII

GAS CHROMATOGRAPHY OF AMINO ACID DERIVATIVES

Column: 1/8" x 5'; 2% neopentyl glycol succinate on Gas Pak S. Initial temperature 100°C; increasing 4°/min; He flow meter 1.35. Flame ionization detector. Peak heights: ++, +, trace; Parenthesis indicates less certainty.

Sample	Peak Seconds	Peak Temp. (C)	Peak Height	Compound identity
STANDARDS				
cysteine	880	156	tr	
	916	158		
methionine	1075	168	++	
(ethionine)	(1125)	(179)	++	
glycine	435	126	++	
alanine	645	140	++	
tyrosine	(1450)	(190)	++	
XII-A	98	103	+	
irradiated,	118	104	++	
sulfide	151	107	++	(amines
	194	109	tr	and
	285	115	tr	esters)
	308	116	++	
	354	120	tr	
	398	122	tr	
	430	124.5	+	
	436	126	+	glycine
	489	129	tr	
	516	131	tr	
	545	132	tr	
	573	133	tr	
	602	136	tr	
	625	138	+	alanine
	660	140	tr	
	702	143	tr	
	720	144	tr	
	761	148	tr	
	848	154	tr	
	891	156	tr	(one is
	922	158	tr	cysteine)
	961	161	tr	
	1018	164	tr	
	1056	168	tr	(one is
	1099	170	tr	methionine)
	1142	173	tr	(ethionine)
	1210	177	tr	
	1261	180	+	
	1307	184	tr	
	1452	193	++	(artifact or
	1497	196	tr	tyrosine)
	2040	end		
Total peaks		33		

TABLE XIII (continued)

Sample	Seconds	Temp. (C)	Height	Compound identity
XII-C	84	103	tr	(amines and esters)
Hydrothermal	106	104	tr	
sulfide	150	107	++	
	-	-	-	
	400	123	tr	
	426	124.5	tr	
	436	125	+	glycine
	460	127	tr	
	484	128	tr	
	511	130	+	
	563	134	tr	
	594	136	tr	
	624	137.5	+	
	702	143	tr	
	733	145	tr	
	762	147	tr	
	854	153	tr	
	893	155	tr	(cysteine)
	1012	164	+	
	1054	167	tr	
	1096	170	tr	
	1141	173	tr	
	1208	178	tr	
	1262	181	+	
	1380	189	tr	
	1442	193	++	(artifact or tyrosine)
	1495	196	tr	
	1600	end		
Total peaks		26		

TABLE XIII (continued)

Sample	Peak Seconds	Peak Temp. (C)	Peak Height	Compound identity
XIII-A	103	103	++	
Irradiated,	130	105	++	(amines
thiocyanate	168	107	++	and
	202	110	++	esters)
	225	111	+	
	286	116	tr	
	303	117	+	
	352	120	+	
	395	122.5	tr	
	426	125	tr	
	455	126	++	glycine
	509	130	+	(leucine)
	540	132.5	tr	
	571	135	tr	
	622	138	+	alanine
	638	139.5	tr	
	655	140	tr	
	690	142.5	tr	
	710	144	tr	
	732	145.5	tr	
	752	146.5	tr	
	781	148.5	tr	
	834	152	++	(serine)
	875	155	tr	(cysteine
	912	157.5	tr	is one)
	926	158.5	tr	
	1005	163.5	tr	
	1042	166	tr	
	1088	169	+	methionine
	1110	171	tr	
	1180	175	tr	(ethionine
	1209	127	tr	is one)
	1236	179	tr	
	1255	180	tr	
	1300	183	tr	
	1404	190	tr	
	1450	193	++	(artifact or
	1497	196	tr	tyrosine)
	1543	199	tr	
	1586	203	tr	
	1640	end		
Total peaks		40		

TABLE XIII (continued)

Sample	Peak Seconds	Peak Temp. (C)	Height	Compound identity
XIII-C	120	105	tr	
Hydrothermal,	153	107	++	(amines
thiocyanate	205	111	tr	and
	266	114.5	tr	esters)
	320	118	tr	
	370	121	++	(glycine)
	466	127	tr	
	528	132	tr	
	590	135	tr	
	640	139	tr	alanine
	672	141	tr	
	685	142	tr	
	707	143	tr	
	740	146	tr	
	822	151	tr	
	870	154	+++	cysteine
	1010	163	tr	
	1092	169	tr	(methionine)
	1160	173	tr	
	1255	179	tr	
	1358	186	tr	
	1398	189	tr	
	1444	200	+	(artifact or tyrosine)
	1490	203	tr	
	1570	209	tr	
	1620	end		
Total peaks		26		

