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REPORT

HIGH RESOLUTION DETECTION

OF RADIATION

By

HENRY J. GOMBERG, Principal Investigator

WILLIAM KERR
MILTON SCHLESINGER
REUBENA WEYANT

PROJECT AT (11-1)-70-No.3

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PROGRESS REPORT

HIGH RESOLUTION DETECTION OF RADIATION
Project AT(11-1)-70-No.3

INTRODUCTION

The project on High Resolution Radiation Detection is directed toward the discovery and development of new techniques for locating microscopic sources of radiation. Existing methods of high resolution autoradiography permit studies of radioactive tracer incorporation into biological and metallurgical specimens, but these techniques are still seriously limited with respect to the resolution obtainable, the chemical stability of the photographic film detector, and the sensitivity. It has been the purpose of this project to search for new methods which are free from some of these limitations.

During the past year, research studies have been carried out in the following areas:

(1) New Techniques

a. Modified Wet Process Film for Electron Microscopy

In extending autoradiographic resolution much beyond that presently obtainable, it becomes necessary to devise a detection method than can be utilized with the electron microscope. Present autoradiographic film is much too thick to be useful, and stretching or melting it out in a thin film leads to poor grain distribution. An approach to this problem that has been studied in this laboratory has been an attempt to form a thin collodion film loaded with silver halide grains which would serve as a detecting medium for the sub-microscopic specimen. The tagged specimen would be placed on the sensitized collodion screen, exposed for a suitable period of time, processed, and examined in the electron microscope. The scattering of the reacted silver would be correlated with specimen morphology and serve as the autoradiograph.

Research in this area has consisted of attempts to form a thin film that is sufficiently loaded with a uniform mono-grain layer of silver halide. A

variety of solvents, plastics, and halides have been tested. Details of this work are noted in section I A. of this report.

b. Radiation-induced Polymerization as a Radiation-Detection System.

Studies have continued on various monomer-polymer systems that will respond to ionizing radiation while otherwise remaining stable to heat and light. These materials, in solution, are capable of penetrating tissue blocks and reacting at the site of radiation, thus providing a three-dimensional mapping of entrained radioactivity in the tagged specimen.

Several systems have been examined with respect to their response to external X-radiation in an attempt to find those conditions which render the system most radiation sensitive. Some initial studies have been carried out on the placing of radioactive sources in the monomer solution. Recently, a highly radiation sensitive copolymer system has been discovered. Results of these studies are noted in Section I B.

c. Spark Discharge Tests

The ionization produced by a particle leaving a radioactive surface can, under proper conditions, be used to initiate a spark discharge. If this spark can be made to strike the surface in the neighborhood of the ionized region produced by the particle, it should mark or burn the surface so that it can be identified later. In this way, a microscopic spot in an otherwise very stable detecting medium would mark a zone of radioactivity. Tests on the application of this principle during the past year have not met with success. The spark counter instrument has been redesigned and further work is contemplated. Additional research results are noted in Section I C.

(2) The Radiation Microscope

This is an instrument which makes possible the direct and immediate measurement of radioactivity within the field of an optical microscope. A given area of a suitable specimen can be examined optically at 1000 diameters and then

the radioactivity of that area determined by scintillation technique. Observation and radioactivity measurement utilize the same optical system without moving the specimen under study. There is therefore no question as to register and correlation of the field observed and source of radioactivity. Resolution to 75 microns is now possible.

Refinements of the optical system and electronic circuitry have been carried out over the past year. More details of this device and recent research are noted in Section II.

(3) Autoradioagraphy and Applications

In keeping abreast with autoradiographic techniques currently in practice, we have continued to undertake co-operative research projects with University groups requiring high resolution radiation detector techniques. Some limited projects utilizing autoradiography have also been undertaken in this laboratory.

In carrying out routine high resolution autoradiography, we have found the Eastman Kodak permeable base stripping film to be the most convenient material to work with. However, some serious difficulties have been encountered with the technique of application, particularly where protecting layers (to prevent chemical artifacts) are necessary. A new type of protective coating for tissue specimens is described in Section III A. The problem of film slippage is also discussed in that section.

The co-operative projects that are currently being carried out are noted below:

The determination of melanin precursors by means of the autoradiographic technique and described in last year's annual report has been continued. Successful autoradiographs of melanin granules synthesized from C-14 tagged precursors have implicated some new pathways of melanin formation. Further details are included in this report under Section III B. This work is being carried out in conjunction with Dr. C. Markert of the University Zoology

Department.

Metallurgical studies on the grain boundary diffusion problems of various binary metal systems such as bismuth-copper, nickel-copper, and others have been investigated using high resolution autoradiography. This work, carried out in co-operation with Prof. Sinnott and S. Yukawa of the Chemical and Metallurgy Department, is further noted in this report (Section III C.) Additional studies of this nature are currently in progress.

An investigation of the penetration of Cobalt-60 into the leaves of plants - as noted by autoradiography - has recently been initiated with Dr. Gustafson of the Botany Department. Autoradiographs of some preliminary experiments have been obtained and results are noted in section III D. of this report.

A study of P-32 incorporation in onion root tips as correlated with their mitotic cycle is continuing. Unfortunate incidents with respect to film fog and film shifting have seriously interfered with this project.

This laboratory has also continued to provide assistance and advice to a variety of persons interested in the autoradiographic technique. During the past year we have had representatives of the following organizations visit our laboratories to note the techniques utilized here: Metals Research Laboratory of Electro Metallurgical Company, Aluminum Corporation of America, Wright-Patterson Air Base, Batelle Memorial Institute, Continental Oil Corporation, Scientific Research Institute of Tokyo, and Wayne University College of Medicine.

A paper on the research carried out under this project entitled "High Resolution Radiation Detectors in Research" has been prepared and accepted for presentation at the United Nations International Atomic Energy Conference to be held in Geneva, August, 1955. An abstract of this paper is included in this report.

A paper on the Radiation Microscope was presented at the International Instrument Congress in Philadelphia.

RESEARCH RESULTS

I. NEW TECHNIQUES

A. Modified Wet Process Film for Electron Microscopy

During the past year, experiments have been undertaken to prepare an ultra-thin, densely loaded silver halide film which would be suitable for electron microscope autoradiography. This film would be similar to the collodion screen now used in electron microscopy but sensitized to detect radioactive tracer materials. The tagged specimen would be placed on the sensitized screen and exposed for a suitable period of time. It would then be processed and examined in the electron microscope. The scattering of the electron beam by the deposited silver would thus serve to represent an autoradiograph of the specimen. With a film sufficiently loaded with very small sensitive and uniform grains, resolution approaching that of the electron microscope itself could be obtained.

