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REPORT

HIGH RESOLUTION DETECTION

OF RADIATION

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

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I. INTRODUCTION

The major purpose of the University of Michigan Project on High Resolution Radiation Detection is the discovery, development and application of new methods of locating microscopic sources of radiation. These sources may be incorporated in organic systems such as radioactive phosphorus used to tag specific cell bodies, or in inorganic systems such as the tagged carbon compounds in steel. In keeping with this purpose, research studies have been continued or initiated in the following areas.

(1) The Radiation Microscope

This is a new device, making possible the direct 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 the radioactivity of the area under observation determined directly and instantaneously. Both observation and radioactivity measurement (by scintillation techniques) make use of the same optical system without moving the object being studied so that there can be no question as to register and correlation between the field observed and the radioactivity measurement.

The first and second of the microscope models have now been built and tested successfully at 970 diameters, with activities of less than one microcurie per square centimeter (Cobalt-60). A third model, suitable for use by laboratory personnel concerned with tracer research and not trained in special instrumentation techniques is now under construction.

(2) Autoradiography

The wet process of autoradiography, in which a radiation sensitive

layer is created chemically directly on the surface to be studied, has been carried as far as it would appear worth going for its use as a general technique. Some advances have been made in improving resolution (now 5 microns or better) and sensitivity, but since the recent development of the commercially available stripping films with comparable resolution, there is less need for the wet process technique as such.

There are still special features of the wet process such as:

a) Very low background fog - as low as one part in 40, compared to stripping film.

b) Extreme mechanical stability and freedom from slipping, peeling, reticulation, etc.

The sensitivity, however, is somewhat lower and the chemical stability is much lower than that of the stripping film. Where short exposures (24-48 hours) are possible there are advantages in the wet process which can outweigh the technical difficulties of its application. However, regardless of its use as an autoradiographic method to be used in connection with optical microscopy, it has opened a new possibility of autoradiography combined with electron microscopy. This is discussed further in section III A.

(3) New Techniques

a) Modified Wet Process Film for Electron Microscopy

The use of tracers in systems requiring electron microscopy for observation has been a continuing challenge. An approach to this problem has been made through the possibility of sensitizing the collodion screens similar to those used for supporting electron microscope specimens. The specimen preparation can be activated, applied to a sensitized collodion screen and left there long enough to cause some direct exposure in the screen. The screen and preparation are now treated to remove any unexposed detector material (silver halide) and then the screen and preparation are studied in an electron microscope.

Because of the excellent scattering power of silver, a very small amount in the support screen brought down by the original tracer radioactivity, should show up well and correlate with the specimen resting on it.

Using techniques based on the original wet process and the dry-film modification described in our last report, preliminary studies have been made on the formation of extremely thin sensitized collodion screens. Results to date are given in section III A.

This work is being carried out in co-operation with Dr. O. H. Bergold, of the Laboratory of Insect Pathology, Canadian Department of Agriculture.

b) Plastic Polymerization as a Method of Detecting Radiation Sources.

Some monomers which are stable at room temperature and under ordinary light conditions will polymerize when subjected to ionizing radiation. These monomers as liquids, or as solids in solution, are capable of penetrating tissue blocks of moderate dimensions. If significant sensitivity to ionizing radiation can be developed, it should be possible to prepare not only two dimensional maps of radioactivity by coating active surfaces, but three dimensional maps by local polymerization of the monomer due to entrained radioactivity in a tissue block.

Early coating tests on activated thallium specimens were very encouraging, but the results had to be discarded when it was found that thallium metal could be catalyzing the polymerization chemically. A new series of tests have been started, using X-rays to determine the best conditions for inducing polymerization. These are reported in section III B. of the report and indicate that, under proper conditions, adequate sensitivity for tracer studies can be achieved. The results of these tests will be used to guide new tissue impregnation tests.

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. Thus, a microscopic burned spot should mark a zone of radioactivity. Preliminary tests using this principle have been made without conclusive results. The conditions under which the spark is initiated primarily by the presence of an ionizing particle have not yet been achieved. Because of the desire to look into some of the other processes described, work on this idea has been temporarily suspended. Description of the tests is given in section III C.

(4) Application and Co-operative Problems

As a continuing test of our methods and ideas, we undertake co-operative research with other University groups requiring high resolution radiation detector techniques, as well as carrying out studies in our own laboratories. The principal problems so studied during the past year were:

(a) Continued study of melanin synthesis from radioactive precursors. Autoradiographic tests lend no support to the hypothesis that tyrosine and tryptophane are not melanin precursors. New tests have been made using C-14 labelled Dopa as the substrate. These tests, carried out in co-operation with Dr. Clement Markert, are reported in more detail in the body of the report.

(b) Continued co-operative research with Watertown Arsenal Metallurgy Study Project.

While no AEC funds were spent directly on this work, it was done in our laboratories, using the same techniques and Phoenix Project equipment made available to the AEC research project.

Detailed studies on the diffusion of nickel into steel, bismuth into copper, nickel into copper, and on the formation of iron sulfide in iron have been made. Some sample autoradiographs and some description is incorporated in the body of the report in section IV B.

(c) Uptake and Localization of P-32 in onion root tips, in correlation with their mitotic cycle.

This is being carried out as an independent study problem by our own laboratory personnel. A similar problem, using Vicia faba seedlings was reported by Howard and Pelc (5). The status and results are given in section IV C.

The activity of the past year also included lectures on autoradiography, given at Purdue University, and the University of Rochester, and also participation through lectures and discussion in the Oak Ridge Institute for Nuclear Studies' intensive course in autoradiography this past summer.

In addition, papers have been presented on the Radiation Microscope at the Nuclear Engineering Conference at Berkeley, California, September 1953, and the Scintillation Counter Conference in Washington in January 1954. A report on the microscope was presented in Science, Volume 119, No. 3080, in January, 1954.

Two doctoral theses were published during the past year. These consisted of the Radiation Microscope (Dr. William Kerr) and Metallurgical Application of Wet Process Autoradiography (Dr. George C. Towe). Two more will be completed this year.

RESEARCH RESULTS

I. BETA RAY MICROSCOPE

The initial development of the Beta Ray Microscope was discussed in a special report (Dr. Kerr's thesis) submitted in September, 1953. On completion of this initial phase, it was decided to expand our activity in this area in order to bring the development to fruition with minimum delay. What follows is a report on work started since October 1, 1953.

Work was begun on improvements in the microscope along two general lines: (1) Improved mechanical packaging, (2) Increased flexibility of the phosphor-optical system. The improved mechanical packaging has been aimed both at the electronic circuitry and the phototube-optical system. A coincidence circuit (Figure 1) has been designed and built which is much more compact than that used in the original model. The preamplifier-phototube mount has been redesigned to allow the photocathode to be more easily aligned with the optical system (Figure 2).

An improved version of the optical system has been constructed which will make the switch from visual viewing to isotope detection easier to accomplish. One revision included an attempt to light the specimen by light transmitted through the objective. At present this attempt has not been successful, apparently because of reflection at the rear surface of the objective. Further investigation is being made.

Design of a background shutter is being considered. Design of a complete light tight system is continuing.

Tests are being run on plastic phosphors furnished by a commercial supplier. The phosphor was supplied in small sheets 75 microns thick. Those tested exhibit an efficiency in detecting betas from Thallium-204 of about 50 per cent of that of anthracene in the same thickness.

Investigation of the growing of thin anthracene crystals by sublimation

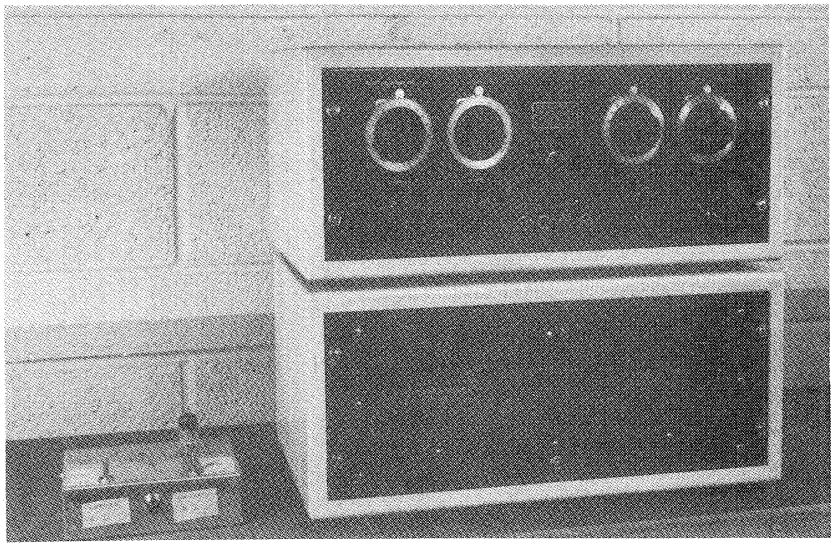


Fig. 1. New Coincidence Analyzer is at left. Unit at right is old Coincidence Analyzer with its power supply.