SF——

FIGURE 1

MAP OF AMINO ACIDS
(Symbols Listed in Table II)

SF—

FIGURE 2

MAP OF SULFUR COMPOUNDS
(Symbols Listed in Table III)

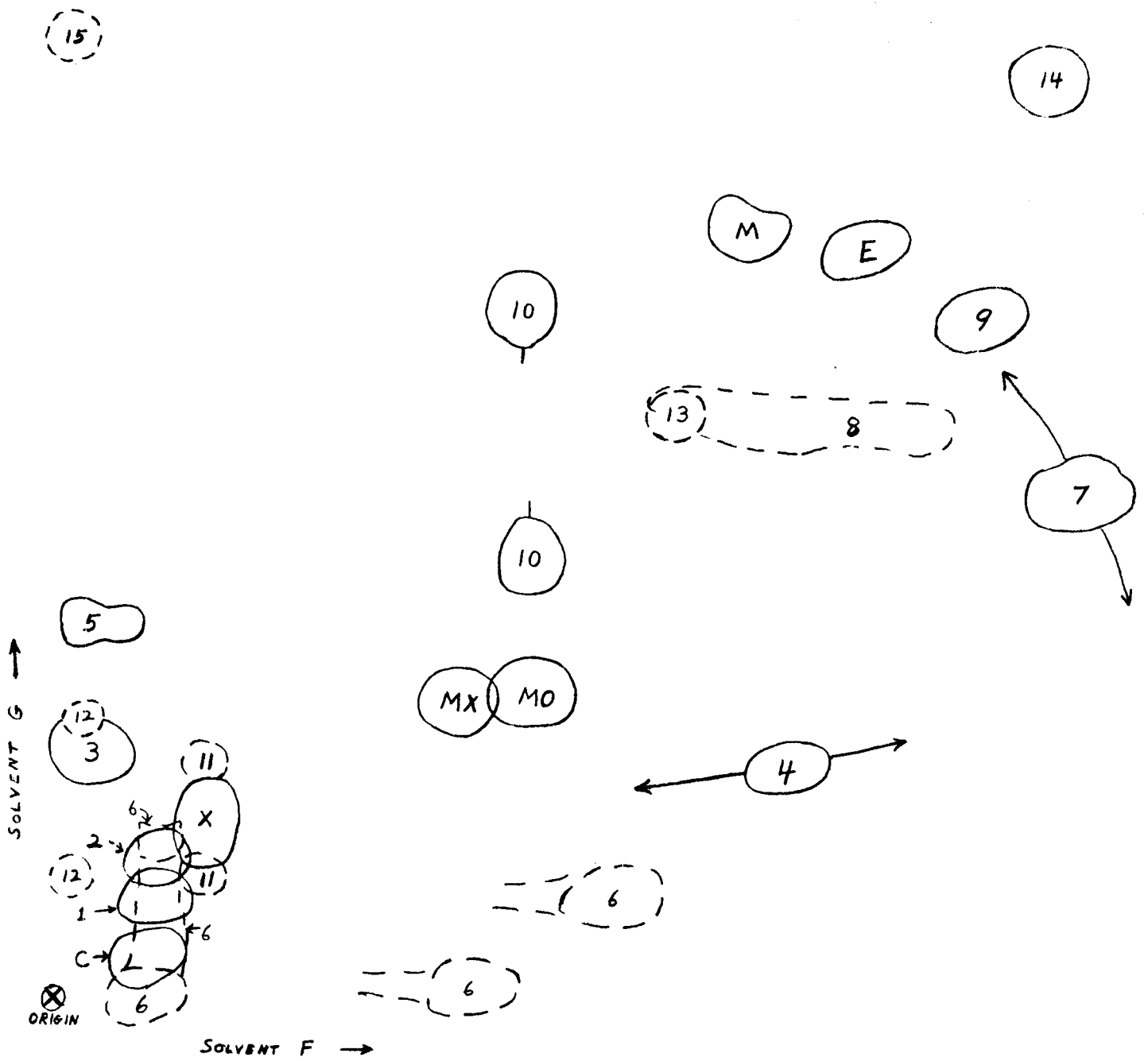
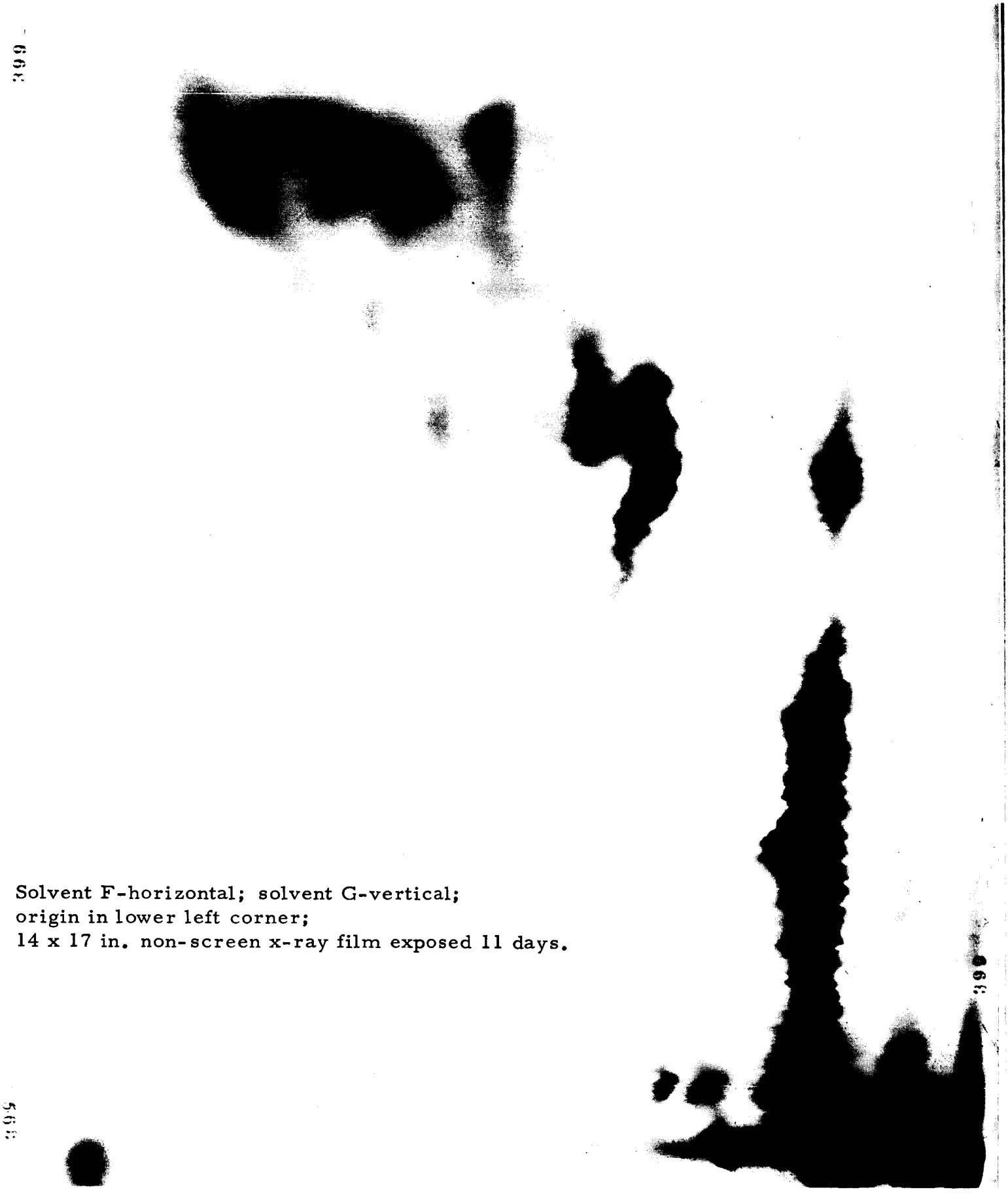