Research has been directed toward obtaining a thin film possessing those properties noted above, and considerable progress has been made over the first films in which a drop of a 2% parlodion-amyl acetate solution containing 5% cadmium iodide was placed on a 5% silver nitrate water solution. Attempts to increase the loading of this film, by increasing the concentration of the salts resulted in a reaction sufficiently violent to tear the thin film apart. However, by carrying the silver nitrate in the plastic-solvent material, it has been possible to form thin, highly loaded films of silver bromide.

A wide variety of solvents and plastics have been tested in preparing this film. It was noted that benzyl alcohol was by far the most suitable solvent for silver nitrate - stable solutions of 15% silver nitrate can be obtained. All other solvents tested have either much lower stability or are unstable to the silver salt. Benzyl alcohol, however, does not dissolve parlodion, and its

low vapor pressure prevents good thin film formation when used with other plastics. It was therefore necessary to find a solvent and plastic which, when mixed with a silver nitrate benzyl alcohol solution, would give a uniform silver halide film.

The following table lists those solvents tested using parlodion as the plastic. Various proportions of the plastic-solvent and benzyl alcohol silver nitrate were investigated and the results tabulated are those corresponding to the best films obtained.

Solvent	Results
acetone	uniform and densely loaded only when thick.
amyl acetate	very nonuniform film, tears apart.
ethyl acetate	uniform film, but low grain loading.
ethyl carbonate	nonuniform, weak film.
ethyl formate	nonuniform, clumping of grains.
methyl aceto acetone	nonuniform weak film.
methyl alcohol	no film formation.
methyl ethyl ketone	good film.
methyl isobutyl ketone	weak film nonuniform.
pentanedione	nonuniform film.
tetrahydrofurane	nonuniform film.

Cellulose was tested with dioxane and gave a poor film. Polystyrene in styrene also did not give a satisfactory film. The vinyl plastics when mixed with the silver nitrate gave a precipitate, and did not yield good films. The chart presented in Figure 1. indicates the relative positions of the solvents noted above with respect to their vapor pressure and water solubility.

On the basis of these experiments, it appeared that the formation of a good film results from solvents having relatively high water solubility and high vapor pressures. The best films to date have been formed when using solutions of parlodion dissolved in methyl ethyl ketone and in acetone.

Experiments have also been carried out using different halide mixtures in water solution. Combinations of potassium bromide, cadmium bromide, ammonium bromide and iodides have been tried. The addition of trace amounts of

iodides yields a good film; however, subsequent work indicated that the iodide drastically reduced the sensitivity of the film. In current work, we have used pure potassium bromide, and addition of other salts did not appear to improve the film.

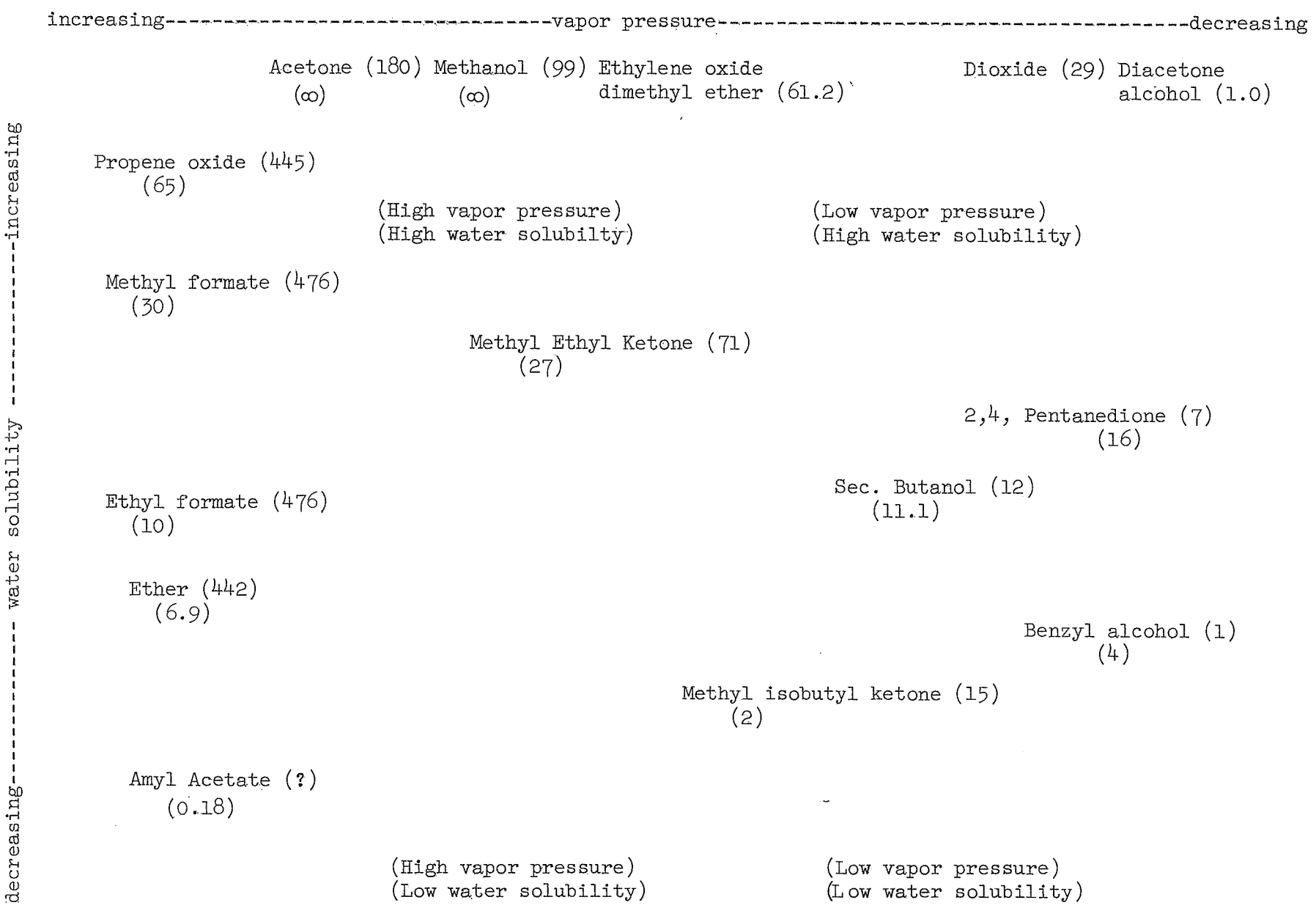
Processing of these thin films can be carried out using commercial chemical developers and fixatives. Physical development does not give good results. Experiments are continuing in this area, and results to date indicate that a 1:3 microdol solution for ten seconds give good results with low background.

The thin film has been found sensitive to light, X-rays, alpha particles of polonium, and C-14 and Tl-204 beta particles. Sensitivity measurements as yet have not been carried out on these films.