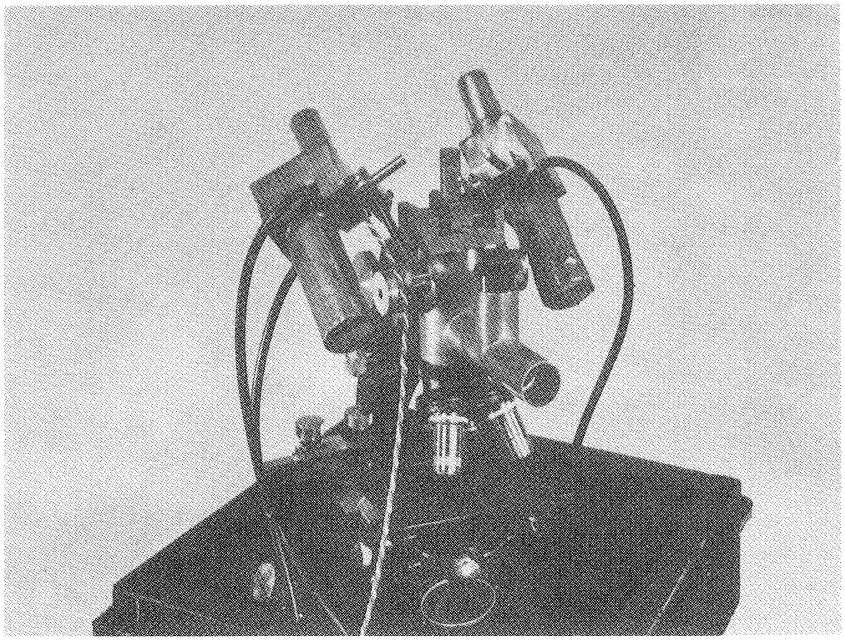


Fig. 2. Optical system and phototube holder for Beta-ray microscope

from a large crystal and condensation of the resulting vapor is being continued.

A new dense plastic phosphor has been secured from Dr. Cowan at Los Alamos and will be tested as soon as the technique of forming thin films has been mastered.

Both RCA and Dumont promise that a small diameter head-on cathode phototube will be available in the near future. This tube should simplify the arrangement of the phototube-optical system.

Consideration is being given to the design of a light tight optical system to eliminate the need for enclosing the phototube-optical system within a light tight box.

II. AUTORADIOGRAPHY

A. Wet Process Autoradiography

Mr. George Towe completed a detailed study of the wet process technique of autoradiography, and his results were subsequently published in a doctoral thesis. An abstract of the thesis follows:

Wet-Process Autoradiography Applied To Metallurgical Studies

The purpose of this investigation was to (1) adapt wet-process autoradiography to metallurgical studies, (2) evaluate and improve the process, and (3) obtain metallurgical information using this high resolution autoradiographic method.

Wet-process autoradiography involves the preparation of a collodion film on the surface of a radioactive metallographic sample. The collodion film contains soluble halides which form silver halides when immersed in a silver nitrate solution. The insoluble silver halide, held in place by the collodion film, is subject to ionization by the radiation from the metal sample. Following the exposure, the radiation affected silver bromide in the collodion emulsion is photographically developed and fixed. The developed silver grains are located directly over the radioactive region.

Physical development is used with the wet collodion process. The developing solution must contain silver ions as well as a reducing agent. The silver of the photographic latent image nucleates the reduction of the silver ions in solution. It is possible to control the developed silver grain size by varying the time and temperature of development and by changing the composition of the developer. The developed silver grains are frequently about one micron in diameter.

Since the photographic chemicals must be kept from contacting the metal specimen, it was necessary to develop suitable protective layers. Gold and silver vacuum evaporated films and many plastic materials were investigated. For high resolution autoradiography, this protective layer must be very thin. The Vinylite layer recommended is less than one micron thick.

High resolution autoradiography requires the use of a thin heavily loaded detecting photographic layer in close contact with the sample surface. The thickness of the collodion "emulsion" is controlled largely by the amount of solvent used to dilute the collodion. Suitable layers, containing about 90 weight per cent silver bromide, were prepared approximately 4 microns thick.

Preparation and processing of the detecting layer were the subject of close study. Sensitizers were added with the hope of improving radiation sensitivity. The temperatures of the several solutions were varied from room temperature to 2° C. Photographic background fog is minimized when the autoradiographic exposure takes place at temperatures near 2° C. A fog level of less than 1000 silver grains per square millimeter is readily achieved. This is lower by a factor of perhaps 10 or 100 than the fog level obtained with commercial emulsions.

A comparison is made of results obtained with wet-process autoradiography and with a commercial autoradiographic emulsion. The metallurgical sample requirements and detecting layer specifications are discussed in detail. The limitations applicable to autoradiography in general and the wet-process in particular are reviewed.

Under favorable conditions of suitable radiation and proper emulsion processing, a resolution of better than ten microns can be obtained with wet-process autoradiography. The autoradiograph is examined in place on the metallurgical specimen at magnifications as high as 1000 X.

Standard radioactive samples were prepared including carbon-14 carburized iron and steel, nickel-63 electroplated on platinum or silver, a nickel-silver powder mixture containing nickel-63, and a copper-antimony alloy containing copper-64. A complex alloy (type N-155) containing tungsten-185 was also prepared. The tungsten distribution in this alloy was unknown.

Figure 3 illustrates an experiment noting the sensitivity of the wet-process technique.

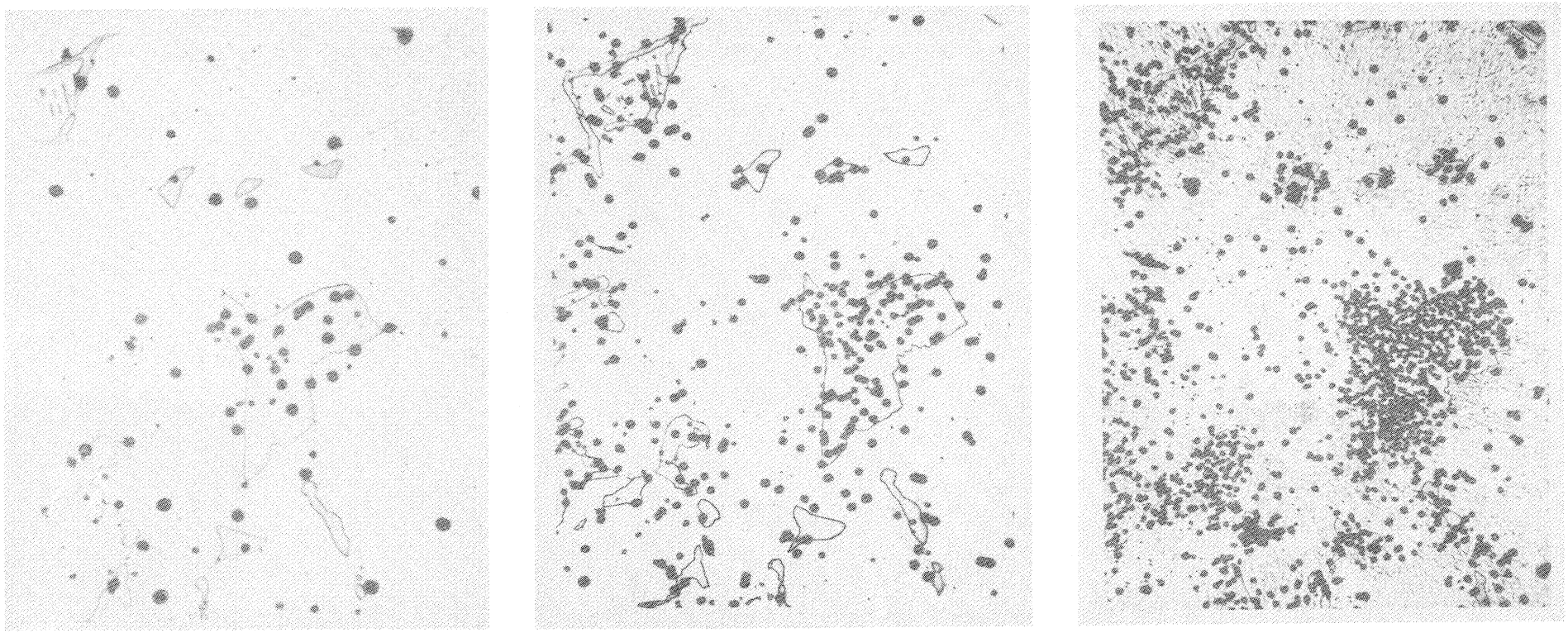
Various experiments were carried out during the past year to attempt to increase the sensitivity of the wet process and to decrease fog level. The description and results of these projects are listed as follows:

(1) Studies with the pyrazole derivative of L. Jenny, as noted in our last report, provided no great difference in its ability to sensitize the system.