FIGURE 3

AUTORADIOGRAM OF PAPER CHROMATOGRAM
OF IRRADIATED SULFIDE-S^{35} SAMPLE
(Sample XII A-1; non-volatile fraction)



Solvent F-horizontal; solvent G-vertical;
origin in lower left corner;
14 x 17 in. non-screen x-ray film exposed 11 days.

FIGURE 4

AUTORADIOGRAM OF PAPER CHROMATOGRAM
OF IRRADIATED SULFIDE : METHANOL-C^{14} SAMPLE
(Sample XII B-1; non-volatile fraction)

Solvent F-horizontal; solvent G-vertical;
origin in lower left corner;
14 x 17 in. non-screen x-ray film exposed 11 days.

FIGURE 5

AUTORADIOGRAM OF PAPER CHROMATOGRAM OF HYDROTHERMAL SULFIDE-S^{35}:METHANOL-C^{14} SAMPLE
(Sample XII C-1; non-volatile fraction)

Solvent F-horizontal; solvent G-vertical;
origin in lower left corner;
14 x 17 in. non-screen x-ray film exposed 11 days.

FIGURE 6

AUTORADIOGRAM OF IRRADIATED THIOCYANATE-S^{35} SAMPLE
(Sample XIII A-1; non-volatile fraction)

FIGURE 7

AUTORADIOGRAM OF PAPER CHROMATOGRAM
OF IRRADIATED THIOCYANATE : METHANOL-C^{14} SAMPLE
(Sample XIII B-1; non-volatile fraction)

FIGURE 8

AUTORADIOGRAM OF PAPER CHROMATOGRAM OF HYDROTHERMAL THIOCYANATE-S^{35}:METHANOL-C^{14} SAMPLE
(Sample XIII C-1; non-volatile fraction)

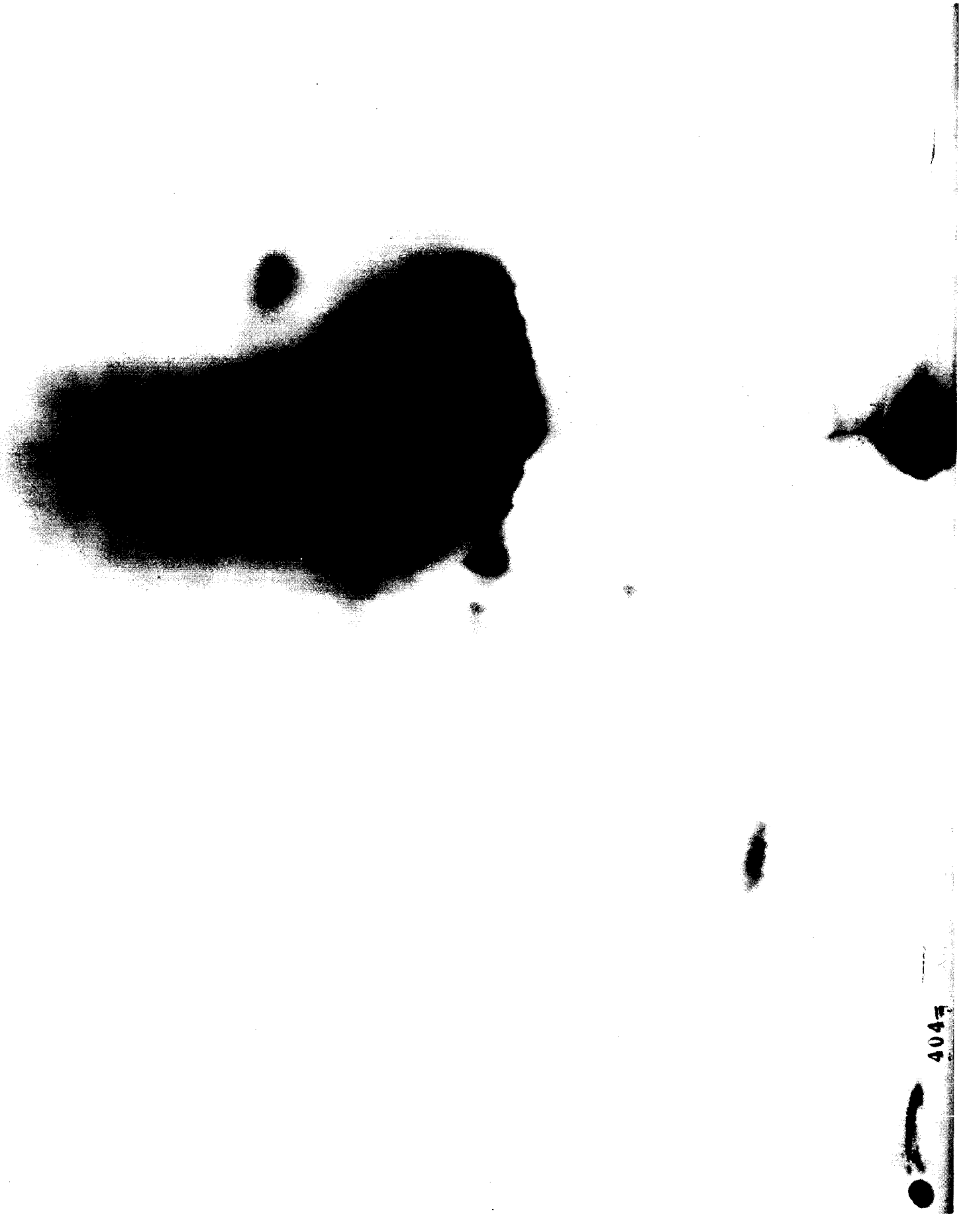


FIGURE 9

MAP OF AREAS OF S^{35}-C^{14} COINCIDENCES