The thickness of these films has been estimated at between 0.2 and 0.5 microns. Commercial stripping film has a 5 micron emulsion on a 5 micron gelatin support.

In Figures 2 and 3, electron photomicrographs of a thin film are compared with a stripping film. The thin film has better uniformity of grain distribution than the stretched stripping film. This thin film had a 0.1% potassium iodide plus bromide in the water and 10% ammonium hydroxide in the silver nitrate. The presence of the ammonium seems to prevent grain clumping but leads to an unstable silver solution and a weakened film. We have since abandoned the use of ammonium. These electron micrographs indicate the need for increased loading and more uniform grain distribution. Experiments designed to perfect this film and study its sensitivity are in progress.

Fig. 1 SOLVENTS FOR PLASTICS IN FORMING THIN FILMS OF SILVER BROMIDE



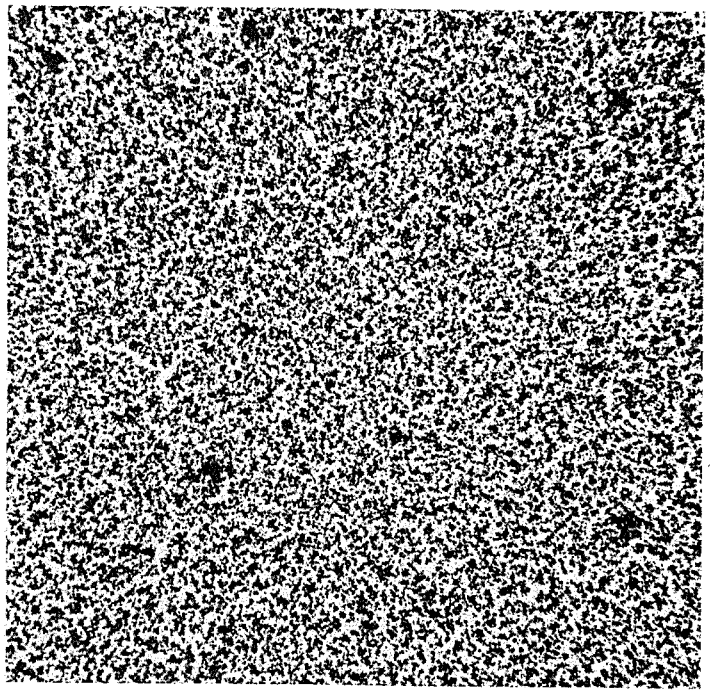


Fig. 2. Electron-micrograph of collodion-silver halide film formed over water bath. Film is a monograin layer 0.2 microns thick. (X2000)

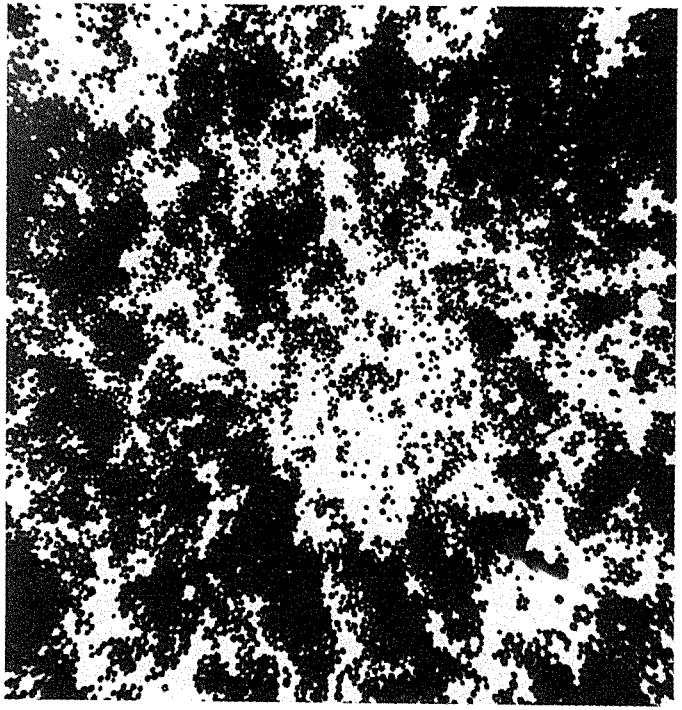


Fig. 3. Electron-micrograph of Eastman permeable base stripping film swelled and stretched by water flotation. (X2000)

B. Radiation Induced Polymerization

In the search for new procedures of radiation detection, systems have been examined that are stable to light yet respond to the higher energies of radioactive particles. A likely mechanism containing an inherent amplification factor is that of a polymerization reaction. Several experiments (1,2,3,) have indicated that certain monomers under special conditions are radiation sensitive while otherwise remaining stable to heat and light. The possibility of using such a system to infuse into a "tagged" specimen and polymerize at the site of radioactivity or to polymerize locally on an irregular solid at the site of radiation suggested itself, and some of these systems have been investigated with this purpose in mind.

The early experiments with these systems, as described in this project's 1954 annual report, were carried out with the liquid monomer tetra-ethylene glycol dimethacrylate (TEGMA) using radioactive metal samples as sources of radiation. Work by Schmitz and Lawton (4) had indicated that TEGMA as a difunctional monomer was more sensitive to radioactive polymerization than monomers such as acrylates, methacrylates, styrene, and acrylonitrile which possess only one functional group. The early successes with this material, however, suggested a chemical reaction and a more complete study of these reactions were undertaken.

During the past year experiments on the TEGMA and other monomer-polymer systems have been investigated using external X-radiation with the object of seeking those conditions for optimum sensitivity while maintaining stability to heat and light. Experimental work by others on polymerization reactions demonstrated that the sensitivity of many of these systems to heat, light, and high energy radiation is reduced in the presence of oxygen (5). It was therefore necessary to work under conditions in which oxygen was excluded.

In proceeding with this work, we have studied five chemicals which appeared likely to respond to a radiation-polymerization mechanism. These substances were purified and sealed in vials under varying conditions of pressure and in the

presence of various sensitizing agents. Samples were subjected to doses of 200 KVP X-rays and also tested for stability to heat and light. Under the conditions tested, it was found that the polymerization of purified tetraethylene glycol dimethacrylate when deaerated and placed under nitrogen proceeds at around 7000 roentgens. Ethylene glycol dimethacrylate polymerized to a solid gel under the same conditions at doses of the order of 30,000 r. Attempts to sensitize these chemicals with peroxides led to self-induced polymerization. Furthermore, neither of these materials, even in an unsensitized condition, remained stable to heat and light at room temperature; and work on these two compounds has been discontinued. An alcohol solution of the solid monomer, methacrylamide, proved to be quite stable to heat and light under a nitrogen atmosphere and was found to polymerize to an insoluble white gel at 4000 r. Another solid monomer, N-N' methylene-bis-acrylamide, was dissolved in an aqueous solution and - under deaerated conditons - reacted at doses of the order of 250 r. This material also proved to be quite stable to heat and light.