(2) Tests with the anti-foggant, 6-nitro-benzidimazole, resulted in smaller grain size, but little or no good anti-fogging action was detectable.

The wet process as it exists now, is useful for biological and metallurgical samples of high activity. The process is relatively simple to carry out (1), but extreme conditions of cleanliness are required, particularly when working with metallurgical samples. Furthermore, exposures are limited to about 48 hours. The process, however, has the advantage of variable grain size and no danger from the film slipping away from the specimen.

This technique has been used with some success in our laboratories in carrying out routine autoradiographic studies. Samples of radioactive thyroid and metal samples bearing radioactive materials gave successful autoradiographs (Figures 4 and 5). Further work on development of the wet process has been



a) 32 minutes b) 95 minutes c) 285 minutes

Fig. 3. Autoradiographs of the same cementite crystal at different exposure times X1000

curtailed, and research has been primarily devoted to working with new techniques.

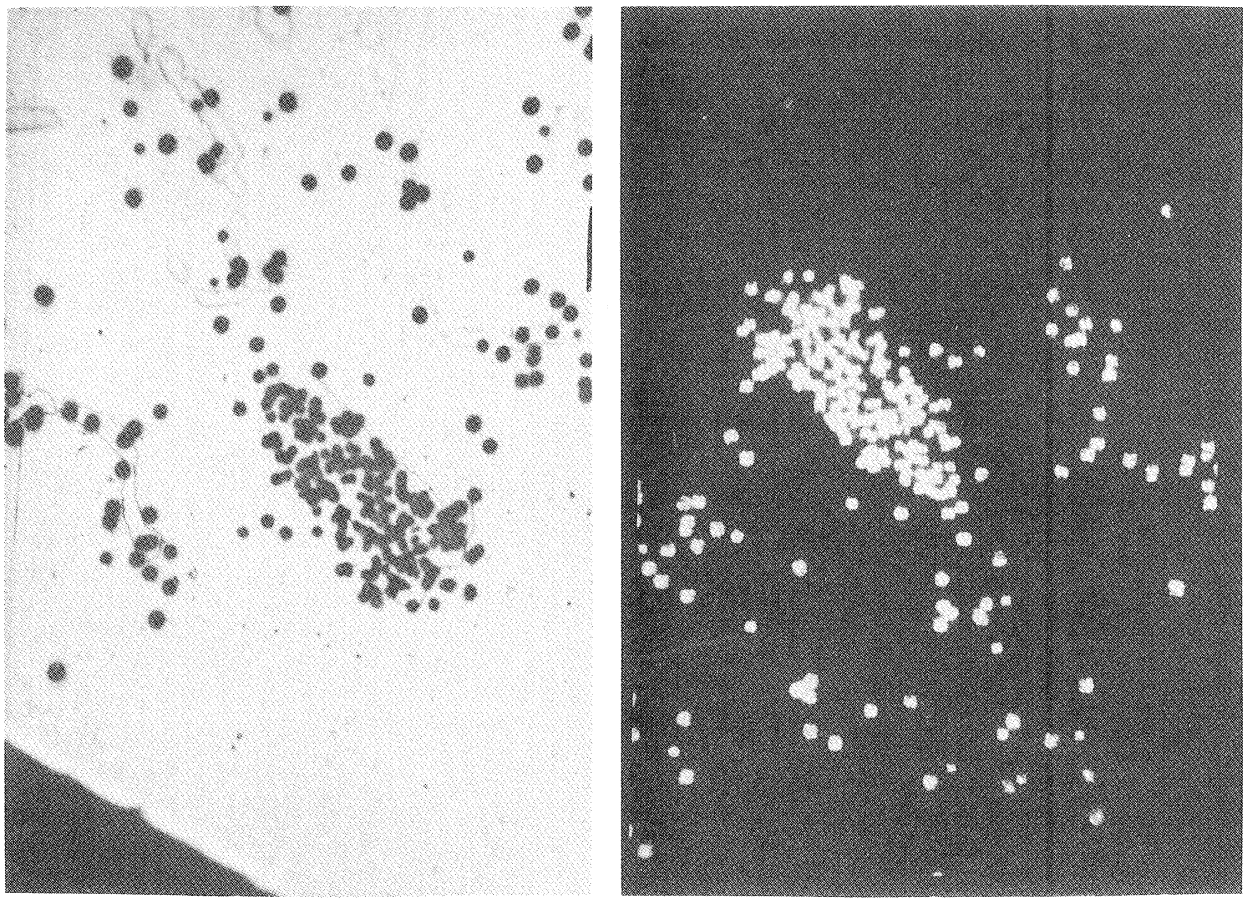
B. Dry Film Version of the Wet Process

During the past year further work was devoted to adapting the wet process autoradiography to a dry film. Carried out by Mr. Marvin Hass and described in part in the last annual report, this technique consisted of coating the collodion wet plates with a preservative so as to render them stable to dryness.

This process uses the solutions of bromidized collodion and silver nitrate described in the wet process (1). The preservative follows the formula of H. O. Klein in "Colloidion Emulsion". A. W Penrose & Co. Ltd. 2nd. Edition, London, 1910, p. 110. It consists of 6 grams sugar, 6 grams gum arabic, and 1 gram Tannin, dissolved in 220 ml of distilled water. The procedure is outlined below:

Emulsions are prepared on a coated slide in a manner similar to the wet process. The slides are immersed in silver nitrate for one hour, then carefully removed and immersed in cold distilled water for two minutes. They are next dipped in fresh cold wash for five minutes, after which the slides are placed in the cold preservative solution for at least five minutes. They are then removed and dried at room temperature for thirty to forty-five minutes. During exposure period they are stored in a cold climate in the dark. Development of the slides is carried out with dilute D-19, 1:3, for thirty seconds. The slides are then dipped in the acid stop bath for a few seconds, followed by hypo for a few seconds, followed by acid stop, or preferably water. The clearing time is quite fast. Figure 6 shows the response of a film prepared in this way to radioactive thallium sources.

Attempts were made to sensitize this dry version with a gold sensitizer, ammonium aurous thiocyanate (2). The anti-foggant, 6-nitro-benzidimazale



Bright field Polarized light

Fig. 4. Autoradiographs showing silver grains over radioactive carbides. Carbon-14 in iron, Picral etch. X1000

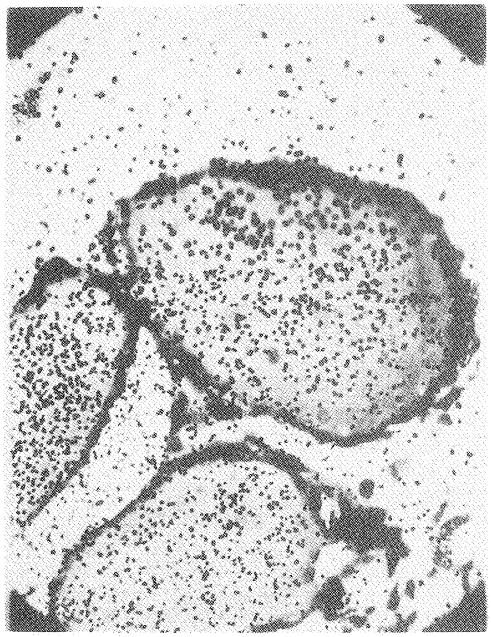


Fig. 5. Wet process autoradiograph of radioactive rat thyroid. Localization of radioactivity is noted in the colloidal tissue of the thyroid follicle. X415

was also tried. Neither of these proved to be successful.

While this process is a satisfactory adaptation of the wet process, it is felt that existing commercial stripping film and liquid emulsions are equally as sensitive and capable of the resolution attained here, in addition to being more reliable and relatively easier to work with. Therefore, further work along the line of that described above has been curtailed.

C. Commercial Stripping Film

In the application of autoradiography as carried out in this laboratory, we have relied on the permeable base autoradiographic stripping film supplied by Eastman Kodak Company. We have found that the following method of application results in good autoradiographs:

(1) Slides to be used are dipped in 2% Saran, dried under an infra-red lamp and then baked at 150°C. for half an hour.

(2) Tissue sections are mounted on the Saran coated slides, stained by routine procedures, dipped in 2% Saran again, and dried under the infra-red lamp. This method of drying avoids any clouding of the Saran as usually occurs on humid days. This technique was found to be superior to a previous method in which the slides were dipped in Saran and dried in vacuo.

(3) The activity of the specimen is determined by use of an end window Geiger counter.

(4) The stripping film is applied, and the slides stored in black bakelite boxes (sealed with black electrical tape) in the refrigerator for the duration of the exposure time. The exposure time is determined primarily by running several specimens and developing them at various times. Through experience we have been able to correlate activity as determined by Geiger counter with exposure time for P-32 and C-14 specimens.

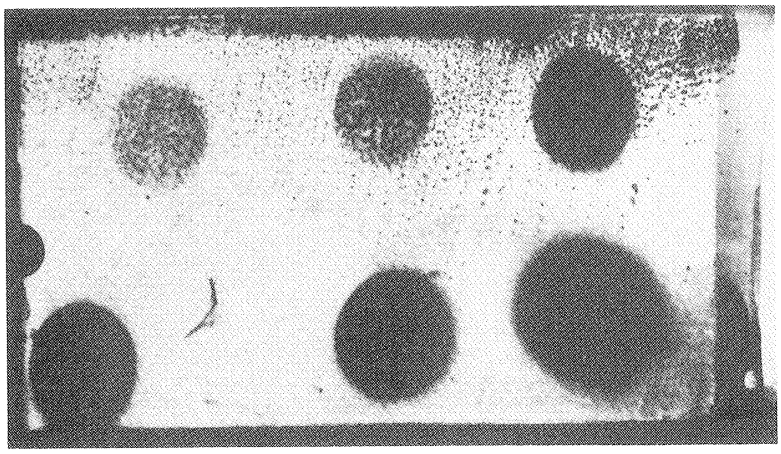


Fig. 6. Response of dry collodion film to radioactive Thallium discs placed immediately over the film. Exposure time was 12 days. Activity of the discs ranged from 0.03 to 10.0 microcuries/cm^2. Some chemical fog is observed in the upper half of the slide. X2

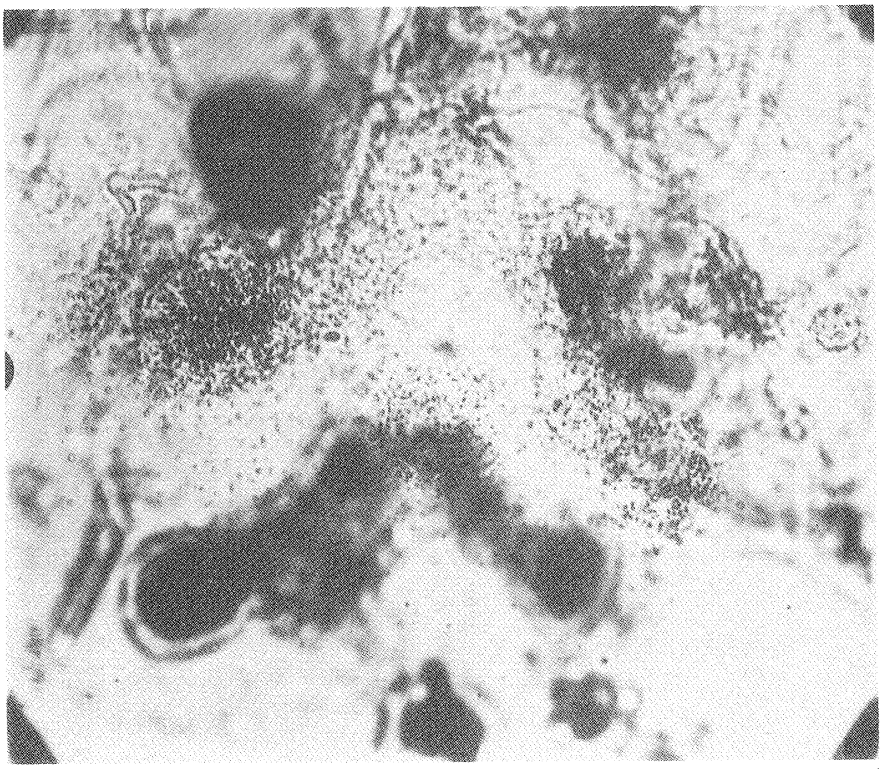


Fig. 7. Stripping film autoradiograph of mouse skin tissue incorporated with carbon-14 Dopa. Picture shows the film having slipped as much as 75 microns. X415

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

In some cases the protective layer of Saran coating (steps 1 and 2) may be eliminated, but the processing of control specimens is essential under these conditions.

The difficulty with stripping film peeling off the specimens during exposure, as noted in our last report, seems to have been overcome. The stripping film is floated on a 5% solution of glycerine, allowed to stand for a minute, and then is picked up on the slide. The film seems to dry more smoothly, although somewhat more slowly, than when pure water is used. We have stored film on slides for two months with this technique without the film peeling off the specimen.

The stripping film still has a tendency to slip during the processing - and specimens must be carefully examined for indication of this action. A good example of this is seen in Figure 7.

Some work was also attempted using commercial liquid emulsion. Exceedingly high fog level was noted in these cases.

III. NEW TECHNIQUES - GENERAL

In the investigation of new techniques of high resolution autoradiography we are seeking the following objectives:

(1) The system should require a vanishingly thin film.

(2) It should respond to high energy particles while having low sensitivity to light photons and chemical artifacts.

(3) It should have high chemical stability in the unexposed state.

A. A Modified Wet Process Film for Electron Microscopy

Late in the year we received a communication from Dr. G. H. Bergold, of the Laboratory of Insect Pathology of the Canadian Department of Agriculture, inquiring as to the possibilities of adapting the wet process to a dry thin film suitable for work with the electron microscope. As presently conceived, the idea is to sensitize, with silver halide, the collodion screen used to hold the sample when placed in the electron microscope. The sample, containing a radioactive tracer, would be placed on the sensitized screen for a sufficient exposure period in the dark. The screen may be developed but this will be done only if the amount of reduced silver produced by direct radiation action is insufficient after fixing to cause electron scatter. "Fixing" with or without development is done to remove the unreacted silver halide. The screen is then placed in the electron microscope and the scattering of electrons by the silver particles correlated with the specimen. This then would be the electron-microscope autoradiograph.

The initial requirements for such a scheme consists of obtaining a very thin film highly loaded with a uniform concentration of silver halide. Thin films of Angstrom thickness can be obtained by applying a drop of 2% parlodion in amyl acetate solution on water. By dissolving cadmium iodide in the amyl acetate and using water containing the silver nitrate, a thin film loaded with silver halide crystals is formed. We have worked with various concentrations

of silver nitrate in water and cadmium iodide in amyl acetate. We have also done some preliminary work varying the temperature of the solutions, and using alcohol-water mixtures of the silver nitrate. Thus far, our best films have been formed using the following solutions:

(1) 5% cadmium iodide in amyl acetate plus 4% parlodion in amyl acetate (3:1)

(2) 5 grams silver nitrate in 20 ml of distilled water.

Ten drops of solution (2) are placed in 25 ml of distilled water in a watch glass and mixed. One drop of solution (1) is placed in the water. The film is allowed to dry, washed with water, and picked up on a glass slide.

Results to date have not produced a high degree of uniformity, nor a sufficiently high density. However, the prospects of this type of dry version of the wet process appear quite promising as a potential method for electron microscope autoradiography, and research is continuing on this problem.

B. Radiation Induced Polymerization

One approach to a method of high resolution detection of radioactive material initiated during the past year was the investigation of monomer-polymer chemical systems that could be influenced by high energy radiation. Considerable evidence exists describing the effects of high energy radiation on the initiation of polymerization. This reaction proceeds as a result of the free radicals formed under the action of the high energy radiation. Of particular interest was work performed by Schmitz and Lawton (3) in which they described a monomer-polymer system in which the polymerization initiated by high energy electrons was found to be dimensionally specific. Although relatively high dose rates are reported for such reactions, it was felt that such a system might prove potentially adaptable for a means of high resolution autoradiography.

The monomer, tetraethylene glycol dimethacrylate was chosen to work with at the outset. This material polymerizes by cross-linking, and its di-

functional grouping leads to a greater susceptibility in polymerization as compared to monomers containing only one double bond. Furthermore, the polymer is insoluble in the monomer and would thus "dissolve" out. This material was obtained from the Monomer-Polymer Company and washed free of inhibitor by running the material through an activated Alumina column. Other materials which are being studied include ethylene glycol dimethacrylate, methacrylamide, and N-N^{1}-methylene bis-acrylamide. The latter two are solids at room temperature. The others are viscous liquids which polymerize to a clear solid material when heated.

The radioactive specimens that were used in the initial experiments consisted of thallium 204 electroplated on copper plates and supplied by Harwell Laboratories in England. Small sections of these plates were mounted edgewise in bakelite blocks along with a strip of plain copper to act as a control (Figure 8). The strips were approximately 300 μ wide and the thallium layer was of the order of 30-40 μ in width. Samples of activities ranging from 0.03 $\mu c/cm^2$ to 100 $\mu c/cm^2$ were used. In later work, discs of these sources were punched out and mounted flat in lucite molds (Figure 9). Other sources utilized consisted of small pieces of uranium in bakelite, and metallurgical mounts of nickel 63 and C-14. Thyroid tissue containing I-131 was used as a biological specimen for this work.