A more detailed study of the N-N' system was undertaken and data was obtained with respect to the extent of polymerization as a function of dose and dose rate. The effect of concentration, temperature, pH, and the presence of various chemical species on the reaction was also investigated. The material was prepared in 15 ml quantities of a 0.1 M (1.5%) solution under high vacuum conditions. Analysis were made by filtering the polymer through fine-porosity gooch-type crucibles and weighing them. Accuracy is considered good to 15%. Figure 4 indicates the response of this system to total dose. The effect of increased temperature may also be noted (the monomer has been found stable at 65°C over a four-week period). The straight line curve for the room temperature reaction is seen to extrapolate to zero point indicating no threshold dose exists. Because of limitations of radiation facilities, data on the dose rate response has not been substantiated, but indications are that the efficiency of the reaction increases with a decrease in dose rate. For a total dose of 500 r, at a

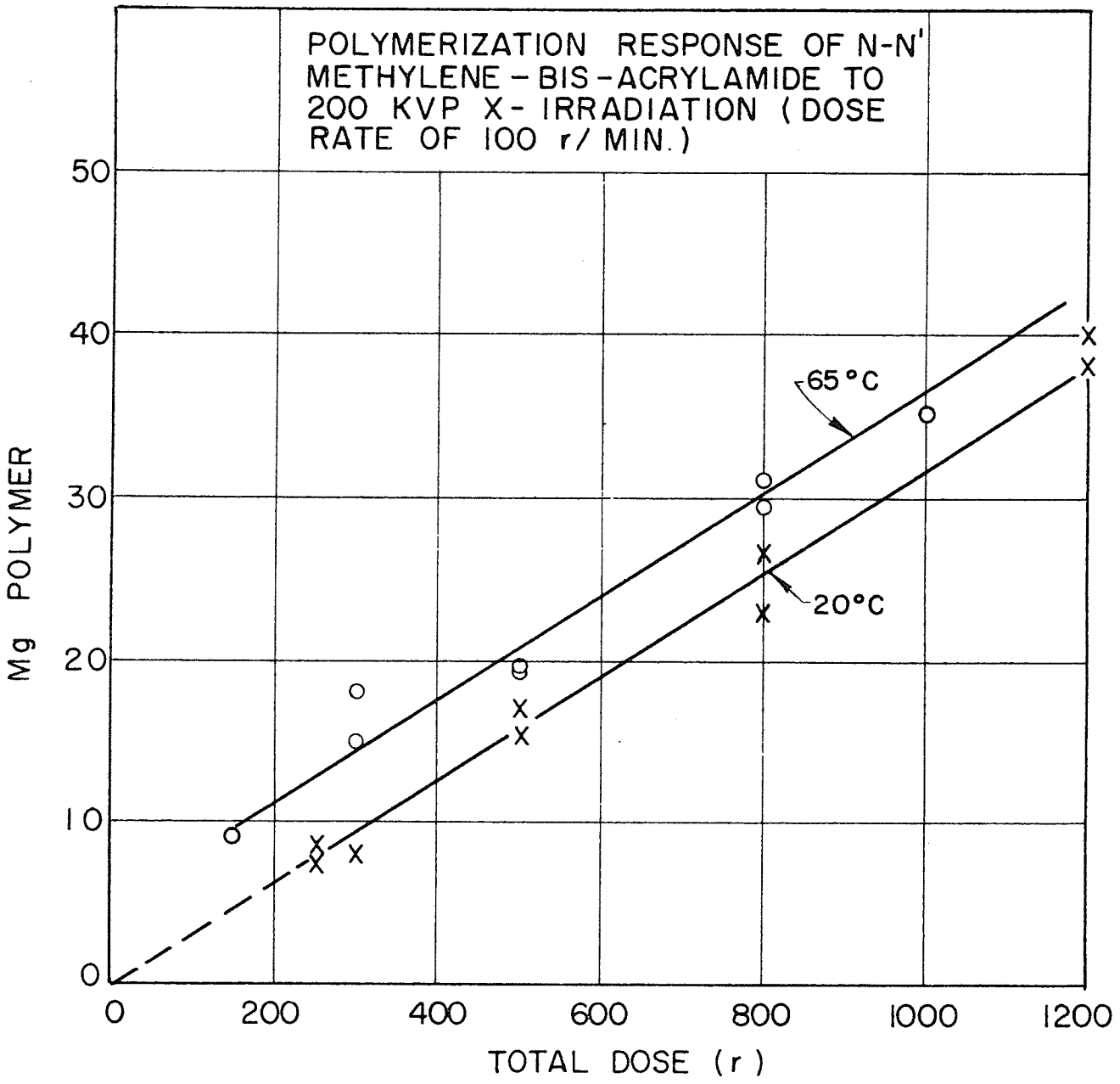


Figure 4

Polymer Graph

rate of 900 r./minute, about 12 mg of material react as compared to some 23 mg at 15 r./minute. Using Cobolt-60 irradiation at a dose rate calculated to be 0.5 r/minute, an average value of 20 mg. was obtained for a total dose of 500 r. Increasing the concentration (the monomer is soluble to 3 gms./100cc at 20°C) does not substantially increase the yield while tending to make the solution unstable at high temperatures. Attempts to adjust the pH with phosphate-citric acid buffers led to unstable solutions. Placing different metallic ions and potential sensitizing agents in the monomer did not appear to be helpful, and the most stable and sensitive system thus far has consisted of the purified monomer alone in distilled water.

Preliminary studies have been carried out, placing radioactive specimens in the evacuated solutions of N-N'. Under conditions where the material is adequately "protected," stability is maintained and response to P-32 and Tl-204 has been noted. Calculations of the dose from the activity of the isotopes used indicates that the visible reaction in these cases occurs at dosages comparable to those noted in the X-ray induced response -- around 200 r.

Experiments at Brookhaven National Laboratories with radiation-induced polymerization of acrylamide in the solid state indicated that this material might prove more reactive than the N-N' (6). Acrylamide is considerably more water soluble (78 gms/100cc.) than the N-N', but it was noted that a 20% solution of acrylamide was quite unstable under high vacuum conditions. However, when nitrogen gas was bubbled through a 20% aqueous solution of acrylamide the X-irradiation induced polymerization proceeded visibly at doses of the order of 100 r. Furthermore, the material appears to be quite stable under these conditions, although further experimentation is needed to confirm these results.