The first experiments were carried out by merely spreading the monomer over the thallium samples that had been polished and cleaned. The samples were stored at room temperature and pressure. After varying exposure times, the unpolymerized material was washed off with benzene. The initial results of this technique indicated that some reaction was proceeding - as indicated by a cloud-like formation over the radioactive edge of the metal strip after about 6-8 hours exposure (Figure 10). These results however, were sporadic and inconsistent with respect to the varying activities of the specimen. Furthermore,

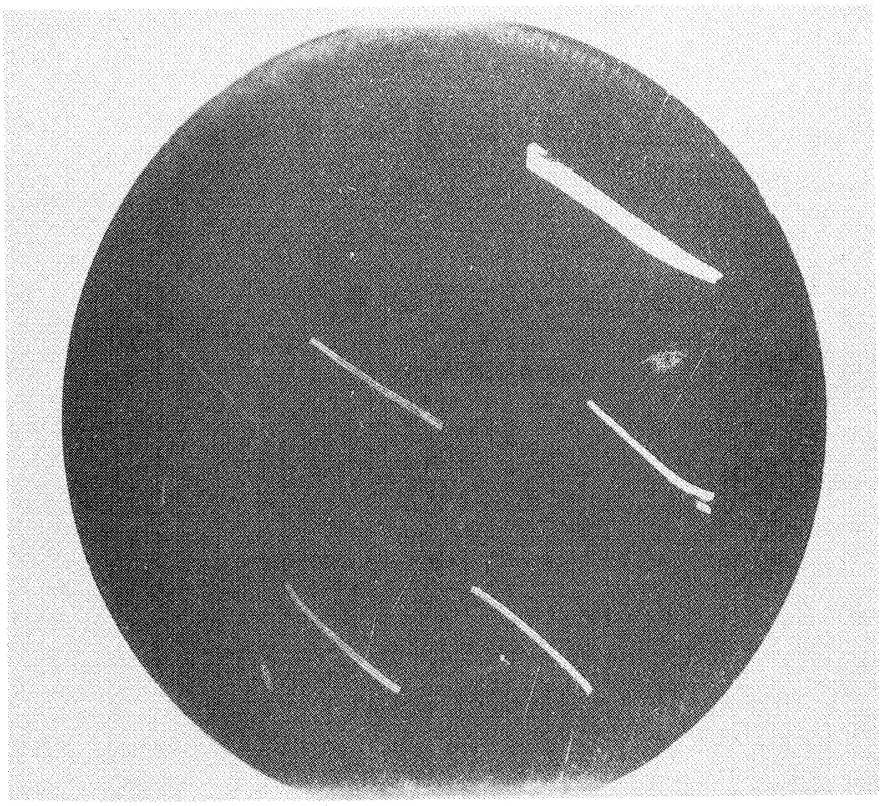


Fig. 8. Typical radioactive Thallium source--mounted on edge--used in the first experiments with radiation-induced polymerization. The strips here are of Thallium 204 with activities from 0.03 to 30 microcuries/cm^2. A plain copper strip serves as a control. X2

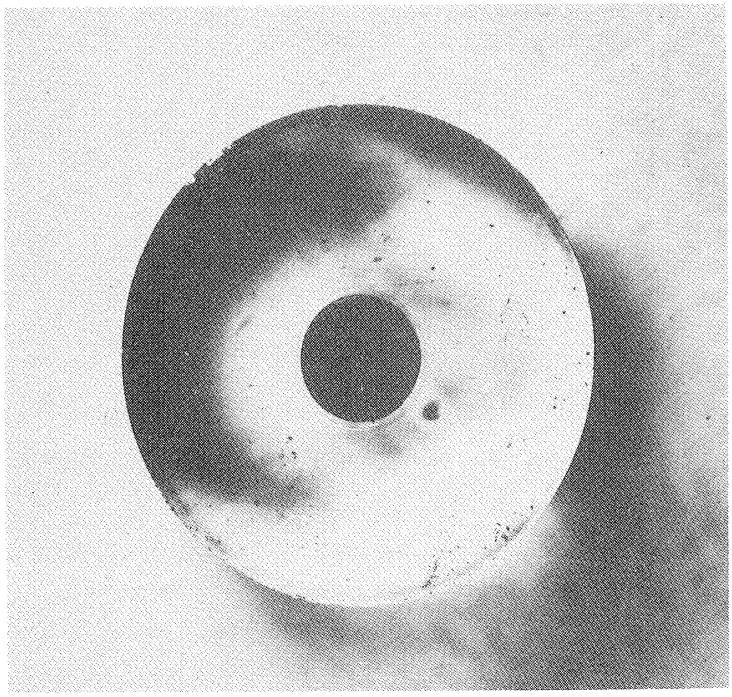


Fig. 9. Radioactive Thallium source mounted flat in lucite. Metal lies directly under surface. X2

no results could be detected when the uranium samples were used as sources. One successful reaction was noted for a nickel 63 specimen (figure 11), but this was not reproducible. Negative results were also forthcoming when placing I-131 thyroid tissue in the monomer and, also when spreading the monomer over radioactive thyroid tissue sections which had a relatively high specific activity. Attempts were then made to first, obtain consistent results with the thallium samples and second, to determine whether the reaction noted was chemical rather than radiation-induced in nature. A variety of techniques and solutions were tried in seeking a consistent reaction. These included working with wetting agents, increasing the viscosity of the monomer, and exposing the material at low temperatures and under nitrogen. To date we have not been able to get consistent results.

Two approaches were undertaken to settle the question of chemical action; one was to use a protective layer over the sources, the other was to run our tests with stable material. A protective layer proved to be difficult to achieve as the monomer dissolves away most materials that have heretofore offered acceptable protection. Recently we have found that mylar film and polyethylene film appear impermeable to the monomer, but these materials must be molded to the surface or the liquid monomer seeps under the layer. Experiments with these protected sources are now in progress. Late in the year we obtained sticks of stable thallium and mounted some of this metal in bakelite. Results of the monomer placed over this specimen show a reaction similar to that seen over the radioactive material, but here again results have been sporadic.

These inconclusive results suggested a more detailed study of the action of solutions of this monomer under various doses of external radiation. We are now working with solutions of this monomer prepared in vials and placed

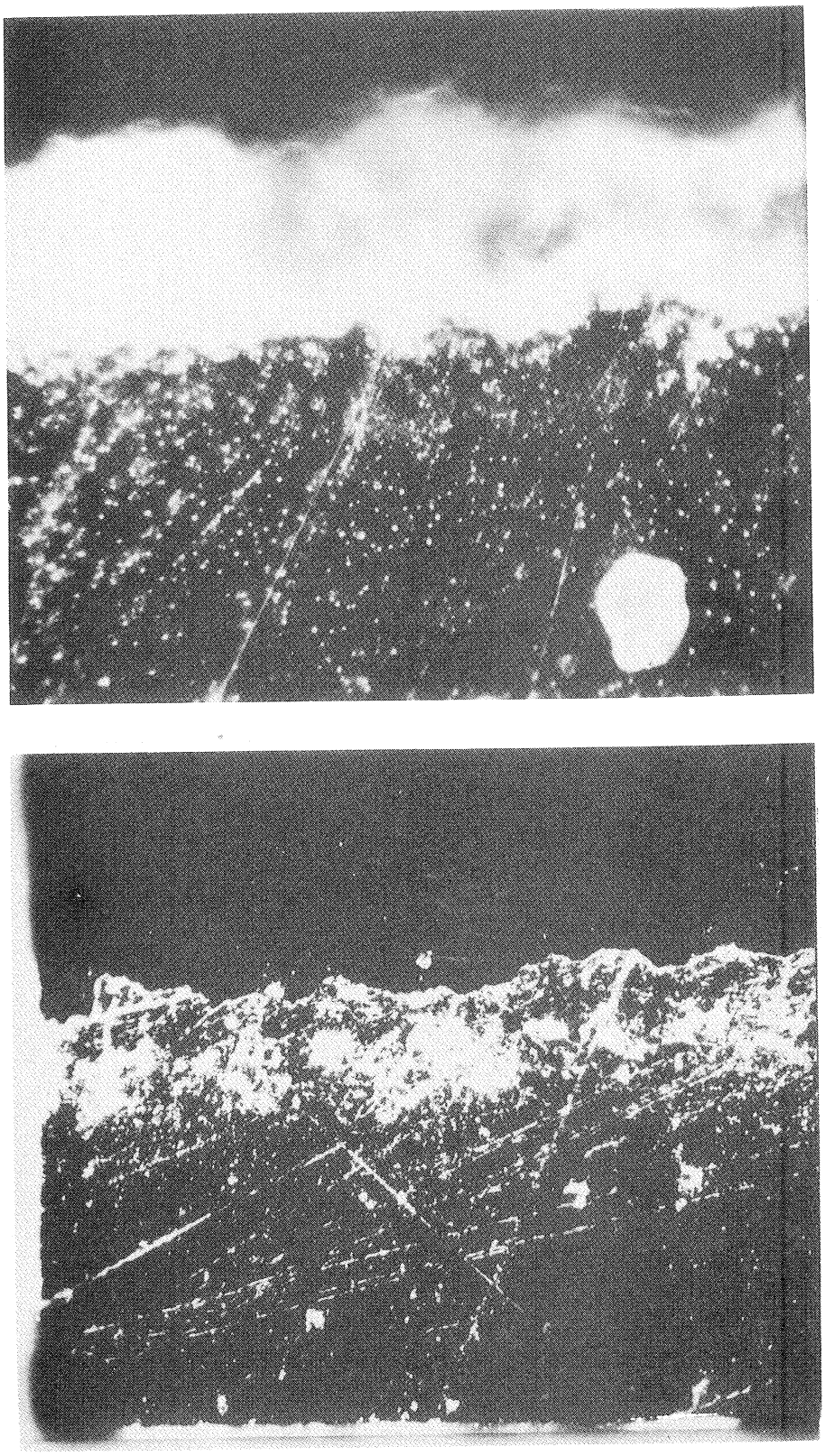


Fig. 10. Top: Selective formation of plastic (Tetraethylene glycol dimethacrylate) over radioactive thallium edge. "Exposure" was for 20 hours, followed by washing in benzene. Dark field (Ultrapak) X780
Bottom: Same specimen before application of plastic. Dark field (Ultrapak) X780

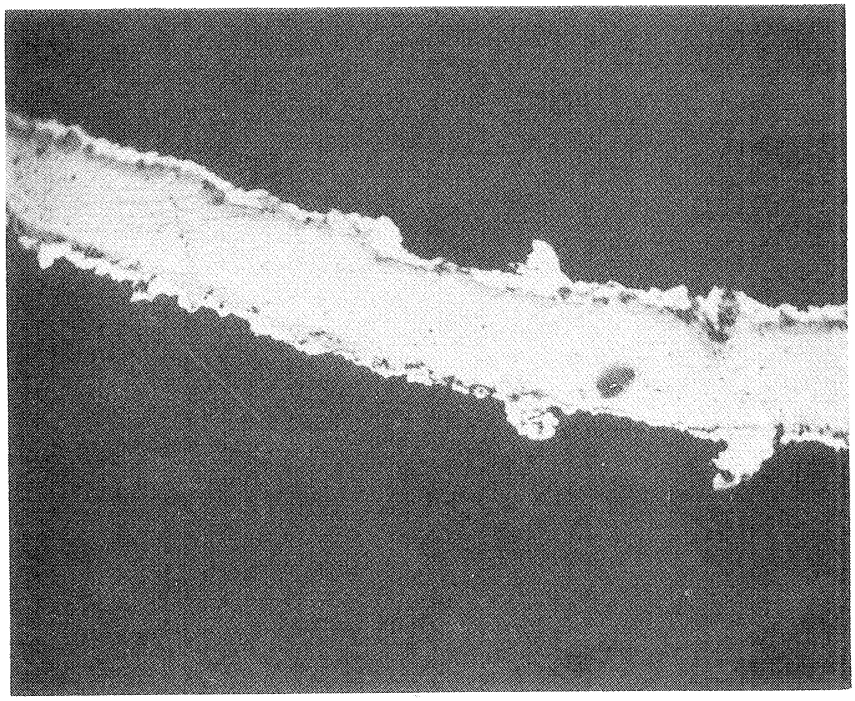


Fig. 11 a) Platinum-Nickel-63 wire specimen before application of monomer (TEGMA). Specimen is made up by plating nickel-63 on platinum wire, followed by a protective silver plate. Dark field (Ultrapak) illumination. X1000

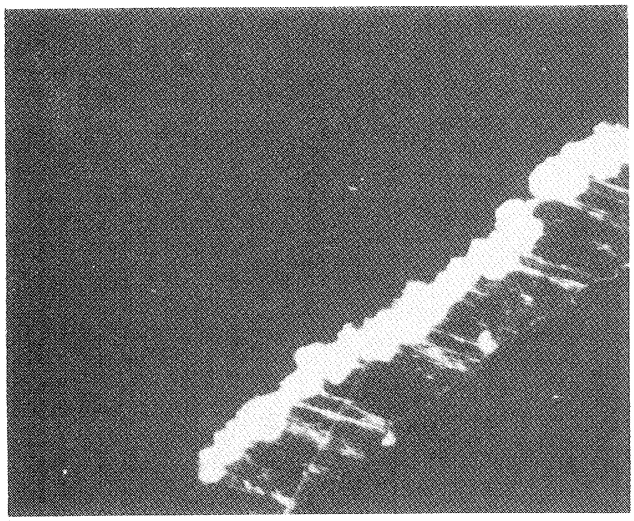


Fig. 11 b) Formation pf plastic (TEGMA) over section of Platinum-Nickel-63 wire. "Exposure" was for six days, followed by benzene wash. Dark field (Ultrapak) illumination. X1000

under various atmospheric conditions. Addition of chemical sensitizers is also being investigated.

The other monomers noted above are also being studied under these conditions, in an attempt to determine the optimum conditions for radiation-induced polymerization. It is then intended to work with the metallurgical and biological materials using the monomers under these conditions.

C. Spark Discharge Tests.

Another potential technique for obtaining high resolution autoradiography was investigated during the past year. This work was concerned with adapting a spark counter to obtain autoradiographs. Work by K. S. Lion (4) at M.I.T. showed that photographic sensitivity could be affected by an electrical discharge. This method essentially makes use of an electrical discharge which is triggered by incident radiation and acts upon a photographic emulsion.

For adapting this principle to our work, we had planned to use the emitting radiation from a radioactive material to initiate the electrical discharge which could then effect some detection device such as sparking a small hole in a thin paper. For the preliminary experiments a piece of photographic paper was used and placed over a radioactive thallium source. These were placed in an airtight lucite box fitted with brass plates - one of them adjustable for varying the plate distances (Figure 12). Experiments were run at various pressures and voltages, in an attempt to determine whether sparking would occur selectively to the radioactive source. These trials proved unsuccessful, as our control specimens were of a different material and, hence, different in their threshold potentials for spark initiation. In addition, we encountered considerable difficulty with respect to field nonuniformity arising from irregular surfaces and edge effects.

Considerable further work needs to be done here with regard to improving the counter itself and finding a suitable means of detecting the spark action. Research was temporarily interrupted in order to devote full attention to the polymerization reactions. However, it is planned to go ahead again with this project in the near future.