A re-examination of all the systems investigated was carried out under the condition of nitrogen bubbling, and the following table indicates the results:

Monomer	Response to 200 KVP X-Irradiation-no filter, 100 r./minute
Tetra-ethyleneglycol dimethacrylate (bulk)	No reaction at 500 r.
Tetra-ethyleneglycol dimethacrylate (aqueous solution)	No reaction at 500 r.
Ethylene dimethacrylate (bulk)	No reaction at 500 r.
Ethylene dimethacrylate (aqueous solution)	No reaction at 50 r.
Acrylonitrile (bulk)[a]	No reaction at 500 r.
Acrylonitrile (1.0 M aqueous soln.)	No reaction at 500 r.
Methacrylamide (20% soln. water)	No reaction at 500 r.
(20% soln. methyl alcohol)	No reaction at 500 r.
(20% soln. 0.1 M N-N' in water)	No reaction at 300 r.
N-N' Methylene-bis-acrylamide (0.1 M soln. water)	Turbidity at 300 r.
Acrylamide (30% soln. water)	71 mg. at 150 r.
(50% soln. water)	100 mg. at 150 r.
(30% soln. 0.1 M N-N' in water)	700 mg. at 150 r. (dose rate of 50 r./minute)

[a] A tracerlab report (7) indicated that bulk acrylonitrile, under high-vacuum air evacuation, is sensitive to radiation doses as low as 50 r. with visible turbidity. Their data show 1.2% polymer formed at 1600 r. of Cobalt-60 at a dose rate of 80 r./minute.

From data thus far obtained with these monomer-polymer reactions, some estimates of the amplification factor associated with these materials have been made. A Q factor (defined here as the number of molecular units reacting per initial ionization event--32ev/ionization) of 2×10^3 has been calculated for the N-N' system. The acrylamide reaction (30% soln. 0.1 M N-N') gives a Q value of 2×10^7. These values may be compared to that of photographic film in which it has been estimated that 10^9 to 10^{11} atoms of silver react in the development process per latent image grain.

On the basis of these very recent developments, the likelihood of radiation localization techniques utilizing the polymerization reaction appears quite encouraging. Further experimentation is being carried out in this area.

C. Spark Counter Experiments

Research has continued on the spark counter technique proposed as a high resolution autoradiographic device. In this technique, it is planned to utilize the electrical discharge triggered by the ionization produced by a particle

leaving a radioactive surface to mark some detecting material, and thus indicate the zone of radioactivity. Early experiments with a spark counter constructed in our laboratories were unsuccessful, and it was proposed that we try to reproduce the experiments of K.S. Lion who showed increased photographic sensitivity to X-rays using a spark counter. With our original spark counter (described in detail in our 1954 Annual Report), experiments similar to those of Lion were carried out. A Nuclear scaler was used as a high voltage and spark detection device. Nitrogen gas bubbled through ether and through the counter acted as a quenching agent. Detection was attempted using Kodabromide paper attached to one of the plates, and the counter was placed in a collimated beam of X-rays. Sparking occurred at 800 volts but was unaffected by the presence of the X-radiation. Further experiments indicated that the counter itself was inadequate in its construction. In later work with a new counter consisting of aluminum plates attached to C-type lucite insulators and enclosed in a glass envelope, the experiments of Lion's were again attempted, but successful results were not obtained.

In late January, one of our group had the opportunity to visit the laboratory of K.S. Lion at M.I.T. and to note the type of counters and conditions employed for obtaining the increased photographic response of X-rays.

As a result of this visit, we have redesigned our counter and experimental set-up. Experiments with this device indicated that the counter itself operates as a radiation detection instrument but no success was obtained when a photographic emulsion was placed on one of the plates. The resistance of the emulsion layer, the distance between the plates, and the parallelism of the plate are quite critical for obtaining the correct response as reported by Lion.

Means for measuring the interplate distance and parallelism of the plates are being worked out, and further experiments with the device are planned.

II. Radiation Microscope

Work has continued on the adapting of the Beta-Ray microscope principle to a convenient working model. Over the past year this has involved both the optical system and the electronic circuitry.

Electronic circuit work has included the design and construction of a line noise filter for the A.C. supply. This filter decreased the background by about a factor of two with a corresponding increase in the sensitivity of the system.

The photo tube circuitry has been modified to allow high voltage to be supplied by a single power supply. Previously two separate supplies had been used.

Work on the optical system has involved experimentation with placement of mirrors and a standard ocular in an effort to achieve a more convenient interchange of the visual viewing and radiation monitoring functions. It is planned that during the current fiscal year a new and more compact model suitable for laboratory use will result from this study.

Some effort has been directed toward growing thin (about 50 - 75 micron) anthracene crystals with moderate success. However, in work on a cognate problem being carried out by another group in the Phoenix Radioisotope Laboratory, a method of growing anthracene crystals a few microns thick has been developed. This technique will be investigated further for its suitability for microscope use. In addition the effect of moisture on the performance of thin anthracene crystals is being studied.

Some additional tests with plastic phosphors have so far not yielded any materials with sensitivities more than about half that of anthracene.

III. Autoradiography and Applications

A. Commercial Stripping Film

In the application of autoradiography as carried out in this laboratory, we are still relying on Eastman Kodak permeable base stripping film.

We have, however, altered somewhat the method of application as outlined in our last annual report (January 1954), and now are using the following procedure:

(1) Tissue sections are mounted on plain glass slides.

(2) The sections are stained by routine procedures, but ending with a water solution of eosin if H and E is being used.

(3) When a protective layer is required to prevent chemical artifact, the slides are removed directly from water into a 2% Elvanol solution and allowed to dry slowly. This Elvanol layer is applied in order to preserve the tissue integrity. When the Elvanol is thoroughly dry, the slide is dipped in a 2% Saran in methyl ethyl ketone solution. This layer serves as the protective coat. We have found it convenient to follow the Saran layer with a 2% parlodion in methyl alcohol layer. Stripping film adheres very well to parlodion, whereas this is not always true with Saran. Unfortunately, parlodion alone has been shown to be inadequate as a protective layer. Figure 5 compares the Elvanol-Saran protective coating with the plain Saran layer previously employed. The Saran alone is noted to destroy tissue detail.

(4) The exposure time for obtaining an autoradiograph is determined by measuring the activity of the specimen with an end window geiger counter and calculating the time required to produce 2×10^6 beta particles per square centimeter. Assuming no isotope decay, uniform distribution, a counter area of 6.15 cm^2, and a counter efficiency of 8.8% for C-14, the following formula is applied: Exposure time (minutes) = $\frac{(2 \times 10^6)\ (6.15)\ (.088)}{\text{counts/minute}}$

Stripping film is applied, and slides are stored in black bakelite boxes (sealed with black electrical tape) in the refrigerator for the duration of the exposure time.

(5) The slides are developed, dried, and cover slips applied using Canadian Balsam.