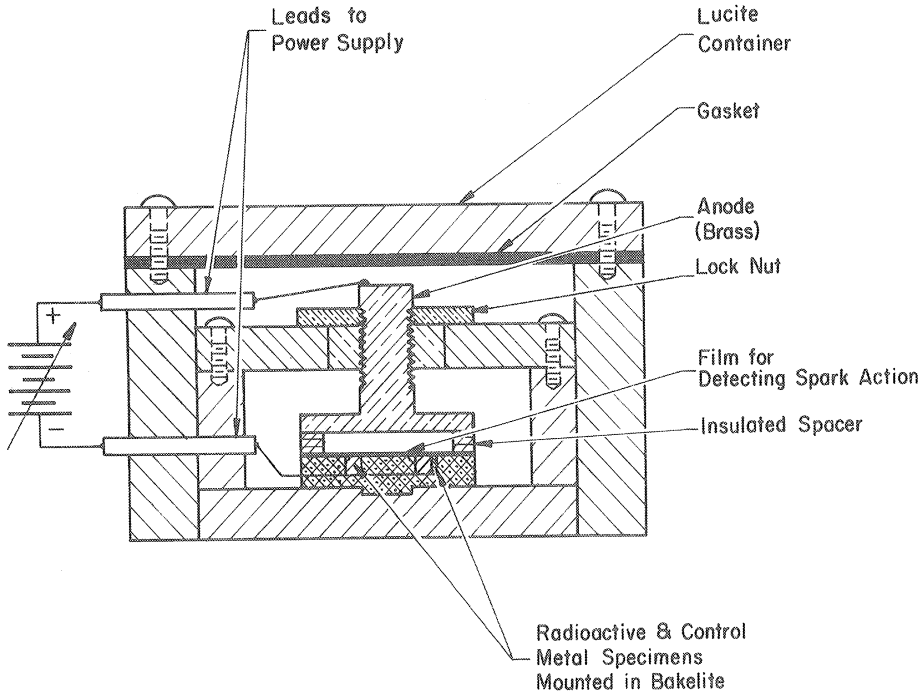


Fig. 12a. Sketch of Spark Counter

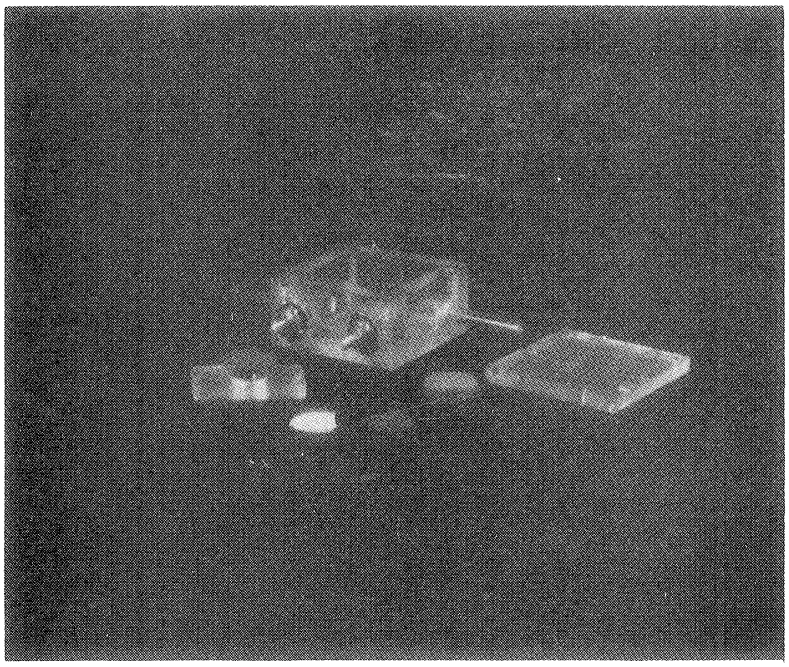


Fig. 12b. Spark counter disassembled showing lucite container, plates, thin film, and photographic paper. The latter two were used for detection of spark action.

IV. APPLICATIONS

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

Work has been continued on the cooperative project with Dr. Clement L. Markert, Director of Phoenix Project No. 56, and Dr. Glenn Fischer. The object of this study, as outlined in last year's report, is to determine the manner in which genes produce their effects during development. It is hoped that by identifying particular substrates used in the synthesis of melanin pigments by cells of different genetic makeup, correlation can be drawn between gene structure and enzyme specificity as revealed by the nature of the substrate acted upon.

Last year's report outlined the experiments using radioactive tyrosine and tryptophane in tissue cultures containing cells which synthesized melanin. Results noted by autoradiography indicated that these substances were not precursors. However an autoradiograph showing resolution of the radioactivity to the parts of the cell containing melanin was obtained (Figure 13). Radioactive tyrosine was incubated with tyrosinase under conditions which should lead to an accumulation of oxidized intermediate products on the way to melanin formation. This radioautograph was obtained when such a solution was cultured with chick skin. The autograph was probably due to a precursor of melanin produced by the enzymatic oxidation of tyrosine.

During the past year, tests for melanin synthesis have been carried out using C-14 Dopa as the substrate. Experiments have been completed using mouse skin in vivo and in vitro, frog embryo, chick epidermis and skin, and pigmented layers of the chick retina. The procedure follows that described in last year's report. Results to date obtained by autoradiography suggest that Dopa is not a precursor of melanin. Research is continuing on the problem.

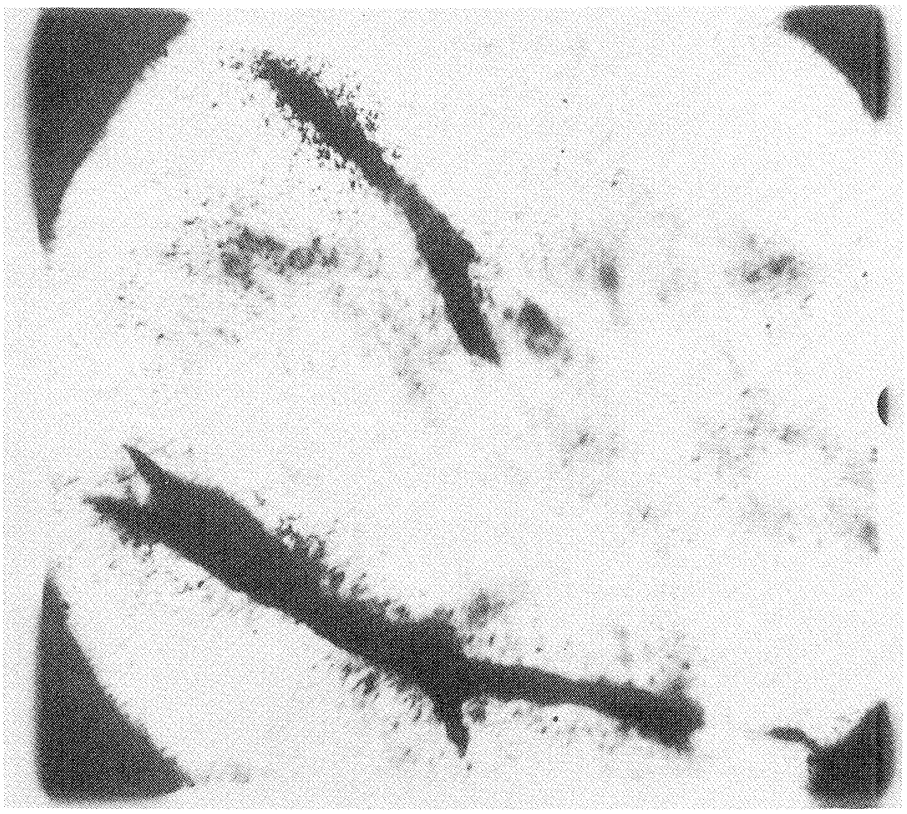


Fig. 13. Autoradiograph localizing carbon-14 to melanin areas of chick-skin cells. The results seen here are believed to be due to a nonmelanin product of tyrosine oxidation by tyrosinase. X960

B. Autoradiography Applied to Metallurgical Problems

The autoradiographic technique has also been widely utilized by groups of the University studying various metallurgical problems. We have been able to offer advice and assistance in adapting the techniques of autoradiography to some of these problems. Two specific investigations are noted below.

In a study of the diffusion of nickel into iron, as noted by autoradiography, the gamma state of the nickel was seen to penetrate through the boundaries of the small grains of austenite. Figures 14 and 15 clearly illustrate this action. No metallographic evidence of this structure sensitive diffusion has been previously detected to as great a depth as is evidenced here.

Work on the copper-bismuth system showed that bismuth exists at the grain boundaries of the copper. On subsequant cold rolling and recrystallization the bismuth did not shift to the new boundaries but remained in situ and appears at the center of new grains. Here again, the autoradiographic technique is far superior to metallographic technique for indicating the presence of bismuth in the system, as noted in figures 16 and 17. Studies on this system have also shown that the grain boundary angle is the controlling factor in determining the depth to which bismuth will diffuse into copper.

C. Studies of Incorporation of P-32 in Onion Root Tip Cells.

In the latter part of the year, an experiment was undertaken in this laboratory to correlate the uptake of P-32 in plant cells with the mitotic activity of these cells, as determined by the techniques of autoradiography.

The original work in this study was reported by Howard and Pelc (5) who used Vicia faba seedlings. We are working with onion root tips and following the general procedure described by Pelc. The cells are incubated in $NaH_2P^{32}O_4$ solution - with 16 mg/l of carrier phosphate. The activity is

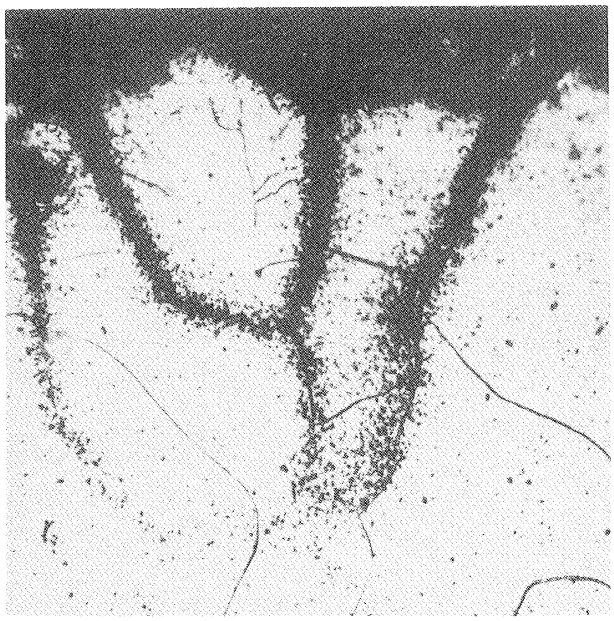


Fig. 14. Stripping film autoradiograph showing grain boundary diffusion of radioactive nickel into iron at 1800°F. X500