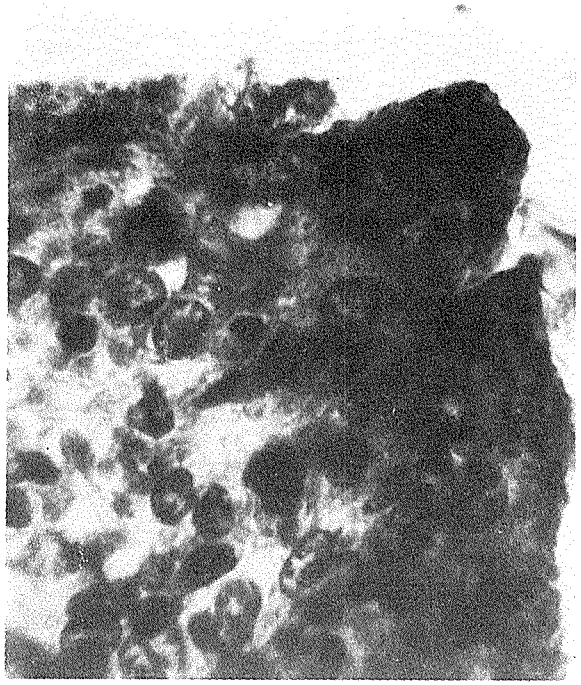
In almost all autoradiographs prepared in this laboratory, a protective coating has been found necessary. Autoradiographs obtained using this procedure may be noted in Figures 6 and 7. We are still not able to completely control peeling and shifting of the film during the development process. In general it appears that the freshly opened roll of film adheres well. Film stored - after initial use - has the tendency to shift and peel, especially if long exposures are required.

B. The Biochemical Synthesis of Melanin Pigment by Cells in Tissue Culture.

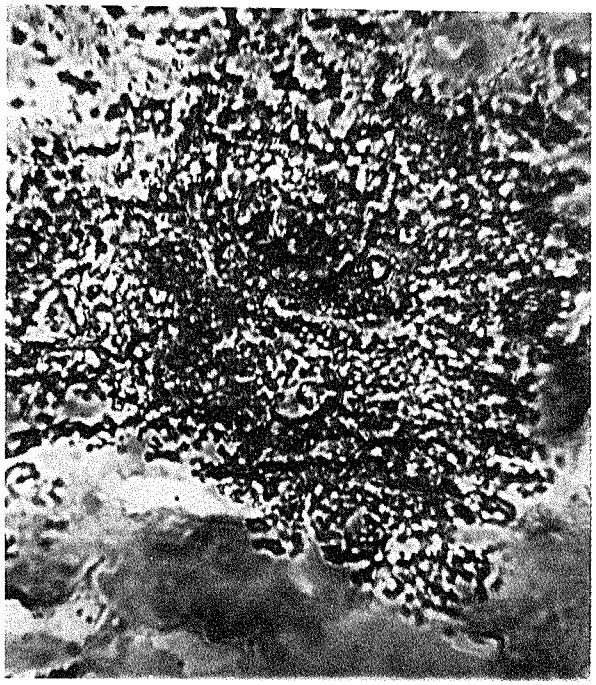
This project has continued its cooperative assistance with Dr. C. L. Markert, Zoology Department, University of Michigan, in the use of autoradiography to determine precursors of melanin synthesis. Professor Markert has been studying the precursors of melanin formation in the hope that by identifying particular substrates used in the synthesis of melanin pigments by cells of different genetic makeup, some correlation can be drawn between gene structure and enzyme specificity. The autoradiographic procedure has provided a unique method for carrying out this investigation. Briefly, the experiments have been conducted as follows: tissues are incubated with radioactively labelled materials thought to be precursors of melanin, and sections are prepared and exposed to stripping film (Eastman Permeable Base). The appearance of radioactivity in the melanin granules as revealed by autoradiographs indicates the extent to which the labelled substrate acts as a precursor in melanin synthesis.

Several interesting results to date have been obtained from this

Normal tissue Mouse skin X1170



Elvanol-Saran layer plus stripping film Mouse skin X1170



Saran layer only plus stripping film. Mouse skin X1170

Figure 5. Protective Coating For Tissue Sections To Be Autoradiographed

study (8). When tyrosine or DOPA (dihydroxy phenylalanine) labelled in the side chain carbon were injected into the tissue cultures of chick epidermis and skin, in tadpoles, and in mouse skin (in vivo and in vitro), no radioactivity was detected in the melanin granules. Figure 6 shows the autoradiographic response of Carbon-14 DOPA polymerized in the gut of the tadpole while no activity is seen over the tissue melanin strip. However, when uniformly labelled tyrosine was incubated with tissue cultures of embryonic chick skin, radioactivity was localized to the melanin particles as noted in Figure 7. This figure also serves to illustrate the problem of the film shifting during processing, since the emulsion containing the silver grains may be noted as having shifted to the right and up from the specimen. In this case, dense areas of silver may be correlated with the dark melanin granules. However, in less well defined situations, such shifting would prevent correlation of autoradiograph with specimen detail.

The implication from these experiments is that the side chain of tyrosine is not involved in melanin formation. An even more recent study of this problem has indicated that yellow and black melanin granules which appear in strains of mice believed to possess only one gene in common, are derived from the same substrate -- the uniformly labelled Carbon-14 tyrosine. Somewhat less activity appeared in the yellow granules than in the black under the same experimental conditions. Thus it would appear from these studies that the site of gene action is not at the stage of initial substrate utilization -- but effective further along the chain to melanin formation.

C. Autoradiography Applied to Metallurgical Problems.

The application of the autoradiographic technique to a variety of metallurgical problems has been noteworthy recently. We have continued to offer assistance to several workers in this field at the University of Michigan.

One such study was carried out by Dr. S. Yukawa and Professor M. J.

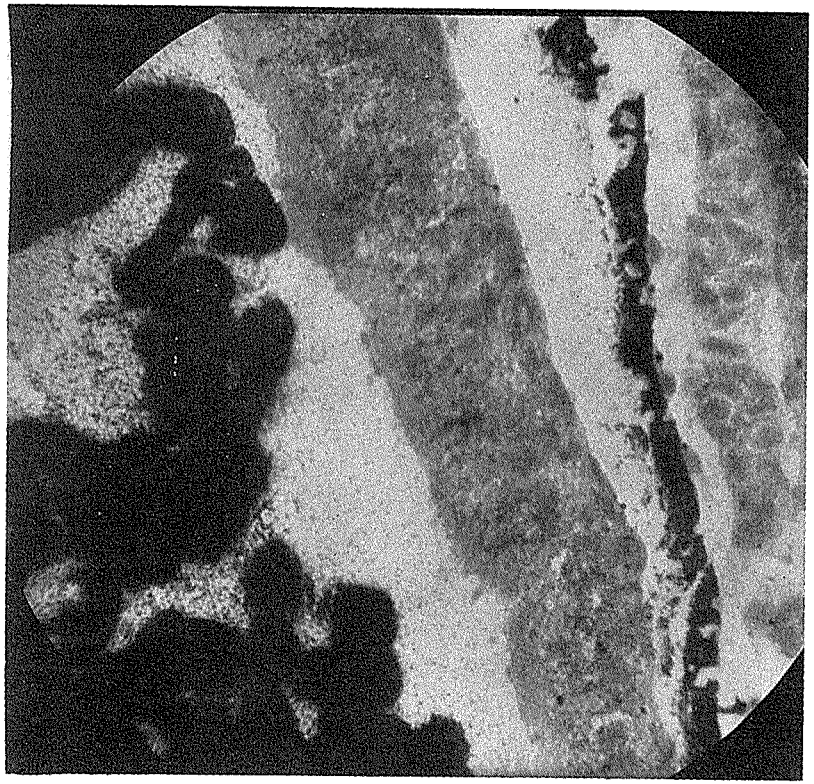


Fig. 6 Stripping film autoradiograph of C-14 labelled DOPA localization in tadpole gut. (X5000)

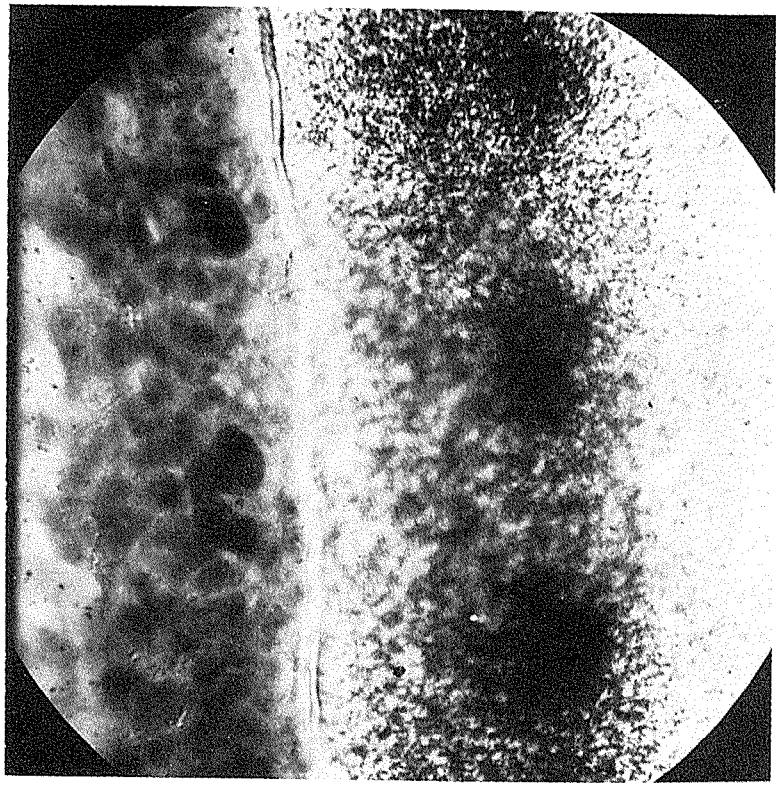


Fig. 7 Stripping film autoradiograph of melanin granules in embryonic chick skin. Film has shifted to the right, uncovering granules in specimen below. (X1000)

Sinnot[9] and is concerned in part with a study of the diffusion of nickel along bicrystal grain boundaries in copper. The relative rate of diffusion through the matrix of the crystal as opposed to that along the boundary and the influence of the angle between the planes forming the boundary on the diffusion rate has been measured.

To measure diffusion of nickel in the copper crystals parallel to the bicrystal face and along the bicrystal face itself, radioactive nickel was used as a tracer. Specimens cut perpendicular to the bicrystal boundary were annealed and cleaned and then radioactive nickel plated onto one of the cut and cleaned faces. A special plating cell and techniques were developed for the plating operation.

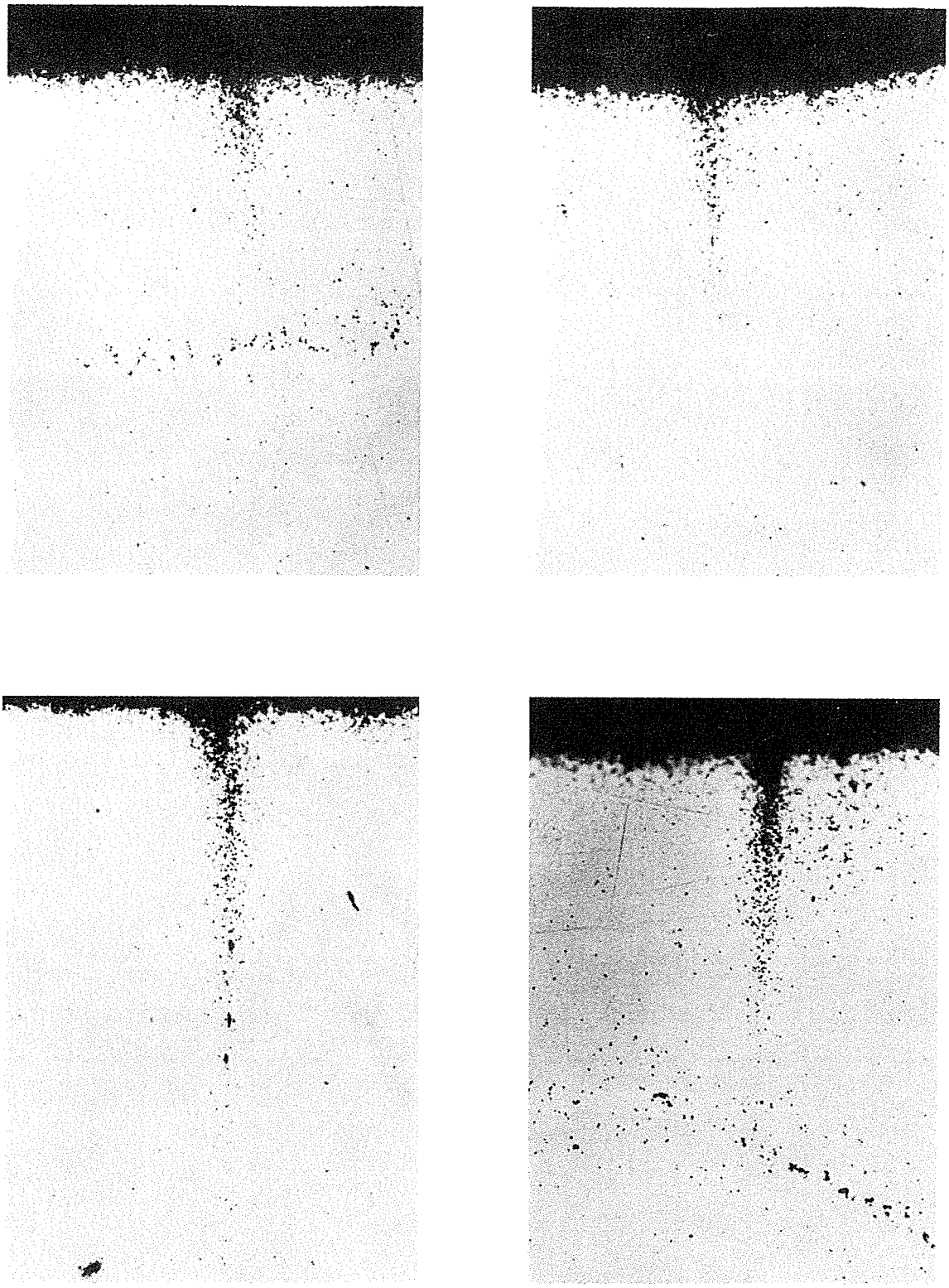
When the metallurgical preparation was completed, the specimen was given a coating of 2 per cent Vinylite VYNS in methyl ethyl ketone as a protective layer. Stripping film was then applied and, after a suitable exposure, developed and fixed.