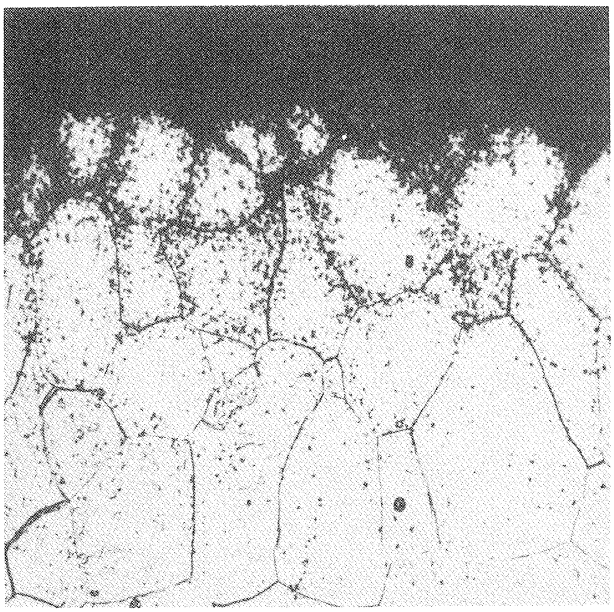


Fig. 15. Diffusion of radioactive nickel into iron at 1300°F. Stripping film autoradiograph. X500

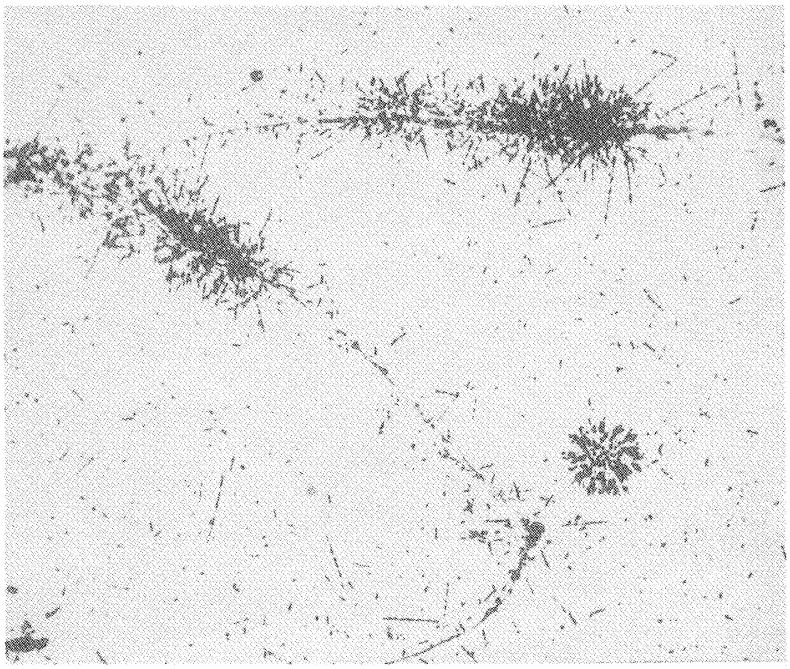


Fig. 16. Stripping film autoradiograph of copper containing radioactive bismuth. X500

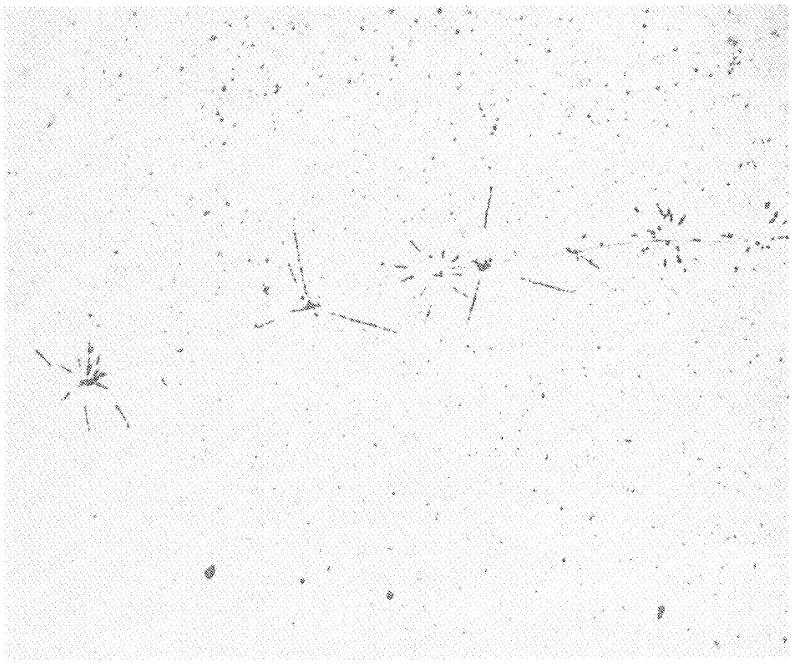


Fig. 17. Stripping film autoradiograph showing grain boundary diffusion of radioactive bismuth into copper at 1200° F. X500

adjusted so that concentration (microcuries) x time (hours) is 4.8. After incubation, the tips are fixed in 3:1 solution of acetic acid and ethanol, hydrolyzed in 1 N HCl in alcohol (1:1), and reimmersed in a fresh solution of 3:1 acetic acid and ethanol. They are then stained with aceto-orcein and squashed on a saran-coated slide under a thin film of polystyrene. When dry the polystyrene is peeled off and the slides are dipped in 2% saran solution. The samples are then counted with an end window G.M. tube and stripping film applied.

Good autoradiographs have been obtained from cells after incubation of 3 and 6 hours. Quantitative analysis and correlation with mitotic activity will be carried out.

III. PROPOSED RESEARCH

General Introduction

The ever widening use of autoradiography in the study of fundamental scientific problems has led to a need for continued research in the improvement and refinement of this technique. Of equal importance is the need for the investigation of new methods of high resolution detection utilizing the mechanisms associated with the high energy radiation of radioactive tracers.

The possibility of adapting the autoradiographic method to electron microscopy work and of obtaining three dimensional type autoradiographs has recently presented itself through research in our laboratories. It is felt that work should be continued on these projects. There is also need for continued research on the radiation microscope presently in the advanced stages of development.

The specific projects planned are presented below:

A. Microscope

Investigation of the Beta-Ray Microscope beyond that described in COO-51 has continued. The details of this investigation to date have been described in two Progress Reports dated February 4, 1954, and March 12, 1954.

It is proposed to continue this investigation along the following lines for the next year:

(1) The optical system will be modified to allow the placement of the field defining diaphragm ahead of the light splitter. This involves forming an image ahead of the light splitter and will probably require a special lens design. This problem has been discussed with the American Optical Company and Bausch and Lomb. Both have indicated that since it is off the beaten path, they are not in a position to undertake it. Correspondence with foreign optical companies is planned.

(2) The investigation of phosphors will continue. This will include

both the characteristics of new phosphors, such as the mercurated polystyrene developed by Cowan, and methods of preparing thin crystals of anthracene.

(3) Work is being done toward making that part of the optical system, used in radiation detection, light tight. This would eliminate the need for enclosing all of the detector in the presently-used, light-tight box.

(4) The electronic circuits used in the system are being consolidated into a more compact arrangement. Additional work will be done on electric filters, designed to decrease the external pickup, and consequently increase the sensitivity of the detector. Effort is being made to secure a small head-on cathode-type phototube from both RCA and Dumont as this should simplify the problem of getting light from optical system to photocathode.

B. Autoradiography

During the coming year we will continue to work with the stripping film technique -- trying to improve our methods for obtaining good autoradiographs. We have recently obtained some stable and radioactive chlorella and plan to use these materials as test specimens for checking techniques. The Saran protecting layer as now used has recently led to poor observation of the tissue preparations -- and work to eliminate this problem is planned.

C. New Techniques

In our research with new techniques of high resolution autoradiography, the following projects are planned for the coming year:

(1) We will continue efforts toward obtaining the very thin dry collodion films for application to electron microscope autoradiography. A variety of solutions and techniques believed useful for loading these films with high uniform densities of silver halide is planned.

(2) Work with the radiation induced polymerization systems will be carried further during the coming year. Two water soluble materials, N-N' methylene-bis-acrylamide and methacrylamide are being investigated, in addition

to the liquids noted earlier. These materials will be studied using x-irradiation, protected radioactive thallium sources, and radioactive metallurgical and biological specimens. We plan to do research with regard to determining the optimum conditions for radiation induced polymerization, such as atmospheric pressure, presence of oxygen, pH of solution and introduction of sensitizers. Various techniques for the application of these materials to radioactive specimens for obtaining autoradiographs will also be considered.

(3) The preliminary research utilizing the principle of spark discharge will be extended. We plan to attempt first to duplicate the results of Lion, as noted earlier, and go on from there to using radioactive sources instead of the x-irradiation used by him.

D. Applications

Applications of the autoradiographic techniques to several biological problems are also planned. We will continue the experiment now in progress concerned with the uptake of P-32 into plant cell nuclei, obtaining quantitative data correlating radioactive cells with the particular stages of the mitotic cycle. Further research related to plant physiology as determined by autoradiography technique is contemplated. An experiment is also planned to study the uptake of C-14 in leukocytes as indicated by autoradiography in work related to that of Skipper (6).

This laboratory, in the past, has offered assistance and advice to members of the various departments of the University who are interested in applying autoradiography to their particular research problems. We will continue to act in this capacity during the coming year.

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