A typical set of results may be seen in Figure 8. The influence of bicrystal angle on diffusion rate of the nickel into the copper along the grain boundary and the very limited diffusion into the crystal lattice proper may be observed directly for the first time. The amount of diffusing metal involved here is well below the limit detectable by etching methods. From autoradiographs such as these, quantitative evaluation of activation energy for grain boundary diffusion as a function of angle has been made.

Work is now proceeding on the study of nickel-nickel diffusion, a problem which can be solved only with the aid of these high resolution autoradiographic methods.

D. Other Studies

In addition to the cooperative projects noted above we have attempted two additional autoradiographic studies during the past year. An experiment related to P-32 incorporation in onion root tip cells and described in detail in



DIFFUSION OF NICKEL INTO COPPER BICRYSTALS
AT 650°C (1200°F)

Autoradiographs 500X

Diffusion Time: 144 hrs.
Autorad. Expos. Time: 5 days

Figure 8

the 1954 annual report has not yet been completed. Difficulties with stripping film slippage in one case and fogged film in another has delayed a complete study of the P-32 incorporation.

An experiment designed to follow the uptake of C-14 Formate in the various types of white cells of rats by autoradiographic technique was unsuccessful due to the high dilution of the isotope in the animal.

Recently, another cooperative project with Professor Gustafson of the Botany Department, University of Michigan, has been initiated. The purpose of this study is to determine the ability of $Co^{60}Cl_2$ to penetrate the leaves of plants under a variety of conditions. Successful autoradiographs of preliminary experiments indicate the autoradiographic technique will be useful in this study.

PROPOSED RESEARCH

During the coming year, the following research is planned on the projects noted earlier in this report:

I. New Techniques.

a. Work will be continued in perfecting the thin silver halide film to be used in electron microscope autoradiography. Additional plastics, solvents, and halides will be examined in an attempt to increase the loading while maintaining a uniform mono-grain dispersion of grains. With better films, sensitivity measurements can be made and preliminary autoradiographic tests run.

b. Further investigation is planned on the copolymer system of acrlyamide and N-N' methylene-bis-acrylamide which has been noted to be quite sensitive to radiation induced polymerization. Response to dose rate, total dose, and other environmental factors will be studied and radioactive sources immersed in the solutions to test its ability for locating centers of radioactivity.

c. Research utilizing the principle of a spark counter will be continued with the newly designed counter. The increased film response from external radiation as a result of sparking will first be sought. Radiation emitters will then be placed on one of the plates, and the spark response noted.

II. Radiation Microscope

The future work in this area will be toward further improvements of the present model. Development of a satisfactory illumination system for visual viewing of the surface of opaque sources is being undertaken. It is planned to secure some of the new RCA 6199 head-on cathode type phototubes for test in this instrument. Work on refining the optical system and on improving the anthracene scintillator crystals is also planned.

III. Autoradiography and Applications

The utilization of high resolution autoradiography to a variety of research problems will be continued. The present cooperative projects with faculty members of the University will be carried on, and some new research studies involving the autoradiographic technique are contemplated.

This laboratory also plans to continue to serve as a center for providing information and suggestions to those workers interested in the application of high resolution autoradiography.

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ABSTRACT

High Resolution Radiation Detectors in Research

by

H. J. Gomberg and M. J. Schlesinger, Jr.

(Paper presented at U.N. International Conference on Atomic Energy, August 1955)

The value of radioactive tracers in research is dependent on our ability to detect them. Two types of information are needed: amount and location. The sensitivity of present instruments leaves little to be desired. However, the accuracy of localization techniques is poor when we consider the inherent resolution of the tracer technique is about one angstrom. The best localization technique to date provides about one micron resolution, indicating the improvement possible. As resolution of radiation detection increases, more direct studies of functional microstructure in living and inorganic systems will be possible. Only slight improvement in present techniques will however exhaust the resolution range of the optical microscope. New techniques are needed which will permit the examination of tracer distribution at magnifications employed in the electron microscope.

The following high resolution radiation detection systems will be discussed:

(1) The best available standard film techniques such as stripping film autoradiography.
(2) New research techniques which are improved versions of the photographic film methods:
    (a) Wet process autoradiography-(Gomberg)
    (b) New silver phosphate technique - (Manfried Siess)
    (c) Super thin high density silver halide films suitable for autoradiography in conjunction with electron microscopy (Gomberg-Schlesinger)
(3) New radiation-sensitive media which are not light-sensitive such as special monomers which polymerize locally in the presence of small radiation sources. This method opens the possibility of three dimensional plastic autoradiography by impregnation of tissues containing tracers with the monomer and subsequent examination for polymerization. (Gomberg-Schlesinger)

All systems are analyzed for resolution, sensitivity and information content. In addition, consideration is given to artifact problems arising from shifting of stripping films and chemical reactions with the surface under study. Included are new surface protection techniques, with very thin formed plastic layers.

In addition to the thin film chemical or physical reaction techniques, other approaches to high resolution detection have been made. These include the beta-ray microscope which provides a direct determination of radioactivity in a field 75 microns in diameter along with visual observation of that field; and a combination of a low sensitivity, low background film with a highly localized gas discharge triggered by microscopic radiation sources. The discharge produces changes in the film which correspond spatially to the radiation sources.

Specific examples of application of high resolution detection methods which have led to unique solutions of difficult problems in biology and solid state will be given. These include determinations on the site and manner of synthesis of melanin from labelled tyrosine in pigmented tissue, and the diffusion of radio-nickel along grain boundaries in copper and iron.